Brazilian Journal of Chemical Engineering (2023) 40:379–388
https://doi.org/10.1007/s43153-022-00245-3

ORIGINAL PAPER

Effect of hydrodynamic parameters on hydrogen production by *Anabaena* sp. in an internal-loop airlift photobioreactor

Zahra Zarei[1] · Peyman Malekshahi[1] · Antoine P. Trzcinski[2] · Mohammad Hossein Morowvat[3,4]

Received: 22 November 2021 / Revised: 11 March 2022 / Accepted: 31 March 2022 / Published online: 12 April 2022

Abstract

Global warming and air pollution caused by fossil fuel emissions have triggered the search for a clean, sustainable, and eco-friendly energy source such as H_2, which can be produced by cyanobacteria and microalgae. In this study, *Anabaena* sp. was used in a photobioreactor to achieve biohydrogen production. To this end, hydrodynamic parameters such as gas holdup, liquid circulation velocity, oxygen mass transfer coefficient, and gas velocity were investigated. Results showed that the gas holdup, liquid circulation velocity, and oxygen mass transfer increased by increasing the inlet gas velocity without causing detrimental shear stress to cyanobacteria. A biomass concentration of 1.2 g L^{-1} and a total H_2 production of 371 mL were recorded after 7 days using an inlet gas velocity of 0.524 cm s^{-1} and a light intensity of 140 µmol photons m^{-2} s^{-1}. Using a superficial gas velocity of 0.524 cm s^{-1} resulted in the optimum gas holdup, mass transfer, and light availability to *Anabaena* sp. The growth of cyanobacteria in an internal-loop airlift photobioreactor was found to be a cost-effective and environmentally friendly technology for hydrogen production.

Keywords Cyanobacteria · Hydrogen · Photobioreactor · Superficial gas velocity

Introduction

The growing consumption of fossil fuels and the associated increase in CO_2 emissions are the main reasons for global warming. Limited fossil fuel supplies have driven the search for an alternative source of energy that is sustainable and environmentally friendly (Cheah et al. 2015; Nagarajan et al. 2017). Hydrogen is considered clean energy due to the high energy output per unit weight (141.6 MJ kg^{-1}) and clean emissions that do not cause air and environmental pollution. It can also be easily converted into electrical energy and used for transportation (Anwar 2019; Mona 2020). In fact, hydrogen has an energy content twice as much as hydrocarbon fossil fuels (Balat 2008; Hossain et al. 2016). The idea of using microalgae and cyanobacteria in the photoproduction of H_2 and the removal of greenhouse gases through photosynthesis is not new (Guo et al. 2016; Schenk et al. 2008). These microorganisms have great potential to convert CO_2 in the atmosphere into various kinds of organic compounds. These raw compounds are appropriate sources for biohydrogen production (Kavitha et al. 2017a, b). About 50% of the dry weight of microalgal biomass contains carbon. Hence, 100 tons of microalgal biomass can fix 183 tons of CO_2 (Cheah et al. 2015). Besides, photobiological hydrogen production compared to other methods of hydrogen generation such as pyrolysis, supercritical gasification, gasification, or steam reforming, has gained attention due to low investment costs, lower energy requirements and high efficiency and less land area for microalgal growth (Guo et al. 2016; Schenk et al. 2008; Kumar 2020; Hu and Chapter, 2021).

Photosynthetic microalgae and cyanobacteria use sunlight and carbon dioxide to carry out biological photolysis to produce hydrogen. Biophotolysis is a light-dependent reaction in photosynthetic systems in which water molecules are converted to hydrogen and oxygen gases under oxidation

✉ Mohammad Hossein Morowvat
mhmorowvat@sums.ac.ir

1 Department of Chemical Engineering, University of Sistan and Baluchestan, Zahedan, Iran

2 School of Civil engineering and Surveying, Faculty of Health Engineering and Sciences, University of Southern Queensland, Toowoomba 4350, Australia

3 Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, P.O. Box 71468-64685, Shiraz, Iran

4 Department of Pharmaceutical Biotechnology, School of Pharmacy, Shiraz University of Medical Sciences, P.O. Box 71468-64685, Shiraz, Iran

reactions (Masukawa et al. 2002; Aoyama et al. 1997; Jeffries et al. 1978). In direct biophotolysis, the reduced form of ferredoxin or NADPH, which is produced during photosynthesis, is used directly in the reduction of protons and the production of hydrogen gas in the presence of hydrogenase (Brentner et al. 2010). In fact, direct biophotolysis refers to the production of hydrogen gas under the influence of light radiation. Light energy is absorbed by photosynthetic systems, increasing the energy level of water electrons, and eventually, some of the light energy is stored directly in molecular hydrogen (Tiwari and Pandey 2012). During indirect photosynthesis, reduced carbon is stored in carbohydrates such as starch and glycogen in microalgae and cyanobacteria, respectively. The stored energy is released as carbohydrates in the dark during the fermentation process, producing molecular hydrogen using the enzyme hydrogenase. The internal storage of carbohydrates is produced by the same cells during photosynthesis. In general, the process of indirect biophotolysis consists of two stages described by Eqs. (1) and (2). The first stage is photosynthesis to produce and store carbohydrates, and the second stage is the fermentation of internal carbohydrates in the dark to produce hydrogen gas (Kruse and Hankamer 2010; Esquível et al. 2011; Kossalbayev et al. 2020).

