INTERNATIONAL JOURNAL OF HYDROGEN ENERGY 47 (2022) 29661–29684

Available online at www.sciencedirect.com

ScienceDirect

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

Review Article

Strategies and economic feasibilities in cyanobacterial hydrogen production

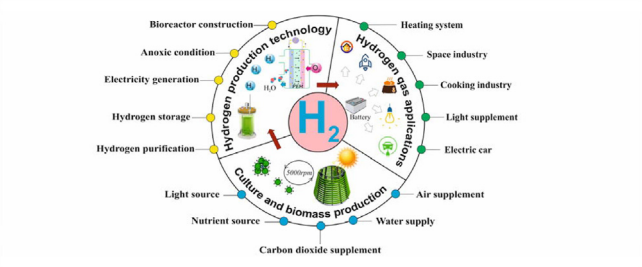
Gulzhanay K. Kamshybayeva [a], Bekzhan D. Kossalbayev [a,b,**], Asemgul K. Sadvakasova [a], Bolatkhan K. Zayadan [a], Ayshat M. Bozieva [c], Dmitry Dunikov [d,e], Saleh Alwasel [f], Suleyman I. Allakhverdiev [a,c,g,*]

[a] Department of Biotechnology, Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Al-Farabi Avenue 71, 050038, Almaty, Kazakhstan
[b] Geology and Oil-gas Business Institute Named After K. Turyssov, Satbayev University, Almaty, Kazakhstan
[c] Controlled Photobiosynthesis Laboratory, K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, Moscow, 127276, Russia
[d] Joint Institute for High Temperatures of the Russian Academy of Sciences, Moscow, Russia
[e] National Research University "Moscow Power Engineering Institute", Moscow, Russia
[f] College of Science, King Saud University, Riyadh, 12372, Saudi Arabia
[g] Department of Plant Physiology, Faculty of Biology, M.V. Lomonosov Moscow State University, Leninskie Gory 1-12, Moscow, 119991, Russia

HIGHLIGHTS

- This review shows hydrogen production mechanisms by cyanobacterial cells.
- Importance of biohydrogen production strategies is discussed.
- Cyanobacterial hydrogen production factors were analyzed.
- Trends and problems for developing cyanobacterial hydrogen production is showed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 April 2022
Received in revised form
14 June 2022

ABSTRACT

Due to the side effects of greenhouse gases, interest in alternative energy sources is growing, and research into hydrogen (H_2) production from cyanobacteria has become a promising direction for the industry. The article provides an overview of cyanobacterial hydrogen production strategies and their current economic efficiency. It also describes

* *Corresponding author*. Department of Biotechnology, Faculty of Biology and Biotechnology, Al-Farabi Kazakh, National University, Al-Farabi Avenue 71, 050038, Almaty, Kazakhstan.
** *Corresponding author*.
E-mail addresses: gkamshebaeva@gmail.com (G.K. Kamshybayeva), kossalbayev.bekzhan@gmail.com (B.D. Kossalbayev), suleyman.allakhverdiev@gmail.com (S.I. Allakhverdiev).
https://doi.org/10.1016/j.ijhydene.2022.06.277
0360-3199/© 2022 Hydrogen Energy Publications LLC. Published by Elsevier Ltd. All rights reserved.

Accepted 27 June 2022
Available online 21 July 2022

Keywords:
Cyanobacteria
Hydrogen
Hydrogen enzymes
Biomass
Economic analysis

metabolic, genetic and technical methods for obtaining H_2 from cyanobacteria. Cyanobacteria are considered potential producers of hydrogen energy that will be economically viable shortly, as they only need cheap salts, water and solar energy to grow. However, producing hydrogen from cyanobacteria still requires extensive work, and the main problem is the small amount of hydrogen energy obtained. To produce large amounts of cyanobacterial hydrogen, the most active wild-type strains must be selected and technological, modular and genetic research must be carried out simultaneously. The low energy efficiency of hydrogen from cyanobacteria also shows the need for comprehensive research through international programs.

© 2022 Hydrogen Energy Publications LLC. Published by Elsevier Ltd. All rights reserved.

Contents

Introduction 29662
Hydrogen production mechanisms 29663
  Direct biophotolysis 29663
  Indirect biophotolysis 29664
Hydrogen production enzymes 29664
  Nitrogenase 29664
  Hydrogenase 29665
Biohydrogen production factors 29666
  Injection of inert gases 29666
  Illumination 29666
  Biomass concentration 29667
  Substrates effect 29667
    Macronutrient starvation 29667
    Micronutrients effect 29667
    Carbon source 29667
    Nitrogen source 29668
    HEPES 29668
  pH effect 29668
  Temperature effect 29668
Biohydrogen production strategies 29668
  Technical approaches 29668
  Metabolic approaches 29670
  Genetic approaches 29671
Active cyanobacterial strains for H_2 production 29672
Trends and prospects for developing biohydrogen production from cyanobacteria 29675
  Policy measures determining the biohydrogen energy development 29675
  Feasibility study of biohydrogen production from cyanobacteria 29675
  Practical significance of this study 29677
Conclusion 29678
CRediT authorship contribution statement 29678
Declaration of competing interest 29678
Acknowledgements 29679
References 29679

Introduction

Nowadays, 35% of the world's energy consumption is met by oil, 25% by natural gas, 28% by coal, 7% by hydroelectric power and the remaining 5% by nuclear energy and alternative energy sources. Consequently, if the use of alternative energy sources does not increase, oil and gas supplies will be exhausted in the future, global temperatures will rise by 2 °C and competition for energy will intensify among countries. These forecasts have led to an increase in demand for alternative energy sources. Moreover, the transition to a renewable

energy economy is a difficult task with numerous unresolved challenges. Hence, the gradual introduction of alternative energy sources is one of the most important tasks facing the global energy sector today [1–3].

In order to optimise the production of biohydrogen as an alternative fuel, numerous studies have been carried out with active cyanobacterial strains, e.g., methods to improve their cultivation and work on the processing of physicochemical growth parameters during direct and indirect biophotolysis [4,5]. Cyanobacteria adapt quickly to their environment and have a high growth rate. Under favourable growth conditions, the species can even double their density within a few hours in closed photobioreactors [6–8].

Some of the factors that enable heterocyst cyanobacteria to grow and increase biomass yield and hydrogen (H_2) production are pH, temperature, light intensity, and organic carbon sources [5,7]. In cyanobacteria, the addition of glucose leads to mixotrophic growth, which contributes to increased biomass productivity [9,10].

Allahverdiyeva et al. [11] conducted extensive studies on H_2 production by *Synechocystis* strains. Yeager et al. [10] and Markov et al. [12] actively produced H_2 using the strains *Anabaena variabilis* and *Anabaena cylindrica*. Accordingly, the best H_2 production was achieved in the presence of light in an argon chamber under anaerobic conditions. Cyanobacteria have an advantage in H_2 production since they can perform both direct biophotolysis in a non-oxidised medium using a bidirectional hydrogenase and indirect biophotolysis using nitrogenase [13].

This review focuses on H_2 production by active cyanobacterial strains and discusses the prospects for various technologies in the future. Furthermore, mechanisms, approaches (technical, metabolic, genetic), responsible enzymes, influencing factors as well as trends and perspectives of cyanobacterial H_2 production are described in the review. Thus, the bioprocesses of H_2 production are highlighted and options for the use of different parameters are given. In addition, the review provides an overview of the studies on cyanobacterial strains published in the last 15 years and describes promising species in more detail. In contrast to other reviews in this field, the review mainly covers the metabolic, technical and genetic areas of cyanobacterial H_2 production and describes the enhancement of hydrogen productivity by using these three approaches simultaneously. The possibility of the commercial use of the biomass of cyanobacterial strains commercially is considered and the importance for industry is described.

Hydrogen production mechanisms

All cyanobacterial species contain chlorophyll *a* and other pigments that use photosynthetic systems (PSII and PSI) to obtain energy from sunlight and carry out oxygenic photosynthesis, as in plants. The species most studied for H_2 production are the model organisms *Synechocystis* and *Anabaena*. At the cellular level, solar energy is absorbed by the antenna pigments in the photosystem 2 (PSII) complex and transferred to photosystem 1 (PSI) via linear electron transport proteins, leading to an increase in the number of electrons and the accumulation of cellular energy in the form of ATP molecules by the protein ferredoxin (Fd). If required, these electrons are used to activate H_2 enzymes [14–22].

In some cases, the cellular protein ferredoxin uses a combination of hydrogenase or nitrogenase to reduce protons and release molecular hydrogen ($2H^+ + 2Fd$ (red) $\leftrightarrow H_2 + 2Fd$ (ox)). Essentially, the enzyme responsible for H_2 production in cyanobacteria is a "reversible hydrogenase" since it can catalyse the reaction in both directions. In addition, the nitrogenase found in cyanobacterial cells is also responsible for H_2 production. This enzyme involved in H_2 production is responsible for the simultaneous fixation of free nitrogen (N_2) from the air [23]. However, hydrogenase and nitrogenase are very sensitive to oxygen (O_2) and nitrogen; O_2, which is also produced during photosynthesis, penetrates the cell wall and reduces/stops the activity of the H_2 enzymes (<0.1% w./w.) [24]. In this context, numerous researches have been conducted with cyanobacterial cells. For example, Khetkorn et al. [25] reported the production of H_2 and O_2 by *A. cylindrica* in an argon chamber within several hours. Kossalbayev et al. [22] also investigated H_2 production by two *Anabaena* strains using a "reversible hydrogenase".

Biophotolysis of cyanobacteria is being actively researched and several methods have already been developed (Table 1). It should be noted that cyanobacteria are physiologically and morphologically diverse and have the ability to produce H_2 by direct and indirect biophotolysis [26].

Direct biophotolysis

Direct biophotolysis of the photosynthetic ability of microalgae and cyanobacteria has been used to split water into O_2 and H_2. During evolution, algae have developed the ability to use solar energy to extract protons and electrons from water. The water splitting reactions occur by absorbing energy from sunlight and transferring electrons to hydrogenases and nitrogenases. Under anaerobic conditions or excessive absorption of solar energy, some microorganisms release excess electrons with the help of hydrogenases, resulting in the conversion of hydrogen ions into H_2 gas. It has been reported that the electrons and protons released during water splitting are recombined by chloroplast hydrogenase to produce up to 98% pure H_2 (Fig. 1) [27–29].

In photosynthesis, light is absorbed by two separate photosynthetic systems connected in series: one system for splitting water and releasing O_2 (PSI or PSII) and a second photosystem (PSII). The absorption of light in the form of photons by PSI (700 nm) and PSII (680 nm) produces a strong oxidant that can oxidise water to protons, electrons, and O_2 [18]. Electrons reduce protons to H_2 according to the following equation (1):

$$2H_2O \rightarrow 2H_2 + O_2 \quad (1)$$

The hydrogen ions produced are converted to H_2 gas by the enzyme hydrogenase (blue-green algae such as cyanobacteria) present in the cells in an environment with electrons (donated by the reduced Fd). The light energy absorbed by PSII generates electrons, which are converted to Fd by the light energy absorbed by PSI, as shown in Fig. 1. Since the hydrogenase is sensitive to O_2, the oxygen content must be kept below 0.1% to maintain H_2 production [30].

Table 1 – Comparison of H_2 production by direct and indirect biophotolysis processes [26].

Nº	Process	Advantages	Disadvantages
1	Direct biophotolysis	Converts solar energy into fuel at maximum efficiency; One-step process, simple installations, easy to use; Uses existing metabolic techniques.	Large waterproof photobioreactors are required; Hydrogen/oxygen compounds have the potential to explode; Occurs near O_2-sensitive hydrogenase.
2	Indirect biophotolysis	Isolation of changes in O_2 and H_2; Reduction of demand for photobioreactors during H_2 production.	During each stage, a little energy is lost.

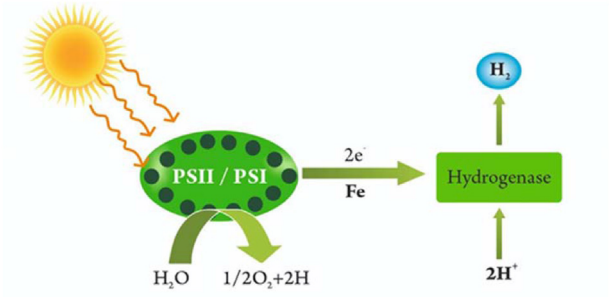


Fig. 1 – Scheme of direct photolysis by cyanobacteria cells. Abbreviations: PSI – photosystem I, PSII – photosystem II, H_2 – hydrogen.

In the literature, *Oscillatoria* sp. 23 and *Oscillatoria chalybea* are reported to produce H_2 via indirect biophotolysis, while *Anabaena* sp. UTEX 1448 produces a considerable amount of H_2 via direct biophotolysis over 12 h [31].

Indirect biophotolysis

Indirect biophotolysis produces H_2 from electrons derived from the breakdown of carbohydrates [32], using mainly green algae and cyanobacteria (which maintain low O_2 concentrations) [33]. This process serves to limit the effect of O_2 on [Fe]-hydrogenase. As shown in Fig. 2, H_2 production is spatially separated from photosynthesis by sulphur depletion/replenishment [34].

In stage 1, CO_2 is bound by oxygenic phototrophs for biosynthesis (sugars) [35] according to the equation:

$$12H_2O + 6CO_2 \rightarrow C_6H_{12}O_6 + 6O_2 \quad (2)$$

In stage 2, the sugar can ferment to H_2 and CO_2 as shown in the following equation:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6O_2 \quad (3)$$

It had also been shown that photosynthetic O_2 depletion enhanced anaerobic H_2 production, as photosynthesis and carbohydrate metabolism were active [36,37]. According to Touloupakis et al. [32], *Synechocystis* sp. PCC6803 actively produced H_2 during indirect biophotolysis at a maximum rate of 40.6 ± 4.9 μmol H_2 mg^{-1} *Chl a* h^{-1}.

Hydrogen production enzymes

Cyanobacterial species contain 2 different enzymes involved in H_2 production: nitrogenase (N_2ase) and hydrogenase (H_2ase) (Fig. 3). Nitrogenase is present in heterocyst cells, while hydrogenase is only found in vegetative cells. Nitrogenase simultaneously absorbs free nitrogen from the air and releases stored energy in the form of H_2 under stress. Accordingly, nitrogenase requires a lot of energy for its operation compared to hydrogenase. Hydrogenase is active in cells under anaerobic conditions and catalyses hydrogen molecules [38,39].

Nitrogenase

Nitrogenase is a complex multisubstrate enzyme responsible for N_2 fixation in cyanobacteria and is mainly found in prokaryotic cells. It catalyses all biochemical N_2 production and mediates the global biogeochemical N_2 cycle. Nitrogenase uses magnesium adenosine triphosphate (MgATP) and electrons to reduce many substrates, including protons [40].

Nitrogenase produces one hydrogen molecule per stable nitrogen molecule, requiring $8e^-$ and 16ATP molecules. Under anaerobic conditions, the cell produces hydrogen by using nitrogenase to release all energy electrons, which determines its important role in H_2 production by cyanobacteria [36,41].

In microorganisms, there are three types of nitrogenase, depending on the metal content in the enzyme active site:

1. [Mo]-nitrogenase;
2. [V]-nitrogenase;
3. [Fe]-nitrogenase.

Nitrogenase consists of three specific protein components: the Mo–Fe protein, which forms α-, β-complexes with P- and Fe–MoCo substrate-reducing clusters, and the Fe reductase protein specific for the Mo–Fe protein. In addition, H_2 production requires the hydrolysis of ADP, which is synthesised by nitrogenase in the absence of N_2 in air: a) *Gap* hydrogenase contains an Fe–Ni component for H_2 oxidation; b) the *Hox* hydrogenase system also consists of [NiFe]-hydrogenase and pyridine dinucleotides (NAD, NADP) and can catalyse the oxidation of H_2 by cell metabolism.

