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Aqueous silica removal from agricultural drainage water and reverse osmosis concentrate by brackish water diatoms in semi-batch photobioreactors

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Abstract

A novel aqueous silica removal process using naturally occurring diatoms for water and wastewater treatment, in particular water reuse and desalination, was developed. Brackish agricultural drainage water containing 39 mg L^{-1} of total silica and 10 g L^{-1} of total dissolved solids was used as a source of silica-assimilating diatoms. The drainage water was statically incubated in 500-mL and 7.5-L photobioreactors at 27 ± 2 °C under continuous illumination using two 13-W compact fluorescent light bulbs as a light source. After 10 days, brown algal biomass became noticeable and silica removal started to occur. Silica removal accelerated as algal biomass accumulated. In the fourth semi-batch cycle, more than 95 % of molybdate reactive silica was removed within 28 h. Removal of nonreactive silica was also confirmed. Reverse osmosis concentrate samples from advanced water reclamation facilities with high silica concentration (>120 mg L^{-1}) were also tested. More than 75 % of silica was removed within 6 days. Microscopic analysis revealed the presence of *Pseudostaurosira*, *Nitzschia*, and *Halamphora* species in the photobioreactors. To the best of our knowledge, this is the first report on the use of diatoms for aqueous silica removal in water treatment.

Keywords Diatoms · Photobioreactor · Silica · Brackish water · Desalination · Water reuse

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Introduction

Silica (SiO_2) is abundant in nature as sand and quartz and has a very complex aqueous chemistry (Iler 1979). In water, silica may be present in a form of crystalline or amorphous solid, colloid, polymeric, or monomeric silica compounds. Silica is soluble in water at concentrations up to 100 to 130 mg L^{-1} as SiO_2, depending on its form. Amorphous SiO_2 is much more soluble than crystalline silica (such as quartz), which has a low aqueous solubility of about 6 mg L^{-1} as SiO_2 (Iler 1979). Average abundance of aqueous silica in surface and groundwater is 14 mg L^{-1} (APHA et al. 2005), while silica concentration in groundwater can be as high as 60 mg L^{-1}. In water, SiO_2 is hydrolyzed to silicic acid [$Si(OH)_4$], which is a very weak acid with the first acid dissociation constant (pK_{a1}) of 9.9 (Ning 2002). Silica is, therefore, more soluble under strong alkaline conditions.

Modern water reuse and desalination projects often employ high-pressure membrane processes, such as reverse osmosis (RO) (Greenleea et al. 2009; Lee et al. 2011; Matin et al. 2011; Kang and Cao 2012; Perez-Gonzalez et al. 2012). The performance of the RO process is affected by a number of factors, including salinity, silt, inorganic scaling, organics, and biological fouling (Greenleea et al. 2009). Scaling by inorganic water constituents is one of the most difficult challenges in RO-based water treatment because it can reduce the permeate flow and potentially cause physical damage to the membrane (Asano et al. 2007). Sources of inorganic scaling can be divided into two categories, silica and hardness metals (e.g., calcium, magnesium, strontium, and barium) combined with carbonate or sulfate. These inorganic substances precipitate on membrane surface when their concentrations in the membrane become higher than their solubility. While hardness removal can be achieved relatively easily and economically by chemical processes, such as cation exchange (Iler 1979) and lime softening (Behrman and Gustafson 1940; Crittenden et al. 2005), silica

removal tends to be more difficult and more expensive. Silica scaling in RO is prevalent and one of the limiting factors in many advanced water reclamation plants, as well as brackish groundwater desalination plants, which requires highly aggressive and toxic chemical cleaning agents such as ammonium bifluoride (NH_4HF_2) for the removal (Gallego et al. 2008).

Removal of silica from water depends on the form of silica. Coagulation with an aluminum or iron coagulant followed by sedimentation and/or filtration can remove colloidal silica, as well as solid amorphous and crystalline silica, while anionic, monomeric, and polymeric silicates can be removed by strong base anion exchange (Iler 1979). Other silica removal processes include activated alumina adsorption and lime-soda softening (Behrman and Gustafson 1940). However, these processes are often very expensive, ineffective, and incomplete due to silica's complex chemistry, such as requirement of strict pH control, as well as the interference by competing anions, including sulfate (SO_4^{2-}), bicarbonate (HCO_3^-), and chloride (Cl^-). Concentrations of these competing anions are often very high in nonconventional water resources such as brackish groundwater and recycled water, which require desalination. These processes also generate waste by-products, such as spent media, brine, and sludge, which require proper disposal. Moreover, these treatment processes can only remove a fraction of aqueous silica, either "dissolved" or "colloidal/particulate" silica. Therefore, it is desirable to develop an efficient and cost-effective silica removal process that can remove both types of these silica compounds.

