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Full Length Article

Physiological and biochemical responses of the green alga *Pachycladella chodatii* (SAG 2087) to sodicity stress

Mustafa A. Fawzy [a,*], Dalia A. Abdel-Wahab [b], Awatief F. Hifney [a]

[a] Botany and Microbiology Department, Faculty of Science, Assiut University, Assiut 71516, Egypt
[b] Botany Department, Faculty of Science (New Valley Branch), Assiut University, Egypt

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ABSTRACT

The effects of various concentrations of different carbon sources (Na_2CO_3 and $NaHCO_3$) as sodicity stress on growth parameters, CO_2 consumption rate, enzyme activity, intracellular lipid content, and fatty acid profiles of *Pachycladella chodatii* were studied. Generally, the total chlorophyll was increased by increasing the concentrations of Na_2CO_3 and $NaHCO_3$. The biomass productivity as well consumption rate of carbon dioxide of *P. chodatii* reached the highest values with increasing concentrations of Na_2CO_3 and $NaHCO_3$. The soluble protein content of *P. chodatii* was highest at the lowest Na_2CO_3 and $NaHCO_3$ concentrations. The addition of different concentrations of Na_2CO_3 and $NaHCO_3$ in the growth media induces lipoxygenase and superoxide dismutase specific activity. Catalase and total antioxidant enzymes were increased by supplementing the growth media with 60 and 45 mg l^{-1} of Na_2CO_3 and $NaHCO_3$, respectively. Hydrogenase uptake activity in *P. chodatii* increased gradually in all treated cultures with the time elapsed recording the maximum activity after 11 days of growth especially at 60, 45 mg l^{-1} of Na_2CO_3 and $NaHCO_3$ respectively. Lipids content was increased at low concentration of Na_2CO_3 (40 and 15 mg l^{-1}) and $NaHCO_3$ (60, 45 mg l^{-1}) respectively. Subsequent to algal cultivation in different concentrations of Na_2CO_3, the cultures were filtered and biodiesel was prepared by direct esterification of dry algal biomass. Methyl esters of palmitic, elaidic and stearic acids represented the major components while myristic, pentadecanoic and 9,12-octadecenoic acids represented a minor component of biodiesel produced from *P. chodatii* treated with different concentrations of Na_2CO_3 and $NaHCO_3$.

1. Introduction

Microalgae, a group of fast-growing unicellular or simple multicellular microorganisms, offer several advantages, including higher photosynthetic efficiency, compared to crop plants. They possess high CO_2 fixation capacities and under optimal culture condition express growth rates several orders of magnitudes higher than conventional crop plants [1,2]. Microalgae can fix CO_2 from different sources, which can be categorized as CO_2 from the atmosphere, industrial exhaust gases, and fixed CO_2 in the form of soluble carbonates ($NaHCO_3$ and Na_2CO_3). Salinization is one of the major environmental factors limiting global crop productivity, because it restricts crop yield particularly in the arid and semi-arid regions [3]. Salinization occurs not only in Na_2CO_3 and $NaHCO_3$ the soil, but also in the surface water and groundwater mainly caused by high evaporation [4,5]. Chloride and carbonate salts, which are the main salts causing salinization, widely exist in aquatic environment. Therefore, algae, the most abundant lower plants living in water, may suffer from salinization stress for high water evaporation [6]. Compared with lots of studies on algae stressed by chloride salt, data on the carbonate stress responses are rather limited. In higher plants, Na_2CO_3 and $NaHCO_3$ stresses can inhibit seed germination [7], seedling growth [8], photosynthesis [9,10], ion absorption [11] and antioxidant enzyme activity [8]. In algae, lower dose of $NaHCO_3$ can promote the photosynthesis as HCO_3^- is the carbon source [12,13], but a higher dose of $NaHCO_3$ and Na_2CO_3 is harmful due to the high pH and Na^+ toxic effects. It has been reported that high pH reduces algal photosynthetic ability and pigment content, because it limits dissolved CO_2 concentration in water [14]. The depletion of dissolved CO_2 can stimulate ROS formation, increase antioxidant enzyme activity [15]. Algal biomass contains all essential amino acids, a variety of unsaturated fatty acids, carbohydrates, dietary fiber as well as numerous vitamins and other bioactive compounds, it is a highly suitable alternative in livestock feeding and rather advantageous (e.g., through aquaculture of food additive) for human nutrition [16,17]. It is also used to produce high-value biofuels, including methane produced by anaerobic digestion of

* Corresponding author.
E-mail address: mostafa.mahmoud@science.au.edu.eg (M.A. Fawzy).

