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# Enhancement of Growth, Lipid, and Carbohydrate Production of the Egyptian isolate *Dunaliella salina* SA20 Using Mozzarella Cheese Whey as a Growth Supplement

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THE growth of *Dunaliella salina* SA20 (Dunal) Teodoresco Egyptian isolate (from El-Bardawil Lake, Sinai, Egypt) in wastewater is a more cost-effective cultivation method. Wastewater, such as mozzarella cheese whey (MCW), is produced in massive quantities as a dairy industry byproduct, and growing *D. salina* in such waste could be used safely in biotechnological applications. Applying different organic MCW ratios to inorganic Loeblich nutrient medium (20, 50, 75% v/v MCW/Loeblich) as growth supplement allowed mixotrophic nutrition, while growth in completely inorganic medium (100% Loeblich medium) allowed for autotrophic nutrition. The optimum growth performance of *D. salina* cells was observed in 20% MCW and was indicated by measured values of chlorophyll *a* (10.51mg/L), dry weight (405mg/L), cell count (6.92 x $10^6$cell/ml), and optical density (1.12). Using 20% MCW, different pH values were applied to explore the pH effect on nutrient availability, and pH 7.5 was found to be optimal for growth. Additionally, a growth curve experiment comparing the mix- and autotrophic conditions presented higher values of the relative mean and maximum growth rates in mixotrophic conditions (0.19, 0.21 $day^{-1}$, respectively) than in the autotrophic conditions (0.16, 0.18 $day^{-1}$, respectively).

**Keywords:** *Dunaliella salina,* Growth supplements, Mixotrophy, Whey.

## Introduction

Whey is a byproduct of cheese production which is formed following the precipitation of milk caseinate (Baldasso et al., 2011). It has been estimated that 10 L of milk produces 8.7 L of whey (De Almeida et al., 2021). The Food and Agricultural Organization (FAO) has announced an expected 13.7% increase in dairy product consumption (Gatamaneni Loganathan et al., 2021) and the whey produced by dairy processing is considered a primary wastewater sources and its treatment is a critical problem for the dairy industry (Zandona et al., 2021). Eutrophication and soil waterproofing are the consequences of the disposal of untreated cheese whey in water, resulting in water quality problems such as increased water purification costs and loss of biodiversity (Carvalho et al., 2013). Despite these factors, in Egypt, biotechnological systems for further whey treatment and useful commercial applications are limited (IndexBox, 2015).

The composition of dairy wastewater varies considerably, but common features are identifiable, including high concentrations of organic matter (especially lactose, oils, and proteins), nitrogen, and suspended solids (Baldasso et al., 2011; Yonar et al., 2018). It also contains minor components such as citric acid, lactic acid, non-proteinic nitrogen compounds

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(urea and uric acid), and B-group vitamins (Tawfik et al., 2008). Additionally, various residues of cleaning products, including alkaline and acidic chemicals, increase its chemical oxygen demand (COD), and it also contains high biodegradable organic loads which increase biological oxygen demand (BOD) (Carvalho et al., 2013). The pH of whey also varies widely from 4.7 to 11 (Ahmad et al., 2019). Therefore, wastewater is a freely available, low cost, and promising alternative medium for enhancing the growth of various algal species (Ding et al., 2015), and the culturing of microalgae in whey wastewater can help in its management. Several recent studies have used microalgae for sewage (El-Sheekh et al., 2005; Reda et al., 2020), industrial (Essa et al., 2018), municipal (Abou-Shanab et al., 2014), and coking wastewater treatment (Wang et al., 2022), and the use of cheese whey wastewater may reduce microalgae production costs to a more economically efficient level (Ende & Noke, 2019).

