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CULTIVATION OF *DUNALIELLA* SP. USING FISH PROCESSING WASTEWATER AS A NUTRIENT SOURCE: EFFECT ON GROWTH, BIOMASS PRODUCTION, AND BIOCHEMICAL PROFILE

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ABSTRACT

Fish processing wastewater (FPWW) has the potential for providing inorganic and organic nutrients, which can be chemically processed and produced as a medium for the production of microalgae. Here the effects of FPWW (1, 3, 5, and 7 mL/L) and Walne medium (as a control) on the growth, biomass, and biochemical content of *Dunaliella* sp. were evaluated. An increase in FPWW concentration enhanced the growth, biomass production, and pigment and protein content of *Dunaliella* sp. There was no significant difference in the growth, biomass, pigment, protein, and lipid content between the FPWW concentration of 7 mL/L and Walne medium (control) ($P > 0.05$). Interestingly, significant 4.4- and 4.1-fold increases in monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), respectively, were observed for *Dunaliella* sp. cultured in FPWW at 7 mL/L vs. Walne medium. The authors conclude that treated FPWW can potentially be used as a medium for the growth of microalgae.

Keywords: Fatty acids, Fish processing wastewater, Microalgae, Pigment, Protein

INTRODUCTION

Microalgae are photoautotrophic microorganisms that use light as an energy source and carbon dioxide as a source of carbon; moreover, they assimilate nutrients such as nitrate, ammonia, and phosphate to generate biomass **(Fakhri *et al*., 2017a; Su, 2021)**. Microalgae have received attention because of their ability to synthesize a valuable biochemical profile, such as lipids, proteins, carbohydrates, and pigments **(Pignolet *et al*., 2013)**. Microalgal biomass is considered as an essential food source for humans and feed supplements in the commercial aquaculture industry **(Nagappan *et al*., 2021)**. Despite the essential benefits of microalgae in aquaculture and human nutrition, the production of microalgal biomass remains economically not competitive **(Borowitzka, 2013)**, with the main problem being that the mass scale of photoautotrophic microalgal culture entails a high cost regarding the culture medium cost, which represents approximately 30%–40% of the total culture cost **(Clarens *et al*., 2010)**.

The application of wastewater as a substitute source of nutrients in microalgal culture has been considered as a practical strategy for rendering microalgal biomass economically viable **(Batista *et al*., 2015; Mohsenpour *et al*., 2021)**. Wastewater contains nitrogen (N), phosphorous (P), and trace elements, for instance iron (Fe), magnesium (Mg), and calcium (Ca), which is vital compounds for algal growth **(Guldhe et al., 2017; Mohsenpour *et al*., 2021)**. Several microalgae have been successfully utilized for the biological treatment of wastewater **(Hawrot-Paw *et al*., 2020; Nugroho *et al*., 2014; Trivedi *et al*., 2019)**. Therefore, the cultivation of microalgae in wastewater is a potentially efficient option not only for producing biomass, but also for polishing the wastewater inexpensively **(Pires *et al*., 2013; Queiroz *et al*., 2013)**.

Fish processing is an activity that requires a significant volume of water, i.e., approximately 11 m^3/ton of fish processed, leading to large amounts of wastewater **(Lim *et al*., 2003)**. The release of the liquid from fish processing activities results in eutrophication and degraded water quality in water bodies **(Ching & Redzwan, 2017)**. Fish processing wastewater (FPWW) has a remarkable variety of macronutrients (carbon, nitrogen, and phosphorus) and microminerals **(Lim *et al*., 2003)**. Moreover, FPWW may be composed of many organic compounds, such as carbonaceous nutrients and nitrogen-containing nutrients (volatile amines, peptides, and proteins) **(Ching & Redzwan, 2017)**. Specifically, it contains 80–1,000 mg/L nitrogen, 0.7–69.7 mg/L ammonia **(Chowdhury *et al*., 2010)**, and 6.1–13.7 mg/L phosphate **(Queiroz *et al*., 2013)**. Furthermore, **Ghaly *et al*. (2013)** reported that fish waste contains 100 ± 42 mg/L iron, 0.17% ± 0.04% magnesium, and 0.68% ± 0.11% potassium. These amounts of nutrients in FPWW are possibly applicable as an alternative low-cost medium for cultivating microalgae **(Lim *et al*., 2003; Queiroz *et al*., 2013)**.

