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**Membrane Bioreactor Pre-treatment of High Salinity O&G Produced
Water**

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32 **Abstract**

33 Produced water (PW) from oil and gas production contains variable constituents
34 which are difficult to remove with conventional treatment processes. The focus of
35 this study was to explore the long-term performance of a membrane bioreactor
36 (MBR) for removal of organic constituents from PW, and how performance and
37 microbial community composition are affected by progressively increasing salinity
38 and introduction of PW from different shale basins around the US. Dissolved organic
39 carbon removal from the PW remained consistent throughout the study, averaging
40 86% from the Denver-Julesburg basin PW and 66% removal from the Permian basin
41 PW. Surfactant removal was less consistent, showing 87% removal of polyethylene
42 glycols (PEGs) at total dissolved solids (TDS) concentration of 40 g/L but only 58%
43 removal at TDS concentration of 100 g/L. Diversity in the microbial community
44 decreased during reactor establishment but increased at TDS concentrations above
45 80 g/L. The results of this study suggest that MBRs can be effective PW
46 pretreatment processes even at high salinities.

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50 **Keywords**

51 Membrane bioreactor; produced water; desalination; wastewater treatment;
52 biological treatment; dissolved organic carbon removal

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56 **Highlights**

- 57 • Up to 95% of dissolved organic carbon (DOC) was removed from non-
58 pretreated O&G produced water by the MBR
- 59 • DOC removal remained consistent throughout the 10-month study
- 60 • DOC removal by the MBR was unhindered as TDS concentrations were raised
61 up to 100 g/L
- 62 • Similar results were observed with produced water from a high salinity basin
- 63 • Microbial community analysis showed decreased diversity with bioreactor
64 establishment, with increased diversity as salinity increased beyond 80 g/L
65 TDS

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68 **1. Introduction**

69 Unconventional oil and gas production generates large volumes of contaminated
70 wastewater. This wastewater, referred to as produced water (PW), is a highly
71 variable mixture of both organic and inorganic constituents, including total
72 suspended solids (TSS), organic matter, metals, anions and cations, and
73 microorganisms.¹ The presence of these contaminants, in combination with a very
74 high level of salinity, makes PW an exceptionally challenging wastewater stream to
75 treat for beneficial reuse. Successful reclamation of PW requires several treatment
76 processes, as many of the above contaminants require unique technologies for
77 removal from PW. For example, coagulation and flocculation are effective at
78 removing low-density TSS and colloids through physical and chemical processes,
79 but not as effective at removing dissolved organic carbon (DOC).^{2, 3} With each
80 additional process added, treatment becomes more complex and expensive,
81 reducing the ability of oil and gas companies to choose between water treatment
82 for reuse and disposal. If the treatment of PW is to be adopted for reuse outside the
83 oil field, the complexity and costs of treatment must be reduced.

84 One way to achieve cost reduction is by using treatment processes that can
85 remove several contaminants in one step, thereby shortening the treatment train.⁴
86 Successful treatment was achieved in one study by combining forward and reverse
87 osmosis systems.⁵ This study also highlighted through life cycle analysis that
88 pretreatment to remove organic foulants could substantially increase efficiency and
89 reduce operating costs of treatment. Several processes can remove organic
90 contaminants from water, including adsorption, chemical oxidation, and
91 biodegradation. Biological processes are usually preferred because they do not
92 require the use and storage of chemicals or require the disposal of the organics that
93 have adsorbed to media or precipitated out. However, using biological processes in
94 the treatment of PW can be challenging. PW total dissolved solids (TDS)
95 concentrations can range from less than 10 g/L to more than 300 g/L.⁶ This
96 complicates treatment because biological degradation of contaminants has been
97 shown to be substantially reduced at TDS levels higher than 10 g/L.⁷⁻⁹ Additionally,
98 biological treatment systems require a stable environment to perform optimally.
99 When parameters such as temperature, pH, salinity, or nutrient concentrations are
100 out of the optimal range, the biological system can be negatively impacted, causing
101 deactivation of the biological community with slow or no potential future recovery.¹⁰

102 The challenges associated with biologically treating PW are substantial; yet,
103 there have been several studies that have demonstrated success at the bench-
104 scale. A review article on biological treatment of PW that surveyed 59 published
105 studies found that on average, 73% of chemical oxygen demand (COD) was
106 removed from PW with TDS concentrations less than 50 g/L.¹¹ One study using
107 biologically active filtration (BAF) in combination with ultra-filtration membranes
108 (UF) as pre-treatment before desalination with nanofiltration (NF) was able to
109 remove over 75% of organic contaminants while reducing the fouling and increasing
110 the efficacy of NF membranes.¹² Another BAF study was able to achieve 95%
111 removal of organic matter.¹³ These studies not only showed the effectiveness of
112 biological systems in treating moderate salinity PW, but also highlighted that
113 combining biological and physical treatment technologies can substantially reduce
114 the number of processes needed.

115 One successful process that combines biological and physical processes into a
116 single system is a membrane bioreactor (MBR). In addition to combining multiple
117 processes, an MBR offers other advantages over other biological treatment systems
118 such as reduced footprint, easy and independent control of hydraulic retention time
119 (HRT) and solid retention time (SRT), and simple control systems that allow for
120 automated operation and maintenance (O&M).¹⁴ Also, because MBRs have been
121 used to successfully treat municipal wastewater for many years, it is a widely
122 accepted commercial process that can be rapidly implemented.¹⁵ These advantages
123 have led to several examinations into treating PW with different TDS concentrations.
124 Frank et al. (2017) explored the treatment of a combined residential wastewater
125 and PW stream using a hybrid sequencing batch reactor-membrane bioreactor
126 process (SBR-MBR) and was able to achieve over 90% soluble COD (sCOD)
127 reduction.¹⁶ Two different studies using the same laboratory-scale membrane
128 sequencing batch reactor (MSBR) system showed removal of total organic carbon
129 (TOC) at 92% and 91% at TDS concentrations of 16 g/L and 35 g/L, respectively,
130 using synthetic and real PW.^{17, 18} In another set of experiments 83% and 95%
131 removal of COD was obtained from synthetic PW with a TDS of 64.4 g/L and 144 g/L,
132 respectively, using a laboratory-scale MBR.^{19, 20}

133 These studies were able to show that an MBR at a laboratory-scale can be
134 effective in the pretreatment of PW; however, they were limited in their overall
135 scope. For example, in three of the four studies reviewed, synthetic PW was used

