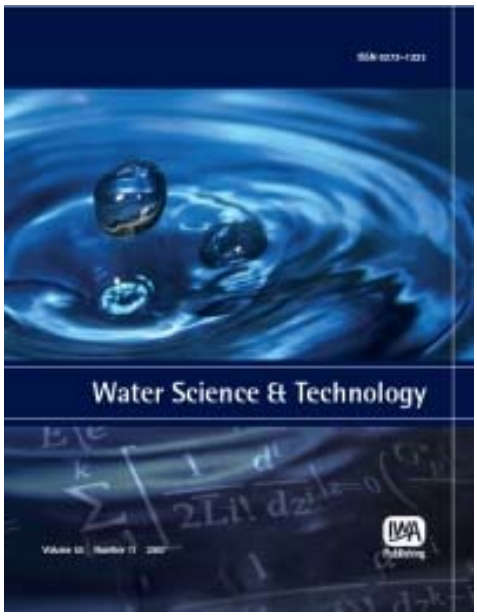

Addition of a magnetite layer onto a polysulfone water treatment membrane to enhance virus removal

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ABSTRACT

The applicability of low-pressure membranes systems in distributed (point of use) water treatment is hindered by, among other things, their inability to remove potentially harmful viruses and ions via size exclusion. According to the USEPA and the Safe Drinking Water Act, drinking water treatment processes must be designed for 4-log virus removal. Batch experiments using magnetite nanoparticle (nano-Fe_3O_4) suspensions and water filtration experiments with polysulfone membranes coated with nano-Fe_3O_4 were conducted to assess the removal of a model virus (bacteriophage MS2). The membranes were coated via a simple filtration protocol. Unmodified membranes were a poor adsorbent for MS2 bacteriophage with less than 0.5-log removal, whereas membranes coated with magnetite nanoparticles exhibited a removal efficiency exceeding 99.99% (4-log). Thus, a cartridge of PSf membranes coated with nano-Fe_3O_4 particles could be used to remove viruses from water. Such membranes showed negligible iron leaching into the filtrate, thus obviating concern about coloured water. Further research is needed to reduce the loss of water flux caused by coating.

Key words | electrostatic adsorption, nanomagnetite, virus removal, water filtration

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INTRODUCTION

The use of low-pressure membranes for water treatment is growing worldwide. However, the application of these technologies faces some limitations that include their inability to remove ionic species and nano-sized particles such as viruses. An estimated 31 million people contract virus-related gastrointestinal illnesses each year, of which only about 9 million are food-related (Mead *et al.* 1999). Groundwater, previously thought to be naturally pristine, has been found to contain enteric viruses (transported from faulty septic tanks, landfills, fields treated with waste sludge, latrines, or contaminated waterways), even in confined aquifers (Borchardt *et al.* 2007). Enteric viruses have also been detected in treated drinking water (Keswick *et al.* 1984; Lee & Kim 2002; Vivier *et al.* 2004; Ehlers *et al.* 2005) and recent studies indicated that enteric viruses were the leading causative agents of waterborne diseases in the USA and worldwide (Griffin *et al.* 2003; Fong & Lipp 2005). Therefore, the presence of enteric viruses in drinking water sources is a growing public health concern necessitating an effective, simple removal technology.

Viral particles are small (20–200 nm in size) and enteric viruses (e.g. *Norovirus*, hepatitis A virus, *Enterovirus*), which are usually sized 20–30 nm diameter, are among the most difficult water-borne microorganisms to remove (Langlet *et al.* 2009). The virion of most enteric viruses consists of nucleic acid genome encapsulated by a capsid composed of proteins containing weak acid and base groups (e.g. carboxyl, sulfhydril and amine groups) that are ionizable (Brown & Sobsey 2009). In natural aquatic environments viruses are charged biocolloidal particles with the ability to adsorb to solid surfaces, which influences viral fate and transport. Factors controlling the adhesion kinetics of viruses include the type of viruses and the associated surface properties: pH, ionic strength, degree of water saturation in soil, and the presence or absence of interfering substances such as natural organic matter (NOM), which may either adsorb the viruses or compete with them for adsorption sites on a surface (Bitton *et al.* 1976; Hurst *et al.* 1980; Schijven & Hassanizadeh 2000; Chu *et al.* 2003; John & Rose 2005).

