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

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Abstract

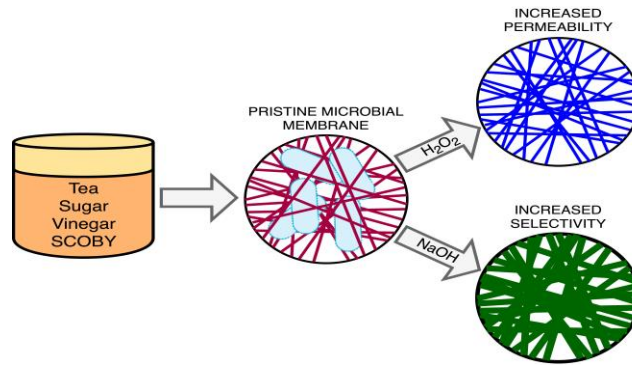
1
2 Water filtration membranes produced sustainably through microbial cellulose production
3 can have filtration properties altered through facile chemical treatments. Microbial
4 cellulose is an effective membrane filtration medium, and pristine microbial membranes
5 can serve as ultrafiltration membranes 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 (H_2O_2) and sodium hypochlorite (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% H_2O_2 for 12 min. Membranes were also treated with sodium
13 hydroxide (NaOH) to increase membrane selectivity, and the particle size cut off was
14 reduced from 35 to 10 nm post-treatment by 0.8% 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 children¹. The World Health Organization estimates that 2 billion people worldwide lack
36 access to a safely-managed drinking service². 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 quality³. 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 water⁴, and aesthetic qualities of chemically
42 disinfected water can deter consumption⁵.

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 formation⁶. 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^{7,8} 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 reprotoxic⁹. 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, environment¹⁰.

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,¹¹ 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 Process¹², a method which involves the use of concentrated chlorine dioxide¹³, an
88 oxidizing agent reported by EPA to cause neurodevelopmental effects in gaseous form
89 at exposures as low as 0.03 ppm/day¹⁴. However, consumer-grade hydrogen peroxide
90 (H₂O₂) 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. H₂O₂ has a long history of use as a
93 cellulose purification agent¹⁵, and sodium hypochlorite is a widely used and effective
94 antimicrobial agent¹⁶. While its longstanding usage as a disinfectant for wounds is
95 controversial¹⁷, H₂O₂ 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 solutions¹⁸.
98 As cellulose does not exhibit a melting temperature¹⁹, 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 dissolution¹⁸. NaOH is used in large-scale food

101 preparation processes,^{20,21} 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 H₂O₂, 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 processed²²;

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 fabrication¹⁰.

132

133 Membrane Post-Processing

134 Liquid bleach (liquid, 6.0%, generic, pH ~12), H₂O₂ (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^{23,24} 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% H₂O₂ (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 2.47×10^{-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°. ²⁵ The scan speed was set to 5 °2 θ ×min⁻¹, 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.²⁶

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⁻¹. 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 nm¹⁰. 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

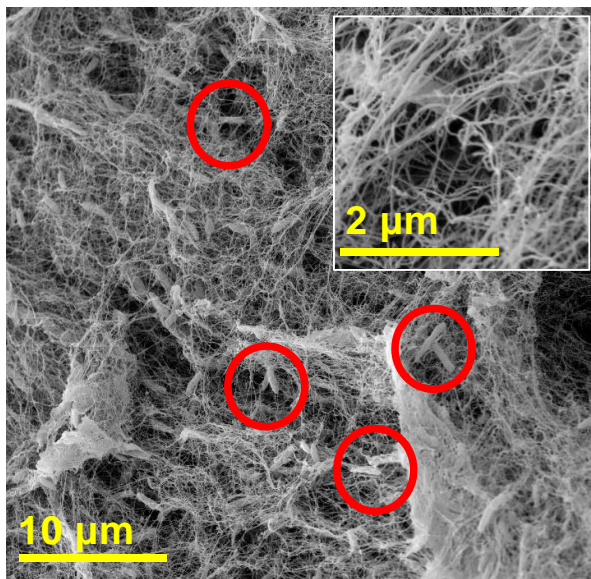
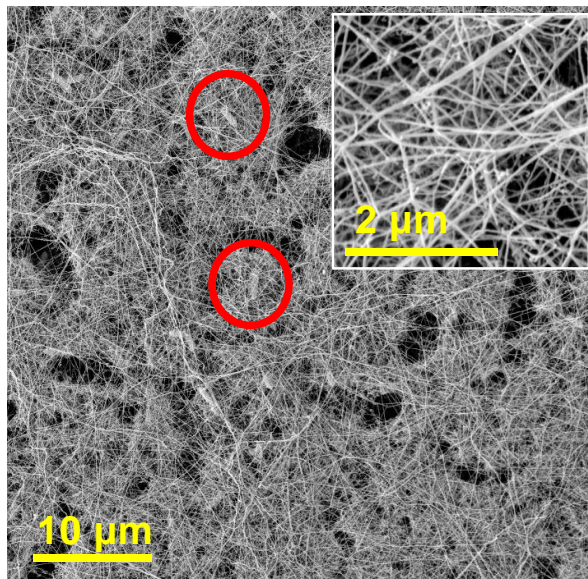
217 Significance of results was determined in MiniTab using a Welch's one-way Analysis of
218 Variance (ANOVA) with a post-hoc Games-Howell test²⁷ with α at 0.05.