Mixotrophic microalgae cultivation is preferable to induce biomass production (Zhan et al., 2017), and although potentially 3–30 times higher biomass yields are possible in mixotrophic conditions compared with autotrophic growth conditions, mixotrophic culturing requires the use of expensive sterilized photobioreactors due to its susceptibility to contamination in open pond systems (Bellou et al., 2014). It is noteworthy that whey has been used as a mixotrophic medium supplement for the cultivation of *Scenedesmus obliquus* (Girard et al., 2014), *Spirulina* spp. (Vieira Salla et al., 2016), *Chlorella variabilis* (Gatamaneni Loganathan et al., 2021), *Tetradesmus* spp. (Ravi Kiran & Venkata Mohan, 2022), and *Oscillatoria* sp. (Kallarakkal et al., 2021).

The organism of interest in this study was *D. salina.* It is the primary producer of natural β-carotene, so is widely used in the food, nutritional supplement, and cosmetics industries (Sathasivam et al., 2012). *D. salina* also represents a potential new target for biofuel feedstock due to its unique lipid-producing features (Ahmed et al., 2017). Moreover, *D. salina* has been successfully enhanced through mixotrophic nutrition for production of lipids (Chavoshi & Shariati, 2019), β-carotene (Morowvat & Ghasemi, 2016), and proteins (Kadkhodaei et al., 2015).

To the best of our knowledge, no previous reports have documented the utilization of mozzarella cheese whey for biomass production of *D. salina*. Special attention has been given to this strain due to its observed survival capability under unfavorable conditions. The present study aimed to explore the influence of complex mozzarella cheese whey (MCW) natural medium on *D. salina* growth and biochemical composition.

## Materials and Methods

*Organism and growth conditions*

*D. salina* was isolated from the salt marshes of El-Bardawil Lake on the northern coast of the Sinai Peninsula, approximately 50 km from El-Arish City. It was kindly supplied by the Phycology Lab., Faculty of Science, Alexandria University, Egypt (Taha et al., 2012). *D. salina* was grown in batch cultures in Loeblich nutrient medium (nutrients used in gram/liter: NaCl, 73.05; $MgSO_4.7H_2O$, 0.5; $MgCl_2.6H_2O$, 1.5; KCl, 0.2; $CaCl_2.2H_2O$, 0.2; $KNO_3$, 1; $NaHCO_3$, 0.043; $KH_2PO_4$, 0.035; and salts used in mg/liter: EDTA, 1.89; $FeCl_3.6H2O$, 2.44; $ZnCl_2.2H_2O$, 0.041; $H_3BO_3$, 0.61; $CoCl_2.2H_2O$, 0.015; $CuCl_2.2H_2O$, 0.041; $MnCl_2.4H_2O$, 0.41; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.3) (Loeblich, 1982). The sodium chloride concentration was modified to 100 g/L to improve growth performance (Shabana et al., 2014). *D. salina* cultures were incubated using continuous white fluorescent lamps with a light intensity of $58.5\mu Em^{-2}s^{-1}$. *Dunaliella* cells were grown in batch cultures of 50mL each in 250mL Erlenmeyer pyrex-glass flasks at 25°C. Each experiment was conducted in triplicate.

*Experimentation*

The first experiment compared the growth of *D. salina* under autotrophic conditions (100% Loeblich inorganic nutrient medium was the control used in all experiments) and under mixotrophic conditions created using various MCW organic medium ratios of 20, 50, and 75% (v/v MCW/Loeblich medium). In 20% MCW/Loeblich medium, an experiment examining the effects of different pH values (5.5, 6.5, 7.5, and 8.5) on the growth of the cells was conducted. The previous two mentioned experiments were harvested after eight days.

In the pH 7.5 experiment, the growth was

compared between cells growing in 20% v/v MCW/Loeblich medium and in cells growing in 100% Loeblich inorganic nutrient medium in two separate experiments. The first experiment lasted for 8 days and analyzed biochemical contents in association with growth parameters. The second experiment continued for 24 days and was conducted as a growth curve experiment.

