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

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Abstract

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

Keywords

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

Highlights

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

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1. Introduction

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

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

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

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

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

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

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

2. Materials and methods

2.1. MBR feed water

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

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

Table 1. Water quality of PW and the dates it was collected from the DJ-Basin and Permian basin throughout this study. Most constituents were observed to remain consistent, with a few outliers seen at each collection date (i.e., DOC concentration 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 TDS	26,800	10,900	14,000	21,420	21,800	111,500
NaCl added	0	16,000	26,000	58,580	78,200	0
Adjusted TDS	26,800	27,000	40,000	80,000	100,000	111,500

2.2. MBR system

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

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

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

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

performance at each of these concentrations.

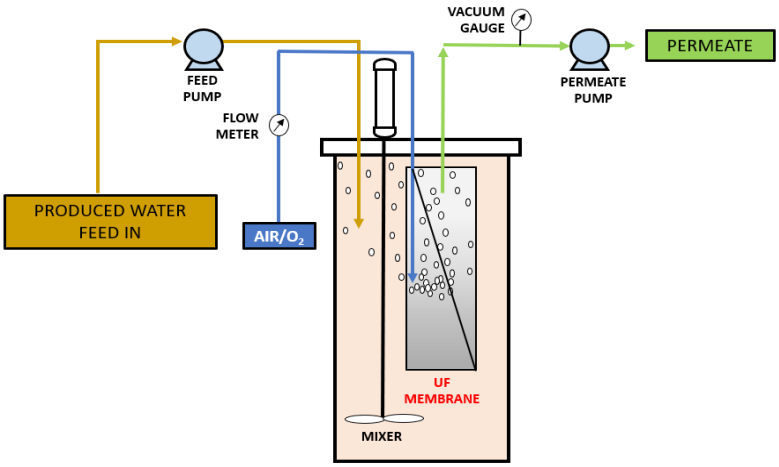

Figure 1. A flow diagram of the MBR system used in this study. PW and air are pumped into the bioreactor where they are mixed with the activated sludge. PW is continuously fed into the reactor, which maintains an average HRT of 48-hours. Treated water is pulled through the UF membrane, keeping all suspended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

2.3. Sampling and bulk analytical procedures

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

concentrated HNO_3, and stored at 3 °C until analysis was performed. TDS, MLSS, and MLVSS quantifications were performed according to standard methods (EPA 160.1, 1684).

2.4. Surfactant analysis

Solid-phase extraction (SPE) was performed on all samples for liquid chromatography and time of flight mass spectrometry (LC-qTOF) analysis for the semi-quantitative abundance and identification of polyethylene glycols (PEGs), polypropylene glycols (PPGs), PEG dicarboxylates (PEG-diCs), and PEG carboxylates (PEG-Cs). SPE cartridges (Oasis HLB 6cc-500mg 60 µm, Waters Corp., Milford, MA) were pre-conditioned with methanol and HPLC water.[26] First, 5 mL of methanol was vacuumed through the cartridge at a rate of 5 mL/min followed by 5 mL of HPLC water at 5 mL/min. Next, 10 mL of sample was pulled through at 5 mL/min. This was followed by 10 mL of HPLC water to flush out any salts that adhered to the cartridge, at a rate of 5 mL/min. Elution of the samples was performed using 10 mL methanol at a rate of 1 mL/min. The eluted samples were then concentrated down to 1 mL by a gentle stream of N_2 gas (XcelVap, Biotage, Uppsala, Sweden). Samples were then pipetted into 2 mL amber vials and stored at -4 °C until analyzed.

