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Original article

Evaluation of halophilic microalgae isolated from Rabigh Red Sea coastal area for biodiesel production: Screening and biochemical studies

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ABSTRACT

In the present study, different water samples from Red Sea coastal area at Rabigh city, Saudi Arabia were studied for their dominant algal species. Microalgal isolation was carried out based on dilution method and morphologically examined using F/2 as a growth medium. Dry weight and main biochemical composition (protein, carbohydrates, lipids) of all species were performed at the end of the growth, and biodiesel characteristics were estimated. *Nannochloropsis* sp., *Dunaliella* sp., *Tetraselmis* sp., *Prorocentrum* sp., *Chlorella* sp., *Nitzschia* sp., *Coscinodiscus* sp., and *Navicula* sp. were the most dominant species in the collected water samples and were used for further evaluation. *Nannochloropsis* sp. surpassed all other isolates in concern of biomass production with the maximum recorded dry weight of 0.89 g L⁻¹, followed by *Dunaliella* sp. (0.69 g L⁻¹). The highest crude protein content was observed in *Nitzschia* sp. (38.21%) and *Dunaliella* sp. (18.01%), while *Nannochloropsis* sp. showed 13.38%, with the lowest recorded lipid content in *Coscinodiscus* sp. (10.09%). Based on the growth, lipid content, and biodiesel characteristics, the present study suggested *Dunaliella* sp. and *Nitzschia* sp. as promising candidates for further large-scale biodiesel production.

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1. Introduction

Due to energy shortage and negative environmental consequences of fossil fuels excessive utilization, scientific research has been motivated towards exploring alternative energy sources that meet the world demand and mitigate the climate change through carbon dioxide sequestration and/or emissions reduction (Jia et al., 2014; Abomohra et al., 2017). Among different resources, biomass-based fuels have been proposed as a sustainable, renewable and eco-friendly alternatives (Jia et al., 2014; Almutairi, 2020a,b). Edible oils, non-edible lignocellulosic biomass, and municipal waste have been discussed as biofuel feedstocks (Ebaid et al., 2019; Abomohra et al., 2021a; Abomohra et al., 2021b; Li et al., 2021). However, edible and non-edible biomass, known as first- and second-generation biofuel feedstocks, have serious economic and environmental impacts, raising critical sustainability and food safety issues (Doan et al., 2011;

Abomohra et al., 2016; El Arroussi et al., 2017; Munisamy et al., 2018). Recently, algal biomass has attracted much attention as a third-generation feedstock for biodiesel production (Wang et al., 2018; Abomohra et al., 2021c; Almutairi et al., 2021). They have numerous recomences in comparison with the other biofuel generations due to the relatively high lipid and biomass production, no need for arable land to grow where they can be grown in brackish or saline water, tolerate the extreme environments, and no need to apply pesticides or herbicides. Moreover, microalgae grow photosynthetically where they can fix carbon dioxide, reducing the greenhouse gases impact (Selvarajan et al., 2015; Li et al., 2015). Also, microalgal cells utilize phosphorus and nitrogen from wastewater, adding extra advantage of bioremediation (Abdelaziz et al., 2014; Shao et al., 2018), with the advantage of genetic transformation and gene editing (Barati et al., 2021). Therefore, microalgal biodiesel is well-thought-out as a potential renewable biofuel to compensate fossil diesel without affecting the crop products or competing with agricultural land.

Nevertheless, biodiesel commercial production from microalgal biomass is not economically feasible yet due to the high production cost (Gumbi et al., 2017; Abomohra et al., 2018) attributed to utilization of freshwater, nutrients, and harvest cost (Khan et al., 2018). Therefore, enhancements of upstream and downstream processes to improve cost-effective biomass production is

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of great importance. These include optimization of cultivation conditions, innovative lipid extraction techniques, co-product development and management, and combining microalgal cultivation with wastewater treatment or seawater desalination (Abdelaziz et al., 2014; Taleb et al., 2016; Li et al., 2015). However, screening of microalgae for high lipid production with a suitable fatty acid (FA) profile using seawater is considered as a bottleneck to enhance the process economy. Therefore, the objective of the present screening study is to isolate and evaluate different indigenous microalgal isolates at Red Sea coastal area for high lipid and biodiesel production. Marine microalgae were isolated and the biochemical composition was determined. Biodiesel yield and characteristics were also determined for all isolates to recommend a promising species.

2. Materials and methods

2.1. Seawater sampling

Seawater samples were collected from Red Sea coastal area at Rabigh city, Saudi Arabia. Using a plankton net of 20- μm mesh, about 1 L of seawater at each location was filtered to remove seaweeds and suspended particles, then water samples were collected in sterile tubes. It was moved to sterilized flasks and incubated under light conditions for enrichment. Microalgal cells were isolated and purified by serial dilution followed by cultivation in Petri plates (Vu et al., 2018) containing sterile F/2 medium (Guillard and Ryther, 1962) and incubated under continuous illumination ($120 \mu\text{mol m}^{-2} \text{s}^{-1}$) at 25 °C.

