Graphene-Based Bacterial Filtration via Electrostatic Adsorption

Rameez Ahmed, Ankita Vaishampayan, Katharina Achazi, Elisabeth Grohmann, Rainer Haag, and Olaf Wagner*

Flexible graphene oxide (GO) microsheets with attached positively charged polymers, termed GOX microsheets, are efficient at bacterial adsorption, as they bind electrostatically to bacterial membranes’ negative surface charge. The authors explore an antimicrobial water filter application for GOX’s extremely high surface area and its previously described efficient bacterial adsorption. Cellulose-fiber carrier material is functionalized with GOX microsheets to create an adsorption-based bacteria filtration material. The morphology and charge density ($7.8 \times 10^{19} \text{ g}^{-1}$) of the prepared GOX fibers are determined by scanning electron microscopy and dye adsorption assay, and widefield fluorescence microscopy is used to visualize the adsorption of stained *Escherichia coli* bacterial cells on the fibers. GOX fibers are tested in filtration setups to investigate their bacteria removal performance. The experimental results, with 100 mg of GOX fibers filtering $2.4 \times 10^9$ colony-forming units (CFU) from an *E. coli* bacterial culture with 99.5% bacterial reduction, demonstrate the fibers’ high bacteria loading capacity. The electrostatic adsorption-based filtration mechanism allows the filter to be operated at higher flow rates than micropore membrane filters, while maintaining 3-log bacterial reduction. GOX filter materials removing bacteria via adsorption are a high flow rate alternative to current water filtration processes that rely on size-exclusion.

1. Introduction

The water crisis is a serious global dilemma of the 21st century. The world’s population is rapidly increasing, placing unprecedented strain on clean water supplies. By 2030, around half the world’s population will be living in extreme water-deficient conditions, according to a recent United Nations World Water Development report.[1] Especially when people in crowded conditions lack clean water, fecal-oral diseases can proliferate rapidly. Diarrhea may not seem deadly to those who have access to improved sanitation, but it kills 750 000 children every year that is more than malaria, AIDS, and measles combined.[2]

Waterborne pathogens are also of great concern in the hospital environment, as the water temperatures and the complex structure of hospital water systems are suitable for bacterial growth and biofilm formation.[3,4] These pathogens in connected devices (such as sinks, showers, ice machines, water baths, eyewash stations, and dental units) can lead to severe infections, especially with the rising number of multidrug-resistant bacteria.[5,6]

The faucet micro pore filters that are used to prevent this outcome must be replaced after a few weeks of use, making them very costly. Novel antibacterial technologies may offer practical and cost-effective prevention strategies for these concerns.

Graphene materials have been reported to exhibit antibacterial activity by physically interacting with bacterial cells.[7–9] These flexible, single-atom-thick, nano-micrometer sized sheets feature an extremely large surface area,[10,11] Among the variety of different graphene materials, graphene oxide (GO) is frequently used due to its inexpensive preparation from graphite[12–16] as well as its hydrophilic functional groups, which enhance its dispersibility in polar solvents and offer multiple options for chemical post-modification.[17,18]

In a previous work we demonstrated that polymer post-modification of micrometer-sized GO can be used to create polycationic microsheets.[19] These flexible GO microsheets matched the size and surface-charge density of opposite charged *E. coli* bacterial cells. Incubating them with gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) and gram-negative *Escherichia coli* (*E. coli*) led to wrapping and immobilization of bacterial cells in both cases.[19] Based on multivalent electrostatic attraction, these GO derivates (GOX) also showed antibacterial activity when they were attached to a carrier material.[20,21]

Although many polycationic polymers like chitosan,[22,23] polyethylene imine,[24,25] ε-polylysine,[26] polysiloxanes,[27,28] polyprenones[29,30] as well as modified GO materials with various conjugated moieties like mannose,[31] lactose,[32] quaternary ammonium compounds[33,34] and zwitterionic systems[35] have been shown to bind and inhibit bacteria, investigation...
of these materials as water purification materials remains quite limited. Ottenhall et al. reported E. coli reduction by cellulose-based filter papers functionalized with polycationic polyvinylamine in single-layer or multilayer systems with polyanionic polyacrylic acid.[36] Musico et al. reported increase in reduction of E. coli and Bacillus subtilis by modifying commercially available membrane filters with poly(N-vinylcarbazole)-graphene oxide.[37]

