Environmental Science
Water Research & Technology

Nitrate Removal from Reverse Osmosis Concentrate in Pilot-Scale Open-Water Unit Process Wetlands

Journal:	*Environmental Science: Water Research & Technology*
Manuscript ID	EW-ART-10-2020-000911.R1
Article Type:	Paper

Nitrate Removal from Reverse Osmosis Concentrate in Pilot-Scale Open-Water Unit Process Wetlands

Rachel C. Scholes[a,c], *Michael A. Vega*[b,c], *Jonathan O. Sharp*[b,c], *David L. Sedlak*[a,c,*]

[a]Department of Civil and Environmental Engineering
University of California
Berkeley, California 94720
United States

[b]Department of Civil and Environmental Engineering
Colorado School of Mines
Golden, Colorado 80401
United States

[c]NSF Engineering Research Center for Reinventing the Nation's Urban Water Infrastructure (ReNUWIt)

Corresponding author
*To whom correspondence should be addressed
E-mail: sedlak@berkeley.edu

Abstract

Biological treatment of nitrate in reverse osmosis (RO) concentrate produced from municipal wastewater effluent is challenging, in part because of the low carbon-to-nitrogen ratio. Open-water unit process wetlands may provide a cost-effective means of removing nitrate because autotrophic production of labile organic carbon supports denitrification in the wetland biomat. To determine the potential for employing open-water unit process wetlands for removing dissolved nitrogen species from RO concentrate, a pilot-scale open-water wetland treatment system was established and studied over a two-year period at a water reuse facility in San Jose, California. The system was operated with a 3-day hydraulic residence time, resulting in removal of up to 30% of the nitrate present in the RO concentrate during the first summer of operation and removal of up to 47% of the nitrate during the second summer. The biomat comprised a diverse algal and heterotrophic bacterial assemblage containing several clades that are putatively capable of denitrification, as well as greater abundances of denitrifying functional genes (*nir*K, *nar*G) in the second year, coincident with higher nitrate removal. In batch reactors, the addition of woodchips increased nitrate removal rates from RO concentrate by approximately a factor of five or more, with rates dependent on the dose of woodchips applied. These results indicate that woodchip amendments could reduce the land area needed for nitrate treatment. This study provides evidence that open-water wetlands can remove nitrate from RO concentrate at the pilot scale, and identifies opportunities to enhance treatment efficiency with low-cost carbon amendments.

Water Impact Statement

Reverse osmosis concentrate from water reuse projects cannot be discharged to many surface waters because it contains high concentrations of contaminants, including nitrate or ammonia. This study provides insight into nitrate removal within a low-cost treatment technology--open-water wetlands. Results from a pilot-scale treatment system, operated for over two years, inform the design of constructed wetlands for concentrate treatment.

Introduction

Reverse osmosis is an important unit process in advanced treatment plants employed for potable water reuse because it removes salts, trace organic contaminants, organic matter, metals, and nutrients.[1,2] Most existing potable water reuse projects simply discharge RO concentrate to coastal waters, estuaries, or rivers in the same location where the effluent from the wastewater treatment plant had been discharged prior to the construction of the potable water reuse facility. This strategy operates under the assumptions that current contaminant discharges from wastewater treatment plants are acceptable and that dilution of the RO concentrate with water from other sources will avoid problems posed by the elevated concentrations of solutes in the RO concentrate.[2] However, the discharge of nutrients from wastewater treatment plants is a growing concern in many ecosystems and it may be necessary to decrease mass loading of nutrients to receiving ecosystems irrespective of the presence of water reuse facilities. In particular, the presence of dissolved nitrogen species in RO concentrate is problematic because nitrogen is often the nutrient that limits algal growth in estuarine and coastal systems.[3,4]

The removal of dissolved nitrogen species from RO concentrate is challenging for most biological treatment systems, in part because insufficient labile carbon is available to fuel denitrification.[5–7] One attractive option, biological aerated filters, removed only about half of the nitrate in RO concentrate with a COD/N ratio of 7.7-8.2.[5] Addition of labile organic carbon is a possible way to increase nitrogen removal. For example, upon addition of glucose to reach a COD/N ratio of 5.8, the removal of nitrate in membrane-aerated biofilm reactors increased from about 45% without added carbon to approximately 80%.[6] An alternative strategy is to increase the bioavailability of wastewater-derived organic carbon by oxidation prior to biological treatment, but the effectiveness of this strategy in RO concentrate is unproven. For example, ozone combined with biological activated carbon (BAC) did not enhance the removal of nitrate from RO concentrate,[8] whereas partial nitrate removal from RO concentrate was observed in BAC treatment following oxidation by photolysis of hydrogen peroxide by ultraviolet light (i.e., the UV/H_2O_2 process).[9,10] Unfortunately, advanced oxidation technologies or the continuous addition of carbon to fuel denitrification adds substantially to operating costs. For instance, the methanol dosed into biologically-treated wastewater to spur denitrification can cost over $16/kg-N removed.[11] At this price, removal of 3.6 mM nitrate (50 mg-N/L) from RO concentrate could add approximately $0.12 per cubic meter to the cost of recycled water, which is substantial considering that the annual operating and maintenance costs for water reuse facilities using RO are typically $0.25-0.50 per cubic meter.[12]

To circumvent the limitations of carbon addition, photosynthetic organisms could be harnessed as an *in situ* carbon source. Natural treatment systems often make use of carbon supplied by photosynthesis, and are a low-cost option for nitrate removal from wastewater

effluent.[13] Recently, up to 60% nitrate removal was reported in a small-scale (9 m^2) subsurface-flow constructed wetland pilot treating RO concentrate,[14,15] indicating the promise of natural treatment systems for concentrate treatment. An alternative natural treatment process, the open-water unit process wetland, is a shallow, unplanted basin that removes nitrate from nitrified municipal wastewater effluent via microbial processes in an autotrophic biomat that forms on the bottom of the water column.[16] Open-water wetlands also provide the added benefit of simultaneous trace organic contaminant removal.[17–19] However, the potential for using the open-water unit process wetland for treatment of RO concentrate is uncertain because the water contains much higher concentrations of nutrients, chromophoric dissolved organic matter, and salts, all of which could result in the establishment of a different microbial community in the biomat. Furthermore, the higher concentrations of nitrate in RO concentrate could result in a higher carbon demand for denitrification, which may exceed the autotrophic capacity of the biomat.[19]

To assess the potential of using open-water unit process wetlands to treat RO concentrate, we built and tested a pilot-scale system consisting of two separate 225-m^2 cells, one of which received RO concentrate that had been subjected to ozonation. This oxidative treatment step was intended both to increase sunlight penetration by oxidizing chromophores and to increase the biodegradable fraction of organic carbon. Monitoring of the chemical and microbiological conditions in the pilot-scale system was complemented by batch experiments designed to assess the potential for enhancing nitrate removal rates through the addition of inexpensive, readily available organic substrates.

Materials and Methods

Pilot-Scale Treatment System

A pilot-scale open-water unit process wetland system that received RO concentrate from a water reuse facility was built at the Silicon Valley Advanced Water Purification Center[20] in July 2017. The system received RO concentrate produced by treatment of nitrified municipal wastewater effluent from the adjacent San Jose/Santa Clara Regional Wastewater Facility between July 2017 and September 2019. The pilot-scale system consisted of two separate open-water unit process wetland cells lined with an impermeable polypropylene liner (Cooley Engineered Membranes, USA) with a water depth of approximately 30 cm (Figure S1). The hydraulic residence time of each of the wetland cells was approximately 3 days, as confirmed by lithium bromide tracer tests (S1.2). Cell 1 received RO concentrate directly from the adjacent advanced water treatment facility. Cell 2 received RO concentrate from the same facility after ozone pre-treatment. Ozone was added at an initial concentration of 20 mg/L (O_3:DOC ~ 0.5), except during a period spanning three sampling events in the summer of 2018, when the initial ozone concentration was increased to 40 mg/L (O_3:DOC ~ 1). All of the ozone decayed prior to discharge of the RO concentrate to the open-water unit process wetland cell.

Throughout the entire study, biomat growth and activity were monitored 3-5 times per week via pH measurements, which were supplemented by periodic measurements of the thickness of the biomat and the collection of samples at different locations within the cells. Ecological assessment of the biomat was conducted on 14 separate occasions using microscopy and 16S rRNA gene sequencing. Biological samples were collected in triplicate approximately one meter from the inlet of each wetland cell and shipped

overnight on ice to the Colorado School of Mines where they were centrifuged, decanted, and archived at -20°C within 24 hours.