136 instead of real PW. Synthetic PW may not be able to accurately represent the
137 chemistry of real PW as not only does the composition change depending on the
138 location of the well and how long it has been in production, but many of the
139 reagents that energy companies use during hydraulic fracturing are proprietary,
140 and therefore not available for use in a synthetic PW. Real PW also contains native
141 microbes which may be well adapted to the salinity and hydrocarbon content of PW.
142 Another limitation seen in these studies is the TDS concentrations of the PW
143 treated. TDS concentrations of real PW can range from 10 g/L to over 300 g/L; yet,
144 only one of the previously referenced studies tests PW with a TDS concentration
145 over 100 g/L, and that was synthetic PW.¹⁹ A third limitation to these studies is their
146 length and/or scale. Most of the studies found in the literature were done for a
147 limited timeframe (weeks to a few months) or performed on a bench-scale setup
148 (approximately 5 liters) or both.²¹⁻²³ And the last limitation these previous studies
149 have is their use of a single PW source, which may not accurately represent the
150 conditions seen during actual well production, particularly the organic chemicals,
151 where the characteristics of PW can vary substantially over the lifetime of the well.³

152 Therefore, the main objective of our study was to evaluate the ability of a small
153 pilot-scale MBR (bioreactor volume of 70 L) to remove various constituents (e.g.,
154 TSS, DOC, nutrients, metals) from PW during 9 months of continuous operation,
155 using real PW from the Denver Julesburg (DJ) basin that had its TDS gradually
156 increased to 100 g/L, and culminating in using real PW from the Permian basin with
157 a natural TDS concentration of 110 g/L. Performance was also evaluated using
158 select water quality indicators and targeted organic constituents (e.g., surfactants).
159 Additionally, the microbial community and its changes were also analyzed over the
160 course of the study using 16S rRNA gene amplicon analysis, revealing that the core
161 microbiome in the MBR is made up of a few key microbial groups that can adapt to
162 varying salinities. As such, this work presents a unique long-term pilot study of the
163 effectiveness of biological treatment for moderate and high salinity PW, illustrating
164 an efficient pretreatment process prior to desalination (e.g., reverse osmosis (RO) or
165 membrane distillation (MD)).

166

167 **2. Materials and methods**

168 **2.1. MBR feed water**

169 The PW used in this study was obtained from multiple well sites in the DJ basin

170 located in the northeastern section of Colorado. The PW was stored in 950 L (250
 171 gal.) water totes at ambient temperature (~ 20 °C) until fed into the MBR. A 950 L
 172 batch of PW from the Permian basin was brought to the laboratory for experiments
 173 with naturally occurring high salinity PW. The water quality of the PW received
 174 throughout this study is summarized in Table 1. No pretreatment was performed on
 175 the PW before use.

176

177 **Table 1.** Water quality of PW and the dates it was collected from the DJ-Basin and
 178 Permian basin throughout this study. Most constituents were observed to remain
 179 consistent, with a few outliers seen at each collection date (i.e., DOC concentration
 180 fluctuating from 83 mg/L to 207 mg/L)

Analytes (mg/L)	Feb. 8th	July 26th	Aug. 14th	Sep. 27th	Dec. 24th	Permian Basin PW
DOC	78	83	207	68	189	71
TN	63	18	114	22	33	455
NH ₃	55	14	103	20	28	405
B	27.3	15.1	19.9	20.1	20.3	48
Ba	30.5	5.32	3.59	12.2	10.9	2.6
Ca	990	97.3	150	239	303	4052
Fe	77.4	BDL	0.22	0.865	1.77	14
K	63.1	20.6	22.3	31.9	42.7	1020
Li	7.52	2.37	3.46	4.03	5.07	36
Mg	126.5	18.9	23.0	40.3	42.8	752
Na	10,288	3,486	4,384	5,823	7,012	45,841
P	3.92	1.4	BDL	BDL	1.36	0
S	7.95	23.8	14.5	13.0	8.30	27
Si	43.3	73.3	46	104	55.3	13
Sr	263.0	75.7	264	1148	67.3	716
Cl	15,000	6,648	8,300	10,702	13,000	69,659
PO ₄	BDL	BDL	BDL	BDL	BDL	BDL
NO ₃	BDL	BDL	BDL	0.8	BDL	BDL
SO ₄	0.09	60.2	32	31.3	16.5	602
Br	26.7	83	46.2	10	128	592
Source	26,800	10,900	14,000	21,420	21,800	111,50
TDS						0
NaCl added	0	16,000	26,000	58,580	78,200	0
Adjusted TDS	26,800	27,000	40,000	80,000	100,00 0	111,50 0

181

182 **2.2. MBR system**

183 A schematic drawing of the MBR system is shown in Figure 1. Raw PW was held
 184 in a 200 L (55 gal) drum and was refilled weekly with fresh PW. The volume of the
 185 bioreactor, including displacement for the submerged ultra-filtration membrane
 186 module and stirring paddle, was 70 L. The peristaltic pump feeding PW into the
 187 continuously stirred MBR was operating at a constant flowrate of 24 mL/min.

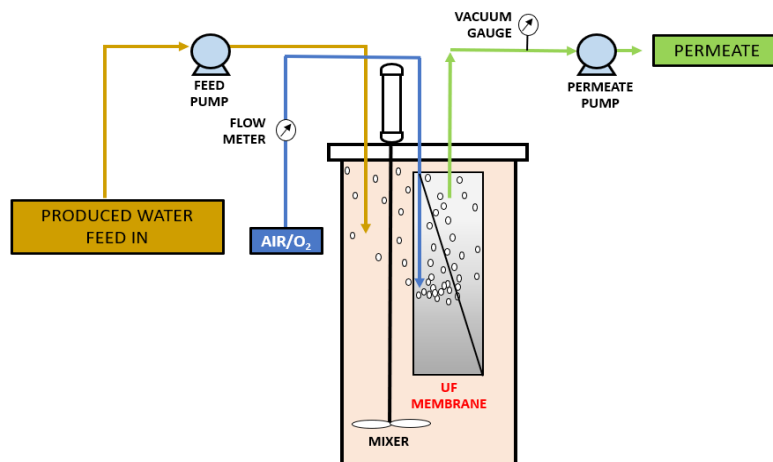
188 Aeration of the bioreactor and air scouring of the membrane was accomplished by
189 pumping air into the membrane aeration port at a rate of 16 L/min using a 5 min on
190 and 2 minutes off cycling of the air pump (AL-15A, Alita Industries, Inc., Arcadia,
191 CA). Permeate was removed from the bioreactor by peristaltic pump at a flowrate of
192 24 mL/min which, when coupled with the 70 L reactor, produced an HRT of 48
193 hours. A constant water flux of 2.9 L per m² per hour (LMH) through the membrane
194 was maintained during the entire study. Backwashing of the membrane was
195 performed for 20 seconds every 10 minutes at a rate of 300 mL/min. To sustain the
196 slow-growing microorganisms in the MBR, no solids were removed from the reactor
197 throughout the entire study, resulting in a theoretically infinite SRT. HRT, water flux,
198 and backwash cycle were chosen based on previous PW biological studies for more
199 straightforward comparisons.^{12, 13, 24, 25}

