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Economic evaluation of inputs for microalgal lab cultures

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Abstract
Economics of microalgae production is highly affected by resource consumption, being nutrients and energy the main direct consumable costs. Moreover, solar radiation is often the cause of poor culture growth. This paper studies resource economization for microalgae upscaling from small-scale batch cultures, including mixing, nutrients, photoperiod in indoors, and solar radiation in outdoors cultures.
Commercial fertilizer concentration of 0.4 mL L^{-1} for *Chlorella* sp. (CMC) and *Scenedesmus* and *Chlorella* mixed cultures (SCMC) led to a maximum production efficiency of 23.8 x10^{12} cell €$^{-1}$ on day 11 and 11.9x10^{12} cell €$^{-1}$ on day 7, respectively. Higher concentrations resulted in inefficient use of nutrients. Mixing interval 12/12 led to the highest production efficiency, with 1.6x10^{12} cell €$^{-1}$ in CMC, almost the double that with continuous mixing. No mixing during dark periods saved energy without decreasing cell concentration. Photoperiod 12/12 also led to higher efficiency than continuous lighting, with 21.1x10^{12} cell €$^{-1}$ on day 9 and 22.1x10^{12} cell €$^{-1}$ on day 14 for CMC and SCMC, respectively. Finally, the use of sunscreens improved solar radiation utilization. Raschel mesh led to 1.1x10^{9} cell (kWh m^{-2})$^{-1}$ on day 11 for CMC, yielding 157% and 224% more biomass than UV filters and direct solar radiation, respectively.

Keywords: Microalgae; economics; resource consumption; production efficiency; solar radiation

1. Introduction

Resource consumption is a bottleneck faced by the microalgae industry focusing on low unit value products such as biofuels. Energy and nutrients are the main direct consumable costs [1], so savings in these resources would result in more efficient and profitable cultures. The most important energy costs when cultures are grown inside arise from mixing, aeration and lighting [1,2], whilst the culture medium requires optimal concentrations of nutrients that depend on the type of microalgae being cultivated and their purpose [3]. Cultures grown outside are frequently affected by photo-saturation, photo-inhibition and UV radiation, which reduce system productivity [1,4,5].

Reducing production costs, while maintaining or increasing culture productivity, requires establishing the appropriate values for these parameters (mixing, photoperiod and nutrients) and assessing whether to use solar radiation filters to increase productivity. Since the optimal value of these parameters is specific to each species [4,6], it is very important to optimize these factors when culture centers are installed in areas without cultivation references or when working with native strains not described in literature. Mixing is a key parameter when designing photo-bioreactors and raceways. Poor mixing results not only in sedimentation, but creates stagnant areas and oxygen accumulation, which directly impacts the availability of nutrients and light for the microalgae, while excessive mixing can damage the cells, reducing system productivity [1,4].

The effect of lighting intensity on cultures grown inside using supplementary lighting is well known [7,8] but the importance of the photoperiod on culture growth has only recently been recognized [7,9]. It is important to assess the optimal photoperiod when working with a new strain in order to reduce production costs, regardless of the final commercial use of microalgal biomass. On the other hand, excessive solar radiation can inhibit photosynthesis and damage cells in cultures grown outside [9]. In particular UV-B radiation (280-315 nm) inhibits photosynthesis, damages DNA, proteins and lipids, generates reactive oxygen species (ROS) and inhibits nutrient uptake [5,10-13]. Given the magnitude of this problem, it is surprising that using solar filters or attenuators has been largely ignored by the industry as an economic solution for microalgae cultures at industrial scale [14,15], contrary to conventional agriculture [16].

Finally replacing microalgae specific nutrient solutions with commercial fertilizers decreases production costs whilst being equally effective [17,18]. It is necessary to find a balance in nutrient dosing, since excessively limiting nutrients can result in nutritional deficiencies that prevent appropriate culture development [6], while an excess can lead to nutrients being lost in the effluent [1] and growth inhibition due to an excess of some compounds, for example ammonium or nitrate [19], which reduce process efficiency.

The aim of this paper is to optimize mixing, nutrient concentration, photoperiod (indoors) and sunscreen usage (outdoors) through small scale batch cultures, and to understand the impact of each of these factors on production costs. Due to the scale of the study, results should be applied at a first step of scaling up

cultures from lab-scale to small-pilot plants operating continuously. Therefore, *Chlorella* and *Scenedesmus* were used, which are microalgae recognized for their wide commercial interest and adaptability to various environments [20-22].

2. Material and Methods

2.1. Microalgae cultures

Cultures of *Chlorella sp*. (CMC) and mixed cultures of *Scenedesmus sp*. and *Chlorella sp*. were used. (SCMC). Microalgae samples were collected in Melipilla (Chile) from treated drinking water ponds and were isolated according to Torrentera and Tacón [23]. The organisms were identified by genus using the descriptions provided by Parra et al. [24].

Chlorella sp. predominated in the initial CMC samples with over 99% of the total culture. The initial participation percentages in SCMC were 35% - 59% for *Chlorella sp*. and 41% - 65% for *Scenedesmus sp.*

2.2. Cultivation system

Experiments were performed in ProCycla's laboratories located in Melipilla (33.7066°S-71.162°W) between August 2014 and January 2015. Tests 1-3 (Table 1) were performed indoors and lit by 20 W white fluorescent light tubes (5,000 K) (Philips, Thailand) with a Photosynthetic Active Radiation (PAR) between 250-300 µmol m^{-2} s^{-1}, measured with a PAR radiation sensor Quantum Meter MQ200 (Apogee Instruments, USA) . In these experiments temperature was not controlled, and it oscillatedbetween 13°C and 20°C as a consequence of indoors environmental conditions. Flasks of 500 mL were constantly mixed with air using an ACO-003 45 W electromagnetic compressor (Yuting, China), with a maximum flow of 50 L min^{-1}.