In biology, dissolved silica uptake by the microorganisms including diatoms and marine protozoan Radiolaria are an important component of the silica cycle (Lewin 1954; Harper and Knoll 1975; Grogera et al. 2008; Lee 2008). Diatoms are a group of unicellular microalgae (Bacillariophyta) that can be found in both freshwater and seawater and are one of the most important sources of biomass in oceans for O_2 production and bioremediators of contaminated water (Bozarth et al. 2009). They have a hard and porous cell wall (frustule), which is composed mostly of silica. Their walls are extremely stable, and they may act as photonic crystals (Sumper and Brunner 2006). Together with the Radiolaria, marine diatoms are responsible for the low silica concentration (<2 mg L^{-1}) in oceanic surface waters (Harper and Knoll 1975) and some of the removal of soluble silica entering the sea (Bien et al. 1958). Egge and Aksnes (1992) showed that diatoms became dominant in seawater if silicate concentration is higher than 2 μM (= 56 μg L^{-1} as Si or 120 μg L^{-1} as SiO_2). The silicon uptake and metabolism of diatoms has been well studied (Grogera et al. 2008; Finkel 2016). Recently, cultivation of various diatom species, such as *Odontella* spp. and *Phaeodactylum tricornutum*, has been attempted for fabricating nanometer-sized silica structures for semiconductor nanolithography and vehicles for drug delivery and biosilica for photoluminescence (Vrieling et al. 1999; Bradbury 2004; Gordon 2010; Lim et al. 2015). With such a special characteristic capable of combining with silica, diatoms have been used as highly attractive silica-based biohybrids (Nassif and Livage 2011). These decades-old biological facts indicate that cultured diatoms may be used to remove aqueous silica from water and wastewater. However, to the best of our knowledge, no report has been published to explore this diatom-based photobiology in water and wastewater treatment. Therefore, the objective of this work is to investigate the feasibility of this diatom-based process for the removal of aqueous silica and discuss its potential implication for water treatment, in particular, desalination and water reuse. Here, the removal of aqueous silica from brackish agricultural drainage water (>30 mg L^{-1} reactive silica) and RO concentrate (>120 mg L^{-1} reactive silica) from advanced water reclamation facilities in Southern California by diatom-based treatment is reported.

Materials and methods

Source of diatoms and aqueous silica

A brackish agricultural drainage water sample collected in the Central Valley of California, USA, during the summer of 2010 was used without any pretreatment as a source of brackish water diatoms, as well as aqueous silica. The drainage water sample was kept in a closed 2500-L high-density polyethylene (HDPE) container at room temperature (25 ± 2 °C) before use. The total dissolved solid (TDS) concentration was about 10 g L^{-1}. Total and molybdate reactive silica concentrations in this water sample were 39 and 30 mg L^{-1}, respectively. The water quality of the drainage water sample is presented in Table 1. The water was aerobic, and ammonia-N content in this sample was negligible (<0.02 mg L^{-1} as N).

An RO concentrate sample was obtained from the Ground Water Replenishment System (GWRS) at the Orange County Water District (OCWD), Fountain Valley, CA, USA, on 24 October 2013. The RO concentrate sample was collected from the third stage of the full-scale RO unit in the GWRS. The sample was characterized in the lab upon arrival and was stored in a refrigerator at 4 °C before use. Table 2 presents the water quality of the RO concentrate sample.

Analytical methods

Reactive silica in the raw and treated water was determined by the silicomolybdate method (HACH Method 8185) using the HACH high range silica reagent set and a DR-2800 Spectrophotometer (HACH Company, USA). Total silica was determined by inductively coupled plasma-atomic spectrometry (ICP-AS) according to the EPA Method 200.7. Alkalinity, chloride, sulfate, nitrate-N, total hardness, calcium hardness, potassium, orthophosphate (*o*-phosphate), iron, manganese,

Table 1 Raw and treated (the end of the fourth cycle) agricultural drainage water sample (collected in the summer of 2010)

Constituents	Raw water	Treated water	% Removal
Cations			
Sodium (mg L^{-1})	2895	2905	–
Potassium (mg L^{-1})	32	28	13
Calcium (mg L^{-1})	232	152	34
Magnesium (mg L^{-1})	248	250	–
Iron (μg L^{-1})	20	20	–
Manganese (μg L^{-1})	85	64	25
Anions			
Chloride (mg L^{-1})	2480	2490	–
Sulfate (mg L^{-1})	4150	3975	4
Bicarbonate (mg L^{-1})	275	204	26
Nitrate-N (mg L^{-1} as N)	37	30	17
Total silica (mg L^{-1})	39	0.14	>99
Reactive silica (mg L^{-1})	30	0.3	99
Orthophosphate (mg L^{-1})	2.3	0.1	96
General parameters			
Total dissolved solids (mg L^{-1})	10380	10036	3
Total hardness (mg L^{-1} as $CaCO_3$)	1600	1410	12
Alkalinity (mg L^{-1} as $CaCO_3$)	450	335	26
Chemical oxygen demand (mg L^{-1})	92	90	2
pH	8.6	9.2	–
Color at 455 nm (PtCo color unit)	31	39	–

total chlorine, color, and chemical oxygen demand (COD) were measured by HACH Methods 8203 (titration), 8207 (silver nitrate), 8051 (USEPA SulfaVer 4), 10020 (chromotropic acid), 8213 (titration with EDTA), 8204 (titration with EDTA), 8049 (tetraphenylborate), 8048 (PhosVer3 ascorbic acid), 8008 (FerroVer®), 8149 [1-(2-pyridylazo)-2-naphtol (PAN)], 8167

Table 2 Raw and treated (the end of first cycle) reverse osmosis concentrate sample water quality (collected on 24 October 2013)