By far the most common technology for addressing bacterial water problems is membrane filtration. It is considered a very effective method, as it removes the targeted contaminants from water via the employed membrane pore size.[38] However, it has been reported that this is not true for bacteria with spirillum-shaped morphology. Wang et al. performed a quantification study to evaluate the filtration ability of commercially available membrane filters with 0.45, 0.22, and 0.1 µm pore sizes against freshwater bacterial communities. They reported that an average of 50% of the spirillum-shaped Hylemonella gracilis strains managed to pass through the 0.45 µm membrane filter.[39] Although this strain is not pathogenic it demonstrates the limitation of size exclusion filtration.

Another drawback of the size exclusion working principle of membrane filters is their low flow rate due to the small pore sizes and continuously decreasing permeability due to accumulated filtrate or biofouling.[40] Commercially available 0.45 µm, 0.2 µm, and 0.1 µm pore size polytetrafluoroethylene (PTFE) filters from Sterlitech have maximum flowrates of 40, 20 and 14 mL min⁻¹ cm⁻², according to the product data sheet.[47] Membrane filters with these low flow rates require very high filter cross sections or specially designed high surface area filter geometries. In addition, membrane filtration systems require regular maintenance, backwash cycles, or membrane replacement to keep them operational.

While size exclusion filtration is used against bacteria, other water contaminants (i.e., heavy metals, biocides, pharmaceuticals) are usually removed by adsorption materials like activated carbon[41] or ion exchange resins.[42]

In this work, we present a universal antimicrobial filter material that removes bacteria by adsorption based on electrostatic interaction. This was realized by covalently coating the above mentioned polycationic GO sheets (GOX) onto cellulose fibers as a carrier material. The GOX-functionalized fibers were tested in bacterial filtration experiments to evaluate their filtration performance in parameters such as flow rate, loading capacity, and reduction of colony-forming units.

2. Results
2.1. Filter Material Synthesis

The polycationic GO sheets (GOX) were prepared by a method described in our previous work.[19] Briefly, GOX was prepared from GO via a two-step synthesis. First, the polymer chains were grafted onto the surface of GO via radical polymerization of a dimethylamino-ethylmethacrylate monomer, based on the work of Kan et al.[43] After the polymerization, the dimethylamine side groups were quaternized by methyl iodide to form quaternary ammonium groups to render GOX polycationic microsheets (Figure 1).

The cellulose fibers were selected as carrier material because it is one of the most abundant natural polymers on earth. It is a renewable, biodegradable, nontoxic material with excellent mechanical properties, which is available in different size.[44,45] Cellulose fibers were initially dried to remove adsorbed water from the surface. Methylene bisphenyl diisocyanate was dissolved in dry DMF and the dried fibers were immersed into the solution to homogeneously distribute the fibers. GOX in a 10 wt% ratio to the fibers was dispersed in dry DMF and then added to the diisocyanate surface-activated cellulose dispersion to covalently bind to them.

2.2. Characterization of Filter Materials
2.2.1. Appearance and Morphology

A change in appearance of the cellulose fibers from a bright white to darker brown to black can be observed after the functionalization with GOX (Figure 2a,d). Scanning electron microscopy (SEM) was used to examine the cellulose fibers before and after immobilization with the GOX microsheets. The cellulose fibers have a smooth surface morphology with random orientation (Figure 2b,c). The GOX-functionalized fibers are also in
random orientation and GOX microsheets are attached along the fiber surfaces increasing their overall surface area (Figure 2e,f).

2.2.2. Polycationic Charge Density

The number of positive charges on GOX ($6.5 \times 10^{20}$ per gram) was quantified in our previous work via a colorimetric method.[19] The method is based on the electrostatic adsorption of a negatively charged dye (fluorescein), which binds to the positive charges of the sample. The concentration decrease via bound dye is determined by UV–Vis spectroscopy and the difference of initial and final concentration is used to calculate the number of positive charges on the sample. The charge density of the 10 wt% GOX-functionalized cellulose fibers was quantified by the same assay (see Supporting Information) and was calculated to be $7.8 \times 10^{19}$ charges per gram. This value is around 10% of the GOX charge density and validates the successful immobilization of 10 wt% GOX microsheets on cellulose fibers (Table 1).