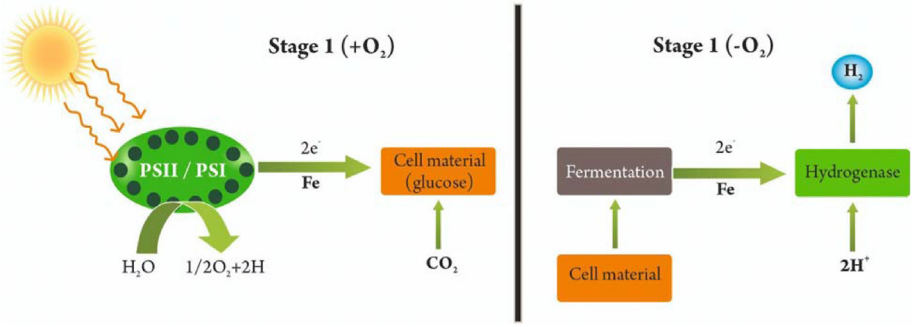


Fig. 2 – Scheme of indirect photolysis by cyanobacteria cells. Abbreviations: PSI – photosystem I, PSII – photosystem II, H_2 – hydrogen, CO_2 – carbon dioxide, $+O_2$ – aerobic condition, $-O_2$ – anaerobic condition.

Alternative nitrogenase ([Mo]-nitrogenase) is the most studied enzyme (Fig. 3). The [Mo]-nitrogenase is a structure consisting of $a_2\beta_2$-heterotetramer with a molecular weight of 220–240 kDa that cleaves nitrogen atoms. Dinitrogenase reductase is a homodimer of about 60–70 kDa and acts as an intermediate carrier in the transfer of electrons from an external electron donor (ferredoxin or flavodoxin) to dinitrogenase. [Mo]-nitrogenase is synthesised in heterocysts as well as in some cases in vegetative cells [42,43]. Nitrogenase synthesis is regulated by metabolism at the ATP level in the cell and is controlled by enzyme activity, which depends on the size of the endogenous redox pool [44,45].

In addition to [Mo]-nitrogenase, active centres of [V]- and [Fe]-nitrogenase have also been found in cyanobacteria [46–48].

The cyanobacterial [V]-nitrogenase was first discovered and described in detail in the strain *A. variabilis* [49,50]. Cells of cyanobacteria in a Mo-poor and V-rich medium reduced acetylene to ethylene, producing high yield of H_2 [51]. In addition, structural genes for [V]-nitrogenase were described in strains of *A. variabilis* and *Nostoc punctiforme* [50]. The V–Fe protein heterodimer of [V]-nitrogenase consists of an additional 2δ-subunit.

Mo or V are not in the active site of [Fe]-nitrogenase and little is known about this type of nitrogenase. The [Fe]-nitrogenase is mainly found in *Azotobacter* species and some strains of purple bacteria. Among cyanobacteria, it is only detected in *A. variabilis*. The rate of hydrogen production by Fe-nitrogenase in the cell may be much lower than that of [Mo]- or [V]-nitrogenase [52,53].

Hydrogenase

The second group of enzymes involved in H_2 production in cyanobacterial species are the hydrogenases. This group of heterogeneous enzymes exhibits a wide range of structures, properties, and functions. An enzyme catalyses a simple chemical reaction, namely the formation of H_2 from protons and electrons. Currently, it is known that all hydrogenases are divided into three classes according to the structure of the active site [54–56]:

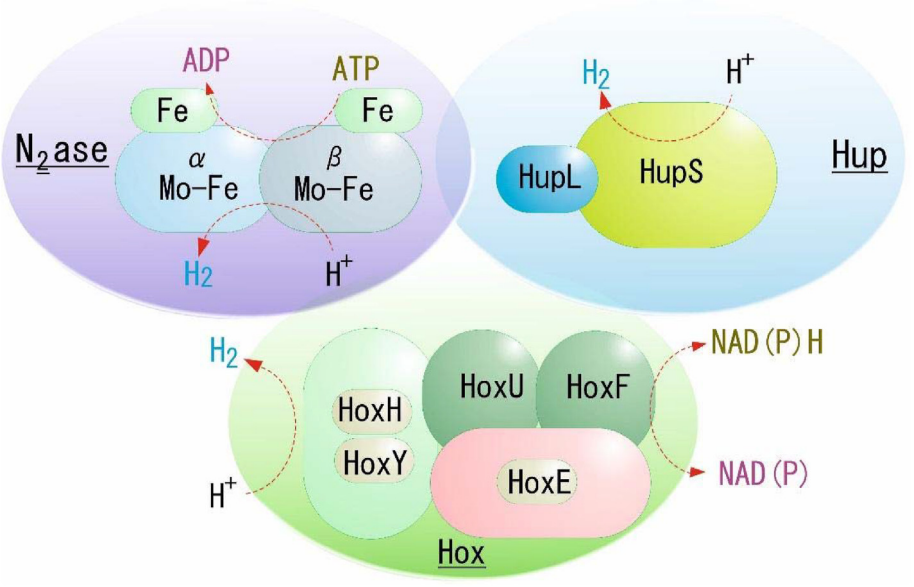


Fig. 3 – Cyanobacterial enzymes involved in hydrogen exchange [37]. Abbreviations: ADP – adenosine diphosphate, ATP – adenosine triphosphate, NAD(P)H – nicotinamide adenine dinucleotide phosphate, NADP – nicotinamide adenine dinucleotide phosphate, N_2ase – nitrogenase, Hup – Hup-hydrogenase, Hox – Hox-hydrogenase, H_2 – hydrogen.

1) [NiFe]-hydrogenase (nickel iron hydrogenase);
2) [FeFe]-hydrogenase (iron hydrogenase);
3) [Fe]-hydrogenases ("non-metallic" hydrogenases).

In many photosynthetic microorganisms, including H_2-producing cyanobacteria, hydrogenases are divided into two groups: (1) oxidative hydrogenases (*Hup*), which catalyse H_2 consumption by nitrogenases, and (2) reversible hydrogenases (*Hox*) with absorption capacity [57].

The bidirectional *Hox* hydrogenase belongs to the [NiFe]-hydrogenases and consists of a complex with the proteins *Hox*E, *Hox*F, *Hox*U, *Hox*Y, and *Hox*H. *Hox*H in the *Hox*YH complex contains a catalytic site that catalyses hydrogen oxidation. *Hox*Y contains the Fe_4S_4 cluster that facilitates electron transfer to the catalytic site. Electrons are donated from the active site in the *Hox*H flux, the *Hox*Y subunit, and the iron-sulphur clusters *Hox*EFU [58,59]. This hydrogenase reacts directly with $NADP^+$ by reducing it in the presence of H_2, or it releases H_2 by reducing pyridine nucleotides. *Hox* hydrogenase has been found in most cyanobacteria, regardless of their morphology and ability to fix N_2. Numerous studies are focused on detection of *Hox* hydrogenase in unicellular cyanobacteria (*Synechocystis*, *Anabaena*, and *Spirulina*). The hydrogenase enzymes in the strains *A. variabilis*, *Anabaena* PCC 7120, *Synechococcus* PCC6301, and *Synechocystis* PCC 6803 have been described in detail [60,61].

The *hup*SL hydrogenase cluster consists of two parts, the large subunit *hup*L and the small subunit *hup*S, which are approximately 60 kDa and 30 kDa in size, respectively (Fig. 3). The large compartment has an active site and is surrounded by a bimetallic NiFe centre, while the small compartment contains iron-sulphur clusters that provide electronic transmission. The *hup*SL cluster, which is present in the cells of nitrogen-forming cyanobacteria, is also present in the cell in a nitrogen-free environment and is closely associated with nitrogenase activity [61–63]. Therefore, *hup*SL hydrogenase is synthesised in heterocysts to absorb H_2 released by the exogenous nitrogenase.

Biohydrogen production factors

Injection of inert gases

As mentioned earlier, [FeFe]-hydrogenase is strongly inhibited by the presence of O_2 and N_2 in the culture medium. Therefore, it is important to remove or replace these gases (0.1% or less of the partial pressure of each gas) to overcome the inhibition of O_2 production. To accomplish this, the culture medium can be continuously enriched with inert gases such as argon, carbon dioxide, or methane [64–66]. It is very important that the pure inert gas, which does not contain O_2, is 100% pure. It is also better to use the correct bubbling method to avoid shear stress in the cells. This method can be implemented well on a laboratory scale but is not suitable for industrial production due to a large amount of inert gas required to increase H_2 consumption adding to process costs [67,68].

In this context, cyanobacterial strains produced large amounts of H_2 after inert gases were introduced [69,70] to induce hydrogen enzyme activity in cyanobacterial cells in GC vials [71]. The use of argon increased nitrogenase activity as well as the number of heterocysts in cyanobacteria. Nitrogen deficiency in the air and the medium leads to a higher number of heterocysts, which causes stress to the cells. The heterocysts activate nitrogenase to fix N_2 from the air, at the same time, nitrogenase releases biohydrogen. *A. variabilis* PK 84 was grown to produce biomass in an atmosphere of 73% argon, 25% N, and 2% CO_2 and aerated with a gas mixture in the ratio of 93% argon, 5% N, and 2% CO_2 during hydrogen production. In this study, the hydrogen yield was 167.6 μmol H_2 mg^{-1} *Chl a* h^{-1} [72].

Illumination

Light is an important growth factor for cyanobacteria. Although most cyanobacteria absorb red light at about 680 nm, the requirement for H_2 production varies among different species of cyanobacteria. The light intensity in biohydrogen production directly affects the activity of hydrogen enzymes in the cells [31].

Many strains of cyanobacteria are adapted to produce H_2 in dark and light environments, which is directly linked to their genes [73,74]. Furthermore, *Spirulina platensis* culture intensively produces H_2 at a temperature of 30 °C under anaerobic and low-light conditions. *Oscillatoria* sp. Miami BG7 produced H_2 under anaerobic conditions in a dark environment [75].

According to Kossalbayev et al. [22], some cyanobacterial strains were characterised by higher H_2 production in the dark than in the light. Under the dark conditions, the highest H_2 yield was recorded for *A. variabilis*. Hydrogen production in light is often closely associated with nitrogenase activity. However, cyanobacteria are adapted to the distribution of H_2 energy in dark and light environments. In the dark, H_2 production occurs in the presence of sugars accumulated during photosynthesis and through an exogenous enzymatic process. Thus, according to our results, it can be assumed that after 24-h incubation, the glycogen supply in the cells is sufficient to produce H_2 under dark anaerobic conditions.

Vargas et al. [31] reported that the optimal illumination for H_2 production by strain *Anabaena* sp. UTEX 1448 was 2220 lux, while Melitos et al. [76] defined 32 W m^{-2} as a favourable light intensity for H_2 production by strain *A. cylindrica*.

In addition, the wild *A. variabilis* ATCC 29413 and the mutant PK84 strains were examined for H_2 production and found to release more active H_2 in the light than in the dark [77]. In a similar study by Rusydi et al. [78], it was reported that the nitrogen activity of the strain *Nostoc muscorum* was directly dependent on light intensity. The light dependence of different cyanobacterial strains was studied under laboratory conditions. The strain *A. cylindrica* produced H_2 in an argon atmosphere for 30 days at low (6.0 W m^{-2}) and 18 days at high (32 W m^{-2}) light intensity. There is also evidence that low light intensity has a positive effect on some cyanobacterial species. For example, H_2 production by strain *A. cylindrica* decreased at a reduced light intensity, thereby increasing the activity of hydrogen enzymes [75]. The effect of light on hydrogen nitrogenase production by most cyanobacteria is well studied. Bolatkhan et al. [79] showed that the optimal light intensity for H_2 production by cyanobacterial strains ranges between 20 and 60 W m^{-2}.

Biomass concentration

Studies have shown that high optical density (OD_{720} = 2–3) increases the rate of O_2 release until PSII is completely inhibited, reducing the transition to the anaerobic phase, which rapidly activates hydrogenase and prolongs H_2 production [80,81]. However, the cell density must be perfectly balanced. If the cultures have a high cell density, the transition to anaerobiosis can be very fast due to self-shading, then only a small amount of starch accumulates inside and the light intensity inside the vessel decreases, which can have a negative effect on the H_2 production rate. On the other hand, cultures do not create anaerobic conditions at a low cell concentration because the total respiration of the cell is not sufficient to absorb all the dissolved O_2 [82].

In H_2 production, it is important to maintain the active growth phase by controlling cell viability. As suggested in many studies, the study should start with a cell concentration of 1–2 × 10^4 mL suspension [82]. Kim et al. [83] performed a study to determine which stage of cell growth (early exponential phase, middle exponential phase, late exponential phase, and stationary phase) is optimal for H_2 production.

Substrates effect

Macronutrient starvation

The normal life cycle of cyanobacterial cells consists of four main phases [84]:

1. "Growth" – an increase in cell density;
2. "Development" – various reactions take place to prepare the cell for division;
3. "Maturation" and fully mature ovaries;
4. "Division".

Macronutrients such as sulphur, nitrogen and phosphorus play a key role in the life cycle of the species [85], and their deficiency leads to significant changes in cell physiology that affect their maturation and division. Sulphur (S) is one of the six macronutrients essential for cell growth and development. It is found in nature primarily in the form of sulphate, which is required in culture for the synthesis of amino acids, sulfolipids, and other S-containing substances [86,87]. One of the earliest methods of H_2 production by cyanobacteria in light was S-starvation, which was pioneered by studying H_2 production by the microalgae *C. reinhardtii* [77]. Zhang et al. [30] pointed out that the removal of the S element from the culture medium is unfavourable for the induction of H_2 photoproduction by cyanobacterial strains. Batyrova et al. [88] also suggested using cheap seawater instead of nutrients for cultivation to reduce the cost of H_2 production.

Furthermore, protein, lipid, and carbohydrate levels can increase several-fold within 24 h of nutrient restriction [89–91]. This phenomenon leads to irreversible inactivation of PSII by enhancing cellular respiration, which creates a chemical environment and results in the transient release of the gases O_2 and H_2 [92].

The H_2 production without macronutrients (N, S) can generally be divided into 4 phases:

1. "aerobic" photosynthetic endogenous cell growth and substrate accumulation;
2. "O_2 consumption" and limitation of macronutrient uptake from the culture medium;
3. "anaerobic";
4. Endogenous gradual lowering of pH and accumulation of enzymatic metabolites, such as succinate, formate, and ethanol.

It should be noted that the restriction of macronutrients to create anaerobic conditions is still considered direct biophotolysis rather than indirect biophotolysis, as PSII is not completely inhibited but its activity is reduced [67]. PSII must therefore be regenerated until a point of no return is reached.

Cyanobacteria can produce H_2 by heterotrophic metabolism using endogenous and exogenous substrates [93]. As for endogenous substrates, the most important is starch, which is formed in the process of half-life and then used to transfer electrons for H_2 production [94]. After all available starch is consumed, the cell must be regenerated with light and nutrients, which activates PSII and initiates a new cycle of biomass growth [95]. The H_2 accumulation can be controlled by the withdrawal of N and S from the culture medium, high light intensity and CO_2 concentration. Other endogenous substrates such as lipids can lead to increased H_2 production by heterotrophic and mixotrophic microorganisms at the expense of higher salt and Fe concentrations.

Micronutrients effect

There are many different theories in the literature about how phototrophic microorganisms produce biohydrogen through the action of various microelements. However, it was found that the addition of various trace elements such as Fe, Cu, Co, Mo, Zn, and Ni to the culture medium significantly increased biofuel production. Zhang et al. [96] reported that low concentrations of Ni^{2+} negatively affected H_2 production in *Anabaena* spp. CA and *Anabaena* spp. 1 F. In the case of *A. variabilis* SPU003, H_2 production was completely inhibited when the concentrations of Co, Cu, Mn, Zn, Ni, and Fe in the culture medium were below 10 mM [97]. *A. cylindrica* grows well at 5.0 mg Fe L^{-1} and produces twice as much H_2 as a culture with 0.5 mg Fe L^{-1} [98].

