Facile Postprocessing Alters the Permeability and Selectivity of Microbial Cellulose Ultrafiltration Membranes

Facile Post-Processing Alters Permeability and Selectivity of Microbial Cellulose Ultrafiltration Membranes

Marcus C. Holland[1], Christina G. Eggensperger[1], Mattia Giagnorio[1,2], Jessica D. Schiffman[3], Alberto Tiraferri[2], Katherine R. Zodrow[1]*

Submitted to Environmental Science & Technology

Affiliations:

[1]Environmental Engineering Department, Montana Technological University, Butte, MT, USA

[2]Department of Environment, Land and Infrastructure Engineering, Politecnico di Torino, Turin, Italy

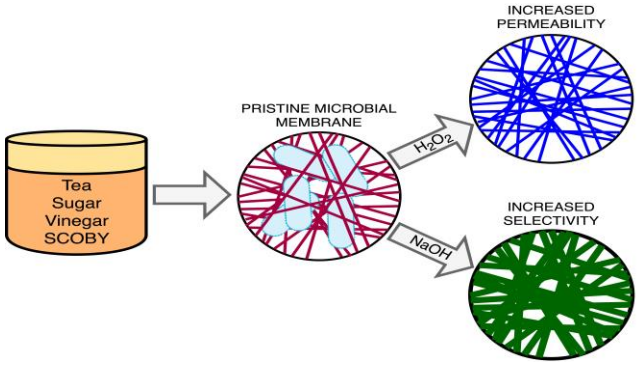
[3]Department of Chemical Engineering, University of Massachusetts, Amherst, MA, USA

*Corresponding author: kzodrow@mtech.edu

Abstract

Water filtration membranes produced sustainably through microbial cellulose production can have filtration properties altered through facile chemical treatments. Microbial cellulose is an effective membrane filtration medium, and pristine microbial membranes can serve as ultrafiltration membranes with a permeability of 143 L $m^{-2}h^{-1}bar^{-1}$ and a particle size cut off of 35 nm. As living biofilms, these membranes consist of microbial cellulose, bacteria, and extracellular polymers. Thus, additional biofilm components may reduce the intrinsic permeability of the cellulose. Here, microbial membranes were treated with hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaOCl, liquid bleach) to remove impurities present in microbial cellulose and increase membrane permeability. For example, permeability increased from 143 to 257 L $m^{-2}h^{-1}bar^{-1}$ with treatment by 0.3% H_2O_2 for 12 min. Membranes were also treated with sodium hydroxide (NaOH) to increase membrane selectivity, and the particle size cut off was reduced from 35 to 10 nm post-treatment by 0.8% NaOH or 20 min. Scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), contact angle goniometry, and X-ray diffraction (XRD) were used to characterize the physical and chemical properties of the membrane matrix. Facile chemical treatments provide a significant degree of flexibility to tailor microbial membranes to meet specific needs. Microbial membrane production is inherently accessible, and this study furthers that accessibility by utilizing only readily-available components to treat microbial membranes and expand their potential applications.

Keywords: sustainable water treatment, accessible water treatment, membrane water treatment, microbial cellulose



Synopsis

Accessible and sustainable water-filtering membranes grown from microbes can be modified through simple chemical treatments.

Introduction

Annually, 1.7 million preventable deaths occur from infectious diseases acquired through consumption of contaminated drinking water. The majority of those that die are children[1]. The World Health Organization estimates that 2 billion people worldwide lack access to a safely-managed drinking service[2]. For these people, drinking water is often collected and stored for later use. Even if the initial water source is relatively clean, storage causes a significant reduction in water quality[3]. Ideally, a point-of-use treatment option would be available. However, current point-of-use chemical treatments can result in the formation of carcinogenic disinfection byproducts when exposed to organic compounds commonly found in untreated water[4], and aesthetic qualities of chemically disinfected water can deter consumption[5].

A more desirable point-of-use treatment option is that of membrane filtration: scalable, robust, and with a small footprint, membranes can provide more consistent water quality than conventional physical, chemical, and biological treatments. Additionally, because membranes provide a physical barrier to the passage of microorganisms, their use for water treatment may not require chlorination, reducing the risk of disinfection byproduct formation[6]. Unfortunately, membranes must eventually be replaced, and membranes can only be manufactured in controlled laboratory environments. Harmful solvents such as such as dimethylacetamide[7,8] are also required for synthetic membrane production. Dimethylacetamide is classified by the California Office of Environmental Health Hazard Assessment (OEHHA) as both carcinogenic and reprotoxic[9]. Therefore, use of membranes as a point-of-use filtration option is hindered by accessibility: users cannot

make new membranes themselves, and are unlikely to be able to afford industrially-produced replacements.

Recently, research has shown that by using microbially-produced cellulose as a medium, “microbial” membranes, can mitigate the aforementioned concerns, including membrane accessibility and using toxic chemicals for synthesis and disinfecting. Requiring only water, tea, sugar, vinegar, and a starter microbial culture, a microbial membrane can be produced in just 7 days in a clean, but non-sterile, environment[10]. Microbial membranes empower individuals or smaller corporations to sustainably produce water treatment membranes, in an environment with less precision than industrial production would require.

However, applications of microbial cellulose membranes are limited to the properties of the as-grown (pristine) membranes, such as permeability and selectivity. Depending on the quality of the source water, changes to membrane permeability and selectivity may be desired. For example, with relatively pure source waters, a membrane with higher permeability, and therefore lower selectivity, may be desired to increase water production rates. Alternatively, in areas with more contaminated source waters, membranes with higher selectivity (and subsequently lower permeability) may be desired. While previous research has shown that microbial cellulose membrane thickness and porosity varies with carbon source and purification treatment parameters,[11] a standardized set of simple, accessible methods for microbial cellulose permeability and selectivity manipulation post-fabrication could allow the development

of “customized” microbial membrane point-of-use water filters. Growing microbial cellulose and post-processing with commonly available chemicals could circumvent current requirements for laboratory environments and laboratory-grade chemicals for cellulose modification, increasing membrane accessibility.

Cellulose cleaning and purification are common processes in the textile industry, and cleaning/purification of pristine microbial cellulose fibers provides an opportunity to tailor the structure of the microbial membranes, increasing permeability and decreasing selectivity. Currently, the most common cellulose purification technique in use is the Kraft Process[12], a method which involves the use of concentrated chlorine dioxide[13], an oxidizing agent reported by EPA to cause neurodevelopmental effects in gaseous form at exposures as low as 0.03 ppm/day[14]. However, consumer-grade hydrogen peroxide (H_2O_2) and sodium hypochlorite (liquid bleach), compounds with greater accessibility and less risk of danger to human health, can also be used to purify cellulose and remove non-cellulose organic matter and microbes. H_2O_2 has a long history of use as a cellulose purification agent[15], and sodium hypochlorite is a widely used and effective antimicrobial agent[16]. While its longstanding usage as a disinfectant for wounds is controversial[17], H_2O_2 remains widely available. Sodium hypochlorite’s ubiquity in the food industry and popularity as a disinfectant makes it similarly widespread. Sodium hydroxide (NaOH) is also a common component in cellulose regeneration solutions[18]. As cellulose does not exhibit a melting temperature[19], effective dissolution agents are essential in cellulose processing; NaOH has been shown to effectively break down hydrogen bonds in cellulose and lead to dissolution[18]. NaOH is used in large-scale food

preparation processes,[20,21] has been used to produce a cellulose-based dialysis membrane, and is widely available.

