Effect of Superficial Gas Velocity on Continuous Hydrogen Production by Anabaena sp. in an Internal-loop Airlift Bioreactor

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Abstract

Global warming and rising air pollution which has been caused by using too much fossil fuel has led to look for a new clean source, sustainable and eco-friendly of energy like H_2, which can be produced by cyanobacteria and microalgae. In this study, *Anabaena* sp. was used in a continuous operation to achieve biohydrogen production. To this end, an airlift photobioreactor (20 L) was considered. The effects of the gas holdup, liquid circulation velocity, and the amount of dissolved oxygen on hydrogen production were investigated. Gas holdup, liquid circulation velocity, and K_La (mass transfer coefficient) showed an upward trend by increasing the velocity of the inlet gas. Maximum biomass concentration of and maximal H_2 production were observed 1.2 g L^{-1} d^{-1} and 371 mL h^{-1} PBR^{-1}, respectively under light intensity of 3500 lux/m^2 applying a light-dark cycle in 7 days, at A_d/A_r of 1.25 and 0.185 and 0.542 cm/s. pH, temperature (30±2 °C), light intensity, and inlet gas flow to the bioreactor (containing 98% air and 2% carbon dioxide) were remained steady. Using the airlift photobioreactor with a good mass transfer and light availability to cyanobacteria growth can be a cost-effective and environmentally technology for biological H_2 production.

Introduction

Climate change is an inevitable phenomenon that occurs under the influence of various factors such as oceanic processes, solar radiation, and volcanic eruptions. These changes are often slow, giving enough time for most of the species to adapt. But faster climate change may cause dangerous and irreversible effects [1]. The increased CO_2 emission is the main reason for the temperature elevation as it can absorb the thermal radiation emitted from the earth's surface. Due to global warming and limited fossil fuel supplies, the use of renewable energy has gained increasing popularity [2, 3]. Biofuel is a type of renewable energy whose energy content is derived from biological sources (organic constituents of the body of living organisms). The idea of using microalgae in the production of biofuels and the removal of greenhouse gases through photosynthesis is not new [4–6]. About 50% of the dry weight of microalgae biomass contains carbon. Hence, 100 tons of microalgae biomass production can fix 183 tons of CO_2 [2]. Moreover, the biological production of hydrogen using these microorganisms is a new horizon to reach hydrogen through renewable sources. The hydrogen gas produced by biological processes can be easily converted into electrical energy; therefore, it can be considered as a clean and suitable fuel for transportation purposes [7, 8]. Biohydrogen is produced through photosynthesis) Biophotolysis direct and indirect) and photofermentation and dark fermentation [8, 9]. A significant number of bacteria, cyanobacteria and green algae such as *Chlamydomonas, Scenedesmus, Chlorella, Tetraspora* are able to produce biological hydrogen [10, 11]. Anaerobic cultures of microorganisms such as *Clostridium*, whose carbon source is supplied by carbohydrates such as glucose, are able to produce hydrogen gas during the fermentation process [12, 13]. Photosynthetic bacteria such as *Rhodobacter* have the ability to use carbon sources such as lactic acid and acetic acid as energy sources and produce hydrogen gas under light conditions [14]. Cyanobacteria among other microorganisms are suitable for hydrogen production due to their simple nutritional, water, light, and carbon dioxide requirements [15]. Some of its species,

especially *Anabaena variabilis* and *Anabaena* sp. are the most potent CO_2 consumers and H_2 producers [16, 17]. Microalgae and cyanobacteria can be grown in open systems and closed systems which called photobioreactors. Photobioreactors provide a closed culture medium that is safe against invasion and competition by other microorganisms and controls the culture of microalgae more effectively [18]. In addition, more diverse species of microalgae can be grown in such closed environments under a large portion of the light not only shines directly on the surface of the culture medium but also passes through the transparent walls of the reactor and reaches the cultured cells [19, 20]. Researchers have been using many photobioreactors in hydrogen production for years [21]. Some studies presented a two-stage photobioreactor alternating between stages of growth and production of hydrogen. In the growth phase, cyanobacteria stabilize atmospheric CO_2 and nitrogen for their own growth and carbohydrate production, and in the hydrogen production phase, they produce hydrogen using stored carbohydrates [22, 23]. It is reported that to produce hydrogen by *Anabaena variabilis* a two-step discontinuous process was used [24]. Among photobioreactors airlift reactors have been widely used in separation processes, especially for the cultivation of microalgae and biohydrogen production [25]. Compared to other similar devices such as stirred tanks and bubble columns, airlift photobioreactors (APBRs) offer lower and more uniform shear stress [26, 27], which has led to their widespread use in biological operations. Besides, the growth of cyanobacteria is significantly affected by superficial gas velocity, which is called aeration rate [28] since the culture medium is fed by a mixture of air and gas with different ratio or with pure gas which increases the mass transfer, avoid scarcity of carbon dioxide, dead zones, microalgae sedimentation [29] and control the amount of O_2 and CO_2 which inhibits cyanobacteria growth [30, 31]; Also, aeration rate improves photosynthesis with an optimized light/dark cycle [32] and influence on Oxygen mass transfer that is an inhibitory factor for biological H_2 production. To achieve high amount of biohydrogen and cyanobacteria growth, the hydrodynamic parameters and volumetric mass transfer coefficient (k_La), which is the essential factor of the PBRs, must provide culture requirements especially sufficient light absorption [33].

On this matter, the present study investigated biohydrogen production in an airlift photobioreactor by *Anabaena* sp. in different superficial gas velocities to find out the impact of bioreactor hydrodynamic on H_2 production. Also, the result of this study might be applied in further researches on biohydrogen production from microalgae.

