

CO₂ Biofixation by *Dunaliella Salina* in Batch and Semi-Continuous Cultivations, Using Hydrophobic and Hydrophilic Poly Ethylene (PE) Hollow Fiber Membrane Photobioreactors

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Abstract

In this work, performance of hollow fiber membrane photobioreactor (HFMPB) on the growth of *Dunaliella Salina* (G26) at various aeration rates (0.1 and 0.2 VVm) and medium re-circulation flow rates (500 and 1000 mL/h) were studied. Cultivation was carried out at both batch and semi-continuous modes in HFMPBs containing neat and hydrophilized in-house fabricated poly ethylene (PE) membranes at fixed light intensity of 300 $\mu\text{mol}/\text{m}^2.\text{s}$ and temperature of 30°C. Microalgae showed better growth in hydrophobic module in both cultivation modes and modules. Maximum biomass concentration, CO₂ biofixation and specific growth rates equal with 0.71g/L, 1.102g/L/d and 0.2241/d were obtained for non-wetted membranes, respectively. Comparing the performance of both modules showed that the impact of cultivation mode on the CO₂ biofixation rate and CO₂ removal is more pronounced than the impact of mass transfer resistance in membrane contactors. The obtained results show that the mean CO₂ biofixation rates in semi-continuous cultivation for both neat and hydrophilized modules are higher than that in batch cultivation in all operating conditions. It was also found that the hydrophobic membranes are much preferable than hydrophilic membrane in HFMPBs.

Key words: Biofixation, Carbon Capture, Microalgae, Hollow Fiber Membrane, Photobioreactor (HFMPB), Membrane Wettability

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1. Introduction

Emission of Carbon dioxide is amongst the most important reasons of global warming. Concentration of CO₂ in flue gas emitted from thermal power stations is about 500 times higher than that in the atmosphere [1]. The energy sector was the most important source, accounting for 71.8% of the total emissions in 2008; energy industries and transports were the most important representing sources, respectively, 24.8% and 24.9% of the GHGs emissions. The atmospheric partial pressure of CO₂ (p_{CO_2}) is predicted to nearly double (i.e., 750ppm) over the coming century. Several chemical and physical techniques have been applied for CO₂ removal from the power plant flue gases; including chemical and physical absorption, adsorption, and cryogenic distillation. Since these methods present some significant challenges, an ideal alternative solution would be to microalgal capture of CO₂ in biomass. Microalgae can uptake the carbon dioxide from effluent gas as the carbon source for photo-synthesis. Microalgae are fast-growing unicellular microorganism that able to divide their cells within 3-4 h, but mostly divide every 1-2 days under favorable growing conditions. Due to their simple cell structure and fast growth rate, microalgae are expected to have CO₂ bio-fixation efficiency of 10-50 times higher than terrestrial plants. Furthermore, their produced biomass contains high amount of lipid, protein, fiber and other valuable components so that they can be utilized in production of medications, food additives and biofuel.

Cultivation of microalgae can be done either in open ponds or closed systems (photo-bioreactors). Closed photobioreactors are advantageous than open ponds for higher biomass productivity because of their reduced contamination, and better control of cultivation conditions [2]. Photobioreactors appear in different configurations: vertical column reactors (bubble columns or air-lift); tubular reactors; flat plate reactors and membrane photo-bioreactors [3]. The integration of a membrane contactor with a photobioreactor serves two major purposes for the mitigation of CO₂ by microalgae, i.e., to enhance the mass transfer and interfacial contact between two different phases and to increase the exchange process of CO₂-O₂ by microalgae in the photobioreactor [4]. Fan *et al.* report that CO₂ retention in a *Chlorella* culture increased over 30% when compared with a sparging unit.

The current conventional injection technique that is used to supply CO₂-enriched air to the culture system creates bubbles that have random sizes [5]. This can impede the uniform dispersion of gas throughout the media culture, which will have an adverse effect on the productivity of the microalgae. In addition, the injection of non-uniform, excessive concentrations of CO₂ can result in high hydrodynamic stress that can kill the microalgae and increase the release of CO₂ to the atmosphere [6]. Thus, well dispersion of CO₂ is essential for the success of the biofixation process. Reminding the fact that membrane contactor had shown high potential to achieve high CO₂ mass transfer efficiencies in small reactor volume.

