

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

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

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## Abstract

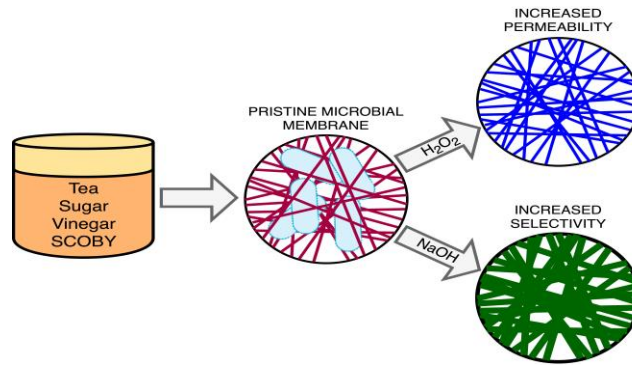
1  
2 Water filtration membranes produced sustainably through microbial cellulose production  
3 can have filtration properties altered through facile chemical treatments. Microbial  
4 cellulose is an effective membrane filtration medium, and pristine microbial membranes  
5 can serve as ultrafiltration membranes with a permeability of  $143 \text{ L m}^{-2}\text{h}^{-1}\text{bar}^{-1}$  and a  
6 particle size cut off of 35 nm. As living biofilms, these membranes consist of microbial  
7 cellulose, bacteria, and extracellular polymers. Thus, additional biofilm components may  
8 reduce the intrinsic permeability of the cellulose. Here, microbial membranes were  
9 treated with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and sodium hypochlorite ( $\text{NaOCl}$ , liquid bleach)  
10 to remove impurities present in microbial cellulose and increase membrane  
11 permeability. For example, permeability increased from 143 to  $257 \text{ L m}^{-2}\text{h}^{-1}\text{bar}^{-1}$  with  
12 treatment by 0.3%  $\text{H}_2\text{O}_2$  for 12 min. Membranes were also treated with sodium  
13 hydroxide ( $\text{NaOH}$ ) to increase membrane selectivity, and the particle size cut off was  
14 reduced from 35 to 10 nm post-treatment by 0.8%  $\text{NaOH}$  or 20 min. Scanning electron  
15 microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), thermogravimetric  
16 analysis (TGA), contact angle goniometry, and X-ray diffraction (XRD) were used to  
17 characterize the physical and chemical properties of the membrane matrix. Facile  
18 chemical treatments provide a significant degree of flexibility to tailor microbial  
19 membranes to meet specific needs. Microbial membrane production is inherently  
20 accessible, and this study furthers that accessibility by utilizing only readily-available  
21 components to treat microbial membranes and expand their potential applications.

22

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

25

26



27

## 28 Synopsis

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

31

## 32 Introduction

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

43

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

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

57

58 Recently, research has shown that by using microbially-produced cellulose as a  
59 medium, “microbial” membranes, can mitigate the aforementioned concerns, including  
60 membrane accessibility and using toxic chemicals for synthesis and disinfecting.

61 Requiring only water, tea, sugar, vinegar, and a starter microbial culture, a microbial  
62 membrane can be produced in just 7 days in a clean, but non-sterile, environment<sup>10</sup>.

63 Microbial membranes empower individuals or smaller corporations to sustainably  
64 produce water treatment membranes, in an environment with less precision than  
65 industrial production would require.

66

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

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

82

83 Cellulose cleaning and purification are common processes in the textile industry, and  
84 cleaning/purification of pristine microbial cellulose fibers provides an opportunity to tailor  
85 the structure of the microbial membranes, increasing permeability and decreasing  
86 selectivity. Currently, the most common cellulose purification technique in use is the  
87 Kraft Process<sup>12</sup>, a method which involves the use of concentrated chlorine dioxide<sup>13</sup>, an  
88 oxidizing agent reported by EPA to cause neurodevelopmental effects in gaseous form  
89 at exposures as low as 0.03 ppm/day<sup>14</sup>. However, consumer-grade hydrogen peroxide  
90 (H<sub>2</sub>O<sub>2</sub>) and sodium hypochlorite (liquid bleach), compounds with greater accessibility  
91 and less risk of danger to human health, can also be used to purify cellulose and  
92 remove non-cellulose organic matter and microbes. H<sub>2</sub>O<sub>2</sub> has a long history of use as a  
93 cellulose purification agent<sup>15</sup>, and sodium hypochlorite is a widely used and effective  
94 antimicrobial agent<sup>16</sup>. While its longstanding usage as a disinfectant for wounds is  
95 controversial<sup>17</sup>, H<sub>2</sub>O<sub>2</sub> remains widely available. Sodium hypochlorite’s ubiquity in the  
96 food industry and popularity as a disinfectant makes it similarly widespread. Sodium  
97 hydroxide (NaOH) is also a common component in cellulose regeneration solutions<sup>18</sup>.  
98 As cellulose does not exhibit a melting temperature<sup>19</sup>, effective dissolution agents are  
99 essential in cellulose processing; NaOH has been shown to effectively break down  
100 hydrogen bonds in cellulose and lead to dissolution<sup>18</sup>. NaOH is used in large-scale food

101 preparation processes,<sup>20,21</sup> has been used to produce a cellulose-based dialysis  
102 membrane, and is widely available.