Test 4 (Table 1) was performed outside, without control of temperature, which oscillated between 7.9°C and 39.7°C, with an average of 18.1°C. Solar radiation was determined using data from the INIA network of meteorological stations (National Agricultural Research Institute of the University of Chile, Government of Chile) with an average value of 226.25 W m^{-2}. PAR radiation was measured throughout the experiment (see section 2.3). Flasks of 1000 mL were constantly mixed with air from the aforementioned compressor.

Table 1. Experimental design used in the study.

Test	Factor	Units	Levels	
1	Nutrient (B)	mL L^{-1}	0; 0.4; 1.0; 3.0; 5.0	
2	Mixing (M)	h	1/1; 3/3; 12/12; 24/0	(ON/OFF)
3	Photoperiod (P)	h	1/1; 12/12; 24/0 (light/dark)	
4	Radiation (R)	-	Raschel mesh; UV filter; direct solar radiation	

2.3. Experimental design

Four factors were sequentially studied in independent tests (Table 1). Factors which could have any influence in microalgal growth but were not studied in a specific test were kept constant or adjusted (when necessary) to minimize their effect in the results (e.g. mixing and photoperiod were synchronized in order to allow similar light availability to all cultures in the photoperiod test, however aeration rate and nutrients were the same to all cultures in order to avoid variation in the results due to variation in these factors).

2.3.1. Concentration of commercial nutrients

Basfoliar® 07/04/10 SL (Compo expert, Germany), an inorganic commercial fertilizer, was diluted with water at four different concentrations (Table 1) and used as culture media for CMC and SCMC, plus a control with distilled water. The composition of each dilution is shown in Table 2. The photoperiod was a 12/12 light/dark interval and t

Table 2. Nutrient content of Basfoliar SL® and the four dilutions studied as culture media.

	Basfoliar (g L^{-1})	B0.4 (mmol L^{-1})	B1.0 (mmol L^{-1})	B3.0 (mmol L^{-1})	B5.0 (mmol L^{-1})
N	100	2.86	7.14	21.41	35.69
P	40	0.52	1.29	3.87	6.46
K	70	0.72	1.79	5.37	8.95
Mg	2	3.29E-02	8.23E-02	0.25	0.41
Fe	0.15	1.07E-03	2.69E-03	8.06E-03	1.34E-02
Zn	0.005	3.06E-05	7.65E-05	2.29E-04	3.82E-04
Mn	0.015	1.09E-04	2.73E-04	8.19E-04	1.37E-03
B	0.02	7.40E-04	1.85E-03	5.55E-03	9.25E-03
Cu	0.025	1.57E-04	3.93E-04	1.18E-03	1.97E-03
Mo	0.003	1.25E-05	3.13E-05	9.38E-05	1.56E-04

2.3.2. Mixing intervals

There were four mixing intervals using air injected at 2vvm in order to cultivate CMC (Table 1). The culture medium for this test was enriched with sufficiently concentrated nutrients to avoid limiting biomass growth until day 14, according to the results of the first test (3 mL L^{-1}). A 12/12 light/dark photoperiod was used for all cultures, without synchronization with the mixing intervals to check if lower mixing times influence light availability at this scale. No tests were performed with SCMC due to genus predominance reversal below 10% by *Scenedesmus sp*.

2.3.3. Photoperiod

Three photoperiods (Table 1) were studied in cultures of CMC and SCMC in a darkroom. To eliminate the interaction between mixing and light availability, mixing was synchronized with the light intervals at a flow rate of 2 vvm. The nutrient concentration was sufficient to avoid limiting biomass growth until day 14, according to the results of the first test (3 mL L^{-1}).

2.3.4. Radiation

The influence of using radiation filters was studied using Raschel mesh (white color, 60% shading) and a UV filter as sunscreen for growing CMC. The UV filter was a neutral colored Optivision 25® (Sun-Gard Inc., USA) with a shading coefficient of 0.43 and UV radiation transmissivity of less than 1%. The Raschel mesh is a low cost mesh used in agriculture [16]. All flasks were distributed randomly to ensure that the solar radiation that reached each flask was only dependent on the presence of radiation filters. The nutrient concentration was sufficient to avoid limiting biomass growth until day 14, according to the results of the first test (3 mL L^{-1}). The mixing was 12/12 (ON / OFF) at 2 vvm.

2.3. Experimental Procedure

Tests 1-3 and 4 used 300 and 500 mL of sterile culture medium, respectively, and 2 mL of the corresponding microalgae culture. Tests 1-3 were done with three replicates and Test 4 with four replicates.

The pH, electrical conductivity (EC) and dissolved oxygen (DO) were recorded every day during all tests between 10.00am and 11.00am with a multi-parameter probe HI9828 (Hanna Instruments, USA). Nutrient, mixing and photoperiod testing required data collection on days 0, 2, 4, 7, 9, 11 and 14. Radiation test required data collection on days 0, 2, 4, 6, 8, 11 and 13. Additionally, it required PAR and UV radiation measurements between 11.00am and 2.00pm, using a PAR radiation sensor Quantum Meter MQ200 (Apogee Instruments, USA) and a UV513AB (General Tools, USA) ultraviolet radiation meter.