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algal biomass, biodiesel derived from oil as well as biohydrogen and bioethanol [18]. These cellular processes could be affected by abiotic stresses such as sodicity. Where, there is information is available about the effects of carbonate stress on algae, although it widely exists in and even dominates water bodies [6]. Therefore, this study was carried out to determine the different effects of carbon sources (Na_2CO_3, $NaHCO_3$) on the growth parameters, CO_2 consumption rate, enzyme activity (LOX, SOD, CAT and Hup), intracellular lipid content, and fatty acid profiles of the green alga *Pachycladella chodatii* in batch culturing technique cultivation.

2. Materials and methods

2.1. Microorganism and culture medium

The culture of *P. chodatii* (SAG 2087) used in this study was kindly donated to Prof. R. Abdel-basset from the Collection of Algal Cultures at the University of Göttingen (Germany). The culture was kept in modified BG11 medium [19]. The alga was grown autotrophically and axenically in batch cultures under 25 ± 1 °C with continuous illumination at intensities of 48.4 μmole photon m^{-2} s^{-1}. Instead of aeration the culture was shaked during the experiment period, pH of the medium was adjusted to pH 7.5 prior to autoclaving.

2.2. Experimental design

Twenty milliliters of exponential cultures were centrifuged, standardized at an optical density at 680 nm of 0.1, and inoculated into 300 ml of BG11 medium in 500 ml Erlenmeyer flasks in triplicate. The effect of different carbon source namely Na_2CO_3 [(control (20 mg l^{-1}), 100% (40 mg l^{-1}), 150% (60 mg l^{-1}) and 200% (80 mg l^{-1})], $NaHCO_3$ [(control (0 mg l^{-1}), (15, 45, 75 mg l^{-1})], on growth and biochemical composition of *P. chodatii* were studied. The cultures were grown as previously mentioned conditions. The alga was harvested by centrifugation at the beginning of stationary phase.

2.3. Monitoring of algal growth

Growth of *P. chodatii* was monitored by determining the dry weight and biomass productivity that was calculated according to Chisti [2]. The biomass productivity (P, mg $l^{-1}d^{-1}$) was calculated using the following equation:

$$P = \Delta X/\Delta t$$

where ΔX is the variation of biomass concentration (mg l^{-1}), during the culture time Δt (d). Biomass was determined as the cellular dry weight and measured gravimetrically at the beginning and end of the study. A known volume of culture was filtered through preweighed GF/C filter paper. The filtered cell mass was oven dried at 105 °C for 24 h until constant weight.

2.4. Estimation of pigments (chlorophylls and carotenoids)

Chlorophyll (a + b) and carotenoids were extracted in methanol (80%) then estimated spectrophotometrically, and determined according to Metzner et al. [20].

2.4.1. Estimation of specific growth rate

The specific growth rate (μ) calculated as chlorophyll *a* was determined using the following formula:

$\mu(h^{-1}) = (\mathrm{Ln}N_2 - \mathrm{Ln}N_1)/(t_2 - t_1)$, where N_2 and N_1 represent the chlorophyll *a* concentrations at times t_1 (day 0) and t_2 (day 11), respectively.

2.5. Determination of the CO_2 consumption rate

The CO_2 consumption rate (P_{CO2}, mg $l^{-1}d^{-1}$) was determined depending the biomass productivity (P) from the following equation as described by Chisti [2].

$$P_{CO2} = 1.88 \times P$$

2.6. Determination of soluble proteins

Protein contents were determined in the algal extract by Folin reagent according to Lowry et al. [21]. A calibration curve was constructed using bovine serum albumin (BSA) and the data were expressed as mg BSA g^{-1} dry weight.

2.7. Assay of enzyme activity

2.7.1. Preparation of enzyme extract

Hundred ml of algal culture were centrifuged at 5000 rpm and the pellet was homogenized in 5 ml of 100 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM of EDTA and 0.1 g polyvinyl pyrrolidone (PVP). The homogenate was centrifuged at 18,000 rpm for 10 min. at 4 °C and the supernatants were collected and used for the assays of Lipoxygenase (LOX), superoxide dismutase (SOD), catalase (CAT) and total antioxidant activity. All colorimetric measurements (including enzyme activities) were made at 20 °C using a Unico UV-2100 spectrophotometer. The specific activity was expressed as units/mg protein.