Constituents	Raw water	Treated water	% Removal
Cations			
Calcium (mg L^{-1})	532	272	49
Magnesium (mg L^{-1})	151	156	–
Iron (mg L^{-1})	0.52	<0.02	>96
Manganese (mg L^{-1})	0.52	0.10	81
Ammonia-N (mg L^{-1})	4.26	<0.02	>99
Anions			
Bicarbonate (mg L^{-1})	1135	378	67
Nitrate-N (mg L^{-1}as N)	58.2	54.6	6
Reactive silica (mg L^{-1})	122.4	3.4	97
Orthophosphate (mg L^{-1})	16.5	0.05	>99
General parameters			
Total dissolved solids (mg L^{-1})	5970	5710	–
Total hardness (mg L^{-1}as $CaCO_3$)	1950	1320	32
Total alkalinity (mg L^{-1}as $CaCO_3$)	930	310	67
Chemical oxygen demand (mg L^{-1})	126	106	16
Total organic carbon (mg L^{-1})	8.0	6.5	19
pH	7.7	8.7	–
Color at 455 nm (PtCo color unit)	176	137	22

[*N*,*N*-diethyl-*p*-phenylendiamine (DPD) method], 8025 (platinum-cobalt standard), and 8000 (USEPA reactor digestion), respectively. Sodium ion concentrations were measured using a HACH Model ISENa38101 sodium ion selective probe, according to the manufacturer's instructions. TDS concentrations were calculated by summing the concentrations of major dissolved ions or derived from the electrical conductivity. Ultrapure water (>18 MΩ) was used for standard and reagent preparation, blanks, and sample dilution for the chemical analyses.

Semi-batch aqueous silica removal experiments

A series of aqueous silica removal experiments were conducted in a bench-scale semi-batch mode using two kinds of plastic containers as the bioreactors, namely clear 500-mL polyethylene terephthalate (PETE) bottles (ø = 65 mm) and 7.5-L white HDPE container (ø = 230 mm). These containers were placed in an illuminating reflective enclosure that was constructed with a 380 mm × 305 mm × 280 mm (= W × D × H) plastic container lined with and covered by aluminum foil and two 13-W compact fluorescent light (CFL) bulbs as a light source. The light source was later replaced with equivalent two 9-W light-emitting diode (LED) light bulbs (light temperature 5000 K, 800 lm each, Cree, Inc., Durham, NC, USA) to avoid excessive heat generation from the CFL bulbs. The light output was also more stable with the LED bulbs.

In the first series of experiments, two 500 mL of silica-rich agricultural drainage water samples were placed in cleaned clear PETE bottles. These bottles were capped with caps with a hole (ø = 5 mm) and incubated statically at 27 ± 2 °C under continuous illumination in the reflective enclosure. The distance between the light source and bottles was approximately 150 mm. The irradiance was approximately 150 μmol photons s^{-1} m^{-2} based on an illuminance measurement (McCree 1972). The drainage water sample initially contained 32 and 2.2 mg L^{-1} of nitrate-N and orthophosphate, respectively. No additional nutrients were added. Aliquots of samples were withdrawn periodically from the bottles and were tested for reactive silica and *o*-phosphate. Once reactive silica concentration was reduced to below 0.8 mg L^{-1}, supernatant was removed from the bottles by decantation while the majority of algal biomass was kept in the bottles. Five hundred milliliter of fresh silica-rich drainage water was added to the bottles for another semi-batch cycle, and the above procedure was repeated several times. Control experiments were performed using the same quality and amount of water in the same containers. The bottles were kept in the dark without illumination. At the end of the last cycle of semi-batch experiment, biomass was collected from the reactors, filtered through a glass fiber filter (934-AH, 1.5 μm particle retention, HACH Company), rinsed thoroughly with ultrapure water to remove dissolved solids, and dried in a desiccator to quantify biomass. In the second series of experiment, approximately 4 L of silica-rich agricultural drainage water was added into a 7.5-L HDPE container. The depth of the water was about 140 mm. The experimental conditions and procedures were same as the first series.

For the treatment of RO concentrate, an aliquot of the RO concentrate sample was first dechlorinated with calcium thiosulfate. Then, 500 mL of dechlorinated RO concentrate was transferred to clear 500-mL PETE bottles where approximately 10 mL of mixed culture diatom biomass obtained from three cycles of preincubation of agricultural drainage water as described above was added as an inoculum. These bottles were incubated at room temperature (25 ± 1 °C) in the reflective enclosure. The irradiance was approximately 94 μmol photons s^{-1} m^{-2} during this experiment. Samples were withdrawn periodically to analyze for color (455 nm), reactive silica, *o*-phosphate, and COD.

Diatom isolation and identification

After several semi-batch experiments using the agricultural drainage water, the presence of a mixture of various diatom species was confirmed by microscopic observations using a light microscope. In an attempt to isolate the diatom species, the serial dilution method was employed. Filter-sterilized agricultural drainage water was used as an isolation medium. After several prefiltration through glass fiber filters with various pore sizes (7 to 0.7 μm), the drainage water was filtered through membrane filters (Osmonics Magna nylon membrane, pore size 0.2 μm, GE Healthcare Bio-Sciences, USA). Then, 9 mL of microfiltered medium was dispensed into a sterile 15-mL plastic culture tube by a 5-mL disposable syringe with a sterile syringe filter (Acrodisc PF syringe filter with 0.8/0.2 μm Supor hydrophilic polyethersulfone membranes, Pall Life Sciences, USA). To start the dilution, 1 mL of the enriched diatom biomass from PETE bottles was transferred into the first sterile culture medium tube (10^{-1}) and gently mixed. After that, 1 mL of the first dilution was transferred into the second culture tube to prepare 10^{-2} dilution. The same procedure was repeated several times to obtain desired dilutions. The culture tubes were statically incubated under the light source until the growth of diatoms was visible. The purity and identity of the diatoms were examined using a light microscope.