$$12H_2O + 6CO_2 \rightarrow C_6H_{12}O_6 + 6O_2 \quad (1)$$

$$C_6H_{12}O_6 + 12H_2O \rightarrow 12H_2 + 6CO_2 \quad (2)$$

A significant number of bacteria, cyanobacteria, and green algae such as *Rhodobacter sphaeroides* O.U. 001, *Rhodopseudomonas palustris*, *Chromatium* sp. Miami PSB 1071, *Rhodospirillum rubnum* (Eroğlu, et al. 2008; Zürrer and Bachofen 1982; Ohta et al. 1071; Otsuki et al. 1998), *Anabaena* sp. UTEX 1448, *Anabaena variabilis* ATCC 29,413, *Anabaena azollae*, *Gleobacter* PCC 7421, *Chroococcidiopsis thermalis* CALU 758, *Oscillatoria limosa*, *Oscillatoria chalybea*, *Oscillatoria* sp. Miami BG7, *Synechocystis* sp. PCC6803, *Microcystis* PCC 7820, (Abdel-Basset and Bader 1997; Berberoğlu et al. 2008; Tsygankov et al. 1998a; Moezelaar et al. 1996; Serebryakova et al. 2000; Heyer et al. 1989; Phlips and Mitsui 1983; Vargas, et al. 1448; Touloupakis, et al. 2016) C*hlamydomonas reinhardtii*, *Scenedesmus*, *Chlorella* sp., *Tetraspora* (Jiménez-Llanos, et al. 2020; Ban et al. 2019; Sivaramakrishnan 2021) can produce hydrogen biologically. Photosynthetic bacteria such as *Rhodobacter* use lactic acid and acetic acid as carbon and energy sources and produce hydrogen gas under light conditions (Eroğlu, et al. 2008). Also, cyanobacteria, especially heterocystous filamentous, are good candidates for hydrogen production due to nitrogenase activity during nitrogen fixation and or bidirectional hydrogenase activity during the photosynthesis process (Khetkorn et al. 2017). Of particular interest are *Anabaena variabilis* and *Anabaena* sp., which are the most potent CO_2 consumers and H_2 producers (Berberoğlu et al. 2008; Vargas, et al. 1448).

Several photobioreactors such as tubular, flat panel, and vertical-column have been designed for photosynthetic biomass growth and hydrogen production. These photobioreactors provide a secure culture medium that is safe against contamination and competition by other microorganisms. Control of the growth condition can be closely adjusted and monitored, enabling a fast and efficient biomass growth (Karemore, et al. 2015; Geada et al. 2017; Xiaogang 2020; Oncel and Kose 2014; Skjånes et al. 2016). Some studies proposed a two-stage photobioreactor alternating between stages of growth and production of hydrogen. In the growth phase, cyanobacteria utilize atmospheric CO_2 and nitrogen for their growth and carbohydrate production, and in the hydrogen production phase, they produce hydrogen using stored carbohydrates (Nayak et al. 2014; Markov et al. 1993; Yoon et al. 2002). Such a two-step process was used for the growth and hydrogen production by *Anabaena* sp. in airlift photobioreactors with different ratios of downcomer to riser cross-section areas (A_d/A_r). The maximum biomass concentration and cumulative hydrogen production were found to be 1.63 g L^{-1} and 1600 mL L^{-1}, respectively, at A_d/A_r of 1.6 (Nayak et al. 2014). Among photobioreactors (PBRs), airlift reactors (ALRs) have been widely used in separation processes, especially for the cultivation of microalgae and biohydrogen production (Ferreira, et al. 2012). Compared to similar devices such as stirred tanks and bubble columns, airlift photobioreactors offer lower and more uniform shear stress (Chisti and Moo-Young 1987; Dasgupta et al. 2010), which has led to their widespread use in biological processes. The growth of cyanobacteria can be affected by superficial gas velocity and the CO_2 content of the gas being sparged in the medium (Anjos et al. 2013). Sparging rate will affect the liquid velocity, which in turn increases the oxygen mass transfer and reduces dead zones and microalgal sedimentation (Pegallapati and Nirmalakhandan 2013). However, high sparging rate and CO_2 content can inhibit cyanobacterial growth (Acién 2017; Carvalho et al. 2006); Also, the aeration rate and light/dark cycle influence photosynthesis (Xue et al. 2013) and affect the mass transfer of oxygen, which can inhibit H_2 production. There is currently a lack of studies trying to link hydrodynamic parameters, growth, and hydrogen production. In order to achieve cyanobacterial growth and high biohydrogen yield, the hydrodynamic parameters and volumetric mass transfer coefficient must be fully understood to provide the best conditions for growth and light absorption (Guo et al. 2015; Babcock et al. 2002).

In this regard, the present study investigated biohydrogen production in an airlift photobioreactor by *Anabaena* sp. under two independent tests carried out at velocities of

0.185 and 0.524 cm/s to find out the impact of bioreactor hydrodynamic on H_2 production.

Materials and methods

Cultivation condition

To cultivate cyanobacteria in the reactor, *Anabaena* sp. was supplied from Science and Technology Park, Bushehr, Iran. The growth medium $BG11_0$ (without supplemented $NaNO_3$) (Nayak et al. 2014; Rippka et al. 1979) contained 0.04 g K_2HPO_4, 0.075 g $MgSO_4{\cdot}7H_2O$, 0.036 g $CaCl_2{\cdot}2H_2O$, 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 $MnCl_2{\cdot}4H_2O$, 0.222 g $ZnSO_4{\cdot}7H_2O$, 0.39 g $Na_2MoO_4{\cdot}2H_2O$, 0.079 g $CuSO_4{\cdot}5H_2O$, and 49.4 mg $Co(NO_3)_2{\cdot}6H_2O$ in one liter (Nayak et al. 2014). The culture medium was then autoclaved at 121 °C for 20 min. *Anabaena* sp. was first cultured for 5–6 days in a 500-mL Erlenmeyer flask containing 100 mL $BG11_0$ medium (without $NaNO_3$) (Nayak et al. 2014; Rippka et al. 1979) at 30 °C and 65 µmol photons m^{-2} s^{-1} of fluorescent lamps under a 12:12 h light–dark cycle and shaking incubator of 150 rpm (Nayak et al. 2014; Masukawa, et al. 2010). After 6 days, 15 mL of the previously cultured solution was added to 4 Erlenmeyer flasks containing 250 mL of culture medium. They were again cultured for 6 days. Then, these 4 Erlenmeyer flasks were used to inoculate the 16L working volume of the photobioreactor.

Before the inoculation, $BG11_0$ medium (without $NaNO_3$) (Rippka et al. 1979) for the working volume of the bioreactor was prepared and autoclaved. The surface of the reactor was illuminated using fluorescent lamps at an intensity of 140 µmol photons m^{-2} s^{-1} with a 12:12 light–dark (LD) cycle (Schütz et al. 2004; Borodin et al. 2000). The reactor was maintained at 30 °C using a 100 W heater (Sobo HC–100 model) installed into the photobioreactor. To adjust the pH at 8, NaOH (0.1 M) was added to the medium (Tsygankov et al. 2002), and a pH meter (Metrohm 827 pH Lab meter) was used.

Construction and operation of photobioreactor

The photobioreactor used in this experiment was an internal-loop airlift photobioreactor with two vertical cylinders made of Plexiglass. The bioreactor height, liquid height (h_l) in the downcomer, and the diameter of the photobioreactor were 200, 110, and 15 cm, respectively (aspect ratio (H/D) = 7.33) as shown in Table 1. The sparger was ring-shaped and installed at a distance of 11 cm from the bottom of the reactor. It was perforated with 18 holes with a diameter of 1 mm (Fig. 1a). The reactor was connected to an air compressor (78% N_2, 21% O_2, and 1% other gases) and a pure carbon dioxide cylinder, and gas flow rates were measured using rotameters. Air containing 2% carbon dioxide was sparged at the bottom of the photobioreactor, according to Borodin et al. (2000).