The green microalgae belonging to the *Dunaliella* genus are widely applied for biomass production using wastewater, including municipal wastewater **(Liu & Yildiz, 2018)**, aquaculture wastewater **(Andreotti *et al*., 2019)**, and digested poultry wastewater **(Han *et al*., 2019)**. Because of their high β-carotene, essential fatty acid, and protein content, *Dunaliella* spp. have been successfully applied to aquatic animal feed **(Morowvat & Ghasemi, 2016; Sui & Vlaeminck, 2020)**. Importantly, these microalgae can also utilize organic carbon in mixotrophic metabolism **(Fakhri *et al*., 2021; Morowvat & Ghasemi, 2016)**.

Few studies have reported the use of FPWW as a nutrient source for microalgae, such as the cyanobacteria *Aphanothece microscopica* **(Queiroz *et al*., 2013)**, *Chlorella vulgaris* **(Riaño *et al*., 2011; Trivedi *et al*., 2019)**, *Microcystis* spp., and *Oocystis* spp. **(Riaño *et al*., 2011)**. The concentration of nutrients in FPWW depends mostly on the raw fish material, additives applied, water utilization, and unit processes **(Chowdhury *et al*., 2010; Parvathy *et al*., 2017)**. In this work, we applied wastewater from the boiling process of the fish *Decapterus* spp. as a nutrient. Therefore, we investigated the effect of different concentrations of FPWW on the growth, biomass, and biochemical profile, including chlorophyll a, β-carotene, protein, and lipid content, of *Dunaliella* sp. We also analyzed the fatty acid profile of *Dunaliella* sp. in the best concentration of FPWW and Walne medium (control).

MATERIAL AND METHODS

Culture of *Dunaliella* sp. and generation of fish processing wastewater

Dunaliella sp. was obtained from the Institute for Mariculture Research and Development, Gondol, Bali, Indonesia. Stock cultures were maintained in Walne medium (Table 1) at a temperature of 28 ± 2°C, a salinity of 20 ppt, an initial pH of 7.8, and a light intensity of 60 µmol/m^2/s under continuous illumination. FPWW was acquired from the boiling process of *Decapterus* spp. in the local Fish Processing Center, Muncar, Banyuwangi.

Table 1 Walne medium composition

No.	Stocks	per 100 mL
1.	**Trace metal solution (TMS)**	
	$ZnCl_2$ (g)	2.1
	$CoCl_2.6H_2O$ (g)	2.0
	$(NH_4)_6Mo_7O_{24}.4H_2O$ (g)	0.9
	$CuSO_4.5H_2O$ (g)	2.0
Made up to 100 mL with distilled water		
2.	**Nutrient solution**	**per liter**
	$FeCl_3.6H_2O$ (g)	1.3
	$MnCl_2.4H_2O$ (g)	0.36
	H_3BO_3 (g)	33.6
	EDTA (g)	45.0
	$NaH_2PO_4.2H_2O$ (g)	20.0
	$NaNO_3$ (g)	100.0
	TMS (mL)	1.0
Made up to 1 L with distilled water		

Pretreatment of the fish processing wastewater

The preparation of wastewater as a growth medium was started by filtration through a Whatman filter paper GF/C with a pore size of 2.5 µm. The medium was then fermented with *Bacillus subtilis* at a density of 1×10^8 cells/mL. The fermentation process was managed for 48 hours under aerobic conditions, to reduce the level of organic compounds in the wastewater. Next, the medium was autoclaved at 121°C for 15 min to prevent contamination with microorganisms. After sterilization, the nutrient characteristics of the medium were determined. The chemical characteristics of the FPWW stock medium are presented in Table 2.

Table 2 Characterization of the fish processing wastewater

Parameter	Average value
NO_3^- (mg/L)	30.30
NH_4^+ (mg/L)	29.84
PO_4^{3-} (mg/L)	20.14
TOC (mg/L)	2,697.69

Experimental medium culture conditions

Dunaliella sp. in the logarithmic phase was used as the inoculum. Cells were cultivated in 350 mL of medium in 500-mL Erlenmeyer flasks containing different concentrations of wastewater (1, 3, 5, and 7 mL/L, v/v) in natural seawater. The experiment was performed in triplicate. The FPWW stock was enriched with essential nutrients, including $ZnCl_2$ (21 g/L), $(NH_4)_6Mo_7O_2.4H_2O$ (9 g/L), $CuSO_4.5H_2O$ (20 g/L), $MnCl_2.4H_2O$ (0.36 g/L), H_3BO_3 (33.6 g/L), and EDTA (45 g/L), to support the growth of microalgae. *Dunaliella* sp. was also grown in Walne medium (Table 1) at a concentration of 1 mL/L, which was used as the control condition. Cultures were started at an initial density of 30×10^4 cells/mL. The cells were cultured for 6 days under aeration with ambient air at 0.5 mL/min and irradiated under continuous light (60 µmol/m^2/s) at 28 ± 2°C and a salinity of 20 ppt.