2.2.3. Adsorption of Bacterial Cells on GOX Fibers

The binding effect of GOX-functionalized cellulose fibers on bacteria was visualized with a widefield microscope using *E. coli* with a live/dead staining kit. The attraction of *E. coli* to GOX cellulose fibers was visible as the *E. coli* cells stuck to the GOX cellulose fibers (Figure 3). The fibers had no immediate toxic effect on the bacteria, as all bacteria appeared alive, showing a fluorescence in the green spectra. However, the membranes of some bacteria seemed to be affected, as some bacteria appeared in the orange/red spectra due to the orange/red fluorescent ethidium homodimer-III (EthD-III) dye that can enter a leaky
bacterial membrane. The unmodified cellulose fibers did not show this interaction with the E. coli cells, which were homogeneously distributed in the suspension. Therefore, the immobilization of E. coli bacterial cells can be attributed to the positive charges of the GOX-functionalized cellulose fibers.

2.2.4. Bacterial Cell Filtration Experiments

To determine the filtration effectivity and capacity of GOX fibers, several experiments have been performed. The initial test was performed to determine the effective amount of material to reduce the number of bacteria in colony forming units (CFUs) by three orders of magnitude (3 logs), which is a common minimal requirement for commercial membrane filters.[46] Different amounts of GOX fibers were loaded in a syringe volume and 2 runs of 10 mL of a 1.6 × 10⁷ CFU mL⁻¹ E. coli bacterial culture was filtered through them. Figure 4 summarizes the reduction of colony-forming E. coli bacteria for the different tested GOX fiber amounts.

The results demonstrate that bacterial reduction increased with increasing amounts of GOX fiber material. 33 mg GOX fibers

| Sample          | Adsorbed dye on fibers [mg] | Cellulose control subtracted [mg][a] | Adsorbed dye [mmol][b] | No. of charges per GOX fibers[c] |
|-----------------|-----------------------------|---------------------------------------|------------------------|---------------------------------
| Cellulose Fibers| 0.0050                      | -                                     | -                      | -                               |
| GOX fibers      | 0.0480                      | 0.0430                                | 0.129                  | 7.8 × 10¹⁹                      |

[a]Amount of adsorbed dye on fibers after subtraction of cellulose control; [b]Amount of adsorbed dye/molecular weight of dye; [c] Adsorbed dye [mol] * Avogadro's constant
caused the lowest CFU reduction at 45%, whereas the filtrate from 600 mg GOX fibers showed a 99.9% reduction of CFU. The amount of 100 mg of GOX fiber was selected for further experiments because of its moderate bacterial reduction performance of 75%, in order to allow the detection of samples with higher CFU reduction, as well as the convenient handling for varying flow rate and pressure experiments, in lab scale environment.

To elucidate to what extent the GOX microsheets on the cellulose fibers were responsible for the filtration of bacteria, control experiments were performed with unfunctionalized cellulose fibers. The procedure is described in detail in the experimental section, and the experimental setup is shown in Figure 5. Briefly, 1000 mL of E. coli bacterial culture (9.1 × 10⁴ CFU mL⁻¹) was filtered through 100 mg of GOX-functionalized fibers and

---

**Figure 4.** Summary of the two E. coli bacterial filtration runs of increasing GOX fiber amounts. The Y-axis values represent the percent reduction of colony-forming bacteria in the filtrate as compared to the initial concentration of 1.6 × 10⁷ CFU mL⁻¹.

**Figure 5.** Scheme of experimental setup for GOX fiber filtration experiment.
control cellulose fibers, at $35 \pm 5 \text{ mL min}^{-1}$ respectively. Samples were collected during filtration at 100 mL, 500 mL and 900 mL flow through and plated on agar to determine the CFU concentration and the percentage of bacterial reduction (Figure 6). GOX-functionalized fibers reduced the CFU concentration more than 90% in all 3 fractions of the 1 L filtered bacterial culture. On the other hand, cellulose fibers reduced the amount of bacteria in the solution by only 2–10%. This result demonstrated that the GOX microsheets on the fibers were responsible for the high bacterial filtration performance.

