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Microalgae Culturing in Thin-layer Photobioreactors

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One of the problems for the high cost of production of microalgal biomass is represented by the productivity rate which is almost one order of magnitude below the theoretical one. There is a general consensus that light saturation effect represents one of the most important factors limiting the microalgal productivity. This work presents an experimental analysis of the flow achieved in a modified cascade photobioreactor, and discusses its basic fluid-dynamic characterization and its growth performances with a microalgal culture (*Chlorella vulgaris* and *Nannochloropsis* sp.) in a laboratory 0.2-m^2 scale and in a outdoor waved-surface 5-m^2 unit.

1. Introduction

The versatility of microalgae metabolism make them a potential source of biochemical products and bioenergy (e.g. hydrogen, biodiesel). Microalgae, are considered as one of the fast-growing photosynthetic microorganisms on Earth, hence it is expected that they can play an important role in CO_2 storage capability, and phytodepuration. So far, however, due to the still too high production cost, only in few cases microalgae have reached a viable commercial production stage (e.g. *Arthrospira*, *Chlorella*, *Dunaliella* and, more recently, *Haematococcus*). One of the problems for the high cost of production is represented by the still relatively low productivity rate which is almost one order of magnitude lower than the theoretical one. Microalgae productivity outdoors is strongly limited by the so called “light saturation effect”. This phenomenon occurs because the growth of the microalgae saturates at a level of light intensity which is roughly 1/10 of the maximum recorded in summer days. The problem of light saturation may be greatly reduced if proper combinations between cell density, and mixing could be achieved. However, it seems difficult to reach this goal with open-ponds and conventional photobioreactors. The aim of this communication was to present an experimental analysis of the flow achieved in a modified cascade system designed by Setlik et al. (1970). The new design of cascade PBR features a thin film of concentrated microalgal suspension falling along a specially designed sloping surface. Several advantages over the conventional culture systems can be gained with this

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configuration: 1) an optically thin culture with a very high cell concentration which are the most important requirements for a better light utilization efficiency, 2) an efficient gas-liquid mass transfer thanks to the moving and extremely shallow layer thickness, and 3) a limited equipment cost. Moreover, in addition to said advantages the modified cascade system allows to improve the light-dark cycles at which cells are exposed. However, like many other PBR designs, culture circulation in cascades requires the use of a pumping system which, if not properly designed, may increase the shear stress with negative effects on the biomass productivity. Here, we discuss the basic fluid-dynamic characterization, and present preliminary data of the growth of a microalgal culture (*Nannochloropsis* sp.) in a outdoor surface-waved 5 m^2 unit. A scheme of the cascade thin layer system used in this study is shown in Figure 1. The reactor is equipped with probes for the measure of the culture parameters such as temperature and pH. CO_2 addition to the culture is controlled by a software designed by Chemitec (Italy).

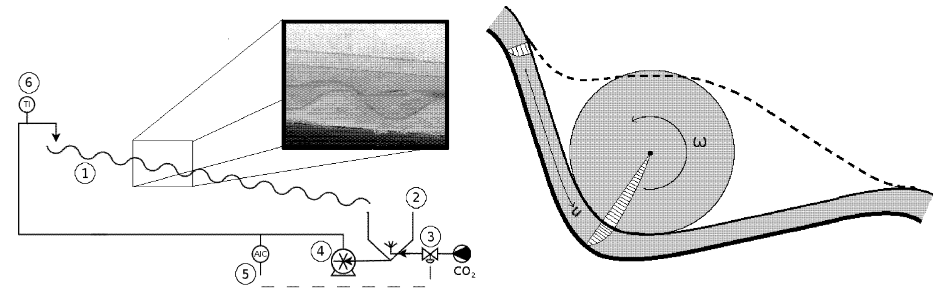

Fig. 1: **left**: Waved-bottom cascade PBR scheme showing all the equipment arrangement. 1. PBR waved surface 2. Night time/rainy days culture hold-up 3. Carbon dioxide sparger 4. Circulating pump 5. pH control loop 6. Temperature probe. In zoomed window a side view of the cascade during operation. **Right**: Schematic representation of fluid compartments with the relevant velocity vectors..