2.2. Morphological identification

After isolation and purification, 8 marine microalgal strains were isolated in axenic culture and used in further experiments. The isolates were identified according to their morphological features (Hoek et al., 1995) using light microscope at 100x objective lens (Olympus BX53).

2.3. Inoculum preparation

The obtained axenic cultures were preliminary scaled up in 300 mL glass tubes, then cultivation was performed in 14 L fully transparent Plexi-Glass columns (El et al., 2015). Finally, the aeration was turned off to allow gravity settling for overnight. The upper layer was discarded, followed by dewatering by centrifugation (4000 rpm, 10 min) of the remained biomass. The obtained cells were washed three times with pre-sterilized artificial seawater and then used in next experiments.

2.4. Microalgal growth

Microalgae were incubated in all experiments at $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ of continuous light intensity at aeration with 3% CO_2 filtered air.

2.4.1. Growth parameter

During the whole cultivation period, growth of the 8 isolates was determined in triplicates as dry weight (g L^{-1}). Samples were centrifuged for 10 min at 3000x g then cell pellet was transfer to a pre-weighed 1.5 mL Eppendorf tube. After that, sample was freeze-dried at $-80 \text{ }^\circ\text{C}$, then cell weight of the dried samples was calculated. Biomass productivity was determined by measuring the wet and dry biomass on the last day of experiment, and was calculated as previously described (Abomohra and Almutairi, 2020).

2.4.2. Chemical and biochemical analysis

Cell metabolites including proteins, carbohydrates and lipids were analyzed. Based on the method of Ma and Zuazaga (1942), total proteins (%) were determined as $\text{T.N} \times 6.25$ and defined as crude protein, while true protein was measured through determination of soluble protein by Trichloroacetic acid (TCA). Then true protein content was calculated by subtracting the soluble protein from total protein. The method of Dubois et al. (1956) was performed to measure total carbohydrate content. Dinitrosalicylic acid (DNS) method was used to estimate the reducing sugars using glucose as a standard (Miller, 1959). Lipid extraction was performed by Soxhlet extraction using *n*-hexane as a solvent (Reda et al., 2020). Fatty acid methyl esters (FAMES) were prepared and analyzed according to the modified method of Christie (1993) as describe previously (Almarashi et al., 2020).

2.5. Biodiesel properties

The main biodiesel characteristics including cetane number (CN), long chain saturated factor (LCSF), saponification value (SV), cold filter plugging point (CFPP), iodine value (IV), and degree of unsaturation (DU) were evaluated for different isolates according to Ramos et al. (2009) and Francisco et al. (2010) as follows;

$$DU = MUFA + (2 \times PUFA)$$

$$LCSF = (0.1 \times C16 : 0) + (0.5 \times C18 : 0) + (1 \times C20 : 0) + (1.5 \times C22 : 0) + (2 \times C24 : 0)$$

$$CFPP = (3.1417 \times LCSF) - 16.477$$

$$SV = \sum 560F / M_w$$

$$IV = \sum 245FD / M_w$$

$$CN = 46.3 + (5458 / SV) - (0.255 \times IV)$$

where *F* represents each fatty acid proportion (as % of total fatty acids); *D* represents the double bonds number, and *M_w* is the fatty acid molecular weight.

3. Results

3.1. Growth and biomass production

The eight strains isolated in the present study were belonging to Chrythophyta (*Nannochloropsis* sp.), Chlorophyta (*Dunaliella* sp., *Chlorella* sp., and *Tetraselmis* sp.) Dinoflagellates (*Proocentrum* sp.) and Bacillariophyta (*Nitzschia* sp., *Navicola* sp., and *Cosinodiscus* sp.), revealing the dominance of Chlorophytes and Bacillariophytes in water samples. Different growth patterns were observed among the studied isolates by measuring the dry weight (Fig. 1). *Nannochloropsis* sp. surpassed all other isolates regarding biomass accumulation (0.89 g L^{-1}) and biomass productivity ($0.05 \text{ g L}^{-1} \text{ d}^{-1}$), while *Nitzschia* sp. showed the lowest dry weight (0.33 g L^{-1}) and biomass productivity ($0.011 \text{ g L}^{-1} \text{ d}^{-1}$). Followed by *Nannochloropsis* sp., *Dunaliella* sp. showed high biomass productivity of $0.047 \text{ g L}^{-1} \text{ d}^{-1}$.