103  
104 Facile treatments with readily-available, consumer-grade compounds may serve as a  
105 means for users to tune microbial membrane parameters as needed. The objective of  
106 this study is to develop methods to adjust the permeability and the selectivity of natural  
107 cellulose membranes, while maintaining their inherent accessibility. Cellulose  
108 membranes obtained from microbial suspensions and with transport properties in the  
109 range of ultrafiltration membranes were subjected to post-treatments using widely  
110 available H<sub>2</sub>O<sub>2</sub>, sodium hypochlorite, and NaOH. Characterization techniques were  
111 applied to characterize the chemical and physical changes that post-treatments  
112 imparted on membranes. Synthetic cellulose membranes produced using  
113 electrospinning were used as a chemical control. This study demonstrates that common  
114 chemical treatments could increase the range of usage of microbial membranes, while  
115 maintaining accessibility.

116  
117

## 118 **Materials and Methods**

### 119 Microbial Membrane Production

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

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

132

### 133 Membrane Post-Processing

134 Liquid bleach (liquid, 6.0%, generic, pH ~12), H<sub>2</sub>O<sub>2</sub> (liquid, 3.0%, generic), and NaOH  
135 (pellets, 97%, Fisher Scientific) were used for membrane treatment. Preliminary trials  
136 were carried out with each treatment type (conditions in Supporting Table S1). Chemical  
137 concentrations were derived from literature<sup>23,24</sup> and obtained by feasible dilutions of  
138 concentrations of consumer-grade chemicals. During treatment, the container was  
139 gently swirled every 30 seconds. Treatment times were determined by placing  
140 membranes into solution until membrane color visibly changed. After treatment, the  
141 membrane was immediately transferred to a new container with DI water. The  
142 membrane was swirled for 30 seconds in the DI water to remove residual treatment  
143 solution. The membrane was removed from the container and added to a new container  
144 with fresh DI water. This rinsing process was performed 3 times. Treated membranes  
145 were used for permeability and selectivity testing immediately, and were not stored.

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

148  
149 After initial testing, 0.1% bleach (10 min), 0.3% H<sub>2</sub>O<sub>2</sub> (12 min), and 0.8% NaOH (20 min)  
150 were chosen for more extensive characterization because they resulted in the largest  
151 shifts in membrane permeability (Supporting Figure S2). Gloves, goggles, and  
152 full-length clothes were worn at all times while working with treatment chemicals.  
153 Concentrated NaOH was handled in a chemical hood.

154

### 155 Membrane Characterization

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

161

162 To confirm that observed FTIR results were due to changes in chemical structure in  
163 treated cellulose and not simply changes in crystallinity, X-ray diffraction (XRD) analysis  
164 was performed and membrane crystallinities were compared. XRD was performed using  
165 an Ultima IV X Ray Diffractometer. Prior to analysis, samples were air-dried at room  
166 temperature and ambient pressure. Samples were analyzed with a theta-theta scan with  
167 a scan range from 10 to 50°. <sup>25</sup> The scan speed was set to  $5$  °2 $\theta$ ×min<sup>-1</sup>, with 40 kV and  
168 40 mA. OriginLab software was used to find the ratio of the area underneath the

169 observed crystalline peaks to the total area for each graph, resulting in crystallinity index  
170 values.<sup>26</sup>

171  
172 Critical point drying (CPD) was used to prepare samples for scanning electron  
173 microscopy. Samples were placed sequentially in conical tubes containing solutions of  
174 5, 15, 30, 50, and 70% isopropyl alcohol (Fisher). Samples were soaked in each  
175 solution for 15 min. The samples were then placed in a 99.97% IPA (Fisher) solution  
176 and left overnight. Then, samples were placed in an Autosamdri-931 CPD 3.175 cm  
177 chamber. The system cycled for 6 h, at which point the unit went into stasis mode. The  
178 samples were removed from the sample tray the next day. Samples were sputter coated  
179 with gold prior to SEM analysis. A TESCAN MIRA3 SEM, using a Schottky source, 5 kV  
180 accelerating voltage, and secondary electron detector was used to image the sample  
181 materials. Working distance of the SEM was in the range 10-14.5 mm.

182  
183 Thermogravimetric analysis (TGA) was performed using a TA Instruments SDT 650  
184 Simultaneous Thermal Analyzer. Argon was utilized as the inert gas. Samples were  
185 brought to 700 °C from room temperature at a rate of 10 °C·min<sup>-1</sup>. Prior to analysis,  
186 samples were lyophilized. Samples were stored at room temperature until analysis.

187  
188 Contact angle was determined using a Biolin Scientific ThetaLite100 contact angle  
189 goniometer with OneAttension software. Samples were inverted in water and an air  
190 bubble was placed on the membrane surface. The inverse of the contact angle of the air  
191 on membrane was taken to determine the contact angle of the water on membrane.

192

### 193 Membrane Filtration Performance

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

198

199 All permeability testing was performed with DI water. Membranes were first compressed  
200 at 3.10 bar for 1 h. After 1 h, the filtration cell was depressurized to 0.69 bar and  
201 allowed to stabilize for 15 min. Permeability was then tested at four pressure intervals:  
202 0.69, 1.34, 2.07, and 3.10 bar. Each interval was tested for 15 min. Mass was recorded  
203 every 60 s, for a total of 15 points for every interval.

