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Efficiency of Microfiltration Systems for the Removal of Bacterial and Viral Contaminants from Surface and Rainwater

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Abstract The aim of this study was to evaluate the efficiency of a passive point-of-use treatment system, namely, a polyvinyl (alcohol) (PVA) nanofiber membrane/activated carbon column, for the treatment of harvested rainwater. The efficiency of SMI-Q10 [quaternized poly (styrene-*co*-maleimide)] nanofiber membrane disks placed in a filtration assembly for the treatment of surface water (Plankenburg River, Western Cape, South Africa) and harvested rainwater was also assessed. Two rainwater harvesting tanks were installed at the Welgevallen Experimental farm, Stellenbosch, South Africa, with the filtration system intermittently attached to the tanks for collection of rainwater samples throughout the study period. Parameters used to monitor the filtration systems included heterotrophic bacteria, *Escherichia coli*, and total coliform enumeration and the presence/absence of adenovirus. When compared to drinking water guidelines, the results indicated that 3 L of potable water could be produced by the synthesized PVA nanofiber membrane/activated carbon column. However, PCR assays indicated that adenovirus and numerous bacteria such as *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., and *Yersinia* spp. were not effectively removed by the filtration system utilized. Additionally, the SMI-Q10 nanofiber membrane disks did not remove viruses from the river or tank water samples as bovine adenovirus 3 strain, simian adenovirus, and human adenovirus A strain were detected in all water samples analyzed. Thus, while the microfiltration system was efficient in reducing the level of indicator organisms to within drinking water standards, further optimization of the electrospun filtration membranes is required as molecular analysis revealed that numerous opportunistic bacterial pathogens and viruses persisted after filtration.

Keywords Microfiltration · Adenovirus · Opportunistic pathogens · Indicator bacteria · Nanofiber membrane · Electrospinning · Rainwater harvesting · Surface water

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1 Introduction

Water resources are becoming increasingly scarce throughout the world due to population increases, climate change, and contamination caused by point- and nonpoint source pollution (Alam Imteaz et al. 2011). Alternative and sustainable sources of water must thus

be considered to supply freshwater for domestic and potable purposes (Lévesque et al. 2008). These alternative water resources include stormwater harvesting, gray water, and wastewater reuse as well as desalination. Stormwater harvesting is utilized most often and includes rainwater harvesting and surface water utilization (Alam Imteaz et al. 2011). While these alternate water sources are routinely utilized in many international countries (De Kwaadsteniet et al. 2013), in South Africa, water sources such as rainwater and surface water are severely contaminated, and it is therefore not advisable to use this water as a untreated primary potable water source (Mwenge Kahinda et al. 2007; Dobrowsky et al. 2014). In addition, untreated water could cause diseases, which poses a threat to newborns, young children, the elderly, immuno-compromised people, and people living in unsanitary conditions (Mwenge Kahinda et al. 2007).

In order to provide clean and safe drinking water to rural communities and informal settlements in urban areas, the development of effective water treatment methods are thus required. Traditional methods for treating contaminated water include chlorination, slow sand filtration, UV radiation, and pasteurization (Burch and Thomas 1998). In addition, four membrane filtration techniques, namely, microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO), are currently utilized for the treatment of water sources. Scientists are also becoming increasingly attentive towards the application of polymer nanofibers (class of nano-material) in microfiltration systems because of their high surface-to-mass volume and other special characteristics that make them attractive for advanced applications.

The scientific, technological, and industrial interest in 1-dimentional (1D) nanostructures has increased in the past few years, as they have been shown to have numerous applications, including in air and water filtration (Gibson et al. 2001; Gopal et al. 2007), drug delivery (Hong et al. 2010; Okuda et al. 2010), tissue engineering, and in the fields of regenerative medicine (Koh et al. 2008; Grafahrend et al. 2011). Numerous production and synthesis methods exist, including polymerization against porous templates, self-assembly, and melt blowing. Recent advances have also allowed for the fabrication of nanofibers through electrostatic spinning or electrospinning, and this technology not only allows for the up-scaling of production, but it is classified as a relatively simple technique (Persano et al. 2013). The benefits of being able to up-scale the electrospinning process includes the ability to produce nanofibers of extreme length (up to kilometers long) (Frenot and Chronakis 2003) along with increased surface area and defined porosity (Bognitzki et al. 2001; Luu et al. 2003; Zhang et al. 2005; Welle et al. 2007), the inherent 3-dimensional (3D) structure (Pagliara et al. 2009; Sambaer et al. 2010, 2011; Dhandayuthapani et al. 2012), and the incorporation of specific functional properties (Yang et al. 2012). The electrospinning process occurs in the presence of an intense electric field, typically ranging from 10^5 to 10^6 V/m, with this electric field formed between the spinneret and a conductive collector. A uni-axial elongated jet, which is ejected from the surface of a charged polymer solution, having enough molecular entanglements, is then captured on a rolling target electrode. In tailoring the specific properties and functionalities of electrospun products, many materials and solvents may be combined.