3.2. Biochemical composition

3.2.1. Protein content

Under similar growth conditions, crude protein in different isolates ranged from 21% to 38% (Fig. 2A). Among different isolates, *Nitzschia* sp. showed the highest crude protein content, while the Chlorophyte *Dunaliella* sp. showed the minimum. *Nitzschia* sp.,

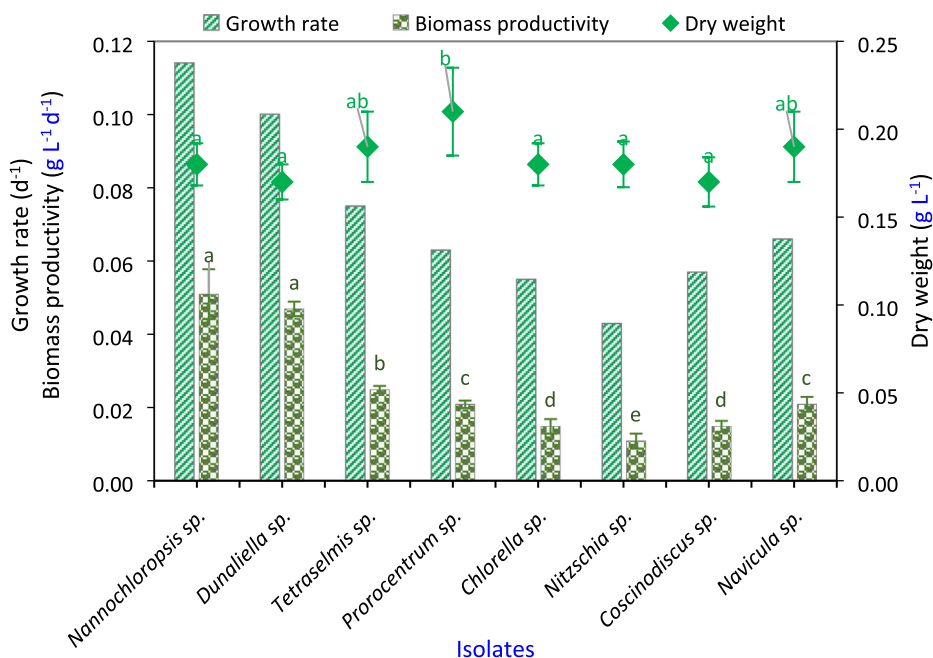


Fig. 1. Dry weight, biomass productivity, and growth rate of different marine water isolates grown in F/2 medium. The same series with different letters showed significant differences ($P < 0.05$).

which exhibited the highest protein content comparing with other examined algae, also showed the highest soluble protein (2.04%) and high true protein (36.17%). A moderate content of *Nannochloropsis* protein was detected (28.19%).

3.2.2. Carbohydrates

Total carbohydrates of Chlorophyta genera were found higher than other genera and such content ranged from 22.06% in *Chlorella* sp. to 38.16% in *Tetraselmis* sp. to the maximum of 39.07% in *Dunaliella* sp. (Fig. 3). The highest carbohydrates content of 42.13% was recorded in *Prorocentrum* sp.; while the lowest carbohydrate content of 19.36% was recorded in *Nitzschia* sp. Reduced sugars are the most important fraction for metabolism of algal cells and for other beneficial industrial uses. *Bacillariophyte* species showed relatively lower reduced sugars content (3.15–5.08% on dry weight basis); while *Chrythophytes* represented a moderate level (5.81%), where the highest content was recorded in Dinoflagellates (8.98%). Overall, based on the relatively high content of carbohydrate/reducing sugars recorded in most of the studied species, they can be used as a proper feedstock for bioethanol production through fermentation.

3.2.3. Lipids

The third algal metabolites (lipids and their fractions) have been discussed during the last years as the most important biomass constituent for renewable energy production in the form of biodiesel. Many factors are affecting such content in microalgal cells. In the current study, 10.09–19.17% of total lipids were recorded in the eight tested species (Fig. 4). Among all, *Nitzschia* sp. showed the maximum lipid content (19.17%); while the lowest was recorded in *Coscinodiscus* sp. (10.09%).

3.2.4. Fatty acids and biodiesel properties

In the present study, FAMES composition was varied based on the isolate (Table 1), ranging from C8:0 to C24:0 carbon chain length. In different isolates, myristic acid (14:0) recorded the most abundance (30.77%) in the Chlorophyte *Tetraselmis* sp., while *Prorocentrum* showed the lowest content of 1.32% (Table 1). The fatty