Cell density was evaluated by cell counting using a Neubahuer chamber with a depth of 0.1 mm (Marienfeld, Germany) under an OMAX 20-1600X (OMAX, South Korea) optical microscope. Dilutions were performed to prevent the number of cells exceeding 200 cells per square.

2.4. Economic production efficiency

The economic production efficiency (η_e) was calculated according to the following equation:

$\eta_e = (CC \cdot V) / (R \cdot t \cdot C)$

where η_e is the economic production efficiency based on the resource R [cell €$^{-1}$; cell (kWh m^{-2})$^{-1}$], CC is the cell concentration at time t (cell mL^{-1}); V is the culture volume (mL); R is resource consumption at time t (N mmol, kWh, kWh m^{-2}); t is the elapsed time (with 1 being the default value for nutrient experimentation, since all nutrients are provided at the beginning of the experiment); C is the unit cost of the resource consumed (€ (N mmol)$^{-1}$, € kWh^{-1}).

One kg of nitrogen in Basfoliar costs € 27.7 [25], while in Chile one kWh of energy costs € 0.1326 [26]. The sunscreen results are shown in [cell (kWh m^{-2})$^{-1}$] basis since the solar radiation resource cannot be economically weighted.

2.5. Statistical Analysis

Statistical analysis was performed independently for each test. Statistically significant differences were determined using heterogeneous mixed models on Infostat v.2015 statistical software [27]. The model results were compared and graphed according to a DGC test ($p < 0.05$) [28] for the formation of exclusive groups. In addition, Pearson correlation analysis was performed between the concentration of biomass and the culture control parameters (EC, pH and DO) throughout the experimental period (days 0 to 13 or 14). Correlations were considered significant at $p < 0.05$.

3. Results and Discussion

This section describes and discusses the effect of the variables on CMC and SCMC biomass growth, as well as the economic study of resources consumption. In order to facilitate reading and understanding, the results will be presented and discussed separately for each variable [nutrient (B), mixing (M), photoperiod (P) and radiation (R)] and in the order in which the tests were conducted.

3.1. Nutrient test

3.1.1. CMC

Figure 1(A) shows CMC growth under laboratory conditions and various concentrations of Basfoliar fertilizer. All phases of growth in B0.4, B3.0 and B5.0 cultures were observed. The control and B1.0 cultures produced a slight yet insignificant increase in biomass (*p> 0.05*). The B0.4 and B3.0 cultures reached the stationary phase on day 9, with $7.8x10^7$ cell $mL^{-1}$and $1.16x10^8$ cell mL^{-1} respectively; whereas the B5.0 culture reached the stationary phase on day 14 with a concentration of $2.95x10^8$ cell mL^{-1}, corresponding to the maximum concentration for this test and significantly higher than the rest (*p <0.05*). Results were as expected, as higher initial nutrient availability resulted in higher biomass production [29] except for culture B1.0, which had virtually no growth, despite having an intermediate concentration of fertilizer. This behavior has been described previously [30], although in this case the results suggest that growth inhibition was not caused by nutrient concentration, as concentrations higher than B1.0 did not result in inhibition.

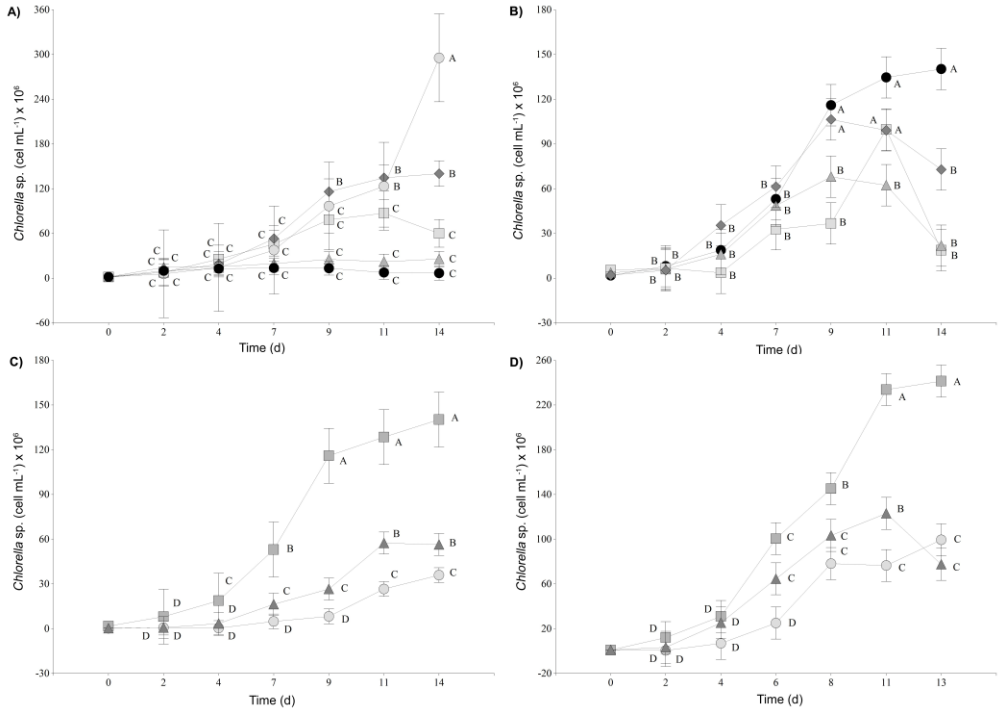

Figure 1. CMC cell concentration with variations in (A) culture media concentration [Control (●); B0.4 (■); B1.0 (▲); B3.0 (♦); B5.0 (○)]; (B) mixing intervals [M1/1 (■); M3/0 (▲); M12/12 (●); M24/0 (♦)]; (Cc) different photoperiods [P1/1 (○); P12/12 (■); P24/0 (▲)]; (D) solar radiation [Control (○);Raschel mesh (■); UV filter (▲)]. Statistically significant differences are shown with capital letters (*p<0.05*), day 0 was used as covariate. Error bars indicate standard error.