Biomass determination

Biomass production was evaluated after 6 days of cultivation. A filter paper (Whatman GF/C, 90-mm diameter) was dried for 2 h at 105°C until the weight was stable [A]. A sample containing 25 mL of the microalgal suspension was filtered, and the resulting filtered biomass was cleaned with distilled water and dried for 2 h at 105°C. Finally, the dry biomass was cooled down in desiccators and subsequently weighed [B]. The calculation of the microalgal biomass (dry weight, g/L) was performed using the following formula **(Fakhri *et al*., 2021b; Janssen *et al*., 1999)**:

$$\text{Biomass production (g/L)} = (B-A) \times 1{,}000/\text{Sample volume} \quad (1)$$

Protein, chlorophyll *a*, and β-carotene determination

Protein, chlorophyll *a*, and β-carotene quantification were determined spectrophotometrically (GENESYS™ 10S UV-Vis, Thermo Scientific, USA). Protein determination was carried out according to the Lowry assay **(Lowry *et al*., 1951)** and calibrated using bovine serum albumin (BSA) standards. A BSA concentration range of 0–2,000 µg/mL ($R^2 = 0.98$) with absorbance readings at 750 nm was applied. The protein content (%) was evaluated using the following equation:

$$\text{Protein content (\%)} = \frac{C \times 100}{DW} \quad (2)$$

where C is the protein concentration (µg/mL) measured by the Lowry assay and DW is the dry weight of the biomass (mg/L).

β-carotene content was determined at a wavelength of 450 nm and analyzed as stated in **Morowvat & Ghasemi (2016)**. In addition, absorbance readings at 652 and 665 nm were applied to the determination of chlorophyll *a* content, which was analyzed as reported by **Ritchie (2006)**.

$$\text{Chlorophyll } a \text{ (µg/mL)} = 16.5169 \times A_{665} - 8.0962 \times A_{652} \quad (3)$$

$$\text{β-carotene (µg/mL)} = 25.2 \times A_{450} \quad (4)$$

Lipid extraction

Eighty milligrams of freeze-dried cells were added to 8 mL of $CHCL_3/CH_3COH$ (1:2 v/v) and stirred into the mixed sample with a solvent for about 30 min at 30°C. The sample was collected by centrifugation at 4,000 rpm for 10 min (MicroCL 21R Microcentrifuge, Thermo Scientific). The supernatant was added to another flask and the extraction and separation steps were repeated twice, as described above. The supernatant containing crude lipids was combined for further evaporation (IKA® RV10 rotary evaporator). After evaporation, 0.5 mL of $CHCl_3/CH_3OH$ (2:1 v/v) was added and the mixture was transferred to Eppendorf tubes for further centrifugation at 12,000 rpm for 10 min. The supernatant was added to a vial that had been previously weighted, for further evaporation.

Fatty acid analysis

Hydrolysis

The sample (10 mL) was added to 10 mL of HCl and heated in a water bath at 80°C until boiling. Then, after the sample had cooled down, the mixture was added to 25 mL of diethyl ether/petroleum ether (1:1), vortexed, and precipitated. The top layer of oil was collected and dried with N_2 for later use in the methylation process.

Methylation

A methanolic natrium solution (1.5 mL) was added to the dried lipid fraction, which was prepared as described above. The mixture was covered and heated at 60°C for 10 min with shaking. Subsequently, the mixture was cooled down, mixed with 2 mL of methyl formate boron trifluoride, and heated for 10 min at 60°C. After the mixture was cooled down, 1 mL of heptane and 1 mL of concentrated NaCl were added to it. The top layer was transferred to an Eppendorf tube for fatty acid methyl ester (FAME) analysis.

