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Optimization of an Experimental Model for Microalgae Cultivation with CO_2 Fixation †

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Abstract: Microalgae cultivation is a promising approach for sustainable CO_2 fixation. This work describes the optimization of a laboratory-scale experimental model for microalgae cultivation under CO_2 supplementation. The experimental model was developed using a stirred clear glass reactor, white LED strips, connection system caps with three ports, tubes, valves, regulators, and N_2-CO_2 compressed gas cylinder. Three microalgae strains were used: *Raphidocelis subcapitata* ATCC22662, *Desmodesmus communis* NIVA-CHL 7, and *Chlorella sorokiniana* NIVA-CHL 176. The appropriate medium for cultivation of each of these strains was selected. The optimized experimental model demonstrated the positive influence of CO_2 supplementation on microalgae growth, particularly for *Chlorella sorokiniana*.

Keywords: stirred glass reactors; connection system caps; white LED strips; microalgae biomass accumulation

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1. Introduction

Among the various technologies and strategies explored for CO_2 capture [1–6], microalgae-based systems offer a sustainable approach [7–11]. Microalgae consume CO_2 during photosynthesis, producing biomass that accumulates bioactive compounds [12–14]. Microalgae's fast growth rates, high photosynthetic efficiency, and potential to thrive in diverse environments make them an attractive candidate for large-scale CO_2 capture and utilization [15,16].

Microalgae photobioreactors, despite their disadvantages related to capital cost and energy consumption for mixing, have a low contamination risk and a high CO_2 feed efficiency [17] and are suitable for the production of microalgae biomass, accumulating high amounts of value-added compounds [18], especially in mixotrophic conditions [19].

This work describes the optimization of a closed photobioreactor type and laboratory-scale experimental model for microalgae cultivation under CO_2 supplementation. The experimental model was developed using a stirred clear glass reactor, white LED strips, connection system caps with three ports, tubes, valves, regulators, and CO_2 compressed gas cylinder. The work explores the various aspects of microalgae utilization for CO_2 fixation, including the selection and screening of suitable strains, cultivation media, and

conditions for enhanced CO_2 uptake. At the end the work, the challenges and perspectives of scaling up the experimental model for microalgae-based CO_2 capture biotechnologies are discussed.

2. Materials and Methods

2.1. Microalgae Strains and Cultivation Media

Three distinct microalgae species were selected: *Raphidocelis subcapitata* ATCC22662, (formerly known as *Selenastrum capricornutum*), *Desmodesmus communis* NIVA-CHL 7, and *Chlorella sorokiniana* NIVA-CHL 176. These unicellular organisms exhibit diverse growth patterns, existing either as individual cells, chains, or clusters.

For the cultivation of the selected microalgae strains, three distinct freshwater media were chosen: BG-11, bold basal medium—BBM, and Z8 [20,21]. These cultivation media were selected due to their suitability for supporting the growth and proliferation of freshwater microalgae [22–25]. The macro- and micronutrient composition of each medium is described in Table 1.

Table 1. Cultivation media (1 L).