*Growth measurements*

Cells were preserved with Lugol's solution and were counted using a Neubauer hemocytometer (Guillard & Sieracki, 2005). Cell density was measured at 678nm (Robert, 1979), and the maximum mean (R) and relative (K) growth rates were calculated according to Littler et al. (1973).

where, $R = [3.322 / t - t_0] [\text{Log } N / N_0]$

$K = \text{Log } N - \text{Log } N_0 / t.$

3.322= growth constant

$t_0$ = time at the beginning of the experiment (days)

t = time at the end of the experiment (days)

$N_0$ = number of cells / ml culture at initial time $t_0$

N = number of cells / ml culture at time t

Chlorophyll *a* and *b*, and β-carotene were extracted using 80% acetone. The optical density of the extracts was measured using a spectrophotometer at 412, 431, 460, and 480nm (Eijckelhoff & Dekker, 1997). Dry weights of algal suspensions were filtered, washed, dried, and weighed as described by Zhu & Lee (1997).

*Determination of biochemical composition*

Total extracted lipids were determined after extraction from cells using a 2:1 chloroform and methanol (v/v) mixture, and gravimetrical estimation of lipid weight as described by Axelsson & Gentili (2014). Protein content was measured and calibrated with standard bovine serum albumin (Lowry et al., 1951). Total carbohydrate content was analyzed using the phenol sulfuric method (Sadasivam & Manickam, 2005).

*Characterization of whey*

The whey was kindly supplied by the Dairy Products Unit, Faculty of Agriculture, Cairo University. The whey pH was adjusted to 7.5 and autoclaved for 10min separately to avoid protein precipitation. It was then cooled to room temperature before it was added to Loeblich medium under aseptic conditions. The characteristics of raw whey were analyzed at the Cairo University Research Park, Faculty of Agriculture.

*Statistical analysis*

The data were analyzed by one way analysis of variance (ANOVA) using IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, NY, USA). A comparison of primary effects was performed using Duncan's test of homogeneity and independent sample *t*-test, with $P < 0.05$ considered significant. The data of the present study were expressed as the means of three replicates ± *SD* (Dytham, 1999).

## Results and Discussion

MCW is a byproduct of mozzarella cheese production. The data in Table 1 show that MCW is nutritionally valuable due to its protein, carbohydrate, and lipid contents. Additionally, MCW contains a high COD. Despite this, processing it into products or the treatment of this effluent product is expensive (Sansonetti et al., 2009) because heating denatures proteins and increases its aggregation and precipitation (Mulvihill & Donovan, 1987). Under neutral pH adjustment, the autoclaving period was limited to 10 min as described by Batal et al. (2000), who claimed that protein precipitation increased with heating and that this hindered protein availability to cultured cells. Moreover, our preliminary studies examined the growth of *D. salina* cells supplemented with sterilized MCW by membrane filtration (pore size, 0.22–0.45µm) compared to autoclaved MCW, and observed improved growth performance in the autoclaved samples. This could result from the loss of important high-molecular weight nutrients through the filtration process (Zydney, 1998). Additionally, extensive heating has a browning effect that was previously attributed to chemical changes induced by thermal heating (Ende & Noke, 2019). The present study considers that this browning effect is directly related to the rennet percentage used in cheese processing.

**TABLE 1. Characterization of mozzarella cheese whey (MCW)**

| Parameter | Value |
|---|---|
| pH | 5.5 |
| COD g/L | 0.728 |
| Total suspended solids g/L | 2.800 |
| Organic carbon g/L | 0.880 |
| Carbohydrate g/L | 4.890 |
| Protein g/L | 4.220 |
| Total phosphorous g/L | 0.015 |
| Total nitrogen g/L | 0.130 |
| Lipid g/L | 0.700 |
| Fe (% wt/v) | 0.080 |
| Ca (% wt/v) | 0.106 |
| Mg (% wt/v) | 0.017 |
| Na (% wt/v) | 0.100 |
| K (% wt/v) | 0.150 |
| Cl meq/L | 20.00 |