Gas chromatography conditions

A gas chromatography Shimadzu 2010 instrument equipped with a cyanopropyl HP-88 column (length, 100 m; i.d., 0.25 mm; film thickness, 0.20 µm) and a flame ionization detector at a temperature of 260°C was used for FAME determination. The sample (1 µL) was administered and the total retention time was 55 min. The fatty acid peak was determined by comparing the relative retention times of FAME with an authentic standard solution (Supelco 37 Component FAME mix).

Statistical analysis

Statistical analysis was performed using the normality test, homogeneity of variance, and one-way ANOVA with a 95% confidence level ($\alpha = 0.05$). Statistical analyses were performed using the SPSS 16.0 software.

RESULTS AND DISCUSSION

Effect of FPWW on the growth and biomass production of *Dunaliella* sp.

In the preliminary study, we cultivated *Dunaliella* sp. cells on only FPWW medium without enriched with additional nutrients as mentioned in the Materials and Methods. We found that the cell growth was inhibited probably due to the insufficient of some nutrients (data not shown). Then, we decided to add some essential nutrients (Zn, Mo, Cu, Mn, Bo, and EDTA) which are probably lacks in the FPWW medium. FPWW-enriched medium (hereafter called as FPWW) was then applied for the whole experiment. The growth characteristics of *Dunaliella* sp. cultured in the presence of different concentrations of FPWW (1, 3, 5, and 7 mL/L) and Walne medium are depicted in Figure 1 and Table 3. A similar growth pattern was observed in FPWW and Walne medium, with the stationary phase being attained on day 6. The lack of a lag phase was noticed on FPWW and Walne medium, which confirmed that the cells adapted well (Figure 1). This phenomenon agrees with that reported by **Trivedi *et al*. (2019)**, who noted that *Chlorella vulgaris* NIOCCV cultivated on fish processing-plant wastewater did not experience a lag phase because the inoculant cells were provided in the exponential phase. Interestingly, Table 3 showed that there was no significant difference between Walne and 7 mL/L of FPWW medium in terms of cells growth.

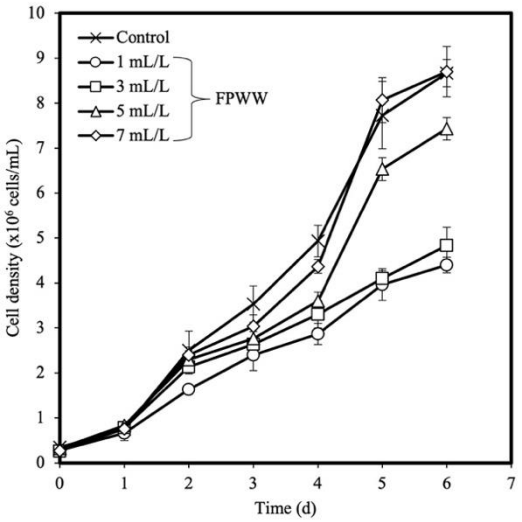

Figure 1 Cell density of *Dunaliella* sp. under Walne and various FPWW concentrations. The cross, open circle, open square, open triangle, and open diamond describe control, and 1, 3, 5, and 7 mL/L FPWW, respectively.

Table 3 Maximum specific growth rates, doubling time, and maximum cell density of *Dunaliella* sp.

Experimental condition	Maximum specific growth rate (/day)	Doubling time (days)	Maximum cell density (× 10^6 cells/mL)
Control	1.198 ± 0.284^c	0.579 ± 0.255	8.667 ± 0.306^c
1 mL/L	0.921 ± 0.192^a	0.753 ± 0.217	4.400 ± 0.173^a
3 mL/L	1.010 ± 0.043^a	0.686 ± 0.043	4.833 ± 0.404^a
5 mL/L	1.100 ± 0.080^b	0.630 ± 0.073	7.433 ± 0.252^b
7 mL/L	1.168 ± 0.114^c	0.593 ± 0.095	8.700 ± 0.557^c

Note: The same superscript indicates the absence of a significant difference; different superscripts indicate significant differences; 95% confidence level ($\alpha = 0.05$)