Facile treatments with readily-available, consumer-grade compounds may serve as a means for users to tune microbial membrane parameters as needed. The objective of this study is to develop methods to adjust the permeability and the selectivity of natural cellulose membranes, while maintaining their inherent accessibility. Cellulose membranes obtained from microbial suspensions and with transport properties in the range of ultrafiltration membranes were subjected to post-treatments using widely available H_2O_2, sodium hypochlorite, and NaOH. Characterization techniques were applied to characterize the chemical and physical changes that post-treatments imparted on membranes. Synthetic cellulose membranes produced using electrospinning were used as a chemical control. This study demonstrates that common chemical treatments could increase the range of usage of microbial membranes, while maintaining accessibility.

Materials and Methods

Microbial Membrane Production

Microbial membranes were produced using a co-culture of yeast and bacteria (Kombucha starter culture, Cultures for Health). To grow a microbial membrane, 15 g microbial cellulose starter culture was placed in 700 mL of growth solution, consisting of sucrose (85 g; granulated; generic), black tea (4.6 g; crush, tear, curl processed[22];

pekoe; filter paper bags), and distilled white vinegar (200 mL; 5% acetic acid; generic), dissolved in sterilized in-house deionized (DI) water (700 mL, Culligan). The mixture was placed, not shaking, in a 25 °C incubator (Low Temperature Incubator 815, Precision Scientific) where a microbial membrane grew at the air-water interface to a thickness of 1.0-1.5 mm over 7-10 days. After fabrication, membranes were kept in a "storage solution" consisting of 4.6 g black tea and 200 mL of 5% acetic acid dissolved in 700 mL sterilized DI water. The microbial membranes were used within 10 days of fabrication[10].

Membrane Post-Processing

Liquid bleach (liquid, 6.0%, generic, pH ~12), H_2O_2 (liquid, 3.0%, generic), and NaOH (pellets, 97%, Fisher Scientific) were used for membrane treatment. Preliminary trials were carried out with each treatment type (conditions in Supporting Table S1). Chemical concentrations were derived from literature[23,24] and obtained by feasible dilutions of concentrations of consumer-grade chemicals. During treatment, the container was gently swirled every 30 seconds. Treatment times were determined by placing membranes into solution until membrane color visibly changed. After treatment, the membrane was immediately transferred to a new container with DI water. The membrane was swirled for 30 seconds in the DI water to remove residual treatment solution. The membrane was removed from the container and added to a new container with fresh DI water. This rinsing process was performed 3 times. Treated membranes were used for permeability and selectivity testing immediately, and were not stored.

Images of membranes treated with undiluted consumer-grade concentrations are presented in Supporting Figure S1.

After initial testing, 0.1% bleach (10 min), 0.3% H_2O_2 (12 min), and 0.8% NaOH (20 min) were chosen for more extensive characterization because they resulted in the largest shifts in membrane permeability (Supporting Figure S2). Gloves, goggles, and full-length clothes were worn at all times while working with treatment chemicals. Concentrated NaOH was handled in a chemical hood.

Membrane Characterization

Chemical composition of membrane surfaces were probed using Fourier transform infrared spectroscopy (FTIR) (Nicolet iS5, iD5, with ATR attachment). Prior to analysis, samples were lyophilized (Labconco FreeZone 2.5) using a pressure of 2.47×10^{-4} bar and a temperature of $-46\ ^{o}C$. Samples were left to sublimate for 2 d and stored at room temperature until analysis.

To confirm that observed FTIR results were due to changes in chemical structure in treated cellulose and not simply changes in crystallinity, X-ray diffraction (XRD) analysis was performed and membrane crystallinities were compared. XRD was performed using an Ultima IV X Ray Diffractometer. Prior to analysis, samples were air-dried at room temperature and ambient pressure. Samples were analyzed with a theta-theta scan with a scan range from 10 to 50°.[25] The scan speed was set to 5 °2θ×min^{-1}, with 40 kV and 40 mA. OriginLab software was used to find the ratio of the area underneath the

observed crystalline peaks to the total area for each graph, resulting in crystallinity index values.[26]

Critical point drying (CPD) was used to prepare samples for scanning electron microscopy. Samples were placed sequentially in conical tubes containing solutions of 5, 15, 30, 50, and 70% isopropyl alcohol (Fisher). Samples were soaked in each solution for 15 min. The samples were then placed in a 99.97% IPA (Fisher) solution and left overnight. Then, samples were placed in an Autosamdri-931 CPD 3.175 cm chamber. The system cycled for 6 h, at which point the unit went into stasis mode. The samples were removed from the sample tray the next day. Samples were sputter coated with gold prior to SEM analysis. A TESCAN MIRA3 SEM, using a Schottky source, 5 kV accelerating voltage, and secondary electron detector was used to image the sample materials. Working distance of the SEM was in the range 10-14.5 mm.

Thermogravimetric analysis (TGA) was performed using a TA Instruments SDT 650 Simultaneous Thermal Analyzer. Argon was utilized as the inert gas. Samples were brought to 700 ^{o}C from room temperature at a rate of 10 ^{o}C·min^{-1}. Prior to analysis, samples were lyophilized. Samples were stored at room temperature until analysis.

Contact angle was determined using a Biolin Scientific ThetaLite100 contact angle goniometer with OneAttension software. Samples were inverted in water and an air bubble was placed on the membrane surface. The inverse of the contact angle of the air on membrane was taken to determine the contact angle of the water on membrane.

Membrane Filtration Performance

Microbial membranes were tested in a 10 or 50 mL unstirred dead-end filtration cell (Amicon, Millipore) connected to a reservoir (1000 mL, Amicon, Millipore), a compressed air cylinder, and a digital scale connected to a computer with software for recording scale readings over time (Software Wedge, WinWedge).

All permeability testing was performed with DI water. Membranes were first compressed at 3.10 bar for 1 h. After 1 h, the filtration cell was depressurized to 0.69 bar and allowed to stabilize for 15 min. Permeability was then tested at four pressure intervals: 0.69, 1.34, 2.07, and 3.10 bar. Each interval was tested for 15 min. Mass was recorded every 60 s, for a total of 15 points for every interval.