200 The ultrafiltration (UF) membrane used in this system was a submersible Puron®
201 0.04 µm pore-size, hollow fiber module with a surface area of 0.5 m² (Koch
202 Separation Solutions, Wilmington, MA). Because flux was kept at a constant 2.9
203 LMH, transmembrane pressure (TMP) was monitored for signs of membrane fouling.
204 Cleaning was performed on the UF membrane whenever the TMP approached 50%
205 of the membrane's maximum filtration TMP of 9 psi, which occurred approximately
206 four times over the course of the study. The cleaning procedure consisted of
207 acid/base wash cycles, including one hour backwashing with HCl solution (pH 2),
208 one hour backwashing with NaOH solution (pH 10), another hour of backwashing
209 with HCl solution, and conclusion with a 30 minutes backwashing rinse with
210 deionized water. It is worth noting that in two separate incidents a single membrane
211 fiber physically detached from the membrane module. In both instances the entire
212 membrane module was replaced.

213 The reactor was seeded with activated sludge from a municipal wastewater
214 treatment facility. Activated sludge was acclimated to the high salinity PW by
215 diluting raw PW with dechlorinated tap water at a starting ratio of 20:1. The fraction
216 of PW in the feed was increased every 48 hours to correspond with an increase of
217 TDS concentration by 2 g/L until 100% of the feed water was raw PW. To further
218 increase TDS levels from the average 40 g/L of the raw PW, sodium chloride
219 (Culinox®, Morton Salt, Chicago, IL) was added to each feed batch at the same
220 acclimation rate as described above to reach the desired salinity. TDS levels were
221 maintained at 40 g/L, 60 g/L, 80 g/L, and 100 g/L for extended time to evaluate MBR

222 performance at each of these concentrations.

223



224

225 **Figure 1.** A flow diagram of the MBR system used in this study. PW and air are
226 pumped into the bioreactor where they are mixed with the activated sludge. PW is
227 continuously fed into the reactor, which maintains an average HRT of 48-hours.
228 Treated water is pulled through the UF membrane, keeping all suspended solids in
229 the bioreactor, and producing a treated permeate stream.

230

231 **2.3. Sampling and bulk analytical procedures**

232 All feed water samples were collected at the point just before the feed water
233 enters the bioreactor. All permeate water samples were collected after the
234 peristaltic pump that draws the permeate through the UF membrane. Conductivity
235 and pH were determined using a handheld digital meter with appropriate probe
236 (HQ40d, PHC10101, CDC40101, Hach Co., Loveland, CO) and conducted once a
237 week. Alkalinity and ammonia were measured using Hach test vials (TNT 870, TNT
238 832, Hach Co., Loveland, CO) and diluted below levels of interference. Analysis for
239 dissolved organic carbon (DOC) and total nitrogen (TN) (TOC-L, Shimadzu,
240 Columbia, MD) was also performed weekly. Samples for DOC and TN analysis were
241 filtered through a 0.45 µm polytetrafluoroethylene (PTFE) filter (VWR International,
242 LLC., Radnor, PA), acidified with concentrated HCl to pH 2, and stored at 3 °C until
243 analysis was performed. Ion chromatography (IC, ICS-900, Dionex, Sunnyvale, CA)
244 for negatively charged ions and inductively coupled plasma-atomic emission
245 spectroscopy (ICP-AES, Optima 5300, Perkin-Elmer, Fremont CA) for positively
246 charged ions were performed monthly. Samples for IC analysis were filtered through
247 a 0.45 µm PTFE filter and stored at -4 °C until analysis was performed. Samples for
248 ICP-AES analysis were filtered through a 0.45 µm PTFE filter, acidified with

249 concentrated HNO₃, and stored at 3 °C until analysis was performed. TDS, MLSS,
250 and MLVSS quantifications were performed according to standard methods (EPA
251 160.1, 1684).

252

253

254 **2.4. Surfactant analysis**

255 Solid-phase extraction (SPE) was performed on all samples for liquid
256 chromatography and time of flight mass spectrometry (LC-qTOF) analysis for the
257 semi-quantitative abundance and identification of polyethylene glycols (PEGs),
258 polypropylene glycols (PPGs), PEG dicarboxylates (PEG-diCs), and PEG carboxylates
259 (PEG-Cs). SPE cartridges (Oasis HLB 6cc-500mg 60 μm, Waters Corp., Milford, MA)
260 were pre-conditioned with methanol and HPLC water.²⁶ First, 5 mL of methanol was
261 vacuumed through the cartridge at a rate of 5 mL/min followed by 5 mL of HPLC
262 water at 5 mL/min. Next, 10 mL of sample was pulled through at 5 mL/min. This was
263 followed by 10 mL of HPLC water to flush out any salts that adhered to the
264 cartridge, at a rate of 5 mL/min. Elution of the samples was performed using 10 mL
265 methanol at a rate of 1 mL/min. The eluted samples were then concentrated down
266 to 1 mL by a gentle stream of N₂ gas (XcelVap, Biotage, Uppsala, Sweden). Samples
267 were then pipetted into 2 mL amber vials and stored at -4 °C until analyzed.

268 Analysis was non-targeted and conducted on a SCIEX X500R QTOF (Framingham,
269 MA) using high resolution liquid chromatography. The operating parameters of the
270 LC-MS were obtained from published methods for PEG, PPG, PEG-diCs, and PEG-Cs
271 identification in PW.²⁷ All organic solvents used throughout this analysis were of
272 HPLC grade or higher (Sigma-Aldrich Corp., St. Louis, MO). For quantification of
273 these compounds, their hydrogen, ammonium, and sodium adducts were extracted
274 from samples and analyzed on the SCIEX OS Analyst Software (Framingham, MA).
275 The peak areas of each of these adducts were summed to give a semi-quantitative
276 concentration of that surfactant.^{26, 28} Removal percentage was then determined
277 using the following equation:

278

$$279 \quad R\% = \frac{C_0 - C_{(t)}}{C_0} * 100$$

280 where $R\%$ is the removal percentage, C_0 is the relative abundance (defined at the
281 counts per second intensity (cps)) of a specific PEG in the feed water, and $C_{(t)}$ is the

282 relative abundance of the same PEG in the permeate. same PEG.

