New Photobioreactor Design for Enhancing the Photosynthetic Productivity of *Chlorella homosphaera* Culture

SANDA VELEA[1], LUCIA ILIE[1]*, EMIL STEPAN[1], RUXANDRA CHIURTU[2]

[1] National Research & Development Institute for Chemistry & Petrochemistry – ICECHIM, 202 Splaiul Independentei, 060021 Bucharest, Romania

[2] National Research & Development Institute of Occupational Safety – INCDPM "Alexandru Darabont", 35A Ghencea, Blv., 061692, Bucharest, Romania

A new hybrid photobioreactor, which combines the advantages of an open system with those of a flat-plate photobioreactor was developed to improve high surface-to-volume ratio of the photobioreactor and the photosynthetic efficiency by enriched CO_2-sequestration via bubbling of CO_2 into the culture medium to achieve high biomass productivities. To evaluate the performance of this photobioreactor, we performed a case study assessing its biomass productivity and the efficiency parameters associated with the conversion of carbon dioxide in the algal photosynthesis process for cultures of Chlorella homosphaera. The biomass concentration of C. homosphaera reached 3.6 g/L on the 11th day of culturing , using a new hybrid photobioreactor, which is 3 fold increased compared to a standard open tank system under similar photoautptrophic conditions.

Keywords:photobioreactor, microalgae, biomass, Chlorella homosphaera, carbon dioxide, bioprocess design

Algae have attracted much interest due to their potential roles in the production of biofuels and other bioactive compounds, and also for mitigation of greenhouse gases, particularly carbon dioxide, associated with global warming. The process of mitigating CO_2 from flue gas emissions by biofixation in a microalgal cultivation system has been extensively studied recently, both in open ponds and closed photobioreactors. Cultivation of algae in open ponds has been extensively studied [1-3], and these may be categorized as either natural waters (*e.g.* lakes, lagoons, ponds) or artificial ponds or containers. Open ponds are easier to construct and operate; however, closed systems allow for better control of the cultivation conditions. Closed photobioreactors can be classified as vertical tubular photobioreactors with either a bubble column or airlift, flat panel photobioreactors, horizontal photobioreactors, stirred tank photobioreactors, and hybrid type photobioreactors [4, 5]. The growth of microorganisms in photobioreactors is a very complex process that couples the effects of photosynthesis, reactor flow dynamics, and irradiance distribution.

In this study, we investigated the performance of a new hybrid photobioreactor [6] that combines the advantages of an open system with those of a flat plate photobioreactor to enhance surface-to-volume ratio of photobioreactor and the photosynthetic efficiency, by enriched CO_2-sequestration via bubbling of CO_2 into the culture medium, by assessing biomass productivity and efficiency parameters of the conversion of carbon dioxide in cultures of *Chlorella homosphaera*.

Experimental part

Materials and methods

Strain and culture conditions

Chlorella homosphaera used in this study are original strains deposited in the Culture Collection of Algae and Protozoa at the Scottish Marine Institute, Scotland, as CCAP 211/121 [7]. The culture medium for *C. homosphaera* was Zarrouk medium, consisting of (g/L) 16.8 $NaHCO_3$, 0.5 K_2HPO_4, 2.5 $NaNO_3$, 1.0 K_2SO_4, 1.0 NaCl, 0.2 $MgSO_4 \cdot 7H_2O$, 0.04 $CaCl_2 \cdot 2H_2O$, 1 mL/L of trace metal solution, and 5 mL of chelated iron.

The culture conditions were maintained at 25 ± 2^0C under continuous illumination from top and both sides totaling 240 $\mu E/m^2s$ white fluorescent light, for 11 days. The cultivations were aerated continuously with synthetic gas mixture rate of 6 L/h consisted of 7% CO_2, 14% O_2, and 79% N_2 (v/v). The cultures were harvested by centrifugation and algal biomass was estimated in terms of dry weight (g/L).