acids within the range of C14–C18 represented from 52.22% in *Coscinodiscus* sp. to 83.05% in *Prorocentrum* sp. The aforementioned fatty acids chemical composition markedly affected the properties of the produced biodiesel, where long chain fatty acids as well as saturated fatty acids responded on the initial energy of biodiesel. Polyunsaturated fatty acids (PUFAs) are the key components maintaining the membrane fluidity of cells avoiding cells rupturing (Hyun et al., 2016). As shown in Table 2, saponification values of different produced FAMES ranged from 200.19 in *Coscinodiscus* sp. to 219.53 in *Nannochloropsis* sp. The high saponification value monitoring the short chain fatty acids, which in turn reduces the net obtained energy compared with those of long chain fatty acids. Cetane number is a main parameter to indicate the biodiesel quality, reflecting its ignition quality (Francisco et al., 2010). Among different isolates, *Dunaliella* sp. showed the maximum cetane number of 32.07 (Table 2). In addition, European biodiesel standards (EN 14214, 2008) recommended iodine value of ≤ 120 g I₂/100 g oil for the best engine performance (Abomohra et al., 2021c). The present study showed compatible iodine value in the isolates *Dunaliella* sp., *Chlorella* sp., *Tetraselmis* sp., *Prorocentrum* sp., and *Navicula* sp. (63.38, 86.57, 111.37, 118.71, and 86.88 g I₂/100 g oil, respectively).

4. Discussions

Within the same microalgal growth conditions, variation in biomass accumulation of different isolates might be attributed to the specific requirements of each species and/or media suitability. Nutritional requirements and salinity margins differ from species to another, which explains the recorded variation in the growth and biochemical composition of different genera under the same growth conditions. Although F/2 has been suggested as a common growth medium for marine microalgae, the current study confirmed that it has significant difference in growth acceleration from a species to another. It also can be noted that *Prorocentrum* sp. has a higher dry weight comparing to *Nannochloropsis* sp., however, it showed lower biomass productivity, which is attributed to the slow growth within a longer incubation period. Regarding *Duna-*

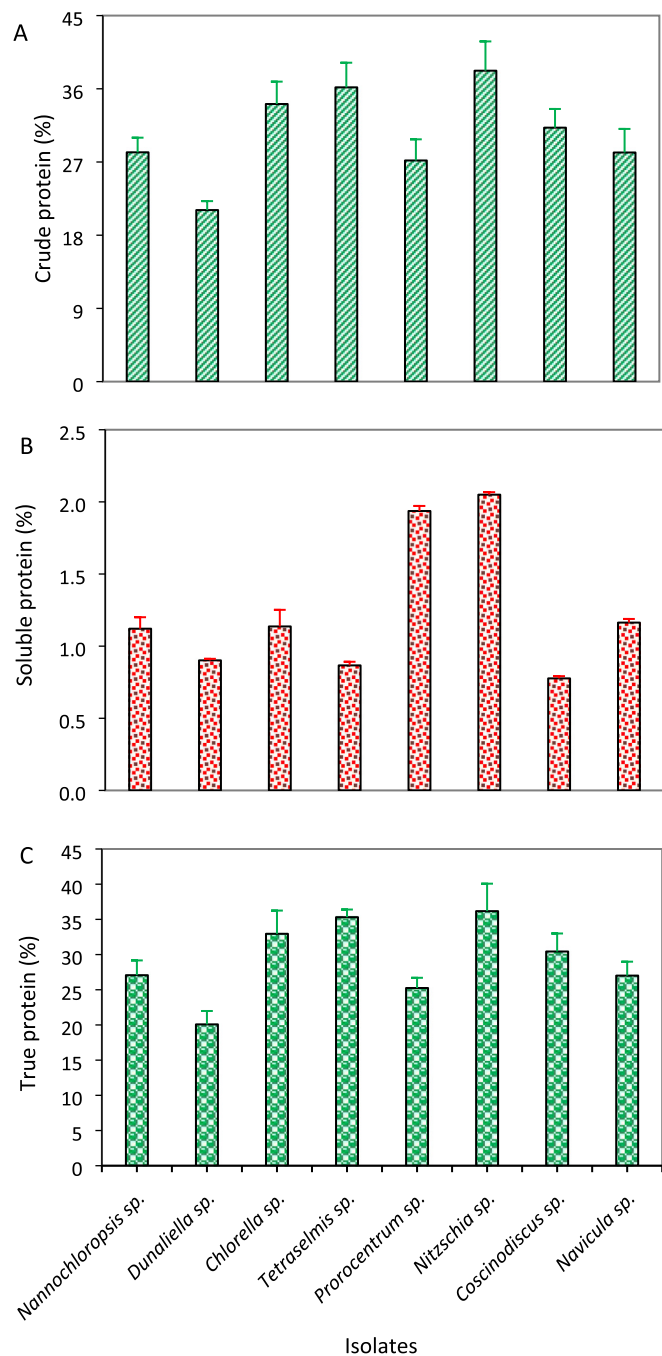


Fig. 2. Soluble protein (B), crude protein (A), and true protein (C) content of different marine water isolates grown in F/2 medium.

liella sp., Costa et al. (2004) stated that growth rate of *Dunaliella* was enhanced by 30% using seawater-enriched F/2 medium. In addition, a recent study (Abomohra et al., 2020a) confirmed the potential of *Dunaliella* sp. to grow at extreme salinity levels up to 250‰. Thus, supplementation of nutrients to the medium could enhance the microalgal growth and further improves the microalgal biomass production rate (Xin et al., 2010). Overall, response of different microalgal species to utilize the growth medium depends mainly on their specific biological requirements which results in considerable differences among different genera/species.