Carbon source

Carbon sources are also known to influence H_2 production by affecting nitrogenase activity [99]. The presence of different carbon sources leads to a change in the ability of electronic factors to bind to nitrogenase, thus affecting H_2 production [100]. The $NaHCO_3$ salt is often used as a carbon source in studies. Kossalbayev et al. [22] reported that a sodium bicarbonate concentration of 100 μmol is optimal for H_2 production. Zayadan et al. [101] showed that N_2-fixing strains produced H_2 intensively at a $NaHCO_3$ concentration of 50–100 mmol. Dickson et al. [102] slightly increased H_2 production in cyanobacterial strains by adding 80 mmol of the above carbon source to the culture medium. Ainas et al. [103] who studied the H_2 production by *S. platensis* in different photobioreactors concluded that the optimal sodium bicarbonate concentration is 300 mmol.

Nitrogen source

Several inorganic nitrogen compounds have a great influence on the rate of H_2 production. Nitrite, nitrate, and ammonia have been reported to inhibit nitrogenase in *A. variabilis* SPU003 and *A. cylindrica* [104]. All exogenous nitrogen sources inhibit nitrogenase synthesis [105]. Addition of ammonium (0.2 mM NH_4) to the strain *A. cylindrica* suppresses H_2 production over time, but moderate additions (0.1 mM ammonium chloride) regularly do not. However, the influence of the nitrogen source is not always strong [106,107]. The formation ratio of H_2 and O_2 (4:1) decreases with the addition of ammonium ions when no total nitrogen is present.

HEPES

HEPES is a compound that maintains the pH of the medium evenly and has a positive effect on cell growth. However, the effect of HEPES salt may vary from strain to strain. In this context, the choice of the optimal exposure concentration is one of the most important researches on H_2 production. Ortega-Ramos et al. [108] showed that a HEPES concentration of 10 mmol was optimal for the growth of the *Synechocystis* strain and achieved a H_2 yield of 3.5 μmol H_2 mg^{-1} *Chl a* h^{-1} in the mutant strain. In this experiment, the H_2 yield of the cells was higher in the presence of HEPES in the dark [108].

pH effect

Cyanobacterial species occur in all ecosystems and under different conditions. They typically grow in the pH range of 4–7 [93,109], and some species inhabit deep-sea environments [68]. pH is an important parameter concerning H_2, and even small changes in the environment affect both biomass growth and the metabolic pathways associated with H_2 production which control the activity of H_2-producing enzymes and prevent the degradation of endogenous substrates [20,110].

During photosynthesis, the initial pH decreases due to the reaction of CO_2 and water, and over time the pH of the medium increases, whereupon the cells produce dissolved CO_2 and by-products [109,111]. At low pH, H_2 is formed by pyruvate-Fd-oxide reductase based on acetate and ATP due to inhibition of [FeFe]-hydrogenase [109]. Vargas et al. [31] reported that the optimal pH for H_2 production in *Anabaena* sp. UTEX 1448 was 8. Zayadan et al. [101] studied 3 different heterocyst strains of *Anabaena* and *Nostoc* and found that the highest H_2 yield was obtained at pH 7.4. Allahverdiyeva et al. [11] reported that the cells of *Anabaena* PCC 7120 (*Hox* mutant) gave a lower yield at high pH. However, the optimal pH for H_2 production was 8.

Temperature effect

The adaptability of cyanobacteria to environmental conditions enables them to survive even at extreme temperatures. For example, *Anabaena* sp., *Synechocystis* sp., and *Oscillatioria* Kutz were found in plant stems in desert areas [112], and their H_2 production and cell growth temperatures were similar [66].

The optimal temperature for H_2 production by cyanobacterial cells is 25–35 °C. However, the values may vary depending on the habitat of the species. For example, the *Nostoc* strain can produce H_2 at temperatures between 22 and 32 °C [112]; furthermore, a higher temperature of 40 °C was found for *Nostoc muscorum* SPU004 [113]. The optimal temperature for growth and H_2 production of *A. variabilis* SPU 003 was 30 °C [75]. Vargas et al. [31] defined the optimal growth temperature for *Anabaena* sp. UTEX 1448 for H_2 production as 24 °C, while Nyberg et al. [114] reported 30 °C as the temperature with the highest H_2 yield (4.85 μmol H_2 mg^{-1} *Chl a* h^{-1}) produced by *Nostoc* (*Anabaena*) PCC 7120.

Biohydrogen production strategies

Implementing strategies for H_2 production from cyanobacteria enables high productivity. In biohydrogen production, each direction has own peculiarities that affect the yield of the final product. Many different approaches are currently being actively explored to improve H_2 production by cyanobacteria (see Table 2). All these approaches can generally be reduced to three main points: metabolic, genetic and technical approaches (Fig. 4) [115,116].

Technical approaches

The development of innovative cultivation methods contributes significantly to increasing biohydrogen production, especially the optimisation of conditions for producers' cultivation

Table 2 – Approaches of H_2 production by cyanobacteria cells.

Approaches		Activities
Metabolic activity	Increasing of biomass yield	- Anoxia; - Creating stress conditions; - The use of exogenous electron donors; - The use of PS inhibitors; - Regulation of external factors.
Genetic activity	Editing of genetic materials	- Mutans with of *Hup*SL, *Hyd*FS genes; - Mutants with *Hox*EFUYH genes; - Mutants with *nif* genes; - Pigment reduced mutants.
Technical activity	Design of biofermentation process	- Improving the characteristic of photobioreactors; - Methods for separation of H_2 and O_2; - Immobilization of cells; - The use of cheap subtracts.

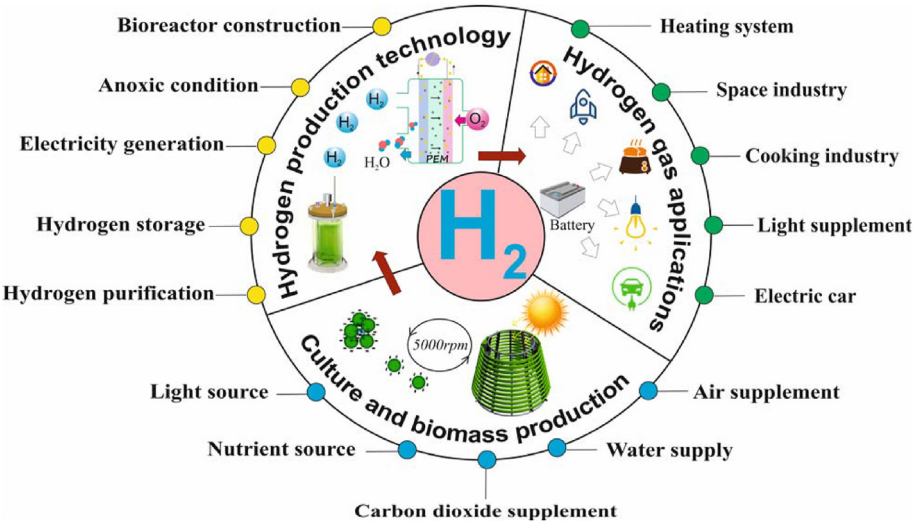


Fig. 4 – Biohydrogen production by cyanobacteria: main stages.

and the design of efficient photobioreactor systems. The development of an optimal photobioreactor depends on the characteristics of the cyanobacterial strain, and its performance is influenced by many physicochemical parameters that determine the biochemical pathway of H_2 production.

A variety of physical and chemical factors influence the H_2 yield. The most important are pH, temperature, light intensity, oxygen and carbon dioxide content, sources and the ratio of carbon and nitrogen. Consequently, great results can be achieved by adjusting these parameters for biohydrogen production. For instance, *Synechocystis* sp. PCC 6803 produced 150 times more H_2 when the glycogen concentration was significantly increased along with the concentration of nutrient sources [117]. Furthermore, the addition of nitrate in the fed-batch process prolonged the exponential growth phase by more than 3 days, resulting in a 68.8% increased carbon fixation rate in cyanobacteria [117,118].

As one of the most important parameters and the main constraint for large-scale H_2 production, the position of the light source is crucial. The photobioreactor contains a photonic zone near the illuminated surface and a dark zone. The dark zone is created by the absorption of light by the cyanobacterial cells and depends on both the illumination conditions and their light absorption properties. Since cyanobacteria prefer to absorb red light in the 680 nm range, it is recommended to construct red light panels in special bioreactors to supply the culture systems with red light to meet this demand. By mixing, the cells circulate at a certain frequency and at regular intervals between the light and dark zones, depending on the design of the reactor and the gas supply. The position of the light and the gas-liquid hydrodynamics also influences cyanobacterial growth and H_2 synthesis [30,77,86,119].

Large-scale biohydrogen production by cyanobacterial cultures requires photobioreactors that meet several conditions:

1) Photobioreactors must be a closed system so that the hydrogen produced can be captured without loss.
2) The design of the reactor should ensure easy operation, cleaning and sterilisation.
3) The reactor construction material should be transparent (so that light can pass through) and durable.
4) Photobioreactors should provide a high surface-to-volume ratio and intensive aeration of the culture medium.

Currently, open and closed systems are known for mass cultivation of phototrophic microorganisms for biohydrogen production. They can be divided into 4 main groups (Table 3):

1) tubular photobioreactor;
2) flat-panel photobioreactor;
3) vertical column photobioreactor;
4) outdoor photobioreactor.

Overall, despite the significant progress made in exploring different approaches and strategies to increase biohydrogen production by cyanobacterial cells, the development of new photobioreactors needs to be further pursued to increase cell concentration and consequently H_2 production. Hydrogen-derived fuels can be used as a clean energy source for highly efficient power generation.

The design and operation of any photobioreactor must take into account the following key points related to the regulation of light intensity: the use of appropriate light sources (intensity and wavelength), increasing the efficiency of light conversion and maintaining an adequate cell concentration during long-term operation. In the proposed design, the sunlight was directed into the photobioreactor through optical fibres with sidelights that were directly immersed in the fermentation culture to ensure high light intensity and uniform distribution. Here, the sunlight was collected with Fresnel lenses and then guided through the edge light optical fibre to the side light optical fibres. Such a light tracking system causes each lens to rotate in the direction of the sun's position so that the sidelight fibre optic cable can absorb the maximum light energy from the sunlight during the day. Internal illumination makes it possible to maintain uniform light distribution and intensity in the photobioreactor as well as to avoid shielding effects due to increased cell density.

Table 3 – The main types of bioreactors used to produce biohydrogen.

Photobioreactors of H_2 production	Characteristics	Refs
Vertical column	Low energy consumption Low shear stress Good mixing Readily tempered	[64,120]
Tubular	Large illumination surface area Monitoring all the indicators High biomass productivity Suitable for outdoor application Relatively cheap High photosynthetic efficiency	[80,121]
Flat panel	Relatively cheap Low power consumption Large illumination surface High biomass productivity	[122]
Open air	Easy maintenance Utilization of land Good light path Low energy inputs Easy to clean up Economically viable	[123,124]

Besides improving the technological properties of photobioreactors, one of the interesting technological approaches to increase H_2 production by cyanobacterial cells is to immobilise their cells on different supports. Since during immobilization the microorganisms are protected from adverse physical (extreme temperatures and ultraviolet radiation), chemical (nutrient deficiencies and toxins) and biological (desiccation) factors and can exist under relatively constant conditions, this approach can be considered a very interesting and important strategy to increase H_2 yield. Thus, immobilising cyanobacteria in artificial systems during biohydrogen production increases their stress resistance and offers advantages over the use of suspension cultures, facilitating in particular the work on scaling up this process.

The technological chain of biohydrogen production with cyanobacteria can generally be summarised as follows: cultivation of cyanobacteria in a bioreactor, concentration and extraction of cyanobacterial biomass, anaerobic cultivation of cyanobacteria in a H_2 photobioreactor (in the dark or in the light), H_2 production and purification from impurities by other gases, storage of H_2 gas in a gas storage tank, conversion of gaseous H_2 into electricity (Fig. 4).

It is believed that the conversion of hydrogen gas into electricity is possible because the hydrogen fuel cell operates via a mechanism similar to the reverse electrolysis of water, where H_2 and O_2 combine to form water and produce an electric current. The cell consists of two electrode surfaces separated by a proton exchange membrane (PEM). Hydrogen enters the fuel cell from one side, called the "anode", and hits a catalytic surface where it splits into protons and electrons. The membrane is only permeable to protons, and the electrons are passed through an external circuit to the "cathode," where they generate an electric current. On the other side of the membrane, oxygen enters the cell and, together with protons and electrons, forms water: $2H_2 + O_2 \rightarrow 2H_2O +$ energy.

Despite the significant progress in exploring different approaches and strategies to increase H_2 production from cyanobacteria, further development of promising photobioreactors is needed to increase cell biomass and thus H_2 yield. Hydrogen as a fuel can be used in various fuel elements (e.g., for green energy) for highly efficient power generation.

Metabolic approaches

Metabolic approaches to increase biohydrogen production are linked to the development of physiological and biochemical approaches to increase the rate and duration of H_2 production by cyanobacteria.

The efficiency of H_2 production by cyanobacterial cells depends on a variety of factors, which is particularly critical when produced on a large scale. In addition to the illumination intensity, temperature, and pH of the medium, the most important factors are molecular oxygen, salinity, nutrient and gas composition of the medium, etc. Different parameters have different effects on the process of H_2 production by different strains of cyanobacteria.

Another effective method to increase the productivity of cyanobacterial cells in biohydrogen production is the use of PSII electron transport inhibitors. Currently, more than 15 different photosystem inhibitors are used, including the widely known diuron (DCMU), carbonyl cyanide-*m*-chlorophenylhydrazone (CCCP), methyl viologen (MV), potassium cyanide (KCN), chloramphenicol, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB), pentachlorophenol (PCP), and malonate (Table 4).

DCMU is the most commonly used inhibitor of the electron transport system, whose molecular structure resembles that of gradually reduced plastoquinone, as these molecules bind to the quinone-binding centre in the reaction zone of PSII. Diuron is used to inhibit the activity of PSI and to create anaerobic conditions that allow molecular hydrogen to be produced. DCMU inhibits QA electron transfer by binding to QB PSI as a quinone analogue. Several studies have shown that DCMU increases the yield of H_2 produced by cyanobacterial cells [125]. Kossalbayev et al. [22] reported that the

Table 4 – PS inhibitors used to increase hydrogen production in cyanobacterial strains.

Name	Abbreviation	Mechanism of action	Refs
Diuron	DCMU	PSII inhibitor, which limits the electron transport from PSII to the PQH_2 pool	[127–129]
Carbonyl cyanide *m*-chlorophenyl hydrazone	CCCP	Inhibits the photochemical activity of PSII	[127]
Dibromothymoquinone	DBMIB	Inhibitor of cytochrome B_6f, which increases the degree of chemical reduction of plastoquinone (PQ) pool	[1]
Potassium cyanide	KCN	Cytochromoxidase inhibitor that inhibits all terminal oxidases	[2]

cells of the cyanobacteria *Synechocystis* sp. PCC 6803 can produce more H_2 after the addition of 10 μmol DCMU under the light in anaerobic conditions. Increasing the concentration of the photosynthesis inhibitor DCMU to 30 or 45 μmol in the medium had no positive effect on the H_2 photoproduction of the cyanobacterial cells. Thus, significantly low values for the maximum H_2 yield of the cyanobacterial cells were obtained at 30 μmol DCMU.

Fig. 5 illustrates the main effects of PS inhibitors and methods for blocking some H_2 exchange systems in cyanobacteria.

Another well-known PSI inhibitor in cyanobacteria is CCCP. Like DCMU, CCCP inhibits the photochemical activity of PSII and reduces O_2 production during the cultivation period. It has been reported that the photochemical activity of PSII is inhibited in *Nostoc* sp. and *Lyngbya* sp. [126], while *Oscillatoria chalybea* and *Synechocystis* sp. PCC 6803 produce increased H_2 yield when exposed to CCCP. Many scientists have also reported that inhibition of ATP synthesis by CCCP leads to an increased respiration rate in *A. variabilis* and *Anacystis nidulans* in the dark [127] (Table 4).

