Short communication

Changes in the gross chemical composition of mass cultures of the marine microalga *Dunaliella tertiolecta* with different aeration rates

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Abstract

The effect of different aeration rates, in the range 0–6·51 l of air min^{-1} l of $culture^{-1}$, and CO_2 supply on the biochemical composition of mass cultures of the marine microalga *Dunaliella tertiolecta* was studied. The biochemical composition of *D. tertiolecta* was strongly affected by the aeration rate. There was a negative correlation between stationary-phase protein cellular content and air flow. Carbohydrate cellular

content also decreased with aeration rate, a minimum being reached with 0·93 l of air min^{-1} l of $culture^{-1}$. Maximum carbohydrate per volume unit was achieved with maximum aeration as the increase of carbohydrates per ml was directly proportional to air flow and therefore to CO_2 availability. Maximum protein per ml was achieved with 1·86 l of air min^{-1} l of $culture^{-1}$, keeping stable with higher air flows. The cultures supplied with CO_2 showed carbohydrate and protein concentrations similar to the cultures with 1·86 l of air min^{-1} l of $culture^{-1}$, indicating a correlation between available CO_2 and not only carbon, but also nitrogen metabolism. Different factors seemed to limit cell division and nitrogen metabolism as maximum nitrogen transformation rate was achieved with an air flow of 1·86 l of air min^{-1} l of $culture^{-1}$, lower than the 3·72 l of air min^{-1} l of $culture^{-1}$needed for maximum cell density.

Keywords

Dunaliella tertiolecta; microalgae; mass culture; aeration; CO_2; gross composition

INTRODUCTION

Species of the genus *Dunaliella* are useful for the production of glycerol and β-carotene (Ben-Amotz & Avron, 1980; Borowitzka, 1991) and some species are widely used in aquaculture (Brown *et al.,* 1989). Recently, anti-cancer (Mokady, 1992; Fujii *et al.,* 1993) and hypocholesterolemic (Fábregas *et al.,* 1995a) effects have been demonstrated.

When nitrate is used as a source of nitrogen in microalgal cultures, the pH of the medium increases gradually with cell growth. The addition of CO_2 to the cultures causes a decrease of pH through a displacement of the buffer system: CO_2-carbonates. The supply of CO_2 for the control of pH in the cultures is one of the main items in the calculation of costs in mass cultures of microalgae. In a previous work, different aeration rates and regimes of pure CO_2 supply were tested in *Dunaliella tertiolecta* cultures, demonstrating that enough CO_2 can be transferred to the culture with high aerations with no need for further supplementation with pure CO_2 (Fáibregas *et al.,* 1994). The aim of the present work was the study of how the different aeration rates,

with the different growth rates and carbon availability derived, affected the chemical composition of the cells of *D. tertiolecta.*

METHODS

The marine microalga *Dunaliella tertiolecta* was grown in sterilized sea water enriched with the commercial inorganic nutrient Algal-1 (Nutrición Avanzada S.A. Avda Cortés 8, Fuentes Nuevas, Ponferrada, León, Spain) (Herrero *et al.,* 1991) in which the source of nitrogen was 2 mM $NaNO_3$ as previously described (Fáibregas *et al.,* 1994). The cultures were grown in 6 l flasks containing 4.3 l of medium at 18°C under a light intensity of 81.04/µE $m^{-2}s^{-1}$ and a light periodicity of 12/12 h light/darkness. Initial inoculum density was 5 x 10^5 cells ml^{-1} from an exponentially-growing culture.

Seven different flows of air were applied to the cultures: 0.11, 0.23, 0.46, 0-93, 1.86, 3.72 and 6.51 l of air min^{-1} l of $culture^{-1}$. Two cultures were set up as controls: one without aeration (named as air flow 0) and another one with no aeration to which different quantities of CO_2 were added by sparging twice a day, in order to keep the pH in the range 7.2-7.9. Two replicates were set-up for each condition.

After 13 days, once all cultures had reached the stationary phase, samples were obtained by centrifugation for immediate analysis. Protein was measured by the dye-binding method (Bradford, 1976) and carbohydrates by the phenol-sulphuric acid method (Kochert, 1978). Chlorophyll-a was measured spectrophotometrically using the formulae proposed by Jeffrey and Humphrey (1975).