Hybrid photobioreactor operation

In the schematic flow (fig.1), the hybrid photobioreactor system appears as an open tank (OT) connected to a variable number of transparent flat plate photosyntetic cells aligned in a parallel (FP) configuration and 2 vertical bubble

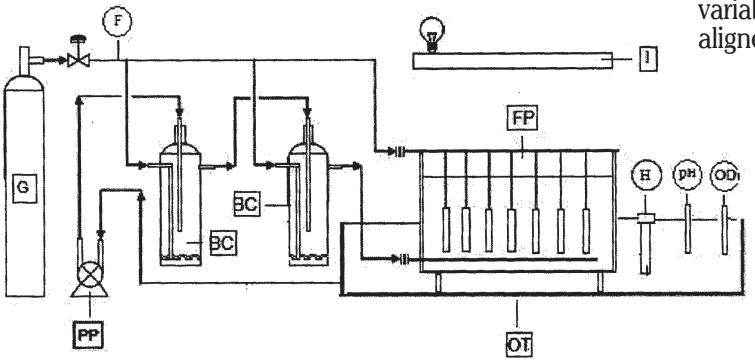


Fig.1 Flow schematic of the hybrid photobioreactor system consisted of open tank (OT), flat plate cells (FP), bubble columns (BC), pump (PP), lighting system (I), synthetic gas with CO_2 (G), gas flow control (F), pH indicator (*p*H), turbidity sensor (OD), electric heater (H)

* *email: luciailie@yahoo.com; Tel.: +40 21 3153299*

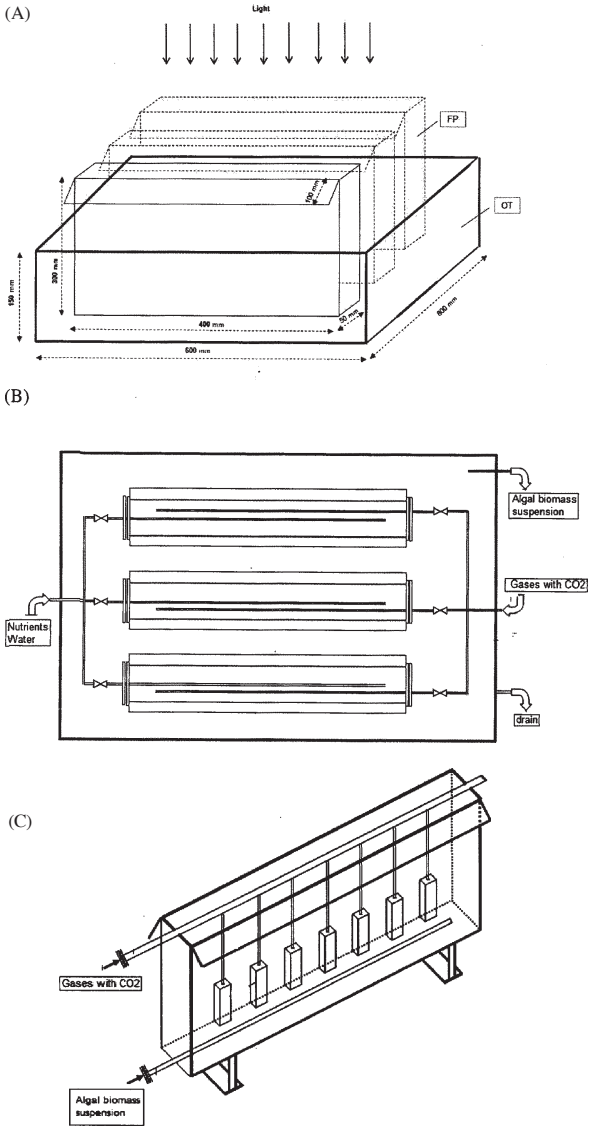


Fig. 2 (A) Front view of hybrid photobioreactor consisted of an open tank (OT) and transparent rectangular flat plate cells (FP); (B) Aerial view of photosynthetic cells inside the tank; (C) Detailed photosynthetic flat plate cell

columns in series (BC), interconnected with pipes for algal suspension recirculation with pump (P) and pipelines for gas bubbling. All system components are made from transparent materials and the lighting system (I) is located above the photobioreactor tank.

The cylinder for synthetic air mixed with CO_2 (G) is fitted with a gas flow control (F) adjustable as a function of pH for the selected culture medium. The open tank also includes a *p*H indicator (*p*H), turbidity sensor (OD), and electric heater (T), to adjust and maintain the microalgae suspension temperature for the selected algal growth.