2.2.5. GOX Fiber Bacterial Loading Capacity

To evaluate the bacterial loading capacity of the GOX fiber material, 3 L of bacterial culture of increasing concentration (1.0, 2.8 and $3.3 \times 10^6 \text{ CFU mL}^{-1}$) was filtered through 100 mg of GOX-functionalized fibers at flowrate of $25 \pm 5 \text{ mL min}^{-1}$. For each filtered liter, two samples were taken from the bacterial culture (at flow points 400 mL and 800 mL) and plated on agar to determine the GFU concentration. The total number of passing bacteria was plotted against the bacterial reduction in the filtrate (Figure 7).

The results show that 100 mg of GOX fibers removed more than 99.5% of bacteria from the first 1.4 L of bacterial culture ($2.4 \times 10^9 \text{ CFU}$). Ottenhallet al. reported maximum 98% bacterial removal by filtering $10^7 \text{ CFU}$ bacterial culture with a stack of 5 sheets of single-layer polyvinylamine-coated filter paper.[36] In comparison to this polyvinylamine cellulose filter paper, GOX fibers have 100-fold greater bacteria loading capacity at a higher bacterial reduction efficiency. After the filtration of 1.8 L of bacterial culture ($3.6 \times 10^9 \text{ CFU}$) the performance dropped to 97%, then further to 83% at 2.4 L ($5.1 \times 10^9 \text{ CFU}$), and finally to 48% at $6.8 \times 10^9 \text{ CFU}$.

The sudden decrease in filtration performance around $5 \times 10^9 \text{ CFU}$ indicates that the filter surface is saturated at this point, which reflects the maximum bacterial loading capacity of 100 mg GOX fibers. The extrapolation of the data shows that the 100 mg GOX fibers will completely lose their filtration ability at $8.25 \times 10^9 \text{ CFU}$. Since the bacteria culture continues passing through the filter, this result further demonstrates that the mechanism of removal is bacterial adsorption and not size exclusion.

2.2.6. Flow Rate and Pressure Dependence

Flow rate and pressure are important parameters for filter applications. Membrane filters have flow rate limitations stemming from the small pore sizes necessary to retain particles. These limitations further increase when retained particles accumulate, tightening the space and clogging the pores, resulting in even lower flow rates and a higher drop in pressure. Therefore we used an experimental setup with increasing water pressure to evaluate the filtration performance of GOX-functionalized fibers at increasing flow rates. In short, 1 L of *E. coli* bacterial culture ($1.7 \times 10^4 \text{ CFU mL}^{-1}$) was prepared, then forced by an air compressor through a cartridge filled with 100 mg of GOX fibers. The flow rate was determined and, in Figure 8, is plotted against the applied pressure. Samples of the filtrate were collected and plated on agar to determine the CFU concentration at the corresponding flow rates.

The results show that increased pressure and flow rate have no negative effect on GOX filtration performance within the range of tested parameters. Even a maximum flow rate of 334 mL min$^{-1}$ allowed for complete removal of bacteria from the filtrate. The maximum flow rate per unit surface area for the GOX fiber filter cartridge is calculated to be 196 mL min$^{-1}$ cm$^{-2}$, by dividing the maximum flow rate (334 mL min$^{-1}$) by cartridge filtration area (1.7 cm$^2$). Commonly used micropore bacterial filters cannot operate at such a high ratio of flow rate to area. Under 0.7 bar of pressure in a clean water environment, commercially available PTFE membrane filters with 0.45 µm pores can reach maximum flow rates of 40 mL min$^{-1}$ cm$^{-2}$.[47] In contrast, the presented filter material based on polycationic graphene oxide sheets provides 3-log bacteria reduction at flow...
rates of 196 mL min⁻¹ cm⁻², which is nearly five times higher than typical membrane filters.

