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Simultaneous biomass production and water desalination concentrate treatment by using microalgae

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ABSTRACT

Environmental effects associated with concentrate disposal have restricted the practical deployment of desalination technologies for inland brackish water, reducing the ability of desalination to alleviate global water shortages. In order to increase the feasibility of deploying desalination processes for inland brackish water sources, a beneficial use for concentrate from inland desalination systems is required. This study purposed the idea of microalgae cultivation in the concentrate stream to solve problems associated with desalination while simultaneously meeting energy needs by providing feedstock for biofuel production. A full factorial experiment was conducted in which two species of algae (*Nannochloropsis oculata* and *Dunaliella tertiolecta*) were cultivated in three different media (concentrate, f/2, and a 50:50 combination of f/2 and concentrate) to investigate the ion removal ability of microalgae from concentrate and examine how well they can grow in the concentrate compared to other conventional media. Based on experimental data, concentrate was found as a better medium for biomass production relative to the conventional f/2 medium. Combination of the concentrate medium with *Dunaliella tertiolecta* produced the highest dry biomass. Furthermore, the contribution of both species of algae to nitrogen, phosphate, and fluoride removal was significant.

Keywords: Concentrate; Desalination; Microalgae; Water treatment; Biomass

1. Introduction

Fresh water is finite and only makes up a small fraction of all water on the earth. Therefore, water reuse and desalination of salty water sources have received attention to address water scarcity [1]. Brackish groundwater is available as a significant resource in many inland and dry places, and has satisfied water demands for different purposes in many countries around the world. In the United States, more than 95% of desalination plants use inland brackish water sources [2]. Since the early 1960s, desalination plants have been constructed and produced a considerable amount of drinking water. In most new designs, membrane processes have been utilized instead of thermal processes. Among membrane processes, reverse osmosis (RO) is the most common method [3] and expected to be expanded notably soon [4]. Despite the substantial potential of desalination methods to alleviate global water shortages, an important environmental and financial problem associated with the process is disposal of the saline waste stream from the process, called concentrate or brine [5–7]. Discharging the concentrate of seawater desalination units has been considered a potential threat to the marine environment by changing the pH value and increasing

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the salinity, temperature, and concentration of heavy metals and antiscalant compounds in the receiving water. Toxicological investigations on discharge sites reveal that eutrophication adversely impacts the marine ecosystem, including seagrass, coral reef, and soft-sediments [8,9]. For inland desalination facilities, the most common methods for disposing the concentrate are deep well injection, surface water discharge, and evaporation ponds [5–7]. The salinity of brines obtained from inland desalination units is lower than from seawater desalination facilities, which makes them more environmentally friendly. However, since the brine of inland desalination units cannot be discharged to seawater, a new concentrate management method is required [8].

The composition and characteristics of a concentrate stream highly depend upon the quality of the feed water and the recovery efficiency of the desalination technique [8]. Since groundwaters commonly contain high concentrations of nitrate and phosphate, mainly due to the usage of fertilizers [10], brines often have a high nitrogen and phosphorus content. One approach to dilute or remove these contaminants is to use concentrate as a medium for microbial growth. Cultivation of microalgae in concentrate may be a feasible way to solve problems associated with desalination while simultaneously meeting energy needs by providing feedstock for biofuel production. Microalgae are fast growing photosynthetic organisms which have the following advantages over conventional biofuel feedstocks: ability to fix CO_2 from industrial flue gases; ability to thrive in highly saline water; pollutant removal capability; and high lipid, carbohydrates, and proteins content [11–14]. Marine algal species are better choices over freshwater species for cultivation in brine as they are adapted to the environments with elevated salinities. Several studies have been undertaken on growing microalgae in wastes from municipal sewage and agricultural manure as a source of nutrients [15–19]. Results showed that microalgae can efficiently grow and remove ions such as nitrate. However, none of these studies discussed the treatment of desalination concentrate.