The modification of electrospun nanofibers is thus receiving increased attention due to the unique properties and applications thereof (Doshi and Reneker 1995; Deitzel et al. 2002; Huang et al. 2003; Dersch et al. 2005; Kim and Park 2006; Gopal et al. 2007; Agarwal et al. 2008; Kiekens et al. 2008; Reneker and Yarin 2008). In addition, nanofibers may be modified before or after electrospinning, and although the modification method does not influence the outcome of affinity studies with regards to microorganisms being captured onto the modified nanofiber, research has indicated that modification after electrospinning (surface modification) has numerous advantages over modification before electrospinning (Cronje and Klumperman 2013). Modification before electrospinning may alter the properties of the nanofiber resulting in a change of the electrospinnability (Kim and Park 2006; Greiner and Wendorff 2007), whereas surface modification will not change the electrospinnability of the polymer as the surface modification occurs after the nanofibers have been electrospun. Surface modification only changes the surface of the polymer, while the bulk properties of the original polymer typically remain intact (Chronakis 2005; Yao et al. 2008; Yoo et al. 2009). Another advantage of surface-modification is that less of the chemical reagent is needed to add the modification to the polymer mat, which is more cost effective and is considered a "green" manufacturing process (Yao et al. 2008). Surface modification of a nanofiber can be achieved in different ways; however, chemical immobilization of

these surface-modification agents are preferred over physical immobilization. This is due to the covalent bond formation achieved with chemical immobilization (Agarwal et al. 2008; Yao et al. 2008; Yoo et al. 2009).

The aim of this study was to evaluate the efficiency of microfiltration systems for the treatment of harvested rainwater and surface water. A polyvinyl (alcohol) (PVA) nanofiber membrane/activated carbon column microfiltration water treatment system was thus constructed and evaluated. The microbial parameters that were investigated included total coliform, *Escherichia coli*, and heterotrophic bacterial counts. Adenovirus is currently the most well-studied DNA virus and is usually found more frequently than other enteric viruses in aquatic environments (Pina et al. 1998; Haramoto et al. 2010). They can thus persist for long periods of time and show high resistance against the physico-chemical treatments implemented by sewage treatment plants (Verheyen et al. 2009). Unfiltered tank water samples and PVA nanofiber membrane/activated carbon column filtered water samples were thus screened for the presence of selected potentially pathogenic bacteria as well as the presence/absence of adenovirus (viral indicator), using molecular techniques. A SMI-Q10 [quaternized poly (styrene-*co*-maleimide)] nanofiber membrane disk was produced and also assessed for the removal of adenovirus from surface water and harvested rainwater samples.

2 Materials and Methods

2.1 Sample Site and Collection

Tank water samples were collected from two polyethylene domestic rainwater harvesting (DRWH) tanks (2000 L) that were installed at the Welgevallen Experimental farm, Stellenbosch University, South Africa (33°56′36.19″ S, 18°52′6.08″ E). The DRWH tanks were situated on the northern side of a well-established building that neighbored the farms' dairy. For microbial (bacterial and viral) analysis, tank water samples were collected before and after filtration. In addition, river water samples were collected from the Plankenburg River (estuary of the Berg River system) at a site (33°55′38.977″ S, 18°51′2.188″ E) close to the municipal substation in the industrial area of Stellenbosch, South Africa. The sampling site was situated downstream from Kayamandi, an informal settlement.

2.2 Microfiltration System One: PVA Nanofiber and Activated Carbon Column (Bacterial and Viral Removal Efficiency)

Polyvinyl (alcohol) nanofibers were produced by a process of needleless electrospinning utilizing a Nanospider 200 Lab (Elmarco, s.r.o., Czech Republic). The substrate material onto which the nanofibers were deposited was a nonwoven poly (propylene) spun-bond material (Tyvek™, Marshall Hinds, Johannesburg, South Africa), which was wound onto a core. A PVA polymer solution was made up by dissolving a PVA powder (Nippongohsei, Japan) in distilled water at 80 °C. The PVA polymer solution (proprietary information) was modified by adding a cross-linker, acid and $CuCl_2$. The PVA polymer solution was then poured into a polypropylene container containing a stainless steel spinning electrode, which was then partially submerged in the polymer solution. In order to create an electric field, a high voltage was connected to the spinning electrode with the collecting wire electrode grounded to create a potential difference. The spinning conditions were as follows; spinning distance was 100 mm, rotation speed of electrode was 3.2 rpm, high voltage was 80 kV, relative humidity was below 40 %, and speed of fabric was 0.1 m/min. Once the nanofibers were spun onto the Tyvek material, the newly synthesized membrane was cross-linked at 140 °C for 15 min. The final product, a PVA nanofiber membrane was then utilized in the column flow through system. A section of the membrane was also analyzed using scanning electron microscopy (SEM) at the Central Analytical Facility (CAF), Stellenbosch University. Microscopy was performed using a LEO 1450VP SEM (Zeiss, Germany).

The column systems were directly attached to two rainwater harvesting tanks located at the Welgevallen Experimental farm. A schematic diagram of the PVA nanofiber membrane column is represented in Fig. 1a, where unfiltered tank water (red arrows) was allowed to flow through the PVA nanofiber membrane to the center of the column where after filtered tank water (blue arrows) was collected. The column systems were designed as follows: an inner cylinder containing holes (Fig. 1b) was fitted inside a larger column (Fig. 1c, d). A sheet of the PVA nanofiber membrane was then wrapped around the inner cylinder twice, which was then covered with red netting (Poly-net, Sigma-Aldrich) (Fig. 1e–f). Activated carbon (Aquasorb Udectrading Pty. Ltd) was then layered around the PVA nanofiber membrane in order to exclude larger