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The net charge of a virus depends on the pH of the medium and the surface chemistry of the virus. The isoelectric point (IEP) is specific to the individual virus type and strain. Typically viruses have IEP in the range of 3–7 (Dowd *et al.* 1998); thus, they may be either positively or negatively charged in natural waters (pH 4–9). In most cases, viruses are negatively charged and positively charged surfaces can adsorb and possibly inactivate them in aqueous systems based on electrostatic interactions.

Virus adsorption onto different solid surfaces such as hematite, clays, activated carbon, ceramic modified materials and iron oxides/hydroxide species is well documented. Non-pathogenic model viruses, such as bacteriophages MS2, PRD1, and phi X174, and the enteric virus Rotavirus have been used to investigate virus transport in different media. Viruses are known to adsorb onto iron oxides commonly present in soil and artificially incorporated into filtration media. Magnetite sand and hematite particles have been shown to be effective filtration media for poliovirus removal (Moore *et al.* 1981). Ryan *et al.* (2002) also reported increased removal of PRD1 and MS2 in a sand column by coating quartz sand with iron oxides. Effluent analysis indicated that the viruses had attached strongly to the medium, the viruses had been inactivated (potentially by the strong attractive force between the capsid and ferric oxyhydroxides), and the remaining virus nucleic acids were released into the effluent. Chu *et al.* (2003) found that viruses are removed effectively in soils that contained iron oxides, and that the most influential environmental factors for virus removal in addition to the presence of iron were pH, NOM, metal oxides contents and soil saturation with water. Bitton *et al.* (1976) suggested that magnetite was a good adsorbent even at low concentrations (300 ppm) and considered various environmental factors affecting adsorption of poliovirus in water and wastewater onto magnetite. Rao *et al.* (1981) used magnetite in conjunction with pH adjustment (to pH3) and 0.0005 mol/L $AlCl_3$ to effectively adsorb and concentrate poliovirus I for coagulation. High retention capacities for MS2 have been reported using magnetite treated with successive cycles of acid and alkali washing (Atherton & Bell 1983).

NOM in particulate and dissolved form decreases the retention capacity of soil for MS2. Gutierrez *et al.* (2009) showed high removal of Rotavirus and MS2 by glass fibre coated with hematite nanoparticles in batch and flow-through experiments. However, virus adsorption decreased in the presence of NOM and bicarbonate ions. Modified media such as ceramics containing Fe and Al oxides can enhance the virus adsorption and inactivation through sorption processes (Brown & Sobsey 2009).

Overall, these past studies show that incorporating iron oxides into water filtration systems could enhance virus removal. Furthermore, manipulations to develop positive surface charges from the protonation of iron oxides (e.g. by decreasing pH) can facilitate electrostatic attraction of negatively charged viruses and enhance their removal efficiency. However, the potential of virus removal by incorporating iron oxides into polymeric low-pressure membranes has received limited attention, and little is known about the effect of water chemistry on this approach.

This paper considers the incorporation of magnetite nanoparticles (nano-Fe_3O_4) into polymeric microfiltration membranes, creating a one-step treatment for virus removal. Two types of experiments were conducted at bench scale (1) batch adsorption experiments using magnetite nanoparticles suspensions to assess virus adsorption capacity in the presence of common inorganic ions (Na^+ and Ca^{2+}) on virus adsorption capacity, and (2) membrane filtration experiments using polysulfone (PSf) membranes coated with nano-Fe_3O_4 to assess virus removal efficiency and iron leaching. Results suggest that nano-Fe_3O_4 coated membranes could potentially be used in point of use devices or small membrane systems for virus removal to avoid formation of harmful disinfection by products associated with the use of chemical disinfectants.