Table 1 Dimensions of the internal-loop airlift reactor

Diameter of the reactor (cm)	15
Diameter of the draft tube (cm)	10
Height of reactor (cm)	200
Liquid height (cm)	110
Riser height (cm)	100
A_d/A_r	1.24
Total liquid volume (L)	16
Total reactor capacity (L)	20

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 the liquid circulation velocity and mixing time, Rhodamine WT ($\rho = 1.1600$ g mL^{-1}) 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. (3).

$$U_{circ} = \frac{X_{circ}}{t_{circ}} \tag{3}$$

In this experiment, t_{circ} was measured when the dye tracer 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 according to Eq. (4) (Chisti and Moo-Young 1987).

$$U_d \times A_d = U_r \times A_r \tag{4}$$

where A_d and A_r are the downcomer and riser cross-section, respectively. Equation (5) was also used to determine the 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 (Chisti and Moo-Young 1987).

$$\varepsilon = \frac{H_2 - H_1}{H_2} \tag{5}$$

The holdup in airlift reactors is affected by liquid circulation velocity, which also depends on the gas–liquid separation zone above the reactor and the height of the reactor (Al-Mashhadani et al. 2015). Therefore, the obtained relationship is highly geometry-dependent.

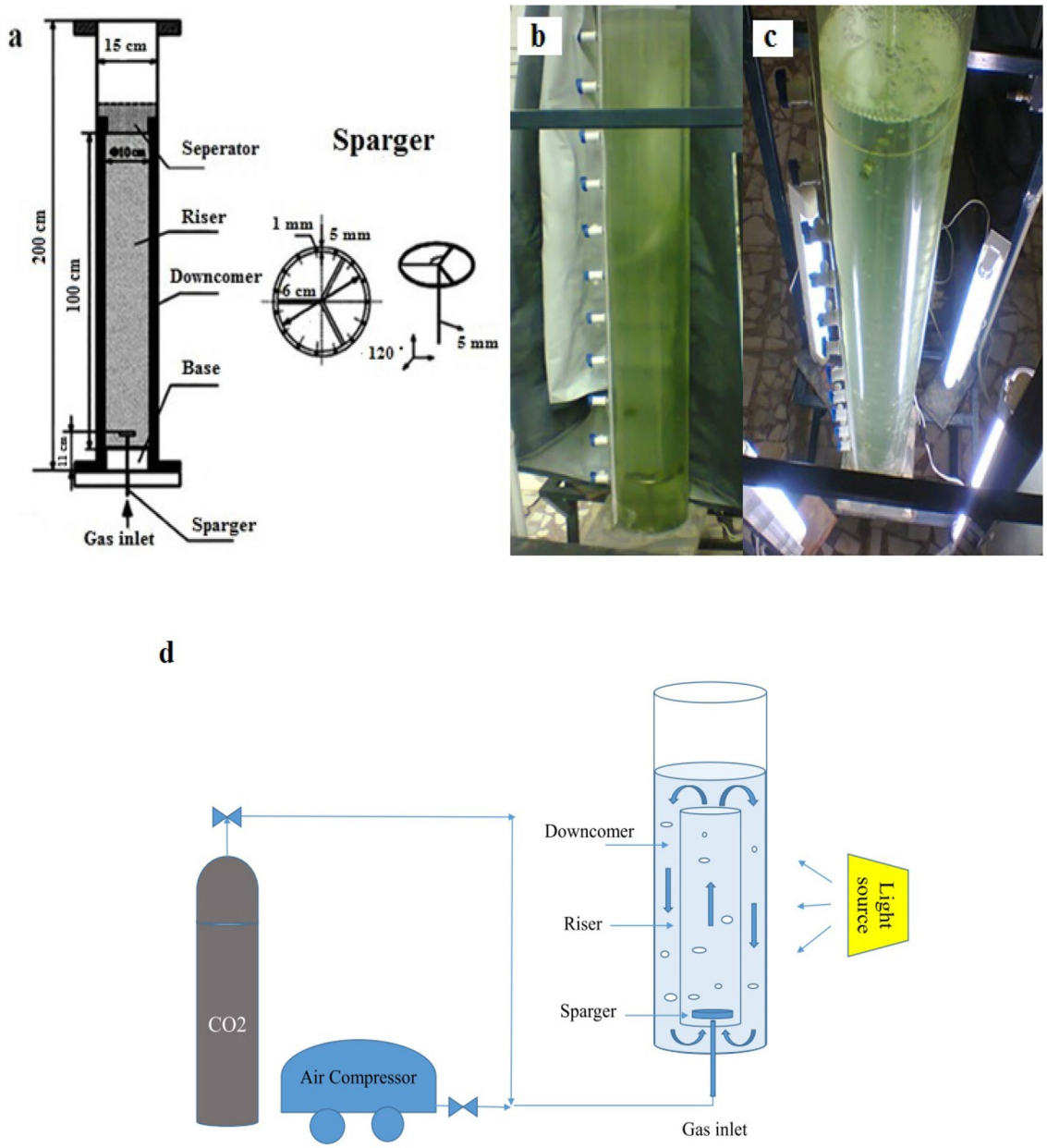

Fig. 1 **a** and **d** Schematics diagram of the photobioreactor, **b** and **c** actual images of (ALR)

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. Equation (6) (Chisti and Moo-Young 1987) was used to calculate the oxygen mass transfer coefficient at superficial velocities of 0.185 and 0.524 cm/s in abiotic condition.

$$\ln\left(\frac{C^* - C_{t0}}{C^* - C_t}\right) = K_l a(O_2) \cdot t \tag{6}$$

By plotting $\ln\left(\frac{C^*-C_{t0}}{C^*-C_t}\right)$ versus time, a straight line was obtained, and its slope is equal to the volumetric oxygen mass transfer coefficient ($K_l a\ (O_2)$). C* is defined as equilibrium concentration; C_t and C_{t0} are concentrations at $t = t_c$ and $t = 0$, respectively.