204

205 Selectivity was tested using gold nanoparticles with hydrodynamic diameters of 8.8 nm,  
206 20.4 nm, and 28.0 nm (NanoComposix) and polypropylene beads with hydrodynamic  
207 diameters of 48.11, 110.0, and 201.3 nm<sup>10</sup>. Their hydrodynamic diameters were  
208 measured via dynamic light scattering (Zetasizer, Malvern). Particle concentration was  
209 determined using UV-vis spectroscopy and a calibration curve previously determined for  
210 each particle type. All gold nanoparticles were analyzed at a wavelength of 519 nm.  
211 Colored polypropylene beads (polybeads) were analyzed at a wavelength of 350 nm.  
212 The dead-end filtration cell was filled with the particle solution, and each test was  
213 performed at 1.34 bar. The first 1.0 g of permeate was discarded, and a conical tube  
214 was used to collect the permeate sample.

215

216 Statistics

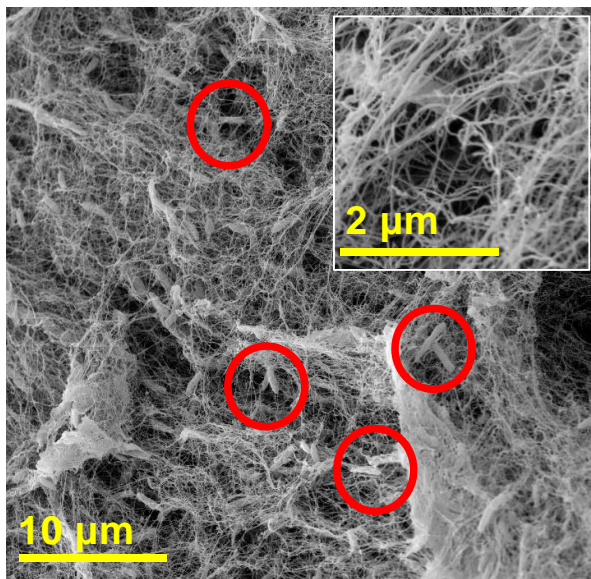
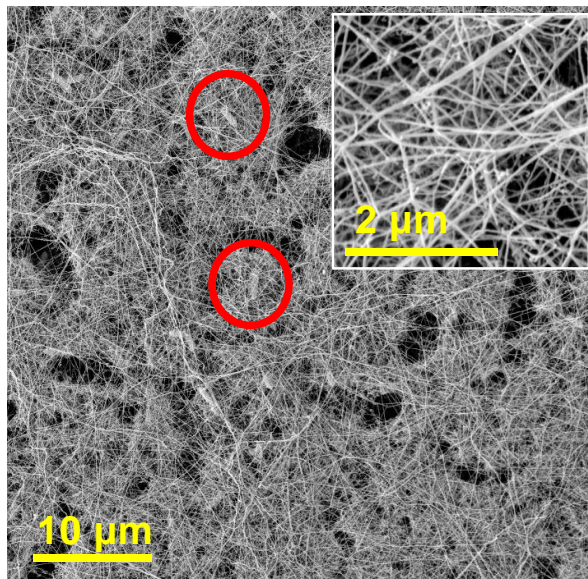
217 Significance of results was determined in MiniTab using a Welch's one-way Analysis of  
218 Variance (ANOVA) with a post-hoc Games-Howell test<sup>27</sup> with  $\alpha$  at 0.05.

219

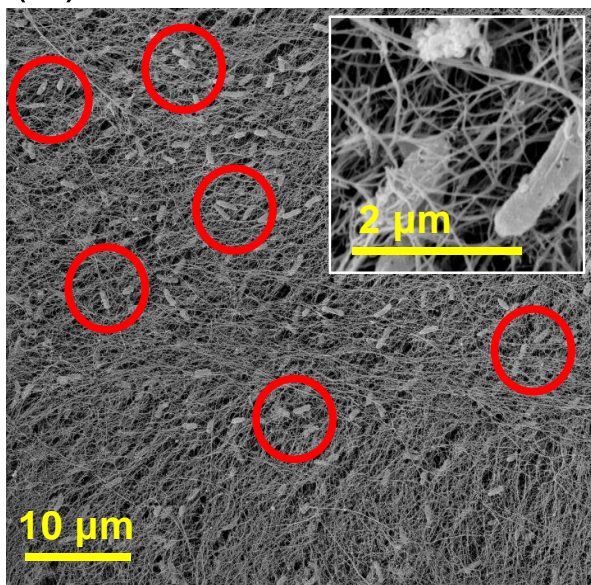
220 **Results and Discussion**

221 Post-Processing Changes Cellulose Structure

(A) Pristine

(B) 0.3% H<sub>2</sub>O<sub>2</sub>

(C) 0.1% Bleach



(D) 0.8% NaOH

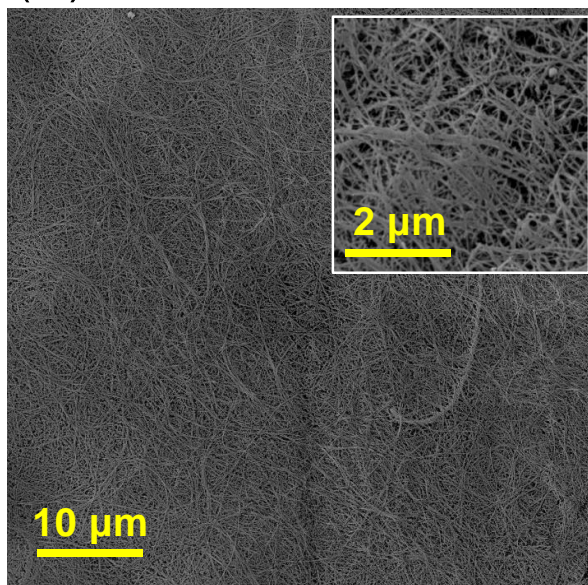


Figure 1: Representative scanning electron micrographs of the (A) pristine membrane, (B) membranes following 0.3% H<sub>2</sub>O<sub>2</sub> treatment, (C) 0.1% bleach treatment, and (D) 0.8% NaOH treatment. Inset micrographs present higher magnification details. Examples of bacteria are circled in red.