BG-11 [26]	**BBM [27]**	**Z8 [28]**
$NaNO_3$: 17.6 µM	$NaNO_3$: 2.94 mM	Stock 1
K_2HPO_4: 0.23 µM	$CaCl_2 \cdot 2H_2O$: 0.17 mM	$NaNO_3$: 5.50 M
$MgSO_4 \cdot 7H_2O$: 0.3 µM	$MgSO_4 \cdot 7H_2O$: 0.3 mM	$Ca(NO_3)_2 \cdot 4H_2O$: 0.254 M
$CaCl_2 \cdot 2H_2O$: 0.24 µM	K_2HPO_4: 0.43 mM	$MgSO_4 \cdot 7H_2O$: 0.101 M
Citric Acid: 0.31 µM	KH_2PO_4: 1.29 mM	Stock 2
Ammonium-iron Citrate: 0.021 µM	NaCl: 0.43 mM	K_2HPO_4: 0.178 M
$Na_2EDTA \cdot 2H_2O$: 2.7×10^{-6} M		Na_2CO_3: 0.198 M
Na_2CO_3: 0.19 µM	EDTA solution: 0.5 mL/L	Stock 3
	EDTA 0.171 M	$FeCl_3 \cdot 6H_2O$: 0.103 M
BG-11 microelement solution	KOH 0.552 M	EDTA: 0.133 M
H_3BO_3: 46 M	$FeSO_4 \cdot 7H_2O$ 0.018M solution	Stock 4
$MnCl_2 \cdot 4H_2O$: 9 M	(H_2SO_4 acidulated): 0.05 mL/L	$Na_2WO_4 \cdot 2H_2O$: 0.010 µM
$ZnSO_4 \cdot 7H_2O$: 0.77 mM		$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$: 0.0071 µM
$Na_2MoO_4 \cdot 2H_2O$: 1.6 M	H_3BO_3—0.178 M: 0.05 mL/L	KBr: 0.101 µM
$CuSO_4 \cdot 5H_2O$: 0.3 M		KI: 0.291 µM
$Co(NO_3)2 \cdot 6H_2O$: 0.17 M	BOLD Stock 50 µL/L:	$ZnSO_4 \cdot 7H_2O$: 0.0997 µM
	H_2SO_4 98%: 9.98M	$Cd(NO_3)_2 \cdot 4H_2O$: 0.0503 µM
	$ZnSO_4 \cdot 7H_2O$: 1.50 µM	$Co(NO_3)_2 \cdot 6H_2O$: 0.0501 µM
	$MnCl \cdot 4H_2O$: 0.36 µM	$CuSO_4 \cdot 5H_2O$: 0.0501 µM
	Na_2MoO_4: 0.26 µM	$NiSO_4(NH_4)_2SO_4 \cdot 6H_2O$: 0.0507 µM
	$CuSO_4 \cdot 5H_2O$: 0.31 µM	$Cr(NO_3)_3 \cdot 9H_2O$: 0.0102 µM
	$Co(N0_3)_2 \cdot 6H_2O$: 0.84 µM	V_2O_5: 0.0049 µM
		$KAl(SO_4)_2 \cdot 12H_2O$: 0.0999 µM
		H_3BO_3: 0.5008 µM

A 1% inoculum of each microalgae strain was introduced into the corresponding selected medium. Subsequently, the cultures were carefully monitored over a span of two weeks. The cultivation was carried out in the AlgaeTron A230 (Photon Systems Instruments, Drásov, Czech Republic), an incubator designed to provide optimal growth conditions for microalgae. The AlgaeTron was maintained at a constant temperature of 25 °C, with a photosynthetically active radiation of 200 µE m^{-2} s^{-1}. The photoperiod was set to 13 h of daylight followed by 11 h of darkness to mimic natural light cycles. Additionally, the cultures were continuously stirred at 100 rpm to ensure homogenous nutrient distribution and prevent settling, using a platform shaker (Unimax 1010, Heidolph, Schwabach, Germany).

During the experimental period, the growth of microalgae was closely observed in the three chosen culture media. The monitoring spanned a total of 15 days, during which the progression of microalgae development was assessed using three essential parameters:

- *Optical Density (OD):* The optical density was measured at specific intervals to track the changes in the concentration of microalgae in each culture medium. OD values served as a quantitative indicator of microalgae growth and population density. This

parameter was measured using an Ocean FX® UV-Vis spectrometer from Ocean Optics (Duiven, The Netherlands).

- *Turbidity:* Turbidity measurements were performed to determine the degree of cloudiness or haziness in the culture media caused by the presence of microalgae. Turbidity served as an additional parameter to assess the growth and aggregation of microalgae in the different media. The used equipment was Grant Bio DEN-1B Turbidimeter (Cambridge, UK).
- *Biomass Accumulation:* At the end of the 15-day cultivation period, the biomass of microalgae in each culture medium was determined. Biomass quantification provided valuable insights into the overall productivity and growth performance of the microalgae strains in their respective environments. The biomass was dried at 105 °C for 4 h in a laboratory oven (Memmert UE200, Buechenbach, Germany) and weighed on an analytical balance (MS105DU, Mettler Toledo, Columbus, OH, USA).