Isolated diatom cells were digested using sodium hypochlorite (household bleach). Diluted bleach solution was prepared by mixing 1:1 ratio (volume) of ultrapure water with a 6 % household bleach. The chlorine concentration in the resulting diluted bleach solution was 2.9 % as Cl_2. Before digestion, 2 mL of biomass suspension was collected from a culture tube and transferred into a 15-mL sterile centrifuge tube. Then, 10 mL of ultrapure water was added to the tube, mixed, and centrifuged at 4000 rpm for 5 min using a VWR Clinical 50 centrifuge (VWR International, USA). The

supernatant was removed and another 10 mL ultrapure water was added for another wash. After the two-time washing, 5 mL of diluted bleach solution was added into the tube. The tube was then shaken vigorously for 30 min to ensure complete digestion of diatom cells. Then, 5 mL of ultrapure water was added into the tube containing digested cell. The content was mixed and centrifuged at 4000 rpm for 5 min. The supernatant was carefully removed. The washing was repeated at least six times using 10 mL of ultrapure water each time followed by centrifugation at 4000 rpm for 5 min to remove residual chlorine. The digested cells were then mounted onto slides using Cargille Meltmount 1.704 (Cargille Laboratories, USA) for microscopic observations.

Results

500-mL PETE photobioreactor experiments

Figure 1 shows the result of the semi-batch experiment to culture silica-assimilating diatoms in the PETE photobioreactor bottles. Very little silica removal or algal growth was noticed in the first 7 days. After about 10 days, brown colonies of diatoms became noticeable at the bottom of the bottles, which started to grow on the side afterward. As shown in Fig. 1, the reactive silica removal rate accelerated after the noticeable algal biomass formed (after day 14). Apparently, the depletion of *o*-phosphate coincided with reactive silica removal. The rates of reactive silica and *o*-phosphate removal accelerated over time and slowed down when their concentrations decreased to low levels (<3 and <0.1 mg L^{-1}, respectively). The change in reactive silica removal rate in the four semi-batch cycles is shown in Table 3. In the fourth cycle, a majority (>75 %) of reactive silica was removed within 18 h. After 28 h, >95 % of the reactive silica was removed. Total silica analysis indicated both molybdate reactive (i.e., monomeric and oligomeric) and molybdate unreactive (i.e., polymeric/colloidal) silica were effectively removed by this process (Fig. 2).

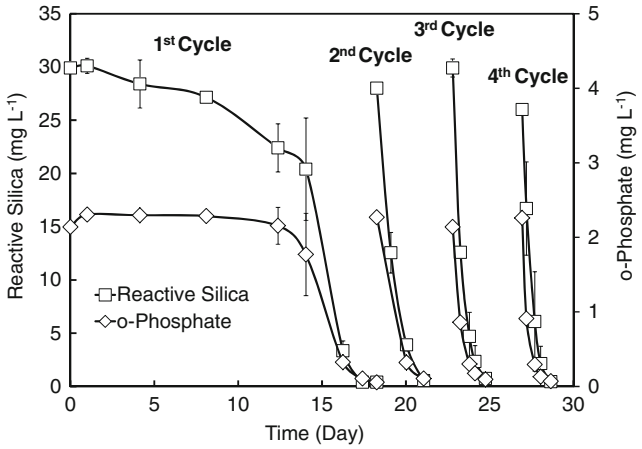

Fig. 1 Reactive silica and orthophosphate removal from brackish agricultural drainage water in 500-mL semi-batch PETE photobioreactors. Light source 2 × 13-W CFL, temperature 27 ± 2 °C

Table 3 Reactive silica removal rate in the four semi-batch cycles in 500-mL semi-batch PETE photobioreactors

Silica removal	First cycle (h)	Second cycle (h)	Third cycle (h)	Fourth cycle (h)
50 %	312	20	11	9
75 %	360	34	24	18
>95 %	408	72	52	28

At the end of the fourth semi-batch cycle, the concentrations of other water constituents were measured as shown in Table 1. Some of the constituents were significantly (>10 %) removed during this process, including calcium (34 %), manganese (25 %), bicarbonate (26 %), and nitrate-N (17 %). Calcium carbonate deposition by microalgae is a well-known phenomenon (Borowitzka 1987). The raised pH was an indication of significant photosynthetic activity, which also facilitated the calcium carbonate precipitation. Manganese might have been precipitated by oxidation by dissolved oxygen. It is important to note that nitrate-N was reduced, but not completely utilized. The water was phosphorous limited. The biomass concentration at the end of the fourth cycle was 0.58 ± 0.07 g dry weight L^{-1} (standard deviation, n = 2).

7.5-L HDPE photobioreactor experiments

Similar to the case of the 500-mL PETE photobioreactors, successful removal of reactive silica was achieved in a larger HDPE photobioreactor. After about 10 days of lag period, the concentration of reactive silica and *o*-phosphate started decreasing rapidly (Fig. 3). In the second cycle, the reactive silica concentration was reduced to below 0.5 mg L^{-1} in