283

284 **2.5. Microbial community analysis: DNA extraction and 16S rRNA gene** 285 **amplicon sequencing**

286 Feed water and sludge samples were collected for analysis at regular intervals
287 during the study. Sludge samples were collected in 50 mL sterile tubes. Feed water
288 samples (50-100 mL) were filtered through a Sterivex™ filter (0.22 µm, PES filters,
289 Millipore-Sigma, MA). Feed and MBR permeate were shipped on ice to LBNL for
290 further analysis. Sludge and feed solids collected on the filters were shipped on dry
291 ice to LBNL, where the samples were stored at -80 °C until DNA extraction.
292 Triplicate 1.5 mL aliquots of MBR sludge collected at each time point were
293 centrifuged at 10,000 x g for 5 mins, followed by DNA extraction of the pellet with
294 DNeasy PowerLyzer PowerSoil kit (Qiagen, Hilden, Germany) as per manufacturer's
295 instructions. Sterivex filters containing feed microbes were extracted using DNeasy
296 PowerWater Sterivex kit (Qiagen, Hilden, Germany).

297 16S rRNA gene amplicons were amplified from DNA extracts using 515F and
298 806R primers targeting V4 hypervariable region, followed by PCR-free short library
299 preparation and sequencing on Novaseq 6000 (Illumina, PE250) at Novogene
300 Corporation Inc. Sequencing reads were processed in QIIME2 v. 2020.8.^{29, 30}
301 Specifically, reads were demultiplexed, quality filtered and denoised with DADA2.³¹
302 Taxonomy was assigned to the amplicon sequence variants (ASVs) using a naive
303 Bayes taxonomy classifier trained on SILVA 138 99% OTUs from 515F/806R region
304 of 16S rRNA gene sequences.^{32, 33} The ASV table generated was then manipulated in
305 R to remove singletons, perform statistical analyses and generate plots using
306 phyloseq package^{34, 35} The raw sequencing reads for MBR sludge and feed samples
307 are deposited at NCBI SRA under the BioProject PRJNA768964 (Individual sample
308 details and accessions are provided in Table S1).

309

310 **3. Results and discussion**

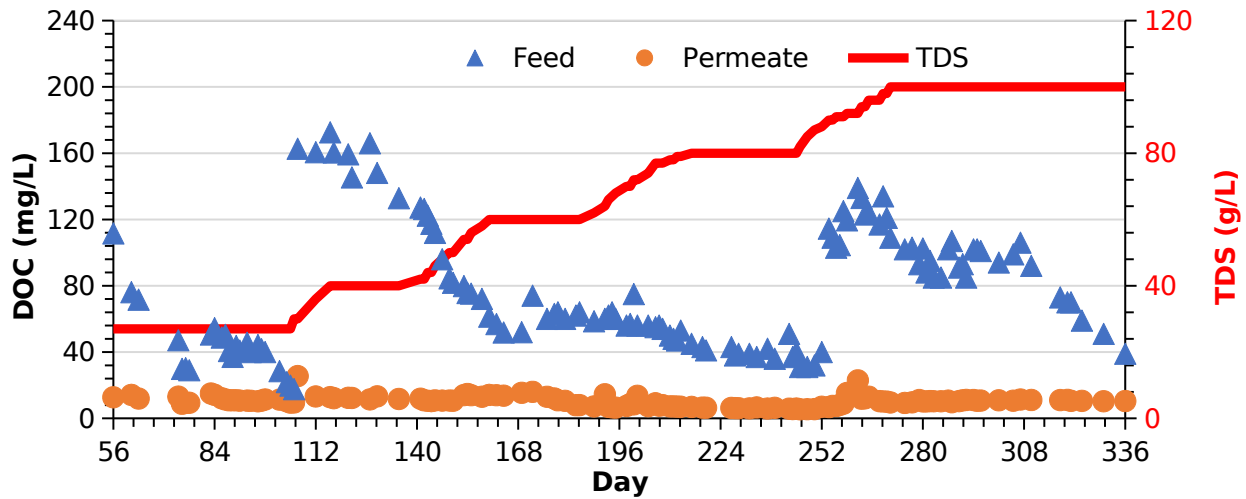
311 **3.1. Removal of organic and inorganic contaminants**

312 DOC and TDS concentrations in the feed and permeate streams of the MBR for
313 the entire study are shown in Figure 2. DOC concentration in the permeate stream
314 remained relatively constant at ~12 mg/L over the almost 10-month testing period,
315 even as TDS concentrations in the feed increased from 27 g/L at the start to 100 g/L

316 by the end; all while the feed DOC concentration fluctuated between 30 and 170
317 mg/L. These results are in contrast to studies that observed a marked decline in
318 biodegradation of organic matter when the salinity of the feed water was
319 increased.^{36, 37} The MBR average effluent DOC concentrations were 12.3 mg/L, 6.2
320 mg/L, 10.5 mg/L, and 10.3 mg/L at bioreactor salinities of 40 g/L, 60 g/L, 80 g/L, and
321 100 g/L TDS, respectively, which translates to 90%, 82%, 87%, and 89% DOC
322 removal over these time periods, respectively. These results compare favorably
323 with several other studies involving biological treatment of PW. Freedman et al.
324 (2017) demonstrated DOC removal of 95% using biologically active filtration (BAF)
325 treating PW with TDS concentrations of ~20 g/L TDS, while Riley et al. (2016) were
326 able to achieve over 75% DOC removal using a similar BAF system.^{12, 13} Pendashteh
327 et al. (2012) observed 91% total organic carbon (TOC) removal with the use of a
328 laboratory-scale MSBR treating PW with TDS concentrations of 35 g/L, and Frank et
329 al. (2017), using a pilot-scale hybrid sequencing batch reactor-membrane
330 bioreactor, were able to remove over 90% sCOD from residential wastewater that
331 was dosed with 6% PW.^{16, 18}

332 While the MBR permeate DOC concentration was relatively constant, the feed
333 DOC concentration declined over time for each of the water batches acquired for
334 the study. This in turn affected the calculated percent removal of DOC over time,
335 with lower feed concentration resulting in lower percent removal of DOC. The
336 changes in influent DOC can be attributed to the slow degradation of organic matter
337 in the PW storage tanks—the longer the PW was kept in the totes after collection
338 from the O&G wells, the more DOC concentrations declined, including in high
339 salinity raw PW.

