Evaluation of high salinity adaptation for lipid bio-accumulation in the green microalga *Chlorella vulgaris*

Adel W. Almutairia,⇑ Abo El-Khair B. El-Sayedb, Marwa M. Redac

*a Biological Sciences Department, Rabigh-Faculty of Science & Arts, King Abdulaziz University, Saudi Arabia
b Algal Biotechnology Unit, National Research Centre, Egypt
c Central Lab for Environmental Quality Monitoring, National Water Research Center, Egypt

**Abstract**

Aiming at the reutilizing wastewater for algal growth and biomass production, a saline water rejected from reverse osmosis (RO) facility (salinity 67.59 g L⁻¹) was used to cultivate the pre-adapted green microalg *Chlorella vulgaris*. The inoculum was prepared by growing cells in modified BG-11 medium, and adaptation was performed by applying a gradual increase in salinity (56.0 g L⁻¹ NaCl and 125 ppm FeSO₄·7H₂O) to the culture in 200 L photobioreactor. Experiments using the adapted alga were performed using original-rejected water (ORW) and treated rejected water (TRW) comparing with the recommended growth medium (BG-11). The initial salinity of ORW was chemically reduced to 39.1 g L⁻¹ to obtain TRW. Vertical photobioreactors (15 L) was used for indoor growth experiments. Growth in BG-11 resulted in 1.23 g L⁻¹, while the next adaptation growth reached 2.14 g L⁻¹ of dry biomass. The dry weights of re-cultivated *Chlorella* after adaptation were 1.49 and 2.19 g L⁻¹ from ORW and TRW, respectively. The cellular oil content was only 12% when cells grown under control conditions verses to 14.3 and 15.42% with original and treated water, respectively. Induction of stress affected the fatty acid methyl esters (FAMEs) profile and the properties of the resulting biodiesel. The present results indicated that induction of stress by high salinity improves the quality of FAMEs that can be used as a promising biodiesel fuel.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Freshwater ecosystems worldwide have experienced increasing salinity mainly as a result of long-term droughts, overly intensive agriculture, rising seawater levels and inappropriate water management strategies (Canedo-Arguelles et al., 2018). Water with high salinity can adversely affect habitats and livestock, although some-aquatic plants, halophytes and microorganisms can survive under such conditions (Hou et al., 2017). Although, second-generation biofuel feedstock doesn’t compete with human food or land, the costly pretreatment is the main disadvantage (Elayed et al., 2018, 2019). In recent years, microalgae have received increasing attention as a sustainable feedstock for biofuel, that can grow in wastewater or saline water without competing with human resources (Abomohra et al., 2019, 2021; Wang et al., 2020; Touliabah and Almutairi, 2021). Extensive research has been carried out to enhance lipid accumulation in microalgae in order to enhance the economic feasibility of biodiesel production. Such strategies include selection and improvement of microalgal strains (Sheehan et al., 1998), metabolic engineering (Sun et al., 2019), salinity optimization (Abomohra et al., 2020a), plant growth regulators (Esakkimuthu et al., 2020), CO₂ supplementation (Tu et al., 2019), and magnetic field stress (Shao et al., 2018). However, extreme high salinity of water adversely affects microorganisms, mainly microalgae, because it inhibits their growth and production of pigments and oils increases protein decomposition, and alters the accumulation of specific bio-active substrates. Algae can be grouped into halophilic and halotolerant based on their tolerance to salt, and they all produce metabolites both for protection from salt injuries and to equilibrate with the surrounding osmotic pressure (Abomohra et al., 2020b). However, many species of microalgae are tolerant to great variations in salinity, and their chemical composition can be affected (Ding et al., 2013). Organisms that

https://doi.org/10.1016/j.jsjbs.2021.04.007
1319-562X/© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
can grow under unfavorable conditions, including saline water, are promising sources of chemical compounds valued by humans (Arora et al., 2019). Indeed, although salinity greatly influences macroalgal growth, many efforts have been made to reduce its impact. The physiological response of microalgae varies among species, and some freshwater microalgae can grow well under marginally saline conditions. For example, Botryococcus sp. can survive in 3.0 M NaCl and has maximal growth at 0.25 M NaCl (Vazquez-Duhalt and Arredondo-Vega, 1991). In addition, a halo-philic Dunaliella salina KSA-HS022 was isolated from a hypersaline lagoon with salinity average of 45.3‰ and was able to grow at elevated salinity up to 250‰ (Abomohra et al., 2020b). High salinity is known to cause both hyper-ionic and hyper-osmotic effects in plants, leading to membrane disorganization, metabolic toxicity and increase of reactive oxygen species (ROS). Thus, DNA mutation, protein denaturation, lipid peroxidation and chlorophyll bleaching as well as the loss of membrane integrity could potentially damage the function of photosystems (Leshem et al., 2007; Almarashi et al., 2020). Salinity stress affects algae and plants through osmotic and ionic stresses. Water deficit can lead to increased osmotic stress due to the increased concentration of Na⁺ and Cl⁻ that subsequently negatively affects the uptake of other mineral nutrients causing ionic imbalances or stress (Ashraf and Harris, 2004). Several plant metabolites were recognized to be used as growth stimulators in which help plant as well algae to overcome adverse effect of biotic and abiotic stress. For instance, nitrogen containing compounds such as amino acids, amides, protein, and quaternary ammonium compounds have been found helpful in osmoregulation (Grumet et al., 1985) and in tolerance of ion toxicity under salt stress (Turhan et al., 2008). Recently, Esakkimuthu et al. (2020) evaluated the application of p-coumaric acid as a suggested novel growth regulator found predominantly in phenolics-rich streams to enhance the biodiesel yield from microalgae, which showed positive impact on biomass and lipid accumulation to overcome the stress conditions. The way these compounds are accumulated is species-specific and spanning from only one to several different compounds (Teixeira and Pereira, 2007).

