RESEARCH ARTICLE

The potential use of stickwater from a kilka fishmeal plant in *Dunaliella salina* cultivation

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Abstract

In this study, the possibility of culturing *Dunaliella salina* in stickwater (SW) as the main effluent of fishmeal plants was evaluated. *D. salina* was grown in different media obtained by replacing standard Guillard medium (F/2) with SW at 0% (control), 10%, 25%, 50%, 75%, and 100% ratios. The cell density, pigment contents, proximate composition, saponification value, and fatty acids (FAs) profiles were measured for 14 days. SW was collected from a kilka fishmeal factory in northern Iran, and the characteristics indicated high concentrations of nitrate (242.00 mg L^{-1}) and phosphate (11.13 mg L^{-1}). A significant increase in the cell density was observed in 14 days when 75% SW was used. Moreover, SW significantly affected the pigment contents. The highest contents of chlorophylls, total carotenoids, and β-carotene (3.64 μg mL^{-1}) were calculated in 75% SW. According to the algal proximate composition, the highest and lowest contents of lipid were accumulated in 75% and 100% SW, respectively ($p < 0.05$). The highest level of saturated FAs was observed in 75% SW compared with the others ($p < 0.05$). In conclusion, replacing F/2 with SW indicated the capability of *D. salina* to grow in a treated medium with 75% SW substitution as a bioremediator.

Keywords Bioremediation · Carotenoid · Fatty acids · Microalgae · Wastewater

Introduction

Stickwater (SW) is a by-product of the fishmeal industry (Kousoulaki et al. 2009) which has high nutritional value because of its high concentrations of protein and other organic materials (Mahdabi and Hosseini Shekarabi 2018). However, this protein-rich syrup is commonly discharged directly into the environment and creates a strong malodor around processing plants due to the proteolytic microbe's activities (Kam et al. 2012; Mahdabi and Hosseini Shekarabi 2018). Although SW can provide a considerable amount of nutrients (i.e., nitrogen and phosphorous), there is limited information about incorporating this valuable by-product in the growth medium of microorganisms.

Marine green algae, *Dunaliella salina* (Polyblepharidacea), is a halophilic flagellate microalga which can grow under various environmental stresses and can be exploited commercially for β-carotene production (Wu et al. 2016). There is a huge demand for the production of *D. salina* in order to obtain high contents of carotenoids, poly-unsaturated fatty acids (PUFAs), minerals, and vitamins (Gomez et al. 2003; Raja et al. 2007). However, the complexity and cost of the pure standard growth medium for producing microalgae on a large scale are serious problems (Gebremedhin et al. 2018). One effective way to overcome these hindrances is to replace conventional methods of growing microalgae with wastewaters. To date, however, there are no reports of using SW in culturing *D. salina* as a halophilic microalga.

Some investigators have reported the possibility of using microalgae species to treat wastewaters from different industries (Costa et al. 2004; Park and Craggs 2010). Hence, many different microalgae species, including *Chlorella sorokiniana* (De Francisci et al. 2017), *C. vulgaris* (Blanco-Carvajal et al. 2018), *Platymonas subcordiformis* (Guo et al. 2013), and *Scenedesmus obliquus* (Gao et al. 2016), have been grown in the wastewater of fish farming to reduce the negative impacts of such aquaculture on the environment. The cultivation

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of freshwater microalgae species, including *Chlorella sorokiniana* and *C. vulgaris*, in dairy wastewaters resulted in more than 80% removal efficiency of the pollutants with the maximum growth performance (Asadi et al. 2019). In one study, the high biological potency for the removal of secondary treated domestic sewage and biodiesel production was observed in the marine microalga *Tetraselmis indica* (Chandra et al. 2017). Moreover, Kumar et al. (2018) explained the maximum growth and lipid production of the various microalgae species such as *C. singularis*, *C. sorokiniana*, and *Micractinium pusillum* in pretreated human and animal wastes. Therefore, nutrients from some industrial wastewaters can be used in the cultivation of microalgae and to reduce the eutrophication process in aquatic ecosystems (Rawat et al. 2011; Qu et al. 2019).

Kilka species (*Clupeonella* spp.) are the most abundant and widespread pelagic fish in the Caspian Sea. They provide raw material to fishmeal factories due to their small size and non-marketability for human consumption (Janbakhsh et al. 2018). Therefore, the objective of this study was to evaluate the possibility of using SW from a kilka fishmeal plant as a growth medium for *D. salina* to examine the algal growth performance, biochemical contents, and fatty acid composition.

Materials and methods

Preparation and physicochemical properties of stickwater

Fresh stickwater was collected at a local kilka fishmeal plant (Fishmeal Company 581 of Babolsar, Mazandaran, Iran) and transported with ice to the Science and Research Laboratory (Tehran). The stickwater was filtered through Whatman No. 1 filter paper, autoclaved (120 °C, 0.1 MPa) for 15 min and then refrigerated at 4 ± 1 °C until further analysis. A spectrophotometer DR/1900 (HACH, Germany) was applied to measure nitrite, nitrate, ammonia, phosphate, potassium, and calcium in the stickwater. Total suspended solids (TSS), total dissolved solids (TDS), total solids (TS), and total volatile solids (TVS) were obtained according to the APHA (1985) method. Also, pH, crude protein (total extracted nitrogen × 6.25), and lipid contents were calculated using the AOAC (1995) method.