Several reports have been published regarding the successful application of various membrane fiber types and distribution specification such as membrane sparger, membrane contactors and bubbling gas [4,7,8]. The obtained results showed that due to the membrane wetting, the overall performance associated with HFMBP is still not satisfactory from engineering point of view. Wetted mode occurs when the membrane pores are filled with liquid, e.g., if the liquid phase is aqueous, pore wetting would be easily happened. It is predictable that utilization of hydrophobic membrane in presence of aqueous phase will show lower mass transfer resistance, however membrane fouling as one of the main drawback of membrane integrity in practical applications in long term operation, would be much lower in case of hydrophilic membranes [9]. Therefore it is critical for engineers to evaluate the impact of the type of membrane on the HFMPB performance versus other parameters.

In our previous work, the performance of hydrophilic and hydrophobic hollow fiber membrane photobioreactors (HFMPBs) in batch and semi-continuous cultivation of *Synochococcus elongatus* was deeply investigated [10]. Despite of the membrane fouling behavior, obtained results confirmed that the hydrophobic membrane works much better than hydrophilic membrane in CO₂ biofixation. *Synochococcus elongates* cyanobacter however is very sensitive to the medium pollution and very severe control of cultivation medium is required. Microalgae *Dunaliella Salina*, a green, single-celled alga, has numerous advantages, has been found to contain various forms of beta-carotene that

are far more powerful and active than the regular type that naturally found in vegetables, plant and even fruits. From cultivation point of view, it easily resists against medium pollution, does not need any fresh water and can assimilate required nitrogen, phosphorus and carbon sources from salty water resources [11]. Therefore, it is worth to look for an efficient method of cultivation with high biomass productivity and at the same time with high degree of CO₂ biofixation.

The main purpose of this study was to determine the effects of various aeration and medium re-circulation flow rates on cultivation of *Dunaliella Salina* in hollow fiber membrane photobioreactor containing hydrophobic and hydrophilized poly ethylene (PE) membranes. In order to prevent self-shading in dense algal culture and optimize the light availability for cells, the performance of HFMPB were examined in both batch and semi-continuous cultivations [12,13].

2. Materials and methods

2-1. Strain and culture conditions

The strain *Dunaliella Salina* was used and kept in modified Johnson medium throughout the experiments according to Hejazi *et al.* [8]. The pH of the medium was adjusted at 7.5 using HCl (37 wt%) and the medium was autoclaved at 121°C for 30 min before inoculation.

2-2. Preparation of hydrophobic and hydrophilized polyethylene (PE) hollow fiber membrane

Similar with our previous work [10], the commercial grade of high density

polyethylene (M_w ca. 119500 g/mol) was provided by Amirkabir Petrochemical Company (Iran) and was used as received. Mineral oil (MO) as diluent, n-hexane as extracting agent and Irganox 1010 as heat stabilizer were purchased from Acros Organics, Merck and Ciba Co., respectively. Measured amounts of PE, MO and Irganox 1010 were fed into the vessel of a house-made batch type extruder. Mixture was heated to 160-170°C and then mixed for 35 min at 40-45 rpm. After preparation a homogeneous dope solution, stirring was stopped and the homogenous solution was left for 25 min to release gas bubbles. Prepared homogenous polymer solution was driven through a spinneret using inert nitrogen gas. The inner and outer diameters of spinneret were about 0.80 mm and 1.50 mm, respectively. Mineral oil was fed into the inner orifice to make a lumen within the hollow fibers. The hollow fiber was extruded throughout the spinneret and wound on a take-up winder after passing through the coagulation bath containing cold water (10°C) to induce phase separation. Fabricated membranes were soaked into n-hexane/acetone mixture to extract remained MO from the membrane and were then washed with di ionized water for several times.

In order to prepare hydrophilized PE hollow fiber membrane, fabricated membranes were soaked in the oxidizing aqueous solution consisting 80 and 1wt% of sulfuric acid and $KMnO_4$ (oxidizing agent), respectively, for 25 min and subsequently were soaked into the deionized water for 10 min to stop the oxidation reaction.