Water quality parameters and concentrations of inorganic nitrogen species were monitored every 2-4 weeks between June and September of 2018 and 2019. Between October and May, when the biological activity decreased due to lower ambient temperatures, the sampling frequency was reduced. Samples were collected at the inlets and outlets of both cells using 24-hr composite autosamplers in 2018. Grab samples were collected at two intermediate locations within the cells approximately 1/3 and 2/3 of the distance along the flow path of each cell, at the ends of the baffles (locations labelled Baffle 1 and Baffle 2, and indicated in Figure S1), and at inlets and outlets in 2019.

The complete monitoring data set is available at: https://doi.org/10.25740/12qf-5243.

Ecological Assessment Methods

Diatoms and green algae were identified by bright-field microscopy, fluorescence microscopy, and environmental scanning electron microscopy (eSEM). Prior to centrifugation, fresh aliquots (~100 μL) of the biomat slurry were wet mounted and visualized under an Olympus BX51 Fluoresence Microscope equipped with an X-Cite 120LED illumination system. For DNA extraction and eSEM analysis, frozen samples were freeze dried using a LabConco FreezeZone.[21] Freeze-dried biomat was placed on carbon tape and gold sputtered using a Hummer IV Sputtering System in preparation for imaging on a Hitachi TM-1000 environmental scanning electing microscope (eSEM).

The relative abundance of bacterial and archaeal clades was assessed using 16S rRNA gene sequencing. DNA was extracted from ~0.05 g of freeze-dried biomat using a ZymoBiomics DNA Miniprep kit (Zymo Research, Irvine, CA, USA). Amplification of DNA for 16S and 18S rRNA gene sequencing was performed with a primer set that broadly represents all three domains of life,[22] however only 16S rRNA gene amplicons were analyzed for this study. Processing of raw reads and bioinformatic methods were performed using R. Quantitative PCR targeting the functional genes *nir*K and *nar*G was performed in accordance with previously published methods.[16,23,24] The Zymo Femto™ Bacterial Quantification Kit was used to determine 16S rRNA gene copies for normalization purposes. Further information regarding sequencing, bioinformatics and PCR methods are available in Section S1.3.

Carbon Amendment Microcosms

Batch microcosm experiments to assess the effect of carbon amendments on nitrate removal rates were conducted with 500-mL samples of unfiltered RO concentrate collected from the inlet to the pilot-scale treatment system amended with 56 g wet weight (~50 mL) of biomat collected from a location approximately 2 m from the entry of the water into the treatment system. Treatments included: (a) a control microcosm without added carbon; (b) a microcosm with 5 mM acetate added at the start of the experiment; and, (c) a microcosm with 6 g of woodchips added at the start of the experiment. Woodchips (untreated Southern longleaf pine bark, *Pinus palustris*) were cut into 1-cm sections and placed in a polypropylene mesh bag as described previously.[25] The bags were placed on the bottom of the microcosm prior to inoculating with biomat. The

204 experiments were conducted in 600-mL Pyrex beakers. Microcosms were maintained in a
205 water bath at 25°C and were irradiated for 8 hours per day with an Oriel solar simulator
206 (Spectra Physics 91194) equipped with a 1000 W Xe lamp and an atmospheric
207 attenuation filter (Spectra Physics 81088 and 81017). A short photoperiod relative to
208 summertime sunlight hours was selected to account for the slightly higher light intensity
209 of the solar simulator relative to average daily sunlight at the latitude of the pilot-scale
210 system. Microcosms were continuously mixed by stir bars suspended from above to
211 avoid suspension of the biomat.[18] Samples taken for water quality analysis resulted in
212 removal of less than 50 mL of the fluid volume (i.e., <10%) over the course of the
213 experiments. Evaporative losses were less than 5%. Dissolved oxygen (DO) and pH were
214 measured at the beginning and end of each photoperiod to track photosynthetic activity
215 (Figure S3). Control experiments with and without biomat were conducted to assess the
216 rate of carbon leaching from woodchips. Further details and results of these control
217 experiments are provided in Section S2.2.
218
219 Additional experiments were carried out to assess the effect of the mass of woodchips on
220 nitrate removal rates. These experiments were conducted in 20-mL glass scintillation
221 vials containing fir (*Abies sp.*) bark chips (0, 100, 200, 500, 750, or 1000 mg) that were
222 dried, milled, and sieved to between 8-mesh and 10-mesh (0.065-0.093 in.); 2 g (wet
223 weight) of biomat; and 20 mL RO concentrate. Fir bark chips were used in these
224 experiments because of their availability in bulk quantities that could be applied in pilot-
225 or full-scale systems. Each treatment condition was run in triplicate. Microcosms were

sampled sacrificially after 8, 24, 48, and 72 hours. Microcosms were maintained in the dark in a shaking incubator at 25°C and 90 rpm.

Analytical Methods

For monitoring of the pilot-scale system, composite and grab samples for nutrients, chloride and dissolved carbon were filtered through 0.7-μm glass fiber filters into amber glass vials in the field. Samples for UV-vis spectral analysis were not filtered prior to placing them in glass vials. Samples were transported to the laboratory on ice. Nutrients and dissolved carbon were analyzed within 48 hours of collection.

Nitrate, phosphate, ammonia, and chloride were analyzed by ion chromatography (Dionex DX-120). Dissolved organic and inorganic carbon, and total nitrogen, were analyzed using a Shimadzu TOC analyzer. Nitrite was quantified using the Griess reagent method.[26] Light absorbance was determined using a UV-visible spectrophotometer (Shimadzu UV-2600). Biodegradable dissolved organic carbon (BDOC) was measured in triplicate 500-mL samples of RO concentrate with and without ozone pre-treatment. BDOC test bottles were inoculated with 5 mL of biomat sampled from the inlet to Cell 1 and were analysed according to the method described by Servais *et. al.*[27]

Calculation of Carbon Fixation Rates

We estimated the potential rate of carbon fixation by photosynthetic diatoms based on light absorption and literature values for photosynthetic quantum yields. First, the amount of photosynthetically active radiation (PAR) reaching the surface of the biomat was

249 determined using irradiance reference spectra for 40 degrees N latitude obtained using the
250 Simple Model of the Atmospheric Radiative Transfer of Sunshine (SMARTS).[28] UV-Vis
251 absorption spectra from water samples were collected at the inlets to both cells in the
252 summer of 2018 (Equation 1).

253 $$PAR\ (Ein\ m^{-2}d^{-1}) = \Sigma_{\lambda=400nm}^{\lambda=700nm}[S(\lambda)Z(\lambda)] \qquad \text{(Equation 1)}$$

254 where S(λ) is a light screening factor defined by Schwarzenbach *et al.*,[29] and Z(λ) is the
255 photon flux at the water surface (Es m^{-2} s^{-1}). This information was then used to estimate
256 the rate of carbon fixation from the product of PAR and the quantum yield for
257 photosynthesis, which was assumed to be 0.065 mol-C/mol-photons absorbed.[30]
258
259 *Calculation of Area Requirements*
260 Based on pilot-scale and microcosm results, the wetland area needed to remove 90% of
261 the nitrate from RO concentrate (A_{90}) was calculated.[16] We considered the effect of
262 recovery during RO treatment on theoretical area requirements. Changes in RO recovery
263 affect both the nitrate concentration and the light absorbance of the RO concentrate. For
264 each scenario, we calculated the depth of the water column at which the flux of PAR
265 incident on the biomat matched the average PAR penetration observed at our pilot-scale
266 system. We then calculated the land area required assuming a 3-day hydraulic retention
267 time, as described in Section S2.3.
268
269 **Results and Discussion**
270 *Nitrogen Removal and Biomat Establishment in Pilot-Scale Wetland Cells*

271 Nitrate removal in the wetland cell without ozone pre-treatment improved over the course
272 of the study. From system startup (July 2017) through April 2018, less than approximately
273 5% of the nitrate was removed from the non-ozonated cell on a mass basis (i.e., correcting
274 for evaporation by normalizing to chloride concentrations, Figure 1). During the summer
275 (June-August) of 2018, between approximately 5 and 30% of the nitrate was removed.
276 Following the first year of operation, nitrate exhibited greater removal during summer and
277 exhibited seasonal fluctuation due to changes in temperature. During the winter (November
278 2018 - January 2019), when outlet water temperatures ranged from 14-15°C, less than 5%
279 of nitrate was removed. From June-August 2019, when outlet water temperatures were
280 22-23°C, between 28 and 47% of the nitrate was removed.