Microorganism and experimental design

The initial purified stock solution of *D. salina* was provided by Zakariya-Razi Laboratory (Tehran, Iran); the microalgae were isolated from the Hoz-e Soltan Salt Lake (Qum province, Iran) using the methods of Tavallaie et al. (2015). Guillard medium (F/2) was used as a control group, and it was replaced by 5 different levels of the stickwater at 10%, 25%, 50%, 75%, and 100% ratios. *D. salina* was grown in all treatments at 10%

NaCl (w/v; 1.7 M NaCl salinity) and 7.5 pH (Tavallaie et al. 2015). The microalgae cultures were maintained in climatic chambers with continuous aeration, at a temperature of 25 ± 1 °C, 55 μmol photons m^{-2} s^{-1} light intensity, and 12D:12L (dark/light) regimen for 14 days. All of the chemical materials were procured from Merck Company (Darmstadt, Germany). The initial stocking density of *D. salina* was 10 mL inoculum of the algae (1×10^4 cells mL^{-1}).

Algal density

A T80 spectrometer (T80+UV/VIS spectrometer PG Instruments Ltd., England) at 680 nm (Rastar et al. 2018) was used to measure the algal density.

Determination of pigments

Chlorophyll (Chl) *a*, *b* and carotenoids were extracted using Yang et al. (1998) method with some modifications. The amount of 2 mL of algae sample was mixed with 5 mL of acetone: distilled water (4:1 v/v) and then stirred for 2 min to create a homogenous mixture. The mixture was centrifuged at 2600×*g* at 20 °C for 5 min (3-30K Sigma, Germany), and the absorption of the supernatants was read at 662, 645, and 470 nm for Chl *a*, *b* and carotenoids, respectively.

$$\text{Chl } a \left(\mu g \text{ mL}^{-1}\right) = 12.25 \times A_{662} - 2.25 \times A_{645}$$

$$\text{Chl } b \left(\mu g \text{ mL}^{-1}\right) = 20.31 \times A_{645} - 2.25 \times A_{662}$$

$$Total carotenoids \left(\mu g m L^{-1}\right) = [Chl a + Chl b]$$
$$+ [(1000 \times A470 - 1.90 \times Chl a - 63.14 \times Chl b)/214]$$

where *A* is the absorption read by the spectrophotometer.

The method of Eijckelhoff and Dekker (1997) with slight modification was used to determine the concentration of β-carotene. In short, 1 mL of the algae was centrifuged at 1274×*g* for 8 min. The precipitates were mixed with acetone 80% (v/v) for an hour and then treated by ultrasonic bath system (Wise Clean, Germany) to extract the pigments. The samples were centrifuged, and the supernatants were measured at 412, 431, 460, and 480 nm. The following equation was used to calculate the β-carotene content:

$$\beta \text{ carotene} \left(\mu g \text{ mL}^{-1}\right) = 0.430 \, A_{412}$$
$$+ 0.251 \, A_{431} - 4.376 \, A_{460}$$
$$+ 13.216 \, A_{480}$$

Proximate chemical composition

After harvesting, the algal biomass was washed three times in deionized water and centrifuged at 6654×*g* for 15 min to

eliminate unwanted materials. Then, the sediment samples were freeze-dried for 24 h using a Christ Alpha 2–4 Plus freeze dryer (Martin Christ Gefriertrocknungsanlagen, Osterode, Germany) and maintained at − 80 °C for a maximum of 2 months before analyzing. Nitrogen contents (N × 6.25) were measured using the Kjeldahl method to determine the crude protein content of the dried samples. The fat content was determined using the method of Folch et al. (1957). Ash content was measured by burning 1 g of the sample over a low flame followed by heating in a muffle furnace at 550 °C for 4 h (AOAC 1995).

Saponification value

The saponification value (SV) was measured based on the method of Sabzi et al. (2018). The extracted oil (3 mL) was mixed with 25 mL of ethanolic KOH. The samples were then enclosed tightly and placed in a water bath at 80 °C for 30 min. Two drops of phenolphthalein were added to the mixture as a pH pointer and titrated with 0.5 M hydrochloric acid to change the color to yellow. A blank sample (all the abovementioned procedures without the oil sample) was also included. The following equation was used to calculate the SV:

$$SV \left(mg\ KOH\ g^{-1}\right) = \frac{56.1 \times V_1 - N \times V_2}{W}$$

where V_1 is the volume of ethanolic KOH used in titration of the blank sample (ml), V_2 is the volume of ethanolic KOH used in titration of the samples (mL), N is the normality of acid (0.5 N), and W is the weight of the oil samples (g).

Fatty acid profile

The fatty acid (FA) composition of the algae samples cultured in different media was measured using the method by Schierholt et al. (2001). In brief, 1 g of the extracted oil was mixed with 20 mL of potassium methoxide 1% for 25 min. Then, 12 mL of 4-fluorobenzyl bromide was added and the solution was boiled for 10 min. After that, 2 mL of sodium chloride was added and the solution was centrifuged at 2150×g for 10 min. FAs methyl esters (1 μL) were injected into a Nexis 2030 GC-chromatography (Shimadzu, Japan). The GC was equipped with an FID detector and a Dikmacap-2330 capillary column with the length of 60 m and the inner diameter of 0.25 mm. Hydrogen gas with a purity of 99.99% and a velocity of 2 mm per minute was used as the carrier gas. The temperatures of the injection and detector were regulated at 250 °C and 260 °C, respectively. The detection of FAs was measured by comparing their peak areas with the standards.