Water contact angel of hydrophobic and hydrophilized PE hollow fiber membranes were about 128 and 82, respectively.

2-3. Hollow fiber membrane photo bioreactor

A schematic of HFMPB is shown in Fig. 1. An aeration gas was supplied to the lumen of the hollow fiber membranes. The reactor was constructed of tubular plexi-glass sealed with epoxy-resin. Overall length of reactor was 42 cm and the total volume was 157 mL. Fabricated membranes, described above, were used to prepare hollow fiber membrane modules. Modules were called PE-HFMPB and HPE-HMPB, when neat and hydrophilized PE hollow fiber membranes were used, respectively. Each module contained 35 fibers with an active length of 38 cm, outer diameter of 790 μm , and inner diameter of 420 μm . The membranes occupied 4% of the photo bioreactor total volume and total surface area for transfer was 318 cm^2 . A water jacket was installed around the modules in order to keep the temperature constant. Liquid re-circulation was provided using a peristaltic pump and a reservoir tank. In each run, fresh culture medium was added to the tank by a 100 mL Burette and circulated in HFMPB by peristaltic pump. On the contrary with conventional airlift and bubble column photobioreactors, there is no direct contact between gas and liquid phases and CO_2 bubble penetrate into the liquid culture via a micron size pores existed on the membrane surface. Therefore, the impact of gas flow rate on the mass transfer resistance and consequently, on the mean growth rate and biomass productivity is much less than other parameters such as liquid re-circulation flow rate.

Detail information regarding previous published work [10]. manufacturing of HFMPB is described in our

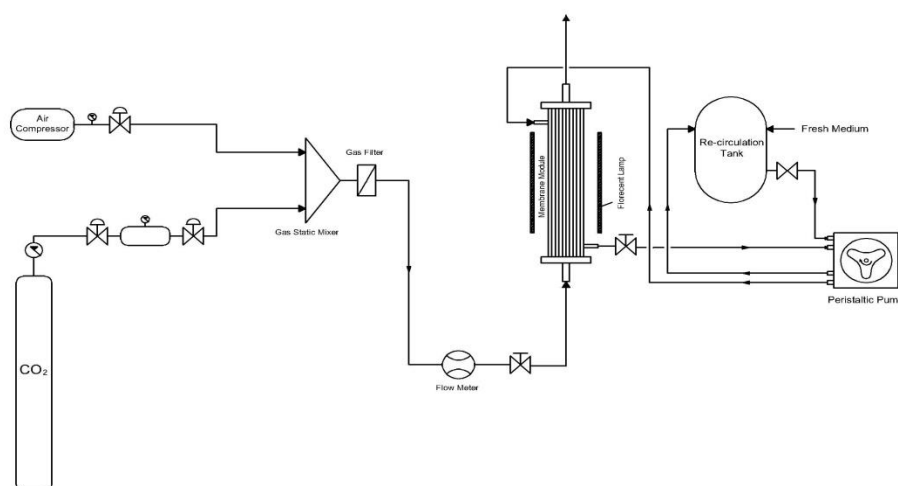


Figure 1. Schematic of hollow fiber membrane photo-bioreactor (HFMPB) experimental set-up.

2-4. Experimental design for batch and semi-continuous cultivations

The photo bioreactor and re-circulation tank was filled with 160 mL Modified-Johnson medium [14]. Medium was inoculated with 20 mL pre-cultured *Dunaliella Salina*. Initial biomass concentration in photo bioreactor was about 0.1 g/L. Various samples were taken in pre-defined time intervals to determine optical density. Continuous illumination of $300 \mu\text{mol}/\text{m}^2\cdot\text{s}$ was provided using fluorescent lamps. For batch cultivations in both PE-HFMPB and HPE-HMPB, inlet feed gas and liquid re-circulation flow rates were fixed at 0.1 VVm and 500 mL/h, respectively.