To examine how well microalgae can grow in desalination concentrate compared to other conventional media, a full factorial experiment with a completely random design arrangement was conducted in which two algal species (*Nannochloropsis oculata* and *Dunaliella tertiolecta*) were cultivated in three different media (concentrate, f/2, and a 50:50 combination of f/2 and concentrate). Although *Chlorella* species have been conventionally used for wastewater treatment due to the efficient nutrient removal [20], for simultaneous water treatment and biomass production, other important factors such as growth rate, algal cell density, and lipid content also must be considered for algal species selection to ensure the biofuel production potential. *Nannochloropsis oculata* and *Dunaliella tertiolecta* are known from marine environments with high salinity [21,22]. *Nannochloropsis oculata* has been considered as a suitable candidate for biofuel production due to its fast reproduction and high oil content, ranging from 31 to 68% of dry weight [13,23]. *Dunaliella tertiolecta* is a very fast growing unicellular green algae species with a high CO_2 fixation rate with oil content of approximately 40% of dry weight [24]. These algal species were selected due to their tolerance of the saline environments, high growth rates, and high lipid contents.

2. Materials and methods

2.1. Algal species and media

Two species of microalgae, *Nannochloropsis oculata* (UTEX- LB 2164) and *Dunaliella tertiolecta* (UTEX-LB 999) were obtained from Culture Collection of Algae at the University of Texas.

Three different media were used: concentrate, f/2, and a 50:50 combination of f/2 and concentrate (50:50). The concentrate was obtained from the RO water desalination process at the Brackish Groundwater National Desalination Research Facility (BGNDRF) in Alamogordo, New Mexico. The f/2 medium [25] is a common enriched seawater medium designed for growing marine algae. Total Dissolved Solids (TDS), Electroconductivity (EC), and pH of all media are available in Table 1. Total nitrogen (TN) content and concentration of some ions vital for algal biomass growth in the concentrate are shown in Table 2.

2.2. Experimental design

A full-factorial design was constructed for testing the various combinations of two types of microalgae and three media, which resulted in six combinations of microalgae and medium (2 × 3). Considering four replications for each combination, 24 runs were conducted to provide the required data. The experimental setup for this study is shown in Fig. 1. The resulting data were analyzed using a General Linear Model (GLM) procedure. Assumptions were checked using SAS 9.1.3. Means were compared using Tukey's Test ($P < 0.05$).

Table 1
Specifications of four media

Medium	pH	EC (μS/cm)	TDS (mg/l)
Concentrate	7.83	10260	6240
f/2	6.97	113.2	59.8
50:50	7.55	5660	3310

Table 2
Total nitrogen content and ion concentrations of concentrate

Ion	Concentration (mg/L)
TN	22.88
K^+	32.93
Na^+	1936.8
Mg^{2+}	608.6
Ca^{2+}	495.25
F^-	16.32
Cl^-	2789.2
SO_4^{2-}	4729.78
PO_4^{3-}	21.9

Fig. 1. Experimental setup for studying growth of algae in concentrate, f/2, and 50:50 media.

2.3. Microalgal cultivation

To avoid any contaminations, all glassware was washed and rinsed with distilled water, and then autoclaved. To autoclave glassware, a SANYO MLS-3751L was used. Both species were cultivated in f/2 for three weeks prior to the experiments to obtain stable characteristics. After preparation of pre-cultures, algae species were inoculated in photobioreactors containing 400 mL of medium. An Eppendorf 1–50 ml pipette was used for inoculation and transfer of algae. Batch cylindrical glass UTEX photobioreactors with working volumes of 500 ml were placed randomly in the racks under 16 h of illumination and 8 h of darkness at 30 ± 2°C for 10 d while air with a volumetric flow rate of 5 ml/s entered each photobioreactor via the air hose inserted through the lid. Each photobioreactor was equipped with five air delivery modules, a water trap, a Fusion Air Pump 200 (1.5 W), an air stone, and one additional access port for sampling and measurements. The lighting device used consisted of four GE, F40PL/AQ-ECO, wide-spectrum, 40 W florescent tubes with a 3100 K color temperature, producing 1900 lumens for each rack. The average distance from the florescent tubes to the top of photobioreactors was 25 cm. The floor of each rack was covered with aluminum foil to enable light from the bottom of each rack to reflect to the underbelly of the photobioreactor.

