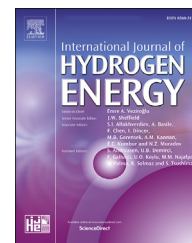


Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/he

Review Article

Biotechnological production of hydrogen: Design features of photobioreactors and improvement of conditions for cultivating cyanobacteria



Bekzhan D. Kossalbayev ^{a,b,**}, Girayhan Yilmaz ^c,
 Asemgul K. Sadvakasova ^b, Bolat Khan K. Zayadan ^b,
 Ayaz M. Belkozhaev ^{a,b,d}, Gulzhanay K. Kamshybayeva ^{a,b,***},
 Gaukhar A. Sainova ^e, Ayshat M. Bozieva ^f, Hesham F. Alharby ^g,
 Tatsuya Tomo ^h, Suleyman I. Allakhverdiev ^{c,f,*}

^a Department of Chemical and Biochemical Engineering, Institute of Geology and Oil-Gas Business Institute Named After K. Turyssov, Satbayev University, Satpayev 22, Almaty, 050043, Kazakhstan

^b Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, 050038, Kazakhstan

^c Faculty of Engineering and Natural Sciences, Bahcesehir University, Istanbul, 34353, Turkey

^d M.A. Aitkhozhin Institute of Molecular Biology and Biochemistry, Almaty, 050012, Kazakhstan

^e Ecology Research Institute, Khoja Akhmet Yassawi International Kazakh-Turkish University, Turkistan, 161200, Kazakhstan

^f K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, Moscow, 127276, Russia

^g Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, 21589, Saudi Arabia

^h Department of Physics, Graduate School of Science, Tokyo University of Science, 1-3 Kagurazaka, Shinjuku-ku, Tokyo 162-8601, Japan

Abbreviations: ATP, adenosine triphosphate; Chl, chlorophyll; CO₂, carbon dioxide; H₂, hydrogen; H₂ase, hydrogenase; LED, light-emitting diode; N₂, nitrogen; N₂ase, nitrogenase; O₂, oxygen; PBR, photobioreactor; SLOF, side-light optical fibres; TCA, tricarboxylic acid cycle.

* Corresponding author. K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, Moscow 127276, Russia.

** Corresponding author. Department of Chemical and Biochemical Engineering, Institute of Geology and Oil-Gas Business Institute Named After K. Turyssov, Satbayev University, Satpayev 22, Almaty, 050043, Kazakhstan.

*** Corresponding author. Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, 050038, Kazakhstan.

E-mail addresses: kossalbayev.bekzhan@gmail.com (B.D. Kossalbayev), gkamshebaeva@gmail.com (G.K. Kamshybayeva), suleyman.allakhverdiev@gmail.com (S.I. Allakhverdiev).

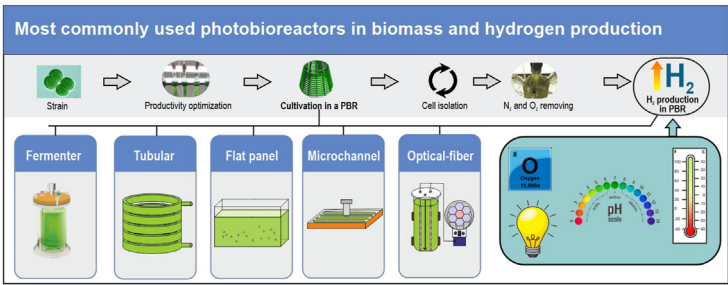
<https://doi.org/10.1016/j.ijhydene.2023.09.001>

0360-3199/© 2023 Hydrogen Energy Publications LLC. Published by Elsevier Ltd. All rights reserved.

HIGHLIGHTS

- Photosynthetic H₂ production processes was discussed.
- Physiochemical factors affecting H₂ production were explained.
- Five PBRs for H₂ production were described.
- Advantages/disadvantages of PBRs used for H₂ production were described.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 June 2023
Received in revised form
9 August 2023
Accepted 1 September 2023
Available online 19 September 2023

Keywords:
Photobioreactors
Cyanobacteria
Biohydrogen
Factors
Perspective

ABSTRACT

Over the last five decades, solar-based hydrogen (H₂) production has been intensively studied. Specifically, the study of biophotolysis by cyanobacteria has received great attention to produce H₂, and promising research approaches have been established. To date, numerous photobioreactors (PBRs) have been built to collect cyanobacterial biomass and generate bioenergy. Additionally, different PBR parameters were adjusted to increase the product yield. PBR development holds great potential not only for cell biomass but also for biological H₂ production. This review aimed to examine the mechanisms involved in H₂ production by cyanobacteria, explore the factors influencing the process, and describe five distinct PBRs known for their high H₂ production. This article examines the pros and cons of the most efficient PBRs for H₂ production and offers insights into strategies for increasing their productivity.

© 2023 Hydrogen Energy Publications LLC. Published by Elsevier Ltd. All rights reserved.

Contents

1.	Introduction	415
2.	Photosynthetic hydrogen production processes	415
3.	Improving the physicochemical parameters of cultivating cyanobacteria to increase hydrogen production	416
4.	Design features of PBRs used to increase hydrogen production	418
4.1.	Fermenter-type photobioreactors	421
4.2.	Tubular photobioreactors	421
4.3.	Flat panel photobioreactors	422
4.4.	Microchannel biofilm photobioreactors	423
4.5.	Optical fibre photobioreactors	424
5.	Current state and prospects of cyanobacterial hydrogen production	425
5.1.	Large-scale cultivation systems	426
6.	Conclusion	427
	Credit authorship contribution statement	427
	Declaration of competing interest	427
	Acknowledgements	427
	References	427

1. Introduction

Mineral resource utilisation poses significant environmental concerns, necessitating the development of new, ecologically benign technologies based on renewable energy sources [1]. Mankind can alleviate the environmental impact on nature, minimise pollution of ecosystems and water bodies, and mitigate the discharge of heavy metals and methane into the atmosphere by expanding the use of biological raw materials as an energy source [2]. Scientific attention is focused on investigating renewable raw materials for biofuel production, particularly in producing hydrogen (H_2) via biophotolysis [3]. Despite the promise to address a variety of future concerns, H_2 production is recognised as the cleanest yet technogenically demanding bioenergy process. The principal methods for producing H_2 involve diverse thermochemical processes [4].

Bio- H_2 produced by cyanobacteria offers new opportunities for replacing conventional fossil fuels and diversifying renewable energy sources [5]. Through diverse metabolic pathways, such as photosynthesis and nitrogen (N_2) fixation, the above microorganisms can convert solar energy into chemical one to produce H_2 [6]. Besides their use in the development of renewable energy sources, cyanobacteria are produced as healthful foods [7].

Since cell mass is primarily important for any production, a well-thought-out design of PBRs is crucial [8]. Therefore, the development of PBRs capable of achieving high cell density is increasingly significant in biofuel and biomass production [9]. To enhance biomass accumulation and biofuel production, PBRs must be designed with the utmost efficiency, as they directly influence the light path within the reactor [10]. In lab-scale research, the concentration of phototrophic microorganism cells has an upper limit of 0.5–2.5 g L⁻¹ in photoautotrophic growth cultures [11,12]. This limitation arises from insufficient light penetration throughout the PBR, thereby hampering cell growth due to insufficient light availability [13].

The production of photobiological H_2 commonly employs five types of PBRs, with other variations being modifications of these types. The widely used PBR types are fermenter, tubular, flat-plate, microchannel biofilm with immobilised cells, and optical fibre [14]. The fermenter PBRs are the most prevalent and effective construction in bioenergy, offering automated control of nutrient medium size and growth, thus increasing H_2 production efficiency [15]. Tubular PBRs come in various configurations such as helical, conical, or fence-like, featuring thin elongated tubes. Their key advantage is the rapid transport of cells within a single tube facilitated by a pump. The conditions within the tubes directly affect the kinetics and biomass density of the culture [16]. Flat-plate PBRs consist of a closed rectangular container with two transparent flat surfaces allowing light beams to pass through. An automated mixer is employed to mix cells, thereby facilitating high-intensity cell growth [17]. Optical fibre PBRs harness solar energy and enable the cultivation of cells in a closed system. They capture and utilise the obtained energy for cell growth [13]. Microchannel biofilm PBRs predominantly employ cell biofilms, resulting in relatively high H_2 yields. In addition, the physiological state of cells can be continuously tracked through microscopy [13].

The review examined the strengths and limitations of the five frequently employed PBRs for cyanobacteria to collect biomass and produce H_2 , as well as the factors that influence photobiological H_2 production enhancement in PBRs.

2. Photosynthetic hydrogen production processes

The utilisation of cyanobacteria is considered the most beneficial among the biological methods of producing H_2 [18]. Phototrophic microorganisms are highly effective H_2 producers since they utilise solar energy to produce H_2 without necessitating costly macro- and micronutrients [6,19]. Recent studies have shown that many species of cyanobacteria can produce H_2 [20]. Hence, the yield and duration of H_2 production vary due to morphological and genetic differences conditioned by their distinct traits and environmental conditions [21]. Heterocyst, trichome, and akinet-forming microorganisms are promising since they do not encounter substantial interference with H_2 production due to intracellular oxygen (O_2) release [22]. Heterocyst cyanobacteria, for instance, can produce H_2 despite molecular O_2 in the surrounding air. The rate of H_2 production by cyanobacteria is governed by the genetic ability to synthesise essential enzymes and the metabolic features that allocate the energy to the internal and external environment [23,24].

Thus, phototrophic microorganisms are promising biological objects for H_2 production by harnessing solar energy. The advancements in the technological features of PBRs used for cultivating producer strains can essentially improve the efficiency of bio- H_2 production. This can be accomplished through diverse genetic and metabolic engineering strategies [25,26].

Bio- H_2 production entails utilising microorganisms to produce H_2 through metabolic processes such as fermentation or photosynthesis [27]. The process occurs within PBRs, where microorganisms are cultivated and supplied with organic matter as an energy source, and H_2 is produced as a by-product. Bio- H_2 production is eco-friendly and releases no greenhouse gases. The process offers the advantage of utilising renewable resources such as plant and animal waste as raw materials [28,29]. Bio- H_2 production is recognised as a key future energy source and is considered a sustainable alternative to conventional H_2 production methods [30,31].

Cyanobacteria primarily utilise photosynthesis as the metabolic pathway of obtaining energy by converting sunlight into chemical energy. Similar to plants, cyanobacteria contain pigments such as chlorophyll *a* and phycobiliproteins that absorb light across a range of wavelengths. These pigments form structures called phycobilisomes and photosystems, optimizing light absorption. During photosynthesis, pigments capture light energy, initiating light-dependent reactions [30]. These reactions involve splitting water into O_2 and protons, transferring electrons along an electron transport chain (ETC), leading to ATP (adenosine triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) formation. ATP and NADPH generated in light-dependent reactions then fuel light-independent reactions, known as the Calvin or C_3 cycle. In this cycle, carbon dioxide (CO_2) is fixed

into organic compounds using ATP and NADPH as energy sources, ultimately producing sugars crucial for cyanobacterial growth and metabolism [32].

Biophotolysis-based H_2 production involves harnessing light energy via photosynthetic systems. Through a series of metabolic events, cyanobacteria convert light energy into chemical energy in the form of H_2 gas [33–35]. The produced H_2 can serve as a renewable energy source for diverse applications, such as fuel cells and other energy-related technologies.

Bio- H_2 production primarily relies on biophotolysis, which is classified into two types. The first type is direct biophotolysis, wherein microorganisms such as cyanobacteria utilise photosynthesis to produce H_2 gas directly. Direct biophotolysis, as opposed to other approaches that rely on the production of organic compounds like glucose or acetate, involves the direct conversion of light energy to H_2 gas facilitated by hydrogenases (H_2 ase), enzymes that catalyse the release of H_2 gas [36]. Direct biophotolysis offers several advantages over alternative methods of producing H_2 . It serves as a sustainable source of H_2 as microorganisms can be cultivated using renewable resources like sunlight and water. In addition, direct biophotolysis is cost-efficient, utilising carbon dioxide (CO_2) as a raw material [37]. However, direct biophotolysis faces certain limitations, including low H_2 production rates and microbial sensitivity to ambient factors such as pH, temperature, and light intensity [38]. Despite these limitations, direct biophotolysis holds considerable potential as a clean and sustainable source of H_2 , making it a significant area of research in renewable energy and sustainable H_2 production [39].

Another microbiological approach of producing H_2 is indirect biophotolysis. The approach relies on photosynthesis to produce organic compounds such as glucose or acetate. These organic molecules are then fermented by H_2 -producing microorganisms, yielding H_2 gas as a by-product [40]. Compared to direct biophotolysis, indirect biophotolysis has various benefits, including a higher yield of H_2 production; the utilisation of cheaper and more readily available carbon sources, such as CO_2 ; a greater overall process efficiency. The latter benefit is attributed to the multi-step process structure, which allows for efficient energy transfer from light to H_2 . These advantages make indirect biophotolysis an appealing alternative for producing H_2 [41]. Indeed, indirect biophotolysis holds significant promise as a sustainable and renewable supply of H_2 gas. The utilisation of microorganisms that may be cultivated using renewable resources like sunlight and water underscores its potential as an ecologically beneficial solution. Indirect biophotolysis is a vital study area within the realm of renewable energy and pursuing sustainable H_2 production [42].

3. Improving the physicochemical parameters of cultivating cyanobacteria to increase hydrogen production

Over the last decade, extensive research has been conducted to explore whether bioreactors can be constructed for large-scale H_2 production and the feasibility of utilising

microorganisms in them [43,44]. Based on the H_2 production approach, bioreactors are classified: some operate in the dark through water-gas shear reaction or fermentation induced by bacteria, while others, known as PBRs, employ cyanobacteria to harness sunlight and produce H_2 from water [45]. Utilising photosynthetic microorganisms like cyanobacteria for direct H_2 production can offer greater energy efficiency at a lower cost. However, it is crucial to effectively manage the physical and physiochemical indicators of phototrophic microorganisms for successful H_2 production [46].