Once culture reached early stationary phase, the semi-continuous regime was

started with a daily renewal of culture. Based on the maximum CO_2 biofixation and related biomass concentration (C_{max}) in batch culture, the value of C_0 was adjusted to start semi-continuous experiments. As shown in table1 semi-continuous cultivations were performed in various aeration and medium re-circulation flow rates. Before adding fresh medium, the culture was sampled and optical density was measured in each time interval. Measured value was used to calculate the amount of fresh medium to keep the cell concentration at initial set value. Depends on final daily biomass concentration, dilution rate was varied for PE-HFMPB and HPE-HFMPB.

Table 1

Operation varied parameters for semi-continuous cultivation of *Dunaliella Salina*.

| No. of Run | Operation Conditions | |
|------------|----------------------|---|
| | Aeration rate (VVm) | circulation rates of culture fluid (mL/h) |
| 1 | 0.1 | 500 |

| | | |
|---|-----|------|
| 2 | 0.2 | 500 |
| 3 | 0.1 | 1000 |
| 4 | 0.2 | 1000 |

2-5. Analytical methods

The optical density was measured at 680 nm wavelength (Unico-2100 spectrophotometer). Then, the correlation between OD and biomass concentration was established and therefore, the biomass concentration was estimated from the optical density (OD) data of the culture. Ash free dry weight (AFDW) was determined by filtering 10 mL of culture through preconditioned Whatman GF/C glass fiber filters (UK), drying at 105°C to constant weight, and heating at 550°C for 1 h [15].

Biomass productivity, Pb (dry g/L/day) in batch mode was estimated from the following equation:

$$Pb = \frac{X_f - X_i}{T} \quad (1)$$

Where X_f and X_i are the final and initial dry biomass concentrations (g/L), respectively, in the batch test period, T (day) [16].

The specific growth rate in batch and semi-continuous cultivation was calculated using the Eq. 2 and Eq.3, respectively as shown below:

$$\mu = \frac{1}{X} \left(\frac{dX}{dt} \right) \quad (2)$$

$$X = X_0 \exp(-\mu t) \quad (3)$$

The rate of growth of the cells in Eq. 2 was estimated numerically by second-order difference method and for Eq. 3 by fitting to the experimental values of X in growth curves.

The carbon fixation rate was calculated from the elemental analysis of the biomass, as shown in Eq. 4 [16]:

$$R_C = C_C \times \left(\frac{X_m - X_0}{T} \right) \times \left(\frac{M_{CO_2}}{M_C} \right) \quad (4)$$

Where C_C is the average carbon content (w/w) in dry biomass, M_{CO_2} and M_C represented the molecular weights of CO₂ and elemental carbon, respectively. X_m and X_0 are the final and initial dry biomass concentrations (g/L), respectively in the batch test period T (day). Due to proposed general structure for microalgae CO_{0.48}H_{1.83}N_{0.11}P_{0.01} [17] carbon content (C_C) is about 50%.

3. Results and discussions

3-1. Effect of wettability on the biomass concentration of culture in batch cultivation of *Dunaliella salina*

Among several influential parameters, the impact of wettability was studied on the biomass concentration of the culture, while other parameters such as light intensity and initial algal density were kept constant. Fig. 2. Shows the growth curves of culture medium as a function of time for the *Dunaliella salina*. According to Fig. 2 in each similar run, PE-HFMPB shows higher biomass concentration of *Dunaliella salina* than HPE-HFMPB. The time that lasted to reach stationary phase in each experiment of HPE-HFMPB is approximately twice in comparison with PE-HFMPB. Since membranes operated in dry mode and mass transfer is suitable, CO₂ transfer is more efficient in PE-HFMPB than HPE-HFMPB.