2.7.2. Assay of lipoxygenase activity

Lipoxygenase (LOX; EC 1.13.11.12) activity was estimated according to the method of Minguez-Mosquera et al. [22].

2.7.3. Assay of antioxidant enzymes activity

2.7.3.1. Superoxide dismutase. Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed by following the autoxidation of epinephrine (adenochrome) as described by Misra and Fridovich [23], with some modifications. Activity was measured in a final volume of 2 ml of the reaction medium containing 50 mM of sodium carbonate buffer (pH 10.2), 0.1 mM EDTA, 100 μl protein extract and 100 μl of 5.5 mg/ml epinephrine (dissolved in 10 mM HCl, pH 2). Autoxidation of epinephrine was determined colorimetrically using a spectrophotometer (Unico UV-2100 spectrophotometer) at 480 nm for 1 min. Activity was reported as specific activity.

2.7.3.2. Catalase. Catalase (CAT; 1.11.1.6) activity was assayed by following the consumption of H_2O_2 for 1 min. as described by Aebi [24] and Matsumura et al. [25].

2.7.3.3. Determination of total antioxidant capacity. Total antioxidant activity of the methanol extracts was evaluated by the phosphomolybdenum method [26]. Methanol (0.3 ml) in the place of extract was used as the blank. Ascorbic acid (AA) was used as standard.

2.8. Assay of hydrogenase activity

The sum uptake activity of Hup (uptake hydrogenase) and the bidirectional hydrogenase assay mixture contained 1 ml algal culture, 2.75 ml phosphate buffer (50 mM), 0.25 ml methyl blue (50 mM), 1 ml sodium dithionite (100 mM), flushed with nitrogen to remove oxygen followed by hydrogen, as conducted by Yu et al. [27] and Colbeau et al. [28]. The reduction of methyl blue by Hup and hydrogen was monitored at 540 nm (spectrophotometer thermoscientific).

2.9. Determination of total lipids

The total lipids were determined by the sulfophosphovanilin method (SPV) Drevon and Schmit [29].

2.10. Fatty acid methyl esters analysis

Fatty acid methyl esters (FAMEs), from the alga was produced by direct acid esterification of its dry biomass according to [30,31], with modification. Algal biomass was air dried at 50 °C. The dry algal biomass (0.05 g) was suspended in 20 ml of mixture A (methanol 2: Chloroform 1: conc. HCl 1) and left overnight at 40 °C with shaking at 120 rpm. *n*-Hexane was used for extraction the produced fatty acid methyl esters and analyzed using GC/MS, Agilent Model 6890N/5975B [Column DB 5 ms, Agilent form (30, 0.25 mm, 0.25 mm)] in the Analytical Chemistry Unit, Chemistry Department, Faculty of Science, Assiut University.

2.11. Statistical analysis

All data obtained were subjected to one-way analysis variance (ANOVA), using the SPSS statistical package. For comparison of the means, the Duncan' multiple range tests ($p < 0.05$) were used.

3. Results and discussion

3.1. Effect of different concentrations of Na_2CO_3 and $NaHCO_3$ on the growth, biomass productivity and CO_2 consumption rate of P. chodatii

The content of chlorophyll a + b in the investigated alga subjected to different concentrations of Na_2CO_3 and $NaHCO_3$ was shown in Table 1. The result showed that, the increase in concentrations of Na_2CO_3 caused significant increment in chl. a + b content for *P. chodatii* compared to the control culture ($p < 0.05$). The high concentration of $NaHCO_3$ (70 mg l^{-1}) caused non-significant increase in the content of chl. a + b. Results obtained dealt with the carotenoids content in *P. chodatii* cleared that, low concentration of Na_2CO_3 (40 mg l^{-1}) caused significant increase in carotenoids content, but increasing of $NaHCO_3$ concentrations led to slight decrease in the carotenoids content at $p > 0.05$.