A schematic front and an aerial view of the flat plate photosynthetic cells inside the open tank are presented in figure 2A and 2B. Each flat plate photosynthetic cell (fig. 2C) has 2 overflows at the top of the assembly arranged at an angle from the vertical, which are designed to direct excess algal suspension and convert it into a fluid sheet designed to increase the illuminated surface and enhance oxygen release resulting from photosynthesis.

The working volumes of the photobioreactor and the standard control tank are given in table 1.

Kinetic parameters calculation

Interpretation of growth rate was based on Guillard's theory [8] on the growth of individual units in phytoplankton populations, later adapted by Wood [9] for algae growth in both continuous and discontinuous photobioreactor. The exponential growth rate is described by the equation (1):

$$R = (\ln X_t - \ln X_o) / \Delta t \quad (1)$$

where R is the exponential growth rate (day^{-1}), X_t is the population size at the time t (day), X_0 is the population size at the beginning of a time interval and Δt is the length of the time interval $(t_t - t_0)$.

Additionally, doubling time T (day) could be calculated from equation (2):

$$T = 0.6931/R \quad (2)$$

In this paper the exponential growth rates were calculated by plotting the semilog graphs according to equation (1) by using different relative units, as substitute for population size (including optical density , absorbance, biomass, nitrogen fixation, phosphorus fixation, and carbon fixation), as function of Δt.

The nitrogen fixation was calculated using the analytical data of g/L NO_3^- in culture medium from equation (3):

$$FN_t = (B_t - B_0) * (N_0 - N_t) * V * (M_N / M_{NO3}) \quad (3)$$

where FN_t is the nitrogen fixation (g) at time t; B_t = g/L biomass at time t; B_0 = g/L biomass at time 0; N_0 = g/L NO_3^- at time *0*; N_t = g/L NO_3^- at time t, V = volume of culture medium (L); M_{NO3} = molecular weight of NO_3; M_N = molecular weight of nitrogen

The phosphorus fixation was calculated using the analytical data of g/L PO_4^{3} in culture medium, from equation (4)

$$FP_t = (B_t - B_0) * (P_0 - P_t) * V * (M_P / M_{PO4}) \quad (4)$$

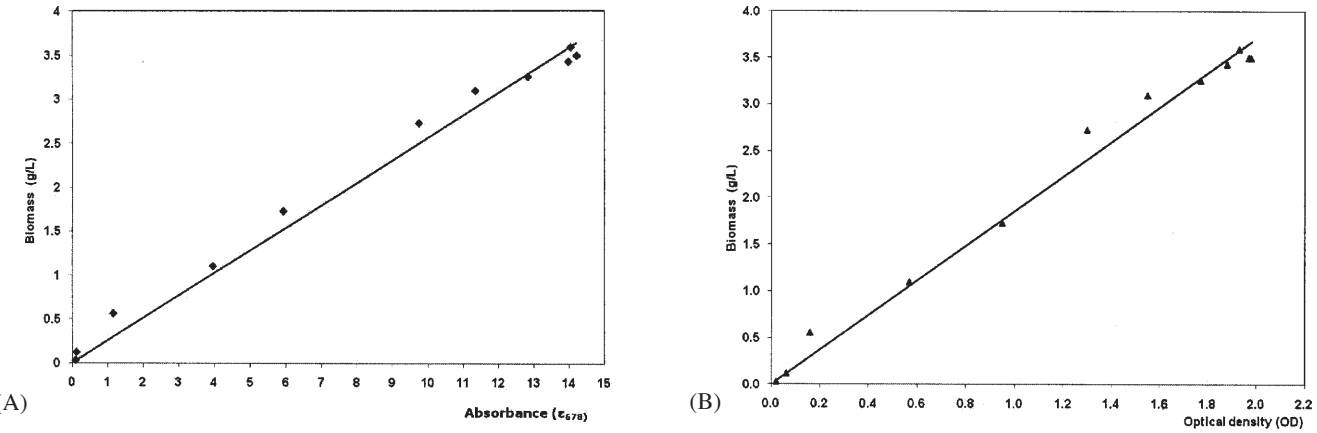
Types	Working volume (L)	Culture depth (mm)	Surface/Volume ratio (m^{-1})	Mixing time (s)
CT[a]	48	100	10	150
HPB-1[b]	66	100	19.5	360
HPB-2[c]	42	50	30.7	240