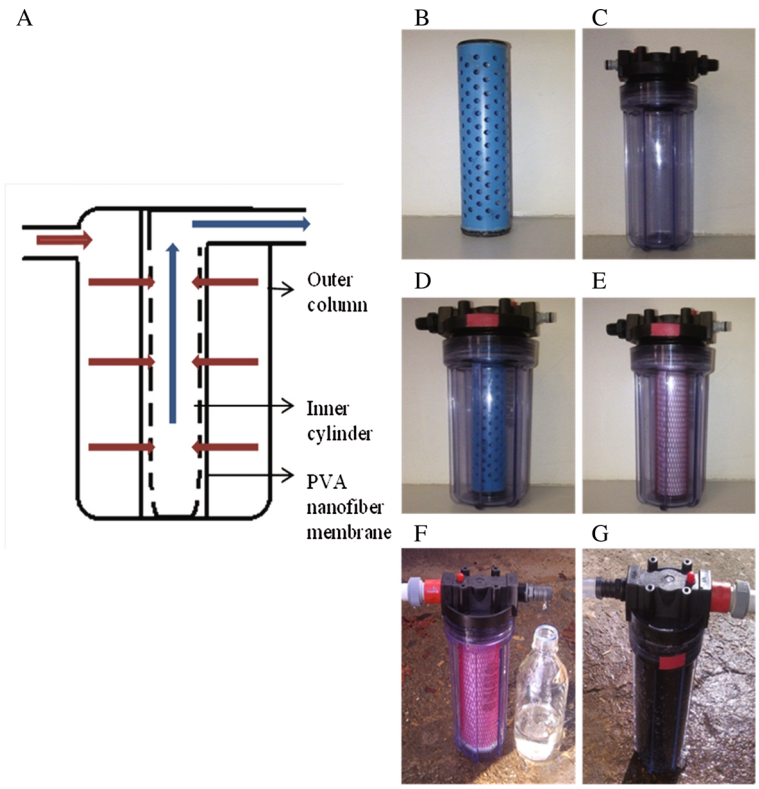

Fig. 1 **a** A schematic diagram of the PVA nanofiber membrane column, where unfiltered rainwater (*red arrows*) was allowed to flow through the PVA nanofiber membrane to the center of the column and then filtered rainwater (*blue arrows*) was collected (**b–f**). A column system containing a PVA nanofiber membrane (**g**). Activated carbon was then layered around the PVA nanofiber membrane

contaminants before passing through the PVA nanofiber membrane (Fig. 1g).

In order to determine the bacterial removal efficiency, a 1-L tank water sample was collected directly from each rainwater tank (unfiltered sample). Five 1-L samples were then individually collected after the tank water had passed through the PVA nanofiber membrane/activated carbon column. The PVA nanofiber membrane/activated carbon column was connected to two rainwater tanks (duplicate samples collected for each tank). In total, four unfiltered tank water samples as well as 20 (4×5 L) filtered tank water samples were assessed for the presence of indicator bacteria (culture based methods) and potentially pathogenic bacteria by utilizing molecular analysis. To monitor for the presence/absence of adenovirus, an unfiltered and filtered tank water sample (500 mL) was collected. The PVA nanofiber membrane/activated carbon column was connected to two rainwater tanks; therefore, in total, two unfiltered tank water samples (500 mL) and two filtered tank water samples (2×500 mL) were collected and analyzed for viral removal. The PVA nanofiber membrane and activated carbon was then replaced and the sampling was repeated with the system connected to each tank.

2.3 Microfiltration System Two: SMI-Q10 Nanofiber Membrane Disks (Viral Removal Efficiency)

Quaternized poly (styrene-*co*-maleimide) nanofibers were produced by the needless electrospinning technique, called ball spinning. The substrate material onto which the nanofibers were deposited was a nonwoven poly (propylene) spunbond material (Tyvek™, Marshall Hinds, Johannesburg, South Africa). The SMI-Q10 nanofibers contain quaternary ammonium groups along the chain of each fiber in relatively large amounts. The SMI-Q10 copolymer is produced from a strongly alternating base polymer of poly (styrene-*alt*-maleic anhydride) (Cloete et al. 2011), and it is considered that all the maleic anhydride units are converted to quaternary ammonium groups during modification of the copolymer prior to electrospinning as described by Bshena and Klumperman (2011). Quaternary ammonium compounds are well known to have antimicrobial properties and have a net positive charge that should be able to attract and retain the negatively charged viruses

in the water samples (Roy et al. 2007). Round disks of SMI-Q10 nanofiber membranes, each with a diameter of 47 mm, were cut out and placed in a Millipore vacuum filtration system (STERIFIL®, Merck). To assess the removal of viral particles only, river water (2 L) was collected from the Plankenburg River site for three consecutive days. A 500-mL aliquot from the 2-L river water sample was analyzed as the initial sample. In addition, two 500-mL samples, of the collected 2-L river water, were passed through the SMI-Q10 nanofiber membrane disks at a flow rate of approximately ≥65 mL min^{-1} cm^{-2} at 0.7 bar (70 kPa, 10 psi). In total, three unfiltered river water samples and six filtered river water samples were analyzed for the presence and removal of adenovirus.

In addition, four tank water samples (1 L), collected from the two DRWH tanks, were each divided into two 500-mL aliquots. To assess the removal of viral particles from harvested rainwater, one aliquot (500 mL) from each tank was filtered through the SMI-Q10 nanofiber membrane disks, and the remaining aliquots (500 mL) from each tank were analyzed as the initial, unfiltered tank water samples. A new SMI-Q10 nanofiber membrane disk was used for each 500 mL water sample. In total, four unfiltered rainwater samples and four filtered rainwater samples were thus analyzed for the presence and removal of adenovirus.