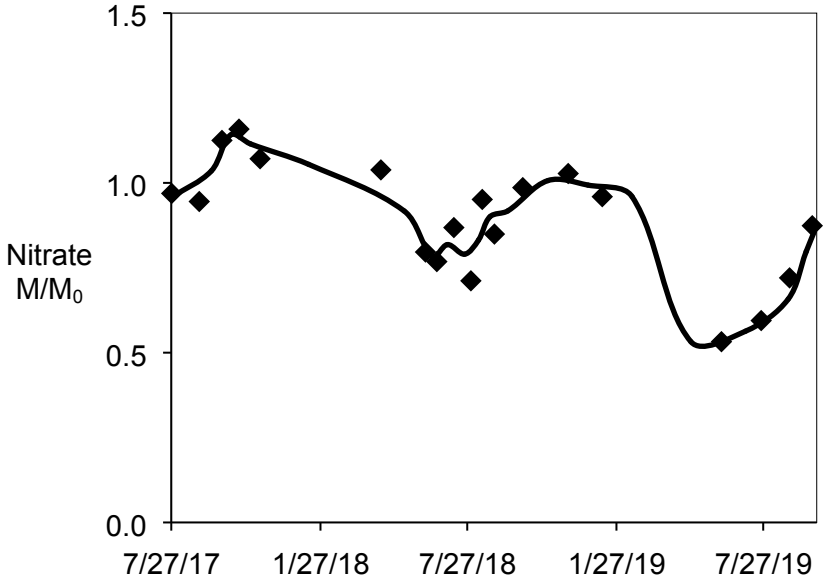

Figure 1. Fraction of nitrate mass remaining after pilot-scale open-water wetland treatment from July 2017 through September 2019 in Cell 1. Line represents the running average.

281

282 Seasonal differences in removal rates are typical in constructed wetlands.[31] However, the
283 observed seasonality of nitrate removal was somewhat greater than predicted by modified

Arrhenius kinetics, which account for the effect of water temperature on denitrification rates[31] and have previously accurately described nitrate removal in open-water wetlands.[16] Using the temperature coefficient derived from open-water wetlands treating wastewater effluent (i.e., $\theta = 1.12$), removal rates were predicted to decrease by 60% as the temperature decreased from 23 to 15°C. The winter removal rates may have been lower than predicted due to carbon limitation, discussed further below, which can result in deviations from modified Arrhenius kinetics by further suppressing denitrification rates, as noted previously in woodchip bioreactors.[32]

Cell 2 exhibited similar nitrate removal to Cell 1 despite the use of ozone pre-treatment (Figure 2). Ozone pre-treatment increased the concentration of BDOC in the RO concentrate from 0.6 to 1.1 mM (Figure 2c) and bleaching of the organic matter resulted in a 26 to 57% increase in photosynthetically active radiation reaching the biomat at the cell inlet (Figure S6). However, no significant differences were observed in nitrate removal in a comparison of the ozonated and non-ozonated cell ($p=0.903$, Wilcoxon Signed Rank Test), suggesting that neither the increase in BDOC nor the decreased screening of PAR improved the treatment process, even when the system was operated with an ozone dose of 40 ppm. In 2019, slightly greater nitrate removal was observed in Cell 1 than in Cell 2. Though the difference was not statistically significant, this trend may have resulted from the longer residence time of Cell 1 compared to Cell 2 (a difference of approximately 0.5-0.7 days was observed in tracer tests) (Section S1.2).

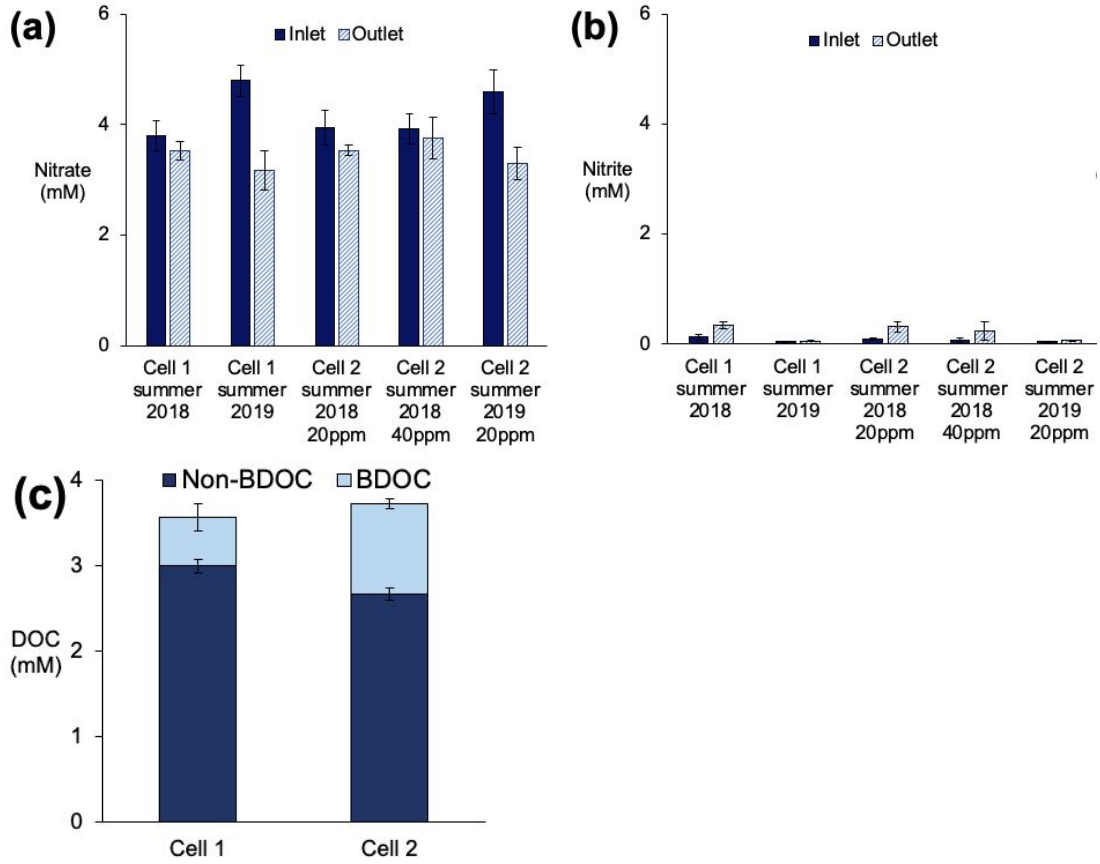

Figure 2. Concentrations of (a) nitrate and (b) nitrite at the inlets and outlets, and (c) dissolved organic carbon at the inlets of the pilot-scale open-water cells. Values in (a) and (b) represent the average over 6 sampling rounds in the summer of 2018, and 3 sampling rounds in the summer of 2019. Values in (c) represent the average of triplicate measurements. Error bars represent the standard deviation.

306

307 Nitrite, a product of partial denitrification, accumulated in the wetland cells (Figure 2b).
308 During the summer of 2018, nitrite concentrations increased from an inlet concentration of
309 0.16 mM to an effluent concentration of 0.37 mM in the non-ozonated cell, which
310 accounted for 80% of the decrease in nitrate concentrations. Effluent concentrations of
311 nitrite were considerably lower in 2019, ranging from 0.04 to 0.07 mM, which accounted
312 for <1 to 3% of the nitrate loss. The accumulation of nitrite during the first year of operation
313 was consistent with partial denitrification, in which the complete reduction of nitrate to
314 nitrogen gas cannot be accomplished, resulting in production of intermediates such as