Differences in growth patterns indicate that the most suitable concentrations for maximum biomass concentration were 3.0 mL L^{-1} and 5.0 mL L^{-1}. The main advantage of the first concentration was that on day 9 it reached the stationary phase, which was significantly better than the rest, without any significant difference to B5.0 until after day 11. Other authors have noted the suitability of other commercial fertilizers for *Chlorella,* where they have obtained growths similar to those in this study [18]. The observed growth dynamics indicate that in B0.4, B3.0 and B5.0 until day 9-11 the limiting nutrient in the culture was CO_2 and/or light [31], since all cultures showed the same growth rate regardless of nutrient content. However, CO_2 was sufficient to support continuous growth of all cultures as indicated by pH values throughout the culture period which oscillated between 7.8 and 9.3 in all flasks without differences between cultures. The pH changes in algal cultures are mainly caused by the CO_2 system (CO_2, HCO_3^-, CO_3^{-2}) [32], therefore a stable pH is consequence of an adequate supply of CO_2 during the whole culture period and ensure that the differences observed are solely consequence of the mineral nutrients in the culture media. In fact, the mineral nutrients were consumed by day 9 in B0.4 and day 11 in B3.0, therefore only B5.0 continued to grow.

However, in a continuous culture system in which the dilution rate is usually between 0.2 and 0.4 d^{-1} to maximize production (HRT = 2.5-5 days) [4], the presence of excess nutrients does not represent an economic or productive advantage since excess nutrients not consumed during the HRT would be wasted in the harvested culture broth. Furthermore, excess nutrients could even slow biomass growth at the

beginning, due to the adaptive processes at high concentrations that cause cellular stress [33], as appears to have happened in B5.0. Therefore, the decision to select a particular concentration in larger production volumes should be subject to economic evaluation and biomass productivity studies in continuous systems. The first of these will be introduced in section 3.5.

Figure 2(A) shows the EC, where a downward trend can be observed during the test. The EC is related to the presence of ions and solutes in the medium, so the rapid decline in conductivity was due to nutrient absorption by the microalgae, which was corroborated by the significant correlation between increased biomass and decreased EC ($p < 0.05$) for cultures with B1.0, B3.0 and B5.0.

3.1.2. SCMC

The development of SCMC cultures under various Basfoliar concentrations are shown in Figure 3(A). All cultures showed the typical growth stages, except the control group, whose biomass remained unchanged throughout the test. The B0.4 culture reached the stationary phase on day 7 with 4.3 x 10^7 cell mL^{-1}; the B1.0 culture reached the stationary phase on day 9 with 8.5 x 10^7 cell mL^{-1}; while the B3.0 and B5.0 cultures reached the stationary phase on day 14 with 1.88 x 10^8 cell mL^{-1} and 2.46 x 10^8 cell mL^{-1}, respectively. Both values were the highest recorded in the test and were significantly different from each other ($p < 0.05$). In this case, the results are as expected in terms of biomass concentration since adding nutrients, produces higher cell concentration [29].

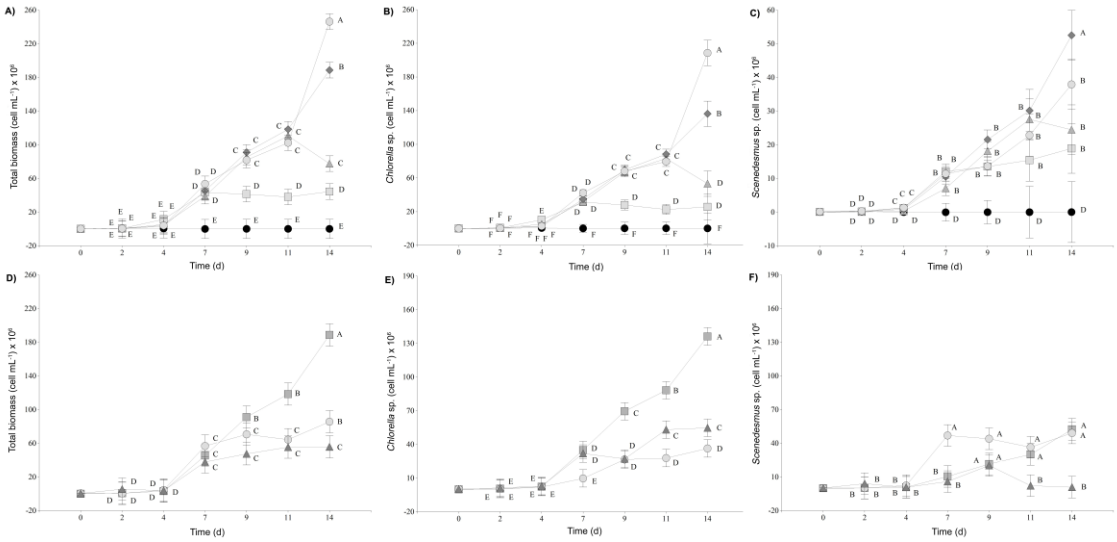

Figure 2. EC in CMC (A) and SCMC (B) during the nutrient concentration assay: Control (●); B0.4 (■); B1.0 (▲); B3.0 (♦); B5.0 (○). Statistically significant differences are shown with capital letters (p<0.05), day 0 was used as covariate. Error bars indicate standard error.