Various physical factors such as a high area-to-volume ratio, temperature control, construction material strength and transparency, and an effective gas exchange and mixing system are important in the functioning of PBRs [47]. However, the performance of PBRs in terms of H_2 production is not solely determined by physical parameters, such as light penetration into the reactor. It also relies on physiochemical parameters that influence the different biochemical pathways involved in H_2 production. pH, temperature, light intensity, dissolved O_2 , dissolved CO_2 , mixing-induced displacement, carbon and nitrogen sources, as well as their specific ratios, are the primary physiochemical indicators affecting H_2 production [48]. Consequently, by appropriately adjusting these parameters, significant advancements can be achieved in bio- H_2 production.

Generally, the optimal pH for photoenzymatic H_2 production by cyanobacteria is around 7 [49]. However, the optimal pH for H_2 production varies amongst phototrophic microbial strains.

Hydrogen production in *Synechococcus* Nag. PCC 7942 was observed even in the dark and under anaerobic conditions. Temperature, similar to pH, significantly influences cyanobacteria's H_2 production capacity by regulating enzymatic reactions and metabolism. Literature indicates varying ideal temperature requirements depending on cyanobacteria species [50]. Dickson et al. [51] found a slight increase in H_2 production by cyanobacterial strains upon adding 80 μmol $NaHCO_3$ (pH = 7.1) to the culture medium. Ainas et al. [52] investigated the H_2 production of *S. platensis* in different PBRs and identified 300 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ of $NaHCO_3$ (pH = 7.5) as the optimal concentration. Similar findings by Leino et al. [53] revealed that *Anabaena* PCC 7120 cells (Hox mutant) had reduced H_2 production at higher pH values. However, the ideal pH for H_2 production with *Anabaena* PCC 7120 is 8.

pH alterations can result in the biodegradation of complex molecules as well as the denaturation of enzymes and proteins essential for bacterial growth and metabolism. Recent studies indicate that the optimal pH range for cultivating the vast majority of cyanobacteria in PBRs is between 7 and 8, with an acceptable temperature range of 25–35 °C [54]. Furthermore, by increasing the glycogen content in the nutrient medium, Burrows et al. (2011) achieved a 150-fold increase in H_2 productivity in *Synechocystis* sp. PCC 6803 cells [55], also detected nitrogen content effects on hydrogen production [56]. While Jin et al. (2006) achieved a 68% increase in the carbon source fixation rate from the air by extending the exponential growth phase with continuous nitrate sources in the culture medium [57]. The key factors influencing photobiological H_2 production are summarized in Table 1.

Table 1 – The main factors affecting photobiological hydrogen production.

Influencing factors	Strain	Concentration or content	H ₂ yield	Ref.
Temperature	<i>Chlorella vulgaris</i> MSU-AGM 14	32 °C	0.0016 g ⁻¹ L ⁻¹ h ⁻¹	[58]
	<i>Anabaena variabilis</i> ATCC 29413	25 °C	18 mL g ⁻¹ DW h ⁻¹	[59]
	<i>Desertifilum</i> sp. IPPAS B-1220	25 °C	0.348 mmol H ₂ mg ⁻¹ Chl h ⁻¹	[60]
pH	<i>Tetraspora</i> sp. CU2551	pH of 7.2	1182 nmol H ₂ mg ⁻¹ DW h ⁻¹	[61]
	<i>Anabaena</i> sp. 7120	pH of 7.5	15.5 μmol H ₂ mg ⁻¹ DW h ⁻¹	[62]
	<i>Nodularia</i> sp. AV33	pH of 7.5	10.2 μmol H ₂ mg ⁻¹ DW h ⁻¹	
Shaking	<i>Synechocystis</i> sp. PSU 1262	150 rpm	1552 nmol H ₂ mg ⁻¹ Chl a h ⁻¹	[63]
	<i>Synechocystis</i> sp. PCC 6803	120 rpm	0.037 μmol H ₂ mg ⁻¹ Chl h ⁻¹	[60]
	<i>Anabaena variabilis</i> A-1	150 rpm	8.67 μmol H ₂ mg ⁻¹ Chl h ⁻¹	[64]
Light intensity	<i>Calothrix</i> sp. BECID 33	70 μmol photons m ⁻² s ⁻¹	0.1 mmol H ₂ mg ⁻¹ Chl h ⁻¹	[62]
	<i>Anabaena variabilis</i>	70 μE m ⁻² s ⁻¹	44 μM H ₂ mg ⁻¹ Chl h ⁻¹	[65]
	<i>Anabaena variabilis</i> IAMM-58	60 μE m ⁻² s ⁻¹	4.2 μmol mg ⁻¹ Chl h ⁻¹	[66]
Oxygen concentration	<i>Anabaena variabilis</i> BTA-1047	2% headspace, v/v	10 μmol H ₂ mg ⁻¹ Chl h ⁻¹	[67]
	<i>Tolypothrix</i> sp. B379	1% headspace, v/v	55 μmol H ₂ mg ⁻¹ Chl h ⁻¹	[68]
	<i>Calothrix</i> sp.	5% headspace, v/v	1.9 mL g ⁻¹	[69]
CO ₂	<i>F. muscicola</i> PCC 7414	3% headspace, v/v	41.2 μmol H ₂ mg ⁻¹ Chl h ⁻¹	[68]
	<i>Calothrix scopulorum</i> 1410/5	3% headspace, v/v	0.128 μmol H ₂ mg ⁻¹ DW h ⁻¹	[70]
	<i>Anabaena variabilis</i> PK84	2% headspace, v/v	167.6 μmol H ₂ mg ⁻¹ Chl h ⁻¹	[71]
Source of nitrogen	<i>Anabaena variabilis</i> PK 84	5% N ₂ headspace, v/v	167.6 μmol H ₂ mg ⁻¹ Chl h ⁻¹	
	<i>Anabaena variabilis</i> ATCC 29413	25% N ₂ headspace, v/v	45.16 μmol H ₂ mg ⁻¹ Chl h ⁻¹	
	<i>Anabaena variabilis</i> BTA-1047	2% N ₂ headspace, v/v	1.2 μmol H ₂ mg ⁻¹ Chl h ⁻¹	[67]
Glucose	<i>Anabaena cylindrica</i> B629	10 mM	49 μmol H ₂ mg ⁻¹ Chl h ⁻¹	[68,72]
	<i>Tolypothrix</i> sp. B379	10 mM	35 μmol H ₂ mg ⁻¹ Chl h ⁻¹	
	<i>Anabaena</i> sp. PCC 7120	10 mM	11.7 μmol H ₂ mg ⁻¹ Chl h ⁻¹	
Cell density	<i>Anabaena</i> PCC 7120	5 mg Chl mL ⁻¹	17 μmol H ₂ mg ⁻¹ Chl h ⁻¹	[73]
	<i>Nodularia</i> sp. TRO31	40 mg Chl mL ⁻¹	~8 μmol H ₂ mg ⁻¹ Chl h ⁻¹	[62]
	<i>Calothrix</i> 336/3	40 mg Chl mL ⁻¹	~4 μmol H ₂ mg ⁻¹ Chl h ⁻¹	
Immobilisation	<i>Synechocystis</i> sp. PCC 6803	3% (w/v) alginate and 2% (w/v) calcium chloride	1 mL h ⁻¹	[74]
	<i>Tetraspora</i> sp. CU2551	4% alginate gel, w/v	1182 nmol H ₂ mg ⁻¹ DW h ⁻¹	[61]
	<i>Lyngbya perelegans</i>	4% alginate gel, w/v	21 nmol H ₂ mg ⁻¹ DW h ⁻¹	[75]
PIs	<i>Synechococcus elongatus</i> PCC 794	10 μM KCN	~20 μmol h ⁻¹	[76]
	<i>Aphanothece halophytica</i>	50 μM DCMU	3.63 μmol H ₂ mg ⁻¹ DW h ⁻¹	[77]
	<i>Synechocystis</i> sp. PSU 1262	500 μmol KCN	1552 nmol H ₂ mg ⁻¹ c Chl h ⁻¹	[63]

In the case of temperature, high degrees greatly interfere with photoenzymatic H₂ production and cell growth and can eventually lead to cell death [78,79]. Fluctuating temperatures force microorganisms to expend excess energy to adapt to the environment [80]. The optimal temperature for biological H₂ production in unicellular photosynthetic cyanobacteria *S. platensis* has been reported to be 32 °C [81]. He et al. (2006) investigated the influence of temperature on bacterial growth, H₂ production, and substrate conversion efficiency, and observed a 33% reduction in photoenzymatic H₂ yield and substrate conversion efficiency when temperature rose from 30 to 34 °C [82]. According to Madamwar et al. (2000), the optimal growth temperature for the bacterium *Synechococcus* Nag. PCC 7942 ranged from 30 °C to 32.5 °C [50], and H₂ production significantly declined as the temperature increased. Hence, maintaining precise control over the culture temperature within the reactor throughout the process is crucial [83,84].

Another important parameter influencing H₂ production during phototrophic microorganisms' cultivation is light [85]. There are two zones within a PBR: the photonic zone, located close to the lit surface, and the dark zone. The dark zone is created as a result of light absorption by the cells and is regulated by illumination conditions as well as the light-absorbing features of the cyanobacterial cells. Since cyanobacteria primarily absorb red light at roughly 680 nm, it is

advisable to incorporate red light panels in specialized bioreactors to provide the necessary red light for the culture systems. The cells circulate between the light and dark zones within the PBR at a certain frequency and regular intervals determined by the reactor design and gas supply. The positioning of the light source and the gas-liquid hydrodynamics influence cyanobacterial development thereby H₂ production [86].

Cyanobacterial cells produce adenosine triphosphate (ATP) through a photosynthetic system that requires light energy for various reactions such as electron transport, ATP synthesis, and H₂ production [87]. Generally, the optimal light intensity ranges from 50 to 200 μE m⁻² s⁻¹, with light intensities over 200 μE m⁻² s⁻¹ causing photoinhibition of phototrophic microorganisms. To counteract this effect, excessive solar absorption and wasteful dispersion of absorbed radiation must be avoided [88]. Combining periods of high light radiation with intervals of darkness can aid in photoinhibition reduction [89]. Light saturation of photosynthesis becomes a crucial parameter influencing efficiency and yield in mass cultivation. Light conversion efficiency has been observed to decrease as light intensity increases [90]. An inverse connection has been discovered between the efficiency of energy conversion in photosynthesis and the intensity of incident sunlight [91]. Light in PBRs plays a crucial role in determining efficiency and performance. In addition, various light sources, including

light-emitting diode (LED) lamps, are used in photoenzymatic H_2 production, with LED lamps being beneficial due to their high operational stability and enhanced performance [92].

In PBRs, the term “dissolved O_2 ” pertains to the amount of unbound molecular O_2 in water or other liquid media. Dissolved O_2 holds significance in evaluating the overall quality of liquid environments, particularly water, as it directly affects the well-being of microorganisms in the reservoir. O_2 enters the water from the surrounding environment through various mechanisms such as diffusion, aeration, or as a by-product of photosynthesis [93,94].

In the case of CO_2 , the CO_2 transfer might be a limiting factor when the concentration of dissolved CO_2 in the liquid phase is insufficient or when inadequate mixing occurs within the PBRs. High concentrations of dissolved CO_2 , on the other hand, might result in low culture pH, impairing microbial cell viability [95].

Culture mixing is another significant factor that should be considered when constructing PBRs for H_2 production. Culture mixing not only reduces temperature gradients, distributes nutrients evenly, and regulates pH levels in culture media, but also prevents cell deposition, dead zone formation, cell adhesion to PBRs walls, and reduces cell accumulation [96,97]. Proper mixing ensures that all cells are equally exposed to light and facilitates mass transfer between phases. However, it is critical to strike a balance in mixing intensity, as excessive mixing can cause cell damage and negatively affect the culture, particularly when cyanobacteria are sensitive to shear forces [98]. Aeration or injection of CO_2 -enriched gas bubbles, mechanical mixing utilising impellers or static mixers, or a combination of these procedures are commonly employed for mixing in PBRs for H_2 production [99].

When considering the H_2 production by cyanobacteria, physicochemical indicators such as carbon and nitrogen sources, as well as their specific ratios, are important. Aside from their relevance in photoenzymatic H_2 production, carbon and nitrogen sources affect cell growth and synthesis, respectively. The type (and concentration) of carbon substrates exerts a substantial influence on H_2 production efficiency due to alterations in electron transport in different metabolic pathways within phototrophic microorganisms [100,101]. Nitrogenase (N_2 ase) is required to produce H_2 by phototrophic microorganisms. Therefore, the type and concentration of nitrogen sources affect H_2 production by influencing the activity of N_2 ase. Notably, the presence of O_2 and ammonium salts suppresses N_2 ase activity. Hence, the H_2 production by photosynthetic microorganisms necessitates conditions, low in O_2 and ammonia. Consequently, in H_2 production utilising ammonium-rich wastewater, adding carbonate was suggested to eliminate ammonium salts [102]. Although ammonium salts negatively affect H_2 production, glutamate, yeast extract, and albumin have been demonstrated to improve the process [103]. The enzyme N_2 ase serves as the primary catalyst for H_2 production in heterocyst cyanobacteria.

Cyanobacteria immobilisation, accomplished by adsorption on solid matrices or encapsulation in alginate gels, has been found to significantly increase their functional lifespan and the number of heterocysts in filamentous cyanobacteria. Immobilisation of cyanobacterial cultures has been shown to improve H_2 photoproduction efficiency. For instance,

Anabaena sp. PCC 7120 immobilised in calcium alginate gels displayed strong H_2 -producing activity under photoautotrophic conditions [104]. Similarly, *Synechocystis* sp. PCC 6803, a model organism for H_2 energy studies, was successfully immobilised in calcium alginate gels [74]. When *Anabaena* N-7363 cells were immobilised in a 2% carrageenan gel, they produced nearly three times more H_2 (up to $3.3 \text{ mmol h}^{-1} \text{ g}^{-1}$ alginate gel) than free cells [105]. Furthermore, when cyanobacteria cells are immobilised in alginate granules, the rate of hydrogen enzyme inactivation by O_2 was lowered because the alginate layer prevents the influx of O_2 into the granules, leading to higher H_2 production [74].