315 nitrite and nitrous oxide. Dissolved organic nitrogen concentrations did not increase during
316 wetland treatment in either summer, which was also consistent with denitrification as the
317 predominant mechanism of nitrogen removal (Figure S7).
318
319 Several factors may have contributed to the faster removal of nitrate and lower nitrite
320 accumulation in the summer of 2019 compared to the summer of 2018. Partial
321 denitrification can be attributed to a variety of conditions, including insufficient carbon for
322 complete denitrification,[33,34] as well as elevated salinity.[35] The presence of a thicker, more
323 established biomat, discussed below, may have contributed by serving as a reservoir of
324 labile organic carbon for denitrifiers, reducing the extent of carbon limitation.
325
326 Nitrate removal in the pilot-scale system coincided with biomat maturation in the open-
327 water cells, as evidenced by measures of photosynthetic activity and biomat accretion.
328 Through the summer of 2018, the biomat grew, and accumulation of biomat solids was
329 observed (Table S2). The biomat was thickest near the cell inlets, with approximately 1 cm
330 of material present in the first 3 m and less than 1 cm of biomat throughout the remainder
331 of Cell 1 in April 2018. By July 2018, the biomat in the area near the inlet was
332 approximately 7.5 cm thick, whereas the biomat had an average thickness of 2.5 cm
333 throughout the remainder of the cell. The accumulation was similar in Cell 2, although
334 slightly more biomat was present near the inlet.
335
336 Throughout the operation of the pilot-scale system, daytime increases in pH and DO, and
337 gradients along a profile from inlet to outlet indicated photosynthetic activity in the open-
338 water cells. Profiles of pH showed regular increases throughout the cells within one month

after starting flow, with consistent increases throughout the cells at midday (Figure S8). Average pH values measured at midday in summer 2018 increased from the inlet (7.5 ± 0.1) to the outlet (8.5 ± 0.3) for both cells. Similarly, during June-August 2019, pH values increased on average from 7.7 ± 0.1 at the inlets to 8.1 ± 0.2 at the outlets of both cells. Furthermore, in March 2018, pH measurements taken approximately every two hours from 8:30 AM to 4:00 PM indicated an increase throughout the day of 0.2 pH units at the outlets of Cell 1 (pH increased from 10.6 to 10.8) and Cell 2 (pH increased from 10.4 to 10.6). On this date, the pH was 9.5 and 9.6 at the inlets to Cell 1 and Cell 2, respectively. The daytime increase of ~0.2 pH units at the cell outlets was smaller in magnitude than the increase observed at an open-water wetland treating municipal wastewater effluent, where pH at the outlet fluctuated from approximately 9.3-9.8.[17] However, a smaller magnitude of fluctuation was expected due to the higher starting pH and the higher alkalinity of the RO concentrate (approximately 650 mg/L as $CaCO_3$) compared to municipal wastewater effluent (typically <200 mg/L as $CaCO_3$). DO concentrations measured throughout the day in March 2018 (on a date when the ozone generator was not operating) increased at the outlets from 19 mg/L (Cell 1) and 14 mg/L (Cell 2) at 8:30 AM to >24 mg/L (i.e., above the quantification limit of the field sensor) in both cells at 4:00 PM. In open-water wetlands treating municipal wastewater effluent, DO at the outlet increased from ~10 mg/L to ~25 mg/L due to photosynthetic activity.[16]

Biomat Ecological Assessment

Ecological assessment of the biomat revealed a microbial community that developed throughout the 2-year study period and differed from previously-studied open-water wetlands. The microbial community consisted of a diverse diatom-rich algal assemblage

(Figure 3a) complemented by bacteria and archaea (Figure 3b). Our analysis did not provide any evidence of differences in species composition between the two cells (further details on this statistical analysis are presented in SI Section 2.7). During the first year of operation, several diatoms and green algae species were identified in both cells. This period coincided with observations of planktonic green algal growth in a holding tank upstream of the open-water wetlands, which introduced green algae to the inlets of both wetland cells. A new, light-impermeable holding tank was installed after six months of operation, after which green algae were not visible in the RO concentrate entering the wetland cells. After one year of operation, several diatoms (e.g., *Navicula*, *Cyclotella, Stauroforma* gen.) and one green algae (i.e., *Desmodesmus* gen.) were prevalent in both cells. However, the diatom *Staurosira construens var. venter*, which was the dominant species in open-water wetlands treating municipal wastewater effluent and an effluent-dominated river,[21] was not observed until the second year of operation.

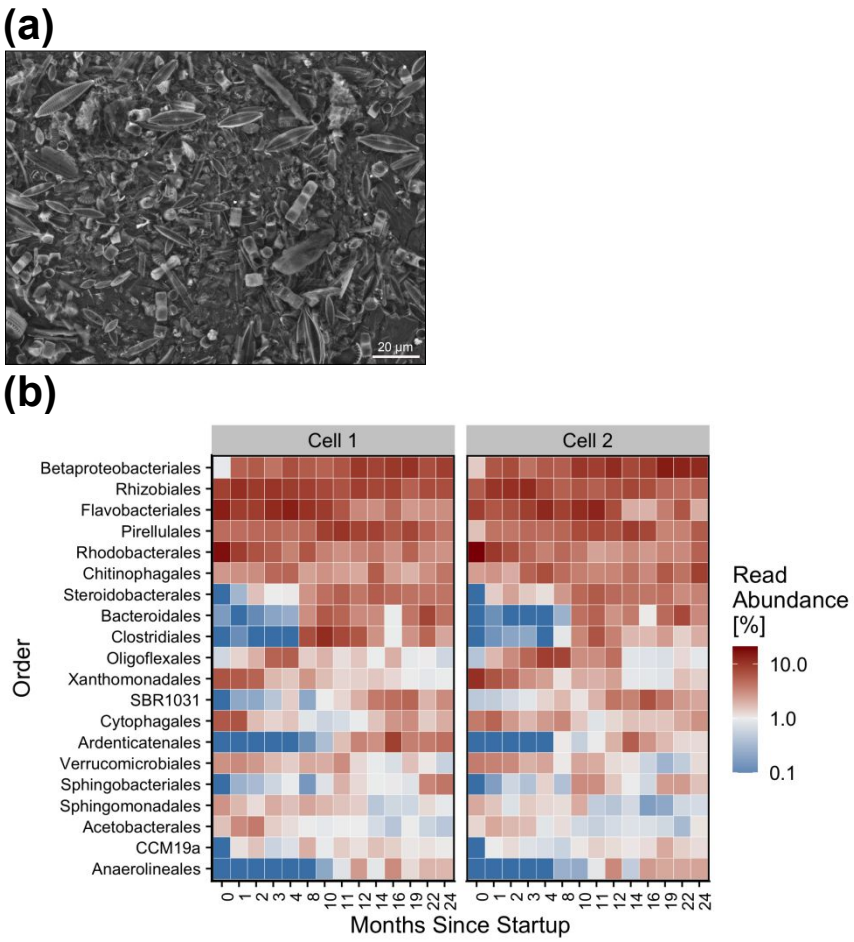

Figure 3. (a) Scanning electron microscopy (SEM) image illustrating diatom diversity (e.g., species of *Navicula*, *Staurosira, Stauroforma*, *Cyclotella,* etc.) within the RO concentrate biomat in March of 2019. (b) Heatmap of the top 20 most abundant bacterial and archaeal taxa within the biomat of Cell 1 and Cell 2 over time, classified at the Order level.

In contrast to open-water wetlands treating a municipal wastewater effluent-dominated river,[21] the microbial community in the pilot-scale wetland continued to change after the first year of operation (Figure S9). Taxa putatively associated with denitrification,[36–38] sulfate reduction,[39,40] and the breakdown of complex organic matter[41,42] were generally present in higher relative abundance in summer 2019 than in summer 2018 (Figure S10), which was consistent with the presence of thicker biomat in 2019.

Phylogenetic and functional gene analyses suggested that denitrification occurred in the biomat and that denitrification potential increased over time. Several of the most abundant bacterial orders present in the biomat include species known to contribute to denitrification (e.g., Betaproteobacteriales, Steroidobacterales, Rhodobacterales, Bacteroidales, etc.).[36,37,43,44] Furthermore, the abundance of organisms from putative family- and genus-level denitrifying lineages (e.g., *Steroidobacteraceae* fam., *Denitratisoma* gen., etc.) increased over time (Figure S11a,b).[43,45] Genes encoding for nitrate and nitrite reductases (*nar*G and *nir*K) were quantified in biomat from the non-ozonated cell during June 2018 and 2019. The abundance of both genes was greater in 2019 relative to 2018 on a dry weight basis (Figure S12; $p<0.001$ with data from each summer pooled, Mann Whitney U Test), consistent with greater nitrate removal and a lack of nitrite accumulation in 2019.

Our analysis did not provide any evidence that anammox played a role in nitrogen cycling, though the anammox hydrazine synthase gene (*hsz*A) was not queried. In open-water wetlands treating nitrified municipal wastewater effluent, anammox bacteria potentially accounted for up to 10% of nitrogen removal with ammonium production contributions from sulfide induced dissimilatory nitrate reduction to ammonium (DNRA).[16,46] Despite high relative abundances of the only phylum known to contain anammox (Planctomycetes; Figure S11c),[47] deeper branching families or genera putatively associated with anammox were not identified in biomat communities from the pilot-scale wetland.