The results in Table 1, indicated that the specific growth rate varied according to the concentration of Na_2CO_3 and $NaHCO_3$. From these data, it concluded that the highest specific growth rate calculated on the basis of chl. *a* in *P. chodatii* was 0.84 that recorded at the control culture. The biomass productivity of *P. chodatii* reached the highest values at 60, 80 mg l^{-1} d^{-1} of Na_2CO_3 and 45, 75 mg l^{-1} mg l^{-1} of $NaHCO_3$, which were 20.9, 21.4 mg l^{-1} d^{-1} and 18.2, 23.6 mg l^{-1} d^{-1}, respectively. Srinivasan et al. [32] observed increase in the biomass of *Dunaliella* sp. grown on media with $NaHCO_3$ in compared to control, the maximum growth and biomass were attained at 100 mM concentration of bicarbonate. Microalgae species have the capacity to use carbonate such as Na_2CO_3 and $NaHCO_3$ for the cell growth [33]. Some of algal species typically have a high extracellular carbon hydrase activity, which is responsible for the conversion of carbonate to free CO_2 and thereby facilitate the assimilation.

The analysis of the carbon dioxide consumption rate of *P. chodatii* confirms that *P. chodatii* has a great capacity utilization of carbon dioxide with an estimated range of 39.3, 40.2 mg l^{-1} d^{-1} in case of the treatment with 60, 80 mg l^{-1} of Na_2CO_3 respectively. While, the highest capacity utilization of carbon dioxide was 34.2, 44.4 mg l^{-1} d^{-1} that obtained for *P. chodatii* treated with 45, 75 mg l^{-1} of $NaHCO_3$, respectively Table 1. In this respect, Elvira-Antonio et al. [33] found that the consumption rate of carbon dioxide of *Neochloris oleoabundans* had a greater capacity and tolerance for using carbon dioxide and carbonate (112.8–115.2 mg l^{-1} d^{-1}) while in case of *Chlorella vulgaris* the values were (95.76–105.75 mg l^{-1} d^{-1}).

3.2. Effect of different concentrations of Na_2CO_3 and $NaHCO_3$ on the soluble proteins content of P. chodatii

Protein content in algae is an important criterion for their use as food. In the present study, addition of 40 mg l^{-1} of Na_2CO_3 induced protein accumulation as shown in Fig. 1. Manjunath and Geeta [34] found that high protein content was recorded in *Spirulina platensis* strains SM, S4 and G1 with higher carbonate levels.

3.3. Effect of different concentrations of Na_2CO_3 and $NaHCO_3$ on lipoxygenase, antioxidant enzymes (LOX, SOD and CAT) and hydrogenase activity of P. chodatii

Under normal growth conditions, reactive oxygen species (ROS), like singlet oxygen, superoxide radical, peroxide and hydroxyl radical are formed at low rate in photosynthetic cells as byproducts of

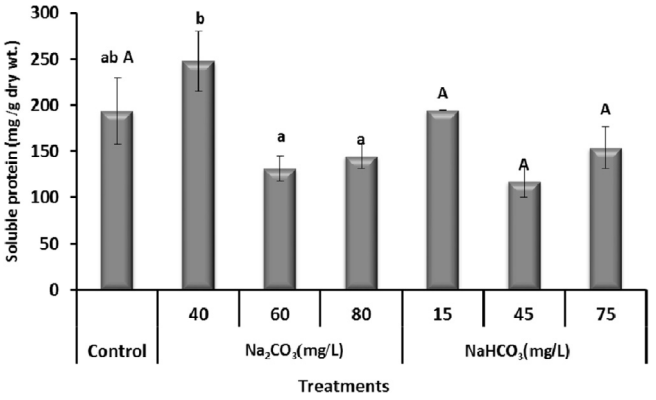

Fig. 1. Effect of different carbon sources on soluble proteins of *P. chodatii*. Data represents mean ± SE of three replicates. Different letters are, Capital for $NaHCO_3$ and small for Na_2CO_3, $p < 0.05$ was considered as significant.

Table 1
Growth parameters, biomass productivity and consumption rate of CO_2 of *Pachycladella chodatii* at various concentrations of Na_2CO_3 and $NaHCO_3$.