Inhibitors such as KCN and PCP block quinol oxidase, resulting in a reduction of the PQ pool. In addition, the KCN completely blocks H_2 production or, like DBMIB, electron transport from the Cyt b_6f complex to PSI. Furthermore, DBMIB has been shown to promote the regulation of nitrate assimilation genes, and inhibition of nitrate assimilation in turn led to an increase in H_2 production [120,128,129].

Another metabolic approach is medium supplementation by various exogenous electron donors. In the presence of simple organic compounds, H_2 is formed more readily because the cofactor compounds attract electrons that activate nitrogenase. Therefore, the combination of different sugars, including mannose, also stimulates H_2 production at a relatively high rate [130]. However, the success of the metabolic approach can only be achieved if it is used simultaneously with technical and genetic approaches.

Genetic approaches

Genetic engineering can be a powerful tool to take cyanobacterial biohydrogen production to a new level, but conducting large-scale analyses requires more theoretical knowledge. Genetic manipulation techniques have recently been used to increase H_2 production in cyanobacterial species, which (1) reduced chlorophyll antennae, (2) increased photosynthetic and respiration rates, (3) increased aerobic phase carbohydrates, and (4) was directly related to high synthesis of xanthophyll cycle pigments that protect against PSII inactivation. The efficiency of solar energy conversion to H_2 can be influenced by pigmentation, cellular antenna composition, light loss, scattering, photosynthetically active radiation, non-photochemical reactions and other environmental factors. Similarly, chlorophyll-free mutant strains can be engineered to be more suitable for large-scale commercial applications and allow more light to penetrate deeper layers of the bioreactor.

The complete genome chains of *Anabaena* sp. PCC 7120 and *Synechocystis* sp. PCC 6803 are now available for genomic studies. Genomic expression studies of heterocysts are closely related to many genes that are strongly and less strongly regulated during heterocyst development. A number of genes have been implicated in heterocyst differentiation and modelling, and mutations in some of these genes have been shown to increase heterocyst frequency [131]. Large number of heterocysts can accelerate H_2 production and N_2 fixation, so it is important to study the effects of heterocysts on H_2 production.

As an alternative method to improve H_2 production in photosynthetic organisms, systematic genetic screening could be conducted to find mutants with increased hydrogen cyanide (HCN) production. Genetic engineering has shown the importance of increasing biohydrogen production in cyanobacteria. Furthermore, cyanobacterial cells can be genetically engineered to be O_2-tolerant or at least reverse inactivated. It can be difficult to determine which organisms are most promising for biofuel production. Therefore, it is important to develop a strategy to reduce the cyanobacterial sensitivity to O_2. Alternatively, cyanobacteria resistant to O_2 inactivation can be catalysed by [FeFe]-hydrogenase, or a gene encoding [NiFe]-hydrogenase can be introduced with increased resistance to photosynthetic cells [132].

Numerous studies are currently underway to develop an O_2-tolerant hydrogenase for sustained H_2 production in cyanobacterial cells [60,61,131,132] as well as to obtain new recombinant strains of cyanobacteria carrying acid-tolerant hydrogenases. For instance, the integration of the necessary structural and auxiliary genes responsible for the synthesis of *Thiocaspa roseoperscina* hydrogenase into the chromosome of *Synechococcus* PCC 7942 has been reported [131]. The introduction of structural genes encoding clostridial hydrogenase Fe into *Synechococcus elongatus* PCC 7942 and their subsequent transcription and translation into a functional H_2-producing enzyme in a photosynthetic microorganism is of interest and deserves further research. There is information on obtaining recombinant strain *Synechocystis* PCC 6803 by introducing two structural hydrogenase and associated genes for its

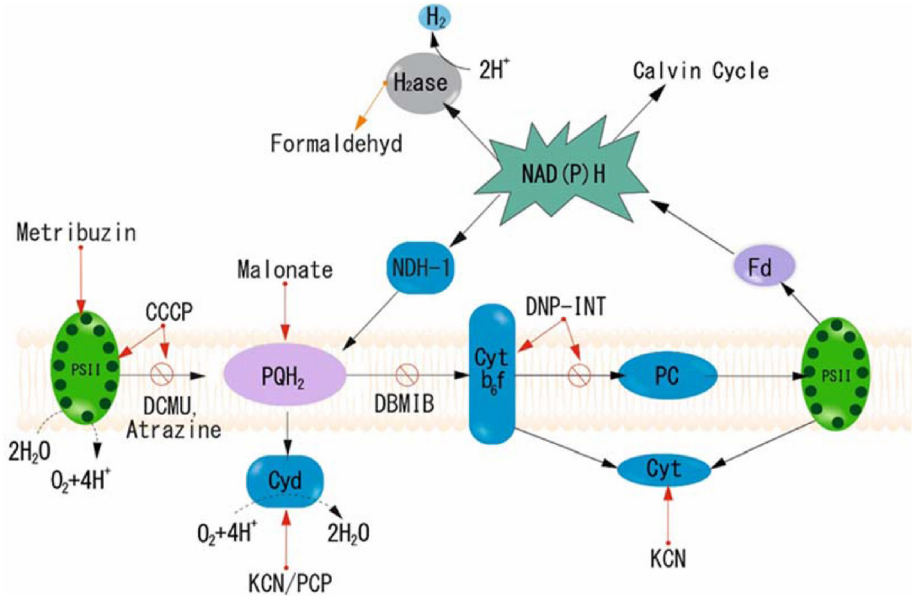


Fig. 5 – The action centres of different inhibitors employed to increase biohydrogen production [3]. Abbreviations: PSI – photosystem I, PSII – photosystem II, DCMU – (3-(3,4-dichlorophenyl)-1,1-dimethylurea), PQH_2 – plastoquinol, Cyt b_6f – cytochrome b_6f complex, DBMIB – dibromothymoquinone, CCCP - Carbonyl cyanide *m*-chlorophenyl hydrazine, KCN – potassium cyanide, PC – plastocyanin, Cyt– cytochrome, Fd – ferrodoxin, NAD(P)H – nicotinamide adenine dinucleotide phosphate, H_2ase-hydrogenase.

maturation from *Rubrivivax gelatinosus*. Obtaining and using such mutant strains of cyanobacteria overexpressing an O_2-tolerant hydrogenase would circumvent the need to bubble the culture medium with an inert gas to create anaerobic conditions.

Genetic engineering approaches to increase H_2 production by redirecting electron flow into H_2 metabolism have recently attracted considerable attention. The technical approach of eliminating competing electron pathways such as the respiratory electron transport system, nitrate uptake, and carbon fixation via the Calvin-Benson cycle could be a very effective and promising method to enhance and optimise H_2 production. Thus, nitrate uptake is a potential competitive pathway that could reduce electron flow for H_2 metabolism. It was found that in *Synechocystis* PCC 6803, a strain mutated in the Δ*Hap* and Δ*Hup* genes with impaired nitrate uptake, deletion of both the Δ*Hap* and Δ*Hup* genes increased H_2 production [57,60]. Inactivation of quinol oxidase also increased H_2 production *in vivo* in *Synechocystis* sp. PCC 6803 [60]. Increasing the NADPH/$NADP^+$ ratio also causes an increase in H_2 production by the NADPH-dependent bidirectional [NiFe]-hydrogenase in *Synechococus* sp. PCC 7002 [22].

H_2 photoproduction processes based on the use of cyanobacteria and other cell cultures have shown relatively low conversion efficiency. In addition, the use of downsized antenna mutants is being investigated to reduce the loss of hydrogen molecules. Increased H_2 production can also be achieved by reducing or eliminating competing biochemical pathways.

In conclusion, each of the genetic approaches described deserves special attention and, in solving many fundamental problems using the methods of modern molecular biotechnology, can be a very effective and promising approach to improve and optimise H_2 production in cyanobacteria.

Active cyanobacterial strains for H_2 production

Many cyanobacterial strains are adapted to produce H_2 in the dark and in the light, which is directly related to their genes [133,134]. The efficiency of H_2 production also depends on the metabolic potential of the microorganisms, which in turn shows how important the search for active H_2-producing strains of cyanobacteria is. So far, there are more than 14 classes of cyanobacteria that produce H_2 under different culture conditions. Table 5 lists active strains of cyanobacteria with the ability to produce bioenergy [135].

In general, H_2 production is higher in N_2-fixing cyanobacteria than in species consisting of vegetative cells only.

In heterocysts cyanobacteria, the nitrogenase system is not disturbed by the release of O_2 from the vegetative cells, thus, they can produce H_2 in the presence of light. In the light, cyanobacteria store carbon dioxide in the form of a polysaccharide and produce O_2. Under the dark conditions of the axons, nitrogenase is formed and the polysaccharide stores are degraded to provide electrons for nitrogen fixation and H_2 production.

Heterocyst cyanobacterial strains with nitrogenase actively excrete molecular H_2 in the first 2–3 days and immediately use the energy stored in the cell. Vegetative species (*Synechocystis*, *Synechococcus*, etc.) can maintain hydrogenase activity for about a week. As these properties are directly related to the energy consumption properties of the two hydrogen enzymes. To date, the genetic and

Table 5 – Active cyanobacterial strains used in H_2 production studies.

Organism name	Organism description	Maximum H_2 production	Growth condition	H_2 production assay condition	H_2 enzymes	Refs
Anabaena siamensis TISTR 8012	Filamentous, *hup*S mutant	3 μmol mg^{-1} *Chl a* h^{-1}	air; under light, BG-11 medium	Ar (100%), 30 μE (photons) $m^{-2}s^{-1}$, at 23 °C	N_2ase, H_2ase	[70]
Anabaena siamensis TISTR 8012	Filamentous, *hup*S-deficient mutant	30 μmol H_2 mg^{-1} *Chl a* h^{-1}	air; under light, BG-11 medium	Ar (100%), under light, at 25 °C	N_2ase, H_2ase	[136]
Anabaena sp. PCC 7120	Filamentous, *hup*L mutant	60 μmol H_2 mg^{-1} *Chl a* h^{-1}	air; under light	Ar (100%), dark, in Ca^{+2}-alginate films	N_2ase, H_2ase	[140]
Anabaena sp. PCC 7120	Filamentous, *hup*L mutant	35 μmol H_2 mg^{-1} *Chl a* h^{-1}	air; under light, BG_0-11 medium	Ar (100%), under light, at 23 °C	N_2ase, H_2ase	[141]
Anabaena sp. PCC 7120	Filamentous, *Hup* mutant	29 μmol H_2 mg^{-1} *Chl a* h^{-1}	air; under light, BG-11 medium	Ar (100%), under light, at 24 °C	N_2ase, H_2ase	[142]
Anabaena sp. PCC 7120	Filamentous, *hup*W mutant	3.3 μmol H_2 mg^{-1} *Chl a* h^{-1}	air; under light, BG-11 medium	Ar (100%), under light, at 28 °C	N_2ase, H_2ase	[138]
Anabaena sp. PCC 7120	Filamentous, *Hup* mutant	63 μmol H_2 mg^{-1} *Chl a* h^{-1}	air; under light, BG-11 medium	Ar (100%), under dark, at 29 °C	N_2ase, H_2ase	[115]
Anabaena sp. PCC 7120	Filamentous, *hup*W mutant	6.2 mL H_2 L^{-1} h^{-1}	air; under light	Ar (100%), under dark, at 25 °C	N_2ase, H_2ase	[138]
Anabaena sp. UTEX 1448	Filamentous, wild-type strain	67 μmol H_2 L^{-1} h^{-1}	air; under light, BG-11 medium	Ar (100%), under light, at 25 °C	N_2ase, H_2ase	[139]
Cyanothece sp. ATCC 51142	N_2-fixing, unicellular, wild-type strain	465 μmol H_2 mg^{-1} *Chl a* h^{-1}	air; ASP2 medium, 25 μE (photons) m^{-2} s^{-1}	Ar (100%); 30 μE (photons) m^{-2} s^{-1}	N_2ase, H_2ase	[27]
Cyanothece sp. Miami BG 043511	N_2-fixing, unicellular, wild-type strain	15.8 mL H_2 L^{-1}	air; ASP2 medium, 30 °C (12 h light, 12 h dark), light intensity of 30 μE (photons) m^{-2} s^{-1}	Ar (100%); 30 μE (photons) m^{-2} s^{-1}	N_2ase, H_2ase	
Cyanothece sp. ATCC 51142	N_2-fixing, unicellular, wild-type strain	300 μmol H_2 mg^{-1} *Chl a* h^{-1}	ASP2 medium, 30 °C, 30 μE (photons) m^{-2} s^{-1}, 50 mmol glycerol	Ar (100%), Ca^{+2} reduced medium with enhanced Fe and at pH-7.4	N_2ase, H_2ase	[143]
Synechocystis M55	unicellular, *ndhB* mutant	200 nmol H_2 mg^{-1} *Chl a* min^{-1}	air; photon fluence rate of 50 μE (photons) m^{-2} s^{-1}, 30 °C	Ar (100%); 2.5 mM NH_4Cl and 5 mM HEPES	H_2ase	[144]
Synechocystis sp. PCC 6803	unicellular, *cta*I/*cyd* mutant	190 nmol H_2 mg^{-1} *Chl a* min^{-1}	air; modified Allen's medium at 34 °C, 70 μmol (photons) m^{-2} s^{-1}	Ar (100%); 300 μmol (photons) m^{-2} s^{-1}	H_2ase	[22]
Synechocystis sp. PCC 6803	unicellular, *cta*I/*cyd* mutant	115 nmol H_2 mg^{-1} *Chl a* min^{-1}	air; cultured at 28 °C; 50 μE (photons) m^{-2} s^{-1}	Ar (100%); 800 μE (photons) m^{-2} s^{-1}	H_2ase	[145]
Synechocystis sp. PCC 6803	unicellular, *nar*B/*nir*A mutant	300 nmol H_2 mg^{-1} *Chl a* h^{-1}	air; 25–40 mmol (photons) m^{-2} s^{-1}	Ar; darkness; N_2 deprivation	H_2ase	[145]
Synechocystis PCC 6308	unicellular, wild-type strain	3.1 μl H_2 mg^{-1} *Chl a* h^{-1}	air; 20 μE (photons) m^{-2} s^{-1}	Ar with CO; 20–30 μE (photons) m^{-2} s^{-1}	H_2ase	[145]
Synechocystis sp. PCC 6803	unicellular, wild-type strain	0.037 mmol H_2 mg^{-1} *Chl a* h^{-1}	air; 20 μE (photons) m^{-2} s^{-1}	Ar; BG-11 medium, pH 7.5; with DCMU, at 25 °C, 30 μE (photons) m^{-2} s^{-1}	H_2ase	[22]
Oscillatoria sp. Miami BG7	filamentous, wild-type strain	5.9 μL H_2 mg^{-1} dry wt h^{-1}	air; NH_4Cl used as combined N_2 source; 100 μE (photons) m^{-2} s^{-1}	Ar (100%); 90 μE (photons) $m^{-2}s^{-1}$, 11 days old cells cultivated at 37 °C	H_2ase	[22]
Oscillatoria limosa 23	filamentous, wild-type strain	19.83 μl H_2 mg^{-1} *Chl a* h^{-1}	air; the nitrate-free medium; 1.2 lux photons	Ar (100%); dark anaerobic incubation	H_2ase	[146]
Oscillatoria sp. Miami BG7	filamentous, wild-type strain	260 μmol H_2 mg^{-1} *Chl a* h^{-1}	air; photon fluence rate 30–50 μE (photons) m^{-2} s^{-1}	Ar; 2 × 30 W fluorescent lamp	H_2ase	[147]
Synechococcus sp. Miami BG 043511	unicellular, wild-type strain	220 μmol H_2 mg^{-1} *Chl a* h^{-1}	air; 150 μE (photons) m^{-2} s^{-1} light intensity, at 30 °C	Ar (100%); medium without Na_2CO_3 (pH-7.6), 150 μE (photons) m^{-2} s^{-1}, at 30 °C	N_2ase, H_2ase	[148]
Synechococcus sp. Miami BG 043511	unicellular, wild-type strain	140 μmol H_2 mg^{-1} *Chl a* h^{-1}	air; 200 μE (photons) m^{-2} s^{-1}	Ar; 25 W m^{-2} (PAR)	H_2ase	[22]

