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Effect of gas holdup on CO_2 biofixation by *Spirulina* sp. in the 20-liter airlift bioreactor

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Abstract

The rise of CO_2 concentration in the Earth is a major environmental problem, which cause global warming. To solve this issue, several methods have been applied, but among these solutions using microalgae is an eco-friendly and cost-effective way of reducing carbon dioxide, as they can efficiently sequestrate CO_2 and produce biomass as valuable products. In this study, hydrodynamic parameters, bubble sizes and carbon dioxide uptake were investigated in an airlift bioreactor. Experiments were studied at two different superficial gas velocities (0.185 and 0.524 cm/s) for *Spirulina* sp. microalgae into a 20-liter airlift bioreactor to find out the amount of carbon dioxide sequestration and cyanobacterial biomass. The highest efficiency of carbon dioxide removal and maximum dry weight of *Spirulina* sp. were achieved 55.48% and 0.86 g/L respectively at 5% CO_2 (v/v) and superficial velocity of 0.185 cm/s. This experiment was conducted in 7 days, light intensity (2600 lux/m^2), temperature (30 ± 2 °C) and a light-dark cycle (12-12), which all were constant. The hydrodynamic parameters studied by *Spirulina* sp. demonstrated a capability of CO_2 sequestration in this airlift photobioreactor.

Keywords Airlift bioreactor . Bubble dynamic . CO_2 biofixation . Gas holdup . Liquid circulation . Velocity . Microalgae biomass

Introduction

Global warming caused by human activities is one of the major environmental problems that has received much attention in the last two decades. One of the reasons for the increase in temperature and global warming is the presence of greenhouse gases, especially the high concentration of carbon dioxide in the Earth's atmosphere (Wang et al. 2017). This rate was 260-280 ppm before the Industrial Revolution, and has increased significantly since then, reaching 381 ppm in 2005 and recording the warmest year of the last century. This increase in the last 100 years is between 0.18 and It was 0.74 °C (Hansen et al. 2006). Due to CO_2 emissions have reached a high record (37%) compared to the late 18^{th} century and the annual increase of 0.2 K over the last 3 decades, the elimination of greenhouse gases, especially CO_2, has become inevitable (Blasing &Smith 2016). Among the various methods for reducing CO_2 physical absorption, chemical fixation, soil carbon sequestration, biochar and CO_2-enhanced oil recovery, biological fixation of CO_2 by photosynthesis of microalgae as an economical and environmentally friendly method is increasingly considered (Chaudhary et al. 2018, Prazeres et al. 2016,Yadav &Sen 2017). The process of removing carbon dioxide using microalgae requires knowledge of cell biology, important factors influencing this process such as: temperature, pH, light, microalgae species, microalgae culture density, CO_2 concentration, photobioreactor hydrodynamic parameters (mass transfer, light distribution) (Almomani et al. 2019a, Ferreira et al. 2012, Karemore et al. 2015). Besides, for the fixation of CO_2 by microalgae on a large and practical scale, there are problems such as limitation of the microalgae growth at high concentrations of CO_2, the need for large amounts of nutrients, light (Sevda et al. 2017) and low CO_2 conversion due to short residence time (Zhao &Su 2014). Therefore, selection of suitable microalgae species for resistance to high concentrations of CO_2 is considered as an important step in the biological removal of CO_2 (Bhola

et al. 2014, Zhao &Su 2014). Among different species of cyanobacteria, *Spirulina* can be used, which not only has ability and resistance to environmental conditions, but also, has the ability to absorb carbon dioxide in different concentrations of CO_2 (Morais et al. 2018) for instance, it is reported that a significant CO_2 biofixation ($R_{CO2} = 0.360\ g_C\ L^{-1}\ d^{-1}$ for *Spirulina platensis (SP.PL)* in (10 v/v%) CO_2 as feed gas (Almomani et al. 2019b). Also, For *Spirulina* sp. at (6% and 12% (v/v)) CO_2, Maximum CO_2 biofixation was 53.29% and 45.61%, respectively (de Morais &Costa 2007). In addition, to grow microalgae for removing carbon dioxide different type of closed photobioreactors (PBRs) are used, but airlift bioreactors (ARLB) are the most common configuration; Important advantages of this type of reactor than the others are better mixing, high mass transfer rate, wider range of inlet gas flow and application for higher viscosity fluids and lower energy consumption (Chisti 1998, Pourtousi et al. 2016, Wadaugsorn et al. 2016). These reactors are actually a modified type of bubble columns that have a specific path for the liquid to move. Like bubble columns, agitation occurs by inlet gas to the device and by providing low shear stress and high mass transfer, they are suitable devices for CO_2 biofixation (Chisti 1989, Ugwu et al. 2008). The overall mass transfer coefficient is a key factor, which the oxygen removed from these bioreactors is calculated with it. Because gas holdup is directly related to the mass transfer of gas and liquid phases in riser and downcomer, calculation of gas holdup plays an important role to performance of (ARLB) (Guo et al. 2015, M et al. 2019). Gas holdup have been studied in different operation condition of airlift photobioreactors as an overall gas holdup, holdup in riser and downcomer (Rengel et al. 2012). Besides, gas holdup is affected by superficial gas velocity, which is called gas flow. By increase gas flow into the reactor, gas holdups in riser and downcomer increase as a result, liquid circulation velocity rise and bubbles which move smoothly in the downcomer and riser and they have spherical shape, despite keeping their shape, larger bubbles

move faster and descend to the downcomer which lead to the better light accessibility to microalgae (Ding et al. 2021, Rengel et al. 2012). However, in higher air flow rates, as liquid circulation velocity increases microalgae cannot stay a long time in downcomer so that they are not able to utilize light efficiently (Sánchez Mirón et al. 2000).

The aim of this present study was investigation of overall gas holdup and bubble sizes influence on CO_2 biofixation by *Spirulina* sp. at two different superficial gas velocities (0.185 and 0.524 cm/s) in an airlift photobioreactor.

Methods

Microalgal culture and growth

To perform this experiment, the desired species of *Spirulina* sp. was prepared from Science and Technology Park, Bushehr, Iran. The solution of culture medium and microalgae should be proportional to the volume of the reactor 20 L and in the form of 5% (v/v) solution. Since this solution was suitable in terms of amount and concentration to propagate algae well in the reactor, for this purpose, 1 L of $BG11_0$ culture medium (0.04 g K_2HPO_4, 0.075g $MgSO_4.7H_2O$, 0.036 g $CaCl_2.2H_2O$, 6.0 mg citric acid, 6.0 mg ferric ammonium citrate, 1.0 mg Na_2EDTA, 0.02 g Na_2CO_3, and 1.0 mL trace metal A5 solution, which contained 2.86 g H_3BO_3, 1.81 g $MnCl_2.4H_2O$, 0.222 g $ZnSO_4,.7H_2O$, 0.39 g $Na_2MoO_4.2H_2O$, 0.079 g $CuSO_4.5H_2O$, and 49.4 mg $Co(NO_3)_2.6H_2O$) was cultured several times in some Erlenmeyer to reach the appropriate volume of the reactor. The culture medium pH was adjusted to 9.8 by adding1 HCl mol/L or 1 NaOH mol/L (Yoon et al. 2002).