Treatments		(Chl. a+b)	Carotenoids	μ (d^{-1})	Biomass productivity	Consumption rate of CO_2
		(μg ml^{-1})			(mg $l^{-1}d^{-1}$)	
	Control	2.5 ± 0.00^{aB}	0.91 ± 0.02^{aB}	0.84	16.4 ± 0.8^{abA}	30.8 ± 1.5^{abA}
Na_2CO_3 (mg/L)	40	3.05 ± 1.03^{b}	1.04 ± 0.00^{b}	0.80	11.1 ± 2.7^{a}	20.9 ± 5.1^{a}
	60	2.99 ± 0.8^{b}	0.88 ± 0.06^{a}	0.72	20.9 ± 2.4^{b}	39.3 ± 4.4^{b}
	80	3.33 ± 0.9 ^{c}	0.91 ± 0.00^{a}	0.78	21.4 ± 0.5^{b}	40.2 ± 0.9^{b}
$NaHCO_3$ (mg/L)	15	2.57 ± 0.7^{A}	0.72 ± 0.00^{A}	0.55	13.6 ± 6.0^{A}	25.6 ± 11.3^{A}
	45	1.63 ± 0.6^{B}	0.63 ± 0.03^{A}	0.17	18.2 ± 3.4^{A}	34.2 ± 6.4^{A}
	75	2.84 ± 0.6 ^{B}	0.60 ± 0.08^{A}	0.51	23.6 ± 3.4^{A}	44.4 ± 6.4^{A}

μ = the specific growth rate, Chl. a + b = chlorophyll a and b

aerobic metabolism, but many stresses can produce a dramatic increase in the ROS production rate. ROS induce the activation of defense enzymes such as lipoxygenases (LOXes) that are key enzymes to adjust the production of hormones and defensive metabolites in plants and algae [35,36]. The results in this study cleared that, in general, LOX enzyme and SOD specific activity were stimulated in *P. chodatii* by increment of $NaHCO_3$ and Na_2CO_3 concentrations in the growth media Fig. 2a, b. In this respect, Wang et al. [37] reported that the activity of SOD under Na_2CO_3 stress was clearly higher than that of NaCl stress in *Puccinellia tenuiflora*. Zuo et al. [6] documented that compared to the NaCl stress, Na_2CO_3 stress induced more ROS production and had more toxic effects on algal photosynthetic pigments and ability, which might be caused by the high pH. Superoxide dismutase is the first enzyme of the enzymatic antioxidative pathway to convert superoxide anion into peroxides, which are scavenged by catalase. In this study, catalase specific activity was increased by supplementing the growth media with 60 and 45 mg l^{-1} of Na_2CO_3 and $NaHCO_3$, respectively Fig. 2c. Catalase, is one of the most important enzymes, scavenges H_2O_2 by directly breaking down to form H_2O and O_2 in peroxisomes and glyoxisomes [38]. Variations in total antioxidant activity of *P. chodatii* affected by sodicity stress are shown in Fig. 2d. Results of the present study show that, all applicable levels stimulate total antioxidant activity especially at (60 and 45 mg l^{-1} of Na_2CO_3 and $NaHCO_3$, respectively). Under various abiotic stresses, the extent of ROS production exceeds the antioxidant defense capability of the cell, resulting in cellular damages. To mitigate and repair damage initiated by ROS, algae have developed a complex antioxidant system, *Chlorella* sp. [39], *Spirulina* sp. [40], *Botryococcus* sp. [41], *Dunaliella* sp. [42] and *Nostoc* sp. [43]. Concerning hydrogenase activity of *P. chodatii*, increased in general with the time and the highest activity recorded at 60, 45 mg l^{-1} of Na_2CO_3 and $NaHCO_3$ respectively Fig. 3. Kapulnik and Phillips [44] showed that