223 Post-processing alters microbial cellulose fiber structure: fiber cleaning and  
224 gelatinization were observed with different treatments (Figure 1). Membranes not  
225 treated before use (pristine) are structurally heterogeneous; there is “webbing” across  
226 the surface of the fibers that may be due to the presence of extracellular polymers  
227 produced by the microorganisms (Figure 1A). Microorganisms are visible in the fiber  
228 network. These microorganisms and impurities are commonly seen in pristine microbial  
229 cellulose<sup>24</sup>. H<sub>2</sub>O<sub>2</sub>-treated membranes have greater homogeneity than pristine  
230 membranes; a more open structure and a smoother fiber morphology is observed in the  
231 Figure 1B inset. Additionally, fewer microorganisms were observed. Bleach-treated  
232 membranes also have an increase in membrane homogeneity similarly to H<sub>2</sub>O<sub>2</sub>, but  
233 microbes are still visible in the pore spaces (Figure 1C). A more open structure is shown  
234 in the inset, but the fibers do not appear as well-defined as those in the H<sub>2</sub>O<sub>2</sub>-treated  
235 membranes. In contrast, the gelatinization that NaOH causes in cellulose<sup>18</sup> is apparent  
236 in Figure 1D; the membrane surface is smoother and the cellulose fibers are also  
237 smoother. The inset suggests that gelatinization results in a drastic decrease in  
238 membrane porosity. Thus, qualitatively, the H<sub>2</sub>O<sub>2</sub> and bleach treatments appeared to  
239 open the structure of the membrane, while the NaOH treatment appeared to tighten the  
240 structure of the membrane.

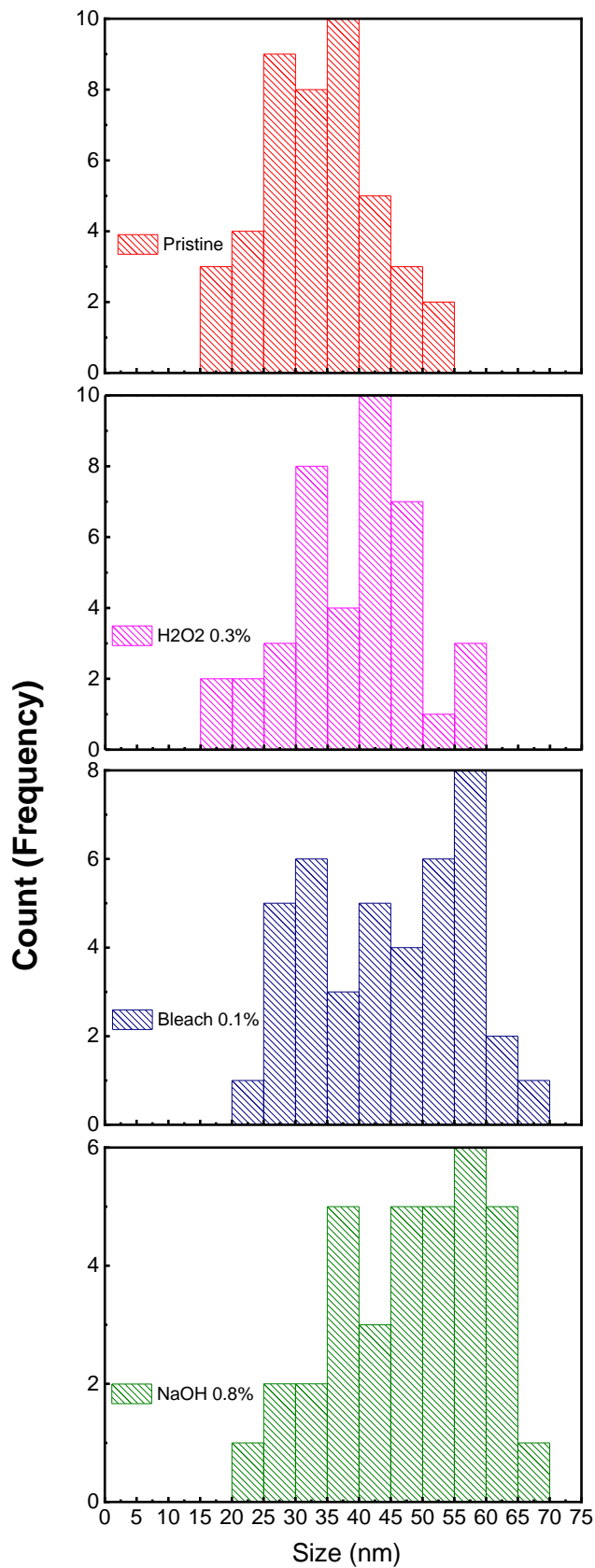


Figure 2: Fiber diameter frequencies of membranes from analyses of SEM images. Fiber diameters were measured for each listed membrane type using ImageJ software. 5