219

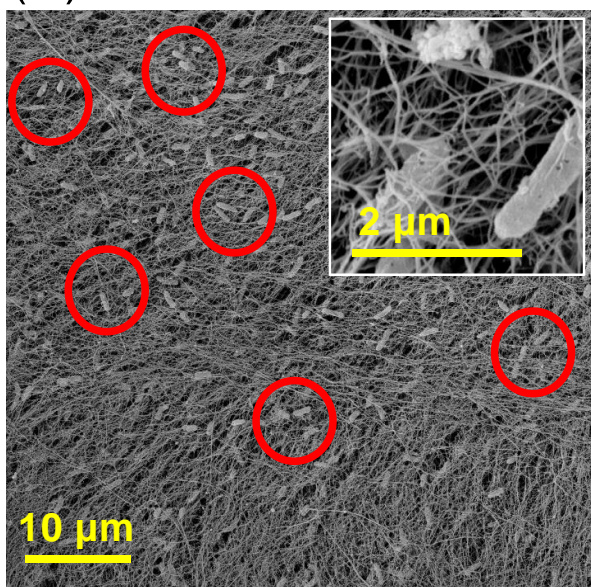
220 **Results and Discussion**

221 Post-Processing Changes Cellulose Structure

(A) Pristine

(B) 0.3% H₂O₂

(C) 0.1% Bleach



(D) 0.8% NaOH

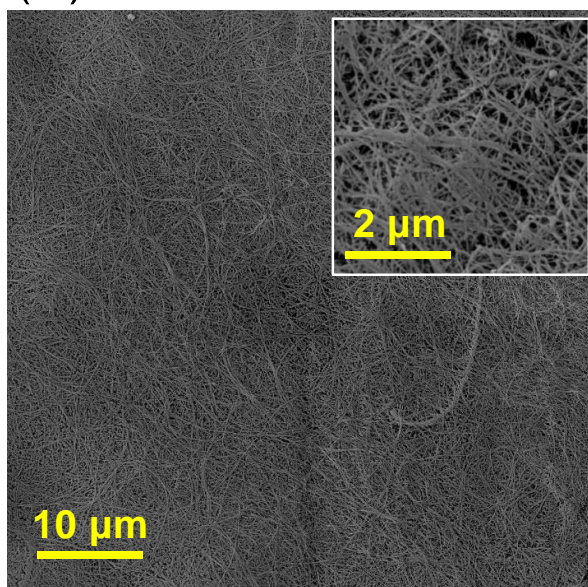


Figure 1: Representative scanning electron micrographs of the (A) pristine membrane, (B) membranes following 0.3% H₂O₂ treatment, (C) 0.1% bleach treatment, and (D) 0.8% NaOH treatment. Inset micrographs present higher magnification details. Examples of bacteria are circled in red.

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 cellulose²⁴. H₂O₂-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 H₂O₂, 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 H₂O₂-treated
235 membranes. In contrast, the gelatinization that NaOH causes in cellulose¹⁸ 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 H₂O₂ 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.