The intersection of membrane water treatment and cyanobacterial cultivation offers significant potential for nutrient recovery. Processes like ultrafiltration and reverse osmosis have proven effective in extracting key nutrients, particularly nitrogen and phosphorus, from nutrient-rich wastewater [106]. This approach extracts essential nutrients, providing an economically viable and sustainable nutrient source for cyanobacterial growth. This reduces reliance on commercial fertilizers and fosters circular nutrient utilisation in H_2 production. Anticipated advancements in membrane technologies are likely to enhance their role in increasing H_2 productivity during cyanobacterial cultivation, unlocking the full potential of this ecologically benign approach to renewable H_2 production. Ongoing research and interdisciplinary cooperation will be crucial in harnessing the synergies between membrane water purification and PBRs for a cleaner, more sustainable future [107].

Fig. 1 illustrates the environmental factors influencing the H_2 production and transpiration mechanism in cyanobacteria. As discussed, H_2 production in cyanobacteria occurs through two pathways: direct and indirect biophotolysis. In both pathways, multiple physicochemical parameters impact the efficiency of H_2 production. Parameters including pH, temperature, light intensity, dissolved O_2 , dissolved CO_2 , and agitation-induced displacement directly influence the biochemical pathways involved in H_2 production. Effective control of these parameters within PBRs is crucial for optimizing bio- H_2 production.

This flowchart outlines the significant contribution of cyanobacteria in phototrophic H_2 production, establishing a direct link between the core principles detailed in Section 2 and the investigated physicochemical parameters from Section 3. This holistic comprehension of the processes offers valuable insights into enhancing H_2 production through cyanobacteria, fostering a sustainable and ecologically conscious alternative energy solution.

4. Design features of PBRs used to increase hydrogen production

Photobioreactors are essential for cultivating cyanobacteria, as the latter requires specific growth media. In recent years, significant efforts in this field have been made, leading to the development of various PBRs with high biomass productivity. These devices are intended to assist biomass accumulation, which may then be modified to produce biofuels. There are now a variety of reactors used in both open and closed



Although numerous types of PBRs have been constructed, a shared objective is the efficient and high-volume production of tailored biofuel. The following conditions should be met by all PBRs despite their specific design [109].

- ❖ PBR construction should be simple and ensure sterility. The system must be easily cleaned and rapidly re-prepared after cultivation. Complex systems can hinder continuous production, and any contamination from other microorganisms can adversely affect product separation;
- ❖ Artificial light or solar energy must be effectively transferred to the PBR's internal environment. The main case should be made of transparent materials such as glass or acrylic. It is crucial to prevent the passage of ultraviolet (UV) light since many microorganisms are UV-intolerant.

Table 2 – Photobioreactors used in photobiological hydrogen production.

PBR type	Strains	H ₂ production	Ref.
Fermenter type	<i>Spirulina platensis</i>	0.17 mL L ⁻¹ h ⁻¹	[86]
Tubular	<i>Anabaena variabilis</i> PK84	8 mL H ₂ L ⁻¹ h ⁻¹	[110]
	<i>Anabaena variabilis</i> PK84	0.82 mol H ₂ m ⁻³ h ⁻¹	[111]
	<i>Anabaena</i> sp. PCC 7120	14.9 mL H ₂ L ⁻¹ h ⁻¹	[112]
	<i>Anabaena azollae</i>	13 mL H ₂ L ⁻¹ h ⁻¹	[110]
Flat-plate	<i>Nostoc</i> PCC 7120 ΔhupW	6.5 mL H ₂ L ⁻¹ h ⁻¹	[113]
	<i>Cyanothece</i> sp. ATCC 51142	1.42 mL L ⁻¹ h ⁻¹	
	<i>Anabaena variabilis</i> ATCC 29413	4.12 mL H ₂ g ⁻¹ DW h ⁻¹	[114]
	<i>Cyanothece</i> sp.	2.33 mL L ⁻¹ h ⁻¹	[115]
	<i>Cyanothece noctigama</i>	0.56 mL L ⁻¹ h ⁻¹	[116]
Microchannel biofilm PBRs	<i>Enterobacter aerogenes</i>	9.44 mmol H ₂ g ⁻¹ glucose	[117]
Optical fibre	<i>Rhodospseudomonas palustris</i> SP-6	1.495 mol H ₂ mol ⁻¹ glucose	[118]

- ◆ The upper part of the PBR, the light travels through, should be sufficiently wide to ensure uniform light dispersion throughout the culture to reach all parts of the biomass and stimulate cell growth.
- ◆ All PBRs require an effective mixing system to enhance cell growth. Proper mixing improves light penetration into the biomass, allowing for optimal growth conditions.

Numerous closed-system PBRs have been developed that fulfil the above conditions. Among them, PBRs tailored to produce bio-H₂ can be divided into five types.

- Fermenter type PBRs
- Tubular PBRs
- Flat panel PBRs
- Microchannel biofilm PBRs
- Optical fibre PBRs with solar collectors.

Table 2 presents an overview of the different PBR types used to produce H₂ from phototrophic microorganisms, along with the corresponding H₂ yield and microbial strain.

Both microchannel biofilm and optical fibre PBRs have been utilized for studying bacterial strains in H₂ production. However, the design of PBRs is also well-suited for investigating H₂ production by cyanobacteria strains. Given that cyanobacterial cells readily form robust biofilms, the microchannel biofilm PBR serves as a convenient setup for H₂ production. Similarly, the optical fibre PBR is apt for the indirect biophotolysis of cyanobacteria strains.

PBRs are essential tools for harnessing cyanobacterial hydrogen production, and different PBR types offer varying advantages and disadvantages (Table 3).

Fermenter-type PBRs offer precise control over environmental conditions, optimizing cyanobacterial growth and H₂ production by regulating factors like temperature, pH, and gas

Table 3 – Comparison of the main photobioreactors used in hydrogen production.

PBR type	H ₂ production capacity	Advantages	Disadvantages
Fermenter	Low efficiency	Cellular photosynthesis can be facilitated by both artificial and natural light sources. Environmental conditions can be controlled easily over the cultivation process.	Using artificial light combined with mixing consumes substantial energy, while limited amounts of light and heat penetrate the central region of the suspension. It is not economically viable to make this fermenter on a large scale.
Tubular	Low efficiency	A substantial biomass may be produced within a relatively short timeframe.	Cleaning the tubes is difficult as they are prone to rapid soiling, and the fragile nature of glass tubes makes the cleaning procedure susceptible to breakage. There is also a risk of clogging if the tubes become too dense or collect impurities.
Flat panel	Efficient	Highly efficient in the transmission of light into the suspension; low operating costs; ease of cleaning; high cell density and H ₂ yield.	The higher the area of cell culture panels, the harder to maintain temperature and pH, also required a considerable land area.
Microchannel biofilm PBRs with immobilised cells	High efficiency	Enhanced capacity of immobilised cells to produce H ₂ , thus strengthening interaction between microbial cells and substrate. The cells show improved resistance against chemical and physical factors. The construction ensures efficient light distribution throughout the biofilm.	The procurement cost of gel and lighting contributes to an increase in the overall cost of produced H ₂ . Maintaining an active biofilm can challenge and may require careful control.
Optical fiber	Efficient	Cell culture harnesses inexhaustible energy sources enabling continuous production of biomass and related products. A design can significantly increase productivity in a relatively small footprint.	Expensive operational costs and requires more technical knowledge.

composition. While effective for research, their implementation can be expensive due to advanced control systems and sterility requirements, and commercial scalability might be challenging and costly.

Tubular PBRs are prized for their scalability and simplicity, making them suitable for large-scale H_2 production. The tube arrangement's spiral or serpentine design enhances illumination and photosynthetic efficiency. However, light limitations in the tube centres could reduce productivity there.

Flat panel PBRs have a straightforward design and cost-effectiveness. While they can achieve moderate H_2 production, they might underutilize light, and substantial land may be needed for significant output.

Microchannel biofilm PBRs leverage biofilm-covered microchannels for improved light distribution, gas exchange, and H_2 production rates. Biofilms protect cyanobacteria from shear stress, extending cell lifespan. Maintaining a stable biofilm can be challenging, requiring meticulous environmental tracking, and constructing microchannel PBRs can be costlier.

Fibre optic PBRs with solar collectors concentrate sunlight onto cyanobacteria via optical fibres and solar collectors, boosting performance in confined spaces. However, this intricate design demands technical knowledge and comes with higher costs, potentially outweighing advantages.

Thus, selecting the proper PBR type for cyanobacterial H_2 production hinges on specific goals and requirements. Each PBR variant carries distinct merits and drawbacks, and comprehending these attributes is pivotal for enhancing H_2 production and driving forward sustainable energy endeavours.

4.1. Fermenter-type photobioreactors

Many designs of PBRs have been developed to accomplish steady and continuous H_2 production [119]. These constructions comprise the main bioreactor case and lighting, mixing, temperature control, and H_2 purification and collection

systems. PBRs function as closed systems to ensure anaerobic conditions since bio- H_2 production relies on cyanobacteria that are sensitive to N_2 and O_2 . Closed systems are more efficient at preserving the purity of photofermentation and facilitating the H_2 collection. Closed PBRs can be divided into two categories: culture suspension and static culture flasks. While the use of suspension culture PBRs is promising, their performance is still limited due to insufficient light supply. Employing a closed system is mandatory to maintain the anaerobic conditions for the extraction and collection of bio- H_2 [120].

The inadequate lighting conditions in PBRs can be attributed to the light reflection and shading effect.

- ❖ When light interacts with the outer surface of PBRs, a portion of the light is reflected and scattered, while some is absorbed by the reactor wall material. These processes lead to a reduction in the total amount of light passing through the PBRs.
- ❖ Cyanobacteria within the PBRs create a spontaneous shading effect due to substrates and products causing light attenuation and limiting the penetration of light into the deeper regions of the PBRs [50].

To enhance light intensity and energy conversion efficiency within PBRs, increasing the surface area to volume ratio showed promise as a possible strategy. Based on physicochemical parameters, various constructions such as tubular, flat-plate, microchannel biofilm, fermenter, and fibre-optic PBRs are being studied for photobiological H_2 production within closed systems [121–123] (Fig. 2).

4.2. Tubular photobioreactors

Tubular PBRs are commonly designed as vertical and horizontal cylinders to intensify the light energy capture. These

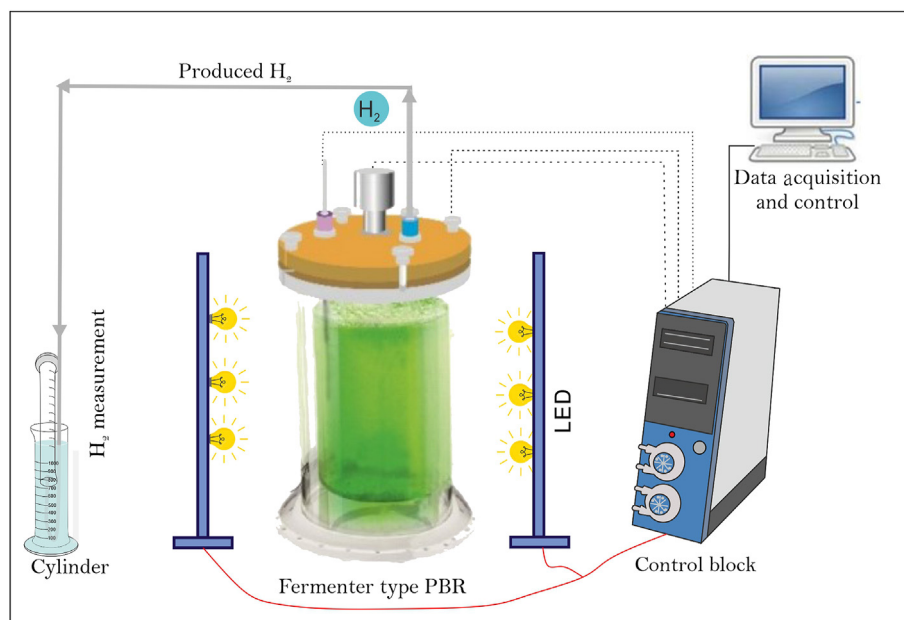


Fig. 2 – Fermenter-type photobioreactor. Modified from [122].

tubular PBRs are constructed using transparent, flexible, and cost-effective materials like glass or polymethyl methyl acrylate [124]. Various configurations of tubular PBRs exist, including simple airlift and bubble column configurations with CO₂ discharge and cross-tube designs where light collection and gas exchange units are separate [125].

Vertical tube PBRs have several advantages over horizontal or serpentine reactors, including the potential to optimize culture mixing and limit cell adherence on the PBR surface [126]. Vertical tube PBRs boast cheaper material costs, high transparency, a high surface area-to-volume ratio, and reduced contamination risk. The CO₂ supply and O₂ discharge are both highly efficient in these reactors. A vertical tube PBR consists of a gas meter, solid gas separator, light source, mixer, and peristaltic pump. Using vertical tube PBRs in H₂ production relies on a mixing system where the introduction of inert gas bubbles causes the H₂ flow to liquefy [127].

Straight-tube PBRs exhibit high light conversion efficiency on their surface due to photon penetration into the culture being limited, resulting in a low photon flux density. These PBRs feature tubes positioned parallel to each other at ground level. In many designs, solar collector tubes, which have a similar shape to the light gradient, are typically made of plastic or glass. These tubes are arranged horizontally on a supporting frame to produce a threaded coil construction (Fig. 3). However, the main drawback of such a reactor is the challenge of temperature control. The accumulation of O₂ due to photosynthetic activity can lead to photoaging and a subsequent reduction in photosynthetic efficiency. An increase in working volume requires higher energy consumption due to elevated flow resistance inside the tubes [128].

Spiral tubular PBRs, on the other hand, utilise straight plastic tubes wound into three-dimensional spiral frames with the desired inclination. These PBRs have been employed for two-stage H₂ production using *Anabaena azollae*, yielding around 12 mL H₂ L⁻¹. The mass transfer features and H₂ productivity of tubular PBRs vary depending on their form and the mixing system [129]. However, these bioreactors face certain drawbacks, such as difficulties in cleaning the tubes due to fouling caused by cyanobacterial culture, as well as the potential for process photoinhibition [130].