(*continued on next page*)

Table 5 – (continued)

Organism name	Organism description	Maximum H_2 production	Growth condition	H_2 production assay condition	H_2 enzymes	Refs
Synechococcus PCC 602	unicellular, wild-type strain	15.77 μl H_2 mg^{-1} *Chl a* h^{-1}	air; 20 μE (photons) m^{-2} s^{-1}	Ar with CO, 20–30 μE (photons) m^{-2} s^{-1}	H_2ase	[22]
Synechococcus PCC7942	unicellular, *hydA* mutant	162.52 μmol H_2 mg^{-1} *Chl a* h^{-1}	air; 20–50 μmol (photons) m^{-2} s^{-1}	Ar; dark adaptation	H_2ase	[22]
Synechococcus sp. I12	unicellular, wild-type strain	0.019 μmol H_2 mg^{-1} *Chl a* h^{-1}	air; cultured at 27 °C; 20 μmol (photons) m^{-2} s^{-1}	Ar (100%); 30 μE (photons) m^{-2} s^{-1}	H_2ase	[22]
Phormidium valderianum BDU 20041	filamentous, wild-type strain	0.22 μl H_2 mg^{-1} dry wt h^{-1}	air; 30 W m^{-2} under fluorescent light	Ar; light/dark cycle	H_2ase	[149]
Phormidium corium B-26	filamentous, wild-type strain	0.003 μmol H_2 mg^{-1} *Chl a* h^{-1}	air; 35 μE (photons) $m^{-2}s^{-1}$	Ar; BG-11 medium, pH 7.5; with DCMU, at 25 °C, 30 μE (photons) $m^{-2}s^{-1}$	H_2ase	[22]
Desertifilum sp. IPPAS B-1220	filamentous, N_2-fixing wild-type strain	0.348 mmol H_2 mg^{-1} *Chl a* h^{-1}	air; 30 μE (photons) m^{-2} s^{-1}	Ar; BG-11 medium, pH 7.5; at 25 °C, 30 μE (photons) m^{-2} s^{-1}, with 10 mM DCMU	N_2ase, H_2ase	[22]

metabolic properties of many strains have been studied for H_2 production, among which there are highly-active species. The cyanobacterial species involved in N_2 fixation are relatively well studied in terms of hydrogen activity. *A. cylindrica*, for example, was able to produce H_2 and O_2 simultaneously for 30 days in an argon chamber under limited light conditions. *Anabaena* sp. is capable of producing a large amount of H_2, with *A. cylindrica* producing the highest H_2 yield (30 mL H_2 L^{-1} *Chl a* h^{-1}) under N_2-starvation [150]. H_2 production by strain *A. cylindrica* IAMM-1 was observed under anaerobic conditions in the dark. According to published data, there are temperature differences in H_2 production in cyanobacterial species [75]. The strain *Desertifilum* sp. IPPAS B-1220, which was investigated for H_2 production for the first time, showed a comparatively high H_2 yield after addition of DCMU [22]. Kossalbayev et al. [22] also studied the strain *Synechococcus* PCC 6307 at 20–30 μE (photons) m^{-2} s^{-1} and obtained 0.47 μmol H_2 mg^{-1} *Chl a* h^{-1} gas within two weeks.

Melnicki et al. [151] reported that actively growing cells of *A. cylindrica* can photolytically split water into H_2 and O_2, slightly suppressing the release of the gases N_2, CO, and CO_2, with the highest H_2 yield, reached after 3 h. After nitrogen removal from the medium, *A. cylindrica* produced 32 μmol H_2 mg^{-1} *Chl a* h^{-1} and continued to produce H_2 for up to 19 days. Furthermore, Skjånes et al. [80] reported that strain *A. cylindrica* B629 was grown with 10 mM $NaHCO_3$ salt in an open photobioreactor, while Park et al. [152] obtained H_2 with this N_2-fixing strain *A. cylindrica* B629. This type of cyanobacterial heterocyst was characterised by high O_2-dependent H_2 production activity. Ferreira et al. [153] investigated the role of hydrogenase in biohydrogen production by photolysis of water with *A. cylindrica* PCC 7122. Kamra et al. [154] studied H_2 accumulation by the strain *Anabaena* N-7363. In a mixed incubation, the H_2 production factor increased from 0.371 to 0.765 to an optimal value and then gradually decreased [154].

Zhang et al. [30] investigated the production of H_2 and O_2 by the marine cyanobacteria *Anabaena* sp. TU 37–1 at high cell density in closed photobioreactors. A density of 300 μg Chl PBR^{-1} proved to be efficient for photosynthesis. Over a period of 12–24 h, the incubation phase was 2.4–2.2% and the H_2 volume collected after 8 h was 8.4 mL PBR^{-1} at atmospheric pressure [30].

Abedi et al. [155] studied H_2 production by the *A. variabilis* ATCC 29413 mutant. In the gas phase containing 25% N_2, 2% CO_2, and 75% air, the PK84 mutant produced 6.91 μmol H_2 μg^{-1} protein h^{-1}, 4.3-fold more than the wild strain. The absence of N_2 and CO_2 in the gas phase improved H_2 yield in both the mutant and wild strains [155].

Mutant strains of *Anabaena* AVM13 (D*hup*SL), *Nostoc punctiforme* NHM5 (D*hup*L) [144], *Anabaena* PCC 7120 (D*hup*L/D*hox*H, D*hup*W) showed high H_2 production rate compared to wild-type strains (Table 5).

Molecular oxygen inhibits the activity of hydrogenase and nitrogenase enzymes [59,155]. Therefore, it is important to study the genes responsible for O_2 release and the production of light-dependent H_2, which is a by-product of H_2O oxidation by the PSII complex. In this regard, the main procedures of metabolic methods to increase the productivity of

cyanobacteria in biological H_2 production, besides genetic studies, are aimed at PSII inhibition, which consists of a system of methods based on creating an O_2-free environment and thus increasing nitrogenase activity.

Trends and prospects for developing biohydrogen production from cyanobacteria

Policy measures determining the biohydrogen energy development

One of the global problems facing many of the world's developing countries is their dependence on imported energy sources, which has led to energy policies aimed at being independent of the volatility of energy source prices and thus not disrupting the supply of raw materials. As price volatility still exists, especially for oil, this encourages and coordinates the use of other energy sources for transport, heating and power generation. As a result, many countries are introducing biofuel policies. For many developing countries, energy tariffs and energy conservation are still a problem. Therefore, producing energy from biofuels can be seen as one of the methods to generate energy and reduce dependence on exporters. One of the energy sources is biohydrogen, which is a very practical and renewable energy source and thus occupies a special position.

Furthermore, the increasing influence of fossil fuels on global climate change is another important factor. Many developed countries are promoting and supporting the use of alternative renewable energy sources to mitigate climate change and reduce greenhouse gas emissions.

The second important feature of bioenergy development is the tendency to reduce air pollution in large cities and urban areas. Compared to the use of fossil fuels, the use of biohydrogen means a significant reduction in the emission of pollutants into the atmosphere, in the areas of electricity generation, heating and vehicles.

In general, biohydrogen production has an impact on many areas such as the economy, energy policy, transport, ecology, trade and agriculture.

Meanwhile, any biofuel on the modern global market leads to the formation of contradictions between subjects at all levels - from states to individual economic structures and consumers, with the financial, environmental and social consequences of the introduction of biofuels remaining a major point of contention. Even though biohydrogen production using the photobiological method through cyanobacterial cells is very promising, the problems of large-scale production and use remain.

Currently, the scale of biohydrogen production by cyanobacterial cells as an energy carrier is not sufficient for commercial production. For this reason, at the current level of technological and scientific development in many countries, production will be expensive compared to conventional fuels [59].

The main problem is the low ability of cyanobacteria to produce H_2. This fact can be a significant obstacle to the photobiological production of hydrogen as fuel. Therefore, it is necessary to thoroughly analyse and consider the process of increasing H_2 production by cyanobacterial cells and the proper distribution of investments and subsidies [39].

The main issue is the implementation of activities to promote bioenergy production and the creation and formation of group programs for the development of biotechnology, in which bioenergy plays an important role. The development of this bioenergy sector will certainly take a lot of time. Many analytics believe that the development of H_2 energy can meet the fuel needs of many people in the world and thus ensure environmental security.

In this regard, the most important subsidies and investments at the moment should mainly go to basic scientific research in H_2 production by cyanobacteria [155].

To increase H_2 production by cyanobacteria, further studies on the genetic and metabolic aspects are essential to improve producers [156,157]. Research using high technology, genetic engineering and advances in biology using common genomic tools offers a great opportunity to obtain cyanobacterial strains with the required genotype and desired H_2 yield. The introduction and development of one of the most important areas of genetic engineering, such as synthetic biology, will potentially allow cyanobacterial cells to be customised to achieve the desired responses. Thus, such cells can be recognised as competitive high-performance bio-H_2 producers [17].

In addition, capital investment is required to build the infrastructure for storage, scaling and transport, as well as the technology to produce biohydrogen, and a significant part of the investment should go into the processes of high-value post-treatment in H_2 production. The expansion of the biohydrogen market will only be possible if there is strong policy support with the right outlets and distribution infrastructure.

Moreover, there are a number of tasks such as the introduction of modern technologies, technical developments, the manufacture of equipment to increase H_2 production for heat and power generation, and the reduction of costs and the need to focus on capital investments, with political support from the state [158].

The main social and political measures required for biohydrogen production in cyanobacterial cells are shown in Fig. 6.

Feasibility study of biohydrogen production from cyanobacteria

A new bioenergy technology requires analyses of a technical and economic nature, an assessment of the costs incurred and the results achieved, and a timetable for the various tasks to be carried out. The technical and economic analysis means studying the level of performance as well as the characteristics of the resource used and the possibility of optimising this process. In terms of socio-economic orientation, hydrogen energy is developing in many countries and is strongly promoted by both the state and the private sector. At this stage, there is an intensive selection of lines for the transition of many energy-intensive industries, including vehicles, to biofuels and chemical generators based on the use of fuel components.

To date, several reviews have been published [3,26] examining comparative analyses of the main H_2 production methods and their feasibility. These range from classical

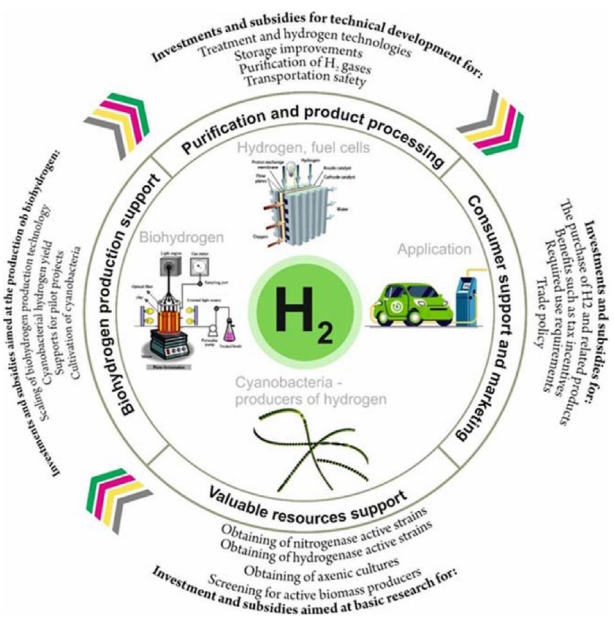


Fig. 6 – Policy measures required to develop the cyanobacteria-based biohydrogen production in the energy industry.

methods such as hydrocarbon conversion and water electrolysis to biological methods.

There are a large number of different technologies for H_2 production [14,115,140], which differ in the process and the origin of the raw materials used for H_2 production. Based on the processes used, H_2 production can be divided into three broad categories:

1. From the various types of fossil fuels and their wastes (fossil coals, wood, plants, natural gas, biomass, etc.) by pyrolysis, steam reforming and gasification of oil residues;
2. By methods of thermochemical water decomposition or electrolysis;
3. From water by cyanobacteria and algae through photosynthesis.

Worldwide, about 60 million tonnes of H_2 are produced industrially. 95% is produced from fossil carbon feedstocks using conventional methods (pyrolysis, steam reforming and gasification of oil residues) [159].

Thus, applying the method of steam reforming of natural gas, it is possible to obtain a H_2-rich gas mixture (about 70–75% of the dry weight). It is an endothermic process that requires an additional heat flux. However, to increase the yield of H_2 produced and purify the impurities, separate operations are required. At a production of one hundred tonnes of hydrogen per day (4170 kg h^{-1}), H_2 productivity of 70–80% could be achieved [160].

The main specific costs in this technological production process per 1 kg of H_2 are natural gas - 5.0–5.5 m^3; water - 4–4.5 kg; electricity - 63.3 kJ mol H_2. In the case of electricity production in the main transport, carbon dioxide emissions amount to 0.3–0.4 m^3, if the loss of natural gas and produced H_2 is taken into account [161].

The H_2 produced by the conventional method described above is the cheapest. For example, the cost of H_2 production at the designed capacity of 379.3 kg per day at 90% productivity is $ 10.00 per MMBtu, i.e., $ 2.27 kg^{-1} and $ 2.08 kg^{-1} with and without carbon capture, respectively (Table 6) [162].

The main drawback of H_2 production by thermochemical methods is the dependence on the supply of old carbonaceous material, the reserves of which are renewable and distributed over certain areas.

Electrolysis is a widely used method for producing H_2 on an industrial scale. Production is based on water, an inexhaustible raw material, but the resulting product accounts for up to 5% of the total H_2 produced.

The cost of H_2 produced by electrolysis is 3-6-fold higher than that of H_2 from natural gas [162].

According to the available calculations, about 53 kWh of electricity is consumed to produce 1 kg of H_2 at a cell efficiency of 75%. The weak point of the method is thus the high cost of the H_2 produced and air pollution [162].

The last approach considered in this article is H_2 production in cyanobacterial cells using methods such as direct and indirect biophotolysis. It is currently only a theoretical method. It is quite difficult to evaluate this method from an economic point of view and to compare it with other methods described above. The main expenses and investments required in this method are aimed at the maintenance of photobioreactors (cultivation of cyanobacteria) and various technical activities for splitting, processing, transporting, and storing the gases [163].

This approach has a number of advantages as it provides a solution to the main problems in obtaining active H_2 producers. It is a very cost-effective and stable method as: firstly, water is used as a renewable source, secondly, the energy of sunlight is used and thirdly, no expensive culture media are needed for the cultivation of cyanobacteria, even dirty water can be used for their cultivation. This method, therefore, lowers the cost of new technologies and generates cheap energy in the processing of waste. Investment in recycling can be ruled out as the risk of air pollution and greenhouse gas emissions is reduced [164].

Compared to electrolysis, H_2 production by thermochemical methods is much cheaper and more productive. However, our observation of the key parameters of cyanobacterial biomass conversion into biofuel shows that, despite the prospects for its use as a renewable biomass source, the industrial production of such biofuels is currently not economically justified and faces significant obstacles. Besides the problem of increasing lipid production by individual strains of cyanobacteria, an important problem is scaling up their cultivation. At the same time, the cultivation of cyanobacteria is one of the most expensive parts of any production process, whether it takes place in photobioreactors or open ponds.

Despite the large energy potential of cyanobacteria, cyanobacteria-based biohydrogen production is currently limited by high production costs due to the high cost of the resources used, including water, nutrient components, and electricity. Since the economic efficiency of biohydrogen production from phototrophic microorganisms depends on their large-scale productivity, the major obstacle to

Table 6 – Technical and economic aspects of H_2 production methods.

Process	Type	Feedstock	Efficiency (%)	Capital cost (M$)	Hydrogen cost [$ kg^{-1}]	Refs
Steam reforming	–	Natural Gas	74–85	226.4	2.27	[162]
Biomass pyrolysis	–	Woody Biomass	35–40	5.4–3.1	2.13	
Bio-photolysis	Direct	Water + Algae	10	50$ m^{-2}	1.13	
	Indirect	Water + Algae	10	135$ m^{-2}	1.42	
Electrolysis	Nuclear	Water	40	–	4.15–7	
	Solar	Water	60	421–22.1	5.10–10.49	

commercialisation is the difficulty in replicating their production potential for large-scale production. Therefore, despite the potential of cyanobacteria for rapid biomass production compared to terrestrial feedstocks, large-scale implementation of this process may require significant effort [165].

