Microalgae biomass cultivation and harvesting optimization in biological carbon capture and utilization systems for biofuels production

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Abstract The boosting of greenhouse gas (GHG) emissions into the atmosphere due to anthropogenic activity contributes significantly to climate change. According to the Green Deal by 2050, net zero greenhouse gas emissions must be achieved. Therefore, actions are needed in order to control GHG emissions. The research presents and discusses the optimization of the microalgae biomass cultivation phase and the harvesting process in an advanced membrane photobioreactor (mPBR) with the aim to improve its production for green fuel generation. Experimental activities are carried out by considering Chlorella vulgaris microalgae as photosynthetic microorganism. A dark/light cycle of 12/12 hours was implemented by varying the light intensity from 100 to 300 µmol m^{-2} s^{-1}. Different L/G rate, by keeping the gas flow rate (G) constant at 100 ml/min and increasing the liquid flow rate recirculation (L) from 500 to 1500 L min^{-1}, has been tested to boost up the productivity of microalgae. Results highlight optimal production of microalgae biomass concentration up to 1.45 g L^{-1}. Then a dynamic membrane module was implemented for the harvesting of the biomass. The work contributes to the field of climate change mitigation actions, by providing useful information to improve green energy production from algae biomass.
Keywords: Photo-bioreactor, Dynamic membrane, Microalgae, Biofuels.

1. Introduction

Nowadays fossil fuels represent the primary energy sources used in the worldwide (Senatore et al., 2020). The burning of fossil fuels caused the boosting of greenhouse gas (GHG) in atmosphere producing the well know global warming phenomena (Oliva et al., 2021; Pires et al., 2011). In 2019 the European Commission emanated the Green Deal with the aim to reduce greenhouse gas emissions above the 70% by 2030. Among the mitigation strategies present in the Green Deal, there is the development of advanced and sustainable technologies capable to immobilize CO_2 and produce valuable compounds in a circular economy point of view (Vermi et al., 2021). Several standalone biotechnologies have been developed for CO_2 capture and bio-product production e.g. open ponds and closed photobioreactor (Senatore et al., 2021). Here, an integrated and cost-effective process for carbon capture and biomass harvesting has been presented.

Here, an integrated photobioreactor, coupling a tubular photobioreactor (PBR) and a membrane module has been presented for production and harvesting the microalgae. Chlorella vulgaris microalgae was chosen as photosynthetic microorganism thanks to the great tolerance in high lipids content. A dark: light cycle of 12:12 hours was implemented by varying the light intensity from 100 to 300 µmol m^{-2} s^{-1}. Several operating conditions were tested to boost up the productivity of microalgae. The harvesting rate was increased by changing the permeate flux of the dynamic membrane module. The L/G was increased from 5 to 15, by keeping the gas flow rate (G) constant at 100 ml/min and increasing the liquid flow rate recirculation (L) from 500 to 1500 L min^{-1}.

Preliminary results showed that it is possible to achieve a biomass concentration of up to 1.45 g L^{-1} and efficiently collect the microalgae biomass by using a dynamic membrane module with an average pore size of 20 µm.

Finally, the algal biomass can be used for many applications such as foods with high protein content, feed, fertilizers, biopolymers and biofuels. Thanks to the energy recovery from algae biomass, it could be possible to make a negative carbon emission process. Further studies are needed for the scale–up of the process.

2. Materials and methods

2.1. Microalgae and culture medium

A freshwater *Chlorella vulgaris* (CCAP 211/11B) was ordered from the Culture Collection of Algae and Protozoa (CCAP) located at the Scottish Association for Marine Science, Scotland. The *Chlorella vulgaris* was pre-