Carbon Sources in the Open-Water Wetlands

To determine whether dissolved organic carbon contributed substantially to nitrate removal in the open-water wetlands, we estimated the fraction of organic carbon available to

microorganisms from the dissolved organic matter in the water entering the cell. Organic carbon concentrations at the inlet to the pilot RO concentrate wetland ranged from 3.0-5.3 mM. However, based on measurements of BDOC, 0.6 mM and 1.1 mM of the carbon was bioavailable in the RO concentrate entering the non-ozonated and ozonated cells, respectively (Figure 2). Because denitrification requires molar C:N ratios of at least 1:1, with approximately 4-5 mM nitrate present in the RO concentrate, the dissolved bioavailable carbon in non-ozonated RO concentrate could fuel denitrification of less than 25% of the incoming nitrate if it were all metabolized under suboxic conditions. The actual fraction of carbon available for denitrification is likely to be lower because some carbon is consumed during aerobic metabolism in the water column and the oxic surface layer of the biomat.

To understand the contribution of photosynthetic diatoms, we calculated carbon fixation rates and estimated the rate at which diatoms would supply organic carbon to heterotrophic microorganisms in the biomat. The calculated rate of uptake of dissolved inorganic carbon due to photosynthesis in the biomat was 2.4 ± 0.8 mol-C/m^2-d. This estimate, calculated using Equation 1, should be considered an upper bound on potential removal because it assumes all incident light was absorbed by the biomat with a maximum quantum yield value, which overestimates the true photosynthesis rate.[33] We therefore took this value as the maximum rate of carbon fixation,[30,48] and we further assumed that 5 to 20% of the fixed carbon was eventually released as exudates by the diatoms.[48–51]

Based on these estimates, the maximum rate of carbon released by the biomat in the RO concentrate wetland cells was equivalent to adding 0.4-1.7 mM organic carbon. Because

light screening varied throughout the cells (representative absorption spectra provided in Figure S6), we repeated the calculation using light absorbance measurements taken at the outlet sampling locations, which resulted in estimates ranging from 0.2-1.4 mM organic carbon equivalent. In all cases, the calculated rate of carbon fixation was lower than what would be necessary to denitrify the 4-5 mM of nitrate entering the wetland cells.

Results from this calculation are consistent with denitrification rates previously observed in an open-water wetland treating municipal wastewater. Using light screening data for municipal wastewater and applying this calculation to secondary effluent,[16] we estimate that the biomat in previous open-water wetlands could have contributed up to an equivalent of 3.1 mM organic carbon to wastewater containing 1.5 mM nitrate, yielding a C:N ratio of 2. In this previously-studied wetland, >90% removal of nitrate was observed during the summer months.[16]

On the basis of this analysis, it is reasonable to assume that the carbon supplied by biomat diatoms was less than the mass necessary to fully denitrify RO concentrate and was approximately equivalent to what would have been required to fuel the decrease in nitrate concentrations observed during the summer of 2019 (approximately 0.3-1.6 mM nitrate). This analysis also implies that nitrate removal rates would not be expected to increase substantially beyond the rates observed in summer 2019 because nitrate removal was likely limited by the rate of carbon fixation. While organic carbon may also be supplied through cell death and decay associated with biomat accretion, in full-scale open-water wetlands treating water from an effluent-dominated river, nitrate removal rates did not measurable increase after the second year of operation,[19,52] consistent with a minor contribution of

456 accreted biomat as a carbon source to fuel denitrification. In addition, porewater sampling
457 in open-water wetland biomats indicates that nitrate is predominantly attenuated in the
458 surficial 1-2 cm of biomat, so further accretion of biomat solids is unlikely to increase
459 nitrate removal rates (unpublished data). Together, these findings indicate that open-water
460 wetlands designed for the treatment of high concentrations of nitrate may require larger
461 surface areas for biomat growth (i.e., a longer hydraulic residence time or a shallower
462 depth), or the addition of a labile carbon source to fuel denitrification.
463
464 *Carbon Amendments*
465 The ability of biomat organisms to remove nitrate from RO concentrate was enhanced in
466 microcosms amended with woodchips or sodium acetate (Figure 4a). In the non-amended
467 control, nitrate removal, which began after the first day of the experiment, resulted in
468 removal of approximately 5% of the nitrate and accumulation of nitrite (up to 0.7 mM)
469 over the course of three days (i.e. in samples taken after 24 and 96 hours). These results
470 were consistent with observations from the pilot-scale wetland under similar conditions in
471 terms of light intensity and temperature. After 10 days, 35% nitrate removal (0.9 mmol-N)
472 was observed in the non-amended microcosm with nitrite accumulation accounting for
473 31% of the nitrate removed. In contrast, in the presence of 6 g woodchips, 96% of the
474 nitrate (2.6 mmol-N) was removed after 10 days, with nitrite accounting for only 10% of
475 the nitrate removed. When 2.5 mmol acetate was used as a carbon amendment, a sharp
476 decrease in nitrate concentration (72% removal, 1.6 mmol-N) was observed during the first
477 two days of the experiment, after which time the rate of removal slowed until 94% removal
478 was reached by day 10. Nitrite concentrations increased to 2.0 mM after two days,
479 representing 55% of nitrate removed over this period. The nitrite concentration then

480 decreased to 1.6 mM after 10 days, accounting for 30% of the nitrate removed in that
481 period. Overall, the fraction of nitrate undergoing partial denitrification to nitrite was
482 lowest in the woodchip-amended microcosm, although nitrate removal was fastest in the
483 presence of acetate. These observations suggest that the added acetate was quickly used by
484 the biomat organisms whereas the woodchip amendment provided a slow-release source
485 of carbon that was not depleted over the course of the 10-day experiment.

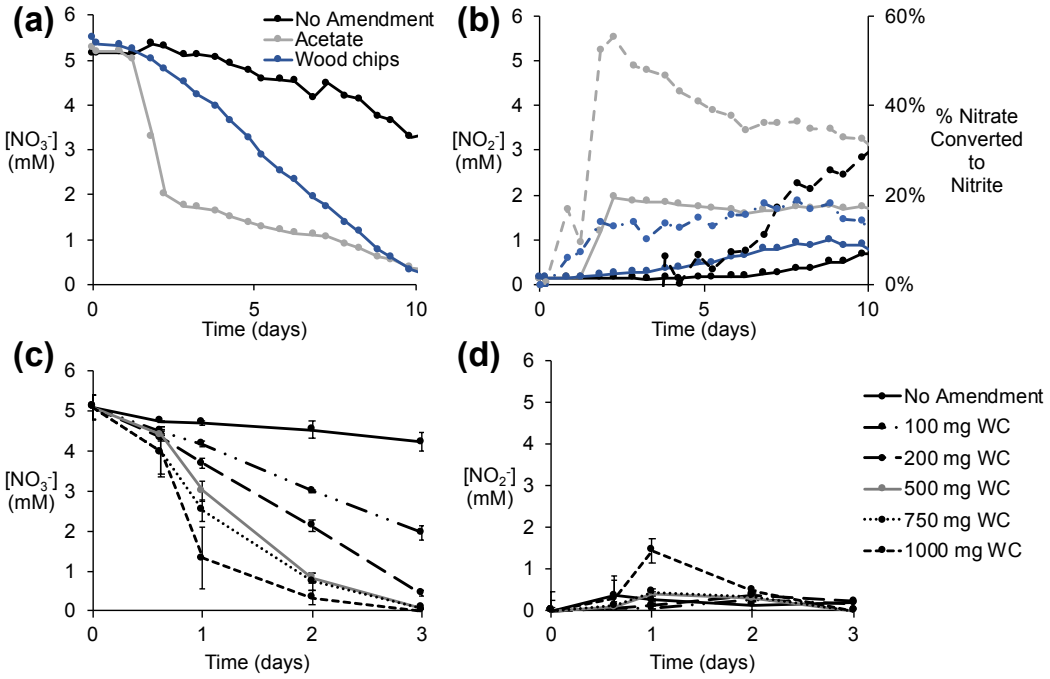

Figure 4. Nitrate and nitrite concentrations in microcosms amended with (a-b) 5mM acetate or 6 g woodchips, maintained under a solar simulator; (c-d) 100-1000 mg woodchips maintained in the dark. In (b) the dotted lines indicate % conversion related to the secondary axis.

486

487 In experiments containing woodchips without biomat, dissolved organic carbon (DOC)
488 concentrations were measured to assess the rate of carbon leaching (S2.2). In deionized
489 water without biomat, DOC concentrations increased over the course of one week: 1.5 g of
490 woodchips released 3.6 mg (0.3 mmol) of carbon within 24 hours, then continued to release
491 carbon at a rate of approximately 0.3 mg/day (0.02 mmol/day) during the following 6 days.