2.4 Recovery of Bacteria Indicator Organisms

To enumerate the heterotrophic bacterial count (HPC) in all the untreated and treated samples, a serial dilution was prepared for each sample (10^{-1}–10^{-2}), and by utilizing the spread plate method, 100 μL of each dilution was cultured onto R2A agar (Difco) in triplicate, with the plates incubated at 37 °C for up to 4 days. Total coliforms (TC) and *E. coli* were then enumerated simultaneously by filtering a total volume of 100 mL (undiluted and 10^{-1}) through a sterile GN-6 Metricel® S-Pack Membrane Disc Filter (Pall Life Sciences, Michigan, USA) with a pore size of 0.45 μm and a diameter of 47 mm. The filtration flow rate was approximately ≥65 mL min^{-1} cm^{-2} at 0.7 bar (70 kPa, 10 psi). The filters were then incubated on membrane lactose glucuronide agar (MLGA) (Oxoid, Hampshire, England) at 35±2 °C for 18–24 h (US Environmental Protection Agency 2009). In all hypothesis tests, a significant level of 5 % was used as standard (Dunn and Clark 1974). In all tests, a $p<0.05$ was considered as statistically significant.

2.5 The Hydraulic Retention Time of the Filtration System

The hydraulic retention time (HRT) is a measurement of the average length of time that a soluble compound remains in a constructed bioreactor. It can be calculated by dividing the total volume of the tank by the flow rate of the influent. The total volume is measured in liters and the flow rate of influent in liters per hour. Hydraulic retention time is then expressed in hours. Equation 1 was used to calculate hydraulic retention time for the PVA nanofiber membrane/activated carbon column filtration system.

$$\text{Hydraulic retention time (h)} = \frac{\text{Volume of tank(L)}}{\text{Flow rate of influent}\left(\text{L/h}^{-1}\right)} \quad (1)$$

2.6 Extraction of Total DNA from Tank Water Samples for the Detection of Opportunistic Pathogenic Bacteria

Each tank water sample was concentrated as outlined in Ndlovu et al. (2013). Total DNA extractions were then performed for each of the 24 tank water samples collected before and after filtration as outlined in Dobrowsky et al. (2014).

2.7 Bacterial Genus Specific PCR Reactions

The primers and PCR conditions for the detection of *Shigella* spp., *Salmonella* spp., *Aeromonas* spp. (Kong et al. 2002), *Pseudomonas* spp. (Spilker et al. 2004), *Yersinia* spp. (Stenkova et al. 2008), *Klebsiella* spp. (Brisse and Verhoef 2001), and *Legionella* spp. (Jonas et al. 1995) are outlined in Dobrowsky et al. (2014).

The following strains were cultured as positive controls after which genomic DNA was extracted: *Legionella pneumophila* ATCC 33152, *Shigella sonnei* ATCC 25931, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Aeromonas hydrophila* (environmental strain), *Klebsiella pneumoniae* ATCC 13385, and *Yersinia enterocolitica* ATCC 27729. All positive control organisms were obtained from Microbiologics®, unless indicated otherwise. The specificity of each primer set was confirmed

using nontarget DNA extracted from all the above-mentioned positive controls and a negative control (sterile distilled H_2O) was also included.

2.8 Concentration of Viruses from Tank Water and River Water Samples

The concentration of viruses from the tank and river water samples was carried out as previously described by Saayman et al. (2012). Briefly, 1 mL of $CaCl_2$ (1 M) and 1 mL of Na_2HPO_4 (1 M) were added to a 500-mL water sample. To allow for flocculation of the viral particles, the mixture was then stirred for 5 min using a magnetic stirrer where after it was filtered through a 47-mm, 0.45-μm pore size noncharged mixed-ester membrane filter (Whatman GmbH, Germany) at a flow rate of approximately ≥65 mL min^{-1} cm^{-2} at 0.7 bar (70 kPa, 10 psi). The membrane was then transferred to a 9-cm Petri dish containing 4 mL of citrate buffer (0.3 M, pH 3.5) and soaked for 3 min. The membrane was subsequently discarded, and the citric acid solution containing the virus particles was then transferred to an Amicon Ultra centrifugal device (Millipore) for concentration. The device was centrifuged (Heraeus, Biofuge Stratos) at 1500×*g* for 2 min, where after the volume was adjusted to 200 μL with phosphate-buffered saline (PBS; 1×).

2.9 Extraction of Viral DNA from Tank Water and River Water Samples

Deoxyribonucleic acid was extracted from 200 μL of the concentrated sample using the QIAmp Ultrasens Virus Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The final volume was approximately 60 μL, which was stored at 4 °C for adenovirus identification.

2.10 The Conditions of the PCR Assays for the Detection of Adenovirus

The PCR mixture for adenovirus was made up to a final volume of 50 μL. The mixture contained 6 μL DNA as template (prepared from Section 2.9), a final concentration of 1× Green GoTaq® Flexi buffer, 0.2 mM dNTP mix, 2 mM $MgCl_2$, and 0.3 μM AQ1 and AQ2 primers (Table 1). All PCRs were performed with 1.25 U of GoTaq® Flexi DNA polymerase (Promega Corp. USA). The amplification was performed with an initial denaturing step at 94 °C for 3 min, after which 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, and elongation at 72 °C for 1 min were completed with a final elongation step at 72 °C for 7 min (Heim et al. 2003; Saayman et al. 2012). An adenovirus positive control (Coris BioConcept, Belgium) and a negative control (sterile distilled H_2O) were also included.

2.11 PCR Purification and Sequencing

The PCR bands corresponding to the respective base pairs were cleaned using the QIAquick® Gel Extraction Kit (Qiagen GmbH, Hilden, Germany) and sent for sequencing at the Stellenbosch University CAF. Sequence identification was completed using the National Centre for Biotechnology Information (NCBI) and The Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences of representative isolates that showed >97 % similarity (<3 % diversity) to organisms were recorded.