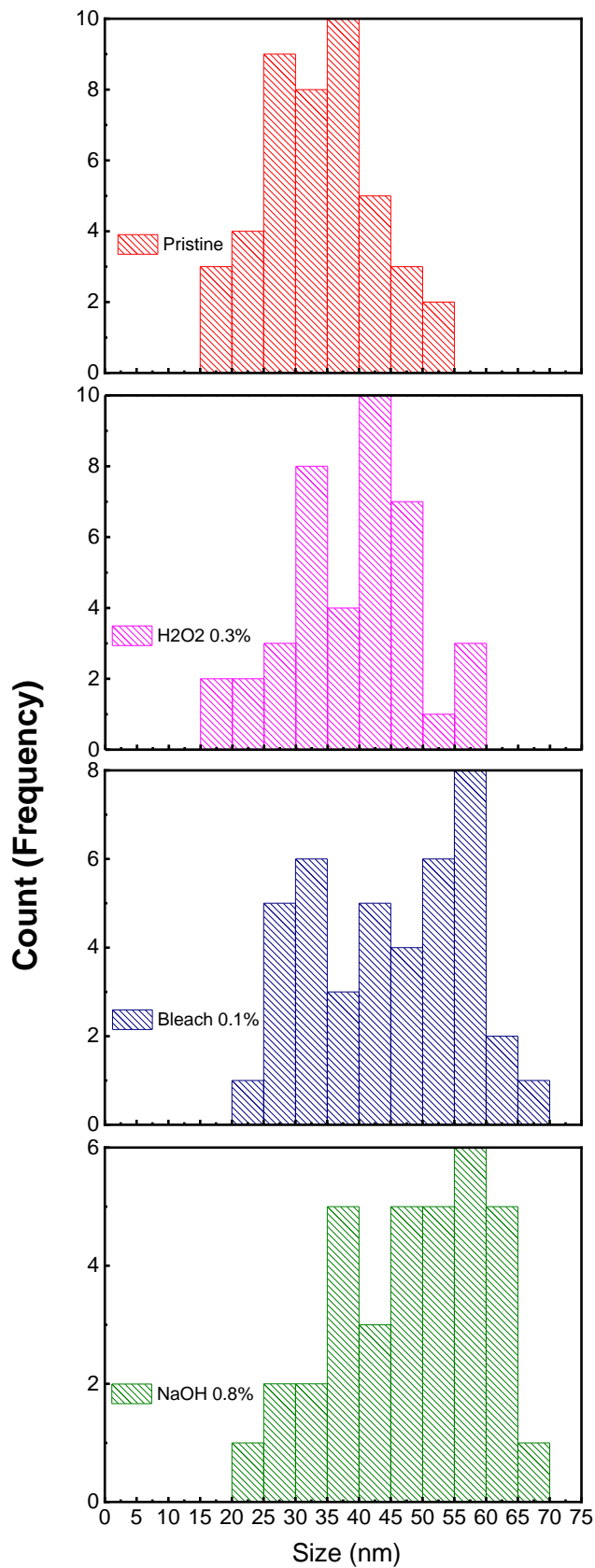


Figure 2: Fiber diameter frequencies of membranes from analyses of SEM images. Fiber diameters were measured for each listed membrane type using ImageJ software. 5

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 ± 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 ± 10 nm. The 0.1% bleach-treated membranes had a maximum fiber diameter of 69 nm
247 and an average fiber diameter of 44 ± 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 ± 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