492 The rate of carbon leaching in the absence of biomat likely underestimates carbon
493 availability because carbon may be released more quickly in the presence of organisms that
494 enzymatically induce additional carbon release.[25] DOC concentrations also increased in the
495 first 24 hours in the presence of biomat (mass of DOC in solution increased by 2.5 mg),
496 then decreased over the following 6 days, indicating that released carbon was consumed
497 by biomat organisms (Figure S4). Similar DOC changes were observed in RO concentrate
498 (i.e., 2.0 mg increase in DOC within 24 hours, followed by DOC removal). In a control
499 experiment containing biomat and gravel, DOC concentrations decreased throughout the
500 week-long experiment.
501
502 Assuming an initial release of 0.2 mmol-C/g woodchips, followed by 0.013 mmol-C/g
503 woodchips-day, the 6 g of woodchips used in the 10-day experiment described above could
504 have released a total of 1.6 mmol-C. In these experiments, an additional 1.7 mmol nitrate
505 was removed in the woodchips-amended microcosms compared to the non-amended
506 control, indicating that the addition of carbon at a C/N ratio of 1:1 was sufficient to fuel
507 additional denitrification, at least over the length of these experiments. In comparison, the
508 addition of 2.5 mmol acetate resulted in initial removal of 1.6 mmol nitrate, indicating a
509 lower yield of nitrate removed per mol of amended carbon.
510
511 To investigate the potential for increasing denitrification by amending the biomat with
512 varying amounts of woodchips, experiments were conducted in sealed containers without
513 exposure to sunlight. The 20-mL vials contained fir bark chips (0-1000 mg), biomat, and
514 RO concentrate. Nitrate removal rates increased with increasing amounts of added
515 woodchips (Figure 4c,d). In the absence of a carbon amendment, 17% of the nitrate was

516 removed after three days. The greater removal in these control experiments compared to
517 experiments conducted in the irradiated microcosms described above is likely attributable
518 to greater solute exchange with biomat porewater in the shaking incubator compared to
519 stirred beakers. In amended microcosms, 62% and 91% of nitrate was removed at initial
520 concentrations of 4.2 mM and 8.3 mM carbon (100 mg and 200 mg woodchips added,
521 assuming 50% carbon by mass), respectively. With 500 mg woodchips (20.8 mM carbon)
522 or more, >95% of the nitrate was removed. Nitrite concentrations remained below 0.5 mM,
523 except in the presence of 1000 mg (41.6 mM-C) woodchips, in which case nitrite
524 concentrations increased to 1.4 mM after 1 day before decreasing to concentrations below
525 the detection limit after 3 days.
526
527 Together, these results indicate that readily available carbon sources could be used to
528 amend open-water unit process wetlands for enhanced nitrate removal. In microcosms
529 containing initial carbon amendments of at least 8 mM woodchips, removal after 3 days
530 was five times higher than in unamended microcosms (17% vs. >90%). This enhancement
531 in nitrate removal rates could result in lower land area requirements to achieve nitrate
532 removal in open-water wetlands. Woodchips are an attractive option for use in future open-
533 water wetland systems because of their availability and low cost. They have also been used
534 in other water treatment applications, such as woodchip bioreactors, and are desirable in
535 part due to their long lifetime that can range from years to decades.[53,54] Other substrates
536 may also be available for designers of open-water wetlands, such as plant matter from
537 managed vegetated wetlands or parks. For instance, leaf-litter extracts from aquatic plants
538 enhanced the rate of wastewater denitrification in freshwater biofilms.[55]
539

The ability to apply these results to wetland design is limited by the nature of the short-term and small-scale microcosm experiments described here. The required mass of woodchips to sustain denitrification in full-scale wetlands may be higher than the doses used here because the initial pulsed release of organic matter from woodchips affected nitrate removal rates in these short-term experiments. Further, carbon amendments conducted at the pilot scale may affect the diversity and functionality of the biomat microbial community and could also increase the initial rate at which biomat organisms establish in open-water systems. Pilot-scale research is needed to assess these questions and to determine the useful lifetime of woodchips in open-water wetlands. In addition, the strong temperature dependence of nitrate removal rates observed at the pilot scale indicates a need to assess the effect of carbon amendments on nitrate removal under winter conditions, in order to achieve year-round nitrate removal.

Implications for Reverse Osmosis Concentrate Treatment

Open-water unit process wetlands could potentially remove similar amounts of nitrate as other RO concentrate treatment options while providing other benefits. In our pilot-scale study, summertime nitrate removal reached 28-47%, but the carbon fixed by the diatoms in a well-conditioned and actively photosynthesizing biomat could potentially fuel further nitrate removal if additional land area was available. For the RO concentrate treated in the pilot-scale open-water wetland, we estimate that 22.4 hectares would have been required to achieve 90% removal of nitrate from 1 m^3/s of RO concentrate discharged to the pilot-scale system (S2.3). Based on our microcosm results, this land requirement could potentially be decreased by adding woodchips as an external carbon source. In microcosms, the nitrate removal rate increased by five times or more, depending on wood chip dose,

564 indicating the potential to decrease the wetland area required by 80% at an intermediate
565 woodchip dose. The use of low-cost carbon amendments or greater land areas could
566 increase nitrate removal by open-water wetlands to levels similar to those observed in other
567 biological treatment technologies tested for RO concentrate, which have also required
568 carbon amendments to remove approximately 50-80% of the nitrate.[5,6]
569
570 Unlike other technologies, open-water wetlands have relatively straightforward
571 maintenance requirements, comprised mainly of managing algae, duckweed, and other
572 vegetation that may establish within the system or along the banks of the wetland cells.
573 Removal of accumulated biomat may also be necessary periodically, depending on
574 accretion rates.[16,19] Another advantage to open-water wetland treatment of RO concentrate
575 is the ability to simultaneously remove trace organic contaminants.[18,19] The pilot-scale
576 system described herein decreased concentrations of several pharmaceuticals and
577 pesticides in RO concentrate through a combination of sunlight photolysis and
578 biotransformation.[56] However, an important consideration for the use of open-water
579 wetlands is the seasonality of treatment. Due to the strong temperature dependence of
580 nitrate removal, open-water wetlands will have the most consistent performance in climates
581 with little seasonal temperature variation. Open-water wetlands may also be applicable in
582 other regions if release of nutrients during the colder months of the year is acceptable, for
583 instance where nitrogen-limited conditions for algal blooms occur only in the summer
584 months.[57]
585
586 The relevance of open-water wetlands for RO concentrate treatment will also depend on
587 future developments in RO membranes for water reuse. Currently, RO membranes

588 employed for water reuse are typically operated at around 85% recovery.[58] Thus, 1 m^3/s
589 corresponds to the production of approximately 6 m^3/s (approximately 23 million gallons
590 per day) of RO permeate (i.e., treated water). As new types of RO membranes are
591 developed, water recoveries may increase, which in turn, might decrease the area of land
592 needed to treat RO concentrate. To provide additional insight into this relationship, we
593 evaluated the effect of recovery on the area needed to treat the concentrate associated with
594 the production of recycled water (Section S2.3). This calculation indicated that the wetland
595 depth would need to decrease from 35 cm at 50% recovery to 18 cm at 95% recovery in
596 order for photosynthetically active radiation to reach the biomat (Fig. S5). However, the
597 volume of concentrate that would need to be treated would decrease by approximately an
598 order of magnitude as the recovery increased, which would decrease the area required to
599 produce recycled water while also treating nitrate. Increasing water recovery from 85% to
600 95% decreased the area needed for the system by 67% (Figure 5). Therefore, in places
601 where salinity is not an issue, such as during discharge to the ocean or an estuary, nutrient
602 removal via open-water wetland treatment may be more space-efficient when reverse
603 osmosis systems operate at a higher water recovery. However, the increased salinity of the
604 resulting RO concentrate could also impact the microbial community that develops in
605 open-water wetlands. Further research is needed to assess potential effects on treatment
606 efficiency at higher RO recoveries.

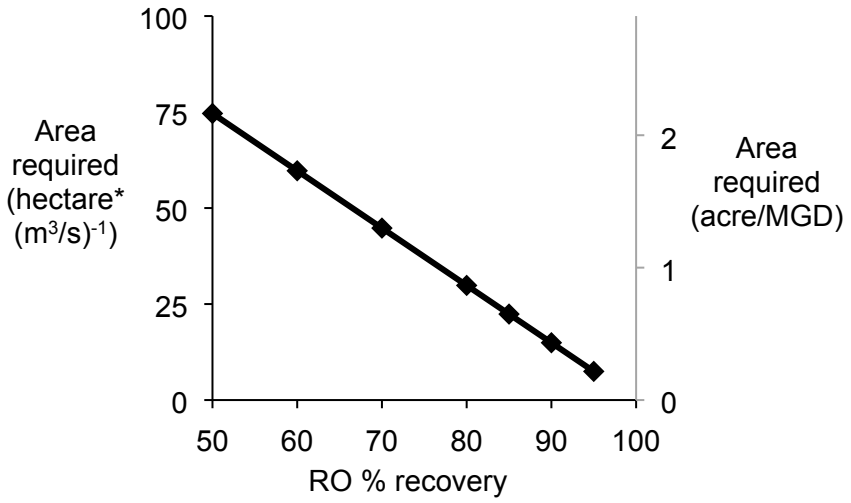

Figure 5. Area required for 90% nitrate removal per m^3/s (MGD, right axis) of RO permeate.