3 Results

3.1 Polymer and Charge Distribution

In order to determine the pore sizes of the PVA membrane filters, scanning electron microscopy (SEM) was performed (Fig. 2a), and the average pore size was calculated at 0.9 μm using the Digimizer Software version 4.25; however, it should be noted that the pore size of the membrane was not uniform throughout and ranged from 0.32 to 1.9 μm.

Once tank water samples were filtered (5×1 L) through the PVA/activated carbon column, the PVA membrane was removed and again analyzed using SEM. The PVA nanofiber membrane was covered with particles varying in size (Fig. 2b). Cocci-shaped particles, rod-shaped particles, as well as other debris and possibly granules of activated carbon were observed. Moreover, the average time recorded to filter 1 L of tank water was recorded as 31.02 min for the first liter, 39.57 min for the second liter, 38.27 min for the third liter, 52.92 min for the fourth liter, and 62.26 min for the fifth liter. The hydraulic retention time for the PVA nanofiber membrane/activated carbon filtration column was calculated at 0.8 h.

Table 1 Primer sequences used for the amplification of adenovirus as previously described by Heim et al. (2003)

Primer	Sequence (5′ – 3′)	Region (product size)
AQ1	GCCACGGTGGGGTTTCTAAACTT	Hexon gene (110 bp)
AQ2	GCCCCAGTGGTCTTACATGCACATC	

In order to verify the even distribution of the quaternary ammonium groups throughout the SMI-Q10 nanofibers membrane surface, the base polymer, styrene-*alt*-maleic anhydride (SMA) was reacted with a fluorescent dye prior to electrospinning. The fluorescent dye, 5-aminofluorescein [Sigma-Aldrich, ~95 % (HPLC)] contains a primary amine, which undergoes a similar nucleophilic addition reaction as dimethylaminopropylamine (DMAPA) to the maleic anhydride units in the polymer backbone. Functionalization with the 5-aminofluorescein was done in the same way as the maleimide modification done to the polymer, prior to spinning, but excluding the quaternization step. As indicated in Fig. 3, the fluorescent dye (green fluorescence) was evenly distributed across the surface of the electrospun membrane (A) compared to the control of nonfunctionalized SMA co-polymer nanofiber (B) showing no fluorescence when placed under UV light. This is significant evidence that the positively charged quaternary ammonium groups should be evenly distributed across the membrane surface.

3.2 The Bacterial and Viral Removal Efficiency of the PVA Nanofiber Membrane/Activated Carbon Column

A column containing two PVA membrane layers surrounded by activated carbon was connected to the two rainwater tanks. Total coliforms, *E. coli*, and heterotrophic bacterial counts were determined in unfiltered and 5 L of filtered tank water samples, collected liter by liter (Table 2).

Total coliform counts in the unfiltered tank water samples collected from the two rainwater tanks had an average of 6×10^2 CFU/100 mL. After filtration, total coliform numbers were reduced significantly ($p=0.008$) as a ≥99 % decrease was observed for all the first liters of filtered tank water samples in comparison to the unfiltered tank water samples. In addition, from the second ($n=4$) to the fifth ($n=4$) liter of filtered tank water, a 99.9 % to a 99.6 % decrease in total coliform counts was observed in comparison to the unfiltered tank water samples. An average of 3 CFU/100 mL *E. coli* was also detected in the unfiltered tank water samples collected from the two rainwater tanks. After filtration, *E. coli* numbers were reduced significantly ($p<0.000006$) to below the detection limit in the subsequent five liters of filtered tank water, as a ≥99 % decrease for all 5 L of filtered tank water samples was observed in comparison to the unfiltered tank water. An average of 3×10^4 CFU/mL heterotrophic bacteria were detected in the unfiltered tank water samples collected from the two rainwater tanks. After filtration, heterotrophic bacterial numbers were reduced significantly ($p=0.008$) and a ≥99 % decrease was

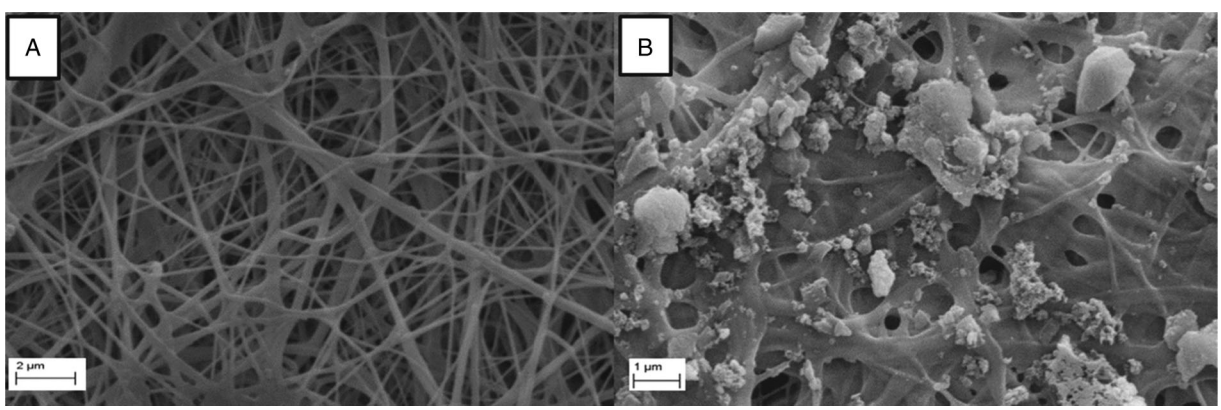

Fig. 2 A scanning electron microscope image of **a** a polyvinyl (alcohol) (PVA) nanofiber membrane before filtration of rainwater and **b** a polyvinyl (alcohol) (PVA) nanofiber membrane examined after rainwater was filtered through the activated carbon and nanofiber membrane system