The production of osmo-protectants or compatible solutes, such as mannitol and proline, can aid the cell to uptake water from the surrounding environment by lowering the internal cell water potential. The later is synthesized intracellularly from glutamate using ATP and NADPH. The trade-off of the energy-demanding process of maintaining proper osmotic conditions is manifested in the reduced growth rates and decreased photosynthetic electron transport activities (Annan, 2014). Photosynthetic organisms possess a number of various antioxidative enzymes that are involved in the detoxification of ROS and the avoidance of damage under salt stress (Hediyse Sekmen et al., 2007). Organisms with high levels of constitutive and induced antioxidants have been reported to have greater resistance to oxidative damage (Shi and Bao, 2007). ROS cause lipid peroxidation and production of highly toxic lipid derivatives, which in turn can modify cell functions and even may lead to cell death (Marnett, 2000; Almarashi et al., 2020).

The use of microalgal cultures as a source of chemical energy products for biofuel feedstocks, including biodiesel (Gomaa, 2018; El-Sheekh et al., 2019; Gomaa et al., 2019; Almutairi et al., 2020), alcohols (El-sayed et al., 2017), methane (Geng et al., 2020), hydrogen (Sharma and Arya, 2017), and crude bio-oil (Wang et al., 2018) was previously evaluated and confirmed as a promising approach. Among different studied microalgal species, C. vulgaris cells can accumulate lipid up to 56% dw when grown under stress conditions (Ilmarinen et al., 2000), which are appropriate feedstock for high-quality biodiesel production (Han et al., 2016). Microalgae are renewable eco-friendly feedstocks as they fix CO₂ that might be useful in reducing the level of the greenhouse gases (Darda et al., 2019; Abomohra et al., 2020b). In recent years, the production of fuel from microalgae has received a great attention due to the increasing energy prices, greenhouse gases emissions, and depletion of fossil fuels (Xiong et al., 2008; Damiani et al., 2010). However, evaluation of the biodiesel potential from microalgae grown on wastes is necessary to obtain a cost-effective microalgal biomass for commercial scale production (Chisti, 2008; Abomohra et al., 2016). Hence, the high cost of biodiesel production and the low lipid content of microalgae are the main bottlenecks for commercial applications (Chi et al., 2019). One of most efficient and economical approaches towards reducing the total cost of biodiesel production is by enhancing lipid accumulation in the microalgal cells by growth on waste- or saline-water (Yahya et al., 2018).

Optimization of growth conditions were reported as effective methods to increase microalgal lipids such as nitrogen limitation, high salinity, high light intensity, static magnetic field, ionized radiation, and high temperature (Converti et al., 2009; Small et al., 2012; Almutairi, 2020a, 2020b; Rugnini et al., 2020). However, the simultaneous occurrence of both high lipid productivity and content is difficult to achieve because the biomass productivity of microalgae is low when the concentration of nutrient is low (Xin et al., 2010). Nevertheless, very few microalgal oils are satisfactory for biodiesel production (Guschina and Harwood, 2006). Gopinath et al. reported that biodiesel produced from unsaturated fatty acids (FA) emits more nitrogen oxides with thermal efficiency lower than the biodiesel produced from saturated FA (Gopinath et al., 2010). The nature of the FA and the alcohol used for fatty acid esters preparation influences the properties of the biodiesel (Abomohra et al., 2020b). Therefore, evaluation of the properties of microalgal oils and FA is essential when trying to develop a suitable biodiesel fuel.

Growth of C. salina was enhanced progressively by increasing salt levels up to 1 M NaCl, where it reached 2-folds of the control value and was approximately comparable to the control at 2 M NaCl. Lipid peroxidation as an oxidative stress parameter showed a marked and progressive increase due to elevation of salinity of C. vulgaris medium reaching about 61% over the control at 0.8 M NaCl. In C. salina, the amount of malondialdehyde (MDA) content decreased up to 1.0 M NaCl and increased marginally by about 17% at 2 M NaCl. In reverse osmosis technique, rejected water contains all of the initial salt of original water and the environment hazarded concerning dilution and safe discarding not fully functioned. Therefore, the objective of this study was to determine the potential of reutilizing rejected water of reverse osmosis process by the green microalga Chlorella vulgaris as a feedstock for biodiesel production.

2. Materials and methods

2.1. Microalga and inoculum preparation

The freshwater microalga C. vulgaris (Chlorophyceae) was provided by the Algal Biotechnology Unit, National Research Centre, Cairo, Egypt. Then cultured to obtain the proper inoculum using modified BG-11 growth medium (Supplementary data) containing 17.6 mM nitrogen (Stanier et al., 1971). All experiments were performed at a nitrogen concentration of 17.6 mM, and urea concentration of 0.53 g L⁻¹ was used instead of 1.5 g L⁻¹ sodium nitrate (El-Shafey et al., 1999). Aeration was supplied by oil-free compressed air and light was provided from one side light bank (12 lamps × 40 W per each one) and the used light intensity at the center of the column was 120 μE. Heterotrophic growth was achieved by addition of 15 mM sodium acetate and 0.25 ml L⁻¹ acetic acid. Sub-culturing was performed within a Plexiglass photobioreactor.
containing 200 L BG-11 growth medium as described in the previous study (El-Sayed and El-Sheekh, 2018).