242 Some of the post-treatments changed fiber diameters (Figure 2). The maximum fiber  
243 diameter of pristine membranes was 60 nm, with an average fiber diameter of  $35 \pm 10$   
244 nm (Figure 2). Membranes treated with 0.3%  $H_2O_2$  had no significant change in fiber  
245 diameter, with a maximum fiber diameter of 57 nm and an average fiber diameter of  $38$   
246  $\pm 10$  nm. The 0.1% bleach-treated membranes had a maximum fiber diameter of 69 nm  
247 and an average fiber diameter of  $44 \pm 12$  nm. Membranes treated with 0.1% bleach  
248 showed an increase in average fiber diameter from pristine membranes of 29%. The  
249 0.8% NaOH-treated membranes had a maximum fiber diameter of 138 nm, and an  
250 average fiber diameter of  $59 \pm 26$  nm. Fiber size distributions of 0.8% NaOH treated  
251 membranes show an increase in fiber diameters of 67%. ANOVA showed significant  
252 differences between fiber diameters of pristine membranes compared to 0.1% bleach-  
253 and 0.8% NaOH-treated membranes. Noticeable gelation was visible in NaOH  
254 membranes during SEM analysis (Figure 1D). Thus,  $H_2O_2$  cleans fibers but cause no  
255 significant change in diameter, while bleach and NaOH treatments result in fiber  
256 gelation and significant increases in diameter (group p value =  $<0.000$ ). It is likely that  
257 changes in fiber diameter will alter membrane performance (discussed below).

258

259 Post-Processing Alters Functional Groups and Hydrophilicity

260

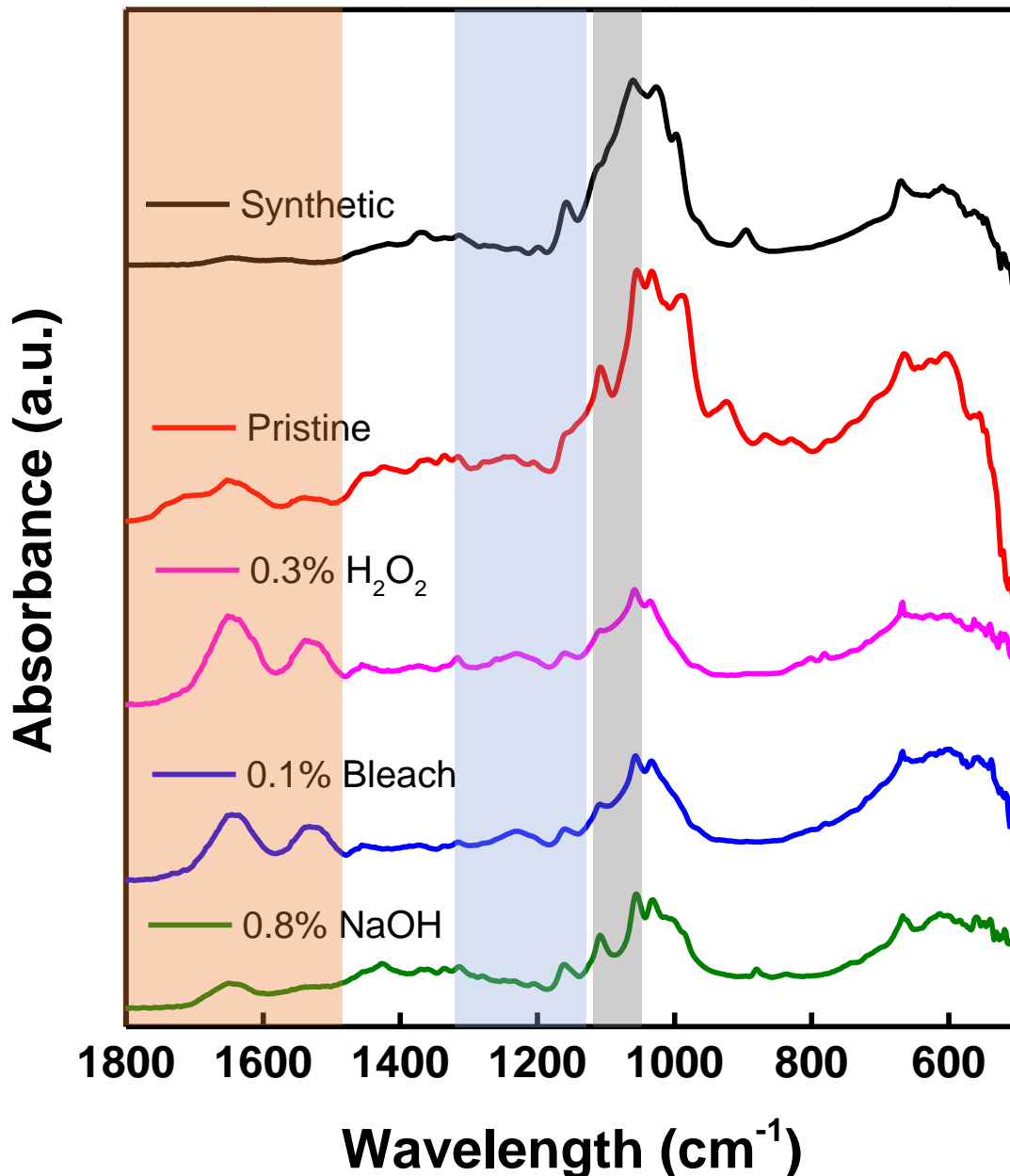


Figure 3: FTIR spectra for pristine and selected treated membranes. Orange corresponds to lipids, proteins, and nucleic acids; blue corresponds to nucleic acids; and gray corresponds to lipids and nucleic acids. Samples were prepared via lyophilization.