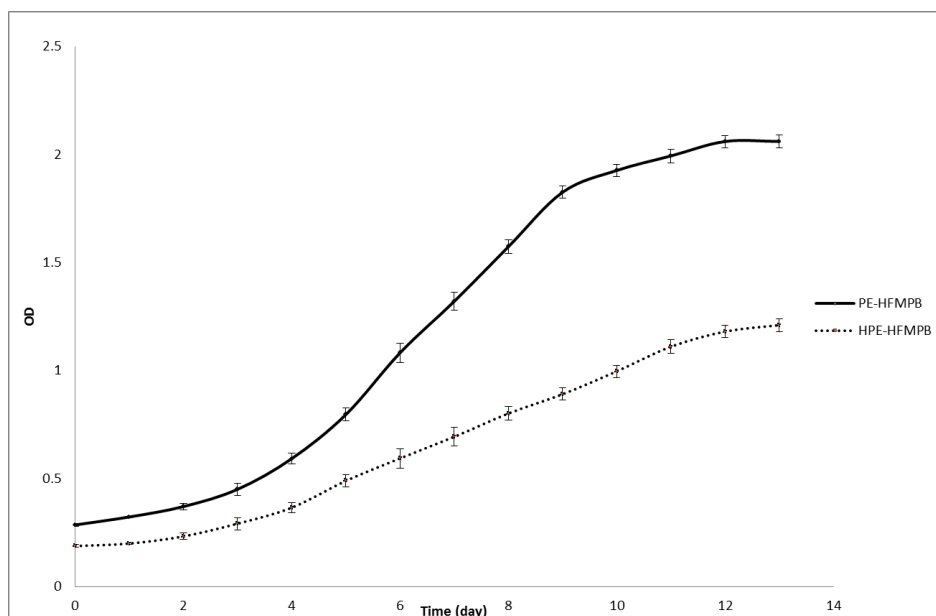


Figure 2. Evolution of ash-free dry weight (AFDW) during the batch experimental growth of *Dunaliella Salina* with bubbling in the HFMPB under 0.1 VVm gas flow rate, 500 mL/h liquid recirculation, fixed light intensity of 300 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ and temperature of 30°C).

Maximum specific growth rate (μ) is a suitable index for determination of initial biomass concentration for semi-continuous

cultivations [18,19]. As shown in Fig. 3 maximum growth rate for PE-HFMPB was achieved in 6th day and was about 0.31 1/d.

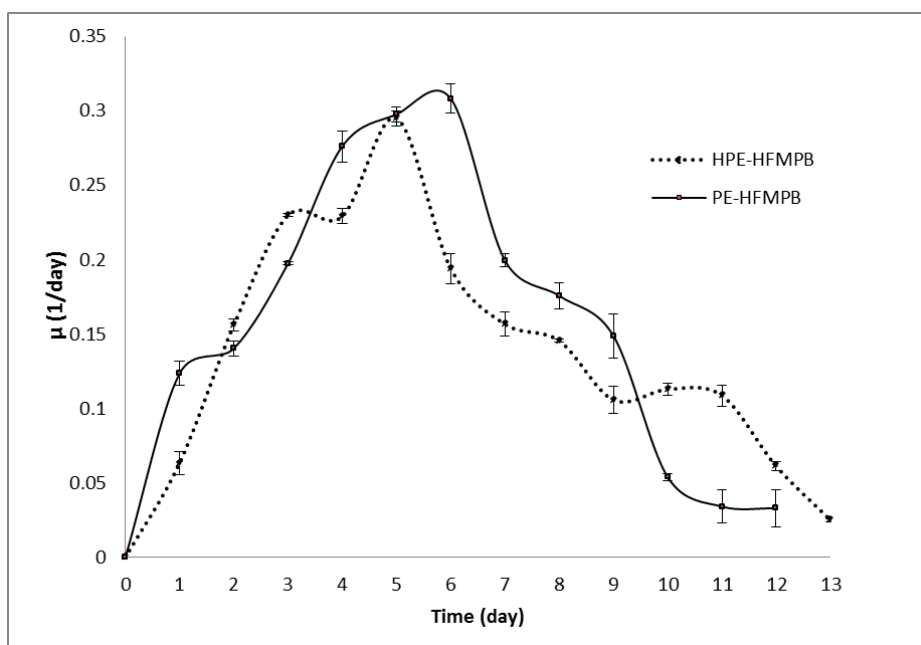


Figure 3. Specific growth rate of *Dunaliella Salina* in HFMPBs during the batch cultivations under 0.1 VVm gas flow rate, 500 mL/h liquid recirculation, fixed light intensity of 300 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ and temperature of 30°C.