2.2. Experimental Model for CO_2 Capture

The experimental setup comprises the following components: a CO_2 source (7% CO_2 + 93% N_2) represented by a gas cylinder (Siad, Bucharest, Romania), a GLS 80 Duran® clear glass reactor with connection caps with three ports and stirring system (DKW Lfe Sciences, Wertheim, Germany), a magnetic agitator (Arex 6, Velp, Usmate Velate, Italy), tubes, valves, regulators, and a white LED strip (MY2250 Myria, Complet Electro Serv, Voluntari, Romania) serving as an additional light source—Figure 1.

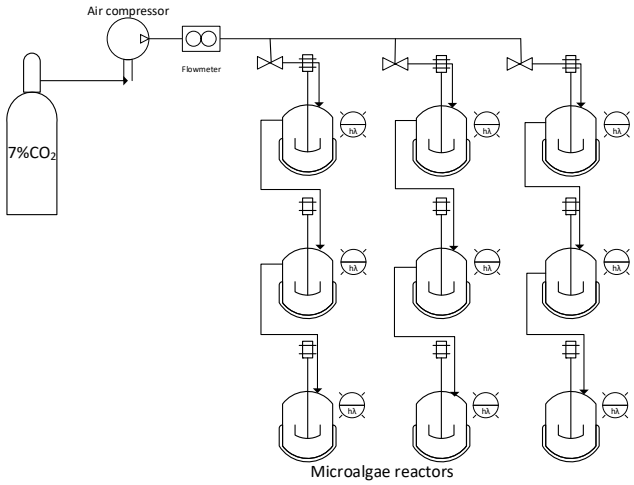

Figure 1. The experimental setup for screening the development of microalgae for CO_2 fixation.

Parallel reactors were employed, with triplicate cultures of selected microalgae and the CO_2 supplementation [29–31]. Reference reactors were included for each microalgae strain, maintaining the microalgae cultures without CO_2 bubbling.

The experimental model consists of a systematic workflow with the following sequential steps:

- *Gas Mixing and Flow Control*: The gases are provided from a gas cylinder through a regulator. The gas flow rate was precisely determined using a flowmeter (Masterflex Variable-Area Flowmeter, Radnor, PA, USA) that ensured consistent and controlled N_2-CO_2 supply.
- *CO_2 Bubbling in the Reactors*: The pre-mixed gas is introduced into the first reactor of each series, where it undergoes bubbling through the culture medium. The stirring system implemented within the photobioreactor allows for prolonged gas–water interaction, promoting efficient CO_2 absorption by the microalgae.
- *Gas Transfer to Subsequent Reactors*: After the initial reactor, the gas exits and proceeds to the second reactor in the series. Here, it again undergoes bubbling through the culture

medium, facilitating further CO_2 absorption. The process is subsequently repeated in the last reactor of each series, ensuring an optimized gas–microalgae interaction.

The development of microalgae was monitored along with the influence of CO_2 by measuring optical density and cell count [32], using a Marienfeld hemocytometer (Lauda-Königshofen, Germany) and an optical microscope, DM1000 LED (Leica Microsystem, Mannheim, Germany).

3. Results and Discussions

3.1. Optimum Cultivation

The microalgae growth was monitored by optical density and turbidity. Analyzing these parameters provides insights into the optimal cultivation medium for each microalgae species. All experiments were conducted in triplicate to ensure statistical rigor and reliable data collection. The optical density and turbidity revealed distinct trends among the microalgae strains under investigation—Figure 2.