Experimental setup and hydrogen production

During the 7 days experiment, the reactor content was sampled daily to determine the growth of *Anabaena* sp. The biomass concentration was estimated by measuring the optical density and dry weight. The growth rate of *Anabaena* sp. was measured in an internal-loop airlift photobioreactor under intermittent culture feeding. 100- mL samples were withdrawn daily from the bioreactor to measure the optical density and dry weight of cyanobacteria. Two samples of 20 mL were used for measuring optical density, while the remaining 60 mL was used to evaluate the dry weight. The optical density measurement experiment was repeated for two samples of 20 mL each time using a spectrophotometer (DR 5000) at 680 nm. Dry weight (DW) was calculated by a calibration plot between DW and OD_{680nm} at two inlet gas velocities (0.185 and 0.524 cm s^{-1}), as shown in Fig. 2. For the dry weight test, a pre-weighed test tube was loaded with 60 ml of the sample and centrifuged (Sigma 3-30KS) at 2200*g* for 10 min (López et al. 2009). Afterward, the sample was dried in an oven for 3 days. The dry weight was finally calculated by re-measuring the weight of the test tube containing the dried cyanobacteria.

Analytical methods

Hydrogen and dissolved oxygen were measured in two different tests; one carried out at the velocity of 0.185 cm s^{-1} and another at 0.524 cm s^{-1} for 7 days. To analyze the concentrations of H_2 and O_2 gases, the headspace gas was collected in a 500 mL Tedlar bag, and 1 mL was injected into a gas chromatograph (HP-5890; Hewlett Packard, USA) equipped with a Carboxen-1000 column (Supelco, USA) and a thermal conductivity detector (HP-3395, Hewlett Packard). The gas chromatography (GC) 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 O_2 and H_2 gases residence times were 8 and 2 min, respectively. Similarly, for CO_2 gas, the oven temperature and residence time were 255 °C and 5 min, respectively. The GC was calibrated with known standard gas containing 10% (by vol.) of hydrogen and 20% (by vol.) of oxygen purchased from Soheil Gas Company, Karaj, Iran. The total hydrogen volume was obtained by multiplying the total headspace volume of the reactor including the Tedlar bag (4.5 L) by the hydrogen content obtained from the GC analysis.

Also, dissolved oxygen was measured with a Clark-type electrode. However, N_2 gas in the photobioreactor and Tedlar bag was not measured.

Results and discussion

Gas holdup and liquid velocity

The local gas holdup test was carried out under abiotic conditions using distilled water and under biotic conditions using *Anabaena* sp. culture on days 1 and 6, as shown in Fig. 3.

The gas holdup in cultivation on the first day was greater than distilled water. It has been reported that this increase is not attributed to the presence of cyanobacteria as they do not sufficiently grow to affect holdup. The difference on the first day is attributed to the presence of ions in the culture medium since dissolved ions prevent larger bubbles from joining (Chisti and Moo-Young 1987). Thus**,** only smaller bubbles could form and increase the gas holdup. The results showed that as the residence time of cyanobacteria in the bioreactor increased, the gas holdup decreased due to the growth of cyanobacteria. When the cyanobacteria reached their maximum growth on the sixth day, the gas holdup was lower than the first day after inoculation. Chisti and Moo-Young (1987) observed that a reduction in the turbulence of

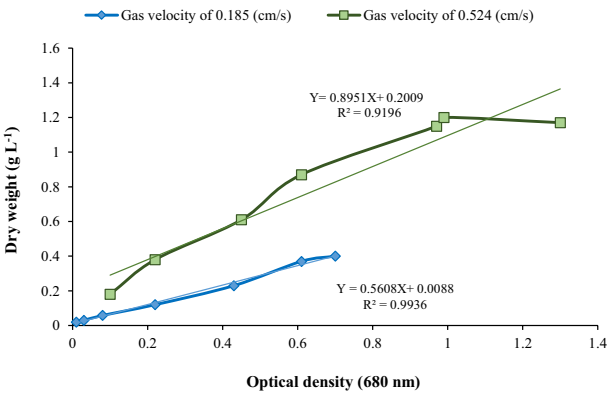

Fig. 2 Relationship between dry weight of *Anabaena* sp. and optical density at 680 nm (number of samples: n = 2 ± standard deviation)

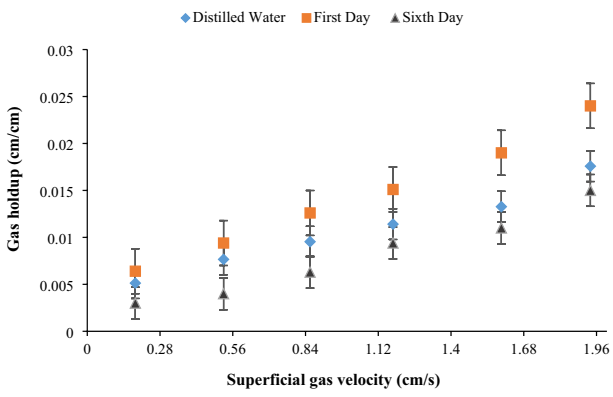

Fig. 3 Gas holdup in distilled water and in cyanobacteria culture on the first day and sixth day; the number of the experiments: n = 3 ± standard deviation

solutions encompassed more solid components, resulting in larger bubbles, which is consistent with our results.

Furthermore, it can be seen in Fig. 4 that the experimental velocity values in the downcomer increased from (3.04 to 10.36 cm/s) with an increase in superficial gas velocity. Similarly, the velocity in the riser increased from 3.8 to 12.96 cm/s, which corroborated Chisti's data due to the similarity of the reactor geometry (Chisti and Moo-Young 1987). This increase in the downcomer and riser velocities was due to the increase in bubbles number, which lowers the drag force on bubbles (Ojha and Al-Dahhan 2018).

Mass transfer coefficient

The volumetric oxygen mass transfer coefficient was calculated using Eq. (4) and compared with Bello et al. (1985a), as illustrated in Fig. 5. It can be seen that with an increase in superficial gas velocity, k_La increased from 1.15×10^{-2} to 3.34×10^{-2} s^{-1}. At low gas velocities, the coefficient obtained from our experimental data was close to Bello et al.'s results. The difference, however, increased 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 fact, k_La is basically determined by the gas holdup when the diameter of bubbles is steady. In other words, with an increase in the gas holdup, the surface area of the bubbles increases; therefore, k_La is higher (Huang et al. 2010). In a similar study with an increase in the superficial gas velocity from 0.3×10^{-3} to 1.35×10^{-3} m/s, k_La increased from 6.91×10^{-3} to 2.69×10^{-2} s^{-1} (Touloupakis, et al. 2016).