Experimental setup

This research consisted of 3 stages. The first step was to prepare the culture medium for *Spirulina* sp. microalgae and introduce it to the working volume of photobioreactor 16 L. In the second stage hydrodynamic parameters were investigated by performing gas holdup tests, liquid circulation velocity and measuring bubble sizes. For this purpose, distilled water was used at 5 different superficial gas velocities, 1.91, 1.21, 0.75, 0.54, 0.27 cm/s were examined and each experiment was repeated 3 times. In the third stage, carbon dioxide uptake and *Spirulina* sp. microalgae growth were studied by inputting the solution of culture medium and microalgae into the device, and entering the mixture of air and carbon dioxide with 5% (v/v), at two superficial gas velocities (0.185 and 0.524 cm/s), and atmospheric pressure under the following conditions: with 12:12 light–dark (LD) cycle, temperature (30 ± 2 °C) (Mousavi et al. 2018), light intensity 2600 lux/m^2 and 7-day experimental period.

Photobioreactor construction and operation

The photobioreactor used in this experiment Fig.1 was an airlift reactor (Sadeghizadeh et al. 2017). The general structure of this reactor was an internal-loop type which had two vertical and concentric cylinders made of Plexiglas and also an internal distributor of stainless steel rings which was installed at the end of the reactor. The distributor had 18 holes with a diameter of 1 mm on which these holes were made at equal intervals.

In order to control the temperature, a 100-watt heater (Sobo HC-100 l) was used inside the reactor, which kept the temperature conditions constant at 30±2 °C. The reactor working volume was 20 L which filled to a height of 110 cm. Also, in order to mix air and carbon dioxide and measure the

rate of inlet flow, two gas rotameters after calibration were used. Moreover, sampling valves were installed in the reactor to sample the solution. To measure the solution pH and CO_2, a pH meter (Metrohm 827 pH Lab meter), a CO_2 meter (Testo 535), which was installed on top of the reactor, were used. The light required for the test was provided by 4 fluorescent lamps and light intensity emitted (2600 lux/m) was measured by the lux meter (TES-1339R, TES Taiwan).

Gas holdup and bubble dynamic

One of the important parameters in determining the liquid circulation velocity, gas residence time, and overall mass transfer coefficient, is gas holdup which is described as a fraction of a gas-filled reactor volume and calculated from the following Eq. (1) (Chisti 1989):

$$\varepsilon = \frac{H_2 - H_1}{H_2} \quad (1)$$

H_2 and H_1 are liquid heights after aeration and initial in the reactor, respectively.

This parameter is important as the residence time of the gas phase in the liquid phase is determined with the gas volume fraction and affects gas-liquid joint surfaces through the bubble size. Since bubbles of different sizes exist in the dispersed phase, the bubble diameter is used in calculations. The average diameter is a sphere diameter with the same volume to surface ratio as the bubble and is determined by Eq. (2) (Chisti 1989, Dejaloud et al. 2018, Zhang et al. 2014).

$$d_{32} = \frac{\sum_{i=1}^{N} N_i d_i^3}{\sum_{i=1}^{N} N_i d_i^2} \quad (2)$$

which N_i is the occurrence of the bubble with diameter d_i.

Superficial gas velocity

In the airlift reactor gas velocity is equal to the ratio of the gas inlet volume to the riser cross-section shown in Eq. (3) (Zhang et al. 2014):

$$U_g = G_g/A_r \quad (3)$$

U_g, G_g, A_r are superficial gas velocity, inlet flow rate to the airlift reactor, riser cross-section. Superficial gas velocity has a direct impact on gas holdup, which is more visible and bigger on bubbly regimes than in turbulent flow. If the proportion of small and large bubbles in turbulence is investigated, it is observed that when superficial gas velocity increases, large bubble number rises, while the amount of small bubbles is constant. But during bubble flow, bubbles respond directly to superficial gas velocity changes. Dependence of gas holdup on superficial gas velocity is generally as follows by Eq. (4) (Chisti 1989):

$$\varepsilon_g = \alpha U_g^n \quad (4)$$

The parameter n depends on flow regime, operational variables, physical properties of a reactor as well as the design of it. In homogeneous regimes, the parameter n varies from 0.7 to 1.2, and in the heterogeneous regime ε_g is a weaker function of U_g, and n changes from 0.4 to 0.7. In this study parameters α and n were measured for both flow regimes.

Liquid circulation velocity

Due to the difference in fluid density between riser and downcomer in reactors, fluid velocity is caused. Liquid velocity affects many hydrodynamic and mass transfer parameters, including average bubble residence time, bubble size, overall mass transfer rate, and mixing time. In

addition, it influences turbulence, wall heat transfer coefficient, mass transfer coefficient and shear stress. liquid velocity is controlled by changing holdup in riser and downcomer, while gas holdup changes with increasing or decreasing bubble velocity. Mean circulation velocity is defined as Eq. (5) (Chisti et al. 1988):

$$U_{\mathrm{circ}} = \frac{\mathrm{X_{circ}}}{\mathrm{t_{circ}}} \quad (5)$$

x_c, t_c are circulation path length and the average time for a complete circulation respectively. However, superficial velocity values in riser (U_r) and downcomer (U_d) were more useful than mean circulation velocity (U_{circ}). Superficial liquid velocity in riser is calculated with the following correlation (6) which A_d and A_r are downcomer and riser cross-sections (Chisti et al. 1988).

$$U_d \times A_d = U_r \times A_r = m_r = m_d \quad (6)$$

Calculation of CO_2 sequestration rate

To estimate the CO_2 uptake Eq. (7) was used;

$$CO_2 \text{ removal efficiency}(\%) = \left(1 - \frac{\mathrm{CO2\ output}}{\mathrm{CO2\ input}}\right) \times 100 \quad (7)$$

CO_2 output is the concentration of carbon dioxide exited (ppm) from the reactor. CO_2 input is carbon dioxide concentration injected to the bioreactor. The carbon capture rate was determined with CO_2 meter (Testo, Germany).