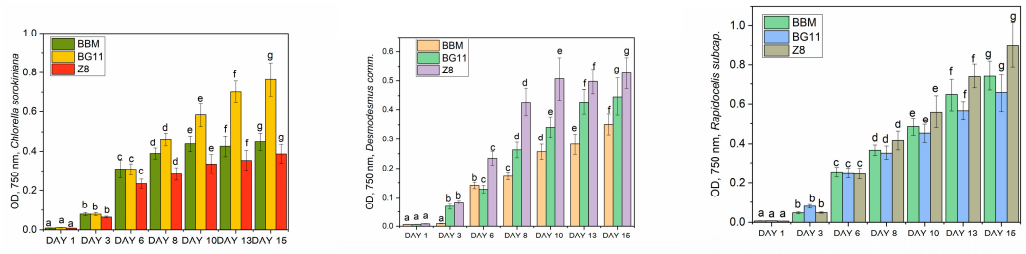

Figure 2. Optical densities for the three microalgae strains in the three cultivation media. The bars height are mean values ± statistical error bars. For the values with the same letter the difference between the means is not statistically significant at $p < 0.05$.

For *Chlorella sorokiniana*, the optical density values (Figure 2) exhibited similar trends to the other two microalgae species. BG-11 and BBM media demonstrated steady growth, with slightly higher OD values observed in BBM medium at day 15. Z8 medium supported relatively slower growth of *Ch. sorokiniana*, as indicated by the lower OD values compared to the other two media.

For *D. communis*, the OD values varied across the three media during the experiment. On day 1, the OD values were relatively low and comparable in all media, with slightly higher values observed in Z8 medium. However, as the cultivation progressed, the OD increased significantly in all media, with the highest growth observed in Z8 medium at day 15. Notably, BG-11 and BBM media also supported considerable growth of *Desmodesmus communis*, but Z8 demonstrated superior performance in promoting cellular development [33].

Similarly, *R. subcapitata* displayed variations in OD values in response to different media. On day 1, the OD values were relatively similar across all three media, but diverged as the cultivation continued. BG-11 medium showed a significant increase in OD values, reaching the highest level at day 8, followed closely by Z8 medium. Though supporting growth, BBM medium displayed a slightly slower growth rate than the other two media. Overall, *R. subcapitata* showed promising growth in all tested media, with the highest OD values recorded in BG-11 and Z8 at day 15.

The evolution of the turbidity values during the experiments is presented in Figure 3.

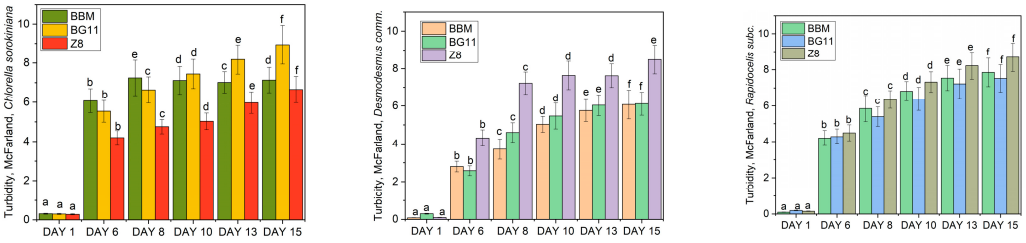

Figure 3. Turbidity (McFarland) for the three microalgae strains in the three cultivation media. The bars height are mean values ± statistical error bars. For the values with the same letter the difference between the means is not statistically significant at $p < 0.05$.

Turbidity values (Figure 3) for *D. communis* increased gradually in all three media from day 1 to day 15. Among the three media, Z8 consistently showed the highest turbidity values, promoting the highest cell growth for *D. communis*. BBM and BG-11 also supported the growth, with BBM showing slightly higher turbidity values than BG-11 throughout the cultivation period.

Turbidity values for *R. subcapitata* increased steadily over time in all three media. Similar to *D. communis*, Z8 medium displayed the highest turbidity values for *R. subcapitata*, indicating its favorable impact on cell growth. BBM and BG-11 also supported the growth, with BBM showing slightly higher turbidity values, especially at later time points.