2.4. Analytical methods

The initial dry weight biomass was defined by taking samples 12 h after the inoculation. To measure dry weight biomass, a 50-ml sample of culture suspension was taken. The sample was transferred to a pre-weighed 50 ml plastic tube. All weights were measured using an Acculab AL-204 scale with an accuracy of +/− 0.0001g. The plastic tube, with algal culture content, was centrifuged for 3 min at 10,000 rpm, after which the supernatant was extracted. An Eppendorf 5804 centrifuge was used to isolate biomass from the medium. Since the dry weight, especially for marine algae, is heavily affected by the salts and nutrients absorbed on the cell surface, the centrifuged content was rinsed with deionized water, based on the suggestion by Lee and Shen [26], to reduce the error in determining the dry weight biomass. Subsequently, the tube was centrifuged again at 10,000 rpm for 3 min after rising with deionized water. The clear supernatant was discarded and the tube containing the biomass was dried in the oven at 80°C for 24 h. A Fisher vacuum oven was used to dry wet biomass. To prevent loss of volatile components in algae cells, the temperature was maintained below 90°C. The dry biomass was determined by the difference between the initial weight and the final weight of the tube. The same procedure was repeated on the final day to determine final dry weight biomass.

For cultures in the concentrate medium, pH, optical density at 750 nm, TDS, EC, and TN were measured daily. Furthermore, ion content was measured on the first and last days of the experiment. The pH was measured using an Accumet AB15/15+ pH meter. Before taking each pH sample, the pH meter was calibrated with the standard solution with pH of 7. Optical density was measured by a HACH DR 5000 Spectrophotometer. A sensION5 Conductivity Meter was used to measure TDS and EC. The total nitrogen (TN) analysis was conducted at the Freeport-McMoRan Water Quality Lab at New Mexico State University with a SHIMADZO TNM-1, which uses the chemiluminescence measurement method to determine TN [27]. Ion content was analyzed using the DIONEX ICS-3000 Ion Chromatography System.

3. Results and discussion

3.1. Biomass production

Two species of microalgae, *N. oculata* and *D. tertiolecta* were used to compare growth of these microalgae in concentrate, with common f/2 medium, and a 50:50 combination of both. Growth rates of microalgae in different media are available in Table 3. Fig. 2 shows that the effect

Table 3
Growth rates of microalgae

Microalgae species	Medium	Concentration (g/l)		
		Initial	Final	μ = Specific growth rate (d^{-1})
D. tertiolecta	Concentrate	0.05224 ± 0.0	0.3576 ± 0.11	0.19
D. tertiolecta	f/2	0.05224 ± 0.0	0.1734 ± 0.02	0.12
D. tertiolecta	50:50	0.05224 ± 0.0	0.211 ± 0.02	0.14
N. oculata	Concentrate	0.04345 ± 0.0	0.2782 ± 0.01	0.18
N. oculata	f/2	0.04345 ± 0.0	0.1118 ± 0.03	0.09
N. oculata	50:50	0.04345 ± 0.0	0.2138 ± 0.02	0.16