MATERIALS AND METHODS

Membrane coating with nano-Fe_3O_4

Nanomagnetite-coated PSf membranes (nFe_3O_4-PSf) were synthesized as follows. Samples of a commercial PSf membrane (0.2 μm mean pore size, 47 mm diameter, HT Tuffryn; Pall Co.) were cut to coupons of 25 mm in diameter and soaked in 100% ethanol solution for 10 min to fully wet the membrane. Then magnetite nanoparticles (Sigma-Aldrich, nanopowder <50 nm particle size, BET (Brunauer, Emmett and Teller) surface area >60 m^2/g, ≥98% purity) were coated onto the membrane surface by filtering 3 mL of a nanomagnetite suspension in ethanol at a concentration of 1 g/L through the membrane at a flow rate of ~1 mL/min. This resulted in a total iron content of 3.9% by weight. Prior to filtration, the nanomagnetite suspension was sonicated for 5 min using a probe sonicator (Sonic Ruptor 250 Ultrasonic Homogenizer, Omni International; Kennesaw, GA) and for 10 min using a bath sonicator (Branson Ultrasonic 5,510; Danbury, CT) to ensure homogeneous magnetite nanoparticle dispersion. The membranes without rinsing

were then dried in the oven at 100 °C for 30 min and stored at 4 °C. To assess the capacity of the membrane to retain the nanomagnetite particles on the membrane surface and in membrane pores during typical microfiltration processes, the coated membrane samples were subject to two different rinsing protocols: superficial rinsing thoroughly with deionized (DI) water for 5 min and/or transversal rinsing by filtering 30 ml of DI water through the membrane for 10 min. These two rinsing protocols simulate the hydraulic condition encountered during cross-flow and dead-end filtration, respectively.

Membrane characterization

Permeability and contact angle

Membrane permeability was determined by measuring the DI water flux at room temperature in an Amicon Stirred cell over a working pressure range of 5–25 psi. The flow was measured using a digital scale that monitors cumulative permeate volume as a function of time. Membrane hydrophobicity was assessed by sessile drop contact angle measurement of DI water using a contact angle analyzer (DROPImage Standard).

Iron concentration in the permeate

Iron leeching from the membrane was evaluated by analyzing the effluent (permeate) for total iron concentration using inductively-coupled plasma optical emission spectrometry (Perkin Elmer Optima 4300DV, Norwalk, CT). Samples (4 mL) were preserved with 1% HNO_3 prior to analysis.

Virus analysis

Preparation and quantification of MS2

Bacteriophage MS2 (ATCC#15597-B1) was used as a model waterborne virus. MS2 has capsid properties similar to those of poliovirus (Badireddy *et al.* 2007) and is commonly used as a surrogate to evaluate human enteric virus removal (You *et al.* 2005). MS2 is a non-enveloped icosahedral single-strand RNA coliphage, with a diameter of 26.0–26.6 nm (VanDuin 1988) and an IEP of 2.2–3.9 (Zerda *et al.* 1985; Yuan *et al.* 2008; Gutierrez *et al.* 2009). A low IEP indicates a high net negative charge on the virus surface at typical pH values of natural water. Langlet *et al.* (2009) discussed the physico-chemical characteristics of MS2 phage, identifying it as a worst-case scenario for the evaluation of virus removal by membrane filtration (i.e. (i) small size, (ii) high negative surface charge, and (iii) high degree of hydrophobicity).

