Wave-mixed and orbitally Shaken Single-Use Photobioreactors for Diatom Algae Propagation

Nicolai Lehmann[a], Heiko Rischer[b], Dieter Eibl[a], and Regine Eibl[a]

[a] Zurich University of Applied Sciences, School of Life Sciences and Facility Management, Institute of Biotechnology

[b] VTT TECHNICAL Research Centre of Finland, Tietotie 2, 02044 VTT, Finland

Zurich University of Applied Sciences

zhaw Life Sciences and Facility Management

VTT

Introduction

Diatoms are one of the largest and most important group of microalgae and are regarded as a potential source of high-value products such as polyunsaturated fatty acids, natural colorants, biopolymers and therapeutic agents [1]. Controlled *in vitro* production processes using diatoms are performed in illuminated such as vertical-column, flat-plate, tubular, bubble column and airlift photobioreactors [2]. However, diatoms can be easily damaged by shear stress which may be induced by mixing and aeration. *Phaeodactylum tricornutum* is particularly sensitive to aeration-induced hydrodynamic stress [3], arising from the bursting of small bubbles introduced by direct aeration (spargers, etc.) in the listed photobioreactors.

To date, the suitability of surface-aerated single-use bioreactors operating with ready-to-use, pre-gamma radiated, light-transmissive flexible plastic bags instead of sterilized glass vessels has not been described for *P. tricornutum*. Such single-use cultivation systems include wave-mixed and orbitally shaken bioreactors whereby the medium containing the cells is efficiently mixed by rocking or shaking the bag, which continuously renews the medium surface, providing bubble-free surface aeration. Wave-mixed and orbitally shaken systems are characterized by homogeneous energy dissipation, resulting in reduced cell damage on shear sensitive cells [4]. In the subsequently described growth experiments, two wave-mixed single-use bag photobioreactor prototypes and one orbitally shaken single-use bag photobioreactor were used. They are equipped with illumination systems providing differing light qualities: cool-white fluorescent tubes (particularly strong at the blue and red ends of the light spectrum), white LEDs, and white and red light LEDs.

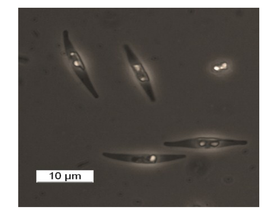


Figure 1: Electron micrograph of *P. tricornutum* cells grown in the BIOSTAT CultiBag RM (1000 fold magnification).

Experimental

Algae Culture Maintenance and Inoculum

An axenic culture of the diatom *P. tricornutum* CCMP2928 (isolated in the North Pacific Yellow Sea, near Dalian, China) was obtained from the National Center for Marine Algae and Microbiota. Maintenance culture and inoculum production for the subsequent bioreactor experiments was carried out in 1-L polycarbonate shake flasks (Corning) with vented caps. Subcultivation of the cultures was performed weekly. The inoculum was cultivated in a Multitron Cell (Infors HT) at 110 rpm with 25 mm shaking diameter.

General cultivation conditions for the inoculum and the propagation experiment:

Irradiance:	95 µmol m $^{-2}$ s^{-1}
Light exposure:	16 h light 8 h darkness
Medium:	Guillard f/2
Initial pH:-value	8.0 not controlled
Salinity:	30%
Temperature:	23°C
Intial cell density:	$5 \cdot 10^5$ cells mL^{-1}
Aeration:	0.5 vvm air with additionally 5% CO_2