To summarise, tubular PBRs are used in both horizontal and vertical orientations. PBRs exhibit a notable area-to-volume ratio, facilitating consistent cell illumination. Furthermore, tubular PBRs demonstrate enhanced rates of H₂ production and solid-liquid separation, attributable to higher mass transfer between the liquid and gas phases. Nevertheless, their primary drawback lies in their challenging geometry, limiting the column length or height due to elevated fluid pressure and suboptimal mixing. Because of the high flow resistance in the tube, increasing the working volume requires a significant rise in energy consumption [131,132].

4.3. Flat panel photobioreactors

Flat-panel PBRs are widely recognised as the most effective cyanobacterial culture reactors [133]. These PBRs consist of rectangular boxes made of transparent materials like glass, plexiglass, or polycarbonate, while the frames can be composed of durable materials such as polymethyl methacrylate, glass, polyvinyl chloride, or stainless steel [134]. The plate orientation might be vertical (East-West), oblique

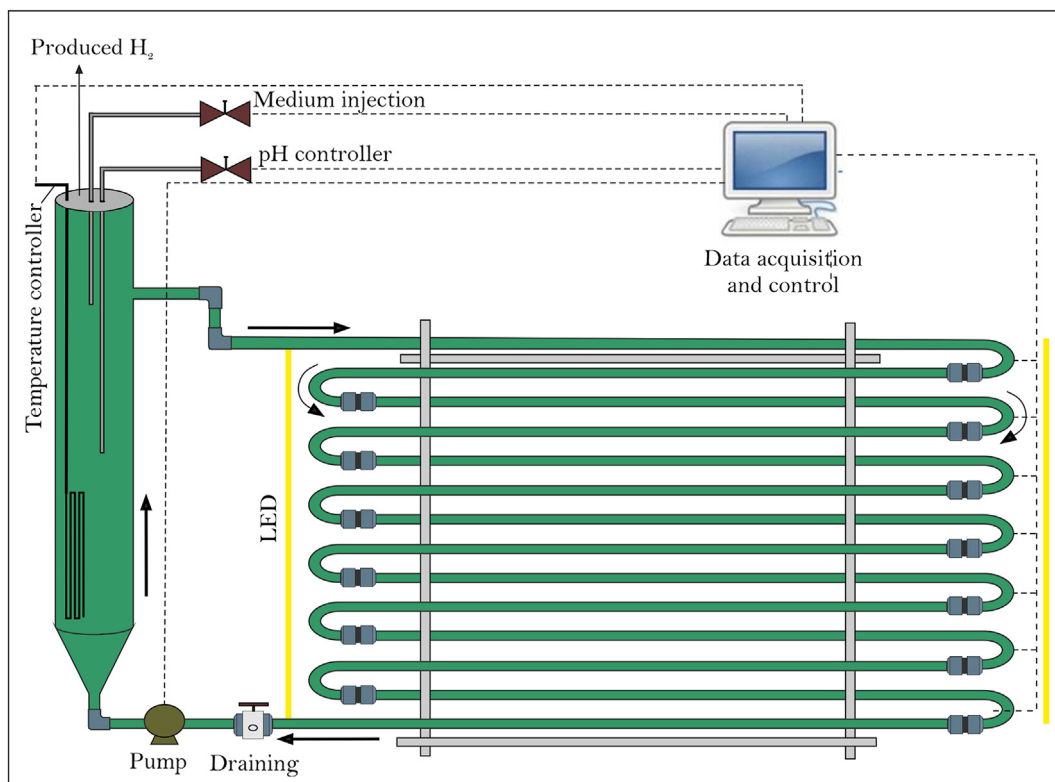


Fig. 3 – Tubular indoor photobioreactor.

(North-South), or horizontal. In large-scale production, multiple plates are arranged in parallel within a designated area.

Flat-panel PBRs provide an open gas transmission area that avoids the requirement for a specialized degassing system. Mixing is typically achieved by introducing air bubbles through nozzles located at the bottom or side of the reactor. The mixing force has minimal impact on the yield and photosynthetic efficiency of low-density cultures but significantly affects high-density cultures by optimizing light utilisation. It is crucial to highlight that excessively low mixing speeds impede maximum light utilisation, whilst high speeds can cause cellular damage (Fig. 4) [135,136].

Flat-panel PBRs offer advantages in terms of contamination control and ease of cleaning, leading to greater cost-effectiveness [137]. Their capacity to collect light, however, is determined by material properties and the area-to-volume ratio. Limited light penetration occurs as a result of photosynthetic inhibition induced by light attenuation along the cell path due to increased cell density caused by photon absorption and cell scattering. Due to mixing intensity restrictions, H_2 production in these PBRs remains unsatisfactory.

Therefore, adjusting the light conditions in these PBRs becomes crucial to further increase H_2 production. For instance, Kondo et al. (2016) developed a multilayer PBR that employed grids and membranes to efficiently attenuate intense incident light and equally disperse it within the PBR, hence improving photo- H_2 production. The PBR design involved four equally sized compartments [138].

Concluding, although these PBRs offer numerous advantages, such as underground performance, flexibility in light construction and modification, and suitability for fundamental research on light propagation and interference, their

H_2 production still falls short of expectations. The primary drawbacks of PBRs can be summarized.

- Dependence on material selection and the area-to-volume ratio for light collection efficiency.
- Limited light penetration along the light path due to the formation of a photosynthetic block induced by photon absorption and cell scattering at larger cell densities.
- Poor mixing performance because of compromised mixing and mass transfer rates, especially under high cell density conditions.

To enhance H_2 production, it is crucial to optimize the light conditions within these PBRs [139].

4.4. Microchannel biofilm photobioreactors

Biofilm-based culture systems are promising technologies that overcome the shortcomings of conventional PBRs. These systems offer the potential for achieving high productivity and biomass yield [14]. Microbial cells in a biofilm are surface-bound and encased in a matrix composed mostly of water, polysaccharides, proteins, and nucleic acids. The spatial organisation of microorganisms and matrix parts determines the formation of cavities and channels, thus altering the nutrition and gas transport [140,141]. The biofilm architecture induces distinct gradients of nutrients, gases, and light throughout its depth, which contributes to cell adaptation or displacement to maintain optimal growth conditions.

Despite the simple structure and online monitoring capabilities, flat panel biofilm PBRs suffer biofilm attachment issues to smooth surfaces such as polymethyl methacrylate and glass (Fig. 5). The H_2 production of immobilised cyanobacteria

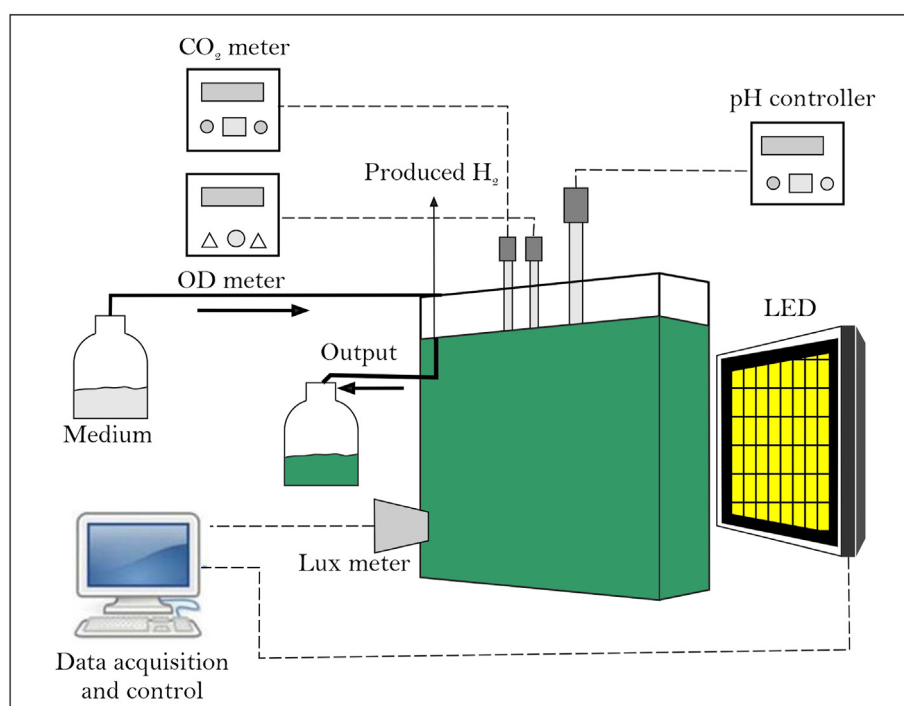


Fig. 4 – Flat panel biofilm photobioreactor. Modified from Ref. [136].

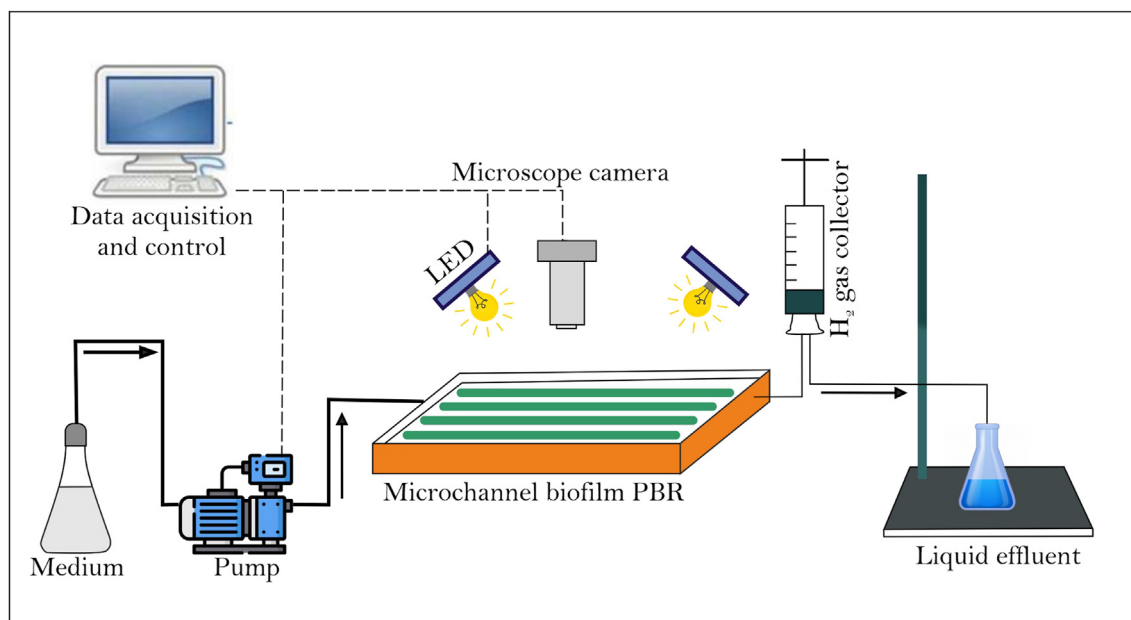
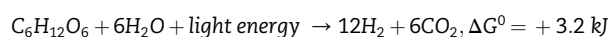


Fig. 5 – Microchannel biofilm photobioreactor. Modified from Ref. [14].

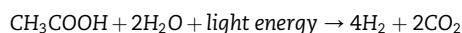
is constrained by limited light penetration and substrate and product movement. To address these challenges while maintaining high H_2 production and substrate transport rates, supporting materials that provide suitable spaces for cyanobacterial immobilisation and allow sufficient light penetration must be used. Therefore, transparent gel granules, silicon chip-based PBRs, and polydimethylsiloxane microchannel PBRs have been developed [14].

Biochemical reactors, particularly fermenter reactors, play a crucial role in H_2 production by phototrophic microorganisms. The performance and economic efficiency of the process are influenced by factors such as reaction rate, ease and duration of reactor operation [142]. Modified commercial bioreactors originally designed for heterotrophic organisms are being utilized as PBRs. These controlled bioreactors offer a high level of parameter control compared to other reactor types. It is critical to have a high reaction rate and a simple reactor setup when choosing the best reactor for industrial use. The reaction rate may be improved by increasing the cell mass concentration in the reactor by optimizing parameters like pH, temperature, substrate concentration, and medium components.

Various cyanobacteria participate in photofermentation by enzymatically transforming diverse substrates. The principal energy source is sunlight, which converts organic materials into H_2 and CO_2 [143]. Molecular H_2 is produced in N_2 -deficient conditions by N_2 ase catalytic action in processes where photosynthetic bacteria are responsible for fermentation. These cyanobacteria can consume organic acids such as acetic acid as electron donors. The released electrons are transferred to N_2 ase via ferredoxin using the energy obtained from ATP. In the absence of N_2 , more ATP energy is used throughout this process by N_2 ase to reduce the protons to H_2 . The common H_2 production reactions are listed below [88]:



The conversion of acetic acid to H_2 and CO_2 through the N_2 -deficient enzymatic process can be represented by the following equation:



Indeed, organic acids can be oxidized using sunlight and the tricarboxylic acid cycle (TCA) as an energy source [82,83].

4.5. Optical fibre photobioreactors

Aside from flat panel PBRs, optical fibre PBRs with solar collectors have been developed to improve solar light utilisation, intensity, and dispersion. Polymer and silica optical fibres that serve as light conduits are widely employed in photofermentation systems and lighting applications [144]. Optical fibres direct sunlight into fluorescent coatings in such reactors, where blue-green light is converted into red light. The reflector within the system recycles internally generated red photons and intensifies fluorescent radiation on the front surface [145].

A solar collector automatically captures sunlight and delivers it over optical fibres to the PBR in the laboratory. Light is diffused from the side of the optical fibre frame within the coated fibres to illuminate neighbouring locations. Side-light optical fibres are stacked in parallel inside the PBR, and their number is determined by the operational needs of the PBR, and the associated light intensity required for cyanobacteria.

