Growth of *Chlorella* in the presence of organic carbon: A photobioreactor study

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Abstract

In this study, the effect of organic carbon supplementation at low light intensity on Chlorella sorokiniana growth was evaluated. Addition of 1 g/L of acetate to media gave the highest growth rate and provided stable high biomass culture during prolonged cultivation time. Glucose at 1 – 5 g/L also improved biomass growth rate, although stability of high biomass culture could not be achieved. Overall, the presence of organic carbon can considerably enhance Chlorella growth when low light intensity is applied.

Keywords: microalgae, growth, organic carbon, biomass

1. Introduction

Microalgae are photoautotrophic microorganisms because they are capable of using light energy to fix carbon dioxide into carbohydrates and discharging oxygen as a waste product during photosynthesis [1]. Microalgae contain valuable compounds such as lipids, proteins and pigments which can find applications in many branches of industry [2]. To make production of target compounds from microalgae a feasible process, high biomass concentrations are necessary. Microalgae are cultivated in lakes, shallow open ponds or closed photo-bioreactors to produce target compounds under selected conditions. However, insufficient illumination of photobioreactors severely affects microalgal cultures in scaled-up systems [3]. Some microalgae such as *Spirulina* [4], *Haematococcus* [5], *Chlorella* [6] and *Nitzschia* [7] can also grow in the presence of organic substrates, using sugars, amino acids or organic acids. Organic substances are assimilated and respired with oxygen to cover energy requirements of microalgal cells. When availability of light in insufficient, high cell densities of microalgae can be attained in large-volume bioreactors during organic carbon based cultivation [8].

In this work, growth of *Chrorella sorokiniana* in the presence of organic carbon and different light intensity was studied.

2. Materials and Methods

2.1 Composition of growth media

Chlorella sorokiniana was cultivated in a medium with basic composition (TMP) as follows: Tris (2.42 g/L), NH_4Cl (0.4 g/L), $MgSO_4 \cdot 7H_2O$ (0.1 g/L), $CaCl_2 \cdot 2H_2O$ (50 mg/L), K_2HPO_4 (93.5 mg/L), KH_2PO_4 (63 mg/L), Na_2EDTA (50 mg/L), H_3BO_3 (11.4 mg/L), $ZnSO_4 \cdot 7H_2O$ (22 mg/L), $MnCl_2 \cdot 4H_2O$ (5.06 mg/L), $FeSO_4 \cdot 7H_2O$ (4.9 mg/L), $CoCl_2 \cdot 6H_2O$ (1.61 mg/L), $CuSO_4 \cdot 5H_2O$ (1.57 mg/L) and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (1.1 mg/L). Additionally, 1 g/L acetate (TAP) or 1 g/L and 5g/L glucose (TGP 1, TGP 5) was addded to TMP. In all media, pH value was set at 7.

2.2 Set-up of photobioreactor study

Experiments with *Chlorella* cultivation were conducted in a photobioreactor (Multi-cultivator MC 1000 – OD) that consists of 8 tubes immersed in a waterbath set at 25°C and independly illuminated by white LEDs (Figure 1). Continuous light irradiance generated by LEDs was set at 20 $\mu E/m^2 \cdot s$ during first 69.7 h of cultivation and then was increased to 150 $\mu E/m^2 \cdot s$ for all tubes. Mixing inside tubes was accomplished by glass spargers that provided pumped air, with a flow rate manually adjustable by valve manifold. During *Chlorella* growth, biomass concentration was estimated by OD detector set at 730 nm (optimal for biomass measurement) and 680 nm (optimal for chlorophyll measurement). Optical density values measured at 10 minute interval were stored in the system memory and displayed on external PC connected to photobioreactor.

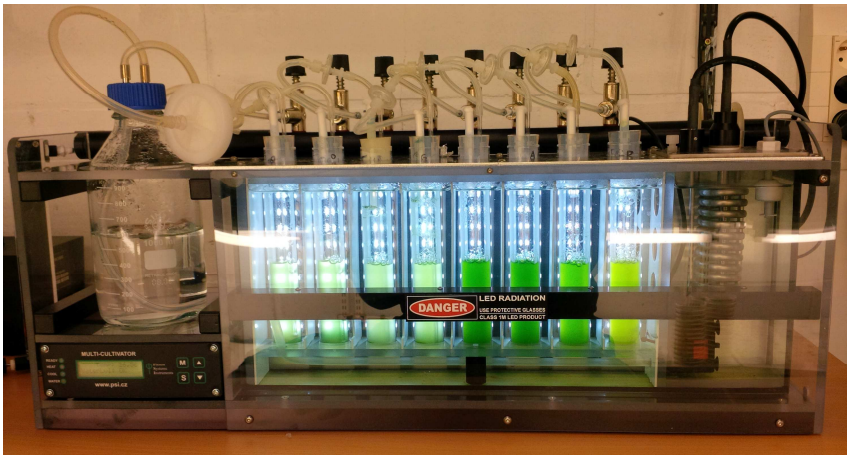

Figure 1. *Cultivation of Chlorella in a tube photobioreactor.*

2.3 Growth rate

Growth of *Chlorella* was evaluated from OD_{730} measurements during cultivation according to equation:

$$\mu = (Lnx_2 - Lnx_1)/(t_2 - t_1)$$

Where:

μ = growth rate within t_2- t_1 cultivation time [h^{-1}]
x_2 = optical density measured at 730 nm at t_2 of cultivation [-]
x_1 = optical density measured at 730 nm at t_1 of cultivation [-]

3. Results

Results of this study show that growth of *Chlorella* can be substantially enhanced in the presence of organic carbon, when low light intensity (20 $\mu E/m^2 \cdot s$) is applied (Figure 2A). Addition of acetate or glucose into medium resulted in respectively, a 3.6 fold and 2.5 fold increase in growth rate within first 31 hours, when compared to medium without organic carbon used (Table 1). After 31 hour cultivation on acetate, growth rate decreased but high biomass density could be maintained for a longer time (117 h). Both glucose concentrations (1 and 5 g/L) exerted the same effect on *Chlorella* growth within first 40 h. However, growth of *Chlorella* in the presence of 1 g/L glucose almost ceased after 40 h, probably due to complete removal of glucose from medium. When 5 g/L of glucose was tested, growth was maintained and biomass concentration achieved at 50 h was higher than in case of *Chlorella* cultivation on acetate. However, after 50 h, foam formation and biomass sedimentation occurred. It resulted in the settle down of culture at the bottom of photobioreactor tube and gradual decrease of optical density values, as cells were not available in detector area measurement (Figure 2B&C). At 69.7 h, light intensity emitted by photobioreactor lamps was increased to 150 $\mu E/m^2 \cdot s$. An increase in light intensity caused that culture depleted from 1 g/L of glucose continued to grow via photosynthesis until 90 h, when foaming effect and biomass sedimentation also appeared. At 69.7 h of *Chlorella* cultivation on acetate, biomass concentration reached the level which was obtained at 50 h during glucose based growth, and after increase in light intensity, acetate based growth was further supported and higher biomass intensity was achieved (117 h). Moreover, although biomass concentration at 50 h was higher in case of using 5 g/L glucose, chlorophyll concentration at 50 h was higher in culture, when acetate was used (Figure 2D). Overall, the presence of organic carbon was necessary to increase growth rate, when low light intensity was applied. In case of low light intensity and a lack of organic carbon, *Chlorella* growth was slow and additional increase of light intensity was necessary (69.7 h) to prevent light limitation and ensure continuation of *Chlorella* growth (Table 2).

Table 1. *Growth rate of Chlorella in TMP, TAP and TGP medium during first 31 h.*

Media	**0 h – 31 h**
TAP 1g/L	$\mu = 0.080\ h^{-1}$
TGP 1g/L	$\mu = 0.057\ h^{-1}$
TGP 5g/L	$\mu = 0.053\ h^{-1}$
TMP	$\mu = 0.022\ h^{-1}$

Table 2. *Growth rate of Chlorella in TMP medium during 117 h.*

	0 h – 69.7 h [20 µE]	**69.7 h – 117 h [150 µE]**
TMP	$\mu = 0.020\ h^{-1}$	$\mu = 0.020\ h^{-1}$