Preliminary experiments were conducted based on different ratios between the nutrient medium (Loeblich) and MCW (20%, 50%, and 75% MCW) applied on *D. salina* cultures. These were conducted to detect the most favorable concentration of MCW to maximize culture growth performance (See Table 2). Among the nutrition levels examined, the 20% MCW concentration was the whey dilution which maximized *D. salina* growth. While whey and its processing are very heterogeneous, multiple preliminary tests (data not shown) found this same concentration of whey to Loeblich (20%) was the most favorable growth medium for the studied organism. Therefore, it was chosen as the dilution for the following experiments. The values of chlorophyll *a* (10.51mg/L), optical density (1.12), cell count ($6.92 \times 10^6$ cell/mL) and dry weight (405mg/L), were higher than their corresponding values in autotrophic growth conditions (0%). Increased whey concentrations of upto 50% showed healthy growth without significant differences in chlorophyll *a*, dry weight, and optical density values compared with the control. While 75% MCW appeared to slow growth, and drastically decreased biomass (Table 2). Table 2 also shows a drop in chlorophyll *a* and dry weight with 75% MCW treatment, whereas using 100% MCW in the preliminary investigation showed a failure of algal cells to adapt and grow. These data agree with the findings of Kothari et al. (2013) for cultures of *Chlamydomonas* grown in the same concentration of dairy waste water.

In the case of the best dilution of growth medium (20% MCW), light is more accessible than in more turbid mediums with higher whey concentrations. This finding agrees with a similar study by Martínez et al. (2018) which examined *Chlorella vulgaris* and illustrated that background turbidity limits light diffusion to microalgae and increases its absorption by non-microalgal constituents of the turbid matter, highlighting that light is the real energy absorbed by cells that is transformed into biomass. Consequently, the average observed growth rate declined in association with increased turbidity. In the present study, MCW dilution improved light access to cell culture and hence increased their growth parameters (Martínez et al., 2018). Nabizadeh et al. (2020) claimed another reason behind the enriched growth in the mixotrophic case; that MCW is rich in minerals which induce the consumption of monosaccharides hydrolyzed from MCW carbohydrates by *Euglena gracilis*.

Figure (1) shows the cell growth profile of the investigated microalgae *D. salina* in autotrophic and mixotrophic conditions using 20% MCW (4.89g/L carbohydrate). A sharp increment in growth rate was observed on the fourth and 12th days; however moderate growth rates were reported on the other intervals. The fast growth which continued until the fourth day was in accordance with a study which examined the growth profile of *C. vulgaris* in cheese whey (CW) (Melo et al., 2018). Consequently, lactose as an organic carbon source may be considered acceptable for the study organism. Similarly, another study confirmed the lactose consumption capability of two microalgal species: *Nannochloropsis oculata* and *Tetraselmis chuii*, with growth performance better than that seen in autotrophic conditions (Velichkova et al., 2016). The modifications in carbon supply present in the mixotrophic cultures were influencing factors that altered the microalgal growth of *D. salina*, the current organism of interest. Under mixotrophic conditions, it did not show an adaptation for growth medium compared with the autotrophic control, but showed better growth performance and faster growth rates. Similar results for *C. vulgaris* supplemented with hydrolyzed CW support these findings (Abreu et al., 2012). In the present work, the reported maximum mean (R) and relative growth rates (K) were 0.16 and 0.18 $day^{-1}$, respectively, in control inorganic medium, and 0.19 and 0.21 in 20% MCW.

**TABLE 2. Effect of different whey concentrations (different trophic levels) on the growth of *D. salina* after 8 days**

| MCW concentration | Chlorophyll a (mg/L) | Dry weight (mg/L) | Cell count x10$^6$ cell/mL | Optical density |
|---|---|---|---|---|
| 0% (control) | 7.25 ± 0.05$^b$ | 311 ± 0.16$^b$ | 4.32 ± 0.12$^c$ | 0.89 ± 0.11$^b$ |
| 20% | 10.51 ± 0.02$^c$ | 405 ± 0.98$^c$ | 6.92 ± 0.65$^d$ | 1.12 ± 0.01$^c$ |
| 50% | 6.77 ± 0.01$^b$ | 290 ± 0.18$^b$ | 3.89 ± 0.25$^b$ | 0.81 ± 0.21$^b$ |
| 75% | 3.98 ± 0.10$^a$ | 115 ± 0.44$^a$ | 2.15 ± 0.41$^a$ | 0.54 ± 0.09$^a$ |

Values superscripted with similar letters are not significantly different (P > 0.05), and values superscripted with different letters are significantly different (P < 0.05). Data are means of three replicates ± SD.