Propagation process parameters

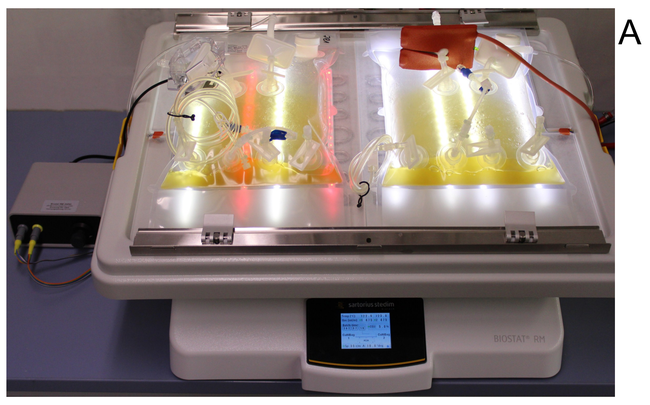
Batch cultivations (as duplicates) of *P. tricornutum* were performed in three single-use photobioreactor types. They differed in their *power input generators*, *bag sizes*, *designs*, *instrumentation* and *illumination* (in particular the light color, light source and its position Fig. 2). For both wave-mixed single-use bioreactors, the BIOSTAT CultiBag RM 20 and AppliFlex (Fig. 2a, b, both with LED illumination), the setup of the process parameters was performed on the basis of our experience with plant and animal cells in these bioreactors at 35 rpm, 10° rocking angle for the BIOSTAT CultiBag RM 20 and 20 rpm, 10° rocking angle for the AppliFlex system, respectively.

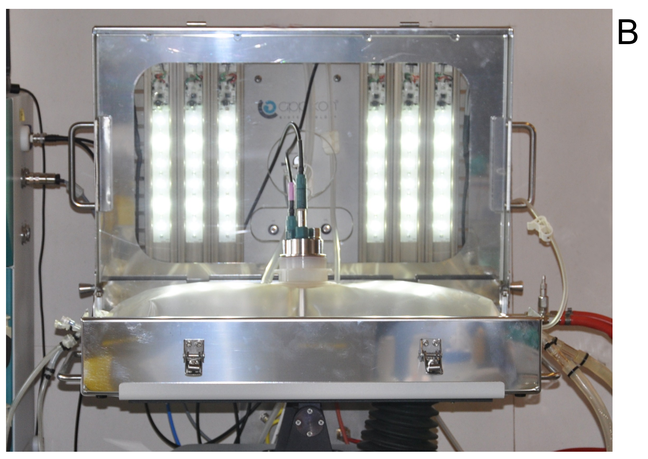
The process parameters for the orbitally shaken bag in the Multitron Cell (Fig. 2c) were selected to ensure a similar fluid flow and a k_La value exceeding 8 h^{-1} like the waved induced bioreactors.

Dry biomass concentrations were followed calculated:

$C_b = 3 \cdot 10^{-8} \cdot n$ [g cells L^{-1}]

pH in the orbitally shaken system and In the wave-mixed system was analyzed offline (Mettler Toledo Five Easy), whereas the pH in the AppliFlex was analyzed online.





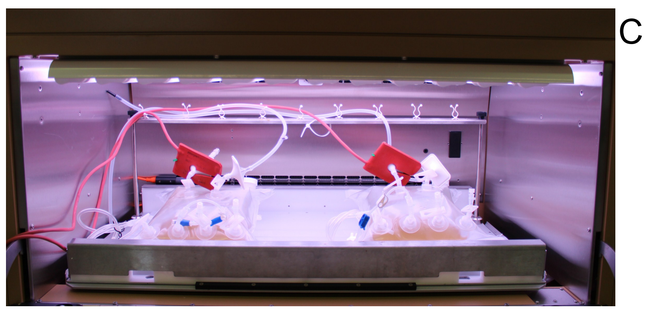


Figure 2. Single-use photobioreactors used in *P. tricornutum* propagation: a) BIOSTAT CultiBag RM 20 prototype with bottom-mounted LEDs, b) AppliFlex prototype with top-mounted LEDs, c) Multitron Cell with shaker bag platform, operating with fluorescent tube-illuminated CultiBag RM 2L.

Results and Discussion

Maximum specific growth rates (0.67 d^{-1}≤ μ_{max} ≥0.98 d^{-1}) and cell division rates (0.71 – 1.04 d^{-1}) were calculated. These results are in the same range as those observed in shake flasks (300 mL culture volume) made from plastic (Corning and ThermoFisher Scientific) and glass (data not shown). Moreover, the results are even up to 17 % higher than or correspond quite well to the maximum specific growth rates and cell division rates reported by other research groups [1, 3, 5].