Due to environmentally friendly cultivation of microalgae without competing with agriculture industry and various unique metabolites with remarkable biological qualities, microalgae are

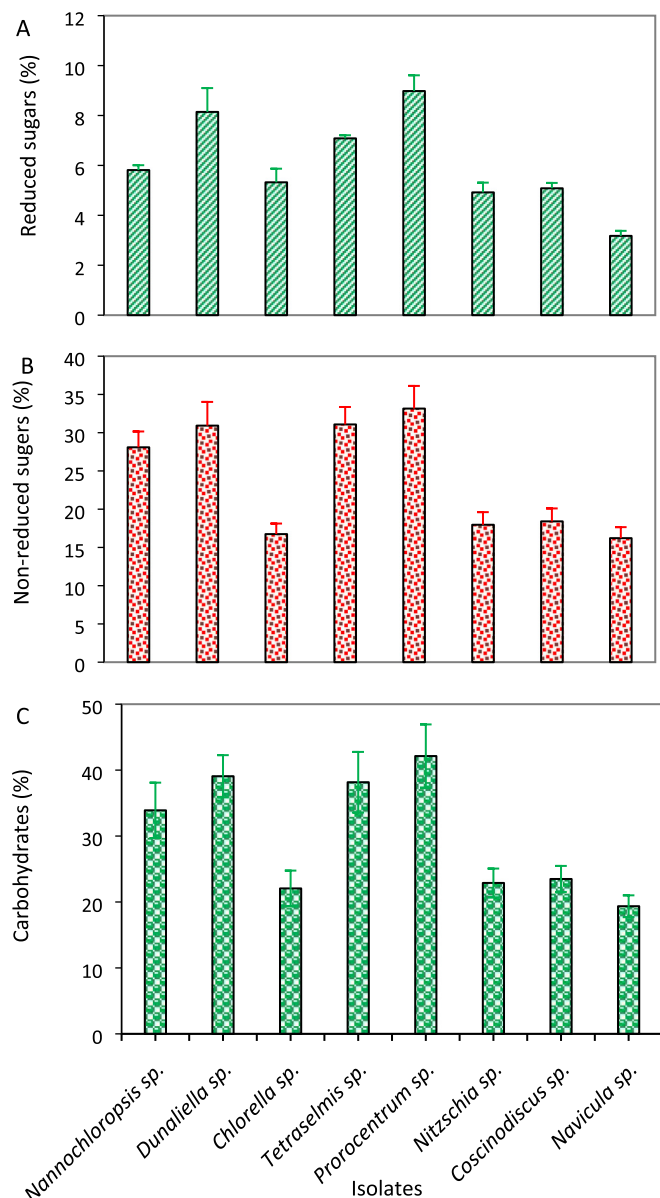


Fig. 3. Reducing sugars (A), non-reducing sugars (B), and carbohydrate (C) content of different marine water isolates grown in F/2 medium.

widely discussed as a potential alternative sustainable source for many industrial products (Bhosale et al., 2010; Khan et al., 2017; Shao et al., 2019). However, selection of the promising candidate for a target product still the main challenge for commercialization. Natural habitats approximately define the biochemical profile within the same margin rather than the belonging taxa. For instance, *Chlorella vulgaris* and *Scenedesmus obliquus* greatly differ in their biochemical composition than *Dunaliella* sp. due to the salinity margin of their natural habitats.

Following carbon, nitrogen represents the second constituent of cellular minerals due to its important role in cell metabolism. Protein is the main constituent belonging to nitrogen-containing compounds, where protein content ranges from 10% to about 60% in microalgae (Salbitani and Carfagna, 2021). Both of environmental conditions and nutritional status markedly influence such content, and the first monitor of drastic conditions is the reduction in protein content (El-Sayed, 1999; Almutairi, 2020a,b). Publications on algal protein content are widely varied even within a sole species, where three different *Dunaliella* sp. (ABRIINW-B1, GT/1, and 11) showed