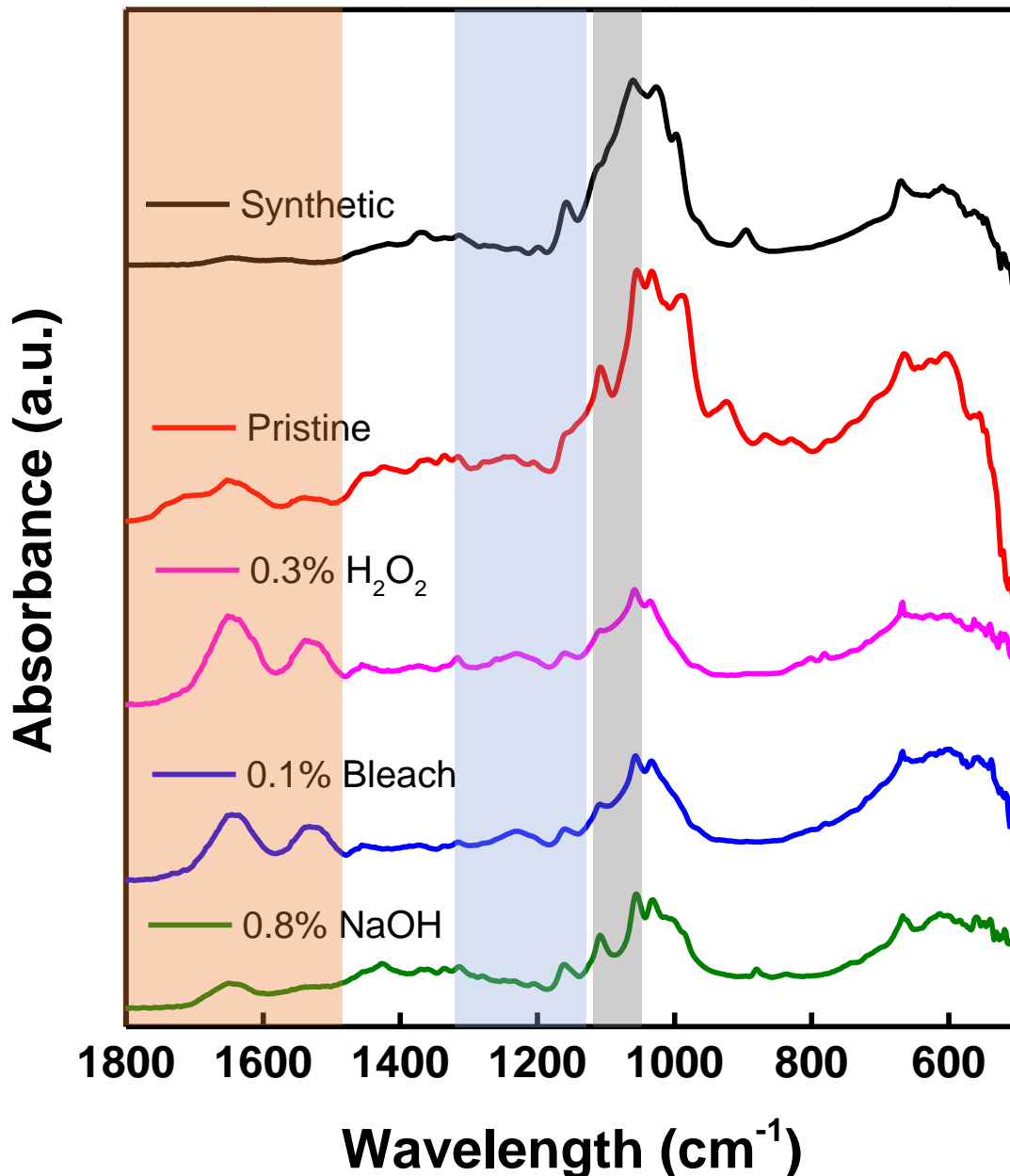


Figure 3: FTIR spectra for pristine and selected treated membranes. Orange corresponds to lipids, proteins, and nucleic acids; blue corresponds to nucleic acids; and gray corresponds to lipids and nucleic acids. Samples were prepared via lyophilization.

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 cm^{-1} (functional group: COC, CCO and CCH

265 deformation and stretching) as well as 1020 and 1046 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 cm^{-1} to 1500 cm^{-1} , a peak at
268 1090 cm^{-1} , and a broad peak at 1261 cm^{-1} . The areas which correspond to microbial
269 cellulose impurities²⁹ are highlighted in Figure 3. The 0.1% bleach and 0.3% H_2O_2
270 treatments led to a reduction in the peak at 1090 cm^{-1} , while 0.8% NaOH treatment did
271 not. The 0.5 M NaOH treatment reduced the peak at 1261 cm^{-1} , whereas 0.1% bleach
272 and 0.3% H_2O_2 treated membranes did not. These results likely indicate that 0.1%
273 bleach and 0.3% H_2O_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 cm^{-1} range have been
279 associated with the presence of bacterial cells on cellulose. These peaks were the most
280 prominent in the H_2O_2 and bleach-treated membranes, membranes with the largest
281 number of cells observed in the SEM images (Figure 1C,D).