3. Conclusion

In this work, a new bacteria-adsorbing material based on electrostatic attraction was developed and investigated for its bacteria reduction performance. The material’s functionality is based on a positively charged high-surface-area graphene derivative. A derivative, a GO microsheet with grafted polyionic polymers (GOX), was covalently immobilized on cellulose fibers. 100 mg of the created GOX fibers adsorbed E. coli bacteria cells, removing up to $2.4 \times 10^9$ CFU with 99.5% filtration efficiency. The filter performance of 3-log CFU reduction was reached even at flow rates of 196 mL min⁻¹ cm⁻², which is around five times higher than membrane filters. Log-3 bacterial reduction at these flow rates per area validates the potential of these GOX sheets as a simple method to efficiently filter bacteria when directly attached to a faucet. Cartridges with GOX-functionalized filter materials could be installed on the faucets of hospitals or public water sources to ensure drinking water quality without falling short of typical faucet flow rates.

4. Experimental Section

Materials and Methods: Used chemicals were purchased from following sources: 2-(dimethylamino) ethyl methacrylate (98%, Aldrich), 2,5-azobis(2-methylpropionitrile) (98%, Aldrich), 4,4'-methylene bis (phenyl isocyanate) (98% Aldrich), aluminum oxide (50–200 µm, Acros Organics), 3-dimethylaminopropylamine (99%, Aldrich), methyl iodide (99%, Acros Organics), dimethylformamide (99.5%, Acros Organics), tetrahydrofuran (99.9%, VWR chemicals), fluorescein sodium salt (Sigma-Aldrich), LB-Broth or LB-Agar (Carl Roth GmbH & Co. KG, Karlsruhe, Germany). The graphene oxide sheets were purchased as paste from “graphene-supermarket.com,” (0.5–5 µm, 80%). Cellulose fibers were from Vitacell LC 1000, JRS, J. Rettenmaier & Söhne GmbH.

Synthesis of GOX Fibers: The detailed synthesis of GOX and its characterization was reported in the previous work.[18] GOX fibers were produced by drying 1 g of cellulose fibers at 80 °C overnight to remove adsorbed water from the surface. 5 mg of 4,4'-methylenebis (phenyl isocyanate) (4,4'MDI) was dissolved in 5 mL dry DMF. The dried fibers were then immersed into the 4,4'MDI solution to homogeneously distribute and activate the surface of the fibers. 100 mg of GOX was dispersed in 45 mL of dry DMF by ultrasonication and added to the reaction mixture to bind the GOX onto the cellulose fibers. The mixture was allowed to react at room temperature under constant stirring for 24 h. The fibers were then filtered, washed with deionized water, and dried by lyophilization.

Scanning Electron Microscopy: The cellulose fibers and GOX fibers were imaged with a field emission scanning electron microscope (FE-SEM, Hitachi SU8030) at 15 kV, a current of 10 µA and a working distance (WD) of around 8.3 mm. The samples were coated with a gold layer by using a sputter coater (Emscope SC 500, Quorum Technologies, UK).

Fluorescent Microscopy of Bacterial Binding to GOX Cellulose Fibers: For analyzing the binding of E. coli bacteria (strain E. coli ORN208, K12 derivative) to cellulose fibers and GOX cellulose fibers, a bacterial culture was prepared and stained with the Bacteria Live/Dead Staining Kit (PromoCell GmbH, Heidelberg, Germany) according to the manufacturer's instructions.

In short, 10 mL LB medium was inoculated with E. coli (strain ORN208, K12 derivative) and incubated overnight at 37 °C in a shaking incubator. The overnight bacterial culture was divided in 2 mL aliquots and centrifuged at room temperature and 10000 × g for 15 min. After discarding the supernatant, 2 mL of 0.85% NaCl solution was added to each aliquot. After resuspending the pellet by vortexing, the aliquots were further incubated at 37 °C under shaking for 1 h and vortexed every 15 min. Then, two times all aliquots were centrifuged at room temperature and 10 000 × g for 15 min, the supernatant was discarded, and the pellet was resuspended by vortexing in 2 mL of 0.85% NaCl solution. The optical density (OD) from all aliquots was measured at 670 nm using 0.85% NaCl solution as blank and if needed adjusted to an OD of 0.25-0.3 corresponding to approximately 2 × 10⁶ CFU mL⁻¹. Then, 200 µL of sample suspension either containing 20 mg mL⁻¹ of bare cellulose fibers or 20 mg mL⁻¹ of GOX-functionalized cellulose fibers was mixed each with 200 µL of the prepared suspension containing living bacteria for at least 15 min at room temperature. 200 µL of each suspension (Bacteria-fiber-mix and bacteria suspension without fibers) was mixed by vortexing 2 µL of freshly prepared staining solution (1 volume of 5 × 10⁻³ M DMAO in DMSO + 2 volumes of 2 × 10⁻² M ethidium homodimer-III in DMSO/H₂O + 8 volumes 0.85% NaCl solution) at room temperature for 15 min in the dark.