The thin layer fluid velocity can be calculated according to Manning's theory on fluid flowing in open channels that has been experimentally validated for a thin layer cascade photobioreactor operated in Trébon in Czech Republic (Masojidek *et al.*, manuscript in preparation). The simplified equations are:

$$\begin{cases} R_h = \dfrac{A}{P} = \dfrac{s \cdot z}{z+2s} \\ \tau_w = \dfrac{K \cdot \rho \cdot u^2}{2} \end{cases} \rightarrow \quad u = \frac{R_h^{2/3} \cdot S_0^{1/2}}{n} \quad \rightarrow \quad Q = \frac{A \cdot R_h^{2/3} \cdot S_0^{1/2}}{n}$$

where, among the relevant parameters, Q is the volumetric flow rate, R_h is called the hydraulic radius, A and P are respectively the flow area and the wetted perimeter, *u* is the fluid velocity and S_0 is the slope of the tilted median plane of the cascade. For a cascade with a volumetric flow rate of $1{,}814 \cdot 10^{-3}$ $m^3 \cdot s^{-1}$, a slope S_0 of 0.0873, assuming a Manning factor *n* of 0.014, the film thickness can be obtained directly from the solution of the third equation and is equal to $3.64 \cdot 10^{-3}$ m. Fluid velocity *u* is then easily obtained by the second equation, for a value of 0.498 $m \cdot s^{-1}$. According to this

hypothesis, this speed can be assumed constant throughout the PBR length and it can also be assumed to be equal to the rotational speed of the swirling fluid cylinder. Being this speed that possessed by fluid elements located on the boundary between the two different fluxes, it is clearly related to the angular speed which can be calculated when geometry of the system is known. In this case, the cylinder radius calculated experimentally by IA is 0.00945 m, while the obtained angular rotational speed is 52.5 rad·s^{-1} which equals a frequency of 8.4 rps.
Bearing in mind the hypothesis of solid rotational movement, it is easy to understand that different cells inside the swirling fluid cylinder are exposed to the same frequency of light/dark phases but to different light intensities. Plotting the results as a combination of position and received light radiation as function of time, we obtained the data depicted in Figure 2.

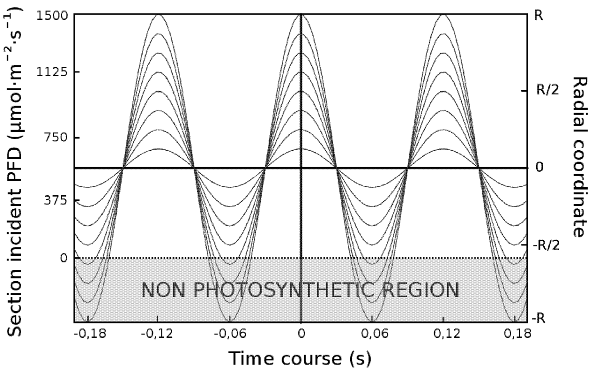

Fig. 2: Light/dark cycles amplitude as a function of time and radial position from the centre of rotation using sinusoidal shaped curves. Incident light intensity is reported for an average 2009 August day at 11.00 UTC in Florence. Linear attenuation with depth is assumed linear owing to the highly concentrated cultures involved in the experiments.

According to the literature, *Nannochloropsis* sp. shows an I_k value close to 73 µmol·m^{-2}·s^{-1} (Fang et al., 2004) that are achieved, according to measurements of light attenuation in microalgal cultures (Cornet et al. 1992) at a depth of about 1.42 cm from the liquid/gas illuminated interface (that is R+R/2 referring to the cylinder geometry as seen in the plot of Fig. 2). Therefore the cells are exposed outdoors to an incident light intensity which is much higher than their saturation value, for the most of the time, a condition that cannot be avoided by simply increasing the cell density. This situation is usually lead to a reduction of the yield as a result of the saturation effect of the photosynthesis, which in some case. e.g. when other additional stress are present, such as low temperature, can lead to photoinhibition of photosynthesis and in some cases to photooxidation (cell lysis). Saturation effect can be avoided by creating a fast light/dark cycle in the culture layers. With this model, each fluid compartment can be analysed separately basing only on the light/dark phases duration to investigate how much of the fluid takes part to the flashing light effect integration phenomena (Kok, 1953), however, using just the frequency calculated above, a first rough estimation of 60 ms L/D cycle can be obtained. Being this value of the same time scale of the plastoquinone pool turnover rate (10 ms) according to Falkowsky & Raven, (2007) it appears reasonable the possibility to benefit at least of a partial intermittent effect of light on photosynthesis. Further investigations are needed to assess the real effect in terms of culture growth and photosynthetic efficiency and to calculate all the flashing light parameters with more accurate mathematics. Finally, the model have to be validated through experimental Image Analysis techniques (still in an early stage) based on dye tracers to calculate and describe thoroughly the fluid pattern on a wave bottomed cascade PBR.