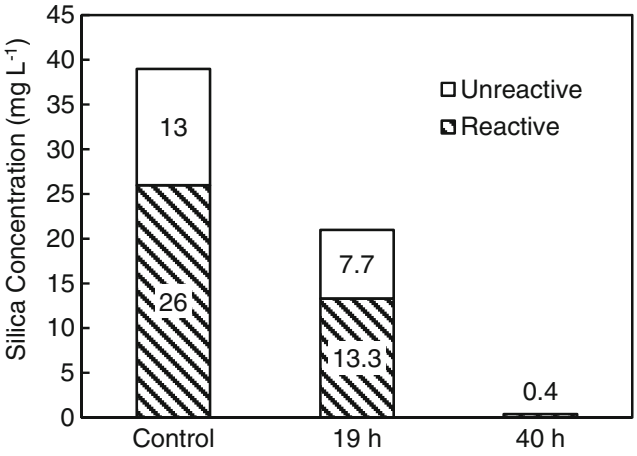

Fig. 2 Removal of reactive and unreactive silica from brackish agricultural drainage water in 500-mL PETE semi-batch photobioreactors (fourth cycle). Light source 2 × 13-W CFL, irradiance 150 μmol photons s^{-1} m^{-2}, temperature 27 ± 2 °C

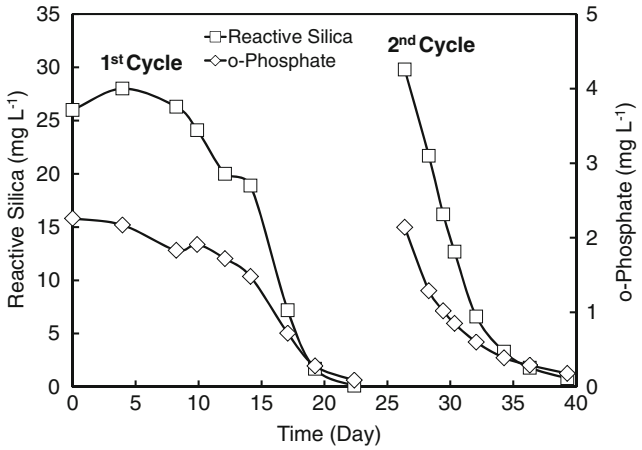

Fig. 3 Reactive silica and orthophosphate removal from brackish agricultural drainage water in a 7.5-L HDPE semi-batch photobioreactor. Light source 2 × 13-W CFL, irradiance 150 μmol photons s^{-1} m^{-2}, temperature 27 ± 2 °C

2 weeks. The rates of silica removal and *o*-phosphate depletion were slower than that in the 500-mL PETE photobioreactor bottles probably because of the lower biomass density (approximately 0.45 g dry weight L^{-1}) and smaller effective surface area. The light intensity per unit volume was also lower in the larger bioreactor. Nevertheless, this experiment demonstrated a scale-up potential of this photobiological silica removal process.

Diatom identification and isolation

Based on the characteristic brown color, as well as the rapid consumption of reactive silica, in the incubated brackish agricultural drainage water in the photobioreactors, it was postulated that the algal biomass were diatoms. A preliminary microscopic analysis confirmed that the majority of the algae were chain-forming and aggregate-forming diatoms, while some unicellular diatoms and occasional green algae cells were also observed. Chain-forming cells were more prevalent in younger cultures (first and second cycles), while more random, aggregate-forming cells became predominant in older cultures (third and fourth cycles). Green algae contamination became problematic when the culture was left for a long time without adding new silica-containing water.

In this study, three strains of brackish water diatoms were isolated from the 500-mL PETE photobioreactors (Fig. 4). Figure 4a, c, e shows the live cells of strains 1, 2, and 3, respectively, while Fig. 4b, d, f shows the corresponding digested ones. Strain 1 formed long-chain colonies which have a centric structure with cylindrical valves. However, after digestion with sodium hypochlorite, the shape of the valves was found to be slightly elliptical. Characteristic striae were present on the valve face, as well as on the valve mantle. Spines were also visible. The diameters of the valves and thickness of the frustules are approximately 4 and 3 μm, respectively. Based on these features, strain 1 was identified as *Pseudostraurosira* sp., most likely *P. trainorii* (Morales 2001; Spaulding and Edlund 2008). The other two diatom strains (strains 2 and 3) were also pennates with a raphe. For strain 2, the raphe was eccentric within a keel which was supported by an internal fibulae, which is a characteristic of *Nitzschia* spp. (AWWA 2010; Kociolek 2011). The raphe of each valve was on opposite sides of the frustule. The width of the valves ranged from 10 to 20 μm. Because of the presence of distinct central nodule, strain 2 could be *Nitzschia amphibia* (Kociolek 2011). Strain 3 was an asymmetrical biraphid. The raphe was eccentric and positioned along ventral margin. The dorsal margin of valve was deeper than ventral margin with absent of fascia and the invisible ventral striae. The width of the valves ranged from 10 to 15 μm. Because of the continuous dorsal striae and rostrate valve ends, strain 3 was most likely *Halamphora veneta* (Stepanek and Kociolek 2010).

Preliminary RO concentrate treatment

Figure 5 presents the removal of silica and *o*-phosphate from an RO concentrate sample from the GWRS of the OCWD by the diatom-based photobiological process. Similar to the experiments performed with the brackish agricultural drainage water, successful removal of reactive silica and *o*-phosphate from the RO concentrate was achieved in the 500-mL PETE photobioreactor. Reactive silica and *o*-phosphate removal started to occur within 3 days after the inoculation of diatom biomass. Within 15 days, 120 mg L^{-1} of reactive silica and 17 mg L^{-1} of *o*-phosphate were completely removed in the first semi-batch cycle. In the second cycle, much faster removal of reactive silica and *o*-phosphate was observed. More than 75 % of reactive silica and 90 % of *o*-phosphate were removed within 5 days. In addition to reactive silica and *o*-phosphate, calcium (49 %), iron (>96 %), manganese (81 %), ammonia-N (>99 %), bicarbonate (67 %), and nitrate (6 %) were removed by the photobiological treatment (Table 2). It could be seen that ammonia-N was used preferentially as a source of nitrogen. A minor removal (about 20 %) of organic carbon was also observed. Color was reduced by about 20 % as well. The biomass concentration at the end of the second cycle was 0.66 ± 0.10 g dry weight L^{-1} (standard deviation, $n = 2$).