Materials And Methods

Construction and Operation of Photobioreactor

The photobioreactor used in this experiment was an internal-loop airlift photobioreactor with two vertical cylinders and a Plexiglas center. The diameter of the bioreactor was 15 cm and its height was 2 m, the cross-section area of its riser and downcomer were 78.54 and 98.17 cm^2, respectively. The distributor was ring-shaped and installed at a distance of 11 cm from the end of the reactor. Also, there were 18 equally-distanced holes with a diameter of 1 mm Fig. 1 [34]. The surface of the reactor was illuminated by

fluorescent lamps at an intensity of 3500 lux/m^2 with a *12:12 light-dark* (LD) *cycle* [35]. To study the filtered airflow and pure carbon dioxide from gas rotameters, they were installed in parallel. Hydrogen and oxygen concentrations were measured for 7 days, during which the parameters were kept constant unless mentioned otherwise.

Microbial Culture Development

For the cultivation of cyanobacteria in the reactor, *Anabaena* sp. was supplied from Science and Technology Park, Bushehr, Iran. 1 L of the culture medium ($BG11_0$) was first prepared. It contained 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 $Co(NO_3)_2.6H_2O$ in one liter [21]. Then, HCl (1 mol/l) was added to the culture to adjust the pH at 8, as measured by a pH-meter (Metrohm 827 pH Lab meter). Finally, the algae biomass was treated at 121°C for 40 min in an autoclave. The solution should be prepared in direct proportion to the volume of the reactor (20 L) [36]. Then, the solution containing cyanobacterial species (5% (v/v)) was added to the reactor. For this purpose, the prototype was cultivated several times to reach the appropriate volume for loading the reactor. To this end, the primary cyanobacteria were first treated in a 500-mL Erlenmeyer flask containing 100 mL $BG11_0$ medium, and kept for 5 to 6 days to grow. After 6 days, the cyanobacteria grew enough and were ready to be used for further propagation. In the next propagation, about 15 mL of the previously-cultured cyanobacterial solution grown was added to 4 Erlenmeyer flasks containing 250 mL of culture medium. They were again treated for 6 days. Now, these cyanobacterial solutions were able to be used to multiply and grow in a reactor containing 20 L of culture medium [24, 22].

Experimental Batch Setup and Hydrogen Production

During the 7 days that the output concentrations of O_2 and H_2 were measured, the reactor content was daily sampled to determine the growth trend of cyanobacteria. The concentration of cyanobacterial biomass was estimated by measuring the light density and dry weight of the cyanobacterial sample. The growth rate of *Anabaena* cyanobacteria was measured in an internal-loop airlift photobioreactor under intermittent culture feeding. 100- mL samples were daily withdrawn from the bioreactor to measure the light density and dry weight of cyanobacteria. 20 mL of the sample was used for measuring light density while the remaining 60 mL was employed to evaluate the dry weight. The optical density measurement experiment was repeated for two samples of 10 mL each time using an optical spectrometer (DR 5000). For the dry weight test, a pre-weighed test tube was loaded with 60 mL of the sample and centrifuged (Sigma 101) at 2000 rpm. Afterward, the sample was dried in an oven for 3 days. The dry weight of the cyanobacterium was finally calculated by re-measuring the weight of the test tube containing the dried cyanobacterium.

Analytical Procedure

The output concentrations of O_2 and H_2 were measured for 7 days as cyanobacterium enters the stationary phase after this time [22]. Therefore, to analyze the concentrations of O_2 and H_2, 500 mL of the gas sample was collected using a gas sampling bag (Tedlar bag) every 24 hours from the bioreactor headspace and injected into gas chromatography (HP-5890; Hewlett Packard, USA), equipped with Carboxen-1000 column (Supelco, USA) and a thermal conductivity detector and an injector (HP-3395, Hewlett Packard). For analyzing O_2 and H_2 gases, the injector and detector temperatures were set to 120°C, Argon gas was used as a carrier gas. The oven temperature was kept at 35°C and residence times of O_2 and H_2 gases were 8 and 2 min, respectively. Similarly, for CO_2 gas, oven temperature and residence time were 255°C and 5 min.

Hydrodynamic Parameters of the Photobioreactor

Liquid Circulation Velocity and Gas Holdup

The tracer response technique was employed to determine the average circulation time. For calculating liquid circulation velocity and mixing time, dye tracer (blue ink) was used. This material was poured on top of the reactor and its travel time was measured between two specific points. The liquid circulation velocity can be calculated by Eq. (1).

$$U_{\mathrm{circ}} = \frac{\mathrm{X}_{\mathrm{circ}}}{\mathrm{t}_{\mathrm{circ}}}$$

1

In this experiment, t_{circ} was measured when the colored material covered a distance of 1 m as obtained from the downcomer at a temperature of 30°C. Having the elapsed time and the distance, the velocity of the fluid inside the riser can be determined a*cc*ording to Eq. (2) [26].

$$U_d \times A_d = U_r \times A_r$$

2

In the above equation, A_d and A_r are downcomer and riser cross-sections, respectively. Eq. (3) was also used to determine gas holdup where H_2 and H_1 denote the height of the liquid after aeration and the initial height of the liquid in the reactor, respectively [26].

$$\epsilon = \frac{H_2 - H_1}{H_2}$$

3

Calculation of Mass Transfer Coefficient

The mass transfer coefficient can be obtained both physically and chemically. In this study, a physical method, oxygen desorption, was used. The amount of dissolved oxygen was recorded by the oxygen meter (AZ 8403, DO accuracy ± 1.5% F.S (in %)) at 5-min intervals for one hour at 30°C. Eq. (4) [26] was used to calculate the mass transfer coefficient at superficial velocities of 0.185 and 0.524 cm/s.

$$ln\left(\frac{C^* - C_{t0}}{C^* - C_t}\right) = \mathrm{K_l a.\,t}$$

4

By plotting $ln\left(\frac{C^*-C_{t0}}{C^*-C_t}\right)$ versus time, a straight line was obtained whose slope indicates the volumetric mass transfer coefficient ($K_l a$). C*, C_t, C_{t0} are equilibrium concentration and concentrations at $t=t_c$ and t=0, respectively.