Turbidity values for *Ch. sorokiniana* increased consistently over the cultivation period in all three media. Interestingly, BG-11 medium demonstrated the highest turbidity values for *Ch. sorokiniana* at later time points, surpassing the values observed in Z8 medium. BBM also supported the growth, with relatively lower turbidity values than BG-11 and Z8.

Overall, the turbidity results suggest that different microalgae species respond differently to the various media used for cultivation. Z8 medium appeared to effectively promote *D. communis* and *R. subcapitata* growth, while BG-11 showed promising results for *Ch. sorokiniana*.

To confirm the selection of the most suitable cultivation media, biomass accumulation (Figure 4) was assessed for each microalgae strain after 15 cultivation days [16].

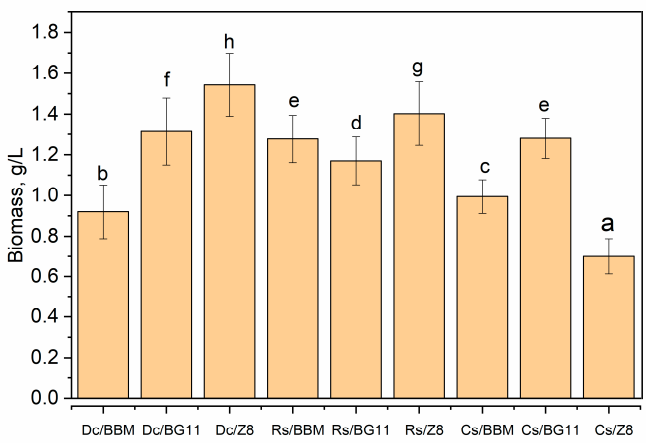

Figure 4. Biomass accumulation of each microalgae strain in different media after 15 days of cultivation. The bars height are mean values ± statistical error bars. For the values with the same letter the difference between the means is not statistically significant at $p < 0.05$.

Consistent with the optical density and turbidity findings, *D. communis* and *R. subcapitata* achieved higher biomass production in the Z8 medium, highlighting its proficiency in supporting their growth. *Chlorella sorokiniana* showed the highest biomass production in the BG-11 medium, while in BBM and Z8 media, it exhibited lower biomass yields. This finding suggests that BG-11 medium is the most suitable for cultivating *Ch. sorokiniana* among the tested media.

Based on the comprehensive evaluation of growth indicators and biomass accumulation, we identified *D. communis* in Z8 and *Ch. sorokiniana* in BG-11 as the most promising candidates for CO_2 biofixation in subsequent experiments. While *R. subcapitata* also exhibited favorable results, the literature analysis [32,34] revealed its heightened sensitivity to water acidulation, which could potentially arise during CO_2 bubbling.

3.2. CO_2 Biofixation Using Microalgae

The growth of microalgae was compared for microalgae with and without supplementation, for microalgae with a higher growing rate, that is, *Ch. sorokiniana* and *D. communis*. Figure 5 illustrates the evolution of the optical density for microalgae growth with and without CO_2.

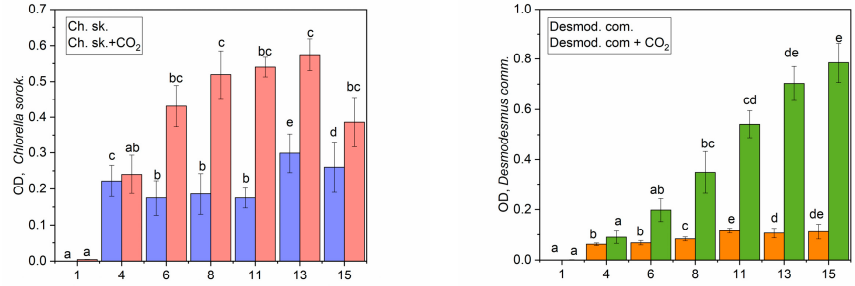

Figure 5. Optical density for microalgae growth with and without CO_2 supplementation. (**Left**) *Chlorella sorokiniana*. (**Right**) *Desmodesmus communis*. The bars height are mean values ± statistical error bars. For the values with the same letter the difference between the means is not statistically significant at $p<0.05$.