2.2. Salinity adaptation

The inoculum grown in 200 L photobioreactor was subjected to hypersaline adaptation. Sodium chloride (2.0 g) and 4.5 ppm FeSO4·7H2O were added to the culture. In addition, nitric acid (0.117 ml L−1) was also added daily, reaching N content to 24.7 ppm which equal 1/10 of the initial nitrogen content of BG-11. Overall, after starting the culture using BG11, culture finally contained 200 L BG-11 growth medium as described in the previous section (Table 1). In addition to the BG-11 medium described in the previous section, two additional growth media were also examined (Table 1). The first medium was the original-rejected water (ORW) from a reverse osmosis (RO) facility and the second medium was treated rejected water (TRW). Both of the examined waters were analyzed as described by Chapman and Pratt (1978). Chemical desalination was performed as described by (El-Sayed, 2004a, 2004b).

Growth experiments were performed in 14 L transparent Plexiglas tubes (2 m long, 11 cm diameter, and 6 mm thickness containing the examined growth medium and pre-adopted microalgae. The pre-adopted cells were harvested and washed twice by the proper examined water. A light bank on one side provided the light irradiation at 120 μmol m−2 s−1. Growth was allowed to proceed for 15 days from the starting time. Biomass was harvested by allowing cells to settle for 24 h and then centrifugation (4000 rpm for 3 min) and drying at 45 °C in a circulated oven until constant weight.

2.4. Growth measurement

Dry weight (g L−1) was determined by filtration of a defined volume of algal culture through a pre-weighted membrane filter (0.45 μm), followed by drying the filters in an oven at 105 °C for 30 min. Growth rate (g L−1 d−1), doubling time (n), and percent of increase (%) were calculated as described by (Pirt, 1975).

2.5. Lipid extraction fatty acid profile

Oil extraction was performed by overnight soaking of de-watered algal samples within cellulose extraction thimbles (33 × 94 mm). The Soxhlet technique (1.0 L flask) was then performed using 750 ml of a non-polar solvent mixture (n-hexane: isopropanol, 3:2, v:v). The thimbles were then water washed, dried at 105 °C for 60 min, and re-weighed. Fatty acid methyl esters were obtained based on acid hydrolysis procedures and gas chromatography (GC) system (GC Perkin Elmer Auto System XL) equipped with a flame ionization detector (FID) was used for fatty acid identification and determination of trans-methylated FA.

2.6. Fuel properties

The cetane number (CN); saponification value (SV); iodine value (IV); degree of unsaturation (DU); long-chain saturated fatty acid (LCSF) and cold filter plugging point (CFPP) were determined using equations from Mittelbach and Remschmidt (2004).

2.7. Statistical analysis

All experiments were carried out in triplicate and results were expressed as mean ± standard deviation. The experimental results were analyzed by one-way ANOVA using the statistical software STATISTICA 8 at P value < 0.05.

3. Results

3.1. Growth adaptation of Chlorella vulgaris

Growth was measured as a dry weight using different growth media of original BG-11 and those adapting media, which showed different growth patterns (Fig. 1). The highest dry weight (2.22 g L−1) was observed in the adapted culture at the 26th day. On the other hand, the dry weight in BG-11 reached a maximum value of 1.06 g L−1 at the 28th day of growth. Growth rate of BG-11 culture possessed a constant increase pattern as compared with the adapted culture, which possessed a negative increase during the initial growth periods then returned to high values. Such observation might be ascribed to the high salinity effect and the algal cells entered a long adaptation time after it received about 32 g L−1 of NaCl.

In addition, Chlorella biomass production was determined as dry weight and biomass productivity at the end of growth period (Fig. 2), where it showed the superior values in the treated rejected water (TRW) comparing to those grown in BG-11 or original rejected water (ORW). Growth of Chlorella in ORW medium led to lower dry weight accumulation (1.49 g L−1). However, adaptation of the cells to high NaCl concentration increased the rate of dry weight accumulation, biomass productivity, and the chlorophyll content. Overall, the dry weight of cultures after vegetative growth was 1.23 g L−1 in control (BG-11) medium, 2.22 g L−1 in the adaptive medium, 1.49 g L−1 in ORW medium, and 2.19 g L−1 in TRW medium. On the other hand, the TRW medium was slightly alkaline (Table 1) and had high nitrogen content due to the desalination processes. These conditions enhanced dry weight accumulation (2.19 g L−1). However, both biomass and lipid content are very important parameters for economical production of biodiesel from microalgae. Therefore, lipid content and production were further evaluated as shown in the next section.