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  $895\text{ cm}^{-1}$  (functional group: COC, CCO and CCH

265 deformation and stretching) as well as 1020 and 1046  $\text{cm}^{-1}$  (C-C, C-OH, C-H ring and  
266 side group vibrations). Before treatment, the pristine microbial membrane displayed  
267 broad peaks not seen in synthetic cellulose from 1800  $\text{cm}^{-1}$  to 1500  $\text{cm}^{-1}$ , a peak at  
268 1090  $\text{cm}^{-1}$ , and a broad peak at 1261  $\text{cm}^{-1}$ . The areas which correspond to microbial  
269 cellulose impurities<sup>29</sup> are highlighted in Figure 3. The 0.1% bleach and 0.3%  $\text{H}_2\text{O}_2$   
270 treatments led to a reduction in the peak at 1090  $\text{cm}^{-1}$ , while 0.8% NaOH treatment did  
271 not. The 0.5 M NaOH treatment reduced the peak at 1261  $\text{cm}^{-1}$ , whereas 0.1% bleach  
272 and 0.3%  $\text{H}_2\text{O}_2$  treated membranes did not. These results likely indicate that 0.1%  
273 bleach and 0.3%  $\text{H}_2\text{O}_2$  treatments are more effective at removing lipids, while NaOH  
274 removes more nucleic acids. The removal of lipids has unclear implications for  
275 membrane filtration performance as the hydrophobicity of lipids depends on their  
276 orientation at the cellulose surface. However, the removal of relatively hydrophobic  
277 nucleic acids could have contributed to the increased hydrophilicity of the NaOH-treated  
278 membrane, discussed below. Finally, peaks in the 1500 to 1700  $\text{cm}^{-1}$  range have been  
279 associated with the presence of bacterial cells on cellulose. These peaks were the most  
280 prominent in the  $\text{H}_2\text{O}_2$  and bleach-treated membranes, membranes with the largest  
281 number of cells observed in the SEM images (Figure 1C,D).

282

283

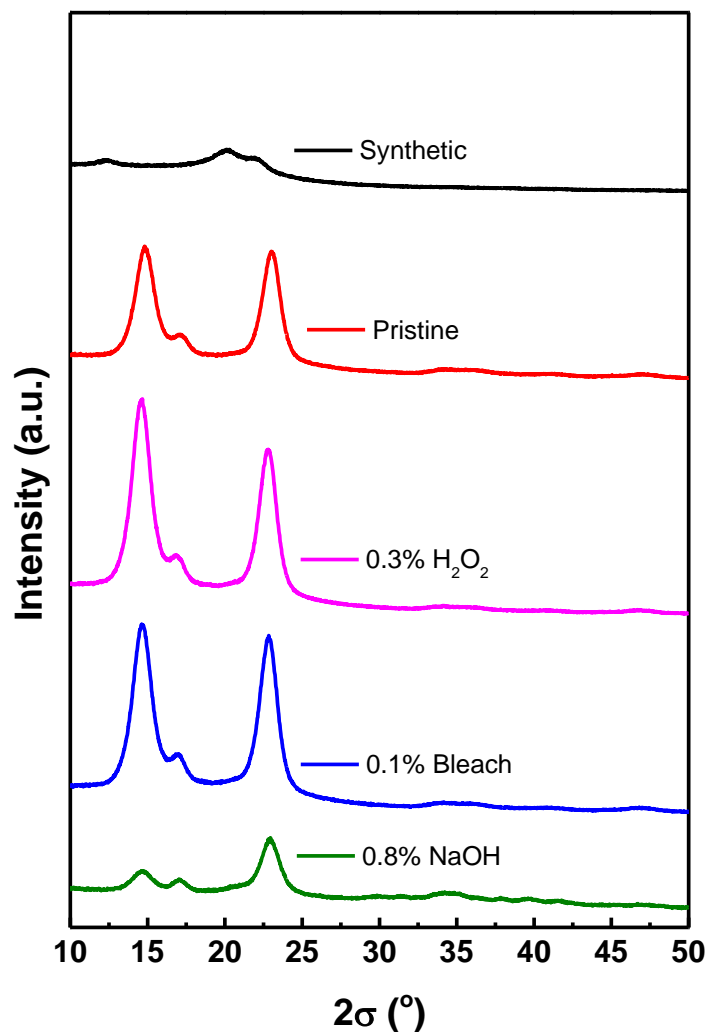


Figure 4: XRD spectra of treated membranes compared to pristine and synthetic membranes. Prior to analysis, samples were air dried at room temperature for 7 days.

284

285 XRD was used to quantify if the facile cleaning treatments altered the crystallinity of the  
 286 membranes. Crystallinity indices were calculated from XRD spectra (Figure 4). A  
 287 pristine membrane has a crystallinity index of 63%, consistent with values reported in  
 288 literature of crystallinity of microbial cellulose<sup>26</sup>. The membrane treated with 0.1%  
 289 bleach had a crystallinity index of 67%, a change compared to pristine of 6%. The 0.3%  
 290 H<sub>2</sub>O<sub>2</sub> and 0.8% NaOH membranes had crystallinity indexes of 69% and 61%,  
 291 respectively, and percent changes from pristine of 10% and 3%. Crystallinity index

292 variability in microbial cellulose is high<sup>26</sup>, and differences in treated membranes  
293 compared to a pristine sample were within observed variability seen in literature.  
294 Therefore, it was assumed that FTIR results are representative of chemical composition  
295 changes and not changes in crystallinity.  
296