Result And Discussions

Gas Holdup and Liquid Velocity

The holdup in airlift reactors is affected by liquid circulation velocity, which also depends on the gas-liquid separation zone above the reactor as well as the height of the reactor [37]. Therefore, the obtained relationship is highly geometry-dependent. The overall gas holdup test was carried out for two different systems.

In the first one, the reactor only contained aerated water, while the second system was a reactor containing a mixture of cyanobacteria and culture medium fed by the air-CO_2 mixture. In this experiment, the overall gas holdup during the culture of Anabaena cyanobacteria was measured on 3 different days Fig. 2.

As shown in Fig. 2, gas holdup in cyanobacterial solution on the first day was greater compared to in distilled water. This increase could not be attributed to the presence of cyanobacteria as they did not sufficiently grow to affect holdup. The difference on the first day could be assigned to the presence of ions in the culture medium since dissolved ions prevent the bubbles from joining [26]. Thus, smaller bubbles increase the gas holdup. The results showed that the longer it takes to put cyanobacteria, the shorter gas holdup due to the growth of cyanobacteria. On the sixth day, when the cyanobacteria reached their maximum growth in the form of solids, the gas holdup was less than the first day when cyanobacteria were not present in solid form. Chisti et al. [26] showed a reduction in the turbulence of solutions encompassing more solid components, resulting in larger bubbles. As a result, the bubbles rise faster which was consistent with the previous work [26]. Also, the obtained velocity values in the downcomer of this experiment were close to the laboratory data due to the similarity of the reactor

geometry which was resemblance with another report [38]. The results of the velocity of the riser were compared with two experimental relationships as depicted in Fig. 3.

Mass Transfer Coefficient

The volumetric mass transfer coefficient was obtained according to Eq. (4) and compared with Bello et al. [39] as illustrated in Fig. 4. At low input gas velocities, the amount of volumetric mass transfer coefficient obtained from the experimental data was close to the empirical results. The difference, however, incremented by increasing the gas velocity. One reason for this is the dependence of the volumetric mass transfer coefficient on superficial velocities of liquid and gas as well as the gas holdup. In a similar study [33], k_La increased with the rise of the superficial gas velocity (from 6.91×10^{-3} to 2.69×10^{-2} s^{-1}). In fact, k_La is basically caused by the gas holdup when the bubbles diameter is steady. In other words, the increase in surface area of the bubbles increases gas holdup, therefore, k_La is higher [40].

Growth of Cyanobacteria

The growth rate of *Anabaena* cyanobacteria was measured in an internal-loop airlift photobioreactor under intermittent culture feeding. The culture medium temperature was 30°C and the light intensity at the surface of the photobioreactor was 3500 lux/m^2. Air containing 2% carbon dioxide entered the culture medium from the bottom of the device. To measure the light density and dry weight of cyanobacteria, 100 mL samples were taken from the reactor every 24 hours. Optical density, which can be used as an indirect method to calculate biomass concentration, is linearly related to dry weight using a standard diagram [41]. This linear relationship is clear in Fig. 4 for the dry weight results for 7 samples in terms of light density obtained at superficial gas velocities of 0.185 and 0.524 cm/s. It was reported that a major increase of *Chlorella vulgaris* concentration from 1.97 to 3.96 g/L was obtained when the superficial gas velocity increased from 2.778×10^{-4} to 8.333×10^{-4} m/s at 2% CO_2 aerated in a rectangular airlift photobioreactor which was an agreement with our results [33].

Using the linear equation between light density and dry weight, the dry weight can be determined at other velocities. Although this method may not lead to accurate results in some cases, as the direct measurement of dry weight is not error-free and may damage the microorganisms, light density measurement can be a proper approach. Also, the biomass density increased during the culture period, with increasing the test time. It reached to its maximal value (1.2 g L^{-1} d^{-1}) at the superficial gas velocity of 0.524 cm/s Fig. 5. In laboratory scales concentration of cyanobacteria usually is limited to 1 g L^{-1} – 3 g L^{-1} in Biophotolysis. This is because microalgae in the whole volume of the bioreactor cannot get adequate light for their growth [42]. It was observed that Flat panel reactors give more and better light to microalgae growth but has higher shear stress than ARLB due to movement of microalgae until bubbles burst. For example, a biomass concentration of *Anabaena platensis* was achieved 1.09 g $L^{-1}d^{-1}$ with 40 m^{-1} A/V ratio [27].

Hydrogen Production

To measure H_2, carbon dioxide at a concentration of 2% entered the reactor some of which were absorbed by the cyanobacterial solution and the rest exited the top of the reactor. In the first experiment, the flow rate of carbon dioxide and the air was 0.824 and 40.376 cm^3/s, respectively. In the second experiment, carbon dioxide and air entered the reactor at the respective flow rates of 0.29 and 14.23 cm^3/s. They were finally combined and entered the reactor at a flow rate of 41.20 cm^3/s and 14.52 cm^3/s. Figure 6 shows the amount of produced H_2 and O_2 as recorded by the gas chromatography in 7 days.