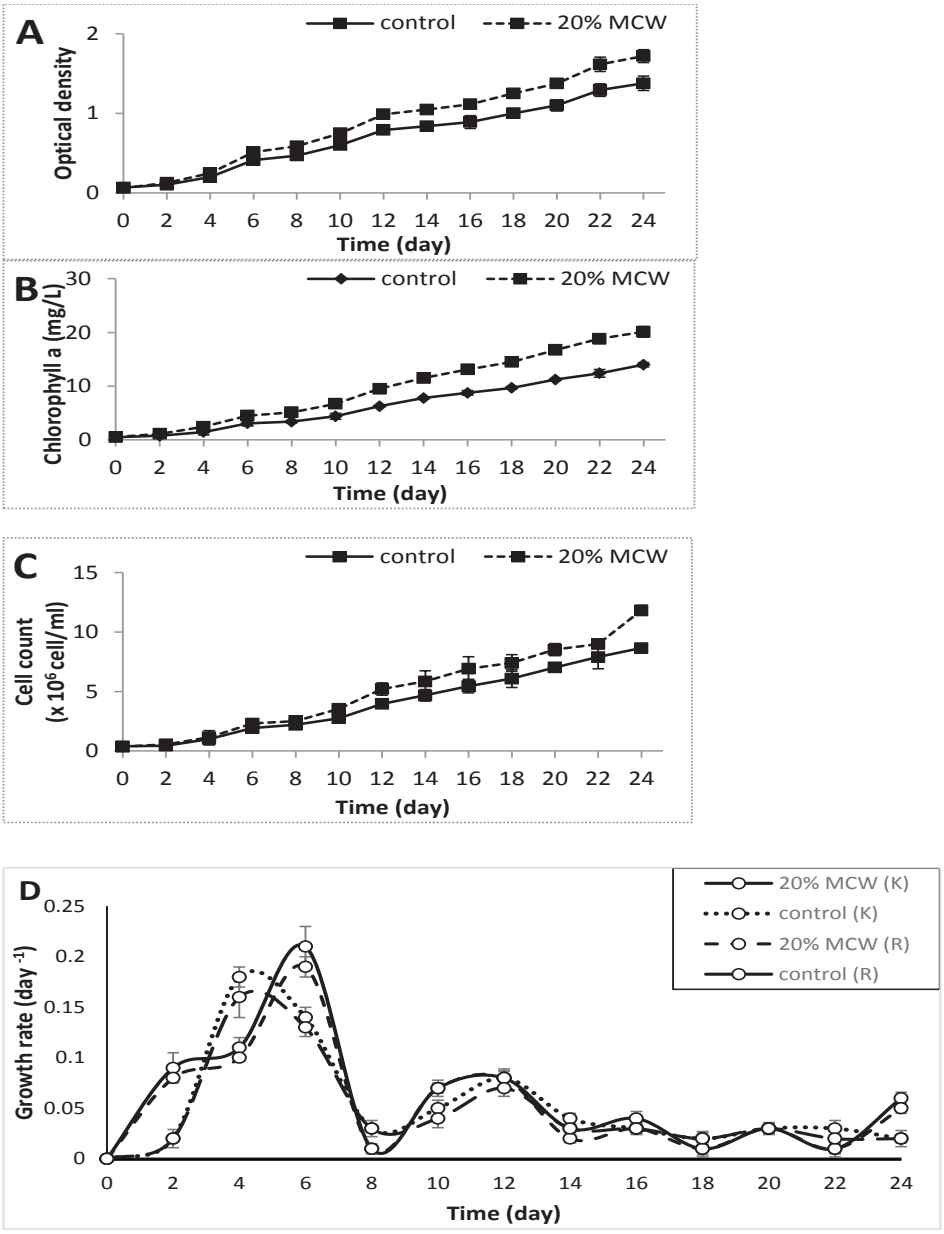


**Fig. 1. compared growth profiles of *D. salina* in 20% MCW and control medium [The measured parameters are optical density (A), chlorophyll content (B), cell count (C), and growth rate (D) at two-day intervals for 24 days]**

The variation of pH can affect the carbon dioxide distribution and its availability through the availability of macro- and micronutrients. Moreover, the culture medium directly responds to variable pH with physiological changes. Consequently, the cell metabolism and growth behavior could be altered (Mostafa et al., 2012). As an example, during microalgae growth, nitrogen uptake is necessary. The frequency of the uptake process is influenced by various factors, and pH is among these factors (Garbisu et al., 1994). In the present study, initial gradient pH changes were established, and the growth was found to be highest at pH 6.5, 7.5, and 8.5 without significant differences between chlorophyll *a*, cell count, and β-carotene production values, as summarized in Table 3. In autotrophic conditions, microalgal cells prefer $HCO_3^-$ uptake over $CO_2$ (Carvalho et al., 2006), while in mixotrophic cultivation, algae utilize combined inorganic and organic carbons, thus producing $OH^-$ and $CO_2$ as metabolites. These induce acceptable natural variations in pH values (Chojnacka & Marquez-Rocha, 2004) which could be seen in the nonsignificant effect of pH changes in the pH 6.5, 7.5, and 8.5 treatments. This study found the highest content of β-carotene at pH 7.0, which concurs with findings of the study by Gonabadi et al. (2022). They found that *Dunalliela salina* grown in mixotrophic cultures enriched with different organic carbon and protein sources at pH 7.5 had the highest β-carotene accumulation.