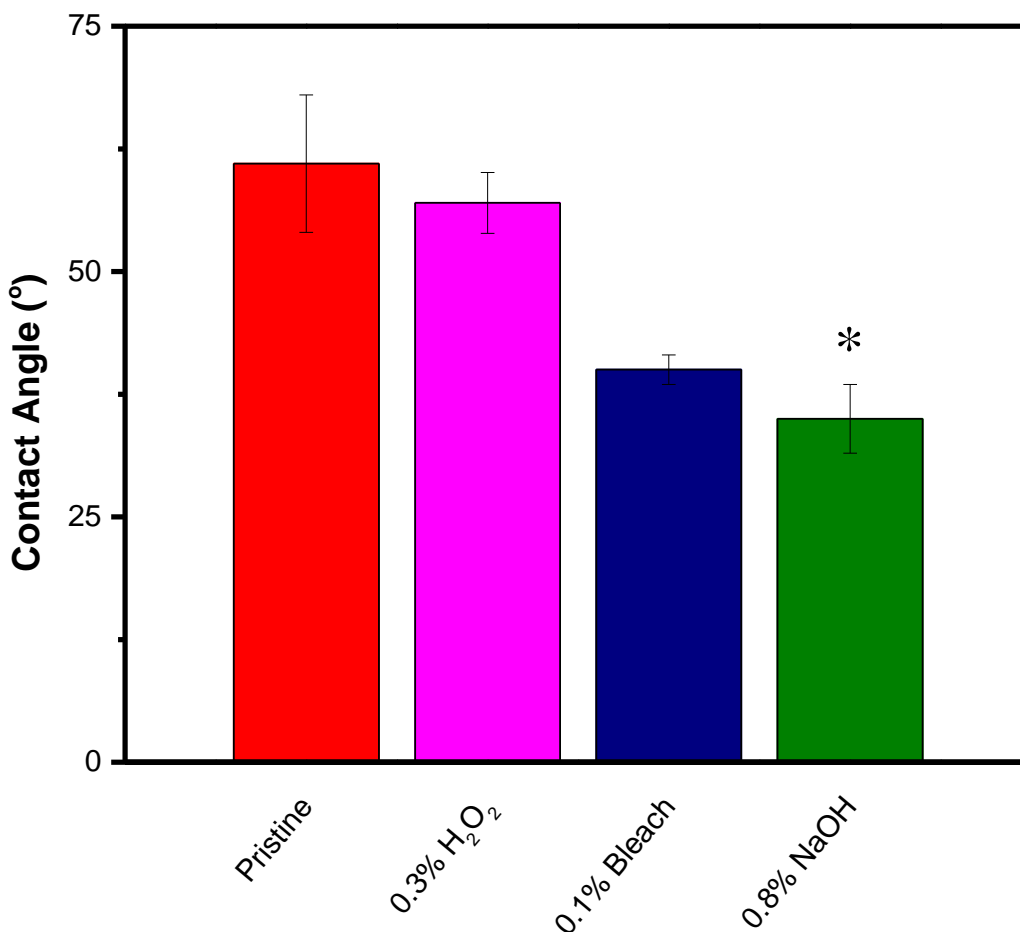


Figure 5: Air in DI water captive bubble contact angles of membranes (asterisk denotes statistically significant results when compared to pristine membrane).

297  
298 Figure 5 shows the contact angle of treated and pristine membranes. An ANOVA test  
299 indicated the contact angle of 0.8% NaOH-treated membranes were significantly  
300 different from the pristine. These results indicate that NaOH treatment causes an

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

306

### 307 Post-Processing Offers Membrane Customization

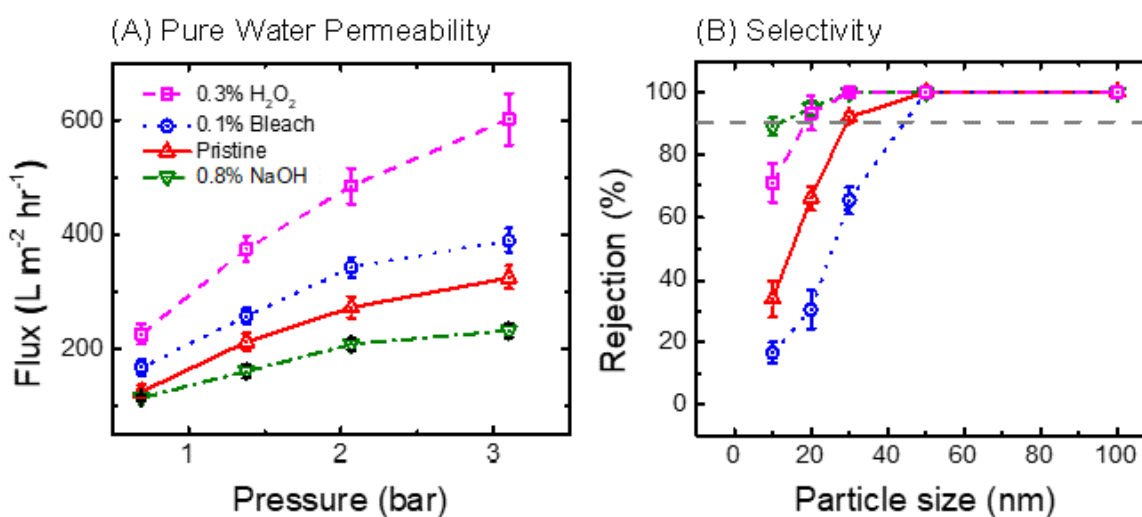


Figure 6: (A) Deionized water flux of membranes, (B) Selectivity of membranes (dashed selectivity lines connecting the experimental data represent expected results). Membranes were tested in a dead-end filtration cell. Prior to flux measurements, membranes were compressed at 3 bar for 1 h. Selectivity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