The results demonstrated that at lower aeration rates (0.185 cm/s), only O_2 production could be achieved while H_2 production was zero. O_2 production could be also increased using high-density culture so that hydrogenase and nitrogenase enzymes of cyanobacteria are sensitive to oxygen produced in photosynthesis. They become inactive even at oxygen concentrations less than 2% [43]. This was one of the problems in the continuous production of hydrogen by photosynthetic cells. However, dissolved Oxygen is not an important concern in airlift, flat plate and bubble column photobioreactors [44]. In these kind of bioreactors during H_2 production, when the value Oxygen production because of (photosynthesis II) PSII is more than amount of cyanobacteria respiration, gas-freeing is really important [27]. *Anabaena* cyanobacteria can also consume CO_2 and produce H_2 at the same time due to their heterocyst cells. These processes occur in opposite directions and can dramatically affect each other [45]. Despite this inhibition by increasing the gas velocity from 0.185 to 0.524 cm/s, O_2 reduced and H_2 production reached at 371 mL h^{-1} PBR^{-1}. In a similar study, hydrogen production of *Anabaena azollae* in a helical photobioreactor with 4.35 L working volume at 2% CO_2 + 98% air (0.5 L min^{-1}), 30°C and 140 µE s^{-1} m^{-2}, after increasing the aeration rate from 0.5 to 1.0 L min^{-1} which led to reduction of O_2 concentration, increased from 0 to 13 mL L^{-1} h^{-1} [46]. Moreover, downcomer and riser cross-sectional area (A_d /A_r) or the width of PBRs also plays an essential role in biohydrogen production. In the range of (1-2.2) A_d/A_r in higher superficial gas velocities was observed that the homogenous regime which lead to better mass transfer rate, more light absorption and cyanobacteria growth. For example in a same work, Maximum dry weight concentration and H_2 production of *Anabaena* sp. PCC 7120 was observed 1.63 g L^{-1} and 600 mL L^{-1}, respectively at A_d /A_r of 1.6 in 9 days under 120 μE m^{-2} s^{-1} light intensity, working volume of 1.4 L, 1% (v/v) CO_2 and 0.25 vvm aeration rate which resemble with our study at A_d /A_r of 1.2. They found that cyanobacteria on the wall of airlift bioreactor had better access to the light, hence cyanobacteria growth in downcomer were higher than ones in riser. Therefore, (A_d /A_r) of 1.6 than A_d /A_r of 3.2 had the better impact on cell growth so that in this cross- sectional area cyanobacteria were prolonged exposure to the light [22].

Conclusion

The results of this research showed that gas holdup and liquid circulation velocity play a decisive role in cyanobacteria growth and H_2 production. The liquid velocity in the riser and downcomer increased by raising the inlet gas velocity, which was more significant at lower gas velocities. Also, the higher the inlet gas velocity of the reactor, the greater the gas holdup. Furthermore, the possibility of H_2 production at two different CO_2 inlet flows, demonstrated that at a superficial gas velocity of 0.185 cm/s, no hydrogen was produced because of photosynthesis. The maximum amount of hydrogen (371 mL h^{-1} PBR^{-1}) was observed at 0.524 cm/s. The process of biological H_2 production in APBR after more investigation of the effect of aeration rate on hydrogen production in different light intensity and pH might be considered to use in large scale.

Declarations

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interests.

Ethics Approval This article does not contain any studies with human participants or animals performed by any of authors.

Authorship Consent All authors read, approved the final manuscript, and agreed for this submission

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Figures

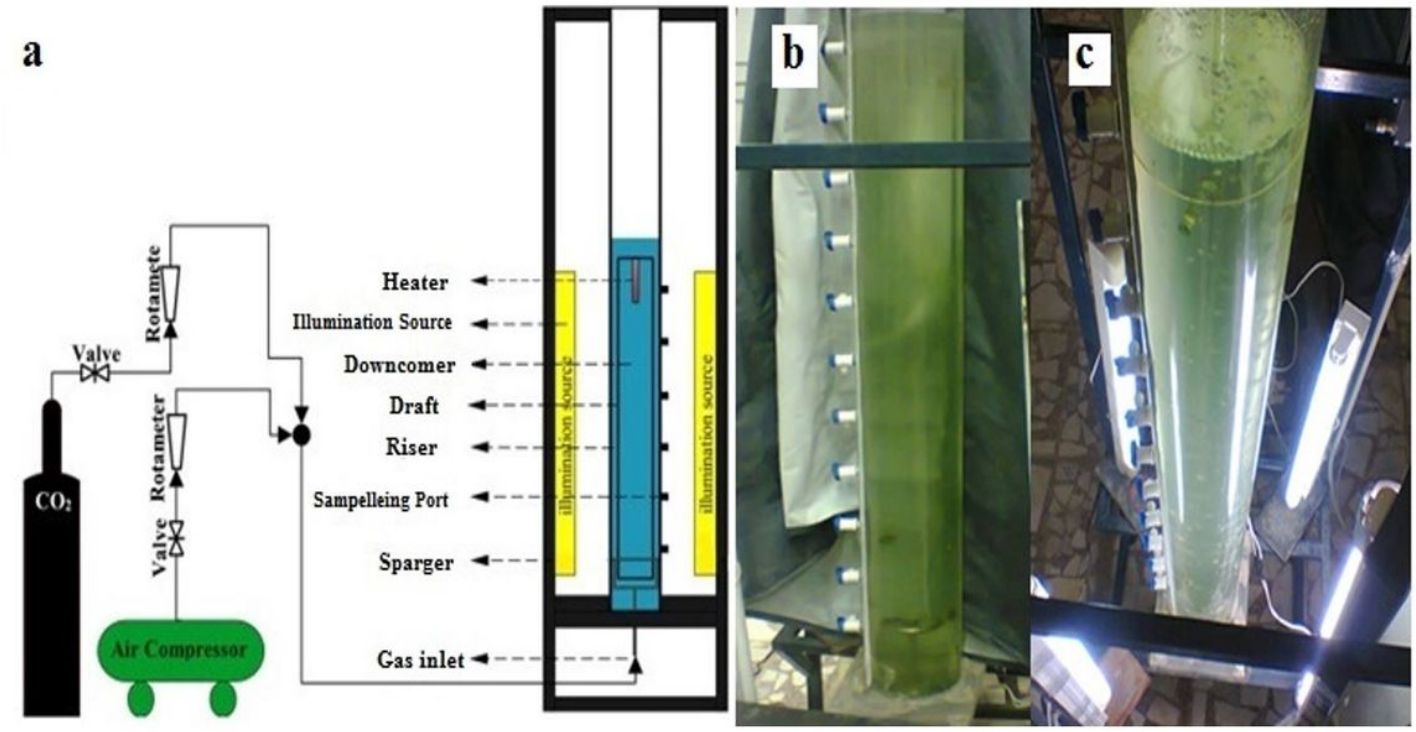

Figure 1

(a) Schematic diagram of the photobioreactor, (b and c): actual images of (APBR)