3-2. Effect of aeration flow rates and medium re-circulation on cell growth in semi-continuous mode using hydrophobic module

Semi-continuous cultivation of *Dunaliella salina* was carried out to examine the ability of cell growth in the HFMPBs using high-density culture. In this first series of experiments, the impacts of aeration rate and liquid re-circulation flow rate on the growth rate and biomass productivity were carried out using hydrophobic (PE-HFMPB) module. Since maximum biofixation was occurred in early exponential phase [20], initial inoculums in semi-continuous mode were determined in the range of that phase. The semi-continuous culture experiments were performed by various aeration and liquid flow rates. Mean specific growth rates are shown in Fig. 4, indicates that increase in

liquid flow rate leads to high cell growth. However increase in aeration flow rate decrease the growth rate since decrease retention time (RT) of gas in medium [21]. It is necessary to mention that, Mean specific growth rate of 0.22 1/d was obtained for PE-HFMPB in 0.1 VVm and 1000 mL/h of aeration and liquid re-circulation flow rate, respectively. Obtained results showed in Fig. 5, indicates that with increasing of aeration rates, biomass productivity decreases. However, the increase in biomass productivity is more pronounced at higher circulation rates of culture fluid. It is mainly due to the decrease in the boundary layer adjacent to the membrane surface, when the liquid flow rate is increased, which decreases the overall mass transfer resistance. Obtained result were in good agreement with the other published works [22].

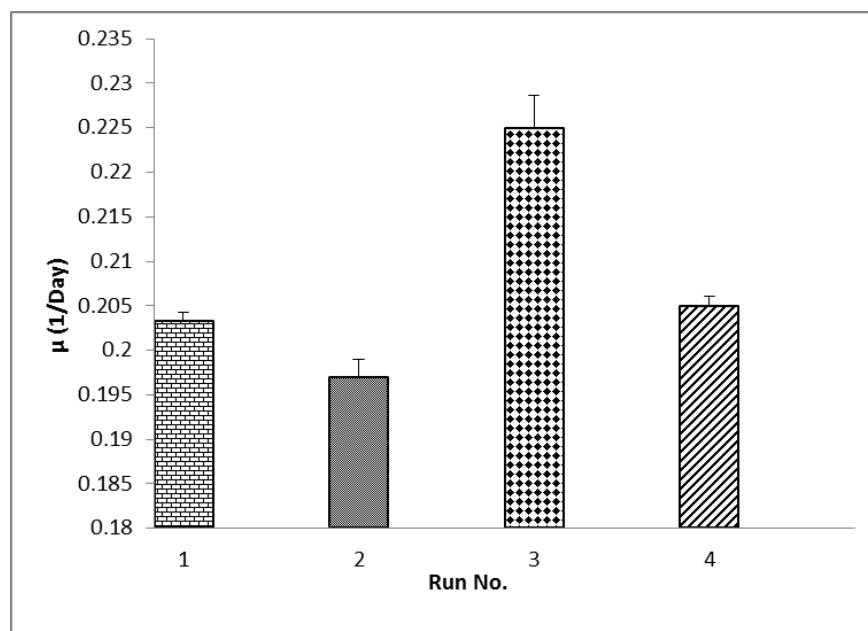


Figure 4. Mean specific growth rate of *Dunaliella Salina* in semi-continuous cultivations in HFMPBs at various operating conditions (mentioned in table1).