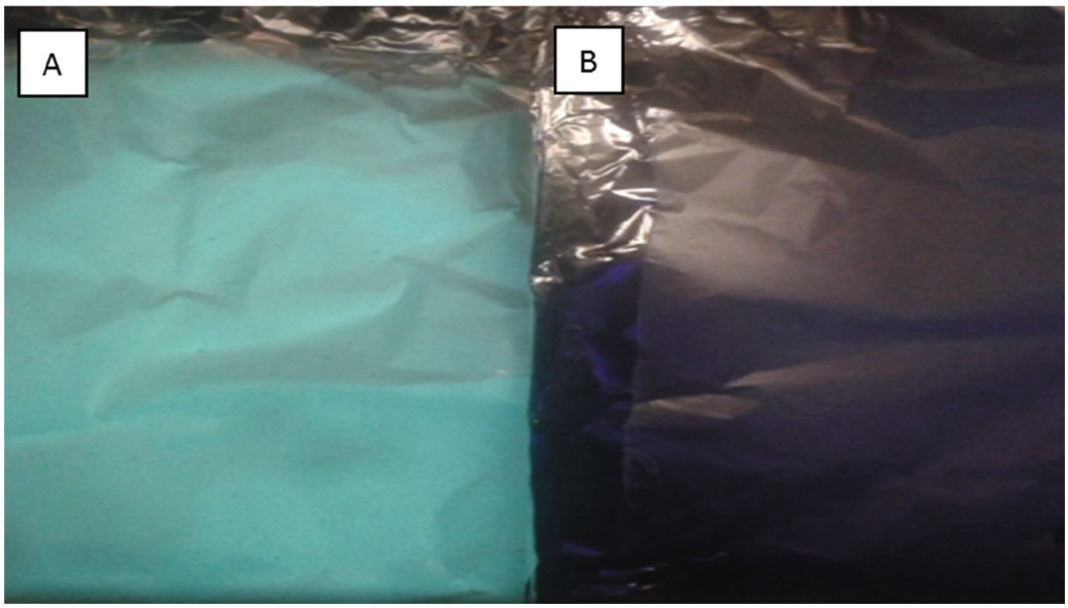

Fig. 3 **a** Styrene-*alt*-maleic anhydride nanofibers functionalized with 5-aminofluorescein showing even distribution of fluorescent functional groups and **b** and nonfunctionalized SMA fibers containing no fluorescing groups under UV light

observed for the first liter (n=4) of filtered tank water sample in comparison to the unfiltered tank water samples. From the second (n=4) till the fifth (n=4) liter of filtered tank water, a 99.7 to a 99.5 % decrease was observed in comparison to the unfiltered tank water samples.

3.2.1 Detection of Bacterial Genera and Viruses in Unfiltered and Filtered Tank Water Samples

The columns containing two PVA membrane layers surrounded by activated carbon were connected to the two DRWH tanks in separate experiments to investigate the bacterial and viral removal efficiencies, respectively, of the system utilizing molecular techniques. For the bacterial detection, the filtration system was analyzed four times (5 L collected liter by liter), while for the virus removal efficiency, the system was analyzed twice for each respective tank. Total DNA was extracted from unfiltered and filtered tank water samples, followed by subsequent bacterial genus and viral specific PCR analysis. A summary of the detection results obtained for all analysis is represented below.

Genus-specific PCR assays revealed the presence of potentially pathogenic bacteria, commonly associated with tank water (Table 3). However, *Salmonella* spp. were not detected in any of the 20 filtered and four unfiltered tank water samples. Similarly, PCR assays

Table 2 Total coliforms, *E. coli*, and heterotrophic bacterial numbers detected in unfiltered rainwater (n=4) and 5 L of rainwater filtered through a column containing two PVA membrane layers surrounded by activated carbon (n=20)

Indicator bacteria	Unfiltered rainwater (n=4)	Filtered liters of rainwater				
		1 L (n=4)	2 L (n=4)	3 L (n=4)	4 L (n=4)	5 L (n=4)
Total coliforms (CFU/100 mL) (% reduction)	6×10^2	≤1 (≥99 %)	≤1 (≥99 %)	≤1 (≥99 %)	1 (99.8 %)	3 (99.6 %)
E. coli (CFU/100 mL) (% reduction)	3	≤1 (≥99 %)	≤1 (≥99 %)	≤1 (≥99 %)	≤1 (≥99 %)	≤1 (≥99 %)
HPC (CFU/mL) (% reduction)	3×10^4	3.3 (≥99 %)	1×10^2 (99.7 %)	7.8×10^1 (99.8 %)	1.5×10^2 (99.5 %)	1.6×10^2 (99.5 %)

indicated the presence of *Shigella* spp. in only one of the filtered tank water samples (Table 3). *Aeromonas* spp. were detected in all the unfiltered tank water samples and were not detected in the first liter (n=4) of tank water filtered through the PVA/activated carbon membrane. In contrast, *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., and *Yersinia* spp. were detected in all the unfiltered tank water samples and were then sporadically detected in the filtered tank water (Table 3). Results indicated that *Legionella* spp. and *Yersinia* spp. were the most persistent genera, as these bacteria were detected in all the unfiltered tank water samples and in 85 and 80 % of the 20 filtered tank water samples, respectively.

The PCR assays and BLAST analysis also confirmed the presence of bovine adenovirus 3 in all of the tank water samples collected before microfiltration for both tanks sampled (results not shown). Other adenovirus strains detected in the rainwater tanks included simian adenovirus B isolate BaAdV-1 and human adenovirus 40 strain M-364. Moreover, once the tank water had undergone filtration through the PVA nanofiber membrane/activated carbon column, the presence of adenovirus was indicated in 75 % (three of the four samples) of the filtered tank water samples (results not shown).