2. Investigation on the Thin-Layer PBR Fluid Dynamics

Dye tracing experiments were carried out in the 5-m^2 wave-bottomed PBR to estimate the entrainment rate of the streamline flux by individual troughs. The nature of the entrainment and the characteristics of the inner motion inside the troughs was investigated by using a small scale replica of the 5-m^2 PBR (1.2 m length x 0.16 cm width) with lateral transparent walls. In both experiments 30-fps, 1280x1024 movies were taken with a commercial camera (Canon Powershot SX 200 IS). Frames were extracted and post-processed by a general purpose image analysis package (ImageJ, http://rsbweb.nih.gov/ij/). During dye tracing experiments the wavy-bottomed cascade was circulated with tap water and injected with 5 mL of a concentrated red dye (Universal Tinting Paste n. 20 by UNIKOL). The spot would rapidly expand during its travel along the 5-m length of the PBR at the same time as it became leaner. Red dye permitted easier background cancellation during image postprocessing. In parallel to the dye tracing runs, a colour recorded intensity vs dye concentration calibration curve was set up (Figure 3, sub-figures B1 and B2) to quantify the local dye concentration on the PBR. Given that the movie was taken from a fixed, central position along the PBR length, for every frame undergoing the IA procedure a view factor was calculated based on the measured contraction of the distance between the wave ridges.

A fairly good (5%) closure of the calculated total material balance was obtained for later frames (Sub-figures C2-C5) but not for the first ones (30% deviation on sub-figure C1). This is considered appropriate for later investigation of the extent of material exchange between the liquid streams over passing the ridges and the liquid hold-up in the troughs. The swirling flow in the entrained volume, evident in the 4-frame sequence in Figure 4, permitted the estimation of the half-period of the rotational motion within the liquid in 100 ms or less which is roughly the same order of magnitude order of that calculated with the theoretical approach (60 ms). A second flashing effect of longer frequency can be expected for the cells following the streamline flow which tracks the bottom, therefore partly exposed to the surface light (in the photic zone) and partly flowing far from it, in the dark zone. Preliminary experiments outdoors to test the performance of the new designed cascade photobioreactor were carried out at the end of Summer 2009, using a *Nannochloropsis gaditana* culture. Total volume of the cascade PBR was 110 litres. The portion of the culture volume maintained in the dark at any time (e.g. receiving vessel, pump, manifold, and tube riser) was about 25%.

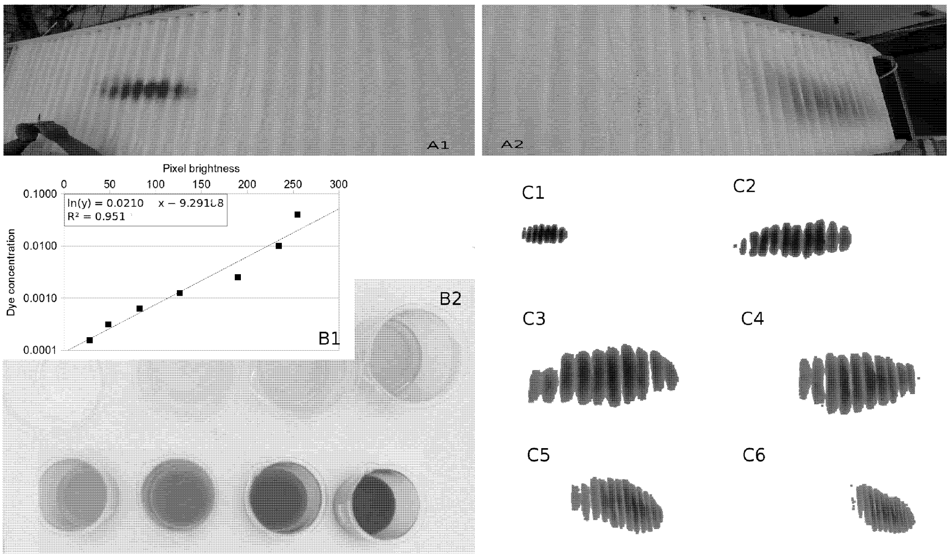

*Figure 3. Fluid dynamic analysis of the 5 m^2 cascade: the beginning (**A1**) and the end (**A2**) of dye tracing experiments; **B1-2**: Calibration curve from dye samples at known concentration. **C1-6***: dye spots displacement analysis by image analysis.