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

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

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

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

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

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

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

3. Results and discussion

3.1. Removal of organic and inorganic contaminants

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

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

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

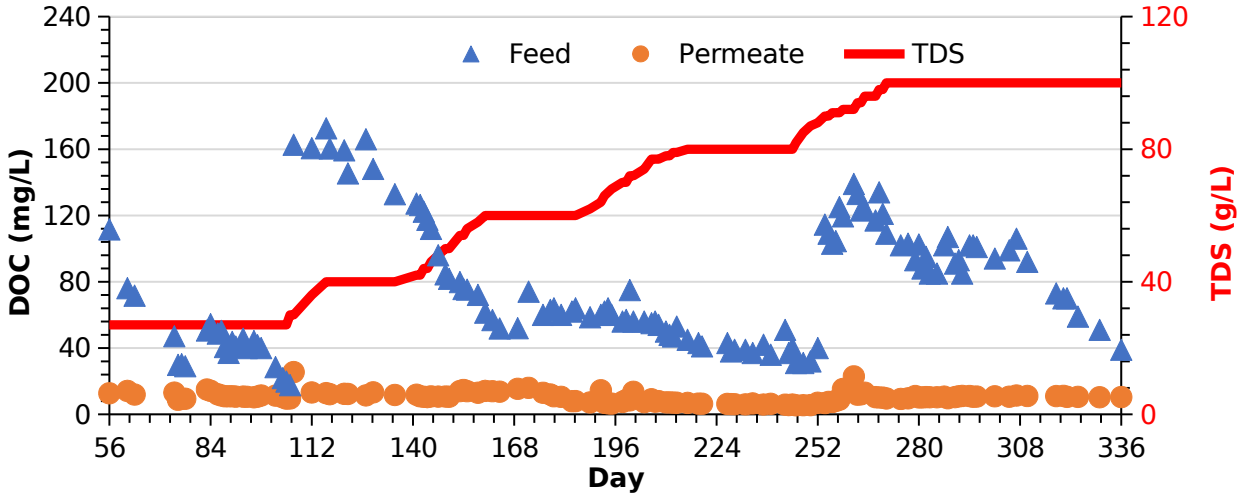

Figure 2. DOC concentration of the feed and permeate of the MBR beginning after the acclimation period (day 56 of operation). DOC concentration is given by the primary Y-axis, with feed represented by blue triangles and permeate represented by orange circles. The solid red line corresponds with the secondary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

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

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

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

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

Analyte (mg/L)	MBR Feed	MBR Permeate
TN	50±23	48±26
NH_3	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
SO_4	19±11	24±10

3.2. Removal of targeted organic compounds

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

use of LC-qTOF analysis and evaluated over the course of the study. Identification of PEGs is determined by the number of ethylene oxide (EO) units, where PEG-EO6 have 6 ethylene oxide units, PEG-EO7 have 7, and so on. The average mass difference between these different PEGs is 44.0262 mass units, which corresponds to the addition or subtraction of an ethylene oxide unit [-CH_2-CH_2-O-]. PPGs have a difference of mass of 58.0419 mass units, which corresponds to the addition or subtraction of a propylene oxide unit [--CH_2-CH(CH_3)-O-]. For convenience, EO6 refers to all surfactant compounds with 6 additional units, i.e., PPG-PO6 will be referred to as "EO6". Quantification of volatile and even semi-volatile chemicals through the MBR is very difficult due to the inability to accurately identify if removal from the PW was due to air stripping or microbial degradation, as demonstrated by Sitterley et al.[40] As such, we opted to evaluate the non-volatile chemicals and target the surfactants present in the raw vs. MBR treated PW.

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

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

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

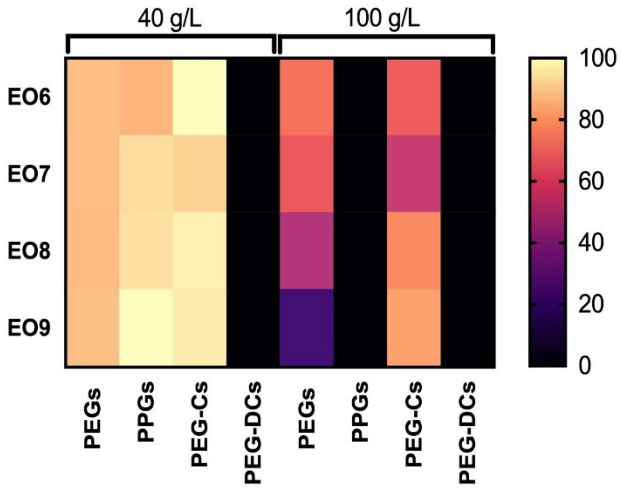

Figure 3. Heatmap of removal percentage of selected PEGs, PPGs, PEG-diCs, and PEG-Cs remaining in the MBR permeate at TDS concentrations of 40 g/L and 100 g/L. The full results for all PEGs, PPGs, PEG-diCs, and PEG-Cs at each TDS concentration are summarized in Table SI-2. Lighter colored boxes represent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

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

3.3. *Transition to treatment of Permian basin PW*

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

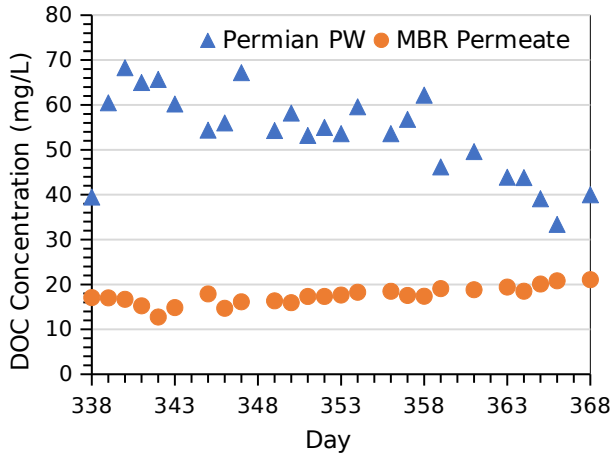

Figure 4. DOC concentrations of feed and permeate, during days 338-368 of operation, using Permian PW. No acclimation period was used with the Permian PW being fed into the MBR immediately after the DJ basin study. The TDS concentration of the Permian PW was naturally 110 g/L. Additionally, this PW contains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

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

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

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

3.4. Microbial community analysis

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

isolate has been reported to degrade alkanes (n-hexadecane).[57, 58]