3.3 Virus Removal of the SMI-Q10 Membrane Disks from River and Tank Water Samples

The SMI-Q10 nanofiber membrane filtration system was analyzed in duplicate, with an initial untreated river water sample also collected. The PCR results and subsequent BLAST analysis confirmed the presence of bovine adenovirus 3 strain, simian adenovirus 3 strain, and human adenovirus A strain in the before river water samples as well as in all the filtered river water samples that were analyzed. As it is assumed that the harvested rainwater contained less organic matter than river water, the SMI-Q10 nanofiber membrane filtration system was then also assessed for the removal of adenovirus from tank water samples. Polymerase chain reaction assays indicated that the SMI-Q10 nanofiber membrane filtration system did not remove the adenovirus particles from the tank water samples, as adenovirus was detected in all the filtered and unfiltered tank water samples.

4 Discussion

Microfiltration, where nanofibers are electrospun onto a substrate, has been utilized in many water filtration applications. As noted previously by Bjorge et al. (2010), the increase in porosity and the pore structures formed offer higher water permeability compared to conventional methods currently used. Based on the results obtained in the current study, the addition of activated carbon to the PVA nanofiber membrane column rendered the treatment system efficient in the removal of total coliforms, *E. coli*, and heterotrophic bacteria, as 3 L of potable water could be produced according to the DWAF (1996) guidelines for indicator bacteria. The addition of activated carbon to the filtration system may have aided in the removal of contaminating substances and improved the water quality as the system relied on the immobilization of bacteria onto the

Table 3 Bacterial genera detected in unfiltered rainwater samples (n=4) and in the first till the fifth tank water samples that had been filtered through a PVA nanofiber membrane/activated carbon column that was connected to two DRWH tanks in duplicate

Organism	Unfiltered rainwater (%) (n=4)	Filtered liters of rainwater (%)				
		1 L (n=4)	2 L (n=4)	3 L (n=4)	4 L (n=4)	5 L (n=4)
Aeromonas spp.	100	ND	25	25	25	25
Klebsiella spp.	100	25	75	50	50	75
Legionella spp.	100	75	100	75	75	100
Pseudomonas spp.	100	50	50	75	25	25
Salmonella spp.	ND	ND	ND	ND	ND	ND
Shigella spp.	ND	ND	ND	25	ND	ND
Yersinia spp.	100	75	50	100	100	75

ND not detected

surface of granular activated carbon, which in turn was able to remove pollutants via processes such as bio-absorption, biodegradation, or bio-regeneration (Wang et al. 2007; Ong et al. 2008). However, for the PVA nanofiber membrane/activated carbon column, the filtering time for the fifth liter (62.26 min) was approximately double that of the filtering time for the first liter (31.02 min), and it is hypothesized that the increase in filtering time was as a result of the PVA nanofiber membrane becoming saturated, with the pores becoming more clogged per liter of tank water filtered. A study conducted by Bjorge et al. (2010) also found that the inability of a polyamide (PA) nanofiber membrane to remove pathogens from contaminated water was due to the trans-membrane pressures that cause deformation of the nanofibers resulting in the pore size to increase, which effectively allows microorganisms to pass through.

Routine analysis methods employed for the detection and enumeration of bacteria in water may include enumeration by plating and culturing (liquid and solid) on media and the use of microscopy. However, the media utilized for culturing methods does not meet all the nutritional and physiological requirements of all microorganisms in water (Nollet and De Gelder 2014). Moreover, it is well understood that microorganisms may occur in a viable, but nonculturable form in water samples (Colwell et al. 1985; Oliver 2005, 2010), and therefore, results may appear as false negatives. Many microorganisms such as *Aeromonas* spp., *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., *Salmonella* spp., and *Shigella* spp. have also been reported to enter a viable but nonculturable state (Oliver 2010). For these reasons, in assessing the efficiency of the PVA nanofiber membrane/activated carbon column, the current study utilized culturable methods and as well as PCR assays for the detection of many of the potentially pathogenic bacteria that had previously been detected in harvested rainwater systems and may have been present in a viable but nonculturable state (Dobrowsky et al. 2014).

Based on genus-specific PCR analysis, utilized to screen the filtered tank water samples for the presence of potential pathogenic bacteria and viruses, *Klebsiella* spp., *Legionella* spp., *Pseudomonas* spp., and *Yersinia* spp. were not removed by the PVA nanofiber/activated carbon column as these organisms where detected in the filtered tank water and all the unfiltered tank water samples. In addition, the PVA nanofiber membrane system with the addition of activated carbon requires further optimization, as only 3 L of potable water can be produced before the components of the system need to be cleaned and the PVA membrane and activated carbon had to be replaced. Moreover, the average time required to filter the first liter of tank water was approximately 31.02 min and increased as more water was filtered, rendering this system impractical for use at the household level.

Microfiltration is currently widely applied in water treatment. Due to size, *Staphylococcus aureus* (0.8× 1 μm) and *E. coli* (2×1 μm) are presumed not likely to pass through the nanofiber membrane with a mean pore size of 0.20–0.45 μm. Daels et al. (2011), however, noted that in hospital wastewater that had been spiked with *S. aureus*, a 1.6×10^{1} CFU/100 mL reduction was observed using a nonfunctionalized membrane. The average pore size of the membrane used in the current study (0.9 μm) was larger than 0.2–0.45 μm (Daels et al. 2011), and for this reason, two layers of the nanofiber membrane was used in the filtration system. It is therefore possible that the pore size did not decrease after doubling the layers of the membrane, and even with the addition of activated carbon, the pore size could possibly not have been reduced to 0.2–0.45 μm. It should however be noted, as indicated by the recovery of indicator bacteria, that the first 3 L of filtered tank water was within drinking water standards as indicated by DWAF (1996).