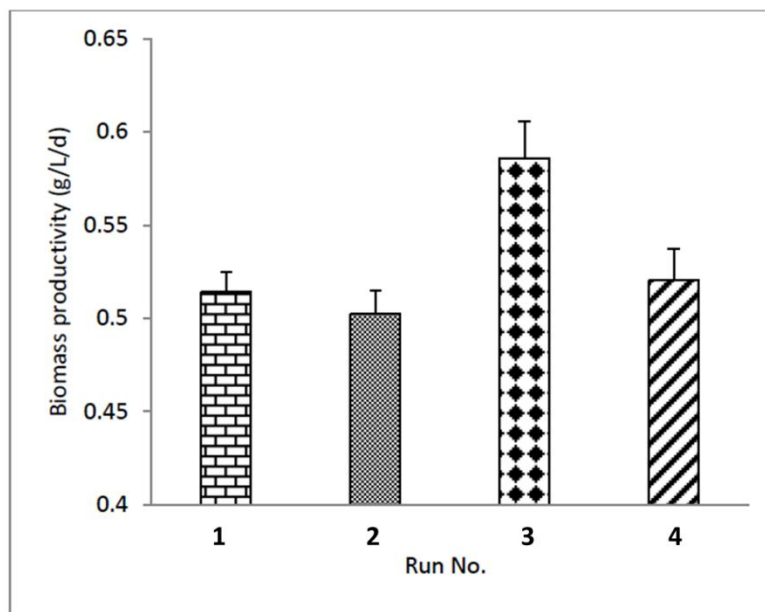


Figure 5. Effect of various aeration and liquid recirculation flow rates on biomass productivity of *Dunaliella Salina* in semi-continuous cultivations.

Figs. 6 and 7 show the growth curves of PE-HFMPB and HPE-HFMPB under above-mentioned conditions. Obtained results in these figures confirm that the effect of varying CO_2 concentration on growth of *Dunaliella Salina* in semi-continuous mode was similar to batch mode. As shown in Fig.

6, higher biomass concentration was obtained in the 0.1 VVm gas flow rate for both modules. In addition, when liquid recirculation flow rate increases, biomass concentration at each time interval also increases.

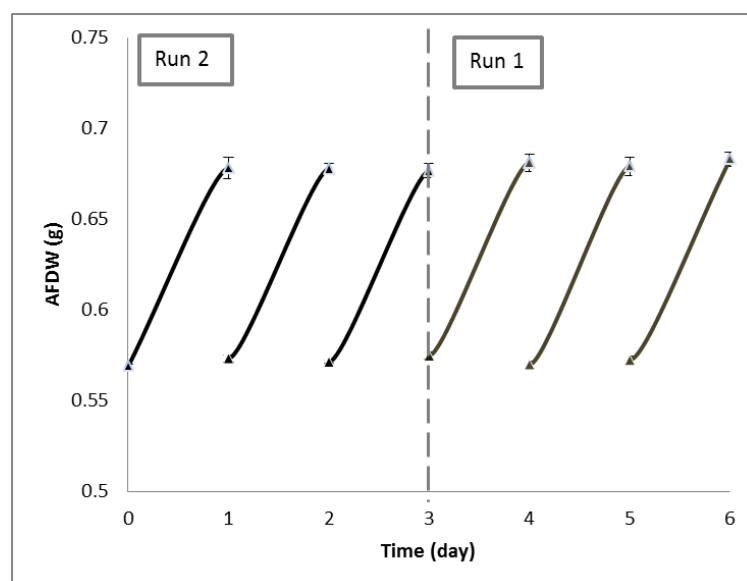


Figure 6. Evolution of ash-free dry weight (AFDW) during the semi-continuous cultivations of *Dunaliella Salina* in the HFMPB under different operating conditions (Run1: 0.1 VVm and 500 mL/h aeration and liquid flow rates, Run2: 0.2 VVm and 500 mL/h aeration and liquid flow rates, respectively).