The biomass yield of *Dunaliella* sp. grown on FPWW and Walne medium is depicted in Figure 2. There was no significant difference ($P > 0.05$) in biomass production of *Dunaliella* sp. between FPWW 7 mL/L and Walne medium. The biomass production of *Dunaliella* sp. cultivated on FPWW at 7 mL/L reached 0.755 g/L, which was significantly higher than that of cells cultivated on FPWW at 1 mL/L (0.329 g/L), 3 mL/L (0.431 g/L), and 5 mL/L (0.611 g/L), but only slightly higher compared with cells cultivated on Walne medium (0.717 g/L). In addition, we found that increasing the wastewater concentration led to an increase in the growth and biomass production of *Dunaliella* sp. Similarly, **Trivedi *et al.* (2019)** observed that enhancing the concentration of fish wastewater from 5% to 30% resulted in an increase in biomass productivity from 67.07 ± 0.22 mg/L/d to 258.30 ± 1.04 mg/L/d. Moreover, those authors reported that the microalgae cultivated in fish wastewater medium produced a higher biomass compared with cultivation in the F/2 medium. Similar findings of an increase in the biomass yield of microalgae in various wastewaters compared with the standard culture medium have been reported extensively **(Hawrot-Paw *et al.*, 2020; Ramanna *et al.*, 2014)**.

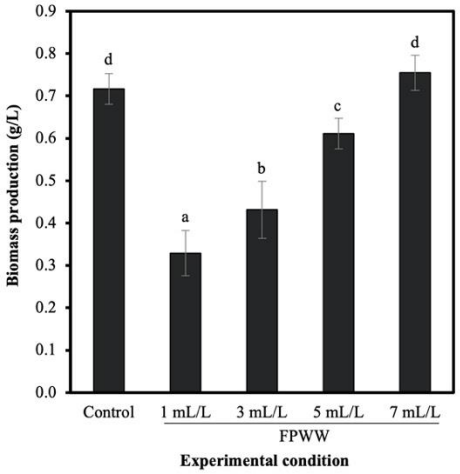

Figure 2 Biomass production of *Dunaliella* sp. under Walne medium as a control and different concentrations of FPWW (1, 3, 5, and 7 mL/L)

We hypothesized that the biomass yield of microalgae was remarkably dependent on the concentration of wastewater in the medium particularly the presence of organic carbon in the medium. In this work, the amount of organic carbon in FPWW stock medium (2,697.69 mg/L) was comparable to the study from **Cristóvão *et al.* (2014)**. According to our calculations, the 7 mL/L FPWW's initial organic content was 18.88 mg/L. It has been noted that adding a small quantity of organic carbon to the medium can enhance the growth and biomass of microalgae **(Cheirsilp & Torpee, 2012; Kim *et al.*, 2013)**. The utilization of wastewater containing organic carbon in microalgal cultivation has remarkably improved both photosynthesis and oxidative phosphorylation; therefore, it may be to the factor underlying the high yield of microalgal biomass **(Yu *et al.*, 2022)**. In addition, ammonium (NH_4^+-N) is the preferred nitrogen source for microalgae, therefore we speculated that its availability in FPWW enhanced the biomass of microalgae especially under mixotrophic condition **(Li *et al.*, 2019)**. Conversely, the lower biomass production observed at lower wastewater concentrations is probably attributable to the small amount of nutrients available in the medium **(Trivedi *et al.*, 2019)**.

Effect of FPWW on the protein content of *Dunaliella* sp.

The protein content of *Dunaliella* sp. cultivated under Walne and FPWW media is reported in Figure 3. The highest protein content (49.438%) of *Dunaliella* sp. was detected in Walne medium (control), whereas the lowest protein content (43.640%) was observed in 1 mL/L FPWW. An increasing concentration of FPWW led to an increase in the protein content of *Dunaliella* sp., with the maximum protein content of 48.940% detected at 7 mL/L FWPP (Figure 3). Moreover, the protein content of microalgae did not differ significantly ($P > 0.05$) between the control and 7 mL/L FPWW. There was a 10.8% increase in protein content in cells grown in 7 mL/L FPWW vs. 1 mL/L FPWW. **Shanthi *et al.* (2021)** proposed that the high protein content observed in fish wastewater medium is related to the availability of organic nitrogen, such as amino acids and short peptides, which trigger nitrogen assimilation in microalgae. Some studies have revealed that the concentration of nitrogen in the medium has a significant effect on the accumulation of proteins in microalgae **(Li *et al.*, 2016; Markou, 2015)**. In addition, the higher protein content detected at a higher FPWW concentration could be attributed to the fact that the higher concentration of nitrogen increased nitrogen assimilation, thus promoting protein synthesis **(Fakhri *et al.*, 2021; Markou & Georgakakis, 2011)**.