Selectivity was tested using gold nanoparticles with hydrodynamic diameters of 8.8 nm, 20.4 nm, and 28.0 nm (NanoComposix) and polypropylene beads with hydrodynamic diameters of 48.11, 110.0, and 201.3 nm[10]. Their hydrodynamic diameters were measured via dynamic light scattering (Zetasizer, Malvern). Particle concentration was determined using UV-vis spectroscopy and a calibration curve previously determined for each particle type. All gold nanoparticles were analyzed at a wavelength of 519 nm. Colored polypropylene beads (polybeads) were analyzed at a wavelength of 350 nm. The dead-end filtration cell was filled with the particle solution, and each test was performed at 1.34 bar. The first 1.0 g of permeate was discarded, and a conical tube was used to collect the permeate sample.

215

216 ### Statistics

217 Significance of results was determined in MiniTab using a Welch's one-way Analysis of

218 Variance (ANOVA) with a post-hoc Games-Howell test[27] with α at 0.05.

219

220 # Results and Discussion

221 ### Post-Processing Changes Cellulose Structure

222

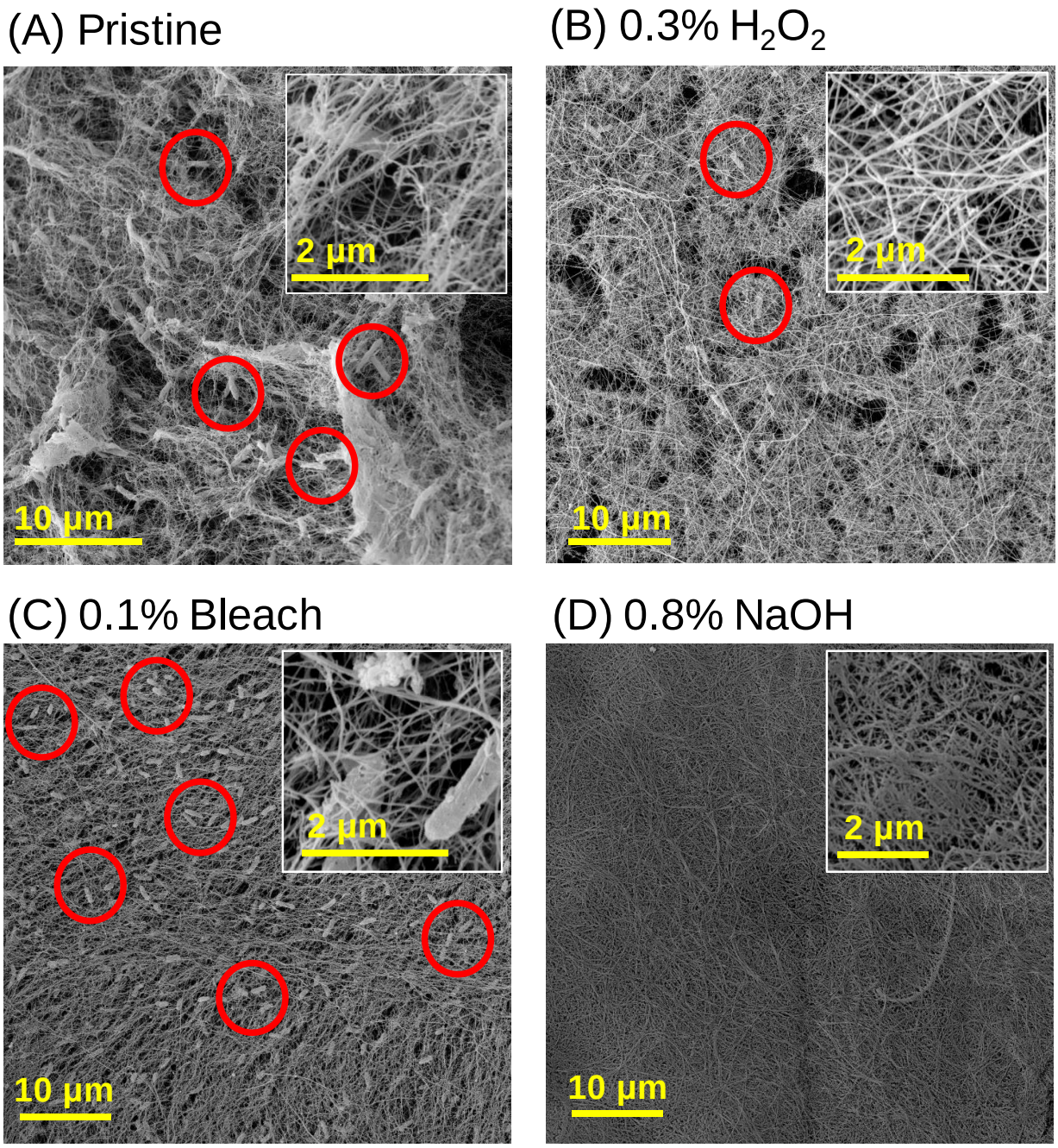
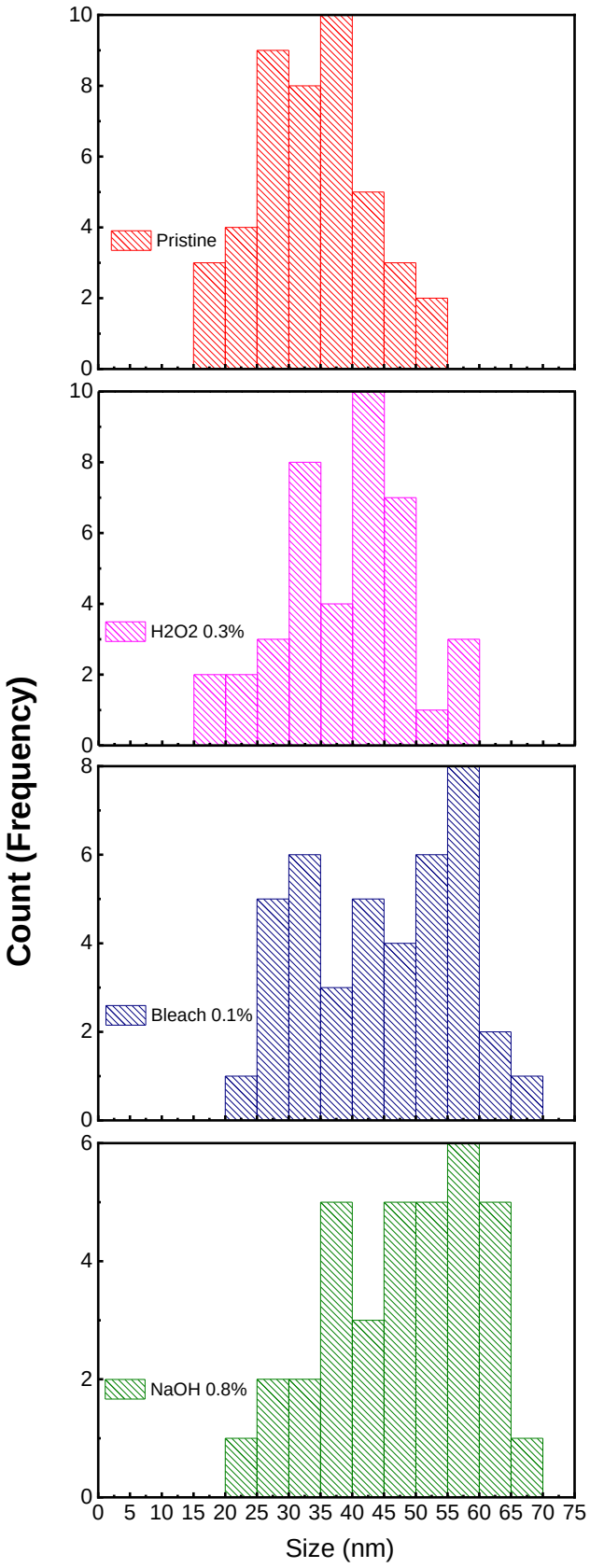


