Physiological and biochemical responses of the green alga 

*Pachycladella chodatii* (SAG 2087) to sodicity stress

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**Abstract**

The effects of various concentrations of different carbon sources (Na₂CO₃ and NaHCO₃) as sodicity stress on growth parameters, CO₂ 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 Na₂CO₃ and NaHCO₃. The biomass productivity as well consumption rate of carbon dioxide of *P. chodatii* reached the highest values with increasing concentrations of Na₂CO₃ and NaHCO₃. The soluble protein content of *P. chodatii* was highest at the lowest Na₂CO₃ and NaHCO₃ concentrations. The addition of different concentrations of Na₂CO₃ and NaHCO₃ 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 Na₂CO₃ and NaHCO₃, 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 Na₂CO₃ and NaHCO₃, respectively. Lipids content was increased at low concentration of Na₂CO₃ (40 and 15 mg l⁻¹) and NaHCO₃ (60, 45 mg l⁻¹) respectively. Subsequent to algal cultivation in different concentrations of Na₂CO₃, 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 Na₂CO₃ and NaHCO₃.

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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 CO₂ fixation capacities and under optimal culture condition express growth rates several orders of magnitudes higher than conventional crop plants [1,2]. Microalgae can fix CO₂ from different sources, which can be categorized as CO₂ from the atmosphere, industrial exhaust gases, and fixed CO₂ in the form of soluble carbonates (NaHCO₃ and Na₂CO₃). 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 Na₂CO₃ and NaHCO₃ 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, Na₂CO₃ and NaHCO₃ 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 NaHCO₃ can promote the photosynthesis as HCO₃⁻ is the carbon source [12,13], but a higher dose of NaHCO₃ and Na₂CO₃ 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 CO₂ concentration in water [14]. The depletion of dissolved CO₂ 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
algal biomass, biodiesel derived from oil as well as biohydrogen and bioethanol [18]. These cellular stresses 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 (Na₂CO₃, NaHCO₃) on the growth parameters, CO₂ 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 Na₂CO₃ [(control (0 mg l⁻¹)], NaHCO₃ [(control (0 mg l⁻¹)], (15, 45, 75 mg l⁻¹)], NaHCO₃ [(control (0 mg l⁻¹)], (15, 45, 75 mg l⁻¹)], on growth and biochemical composition of the green alga 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:

\[ P = \frac{\Delta X}{\Delta t} \]

where \( \Delta X \) is the variation of biomass concentration (mg l⁻¹), during the culture time \( \Delta t \) (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:

\[ \mu (h^{-1}) = \frac{\ln N_{t_{2}} - \ln N_{t_{1}}}{(t_{2} - t_{1})} \]

where \( N_{t} \) and \( N_{t_{1}} \) represent the chlorophyll a concentrations at times \( t_{1} \) (day 0) and \( t_{2} \) (day 11), respectively.

2.5. Determination of the CO₂ consumption rate

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

\[ P_{CO_{2}} = 1.88 \times P \]

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 Mínguez-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 H₂O₂ 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.

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 Na2CO3 and NaHCO3 on the growth, biomass productivity and CO2 consumption rate of P. chodatii

The content of chlorophyll a + b in the investigated alga subjected to different concentrations of Na2CO3 and NaHCO3 was shown in Table 1. The result showed that, the increase in concentrations of Na2CO3 caused significant increment in chl. a + b content for P. chodatii compared to the control culture (p < 0.05). The high concentration of NaHCO3 (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 Na2CO3 (40 mg l⁻¹) caused significant increase in carotenoids content, but increasing of NaHCO3 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 Na2CO3 and NaHCO3. 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 Na2CO3 and 45, 75 mg l⁻¹ d⁻¹ of NaHCO3, 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 NaHCO3 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 Na2CO3 and NaHCO3 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 CO2 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 Na2CO3 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 NaHCO3, 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 Na2CO3 and NaHCO3 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 Na2CO3 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 Na2CO3 and NaHCO3 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 NaHCO3 and small for Na2CO3, p < 0.05 was considered as significant.](image)

Table 1

| Treatments | (Chl. a+b) (µg ml⁻¹) | Carotenoids (µg ml⁻¹) | µ (d⁻¹) | Biomass productivity (mg l⁻¹ d⁻¹) | Consumption rate of CO₂ (mg l⁻¹ d⁻¹) |
|------------|----------------------|-----------------------|---------|-----------------------------------|-------------------------------------|
| Na₂CO₃ (mg/L) |          |                      |       |                                   |                                     |
| Control    | 2.5 ± 0.00a          | 0.91 ± 0.02a          | 0.84   | 16.4 ± 0.3a                       | 30.8 ± 1.5a                        |
| 40         | 3.05 ± 1.03b         | 1.04 ± 0.00b          | 0.80   | 11.1 ± 2.7a                       | 20.9 ± 5.1a                        |
| 60         | 2.99 ± 0.8a          | 0.88 ± 0.00a          | 0.72   | 20.9 ± 2.4b                       | 39.3 ± 4.4b                        |
| 80         | 3.33 ± 0.9a          | 0.91 ± 0.00b          | 0.78   | 21.4 ± 0.5a                       | 40.2 ± 0.5a                        |
| NaHCO₃ (mg/L) |          |                      |       |                                   |                                     |
| 15         | 2.57 ± 0.3a          | 0.72 ± 0.00a          | 0.55   | 13.6 ± 6.9a                       | 25.6 ± 11.3a                       |
| 45         | 1.63 ± 0.6a          | 0.63 ± 0.003a         | 0.17   | 18.2 ± 3.4a                       | 34.2 ± 6.4a                        |
| 75         | 2.84 ± 0.6b          | 0.60 ± 0.004a         | 0.51   | 23.6 ± 3.4a                       | 44.4 ± 6.4a                        |