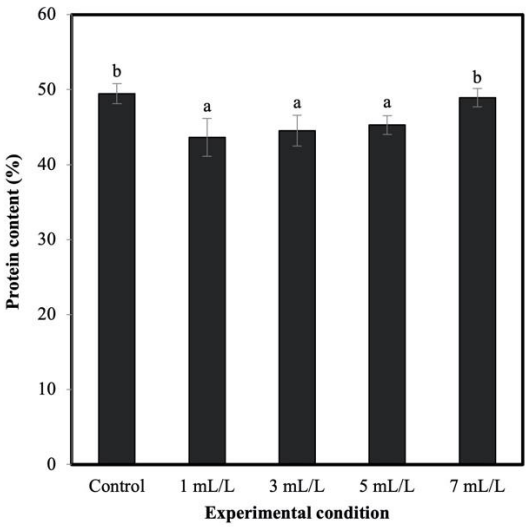

Figure 3 Protein content of *Dunaliella* sp. under Walne medium as a control and different concentrations of FPWW (1, 3, 5, and 7 mL/L)

Effect of FPWW on the chlorophyll *a* and β-carotene content of *Dunaliella* sp.

The chlorophyll *a* and β-carotene content of *Dunaliella* sp. cultured in control and FPWW media is reported in Figure 4. *Dunaliella* sp. cultured in control Walne medium and FPWW at 7 mL/L did not exhibit a significant difference ($P > 0.05$) in chl *a*; however, it was significantly higher than that observed at 1, 3, and 5 mL/L FPWW. An increasing FPWW concentration enhanced the chl *a* content of *Dunaliella* sp. Similarly, **Shanthi *et al.* (2021)** reported that enhancing the fish waste concentration up to 0.5% increased chl *a* production in *Spirulina platensis*. In this study, the chl *a* content exhibited a similar pattern to that of the microalgal growth, biomass production, and protein content. This result agrees with **Shanthi *et al.* (2021)**, who reported that the chl *a* content is linearly correlated with microalgal growth and protein content. Moreover, **Danesi *et al.* (2011) and Fakhri *et al.* (2017b)** revealed that chl *a* production is highly correlated with microalgal growth, because the synthesis of chlorophyll is part of its primary metabolism. The lowest chl *a* content was detected in the medium containing 1 mL/L FPWW,

probably because of the lower nitrogen concentration and assimilation that occurred at low FPWW concentrations.

Figure 4 also reveals a similar trend for the chl *a* and β-carotene content in *Dunaliella* sp. among all treatments. This result agrees with **Liu *et al.* (2021)**, who reported a linear correlation between chlorophyll and carotenoid content in *Scenedesmus obliquus*. Those authors also revealed that the composition and quantity of pigments are highly affected by the culture conditions.

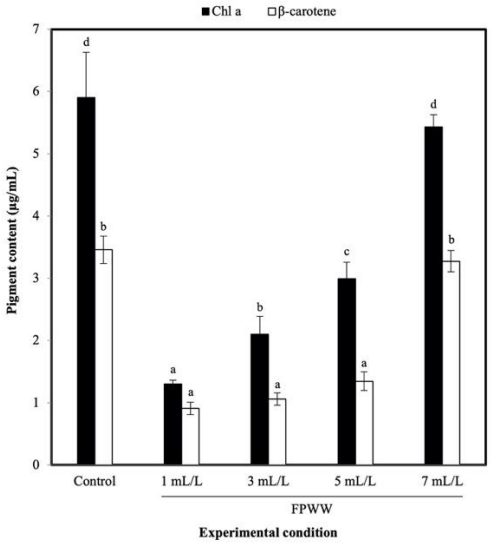

Figure 4 Chlorophyll *a* and β-carotene content of *Dunaliella* sp. grown in Walne medium (control) and in different concentrations of FPWW

Table 4 Fatty acid profile (relative %) and lipid content of *Dunaliella* sp. cultured in control Walne and 7 mL/L FPWW media