Optical density and dry weight

Optical density can be used as an indirect method to calculate biomass concentration, which is directly and linearly related to dry weight using a standard diagram. In this experiment to determine biomass concentration a spectrophotometer (DR 5000), centrifuge (Sigma 101) were used and *Spirulina* sp. dry weight was measured by changing OD 680nm of cyanobacteria. Eq. (8, 9) were established suitable relationships between *Spirulina* sp. dry weight and OD 680nm at two superficial gas velocities.

$C_b = 1.071x + 0.1011$ ($R^2 = 0.835$, $U_g = 0.185$) (8)

$C_b = 0.718x + 0.2869$ ($R^2 = 0.9136$, $U_g = 0.524$) (9)

C_b was biomass concentration (g L^{-1}) and x was OD 680nm.

Results and discussion

Gas holdup and bubble passage in reactor

In this study the total gas holdup test was performed for two different systems. In the first system, there was distilled water inside the reactor, which was aerated by air. In the second system, inside the reactor was filled with a mixture of microalgae and culture medium, which was aerated with a mixture of air and carbon dioxide. In airlift reactors, gas holdup is affected by liquid circulation velocity, which depends on the gas-liquid separation area in reactor head-space and also reactor height, as a result, the relationship obtained is highly dependent on geometry (Wadaugsorn et al. 2016).

Fig.2 shows that with increasing superficial gas velocity (0-2 cm/s), gas holdup rises. On one hand, in this study, factors such as gas and liquid densities ρ_g and ρ_l, surface tension of the liquid σ, gas

and liquid viscosities $\mu_g$and μ_l and gravitational constant g, which impacted on gas holdup in Hikita, Hikita and Kikukaw, Reilly et al., Hughmark and Joshi and Sharma correlations, were neglected as in this study Eq.1 was used. Therefore, a difference between gas holdup experimental data and empirical relationships was demonstrated. On the other hand, experimental data by neglecting factors gave errors about 15% were closely consistent with Hikita data (Hikita et al. 1980), which total gas holdup changes linearly with inlet gas velocity in this range of velocity. The reason for this was the change in the shape and nature of gas bubbles at different superficial velocities, which were noticed in the higher velocity of the inlet gas, therefore larger bubbles were formed and due to turbulent flow these bubbles were broken into smaller bubbles. Besides the higher inlet gas, the faster the bubbles broke then the quicker they coalesced, and the gas holdup increased (Blažej et al. 2004). In general, as gas velocity rises, bubble sizes also increase, and the accumulation of large bubbles in the center and small ones along the walls of the reactor are observed. Also, in total gas holdup the percent of small bubbles is more than large bubbles. What is more, rise of small bubbles gradually decreases, and if gas velocity increases, the rate of small bubble rise reaches a constant value after a while. While the rate of ascent of large bubbles rises continuously with increasing gas velocity, this growth will continue as long as it is the bubble flow, after which the changes decrease and even stop (Chisti 1989, Ojha &Al-Dahhan 2018).

In other words, the type of flow tends from bubble to turbulence. According to Eq. (4), dependence of gas holdup on superficial velocity in airlift reactors for the homogeneous regime is calculated as $\varepsilon_g = 0.0045U_g^{0.907}$ and for the heterogeneous regime $\varepsilon_g = 0.0053U_g^{0.58}$. Fig.3 shows gas bubbles at different superficial velocities at a height of 75 cm from the distributor.

In Fig.4, the results of total gas holdup in cyanobacterial solution were compared with the holdup result in distilled water and the mean bubble velocity in distilled water, on the first and sixth days

10.997 was, 65.89 and 87.98 cm/s, respectively. Results showed that with increasing superficial gas velocity, the bubble velocity in both distilled water and cyanobacteria culture increased and bubbles distributed widely. However, as the bubble velocity is directly influenced with the bubbles size, higher bubble velocity was observed in distilled water compared to cyanobacteria culture due to lower viscosity of water. This observation was similar to other scientists reports (Deng et al. 2010, Ojha 2016). In another experiment performed during cyanobacterial culture to remove CO_2, the total gas holdup was measured at 3 different days. Since, gas holdup in cyanobacterial solution on the first day was more than the gas holdup in distilled water, and with the passage of time and microalgae growth in the airlift reactor, holdup decreased. On the sixth day, when *Spirulina* sp. was at its highest amount, the gas holdup was less than on the first day, when cyanobacteria were absent; Because turbulence reduced in solutions where more solids were present. As a result, larger bubbles could form, which in turn cause the bubbles to rise more rapidly, which was consistent with other works (Chisti 1998, Zhang et al. 2014). Also, the ions dissolved in the cyanobacterial culture medium prevented the bubbles from joining together and forming larger bubbles, but made smaller bubbles, which increased gas holdup. Therefore, holdup on the first day in a solution containing cyanobacteria was higher than in distilled water, and with the increase of *Spirulina* sp. microalgae in the following days, turbulence decreased and larger bubbles were formed, as a result gas holdup increased in the following days, which was a close resemblance to other reports (Blažej et al. 2004, Chisti 1989).

The following Equation clearly shows that the total persistence decreases as the bubble rises at a constant superficial velocity (Chisti 1989).

$$U_b = \frac{U_g}{\varepsilon} \quad (7)$$

Liquid circulation velocity

movement of gas bubbles upwards caused liquid to go up in riser and fall in downcomer. As superficial gas velocity entering the reactor increased, liquid velocity in riser and downcomer and holdup in riser soared, but these changes were quicker in low gas velocities. Also, velocity in riser compared to inlet superficial gas velocity increased, which was higher at lower velocities of the intake gas. This observation was found to be similar with another work (Kilonzo et al. 2010) Fig.5.

Growth measurement analysis

To evaluate biomass concentration 20% of the 0.1 L sample of the bioreactor was daily used to measure optical density. In two steps, optical density of the 10% fraction was measured. Also, to obtain dry weight, 60% of the sample solution at 2000 rpm was centrifuge, then placed in the oven for 72 hours to absorb microalgae moisture completely. Finally, the total dry weight of *Spirulina* sp. was measured.