Statistical analysis

All experiments were conducted in three replicates and data was expressed as mean ± standard error of the mean. SPSS software, version 20.0 (SPSS, Chicago, IL, USA) was used to analyze the obtained data. Data were subjected to a one-way analysis of variance (one-way ANOVA) followed by Tukey's post-hoc test to identify the significance of differences between the means at a confidence level of 5%.

Results

Physicochemical properties of SW

Some physical and chemical characteristics of the SW used in this study are summarized in Table 1. High concentrations of nitrate (242.00 mg L^{-1}) and phosphate (11.13 mg L^{-1}) were measured in SW.

Algal density

As shown in Fig. 1, the density of cultured D. salina was affected by different levels of SW substitution. Although there were significant differences between the treatments on different days, the density of microalgae had an increasing trend during the trial. The highest cell density was observed by replacing F/2 medium with SW up to 75% from 22.05 × 10⁴ to 112.60 × 10⁴ cell mL⁻¹ at the end of the trial ($p < 0.05$). However, the lowest cell density was calculated in the treatment with 100% replacement by SW ($p < 005$).

Table 1 Properties of the stickwater prepared from kilka fishmeal plant

Characteristic	Value
Calcium (mg L^{-1})	2062.50 ± 3.50
Potassium (mg L^{-1})	1441.50 ± 10.50
Phosphate (mg L^{-1})	11.13 ± 0.02
Ammonia (mg L^{-1})	0.12 ± 0.003
Nitrate (mg L^{-1})	242.00 ± 3.00
Nitrite (mg L^{-1})	0.65 ± 0.02
Lipid (g in 100 g dry weight)	0.084 ± 0.001
Protein (g in 100 g dry weight)	40.45 ± 0.01
pH	6.63 ± 0.03
TDS (mg L^{-1})	4.951 ± 0.003
TSS (mg L^{-1})	0.484 ± 0.006
TVS (mg L^{-1})	0.316 ± 0.001
TS (mg L^{-1})	0.484 ± 0.006

Data are expressed as mean values ± standard deviations ($n = 3$)
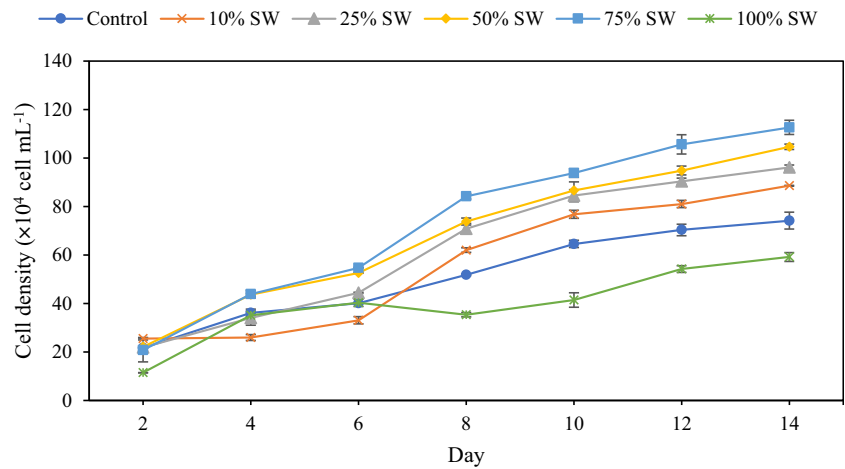
Chlorophyll content

Substitution with SW had a significant impact on Chl *a* and *b* contents (Figs. 2 and 3). The highest amount of Chl *a* was observed at 10 days in 50% SW (4.591 μg mL^{-1}). However, the Chl *a* concentration on days 12 and 14 of the experiment were at the highest level in 75% SW with 5.741 and 6.540 μg mL^{-1}, respectively. In addition, the minimum content of Chl *a* was seen in 100% replacement of F/2 medium with SW. At the end of the trial, the highest and lowest Chl *b* contents were observed in 75% SW (3.334 μg mL^{-1}) and 100% SW (1.639 μg mL^{-1}), respectively (Fig. 3).

Carotenoid concentration

The amount of total carotenoids was increased during the trial in all treatments (Fig. 4). The highest carotenoid concentration was observed on day 14 (5.30 μg mL^{-1}) in 75% SW, while the lowest value (2.33 μg mL^{-1}) was measured in the control group ($p < 0.05$).

The β-carotene contents of *D. salina* in different concentrations of SW are shown in Fig. 5. The highest and lowest β-carotene levels were observed in 75% SW (3.64 μg mL^{-1}) and the control group (0.51 μg mL^{-1}), respectively ($p < 0.05$).

Approximate chemical composition

The amount of moisture showed no significant differences among treatments (Table 2). The highest and lowest levels of protein were observed in 75% SW (59.18%, dry basis) and the control (46.63%, dry basis), respectively ($p < 0.05$). The highest fat and ash contents were calculated in 75% SW (16.3%, dry basis) and 10% SW (55.31%, dry basis), respectively ($p < 0.05$). The lowest fat (14.8%, dry basis) and ash (39.17%, dry basis) contents were seen in 100% SW ($p < 0.05$; Table 2).