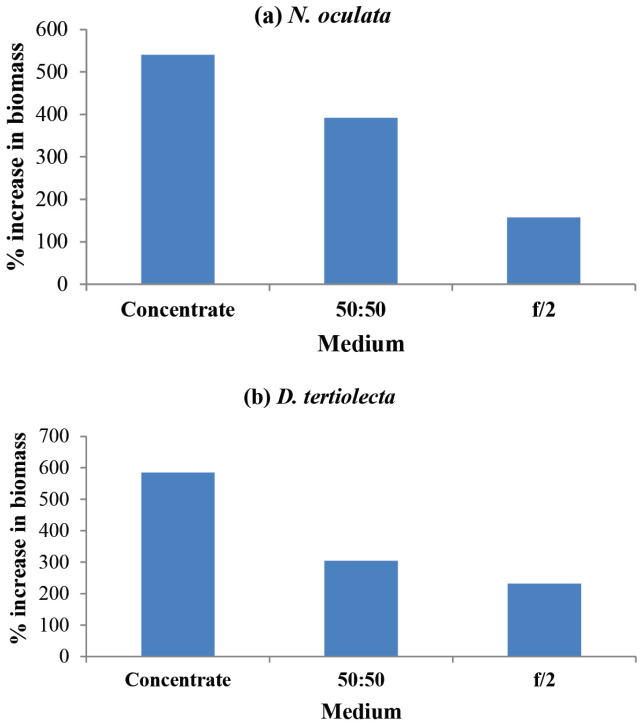

Fig. 2. Percentage increase in biomass in different media: (a) *N. oculate*, (b) *D. tertiolecta*.

of medium on biomass production was significant. For both species, concentrate was the medium that maximized the biomass production, followed by 50:50 and f/2. This indicates that the high concentrations of nitrogen, phosphate, and NaCl in the concentrate provided a better environment for these species to grow compared to f/2. The 50:50 medium performed better than f/2 because the nutrients available in concentrate could still contribute to algae growth when the nutrients in f/2 were diminishing. This is consistent with the results from Ridley, et al. [28] in which five marine microalgae produced more biomass in modified f/2 media supplemented with the appropriate amount of brine wash (as a rich source of nitrate) compared to neat f/2.

The full factorial experiment with two levels for algae and three levels for medium considered the interaction of these two factors. No significant difference in biomass production between *N. oculata* and *D. tertiolecta* was observed (P-value = 0.35), while the effect of medium was notable, as shown in Fig. 3 (P-value < 0.0001). No significant difference was observed among interactions (P-value = 0.2470). The combination of the concentrate medium with *D. tertiolecta* produced the highest dry biomass. Combination of the concentrate medium with *N. oculata* was also substantial. Combination of 50:50 medium with both algal species yielded considerable amounts of dry biomass, but significantly less than the biomass produced in the concentrate medium.

Figs. 4 and 5 depict the growth curves of both algae species in three different media during the ten-day experimentation. In the case of *D. tertiolecta*, during the first three days (lag phases), the growth was slow in all

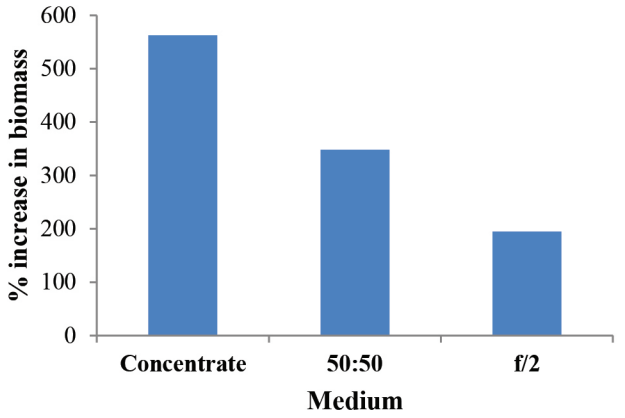

Fig. 3. Effect of medium on dry weight biomass production (P-value < 0.0001).

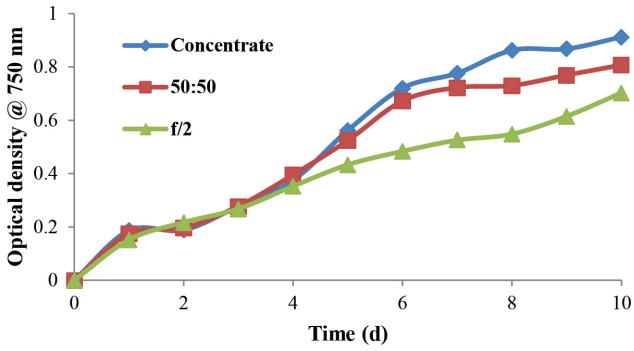

Fig. 4. Growth curves of *D. tertiolecta* in three different media.