Practical significance of this study

Hydrogen production by cyanobacterial species has so far only been published under laboratory conditions, but there are no data on pilot trials in factories. This is because the following key issues remain unresolved: light environment, climate and space, reactor construction materials, and mixing mechanism of cultures.

At the laboratory scale, H_2 production has improved rapidly and shown promising results, but the scientific findings need to be linked to industrial needs to enable appropriate optimisation and scaling for specific applications. Compared to chemically produced hydrogen, H_2 production is associated with major difficulties, e.g.: maintenance of monoculture during cultivation, low productivity and energy efficiency, O_2 inhibition, the sensitivity of cyanobacteria to external factors, stable gas production, design of appropriate photoreactor system (light (mixing), temperature, control devices, size, maintenance and disposal of H_2, consideration of capital costs), flocculation, collection methods, gas purification methods, appropriate systems for storage, transportation, delivery, commercialisation, process control, well-defined cost and reliability, and lack of production technology.

All authors noted that the price of the final product is lower than that of other commercial fuels, given the technical, social and political obstacles mentioned above. Therefore, it is necessary to focus on the development of low-cost photoreactors and the optimisation of photosynthesis.

Despite the promising possibilities offered by H_2 production today, this technology is still in the early stages of its development and is only used in laboratories and semi-technical plants. The main reason for the lack of industrial-scale production is the slow H_2 production and, of course, the low H_2 yield, which consequently has a strong impact on the initial costs. The analysis of the publications of the last 10 years shows that the technology of biohydrogen production by cyanobacteria and algal cells is of interest to many research societies, although it has not yet been put into practice.

As a result of the review, based on the identification of the strengths and weaknesses of the H_2 production method under consideration, a SWOT analysis has been prepared, showing possible prospects and threats for the introduction of this technology into production (Fig. 7).

Currently, much research is being done on the processes of H_2 production by cyanobacterial cells, but a complete, generalised and also theoretical analysis of the experimental data is still pending, which will eventually be useful for practical applications and pave the way for a new research approach. The analysis, based on the research material published so far, summarises and highlights the scientific achievements in the field of bioenergy.

For example, photobiological H_2 production based on an integrated approach requires an increase in the metabolic capabilities of H_2 producers. It can be concluded that the commercialisation of biohydrogen production is currently facing difficulties, but the prospects of H_2 as a "clean energy" will further develop bioenergy and genetic engineering.

There are many experimental studies on the effects of biochemical and physiological approaches and the use of various enzyme inhibitors and transporters, creating stress conditions by regulating the external environment, etc., aimed at increasing the rate and duration of H_2 production by cyanobacterial cells. However, it is not yet efficient enough to produce H_2 on a large scale.

The development of genetic mutants of cyanobacteria could offer great potential for the development of biohydrogen energy. The purpose of the genetic engineering approach in modifying cyanobacteria is to increase H_2 production by

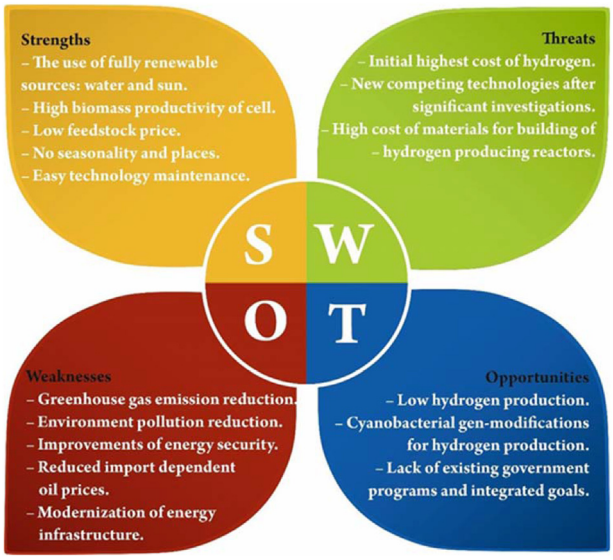


Fig. 7 – SWOT analysis of cyanobacteria-based biohydrogen production.

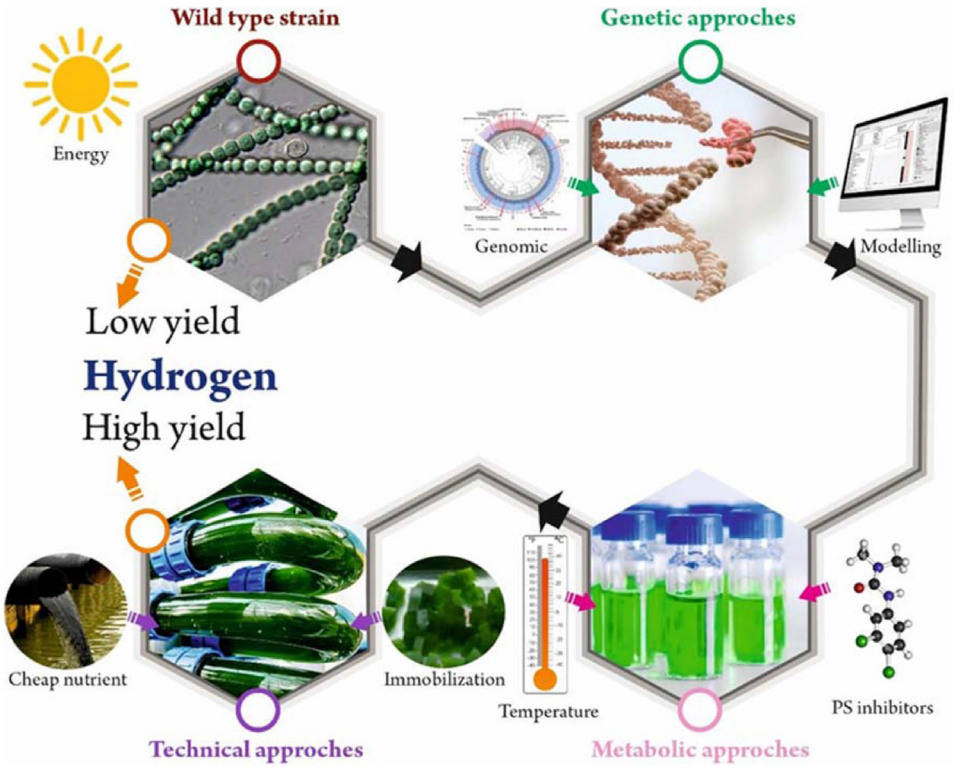


Fig. 8 – Main approaches and factors contributing to the increase in H_2 yield in cyanobacteria cells.

suppressing and introducing expressive and novel genes. This manipulation eliminates the main problems:

1) the low photochemical efficiency of the collecting antennae.
2) the low frequency of heterocyst formation;
3) the presence and activity of the absorbing hydrogenase;
4) the competition for reducing agents and the presence of other assimilation pathways.

Our review highlights the mechanisms of the processes, as understanding them will allow researchers to pool all the data to develop strategies to obtain mutants that significantly increase H_2 production.

The next factor is the design of the photobioreactors used to cultivate cyanobacteria for H_2 production on a large scale. They should differ from the regular fermenters used for biomass production.

Genetic modification of cyanobacterial strains can increase the product yield if the right metabolic approach is chosen, allowing the development of biochemical hydrogen production as an innovative method. And under the influence of policy, it is possible to improve a new technology with large investments leading to the development of biohydrogen energy.

A summary of the results is shown graphically in Fig. 8.

Conclusion

A review of many studies shows that the energy of hydrogen cyanobacteria has been intensively investigated over the last 20 years. The vast majority of studies have been conducted using photosynthetic activity and optimisation methods. In addition, fundamental research on the modification of subunits of cyanobacterial enzymes shows the possibility of the practical application of this method in the biotechnological industry. In addition, the modulation of cells with various biochemical substances to obtain H_2 is being carried out on a large scale. In recent years, much has been done to develop numerous photobioreactors for obtaining biomass from cyanobacterial cells. Despite so much research, the practical application still lags behind theoretical research. By joining forces of international scientists, attracting the attention of international organisations in this field and increasing investment support, it is possible to determine the true future of cyanobacterial H_2 production.

CRediT authorship contribution statement

AKS, GKK, analysis, writing original draft preparation; BZK, AKS, SA, SIA, project administration, idea construction; GKK, BKD, AMB, DD literature survey. BKD, AKS, picture construction, drawing. BZK, AKS, DD, SA, SIA, final preparation of the manuscript. All authors have read and agreed to the published version of 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 work was funded by the Ministry of Education and Science of the Republic of Kazakhstan under projects: AP09260077, AP13067574 and AP09260785. SA gratefully acknowledges the support of the Distinguished Scientists Fellowship Program, King Saud University, Saudi Arabia. AMB and SIA were supported by grants from the Russian Science Foundation (19-14-00118) and in particular, results shown in Fig. 5 and Table 3 by grants 22-19-00516 and 22-44-08001, respectively. The part of the results (Fig. 7) was obtained under the state contract of the Ministry of Science and Higher Education of the Russian Federation (Project No. 122050400128–1).

REFERENCES

[1] Burrows E, Chaplen F, Ely R. Effects of selected electron transport chain inhibitors on 24-h hydrogen production by *Synechocystis* sp. PCC 6803. Bioresour Technol 2010;102:3062–70.

[2] Abdelwahab HEM. Hydrogen production in the cyanobacterium *Synechocystis* sp. PCC 6803 with engineered subunit of the bidirectional H_2-ase. Adv Life Sci Technol 2014;18:7–19.

[3] Sadvakasova AK, Kossalbayev BD, Zayadan BK, Bolatkhan K, Alwasel S, Najafpour MM, Tatsuya T, Allakhverdiev SI. Bioprocesses of hydrogen production by cyanobacteria cells and possible ways to increase their productivity. Renew Sustain Energy Rev 2020;133:110054. https://doi.org/10.1016/j.rser.2020.110054.

[4] Tiwari A, Pandey A. Cyanobacterial hydrogen production – a step towards clean environment. Int J Hydrogen Energy 2012;37:139–50.

[5] Kaushik A, Anjana K. Biohydrogen production by *Lyngbya perelegans*: influence of physic-chemical environment. Biomass Bioenergy 2011;35:1041–5.

[6] Razeghifard R. Algal biofuels. Photosynth Res 2013;117:207–19.

[7] Tamburic B, Zemichael FW, Maitland GC, Hellgardt K. Parameters affecting the growth and hydrogen production of the green alga *Chlamydomonas reinhardtii*. Int J Hydrogen Energy 2011;36:7872–6.

[8] Antal TK, Krendeleva TE, Tyystjärvi E. Multiple regulatory mechanisms in the chloroplast of green algae: relation to hydrogen production. Photosynth Res 2015;125(3):357–81.

[9] Bolatkhan K, Sadvakasova AK, Zayadan BK, Kakimova AB, Sarsekeyeva FK, Kossalbayev BD, et al. Prospects for the creation of a waste-free technology for wastewater treatment and utilization of carbon dioxide based on cyanobacteria for biodiesel production. J Biotechnol 2020;324:162–70. https://doi.org/10.1016/j.jbiotec.2020.10.010.

[10] Yeager CM, Milliken CE, Bagwell CE, Staples L, Berseth PA, Sessions HT. Evaluation of experimental conditions that influence hydrogen production among heterocystous cyanobacteria. Int J Hydrogen Energy 2011;36:7487–99.

[11] 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. Int J Hydrogen Energy 2010;35:1117–27.

[12] Markov SA, Protasov ES, Bybin VA, Eivazova ER, Stom DI. Using immobilized cyanobacteria and culture medium contaminated with ammonium for H_2 production in a hollow-fiber photobioreactor. Int J Hydrogen Energy 2015;40:4752–7.

[13] Sadvakasova AK, Kossalbayev BD, Zayadan BK, Kirbayeva DK, Alwasel S, Allakhverdiev SI. Potential of cyanobacteria in the conversion of wastewater to biofuels. World J Microbiol Biotechnol 2021;37. https://doi.org/10.1007/s11274-021-03107-1.

[14] Bothe H, Schmitz O, Yates MG, Newton WE. Nitrogen fixation and hydrogen metabolism in cyanobacteria. Microbiol Mol Biol Rev 2010;74:529–51. https://doi.org/10.1128/MMBR.00033-10.

[15] Phunpruch S, Taikhao S, Incharoensakdi A. Identification of bidirectional hydrogenase genes and their co-transcription in unicellular halotolerant cyanobacterium *Aphanothece halophytica*. J Appl Phycol 2016;28:967–78. https://doi.org/10.1007/s10811-015-0664-8.

[16] Voloshin RA, Rodionova MV, Zharmukhamedov SK, Nejat Veziroglu T, Allakhverdiev SI. Review: biofuel production from plant and algal biomass. Int J Hydrogen Energy 2016;41:17257–73. https://doi.org/10.1016/j.ijhydene.2016.07.084.

[17] Srirangan K, Pyne ME, Perry Chou C. Biochemical and genetic engineering strategies to enhance hydrogen production in photosynthetic algae and cyanobacteria. Bioresour Technol 2011;102:8589–604. https://doi.org/10.1016/j.biortech.2011.03.087.

[18] Oh Y-K, Raj SM, Jung GY, Park S. Current status of the metabolic engineering of microorganisms for biohydrogen production. Bioresour Technol 2011;102:8357–67. https://doi.org/10.1016/j.biortech.2011.04.054.

[19] Skizim NJ, Ananyev GM, Krishnan A, Dismukes GC. Metabolic pathways for photobiological hydrogen production by nitrogenase- and hydrogenase-containing unicellular cyanobacteria *cyanothece*. Int J Biol Chem 2012;287:2777–86. https://doi.org/10.1074/jbc.M111.302125.

[20] Show K-Y, Lee D-J, Chang J-S. Bioreactor and process design for biohydrogen production. Bioresour Technol 2011;102:8524–33. https://doi.org/10.1016/j.biortech.2011.04.055.

[21] Kufryk G. Advances in utilizing cyanobacteria for hydrogen production. Adv Microbiol 2013;3:60–8.

[22] Kossalbayev BD, Tomo T, Zayadan BK, Sadvakasova AK, Bolatkhan K, Alwasel S, Allakhverdiev SI. Determination of the potential of cyanobacterial strains for hydrogen production. Int J Hydrogen Energy 2020;45(4):2627–39. https://doi.org/10.1016/j.ijhydene.2019.11.164.

[23] Masirbaeva AD, Baidyldaeva ZA, Sadanov AK, Baigonusova ZA, Ultanbekova GD. Study of nitrogen-fixing activity and competitive ability of nodule bacteria of the genus *Rhizobium*. Biol med series 2014;2:3252–62.

[24] Zhakeeva MB, Bekenova US, Zhumadilova ZS, Shorabaev EZ, Sadanov AK. Study of ecological and trophic groups of soil microorganisms under alfalfa and soybeans using biological products of the Rizovit-AKS series. Succ of Modern Nat Sci 2015;2:144–7.

[25] Khetkorn W, Rastogi RP, Incharoensakdi A, Lindblad P, Datta M, Pandey A, Larroche C. Microalgal hydrogen production – a review. Bioresour Technol 2017;243:1194–206.

[26] Bolatkhan K, Kossalbayev BD, Zayadan BK, Tomo T, Veziroglu VT, Allakhverdiev SI. Hydrogen production from phototrophic microorganisms: reality and perspectives. Int J Hydrogen Energy 2019;44(12):5799–811. https://doi.org/10.1016/j.ijhydene.2019.01.092.

[27] Bandyopadhyay A, Stöckel J, Min H, Sherman LA, Pakrasi HB. High rates of photobiological H_2 production by a cyanobacterium under aerobic conditions. Nat Commun 2010;1:139. https://doi.org/10.1038/ncomms1139.