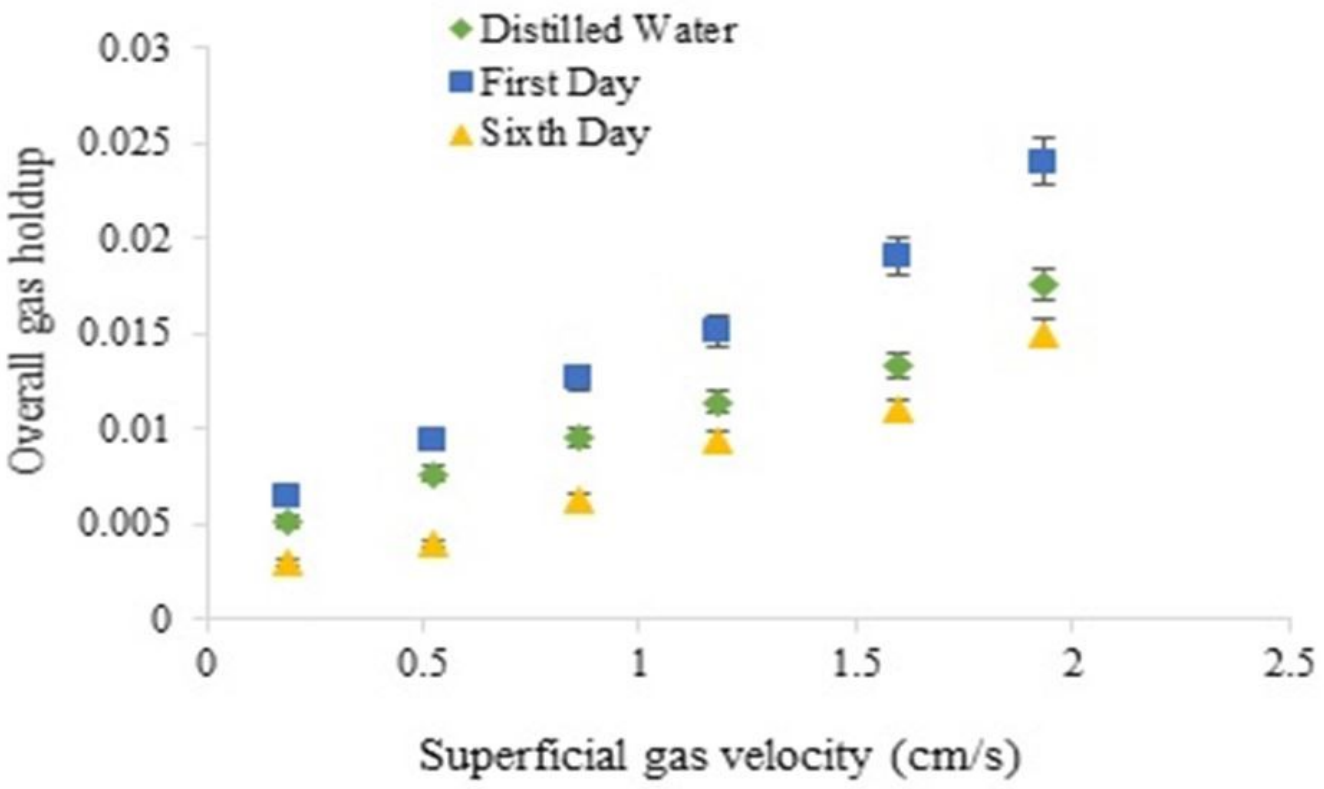

Figure 2

Overall gas holdup in algal solution on the first day and sixth day with distilled water; the number of the experiment (n= 3 + standard deviation)