Fig. 6 shows the dry weight results for 7 samples in terms of optical density at the superficial gas velocity (0.185, 0.524 cm/s) which biomass concentration increased during the culture period with expansion test time and reached a maximum dry weight of 1.62 g/L at the superficial velocity of 0.524 cm/s. This result showed that by increasing superficial gas velocity the cell growth increased, perhaps as aeration rate rose the gas holdup increased in the reactor. Therefore, the sedimentation rate reduced and more microalgae were able to be suspended in the culture medium. Besides, the mean bubble size is quite important for better cyanobacteria growth (Chisti 1989). Thus, 0.524 cm/s was considered as a more suitable gas velocity for *Spirulina* sp. growth, which gave

268 microalgae growth a better support without damage to cells. Moreover, increasing in the gas
269 velocity improved a better mixing in bioreactor so that microalgae could move faster toward the
270 light, which caused a better light absorption hence, the microalgae had a better photosynthesis and
271 growth rate. In a similar study (Ding et al. 2021) which carried out in a 50 L airlift photobioreactor
272 with 38 L working volume and 5.0% input CO_2, at air flow rates of 8.0, 12.0 the maximum dry
273 cell of *Chlorella protothecoides* was achieved 0.24 g/L at 12.0 L/min and 300 h. They found that
274 when the aeration rate was 8.0 L/min, the final biomass concentration of *C. protothecoides* reached
275 0.32 g/L at 500h, in contrast, in the aeration rate of 12.0 L/min for 300 h the dry weight was
276 0.24 g/L, which was higher than at 8.0 L/min and 300 h. Also, it was observed that the maximum
277 biomass concentration of *Spirulina platensis* in the batch cultivation and continuously pre-
278 harvesting (different operation mode) was 3.383 and 4.893 g L^{-1} after 11 days' cultivation
279 respectively (Liu et al. 2018). whereas this experiment carried out (5% CO_2 in air) and 100 mL
280 min^{-1} gas flow rate in one hollow cylinder with total working volume 1L and in Zarrounk culture
281 medium. The difference of amount of biomass concentration between this present study and
282 previous report was affected by 2 factors the first one was culture medium (nutrient supply) which
283 had a major impact on high density microalgae production (Karemore et al. 2015) and gas hold up
284 which is affected by liquid circulation velocity that rely on the flow path geometry, the ability of
285 the reactor head zone (Chisti &Moo-Young 1993) and the height of the airlift photobioreactor
286 (Chisti 1989) which provide a suitable light availability for microalgae growth. Moreover, batch
287 culture is a suitable operation mode which generally used for microorganisms that must kept away
288 any contamination although in this mode we are not able to take measurements regularly as the
289 risk of decreasing in the culture medium volume (Tebbani et al. 2014).

290

CO_2 uptake

to better investigate the effect of algae concentration on carbon dioxide sequestration, the removal efficiency for each velocity during 7 days was calculated. Fig. 7 shows the relationship between carbon dioxide removal efficiency, superficial gas velocity and dry weight.

The results showed that although the superficial velocity of 0.524 cm/s provided sufficient gas-liquid mixing, better circulation and mass transfer for microalgae growth and maximum photosynthesis, the highest efficiency of carbon dioxide removal which was equal to 55.48%, achieved at superficial gas velocity 0.185 cm/s and in dry weight 0.86 g/L, that due to two reasons for lower aeration rate and high density microalgae culture, higher CO_2 removal efficiency can be achieved (Chisti 1998, Chiu et al. 2008). 1) Reduce carbon capture efficiency by increasing the superficial gas velocity due to bubbles interconnect; Because bubble surface area per unit volume of gas decreased and larger bubbles rose faster than smaller which was agreement with previous report (Blažej et al. 2004). As a result, CO_2 uptake from gas bubbles would reduce. 2) The longer the initial culture, the lower the CO_2 content due to the growth of Cyanobacterium *Spirulina* sp., which used CO_2 as the main feed.

In similar studies (Duarte et al. 2020) the maximum dry cell and CO_2 biofixation efficiency of *Spirulina* sp. in a tubular (1.8 L) photobioreactor with 10% (v/v) CO_2 and at 0.05 vvm (volume per working volume per minuet) for 10 days was found to be 1.22 ± 0.05 and 21.8 respectively in Zarrouk's culture. The main reason for this difference in our results and this study was related to the amount of injected CO_2 and the geometry of PBRs especially in large scale which influences on the movement of bubbles and as a result, the amount of light absorption. Other studies reported that microalgae strains had lower ability to capture carbon dioxide in CO_2 high concentrations (>5% v/v) as in these volume of CO_2, sensitivity of microalgae to O_2 increased as a result, the

enzyme activity to utilize carbon dioxide reduced (Almomani et al. 2019b, Gonçalves et al. 2016). In addition, it was reported that the highest biomass concentration and CO_2 removal efficiency by *Spirulina platensis* 4.1 g L^{-1} and 99% in 12 days at 2.5% CO_2, 0.5 L min^{-1} aeration rate for 2 L working volume of 5 Duran glass bottles as photobioreactors in semi-continuous process (Ramirez-Perez &Janes 2021). Another explanation is different strains of one species show different behavior in CO_2 biofixation (Yang &Gao 2003). For example, maximum CO_2 removal efficiency of *Spirulina* sp. was found 53.3% in a three-stage vertical tubular bioreactor for 6% CO_2 at 30 °C which had a close agreement to the result of this present study (de Morais &Costa 2007).

Conclusions

This study demonstrated that gas holdup in airlift photobioreactor was reliance on gas velocity to uptake CO_2 as it can be seen the higher gas velocity entering the reactor, the higher gas holdup. In addition, with increasing cyanobacterial growth, the flow turbulence decreased and larger bubbles were formed, which increased the holdup. However, gas holdup which conceivably is affected by fluid physical properties were neglected. Although to improve CO_2 removal efficiency needs more investigation on different hydrodynamic parameters in airlift photobioreactor, This CO_2 biofixation process might be scaled up and *Spirulina* sp. is a good choice for this purpose.

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Authors' contributions **Zahra Zarei:** Methodology, Data collection, Preliminary analysis, Writing, Original draft. **Peyman Malekshahi:** Methodology, Data collection, Preliminary analysis. **Mohammad Hossein Morowvat:** Investigation, Writing, Review, Data curation, Editing, Visualization, Formal analysis. **Rahbar Rahimi:** Resources, Conceptualization, Methodology, Project administration, Visualization, Funding acquisition, Supervision, Validation. **Seyyed Vahid Niknezhad:** Investigation, Data curation, Formal analysis, Writing, Review, Editing.