For imaging, 5 µL from each stained suspension was mounted on a glass slide and covered with a coverslip. Imaging was done with a Zeiss Axio Observer.Z1 epifluorescence microscope (inverted) with an 40x objective and the Zeiss ZEN software using the fluorescence filter sets for GFP/FITC (live bacteria) and Cy3/Texas Red (dead bacteria) and using also brightfield and phase-contrast imaging.

GOX Fibers Filtration Test: An overnight culture of E. coli BL21 (DE3) was prepared in LB broth and incubated at 37 °C with constant agitation at 150 rpm. The culture was then diluted in LB broth to OD₆₀₀ = 0.05, approximately, 10⁷ colony forming units (CFU mL⁻¹). Initially, the syringe filter was wetted by passing 5 mL deionized water. 10 mL of the diluted culture was passed through syringe filters containing, 33, 66, 100, 200, or 600 mg GOX fibers. The filtrate was collected, serially diluted, and spread on LB agar plates (100 µL culture was spread). The filtration step was replicated by filtering 10 mL of the diluted culture. The LB agar plates were incubated for 16–24 h at 37 °C, colonies were counted, and CFU mL⁻¹ was calculated. To flush out all the bacteria that were retained in the filter, a final step in the filtration process was performed by filtering 5 mL LB broth containing 1% NaCl. The filtrate was serially diluted and plated on LB agar plates to calculate the CFU mL⁻¹ as described above.

Filter Cartridge Preparations: Cylindrical filter cartridges of 1.7 cm² surface area consist of 100 µm stainless steel mesh as base layer. Thin layer of cellulose fleece was placed on top of it as support layer for the fibers. 100 mg of GOX fibers or cellulose fibers were dispersed in water and filtered through the cartridge to load the fibers. Cellulose fleece layer was then deposited on top to sandwich the fibers. 300 mL of water was...
was further diluted 1:1000 in LB broth to obtain the desired 10⁴ CFU mL⁻¹. Three samples were collected from the bacterial culture was further diluted in deionized water to yield 3 L of a solution with 10⁶ CFU mL⁻¹. The bacterial culture was prepared in LB broth (devoid of salt) and incubated at 37 °C with constant agitation at 150 rpm. The overnight culture of E. coli BL21 (DE3) was prepared in LB broth without salt and incubated at 37 °C with constant agitation at 150 rpm. The overnight culture was diluted to OD₆₀₀ = 0.1 (in LB broth without salt) corresponding to a bacteria concentration of approximately 10⁶ CFU mL⁻¹. The bacterial culture was further diluted in deionized water to yield 3 L of a solution with 10⁷ CFU mL⁻¹. Three liters of bacterial culture were then filtered through the cartridge by applying external pressure with the help of an air compressor at steady flow rate. Two samples were collected from the filtrate for each liter, after passing 400 mL and 800 mL bacterial culture. The filtrate for each liter was filtered through a 100 mg GOX fibers filter cartridge. Pressure was applied to the culture by using an air compressor at steady flow rate. Two samples were collected from the filterate for each liter, after passing 400 mL and 800 mL bacterial culture. The samples were serially diluted and spread on LB agar plates. They were incubated for 16–24 h at 37 °C before colonies were counted and the corresponding CFU mL⁻¹ was calculated.

Flow Rate and Pressure Dependence: An overnight culture of E. coli BL21 (DE3) was prepared in LB broth (devoid of salt) and incubated at 37 °C with constant agitation at 150 rpm. The overnight culture was diluted in LB broth to OD₆₀₀ = 0.05, equal to approximately 10⁷ CFU mL⁻¹. This diluted culture was further diluted 1:1000 in deionized water to obtain the desired 10⁵ CFU mL⁻¹ cell density. One liter of diluted culture of 10⁵ CFU mL⁻¹ was passed through the cartridge filter containing 100 mg GOX fibers or 100 mg cellulose fibers. Three fractions of the flow-through were collected after 100 mL, 500 mL, and 900 mL of bacterial culture and spread on LB agar plates. The plates were incubated for 16–24 h at 37 °C, colonies were counted, and CFU mL⁻¹ was calculated.