282

283

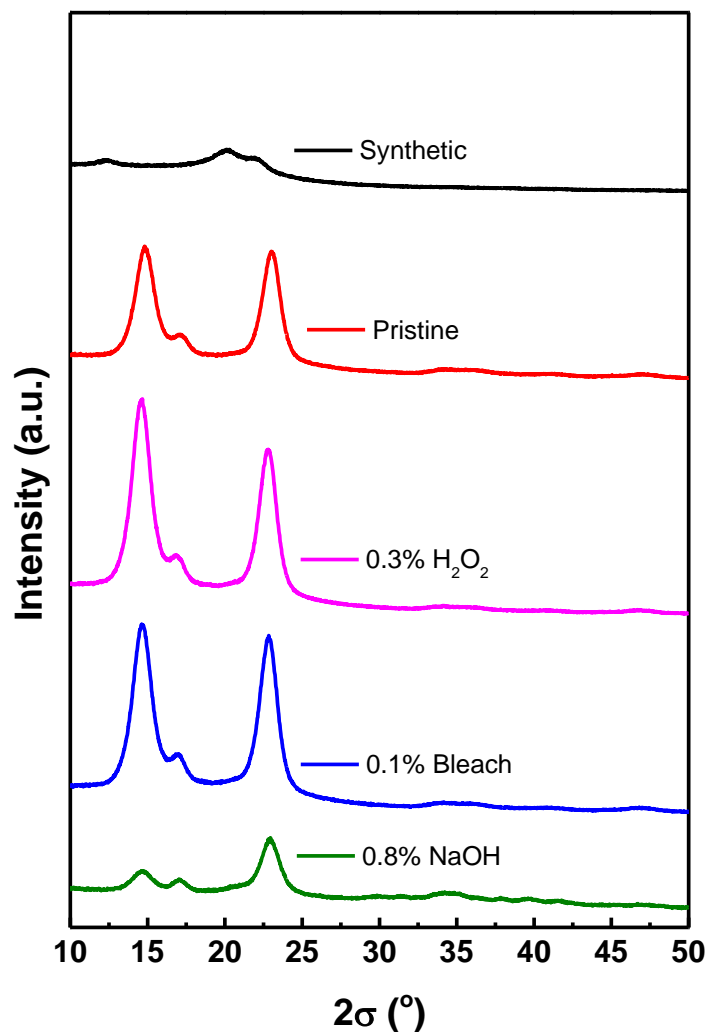


Figure 4: XRD spectra of treated membranes compared to pristine and synthetic membranes. Prior to analysis, samples were air dried at room temperature for 7 days.

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 cellulose²⁶. 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 H₂O₂ 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 high²⁶, 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