At the high pH of 9.5, growth was hindered, and this could be attributed to the reduced percentage of phosphorus, as Mujtaba & Lee (2017) confirmed phosphorous precipitation at pH 9. Phosphorus is a major macronutrient which plays a fundamental role in cellular metabolism and is therefore a structural functional component essential for normal growth and development (Zmora & Richmond, 2004). The growth measurements in the MCW at pH 5.5 were higher compared with values seen in the autotrophic growth conditions at pH 5.5, with the phosphorus and calcium levels of the MCW used in the current study reported as 0.015 g/L and 0.106 (% wt/v), respectively. This is in agreement with Mavropoulou & Kosikowski (1973), who claimed that acidic CW powder improves growth parameters due to its higher content of calcium and phosphorus.

Growth criteria and lipid, protein, and carbohydrate contents of *D. salina* were compared and summarized in Table 4. Remarkable increases in the growth parameters, total chlorophyll, and cell count in 20% MCW were observed. Moreover, the biochemical analysis revealed an increase in the contents of total lipids and carbohydrates, and a decrease in protein content, while the productivity of the culture was higher in mixotrophic nutrition for all the biochemical ingredients.

The changes in physiological conditions, culture medium composition, and surrounding physiological state, lead to modifications in biomass composition, and lipid, protein, and carbohydrate levels. The results showed significant 2.44- and 1.32-fold increases in carbohydrate and lipid contents, respectively. Therefore, growth in 20% MCW could be used to enhance the lipid and carbohydrate production of *D. salina*. The study by Chavoshi & Shariati (2019) supports these results as they reported an increase in lipid production by *D. salina* under mixotrophic growth conditions using glucose and acetate as carbon sources. Similarly, Liang et al. (2009) reported lower lipid content in the autotrophic growth of *C. vulgaris* compared with mixotrophic conditions. In contrast, Fan et al. (2015) reported that the highest lipid content was observed in autotrophic cultures of *Chlorella pyrenoidosa*. This demonstrates that lipid accumulation by algal cells is influenced by algal species and growth conditions.