MS2 was propagated according to the method described by Zhu *et al.* (2005). To propagate MS2, the bacteriophage (100 μL) was incubated with 100 μL of its *Escherichia coli* host (ATCC 15597) with a concentration of 4×10^8 CFU/mL for 10 min in 900 μL 0.1 mol/L bicarbonate buffer (pH 8.3). Then, warm tryptic soy soft agar was added to the suspension and the mixture was deposited onto a Luria-Bertani agar plate using the agar-overlay technique (Kennedy *et al.* 1986). After incubation overnight at 37 °C, the viruses were removed from the plate with bicarbonate buffer. Approximately 5 mL of the viral suspension was added to the plate and left to incubate for 10 min. This suspension was then removed and centrifuged at 5,000 rpm for 1 min, and the supernatant was filtered through a 0.2 μm polyethersulfone filter. The resulting viral suspension was stored at 4 °C until use. The viral stock concentration was determined by the standard plaque forming units (PFU) assay (ISO-10705-1 1995). Viruses were detected by the formation of clear zones (plaques) on the bacterial mat. Dilutions exhibiting 20–300 plaques per plate were considered for MS2 enumeration. All virus assays were performed in duplicate, and the virus concentration was reported by averaging the number of plaques from two replicate plates. Removal was calculated as a logarithm of the ratio of infectious units (PFUs) in the permeate to those in the feed solution.

Virus adsorption onto magnetite nanoparticles

Magnetite nanoparticles for batch adsorption experiments were purchased from READE advanced materials, Reno, NV. The nominal size range of the nanoparticles was from 20 to 30 nm. Surface area of the magnetite nanoparticles was determined by BET surface analyzer to be 69.4 m^2/g. Suspensions of magnetite at different concentrations were prepared by adding different amounts of magnetite powder to 15–100 mg/L background electrolyte solution to a final concentration of 1 g/L. The pH of the suspension was then adjusted to pH 6 by adding 0.034 mol/L of HCl or NaOH. The suspensions were ultrasonicated for 30 s using a bath sonicator (Branson Ultrasonic 5510; Danbury, CT) immediately prior to the adsorption experiments. To assess electrostatic attraction as a potential virus removal mechanism, the zeta potential of the magnetite nanoparticles in all test solutions was measured using a *ZetaSizer* Nano ZS (Malvern, Inc., Southborough, MA). The magnetite nanoparticles were found to be positively

charged with the surface zeta potential in the range of +12.5 to +16 mV, suggesting electrostatic attraction between the magnetite nanoparticles and the negatively charged MS2.

The MS2 stock was diluted into the magnetite suspensions to a final concentration of 10^6 PFU/mL in 20 mL glass vials. These vials were shaken at 250 rpm at room temperature for 1 h. At predetermined time intervals, 1 mL samples were taken from the suspension and placed into a 1.5 mL vial. A horseshoe magnet was placed under the vial to separate the magnetite nanoparticles from the aqueous phase. The supernatant was then serially diluted, and the virus titre quantified using the agar overlay technique. Control experiments were conducted simultaneously using four different buffer solutions at pH 6: 1.7 mmol/L NaCl + 1 mmol/L $CaCl_2$, 1.7 mmol/L NaCl + 2 mmol/L $CaCl_2$, 3 mmol/L NaCl or 4 mmol/L NaCl.

Virus adsorption onto magnetite-PSf membranes

Membrane filtration experiments were conducted to evaluate the MS2 adsorption capacity of the PSf UF membranes coated with magnetite nanoparticles (nFe_3O_4-PSf). For every test, 3 mL of viral suspension in 0.1 mol/L bicarbonate buffer (adjusted to pH 8.3) were used. The viral suspension with a concentration between 10^6 and 10^7 PFU/mL was filtered at a flow rate of ~1.5 mL/min through a 25 mm nanomagnetite coated membrane coupon using a membrane syringe filter, corresponding to a volumetric flux of 3×10^{-3} m^3/m^2-min. Prior to each test the membrane sample was rinsed superficially and transversally by filtering DI water through the membrane. Samples of viral solution were taken from the feed (influent) and permeate (effluent) streams, and were subsequently serial-diluted according to the protocol described by Zodrow *et al.* (2008) and quantified by the PFU method. Each filtration experiment was carried out at least in duplicate. Control experiments for MS2 filtration on non-coated PSf membranes were performed to provide a baseline for virus removal by the PSf membrane alone.