GOX Fiber Bacterial Loading Test: An overnight culture of E. coli BL21 (DE3) was prepared in LB broth and incubated at 37 °C with constant agitation at 150 rpm. The culture was diluted in LB broth to OD₆₀₀ = 0.05, equal to approximately 10⁷ CFU mL⁻¹. The diluted culture of OD₆₀₀ = 0.05 was further diluted 1:1000 in LB broth to obtain the desired 10⁵ CFU mL⁻¹ cell density. One liter of diluted culture of 10⁵ CFU mL⁻¹ was passed through the cartridge filter containing 100 mg GOX fibers or 100 mg cellulose fibers. Three fractions of the flow-through were collected after 100 mL, 500 mL, and 900 mL of bacterial culture and spread on LB agar plates. The plates were incubated for 16–24 h at 37 °C, colonies were counted, and CFU mL⁻¹ was calculated.

Keywords
flow rate, graphene oxide, polycationic microsheets, water filter, water purification

Received: October 5, 2021
Revised: December 10, 2021
Published online: January 23, 2022

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements
Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest
The authors declare no conflict of interest.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

[1] A. Otte, D. Coates, R. Connor, G. Roder, D. Hebart-Coleman, M. Klimes, E. Yaari, M. Gutierrez, N. Crawhall, R. Kinna, The United Nations World Water Development Report 2021, pp. 97–106.
[2] L. Liu, H. L. Johnson, S. Cousens, J. Perin, S. Scott, J. E. Lawn, I. Rudan, H. Campbell, R. Cibulskis, M. Li, Lancet 2012, 379, 2151.
[3] B. K. Decker, T. N. Palmore, Curr. Opin. Infect. Dis. 2013, 26, 345.
[4] A. Breathnach, M. Cubbon, R. Karunaharan, C. Pope, T. Planche, J. Hosp. Infect. 2012, 82, 19.
[5] F.-A. Pitten, B. Panzig, G. Schröder, K. Tietze, A. Kramer, J. Hosp. Infect. 2001, 47, 125.
[6] H. Kanamori, D. J. Weber, W. A. Rutala, Clin. Infect. Dis. 2016, 62, 1423.
[7] M.-Y. Xia, Y. Xie, C.-H. Yu, G.-Y. Chen, Y.-H. Li, T. Zhang, Q. Peng, J. Controlled Release 2019, 307, 16.
[8] O. Akhavan, E. Ghaderi, A. Esfandiar, J. Phys. Chem. B 2011, 115, 6279.
[9] S. Liu, M. Hu, T. H. Zeng, R. Wu, R. Jiang, J. Wei, L. Wang, J. Kong, Y. Chen, Langmuir 2012, 28, 12364.
[10] A. Tiwari, M. Syväjärvi, Graphene Materials: Fundamentals and Emerging Applications, John Wiley & Sons, New York 2015.
[11] S. Liu, T. H. Zeng, M. Hofmann, E. Burcombe, J. Wei, R. Jiang, J. Kong, Y. Chen, ACS Nano 2011, 5, 6971.
[12] S. Hermanová, M. Zarevická, D. Bouša, M. Pumera, Z. Sofer, Nanoscale 2015, 7, 5852.
[13] S. You, J. Yu, B. Sundqvist, A. V. Talyzin, Phys. Status Solidi 2012, 249, 2568.
[14] W. S. Hummers, J. R. Offeman, J. Am. Chem. Soc. 1958, 80, 1339.
[15] C. K. Chua, Z. Sofer, M. Pumera, Chem. - Eur. J. 2012, 18, 13453.
[16] D. R. Dreyer, S. Park, C. W. Bielawski, R. S. Ruoff, Chem. Soc. Rev. 2010, 39, 228.