340



341
 342 **Figure 2.** DOC concentration of the feed and permeate of the MBR beginning after
 343 the acclimation period (day 56 of operation). DOC concentration is given by the
 344 primary Y-axis, with feed represented by blue triangles and permeate represented
 345 by orange circles. The solid red line corresponds with the secondary Y-axis to show
 346 the gradual increase in TDS concentration from 27 g/L to 100 g/L.

347 As expected, the MBR's DOC removal is heavily dependent on the DOC
 348 concentrations in the PW used in this study. Throughout this study, when influent
 349 DOC levels exceeded 100 mg/L, percent removal averaged 91% at all TDS
 350 concentrations. Considering the relatively long HRT of 48 hours in the bioreactor,
 351 most of the labile organic compounds were likely degraded, leaving behind
 352 biologically recalcitrant organic compounds, which remained in the permeate and
 353 consisted of an average of 10.4 mg/L DOC throughout the study. It is possible that
 354 with the addition of supplemental nutrients, the microorganisms would perform
 355 better and reduce the permeate DOC concentration even further. Nicholas et al.,³⁸
 356 using a similar sequencing batch reactor (SBR), were able to further reduce sCOD
 357 concentrations in PW by an additional 20% with the addition of phosphorus at a
 358 level of 7.5 mg-P/L. Since the completion of this study, additional testing with
 359 supplemental phosphorus has been performed in the MBR, with preliminary results
 360 showing no additional DOC removal. It is likely that despite the addition of
 361 phosphorus, the lack of additional DOC removal is due to the very high PW salinity
 362 in this study.

363 A comparison of the concentrations of inorganic constituents between the PW
 364 feed and MBR permeate observed for bioreactor salinities ranging from 40 g/L to

365 100 g/L is summarized in Table 2. Except for iron, little to no reduction was
 366 observed in any of these constituents. Riley et al. reported similar results using a
 367 BAF and NF treatment processes on similar PW.⁴ The reduced iron concentrations
 368 are most likely due to oxidation from aeration in the reactor, causing the iron to
 369 precipitate out. While no removal was observed, Table 2 does show the substantial
 370 variability in the concentration of constituents typically seen in PW over time. This
 371 characteristic of PW has been well documented in previous studies and again
 372 highlights the challenges associated with PW treatment.³

373

374 **Table 2.** Average feed and permeate inorganic concentrations throughout the
 375 study. Little to no reduction in inorganics was observed during this study. However,
 376 the accumulation of these ions in the bioreactor over the 10-month study did not
 377 hinder organic biodegradation. Phosphate and nitrate levels were below detection
 378 limits.

Analyte (mg/L)	MBR Feed	MBR Permeate
TN	50±23	48±26
NH₃	45±21	40±18
B	21±1.5	20±2.7
Ba	11±4.3	9±3.3
Ca	222±51	232±74
Fe	2±1.1	0.1±0.14
K	47±18	46±27
Li	5±1.3	4±1
Mg	34±7.2	33±6.6
Na	24,198±11,152	25,025±11,651
P	1±0.6	1±1.2
S	15±10	27±11
Si	41±6.5	49±19
Sr	50±17	44±5.2
F	4±2.5	2±1
Cl	38,893±14,178	38,066±13,882
Br	123±29	117±30
SO₄	19±11	24±10

379

380 **3.2. Removal of targeted organic compounds**

381 The MBR's ability to remove targeted organic chemicals was also evaluated.
 382 Several surfactants were targeted that are commonly found in PW, which included
 383 PEGs, PPGs, PEG-diCs, and PEG-Cs.^{26, 27} PEGs and PPGs are used as surfactants to
 384 enhance recovery of O&G, and they can remain in PW after it is brought to the
 385 surface for over a year.³⁹ Several PEGs and PPGs were identified in the PW with the

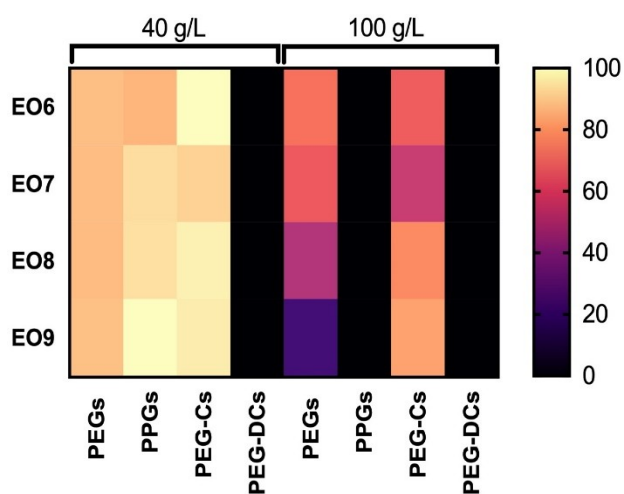
386 use of LC-qTOF analysis and evaluated over the course of the study. Identification of
387 PEGs is determined by the number of ethylene oxide (EO) units, where PEG-EO6
388 have 6 ethylene oxide units, PEG-EO7 have 7, and so on. The average mass
389 difference between these different PEGs is 44.0262 mass units, which corresponds
390 to the addition or subtraction of an ethylene oxide unit [-CH₂-CH₂-O-]. PPGs have a
391 difference of mass of 58.0419 mass units, which corresponds to the addition or
392 subtraction of a propylene oxide unit [--CH₂-CH(CH₃)-O-]. For convenience, EO6
393 refers to all surfactant compounds with 6 additional units, i.e., PPG-PO6 will be
394 referred to as "EO6". Quantification of volatile and even semi-volatile chemicals
395 through the MBR is very difficult due to the inability to accurately identify if removal
396 from the PW was due to air stripping or microbial degradation, as demonstrated by
397 Sitterley et al.⁴⁰ As such, we opted to evaluate the non-volatile chemicals and target
398 the surfactants present in the raw vs. MBR treated PW.