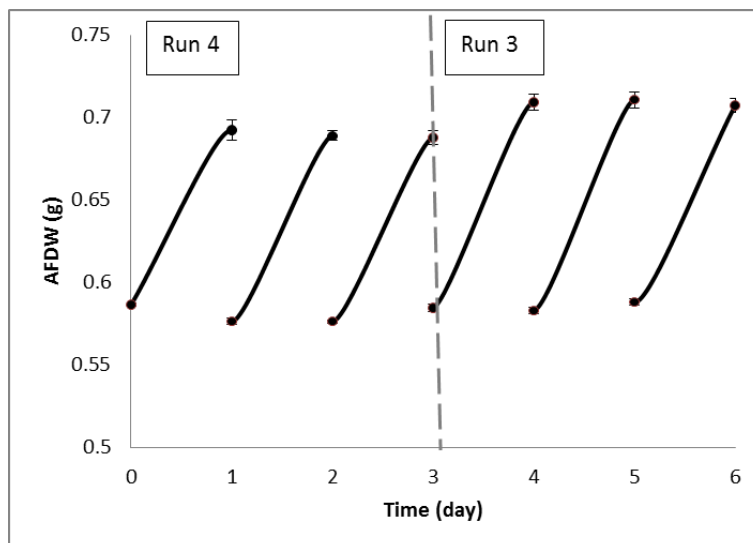


Figure 7. Evolution of ash-free dry weight (AFDW) during the semi-continuous cultivations of *Dunaliella Salina* in the HFMPB under different operating conditions (Run3: 0.1 VVm and 1000 mL/h aeration and liquid flow rates, Run4: 0.2 VVm and 1000 mL/h aeration and liquid flow rates, respectively).

3-3. Comparison the performance of PE-HFMPB and HPE-HFMPB in CO₂ biofixation in various operational conditions and cultivation modes

The performance of hydrophobic and hydrophilic PE hollow fiber membrane photobioreactors, PE-HFMPB and HPE-HFMPB, in batch and semi-continuous cultivation of *Dunaliella Salina* in various aeration and medium re-circulation flow rates was also examined. Fig. 8 compares the mean CO₂ biofixation rates for both modules. The obtained results show that the mean CO₂ biofixation rates in semi-continuous cultivation for both neat and hydrophilized HFMPBs are higher than that in batch

cultivation in all operating conditions. These results confirm that the impact of cultivation mode on the CO₂ biofixation rate is more pronounced than the impact of mass transfer resistance in membrane contactors. Interestingly, the mean CO₂ biofixation rates in PE-HFMPB modules are considerably higher than of HPE-HFMPB which imply that hydrophobic membranes are much preferable than hydrophilic membrane. Since most of cheap and locally produced polymers are inherently hydrophobic, it gives good impression to deeply investigate the performance of HFMPB fabricated by locally produced polymers.

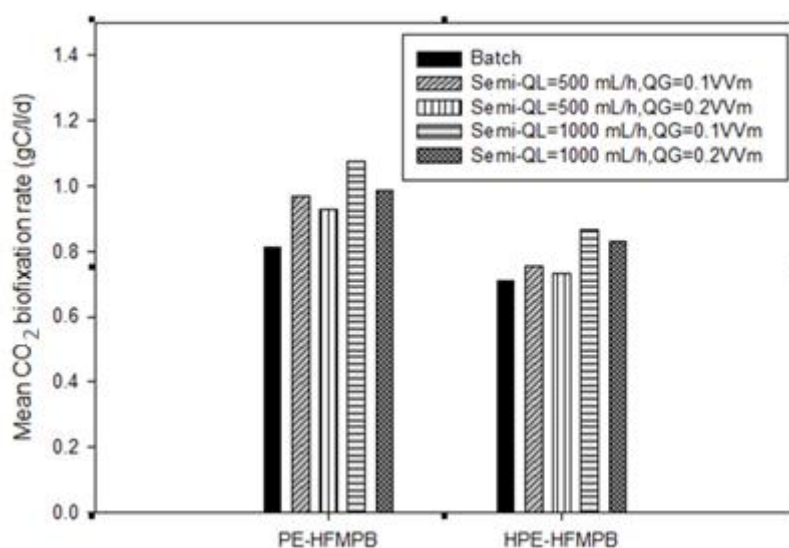


Figure 8. Comparison the performance of PE-HFMPB and HPE-HFMPB in CO₂ biofixation at various operation conditions and cultivation modes.

4. Conclusions

HFMPBs have the potential to achieve high CO₂ mass transfer efficiencies and biomass productivity in small volume of reactors. Semi-continuous method showed high potential as an effective approach for producing further biomass than batch culture. It was confirmed that the impact of aeration flow rate and medium re-circulation on cell growth are important parameters on cell growth rate. It can be concluded that all feed gas flow rates and liquid re-circulation flow rates, the performance of hydrophobic module is better than the hydrophilic one.

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