It should be noted that the diatom biomass used in this preliminary experiment was a mixed culture from the agricultural drainage water without isolation or separation of individual strains. The treatment conditions can be further optimized with respect to the light source, intensity and duration, diatom species, mono or mixed culture, biomass concentration, temperature, pH, and aeration/carbonation. Another RO concentrate sample obtained from the Leo J. Vander Lans Advanced Water Treatment Facility owned and operated by the Water

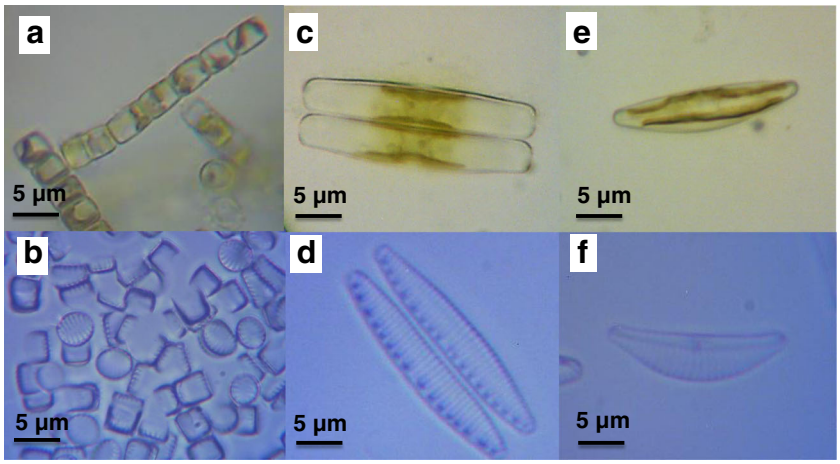

Fig. 4 Three diatom strains isolated from the brackish agricultural drainage water using the 500-mL PETE semi-batch photobioreactors [**a**, **b** strain 1, *Pseudostaurosira* sp.; **c**, **d** strain 2, *Nitzschia* sp.; **e**, **f** strain 3, *Halamphora* sp.; **a**, **c**, **e** live cells; **b**, **d**, **f** digested cells]

Replenishment District of Southern California has been successfully treated as well (data not shown).

Discussion

Silica uptake and other constituent removal

The photobiological process described in this paper was able to remove aqueous silica, phosphorus, nitrogen, and potassium, and several other constituents from the agricultural drainage water and RO concentrate (Tables 1 and 2). Based on the visual observation and microscopic analysis, silica uptake by a mixture of brackish water diatoms native to the evaporation ponds where the drainage water was sampled was evident. Silica is essential for diatoms if cell division is taking place. Since the pioneering work performed by Lewin (1954), numerous studies have been carried out to investigate the silica uptake and diatom growth conditions (Paasche 1973a, 1973b; Martin-Jézéquel et al. 2000; Finkel 2016). Silica is mainly taken up into the diatom cells as silicic acid at the expense of metabolic energy, which is primarily coming from aerobic respiration, while the involvement of photosynthetic energy in the silicificatoin process is minimal (Martin-Jézéquel et al. 2000). In this study, different rates of silica uptake were observed in the semi-batch reactors (Table 4), although the biomass concentrations at the end of the last cycle were comparable (0.45 to 0.66 mg L^{-1} dry weight). In general, once sufficient population of diatom biomass was established, the silica uptake became rapid, although limited numbers of cycles were attempted in the 7.5-L semi-batch reactor run using the agricultural drainage water and the 500-mL semi-batch reactor run using RO concentrate sample. In these experiments, the growth of other algae species, primarily green algae that was present in the agricultural drainage water, became problematic. This was probably one of the most significant factors that affected the relatively slow silica uptake rates in these experiments. More experiments using isolated strains are currently underway.

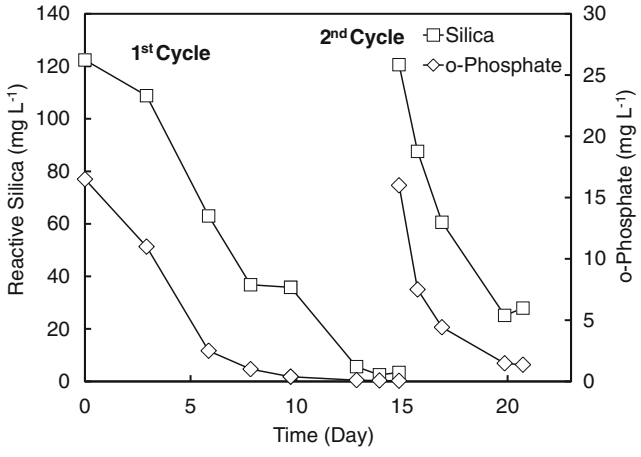

Fig. 5 Removal of reactive silica and orthophosphate from a reverse osmosis concentrate sample from an advanced water reclamation facility in 500-mL PETE semi-batch photobioreactors. Light source 2 × 9-W LED, irradiance 94 μmol photons s^{-1} m^{-2}, temperature 25 ± 1 °C

In addition to the green algae contamination, there might be additional factors affected the growth rate and silica uptake, such as modes of silicic acid uptake, dissolved silica concentration, light, temperature, nutrient concentrations, and trace metal ions (Paasche 1973a; Harrison et al. 1976; Martin-Jézéquel et al. 2000; Gilpina et al. 2004). The irradiance was 37 % lower in the RO concentrate experiment (94 μmol photons m^{-2} s^{-1}). A minor difference in temperature (−2 °C) might have affected the silica uptake rate in the RO concentrate as well. It was suspected that the available number of photons per unit volume for the diatom cells was smaller in the 7.5-L photobioreactor than in the 500-mL ones because the former reactor has white opaque sidewall and the light only entered from the water surface.