Influence of superficial gas velocity on *Anabaena* sp. growth

The growth rate of *Anabaena* sp. was monitored in the internal-loop airlift photobioreactor in order to investigate the impact of superficial gas velocity. It can be seen from

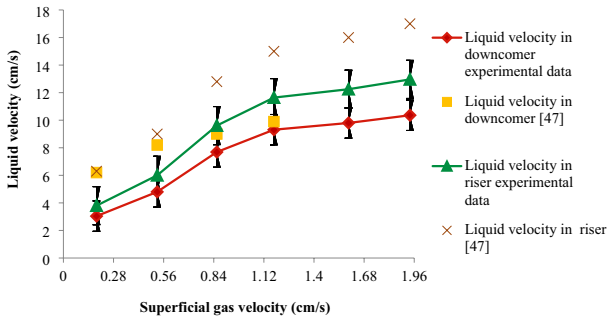

Fig. 4 Comparison of experimental data of liquid velocity in riser and downcomer with Chisti's work; the number of experiments: n = 3 ± standard deviation

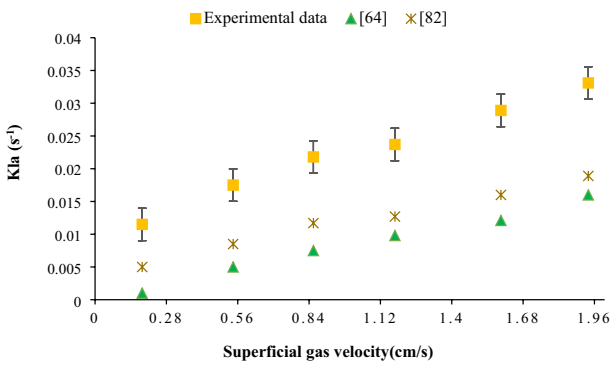

Fig. 5 Comparison of experimental data of K_La with Bello et al.'s results (Bello et al. 1985a, b)

Fig. 6 that a superficial gas velocity of 0.524 cm s^{-1} resulted in better growth and greater biomass density of 1.2 g L^{-1} after 7 days. It was observed that the increase in superficial gas velocity was beneficial to the light/dark cycle in the reactor. Janssen et al. (2003) studied the light/dark cycles frequency in the photobioreactors and found that increasing liquid circulation velocity, which depends on the inlet gas velocity, increased this parameter. Moreover, higher light/dark cycles frequencies resulted in a greater biomass concentration due to a decrease in the mean residence time of cells in the riser and downcomer (Sarat Chandra et al. 2017). The same authors reported that shorter light/dark cycles time of microsecond or millisecond led to higher photosynthetic efficiencies. They observed the improvement of photosynthetic efficiencies with light/dark cycles of 1–4 s. Besides, at higher gas velocity, there is more liquid circulation and mixing, and higher turbulence makes microalgae move faster toward the light sources and between the light regions in the photobioreactor, which is the main factor to achieve higher productivity (Cañedo and Lizárraga 2016). It was reported that a significant increase of *Chlorella vulgaris* concentration from 1.97 to 3.96 g L^{-1} was obtained

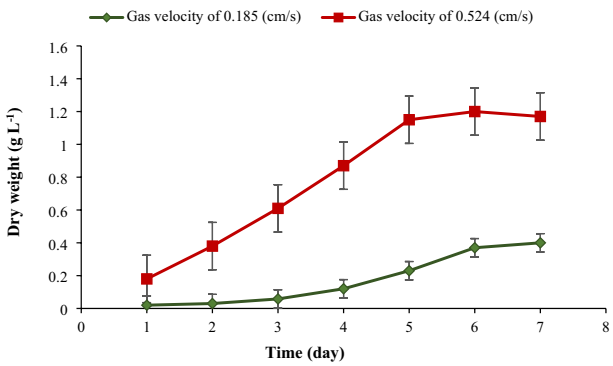

Fig. 6 Dry weight of *Anabaena* cyanobacteria over 7 days of growth in the photobioreactor sparged at gas velocities of 0.185 and 0.524 cm/s. (number of experiments: n = 3 ± standard deviation

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 is in agreement with our results (Touloupakis et al. 2016). According to Fernandes et al., increasing the aeration rate from 0.11 to 0.44 cm/s in a bubble column reactor resulted in a maximum *C. vulgaris* yield of 0.52–0.6 g L^{-1} d^{-1} (Fernandes et al. 2014). Other researchers investigated the biomass productivity of *Chaetoceros calcitrans* in a bubble column and airlift PBR when the inlet gas velocity increased, and the productivity was nearly half in the bubble column (Krichnavaruk et al. 2007). In another study in a 3.4 L airlift photobioreactor inoculated with *Scenedesmus obtusus*, the increase in the gas flow rate resulted in an increase in biomass productivity from 0.04 to 0.07 g $L^{-1}d^{-1}$ (Sarat Chandra et al. 2017). However, in laboratory studies, the concentration of cyanobacteria is usually limited to 1–3 g L^{-1} due to excessive shear stress in the photobioreactor at a gas velocity greater than 1 cm s^{-1}, which limits biomass concentration (Guo et al. 2015).

Hydrogen production

During the operation, 2% CO_2 and 98% air were sparged in the reactor. Some of the gases are absorbed by cyanobacteria through photosynthesis, while the rest exits at the top of the reactor. As it can be seen in Fig. 7, the net concentration of O_2 was due to the balance of O_2 produced from direct photolysis and O_2 consumed by respiration.

The results demonstrated that at the superficial gas velocity of 0.185 cm/s, the density of cyanobacteria was much less than at 0.524 cm/s due to the lower metabolism; as a result, H_2 production was insignificant (data not shown) due to O_2 obtained from photolysis which inhibited nitrogenase and hydrogenase activities (Lazaro et al. 2015). During the first four days, when *Anabaena* sp. started to grow, the rate of O_2 concentration also increased, indicating that photosynthesis was prevailing. However, after four days, the O_2 concentration remained steady.