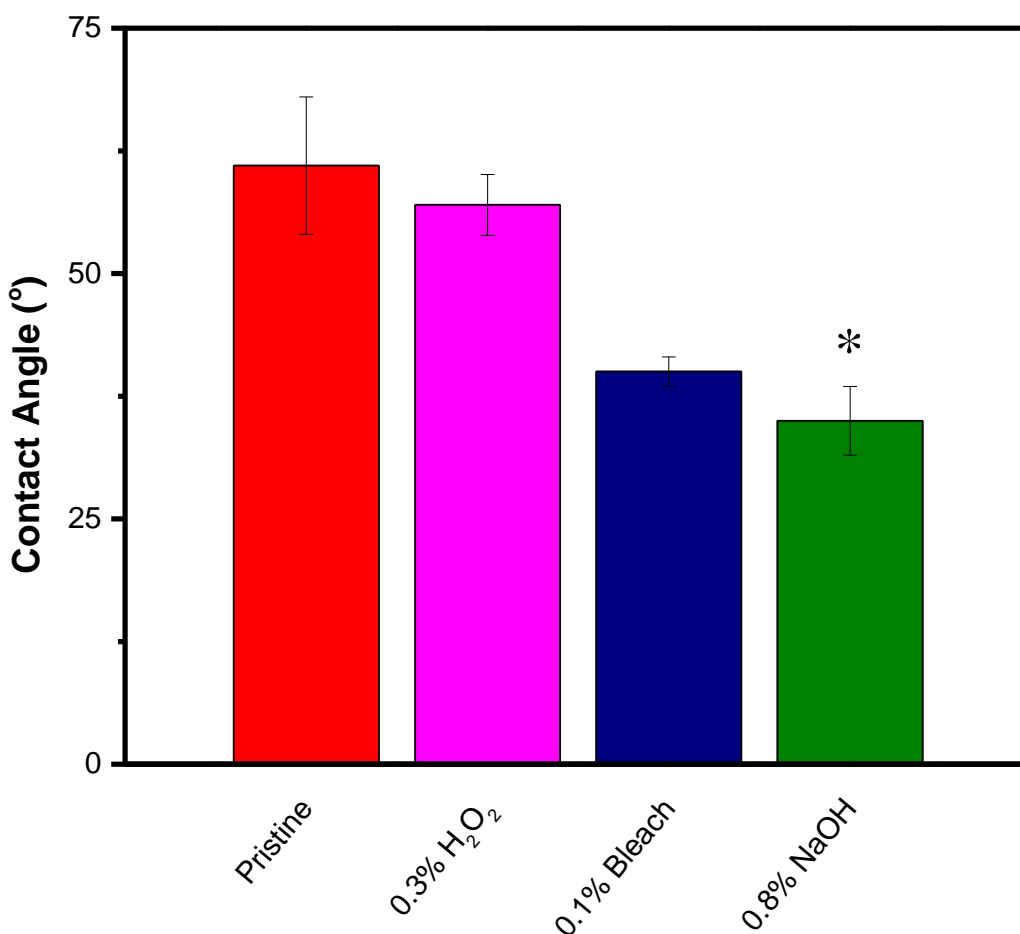


Figure 5: Air in DI water captive bubble contact angles of membranes (asterisk denotes statistically significant results when compared to pristine membrane).

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

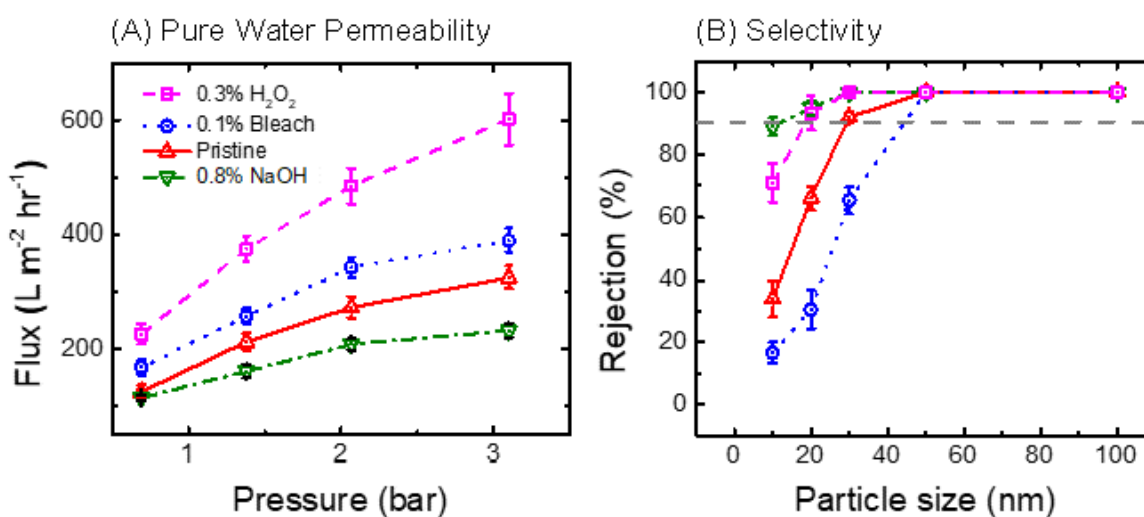


Figure 6: (A) Deionized water flux of membranes, (B) Selectivity of membranes (dashed selectivity lines connecting the experimental data represent expected results). Membranes were tested in a dead-end filtration cell. Prior to flux measurements, membranes were compressed at 3 bar for 1 h. Selectivity 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% H₂O₂ showed the greatest
 312 increase in permeability (Figure 6A). Pure water permeability increased from an
 313 average of 143 L·m⁻²·hr⁻¹·bar⁻¹ for pristine membranes to 257 L·m⁻²·hr⁻¹·bar⁻¹ for 0.3%