[28] Androga DD, Özgür E, Eroglu I, Gündüz U, Yücel M. Photofermentative hydrogen production in outdoor conditions. In: Minic D, editor. Hydrogen energy-challenges and perspective. Intech; 2012. p. 77–120. https://doi.org/10.5772/50390.
[29] Hüner NPA, Grodzinski B. 1.23 - photosynthesis and photoautotrophy. In: Moo-Young M, editor. Comprehensive biotechnology. 2nd ed. Burlington: Academic Press; 2011. p. 315–22. https://doi.org/10.1016/B978-0-08-088504-9.00036-2.
[30] Zhang L, He M, Liu J, Li L. Role of the mitochondrial alternative oxidase pathway in hydrogen photoproduction in *Chlorella protothecoides*. Planta 2015;241:1005–14. https://doi.org/10.1007/s00425-014-2231-y.
[31] Vargas S, Santos P, Zaiat M, Calijuri M. Optimization of biomass and hydrogen production by *Anabaena* sp. (UTEX 1448) in nitrogen-deprived cultures. Biomass Bioenergy 2018;111:70–6. https://doi.org/10.1016/j.biombioe.2018.01.022.
[32] Touloupakis E, Torzillo G. Chapter 14 - photobiological hydrogen production. In: Calise F, D'Accadia MD, Santarelli M, Lanzini A, Ferrero D, editors. Solar hydrogen production. Academic Press; 2019. p. 511–25. https://doi.org/10.1016/B978-0-12-814853-2.00014-X.
[33] Chandrasekhar K, Lee Y-J, Lee D-W. Biohydrogen production: strategies to improve process efficiency through microbial routes. Int J Mol Sci 2015;16:8266–93. https://doi.org/10.3390/ijms16048266.
[34] Amaro-Gahete J, Pavliuk MV, Tian H, Esquivel D, Romero-Salguero FJ, Ott S. Catalytic systems mimicking the [FeFe]-hydrogenase active site for visible-light-driven hydrogen production. Coord Chem Rev 2021;448:214172. https://doi.org/10.1016/j.ccr.2021.214172.
[35] Harwood CS. Iron-only and vanadium nitrogenases: fail-safe enzymes or something more? Annu Rev Microbiol 2020;74:247–66. https://doi.org/10.1146/annurev-micro-022620-014338.
[36] Allakhverdiev SI, Thavasi V, Kreslavski VD, Zharmukhamedov SK, Klimov VV, Ramakrishna S, et al. Photosynthetic hydrogen production. J Photochem Photobiol C Photochem Rev 2010;11:101–13. https://doi.org/10.1016/j.jphotochemrev.2010.07.002.
[37] Hallenbeck PC. Hydrogen production by cyanobacteria. In: Book: microbial technologies in advanced biofuels production publisher. Springer US; 2011. p. 15–28.
[38] Magnuson A, Cardona T. Thylakoid membrane function in heterocysts. BBA - Bioenergetics 2016;1857:309–19. https://doi.org/10.1016/j.bbabio.2015.10.016.
[39] Sakurai H, Masukawa H, Kitashima M, Inoue K. Photobiological hydrogen production: bioenergetics and challenges for its practical application. J Photochem Photobiol C Photochem Rev 2013;17:1–25. https://doi.org/10.1016/j.jphotochemrev.2013.05.001.
[40] 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.
[41] Hoffman BM, Lukoyanov D, Yang Z-Y, Dean DR, Seefeldt LC. Mechanism of nitrogen fixation by nitrogenase: the next stage. Chem Rev 2014;114:4041–62. https://doi.org/10.1021/cr400641x.
[42] Choudhary KK. Occurrence of nitrogen fixing cyanobacteria during different stages of paddy cultivation. Bangladesh J Plant Taxon 2011;18:73–6.
[43] Alberto AEF, Henrique CJ, Marcelo V, Alvarenga V, Nunes-Adriano N, Wagner A. Cyanobacterial nitrogenases: phylogenetic diversity, regulation and functional predictions. Genet Mol Biol 2017;40:261–75.
[44] Signorelli S, Sainz M, Tabares-da Rosa S, Monza J. The role of nitric oxide in nitrogen fixation by legumes. Front Plant Sci 2020;11.
[45] Burén S, Jiménez-Vicente E, Echavarri-Erasun C, Rubio LM. Biosynthesis of nitrogenase cofactors. Chem Rev 2020;120:4921–68. https://doi.org/10.1021/acs.chemrev.9b00489.
[46] Sippel D, Einsle O. The structure of vanadium nitrogenase reveals an unusual bridging ligand. Nat Chem Biol 2017;13:956–60. https://doi.org/10.1038/nchembio.2428.
[47] Koirala A, Brözel VS. Phylogeny of nitrogenase structural and assembly components reveals new insights into the origin and distribution of nitrogen fixation across bacteria and archaea. Microorganisms 2021;9:1662. https://doi.org/10.3390/microorganisms9081662.
[48] Mcglynn S, Boyd E, Peters J, Orphan V. Classifying the metal dependence of uncharacterized nitrogenases. Front Microbiol 2013;3.
[49] Thiel T, Pratte BS. Regulation of three nitrogenase gene clusters in the cyanobacterium *Anabaena variabilis* ATCC 29413. Life 2014;4:944–67. https://doi.org/10.3390/life4040944.
[50] Pratte BS, Thiel T. Comparative genomic insights into culturable symbiotic cyanobacteria from the water fern Azolla. Microb Genom 2021;7:595. https://doi.org/10.1099/mgen.0.000595.
[51] Park J-J, Lechno-Yossef S, Wolk CP, Vieille C. Cell-specific gene expression in *Anabaena variabilis* grown phototrophically, mixotrophically, and heterotrophically. BMC Genom 2013;14:759. https://doi.org/10.1186/1471-2164-14-759.
[52] Maeda I. Potential of phototrophic purple nonsulfur bacteria to fix nitrogen in rice fields. Microorganisms 2022;10:28. https://doi.org/10.3390/microorganisms10010028.
[53] Poudel S, Colman DR, Fixen KR, Ledbetter RN, Zheng Y, Pence N, et al. Electron transfer to nitrogenase in different genomic and metabolic backgrounds. J Bacteriol 2018;200. https://doi.org/10.1128/JB.00757-17. e00757-17.
[54] Teng Y, Xu Y, Wang X, Christie P. Function of biohydrogen metabolism and related microbial communities in environmental bioremediation. Front Microbiol 2019;10.
[55] Yagi T, Higuchi Y. Studies on hydrogenase. Proc Jpn Acad Ser B Phys Biol Sci 2013;89:16–33. https://doi.org/10.2183/pjab.89.16.
[56] Preissler J, Wahlefeld S, Lorent C, Teutloff C, Horch M, Lauterbach L, et al. Enzymatic and spectroscopic properties of a thermostable [NiFe]-hydrogenase performing H_2-driven NAD^+-reduction in the presence of O_2. BBA – Bioenergetics 2018;1859:8–18. https://doi.org/10.1016/j.bbabio.2017.09.006.
[57] Greening C, Biswas A, Carere CR, Jackson CJ, Taylor MC, Stott MB, et al. Genomic and metagenomic surveys of hydrogenase distribution indicate H_2 is a widely utilised energy source for microbial growth and survival. ISME J 2016;10:761–77. https://doi.org/10.1038/ismej.2015.153.
[58] Artz JH, Tokmina-Lukaszewska M, Mulder DW, Lubner CE, Gutekunst K, Appel J, et al. The structure and reactivity of the *HoxEFU* complex from the cyanobacterium *Synechocystis* sp. PCC 6803. J Biol Chem 2020;295:9445–54. https://doi.org/10.1074/jbc.RA120.013136.
[59] Khanna N, Lindblad P. Cyanobacterial hydrogenases and hydrogen metabolism revisited: recent progress and future prospects. Int J Mol Sci 2015;16:10537–61. https://doi.org/10.3390/ijms160510537.
[60] Puggioni V, Tempel S, Latifi A. Distribution of hydrogenases in cyanobacteria: a phylum-wide genomic survey. Front Genet 2016;7.
[61] Kumar K, Mella-Herrera RA, Golden JW. Cyanobacterial heterocysts. Cold Spring Harbor Perspect Biol

2010;2:a000315. https://doi.org/10.1101/cshperspect.a000315.
[62] Liu D, Liberton M, Yu J, Pakrasi HB, Bhattacharyya-Pakrasi M. Engineering nitrogen fixation activity in an oxygenic phototroph. mBio 2018;9:e01029-18. https://doi.org/10.1128/mBio.01029-18.
[63] Singh H, Das D. Chapter 15 - biohydrogen from microalgae. In: Jacob-Lopes E, Maroneze MM, Queiroz MI, Zepka LQ, editors. Handbook of microalgae-based processes and products. Academic Press; 2020. p. 391–418. https://doi.org/10.1016/B978-0-12-818536-0.00015-4.
[64] 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.
[65] Kosourov S, Jokel M, Aro E-M, Allahverdiyeva Y. A new approach for sustained and efficient H_2 photoproduction by *Chlamydomonas reinhardtii*. Energy Environ Sci 2018;11:1431–6. https://doi.org/10.1039/C8EE00054A.
[66] Kaushik A, Sharma M. Exploiting biohydrogen pathways of cyanobacteria and green algae: an industrial production approach. In: Singh A, Rathore D, editors. Biohydrogen prod. Sustain. Curr. Technol. Futur. Perspect. 10.1007/97. New Delhi: Springer India; 2017. p. 97. https://doi.org/10.1007/978-81-322-3577-4_5.
[67] Lam MK, Lee KT. Biohydrogen production from algae. Biohydrogen. 1st ed. Elsevier; 2013. p. 161–84. https://doi.org/10.1016/B978-0-444-59555-3.00008-8.
[68] Skjanes K, Rebours C, Lindblad P. Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. Crit Rev Biotechnol 2013;33:172–215. https://doi.org/10.3109/07388551.2012.681625.
[69] Taikhao S, Incharoensakdi A, Phunpruch S. Dark fermentative hydrogen production by the unicellular halotolerant cyanobacterium *Aphanothece halophytica* grown in seawater. J Appl Phycol 2014;27:187–96.
[70] Taikhao S, Phunpruch S. Increasing hydrogen production efficiency of N_2-fixing cyanobacterium *Anabaena siamensis* TISTR 8012 by cell immobilization. Energy Proc 2017;138:366–71.
[71] Weyman PD, Pratte B, Thiel T. Hydrogen production in nitrogenase mutants in *Anabaena variabilis*. FEMS Microbiol Lett 2010;304:55–61.
[72] Singh P, Khan A, Srivastava A. Advances in Cyanobacterial Biology: chapter 16 – heterocyst and akinete differentiation in cyanobacteria: a view toward cyanobacterial symbiosis. Elsevier: Academic Press; 2020. p. 235–48.
[73] Zayadan B, Usserbayeva A, Bolatkhan K, Akmukhanova N, Kossalbayev B, Baizhigitova A, et al. Screening of isolated and collection strains of cyanobacteria on productivity for determining their biotechnological potential. Eur J Entomol 2018;55:121–30.
[74] Fishtik I, Alexander A, Datta R, Geană D. A thermodynamic analysis of hydrogen production by steam reforming of ethanol via response reactions. Int J Hydrogen Energy 2000;25:31–45. https://doi.org/10.1016/S0360-3199(99)00004-X.
[75] Zayadan B, Kakimova A, Bolatkhan K, Sandybayeva S, Kosalbayev B, Nurabayeva D. Production of biohydrogen from cyanobacteria: current challenges and opportunities. Int J Biol Chem 2021;14:4–20. https://doi.org/10.26577/ijbch.2021.v14.i1.01.
[76] Melitos G, Voulkopoulos X, Zabaniotou A. Waste to sustainable biohydrogen production via photo-fermentation and biophotolysis – A systematic review. Renew Energy Environ Sustain 2021;6:45. https://doi.org/10.1051/rees/2021047.
[77] Kosourov S, Murukesan G, Seibert M, Allahverdiyeva Y. Evaluation of light energy to H_2 energy conversion efficiency in thin films of cyanobacteria and green alga under photoautotrophic conditions. Algal Res 2017;28:253–63.
[78] Rusydi R, Rusydi R, Yakupitiyage A, Gallardo WG, Dabbadie L, Anal AK. Potential of *Nostoc muscorum* cultured in BG-II medium as biodiesel feedstock source: evaluation of nutrient requirement for culture and its daily lipid content. KnE life sci 2015:103–13. https://doi.org/10.18502/kls.v1i0.93.
[79] Sadvakasova AK, Kossalbayev BD, Token AI, Bauenova MO, Wang J, Zayadan BK, et al. Influence of Mo and Fe on photosynthetic and nitrogenase activities of nitrogen-fixing cyanobacteria under nitrogen starvation. Cells 2022;11:904. https://doi.org/10.3390/cells11050904.
[80] Oey M, Sawyer AL, Ross IL, Hankamer B. Challenges and opportunities for hydrogen production from microalgae. Plant Biotechnol J 2016;14:1487–99. https://doi.org/10.1111/pbi.12516.
[81] Fan X, Wang H, Guo R, Yang D, Zhang Y, Yuan X, et al. Comparative study of the oxygen tolerance of *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* CC124 in photobiological hydrogen production. Algal Res 2016;16:240–4. https://doi.org/10.1016/j.algal.2016.03.025.
[82] Zerveas S, Kydonakis E, Moutidis P, Maragkoudakis A, Kotzabasis K. Microalgae strategy in anoxic atmospheres with various CO_2 concentrations – environmental and (astro)biotechnological perspectives. EEB 2021;187:104474. https://doi.org/10.1016/j.envexpbot.2021.104474.
[83] Kim D, Kim G, Nam S-J, Yin J, Yoon J. Visualization of endogenous and exogenous hydrogen peroxide using A lysosome-targetable fluorescent probe. Sci Rep 2015;5:8488. https://doi.org/10.1038/srep08488.
[84] Bayne BL. Chapter 9 - reproduction. In: Bayne B, editor. Developments in aquaculture and fisheries science, vol. 41. Elsevier; 2017. p. 565–701. https://doi.org/10.1016/B978-0-12-803472-9.00009-1.
[85] Tian J, Ge F, Zhang D, Deng S, Liu X. Roles of phosphate solubilizing microorganisms from managing soil phosphorus deficiency to mediating. Biogeochemical P Cycle Biology 2021;10:158. https://doi.org/10.3390/biology10020158.
[86] Oncel S. Biohydrogen from microalgae, uniting energy, life, and green future. Handb. Mar. Microalgae. Elsevier; 2015. p. 159–96. https://doi.org/10.1016/B978-0-12-800776-1.00011-X.
[87] Aarabi F, Kusajima M, Tohge T, Konishi T, Gigolashvili T, Takamune M, et al. Sulfur deficiency–induced repressor proteins optimize glucosinolate biosynthesis in plants. Sci Adv 2016;2:e1601087. https://doi.org/10.1126/sciadv.1601087.
[88] Batyrova K, Hallenbeck P. Hydrogen production by a *Chlamydomonas reinhardtii* strain with inducible expression of photosystem II. Int J Mol Sci 2017;18:647.
[89] Ramanan R, Kim B-H, Cho D-H, Oh H-M, Kim H-S. Algae–bacteria interactions: evolution, ecology and emerging applications. Biotechnol Adv 2016;34:14–29. https://doi.org/10.1016/j.biotechadv.2015.12.003.
[90] Pongpadung P, Liu J, Yokthongwattana K, Techapinyawat S, Juntawong N. Screening for hydrogen-producing strains of green microalgae in phosphorus or sulphur deprived medium under nitrogen limitation. Sci Asia 2015;41:97. https://doi.org/10.2306/scienceasia1513-1874.2015.41.097.
[91] Antal TK, Krendeleva TE, Rubin AB. Acclimation of green algae to sulfur deficiency: underlying mechanisms and application for hydrogen production. Appl Microbiol Biotechnol 2011;89:3–15. https://doi.org/10.1007/s00253-010-2879-6.