cultured following the condition reported in Senatore et al., (2021) for 14 days using the modified Bold Basal Medium solution with the following composition: a stocks solution per 400 mL of (1) 10.0 g of $NaNO_3$; (2) 3.0 g of $MgSO_4 \cdot 7H_2O$; (3) 1.0 g of NaCl; (4) 3.0 g of K2HPO4; (5) 7.0 g of KH_2PO_4; (6) 1.0 g of $CaCl_2 \cdot 2H_2O$; (7) trace elements solution (g/L); $ZnSO_4 \cdot 7H_2O$ (8.82 g), $MnCl_2 \cdot 4H_2O$ (1.44 g), $(NH_4)_6MO_7)_4 \cdot 4H_2O$ (0.87 g), CuSO4·5H2O (1.57 g), CoCl2·6H2O (0.38); (8) H_3BO_3 11.42 g/L; (9) 50.0 g/L of EDTA and 31.0 g/L of KOH; and (10) 4.98 g/L of $FeSO_4 \cdot 7H_2O$.

2.2. Operating condition

In the present study three different concentration CO_2 gas (5,10 and 15%) were prepared by mixing pure CO_2 with atmospheric. The gas flow rate was kept 100 mL min^{-1} by using a flow meter (OMEGA) and was fed in the system starting from the absorption column made from PVC and subsequently injected to a cylindrical PBR which is made of Plexiglas. Four white LED bulbs placed around the cylindrical PBR were utilized as light sources. Three PPDF (Photosynthetic photon flux density) (100, 200 and 300 µmol m^{-2} s^{-1}) were tested. A dark-light cycle of 12:12 hours was chosen. The mixture of CO_2 gas and atmospheric air was feed in the system for 6 h (during the 12 hours of light period). During the feeding period the concentration of the CO_2 in gas mixture was continuous measured with a gas analyzer GA 2000 (Geotechnical instrument). The system was filled with Bold's Basal Medium (BBM) and *C. vulgaris* with a working volume of 40 L, and with the continuous mixing using a magnetic stirrer. The BBM was recirculated using a peristaltic pump from the PBR to the column and vice versa. In Table 1 are summarized the operating condition utilized in the present study.

Table 1. Operational conditions evaluated in the photobioreactor.

Stage	CO_2 [%]	Q_{gas} [ml·min^{-1}]	Q_{liq} [ml·min^{-1}]	PPDF [µmol·m^{-2}·s^{-1}]
I	5	100	500	100
II	10	100	1000	200
III	15	100	1500	300

During the acclimation period, the culture was pre-adapted with 5% of CO_2 gas to overcome environmental stress. The initial pH was 7 and was continuous monitored for insure acid condition. Liquid samples were taken three times per day for the measurement of total suspended solid (TSS) (once a day), dissolved oxygen (DO), conductivity, turbidity, temperature and pH.

2.3. Instrumentation

The measurement of CO_2 concentration was determined using the gas analyzer GA 2000 (Geotechnical instrument). The pH, DO, temperature and conductivity were measured using the multiparameter probe (Hanna HI9829 Multiparameter) while the turbidity was measured using 2100N Turbidimeter-Hach. The biomass concentration was determined by getting the total suspended solids (TSS) according to the standard method 2540 D.

3. Results and discussions

3.1. Biomass concentration

During the second (II) and third (III) stage the best algae growth conditions were obtained. In stage II the mineral medium BBM was modified in order to balance the increased carbon load and the nutrients. The third (III) stages were carried out by feeding respectively 10 and 15 % of CO_2 concentrations into the photo-bioreactor. Each stage was performed for 7 days, and the amount of microalgae biomass observed daily at various stages is presented in Figure 1. According to this diagram, it can be seen that by increasing the light intensity from 100 (stage I) to 300 µmol m^{-2} s^{-1} (stage III), the microalgae biomass also increased. Microalgae are photoautotrophic organism, so the availability of light (as energy source) for the biofixation of inorganic carbon sources (e.g. carbon dioxide) is necessary. Figure 1 shows that in the II and III stages a biomass concentration of 762.1 mg L^{-1} and 1450 mg L^{-1}, respectively, was achieved at 200 and 300 µmol m^{-2} s^{-1}. Chlorella vulgaris strains are capable of producing high-valuable compounds, which can contribute to the economic sustainability of the photosynthetic CO_2 fixation and utilization process. Carbohydrates, protein and lipids accumulation triggered by nitrogen starvation is an effective ways to obtain added-value biomass (Nayak et al., 2019). Carbohydrate, lipids and protein content in C. vulgaris are 7 ± 1%, 8 ± 2% and 15 ± 3%, respectively (Kong et al., 2013).