In the tested system, phosphorus was the limiting nutrients that depleted first, followed by reactive silica. Nitrogen was

Table 4 Initial reactive silica uptake rates in different experiments performed in this study

Experiment	Initial reactive silica uptake rate (mg L^{-1} h^{-1})			
	First Cycle	Second cycle	Third cycle	Fourth cycle
Agricultural drainage water (500-mL reactor)	0.38	0.78	1.57	1.55
Agricultural drainage water (7.5-L reactor)	0.17	0.18	–	–
RO concentrate (500-mL reactor)	0.61	1.23	–	–

abundant in both the agricultural drainage water and RO concentrate samples. The RO concentrate was more phosphorus rich and the silica to phosphorus (measured as *o*-phosphate) to nitrogen (measured as ammonia and nitrate) molar ratio was 1:0.09:2.2 whereas the ratio was 1:0.05:5.2 in the agricultural drainage water. However, in RO concentrate, *o*-phosphate was depleted first and silica uptake seized for a brief period of time from the eighth until tenth days in the first cycle (see Fig. 5); then, the silica removal resumed and a complete (>99 %) silica removal was achieved. This might be due to the presence of ammonia in RO concentrate, which was utilized first (Table 2). This phenomenon was repeatable when different RO concentrate samples were tested (data not shown). Since it is still unclear how the Si/P/N ratio and the forms of nitrogen affect the silica uptake, more experiments using more controlled culture conditions would be needed to optimize the silica removal in the photobioreactors. It is also desirable to screen different species of brackish water diatoms for silica removal efficiency and compare their silica uptake kinetics (Michaelis-Menten or Monod functions) with published values (Paasche 1973b; Martin-Jézéquel et al. 2000).

In addition to silica, *o*-phosphate, ammonia, and nitrate, a number of dissolved water constituents, such as calcium, bicarbonate, iron, and manganese, were effectively removed in this photobiological process (Tables 1 and 2). As discussed in the "Results" section, bicarbonate and calcium was probably removed via precipitation of calcium carbonate ($CaCO_3$) as calcite or aragonite (Borowitzka 1987) due to the removal of carbon dioxide (CO_2) by algal photosynthesis:

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 \downarrow + H_2O + CO_2 \uparrow \quad (1)$$

Dissolved iron and manganese were likely oxidized by a high concentration of dissolved oxygen due to photosynthesis either chemically or biologically and adsorbed by the biomass or precipitated. Some portions of iron and manganese were probably utilized by algae.

Implications for desalination and water reuse

As discussed earlier, dissolved and colloidal silica constitutes one of the most difficult challenges facing RO desalination and advanced water reclamation because it precipitates on the membrane surface, causes increasing pressure across the membrane, and reduces the permeate flow rate and water recovery (Asano et al. 2007; Ning 2002). In typical RO system designs, a silica saturation concentration of 120 mg L^{-1} is typically used (e.g., Hydranautics IMS Design Software). Assuming the feed silica concentration of 20 mg L^{-1} and the silica rejection rate of ~100 % by RO, silica precipitation will become a problem when the permeate recovery rate is higher than 83 % based on the following equations:

$$\begin{aligned}\text{Permeate recovery}(\%) &= Q_{\text{permeate}} \div Q_{\text{feed}} \times 100\% \\ &= (Q_{\text{feed}} - Q_{\text{concentrate}}) \div Q_{\text{feed}} \times 100\% \\ &= (1 - Q_{\text{concentrate}} \div Q_{\text{feed}}) \times 100\%\end{aligned} \quad (2)$$

$$[\text{SiO}]_{\text{concentrate}} = [\text{SiO}_2]_{\text{feed}} \times Q_{\text{feed}} \times \text{rejection rate} \div Q_{\text{concentrate}} \quad (3)$$

$$\alpha = [\text{SiO}_2]_{\text{concentrate}} \div [\text{SiO}_2]_{\text{feed}} \div \text{rejection rate} = Q_{\text{feed}} \div Q_{\text{concentrate}} \quad (4)$$

where Q_{permeate}, Q_{feed}, and $Q_{\text{concentrate}}$ are the flow rates of RO permeate, feed, and concentrate streams, respectively; $[\text{SiO}_2]_{\text{concentrate}}$ and $[\text{SiO}_2]_{\text{feed}}$ are the silica concentration in the concentrate stream and RO feed, respectively; the rejection rate is the rejection of silica by RO (~1 or 100 %); and α is a concentration factor. For the maximum permeate recovery, the concentration factor shall be:

$$\alpha = 120 \text{ mg L}^{-1} \div 20 \text{ mg L}^{-1} \div 1 = 6 \quad (5)$$

From Eqs. 2 and 5,

$$Q_{\text{concentrate}} \div Q_{\text{feed}} = 1 \div \alpha = 1 \div 6 = 0.167 \quad (6)$$

$$\text{Maximum permeate recovery}(\%) = (1 - 0.167) \times 100\% = 83.3\% \quad (7)$$

Silica precipitation may limit the permeate water recovery when the feed water contains a high level (>30 mg L^{-1}) of silica, which is very common in brackish groundwater and

treated wastewater (Gallego et al. 2008). In order to increase the permeate recovery in such a situation, several approaches may be used, such as alkaline injection to adjust the feed water pH to 10–11, application of antiscalants to prevent precipitation and inhibit silica polymerization, and the pretreatment to remove silica prior to RO desalination (Ning 2002; Gallego et al. 2008). This diatom-based photobiological process can be used as an efficient and cost-effective pretreatment alternative for the removal of aqueous silica from feed water and increase the permeate water recoverability.