Figure 1: Representative scanning electron micrographs of the (A) pristine membrane, (B) membranes following 0.3% H_2O_2 treatment, (C) 0.1% bleach treatment, and (D) 0.8% NaOH treatment. Inset micrographs present higher magnification details. Examples of bacteria are circled in red.

Post-processing alters microbial cellulose fiber structure: fiber cleaning and gelatinization were observed with different treatments (Figure 1). Membranes not treated before use (pristine) are structurally heterogeneous; there is “webbing” across the surface of the fibers that may be due to the presence of extracellular polymers produced by the microorganisms (Figure 1A). Microorganisms are visible in the fiber network. These microorganisms and impurities are commonly seen in pristine microbial cellulose[24]. H_2O_2-treated membranes have greater homogeneity than pristine membranes; a more open structure and a smoother fiber morphology is observed in the Figure 1B inset. Additionally, fewer microorganisms were observed. Bleach-treated membranes also have an increase in membrane homogeneity similarly to H_2O_2, but microbes are still visible in the pore spaces (Figure 1C). A more open structure is shown in the inset, but the fibers do not appear as well-defined as those in the H_2O_2-treated membranes. In contrast, the gelatinization that NaOH causes in cellulose[18] is apparent in Figure 1D; the membrane surface is smoother and the cellulose fibers are also smoother. The inset suggests that gelatinization results in a drastic decrease in membrane porosity. Thus, qualitatively, the H_2O_2 and bleach treatments appeared to open the structure of the membrane, while the NaOH treatment appeared to tighten the structure of the membrane.



241

Figure 2: Fiber diameter frequencies of membranes from analyses of SEM images. Fiber diameters were measured for each listed membrane type using ImageJ software.

Some of the post-treatments changed fiber diameters (Figure 2). The maximum fiber diameter of pristine membranes was 60 nm, with an average fiber diameter of 35 ± 10 nm (Figure 2). Membranes treated with 0.3% H_2O_2 had no significant change in fiber diameter, with a maximum fiber diameter of 57 nm and an average fiber diameter of 38 ± 10 nm. The 0.1% bleach-treated membranes had a maximum fiber diameter of 69 nm and an average fiber diameter of 44 ± 12 nm. Membranes treated with 0.1% bleach showed an increase in average fiber diameter from pristine membranes of 29%. The 0.8% NaOH-treated membranes had a maximum fiber diameter of 138 nm, and an average fiber diameter of 59 ± 26 nm. Fiber size distributions of 0.8% NaOH treated membranes show an increase in fiber diameters of 67%. ANOVA showed significant differences between fiber diameters of pristine membranes compared to 0.1% bleach- and 0.8% NaOH-treated membranes. Noticeable gelation was visible in NaOH membranes during SEM analysis (Figure 1D). Thus, H_2O_2 cleans fibers but cause no significant change in diameter, while bleach and NaOH treatments result in fiber gelation and significant increases in diameter (group p value = <0.000). It is likely that changes in fiber diameter will alter membrane performance (discussed below).