Table 1
TECHNICAL CHARACTERISTICS OF NEW HYBRID PHOTOBIOREACTOR AND STANDARD CONTROL TANK

[a] CT – Control tank is an open tank, working volume of 48 L and culture depth of 100 mm

[b] HPB 1 – Hybrid photobioreactor, , working volume of 66 L and culture depth of 100 mm

[c] HPB 2 – Hybrid photobioreactor, , working volume of 42 L and culture depth of 50 mm



(A) (B)

Fig. 3 Linear calibration curve of biomass concentration as function of (A) optical density (OD) and (B) absorbance (ε)

where FP_t is the phosphorus fixation (g) at time *t*; B_t = g/L biomass at time *t*; B_0 = g/L biomass at time *0*; P_0 = g/L PO_4^{3-} at time *0*; P_t = g/L PO_4^{3-} at time *t*, *V* = volume of culture medium (L); M_{PO4} = molecular weight of PO_4^{3-}; M_p = molecular weight of phosphorus.

The carbon dioxide fixation was calculated using the CHNS data, by developing the equation (5) of Morais and Costa [10].

$$FC_t = (B_t - B_0) * (C_t/100) * V * (M_{CO2}/M_C) \quad (5)$$

where FC_t is the carbon dioxide fixation (g) at time *t*; B_t = g/L biomass at time *t*; B_0 = g/L biomass at time *0*; C_t = % carbon at time *t*; *V* = volume of culture medium (L); M_{CO2} = molecular weight of CO_2; M_c = molecular weight of carbon.

The percentage of carbon dioxide fixation was calculated as g CO_2 fixed per g CO_2 injected per day, from equation (6):

$$FD_{CO2} = (FC_{t+1} - FC_t) / g_{CO2} * 100 \quad (6)$$

where FD_{CO2} is percentage of carbon dioxide fixation (%), FC_{t+1} is the accumulation of CO_2 fixed (g) at time t+*1*, FC_t is the accumulation of CO_2 fixed (g) at time *t* and g_{CO2} is grams of CO_2 injected each day.

The specific consumption of carbon dioxide per unit of algal biomass, produced daily throughout the growth process, could be calculated from equation (7):

$$CS = FC_t/B_t \quad (7)$$

where CS is specific consumption (g/g day^{-1}), FC_t is the accumulation of CO_2 fixed (g) at time *t* and B_T is g/L biomas at time *t*.

Analytical methods

The concentration of of *C. homosphaera* biomass in culture medium was measured, based on the calibration curves plotted as linear functions of optical density (OD), measured by using a turbidity measurement system model FUNDALUX II–Sartorius,(Germany) and absorbance (ε) at 678 nm measured using a model M400 Karl Zeiss spectrophotometer (SPECORD M85 UV-VIS, Zeiss Yena, Germany) with a Jena microprocessor, as seen in figure 3.

The relationships between dry weight biomass (*B*) in g/L and optical density (OD) or absorbance (ε_{678}) are given by the equations (8) and (9):

$$B = 1.8568 * OD \quad (8)$$
$$B = 0.2561 * \varepsilon_{678} \quad (9)$$

Both temperature and *p*H of the culture medium were measured hourly during the daytime for a period of 11 days. The *p*H was measured using a Consort C931 model pH meter (Belgium). The irradiance and photosynthetically active radiation (PAR) of the artificial light source to the hybrid photobioreactor were measured by using a portable lux meter, model HD 2102.2 and a LP 471 Quantum radiometric probe (Italy). The concentration of constituents of the culture medium, specifically NO_3^-, PO_4^{3-}, SO_4^{2-}, Cl^- (g/L) were determined by ion chromatography using an ICS-3000 Dionex instrument (USA), and the ratio of $NaHCO_3$/Na_2CO_3 (g/L) was determined by chemical analysis. Carbon, hydrogen, nitrogen, and sulfur (CHNS) content, in algal dried biomass, were determined by elemental analysis using a Perkin-Elmer apparatus 2400 Series II CHNS/O Analyzer (USA).