Saponification value of the extracted oil

The highest SV was obtained in 75% SW (195.000 mg KOH g^{-1}), while the lowest value was observed in 100% SW (177 mg KOH g^{-1}) ($p < 0.05$; Fig. 6).
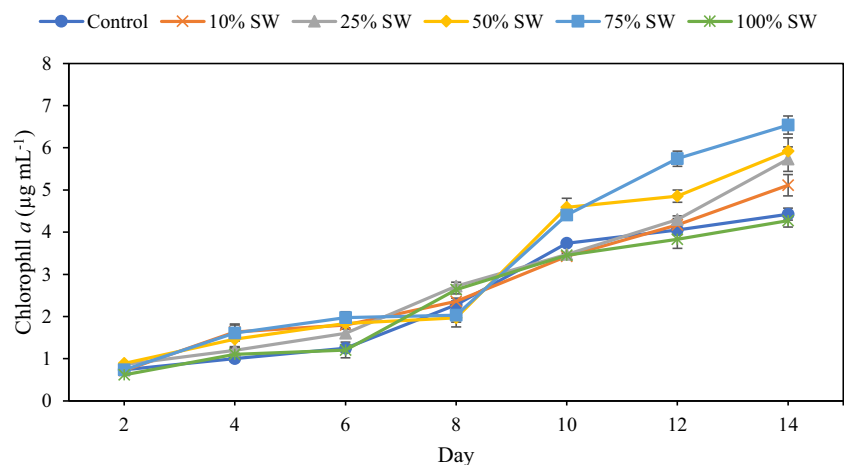
Fig. 3 Chlorophyll *b* concentration of *Dunaliella salina* microalgae grown in different concentrations of stickwater (SW) as a substitute for F/2 medium. The vertical error bars correspond to standard deviation of the mean, and different letters represent statistically significant differences between groups ($n = 3$, $p < 0.05$)
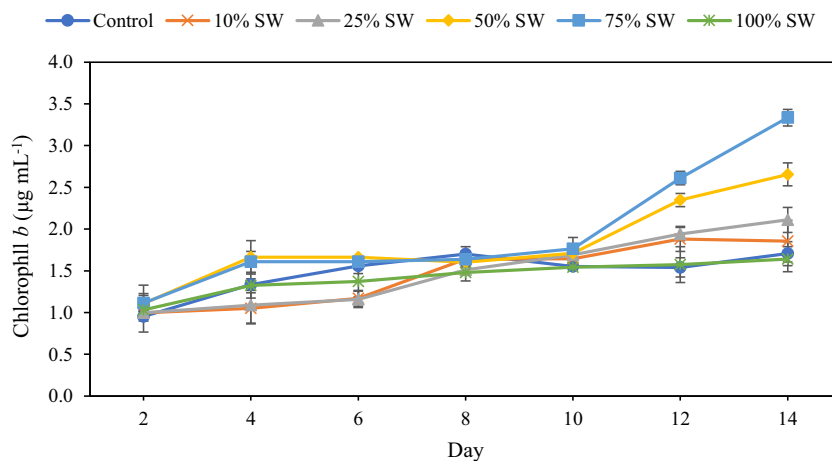

Fatty acid composition

Table 3 shows the FA composition of the extracted oil from *D. salina* which was grown in different concentrations of SW. The highest myristic acid (C14:0) was obtained in the control group (5.84%), while the minimum level was measured in 100% SW (1.31%) ($p < 0.05$). In all samples, palmitic acid (C16:0) was the main FAs and had the highest value in 75% SW (33.59%) compared with the others ($p < 0.05$). The highest and lowest contents of pentadecanoic acid (C15:1) as a dominant mono-unsaturated FAs (MUFAs) were in 10% SW (13.16%) and 100% SW (0.29%) treatments, respectively ($p < 0.05$). Also, the highest value of vaccenic acid (C18:1t) was achieved in the 100% SW group, while the minimum level was seen in the 75% SW treatment ($p > 0.05$). The highest amount of oleic acid (C18:1c) was observed in 100% SW compared with the control group ($p < 0.05$). The maximum amount of linoleic acid (C18:2c) was measured in the control group. The content of other n-6 FAs family members, including gamma linolenic (GLA, C18:3), was changed in a parallel manner, but reached the minimum of 4.84% in 100% SW. There were no significant differences among

experimental treatments for eicosapentaenoic acid (EPA, C20:5) content ($p > 0.05$).

The amounts of MUFAs, saturated FAs (SFAs), and PUFAs were significantly varied among the treatments, and the highest level of SFAs (40.470%) was found in the 75% SW group among other groups ($p < 0.05$). However, the control group showed the highest contents of MUFA (30.445%) and PUFA (27.395%) ($p < 0.05$).

