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Full Length Article

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 (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

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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).

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