Results and discussions

Cultivations of C. homosphaera in HPB-1 and HPB-2 (100 mm and 50 mm culture broth depths, respectively) were carried out to examine the performance of this hybrid photobioreactor model, by assessing biomass concentration and productivity, exponential growth rates expressed as optical density, absorbance, carbon dioxide, nitrogen, phosphorus, biofixation and efficiency of the conversion of carbon dioxide in the algal photosynthesis process.

Time courses of cell growth and biomass concentration of HPB-1 and HPB-2 compared to standard open tank are shown in figure 4.

C. homosphaera produced a maximum biomass yield of 3.6 g/L in HPB-2, which is higher than 2.5 g/L in HPB-1 and 1.2 g/L in standard control tank, on the 11th day. The

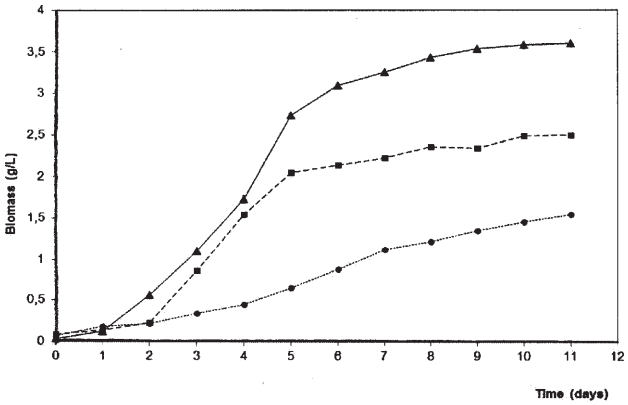


Fig. 4 Biomass concentration (g/L) in batch culture versus time (days) in HPB-1 (- ■ -), HPB-2 (-▲-) against control tank (-- ● --)

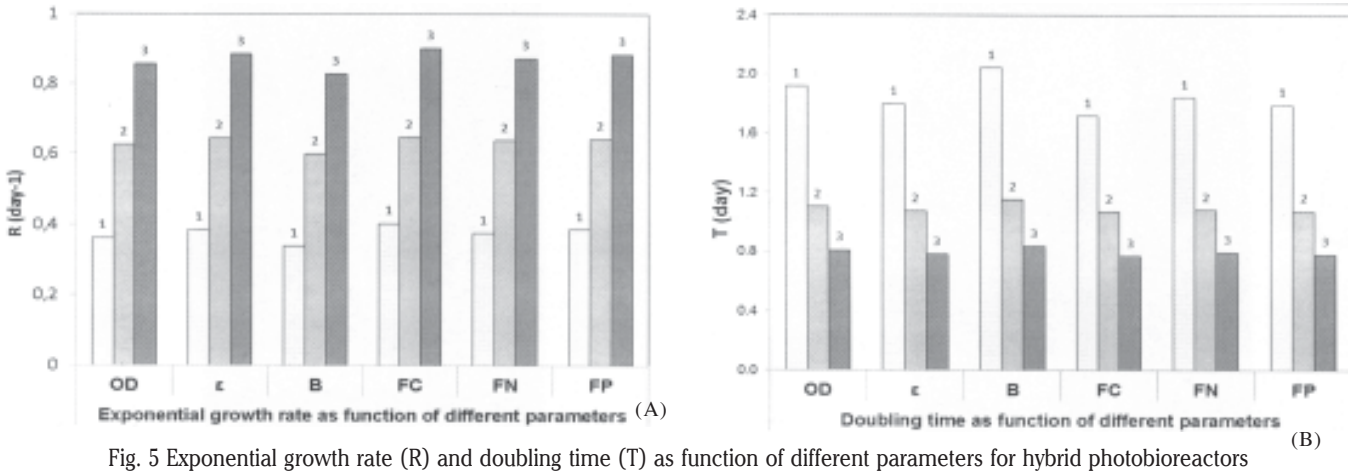


Fig. 5 Exponential growth rate (R) and doubling time (T) as function of different parameters for hybrid photobioreactors HPB-1 and HPB-2 compared with control tank (CT) 1 - CT , 2 - HBP-1, 3 - HBP-2, OD - Optical density, ε - Absorbance, B - Biomass, FC - Carbon dioxide fixation, FN - Nitrogen fixation, FP - Phosphorous fixation