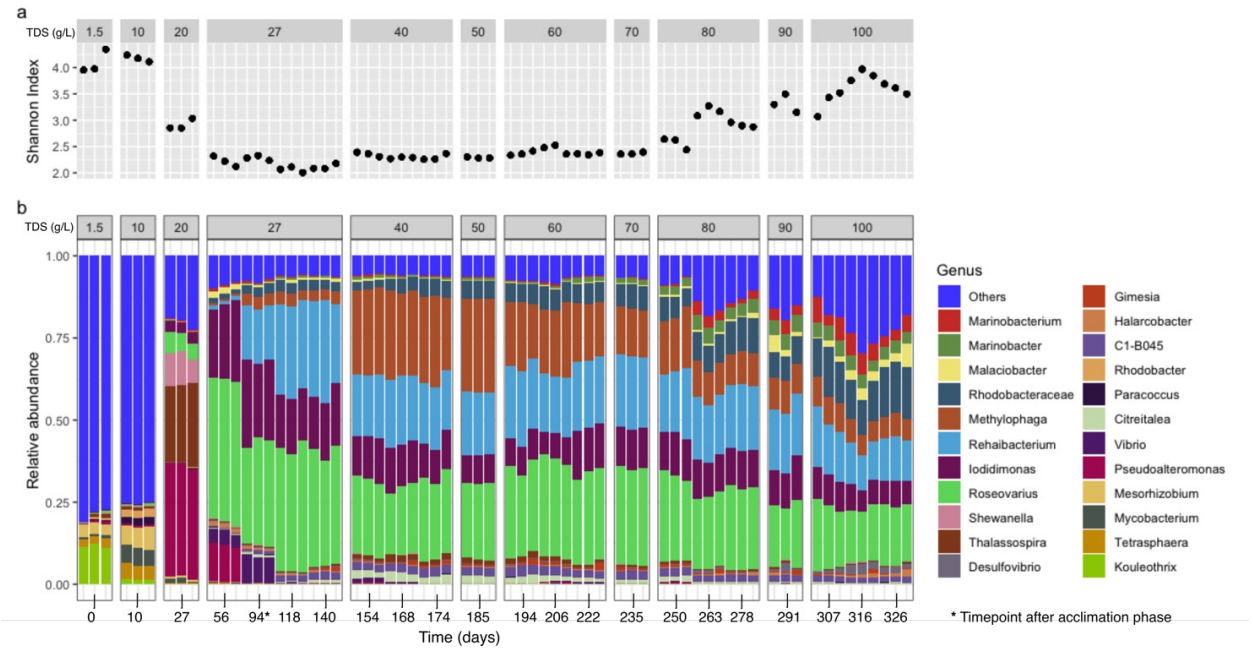

Figure 5. Microbial community diversity in MBR sludge. (a) Shannon diversity index and (b) relative abundance of dominant genera based on 16S rRNA gene amplicon sequencing reads. Sludge samples collected at each timepoint were processed as three technical replicates. Genera with relative abundance greater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

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

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

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

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

increase of Desulfobacterota in the sludge is unclear.[63]

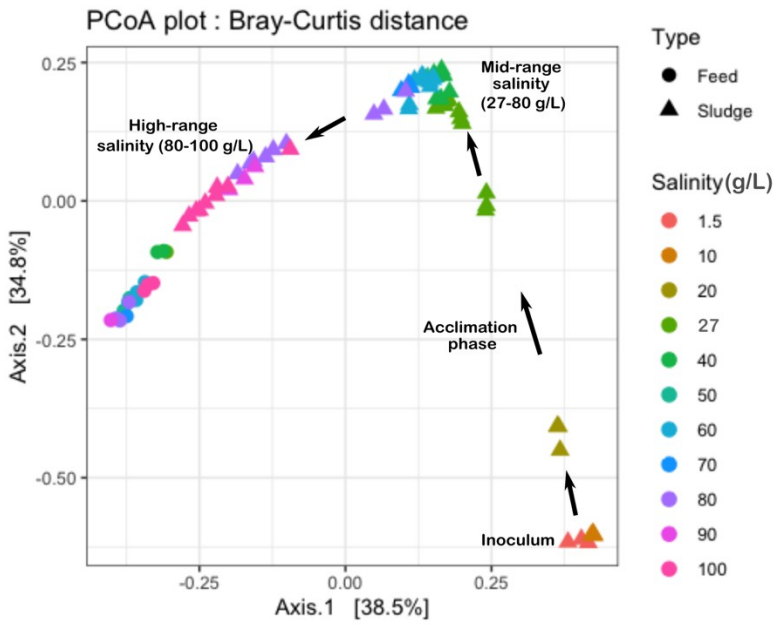

Figure 6. PCoA plots showing microbial communities in sludge and feed samples during reactor operation. The data were Hellinger transformed and distance was calculated using Bray-Curtis distance; black arrows show the evolution of sludge communities with time and salinity of the feed.

4. Conclusion

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

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