607

608 **Conclusion**

609 Overall, the pilot-scale treatment system and microcosm experiments described herein
610 indicated that open-water wetlands could provide treatment of nitrate from RO
611 concentrate generated during potable water reuse. Nitrate removal depended on the
612 availability of carbon to fuel denitrification, which was provided by photosynthetic
613 diatoms. The reliance on carbon fixation resulted in a large footprint requirement that is
614 likely to be a major limitation for the adoption of these systems. However, the addition of
615 inexpensive carbon sources, such as woodchips, or the use of RO membranes that allow
616 for higher water recovery could reduce the land area required for treatment. The low cost
617 and operational simplicity of open-water wetlands, as well as the ability to
618 simultaneously remove trace organic contaminants, make these systems advantageous for
619 RO concentrate treatment.

620

621 **Conflicts of Interest**

There are no conflicts to declare.

Acknowledgements

This research was supported by the National Science Foundation (NSF) through the Engineering Research Center for Reinventing the Nation's Urban Water Infrastructure (ReNUWIt; EEC-1028968), by the U.S./China Clean Energy Research Center for Water-Energy Technologies (CERC-WET, DE-IA 0000018), by an NSF Graduate Research Fellowship, and by Valley Water through the Reverse Osmosis Concentrate Management Project with support from the California State Water Resources Control Board (D190503100). Additional collaborative funding was provided from the National Institutes for Water Resources and U.S. Geological Survey (NIWR-USGS) subaward G-62991-01, and NSF grant number CBET-1055396. We would like to thank Valley Water, GHD, and the San Francisco Estuary Institute for supporting the pilot-scale project. We'd also like to thank Cayla Anderson (UC Berkeley) for assisting with microcosm setup and Kristin Mikkelson (CSM) for initial microbial data analysis.

1 E. L. Marron, W. A. Mitch, U. Von Gunten and D. L. Sedlak, A Tale of Two Treatments: The Multiple Barrier Approach to Removing Chemical Contaminants During Potable Water Reuse, *Acc. Chem. Res.*, 2019, **52**, 615–622.

2 M. Umar, F. Roddick and L. Fan, Recent Advancements in the Treatment of Municipal Wastewater Reverse Osmosis Concentrate An Overview, *Crit. Rev. Environ. Sci. Technol.*, 2014, **25**, 193–248.

3 D. J. Conley, H. W. Paerl, R. W. Howarth, D. F. Boesch, S. P. Seitzinger, K. E. Havens, C. Lancelot and G. E. Likens, Controlling eutrophication: Nitrogen and phosphorus, *Science*, 2009, **323**, 1014–1015.

4 R. W. Howarth and R. Marino, Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades, *Limnol. Oceanogr.*, 2006, **51**, 364–376.

5 H. J. Choi, Nutrient removal in reverse osmosis concentrates using a biological aerated filter, *Water Sci. Technol. Water Supply*, 2015, **15**, 302–307.

6 X. Quan, K. Huang, M. Li, M. Lan and B. Li, Nitrogen removal performance of municipal reverse osmosis concentrate with low C/N ratio by membrane-aerated biofilm reactor, *Front. Environ. Sci. Eng.*, 2018, **12**, 1–11.

7 P. Westerhoff, H. Moon, D. Minakata and J. Crittenden, Oxidation of organics in retentates from reverse osmosis wastewater reuse facilities, *Water Res.*, 2009, **43**, 3992–3998.

8 L. Y. Lee, H. Y. Ng, S. L. Ong, J. Y. Hu, G. Tao, K. Kekre, B. Viswanath, W. Lay and H. Seah, Ozone-biological activated carbon as a pretreatment process for reverse osmosis brine treatment and recovery, *Water Res.*, 2009, **43**, 3948–3955.

9 S. Pradhan, L. Fan and F. A. Roddick, Removing organic and nitrogen content

661 from a highly saline municipal wastewater reverse osmosis concentrate by
662 UV/H2O2-BAC treatment., *Chemosphere*, 2015, **136**, 198–203.
663 10 J. Lu, L. Fan and F. A. Roddick, Potential of BAC combined with UVC/H2O2 for
664 reducing organic matter from highly saline reverse osmosis concentrate produced
665 from municipal wastewater reclamation, *Chemosphere*, 2013, **93**, 683–688.
666 11 V. Rocher, C. Paffoni, A. Gonçalves, S. Azimi and A. Pauss, Municipal
667 wastewater treatment by biofiltration: Comparisons of various treatment layouts.
668 Part 2: Assessment of the operating costs in optimal conditions, *Water Sci.*
669 *Technol.*, 2012, **65**, 1713–1719.
670 12 R. S. Raucher and G. Tchobanoglous, *The Opportunities and Economics of Direct*
671 *Potable Reuse*, 2014.
672 13 R. H. Kadlec and S. Wallace, *Treatment Wetlands*, CRC Press, Boca Raton, FL,
673 2nd edn., 2009.
674 14 R. K. Chakraborti and J. S. Bays, Natural treatment of high-strength reverse
675 osmosis concentrate by constructed wetlands for reclaimed water use, *Water*,
676 DOI:10.3390/w12010158.
677 15 R. K. Chakraborti, J. S. Bays, T. Ng, L. Balderrama and T. Kirsch, A pilot study of
678 a subsurface-flow constructed wetland treating membrane concentrate produced
679 from reclaimed water, *Water Sci. Technol.*, 2015, **72**, 260–268.
680 16 J. T. Jasper, Z. L. Jones, J. O. Sharp and D. L. Sedlak, Nitrate removal in shallow,
681 Open-water treatment wetlands, *Environ. Sci. Technol.*, 2014, **48**, 11512–11520.
682 17 J. T. Jasper and D. L. Sedlak, Phototransformation of wastewater-derived trace
683 organic contaminants in open-water unit process treatment wetlands., *Environ. Sci.*
684 *Technol.*, 2013, **47**, 10781–90.

18 J. T. Jasper, Z. L. Jones, J. O. Sharp and D. L. Sedlak, Biotransformation of trace organic contaminants in open-water unit process treatment wetlands., *Environ. Sci. Technol.*, 2014, **48**, 5136–44.

19 S. E. Bear, M. T. Nguyen, J. T. Jasper, S. Nygren, K. L. Nelson and D. L. Sedlak, Removal of nutrients, trace organic contaminants, and bacterial indicator organisms in a demonstration-scale unit process open-water treatment wetland, *Ecol. Eng.*, 2017, **109**, 76–83.

20 ValleyWater, Silicon Valley Advanced Water Purification Center, https://www.purewater4u.org/.

21 Z. L. Jones, K. M. Mikkelson, S. Nygren, D. L. Sedlak and J. O. Sharp, Establishment and convergence of photosynthetic microbial biomats in shallow unit process open-water wetlands, *Water Res.*, 2018, **133**, 132–141.

22 A. E. Parada, D. M. Needham and J. A. Fuhrman, Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples, *Environ. Microbiol.*, 2016, **18**, 1403–1414.

23 S. Henry, E. Baudoin, J. C. López-Gutiérrez, F. Martin-Laurent, A. Brauman and L. Philippot, Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR, *J. Microbiol. Methods*, 2004, **59**, 327–335.

24 J. C. López-Gutiérrez, S. Henry, S. Hallet, F. Martin-Laurent, G. Catroux and L. Philippot, Quantification of a novel group of nitrate-reducing bacteria in the environment by real-time PCR, *J. Microbiol. Methods*, 2004, **57**, 399–407.