Figure 3(B) and Figure 3(C) show in detail the variation of *Chlorella sp.* and *Scenedesmus sp*. in the SCMC culture with various nutrient concentrations. There was a reversal in predominance within the culture, as initially, *Scenedesmus sp*. was slightly predominant with 53.7 ± 5.7% participation. From day two *Chlorella* became predominant with an average participation of 73.9 ± 5.9%. *Scenedesmus sp*. recorded its lowest participation between days 4 and 7. The cell concentration of *Chlorella sp*. determined the behavior of the total biomass, especially until day 11. Both genus had similar growth patterns in all cultures except the B1.0 culture, where *Scenedesmus* reached the stationary phase on day 11 and *Chlorella* on day 9. As in the case of CMC, pH was similar in all cultures throughout the whole culture period and it oscillated between 9.6 and 7.2. Therefore, CO_2 addition was enough to keep biomass growth regardless of the nutrient concentration.

The behavior of the total biomass suggests that all dilutions can be used for the development of a commercial culture. Given the influence of the dilution rate on the productivity of a culture system operating continuously, the B0.4 and B1.0 dilutions could provide a substantial economic advantage over the other tests, as, they showed the same biomass concentration as B3.0 and B5.0 up until day 7 and 11 respectively. However, B3.0 or B5.0 can be recommended specifically for hyper-concentrated cultures. Brito et al. [17] used the B0.4 dilution for a *C. vulgaris* culture and a mixed culture of *C. vulgaris* and *S. capricornutu*, observing biomass concentrations of 8.0 x 10^6 cell ml^{-1} and 1.0 x 10^7 cell ml^{-1} respectively; lower than those obtained in this study with similar dilutions.

The EC for SCMC showed a completely different pattern than the EC in CMC, as shown in Figure 2(B). As in CMC, the EC at day 0 was higher with higher concentrations of Basfoliar. However, from day 4 the

behavior of B1.0, B3.0 and B5.0 cultures did not show a negative trend in the EC. The increase of EC in microalgae cultures has been associated with cell damage that release intracellular electrolytes into the medium [34].

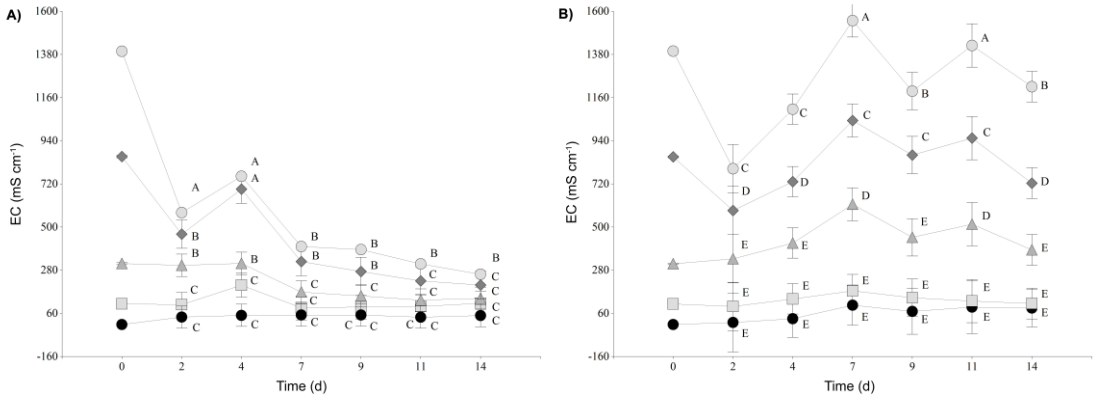

Figure 3. SCMC cell concentration with variations in: (first row) culture media concentration [Control (●); B0.4 (■); B1.0 (▲); B3.0 (♦); B5.0 (○)] (A) total cells, (B) *Chlorella* sp., (C) *Scenedesmus* sp.; (second row) photoperiods [P1/1 (○); P12/12 (■); P24/0 (▲)] (D) total cells,, (E) *Chlorella* sp., (F) *Scenedesmus* sp. Statistically significant differences are shown with capital letters ($p<0.05$), day 0 was used as covariate. Error bars indicate standard error.

3.2. Mixing test

Biomass growth for CMC is shown in Figure 1(B). Various behaviors were observed at these mixing intervals, although all cultures had typical growth stages. The M3/3, M12/12 and M24/0 cultures reached the stationary phase on day 9 with concentrations of $6.8x10^7$, $1.34x10^8$ and $1.06x10^8$ cell mL^{-1}, respectively. The M1/1 culture reached the stationary phase on day 11 with $9.9x10^7$ cell mL^{-1}. The M12/12 culture continued to grow after day 9, and reached maximum cell concentrations on day 14.

The M12/12 and M24/0 cultures had higher biomass concentrations and higher growth rates because lighting periods coincided with mixing periods, while the M1/1 and M3/3 cultures had lower biomass growth because light was not efficiently distributed throughout the lighting period (the photoperiod was 12/12 light/dark). In addition, the low growth observed in the M1/1 and M3/3 cultures could be associated with difficulties in accessing nutrients and an accumulation of toxic products such as OD, which could cause cell death [35]. In fact, the M1/1 / and M3/3 cultures generally showed the highest OD concentrations in the experiment (data not shown).