3.2. Oil content and FAMEs profile

The oil content also varied according to the culture medium. Oil content of the optimally grown C. vulgaris was reduced to be about 12.0%, comparing to 14.3% and 15.4% for those grown in original rejected and treated rejected-water; respectively (Fig. 3). In particular, the total SFA content was about 20% in control medium,

| Table 1 | The main chemical components (g L−1) of the different media and wastewater used in the present study. |
|---------|----------------------------------------------------------------------------------------------------------|
| Medium   | pH      | TDS    | N     | P    | Na    | Cl     |
| BG-11    | 7.3     | 1.408  | 0.2465| 0.0297| 0.0340| 0.0272 |
| ORW      | 7.5     | 67.59  | 0.039 | 0.042 | 12.8  | 37.79  |
| TRW      | 8.2     | 39.1   | 0.205 | 0.09  | 9.13  | 11.05  |

Original-rejected water (ORW); Treated rejected water (TRW)

3983
but exceeded 28% for the other media. Thus, the reduction of nitrogen stress during growth appeared to increase the oil content. Regarding fatty acid profile, the present results showed relatively lower concentrations of saturated fatty acids (SFAs) in *C. vulgaris* oil, with a maximum of 29.53% lipids in the TRW, when the P level was 50% lower (Table 2). While the lowest SFAs of 19.7% was recorded in the control medium. In addition, the present results showed C16:1, C18:1, and C14:0 ratio of 3:4:1 in the control medium. The present study showed that *Chlorella* cells produced a considerable amount of oleic acid, suggesting its bio-oil suitability for good biodiesel quality (Section 4).

3.3. Biodiesel properties

Biodiesel properties produced by *C. vulgaris* when grown under different conditions were evaluated (Table 3). The DU represents the weight percentage of monounsaturated and polyunsaturated fatty acids. Growth of *C. vulgaris* in ORW and TRW reduced the DU from 146.78 to 78.04 and 80.37, respectively. Results indicated that biodiesel of *Chlorella* grown in control medium has an IV of 100 g I2/100 g oil, with lower IVs when grown in ORW and TRW (94.7 and 90.2 g I2/100 g oil, respectively). On the other hand, growth in ORW media increased the SV (194.4) above that of the control medium (182.77021). Interestingly, growth of *C. vulgaris* in TRW showed increase of CN from 53.6 in the control BG-11 medium to 55.8. In addition, CFPP increased from 0.7497 to 1.6513 in BG-11 and TRW, respectively (Table 3).

4. Discussion

The growth of microalgae is widely affected by the composition of the growth medium. The maximum dry weight was observed in the adapted culture by the 11th day. The continuous enhancing effect on algal growth could be attributed to the nutrition strategy
in concord nitric acid as continuous nitrogen feeding with buffering action and also due to the presence of vitamin mixture. The fast nitrogen utilization might lead to retardation of growth as present in cultures grown under the recommended growth medium (BG-11). In addition, high salinity results in algal cells entered a long adaptation time, which resulted in longer lag phase in the adapted culture. However, implementation of vitamin in algal growth medium might mitigate the adverse effect of hyper-salinity of the medium. It was previously reported that some algal species have lost a gene of the biosynthetic pathway of this cofactor and as a result cobalamin comes from a symbiotic relationship with bacteria (Croft et al., 2005). Chlorella growth was superior in treated rejected water (TRW) comparing with those grown in BG-11 or original rejected water (ORW). The sustainable enhancing effect on growth dry weight accumulation during adaptation process could be ascribed to the continuous supplementation of nitrogen through daily nitric acid addition besides buffering action and hardness solubility. Moreover, vitamin B mixture might play an important role against the injury effect of hyper-salinity and algae stimulate vitamins parallel to growth conditions, where optimum growth, biomass and total B vitamins of the marine diatom Skeletonema costatum produced was achieved by culturing at salinity of 24 g L\(^{-1}\) and light intensity 34 μmol m\(^{-2}\) sec\(^{-1}\) (Suantika et al., 2016). Further, many marine algae are vitamin B12-rich, however the source is unknown. Previous reports point that certain algae can de novo synthesize cobalamin (Carlucci and Bowes, 1970); others have been shown to require exogenous cobalamin for growth in culture (Kraepelin, 1977); implying that many algae cannot synthesize it. As early mentioned by El-Sayed et al. (2010); chemical desalination leads to precipitation of some salts mainly ammonia, sodium, magnesium and chlorine and the remainder of desalination contain an adequate amount of them to save the next algal growth. It was reported that dissolved vitamin B12, thiamine, and biotin can be found in many seawaters samples within the ranges which influence the growth rate, cell size, and chlorophyll content of Cyclotella nana, Monochrysis lutheri, and Amphidinium carterae, respectively. The response of algae in situ to vitamins may be similar (Carlucci and Silbernagel, 1969). Type of the salt stress based on nutrients balance could increase the obtained biomass of algae (El-Sayed et al., 2008); where the green alga Scenedesmus sp. successfully grown under 4 folds salinity of the recommended growth medium (El-Sayed et al., 2001).

NaCl led to delays in growth, especially at higher concentrations (1 to 2%), and to chlorophyll and protein decomposition and massive accumulation of carotenoids and oils. In addition, nitrogen limitation, high light irradiation, and free radical production can stimulate carotenogenesis (El-Sayed, 2004a, 2004b). The present results indicated that use of adequate nitrogen content, a regular light regime, and slow adaptation to high NaCl ameliorated the adverse effects of high NaCl concentration on growth. Lower dry weight was recorded in ORW medium, while adaptation to high NaCl concentration increased the dry weight accumulation and the chlorophyll content. This might be attributed to the utilization of sodium ions by microalgal cells within organic metabolites to overcome the adverse effect of sodium.