RESULTS AND DISCUSSION

Maximum cell density in the stationary phase was achieved with an aeration of 3.72 l of air min^{-1} l of $culture^{-1}$, no statistical difference being recorded in the cell densities between this air flow and 6.51 1 of air min^{-1} l of $culture^{-1}$ (Fábregas *et al.*, 1994). However, when the conversion rate of nitrate into protein is considered, a maximum around 80% conversion was achieved with a lower air flow than that needed for maximum final cell density: 1.86 1 of air min^{-1} l of $culture^{-1}$ (Fig. 1). The non-aerated cultures that were supplied with CO_2 had a final cell density between the

densities obtained with aeration rates 0.93 and 1.86 l of air min^{-1} l of $culture^{-1}$ (Fábregas *et al.*, 1994), but the nitrogen conversion rate was almost maximum (79%) and similar to the conversion rates obtained with air flows between 1.86 and 6.51. These data indicate that different factors were limiting cell division and nitrogen metabolism. Similar results were obtained in semicontinuous cultures of *D. tertiolecta* under a cyclostat regime (Fábregas *et al.*, 1995b).

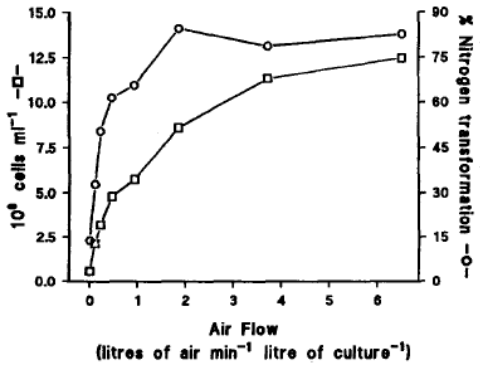

Fig. l. Stationary phase cell density (10^6 cells $m1^{-1}$) and percentage of conversion of nitrate into protein in mass cultures of *D. tertiolecta* with different air flows. Values are the averages of two replicates.

Maximum cellular contents of protein, 53.38 pg $cell^{-1}$, and carbohydrates, 20.14 pg $cell^{-1}$, were found in the non-aerated cultures [Fig. 2(a)], that had a growth rate, measured as the increase in cell number, of effectively zero during the period of study (Fábregas *et al.*, 1994). Values of protein and carbohydrate contents in the non-aerated cultures were much higher than the values of the logarithmic-phase inoculum which were 21.46 and 4.36 pg $cell^{-1}$, respectively (Fábregas *et al.*, 1986). There was a negative correlation between stationary-phase cellular protein content and growth rate. For carbohydrates, however, the decrease was not continuous, but reached a minimum with 0.93 l of air min^{-1} l of culture -1 and increased slightly with higher air flows. The range of variation recorded between the lowest and the highest values was 351 and 205% for protein and carbohydrate content, respectively; higher than the range generated in mass cultures of this species by increasing the nutrient concentration from 2 mmol N l^{-1} to 16 mmol N l^{-1} (Fábregas *et al.*, 1986). In the cultures with pure CO_2 supply, protein content was 18.24 pg cell $^{-1}$, between the values obtained for 0.46 and 0.93 1 of air min^{-1} 1 of $culture^{-1}$, and carbohydrate content was 9.52 pg $cell^{-1}$.

The range of variation of chlorophyll-a content was much narrower than for the other two organic fractions studied [Fig. 2(a)], the maximum cellular content being achieved with 0.93 l of air min^{-1} l of $culture^{-1}$, decreasing with higher air flows. Maximum chlorophyll-a content coincided with minimum carbohydrate content [Fig. 2(a)]. A lower cellular chlorophyll content in cultures with low cellular density would be expected, as more light would be available in these cultures. The decrease in chlorophyll content recorded with air flows higher than 0.93 1 of air min^{-1} l of $culture^{-1}$ might have reflected a lower availability of nitrogen in the medium, which might have reduced the pigment content of the cells.

