***Scenedesmus Obliquus* Growth in Semi-Batch Microphotobioreactor under Non-Limiting CO2 Supply**

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**Highlights**

* Microalgae growth monitoring assessed with fluorescence measurements.
* Semi-batch microphotobioreactor for non-limiting nutrients condition.
* The system is effective in CO2 supply.
* It will be possible to assess different light conditions simultaneously.

**1. Introduction**

Microalgae are nowadays widely used as a source for different chemicals and products of interest. However, the industrial optimization of biomass production is hard to achieve since many factors contribute to their growth, such as nutrients availability and light intensity and many experiments are needed to characterize their impact. A microscale platform would strongly aid with this purpose, allowing the simplification and the parallelization of the reactors [1].

One of the main challenge in micro-photobioreactor (mPBR) technologies is linked to CO2 supply, as it is the main nutrient required for photosynthesis and, even if the mPBR materials are somewhat permeable with gases, the CO2 concentration in air is far to be non-limiting for microalgal growth. Accordingly, there is the need to provide CO2 directly into the solution, through an absorption step in a sodium carbonate enriched medium [2].

In this work, a new device with non-limiting CO2 supply was proposed. Here *Scenedesmus obliquus* was cultivated at constant light intensity by monitoring growth through in vivo chlorophyll fluorescence measurements [3] with the aim to assess the efficiency of CO2 supply.

**2. Methods**

The device is made in polydimethylsiloxane (PDMS) which is a polymer with high compatibility with biological application and is transparent to light. The device has been designed to allow up to three different channel, each channel has 8 independent wells (non-mixed photobioreactors). A CFD simulation has been performed in order to ensure homogeneous fluid pattern at the entrance of each well. Since here velocity is almost zero, nutrients enter continuously via molecular diffusion only, avoiding limiting conditions. This device allow up to 24 parallel experiments. A syringe pump is needed to guarantee low and constant flowrates of fresh medium.

To efficiently absorb CO2 in the liquid phase, the equilibrium of carbonate was exploited [2]. A sensitivity analysis (performed on Aspen Plus using ELECNRTL activity coefficient model), was carried out to select the carbonate concentration needed in order to obtain a suitable pH value in a liquid which is bubbled whit 100% CO2. This means that by slightly increasing pH in a buffered solution, is possible to shift equilibrium to increase carbon capture. Enriched medium are loaded into 50 mL syringe that allow to run 5-days long experiments.

*S. obliquus* was pre-cultured in agitated flask at illumination of 40 µmol photons m-2s-1 at 24±1 °C in BG11 medium, cells were then collected in the middle of exponential phase. A correlation line between biomass concentration and F0 has been set. Measurements were performed with Open FluorCam FC800 (Photon System Instruments, Czech Republic). Microalgae growth has been carried out at ambient temperature with a constant incident light of 40 µmol photons m-2s-1. Growth rate was obtained as the slope of logarithmic variation of the concentration over time.

**3. Results and discussion**

Using a 2.5 g L-1 carbonate-enriched BG11, microalgae growth has been evaluated in two cases, with and without CO2 in Figure 1 (left). The corresponding growth rates, calculated from eight wells replicates each, lead to a µ=0.14±0.12 day-1 without CO2 and µ=0.53±0.03 day-1 in the opposite case. These results are statistically different by 1-way ANOVA and a Tukey-Kramer significant difference criterion since p-value<0.05. In the latter case, µ is comparable to traditional cultures where no CO2 limitation is observed [4].



**Figure 1**. *S. obliquus* growth in mPBR, the lines represent the trend of the exponential phase (left). *S. obliquus* growth rate comparisons (right).

**4. Conclusions**

A new method to supply CO2 efficiently in mPBR for photosynthetic organisms was proposed. Microalga *S. obliquus* cultivated in this device showed an increased growth rate of more than three times with respect to air diffusion condition. As the carbon is not limiting, this mPBR can be used in future to assess the effect of other variables such as light intensity.

**References**

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