Optical fibres serve as efficient means of delivering light, allowing sunlight to be captured from external sources and distributed anywhere within the reactor area. Fibre optic materials may filter out a considerable fraction of UV and infrared radiation, making them particularly suitable for photofermentation applications. Since the light source is the sun, optical fibre PBRs consume less energy and have a favourable environmental impact (Fig. 6). Optical fibres embedded in the PBRs can improve the mixing of culture

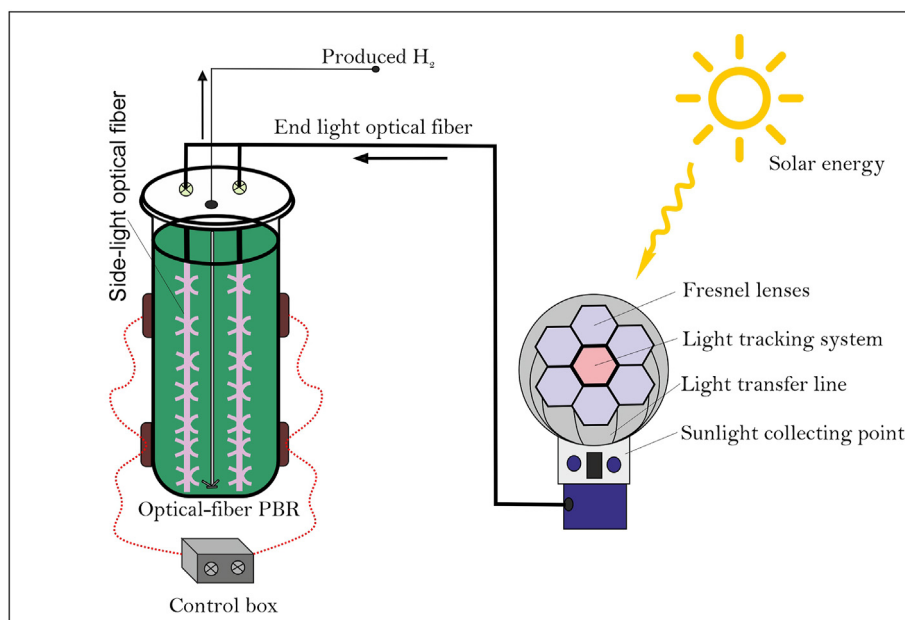


Fig. 6 – Optical fibre photobioreactor. Modified from Ref. [14].

media and cyanobacteria [87,88]. Even if such a device has restricted light and a low cell concentration, its operation is not entirely satisfying.

Therefore, improving lighting conditions and increasing biofilm mass entails employing optical fibres as biofilm support material. Several optical fibre biofilm PBR models have been constructed, including ring fibre lighting PBRs, biofilm PBRs with rough surfaces, and hollow optical fibres with interior rough surfaces [14].

The described system uses solar-excited side light optical fibres (SLOF) and an artificial light source (tungsten fibre lamp). Sunlight is primarily used to provide natural daylight energy, however, when the intensity of sunlight is insufficient for cyanobacterial growth, particularly at night, artificial light sources are employed.

To ensure high light intensity and uniform dispersion, two optical fibres, encased within a glass tube, are submerged in the liquid medium within the PBR. These SLOFs are constructed using quartz glass with dimensions of 11 mm in diameter and 50 cm in length, which also influences the light distribution. Common approaches to address this issue include raising the light intensity and optimizing the distance between the source and the reactor, which might result in higher surface temperatures. However, excessive temperatures can lead to cell damage. By implementing a bundle of optical fibres, the challenges associated with light intensity, dispersion, and heat dissipation can be mitigated.

5. Current state and prospects of cyanobacterial hydrogen production

Given the direct economic ties to petroleum products, many countries hesitate to curtail environmentally damaging energy sources. Simultaneously, the complex landscape of energy policies poses challenges in diversifying energy supplies,

causing price instability. In response, heightened prices of petroleum products and growing interest in alternative energy sources have spurred the development of electric-based solutions. To date, transportation, heating systems, and electricity generators are evolving with renewable energy at their core. Numerous nations have launched extensive programs to ramp up renewable energy production in the 21st century. Rapidly advancing economies are concerned about petroleum price hikes, recognizing that stable prices hinge on bolstering renewable energy output. Intensive adoption of favourable renewable energy strategies not only reduces dependence on exporters but also drives sustainable economic growth.

Hydrogen energy, offering rapid and clean power, is essentially limitless and can be derived from water, coal, gas, and biological materials [146]. This renewable H_2 energy stands as a leading and promising initiative for the future. In envisioning future systems, H_2 energy takes centre stage, with active efforts underway. Although H_2 -based systems are on the horizon, H_2 energy already plays a pivotal role in integrated systems. H_2 finds applications in battery development, heating systems, and space technologies. Using biological cell-derived H_2 in public transportation to enhance a nation's economic well-being represents a global positive trend.

An urgent concern arises from the substantial environmental harm caused by fossil fuel-based energy, which releases significant CO_2 into the atmosphere. This has led to rising average surface temperatures, causing irreversible harm to terrestrial and aquatic flora and fauna. Escalating greenhouse gas (GHG) emissions in urban regions have spurred initiatives to curtail harmful emissions. A growing number of states are embracing this movement, undertaking projects to harness energy from renewable biological sources. Energy types derived from biological entities are seen as a pivotal solution to the contemporary challenges at hand [147].

Severe air pollution in densely populated urban areas has become increasingly pressing. The widespread adoption of H_2

energy has shown a capacity to lower toxic heavy metal levels, thus benefiting residents' well-being. Some cities have transitioned their heating systems and transportation to rely on H_2 energy.

The favourable government stance on biological energy sources paves the path for the growth of multiple economic sectors. Prominent domains benefiting from H_2 energy encompass heavy industry, energy production, transportation, environmental conservation, and commerce. Continuously pursuing a comprehensive policy, assessing the impact of renewable energy on specific economic sectors, offers a lasting solution to existing challenges. State-sponsored incentives for renewable energy sources will enhance the quality and use of biological H_2 .

Nonetheless, it's important to acknowledge that many challenges hinder the seamless integration of bio-derived energy into the global trade landscape. Obstacles and biases persist against a complete substitution of biological energy for fossil fuels. Despite ongoing intensive efforts in H_2 energy research based on cyanobacteria, the practical significance is dampened by the low cost of the product. While various PBRs for H_2 production exist, a strain yielding active H_2 production has not yet been developed, raising process costs and impeding commercialization. The lack of market competitiveness makes large-scale H_2 production economically unviable. The lower cost of bio- H_2 compared to petroleum products fosters consumer interest. However, ongoing research aims to enhance cyanobacterial strains' H_2 yield through advanced technologies.

In the forthcoming years, a crucial step is to enhance H_2 production within cells, primarily achieved through genetic manipulation, to address current challenges. The limited H_2 yield of strains underscores the substantial potential for large-scale production. Prior to subsidizing cyanobacteria-based H_2 projects and programs, a thorough analysis of this direction is essential. Positive outcomes stem from investing in promising ventures. Stimulating bioenergy is paramount for achieving favourable outcomes, necessitating the implementation of programs wherein bioenergy plays a pivotal role. Employing cutting-edge biotechnological advancements is vital for project execution. Analysts emphasize that significant investment and time are necessary for the rapid advancement of the bioenergy sector. Boosting bio- H_2 production will satisfy current energy demands and optimize environmental sustainability.

Ramping up H_2 production from cyanobacteria demands substantial investment in fundamental scientific research. This sector has yet to yield profits even in laboratory settings, underscoring the need for significant financial input. To effectively enhance productivity, optimizing all conditions essential for strain-based H_2 production is vital. Employing advanced genetic techniques and leveraging core tools of systematic biotechnology are the primary pathways to augmenting H_2 output from cyanobacterial strains. The cost of bio- H_2 transportation and storage significantly affects the price. State subsidies should target the optimization of storage and transportation, requiring an expansion of structural infrastructure and intensified efforts to discover cost-effective storage methods.

5.1. Large-scale cultivation systems

The current status of large-scale cyanobacterial culture systems, particularly in the realm of cyanobacterial H_2 production, has made notable advancements. Laboratory techniques have demonstrated encouraging outcomes in terms of H_2 yield and efficiency. Yet, several obstacles hinder the widespread application of these methods in industrial or commercial settings [148].

One of the primary challenges lies in achieving consistent light distribution. While uniform light exposure can be relatively straightforward in laboratory-scale PBRs, it becomes more complex as systems increase in size. This inconsistency in light distribution impacts the growth rate of cyanobacteria and H_2 production. Nutrient supply is also crucial [149]. Smaller systems can manage nutrient provision, but larger setups face difficulties in maintaining balanced nutrient levels, leading to gradients that affect biomass productivity. Temperature control is another hurdle. Precise control is achievable at the lab scale, but maintaining uniform temperature across larger systems is challenging and temperature fluctuations can affect cyanobacterial growth. O_2 buildup poses a significant issue in larger cultivation. Closed PBR designs can lead to O_2 accumulation, hindering H_2 production by competing with H_2 ase enzymes. Scaling up from lab conditions to engineering environments requires overcoming various technical challenges in designing efficient, economical, and reliable large-scale PBRs. For example, Nagy et al. (2021) increased the hydrogen production of the pgr5 mutant by 250% in a thin cell layer photobioreactor and at day 6 the hydrogen production was 1.2 mL H_2 /mL. An automated lighting system was used during hydrogen production in a larger cultivation PBRs [150].

Utilising advanced automation and control systems can optimize environmental conditions, enhancing performance and stability in large-scale growth setups. Genetic engineering of cyanobacteria offers the potential to boost H_2 production capability and resilience against environmental pressures, rendering them more apt for extensive cultivation. Integrating these systems with other processes like wastewater treatment or CO_2 capture enhances resource efficiency and system robustness. It requires collaborative efforts among researchers, engineers, and industry experts to innovate, exchange insights, and tackle the complexities of large-scale cyanobacteria cultivation.

In terms of proximity to real engineering, while laboratory studies indicate the potential for cyanobacterial H_2 production, practical engineering and economic challenges must be overcome for successful large-scale implementation. Pilot and demo projects show promise, but broad commercial implementation remains in progress. Enhanced PBR designs play a crucial role in advancing large-scale culture systems. Innovations targeting improved light distribution, nutrient supply, and temperature control can enhance overall efficiency. Novel designs like microchannel and fibre optic PBRs with solar collectors hold promise for realizing these improvements.

Large-scale cyanobacterial culture systems are in their nascent phases of development. By addressing challenges and

enhancing these systems through continuous research, technological innovations, and collaborative efforts, the path may be paved for cyanobacterial H₂ production to emerge as a feasible and environmentally sustainable source of renewable energy.

6. Conclusion

Despite the successful discovery of active H₂-producing species in laboratory settings, attaining significant H₂ production rates for industrial purposes remains a challenge. Thus, numerous PBRs have been developed to enhance bio-H₂ production on a large scale. Tubular PBRs enable rapid biomass production, whereas biofilm PBRs allow for immobilised culture and efficient H₂ production. However, several thermodynamic factors such as fluid flow, heat and mass transfer, and light distribution all have a substantial influence on PBR efficiency. Optimizing cell growth parameters and implementing continuous photobiological enzymatic H₂ production using sunlight are critical aims for developing an effective PBR system for bio-H₂ production. The support and financing of promising ventures are pivotal for the advancement of hydrogen production through cyanobacteria. The development of an efficient photobioreactor to enhance hydrogen generation stands as an immediate solution to prevailing issues in this field.

Credit authorship contribution statement

BDK, AKS, AMB, literature, experimental works and writing; BDK, TT, SIA writing and main idea; AKS, BKZ, GKK, AMB consulting and analysis; GAS, GKK, GY, AMB consulting and language checking; BDK, GY, HFA, TT, SIA consulting and writing; All the authors contributed in the writing of the final version and approved the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was supported by the Ministry of Higher Education and Science of the Republic of Kazakhstan in the framework of the following projects: AP14869048, AP09260785, AP13067574 and AP09260077. HFA gratefully acknowledges support from the Distinguished Scientists Fellowship Programme, King Abdulaziz University, Saudi Arabia. AMB and SIA were supported by a grant from Russian Science Foundation (22-44-08001). Fig. 1 was supported under the state contract of the Ministry of Science and Higher Education of the Russian Federation (theme No. 122050400128-1). The present

work was also financially supported in part by Grants-in-Aid for Scientific Research (No. 21K06101 (TT)).