For economical production of biofuel from microalgae, biomass as well as lipid content are very important parameters. Growth medium should provide the inorganic elements that constitute

### Table 2

| Fatty acid methyl esters produced by C. vulgaris grown in different water types. |
|------------------|------------------|------------------|------------------|
| **Fat. acid** | **C No.** | **BG-11** | **ORW** | **TRW** |
| Caprylic | CB:0 | 0.54 ± 0.08 | 0 ± 0.00 | 0.09 ± 0.01 |
| Capric | CI0:0 | 0.26 ± 0.01 | 0.18 ± 0.01 | 0.19 ± 0.02 |
| Lauric | CI2:0 | 0.46 ± 0.01 | 0.19 ± 0.03 | 0.22 ± 0.02 |
| Myristic | C14:0 | 1.29 ± 0.13 | 0.54 ± 0.10 | 0.65 ± 0.10 |
| Myristoleic | C14:1 | 6.42 ± 0.43 | 6.04 ± 0.90 | 6.22 ± 0.80 |
| Palmitic | C16:0 | 12.16 ± 1.10 | 26.03 ± 2.10 | 24.29 ± 3.60 |
| Palmitoleic | C16:1 | 2.88 ± 0.43 | 2.12 ± 0.09 | 1.59 ± 0.09 |
| Stearic | C18:0 | 3.64 ± 0.42 | 0.58 ± 0.05 | 3.69 ± 0.23 |
| Oleic | C18:1 | 32.81 ± 2.50 | 34.05 ± 3.70 | 22.6 ± 1.60 |
| Linoleic | C18:2 | 12.14 ± 1.40 | 9.42 ± 1.10 | 13.6 ± 1.20 |
| Linolenic | C18:3 | 1.32 ± 0.18 | 1.03 ± 0.10 | 2.12 ± 0.09 |
| Arachidic | C20:0 | 0.26 ± 0.02 | 0.24 ± 0.03 | 0.31 ± 0.00 |
| Gadoleic | C20:1 | 2.74 ± 0.43 | 2.27 ± 0.21 | 3.06 ± 0.20 |
| Arachidonic | C20:4 | 4.12 ± 0.53 | 4.37 ± 0.76 | 2.19 ± 0.18 |
| Behenic | C22:0 | 1.14 ± 0.16 | 1.16 ± 0.05 | 0.09 ± 0.00 |
| Erucic | C22:1 | 2.03 ± 0.21 | 1.54 ± 0.06 | 2.36 ± 0.20 |
| Docosahexaenoic | C22:6 | 4.09 ± 0.64 | 4.21 ± 0.67 | 4.36 ± 0.50 |
| SFA | 19.75 ± 1.60 | 28.92 ± 2.10 | 29.53 ± 2.50 |
| USFA | 68.55 ± 3.80 | 65.05 ± 4.50 | 58.10 ± 3.90 |

Original-rejected water (ORW), Treated rejected water (TRW)

---

**Fig. 3.** Lipid content of C. vulgaris grown in different water types. The error bars show the standard deviation of three replicates. A single (*) and double (**) asterisk indicate respectively significant (P < 0.05) and highly significant (P < 0.01) differences with the control.
the algal cell. Many of freshwater algae showed a good defense strategy towards the increased levels of ROS under salinity stress by increasing the activity of protective enzymes responsible for the detoxification and elimination of ROS. For example, Farghi et al., (2015) indicated the importance of antioxidations induction for the tolerance mechanism of Chlorella species to salinity as demonstrated by growth behavior. The satisfactory growth during the vegetative phase seems to be the main reason for the greater growth during carotenogenesis due to the presence of large quantities of organic matter. Similarly, Xu et al. (2012) reported the induction of osmotic pressure by NaCl and the effects of salt stress on cell growth and lipid production in C. vulgaris which depended on time of exposure and concentration of NaCl. In particular, NaCl greatly affected the cellular growth since it was added at the beginning of cultivation, which consequently had a significant negative effect on lipid accumulation. Furthermore, a gradual increase of NaCl concentration, with a low salt concentration used during the growth phase and high concentration during the stationary phase, can maximize lipid yield and minimize the negative effects of NaCl on cell growth. On the other hand, the TRW medium was slightly alkaline and showed high nitrogen content due to the desalination processes. These conditions enhanced the dry weight accumulation in this medium. In that context, the elevated levels of nitrogen, phosphorus, and potassium are known to increase microalgal growth (El-Sayed et al., 2008).