A continuous flow experiment was also conducted to evaluate virus breakthrough at a flow rate of ~1.5 mL/min through a 25 mm nanomagnetite coated membrane coupon using a membrane syringe filter (volumetric flux of 3×10^{-3} m^3/m^2-min). About 20 mL of viral suspension was filtered continuously through the membrane, and 3 mL permeate samples were collected. Virus removal was calculated as a logarithm of the ratio of infectious units (PFUs) in the permeate to those in the feed solution.

RESULTS AND DISCUSSION

Virus adsorption onto magnetite nanoparticles (Batch) experiments

The effects of the divalent Ca^{2+} versus monovalent Na^+ cations on MS2 removal were compared (at pH 6) to investigate the effect of common inorganic cations on virus adsorption. The effect of ionic strength was also investigated by varying the salt concentration. Increasing the ionic strength with NaCl from 3 to 4 mmol/L decreased removal (Figure 1). This is likely due to the greater charge screening effect at higher ionic strength, and consequently reduced electrostatic attraction between the negatively charged viruses and the positively charged magnetite nanoparticles. The removal of MS2 by 1 g/L magnetite nanoparticles increased when the divalent calcium ion Ca^{2+} was present. Ca^{2+} was more effective than Na^+ in promoting virus removal (i.e. 2.7-log removal of MS2 was achieved in the presence of Ca^{2+} compared to less than 0.5-log removal in the presence of Na^+ alone). This could be attributed to two factors: (1) Ca^{2+} promotes virus coagulation to form complexes, and (2) a small number of negatively charged sites exist on the overall positively charged magnetite surface. Ca^{2+} forms ionic bridges between the few negative charge sites on the magnetite surface and those on the MS2 capsid.

These results corroborate previous studies reporting that divalent cations enhanced the deposition of MS2 on to NOM-coated silica surface and silica (Pham *et al.* 2009). Apparently, Ca^{2+} promotes electrostatic attraction due to its tendency to form complexes on the adsorbent surface, which bind to negatively charged carboxylate groups on the viral capsid proteins.

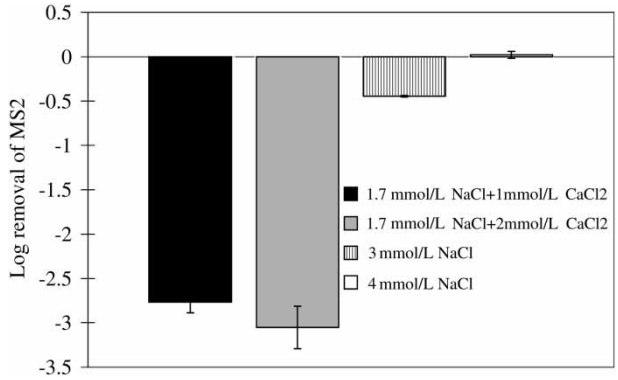

Figure 1 | Removal of MS2 by 1 g/L magnetite nanoparticles at pH 6 after 1 h incubation in different electrolyte solutions. Initial virus concentration was 10^6 PFU/mL.

Membrane characterization

PSf membranes coated with ~3 mg of nanomagnetite (nFe_3O_4-PSf) had permeability three times lower than the control membrane (PSf) without nFe_3O_4 (Table 1). The decrease in membrane permeability due to the hydraulic resistance imposed by the nanomagnetite coating layer represents a concern for the higher energy requirement for membrane filtration. On the other hand the nFe_3O_4 membrane was slightly more hydrophilic than the control sample with a contact angle 7% smaller than that of the control PSf membrane. This decrease in hydrophobicity has potential benefits in preventing membrane fouling (Cheryan 1998).