Fatty acid profile	Relative %	
	Control	7 mL/L FPWW
C4:0	2.645 ± 0.177	1.740 ± 0.113
C6:0	0.225 ± 0.035	0.515 ± 0.007
C10:0	0.410 ± 0.113	0.710 ± 0.028
C12:0	1.845 ± 2.298	0.170 ± 0.014
C14:0	2.885 ± 2.185	1.250 ± 0.014
C14:1	3.135 ± 05.02	2.955 ± 0.063
C15:0	-	0.890 ± 0.254
C16:0	72.490 ± 3.168	40.550 ± 0.579
C16:1	2.740 ± 0.170	5.860 ± 1.004
C17:0	2.345 ± 2.185	1.370 ± 0.191
C18:0	3.29 ± 0.212	2.610 ± 0.297
C18:1	-	7.215 ± 0.144
C18:2	-	2.460 ± 0.296
C20:0	-	0.710 ± 0.141
C18:3	-	1.000 ± 0.014
C20:1	-	2.900 ± 0.056
C22:0	3.330 ± 4.455	2.900 ± 0.113
C20:3	0.660 ± 0.255	1.635 ± 0.007
C23:0	-	1.375 ± 0.035
C20:5	3.995 ± 1.039	10.930 ± 0.395
C24:1	-	6.980 ± 0.007
C22:6	-	3.275 ± 0.021
SFAs (%)	**89.465**	**54.79**
MUFAs (%)	**5.875**	**25.91**
PUFAs (%)	**4.655**	**19.3**
Lipid content (%)	**6 ± 0.28**	**6.1 ± 0.49**

Effect of FPWW on the lipid content and fatty acid profile of *Dunaliella* sp.

The lipid content of *Dunaliella* sp. grown in control medium and 7 mL/L FPWW is reported in Table 4. The difference in lipid content observed between the control (6%) and 7 mL/L FPWW (6.1%) media was insignificant ($P > 0.05$). The lipid content detected in this study is in accordance with that described by **Almutairi (2020)**, who reported a lipid content of 5.88% ± 0.85% for *D. salina* grown in a standard medium. In addition, the difference in lipid content detected here between the control and FPWW media is consistent with **Shanthi *et al.* (2021)**, who showed that the lipid content of *S. platensis* was almost equal between the control and fish-waste media.

The composition of fatty acids of *D. salina* cultivated in Walne medium and 7 mL/L FPWW is shown in Table 4. The highest fatty acid content in both conditions was detected for palmitic acid (C16:0). However, a remarkable decrease in C16:0 was observed in microalgae grown in FPWW (40.550% ± 0.579%) compared with Walne medium (72.490% ± 3.168%). **Ferreira *et al.* (2019)** explained that C16:0 is the most abundant saturated fatty acid (SFA) in microalgae. Moreover, **Abd El Baky *et al.* (2014) and Hosseinzadeh Gharajeh *et al.* (2020)** reported that palmitic acid was the most abundant fatty acid in *Dunaliella* strain. Interestingly, here, several unsaturated fatty acids, including oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), and docosahexaenoic acid (C22:6), were only produced in *Dunaliella* sp. cultivated in FPWW medium. In addition, the eicosatetraenoic acid (C20:5) content increased by 2.7-fold in *Dunaliella* sp. grown in FPWW compared with Walne medium. The assessment of the overall fatty acid composition revealed a 4.4- and 4.1-fold enhancement in monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), with a 1.6-fold decrease in SFAs detected in microalgae cultivated in FPWW vs. Walne medium. We hypothesized that the shift in fatty acid content in FPWW was caused by organic carbon in the medium. Increasing PUFA concentration in the presence of organic carbon is consistent with **Liu *et al.* (2021)**, who found that adding glucose and maltose enhanced C16:3 and C22:6 content in *Micractinium reisseri* much more than photoautotrophic culture. **Chia *et al.* (2013) and Ferreira *et al.* (2019)** suggested that the fatty acid composition and their degree of saturation in microalgae are species specific and dependent on the cultivation medium, culture conditions, nutrient concentrations, and other environmental factors.

CONCLUSION

In conclusion, our study demonstrated that different doses of treated FPWW remarkably affected the growth, biomass, chlorophyll *a*, β-carotene, and protein content of *Dunaliella* sp. Finally, there were no differences in the growth, biomass; and pigment, protein, and lipid yield between the FPWW concentration of 7 mL/L and Walne medium. The cultivation of cells with FPWW results in a higher production of unsaturated fatty acids in comparison to the use of Walne medium. Therefore, the utilization of FPWW-enriched medium can be considered as a prospective nutrient source for microalgal culture.

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