Lipid content also varied according to the culture medium, where original rejected and treated rejected-waters enhanced the lipid content over the control. The enhancing effect of treated rejected water on lipid accumulation might be attributed to the initial nutrients mainly nitrogen with high media reaction value (pH) as a result of desalination process (El-Sayed et al., 2010). Results also confirm that reduction of nitrogen stress during growth appeared to increase the oil production (El-Sayed et al., 2008). Moreover, a low CN in the control culture is related to more highly unsaturated components (C18:2 and C18:3). Furthermore, oxidation stability decreases as the content of polyunsaturated fatty acids is that their combustion leads to polymerization of glycerides and the development of deposits or worsening of lubrication (Schenk et al., 2008). The present results indicated lower IVs when C. vulgaris is grown in ORW or TRW, which is advantageous for higher biodiesel quality. Microalgae commonly produce polyunsaturated fatty acids with at least four double bonds, which are susceptible to oxidation, emit more nitrogen oxides, and have a thermal efficiency lower than biodiesel produced from saturated fatty acids, which restricts the acceptability of microalgae bio-oil for biodiesel production (Chisti, 2007). The oxidative potential of such biodiesel would be very low (Schenk et al., 2008). Moreover, a low CN in the control culture is related to more highly unsaturated components (C18:2 and C18:3). Furthermore, oxidation stability decreases as the content of polyunsaturated methyl esters increases. However, the worst CPFF values were reported for biodiesels rich in long carbon chains with saturated methyl esters. Therefore, it is important to generate the proper percentages of saturated and unsaturated FA when using microalgae as a biodiesel feedstock. Overall, the present study concluded that C. vulgaris is a suitable feedstock for biodiesel because of its high oil content and high levels of palmitic and oleic acids. In addition, cultivation of C. vulgaris on TRW could enhance the biodiesel characteristics.

5. Conclusion

The energy crisis and the increasing salinization of fresh water are closely related problems. It may be possible to resolve both problems by using hyper-saline water after chemical desalination to stimulate bio-oil production in microalgae. Biofuel matrix can

| Medium   | SV     | IV     | CN    | DU    | LCSF   | CFPP   |
|----------|--------|--------|-------|-------|--------|--------|
| BG-11    | 182.77 | 100.095| 53.6414| 146.78| 5.006  | 0.7497 |
| ORW      | 194.411| 94.6807| 53.0714| 78.04 | 4.873  | 1.1675 |
| TRW      | 182.755| 90.1689| 55.8771| 80.37 | 4.719  | 1.6513 |
| Europe (EN 14214) | ≤ 120 | ≥ 51 | – 20 to – 5 |
| US (ASTM D6751-08) | ≥ 47 | – 5 to – 13 |

ORW = original rejected water and TRW = treated rejected water.
CN = cetane number; SV = saponification value; IV = iodine value; DU = degree of unsaturation; LCSF = long-chain saturated fatty acid and CFPP = cold filter plugging point.
be obtained from algal biomass through the production of bioalcohol from carbohydrates, biogas from proteins and biodiesel from oils. In addition, biogas can be obtained from all of the mentioned fractions. The main goals of future research are to optimize the desalination method using proper microalgal strain and strategy used to stimulate carotenogenesis. In addition, large-scale open cultivation of microalgae using seawater might be an efficient approach for dual purpose of seawater desalination and biofuel production in arid and semi-arid regions such as Kingdom Saudi Arabia.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Acknowledgments**

This work was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University (Jeddah, Saudi Arabia) by grant No. D-122-662-1441. The authors acknowledge the DSR for technical and financial support.

**Appendix A. Supplementary material**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sbsj.2021.04.007.

**References**

Abomohra, A.E.-F., El-Naggar, A.H., Alowad, S.O., Elsayed, M., Li, M., Li, W., 2020a. Enhancement of biodiesel yield from a halophic green microalga isolated under extreme hypersaline conditions through stepwise salinity adaptation strategy. Bioresour. Technol. 310, 123462.

Abomohra, A.E.-F., Elsayed, M., Esakkimuthu, S., El-Sheekh, M., Hanelt, D., 2020b. Potential of fat, oil and grease (FOG) for biodiesel production: A critical review on the recent progress and future perspectives. Prog. Energy Combust. Sci. 81, 100868.

Abomohra, A.E.-F., Jin, W., Tu, R., Han, S.-F., Eid, M., Eladet, H., 2016. Microalgal biomass production as a sustainable feedstock for biodiesel: Current status and perspectives. Renew. Sustain. Energy Rev. 64, 596–606.

Abomohra, A.E.-F., Shang, H., El-Sheekh, M., Eladet, H., Elbaid, R., Wang, S., Wang, Q., 2019. Night illumination using monochromatic light-emitting diodes for enhanced microalgal growth and biodiesel production. Bioresour. Technol. 288, 121514.

Abomohra, A.E.-F., Zheng, X., Wang, Q., Huang, J., Elbaid, R., 2021. Enhancement of biodiesel yield and characteristics through in-situ solvothermal co-transesterification of wet microalgae with spent coffee grounds. Bioresour. Technol. 323, 124640.

Almarashi, J.Q.M., El-Zohary, S.E., Ellabban, M.A., Abomohra, A.E.-F., 2020. Enhancement of lipid production and energy recovery from the green microalga Chlorella vulgaris by inoculum pretreatment with low-dose cold atmospheric pressure plasma (CAPP). Energy Convers. Manage. 204, 112314.

Almutairi, A.W., 2020a. Effects of nitrogen and phosphorous limitations on fatty acid methyl esters and fuel properties of Dunaliella salina. Environ. Sci. Pollut. Res. 27, 32296–32303.

Almutairi, A.W., 2020b. Improvement of Chemical Composition of Tisochrysis lutea Grown Mixotrophically under Nitrogen Depletion towards Biodiesel Production. Molecules 25, 4609.

Almutairi, A.W., El-Sayed, A.E.-K.B., Reda, M.M., 2020. Combined effect of salinity and financial support. No. D-122-662-1441. The authors acknowledge the DSR for technical and financial support.