25 D. M. Drennan, R. Almstrand, I. Lee, L. Landkamer, L. Figueroa and J. O. Sharp, Organoheterotrophic Bacterial Abundance Associates with Zinc Removal in Lignocellulose-Based Sulfate-Reducing Systems, *Environ. Sci. Technol.*, 2016, **50**,

709 378–387.
710 26 K. B. Benedict, A. S. McFall and C. Anastasio, Quantum Yield of Nitrite from the
711 Photolysis of Aqueous Nitrate above 300 nm, *Env. Sci Technol*, 2017, **51**, 4387–
712 4395.
713 27 P. Servais, A. Anzil and C. Ventresque, Simple Method for Determination of
714 Biodegradable Dissolved Organic Carbon in Water, *Appl Env. Microbiol*, 1989,
715 **55**, 2732–2734.
716 28 J. N. Apell and K. Mcneill, Updated and validated solar irradiance reference
717 spectra for estimating environmental photodegradation rates, *Env. Sci Process.*
718 *Impacts*, 2019, **21**, 427–437.
719 29 R. P. Schwarzenbach, P. M. Gschwend and D. M. Imboden, *Environmental*
720 *Organic Chemistry*, Wiley, New Jersey, 2nd edn., 2003.
721 30 T. B. Bittar, Y. Lin, L. R. Sassano, B. J. Wheeler, S. L. Brown, W. P. Cochlan and
722 Z. I. Johnson, Carbon allocation under light and nitrogen resource gradients in two
723 model marine phytoplankton1, *J. Phycol.*, 2013, **49**, 523–535.
724 31 R. H. Kadlec, Constructed Marshes for Nitrate Removal, *Crit. Rev. Environ. Sci.*
725 *Technol.*, 2011, 934–1005.
726 32 B. J. Halaburka, G. H. Lefevre and R. G. Luthy, Quantifying the temperature
727 dependence of nitrate reduction in woodchip bioreactors: experimental and
728 modeled results with applied case-study, *Environ. Sci. Water Res. Technol*, 2019,
729 **5**, 782–797.
730 33 D. Chen, X. Chen, X. Huang, S. He, J. Huang and W. Zhou, Controlling
731 denitrification accompanied with nitrite accumulation at the sediment-water
732 interface, *Ecol. Eng.*, 2017, **100**, 194–198.

34 X. Yang, S. Wang and L. Zhou, Effect of carbon source, C/N ratio, nitrate and dissolved oxygen concentration on nitrite and ammonium production from denitrification process by Pseudomonasstutzeri D6, *Bioresour. Technol.*, 2012, **104**, 65–72.

35 W. Zhao, Y. Wang, S. Liu, M. Pan, J. Yang and S. Chen, Denitrification activities and N2O production under salt stress with varying COD/N ratios and terminal electron acceptors, *Chem. Eng. J.*, 2013, **215**–**216**, 252–260.

36 W. G. Zumft, Cell biology and molecular basis of denitrification., *Microbiol. Mol. Biol. Rev.*, 1997, **61**, 533–616.

37 M. I. Bellini, D. Kumaresan, S. Tarlera, J. C. Murrell and A. Fernández-Scavino, Identification of active denitrifiers by DNA-stable isotope probing and amplicon sequencing reveals Betaproteobacteria as responsible for attenuation of nitrate contamination in a low impacted aquifer, *FEMS Microbiol. Ecol.*, 2018, **94**, 1–13.

38 H. R. Beller, P. S. G. Chain, T. E. Letain, A. Chakicherla, F. W. Larimer, P. M. Richardson, M. A. Coleman, A. P. Wood and D. P. Kelly, The genome sequence of the obligately chemolithoautotrophic, facultatively anaerobic bacterium Thiobacillus denitrificans, *J. Bacteriol.*, 2006, **188**, 1473–1488.

39 M. Pester, K. H. Knorr, M. W. Friedrich, M. Wagner and A. Loy, Sulfate-reducing microorganisms in wetlands - fameless actors in carbon cycling and climate change, *Front. Microbiol.*, 2012, **3**, 1–19.

40 H. F. Castro, N. H. Williams and A. Ogram, Phylogeny of sulfate-reducing bacteria, *FEMS Microbiol. Ecol.*, 2000, **31**, 1–9.

41 S. J. McIlroy and P. H. Nielsen, in *The Prokaryotes: Other Major Lineages of Bacteria and The Archaea*, eds. E. Rosenberg, E. F. DeLong, S. Lory, E.

Stackebrandt and F. Thompson, Springer Berlin Heidelberg, Berlin, Heidelberg, 2014, pp. 863–889.

42 E. Ransom-Jones, D. L. Jones, A. J. McCarthy and J. E. McDonald, The Fibrobacteres: An Important Phylum of Cellulose-Degrading Bacteria, *Microb. Ecol.*, 2012, **63**, 267–281.

43 M. Fahrbach, J. Kuever, M. Remesch, B. E. Huber, P. Kämpfer, W. Dott and J. Hollender, Steroidobacter denitrificans gen. nov., sp. nov., a steroidal hormone-degrading gammaproteobacterium, *Int. J. Syst. Evol. Microbiol.*, 2008, **58**, 2215–2223.

44 G. P. Robertson and P. M. Groffman, in *Soil Microbiology, Ecology and Biochemistry*, Elsevier, 2007, pp. 341–364.

45 M. Fahrbach, J. Kuever, R. Meinke, P. Kämpfer and J. Hollender, Denitratisoma oestradiolicum gen. nov., sp. nov., a 17 β-oestradiol-degrading, denitrifying betaproteobacterium, *Int. J. Syst. Evol. Microbiol.*, 2006, **56**, 1547–1552.

46 Z. L. Jones, J. T. Jasper, D. L. Sedlak and J. O. Sharp, Sulfide-induced dissimilatory nitrate reduction to ammonium supports anaerobic ammonium oxidation (anammox) in an openwater unit process wetland, *Appl. Environ. Microbiol.*, 2014, **83**, 1–14.

47 J. G. Kuenen, Anammox bacteria: From discovery to application, *Nat. Rev. Microbiol.*, 2008, **6**, 320–326.

48 M. S. Wetz and P. A. Wheeler, Release of dissolved organic matter by coastal diatoms, *Limnol. Ocean.*, 2007, **52**, 798–807.

49 A. Barofsky, C. Vidoudez and G. Pohnert, Metabolic profiling reveals growth stage variability in diatom exudates, *Limnol. Ocean. Methods*, 2009, **7**, 382–390.

50 K. Matusiak-Mikulin, C. Tukaj and Z. Tukaj, Relationships between growth, development and photosynthetic activity during the cell cycle of Desmodesmus armatus (Chlorophyta) in synchronous cultures Relationships between growth, development and photosynthetic activity during the cell cycle of Desmode, *Eur. J. Phycol*, 2006, **41**, 29–38.

51 G. Hu, Y. Fan, L. Zhang, C. Yuan and J. Wang, Enhanced Lipid Productivity and Photosynthesis Efficiency in a Desmodesmus sp. Mutant Induced by Heavy Carbon Ions, *PLoS One*, 2013, **8**, 60700.

52 A. P. Reed, Colorado School of Mines, 2019.

53 L. A. Schipper, W. D. Robertson, A. J. Gold, D. B. Jaynes and S. C. Cameron, Denitrifying bioreactors - An approach for reducing nitrate loads to receiving waters, *Ecol. Eng.*, 2010, **36**, 1532–1543.

54 D. M. Drennan, R. Almstrand, J. Ladderud, I. Lee, L. Landkamer, L. Figueroa and J. O. Sharp, Spatial impacts of inorganic ligand availability and localized microbial community structure on mitigation of zinc laden mine water in sulfate-reducing bioreactors, *Water Res.*, 2017, **115**, 50–59.

55 M. Ribot, J. Cochero, T. N. Vaessen, S. Bernal, E. Bastias, E. Gacia, A. Sorolla, F. Sabater and E. Martí, Leachates from helophyte leaf-litter enhance nitrogen removal from wastewater treatment plant effluents, *Environ. Sci. Technol.*, 2019, **53**, 7613–7620.

56 R. C. Scholes, J. F. King, W. A. Mitch and D. L. Sedlak, Transformation of Trace Organic Contaminants from Reverse Osmosis Concentrate by Open-Water Unit Process Wetlands with and without Ozone Pre-Treatment, *Environ. Sci. Technol.*, DOI:10.1021/acs.est.0c04406.

805 57 I. M. Andersen, T. J. Williamson, M. J. González and M. J. Vanni, Nitrate,
806 ammonium, and phosphorus drive seasonal nutrient limitation of chlorophytes,
807 cyanobacteria, and diatoms in a hyper-eutrophic reservoir, *Limnol. Oceanogr.*,
808 2020, **65**, 962–978.
809 58 D. Solley, C. Gronow, S. Tait, J. Bates and A. Buchanan, Managing the reverse
810 osmosis concentrate from the Western Corridor Recycled Water Scheme, *Water*
811 *Pract. Technol.*, DOI:10.2166/WPT.2010.018.
812