Growth patterns under various mixing intervals indicate that M12/12 and M24/0 were most suitable for developing biomass. The results of this study agree with Cuello et al. [36] who observed that stopping mixing during dark periods in raceway systems does not affect culture growth while producing significant energy savings.

3.3. Photoperiod test

3.3.1. CMC

Biomass growth in CMC under various photoperiods is shown in Figure 1(C). The P12/12 period was significantly better ($p < 0.05$), as it reached the stationary phase earlier and achieved higher cell concentration ($1.16x10^8$ cell mL^{-1}). The P24/0 culture with continuous lighting, reached the stationary phase on day 11 with $5.7x10^7$ cell mL^{-1}. Culture yield for P1/1 was well below that for P12/12 despite sharing the same total period of light exposure (12 hours per day).

Scientific literature contains conflicting information regarding the suitability of continuous lighting or lighting intervals. Bouterfas et al. [37] obtained better results under continuous lighting indicating that growth depends on the amount of energy received by cultures, although they recognized that a dark period is required to regenerate cofactors (NAD^+, $NADP^+$) that are required for Phase I photosynthesis. Therefore, as lighting duration lengthens, cell division is shorter and therefore cell concentration increases. Also Krzeminska et al. [38] observed better yields of *Scenedesmus obliquus* with continuous lighting, but the 12/12 photoperiod was most suitable for three species of *Neochloris sp*. suggesting that the differences arose from the reproduction mechanism for each genus. In another study, Jacob-Lopes et al. [7] found that longer lighting periods resulted in increased *Aphanothece* biomass productivity and cell concentration, except for photoperiod 12/12, which produced the best results. Finally, Khoeyi et al. [39] observed an increase in biomass production and cell concentration in cultures of *C. vulgaris* when the lighting duration was increased from 8/16 to 16/8 (light/dark). The need for a dark phase together with the resulting energy savings when using photoperiod intervals suggest the suitability of light/dark intervals to increase the economic efficiency of microalgae cultures.

3.3.2. SCMC

The SCMC cultures (Figure 3(D)) had improved development for P12/12. This culture reached the stationary phase on day 14 with the maximum cell concentration ($1.88x10^8$ cell mL^{-1}). Both P24/0 and P1/1 reached the stationary phase on day 7 with cellular concentrations of $3.8x10^7$ cell mL^{-1} and $5.6x10^7$ cell mL^{-1}, respectively.

Chlorella sp. (Figure 3(E)) was predominant during the photoperiod test, with an initial average participation of 56.8 ± 2.3%. This pattern was maintained for P12/12 and P24/0, with maximum *Chlorella sp*. predominance of 76.2 ± 3.9% (day 7) and 98.2 ± 0.2% (day 14), respectively. The P1/1 cultures recorded predominance reversal from day 4 with a *Scenedesmus* maximum on day 7 (75.8 ± 7.3%), which subsequently decreased to 55.7 ± 3.4% by day 14 (Figure 3(F)).

As in the nutrient test, *Chlorella* beat *Scenedesmus* in all tests except P1/1. The largest and fastest *Chlorella* development was due to physiological and metabolic processes as well as its size and structure, as these smaller cells assimilate nutrients and carbon faster than larger cells [40]. The superiority of *Chlorella* in limited light intensity conditions has also been demonstrated [41]. This study observed that *Scenedesmus* predominated over *Chlorella* in P1/1, suggesting an initial competitive advantage when light limitation was controlled using the duration of the lighting period, which may be due to the shading effect of *Scenedesmus* over *Chlorella* because of its larger size [42].

3.4. Radiation test

Figure 1(D) shows growth for CMC with and without solar radiation filters. The Raschel mesh gave better results reaching the stationary phase on day 11 with the highest cell concentration at 2.41×10^8 cell mL^{-1} ($p < 0.05$). The culture with a UV filter behaved very similarly to the control group (direct sunlight), both reaching the stationary phase on day 8 with a cell concentration of 1.03×10^8 cell mL^{-1} and 7.8×10^7 cell mL^{-1}, respectively.

Light is generally the limiting factor in outdoors microalgae cultures [15]. Cultures developed under ambient conditions suffer significant light intensity changes. The lack of light availability throughout the culture could limit the development of microalgae; whereas high solar radiation levels at noon could inhibit the growth due to photoinhibition phenomena [43,44]. The solution to the last problem is to increase the cell concentration in the culture creating a mutual shading effect that decrease photo-inhibition and the cell damage associated to photo-oxidation [15,45]. A higher cell concentration implies a lower average irradiance throughout the culture and therefore a higher protection against the negative effects of the excess solar radiation. In the cultures used in this study (flasks of 1 liter with 10 cm diameter) and with the solar radiation measured at the beginning of the experiment (between 1,200-1,500 µmol $m^{-2} \cdot s^{-1}$, see Figure 4(A)) the mutual shading effect could not be enough to protect microalgae from photo-inhibition [46].