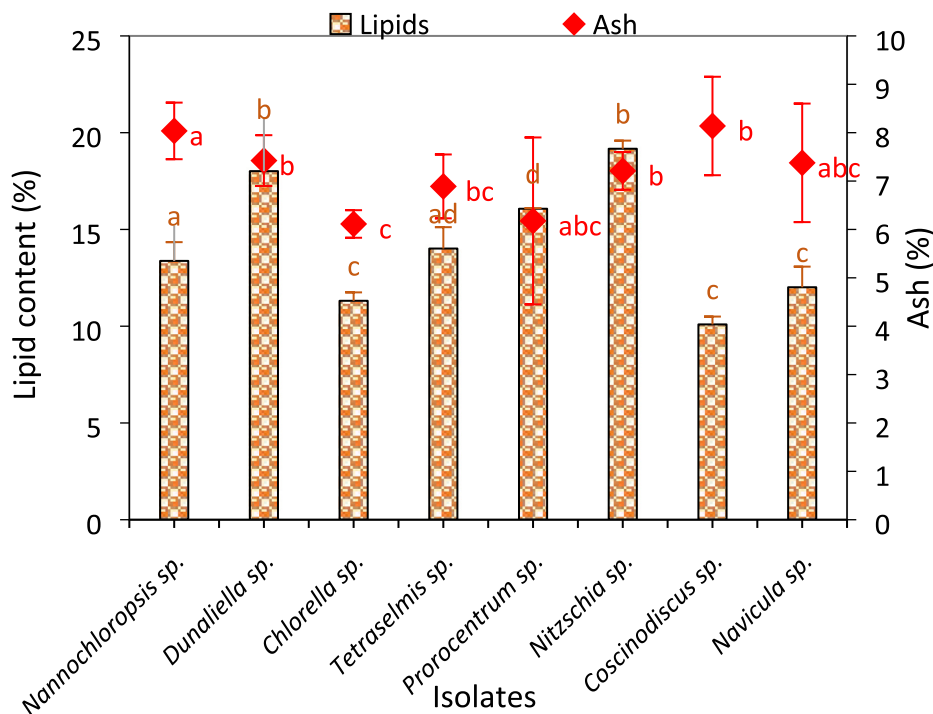


Fig. 4. Lipid and ash contents of different marine water isolates grown in F/2 medium. The same series with different letters showed significant differences ($P < 0.05$).

Table 1

Fatty acids methyl ester of different marine water isolates grown with F2 growth medium.