The South African Drinking Water guidelines (DWAF 1996) recognize that viruses, protozoa, and bacteria may be transmitted by water, and for technical and economic reasons, it may be impractical to assess various water sources for all the pathogens that may be present. It is therefore recommended by the guidelines that assessment practices involving routine quality surveillance should include appropriate combinations of indicator organisms and that the pathogens of major importance should also be monitored as a reliable indication of potential risks of infection. Thus, in addition to monitoring for the indicator bacterial groups and the PCR assays which indicated the presence/absence of the opportunistic pathogens generally associated with tank water, in the current study, tank and river water samples were also screened and monitored for viral removal efficiency utilizing the filtration systems.

Throughout the study period, adenovirus was consistently detected in the rain- and river water samples analyzed. Human adenovirus 40 strain M-364, simian adenovirus B isolate BaAdV-1, and bovine adenovirus 3

strain HLJ0955 were detected in the rainwater samples (unfiltered and filtered), and the source of the simian strains could have been due to primates that inhabit the environment surrounding the farm. In addition, it is hypothesized that adenovirus from human feces, originating from settlements where many inhabitants do not have access to flushable toilets, could have been transferred by small animals and rodents to the roof surface (Ahmed et al. 2012). The consistent detection of adenovirus in the river water samples was most probably due to the fact that adenovirus is more persistent and is usually found more frequently than other enteric viruses in aquatic environments (Pina et al. 1998; Haramoto et al. 2010).

The inability of the PVA nanofiber membrane/activated column to remove adenovirus from three of the tank water samples could be explained by the fact that the adenovirus is small enough (90–100 nm) to pass through the pores and was not retained by means of a charge or other interaction between the virus and the membrane. Furthermore, no electrostatic interactions between the viruses and the membrane are present to ensure removal. A study conducted by Wang et al. (2013) showed that a novel microfiltration membrane consisting of a two-layered nanoscale polyacrylonitrile (PAN)/microscale polyethelyne terephthalate (PET) fibrous scaffold with an ultra-fine functional cellulose nanofiber could simultaneously remove bacteria, viruses, and toxic heavy metals. The PAN/PET scaffold membrane could remove viruses and ions from polluted water with relatively large pore sizes that could originally only be used to remove bacteria by size exclusion. It was thus the electrostatic interactions between the viruses and the membrane that were found to be the reason for removal and not size exclusion as for bacterial removal. However, the study conducted by Wang et al. (2013) utilized culture-based methods, including the colony assay procedure for the detection of bacteria and the plaque assay procedure to determine the number of viruses present. Contrasting to the current study, Wang et al. (2013) did thus not account for viable but nonculturable organisms that may be detected utilizing molecular methods.

As indicated in Section 2.3, quaternary ammonium compounds are well known to have antimicrobial properties and have a net positive charge that should be able to attract and retain the negatively charged viruses in the water samples (Roy et al. 2007). The SMI-Q10 nanofiber membrane disks were thus immobilized with these quaternary ammonium groups along the chain of each fiber. Results of the filtration study utilizing the SMI-Q10 nanofiber membrane disks, however, showed that this membrane was not effective in removing adenovirus from the river water samples, possibly due to damage to the nanofibers. The damage may have been caused by the debris in the river water causing fouling of the membrane surface and the quaternary ammonium groups along the chain of each fiber (Bjorge et al. 2010). To confirm the theory, the efficiency of the SMI-Q10 nanofiber membrane disks was analyzed using tank water samples, where it is hypothesized that a lower level of organic matter and debris should be present. However, results showed that adenovirus was present in all the unfiltered and SMI-Q10 nanofiber filtered tank water samples. It is, therefore, recommended that the water be pre-filtered through a coarse filter such as a polypropylene membrane to separate the larger contaminants. Further studies are, however, required in order to determine whether the SMI-Q10 nanofibers would be effective in adsorption of the viruses.

5 Conclusions

Results indicate that 3 L of potable water may be produced by the PVA nanofiber membrane/activated column as *E. coli*, total coliform, and heterotrophic bacterial numbers were reduced to within drinking water guidelines according to the DWAF (1996). However, 5 L of potable water may be produced by the system according to the SANS 241 (SABS 2005) and the ADWG (NHMRC and NRMMC 2011) standards, as these standards do not recommend a value for total coliforms or heterotrophic bacteria. However, molecular methods should be included in analyzing the efficiency of filtration systems as PCR assays indicated the presence of potentially pathogenic bacteria, such as *Legionella* spp. and *Klebsiella* spp., and the virus, adenovirus, in some of the filtered water samples, and this may be an indication of a serious health risk for humans, especially the young and immuno-compromised individuals. Moreover, the positively charged SMI-Q10 nanofibers did not aid in the removal of negatively charged adenovirus. It is therefore recommended that the electrospinning process be optimized further in order to produce PVA nanofiber membrane sheets that exhibit reduced pore sizes, therefore aiding in the removal of opportunistic pathogenic bacteria from harvested

rainwater. Moreover, an examination of the different porous supports that allow for higher flux to support the nanofibers could be utilized as a substrate during the electrospinning process. In addition, as the column filtration systems require approximately half an hour to produce 1 L of potable water, a solution would entail connecting a velocity pump to the system to enhance the pressure and decrease the time required for filtration.

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