Post-Processing Alters Functional Groups and Hydrophilicity

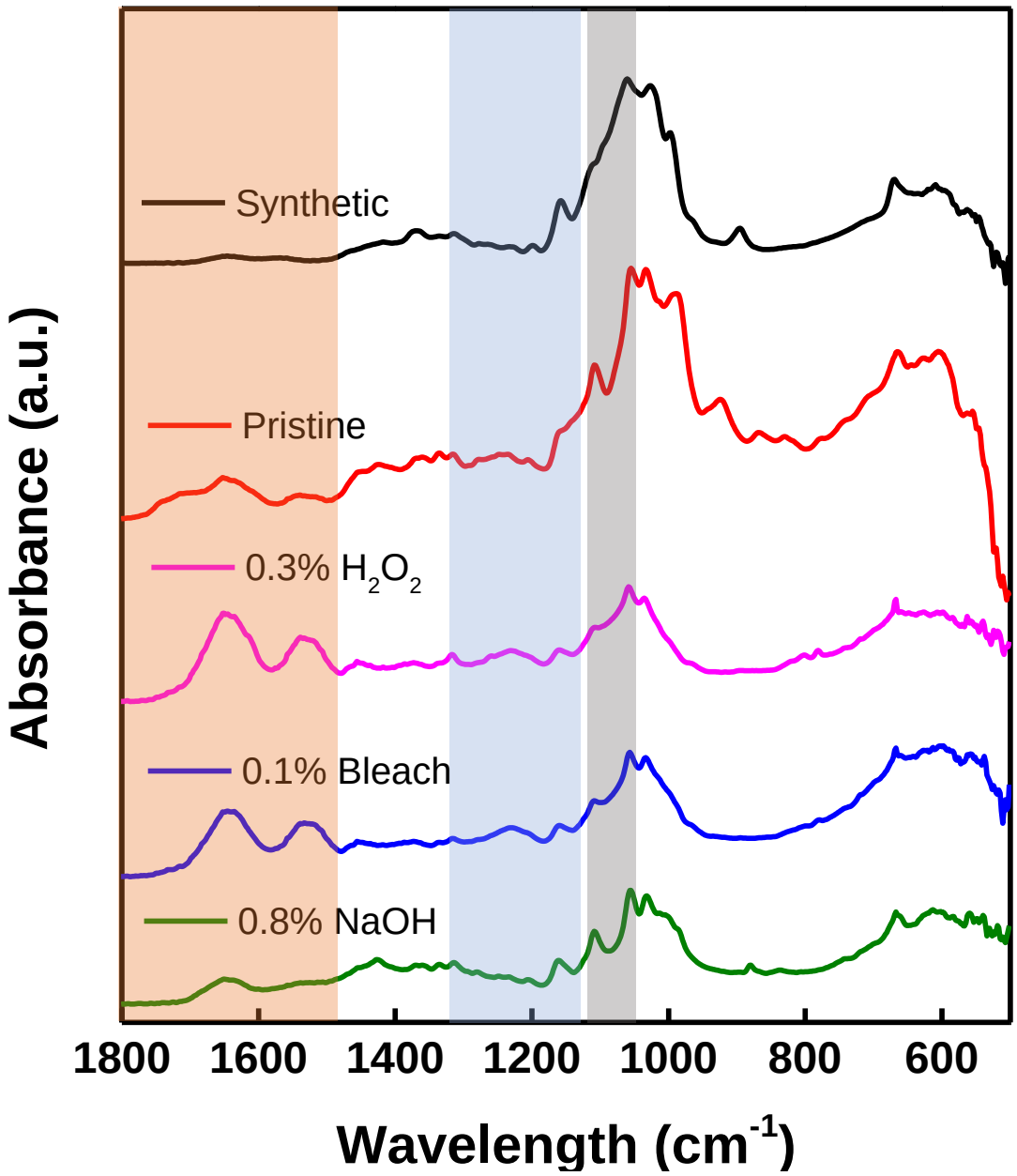


Figure 3: FTIR spectra for pristine and selected treated membranes. Orange corresponds to lipids, proteins, and nucleic acids; blue corresponds to nucleic acids; and gray corresponds to lipids and nucleic acids. Samples were prepared via lyophilization.

261

262 FTIR spectra of the membranes indicate that some of the treatments decreased

263 cellulose impurities (Figure 3). The synthetic membrane displayed peaks characteristic

264 to cellulose, namely, at wavelength 895 cm^{-1} (functional group: COC, CCO and CCH

deformation and stretching) as well as 1020 and 1046 cm^{-1} (C-C, C-OH, C-H ring and side group vibrations). Before treatment, the pristine microbial membrane displayed broad peaks not seen in synthetic cellulose from 1800 cm^{-1} to 1500 cm^{-1}, a peak at 1090 cm^{-1}, and a broad peak at 1261 cm^{-1}. The areas which correspond to microbial cellulose impurities[29] are highlighted in Figure 3. The 0.1% bleach and 0.3% H_2O_2 treatments led to a reduction in the peak at 1090 cm^{-1}, while 0.8% NaOH treatment did not. The 0.5 M NaOH treatment reduced the peak at 1261 cm^{-1}, whereas 0.1% bleach and 0.3% H_2O_2 treated membranes did not. These results likely indicate that 0.1% bleach and 0.3% H_2O_2 treatments are more effective at removing lipids, while NaOH removes more nucleic acids. The removal of lipids has unclear implications for membrane filtration performance as the hydrophobicity of lipids depends on their orientation at the cellulose surface. However, the removal of relatively hydrophobic nucleic acids could have contributed to the increased hydrophilicity of the NaOH-treated membrane, discussed below. Finally, peaks in the 1500 to 1700 cm^{-1} range have been associated with the presence of bacterial cells on cellulose. These peaks were the most prominent in the H_2O_2 and bleach-treated membranes, membranes with the largest number of cells observed in the SEM images (Figure 1C,D).

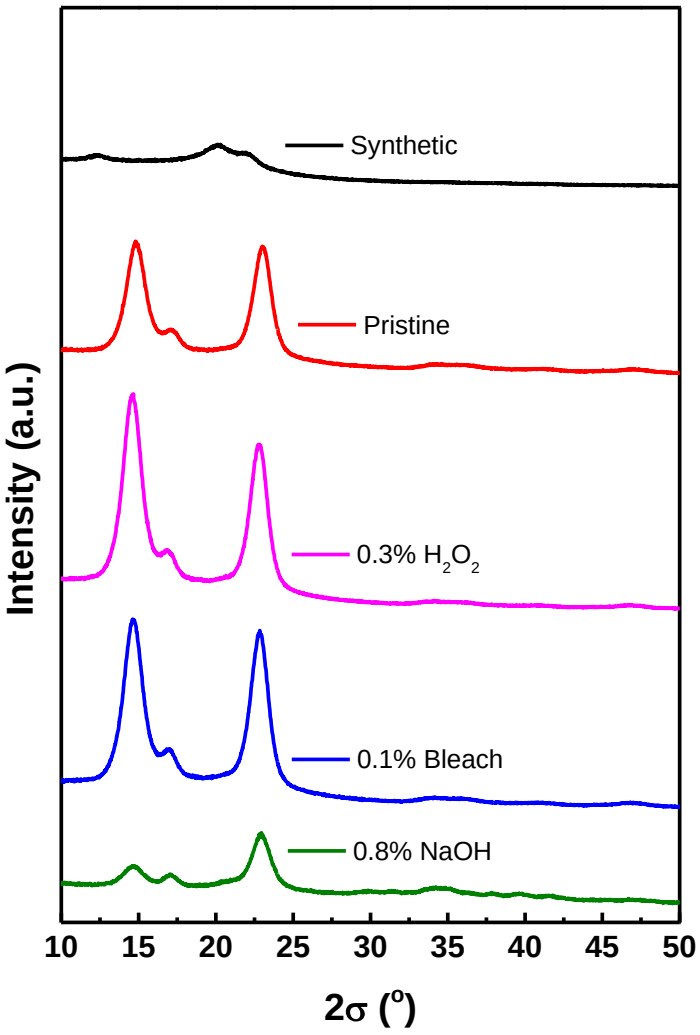


Figure 4: XRD spectra of treated membranes compared to pristine and synthetic membranes. Prior to analysis, samples were air dried at room temperature for 7 days.

XRD was used to quantify if the facile cleaning treatments altered the crystallinity of the membranes. Crystallinity indices were calculated from XRD spectra (Figure 4). A pristine membrane has a crystallinity index of 63%, consistent with values reported in literature of crystallinity of microbial cellulose[26]. The membrane treated with 0.1% bleach had a crystallinity index of 67%, a change compared to pristine of 6%. The 0.3% H_2O_2 and 0.8% NaOH membranes had crystallinity indexes of 69% and 61%, respectively, and percent changes from pristine of 10% and 3%. Crystallinity index

variability in microbial cellulose is high[26], and differences in treated membranes compared to a pristine sample were within observed variability seen in literature. Therefore, it was assumed that FTIR results are representative of chemical composition changes and not changes in crystallinity.