In the experimental treatment without CO_2 supplementation, *Ch. sorokiniana* exhibited steady growth throughout the 15 days. The optical density (Figure 5) increased gradually but remained relatively low, reaching a maximum of 0.299 at day 13. In the variant with CO_2 supplementation, *Ch. sorokiniana* showed enhanced growth compared to the control variant. The OD increased more rapidly, reaching a higher value of 0.784 at day 15. The CO_2 supplementation significantly stimulated the growth of *Chlorella sorokiniana*, leading to a substantial development.

In the control (without CO_2 supplementation), *D. communis* also displayed gradual growth over the 15 days, with the optical density reaching 0.116 at day 11. In the CO_2 supplementation variant, *D. communis* showed a similar growth pattern to the control culture until day 11. However, after day 11, the OD increased rapidly in the presence of CO_2, reaching a higher value of 0.703 at day 13. The CO_2 supplementation had a notable impact on the growth of *D. communis* during the last two days of cultivation.

In both cases, the addition of CO_2 positively influenced the growth of the microalgae. The CO_2 supplementation accelerated biomass production and resulted in higher final OD values than non-CO_2 variants. The results suggest that CO_2 supplementation plays a crucial role in enhancing the growth of both *Ch. sorokiniana* and *D. communis*, especially during the later stages of cultivation.

Figure 6 represents the evolution of cell number for microalgae growth using CO_2.

In the control (without CO_2 supplementation), both strains started with no detectable cells on day 1 and gradually increased in terms of cell number (Figure 6). CO_2 supplementation significantly increased microalgae cell multiplications.

Overall, CO_2 supplementation increases both cell number and cell density. The experimental model optimized during this work allows an efficient CO_2 sequestration in microalgae biomass. Upscaling of the model should enhance the benefits of sequential gas transfer and reduce the costs of mixing and agitation. Exposure to the sunlight in shadow conditions, i.e., photosynthetically active radiation lower than 300 $\mu E\ m^{-2}\ s^{-1}$, could further reduce operating cost and improve yields.

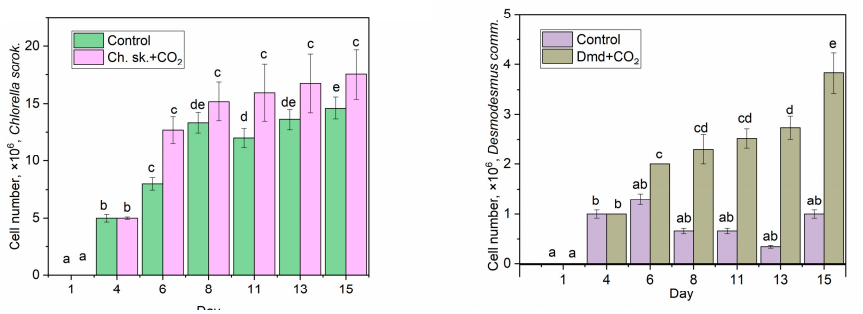

Figure 6. Cell number for microalgae growth using CO_2. (**Left**) *Chlorella sorokiniana* (Ch. sk.). (**Right**) *Desmodesmus communis* (Dmd). The bars height are mean values ± statistical error bars. For the values with the same letter the difference between the means is not statistically significant at $p<0.05$.

4. Conclusions

Optimal growth media were determined. For *D.* communnis and *R. subcapitata*, the Z8 medium demonstrated the highest growth-promoting capabilities, yielding superior results compared to other media. On the other hand, *Chlorella sorokiniana* exhibited the best performance in the BG-11 medium.

The workflow and parallel reactor configuration allow a comparison of microalgae strains and CO_2 supplementation. The sequential gas transfer from one reactor to another increases the efficiency of CO_2 sequestration.

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