399 Contrary to DOC removal, it appears that TDS concentration did have a negative
400 impact on the MBR's ability to degrade PEG, PPG, PEG-diCs, and PEG-Cs surfactants.
401 Kawai showed that PEGs are aerobically metabolized first through the
402 oxidation of a PEG compound to a carboxylated PEG. This is done through the
403 microorganism's use of alcohol and aldehyde dehydrogenases enzymes. In a
404 second step, the terminal ether bond is cleaved, reducing the PEG by one
405 glycol unit.⁴¹ Figure 3 illustrates the negative impact TDS concentration had on
406 biodegradation of PEGs by using the relative abundances obtained from LC-qTOF
407 analysis to show the removal percentage from feed to permeate for PEGs, PPGs,
408 PEG-Cs, and PEG- diCs of size E06-E09 in PW with TDS concentration of 40 g/L and
409 100 g/L. The percent removal is semi-quantitative because relative abundance was
410 used. This is due to individual PEG standards not being easily obtained or readily
411 available. Additionally, this semi-quantitative method was used for this study
412 because, as Rosenblum et al. reported, "...a quantitative measure would be
413 challenging due to matrix-induced ionization effects and specific response factors
414 for these types of compounds, relative abundance was used as a way to compare
415 these compound levels over time".^{39, 42} The results from each TDS level on all
416 targeted surfactants can be found in the SI and are summarized in Table SI-2. As
417 shown in Figure 3, PEG removal averaged 87% when TDS concentrations were 40 g/
418 L but only 58% when TDS concentrations were at 100 g/L. This trend was also

419 observed with PPG removal, which averaged 81% at 40 g/L but averaged only 2.3%
 420 removal at 100 g/L; and also with PEG-Cs, where removal averaged 96% at 40 g/L
 421 but dropped down to 67% at 100 g/L. These results are possibly due to two
 422 previously described phenomena. The first is that biodegradation of PEGs is
 423 compromised in salty environments. Bernhard et al. showed that short-chain PEGs,
 424 while remaining completely biodegradable, require a much longer time of treatment
 425 in saline environments compared to a freshwater environment.⁴³ In artificial
 426 seawater (TDS of 35 g/L), short-chain PEGs did not fully biodegrade until after 37
 427 days of treatment. The second phenomena that might explain these results is the
 428 biodegradation of other ethoxylated additives present in the PW that have a mass
 429 that falls outside of the mass range analyzed in this study. Sitterley et al.
 430 demonstrated the presence of these compounds in PW (same source that was used
 431 in the current study) and that their aerobic biodegradation can lead to the
 432 formation of straight-chain PEGs.²⁶ McAdams et al. showed how alkyl ethoxylates
 433 used in fracturing fluid transform to PEGs through cleavage of the alkyl group from
 434 the polyethoxylated chain as a result of aerobic biodegradation.⁴⁴

435 The inability to remove surfactants from PW with high TDS levels is a concern
 436 and additional treatment steps may be necessary if treated PW is to be reused in
 437 applications that require these residual surfactants to be removed to a greater
 438 degree. For every TDS level, PEG-diCs concentration increased after MBR treatment.
 439 This is due to PEGs biodegrading first to singly and then to doubly carboxylated
 440 metabolites during treatment as previously described by Sitterley et al.⁴⁰

441



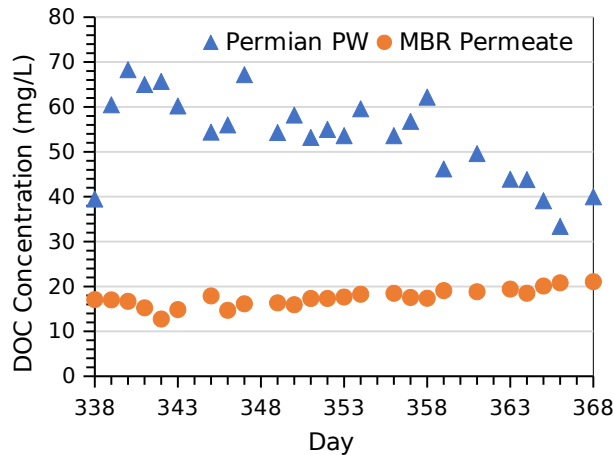
442

443 **Figure 3.** Heatmap of removal percentage of selected PEGs, PPGs, PEG-diCs, and
444 PEG-Cs remaining in the MBR permeate at TDS concentrations of 40 g/L and 100
445 g/L. The full results for all PEGs, PPGs, PEG-diCs, and PEG-Cs at each TDS
446 concentration are summarized in Table SI-2. Lighter colored boxes represent higher
447 removal percentage, with increasingly darker boxes representing lower removal
448 percentages.
449

450 Overall, these results illustrate the variable nature of constituent removal, and
451 shed some light into the 10.4 mg/L of remaining DOC present in the PW studied.
452 Unfortunately, without the ability to obtain individual PEG standards (e.g., an
453 analytical HPLC standard of PEG-E06), only semi-quantitative analysis was
454 performed.⁴⁵ However, these compounds are only expected to make up a small
455 percentage of the remaining DOC, as shown by Thurman et al.⁴⁶ Regardless, the
456 persistence of these chemicals in the permeate suggests that additional treatment
457 processes may be needed for complete removal. For example, research on PW has
458 shown the removal of surfactants through other processes, like activated carbon,
459 which could be utilized in a water reuse treatment train.⁴⁷
460

461 **3.3. Transition to treatment of Permian basin PW**

462 PW characteristics can vary dramatically depending on the source basin. To test
463 the MBR's ability to treat naturally occurring high salinity PW, once all experiments
464 had been completed using DJ-Basin PW, Permian basin PW was fed into the MBR for
465 one month. The characteristics of this new feed stream are summarized in Table 1.
466 Because the microorganisms in the MBR had already been acclimated to a TDS
467 concentration of 100 g/L, no acclimation period was given for the Permian PW that
468 had 110 g/L TDS. The performance of the MBR, during days 338-368 of operation, in
469 removing DOC from Permian basin PW is shown in Figure 4. Like treatment of DJ
470 basin PW, consistent DOC concentrations in the MBR permeate were observed over
471 the entire one-month study period (average of 17.5 mg/L vs. 10.4 mg/L when
472 operating with DJ basin PW).



473

474 **Figure 4.** DOC concentrations of feed and permeate, during days 338-368 of
 475 operation, using Permian PW. No acclimation period was used with the Permian PW
 476 being fed into the MBR immediately after the DJ basin study. The TDS concentration
 477 of the Permian PW was naturally 110 g/L. Additionally, this PW contains much higher
 478 concentrations of several other constituents than the DJ basin PW, such as
 479 ammonia, calcium, and potassium.

480

481 While consistent, DOC removal from the Permian basin water was lower
 482 compared to DJ basin water (66% vs. 86%). This was due to the combination of the
 483 lower DOC concentration found in Permian PW (average of 54 mg/L vs. 76.2 mg/L in
 484 the DJ water) and the lower average DOC concentrations of the treated DJ basin PW.
 485 The higher DOC concentrations observed in the permeate during the 31-day period
 486 of testing with Permian basin PW feed may be due to one or a combination of
 487 several factors. As shown in Table 1, the PW from the Permian basin contained
 488 substantially higher concentrations of many inorganic constituents compared to DJ
 489 basin PW, including ammonia, bromide, calcium, iron, potassium, magnesium,
 490 lithium, and sulfate. These substantial increases, without the benefit of an
 491 acclimation period, might have shocked the microorganisms in the bioreactor,
 492 creating a less than ideal environment that hindered their ability to biodegrade the
 493 DOC in the feed. Additionally, the amount of recalcitrant organics in the Permian PW
 494 may be naturally higher than that found in the DJ basin PW, leading to overall higher
 495 concentrations remaining in the permeate.