Discussion

The main cost of microalgae production is related to the ingredients in the growth media. However, it is possible to produce a cheaper microalga by replacing the culture medium with low-cost wastewaters, like stickwater from fishmeal factories. Growth is the most important way to express the ecological success or the ability of adaptation of an algae species to the environmental conditions. An increase in the density of *D. salina* microalgae in treated media with partial replacement with SW showed the

Fig. 4 Carotenoid concentration of *Dunaliella salina* microalgae grown in different concentrations of stickwater (SW) as a substitute for F/2 medium. The vertical error bars correspond to standard deviation of the mean, and different letters represent statistically significant differences between groups ($n = 3$, $p < 0.05$)
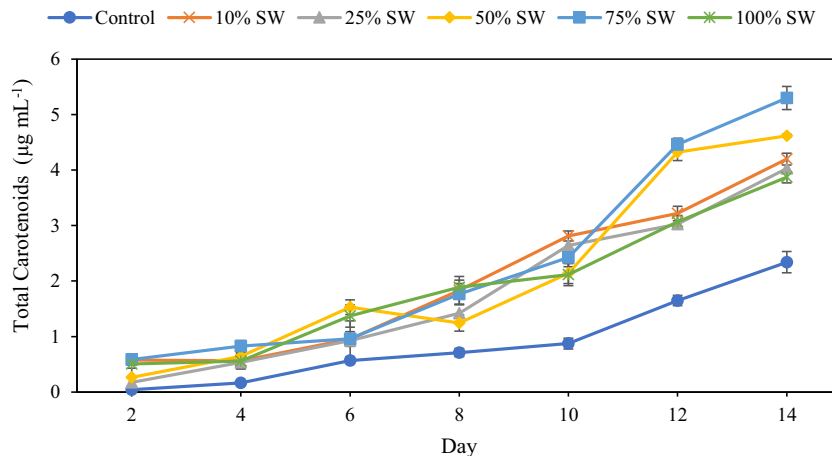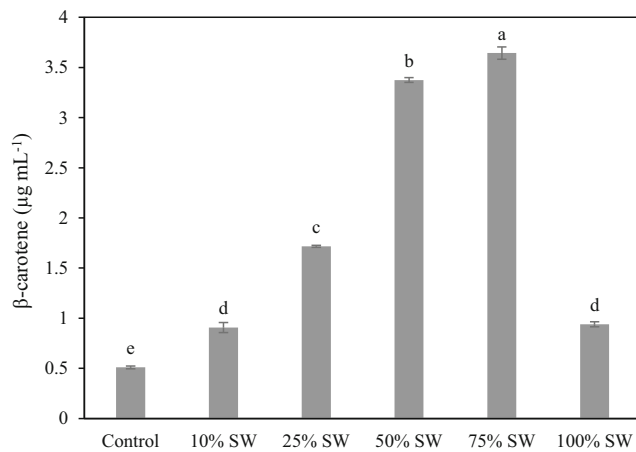
Fig. 5 β-Carotene concentration of *Dunaliella salina* microalgae grown in different concentrations of stickwater (SW) as a substitute for F/2 medium. The vertical error bars correspond to standard deviation of the mean, and different letters represent statistically significant differences between groups ($n = 3$, $p < 0.05$)

Fig. 6 Saponification value of *Dunaliella salina* microalgae grown in different concentrations of stickwater (SW) as a substitute for F/2 medium. The vertical error bars correspond to standard deviation of the mean, and different letters represent statistically significant differences between groups ($n = 3$, $p < 0.05$)
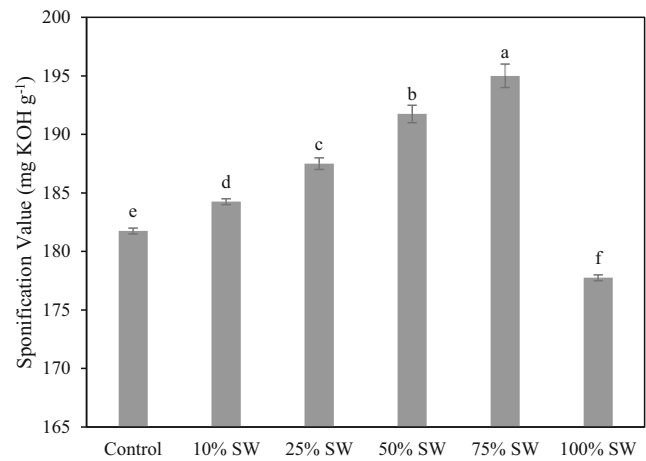
compatibility of these microalgae to growing in wastewater to become a potential bioremediator. Therefore, SW is a liquid nutrient substance that probably can provide a high concentration of nutrients for the microbial biomass.

This study showed that the standard cultivation medium of *D. salina* can be replaced with SW up to 75% without negative effects. However, the microalgae growth performance in a treatment which is totally replaced with SW was significantly decreased possibly due to the inability to properly meet the needs of the algae. Various factors have been proposed to reduce the growth rate of algae, including salinity changes, optical limitations, carbon dioxide or mineral carbon limitations, and undesirable ratios of nitrogen to phosphorus (Tam et al. 1994). Similarly, Kam et al. (2012) explained that the standard growth mediums of *Chlorella* sp., *Pseudomonas aeruginosa*, and *Saccharomyces cerevisiae* could be partially replaced by SW from a kilka fishmeal plant. *Spirulina platensis* was also cultured in agro-industrial wastewaters, and the results showed that producing the microalgal biomass