The highest dry biomass concentration (0.65 g L^{-1}), cell density ($2.11 \cdot 10^7$ cells mL^{-1}) and maximum specific growth rate (μ_{max} = 0.98 d^{-1}) were found in the Multitron Cell, operating with cool-white fluorescent tube illuminated CultiBags RM 2 L. Here the peak dry biomass was between 38 and 40 % higher (Fig. 3c), and the maximum specific growth rate was 20 % higher than in the BIOSTAT CultiBag RM and the AppliFlex. This observation may be explained by the main difference between the cultivations, which was the higher red and blue light spectrum ranges provided by the Multitron Cell. Unexpectedly, a extended lag phase (3 days) and the lowest biomass growth occurred in the BIOSTAT CultiBag RM with red and white light LEDs. The peak cell density ($1.09 \cdot 10^7$ cells mL^{-1}) as well as dry biomass concentration (0.326 g L^{-1}) were measured one day later than in the other experiments (Fig. 3a). The maximum specific growth rate was 14 % lower (μ_{max} = 0.67 d^{-1}) and the maximum dry biomass concentration was 19 % lower than in the BIOSTAT CultiBag RM, which was only illuminated with white light LEDs. If similar fluid flow, oxygen supply, average illumination intensity and light quality was guaranteed, as in the case of the BIOSTAT CultiBag RM (Fig. 3a) and the AppliFlex (Fig. 3b), identical propagation curves resulted.

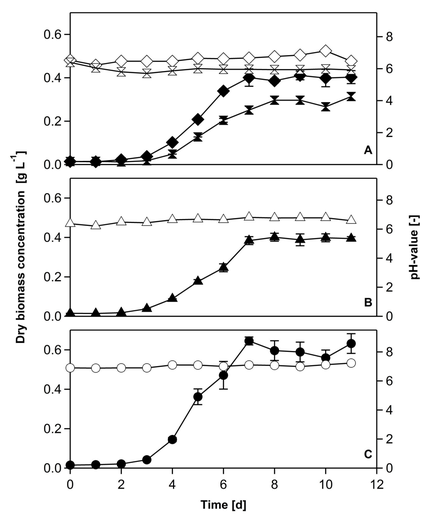


Figure 3: Dry biomass concentration (black symbols) and pH value (white symbols) as duplicates vs. time in batch culture: a) wave-mixed BIOSTAT CultiBag RM with white and red LEDs (sand clock) and single white LEDs (diamond), b) wave-mixed AppliFlex, and c) orbitally shaken CultiBag RM operated in the Multitron Cell.

Conclusion

The study clearly indicates that *P. tricornutum* cells can be successfully grown in wave-mixed and orbitally shaken single-use photobioreactors operating with cultivation bags at benchtop scale, suitable for diatom-based manufacture of high-value products. Moreover, *P. tricornutum* can attain maximum cell densities, dry biomass concentrations and specific growth rates that are comparable to values typically achieved in reusable stirred, helical tubular and airlift photobioreactors. However, a current weakness of the tested single-use systems – excluding the AppliFlex prototype in which reusable sensors to control and measure pH and DO were installed – is the lack of single-use sensors that are suitable for phototrophic applications. Since higher red and blue light spectrum ranges not only contribute to improved biomass growth, but also have the potential to support protein and metabolite expression of diatoms, additional illumination of both of the wave-mixed photobioreactor prototypes with blue light LEDs would appear to be advantageous.

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Contact:
ZHAW, Institute of Biotechnology
Nicolai Lehmann
Grüental, CH-8820 Wädenswil
Tel: +41 (0) 58 934 57 55
Mail: nicolai.lehmann@zhaw.ch
Web: www.ibt.zhaw.ch/bioverfahrenstechnik
www.ibt.zhaw.ch/zellkulturtechnik
www.lsfm.zhaw.ch

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