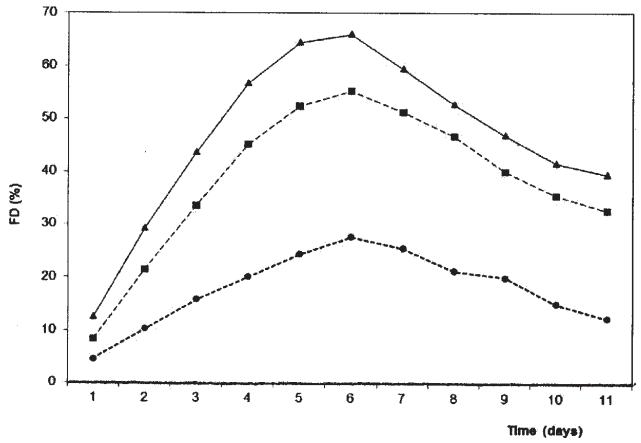


Fig. 6 Daily carbon dioxide fixation rate (FD) for hybrid photobioreactors HPB-1 (- ■ -); HPB-2 (-▲-) and control tank CT (-- ● --)

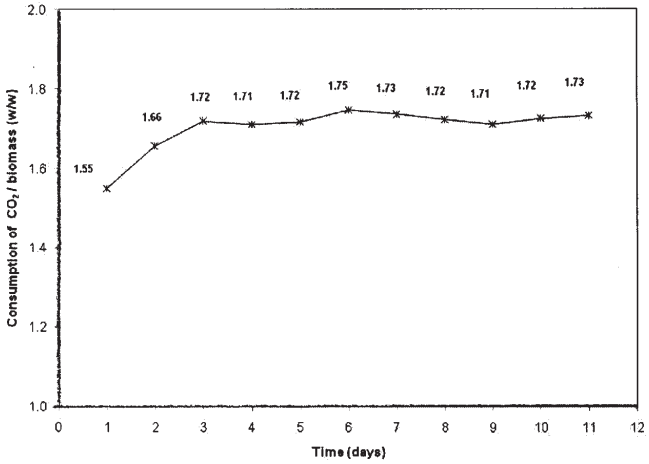


Fig. 7 Specific consumption of CO_2 per algal biomass (w/w) as function of time (days)

biomass productivity increased with the decreasing of culture broth depth to due to better illumination surface to volume ratio and a short mixing time, considered a critical factor for the performance of cultivation in photobioreactor. The biomass concentration in the HPB-2 was 3 fold increased compared to a standard open tank system, hence the HPB-2 provides a simple and economical means of improving microalgae cultivation.

The exponential growth rates for *C. homosphaera* cultivated in HPB-1 and HPB-2 compared to standard open tank were calculated using 2 data points for paired values, in the lag phase, according to Wood [9], and are presentated in figure 5A.

The runs in HPB-2 showed higher values for exponential growth rates ($R=0.87\ day^{-1}$) than the runs in HPB-1 ($R=0.63\ day^{-1}$) and standard open tank ($R=0.37\ day^{-1}$) for the same cultures grown in the same media and under the same culture conditions. The results regarding the developed exponential growth rates were comparable using different parameters. The corresponding doubling time are illustrated in figure. 5B and the values reported in HPB-2 are approximately 0.79 day, in comparison with those of 1.85 day in to standard open tank.

The hybrid photobioreactor HPB-2 caused a rapid growth increase in microalgae *C. homosphaera* due to optimizing the light transmission, 30 fold higher surface to volume ratio compared to standard open tank and a better gas exchange in the water–air interface, both in two vertical bubble columns and flat plate cells.

These results indicate that more biomass with a higher exponential growth rate can be produce in HPB-2 with a lower culture depth than in HPB-1 due to a better mixing time which increases average irradiance.

System configuration efficiency was quantified by calculating an indicator expressing maximum percentage daily carbon dioxide fixation (FD) which is shown in figure 6.

The highest FD values produced in HPB-2 are 65 % fold increased compared to 27 % in standard open tank.

The algal dry biomass composition contains up to 48% carbon, 12% nitrogen, 1.5% sulfur and 1% phosphorus . For *C. homosphaera* cultivated in HPB-2, we have evaluated that one kilogram of dry algal biomass utilizes up to 1.7 kg carbon dioxide, as it is presented in figure 7.