314 H₂O₂-treated membranes (79.7% increase). This increase was found to be statistically
315 significant in ANOVA testing. This change is likely due to H₂O₂ being an effective
316 membrane purification agent; as a strong oxidizer, H₂O₂ is widely utilized for removal of
317 non-cellulose organic matter in wood pulp. By removing non-cellulose membrane
318 constituents, H₂O₂ 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 H₂O₂ cellulose purification
322 0.3% H₂O₂ 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, H₂O₂ removes non-cellulose components of
325 microbial membranes while leaving cellulose intact (Figure 3). Interestingly, the H₂O₂
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 H₂O₂ 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⁻²·hr⁻¹·bar⁻¹ to 181
336 L·m⁻²·hr⁻¹·bar⁻¹ (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 H₂O₂ 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% H₂O₂ 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⁻²hr⁻¹bar⁻¹ to 115 L·m⁻²hr⁻¹bar⁻¹ (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 H₂O₂ 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% H₂O₂
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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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-](https://www.who.int/news-room/fact-sheets/detail/drinking-water)
417 [sheets/detail/drinking-water](https://www.who.int/news-room/fact-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 Scilimenti, 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**,

- 429 613–614, 840–846. <https://doi.org/10.1016/j.scitotenv.2017.09.135>.
- 430 (6) Fane, A. G.; Wang, R.; Hu, M. X. Synthetic Membranes for Water Purification:
431 Status and Future. *Angew. Chemie Int. Ed.* **2015**, *54* (11), 3368–3386.
432 <https://doi.org/10.1002/anie.201409783>.
- 433 (7) Song, X.; Chen, F.; Liu, F. Preparation and Characterization of Alkyl Ketene
434 Dimer (Akd) Modified Cellulose Composite Membrane. *Carbohydr. Polym.* **2012**,
435 *88* (2), 417–421. <https://doi.org/10.1016/j.carbpol.2011.10.062>.
- 436 (8) Lalia, B. S.; Kochkodan, V.; Hashaikeh, R.; Hilal, N. A Review on Membrane
437 Fabrication: Structure, Properties and Performance Relationship. *Desalination*
438 **2013**, *326*, 77–95. <https://doi.org/10.1016/j.desal.2013.06.016>.
- 439 (9) California Office of Environmental Health Hazard Assessment. N,N-
440 Dimethylacetamide.
- 441 (10) Eggensperger, C.; Zodrow, K. Sustainable Living Filtration Membranes. *Environ.*
442 *Sci. Technol. Lett.* **2020**, *7* (3), 213–218.
443 <https://doi.org/10.1021/acs.estlett.0c00019>.
- 444 (11) Al-Shamary, E.; Khalaf, A. Influence of Fermentation Condition and Alkali
445 Treatment on the Porosity and Thickness of Bacterial Cellulose Membranes.
446 2013.
- 447 (12) Aguayo, M. G.; Pérez, A. F.; Reyes, G.; Oviedo, C.; Gacitúa, W.; Gonzalez, R.;
448 Uyarte, O. Isolation and Characterization of Cellulose Nanocrystals from Rejected
449 Fibers Originated in the Kraft Pulping Process. *Polymers (Basel)*. **2018**, *10* (10),
450 1145.
- 451 (13) Das, S.; Lachenal, D.; Marlin, N. Production of Pure Cellulose from Kraft Pulp by