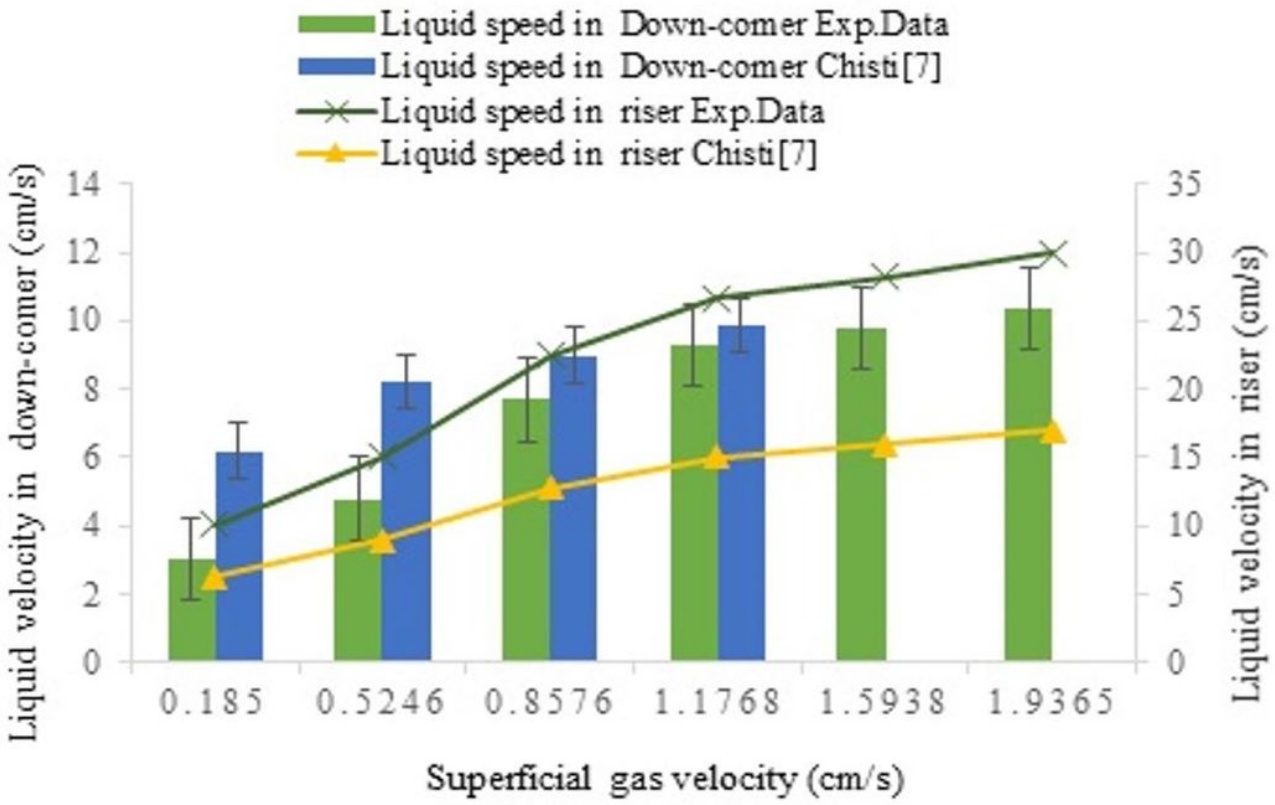

Figure 3

Comparison of experimental data of liquid velocity in riser and downcomer with Chisit's work; the number of experiment (n= 3 + standard deviation)

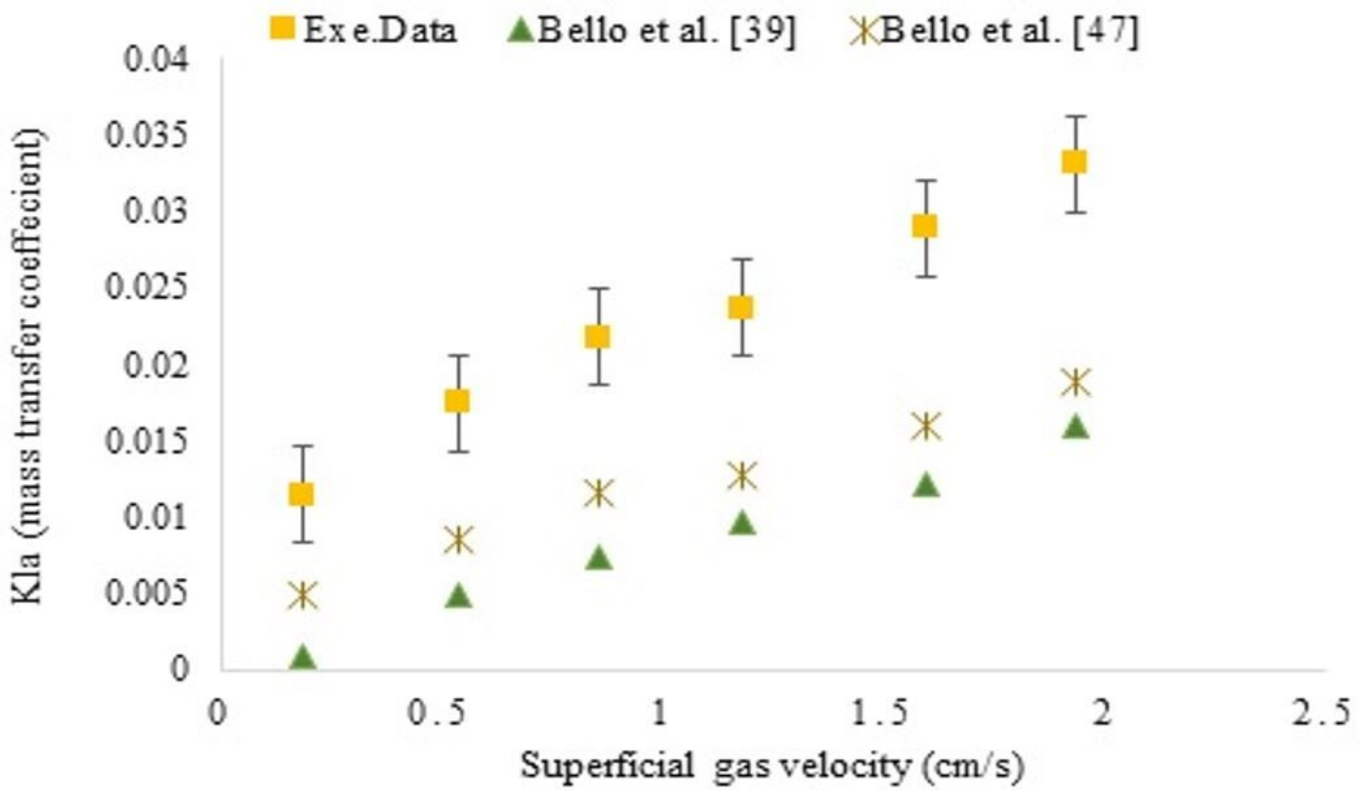

Figure 4

Comparison of Experimental data of KLa with Bello et al. results [39,47]