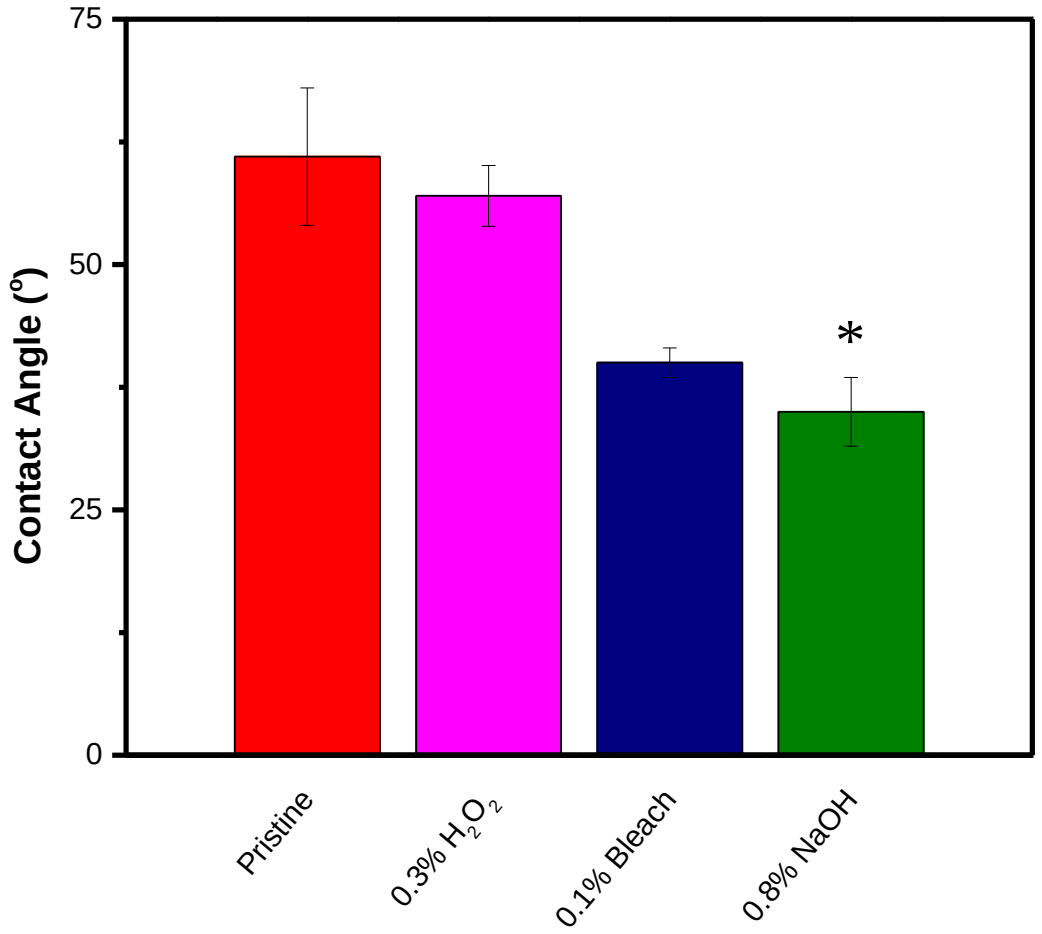


Figure 5: Air in DI water captive bubble contact angles of membranes (asterisk denotes statistically significant results when compared to pristine membrane).

Figure 5 shows the contact angle of treated and pristine membranes. An ANOVA test indicated the contact angle of 0.8% NaOH-treated membranes were significantly different from the pristine. These results indicate that NaOH treatment causes an

increased affinity for water in the membranes, possibly as a result of gelation, which may have been due to changes in the membrane's chemistry (Figure 3). Increased hydrophilicity in the NaOH-treated membrane may be due to a decrease in nucleic acid content in this membrane. Contact angle measurements on membranes with different treatment intensities are reported in Supporting Figure S3.

Post-Processing Offers Membrane Customization

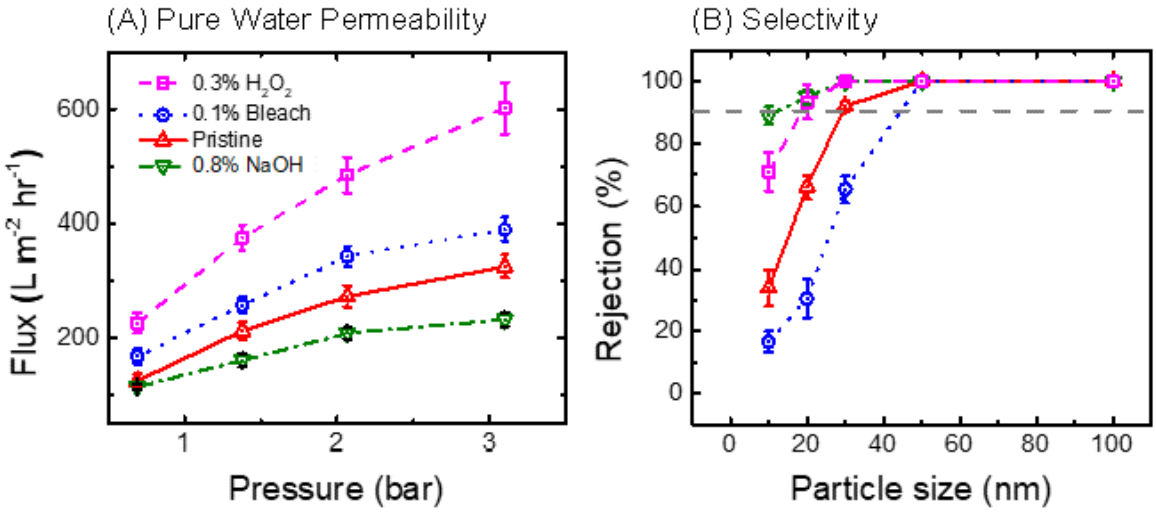


Figure 6: (A) Deionized water flux of membranes, (B) Selectivity of membranes (dashed selectivity lines connecting the experimental data represent expected results). Membranes were tested in a dead-end filtration cell. Prior to flux measurements, membranes were compressed at 3 bar for 1 h. Selectivity tests were run at 1.38 bar using gold and polymer nanoparticles. Lines are intended only as a guide for the eye.

Microbial membrane treatments enable permeability and selectivity property customization (Figure 6). Membranes treated with 0.3% H_2O_2 showed the greatest increase in permeability (Figure 6A). Pure water permeability increased from an average of 143 $L \cdot m^{-2} hr^{-1} bar^{-1}$ for pristine membranes to 257 $L \cdot m^{-2} hr^{-1} bar^{-1}$ for 0.3%

H_2O_2-treated membranes (79.7% increase). This increase was found to be statistically significant in ANOVA testing. This change is likely due to H_2O_2 being an effective membrane purification agent; as a strong oxidizer, H_2O_2 is widely utilized for removal of non-cellulose organic matter in wood pulp. By removing non-cellulose membrane constituents, H_2O_2 frees up pore spaces and allows for an increase in rate of water filtration. Cleaner cellulose fibers with more open pore space were observed in the scanning electron micrographs (Figure 1B). The thermogravimetric analysis shown in Supporting Figure S4 also indicates the effectiveness of H_2O_2 cellulose purification 0.3% H_2O_2 treatment resulted in membrane decomposition closer to that of synthetic cellulose, indicating a greater similarity in chemical composition to synthetic cellulose than microbial cellulose. Therefore, H_2O_2 removes non-cellulose components of microbial membranes while leaving cellulose intact (Figure 3). Interestingly, the H_2O_2 treatment also increased the selectivity of the membrane, decreasing the particle size cutoff from 30 to 20 nm. This simultaneous increase in permeability and selectivity was not due to an increase in hydrophilicity (Figure 5). We also considered possible adsorption of the nanoparticles to the membrane during testing. However, no adsorption of nanoparticles in batch testing was observed (Supporting Figure S4). Thus, the reason H_2O_2 increases both membrane permeability and selectivity is unknown and warrants further investigation.