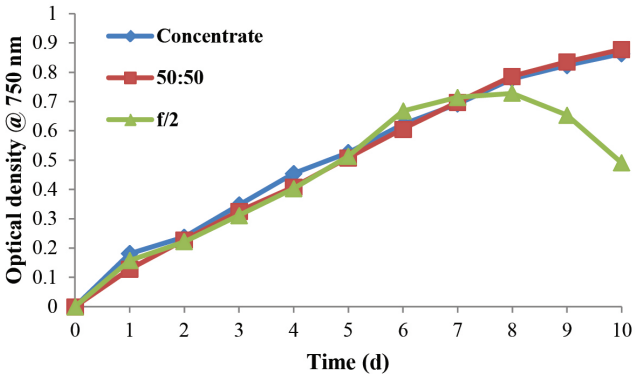

Fig. 5. Growth curve of *N. oculata* in three different media.

media. However, on the fourth day, the cultures began their exponential phases and the differences between media manifested themselves. In the exponential phase, the growth rates in the concentrate and 50:50 media were significantly higher than f/2 medium due to the high nitrogen and phosphate content of the concentrate. On the eighth day, the algae growth in the concentrate slowed, mainly because of nutrient deficiency. The role of light was also important at this stage because the cultures in the concentrate and 50:50 media became very dense and turbid, which inhibits

light penetration, especially in the middle of the reactor [29]. However, since the f/2 cultures did not become overly dense, light penetration was more convenient. It is anticipated that the continuation of the experiment for a few more days would have resulted in a similar outcome for f/2 cultures. In the case of *N. oculata*, the vertex point occurred on the seventh day of the experiment for f/2, indicating that the nutrients in f/2 were diminishing. The lack of nutrients in f/2 media caused the stationary phase to be approximately one day, a duration that, compared to the other media, was considerably short. However, *N. oculate* in the concentrate and 50:50 media remained in the exponential phase and kept growing up to the tenth day because concentrate and, accordingly, 50:50 are nutrient rich media and microalgae can still grow.

3.2. Ion removal from the concentrate

Nitrogen is the main nutrient that supports the reproduction of microalgae cells [30]. In a batch system, the nitrogen concentration in growth media is eventually depleted without new inputs and remains at a level that only supports the synthesis of enzymes and critical cell formation [31]. Under this condition, available carbons are converted into lipids rather than proteins, which slows algal growth because proteins are necessary for continued algal growth. Microalgae collect useful nitrogenous compounds in the biomass, which is safer ecologically compared to the other bacterial nitrogen removal methods in which most of the nitrogen is removed as nitrogen gas [32–35]. Fig. 6 shows total nitrogen (TN) concentration over the period of the experiment for both algae in the concentrate. This graph reveals that both species significantly reduced the nitrogen levels in the concentrate. The nitrogen removal yield by *D. tertiolecta* and *N. oculata* were 0.93 and 0.91, respectively. Given the negative impacts on water quality, wildlife, and humans resulting from excess levels of nitrogen (especially nitrate) in the concentrate disposal pond [36], these results seem to be promising.

In addition to nitrogen, the concentration of certain ions in the concentrate medium was measured on the first and final days. Fig. 7 and Table 4 show the initial concentration of these ions and their removal yield by two species of algae. The contribution of both algae to fluoride,

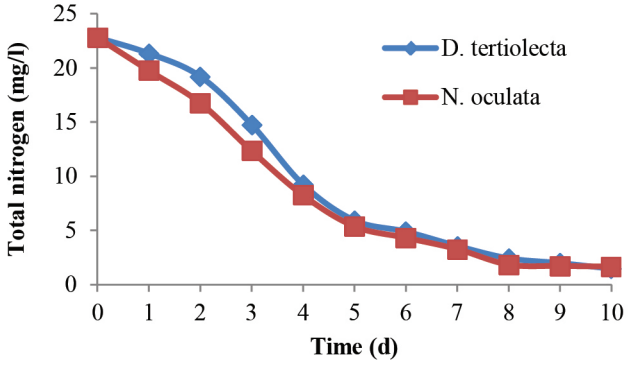

Fig. 6. Total nitrogen concentration over the period of the experiment for both algae in the concentrate medium.