The results are consistent with reported data about *Chlorella sp* cultivated in a photobioreactor with transparent rectangular chamber [11], *Nannochloropsis sp* cultivated in a flat plate photobioreactor [12], *C. vulgaris* cultivated in a reactor with inserted helical static mixer and plates [13], and *Chlorella zofingiensis* cultivated in flat plate photobioreactor [14].

All the results of the present study regarding the performances of carbon dioxide sequestration in a new hybrid photobioreactor for *C. homosphaera* culture are summarized in the table 2.

Types	Biomass concentration (g/L)	Volumetric productivity (g/L day)	Total biomass (g)	Exponential growth rate (day^{-1})	Doubling time (day)	Maximum carbon dioxide fixation (%)
CT[a]	1,2 ± 0.2	0.150	57.6	0.37	1.85	27
HPB-1[b]	2.5 ± 0.2	0.312	165.0	0.63	1.09	55
HPB-2[c]	3.6 ± 0.2	0.450	151.2	0.87	0.79	65

Table 2
PERFORMANCES OF NEW HYBRID PHOTOBIOREACTOR COMPARED TO STANDARD CONTROL TANK AFTER 11 DAYS OF CULTIVATION

[a]CT – Control tank is an open tank, working volume of 48 L and culture depth of 100 mm

[b] HPB 1 – Hybrid photobioreactor, , working volume of 66 L and culture depth of 100 mm

[c] HPB 2 – Hybrid photobioreactor, , working volume of 42 L and culture depth of 50 mm

Conclusions

The new hybrid photobioreactor investigated in this study is a very promising microalgae production system, in which the combination of an open system with a flat plate photobioreactor enhances irradiance distribution, flow dynamics, and gaseous transfer, and thereby produces increased biomass, dense algal cultures and a very good sequestration of carbon dioxide. The kinetics data and CO_2 fixation rates for *C. homosphaera* demonstrate that the new hybrid photobioreactor could be used to mitigate the effects of CO_2, by reducing emissions of flue gases.

Acknowledgements: This research work was supported by the LIFE+ Environment Policy and Governance Program of European Union Commission, Grant Number LIFE 10/ENV/RO/000734, and by UEFISCDI, Romania,, in the framework of National Partnership Program, financing contract no. 44/2012

References

1. BOUSSIBA, S., SANDBANK, E., SHELEF, G., COHEN, Z., VONSHAK, A., BEN-AMOTZ, A., ARAD, S., RICHMOND, A., Aquaculture, 72, 1988, p. 247–253.
2. TREDICI, M.R., MATERASSI, R., J. Appl. Phycol., 4, 1992, p. 221–231.
3. HASE, R.. OIKAWA, H., SASAO, C., MORITA, M., WATANABE, Y., J. Biosci. Bioeng., 89, 2000, p. 157–163.
4. SINGH, R.N., SHARMA, S., Renewable and Sustainable Energy Rev., 16, 2012, p. 2347–2353.
5.CARVALHO, A.P., MEIRELES, L.A., MALCATA, F.X., Biotechnol. Prog., 22, 2006, p. 1490–1506.
6. VELEA, S., STEPAN, E., RO Patent 123,480, 2012.
7. DRAGOS, N., ROVINARU, C., BICA, A., OANCEA, F., Patent application A01374, 2011.
8.GUILLARD, R.R.L., Stein, J.R. (ed), Handbook of Phycological methods: culture methods and growth measurements, Cambridge University Press, Cambridge,1973, p. 289–312.
9. WOOD, A.M., EVERROAD, R.C., WINGARD, L.M., In: Andersen, R.A. (ed,), Algal culturing techniques, Elsevier Acad. Press, Amsterdam, 2005.
10. MORAIS, M.G., COSTA, J.A.V., Biotechnol. Lett., 29, 2007, p. 1349–1352.
11. HSIEH, C.H., WU, Z.C., Biochem. Eng. J., 46, 2009, p. 300-305.
12. RICHMOND, A., WU, Z.C., J. Biotechnol., 85, 2001, p. 259-269
13. NOCHTA, P., LUCUMI, A., POSTEN, C., Eng. Life Sci., 7, 2007, p. 127–135.
14. FENG, P., DENG, Z., HU, Z., FAN, L., Bioresour. Technol., 102, 2011, p. 10577-10584

Manuscript received: 19.05.2012