Membranes treated with 0.1% bleach offered a more modest increase in permeability, bringing membrane pure water permeability from 147 $L \cdot m^{-2} hr^{-1} bar^{-1}$ to 181 $L \cdot m^{-2} hr^{-1} bar^{-1}$ (26.1% increase). This increase was not found to be statistically

significant in ANOVA testing. As seen in Figure 1D, 0.1% bleach-treated membranes were more homogeneous than pristine membranes, but bacteria were retained in fibers. These bacteria, while not dangerous to human health, may occupy pore spaces that could otherwise be utilized for filtration. It is possible that the bleach treatment was less effective than the H_2O_2 treatment at removing bacteria from the surface of the membrane due to bleach having a lower oxidation potential, i.e., being a less powerful oxidizer. Based on fiber diameters analyzed in Figure 2, it is possible that 0.1% bleach treatment caused small amounts of gelatinization or dissolution of cellulose similar to that seen in 0.8% NaOH treatment. However, the decrease in membrane permeability and increase in selectivity seen in 0.8% NaOH treatment was not observed in 0.1% bleach treatment; instead, a slight increase in permeability and slight decrease in selectivity was seen (Figure 6A,B). In Supporting Figure S4, TGA results indicate that 0.1% bleach-treated membranes are more similar in composition to synthetic membranes compared to pristine cellulose membranes. Thus, 0.1% bleach treatment appears to purify cellulose, but to a lesser degree than 0.3% H_2O_2 treatment.

The NaOH treatment was explored for its ability to tighten the pores of the pristine microbial membrane, and the NaOH treatment decreases membrane pure water permeability from 143 $L \cdot m^{-2} hr^{-1} bar^{-1}$ to 115 $L \cdot m^{-2} hr^{-1} bar^{-1}$ (24.7%) (Figure 6A). This change was found to be statistically significant in ANOVA testing. Likewise, NaOH increased membrane selectivity to a particle size cutoff of 10 nm (Figure 6B). This result is attributed to a reduction in pore size from cellulose gelatinization. The gelatinization reaction that caused this change in pore size and increase in selectivity is apparent in

Figure 1D, where scanning electron micrographs show the surface of the NaOH-treated membrane was smoother and had larger-diameter cellulose strands than other treatments (Figure 2). Altogether, NaOH has a distinctly different impact on cellulose compared with H_2O_2 or bleach; rather than removing impurities and freeing up pore spaces, NaOH instead causes partial cellulose dissolution and significantly shrinks the sizes of pores.

Significance

The inherent accessibility of microbial membrane production opens up meaningful new avenues of point-of-use water treatment. By providing a means of manufacture of a viable water filtration medium requiring only readily-available components, and one in which workspace sterility and formal laboratory skills are not required, microbial membranes allow individuals who may otherwise have no means of safely storing water to develop their own treatment system which can be utilized immediately before consumption. In this way, microbial membranes can mitigate the dangers of unclean water storage. Likewise, they offer an alternative avenue for commercial membrane manufacturers interested in green manufacturing processes. However, with a fixed selectivity and low permeability relative to similar synthetic cellulose membranes, these membranes have a limited range of use. These restrictions reduce the applicability of microbial membranes and hinder their accessibility. Our research indicates that simple treatments using readily available and relatively safe concentrations of chemical compounds can significantly alter microbial membrane properties. Use of 0.3% H_2O_2 can increase membrane permeability by 80%. Use of 0.8% NaOH can decrease the

nominal pore size from 35 nm to 10 nm. While the pristine and treated membranes may be incorporated easily into a plate-and-frame membrane module, as we have done in laboratory experiments, further study is needed to incorporate these membranes into hollow fiber or spiral wound modules and study their long-term filtration properties. By allowing substantial modification of membrane properties with widely available compounds used in a facile manner, we hope to expand accessibility to membrane materials and encourage manufacturers to explore green manufacturing options for membrane production.

Supporting Information: Concentrations and times of membrane treatments, visual comparison of pristine and treated membranes, permeability data for all membranes tested, contact angle measurements for all membranes tested, gold nanoparticle adsorption test results, thermogravimetric analysis.

Acknowledgements

The authors would like to thank Jeanne Larson, Gary Wyss, Cristina Stefanescu, John "JP" Murphy, Jordan Foster, Isaiah Robertson, Akua Oppong-Anane, Raja Nagisetty, and Alysia Cox for their invaluable assistance in this research. Research was sponsored by a Montana Tech Seed Grant, the Combat Capabilities Development Command Army Research Laboratory (Cooperative Agreement Number W911NF-15-2-0020), and NSF EPSCoR CREWS (Grant No. OIA- 1757351). The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the official policies, either expressed or implied, of the

406 Combat Capabilities Development Command Army Research Laboratory, the National
407 Science Foundation, or the U.S. Government. The U.S. Government is authorized to
408 reproduce and distribute reprints for Government purposes notwithstanding any
409 copyright notation herein.
410

411 # References

412

413 (1) Ashbolt, N. J. Microbial Contamination of Drinking Water and Disease Outcomes
414 in Developing Regions. *Toxicology* **2004**, *198* (1–3), 229–238.
415 https://doi.org/10.1016/j.tox.2004.01.030.
416 (2) World Health Organization. Drinking-Water https://www.who.int/news-room/fact-
417 sheets/detail/drinking-water (accessed Apr 22, 2019).
418 (3) Wright, J.; Gundry, S.; Conroy, R. Household Drinking Water in Developing
419 Countries: A Systematic Review of Microbiological Contamination between
420 Source and Point-of-Use. *Trop. Med. Int. Heal.* **2004**, *9* (1), 106–117.
421 https://doi.org/10.1046/j.1365-3156.2003.01160.x.
422 (4) Krasner, S. W.; Weinberg, H. S.; Richardson, S. D.; Pastor, S. J.; Chinn, R.;
423 Sclimenti, M. J.; Onstad, G. D.; Thruston, A. D. Occurrence of a New Generation
424 of Disinfection Byproducts. *Environ. Sci. Technol.* **2006**, *40* (23), 7175–7185.
425 https://doi.org/10.1021/es060353j.
426 (5) Crider, Y.; Sultana, S.; Unicomb, L.; Davis, J.; Luby, S. P.; Pickering, A. J. Can
427 You Taste It? {Taste} Detection and Acceptability Thresholds for Chlorine
428 Residual in Drinking Water in {Dhaka}, {Bangladesh}. *Sci. Total Environ.* **2018**,

613–614, 840–846. https://doi.org/10.1016/j.scitotenv.2017.09.135.