**Appendix A. Supplementary material**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sbsj.2021.04.007.

**References**

Abomohra, A.E.-F., El-Naggar, A.H., Alowad, S.O., Elsayed, M., Li, M., Li, W., 2020a. Enhancement of biodiesel yield from a halophic green microalga isolated under extreme hypersaline conditions through stepwise salinity adaptation strategy. Bioresour. Technol. 310, 123462.

Abomohra, A.E.-F., Elsayed, M., Esakkimuthu, S., El-Sheekh, M., Hanelt, D., 2020b. Potential of fat, oil and grease (FOG) for biodiesel production: A critical review on the recent progress and future perspectives. Prog. Energy Combust. Sci. 81, 100868.

Abomohra, A.E.-F., Jin, W., Tu, R., Han, S.-F., Eid, M., Eladet, H., 2016. Microalgal biomass production as a sustainable feedstock for biodiesel: Current status and perspectives. Renew. Sustain. Energy Rev. 64, 596–606.

Abomohra, A.E.-F., Shang, H., El-Sheekh, M., Eladet, H., Elbaid, R., Wang, S., Wang, Q., 2019. Night illumination using monochromatic light-emitting diodes for enhanced microalgal growth and biodiesel production. Bioresour. Technol. 288, 121514.

Abomohra, A.E.-F., Zheng, X., Wang, Q., Huang, J., Elbaid, R., 2021. Enhancement of biodiesel yield and characteristics through in-situ solvothermal co-transesterification of wet microalgae with spent coffee grounds. Bioresour. Technol. 323, 124640.

Almarashi, J.Q.M., El-Zohary, S.E., Ellabban, M.A., Abomohra, A.E.-F., 2020. Enhancement of lipid production and energy recovery from the green microalga Chlorella vulgaris by inoculum pretreatment with low-dose cold atmospheric pressure plasma (CAPP). Energy Convers. Manage. 204, 112314.

Almutairi, A.W., 2020a. Effects of nitrogen and phosphorous limitations on fatty acid methyl esters and fuel properties of Dunaliella salina. Environ. Sci. Pollut. Res. 27, 32296–32303.

Almutairi, A.W., 2020b. Improvement of Chemical Composition of Tisochrysis lutea Grown Mixotrophically under Nitrogen Depletion towards Biodiesel Production. Molecules 25, 4609.

Almutairi, A.W., El-Sayed, A.E.-K.B., Reda, M.M., 2020. Combined effect of salinity and financial support. No. D-122-662-1441. The authors acknowledge the DSR for technical and financial support.
Gomaa, M.A., 2018. Ethyl methane sulfonate effect on total lipids of chlorella vulgaris isolated from nile river Egypt. Ann. Agric. Sci. Moshtohor 56, 669–674.

Gopinath, A., Puhun, S., Govindan, N., 2010. Effect of biodiesel structural configuration on its ignition quality. Int. J. Energy Environ. 1.

Gouveia, L., Oliveira, A.C., 2009. Microalgae as a raw material for biofuels production. J. Ind. Microbiol. Biotechnol. 36, 269–274.

Grumet, R., Isleib, T.G., Hasson, A.D., 1985. Genetic control of glycinobetaine level in Barley. Crop Sci. 25, 618–622.

Guschina, I.A., Harwood, J.L., 2006. Lipids and lipid metabolism in eukaryotic algae. Prog. Lipid Res. 45, 160–186.

Han, S.F., Jin, W., Tu, R., Abomohra Ael, F., Wang, Z.H., 2016. Optimization of aeration for biodiesel production by Scenedesmus obliquus grown in municipal wastewater. Bioprocess Biosyst. Eng. 39, 1073–1079.

Hediey Sekmen, A., Türkcan, İ., Takio, S., 2007. Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt-tolerant Plantago maritima and salt-sensitive Plantago media. Physiol. Plant. 131, 399–411.

Hou, D., Huang, Z., Zeng, S., Liu, J., Wei, D., Deng, X., Weng, S., He, Z., He, J., 2017. Environmental factors shape microbial community structure and function in shrimp culturable enclosure ecosystems. Front. Microbiol. 8, 2359.

Illum, A.M., Scragg, A.H., Shales, S.W., 2000. Increase in Chlorella strains caloric values when grown in low nitrogen medium. Enzyme Microb. Technol. 27, 631–635.

Kraepelin, G., 1977. W. D. P. STEWART (Editor), Algal Physiology and Biochemistry (Reihe: Botanical Monographs, Vol. 10). 989 S., 76 Abb., 78 Tab., 26 Tafeln. Oxford-London-Edinburgh-Melbourne 1974. Black-well Scientific Publ. £ 15.50. Zeitschrift für allgemeine Mikrobiologie. 17, 88–88.

Lardon, L., Hélias, A., Salive, B., Steyer, J.-P., Bernard, O., 2009. Life-cycle assessment of biodiesel production from microalgae. Environ. Sci. Technol. 43, 6475–6481.

Leshem, Y., Seri, L., Levine, A., 2007. Induction of phosphatidylinositol 3-kinase-mediated endocytosis by salt stress leads to intracellular production of reactive oxygen species and salt tolerance. Plant J. Cell Mol. Biol. 51, 183–197.

Marnett, L.J., 2000. Oxyradicals and DNA damage. Carcinogenesis 21, 361–370.