Table 2 Proximate composition (%, wet basis) of *Dunaliella salina* microalgae grown in different concentrations of stickwater (SW) as a substitute for F/2 medium

Treatment	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Control	98.30 ± 0.10	48.67 ± 0.01d	14.8 ± 0.1d	51.02 ± 0.22b
10% SW	98.29 ± 0.13	54.58 ± 0.44c	15.1 ± 0.0c	55.31 ± 0.32a
25% SW	98.22 ± 0.17	55.67 ± 0.61bc	15.3 ± 0.0c	44.38 ± 0.68c
50% SW	98.21 ± 0.24	56.48 ± 0.57b	15.6 ± 0.1b	42.78 ± 0.06d
75% SW	98.20 ± 0.13	59.18 ± 0.21a	16.3 ± 0.1a	34.57 ± 0.61f
100% SW	98.29 ± 0.20	46.63 ± 0.97e	14.8 ± 0.0d	39.17 ± 0.01e

Data are expressed as mean values ± standard deviations. Different lowercase letters within the same column indicate significant differences among treatments ($n = 3$, $p < 0.05$)

was increased by increasing the nitrogen concentration (Markou and Georgakakis 2011). A study conducted by Lv et al. (2010) showed that low nitrogen concentrations (0.2–0.3 mM) led to a significant reduction in *Chlorella vulgaris* cell growth, while a high concentration of nitrogen (up to 0.5 mM) improved the growth performance. Safafar et al. (2016) examined industrial process water (IPW) as a *C. vulgaris* cultivation medium and indicated that the algal biomass which had been cultured in 67% of IPW was higher than 100% of ICW (internal circulation water of sludge tower reactor). Similar to the current results, the high growth rate and biomass productivity of *C. sorokiniana* were reported when it was grown in the secondary treated effluent of dairy plants (Asadi et al. 2019).

The photosynthetic pigments are specific markers for microalgae cells which are mostly affected by the availability of essential nutrients (Kaya et al. 1995). The present study showed that by increasing the substitution level of F/2 with SW up to 75%, chlorophyll concentrations were increased. The main reason for this increasing trend was the high levels of nitrogen and phosphorus in SW (Mahdabi and Hosseini Shekarabi 2018). Kiran et al. (2016) examined the effects of various concentrations of nitrogen on *Chlorella* growth performance and showed that the algae could not increase the amount of chlorophyll pigments properly under conditions with nitrogen deficiency due to the reduction in electron receptors such as $NADP^+$ at low nitrogen levels (Da Silva et al. 2009; Pruvost et al. 2011; Kiran et al. 2016). Carotenoids are produced in the chloroplasts of microalgae and reach the highest concentrations under stress environmental conditions (Zhu and Jiang 2008). β-Carotene also plays a vital role in protecting chlorophyll from the destructive effects of oxidation and optical suppression in the microorganisms (Ben-Amotz 1981). *D. salina* is a proper candidate for producing

Table 3 Fatty acid content (% of total fatty acid) of *Dunaliella salina* microalgae grown in different concentrations of stickwater (SW) as a substitute for F/2 medium