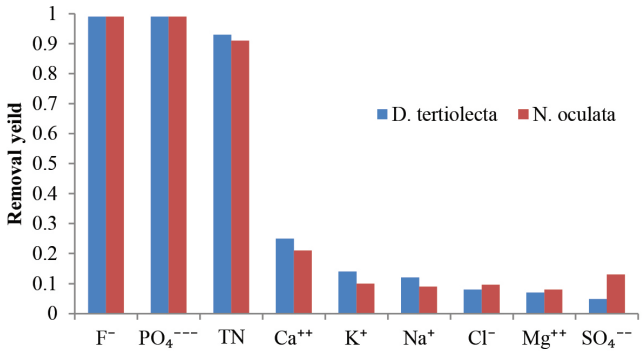

Fig. 7. Ion removal yield from concentrate by two species of algae.

nitrogen, and phosphate removal was significant. A slight difference in ion removal yield between the two algae can be explained by the following: the elementary composition and C:P:N ratio of microalgae cells usually varies with the species type [37]. Thus, the ability to absorb nitrogen and phosphorous may be different for different species of microalgae. In marine algae, the molecular ratios of carbon, phosphorus, and nitrogen allow the algae to grow quickly by uptaking the nutrients available in wastewater and salty water [37]. This uptake can occur quickly in bodies of water with high concentrations of N and P [37], such as the concentrate of water desalination units used as the medium in this experiment.

Fig. 8 shows that the TDS of the concentrate was decreased by both algae species. Mainly ions such as potassium, chloride, sodium, calcium, and sulphate are responsible for high TDS [38]. Since *D. tertiolecta* removed calcium, sodium, and potassium ions more than *N. oculate* (Table 4 and Fig. 7), the TDS was decreased more with *D. tertiolecta*.

4. Conclusion

Given the negative environmental impacts associated with discharging of desalination brine, an appropriate management technique for efficiently using brine is of

Table 4
Initial concentration of important ions and their removal yield by two species of algae

	Initial concentration (mg/L)	Removal yield of *N. oculata*	Removal yield of *D. tertiolecta*
F^-	15.2	≈1	≈1
Cl^-	2754.2	0.1	0.09
SO_4^{2-}	3598.3	0	0.1
PO_4^{3-}	20.8	≈1	≈1
K^+	29.6	0.1	0.1
Na^+	1987.6	0.1	0.09
Mg^{2+}	595.4	0.1	0.1
Ca^{2+}	445.4	0.2	0.2

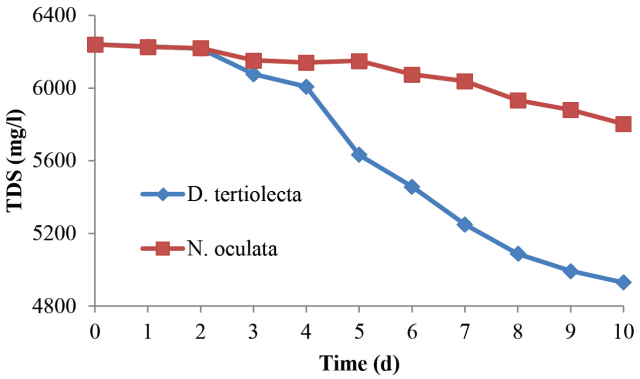

Fig. 8. TDS of two algae cultures in concentrate medium during the experiment.

interest. Cultivation of marine algae species in the waste concentrate of water desalination units could be a promising approach to remove pollutants, while also producing feedstock for biofuel production. Based on the results, the concentrate medium showed the highest increase in biomass, even more so than f/2, which is a conventional medium for growing marine algae. The contributions of both microalgae species, *N. oculata* and *D. tertiolecta*, to biomass production, ion removal, and TDS reduction were significant. In conclusion, this approach can be considered as a potential way to reduce the cost of desalination by creating revenue from biofuel production with positive environmental benefits, such as CO_2 mitigation and concentrate disposal treatment.

Acknowledgments

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