**TABLE 3. Effect of pH on Chl. a, Cell count, and β-carotene production of *D. salina* under autotrophic and mixotrophic (20% MCW) conditions after 8 days**

| pH | Chlorophyll a (mg/L) | | Cell count (x10$^6$ cell/mL) | | β-Carotene (mg/L) | |
|---|---|---|---|---|---|---|
| | C | T | C | T | C | T |
| 5.5 | $6.87 \pm 0.23^b$ | $7.33 \pm 0.18^b$ | $3.75 \pm 0.42^a$ | $4.91 \pm 0.14^b$ | $3.84 \pm 0.30^a$ | $4.71 \pm 0.11^b$ |
| 6.5 | $8.12 \pm 0.17^c$ | $9.15 \pm 0.09^c$ | $5.25 \pm 0.73^b$ | $6.12 \pm 0.54^c$ | $4.21 \pm 0.57^b$ | $5.34 \pm 0.15^c$ |
| 7.5 | $8.21 \pm 0.21^c$ | $9.27 \pm 0.11^c$ | $5.68 \pm 0.22^b$ | $6.32 \pm 0.23^c$ | $4.83 \pm 0.62^b$ | $5.21 \pm 0.12^c$ |
| 8.5 | $8.17 \pm 0.40^c$ | $9.01 \pm 0.31^c$ | $5.83 \pm 0.14^b$ | $6.45 \pm 0.17^c$ | $4.66 \pm 0.15^b$ | $5.34 \pm 0.22^c$ |
| 9.5 | $5.93 \pm 0.12^a$ | $5.23 \pm 0.10^a$ | $3.25 \pm 0.89^a$ | $4.12 \pm 0.47^a$ | $3.77 \pm 0.17^a$ | $4.21 \pm 0.41^a$ |

C, control (absence of MCW); T, treatment (20% MCW). Values superscripted with similar letters are not significantly different ($P > 0.05$), and values superscripted with different letters are significantly different ($P < 0.05$). Data are means of three replicates ± SD.

**TABLE 4. Effect of 20% MCW on the growth and biochemical composition of *D. salina* after 8 days**

| Parameters | Growth condition autotrophic | Growth condition Mixotrophic | t- test |
|---|---|---|---|
| Chlorophyll *a* (mg/L) | 15.69 ± 0.23 | 21.22 ± 0.28 | s |
| Chlorophyll *b* (mg/L) | 2.37 ± 0.35 | 3.93 ± 0.37 | ns |
| Total chlorophyll (mg/L) | 18.06 ± 0.13 | 25.15 ± 0.52 | s |
| β-Carotene content | 5.14 ± 0.09% | 3.94 ± 0.11% | ns |
| Cell count (x$10^7$ cell/ml) | 2.10 ± 0.11x$10^7$ | 2.70 ± 0.45 x $10^7$ | ns |
| Carbohydrate content | 9.01 ± 0.12% | 22.00 ± 0.19% | s |
| Lipid content | 12.81 ± 0.54% | 16.93 ± 0.77% | s |
| Protein content | 22.52 ± 0.65% | 19.11 ± 0.44% | ns |

Data are means of three replicates ± SD, two-sample independent t-test ($P < 0.05$). s; significant, ns; nonsignificant.

The observed increase in carbohydrate content could be attributed to the supply of disaccharide lactose as the major carbohydrate source of MCW (Abreu et al., 2012). Similarly, *Spirulina platensis* grown in 20% diluted Zarrouk medium and the addition of 2.5% residue from the ultra- and nanofiltration of whey protein accumulates high carbohydrate and biomass concentrations (Vieira Salla et al., 2016). In contrast, the sugars contained in CW were insufficient to achieve a higher rate of carbohydrates in *Chlamydomonas* sp. as reported by Calixto et al. (2016). While, it was reported that *Dunaliella parva* is able to grow heterotrophically utilizing glucose as an organic C-substrate for its growth (Hard & Gilmour, 1996).