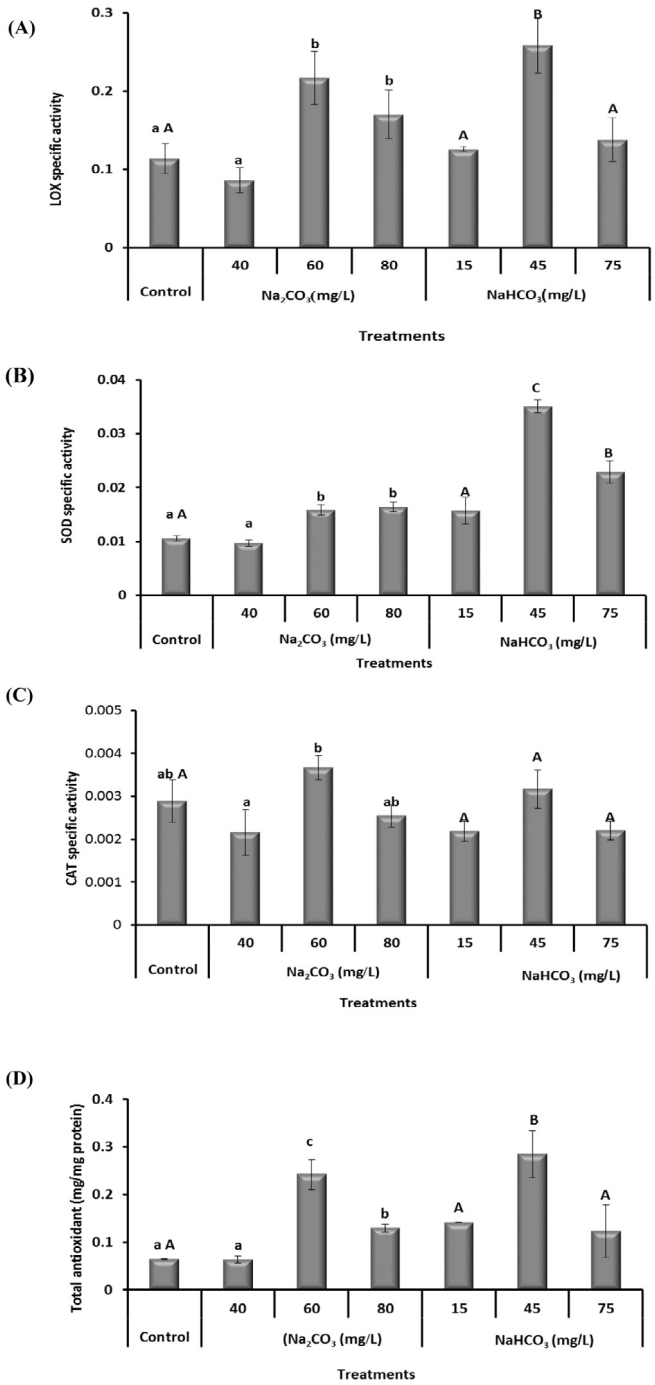

Fig. 2. Lipoxygenase specific activity (A), superoxide dismutase specific activity (B), catalase specific activity (C), total antioxidant activity (D) of *P. chodatii* as influenced by the addition of different carbon sources. Data represents mean ± SE of three replicates. Different letters are, Capital for $NaHCO_3$ and small for Na_2CO_3, $p < 0.05$ was considered as significant.

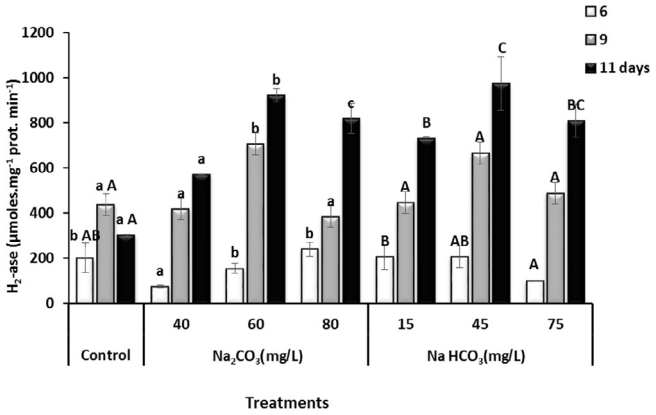

Fig. 3. Hydrogenase activity (Hup) of *P. chodatii* as influenced by the addition of different carbon sources. Data represents mean ± SE of three replicates. Different letters are, Capital for $NaHCO_3$ and small for Na_2CO_3, $p < 0.05$ was considered as significant.