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References

Almomani F, Al Ketife A, Judd S, Shurair M, Bhosale RR, Znad H, Tawalbeh M (2019a): Impact of CO_2 concentration and ambient conditions on microalgal growth and nutrient removal from wastewater by a photobioreactor. Sci Total Environ 662, 662-671

Almomani F, Judd S, Bhosale RR, Shurair M, Aljaml K, Khraisheh M (2019b): Intergraded wastewater treatment and carbon bio-fixation from flue gases using *Spirulina platensis* and mixed algal culture. Process Saf Environ Prot 124, 240-250

Bhola V, Swalaha F, Ranjith Kumar R, Singh M, Bux F (2014): Overview of the potential of microalgae for CO_2 sequestration. Int J Environ Sci Technol 11, 2103-2118

Blasing T, Smith K (2016): Recent greenhouse gas concentrations. Department of Energy. doi: 10.3334/CDIAC/atg.032

Blažej M, Kiša M, Markoš J (2004): Scale influence on the hydrodynamics of an internal loop airlift reactor. Chem Eng Proc: Proc Intensif 43, 1519-1527

Chaudhary R, Dikshit AK, Tong YW (2018): Carbon-dioxide biofixation and phycoremediation of municipal wastewater using *Chlorella vulgaris* and *Scenedesmus obliquus*. Environ Sci Pollut Res 25, 20399-20406

Chisti M, Halard B, Moo-Young M (1988): Liquid circulation in airlift reactors. Chem Eng Sci 43, 451-457

Chisti MY (1989): Airlift bioreactors. Elsevier Applied Science, London; New York

Chisti Y, Moo-Young M (1993): Improve the performance of airlift reactors. Chem Eng Prog 89, 38-38

Chisti Y (1998): Pneumatically agitated bioreactors in industrial and environmental bioprocessing: hydrodynamics, hydraulics, and transport phenomena. Appl Mech Rev 51(1), 33-112

373 Chiu S-Y, Kao C-Y, Chen C-H, Kuan T-C, Ong S-C, Lin C-S (2008): Reduction of CO_2 by a high-
374 density culture of *Chlorella* sp. in a semicontinuous photobioreactor. Bioresour Technol
375 99, 3389-3396
376 de Morais MG, Costa JAV (2007): Biofixation of carbon dioxide by *Spirulina* sp. and
377 *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. J
378 Biotechnol 129, 439-445
379 Dejaloud A, Vahabzadeh F, Habibi A (2018): Hydrodynamics and oxygen transfer
380 characterization in a net draft tube airlift reactor with water-in-diesel microemulsion. Fuel
381 Proc Technol 171, 265-276
382 Deng Z, Wang T, Zhang N, Wang Z (2010): Gas holdup, bubble behavior and mass transfer in a
383 5m high internal-loop airlift reactor with non-Newtonian fluid. Chem Eng J 160, 729-737
384 Ding N, Li C, Wang T, Guo M, Mohsin A, Zhang S (2021): Evaluation of an enclosed air-lift
385 photobioreactor (ALPBR) for biomass and lipid biosynthesis of microalgal cells grown
386 under fluid-induced shear stress. Biotechnol Biotechnol Equip 35, 139-149
387 Duarte JH, Fanka LS, Costa JAV (2020): CO_2 Biofixation via *Spirulina* sp. Cultures: Evaluation
388 of Initial Biomass Concentration in Tubular and Raceway Photobioreactors. BioEnergy
389 Res 13, 939-943
390 Ferreira AF, Marques AC, Batista AP, Marques PA, Gouveia L, Silva CM (2012): Biological
391 hydrogen production by *Anabaena* sp.–yield, energy and CO_2 analysis including
392 fermentative biomass recovery. Int J Hydrog Energy 37, 179-190
393 Gonçalves AL, Rodrigues CM, Pires JC, Simões M (2016): The effect of increasing CO_2
394 concentrations on its capture, biomass production and wastewater bioremediation by
395 microalgae and cyanobacteria. Algal Res 14, 127-136

396 Guo X, Yao L, Huang Q (2015): Aeration and mass transfer optimization in a rectangular airlift
397 loop photobioreactor for the production of microalgae. Bioresour Technol 190, 189-195
398 Hansen J, Sato M, Ruedy R, Lo K, Lea DW, Medina-Elizade M (2006): Global temperature
399 change. Proc Nat Acad Sci 103, 14288-14293
400 Hikita H, Kikukawa H (1974): Liquid-phase mixing in bubble columns: Effect of liquid properties.
401 Chem Eng J 8, 191-197
402 Hikita H, Asai S, Tanigawa K, Segawa K, Kitao M (1980): Gas hold-up in bubble columns. Chem
403 Eng J 20, 59-67
404 Hughmark G (1967): Holdup and mass transfer in bubble columns. Ind Eng Chem Process Des
405 Dev 6, 218-220
406 Joshi J, Parasu U, Prasad C, Phanikumar D, Deshpande N, Thorat B (1998): Gas hold-up structures
407 in bubble column reactors. Proc Ind Nat Sci Acad 64, 441-567
408 Karemore A, Ramalingam D, Yadav G, Subramanian G, Sen R (2015): Photobioreactors for
409 Improved Algal Biomass Production: Anal Des Consid, pp. 103-124
410 Kilonzo PM, Margaritis A, Bergougnou M (2010): Hydrodynamics and mass transfer
411 characteristics in an inverse internal loop airlift-driven fibrous-bed bioreactor. Chem Eng
412 J 157, 146-160
413 Liu H, Chen H, Wang S, Liu Q, Li S, Song X, Huang J, Wang X, Jia L (2018): Optimizing light
414 distribution and controlling biomass concentration by continuously pre-harvesting
415 *Spirulina platensis* for improving the microalgae production. Bioresour Technol 252, 14-
416 19

M M, A S, P P (2019): Bubble column photobioreactor for *Chlorella pyrenoidosa* cultivation and validating gas hold up and volumetric mass transfer coefficient. Energy Sources A: Recov Util Environ Effect 1-15

Morais EGd, Cassuriaga APA, Callejas N, Martinez N, Vieitez I, Jachmanián I, Santos LO, Morais MGd, Costa JAV (2018): Evaluation of CO_2 biofixation and biodiesel production by *Spirulina* (*Arthospira*) cultivated in air-lift photobioreactor. Braz Arch Biol Technol 61

Mousavi S, Najafpour GD, Mohammadi M (2018): CO_2 bio-fixation and biofuel production in an airlift photobioreactor by an isolated strain of microalgae *Coelastrum* sp. SM under high CO_2 concentrations. Environ Sci Pollut Res 25, 30139-30150

Ojha A (2016): Advancing microalgae culturing via bubble dynamics, mass transfer, and dynamic growth investigations. Doctoral Dissertations. 2542., Missouri University of Science and Technology

Ojha A, Al-Dahhan M (2018): Local gas holdup and bubble dynamics investigation during microalgae culturing in a split airlift photobioreactor. Chem Eng Sci 175, 185-198

Pourtousi M, Ganesan P, Sandaran SC, Sahu JN (2016): Effect of ring sparger diameters on hydrodynamics in bubble column: A numerical investigation. J Taiwan Inst Chem Eng 69, 14-24