308

309

310 Microbial membrane treatments enable permeability and selectivity property  
311 customization (Figure 6). Membranes treated with 0.3% H<sub>2</sub>O<sub>2</sub> showed the greatest  
312 increase in permeability (Figure 6A). Pure water permeability increased from an  
313 average of 143 L·m<sup>-2</sup>·hr<sup>-1</sup>·bar<sup>-1</sup> for pristine membranes to 257 L·m<sup>-2</sup>·hr<sup>-1</sup>·bar<sup>-1</sup> for 0.3%

314 H<sub>2</sub>O<sub>2</sub>-treated membranes (79.7% increase). This increase was found to be statistically  
315 significant in ANOVA testing. This change is likely due to H<sub>2</sub>O<sub>2</sub> being an effective  
316 membrane purification agent; as a strong oxidizer, H<sub>2</sub>O<sub>2</sub> is widely utilized for removal of  
317 non-cellulose organic matter in wood pulp. By removing non-cellulose membrane  
318 constituents, H<sub>2</sub>O<sub>2</sub> frees up pore spaces and allows for an increase in rate of water  
319 filtration. Cleaner cellulose fibers with more open pore space were observed in the  
320 scanning electron micrographs (Figure 1B). The thermogravimetric analysis shown in  
321 **Supporting Figure S4** also indicates the effectiveness of H<sub>2</sub>O<sub>2</sub> cellulose purification  
322 0.3% H<sub>2</sub>O<sub>2</sub> treatment resulted in membrane decomposition closer to that of synthetic  
323 cellulose, indicating a greater similarity in chemical composition to synthetic cellulose  
324 than microbial cellulose. Therefore, H<sub>2</sub>O<sub>2</sub> removes non-cellulose components of  
325 microbial membranes while leaving cellulose intact (Figure 3). Interestingly, the H<sub>2</sub>O<sub>2</sub>  
326 treatment also increased the selectivity of the membrane, decreasing the particle size  
327 cutoff from 30 to 20 nm. This simultaneous increase in permeability and selectivity was  
328 not due to an increase in hydrophilicity (Figure 5). We also considered possible  
329 adsorption of the nanoparticles to the membrane during testing. However, no adsorption  
330 of nanoparticles in batch testing was observed (Supporting Figure S4). Thus, the reason  
331 H<sub>2</sub>O<sub>2</sub> increases both membrane permeability and selectivity is unknown and warrants  
332 further investigation.

333

334 Membranes treated with 0.1% bleach offered a more modest increase in permeability,  
335 bringing membrane pure water permeability from 147 L·m<sup>-2</sup>·hr<sup>-1</sup>·bar<sup>-1</sup> to 181  
336 L·m<sup>-2</sup>·hr<sup>-1</sup>·bar<sup>-1</sup> (26.1% increase). This increase was not found to be statistically

337 significant in ANOVA testing. As seen in Figure 1D, 0.1% bleach-treated membranes  
338 were more homogeneous than pristine membranes, but bacteria were retained in fibers.  
339 These bacteria, while not dangerous to human health, may occupy pore spaces that  
340 could otherwise be utilized for filtration. It is possible that the bleach treatment was less  
341 effective than the H<sub>2</sub>O<sub>2</sub> treatment at removing bacteria from the surface of the  
342 membrane due to bleach having a lower oxidation potential, i.e., being a less powerful  
343 oxidizer. Based on fiber diameters analyzed in Figure 2, it is possible that 0.1% bleach  
344 treatment caused small amounts of gelatinization or dissolution of cellulose similar to  
345 that seen in 0.8% NaOH treatment. However, the decrease in membrane permeability  
346 and increase in selectivity seen in 0.8% NaOH treatment was not observed in 0.1%  
347 bleach treatment; instead, a slight increase in permeability and slight decrease in  
348 selectivity was seen (Figure 6A,B). In Supporting Figure S4, TGA results indicate that  
349 0.1% bleach-treated membranes are more similar in composition to synthetic  
350 membranes compared to pristine cellulose membranes. Thus, 0.1% bleach treatment  
351 appears to purify cellulose, but to a lesser degree than 0.3% H<sub>2</sub>O<sub>2</sub> treatment.

352

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

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

366

## 367 **Significance**

368 The inherent accessibility of microbial membrane production opens up meaningful new  
369 avenues of point-of-use water treatment. By providing a means of manufacture of a  
370 viable water filtration medium requiring only readily-available components, and one in  
371 which workspace sterility and formal laboratory skills are not required, microbial  
372 membranes allow individuals who may otherwise have no means of safely storing water  
373 to develop their own treatment system which can be utilized immediately before  
374 consumption. In this way, microbial membranes can mitigate the dangers of unclean  
375 water storage. Likewise, they offer an alternative avenue for commercial membrane  
376 manufacturers interested in green manufacturing processes. However, with a fixed  
377 selectivity and low permeability relative to similar synthetic cellulose membranes, these  
378 membranes have a limited range of use. These restrictions reduce the applicability of  
379 microbial membranes and hinder their accessibility. Our research indicates that simple  
380 treatments using readily available and relatively safe concentrations of chemical  
381 compounds can significantly alter microbial membrane properties. Use of 0.3% H<sub>2</sub>O<sub>2</sub>  
382 can increase membrane permeability by 80%. Use of 0.8% NaOH can decrease the

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

391

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

396

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