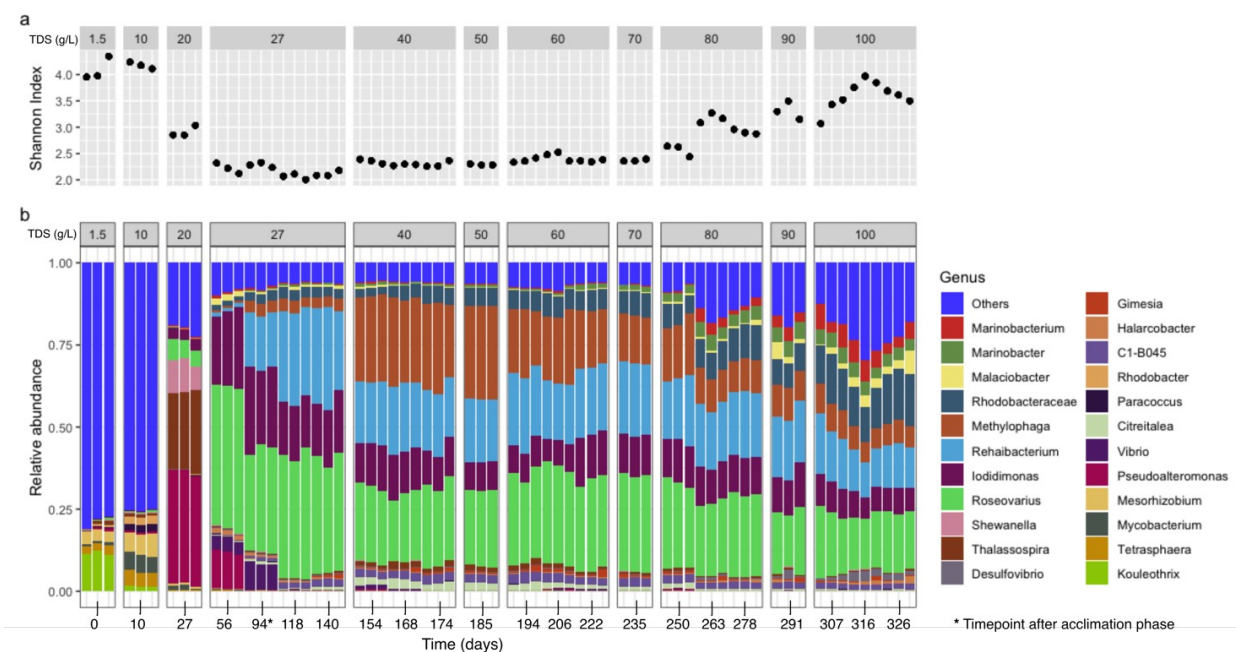
496 Upon conclusion of the experiment with Permian basin PW feed stream,
 497 operation of the MBR was resumed with DJ basin PW feed having an artificially
 498 raised TDS concentration of 100 g/L. This TDS concentration was maintained for the
 499 next 6 weeks. At that point, in an attempt to further test the robustness of the MBR

500 to maintain performance during a sudden and substantial change in salinity, the
501 TDS concentration was reduced to 75 g/L without any acclimation period. This
502 procedure was repeated 6 weeks later when the TDS concentration was reduced to
503 50 g/L without an acclimation phase. The final phase of this experiment involved the
504 raising of the TDS concentration from 50 g/L to 100 g/L with no acclimation period.
505 The DOC removal percentage results observed were 77%, 85%, and 84% for each
506 phase, respectively. The complete DOC removal data for this testing can be found in
507 the supporting information (SI) document (Figures SI-2, SI-3, and SI-4). The
508 microbial community analysis for this testing is shown in Figures SI-5, SI-8, and SI-9.
509

510 **3.4. Microbial community analysis**

511 The diversity of the microbial community in MBR sludge declined dramatically
512 during the acclimation phase, as indicated by the decrease in Shannon index and
513 increase in abundance of a few key taxa (Figure 5). This is likely attributable to the
514 inability of bacteria originating from the inoculum (activated sludge) to adapt to the
515 increased salinity. The core community composition of the MBR was relatively
516 stable beyond the acclimation phase, with a few persistent taxa constituting greater
517 than 50% of the reads. Specifically, members of the genera *Roseovarius* and
518 *Iodidimonas* first became established in the reactor when it was fed with 100% PW,
519 followed by other genera like *Rehaibacterium*, *Methylophaga*, and unclassified
520 *Rhodobacteraceae*, which together constituted close to 80% of the reads when the
521 reactor was fed with 100% PW in the salinity range of 40-70 g/L. Both *Roseovarius*
522 and *Iodidimonas* have been reported/isolated from PWs/hydrocarbon contaminated
523 environments from around the world; however, experimental evidence for
524 hydrocarbon degradation by *Roseovarius* and *Iodidimonas* isolates is not
525 available.⁴⁸⁻⁵¹ Isolates belonging to these genera have salinity tolerance in the range
526 of 5-100 g/L.^{52, 53} *Roseovarius* and *Iodidimonas* have been identified as Iodide
527 Oxidizing Bacteria (IOB) because they can produce iodine from iodide in the
528 presence of oxygen.^{48, 54} A wide range of salt tolerance, coupled with the reported
529 bactericidal properties of iodine, may allow these genera to colonize the reactor first
530 by inhibiting susceptible bacteria.⁹ *Rehaibacterium* is a newly described genus with
531 one reported isolate so far, but a recent study correlated this genus with DOC
532 removal in a BAF treating PW from Sichuan Basin, China.^{55, 56} Members of genus
533 *Methylophaga* are primarily known for metabolism of C1 compounds; however, one

534 isolate has been reported to degrade alkanes (n-hexadecane).^{57, 58}



535

536 **Figure 5.** Microbial community diversity in MBR sludge. (a) Shannon diversity index
537 and (b) relative abundance of dominant genera based on 16S rRNA gene amplicon
538 sequencing reads. Sludge samples collected at each timepoint were processed as
539 three technical replicates. Genera with relative abundance greater than 2% at any
540 time point are shown and the remaining minor taxa are grouped together as
541 “Others.”