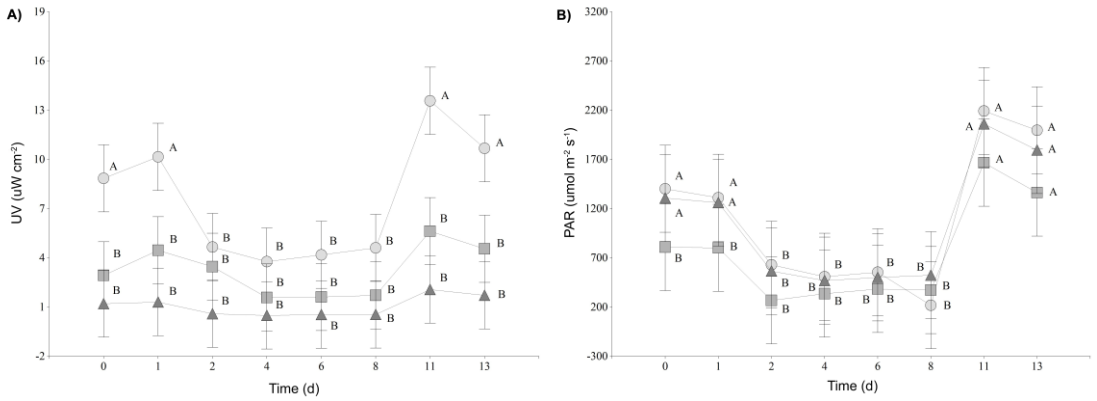

Figure 4. Radiation measurements (A) UV (UV-A y UV-B); (B) PAR [Control (○); Raschel mesh (■); UV filter (▲)]. Statistically significant differences are shown with capital letters ($p<0.05$), day 0 was used as covariate. Error bars indicate standard error.

Light control in microalgal cultures is complicated due to the large numbers of factors that have to be taken into account, such as light intensity, light-dark fluctuations, duration of light-dark cycles and hydrodynamics of the reactor [47]. Solar radiation is given by ambient conditions and therefore cannot be modified. However, with systems such as the used in this study solar radiation received by microalgal cultures could be altered, avoiding photo-inhibition and therefore increasing biomass growth rates and productivities [48]. In this study, solar filters caused a higher positive impact at the beginning of the experiment with low cell concentrations, favoring the development of the cultures using the Raschel mesh and the UV-filter. As cell concentration increased, the cultures were further favored by the mutual shading effect. Indeed, once the

control culture increased its concentration it reached cell concentration similar to the culture under the UV-filter. On the other hand, the culture under the Raschel mesh exhibited a much higher cell concentration during the whole culture period, revealing that the Raschel mesh is adequate for the development of new cultures with low concentrations but also in order to protect high concentrated microalgae cultures against photo-inhibition and photo-oxidation.

The results suggest that the Raschel mesh has an interesting potential for microalgae cultivation, especially in areas where there is a high level of solar radiation. The slower growth of cultures with a UV filter could be due to photo-inhibition processes, as PAR radiation measured under the Optivision 25® filter was similar to that measured in the control group (Figure 4(B)).

In both cases the sunscreens were observed to positively favor the development of culturescompared to direct solar radiation, as observed in similar studies using specific wave length filters and shading nets [14,48]. It is significant that the Raschel mesh is easy to procure and install and its cost is much lower than that of UV filters (≈US$ 0.6 m^{-2} and US$ 6.9 m^{-2}, respectively). Therefore, the Raschel mesh could be potentially used at larger scales in order to decrease the photoinhibition phenomena.

3.5. Economic production efficiency

This research studied the resource consumption and savings for microalgal cultures with the aim to potentially scale up the culture to small-pilot scale cultures. The results are shown in Figure 5.

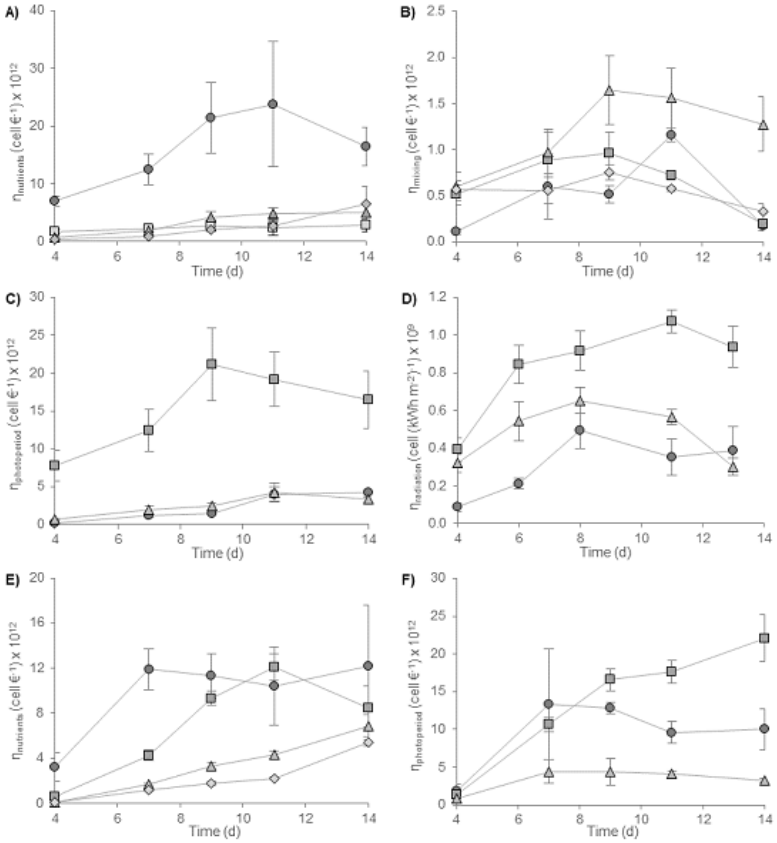

Figure 5. Efficiency in biomass production in (A) CMC culture media concentration test [B0.4 (●), B1.0 (■), B3.0 (▲), B5.0 (♦)], (B) CMC mixing test [M1/1 (●), M3/3 (■), M12/12 (▲), M24/0 (♦)]; (C) CMC photoperiod test [P1/1 (●), P12/12 (■), P24/0 (▲)]; (D) CMC solar radiation [Control (●), Raschel (■), Filtro-UV (▲)]; (E) SCMC culture media concentration test [B0.4 (●), B1.0 (■), B3.0 (▲), B5.0 (♦)]; (F) SCMC photoperiod test [P1/1 (●), P12/12 (■), P24/0 (▲)]. Error bars indicate standard error.