REFERENCES

- [1] Thompson RC, Moore CJ, vom Saal FS, Swan SH. Plastics, the environment and human health: current consensus and future trends. *Philos Trans R Soc Lond B Biol Sci* 2009;364:2153–66. <https://doi.org/10.1098/rstb.2009.0053>.
- [2] Siddiqua A, Hahladakis JN, Al-Attia WAKA. An overview of the environmental pollution and health effects associated with waste landfilling and open dumping. *Environ Sci Pollut Res Int* 2022;29:58514–36. <https://doi.org/10.1007/s11356-022-21578-z>.
- [3] Malode SJ, Gaddi SAM, Kamble PJ, Nalwad AA, Muddapur UM, Shetti NP. Recent evolutionary trends in the production of biofuels. *Mater Sci Technol* 2022;5:262–77. <https://doi.org/10.1016/j.mset.2022.04.001>.
- [4] Akimbekov NS, Digel I, Tastambek KT, Marat AK, Turaliyeva MA, Kaiyrmanova GK. Biotechnology of microorganisms from coal environments: from environmental remediation to energy production. *Biology* 2022;11:1306. <https://doi.org/10.3390/biology11091306>.
- [5] Li S, Li F, Zhu X, Liao Q, Chang J-S, Ho S-H. Biohydrogen production from microalgae for environmental sustainability. *Chemosphere* 2022;291:132717. <https://doi.org/10.1016/j.chemosphere.2021.132717>.
- [6] Tanvir RU, Zhang J, Canter T, Chen D, Lu J, Hu Z. Harnessing solar energy using phototrophic microorganisms: a sustainable pathway to bioenergy, biomaterials, and environmental solutions. *RSER* 2021;146:1–111181. <https://doi.org/10.1016/j.rser.2021.111181>.
- [7] Moreira JB, Vaz B da S, Cardias BB, Cruz CG, Almeida ACA de, Costa JAV, et al. Microalgae polysaccharides: an alternative source for food production and sustainable agriculture. *Polysaccharides* 2022;3:441–57. <https://doi.org/10.3390/polysaccharides3020027>.
- [8] Huang Q, Jiang F, Wang L, Yang C. Design of photobioreactors for mass cultivation of photosynthetic organisms. *Engineering* 2017;3:318–29. <https://doi.org/10.1016/j.ENG.2017.03.020>.
- [9] Chanquia SN, Vernet G, Kara S. Photobioreactors for cultivation and synthesis: specifications, challenges, and perspectives. *Eng Life Sci* 2021;22:712–24. <https://doi.org/10.1002/elsc.202100070>.
- [10] Assunção J, Malcata FX. Enclosed “non-conventional” photobioreactors for microalga production: a review. *Algal Res* 2020;52:102107. <https://doi.org/10.1016/j.algal.2020.102107>.
- [11] Clark RL, McGinley LL, Purdy HM, Korosh TC, Reed JL, Root TW, et al. Light-optimized growth of cyanobacterial cultures: growth phases and productivity of biomass and secreted molecules in light-limited batch growth. *Metab Eng* 2018;47:230–42. <https://doi.org/10.1016/j.ymben.2018.03.017>.
- [12] Barbera E, Grandi A, Borella L, Bertucco A, Sforza E. Continuous cultivation as a method to assess the maximum specific growth rate of photosynthetic organisms. *Front Bioeng Biotechnol* 2019;7:274. <https://doi.org/10.3389/fbioe.2019.00274>.
- [13] Wang S-K, Stiles AR, Guo C, Liu C-Z. Microalgae cultivation in photobioreactors: an overview of light characteristics. *Eng Life Sci* 2014;14:550–9. <https://doi.org/10.1002/elsc.201300170>.
- [14] Reungsang A, Zhong N, Yang Y, Sittijunda S, Xia A, Liao Q. Hydrogen from photo fermentation. In: Liao Q, Chang J,

- Herrmann C, Xia A, editors. Bioreactors for microbial biomass and energy conversion. Singapore: Springer; 2018. p. 221–317. https://doi.org/10.1007/978-981-10-7677-0_7.
- [15] Khetkorn W, Rastogi RP, Incharoensakdi A, Lindblad P, Madamwar D, Pandey A, et al. Microalgal hydrogen production – a review. *Bioresour Technol* 2017;243: 1194–206. <https://doi.org/10.1016/j.biortech.2017.07.085>.
 - [16] Oncel S, Kose A. Comparison of tubular and panel type photobioreactors for biohydrogen production utilizing *Chlamydomonas reinhardtii* considering mixing time and light intensity. *Bioresour Technol* 2014;151:265–70. <https://doi.org/10.1016/j.biortech.2013.10.076>.
 - [17] Skjånes K, Andersen U, Heidorn T, Borgvang SA. Design and construction of a photobioreactor for hydrogen production, including status in the field. *J Appl Phycol* 2016;28:2205–23. <https://doi.org/10.1007/s10811-016-0789-4>.
 - [18] Carbas BB, Güler M, Yücel K, Yildiz HB. Construction of novel cyanobacteria-based biological photovoltaic solar cells: hydrogen and photocurrent generated via both photosynthesis and respiratory system. *J Photochem Photobiol, A* 2023;442:114764. <https://doi.org/10.1016/j.jphotochem.2023.114764>.
 - [19] Khan MI, Shin JH, Kim JD. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microb Cell Factories* 2018;17:36. <https://doi.org/10.1186/s12934-018-0879-x>.
 - [20] Kosourov S, Böhm M, Senger M, Berggren G, Stensjö K, Mamedov F, et al. Photosynthetic hydrogen production: novel protocols, promising engineering approaches and application of semi-synthetic hydrogenases. *Physiol Plantarum* 2021;173:555–67. <https://doi.org/10.1111/ppl.13428>.
 - [21] Touloupakis E, Faraloni C, Silva Benavides AM, Torzillo G. Recent achievements in microalgal photobiological hydrogen production. *Energies* 2021;14:7170. <https://doi.org/10.3390/en14217170>.
 - [22] Esteves-Ferreira AA, Cavalcanti JHF, Vaz MGMV, Alvarenga LV, Nunes-Nesi A, Araújo WL. Cyanobacterial nitrogenases: phylogenetic diversity, regulation and functional predictions. *Genet Mol Biol* 2017;40:261–75. <https://doi.org/10.1590/1678-4685-GMB-2016-0050>.
 - [23] Issa AA, Abd-Alla MH, Ohyama T. Nitrogen fixing cyanobacteria: future prospect. In: *Advances in biology and ecology of nitrogen fixation*. IntechOpen; 2014. <https://doi.org/10.5772/56995>.
 - [24] Satta A, Esquirol L, Ebert BE. Current metabolic engineering strategies for photosynthetic bioproduction in cyanobacteria. *Microorganisms* 2023;11:455. <https://doi.org/10.3390/microorganisms11020455>.
 - [25] Sharma A, Arya SK. Hydrogen from algal biomass: a review of production process. *Biotechnol Rep* 2017;15:63–9. <https://doi.org/10.1016/j.btre.2017.06.001>.
 - [26] Thanigaivel S, Rajendran S, Hoang TKA, Ahmad A, Luque R. Photobiological effects of converting biomass into hydrogen – challenges and prospects. *Bioresour Technol* 2023; 367:128278. <https://doi.org/10.1016/j.biortech.2022.128278>.
 - [27] Arimbrathodi SP, Javed MA, Hamouda MA, Aly Hassan A, Ahmed ME. BioH₂ production using microalgae: highlights on recent advancements from a bibliometric analysis. *Water* 2023;15:185. <https://doi.org/10.3390/w15010185>.
 - [28] Singh H, Paritosh K, Vivekanand V, Pareek N. Microorganism-Assisted biohydrogen production and bioreactors. *Chem Eng Technol* 2023;46:204–17. <https://doi.org/10.1002/ceat.202000561>.
 - [29] Agyekum EB, Nutakor C, Agwa AM, Kamel S. A critical review of renewable hydrogen production methods: factors affecting their scale-up and its role in future energy generation. *Membranes* 2022;12:173. <https://doi.org/10.3390/membranes12020173>.
 - [30] Pareek A, Dom R, Gupta J, Chandran J, Adepu V, Borse PH. Insights into renewable hydrogen energy: recent advances and prospects. *Mater Sci Technol* 2020;3:319–27. <https://doi.org/10.1016/j.mset.2019.12.002>.
 - [31] 7.3. Technologies for hydrogen production. NetlDoeGov. <https://www.netl.doe.gov/research/carbon-management/energy-systems/gasification/gasifipedia/technologies-hydrogen> (accessed May 19, 2023).
 - [32] Chew YE, Cheng XH, Loy ACM, How BS, Andiappan V. Beyond the colours of hydrogen: opportunities for process systems engineering in hydrogen economy. *Process Integr Optim Sustain* 2023. <https://doi.org/10.1007/s41660-023-00324-z>.
 - [33] Ahmed SF, Rafa N, Mofijur M, Badruddin IA, Inayat A, Ali MS, et al. Biohydrogen production from biomass sources: metabolic pathways and economic analysis. *Front Energy Res* 2021;9. <https://doi.org/10.3389/fenrg.2021.753878>.
 - [34] Nagarajan D, Dong C-D, Chen C-Y, Lee D-J, Chang J-S. Biohydrogen production from microalgae - major bottlenecks and future research perspectives. *Biotechnol J* 2021;16:2000124. <https://doi.org/10.1002/biot.202000124>.
 - [35] Alternative Fuels Data Center: Hydrogen benefits and considerations. https://afdc.energy.gov/fuels/hydrogen_benefits.html (accessed May 16, 2023).
 - [36] Benemann JR, Weissman JC. Biophotolysis: problems and prospects. In: Schlegel HG, Barnea J, editors. *Microbial energy conversion*. Pergamon; 1977. p. 413–26. <https://doi.org/10.1016/B978-0-08-021791-8.50038-X>.
 - [37] Javed MA, Zafar AM, Aly Hassan A, Zaidi AA, Farooq M, El Badawy A, et al. The role of oxygen regulation and algal growth parameters in hydrogen production via biophotolysis. *J Environ Chem Eng* 2022;10:107003. <https://doi.org/10.1016/j.jece.2021.107003>.
 - [38] Woon JM, Khoo KS, AL-Zahrani AA, Alanazi MM, Lim JW, Cheng CK, et al. Epitomizing biohydrogen production from microbes: critical challenges vs opportunities. *Environ Res* 2023;227:115780. <https://doi.org/10.1016/j.envres.2023.115780>.
 - [39] Singh RP, Yadav P, Gupta RK. Chapter 11 – modern strategy of cyanobacterial biohydrogen production and current approaches toward its enhancement. In: Singh PK, Fillat MF, Sittler V, Kumar A, editors. *Expanding horizon of cyanobacterial biology*. Academic Press; 2022. p. 219–38. <https://doi.org/10.1016/B978-0-323-91202-0.00002-6>.
 - [40] Vinayak V, Sirotiya V, Khandelwal P, Rai A, Jadhav DA, Pugazhendhi A, et al. Recent trends in engineering algae for biohydrogen production: state of art strategies. *Fuel* 2023;348:128636. <https://doi.org/10.1016/j.fuel.2023.128636>.
 - [41] Badawi EY, Elkharsa RA, Abdelfattah EA. Value proposition of bio-hydrogen production from different biomass sources. *Energy Nexus* 2023;10:100194. <https://doi.org/10.1016/j.nexus.2023.100194>.
 - [42] Gautam R, Nayak JK, Ress NV, Steinberger-Wilckens R, Ghosh UK. Bio-hydrogen production through microbial electrolysis cell: structural components and influencing factors. *J Chem Eng* 2023;455:140535. <https://doi.org/10.1016/j.cej.2022.140535>.
 - [43] Park J-H, Chandrasekhar K, Jeon B-H, Jang M, Liu Y, Kim S-H. State-of-the-art technologies for continuous high-rate biohydrogen production. *Bioresour Technol* 2021;320:124304. <https://doi.org/10.1016/j.biortech.2020.124304>.
 - [44] Vavra C. Smart instrumentation helps with green hydrogen production, sustainability. *Control Engineering*; 2023. <https://www.controleng.com/articles/smart-instrumentation-helps-with-green-hydrogen-production-sustainability/>. [Accessed 19 May 2023].