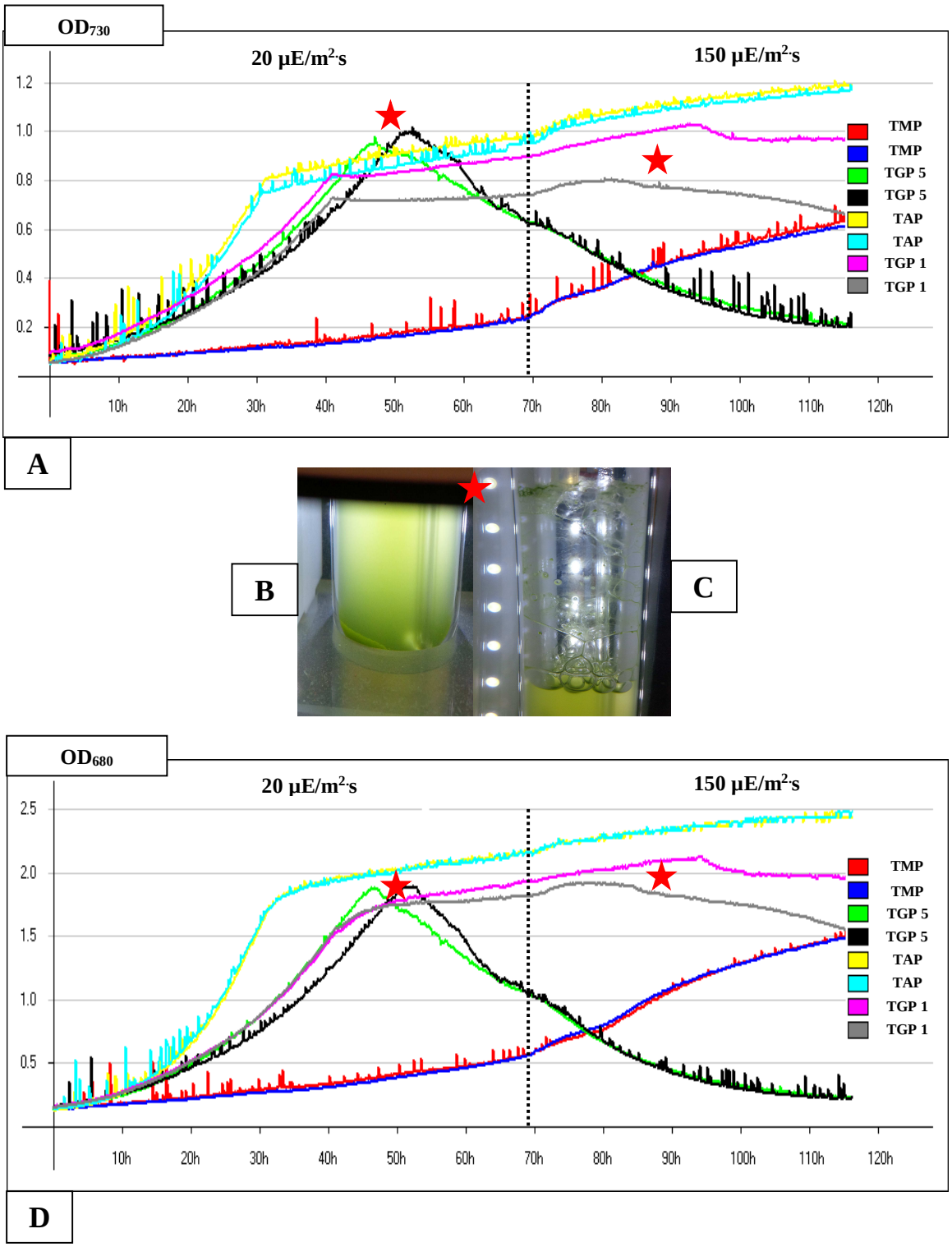

Figure 2. *Growth of Chlorella measured at OD_{730nm} [A] and OD_{680nm} [D] & Sedimentation [B] and Foaming [C] effect.*

4. Discussion & Conclusions

Both acetate and glucose increased the growth of *Chlorella sorokiniana* in conducted experiments. In literature, acetate at concentration up to 3.28 g/L increased by 30 % the growth of *Chlorella sorokiniana* when light intensity applied was 460 $\mu E/m^2 \cdot s$ [9]. In our study, low light intensity 20 $\mu E/m^2 \cdot s$ was decided to favour the uptake of organic carbon and 1 g/L acetate improved *Chlorella* growth 3.6 times. In another study [10], 4 g/l of glucose caused a 5.4 fold increase in *Chlorella sorokiniana* growth at an irradiance of 100 $\mu E/m^2 \cdot s$ and for 2 g/L glucose, the growth of *Chlorella* stopped after 2 days. In our work, a 2.5 fold increase in *Chlorella sorokiniana* growth with 1 – 5 g/L glucose was achieved with a cease of growth for 1 g/L glucose after 40 h. 5 g/L glucose enabled continuation of growth till the biomass sedimentation and foam formation appeared. The explanation of this phenomena is that cells use nutrient from media during growth and when high biomass density is achieved, a nutrient depletion appears. A lack of microelements in media causes cell lysis [11] and release of cellular content such as proteins, lipids and polysaccharides into medium. Such macromolecules can act as flocculants and enhance agregation of ramaining cells [12] followed by their sedimentation. Moreover, proteins, lipids and carbohydrates can also act as surfactants and the combination of intensive bubbling and extracellular surfactants present in media can result in foam formation [13]. Such effects did not occur when acetate as organic carbon was used. Presumably, a change in medium composition should be made in order to support high cell densities and prevent nutrient depletion during glucose based growth. It is also noteworthy that although glucose gave high biomass density, chlorophyll concentration in culture was smaller, when compared to *Chlorella* cultivation on acetate. It shows that content of microalgae cells changes according to type of organic carbon available in medium. Hence, the use of organic substances for growth acceleration should be strictly adjusted with a target compound, that is to be produced from microalgae culture.

5. Literature

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