Fatty acid	Treatments					
	Control	10% SW	25% SW	50% SW	75% SW	100% SW
C14:0	5.84 ± 0.233a	3.69 ± 0.042b	2.26 ± 0.106c	3.64 ± 0.587b	4.21 ± 0.163b	5.61 ± 0.233a
C15:0	0.47 ± 0.014b	0.68 ± 0.233b	0.59 ± 0.120b	0.56 ± 0.205b	0.58 ± 0.24b	1.75 ± 0.049a
C16:0	23.99 ± 0.12e	28.90 ± 0.311d	31.10 ± 0.212b	29.77 ± 0.297c	33.59 ± 0.502a	27.39 ± 0.382e
C17:0	0.39 ± 0.021b	0.36 ± 0.000b	0.31 ± 0.071b	0.55 ± 0.049a	0.36 ± 0.007b	0.20 ± 0.000c
C18:0	1.74 ± 0.198ab	2.01 ± 0.297a	1.24 ± 0.134b	1.31 ± 0.014b	1.29 ± 0.148b	2.29 ± 0.354a
C21:0	0.34 ± 0.042c	0.34 ± 0.057c	0.72 ± 0.049b	0.36 ± 0.064c	0.37 ± 0.021c	1.23 ± 0.233a
C22:0	0.21 ± 0.021a	0.24 ± 0.021a	0.11 ± 0.007b	0.19 ± 0.049a	0.10 ± 0.014b	0.17 ± 0.035ab
C24:0	0.27 ± 0.141a	0.22 ± 0.014ab	0.08 ± 0.014b	0.12 ± 0.078ab	0.08 ± 0.021b	0.14 ± 0.028ab
C14:1	0.58 ± 0.049c	0.26 ± 0.198c	1.51 ± 0.017ab	0.63 ± 0.262bc	0.50 ± 0.148c	1.36 ± 0.403a
C15:1	11.87 ± 0.474b	13.16 ± 0.537a	5.794 ± 0.361d	11.21 ± 0.198b	10.19 ± 0.240c	0.29 ± 0.099e
C16:1	5.32 ± 0.339 ab	3.01 ± 0.622c	5.07 ± 0.891ab	6.27 ± 0.071a	5.12 ± 0.375ab	4.14 ± 0.113bc
C17:1	045 ± 0.007a	0.39 ± 0.007a	0.82 ± 0.212a	0.61 ± 0.042a	0.52 ± 0.021a	0.41 ± 0.424a
C18:1t	3.29 ± 0.085b	1.48 ± 0.184e	2.72 ± 0.092c	2.03 ± 0.000d	1.32 ± 0.057e	6.13 ± 0.014a
C18:1c	8.630 ± 0.170b	10.08 ± 2.036 b	13.18 ± 0.39 a	8.51 ± 0.099 b	10.33 ± 0.099b	14.61 ± 1.577a
C20:1	0.22 ± 0.064ab	0.08 ± 0.014 ab	0.03 ± 0.007 b	0.14 ± 0.170 ab	0.04 ± 0.014b	0.35 ± 0.219a
C22:1	0.06 ± 0.021c	0.25 ± 0.014 a	0.04 ± 0.028 c	0.04 ± 0.007 c	0.25 ± 0.014a	0.20 ± 0.007b
C24:1	0.05 ± 0.014b	0.06 ± 0.042 b	0.03 ± 0.000b	0.03 ± 0.014b	0.06 ± 0.035b	0.25 ± 0.156a
C18:2t	0.40 ± 0.042b	0.81 ± 0.240a	0.42 ± 0.049b	0.29 ± 0.007b	0.34 ± 0.021b	0.25 ± 0.007b
C18:2c	13.45 ± 0.502a	13.05 ± 0.226ab	13.17 ± 0.078b	12.48 ± 0.339c	11.24 ± 0.148d	13.19 ± 0.304b
C18:3t	0.58 ± 0.000ab	0.64 ± 0.198ab	0.82 ± 0.191a	0.45 ± 0.028bc	0.23 ± 0.148cd	0.05 ± 0.014d
C18:3n3	4.89 ± 0.198a	4.12 ± 0.007b	3.51 ± 0.057d	3.77 ± 0.220c	3.90 ± 0.07c	3.21 ± 0.049e
C18:3n6	7.94 ± 0.170a	7.35 ± 0.495a	6.76 ± 0.127b	6.95 ± 0.271b	6.76 ± 0.149b	5.84 ± 0.191c
C20:5	0.12 ± 0.028a	0.11 ± 0.057a	0.13 ± 0.42a	0.10 ± 0.000a	0.10 ± 0.071a	0.11 ± 0.007a
C22:2	0.02 ± 0.028a	0.06 ± 0.021a	0.03 ± 0.000a	0.05 ± 0.007a	0.05 ± 0.014a	0.04 ± 0.007a
SFAs	33.230 ± 0.396e	35.620 ± 0.198d	36.385 ± 0.573c	36.480 ± 0.480c	40.470 ± 0.311a	38.755 ± 1.789b
MUFAs	30.455 ± 0.205a	27.765 ± 0.544bc	28.820 ± 0.509c	29.460 ± 0.242b	28.310 ± 0.085cd	27.720 ± 0.253d
PUFAs	27.395 ± 0.912a	26.130 ± 0.651b	24.825 ± 0.290c	24.075 ± 0.580c	22.600 ± 0.263d	22.665 ± 0.184d

Data are expressed as mean values ± standard. Different lowercase letters within the same row indicate significant differences among treatments ($n = 3$, $p < 0.05$)

natural β-carotene on an industrial scale (Ribeiro et al. 2011). In the current study, total carotenoids and β-carotene were increased by increasing the replacement of cultivation medium of the algae with SW. Although this increase in 100% stickwater treatment was lower than other SW treatments, it was higher than the control group. This could be due to stressful conditions for the algal biomass (Nikokar et al. 2004; Ak et al. 2008). There are many probable reasons to explain the highest amount of pigments in 75% SW treatment including (1) cell growth and multiplication (Kaya et al. 1995; Tam and Wang 2000); (2) decreased incident light by immobilization, self-shading of light, and screen effect by gel, thus increasing the synthesis of pigments (Vichez and Vega 1994); and (3) immobilization which promotes the algal anabolism and physiological activity leading to enhanced removal efficiency of inorganic nutrients (Yan et al. 1995).

In general, microalgae produce protein, lipid, and carbohydrates by consuming the energy in the photosynthesis process (Liu et al. 2012). The present study showed the highest and lowest amounts of protein in *D. salina* in 75% SW and control treatments, respectively. The positive relation between the protein content of algae and the nitrogen concentration of the growth media has been proven by many investigations (Lv et al. 2010; He et al. 2013; Guccione et al. 2014). This may mean that appropriate amounts of nitrogen in the growth medium can lead to stabilized carbon and synthesis of nitrogen-based compounds such as proteins by the cells in the photosynthesis process (Griffiths et al. 2014). Because stickwater has a high level of nitrogen, it uptakes during metabolic activities and increases the nitrogen concentration of the microalgae cells in parallel by increasing the content of cell protein (Djaghoubi et al. 2015; Venckus et al. 2017). The