[17] A. M. Dimiev, S. Eigler, Graphene Oxide: Fundamentals and Applications, John Wiley & Sons, New York 2016.
[18] H. Ji, H. Sun, X. Qu, Adv. Drug Delivery Rev. 2016, 105, 176.
[19] R. Ahmed, A. Vasihampayan, J. L. Cuellar-Camacho, D. J. Wight, I. Donský, W. Unger, E. Grohmann, R. Haag, O. Wagner, Adv. Mater. Interfaces 2020, 7, 1902066.
[20] A. Vasihampayan, R. Ahmed, O. Wagner, A. de Jong, R. Haag, J. Kok, E. Grohmann, Mater. Sci. Eng., C 2021, 119, 11578.
[21] D. Wischer, D. Schneider, A. Poehlein, F. Herrmann, H. Oruc, J. Meinhardt, O. Wagner, R. Ahmed, S. Kharin, N. Novikova, Front. Microbiol. 2020, 11, 1626.
[28] Q. Zhang, H. Liu, X. Chen, X. Zhan, F. Chen, *J. Appl. Polym. Sci.* 2015, 132, 14.
[29] S. R. Williams, T. E. Long, *Prog. Polym. Sci.* 2009, 34, 762.
[30] S. Liu, R. J. Ono, H. Wu, J. Y. Teo, Z. C. Liang, K. Xu, M. Zhang, G. Zhong, J. P. Tan, M. Ng, *Biomaterials* 2017, 127, 36.
[31] W. Zhan, T. Wei, L. Cao, C. Hu, Y. Qu, Q. Yu, H. Chen, *ACS Appl. Mater. Interfaces* 2017, 9, 3505.
[32] P. Subramanian, F. Barka-Bouaifel, J. Bouckaert, N. Yamakawa, R. Boukherroub, S. Szunerits, *ACS Appl. Mater. Interfaces* 2014, 6, 5422.
[33] L. Xiao, J. Sun, L. Liu, R. Hu, H. Lu, C. Cheng, Y. Huang, S. Wang, J. Geng, *ACS Appl. Mater. Interfaces* 2017, 9, 5382.
[34] S. Omidi, A. Kakanejadifard, F. Azarbari, J. Mol. Liq. 2017, 242, 812.
[35] K. H. Tan, S. Sattari, I. S. Donskyi, J. L. Cuellar-Camacho, C. Cheng, K. Schwibbert, A. Lippitz, W. E. Unger, A. Gorbushina, M. Adeli, *Nanoscale* 2018, 10, 9525.
[36] A. Ottenhall, J. Henschen, J. Illergård, M. Ek, *Environ. Sci.: Water Res. Technol.* 2018, 4, 2070.
[37] Y. L. F. Musico, C. M. Santos, M. L. P. Dalida, D. F. Rodrigues, *ACS Sustainable Chem. Eng.* 2014, 2, 1559.
[38] M. R. Esfahani, S. A. Aktij, Z. Dabaghian, M. D. Firouzjaei, A. Rahimpour, J. Eke, I. C. Escobar, M. Abolhassani, L. F. Greenlee, A. R. Esfahani, *Sep. Purif. Technol.* 2019, 213, 465.
[39] Y. Wang, F. Hammes, N. Boon, T. Egli, *Environ. Sci. Technol.* 2007, 41, 7080.
[40] R. Zhang, Y. Liu, M. He, Y. Su, X. Zhao, M. Elimelech, Z. Jiang, *Chem. Soc. Rev.* 2016, 45, 5888.
[41] L. Li, P. A. Quinlivan, D. R. Knappe, *Carbon* 2002, 40, 2085.
[42] S. D. Alexandratos, *Ind. Eng. Chem. Res.* 2009, 48, 388.
[43] L. Kan, Z. Xu, C. Gao, *Macromolecules* 2011, 44, 444.
[44] T. G. Van De Ven, A. Sheikhi, *Nanoscale* 2016, 8, 15101.
[45] J. K. Pandey, A. N. Nakagaito, H. Takagi, *Polym. Eng. Sci.* 2013, 53, 1.
[46] National systems to support drinking-water, sanitation and hygiene: global status report, W. H. Organization, 2019.
[47] https://www.sterlitech.com/ptfe-unlaminated-membrane-filters-hydrophilic-02-micron-90mm-25pk.html (accessed: June 2021).