Mittelbach, M., Remischmidt, C., 2004. Biodiesel: the comprehensive handbook. Martin Mittelbach, Austria.

Pir, S.J., 1975. Principles of microbe and cell cultivation. Wiley, New York.

Prabakaran, P., Ravindran, A.D., 2012. Scenedesmus as a potential source of biodiesel among selected microalgae. Curr. Sci. 102, 616–620.

Rugnini, L., Rossi, C., Antonaroli, S., Rakaj, A., Bruno, L., 2020. The influence of light concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga Scenedesmus sp. Bioresour. Technol. 101, 5494–5500.

Sun, X.M., Ren, L.J., Zhao, Q.Y., Ji, X.J., Huang, H., 2019. Enhancement of lipid accumulation in microalgae by metabolic engineering. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 1864 (4), 552–566.

Tandon, P., 2017. A promising approach to enhance microalgae productivity by exogenous supply of vitamins. Microb. Cell Fact. 21.

Teixeira, J., Pereira, S., 2007. High salinity and drought act on an organ-dependent manner on potato glutamine synthetase expression and accumulation. Environ. Exp. Bot. 60, 121–126.

Touliahab, H.E., Almutairi, A.W., 2021. Effect of phytohormones supplementation under nitrogen depletion on biomass and lipid production of nannochloropsis oocorica for integrated application in nutrition and biodiesel. Sustainability 13.

Tu, R., Jin, W., Han, S.F., Zhou, X., Wang, J., Wang, Q., He, Z., Ding, W., Che, L., Feng, X., 2019. Enhancement of microalgal lipid production in municipal wastewater: Fixation of CO2 from the power plant tail gas. Biomass Bioenergy 131, 105400.

Turhan, E., Gulen, H., Eris, A., 2008. The activity of antioxidative enzymes in three strawberry cultivars related to salt-stress tolerance. Acta Physiologiae Plantarum 30, 201–208.

Vazquez-Duhalt, R., Arredondo-Vega, B.O., 1991. Haloadaptation of the green alga Botryococcus braunii (race a). Phytochemistry 30, 2919–2925.

Wang, S., Uzoejinnah, B.B., Abomohra, A.E.-F., Wang, Q., He, Z., Feng, Y., Zhang, B., Hui, C.-W., 2018. Characterization and pyrolysis behavior of the green microalga Microcystis aeruginosa grown in lab-scale tubular photobioreactor using Py-GC/MS and TGA/MS. J. Anal. Appl. Pyrol. 135, 340–349.

Wang, S., Zhao, S., Uzoejinnah, B.B., Zeng, A., Wang, Q., Huang, J., Abomohra, A.E.-F., 2020. A state-of-the-art review on dual purpose seaweed utilization for wastewater treatment and crude bio-oil production. Energy Convers. Manage. 223, 112352.

Xin, L., Hu, H.Y., Ke, G., Sun, Y.X., 2010. Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalg Scenedesmus sp. Bioreour Technol. 101, 5494–5500.

Xiong, W., Li, X., Xiang, J., Wu, Q., 2008. High-density fermentation of microalg Chlorella protothecoides in bioreactor for microbio-diesel production. Appl. Microbiol. Biotechnol. 78, 29–36.

Xu, K., Babcock, H.P., Zhuang, X., 2012. Dual-objective STORM reveals three-dimensional filament organization in the actin cytoskeleton. Nat. Methods 9, 185–188.

Yahya, N.A., Suhaimi, N., Kaha, M., Hara, H., Zakaria, Z., Sugiura, N., Othman, N.A., 2018. Influence of fatty acid composition of raw materials on biodiesel properties. Bioresour. Technol. 265, 163–169.

Sharma, A., Arya, S.C.K., 2017. Hydrogen from algal biomass: A review of production process. Biotechnol. Rep. 15, 63–69.

Sheehan, J., Dunahay, T., Benemann, J., Roessler, P., 1998. Look Back at the U.S. Department of Energy’s Aquatic Species Program: Biodiesel from Algae. Close- Out Report.; National Renewable Energy Lab., Golden, CO (US). p. Medium: ED: Size: 325 p.

Shi, F., Bao, F., 2007. Effects of salinity and temperature stress on ecophysiological characteristics of exotic cordgrass. Spartina alterniflora. Acta Ecologica Sinica 27, 2733–2741.

Small, D., Huner, N., Wan, W., 2012. Effect of static magnetic fields on the growth, photosynthesis and ultrastructure of Chlorella kessleri microalgae. Bioelectromagnetics 33.

Stanier, R.Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G., 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol. Rev. 35, 171–205.

Stournas, S., Lois, E., Serdani, A., 1995. Effects of fatty acid derivatives on the ignition quality and cold flow of diesel fuel. J. Am. Oil Chem. Soc. 72, 433–437.

Suantika, G., Putri, A., Djobhan, Y., Nur Azziah, F., Astuti, D., Aditiaswati, P., 2016. Impact of salinity and light intensity stress on B vitamins content in marine diatom skeletonema costatum. J. Fish. Aquat. Sci. 12, 22–28.

Sun, X.M., Ren, L.J., Zhao, Q.Y., Ji, X.J., Huang, H., 2019. Enhancement of lipid accumulation in microalgae by metabolic engineering. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 1864 (4), 552–566.