- 452 a Totally Chlorine-Free Process Using Catalyzed Hydrogen Peroxide. *Ind. Crop.*
453 *Prod.* **2013**, *49*, 844.
- 454 (14) Environmental Protection Agency. Chlorine dioxide; CASRN 10049-04-4.
- 455 (15) Zeronian, S. H.; Inglesby, M. K. Bleaching of Cellulose by Hydrogen Peroxide.
456 *Cellulose* **1995**, *2* (4), 265–272. <https://doi.org/10.1007/BF00811817>.
- 457 (16) Rossoni, E. M. M.; Gaylarde, C. C. Comparison of Sodium Hypochlorite and
458 Peracetic Acid as Sanitising Agents for Stainless Steel Food Processing Surfaces
459 Using Epifluorescence Microscopy. *Int. J. Food Microbiol.* **2000**, *61* (1), 81–85.
460 [https://doi.org/10.1016/S0168-1605\(00\)00369-X](https://doi.org/10.1016/S0168-1605(00)00369-X).
- 461 (17) Drosou, A.; Falabella, A.; Kirsner, R. Antiseptics on Wounds: An Area of
462 Controversy. *Wounds* **2003**, *15* (5), 149–166.
- 463 (18) Zhang, S.; Li, F.-X.; Yu, J.; Hsieh, Y.-L. Dissolution Behaviour and Solubility of
464 Cellulose in NaOH Complex Solution. *Carbohydr. Polym.* **2010**, *81* (3), 668–674.
465 <https://doi.org/10.1016/j.carbpol.2010.03.029>.
- 466 (19) Budtova, T.; Navard, P. Cellulose in NaOH–Water Based Solvents: A Review.
467 *Cellulose* **2016**, *23* (1), 5–55. <https://doi.org/10.1007/s10570-015-0779-8>.
- 468 (20) Javier Benítez, F.; Acero, J. L.; González, T.; García, J. Application of Ozone and
469 Advanced Oxidation Processes to the Treatment of Lye-Wastewaters from the
470 Table Olives Industry. *Ozone Sci. Eng.* **2002**, *24* (2), 105–116.
471 <https://doi.org/10.1080/01919510208901601>.
- 472 (21) Das, D. J.; Barringer, S. A. Potassium Hydroxide Replacement for Lye (Sodium
473 Hydroxide) in Tomato Peeling: Potassium Hydroxide Peeling. *J. Food Process.*
474 *Preserv.* **2006**, *30* (1), 15–19. <https://doi.org/10.1111/j.1745-4549.2005.00043.x>.

- 475 (22) Zhang, L.; Zhang, J.; Chen, L.; Liu, T.; Ma, G.; Liu, X. Influence of Manufacturing
476 Process on the Contents of Iron, Copper, Chromium, Nickel and Manganese
477 Elements in Crush, Tear and Curl Black Tea, Their Transfer Rates and Health
478 Risk Assessment. *Food Control* **2018**.
479 <https://doi.org/10.1016/j.foodcont.2018.01.030>.
- 480 (23) Lee, Y.-J.; Chung, C.-H.; Day, D. F. Sugarcane Bagasse Oxidation Using a
481 Combination of Hypochlorite and Peroxide. *Bioresour. Technol.* **2009**, *100* (2),
482 935–941.
- 483 (24) Meftahi, A.; Khajavi, R.; Rashidi, A.; Rahimi, M. K.; Bahador, A. Effect of
484 Purification on Nano Microbial Cellulose Pellicle Properties. *Procedia Mater. Sci.*
485 **2015**, *11* (C), 206–211.
- 486 (25) Zhao, H.; Kwak, J.; Conradzhang, Z.; Brown, H.; Arey, B.; Holladay, J. Studying
487 Cellulose Fiber Structure by {SEM}, {XRD}, {NMR} and Acid Hydrolysis.
488 *Carbohydr. Polym.* **2007**, *68* (2), 235–241.
489 <https://doi.org/10.1016/j.carbpol.2006.12.013>.
- 490 (26) Park, S.; Baker, J. O.; Himmel, M. E.; Parilla, P. A.; Johnson, D. K. Cellulose
491 Crystallinity Index: Measurement Techniques and Their Impact on Interpreting
492 Cellulase Performance. *Biotechnol. Biofuels* **2010**, *3* (1), 10.
493 <https://doi.org/10.1186/1754-6834-3-10>.
- 494 (27) McDonald, J. H. *Handbook of Biological Statistics*, 3rd ed.; 2014.
- 495 (28) Coenen, K. T.; Gallucci, F.; Cobden, P.; van Dijk, E.; Hensen, E. J. M.; van Sint
496 Annaland, M. Chemisorption of H₂O and CO₂ on Hydrotalcites for Sorption
497 Enhanced Water-Gas-Shift Processes. *Energy Procedia* **2017**, *114*, 2228–2242.

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

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