Fatty acids	Carbon length	<i>Nannochloropsis</i> sp.	<i>Dunaliella</i> sp.	<i>Chlorella</i> sp.	<i>Tetraselmis</i> sp.	<i>Prorocentrum</i> sp.	<i>Nitzschia</i> sp.	<i>Coscinodiscus</i> sp.	<i>Navicula</i> sp.
Acrylic	8:0	4.19 ± 0.19	0.61 ± 0.03	ND	ND	0.91 ± 0.08	1.96 ± 0.07	ND	ND
Caprice	10:0	5.57 ± 0.23	0.19 ± 0.01	ND	ND	4.59 ± 0.19	3.14 ± 0.19	ND	ND
Laurie	12:0	6.14 ± 0.31	0.41 ± 0.12	ND	ND	4.71 ± 0.21	2.09 ± 0.11	6.12 ± 0.25	0.37 ± 0.02
Meristic	14:0	4.17 ± 0.15	16.04 ± 0.81	30.77 ± 1.51	1.32 ± 0.09	12.61 ± 0.37	8.05 ± 0.24	9.69 ± 0.42	3.21 ± 0.11
Myristoleic	14:1	5.13 ± 0.11	4.51 ± 0.17	4.78 ± 0.13	ND	2.44 ± 0.11	3.66 ± 0.15	ND	20.1 ± 0.78
Palmitic	16:0	11.32 ± 0.48	10.23 ± 0.51	14.03 ± 0.62	24.8 ± 1.2	8.52 ± 0.24	9.25 ± 0.28	9.64 ± 0.38	24.9 ± 0.88
Palmitoleic	16:1	9.18 ± 0.32	14.91 ± 0.74	12.61 ± 0.41	7.09 ± 0.32	11.32 ± 0.48	19.6 ± 0.62	16.4 ± 0.52	29.1 ± 0.92
Stearic	18:0	3.94 ± 0.12	2.85 ± 0.14	6.04 ± 0.22	12.4 ± 0.44	7.51 ± 0.32	2.08 ± 0.04	3.49 ± 0.14	3.02 ± 0.17
Oleic	18:1	2.78 ± 0.09	22.91 ± 1.12	2.54 ± 0.08	22.3 ± 1.09	9.41 ± 0.29	2.27 ± 0.02	2.15 ± 0.09	3.4 ± 0.21
Linoleic	18:2	13.41 ± 0.51	13.04 ± 0.47	6.41 ± 0.34	15.14 ± 0.72	5.41 ± 0.23	11.52 ± 0.78	5.94 ± 0.15	2.25 ± 0.09
Linolenic	18:3	9.02 ± 0.33	2.61 ± 0.11	5.02 ± 0.12	ND	3.97 ± 0.08	9.21 ± 0.41	4.91 ± 0.19	0.97 ± 0.04
Arachidic	20:0	2.17 ± 0.11	1.15 ± 0.05	2.18 ± 0.09	1.14 ± 0.04	3.62 ± 0.14	3.27 ± 0.11	ND	ND
Eicospentanoic	20:1	3.28 ± 0.17	1.14 ± 0.04	1.01 ± 0.06	1.06 ± 0.03	3.09 ± 0.09	6.41 ± 0.19	4.65 ± 0.19	4.01 ± 0.14
Dihomo- γ -linolenic	20:3	ND	ND	1.17 ± 0.03	ND	1.06 ± 0.03	1.87 ± 0.04	ND	0.38 ± 0.01
Arachidonic	20:4	7.19 ± 0.24	ND	2.64 ± 0.07	ND	9.32 ± 0.45	4.51 ± 0.14	3.07 ± 0.23	2.17 ± 0.15
Eicospentanoic	20:5	10.42 ± 0.37	ND	ND	2.09 ± 0.11	2.09 ± 0.18	3.58 ± 0.21	23.1 ± 0.89	3.25 ± 0.19
Docosanoic	22:0	ND	2.4 ± 0.12	0.41 ± 0.01	ND	ND	0.19 ± 0.02	ND	0.18 ± 0.01
Docosaenoic	22:1	ND	5.07 ± 0.21	ND	2.74 ± 0.13	1.04 ± 0.05	1.08 ± 0.08	3.52 ± 0.14	ND
Docosahexanoic	22:6	ND	ND	8.61 ± 0.22	9.17 ± 0.44	6.21 ± 0.31	3.87 ± 0.32	3.27 ± 0.21	0.54 ± 0.02
Lignoceric	24:0	ND	ND	ND	ND	1.09 ± 0.08	1.01 ± 0.03	2.14 ± 0.13	ND
SFA (%)		37.5 ± 1.59	33.88 ± 1.79	53.43 ± 2.45	39.66 ± 1.77	43.56 ± 1.63	31.04 ± 1.09	31.08 ± 1.32	31.68 ± 1.19
MUFA (%)		20.37 ± 0.69	48.54 ± 2.28	20.94 ± 0.68	33.19 ± 1.57	27.3 ± 1.02	33.02 ± 1.06	26.72 ± 0.94	56.61 ± 2.05
PUFA (%)		40.04 ± 1.45	15.65 ± 0.58	23.85 ± 0.78	26.4 ± 1.27	28.06 ± 1.28	34.56 ± 1.9	40.29 ± 1.67	9.56 ± 0.5

ND = not detectable.

protein content of 19%, 41% and 31%, respectively, under the same conditions (Gharajeh et al., 2020). Other species of *Dunaliella* are rich in protein, where 40–57% was found in *Dunaliella salina* (Milledge, 2011) and 49% in *Dunaliella bioculata* (Van Krimpen et al., 2013). Microalgal proteins are currently used in aquaculture feed and provided as a health functional food due to the complete profile of essential amino acids (EAAs) (Schwenzfeier et al., 2011; Koyande et al., 2019). In that context, Xu-xiong et al. (Xu-xiong et al., 2004) found that exponential phase of *Nannochloropsis* have the highest

crude protein content (33.99%), while the lowest (28.33%) was recorded in the stationary phase. However, it showed higher amino acid content (225.02 mg g⁻¹ and 214.82 mg g⁻¹) at stationary phase and phase of declining relative growth, respectively, with lowest amino acid content (98.87 mg g⁻¹) in the exponential phase. Therefore, the total lipid content of algae in phase of declining relative growth was significantly higher than those in exponential phase and stationary phase. Under unfavorable conditions of high salinity, nitrogen depletion, high light irradiation, protein decomposition

Table 2

The main fuel properties of biodiesel produced from the different marine water isolates grown in F/2 medium.

Properties	<i>Nannochloropsis</i> sp.	<i>Dunaliella</i> sp.	<i>Chlorella</i> sp.	<i>Tetraselmis</i> sp.	<i>Prorocentrum</i> sp.	<i>Nitzschia</i> sp.	<i>Coscinodiscus</i> sp.	<i>Navicula</i> sp.
SV	219.53	210.99	214.76	202.68	212.65	212.52	200.19	214.83
IV	123.28	63.38	86.57	111.37	118.71	132.48	172.34	86.88
CN	18.59	32.07	26.85	21.27	19.62	16.52	7.55	26.78
DU	89.65	79.96	56.85	85.99	80.62	102.14	107.30	74.65
LCSF	5.272	7.20	7.22	7.34	8.23	5.52	2.71	4.27
CEPP	0.086	6.14	6.20	6.58	9.37	0.87	0.62	-3.06

CN = cetane number; SV = saponification value; IV = iodine value; DU = degree of unsaturation; LCSF = long-chain saturated fatty acid; and CEPP = cold filter plugging point.

take place in many ways coupling with growth failure. In this case, the net gain of protein decomposition to other nitrogenous compounds takes place which are easy to loss from the algal growth media. To avoid the loss of dry weight regardless the protein decomposition, correct extending photosynthesis should be employed through carotenogenesis, which is the main method used to increase the lipid content of microalgae (El-Sayed, 2010).