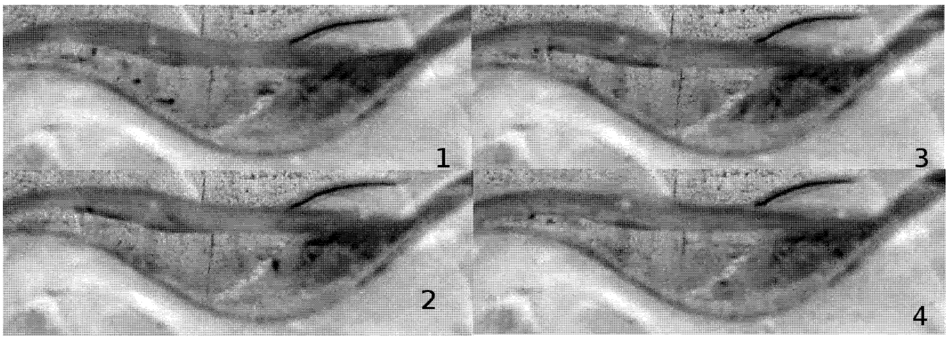

Figure 4. Sequence showing coloured particles tracking the liquid flow.

3. Biomass productivity in outdoor PBR and pumping stress

Incident solar light was measured perpendicularly to the fluid surface and reached 1500 µmol photons m^{-2} s^{-1} in middle of day. The dry weight increase during 10 days of culture is shown in Figure 6. The average net productivity during the first 4 days of culture was 125 mg/l/day and increased to 183 mg/l/day during the following 6 days of cultivation. These productivity translated on areal basis amounted to 3.2 and 5.8 g/m2/day respectively. The higher cell concentration of the second part of the growth accounts for the better yield achieved. However, biomass yields were significantly lower to that expected most likely because of the high temperature stress experienced by the *Nannochloropsis* culture during the sunny days which increased up to 35 °C, that is, 10 degrees about the optimum for this species. High temperature may have exacerbated photoinhibition. In the future, we aim at increasing cell concentration (up to 10 g/l) in order to take advantage of intermittent light phenomena. Furthermore, the performance of the waved PBR needs to be evaluated with cultures more resistant to high light, with possible candidate being *Chlorella* and *Arthrospira* sp..

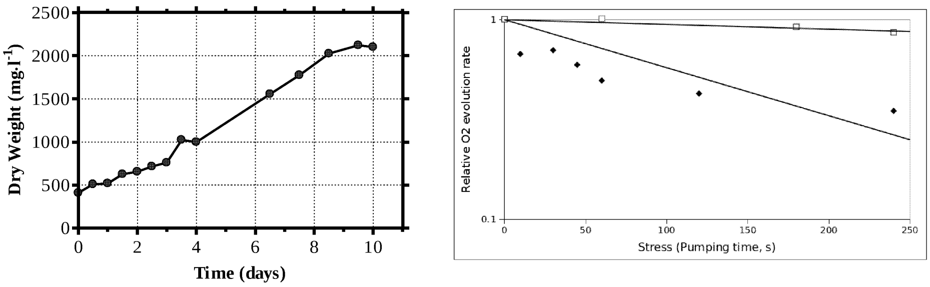

Figure 5. ***Left****: Outdoor cultures of Nannochloropsis sp. grown outdoors on a waved cascade* ***Right****: Relative O_2 evolution rate of cultures of C. vulgaris (filled symbols) and Nannochloropsis sp (unfilled symbols).*

The effect of the stress due to pumping the culture was studied by measuring the reduction in the photosynthesis oxygen evolution rate of the culture at different times. The investigation was carried out by circulating a fixed culture volume through a small (aquarium) centrifugal pump (Aquarium Systems New-Jet 1200). Max discharge head 1.45 m, max capacity 1200 L/h). The O_2 dissolved in the culture was stripped with a 95%/5% N_2/CO_2 mixture, while O_2 evolution rate under controlled illumination was monitored by using a chemiluminescence probe.

4. Conclusions

A preliminary analysis of wave-bottomed cascades has been reported. A model-based analysis, based on an experimental fluid dynamic analysis, permitted the calculation of the flashing effect undergone by the cultured microalgal cells. Biomass productivity in outdoor culturing and expected cell damage during centrifugal pumping has also been experimentally investigated.

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