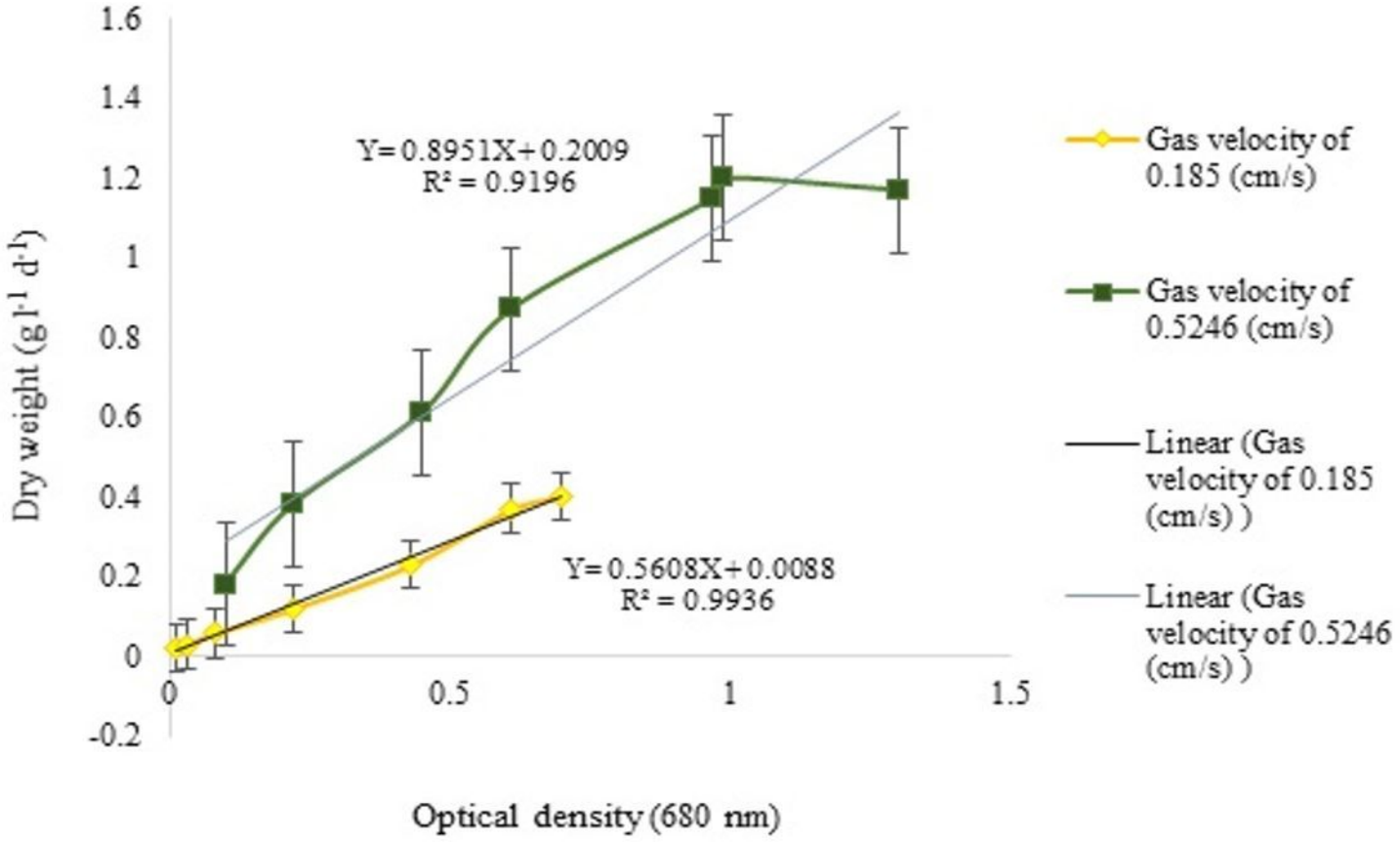

Figure 5

Dry weight of Anabaena sp. under 680 nm Optical density, gas velocities of 0.185, 0.524 cm/s, for 24 h; number of experiment (n= 3 + standard deviation)