Virus adsorption onto nanomagnetite-coated (nFe_3O_4-PSf) membranes

Significant virus removal (>99.99% with average virus log removal of 4.4 ± 0.5 ($n = 9$)) was observed when 3 mL of viral suspension was filtered through membranes coated with magnetite nanoparticles, a dramatic improvement relative to the control membranes without magnetite, which only showed less than 0.5 log removal (Figure 2). The removal of virus by nano-Fe_3O_4-PSf membrane is a novel significant finding.

Two mechanisms of virus removal were considered (1) size exclusion through the nanomagnetite coating (potentially clogging pores or decreasing the pore size), and (2) electrostatic adsorption of the viruses to the magnetite nanoparticles. A continuous flow experiment was carried out to evaluate n-Fe_3O_4-PSf membrane performance for MS2 removal and to determine the main virus removal mechanism. A 4.5-log removal was initially observed (Figure 3(a)). The MS2 breakthrough curve is presented in Figure 3(b). The allowable breakthrough concentration (representing 4-log virus removal) was reached after 18 mL of filtrate was collected. The decreasing removal efficiency and increasing effluent virus concentration with increasing cumulative filtrate volume suggests that adsorption likely via electrostatic interaction instead of size exclusion is the major removal mechanism. Adsorptive removal efficiency decreased as more and more adsorption sites were occupied. Size exclusion, which would result in a stable or increasing (due to pore blockage) removal, did not seem to be the predominant mechanism.

The permeate volume at breakthrough corresponds to approximately 25 min of filtration time at a typical permeate flux of 50 gallons per square foot per day (GFD), suggesting that the n-Fe_3O_4 coating approach could be sustainable if the magnetite nanoparticles can be regenerated at each backwash by adjusting the solution chemistry (e.g. pH) of the backwash water. Adsorption capacity can be defined as the number of infectious virus particles (PFU) adsorbed

Table 1 | Basic properties of the PSf and nFe_3O_4-PSf membranes. (Values presented as average ± range; $n = 2$)

	PSf	nFe_3O_4-PSf
Permeability ($L/m^2/h/psi$)	222 ± 6.4	70 ± 5.1
Contact angle (°)	70 ± 6.8	65 ± 6.3

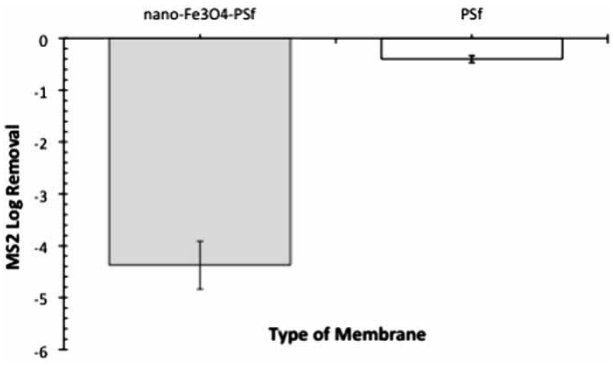

Figure 2 | Virus removal by the addition of nano-Fe_3O_4-PSf membranes. Initial virus concentration ~10^7 PFU/mL.