Prazeres AR, Rivas J, Paulo Ú, Ruas F, Carvalho F (2016): Sustainable treatment of different high-strength cheese whey wastewaters: an innovative approach for atmospheric CO_2 mitigation and fertilizer production. Environ Sci Pollut Res 23, 13062-13075

Ramirez-Perez JC, Janes H (2021): Impact of salinity on the kinetics of CO_2 fixation by *Spirulina platensis* cultivated in semi-continuous photobioreactors. Eclét Quím J 46, 21-34

439 Reilly I, Scott D, De Bruijn T, MacIntyre D, Piskorz J (1990): Axial solids concentrations in three-
440 phase bubble columns. Chem Eng Sci 45, 2293-2299
441 Rengel A, Zoughaib A, Dron D, Clodic D (2012): Hydrodynamic study of an internal airlift reactor
442 for microalgae culture. Appl Microbiol Biotechnol 93, 117-129
443 Sadeghizadeh A, Moghaddasi L, Rahimi R (2017): CO_2 capture from air by *Chlorella vulgaris*
444 microalgae in an airlift photobioreactor. Bioresour Technol 243, 441-447
445 Sánchez Mirón A, García Camacho F, Contreras Gómez A, Grima EM, Chisti Y (2000): Bubble-
446 column and airlift photobioreactors for algal culture. AIChE J 46, 1872-1887
447 Sevda S, Bhattacharya S, Reesh IMA, Bhuvanesh S, Sreekrishnan TR (2017): Challenges in the
448 Design and Operation of an Efficient Photobioreactor for Microalgae Cultivation and
449 Hydrogen Production. In: Singh A , Rathore D (Editors), Biohydrogen Production:
450 Sustainability of Current Technology and Future Perspective. Springer India, New Delhi,
451 pp. 147-162
452 Tebbani S, Lopes F, Filali R, Dumur D, Pareau D (2014): Bioprocess Modeling, CO_2 Biofixation
453 by Microalgae, pp. 33-63
454 Ugwu CU, Aoyagi H, Uchiyama H (2008): Photobioreactors for mass cultivation of algae.
455 Bioresour Technol 99, 4021-4028
456 Wadaugsorn K, Limtrakul S, Vatanatham T, Ramachandran PA (2016): Hydrodynamic behaviors
457 and mixing characteristics in an internal loop airlift reactor based on CFD simulation.
458 Chem Eng Res Des 113, 125-139
459 Wang X, Jiang D, Lang X (2017): Future extreme climate changes linked to global warming
460 intensity. Sci Bull 62, 1673-1680

461 Yadav G, Sen R (2017): Microalgal green refinery concept for biosequestration of carbon-dioxide
462 vis-à-vis wastewater remediation and bioenergy production: Recent technological
463 advances in climate research. J CO_2 Util 17, 188-206
464 Yang Y, Gao K (2003): Effects of CO_2 concentrations on the freshwater microalgae,
465 *Chlamydomonas reinhardtii, Chlorella pyrenoidosa and Scenedesmus obliquus*
466 (*Chlorophyta*). J Appl Phycol 15, 379-389
467 Yoon JH, Sim SJ, Kim M-S, Park TH (2002): High cell density culture of *Anabaena variabilis*
468 using repeated injections of carbon dioxide for the production of hydrogen. Int J Hydrog
469 Energy 27, 1265-1270
470 Zhang W, Yong Y, Zhang G, Yang C, Mao Z (2014): Mixing Characteristics and Bubble Behavior
471 in an Airlift Internal Loop Reactor with Low Aspect Ratio. Chin J Chem Eng 22, 611-621
472 Zhao B, Su Y (2014): Process effect of microalgal-carbon dioxide fixation and biomass
473 production: A review. Renew Sust Energ Rev 31, 121-132

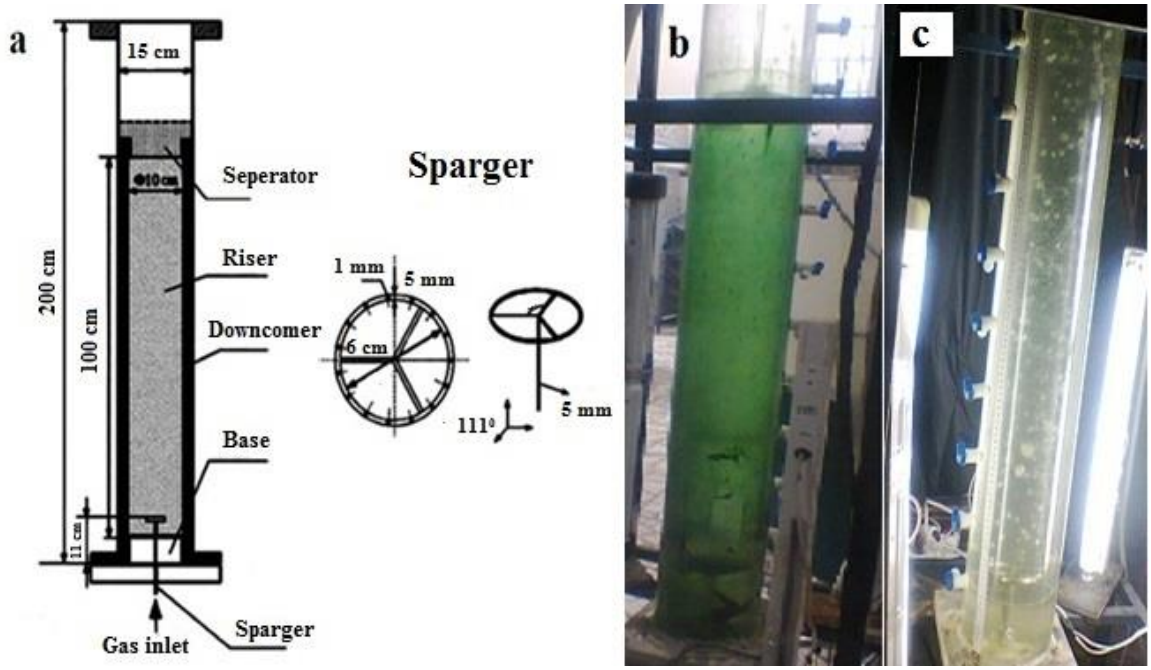


Fig. 1 **a** Schematic diagram of experimental setup; **b** and **c** Real diagrams (Sadeghizadeh et al. 2017)