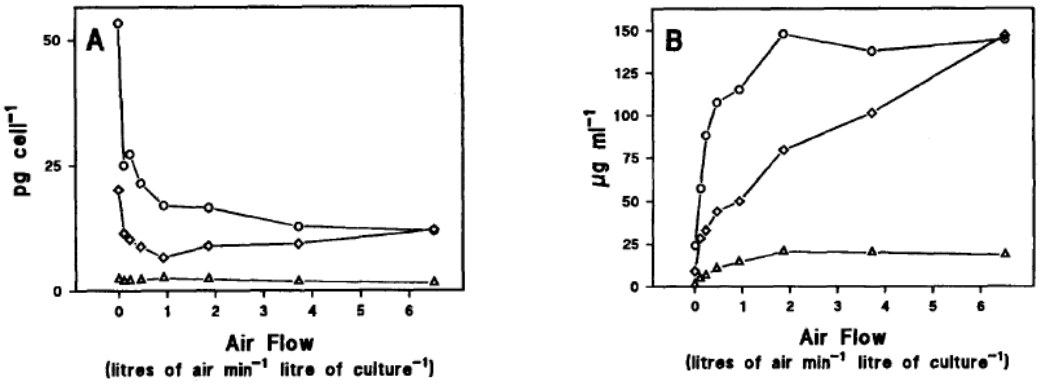

Fig. 2. (a) Cellular content (pg $cell^{-l}$) and (b) concentration (µg $m1^{-1}$) of protein (o), carbohydrates (◊) and chlorophyll-a (⌂) in mass cultures of *D. tertiolecta* with different air flows.

The chlorophyll-a cellular content in the cultures under CO_2 was also lower than the chlorophyll-a cellular content recorded with a flow rate 0.93 1 of air min^{-1} l of $culture^{-1}$, and similar to those with 0-46 and 1.86 l of air min^{-1} l of $culture^{-1}$.

The evolution of protein and carbohydrate concentration per volume unit was opposite to the evolution of cellular contents, increasing with increasing aeration rates [Fig. 2(b)]. Carbohydrate per culture volume unit was directly proportional to the carbon introduced by aeration, the increase being linear (r^2 = 0.95), meanwhile protein concentration reached a plateau at 1.86 l of air min^{-1} l of $culture^{-1}$ [Fig. 2(b)]. The concentration of chlorophyll-a per volume unit stabilized at the same aeration rate as protein concentration [Fig. 2(b)].

The concentration of carbohydrate per ml in the cultures supplied with pure CO_2 was 71.9 µg/ml, similar to the carbohydrate concentration obtained with an air flow of 1.86 l of air min^{-1} l of $culture^{-1}$, although the amount of CO_2 supplied to the cultures with 1.86 l of air min^{-1} l of $culture^{-1}$ was 6.5 times higher than the CO_2 sparged in the first case. The higher efficiency of transference to culture media when pure CO_2 was bubbled may explain this difference. Protein per ml was also similar for cultures supplied with CO_2 (136.2/µg ml^{-1}) and cultures with 1.86 l of air min^{-1} l of $culture^{-1}$ (146.7 µg ml^{-1}) indicating a correlation between available CO_2 and not only carbon, but also nitrogen, metabolism. Despite the similar concentrations of protein and carbohydrate in cultures with 1.86 l of air min^{-1} l of $culture^{-1}$ and cultures with CO_2 supply, cell density was significantly higher in the first case: 8.59 x 10^6 cell $m1^{-1}$, against 7x 10^6 cell $m1^{-1}$ in the cultures with CO_2 (Fábregas *et al.,* 1994) and, therefore, when expressed as cellular content, higher protein and carbohydrate cellular contents were found in the cultures supplied with pure CO_2.

Despite carbohydrates being preferentially accumulated as a response to nitrogen limitation in cultures of *D. tertiolecta* (Borowitzka, 1988), a parallel increase in lipid content could not be ruled out. The addition of CO_2 increased the lipid contents of several species of algae by increasing fatty acids (Hamza & Robin, 1992), although the degree of unsaturation decreased in *Dunaliella* at high CO_2 levels (Tsuzuki *et al.,* 1990).

Considering nitrogen conversion into protein, the culture with 1-86 l of air min^{-1} l of $culture^{-1}$ could be considered optimal, as this was the minimum air flow producing maximum conversion and minimum medium evaporation. When carbohydrate production is to be optimized, an air flow of 6.51 1 of air min^{-1}l of $culture^{-1}$ must be applied.

ACKNOWLEDGEMENT

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