- [45] Androga DD, Özgür E, Eroglu I, Gündüz U, Yücel M, Androga DD, et al. Photofermentative hydrogen production in outdoor conditions. *Hydrogen energy – challenges and perspectives*. IntechOpen; 2012. <https://doi.org/10.5772/50390>.
- [46] Agarwal P, Soni R, Kaur P, Madan A, Mishra R, Pandey J, et al. Cyanobacteria as a promising alternative for sustainable environment: synthesis of biofuel and biodegradable plastics. *Front Microbiol* 2022;13.
- [47] Borowiak D, Krzywonos M. Bioenergy, biofuels, lipids and pigments – research trends in the use of microalgae grown in photobioreactors. *Energies* 2022;15:5357. <https://doi.org/10.3390/en15155357>.
- [48] Sivagurunathan P, Sen B, Lin C-Y. High-rate fermentative hydrogen production from beverage wastewater. *Appl Energy* 2015;147. <https://doi.org/10.1016/j.apenergy.2015.01.136>.
- [49] Reyna-Gómez LM, Molina-Guerrero CE, Alfaro JM, Suárez Vázquez SI, Robledo-Olivo A, Cruz-López A. Effect of carbon/nitrogen ratio, temperature, and inoculum source on hydrogen production from dark codigestion of fruit peels and sewage sludge. *Sustainability* 2019;11:2139. <https://doi.org/10.3390/su11072139>.
- [50] Madamwar D, Garg N, Shah V. Cyanobacterial hydrogen production. *World J Microbiol Biotechnol* 2000;16:757–67. <https://doi.org/10.1023/A:1008919200103>.
- [51] Dickson DJ, Page CJ, Ely RL. Photobiological hydrogen production from *Synechocystis* sp. PCC 6803 encapsulated in silica sol–gel. *IJHE* 2009;34:204–15. <https://doi.org/10.1016/j.ijhydene.2008.10.021>.
- [52] Ainas M, Hasnaoui S, Bouarab R, Abdi N, Drouiche N, Mameri N. Hydrogen production with the cyanobacterium *Spirulina platensis*. *IJHE* 2017;42:4902–7. <https://doi.org/10.1016/j.ijhydene.2016.12.056>.
- [53] Leino H, Kosourov S, Saari L, Sivonen K, Tsygankov A, Aro E-M, et al. Extended H₂ photoproduction by N₂-fixing cyanobacteria immobilized in thin alginate films. *IJHE* 2012;37:151–61. <https://doi.org/10.1016/j.ijhydene.2011.09.088>.
- [54] Rai SV, Rajashekhar M. Effect of pH, salinity and temperature on the growth of six species of cyanobacteria isolated from arabian sea coast of Karnataka. *Int J Biosci Technol* 2016;9:1–6.
- [55] Burrows EH, Chaplen FWR, Ely RL. Effects of selected electron transport chain inhibitors on 24-h hydrogen production by *Synechocystis* sp. PCC 6803. *Bioresour Technol* 2011;102:3062–70. <https://doi.org/10.1016/j.biortech.2010.10.042>.
- [56] Burrows EH, Wong W-K, Fern X, Chaplen FWR, Ely RL. Optimization of pH and nitrogen for enhanced hydrogen production by *Synechocystis* sp. PCC 6803 via statistical and machine learning methods. *Biotechnol Prog* 2009;25:1009–17. <https://doi.org/10.1002/btpr.213>.
- [57] Jin H-F, Lim B-R, Lee K. Influence of nitrate feeding on carbon dioxide fixation by microalgae. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 2006;41:2813–24. <https://doi.org/10.1080/10934520600967928>.
- [58] Lakshmikanandan M, Murugesan AG. Enhancement of growth and biohydrogen production potential of *Chlorella vulgaris* MSU-AGM 14 by utilizing seaweed aqueous extract of *Valoniopsis pachynema*. *Ren Energy* 2016;96:390–9. <https://doi.org/10.1016/j.renene.2016.04.097>.
- [59] Markov SA, Protasov ES, Bybin VA, Eivazova ER, Stom DI. Using immobilized cyanobacteria and culture medium contaminated with ammonium for H₂ production in a hollow-fiber photobioreactor. *IJHE* 2015;40:4752–7. <https://doi.org/10.1016/j.ijhydene.2015.02.053>.
- [60] Kossalbayev BD, Tomo T, Zayadan BK, Sadvakasova AK, Bolatkhan K, Alwasel S, et al. Determination of the potential of cyanobacterial strains for hydrogen production. *IJHE* 2020;45:2627–39. <https://doi.org/10.1016/j.ijhydene.2019.11.164>.
- [61] Maswanna T, Phunpruch S, Lindblad P, Maneeruttanarungroj C. Enhanced hydrogen production by optimization of immobilized cells of the green alga *Tetraspora* sp. CU2551 grown under anaerobic condition. *Biomass Bioenergy* 2018;111:88–95. <https://doi.org/10.1016/j.biombioe.2018.01.005>.
- [62] Allahverdiyeva Y, Leino H, Saari L, Fewer DP, Shunmugam S, Sivonen K, et al. Screening for biohydrogen production by cyanobacteria isolated from the Baltic Sea and Finnish lakes. *IJHE* 2010;35:1117–27. <https://doi.org/10.1016/j.ijhydene.2009.12.030>.
- [63] Kamshybayeva GK, Kossalbayev BD, Sadvakasova AK, Bauenova MO, Zayadan BK, Krapivina AA, et al. Effect of the photosynthesis inhibitors on hydrogen production by non-heterocyst cyanobacterial strains. *IJHE* 2023. <https://doi.org/10.1016/j.ijhydene.2023.03.453>.
- [64] Kossalbayev BD, Kakimova AB, Bolatkhan K, Zayadan BK, Sandybayeva SK, Bozieva AM, et al. Biohydrogen production by novel cyanobacterial strains isolated from rice paddies in Kazakhstan. *IJHE* 2022;47:16440–53. <https://doi.org/10.1016/j.ijhydene.2022.03.126>.
- [65] Salleh SF, Kamaruddin A, Uzir MH, Mohamed AR. Effects of cell density, carbon dioxide and molybdenum concentration on biohydrogen production by *Anabaena variabilis* ATCC 29413. *Energy Convers Manag* 2014;87:599–605. <https://doi.org/10.1016/j.enconman.2014.06.069>.
- [66] Bandyopadhyay A, Stöckel J, Min H, Sherman LA, Pakrasi HB. High rates of photobiological H₂ production by a cyanobacterium under aerobic conditions. *Nat Commun* 2010;1:139. <https://doi.org/10.1038/ncomms1139>.
- [67] Kamshybayeva GK, Kossalbayev BD, Sadvakasova AK, Bauenova MO, Zayadan BK, Bozieva AM, et al. Screening and optimisation of hydrogen production by newly isolated nitrogen-fixing cyanobacterial strains. *IJHE* 2023;48:16649–62. <https://doi.org/10.1016/j.ijhydene.2023.01.163>.
- [68] Yeager CM, Milliken CE, Bagwell CE, Staples L, Berseth PA, Sessions HT. Evaluation of experimental conditions that influence hydrogen production among heterocystous Cyanobacteria. *IJHE* 2011;36:7487–99. <https://doi.org/10.1016/j.ijhydene.2011.03.078>.
- [69] Hwang J-H, Kim H-C, Choi J-A, Abou-Shanab RaI, Dempsey BA, Regan JM, et al. Photoautotrophic hydrogen production by eukaryotic microalgae under aerobic conditions. *Nat Commun* 2014;5:3234. <https://doi.org/10.1038/ncomms4234>.
- [70] Lambert GR, Smith GD. Hydrogen formation by marine blue-green algae. *FEBS Lett* 1977;83:159–62. [https://doi.org/10.1016/0014-5793\(77\)80664-9](https://doi.org/10.1016/0014-5793(77)80664-9).
- [71] Sveshnikov DA, Sveshnikova NV, Rao KK, Hall DO. Hydrogen metabolism of mutant forms of *Anabaena variabilis* in continuous cultures and under nutritional stress. *FEMS Microbiol Lett* 1997;147:297–301. <https://doi.org/10.1111/j.1574-6968.1997.tb10257.x>.
- [72] Marques AE, Barbosa AT, Jotta J, Coelho MC, Tamagnini P, Gouveia L. Biohydrogen production by *Anabaena* sp. PCC 7120 wild-type and mutants under different conditions: Light, nickel, propane, carbon dioxide and nitrogen. *Biomass and Bioenergy* 2011;35:4426–34. <https://doi.org/10.1016/j.biombioe.2011.08.014>.
- [73] Leino H, Shunmugam S, Isojärvi J, Oliveira P, Mulo P, Saari L, et al. Characterization of ten H₂ producing cyanobacteria isolated from the Baltic Sea and Finnish lakes. *IJHE* 2014;39:8983–91. <https://doi.org/10.1016/j.ijhydene.2014.03.171>.

- [74] Touloupakis E, Rontogiannis G, Silva Benavides AM, Cicchi B, Ghanotakis DF, Torzillo G. Hydrogen production by immobilized *Synechocystis* sp. PCC 6803. *Int J Hydrogen Energy* 2016;41:15181–6. <https://doi.org/10.1016/j.ijhydene.2016.07.075>.
- [75] Anjana K, Kaushik A. Enhanced hydrogen production by immobilized cyanobacterium *Lyngbya perelegans* under varying anaerobic conditions. *Biomass Bioenergy* 2014; 63:54–7. <https://doi.org/10.1016/j.biombioe.2014.01.019>.
- [76] Salem K, van Waasbergen LG. Photosynthetic electron transport controls expression of the high light inducible gene in the cyanobacterium *Synechococcus elongatus* strain PCC 7942. *Plant Cell Physiol* 2004;45:651–8. <https://doi.org/10.1093/pcp/pch072>.
- [77] Pansook S, Incharoensakdi A, Phunpruch S. Effects of the photosystem II inhibitors CCCP and DCMU on hydrogen production by the unicellular halotolerant cyanobacterium *Aphanthece halophytica*. *Sci World J* 2019;2019:e1030236. <https://doi.org/10.1155/2019/1030236>.
- [78] Sadvakasova AK, Kossalbayev BD, Zayadan BK, Bolatkhan K, Alwasel S, Najafpour MM, et al. Bioprocesses of hydrogen production by cyanobacteria cells and possible ways to increase their productivity. *RSER* 2020;133:110054. <https://doi.org/10.1016/j.rser.2020.110054>.
- [79] Ali M, Elreedy A, Ibrahim MG, Fujii M, Nakatani K, Tawfik A. Regulating acidogenesis and methanogenesis for the separated bio-generation of hydrogen and methane from saline-to-hypersaline industrial wastewater. *J Environ Manag* 2019;250:109546. <https://doi.org/10.1016/j.jenvman.2019.109546>.
- [80] Tehei M, Franzetti B, Madern D, Ginzburg M, Ginzburg BZ, Giudici-Orticoni M-T, et al. Adaptation to extreme environments: macromolecular dynamics in bacteria compared in vivo by neutron scattering. *EMBO Rep* 2004;5:66–70. <https://doi.org/10.1038/sj.embor.7400049>.
- [81] Aoyama K, Uemura I, Miyake J, Asada Y. Fermentative metabolism to produce hydrogen gas and organic compounds in a cyanobacterium, *Spirulina platensis*. *J Ferment Bioeng* 1997;83:17–20.
- [82] He D, Bultel Y, Magnin J-P, Willison JC. Kinetic analysis of photosynthetic growth and photohydrogen production of two strains of *Rhodospirillum rubrum*. *Enzyme Microb* 2006; 38:253–9. <https://doi.org/10.1016/j.enzymictec.2005.06.012>.
- [83] Wang Y-Z, Liao Q, Zhu X, Tian X, Zhang C. Characteristics of hydrogen production and substrate consumption of *Rhodospirillum rubrum* CQK 01 in an immobilized-cell photobioreactor. *Bioresour Technol* 2010;101:4034–41. <https://doi.org/10.1016/j.biortech.2010.01.045>.
- [84] Alvarez AJ, Fuentes KL, Alberto Arias C, TatianaR Chaparro. Production of hydrogen from beverage wastewater by dark fermentation in an internal circulation reactor: effect on pH and hydraulic retention time. *Energy Convers Manag* 2022;15:100232. <https://doi.org/10.1016/j.ecmx.2022.100232>.
- [85] Shui X, Jiang D, Zhang H, Zhang X, Yang J, Lei T, et al. Effect of light perturbation on the photo-fermentative hydrogen production of biomass from giant reed. *J Clean Prod* 2022;351:131481. <https://doi.org/10.1016/j.jclepro.2022.131481>.
- [86] Dutta D, De D, Chaudhuri S, Bhattacharya SK. Hydrogen production by cyanobacteria. *Microb Cell Factories* 2005;4:36. <https://doi.org/10.1186/1475-2859-4-36>.
- [87] Mohan SV, Pandey A. Chapter 1 - biohydrogen production: an introduction. In: Pandey A, Chang J-S, Hallenbeck PC, Larroche C, editors. *Biohydrogen*. Amsterdam: Elsevier; 2013. p. 1–24. <https://doi.org/10.1016/B978-0-444-59555-3.00001-5>.
- [88] Akkerman I, Janssen M, Rocha J, Wijffels RH. Photobiological hydrogen production: photochemical efficiency and bioreactor design. *IJHE* 2002;27:1195–208. [https://doi.org/10.1016/S0360-3199\(02\)00071-X](https://doi.org/10.1016/S0360-3199(02)00071-X).
- [89] González-Camejo J, Viruela A, Ruano MV, Barat R, Seco A, Ferrer J. Effect of light intensity, light duration and photoperiods in the performance of an outdoor photobioreactor for urban wastewater treatment. *Algal Res* 2019;40:101511. <https://doi.org/10.1016/j.algal.2019.101511>.
- [90] Torzillo G, Zittelli GC, Cicchi B, Diano M, Parente M, Benavides AMS, et al. Effect of plate distance on light conversion efficiency of a *Synechocystis* culture grown outdoors in a multiplate photobioreactor. *Sci Total Environ* 2022;842:156840. <https://doi.org/10.1016/j.scitotenv.2022.156840>.
- [91] Negi S, Perrine Z, Friedland N, Kumar A, Tokutsu R, Minagawa J, et al. Light regulation of light-harvesting antenna size substantially enhances photosynthetic efficiency and biomass yield in green algae. *Plant J* 2020;103:584–603. <https://doi.org/10.1111/tpj.14751>.
- [92] Seo YH, Lee Y, Jeon DY, Han J-I. Enhancing the light utilization efficiency of microalgae using organic dyes. *Bioresour Technol* 2015;181:355–9. <https://doi.org/10.1016/j.biortech.2015.01.031>.
- [93] Kazbar A, Cogne G, Urbain B, Marec H, Gouic B, Tallec J, et al. Effect of dissolved oxygen concentration on microalgal culture in photobioreactors. *Algal Res* 2019;101. <https://doi.org/10.1016/j.algal.2019.101432>.
- [94] Carvalho AP, Meireles LA, Malcata FX. Microalgal reactors: a review of enclosed system designs and performances. *Biotechnol Prog* 2006;22:1490–506. <https://doi.org/10.1021/bp060065r>.
- [95] Del Campo JA, García-González M, Guerrero MG. Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. *Appl Microbiol Biotechnol* 2007;74:1163–74. <https://doi.org/10.1007/s00253-007-0844-9>.
- [96] Hawkes FR, Hussy I, Kyazze G, Dinsdale R, Hawkes DL. Continuous dark fermentative hydrogen production by mesophilic microflora: principles and progress. *IJHE* 2007; 32:172–84. <https://doi.org/10.1016/j.ijhydene.2006.08.014>.
- [97] Acien Fernández FG, Fernández Sevilla JM, Molina Grima E. Photobioreactors for the production of microalgae. *Rev Environ Sci Biotechnol* 2013;12:131–51. <https://doi.org/10.1007/s11157-012-9307-6>.
- [98] Benner P, Meier L, Pfeffer A, Krüger K, Oropeza Vargas JE, Weuster-Botz D. Lab-scale photobioreactor systems: principles, applications, and scalability. *Bioproc Biosyst Eng* 2022;45:791–813. <https://doi.org/10.1007/s00449-022-02711-1>.
- [99] Ugwu U, Ogbonna J, Tanaka H. Design of static mixers for inclined tubular photobioreactors. *J Appl Phycol* 2003;15:217–23. <https://doi.org/10.1023/A:1023837400050>.
- [100] Vasiliadou IA, Melero JA, Molina R, Puyol D, Martínez F. Optimization of H₂ production through minimization of CO₂ emissions by mixed cultures of purple phototrophic bacteria in aqueous samples. *Water* 2020;12:2015. <https://doi.org/10.3390/w12072015>.
- [101] Natzke J, Noar J, Bruno-Bárcena JM. *Azotobacter vinelandii* nitrogenase activity, hydrogen production, and response to oxygen exposure. *Appl Environ Microbiol* 2018;84:e01208-e01218. <https://doi.org/10.1128/AEM.01208-18>.
- [102] Fascetti E, Todini O. *Rhodospirillum rubrum* cultivation and hydrogen production in a one- and two-stage chemostat. *Appl Microbiol Biotechnol* 1995;44:300–5. <https://doi.org/10.1007/BF00169920>.
- [103] Oh Y-K, Seol E-H, Kim M-S, Park S. Photoproduction of hydrogen from acetate by a chemoheterotrophic bacterium *Rhodospirillum rubrum* palustris P4. *IJHE* 2004;29:1115–21. <https://doi.org/10.1016/j.ijhydene.2003.11.008>.
- [104] Kosourov S, Murukesan G, Seibert M, Allahverdiyeva Y. Evaluation of light energy to H₂ energy conversion