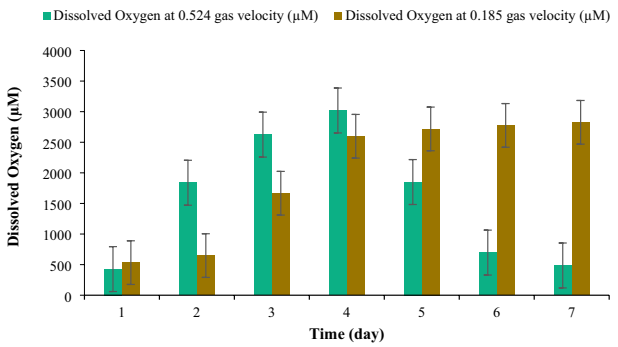

Fig. 7 Dissolved oxygen at two gas velocities (0.185, 0.524 cm/s); temperature: (30 ± 2 °C); pH = 8; the number of experiments: 3 ± standard deviation

When the inlet gas velocity was 0.185 cm s^{-1}, some cells could not move from the downcomer to the riser and remained on the wall of the downcomer, as a result, less light penetrated the reactor, but this did not have a significant effect on dissolved oxygen. It means that the light intensity influences the compensatory point between photosynthesis and respiration, as well as higher cell density due to the lower light penetration. These factors influence the amount of O_2 produced and how much is consumed (Volgusheva et al. 2015; Prabina and Kumar 2010). This observation is consistent with Tsygankov et al.'s study in which H_2 production at low gas velocity was inhibited and remained low because of photosynthetically produced O_2 (Tsygankov et al. 1998b). On the other hand, at higher aeration rates (0.524 cm s^{-1}), the amount of O_2 increased gradually from day 1 to 4 due to the increase in microalgal metabolism. As the density of cyanobacteria increased, photosynthesis and O_2 concentration also increased. However, after four days, due to increasing cell density, the light penetration declined through the center of the ALR (riser or dark zone), leading to a higher respiration rate and reduced photosynthesis. Consequently, the production of O_2 also dropped, which decreased the inhibition of hydrogenase, and led to a H_2 content of 8.24% in the headspace and a total H_2 production of 371 mL after 7 days (at 30 °C and 1 atm). A similar study reported that hydrogen evolution in darkness by *Anabaena* sp. strain N-7363 occurred by hydrogenases, and they did not observe hydrogen uptake activity (Asada and Kawamura 1986). The previous study also studied the impact of the increase of dissolved oxygen on H_2 production by *Anabaena variabilis* ATCC 29413, and they found a sharp decline in hydrogen production to 7% (Tsygankov et al. 1998b).

However, O_2 production can increase in high-density cultures (2.5–3.0 g L^{-1}), and it is known that hydrogenase and nitrogenase enzymes secreted by cyanobacteria are sensitive to oxygen produced during photosynthesis (Tsygankov et al. 2002). Hydrogen, which is a by-product of nitrogen fixation in filamentous cyanobacterium such as *Anabaena* sp., is mainly produced via nitrogenase enzyme in its heterocyst cells (Weber et al. 2014). In contrast to the bidirectional hydrogenase enzyme, which can produce and consume hydrogen, nitrogenase only produce H_2 in the unidirectional (Esteves-Ferreira et al. 2017). Hydrogen is formed by an optimal nitrogen-fixation reaction (Rosenbaum and Schröder 2010):

$$N_2 + 8e^- + 8H^+ + 16ATP \rightarrow H_2 + 2NH_3 + 16\left(ADP + P_i\right) \quad (7)$$

Or without N_2:

$$8H^+ + 8e^- + 16ATP \rightarrow 4H_2 + 16ADP + 16P_i \quad (8)$$

where as nitrogenase is protected by heterocysts against inactivation by oxygen, rising O_2 partial pressure reduces nitrogenase activity significantly. This is often a problem

encountered during the hydrogen production by *Anabaena* sp. (Su et al. 2010). However, Masukawa et al. (Masukawa et al. 2010, 2014) observed that the lowest level of H_2 production by *Anabaena* sp. PCC7120 took place in the presence of high N_2 concentrations. A similar study also reported that *Anabaena* sp. PCC7120 and *Anabaena cylindrica* UTEX B629 produced the lowest amount of hydrogen under air due to the presence of N_2, which decreased nitrogenase activity (Yeager et al. 2011).

Conclusions

The results of this research showed that gas holdup and liquid circulation velocity played a decisive role in cyanobacterial 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, it was demonstrated that at the superficial gas velocity of 0.185 cm s^{-1}, far less hydrogen was produced than 0.524 cm s^{-1}. The total amount of hydrogen (371 mL after 7 days) was observed at 0.524 cm s^{-1}. However, the process of biological H_2 production in the ALR requires more investigation related to the aeration rate under air or in the absence of N_2 or O_2 and under different light intensities. The results of this study might be applied in further research on biohydrogen production from microalgae.

Acknowledgements This work was supported by the Department of Chemical Engineering of the University of Sistan & Baluchestan, Zahedan and Department of Pharmaceutical Biotechnology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. The authors would like to thank Professor Rahbar Rahimi for his help with bioreactor design.

Author contributions ZZ methodology, data collection, preliminary analysis, writing, original draft. PM methodology, data collection, preliminary analysis. AT writing, review, editing. MHM investigation, writing, review, data curation, editing, visualization, formal analysis, conceptualization, methodology, supervision.

Funding Not applicable.

Availability of data and material All generated or analyzed data, the exploited software, and materials were included in this published article. The generated results during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

Abdel-Basset R, Bader KP (1997) Characterization of hydrogen photoevolution in oscillatoria chalybea detected by means of mass spectrometry. Zeitschrift Für Naturforschung C 52(11–12):775–781

Acién F et al (2017) Photobioreactors for the production of microalgae. Microalgae-based biofuels and bioproducts. Elsevier, pp 1–44

Al-Mashhadani MKH, Wilkinson SJ, Zimmerman WB (2015) Airlift bioreactor for biological applications with microbubble mediated transport processes. Chem Eng Sci 137:243–253

Anjos M et al (2013) Optimization of CO_2 bio-mitigation by *Chlorella vulgaris*. Biores Technol 139:149–154

Anwar M et al (2019) Recent advancement and strategy on bio-hydrogen production from photosynthetic microalgae. Bioresour Technol 292:121972

Aoyama K et al (1997) Fermentative metabolism to produce hydrogen gas and organic compounds in a cyanobacterium, *Spirulina platensis*. J Ferment Bioeng 83(1):17–20

Asada Y, Kawamura S (1986) Aerobic hydrogen accumulation by a nitrogen-fixing cyanobacterium *Anabaena* sp. Appl Environ Microbiol 51(5):1063–1066

Babcock RW, Malda J, Radway JC (2002) Hydrodynamics and mass transfer in a tubular airlift photobioreactor. J Appl Phycol 14(3):169–184

Balat M (2008) Potential importance of hydrogen as a future solution to environmental and transportation problems. Int J Hydrogen Energy 33(15):4013–4029