3.2. CO_2 capture efficiency

During the first (I) and second (II) stage, a CO2 concentration of 5% and 10 % was supplied. As reported in Table 1, during the third (III) a higher CO_2 concentration was supplied (15 %), corresponding to an inlet load (IL) of 52.65 g $m^{-3}h^{-1}$. The L/G ratio was adjusted at 10, corresponding to a liquid recirculation from the photo-bioreactor and the vertical adsorption column of 1000 ml min^{-1} and a gas flow of 100 ml min^{-1}. During the II stage a 65.1% capture of CO_2 was achieved with a biomass concentration of 762.1 mg L^{-1}. When a microalga biomass concentration of 1450 mg L^{-1} was achieved, a removal efficiency of 80.8 % was obtained, at an IL of 52.65 gm^{-3} h^{-1}.

3.3. Harvesting

Preliminary results showed that it was possible to efficiently collect the microalgae biomass by using a dynamic membrane module with an average pore size of 20 µm. The average harvesting rate was 50 g m^{-2} h^{-1} when the biomass concentration was 1450 mg L^{-1}.

4. Conclusion

CO_2 removal efficiency (RE) by Chlorella vulgaris was boosted through obtaining the best condition in an advanced sustainable biotechnology-based control system considering four parameters: pH, light intensity, inlet load and liquid recirculation rate. The results highlighted that by increasing the CO_2 concentration from 5% to 15%, the maximum concentration of biomass and CO_2 removal

efficiency (RE) were increased up to 1450 mg L^{-1} and 80.8 %, respectively. The maximum CO_2 elimination capacity (EC) of *Chlorella vulgaris* at CO_2 concentration of 15% was 42.5 g m^{-3} h^{-1}. Finally, an optimal L/G ratio of 15 and a PPDF of 300 µmol m^{-2} s^{-1} allowed increasing both CO_2 capture and biomass productivity. The present study shows that the investigated technology is useful to capture CO_2 and produce biomass. In addition to that, several studies reported that it is possible to biodegrade aromatic substances such as volatile organic compounds (VOCs) thanks to the oxidation capability of the microalgae, thus creating a highly environmental friendly system that potentially could treat GHGs and pollutants.

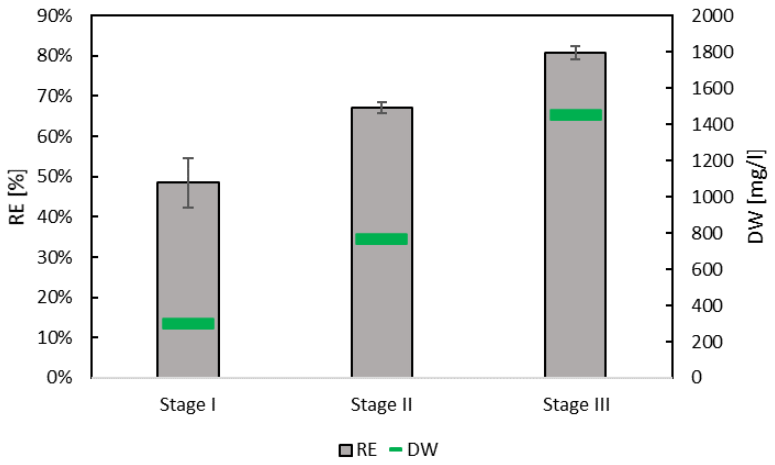

Figure 1. Dry weight (DW) of biomass and CO_2 removal efficiency (RE (%)) during the tree stages investigated.

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