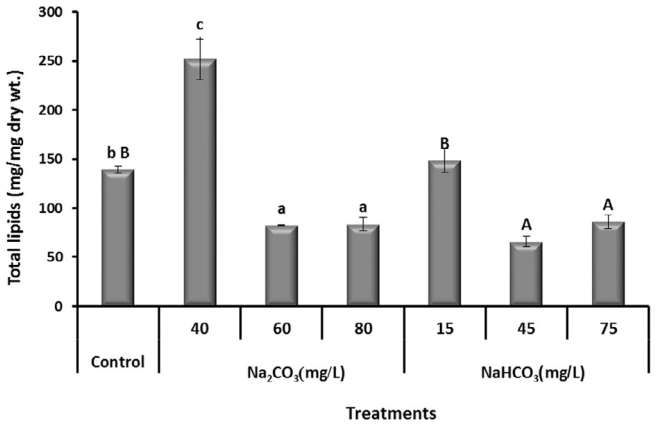

Fig. 4. Total lipids of *P. chodatii* as influenced by the addition of different carbon sources. Data represents mean ± SE of three replicates. Different letters are, Capital for $NaHCO_3$ and small for Na_2CO_3, $p < 0.05$ was considered as significant.

Table 2
Fatty acid methyl ester (FAME) profile of *P. Chodatii* grown under various concentrations of Na_2CO_3 and $NaHCO_3$.

FAME	Control	Na_2CO_3			$NaHCO_3$		
		40	60	80	15	45	75
		(mg l^{-1})					
	FAME (%)						
Lauric acid	0.57	2.05	–	0.69	–	–	–
Hexanoic anhydride	–	–	1.29	–	–	–	–
Myristic acid	5.42	6.62	1.4	4.73	0.89	1.19	0.46
Pentadecanoic acid	0.87	0.85	1.99	0.67	084	0.52	1.49
13-Methyltetradecanoic acid	–	–	–	–	–	1.39	–
Palmitic acid	27.41	29.3	26.52	30.45	26.22	25.56	24.43
Methyl isohexadecanoate	–	–	1.88	1.32	–	–	–
Stearic acid	2.66	27.6	5.88	24.33	1.44	3.35	1.13
Pentadecyl 2-chlorpropanoate	–	–	1.99	–	–	–	–
Heptadecanoic acid	–	–	–	1.07	–	–	–
Palmitic acid β-monoglyceride	3.42	4.35	–	–	–	–	–
Propanoic acid, 2-chloro-, hexadecyl ester	–	–	–	–	–	–	0.81
Pentadecan-4-yl pentanoate	–	–	2.22	–	–	–	0.46
Arachidic acid	0.86	1.25	–	0.94	–	–	–
Propanoic acid,2-chloro-,octadecyl ester	–	–	–	–	0.84	–	–
Heptadecylperfluorobutyrate	–	–	–	1.26	–	–	–
2-Hydroxy-1-(hydroxymethyl) Octadecanoic acid ethyl ester	3.26	5.76	–	–	–	–	–
Heptafluorobutyric acid	0.57	–	–	–	–	–	–
Crotonic acid	–	0.59	–	–	–	–	–
2-Maleic acid, monomethyl ester	–	–	–	–	–	–	2.03
Palmitoleic acid	.870	.950	–	–	–	3.16	9.94
Valeric acid, undec-2-enyl ester	1.23	–	–	–	2.52	–	–
Methyl palmitoleate	–	–	–	2.68	4.65	–	–
Elaidic acid	6.52	3.01	2.85	22.43	5.16	38.83	2.33
8-Octadecenoic acid	4.55	–	–	–	–	–	–
Octadec-11-enoic acid	–	–	–	–	–	1.55	–
1-Nonadecenoic acid	0.87	–	–	–	–	–	–
9,12-Octadecenoic acid	2.67	2.76	9.39	4.76	7.61	9.04	6. 0
Hexadecatrienoic acid	1.1	1.16	–	1.261	2.64	–	2.06
9,12,15-Octadecatrien-1-ol, (*Z,Z,Z*)	–	–	–	–	–	1.49	–
cis,cis,cis-9,12,15 Octadecatrienoic acid	23.95	–	39.04	–	–	–	40.48
Linolenic acid	12.42	12.48	–	–	46.37	–	–
γ-Linolenic acid	–	–	–	.380	.780	–	–
11,14,17-Eicosatrienoic acid	–	1.76	–	–	–	–	–
2-Linolenoylglycerol	–	1.75	–	–	–	–	–
Methyl eicosapentaenoate	3.42	–	–	–	–	7.18	8.41
Methyl eicosa-5,8,11,14,17-pentaenoate	–	–	–	–	–	–	3.43

the sodium ion stimulates hydrogenase activity in pea root nodules containing *Rhizobium leguminosarum* bacteria.