- efficiency in thin films of cyanobacteria and green alga under photoautotrophic conditions. *Algal Res* 2017. <https://doi.org/10.1016/j.algal.2017.09.027>.
- [105] Kayano H, Karube I, Matsunaga T, Suzuki S, Nakayama O. A photochemical fuel cell system using *Anabaena* N-7363. *Appl Microbiol Biotechnol* 1981;12:1–5. <https://doi.org/10.1007/BF00508110>.
- [106] Gienau T, Ehrmanntraut A, Kraume M, Rosenberger S. Influence of ozone treatment on ultrafiltration performance and nutrient flow in a membrane based nutrient recovery process from anaerobic digestate. *Membranes* 2020;10:64. <https://doi.org/10.3390/membranes10040064>.
- [107] Mehta CM, Khunjar WO, Nguyen V, Tait S, Batstone DJ. Technologies to recover nutrients from waste streams: a critical review. *Crit Rev Environ Sci Technol* 2015; 45:385–427. <https://doi.org/10.1080/10643389.2013.866621>.
- [108] Leong YK, Chang J-S, Lee D-J. Chapter 3 - types of photobioreactors. In: Sirohi R, Pandey A, Sim S, Chang J-S, Lee D-J, editors. *Current developments in biotechnology and bioengineering*. Elsevier; 2023. p. 33–58. <https://doi.org/10.1016/B978-0-323-99911-3.00007-5>.
- [109] Markov SA. Hydrogen production in bioreactors: current trends. *Energy Proc* 2012;29:394–400. <https://doi.org/10.1016/j.egypro.2012.09.046>.
- [110] Tsygankov AA, Fedorov AS, Kosourov SN, Rao KK. Hydrogen production by cyanobacteria in an automated outdoor photobioreactor under aerobic conditions. *Biotechnol Bioeng* 2002;80:777–83. <https://doi.org/10.1002/bit.10431>.
- [111] Chen C-Y, Lee C-M, Chang J-S. Feasibility study on bioreactor strategies for enhanced photohydrogen production from *Rhodospseudomonas palustris* WP3-5 using optical-fiber-assisted illumination systems. *IJHE* 2006; 31:2345–55. <https://doi.org/10.1016/j.ijhydene.2006.03.007>.
- [112] Lindberg P, Schütz K, Happe T, Lindblad P. A hydrogen-producing, hydrogenase-free mutant strain of *Nostoc punctiforme* ATCC 29133. *IJHE* 2002;27:1291–6. [https://doi.org/10.1016/S0360-3199\(02\)00121-0](https://doi.org/10.1016/S0360-3199(02)00121-0).
- [113] Nyberg M, Heidorn T, Lindblad P. Hydrogen production by the engineered cyanobacterial strain *Nostoc* PCC 7120 Δ hupW examined in a flat panel photobioreactor system. *J Biotechnol* 2015;215:35–43. <https://doi.org/10.1016/j.jbiotec.2015.08.028>.
- [114] Golden JW, Yoon H-S. Heterocyst development in *Anabaena*. *Curr Opin Microbiol* 2003;6:557–63. <https://doi.org/10.1016/j.mib.2003.10.004>.
- [115] Dechatiwongse P, Srisamai S, Maitland G, Hellgardt K. Effects of light and temperature on the photoautotrophic growth and photoinhibition of nitrogen-fixing cyanobacterium *Cyanothece* sp. ATCC 51142. *Algal Res* 2014;5:103–11. <https://doi.org/10.1016/j.algal.2014.06.004>.
- [116] Skjånes K, Knutsen G, Källqvist T, Lindblad P. H₂ production from marine and freshwater species of green algae during sulfur deprivation and considerations for bioreactor design. *IJHE* 2008;33:511–21. <https://doi.org/10.1016/j.ijhydene.2007.09.040>.
- [117] Satar I, Ghasemi M, Aljlil SA, Isahak WNRW, Abdalla AM, Alam J, et al. Production of hydrogen by *Enterobacter aerogenes* in an immobilized cell reactor. *IJHE* 2017;42:9024–30. <https://doi.org/10.1016/j.ijhydene.2016.04.150>.
- [118] Liu W, Yuan L, Wei B. Study on improvement of continuous hydrogen production by photosynthetic biofilm in interior illuminant reactor. *J Environ Biol* 2016;37:999–1006.
- [119] Elkhallout K, Sagor E, Alipour S, Koku H, Gunduz U, Eroglu I, et al. Long-term stable hydrogen production from acetate using immobilized *Rhodobacter capsulatus* in a panel photobioreactor. *IJHE* 2019;44:18801–10. <https://doi.org/10.1016/j.ijhydene.2018.10.133>.
- [120] Boran E, Özgür E, Yücel M, Gündüz U, Eroglu I. Biohydrogen production by *Rhodobacter capsulatus* Hup[−] mutant in pilot solar tubular photobioreactor. *IJHE* 2012;37:16437–45. <https://doi.org/10.1016/j.ijhydene.2012.02.171>.
- [121] Eroglu İ, Aslan K, Gündüz U, Yücel M, Türker L. Continuous hydrogen production by *rhodobacter sphaeroides* O.U.001. In: Zaborsky OR, Benemann JR, Matsunaga T, Miyake J, San Pietro A, editors. *BioHydrogen*. Boston, MA: Springer US; 1999. p. 143–9. https://doi.org/10.1007/978-0-585-35132-2_18.
- [122] Biotech removable porous frit micro sparger for mini bioreactor system and fermentors. <https://www.hengko.com/biotech-removable-porous-frit-micro-sparger-for-mini-bioreactor-system-products/> (accessed June 15, 2023).
- [123] Chen CY, Lu WB, Liu CH, Chang JS. Improved phototrophic H₂ production with *Rhodospseudomonas palustris* WP3-5 using acetate and butyrate as dual carbon substrates. *Bioresour Technol* 2008;99:3609–16.
- [124] Markov SA, Weaver PF, Seibert M. Spiral tubular bioreactors for hydrogen production by photosynthetic microorganisms : design and operation. *Appl Biochem Biotechnol* 1997;63–65:577–84. <https://doi.org/10.1007/BF02920455>.
- [125] Huang J, Ying J, Fan F, Yang Q, Wang J, Li Y. Development of a novel multi-column airlift photobioreactor with easy scalability by means of computational fluid dynamics simulations and experiments. *Bioresour Technol* 2016; 222:399–407. <https://doi.org/10.1016/j.biortech.2016.09.109>.
- [126] Zittelli GC, Rodolfi L, Bassi N, Biondi N, Tredici MR. Photobioreactors for microalgal biofuel production. In: Borowitzka MA, Moheimani NR, editors. *Algae for biofuels and energy*. Dordrecht: Springer Netherlands; 2013. p. 115–31. https://doi.org/10.1007/978-94-007-5479-9_7.
- [127] Alvarado-Gutiérrez ML, Ruiz-Ordaz N, Galíndez-Mayer J, Santoyo-Tepole F, Curiel-Quesada E, García-Mena J, et al. Kinetics of carbendazim degradation in a horizontal tubular biofilm reactor. *Bioproc Biosyst Eng* 2017;40:519–28. <https://doi.org/10.1007/s00449-016-1717-3>.
- [128] Sánchez Mirón A, Contreras Gómez A, García Camacho F, Molina Grima E, Chisti Y. Comparative evaluation of compact photobioreactors for large-scale monoculture of microalgae. *J Biotechnol* 1999;70:249–70. [https://doi.org/10.1016/S0168-1656\(99\)00079-6](https://doi.org/10.1016/S0168-1656(99)00079-6).
- [129] Markov SA, Bazin MJ, Hall DO. Hydrogen photoproduction and carbon dioxide uptake by immobilized *Anabaena variabilis* in a hollow-fiber photobioreactor. *Enzym Microb Technol* 1995;17:306–10. [https://doi.org/10.1016/0141-0229\(94\)00010-7](https://doi.org/10.1016/0141-0229(94)00010-7).
- [130] Markov SA, Bazin MJ, Hall DO. The potential of using cyanobacteria in photobioreactors for hydrogen production. In: Hiroto M, Nishimura H, Kodera Y, Kawai F, Rusin P, Ehrlich HL, et al., editors. *Microbial and enzymatic bioproducts*. Berlin, Heidelberg: Springer; 1995. p. 59–86. <https://doi.org/10.1007/BFb0102316>.
- [131] Krujatz F, Illing R, Krautwer T, Liao J, Helbig K, Goy K, et al. Light-field-characterization in a continuous hydrogen-producing photobioreactor by optical simulation and computational fluid dynamics. *Biotechnol Bioeng* 2015;112:2439–49. <https://doi.org/10.1002/bit.25667>.
- [132] Wang Y-Z, Liao Q, Zhu X, Chen R, Guo C-L, Zhou J. Bioconversion characteristics of *Rhodospseudomonas palustris* CQK 01 entrapped in a photobioreactor for hydrogen production. *Bioresour Technol* 2013;135:331–8. <https://doi.org/10.1016/j.biortech.2012.09.105>.
- [133] Van T Do C, Dinh CT, Dang MT, Dang Tran T, Giang Le T. A novel flat-panel photobioreactor for simultaneous production of lutein and carbon sequestration by *Chlorella sorokiniana* TH01. *Bioresour Technol* 2022;345:126552. <https://doi.org/10.1016/j.biortech.2021.126552>.

- [134] Gilbert JJ, Ray S, Das D. Hydrogen production using *Rhodobacter sphaeroides* (O.U. 001) in a flat panel rocking photobioreactor. *IJHE* 2011;36:3434–41. <https://doi.org/10.1016/j.ijhydene.2010.12.012>.
- [135] Qiang H, Richmond A. Productivity and photosynthetic efficiency of *Spirulina platensis* as affected by light intensity, algal density and rate of mixing in a flat plate photobioreactor. *J Appl Phycol* 1996;8:139–45. <https://doi.org/10.1007/BF02186317>.
- [136] Zúñiga H, Vergara C, Donoso-Bravo A, Jeison D. Effect of delays on the response of microalgae when exposed to dynamic environmental conditions. *Processes* 2020;8:87. <https://doi.org/10.3390/pr8010087>.
- [137] Ruiz J, Alvarez P, Arbib Z, Garrido-Pérez C, Barragan J, Perales J. Performance of a flat panel reactor in the continuous culture of microalgae in urban wastewater: prediction from a batch experiment. *Bioresour Technol* 2012;127C:456–63. <https://doi.org/10.1016/j.biortech.2012.09.103>.
- [138] Kondo T, Wakayama T, Miyake J. Efficient hydrogen production using a multi-layered photobioreactor and a photosynthetic bacterium mutant with reduced pigment. *IJHE* 2006;11:1522–6. <https://doi.org/10.1016/j.ijhydene.2006.06.019>.
- [139] Das A, Basu S, Ghosh S, Dairkee U, Chowdhury R. Mathematical modelling of flat plate biofilm photobioreactors with circular and rectangular configurations. *Biosyst Eng* 2018;174. <https://doi.org/10.1016/j.biosystemseng.2018.06.004>.
- [140] Liao Q, Chang J, Herrmann C, Xia A. *Bioreactors for microbial biomass and energy conversion*. Springer; 2018.
- [141] Sutherland IW. The biofilm matrix – an immobilized but dynamic microbial environment. *Trends Microbiol* 2001;9:222–7. [https://doi.org/10.1016/S0966-842X\(01\)02012-1](https://doi.org/10.1016/S0966-842X(01)02012-1).
- [142] Jung K-W, Kim D-H, Kim S-H, Shin H-S. Bioreactor design for continuous dark fermentative hydrogen production. *Bioresour Technol* 2011;102:8612–20. <https://doi.org/10.1016/j.biortech.2011.03.056>.
- [143] Zhang Q, Zhang Z. Chapter four - biological hydrogen production from renewable resources by photofermentation. In: Li Y, Ge X, editors. *Advances in bioenergy*, vol. 3. Elsevier; 2018. p. 137–60. <https://doi.org/10.1016/bs.aibe.2018.03.001>.
- [144] Chen C-Y, Saratale GD, Lee C-M, Chen P-C, Chang J-S. Phototrophic hydrogen production in photobioreactors coupled with solar-energy-excited optical fibers. *IJHE* 2008;33:6886–95. <https://doi.org/10.1016/j.ijhydene.2008.09.014>.
- [145] Zhubanova A, Qiao X, Ualiev P, Abdieva G, Tastambek K, Kayrmanova GK, et al. Metagenomic analysis reveals correlation between microbiome structure and leonardite characteristics from Kazakhstan coal deposits. *Eurasian Chemico-Technological J* 2019;21:135. <https://doi.org/10.18321/ectj823>.
- [146] Xu X, Zhou Q, Yu D. The future of hydrogen energy: bio-hydrogen production technology. *Int J Hydrogen Energy* 2022;47:33677–98. <https://doi.org/10.1016/j.ijhydene.2022.07.261>.
- [147] Santos FD, Ferreira PL, Pedersen JST. The climate change challenge: a review of the barriers and solutions to deliver a Paris solution. *Climate* 2022;10:75. <https://doi.org/10.3390/cli10050075>.
- [148] Sakurai H, Masukawa H, Kitashima M, Inoue K. How close we are to achieving commercially viable large-scale photobiological hydrogen production by cyanobacteria: a review of the biological aspects. *Life* 2015;5:997–1018. <https://doi.org/10.3390/life5010997>.
- [149] Wheaton ZC, Krishnamoorthy G. Modeling radiative transfer in photobioreactors for algal growth. *Comput Electron Agric* 2012;87:64–73. <https://doi.org/10.1016/j.compag.2012.05.002>.
- [150] Kossalbayev BD, Sadvakassova AK, Zaletova DE, Belkozhayev AM, Kamshybayeva GK, Bauyenova MO. Photobioreactor for the cultivation of phototrophic microorganisms. Patent for utility model - No. 8207. 2023. <https://qazpatent.kz/ru>.