Microalgae behave different at small scale batch cultures than at large scale commercial cultures [49], but the development of any commercial process should follow certain steps that are initiated at small-scale laboratory trials, followed by pilot plant designs and final scale-up to commercial scale [50]. Results obtained in this study should therefore be verified first at small-pilot cultures before extrapolating to commercial production, but these results should at least allow cultivation to be scaled up to the next step based on the most economically efficient options, which have been estimated in accordance with the cost of each resource and the biomass produced. This next step of scale-up is considered to be microalgae cultures up to 200 L that can be used as inoculum for commercial scale plant production, such as stated in Helm et al. [51] for microalgae production for hatcheries.

In order to calculate the efficiency of nutrients use, the cellular concentration was divided by the concentration of nitrogen (in mmol) at the beginning of the experiment. Nitrogen was used since it is the main nutrient consumed by the microalgae and it largely determines the growth of both *Chlorella* and *Scenedesmus* [52]. In CMC the highest efficiency was obtained for B0.4 with 23.8 $x10^{12}$ cell €$^{-1}$ (Figure 5(A)). This emphasizes the fact that cultures B3.0 and B5.0 had an excess of nutrients, with CO_2 and light being the growth limiting factors. In addition, under excess nutrient conditions, microalgae are able to adapt reaching "luxury uptake" and accumulating nutrients without increased cell concentration [6]. In the case of SCMC there was also greater efficiency in B0.4 (Figure 5(E)) with 11.9$x10^{12}$ cell €$^{-1}$ on day 7, surpassed only by B1.0 on day 11, which again suggests an inefficient use of nutrients in B3.0 and B5.0.

In the mixing test, M12/12 was clearly the most efficient (Figure 5(B)). The M24/0 culture showed lower yields, as its energy consumption, and therefore cost, was double that in other cases. In this case, it was demonstrated that it was more efficient to mix during lighting periods for CMC, and stop mixing in dark periods. Cuello et al. [36] estimated energy savings of 37% can be produced using this strategy in a raceway culture system. This study also confirmed that stopping mixing during dark periods increases the system efficiency, reaching 1.6$x10^{12}$ cell €$^{-1}$ for M12/12 in comparison to 1.0$x10^{12}$ cell €$^{-1}$ for M24/0.

In photoperiod tests for both CMC (Figure 5(C)) and SCMC (Figure 5(F)) the P12/12 interval had a higher efficiency, with ratios of 21.1$x10^{12}$ cell €$^{-1}$on day 9 and 22.1$x10^{12}$ cell €$^{-1}$ on day 14, respectively. Bouterfas et al. [37] emphasizes the practical and economic advantages of a system with light intervals versus continuous lighting due to its higher energy savings, as observed in this study.

Finally, in the sunscreen tests the best efficiency was recorded when using Raschel meshes, with 1.1$x10^{9}$ cell (kWh m^{-2})$^{-1}$ on day 11 (Figure 5(D)). The best result for the UV filter was on day 8, with a value of 0.7$x10^{9}$ cell (kWh m^{-2})$^{-1}$. This confirms the Raschel mesh as the best choice for cultivating microalgae under direct sunlight. This is especially relevant in regions where photo-inhibition and UV radiation are intense for large-scale cultivation.

The results suggest that energy expenditure in mixing is more important than expenditure on nutrients and lighting. The search for significant savings in resource consumption and microalgae production costs must initially focus on energy expenditure, followed by nutrients. Estimates such as those in this study have not been found in literature. Therefore this study is the first to address economic scaling up using laboratory cultures.

4. Conclusions

In general, it can be concluded that the objective of rationalizing resources in order to scale up microalgae from small-scale batch cultures to the next step of development has been satisfactorily achieved. For both cultures studied the B0.4 dilution (0.4mL L^{-1} of Basfoliar) was the most efficient, as similar cellular concentrations were achieved in cultures with higher nutrient concentrations through to day 9 and 11 for CMC and SCMC respectively.

The 12/12 (ON/OFF) mixing interval was the most efficient for CMC, well above the 24/0 (ON/OFF) interval. This shows that when scaling up microalgae cultures, it is possible to stop mixing during dark periods in order to reduce energy consumption while maintaining biomass yield. The photoperiod test showed that the P12/12 (light/dark) interval was superior to continuous lighting (24/0) and intermittent lighting (P1/1) in both cell concentration and production efficiency for both cultures, CMC and SCMC.

Finally, CMC performed better under the Raschel mesh, with a cell concentration almost double that obtained under UV filters and almost three times that obtained under direct sunlight. This may mean a major breakthrough in outdoors microalgae cultures due to the low cost of Raschel mesh, which generates a significant decrease in photo-inhibition phenomena in microalgae cultures.

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