Ban S et al (2019) Improving hydrogen production of *Chlamydomonas reinhardtii* by reducing chlorophyll content via atmospheric and room temperature plasma. Bioresour Technol 275:425–429

Bello RA, Robinson CW, Moo-Young M (1985a) Prediction of the volumetric mass transfer coefficient in pneumatic contactors. Chem Eng Sci 40(1):53–58

Bello RA, Robinson CW, Moo-Young M (1985b) Gas holdup and overall volumetric oxygen transfer coefficient in airlift contactors. Biotechnol Bioeng 27(3):369–381

Berberoğlu H, Jay J, Pilon L (2008) Effect of nutrient media on photobiological hydrogen production by *Anabaena variabilis* ATCC 29413. Int J Hydrogen Energy 33(4):1172–1184

Borodin VB et al (2000) Hydrogen production by Anabaena variabilis PK84 under simulated outdoor conditions. Biotechnol Bioeng 69(5):478–485

Brentner LB, Peccia J, Zimmerman JB (2010) Challenges in developing biohydrogen as a sustainable energy source: implications for a research agenda. Environ Sci Technol 44(7):2243–2254

Cañedo JCG, Lizárraga GLL (2016) Considerations for photobioreactor design and operation for mass cultivation of microalgae. Algae-organisms for imminent biotechnology. InTech

Carvalho AP, Meireles LA, Malcata FX (2006) Microalgal reactors: a review of enclosed system designs and performances. Biotechnol Prog 22(6):1490–1506

Cheah WY et al (2015) Biosequestration of atmospheric CO_2 and flue gas-containing CO_2 by microalgae. Bioresour Technol 184:190–201

Chisti M, Moo-Young M (1987) Airlift reactors: characteristics, applications and design considerations. Chem Eng Commun 60(1–6):195–242

Dasgupta CN et al (2010) Recent trends on the development of photobiological processes and photobioreactors for the improvement of hydrogen production. Int J Hydrogen Energy 35(19):10218–10238
Eroğlu İ et al (2008) Hydrogen production by *Rhodobacter sphaeroides* O.U.001 in a flat plate solar bioreactor. Int J Hydrogen Energy 33(2):531–541
Esquível MG et al (2011) Efficient H2 production via *Chlamydomonas reinhardtii*. Trends Biotechnol 29(12):595–600
Esteves-Ferreira AA et al (2017) Cyanobacterial nitrogenases: phylogenetic diversity, regulation and functional predictions. Genet Mol Biol 40:261–275
Fernandes BD et al (2014) Characterization of split cylinder airlift photobioreactors for efficient microalgae cultivation. Chem Eng Sci 117:445–454
Ferreira AF et al (2012) Biological hydrogen production by *Anabaena* sp–yield, energy and CO_2 analysis including fermentative biomass recovery. Int J Hydrogen Energy 37(1):179–190
Geada P et al (2017) Chapter 13—microalgal biomass cultivation. In: Rastogi RP, Madamwar D, Pandey A (eds) Algal green chemistry. Elsevier, Amsterdam, pp 257–284
Guo X, Yao L, Huang Q (2015) Aeration and mass transfer optimization in a rectangular airlift loop photobioreactor for the production of microalgae. Bioresour Technol 190:189–195
Guo Z, Li Y, Guo H (2016) Characterization of H_2 photoproduction by marine green alga Tetraselmis subcordiformis integrated with an alkaline fuel cell. Biotech Lett 38(3):435–440
Heyer H, Stal L, Krumbein WE (1989) Simultaneous heterolactic and acetate fermentation in the marine cyanobacterium *Oscillatoria limosa* incubated anaerobically in the dark. Arch Microbiol 151(6):558–564
Hossain MA, Jewaratnam J, Ganesan P (2016) Prospect of hydrogen production from oil palm biomass by thermochemical process—a review. Int J Hydrogen Energy 41(38):16637–16655
Hu J (2021) Chapter 4—comparisons of biohydrogen production technologies and processes. In: Zhang Q et al (eds) Waste to renewable biohydrogen. Academic Press, pp 71–107
Huang Q et al (2010) CFD simulation of hydrodynamics and mass transfer in an internal airlift loop reactor using a steady two-fluid model. Chem Eng Sci 65(20):5527–5536
Janssen M et al (2003) Enclosed outdoor photobioreactors: light regime, photosynthetic efficiency, scale-up, and future prospects. Biotechnol Bioeng 81(2):193–210
Jeffries TW, Timourian H, Ward RL (1978) Hydrogen production by Anabaena cylindrica: effects of varying ammonium and ferric ions, pH, and light. Appl Environ Microbiol 35(4):704–710
Jiménez-Llanos J et al (2020) Sustainable biohydrogen production by *Chlorella* sp microalgae: a review. Int J Hydrogen Energy 45(15):8310–8328
Karemore A et al (2015) Photobioreactors for improved algal biomass production: analysis and design considerations. Springer, Cham, pp 103–124
Kavitha S et al (2017a) Low temperature thermochemical mediated energy and economically efficient biological disintegration of sludge: simulation and prediction studies for anaerobic biodegradation. Chem Eng J 317:481–492
Kavitha S et al (2017b) Liquefaction of food waste and its impacts on anaerobic biodegradability, energy ratio and economic feasibility. Appl Energy 208:228–238
Khetkorn W et al (2017) Microalgal hydrogen production—a review. Bioresour Technol 243:1194–1206
Kossalbayev BD et al (2020) Determination of the potential of cyanobacterial strains for hydrogen production. Int J Hydrogen Energy 45(4):2627–2639
Krichnavaruk S, Powtongsook S, Pavasant P (2007) Enhanced productivity of *Chaetoceros calcitrans* in airlift photobioreactors. Bioresour Technol 98(11):2123–2130
Kruse O, Hankamer B (2010) Microalgal hydrogen production. Curr Opin Biotechnol 21(3):238–243
Kumar G et al (2020) Thermochemical conversion routes of hydrogen production from organic biomass: processes, challenges and limitations. Biomass Convers Biorefin. https://doi.org/10.1007/s13399-020-01127-9
Lazaro CZ, Varesche MBA, Silva EL (2015) Effect of inoculum concentration, pH, light intensity and lighting regime on hydrogen production by phototrophic microbial consortium. Renew Energy 75:1–7
López CG et al (2009) Utilization of the cyanobacteria *Anabaena* sp ATCC 33047 in CO_2 removal processes. Bioresour Technol 100(23):5904–5910
Markov SA et al (1993) A hollow fibre photobioreactor for continuous production of hydrogen by immobilized cyanobacteria under partial vacuum. Int J Hydrogen Energy 18(11):901–906
Masukawa H, Mochimaru M, Sakurai H (2002) Hydrogenases and photobiological hydrogen production utilizing nitrogenase system in cyanobacteria. Int J Hydrogen Energy 27(11):1471–1474
Masukawa H et al (2010) Site-directed mutagenesis of the *Anabaena* sp Strain PCC 7120 nitrogenase active site to increase photobiological hydrogen production. Appl Environ Microbiol 76(20):6741–6750
Masukawa H et al (2014) Sustained photobiological hydrogen production in the presence of N2 by nitrogenase mutants of the heterocyst-forming cyanobacterium *Anabaena*. Int J Hydrogen Energy 39(34):19444–19451
Moezelaar R, Bijvank SM, Stal LJ (1996) Fermentation and sulfur reduction in the mat-building cyanobacterium Microcoleus chthonoplastes. Appl Environ Microbiol 62(5):1752–1758
Mona S et al (2020) Green technology for sustainable biohydrogen production (waste to energy): a review. Sci Total Environ 728:138481
Nagarajan D et al (2017) Recent insights into biohydrogen production by microalgae—from biophotolysis to dark fermentation. Bioresour Technol 227:373–387
Nayak BK, Roy S, Das D (2014) Biohydrogen production from algal biomass (*Anabaena* sp PCC 7120) cultivated in airlift photobioreactor. Int J Hydrogen Energy 39(14):7553–7560
Ohta Y, Frank J, Mitsui A (1981) Hydrogen production by marine photosynthetic bacteria: effect of environmental factors and substrate specificity on the growth of a hydrogen-producing marine photosynthetic bacterium, *Chromatium* sp Miami PBS 1071. Int J Hydrogen Energy 6(5):451–460
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
Oncel S, Kose A (2014) Comparison of tubular and panel type photobioreactors for biohydrogen production utilizing *Chlamydomonas reinhardtii* considering mixing time and light intensity. Bioresour Technol 151:265–270
Otsuki T et al (1998) Hydrogen production by a floating-type photobioreactor. Biohydrogen. Springer, pp 369–374
Pegallapati AK, Nirmalakhandan N (2013) Internally illuminated photobioreactor for algal cultivation under carbon dioxide-supplementation: performance evaluation. Renew Energy 56:129–135
Phlips E, Mitsui A (1983) Role of light intensity and temperature in the regulation of hydrogen photoproduction by the marine cyanobacterium *Oscillatoria* sp strain Miami BG7. Appl Environ Microbiol 45(4):1212–1220
Prabina BJ, Kumar K (2010) Studies on the optimization of cultural conditions for maximum hydrogen production by selected cyanobacteria. J Agric Biol Sci 5(5):22–31
Rippka R et al (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Microbiology 111(1):1–61
Rosenbaum M, Schröder U (2010) Photomicrobial solar and fuel cells. Electroanalysis 22(7–8):844–855