In terrestrial plants and microalgae, polysaccharides are used as structural elements and for energy storage. Comparing to treated lignocellulosic biomass or conventional sugars resources, microalgal carbohydrates are preferred as an alternative in fermentation processes (Xu et al., 2019). Microalgal biomass and their less-value residues can be converted into high-quality bioethanol through fermentation (Lam and Lee, 2012; Chew et al., 2017). Naturally, many enzymes can degrade the lignocellulosic starch to simple sugars for easier transportation across the gut lumen, but cannot digest many more complex polysaccharides, known as dietary fibers (Saiki, 1906), that can be fermented in the large intestine based on the enzymatic production of the microbiome (Cian et al., 2015). Algal cell walls are different since they contain unique polysaccharides and polyuronides that may be acetylated, methylated, sulfated, or pyruvylated (Stiger-Pouvreau et al., 2016). Mostly, carbohydrate serves as one of the main growth metabolites of algal biomass and certain sugars are much desired for different purposes. A saline alga also surpasses those of freshwater or brackish water grown algae as a functional component on the expense of protein content. For instance, 37.6% available carbohydrates of *Nannochloropsis* sp. was obtained by Reboloso-Fuentes et al. (2001) compared to 28.8% protein content. In addition, saccharides from algae have various antitumor, antimicrobial, antiviral, anticoagulant, and fibrinolytic properties (Dere et al., 2003). Thus, the present study can be served as information source for further wide-range applications of the studied marine microalgae for different carbohydrates-based industrial purposes. The rise in reduced sugars content was found to be positively correlated to the total carbohydrates content, in which reducing sugars from microalgae is a good feedstock for bioethanol production (El et al., 2015; El-Sayed et al., 2017; El-Sayed et al., 2020).

Comparing to other biodiesel feedstocks, microalgae can accumulate higher amounts of cellular lipids that can be further converted to biodiesel by transesterification. Thus, high lipid content of a certain species is one main criterion to select a proper microalgal strain as a renewable biodiesel source (Wang et al., 2022). Lipid content is widely varied within the same genus, as for example, lipid contents of 42%, 47%, and 36% were recorded in different isolates of *Dunaliella* sp. (Ahmed et al., 2017; Khan et al., 2017). Concerning *Dunaliella*, lipid content in the present study was above the reported middle data range of this genus, while it is similar to that of *Dunaliella salina* reported by Adarme-Vega et al. (2014). Regarding fatty acids, microalgae are considered as the original long chain and very-long-chain PUFAs producers (>18C and > 20C, respectively) including n3 and n6 fatty acids. These fatty acids enter the human body through the food chain by fish feeding on phytoplankton, which is considered as the subsequent nutritional source

(Kainz et al., 2009). These fatty acids can be used for nutrition, which promote the biorefinery approach of microalgal biomass. In addition, fatty acid profile significantly influences the main biodiesel characteristics (Abomohra et al., 2020b). Due to the varied fatty acid profile among different studied species, they showed different biodiesel characteristics. However, most of the estimated characteristic were within the recommended ranges by international standards. Putting altogether, the present study suggests *Dunaliella* sp. and *Navicula* sp. as potential candidates for biodiesel production, which require future studies on the optimization of growth conditions for enhanced biodiesel and biomass production.

5. Conclusions

This work aimed to evaluate different dominant microalgal species isolated from marine water at the coastal area of the Red Sea for possible application as biodiesel feedstocks. Results showed that *Nannochloropsis* sp. has the highest biomass yield, but it showed low lipid content (13.38%) and non-desirable biodiesel properties regarding cetane number and iodine value. On the other hand, *Dunaliella* sp. and *Navicula* sp. showed the highest lipid contents among the studied species (18.01% and 19.17%, respectively) with biodiesel characteristics complied with the international standards. Therefore, these two isolates are recommended as promising candidates for further studies and large-scale lipid production which could enhance the biodiesel production from marine microalgae. In addition to biodiesel production, the present screening study could be served as information pool for further wide-range applications of the studied marine microalgae at Rabigh coastal area for different industrial purposes.

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.

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