One of the potential advantages of this algal process over other approaches described above is that it can be used to treat RO concentrate that may contain up to 120 mg L^{-1} of silica, in conjunction with other treatment process such as cation exchange for additional hardness removal, to recover more water in the second RO process (Fig. 6). This application may be more feasible than pretreating the feed water because TDS and salinity can be high (>2000 mg L^{-1}, if the feed water contains 500 mg L^{-1} of TDS and RO is working at >75 % recovery) in the concentrate flow from the first RO and can be more suitable for brackish water diatoms. In addition, the concentrate tends to contain high levels of nutrients, such as phosphate and nitrate, which may be used by in the photobiological assimilation of aqueous silica by diatoms.

In addition to silica and orthophosphate, this algal process can remove calcium, iron, and manganese, which are also known scalants in RO process (Asano et al. 2007). This is an additional benefit of this diatom-based treatment of RO concentrate. Although COD did not change significantly (see Tables 1 and 2) due to this treatment, color measured at 455 nm increased or decreased depending on the feed water. This indicates that algal growth may be producing and/or modifying the composition of dissolved organics, which are another source of membrane fouling. In addition, the possible generation of organic particulate matter, such as transparent exopolymer particles (TEP) and protein-containing particles, was suspected. This organic particulate matter is known to be released by algae including diatoms (Grossart et al. 2006). Recently, TEP have been recognized as a cause of membrane fouling in seawater RO affected by harmful algal blooms (Caron et al. 2010; Villacorte et al. 2013). Thus, organic by-product production during this photobiological silica removal process needs to be studied further and a control strategy may need to be developed.

Conclusions

The results of this work demonstrated the use of diatom-based photobioreactors for silica removal in water and wastewater treatment for the first time. Multiple-cycle semi-batch treatment was successfully performed in 500-mL and 7.5-L photobioreactors using a brackish agricultural drainage water from the Central Valley of California as a source of diatoms. In one experiment, 39 mg L^{-1} of total silica in the drainage water was completely removed within 40 h. Several other water constituents, including *o*-phosphate, calcium, manganese, bicarbonate, and nitrate, were removed by 95, 34, 25, 26, and 19 %, respectively, in this process. A microscopic analysis of isolated and digested cells confirmed the presence of several diatom species, including *Pseudostaurosira* sp. (likely *P. trainorii*), *Nitzschia* sp. (likely *N. amphibia*), and *Halamphora* sp. (likely *H. veneta*). A mixture of the brackish water diatoms obtained from the agricultural drainage water was also successfully used for the treatment of silica-rich RO concentrate samples from advanced water reclamation plants in Southern California. More than 75 % of silica and 90 % of *o*-phosphate were removed in 5 days. Additional inorganic cations, including calcium (49 %), iron (>96 %), and manganese (81 %), which also cause RO scaling, were effectively removed by the photobiological process. Further research is needed, and some is currently underway, to optimize and explore this unique algal process, such as the effect of water constituents (e.g., salinity, initial silica concentration, dissolved carbon dioxide and bicarbonate, macro- and micro-nutrient levels and their ratios, and presence of heavy metals and other toxic substances) and cultivation conditions (e.g., pH, light source, intensity, and duration, and biomass concentrations), as well as the characteristics of dissolved and particulate organic by-products. This new algal process has a potential for application in desalination and water reuse.

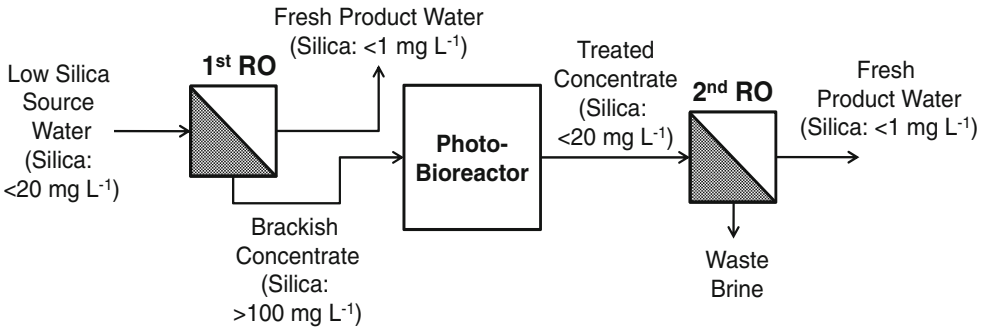

Fig. 6 Conceptual flow scheme of the enhanced-recovery RO desalination using the diatom-based photobioreactors

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Conflict of interest Two of the authors (Keisuke Ikehata and Andrew T. Komor) have applied for a US patent (application no. 20120175301) based on this discovery. The authors declare that there is no other conflict of interest.

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