Sarat Chandra T et al (2017) Growth and biochemical characteristics of an indigenous freshwater microalga, *Scenedesmus obtusus*, cultivated in an airlift photobioreactor: effect of reactor hydrodynamics, light intensity, and photoperiod. Bioprocess Biosyst Eng 40(7):1057–1068

Schenk PM et al (2008) Second generation biofuels: high-efficiency microalgae for biodiesel production. BioEnergy Res 1(1):20–43

Schütz K et al (2004) Cyanobacterial H2 production—a comparative analysis. Planta 218(3):350–359

Serebryakova LT, Sheremetieva ME, Lindblad P (2000) H2-uptake and evolution in the unicellular cyanobacterium *Chroococcidiopsis thermalis* CALU 758. Plant Physiol Biochem 38(6):525–530

Sivaramakrishnan R et al (2021) Insights on biological hydrogen production routes and potential microorganisms for high hydrogen yield. Fuel 291:120136

Skjånes K et al (2016) Design and construction of a photobioreactor for hydrogen production, including status in the field. J Appl Phycol 28(4):2205–2223

Su Z et al (2010) An effective device for gas–liquid oxygen removal in enclosed microalgae culture. Appl Biochem Biotechnol 160(2):428–437

Tiwari A, Pandey A (2012) Cyanobacterial hydrogen production—a step towards clean environment. Int J Hydrogen Energy 37(1):139–150

Touloupakis E et al (2016) Hydrogen production by immobilized *Synechocystis* sp PCC 6803. Int J Hydrogen Energy 41(34):15181–15186

Tsygankov AA et al (1998a) An automated helical photobioreactor incorporating cyanobacteria for continuous hydrogen production. Biohydrogen. Springer, pp 431–440

Tsygankov AA et al (1998b) Acetylene reduction and hydrogen photoproduction by wild-type and mutant strains of *Anabaena* at different CO_2 and O_2 concentrations. FEMS Microbiol Lett 167(1):13–17

Tsygankov A et al (2002) Hydrogen production by cyanobacteria in an automated outdoor photobioreactor under aerobic conditions. Biotechnol Bioeng 80(7):777–783

Vargas SR et al (2018) Optimization of biomass and hydrogen production by *Anabaena* sp (UTEX 1448) in nitrogen-deprived cultures. Biomass Bioenergy 111:70–76

Volgusheva A et al (2015) Hydrogen photoproduction in green algae *Chlamydomonas reinhardtii* under magnesium deprivation. RSC Adv 5(8):5633–5637

Weber J et al (2014) Biotechnological hydrogen production by photosynthesis. Eng Life Sci 14(6):592–606

Xiaogang H et al (2020) Microalgal growth coupled with wastewater treatment in open and closed systems for advanced biofuel generation. Biomass Convers Biorefin. https://doi.org/10.1007/s13399-020-01061-w

Xue S et al (2013) A novel photobioreactor structure using optical fibers as inner light source to fulfill flashing light effects of microalgae. Bioresour Technol 138:141–147

Yeager CM et al (2011) Evaluation of experimental conditions that influence hydrogen production among heterocystous *Cyanobacteria*. Int J Hydrogen Energy 36(13):7487–7499

Yoon JH et al (2002) High cell density culture of *Anabaena variabilis* using repeated injections of carbon dioxide for the production of hydrogen. Int J Hydrogen Energy 27(11):1265–1270

Zürrer H, Bachofen R (1982) Aspects of growth and hydrogen production of the photosynthetic bacterium *Rhodospirillum rubrum* in continuous culture. Biomass 2(3):165–174