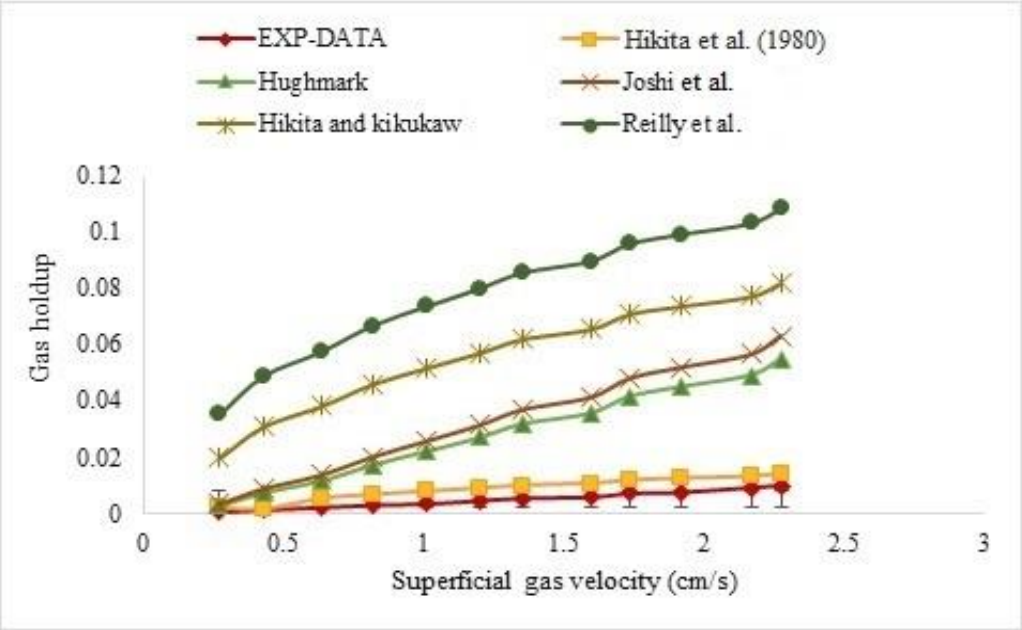


Fig. 2 Comparison of gas holdup experimental data with empirical relationships in the airlift bioreactor (Hikita et al. 1980, Hikita &Kikukawa 1974, Hughmark 1967, Joshi et al. 1998, Reilly et al. 1990)

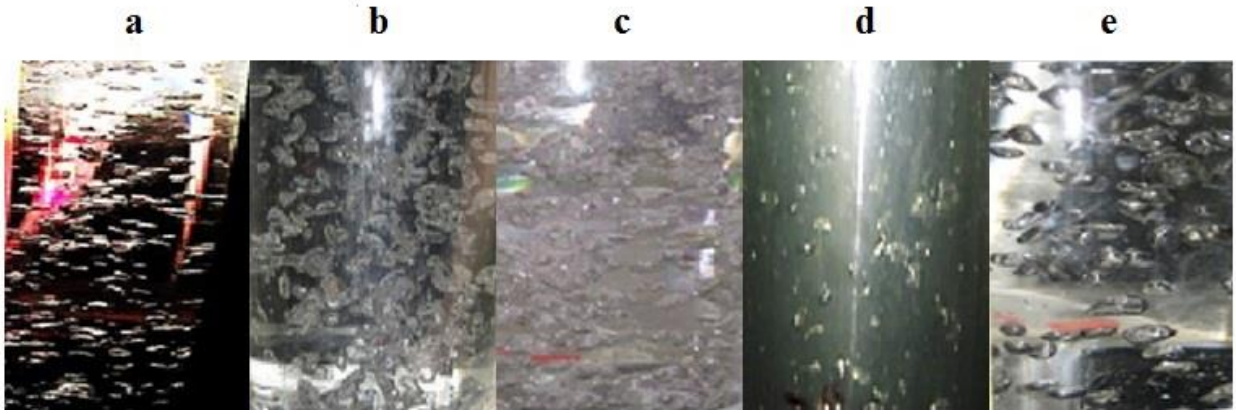
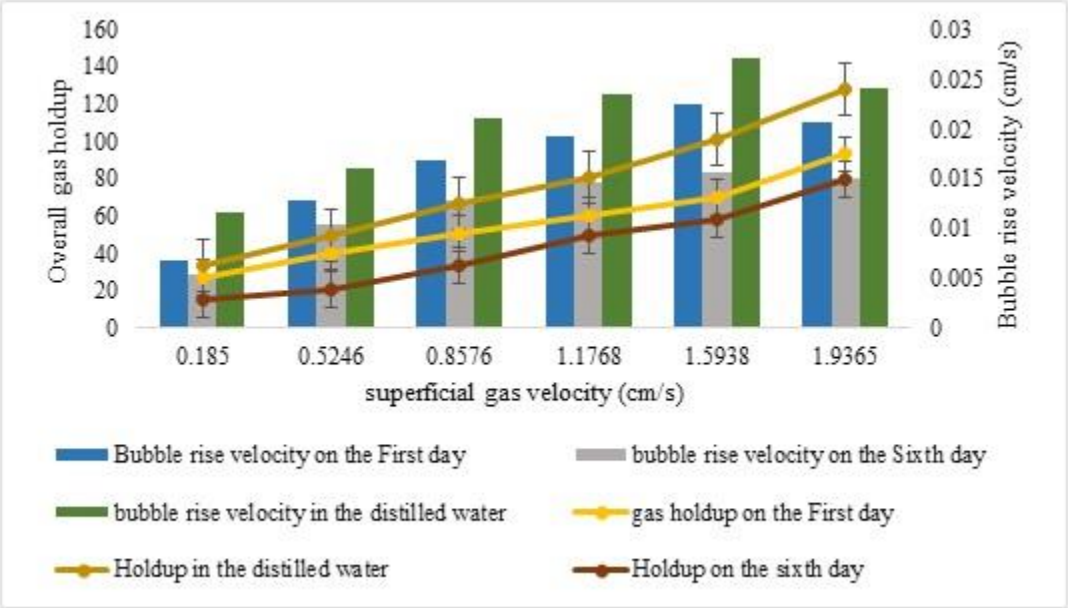


Fig. 3 Bubbles gas in 30± 2 °C and 5 different gas velocities: **a** 0.27 (cm/s) there was laminar flow and bubbles go up slowly, also in **b** 0.54 and **c** 0.75 (cm/s) was seen homogenous regime but by increasing gas velocities to **d** 1.91 and **e** 1.21(cm/s) bubbles started to burst and make turbulence



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493 **Fig. 4** Comparison of gas holdup and the rate of bubble rise at different superficial gas velocities

494 and the first and sixth days of experiment in the reactor with and without *Spirulina* sp.