amount of lipid storage in microalgae can be changed by manipulating some environmental conditions such as light (Yeesang and Cheirsilp 2011), nutrients availability (Gouveia and Oliveira 2009), and temperature (Zhu et al. 2009; Bellou and Aggelis 2012). In this study, the highest content of fat accumulation was observed in 75% SW on day 14. Similar results have been reported regarding the direct effect of nitrogen levels on the fat storage of *Ellipsoidion* sp. and *Isochrysis zhangjiangensis* microalgae (Xu et al. 2001; Feng et al. 2011). Generally, lipid storage level in algae species is estimated to be about 50% of their dry weight, but some types of algae can store fat up to 70% of their dry weight in stressful conditions (Xin et al. 2010). Increases in the nitrogen concentration (NaNO$_3$) of the growth medium lead to increases in the cellular fat accumulation of *Chlorella*. However, fat storage in *Nannochloropsis gaditana* is doubled in comparison with *Chlorella* under nutrient-replete conditions (Ajjawi et al. 2017). Therefore, the response of algae cells to accumulating lipids in order to change the nitrogen budget (increase or decrease) differs among algae species (Sreedharan et al. 2018).

Since microalgae include high levels of essential fatty acids, it is possible to be soaped (El-Mashad et al. 2008). In this study, the highest soap number was seen in the 75% SW treatment (414.1 mg KOH g^{-1}). High levels of SV indicate a higher quality of biofuels (Predojević et al. 2012). Kirk and Sawyer (1991) stated that high levels of SV are mostly due to the high contribution of carbon in short-chain fatty acids. In other words, a lower soap number indicates a longer chain fatty acid (Nielson 1994).

Fatty acids play a crucial role in the algal cell membrane function, metabolism, and adaptation processes against environmental changes (Zhukova and Titlyanov 2006; Sabzi et al. 2018). One protective mechanism of algae for overcoming environmental fluctuations is the synthesis and accumulation of lipids in the form of saturated FAs (Stansell et al. 2011). Stickwater is a rich source of nitrogen and phosphate, which are needed to grow microalgae (Shi et al. 2000). However, kilka fish have non-protein nitrogenous compounds (NPNs), and the stickwater may possibly have high amounts of NPNs. In fact, the NPNs could not be fully absorbed by the algae and negatively impacted the fatty acid composition of the microalgae. The types and concentrations of NPNs differ in various aquatic organisms and generally include free amino acids, peptides, nucleotides, guanidine, quaternary ammonium, and urea (Gram and Huss 1996). Some researchers have shown that microalgae can store high levels of NPN compounds (Dortch et al. 1984), and most marine microalgae species can store 15–30% of the total intracellular nitrogen as NPNs (Lourenço et al. 2004; González López et al. 2010). The possible amount of nitrogen available to *D. salina* algae is decreased because of the high amount of NPNs. By increasing the replacement of the culture medium with SW up to 100%, cell density, pigments, and lipid accumulation were markedly reduced.

In this research, C16:0 level was the major FAs in the 75% SW treatment, a higher rate than those of *Botryococcus braunii*, *Scenedesmus* sp., and *Chlorella* sp. (Lee et al. 2010; Matsumoto et al. 2010). The major FAs in *D. salina* are C16:0 and C18:0 in the standard growth medium (Akter et al. 2016). For biodiesel production, C16:0 is one of the greatest thermal and oxidative stability FAs components among SFAs, which can delay the deterioration of its fuel in high-temperature conditions or long-term storage (Hoekman et al. 2012). The highest SFA content was measured in the 75% SW treatment, while the control group showed the highest amounts of MUFAs and PUFAs. In agreement with the current results, Chandra et al. (2017) reported the highest amount of SFAs from *T. indica* which was grown in secondary treated domestic sewage for 14 days.

The results showed that the lowest GLA content was obtained in 100% SW in comparison with the other treatments. This reduction may be explained by the fact that the microalgae were not able to meet their nutritional demands in order to produce bioactive compounds from SW when F/2 was replaced with 100% SW. GLA from PUFAs plays a key role as the precursor of eicosanoids that are implicated in many physiological functions of biological systems (Bellou et al., 2016). High GLA content is found in most commercial microbial oils of some fungi species (Ratledge 2013). Producing these FAs is highly interesting for nutritional and pharmaceutical applications due to its anti-inflammatory properties and therapeutic values (Gunstone 1992). The quantity and quality of microalgae FAs could be changed and promoted by manipulating the media and environmental conditions (Bougaran et al. 2012; Draaisma et al. 2013; Vinayak et al. 2015). In fact, algae produce FAs as building blocks in their plastids to form different types of fat for construction of the cell membrane or to be stored in its cells (Ohlrogge and Browse 1995).

Conclusion

Stickwater is produced in fishmeal plants, and, despite the high concentrations of nutrients, it is directly discharged into the environment. In this study, replacing *D. salina* microalgae standard growth medium (F/2) with different concentrations of SW showed the highest cell density, chlorophyll, beta-carotene, carotenoid, lipid accumulation, and protein contents in the treated medium with 75% SW. Moreover, the highest amounts of fat storage and SFA composition were observed in the 75% SW treatment. However, complete substitution (100%) of F/2 with SW negatively influenced the microalgae growth performance and biochemical compositions. Therefore, *D. salina* growth medium can be replaced with

75% SW for the production of biofuel and some bioactive compounds, like carotenoids, with many economic and environmental advantages. Further supplementary studies are needed to assess how effective *D. salina* is in removing the nutrients of SW.

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