(6) Fane, A. G.; Wang, R.; Hu, M. X. Synthetic Membranes for Water Purification: Status and Future. *Angew. Chemie Int. Ed.* **2015**, *54* (11), 3368–3386. https://doi.org/10.1002/anie.201409783.

(7) Song, X.; Chen, F.; Liu, F. Preparation and Characterization of Alkyl Ketene Dimer (Akd) Modified Cellulose Composite Membrane. *Carbohydr. Polym.* **2012**, *88* (2), 417–421. https://doi.org/10.1016/j.carbpol.2011.10.062.

(8) Lalia, B. S.; Kochkodan, V.; Hashaikeh, R.; Hilal, N. A Review on Membrane Fabrication: Structure, Properties and Performance Relationship. *Desalination* **2013**, *326*, 77–95. https://doi.org/10.1016/j.desal.2013.06.016.

(9) California Office of Environmental Health Hazard Assessment. N,N-Dimethylacetamide.

(10) Eggensperger, C.; Zodrow, K. Sustainable Living Filtration Membranes. *Environ. Sci. Technol. Lett.* **2020**, *7* (3), 213–218. https://doi.org/10.1021/acs.estlett.0c00019.

(11) Al-Shamary, E.; Khalaf, A. Influence of Fermentation Condition and Alkali Treatment on the Porosity and Thickness of Bacterial Cellulose Membranes. 2013.

(12) Aguayo, M. G.; Pérez, A. F.; Reyes, G.; Oviedo, C.; Gacitúa, W.; Gonzalez, R.; Uyarte, O. Isolation and Characterization of Cellulose Nanocrystals from Rejected Fibers Originated in the Kraft Pulping Process. *Polymers (Basel).* **2018**, *10* (10), 1145.

(13) Das, S.; Lachenal, D.; Marlin, N. Production of Pure Cellulose from Kraft Pulp by

a Totally Chlorine-Free Process Using Catalyzed Hydrogen Peroxide. *Ind. Crop. Prod.* **2013**, *49*, 844.

(14) Environmental Protection Agency. Chlorine dioxide; CASRN 10049-04-4.

(15) Zeronian, S. H.; Inglesby, M. K. Bleaching of Cellulose by Hydrogen Peroxide. *Cellulose* **1995**, *2* (4), 265–272. https://doi.org/10.1007/BF00811817.

(16) Rossoni, E. M. M.; Gaylarde, C. C. Comparison of Sodium Hypochlorite and Peracetic Acid as Sanitising Agents for Stainless Steel Food Processing Surfaces Using Epifluorescence Microscopy. *Int. J. Food Microbiol.* **2000**, *61* (1), 81–85. https://doi.org/10.1016/S0168-1605(00)00369-X.

(17) Drosou, A.; Falabella, A.; Kirsner, R. Antiseptics on Wounds: An Area of Controversy. *Wounds* **2003**, *15* (5), 149–166.

(18) Zhang, S.; Li, F.-X.; Yu, J.; Hsieh, Y.-L. Dissolution Behaviour and Solubility of Cellulose in NaOH Complex Solution. *Carbohydr. Polym.* **2010**, *81* (3), 668–674. https://doi.org/10.1016/j.carbpol.2010.03.029.

(19) Budtova, T.; Navard, P. Cellulose in NaOH–Water Based Solvents: A Review. *Cellulose* **2016**, *23* (1), 5–55. https://doi.org/10.1007/s10570-015-0779-8.

(20) Javier Benítez, F.; Acero, J. L.; González, T.; García, J. Application of Ozone and Advanced Oxidation Processes to the Treatment of Lye-Wastewaters from the Table Olives Industry. *Ozone Sci. Eng.* **2002**, *24* (2), 105–116. https://doi.org/10.1080/01919510208901601.

(21) Das, D. J.; Barringer, S. A. Potassium Hydroxide Replacement for Lye ({Sodium} Hydroxide) in Tomato Peeling: Potassium Hydroxide Peeling. *J. Food Process. Preserv.* **2006**, *30* (1), 15–19. https://doi.org/10.1111/j.1745-4549.2005.00043.x.

(22) Zhang, L.; Zhang, J.; Chen, L.; Liu, T.; Ma, G.; Liu, X. Influence of Manufacturing Process on the Contents of Iron, Copper, Chromium, Nickel and Manganese Elements in Crush, Tear and Curl Black Tea, Their Transfer Rates and Health Risk Assessment. *Food Control* **2018**. https://doi.org/10.1016/j.foodcont.2018.01.030.

(23) Lee, Y.-J.; Chung, C.-H.; Day, D. F. Sugarcane Bagasse Oxidation Using a Combination of Hypochlorite and Peroxide. *Bioresour. Technol.* **2009**, *100* (2), 935–941.

(24) Meftahi, A.; Khajavi, R.; Rashidi, A.; Rahimi, M. K.; Bahador, A. Effect of Purification on Nano Microbial Cellulose Pellicle Properties. *Procedia Mater. Sci.* **2015**, *11* (C), 206–211.

(25) Zhao, H.; Kwak, J.; Conradzhang, Z.; Brown, H.; Arey, B.; Holladay, J. Studying Cellulose Fiber Structure by {SEM}, {XRD}, {NMR} and Acid Hydrolysis. *Carbohydr. Polym.* **2007**, *68* (2), 235–241. https://doi.org/10.1016/j.carbpol.2006.12.013.

(26) Park, S.; Baker, J. O.; Himmel, M. E.; Parilla, P. A.; Johnson, D. K. Cellulose Crystallinity Index: Measurement Techniques and Their Impact on Interpreting Cellulase Performance. *Biotechnol. Biofuels* **2010**, *3* (1), 10. https://doi.org/10.1186/1754-6834-3-10.

(27) McDonald, J. H. *Handbook of Biological Statistics*, 3rd ed.; 2014.

(28) Coenen, K. T.; Gallucci, F.; Cobden, P.; van Dijk, E.; Hensen, E. J. M.; van Sint Annaland, M. Chemisorption of H2O and CO2 on Hydrotalcites for Sorption Enhanced Water-Gas-Shift Processes. *Energy Procedia* **2017**, *114*, 2228–2242.

(29) Fuller, M. E.; Andaya, C.; McClay, K. Evaluation of ATR-FTIR for Analysis of Bacterial Cellulose Impurities. *J. Microbiol. Methods* **2018**, *144*, 145–151. https://doi.org/10.1016/j.mimet.2017.10.017.