542

543 As the salinity of the feed was further increased beyond TDS of 80 g/L, there was
544 an increase in diversity, which coincided with an increase in abundance of other
545 genera, including *Marinobacter*, *Malaciobacter*, and *Marinobacterium*. *Marinobacter*
546 and *Malaciobacter* (formerly classified as *Arcobacter*) were predominant groups in
547 early flowback period natural gas brines from Utica and Marcellus Shale, and
548 isolates belonging to these genera demonstrated salinity tolerance up to 150 g/L.⁵⁹
549 *Marinobacterium* has been detected as a dominant genus in several PWs, and
550 studied isolates have a wide salinity growth range (5-180 g/L).^{49, 50, 60, 61} Thus, the
551 increase in diversity at higher salinities could be linked to an increase in these
552 microbial groups that are better adapted to higher salinity. Additionally,
553 *Roseovarius*, *Iodidimonas*, *Rehaibacterium*, and *Marinobacterium* have been
554 reported as dominant genera in the effluent of an aerated BAF treating DJ-Basin PW,
555 indicating that these microbes can consistently grow in DJ-Basin PW under aerobic
556 conditions.⁶²

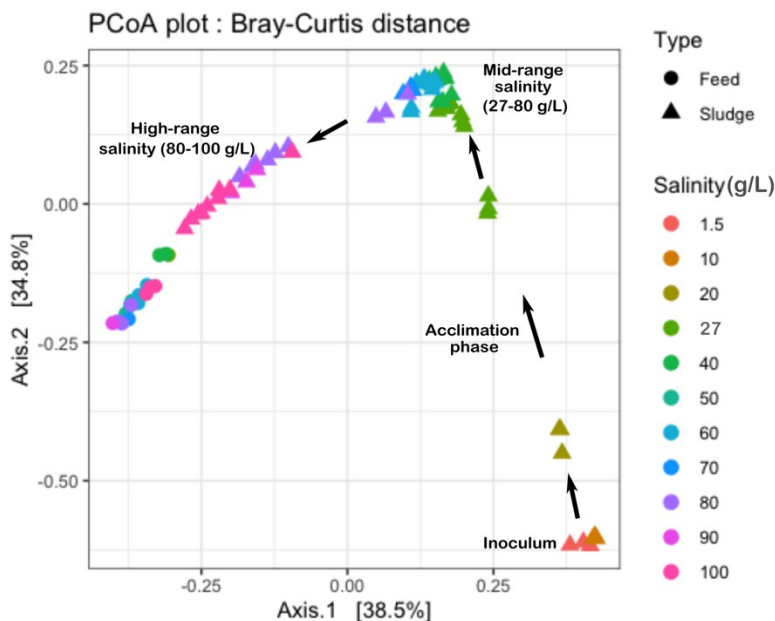
557 In contrast to the sludge community, the microbial community of the feed
558 (Figure SI-6) had higher diversity and included several obligate nitrate and sulfate-
559 reducing anaerobes like *Denitrovibrio*, *Desulfotignum*, *Desulfomicrobium*,
560 *Desulfovibrio*, and *Desulfuromonas*, suggesting the presence of anoxic zones in the
561 feeding tank. The dominant and prevalent genera in feed samples were
562 *Marinobacterium* and *Malaciobacter*. The dominant genera found in the MBR sludge
563 (*Roseovarius*, *Iodidimonas*, *Rehaibacterium*, *Methylophaga*, unclassified
564 *Rhodobacteraceae* and *Marinobacter*) are also present in the feed at relatively low
565 read abundances, indicating these organisms likely derive from the PW rather than
566 the sludge inoculum.

567 In contrast to the feed, aerobes dominate in the aerated sludge. Thus, a
568 combination of aeration, salinity, and addition of PW shapes the evolution of the
569 microbial community in the MBR from the conventional sludge inoculum to a
570 community adapted to PW treatment (Figure 6). The microbial community in the
571 MBR sludge falls into two major clusters in a principal coordinates analysis (PCoA)
572 plot—one at TDS of 29-80 g/L that shows little similarity to inoculum or feed, and
573 another at higher salinities (80-100 g/L TDS), which is closer to the community of
574 the feed.

575 While the reason for this is not entirely clear, relative abundance at phylum level
576 of sludge and feed paints a simpler picture (Figure SI-7). Proteobacteria (35.3-
577 66.1%), Firmicutes (13.6-7.4%), Campilobacterota (6.2-35.8%) Desulfobacterota
578 (5.2-21.9%) and Bacteroidota (1.1-7.4%) were major phyla in the feed. The
579 inoculum (activated sludge from a municipal wastewater treatment facility) has
580 Proteobacteria (26.5-27.9%), Firmicutes (1.8-3.8%), Bacteroidota (26.3-33.3%),
581 Chloroflexi (16.6-20.4%), Actinobacteriota (4.4-6.7%), and Patescibacteria (13.1-
582 15.2%) as major phyla. On acclimation, Proteobacteria (92.6-95.8%) becomes the
583 dominant phylum in the sludge. However, when the salinity is increased to 80 g/L,
584 other phyla specifically Firmicutes (0.7-6.8%), Campilobacterota (0.8-12.6%; genus
585 *Malaciobacter* belongs to this phylum) and Desulfobacterota (0.4-8.5%) increase in
586 abundance, making the composition of the community closer to the feed. These
587 other populations are likely better adapted to the higher salinities, but the design of
588 the reactor allows for retention of all microbial biomass so the dominant members
589 are likely to persist even under suboptimal conditions. Cultured members of
590 Desulfobacterota are known to prefer anoxic conditions, hence the reason for this

591 increase of Desulfobacterota in the sludge is unclear.⁶³

592



593

594 **Figure 6.** PCoA plots showing microbial communities in sludge and feed samples
595 during reactor operation. The data were Hellinger transformed and distance was
596 calculated using Bray-Curtis distance; black arrows show the evolution of sludge
597 communities with time and salinity of the feed.

598

599

600 4. Conclusion

601 This study investigated the ability of a pilot-scale MBR to effectively pre-treat PW
602 as the singular process before membrane-based desalination treatment. Despite
603 changes to TDS concentrations (from 27 g/L to over 100 g/L), the MBR's
604 performance in removing suspended solids and DOC remained consistent,
605 averaging 86%. After an initial acclimation period, the microbial community was
606 largely stable up to ~80 g/L TDS; at higher salinities, a more diverse community
607 with a higher prevalence of PW-derived organisms was observed but was not
608 associated with any change in performance. DOC removal remained consistent
609 during a 31-day period when PW from the Permian basin was treated, averaging
610 66%, with little change to the dominant microbial genera.

611

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621

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