3.4. Effect of different concentrations of Na_2CO_3 and $NaHCO_3$ on the total lipids content and fatty acid methyl ester (FAME) of P. chodatii

Results concerning the influence of addition of different carbon sources on the total lipid contents of *P. chodatii* are depicted in Fig. 4. The results indicated that, the low concentration (40 and 15 mg l^{-1}) of Na_2CO_3 and $NaHCO_3$, respectively, led to increasing the total lipids, but reversible trend was observed when the culture of *P. chodatii* treated with higher concentrations of Na_2CO_3 and $NaHCO_3$. Gardner et al. [45] reported that, inorganic carbon sources mostly could be one of the chief factors that help improve the carotenoids and lipids content in the algal cells by improving photosynthetic efficiency and growth rate. Zheng et al. [46] demonstrated that, lipid yield of *C. vulgaris* reached its peak with the concentration increase of the inorganic carbon source after 10 days cultivation, but dropped again by further increase of the concentration. Inorganic carbon, in the form of bicarbonate (HCO_3^-), is an effective lipid accumulation trigger [45]. Furthermore, it was recently shown that the addition of sodium bicarbonate is a viable strategy to increase lipid accumulation in marine Chlorophytes [47] and *Dunaliella* sp. [32].

A systematic analysis of the fatty acid methyl ester composition is very important for species selection for biodiesel production. The most common fatty acids of microalgae are palmitic, stearic, linolenic acids [48]. Most algae have only small amounts of eicosapentaenoic acid and docosahexaenoic acid; however, in some species of particular genera these polyunsaturated fatty acids can accumulate in appreciable quantities depending on cultivation conditions [49].

In this study, the direct esterification of dry mass was applied to *P. chodatii* and the produced fatty acid methyl esters (biodiesel) were analyzed by GC/MS as shown in Table 2. Methyl esters of palmitic, elaidic and stearic acids represented a major amount of biodiesel produced from *P. chodatii* treated with all concentration of Na_2CO_3 and $NaHCO_3$; while, myristic, pentadecanoic and 9,12-octadecenoic acids represented a minor component of biodiesel produced from all treatments in this study. Low concentration of $NaHCO_3$ (15 mg l^{-1}) stimulated a giant production of linolenic acid about four fold compared with control. As well, cis,cis,cis-9,12,15-Octadecatrienoic acid was improved and recorded 39.04, 40.48% in the algal culture grown in 60 mg l^{-1} of Na_2CO_3 and 75 mg l^{-1} of $NaHCO_3$, respectively, compared with the control culture that recorded 23.95%. In this respect, the composition of fatty acids of *Chlamydomonas mexicana* and *Scenedesmus obliquus* was also enhanced by the increased NaCl concentration. Whereas, at 50 mM NaCl palmitic acid (35%) and linoleic acid (41%) were the

predominant fatty acids in *C. mexicana*, while oleic acid (41%) and α-linolenic acid (20%) were the major fraction found in *S. obliquus* [50]. The degree of membrane fatty acids is an important parameter in the algal adaptation to the environmental conditions [51]. Generally, the compositional profiles of fatty acid for the algal strains are influenced by the conditions of growth such as nutrient levels, light intensities and temperatures [52]. This makes it more difficult to define a single compositional profile for algal-based biodiesel [53]. As well, clear changes in the carbon chain length and degree of unsaturation are important algal oil features for the biodiesel production and may influence its properties and performance [54].

4. Conclusion

The current study tends to investigate the effects of various concentrations of Na_2CO_3 and $NaHCO_3$ on the growth parameters, CO_2 consumption rate, enzyme activity, intracellular lipid content and fatty acid profiles of *P. chodatii*. The biomass productivity as well consumption rate of carbon dioxide of *P. chodatii* were increased by increasing Na_2CO_3 and $NaHCO_3$ concentrations. Similarly, lipoxygenase and superoxide dismutase specific activity were enhanced with different concentrations of Na_2CO_3 and $NaHCO_3$. Catalase and total antioxidant enzymes of *P. chodatii* was increased with 60 and 45 mg l^{-1} of Na_2CO_3 and $NaHCO_3$, respectively. The low concentration of Na_2CO_3 and $NaHCO_3$ increased the lipid content of *P. chodatii*. The concentration of fatty acid methyl ester produced from *P. chodatii* were altered by the treatment with different concentrations of Na_2CO_3 and $NaHCO_3$.

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