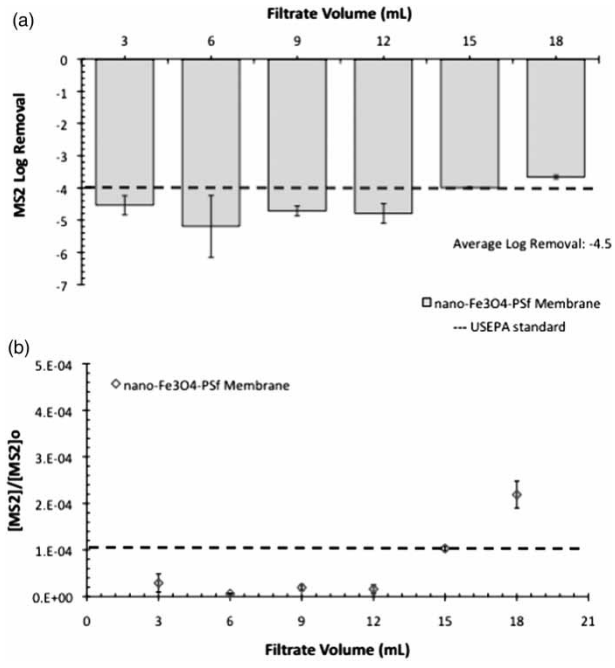

Figure 3 | (a) Removal of MS2 by nFe_3O_4-PSf membranes. Initial virus concentration ~10^7 PFU/mL. (b) MS2 breakthrough curve from flow-through experiments with nFe_3O_4-PSf membranes. The 4-log removal USEPA (United States Environmental Protection Agency) requirement is depicted as a dotted line.

Table 2 | Virus removal by nFe_3O_4-PSf membranes with different rinsing methods

Rinsing membrane method	Log MS2 removal
No rinse	6.0
Superficial	4.3
Superficial/transversal	4.4 ± 0.5

per gram of magnetite nanoparticles in solution (Gutierrez *et al.* 2009). Accordingly, the adsorption capacity for MS2 in a 0.1 mol/L bicarbonate buffer solution (pH 8.3) for 4.5-log (average Log removal of MS2 in our continuous flow experiment) was 3.3×10^9 PFU/g.

Analysis of iron in the filtrate and implications for long-term performance

The average total Fe in the membrane permeate (after filtration of 3 mL MS2 suspension) was 10.6 ± 0.006 μg/L, corresponding to 0.00106% over the total estimated amount of nanomagnetite coated on the membrane (3 mg). The effluent iron concentration was significantly below the 0.3 mg/L secondary standard for drinking water, which was set to prevent aesthetic impacts on colour and taste.

During the operation of a membrane system, the membrane surface and the pore wall are subject to hydraulic shear, which can potentially disturb the nanomagnetite coating layer and release magnetite nanoparticles. Therefore, superficial and transversal rinsing protocols were performed to remove loosely attached magnetite nanoparticles before the filtration experiments. These two rinsing protocols simulate the hydraulic condition encountered in cross-flow and dead-end filtration, respectively. As shown in Table 2, MS2 removal after both rinsing protocols decreased compared to that without rinsing, suggesting the loss of some magnetite nanoparticles during the rinse. However, the rinsed membranes were still able to achieve greater than 4-log removal of MS2, indicating that an effective coating layer remained. These results suggest that the simple coating procedure used in this study may be effectively applied to industrial membrane units. Long term durability testing is needed to determine the lifetime of the coating.

CONCLUSIONS

In agreement with the literature, we showed that magnetite successfully removes viruses by adsorption. Furthermore, PSf membranes coated with magnetite nanoparticles were effective in removing bacteriophage MS2, potentially obviating the need for pre- or post-treatment to remove viruses in a membrane based system. Commercial PSf membranes are notable for their widespread application in water filtration. Cartridges of PSf membranes coated with nano-Fe_3O_4 particles may be an option to consider as point-of-use devices and a modification to existing membrane filtration processes to remove viruses from water. Advantages of nanomagnetite coating of PSf membranes include the simple coating protocol, avoidance of harmful disinfection byproducts, and negligible iron leaching into the filtrate. Due to the limited virus adsorption capacity of membranes prepared in this fashion, regeneration of the nanomagnetite material is necessary for this approach to be sustainable.

Although these results are promising, much research remains to be conducted to determine the feasibility of such membranes to treat different source waters. Key challenges include coating methods that lower the filtration energy requirement, and backwash processes (e.g. with a basic solution) to regenerate adsorption capacity.

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