In contrast, the contents of β-carotene and protein (5.14 and 22.52%, respectively) in autotrophic cultivation were slightly higher than those under mixotrophic conditions (3.94 and 19.11%, respectively) with nonsignificant differences. Similarly, the dilution of Zarrouk medium to concentrations of 20% reduced the concentrations of all nutrients, including nitrogen, and decreased protein content in *spirulina* (Vieira Salla et al., 2016). This concurs with the results of Kong et al. (2012) who reported the same trend for *C. vulgaris* under mixotrophic conditions. Xie et al. (2017) confirmed stimulated protein synthesis in *C. vulgaris* in an inorganic (nitrate) nitrogen rich medium. Conversely, when the availability of nitrogen for microalgae is low, the concentrations of protein and chlorophyll tend to decrease (Lourenço, 2006; Vieira Salla et al., 2016). In contrast, Fabregas et al. (1989) reported that organic nitrogen consumption by *D. salina* cells regulates metabolic pathways. Fan et al. (2015) illustrated that exogenous carbon sources increased starch content and decreased protein content compared with cells cultured in autotrophic conditions, as the high C/N ratio reduces nitrogen available for protein synthesis. An interesting finding of Capa-Robles et al. (2021) is a contrary behavior of *D. salina* when using glycerol as an organic carbon source with higher β-carotene accumulation than seen in autotrophic growth conditions.

## Conclusions

The present study confirms that the mixotrophic cultivation of *D. salina* using 20% MCW is a successful and economically efficient process that stimulates renewable and nontoxic algal biomass production. Additionally, this type of cultivation can solve the environmental problems caused by CW disposal using a clean bio-recycling technology. In the future, comprehensive large-scale approaches are needed to balance the costs of bioreactors used for mixotrophic culture production with the supplemental income generated.

*Disclosure statement:* No conflicts of interest are reported by the authors.

*Authors' contributions*: Dr. Tanahy suggested the research idea, while Hend conducted the practical work. Dr. Fatma and Dr. Tamer contributed to the discussion of the results and Dr. Khalil suggested the protocol and supervised both the lab work and the scientific writing.

*Ethics approval*: Not applicable.

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## تعزيز النمو وإنتاج الدهون والكربوهيدرات لعزلة الدوناليلا سالينا المصرية باستخدام الشرش كمكمل غذائي

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تم استخدام شرش الموتزريلا والناتج بكميات كبيرة من صناعة الألبان كوسط غذائي لنمو طحلب دوناليلا سالينا بطريقة آمنة فى تطبيقات التكنولوجيا الحيوية وذلك باضافته إلى الوسط الغذائي الغير عضوى (لوبلش) بتركيزات مختلفة (20 و 50 و 75 % شرش/لوبلش) كمثال للتغذية المختلطة بينما يعتبر النمو فى الوسط الغير عضوى (100% لوبلش) هو مثال للتغذية الذاتية وقد دلت النتائج على ان التغذية المختلطة بتركيز 20% قد سجلت أعلى النتائج (كلوروفيل أ 10.51 مجم / لتر والوزن الجاف 405 مجم/ لتر وعدد الخلايا 6.92 × $10^6$ خلية / مل)، والكثافة البصرية (1.12). كما تم استخدام قيم مختلفة للاس الهيدروجينى لدراسة تأثير تلك القيم المختلفة حيث وجد أن pH 7 هو القيمة المثلى للنمو. بالإضافة إلى ذلك، قدمت تجربة منحنى النمو التي تقارن بين ظروف التغذية المختلطة والتغذية الذاتية قيمًا أعلى للمتوسط النسبي ومعدلات النمو القصوى في ظروف التغذية المختلطة (0.19، 0.21 / يوم، على التوالي) مقارنة بظروف التغذية الذاتية (0.16، 0.18 / يوم، على التوالى).