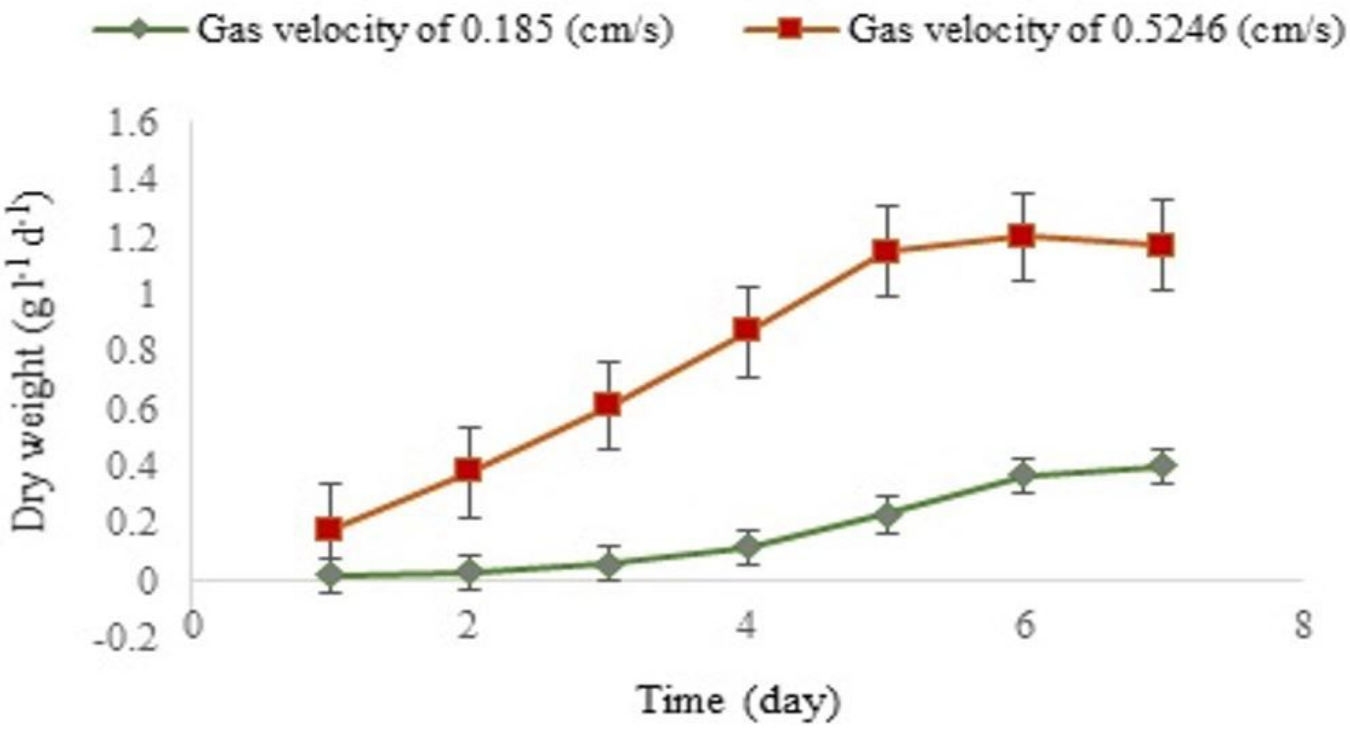

Figure 6

Dry weight of Anabaena cyanobacteria in 7 days under two gas velocities, number of the experiment (n= 3 + standard deviation)

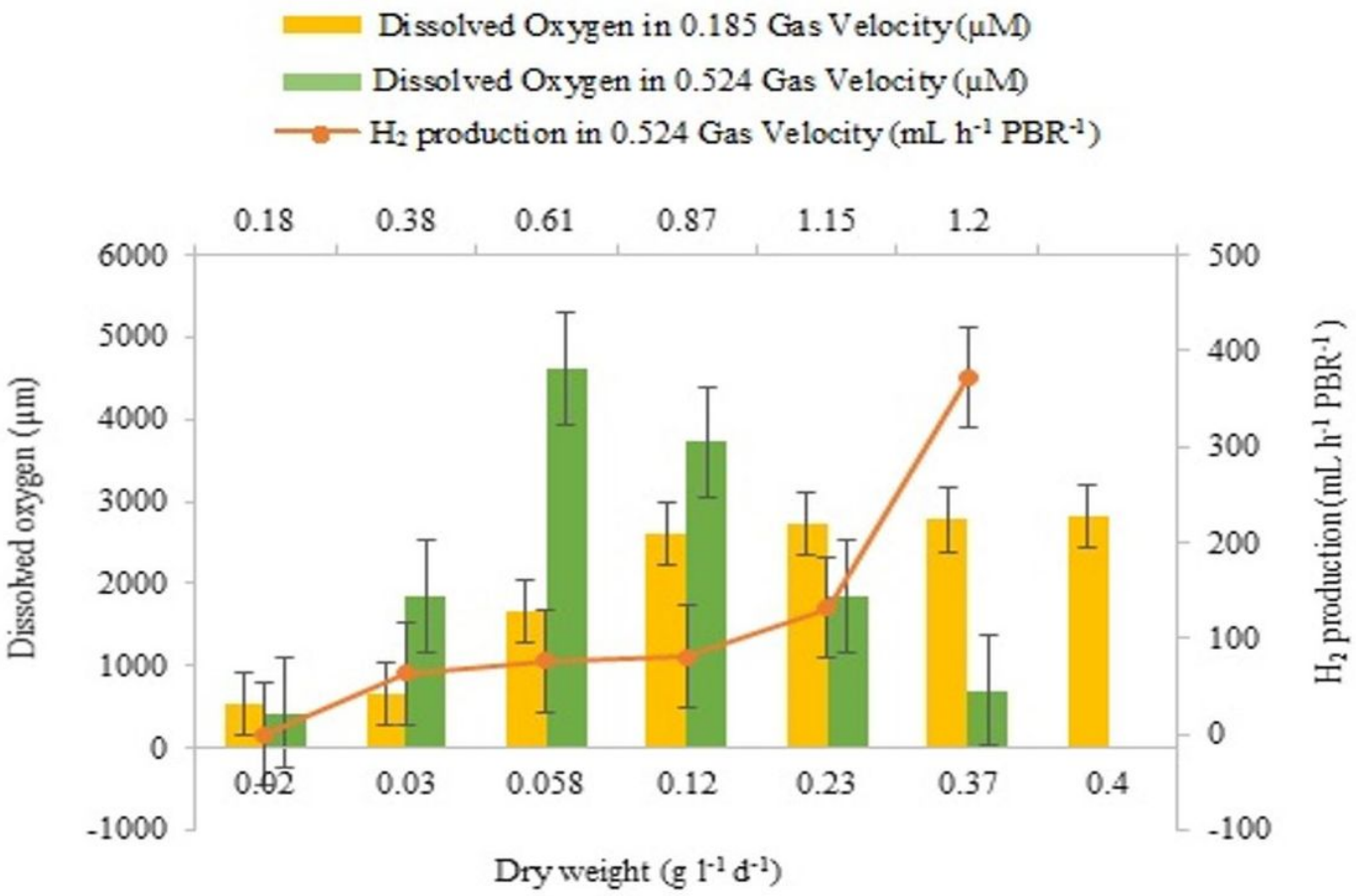

Figure 7

Hydrogen production and dissolved oxygen in two gas velocity (0.185, 0.542 cm/s), for 24 h; temperature: (30+2 °C); pH= 8; number of experiments: 3 + standard deviation