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 NaHCO3 and Na2CO3 concentrations in the growth media Fig. 2a, b. In this respect, Wang et al. [37] reported that the activity of SOD under Na2CO3 stress was clearly higher than that of NaCl stress in Puccinellia tenuiflora. Zuo et al. [6] documented that compared to the NaCl stress, Na2CO3 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 Na2CO3 and NaHCO3, respectively Fig. 2c. Catalase, is one of the most important enzymes, scavenges H2O2 by directly breaking down to form H2O and O2 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 Na2CO3 and NaHCO3, 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 Na2CO3 and NaHCO3 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 NaHCO3 and small for Na2CO3, 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 NaHCO3 and small for Na2CO3, 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 NaHCO3 and small for Na2CO3, p < 0.05 was considered as significant.
the sodium ion stimulates hydrogenase activity in pea root nodules containing *Rhizobium leguminosarum* bacteria.

3.4. Effect of different concentrations of Na$_2$CO$_3$ 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$_2$CO$_3$ and NaHCO$_3$, respectively, led to increasing the total lipids, but reversible trend was observed when the culture was treated with higher concentrations of Na$_2$CO$_3$ 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 Na$_2$CO$_3$ and NaHCO$_3$, respectively, compared with the control culture that recorded 23.95%. In this respect, the composition of fatty acids of *P. chodatii* grown under various concentrations of Na$_2$CO$_3$ and NaHCO$_3$ are depicted in Table 2. Methyl esters of palmitic, stearic, linoleic, elaidic and stearic acids represented a major amount of biodiesel produced from *P. chodatii* treated with increased NaCl concentration. Whereas, at 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$_2$CO$_3$ 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 *C. vulgaris* reached its peak with 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 *C. vulgaris* reached its peak with 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 *C. vulgaris* reached its peak with 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 *C. vulgaris* reached its peak with 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 *C. vulgaris* reached its peak with 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

### Table 2

| FAME | Control | 40 | 60 | 80 | 15 | 45 | 75 |
|------|---------|----|----|----|----|----|----|
|      | Na$_2$CO$_3$ | Mg l$^{-1}$ | NaHCO$_3$ | Mg l$^{-1}$ | FAME (%) | FAME (%) | FAME (%) |
| Lauric acid | 0.57 | 2.05 | – | 0.69 | – | – | – |
| Hexanoic anhydride | – | – | 1.29 | – | 0.89 | 1.19 | 0.46 |
| Myristic acid | 5.42 | 6.62 | 1.4 | 4.73 | 0.89 | 1.19 | 0.46 |
| Palmitic acid | 27.41 | 29.3 | 26.52 | 30.45 | 26.22 | 25.56 | 24.43 |
| Myristic acid | – | – | 1.88 | 1.32 | – | – | – |
| Stearic acid | 2.66 | 27.6 | 5.88 | 24.33 | 1.44 | 3.35 | 1.13 |
| Palmitic acid | – | – | 1.99 | – | – | – | – |
| Pentadecanoic acid | – | – | 1.07 | – | – | – | – |
| Palmitic acid | 3.42 | 4.35 | – | – | – | – | – |
| Propanoic acid, 2-hexadecyl ester | – | – | 2.22 | – | – | – | – |
| Pentadecane-4-yl pentanoate | – | – | 2.22 | – | – | – | – |
| Arachidic acid | 0.86 | 1.25 | – | 0.94 | – | – | – |
| Propanoic acid,2-chloro-octadecyl ester | – | – | – | – | 0.84 | – | – |
| Heptadecylfluorobutyrate | – | – | – | 1.26 | – | – | – |
| 2-Hydroxy-1-(hydroxymethyl) | 3.26 | 5.76 | – | – | – | – | – |
| Octadecanoic acid ethyl ester | – | – | – | – | – | – | – |
| Heptadecanoic acid | – | – | – | – | – | – | – |
| Crotonic acid | – | 0.59 | – | – | – | – | – |
| 2-Maleic acid, mononethyl ester | – | – | – | – | – | – | – |
| Palmitoleic acid | 1.23 | .950 | – | – | 3.16 | 9.94 |
| Valeric acid, undec-2-enyl ester | – | – | – | – | 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, (ZZZ) cis,cis,cis-9,12,15-Octadecatrienoic acid | 23.95 | 39.04 | – | – | – | – | – |
| 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 | – | – | – | – | – | – | – |

**Table 2** Fatty acid methyl ester (FAME) profile of *P. Chodatii* grown under various concentrations of Na$_2$CO$_3$ and NaHCO$_3$. 

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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 Na2CO3 and NaHCO3 on the growth parameters, CO2 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 Na2CO3 and NaHCO3 concentrations. Similarly, lipoxygenase and superoxide dismutase specific activity were enhanced with different concentrations of Na2CO3 and NaHCO3. Catalase and total antioxidant enzymes of *P. chodatii* was increased with 60 and 45 mg l−1 of Na2CO3 and NaHCO3, respectively. The low concentration of Na2CO3 and NaHCO3 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 Na2CO3 and NaHCO3.

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