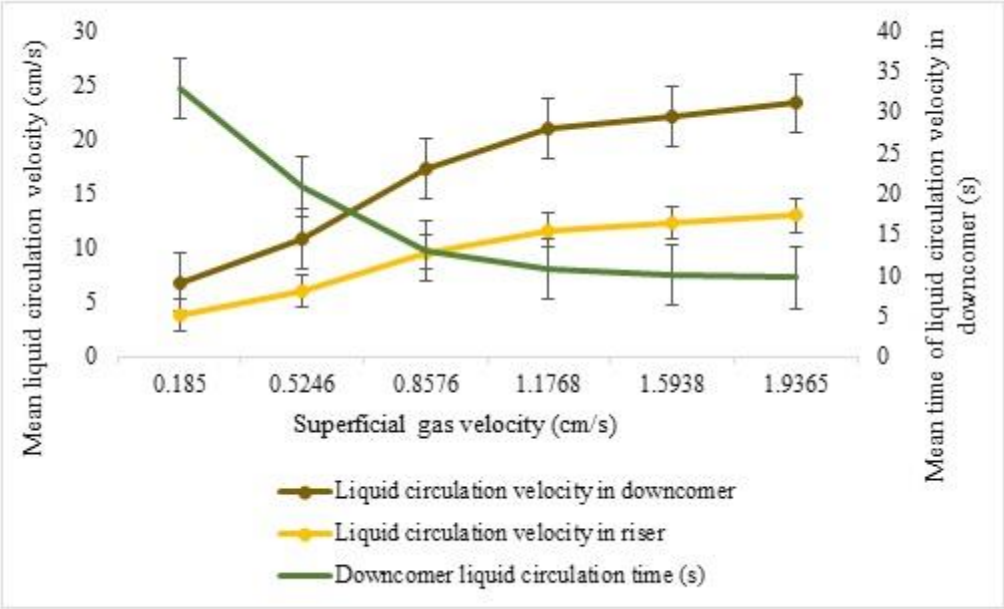

Fig. 5 Comparison of liquid circulation velocity in riser and downcomer and mean time of circulation at 6 different superficial gas velocities which was resemble with Chisti's data (Chisti et al. 1988)

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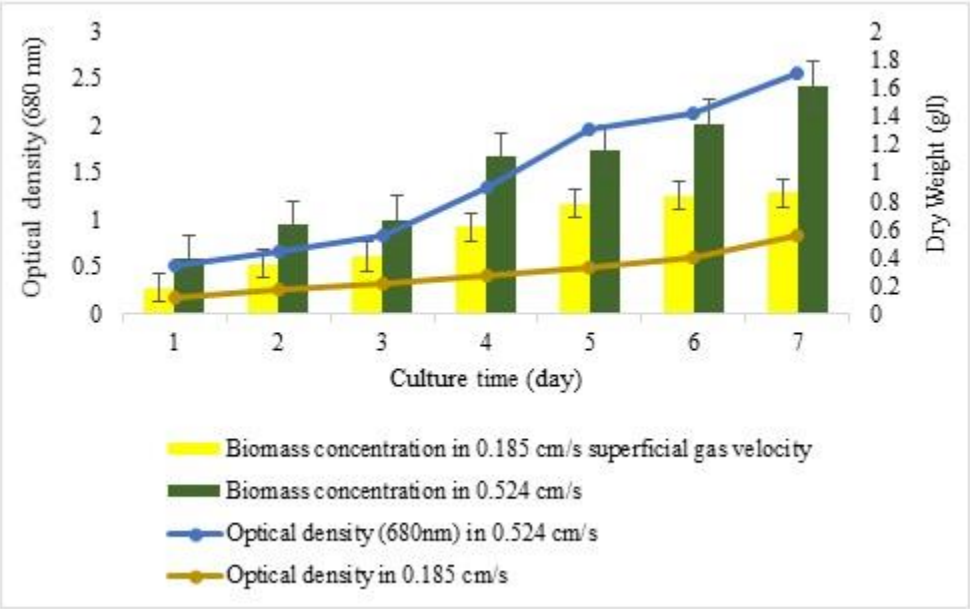

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503 **Fig. 6** Biomass concentration and dry weight of *Spirulina* sp. at two superficial gas velocities

504 (0.185, 0.524 cm/s), OD (680 nm), temperature 30 ± 2 °C and pH = 10

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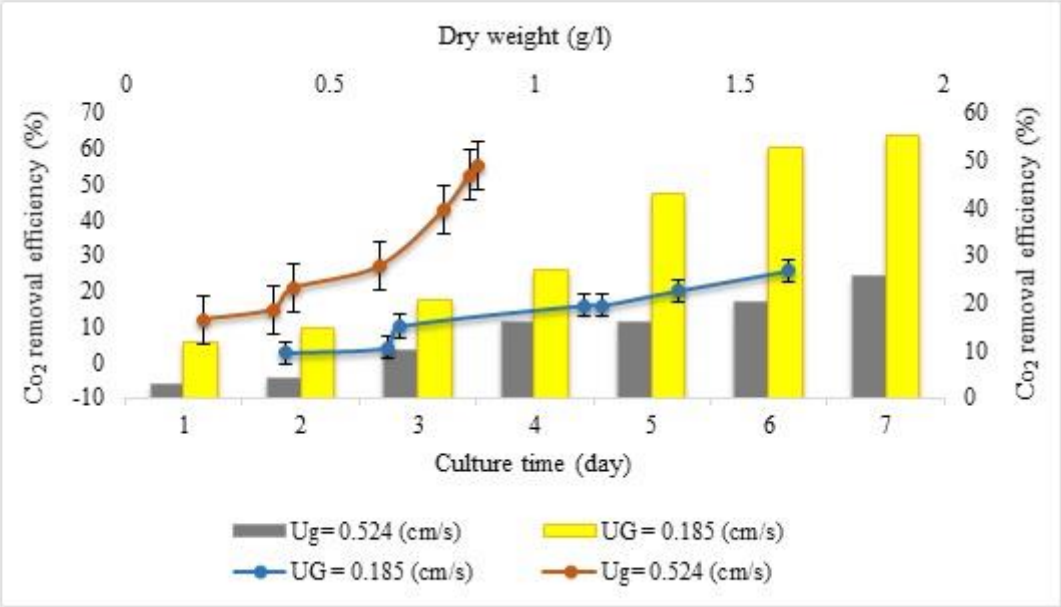

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507 **Fig. 7** The amount of CO_2 removal efficiency compared at the superficial gas velocities (0.185,

508 0.524 cm/s) and different concentrations of cyanobacteria (temperature 30 ± 2 °C; pH = 10)

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