[92] Shastik E, Li L, Liu J. New methods for hydrogen production by marine microalga *Chlorella pyrenoidosa* in natural seawater. Int J Hydrogen Energy 2019;44:14707–14. https://doi.org/10.1016/j.ijhydene.2019.04.178.
[93] Alalayah WM, Alhamed YA, Al-zahrani A, Edris G. Influence of culture parameters on biological hydrogen production using green algae *Chlorella vulgaris*. Rev Chim (Bucharest) 2015;66:788–91.
[94] Song W, Rashid N, Choi W, Lee K. Biohydrogen production by immobilized *Chlorella* sp. using cycles of oxygenic photosynthesis and anaerobiosis. Bioresour Technol 2011;102:8676–81. https://doi.org/10.1016/j.biortech.2011.02.082.
[95] Melis A. Photosynthesis-to-fuels: from sunlight to hydrogen, isoprene, and botryococcene production. Energy Environ Sci 2012;5:5531–9. https://doi.org/10.1039/C1EE02514G.
[96] Zheng Y, Harwood CS. Influence of energy and electron availability on *in vivo* methane and hydrogen production by a variant molybdenum nitrogenase. Appl Environ Microbiol 2019;85. https://doi.org/10.1128/AEM.02671-18. e02671-18.
[97] Wang L, Chen M, Wei L, Gao F, Lv Z, Wang Q, et al. Treatment with moderate concentrations of $NaHSO_3$ enhances photobiological H_2 production in the cyanobacterium *Anabaena* sp. strain PCC 7120. Fuel Energy Abstr 2010;35:12777–83. https://doi.org/10.1016/j.ijhydene.2010.08.115.
[98] Ghimire A, Frunzo L, Pirozzi F, Trably E, Escudie R, Lens PNL, et al. A review on dark fermentative biohydrogen production from organic biomass: process parameters and use of by-products. Appl Energy 2015;144:73–95. https://doi.org/10.1016/j.apenergy.2015.01.045.
[99] Kossalbayev BD, Kakimova AB, Bolatkhan K, Zayadan BK, Sandybayeva SK, Bozieva AM, Sadvakasova AK, Alwasel S, Allakhverdiev SI. Biohydrogen production by novel cyanobacterial strains isolated from rice paddies in Kazakhstan. Int J Hydrogen Energy 2022;47:16440–53. https://doi.org/10.1016/j.ijhydene.2022.03.126.
[100] Yodsang P, Raksajit W, Aro E-M, Mäenpää P, Incharoensakdi A. Factors affecting photobiological hydrogen production in five filamentous cyanobacteria from Thailand. Photosynthetica 2018;56:334–41. https://doi.org/10.1007/s11099-018-0789-5.
[101] Zayadan BK, Kossalbayev BD, Tomo T, Allakhverdiev SI, Sadvakasova AK, Bolatkhan K, Kakimova A. Study of promising heterocystic cyanobacterial strains for biohydrogen production. Series of biological and medical 2020;3(339):41–8.
[102] Dickson D, Ely R. Silica sol-gel encapsulation of cyanobacteria: lessons for academic and applied research. Appl Microbiol Biotechnol 2013;97. https://doi.org/10.1007/s00253-012-4686-8.
[103] Ainas M, Hasnaoui S, Bouarab R, Abdi N, Drouiche N, Mameri N. Hydrogen production with the cyanobacterium *Spirulina platensis*. Int J Hydrogen Energy 2017;42. https://doi.org/10.1016/j.ijhydene.2016.12.056.
[104] Zulkefli NS, Hwang S-J. Heterocyst development and diazotrophic growth of *Anabaena variabilis* under different nitrogen availability. Life 2020;10:279. https://doi.org/10.3390/life10110279.
[105] Yin TT, Pin UL, Ghazali AHA. Influence of external nitrogen on nitrogenase enzyme activity and auxin production in *Herbaspirillum seropedicae* (Z78). Trop Life Sci Res 2015;26:101–10.
[106] El-Shafie M, Kambara S, Hayakawa Y. Hydrogen production technologies overview. JPEE 2019;7:107–54. https://doi.org/10.4236/jpee.2019.71007.
[107] Brodsky AN, Zhang J, Visconti RP, Harcum SW. Expansion of mesenchymal stem cells under atmospheric carbon dioxide. Biotechnol Prog 2013;29:1298–306. https://doi.org/10.1002/btpr.1782.
[108] Ortega-Ramos M, Jittawuttipoka T, Saenkham P, Czarnecka-Kwasiborski A, Bottin H, Cassier-Chauvat C, et al. Engineering *Synechocystis* PCC6803 for hydrogen production: influence on the tolerance to oxidative and sugar stresses. PLoS One 2014;9:e89372. https://doi.org/10.1371/journal.pone.0089372.
[109] Rashid N, Lee K, Han JI, Gross M. Hydrogen production by immobilized *Chlorella vulgaris*: optimizing pH, carbon source and light. Bioproc Biosyst Eng 2013;36:867–72. https://doi.org/10.1007/s00449-012-0819-9.
[110] Lakshmikandan M, Murugesan AG. Enhancement of growth and biohydrogen production potential of *Chlorella vulgaris* MSU-AGM 14 by utilizing seaweed aqueous extract of *Valoniopsis pachynema*. Renew Energy 2016;96:390–9. https://doi.org/10.1016/j.renene.2016.04.097.
[111] Bakonyi P, Kumar G, Belafi-Bak o K, Kim S-H, Koter S, Kujawski W, et al. A review of the innovative gas separation membrane bioreactor with mechanisms for integrated production and purification of biohydrogen. Bioresour Technol 2018;270:643–55. https://doi.org/10.1016/j.biortech.2018.09.020.
[112] Meng SL, Chen X, Wang J, Fan LM, Qiu LP, Zheng Y, et al. Interaction effects of temperature, light, nutrients, and pH on growth and competition of *Chlorella vulgaris* and *Anabaena* sp. strain PCC. Front Environ Sci 2021;9.
[113] Ortiz-Moreno ML, Solarte-Murillo LV, Sandoval-Parra KX, Ortiz-Moreno ML, Solarte-Murillo LV, Sandoval-Parra KX. Analysis of *Nostoc muscorum* biomass production in a hydroponic system. Orinoquia 2020;24:23–31. https://doi.org/10.22579/20112629.599.
[114] Nyberg M, Heidorn T, Lindblad P. Hydrogen production by the engineered cyanobacterial strain *Nostoc* PCC 7120 *ΔhupW* examined in a flat panel photobioreactor system. J Biotechnol 2015;215:35–43.
[115] 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.
[116] Singh JS, Kumar A, Rai AN, Singh DP. Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. Front Microbiol 2016;7.
[117] 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.
[118] Jin Q, Zhu K, Cui W, Xie Y, Han B, Shen W. Hydrogen gas acts as a novel bioactive molecule in enhancing plant tolerance to paraquat-induced oxidative stress via the modulation of heme oxygenase-1 signalling system. Plant Cell Environ 2013;36:956–69. https://doi.org/10.1111/pce.12029.
[119] Kosourov S, Leino H, Murukesan G, et al. Hydrogen photoproduction by immobilized N_2-fixing cyanobacteria: understanding the role of the uptake hydrogenase in the long-term process. Appl Environ Microbiol 2014;80(18):5807–17. https://doi.org/10.1128/AEM.01776-14.
[120] Chen X. Characterization of *Synechocystis* sp. PCC 6803 under mixotrophic conditions. Online unter, https://macau.uni-kiel.de/receive/diss_mods_00014133; 2014.
[121] Tapia E, González-Pardo A, Iranzo A, Romero M, González-Aguilar J, Vidal A, et al. Multi-tubular reactor for hydrogen production: CFD thermal design and experimental testing. Processes 2019;7:31. https://doi.org/10.3390/pr7010031.
[122] Wang Y, Tahir N, Cao W, Zhang Q, Lee D-J. Grid columnar flat panel photobioreactor with immobilized photosynthetic bacteria for continuous photofermentative

hydrogen production. Bioresour Technol 2019;291:121806. https://doi.org/10.1016/j.biortech.2019.121806.
[123] Nemestóthy N, Bélafi-Bakó K, Bakonyi P. Enhancement of dark fermentative H_2 production by gas separation membranes: a review. Bioresour Technol 2020;302:122828. https://doi.org/10.1016/j.biortech.2020.122828.
[124] Saper G, Kallmann D, Conzuelo F, Zhao F, Tóth TN, Liveanu V, et al. Live cyanobacteria produce photocurrent and hydrogen using both the respiratory and photosynthetic systems. Nat Commun 2018;9:2168. https://doi.org/10.1038/s41467-018-04613-x.
[125] Batyrova K, Gavrisheva A, Ivanova E, Liu J, Tsygankov A. Sustainable hydrogen photoproduction by phosphorus-deprived marine green microalgae *Chlorella* sp. Int J Mol Sci 2015;16:2705–16. https://doi.org/10.3390/ijms16022705.
[126] Seabra R, Santos A, Pereira S, Moradas-Ferreira P, Tamagnini P. Immunolocalization of the uptake hydrogenase in the marine cyanobacterium *Lyngbya majuscula* CCAP1446/4 and two *Nostoc* strains. FEMS Microbiol Lett 2009;292:57–62. https://doi.org/10.1111/j.1574-6968.2008.01471.x.
[127] Pansook S, Incharoensakdi A, Phunpruch S. Effects of the photosystem II inhibitors CCCP and DCMU on hydrogen production by the unicellular halotolerant cyanobacterium *Aphanothece halophytica*. Sci World J 2019;10:302–36.
[128] Antal T, Galina K, Volgusheva A, Krendeleva T, Tyystjärvi E, Rubin A. Hydrogen photoproduction by immobilized S-deprived *Chlamydomonas reinhardtii*: effect of light intensity and spectrum, and initial medium pH. Algal Res 2016;17:38–45.
[129] Antal T, Matorin D, Kukarskikh G, Lambreva M, Tyystjärvi E, Krendeleva T, Tsygankov A, Rubin A. Pathways of hydrogen photoproduction by immobilized *Chlamydomonas reinhardtii* cells deprived of sulfur. Int J Hydrogen Energy 2014;39:18194–203.
[130] Carrieri D, Wawrousek K, Eckert C, Yu I, Maness P-J. The role of bidirectional hydrogenases in cyanobacteria. Bioresour Technol 2011;102:8368–77.
[131] Wang Y, Gao Y, Li C, Gao H, Zhang C-C, Xu X. Three substrains of the cyanobacterium *Anabaena* sp. strain PCC 7120 display divergence in genomic sequences and hetC function. J Bacteriol 2018;200. https://doi.org/10.1128/JB.00076-18. e00076-18.
[132] Masukawa H, Kitashima M, Inoue K, Sakurai H, Hausinger RP. Genetic engineering of cyanobacteria to enhance biohydrogen production from sunlight and water. Ambio 2012;41:169–73. https://doi.org/10.1007/s13280-012-0275-4.
[133] Allakhverdiev ES, Khabatova VV, Kossalbayev BD, Zadneprovskaya EV, Rodnenkov OV, Martynyuk TV, et al. Raman spectroscopy and its modifications applied to biological and medical research. Cells 2022;11. https://doi.org/10.3390/cells11030386.
[134] Issa AA, Abd-Alla MH, Ohyama T. Nitrogen fixing cyanobacteria: future prospect. In: Ohyama T, editor. Advances in biology and ecology of nitrogen fixation. Intech; 2014. p. 23–48. https://doi.org/10.5772/56995.
[135] Quintana N, Van der Kooy F, Van de Rhee MD, Voshol GP, Verpoorte R. Renewable energy from Cyanobacteria: energy production optimization by metabolic pathway engineering. Appl Microbiol Biotechnol 2011;91:471–90. https://doi.org/10.1007/s00253-011-3394-0.
[136] Khetkorn W, Lindblad P, Incharoensakdi A. Inactivation of uptake hydrogenase leads to enhanced and sustained hydrogen production with high nitrogenase activity under high light exposure in the cyanobacterium *Anabaena siamensis* TISTR 8012. J Biol Eng 2012;6:19.
[138] Lindberg P, Devine E, Stensjö K, Lindblad P. *HupW* protease specifically required for processing of the catalytic subunit of the uptake hydrogenase in the cyanobacterium *Nostoc* sp. strain PCC 7120. Appl Environ Microbiol 2012;78:273–6.
[139] Marques AE, Barbosa AT, Jotta J, Coelho MC, Tamagnini P, Gouveia L. Biomass Bioenergy 2011;35:4426–34.
[140] Boison G, Bothe H, Schmitz O. Transcriptional analysis of hydrogenase genes in the Cyanobacteria *Anacystis nidulans* and *Anabaena variabilis* monitored by RT-PCR. Curr Microbiol 2000;40:315–21. https://doi.org/10.1007/s002849910063.
[141] Khetkorn W, Lindblad P, Incharoensakdi A. Enhanced biohydrogen production by the N_2-fixing cyanobacterium *Anabaena siamensis* strain TISTR 8012. Int J Hydrogen Energy 2010;35:12767–76. https://doi.org/10.1016/j.ijhydene.2010.08.135.
[142] Bauer CC, Buikema WJ, Black K, Haselkorn R. A short-filament mutant of *Anabaena* sp. strain PCC 7120 that fragments in nitrogen-deficient medium. J Bacteriol 1995;177:1520–6.
[143] Min H, Sherman LA. Hydrogen production by the unicellular, diazotrophic cyanobacterium *Cyanothece* sp. strain ATCC 51142 under conditions of continuous light. Appl Environ Microbiol 2010;76:4293–301.
[144] Ekman M, Ow SY, Holmqvist M, Zhang X, van Wagenen J, Wright PC, et al. Metabolic adaptations in a H_2 producing heterocyst-forming cyanobacterium: potentials and implications for biological engineering. J Proteome Res 2011;10:1772–84. https://doi.org/10.1021/pr101055v.
[145] Baebprasert W, Jantaro S, Khetkorn W, Lindblad P, Incharoensakdi A. Increased H_2 production in the cyanobacterium *Synechocystis* sp strain PCC 6803 by redirecting the electron supply via genetic engineering of the nitrate assimilation pathway. Metab Eng 2011;13:610–6. https://doi.org/10.1016/j.ymben.2011.07.004.
[146] Heyer H, Stal L, Krumbein WE. Simultaneous heterolactic and acetate fermentation in the marine cyanobacterium *Oscillatoria limosa* incubated anaerobically in the dark. Arch Microbiol 1989;151:6558–64.
[147] Phlips EJ, Mitsui A. Characterization and optimization of hydrogen production by a salt water blue-green alga *Oscillatoria* sp. Miami BG 7. II – use of immobilization for enhancement of hydrogen production. Int J Hydrogen Energy 1986;11.
[148] Asada Y, Miyake M, Miyake J. Production of bioplastics and hydrogen gas by photosynthetic microorganisms. Chin J Oceanol Limnol 1998;16:91. https://doi.org/10.1007/BF02849086.
[149] Prabaharan D, Subramanian G. Oxygen-free hydrogen production by the marine cyanobacterium. *Phormidium valderianum* BDU 1996;57:11–116. https://doi.org/10.1016/0960-8524(96)00050-8.
[150] Wilson ST, Caffin M, White AE, Karl DM. Evaluation of argon-induced hydrogen production as a method to measure nitrogen fixation by cyanobacteria. J Phycol 2021;57:863–73. https://doi.org/10.1111/jpy.13129.
[151] Melnicki MR, Pinchuk GE, Hill EA, Kucek LA, Fredrickson JK, Konopka A, et al. Sustained H_2 production driven by photosynthetic water splitting in a unicellular cyanobacterium. mBio 2012;3. https://doi.org/10.1128/mBio.00197-12. e00197-12.
[152] Park J-W, Nam SW, Kim HS, Youn S-H, Yih W. Enhanced photobiological H_2 production by the addition of carbon monoxide and hydrogen cyanide in two unicellular N_2-fixing cyanobacterial strains isolated from Korean coasts. Ocean Sci J 2014;49:11–8. https://doi.org/10.1007/s12601-014-0002-0.

[153] Ferreira A, Gouveia L. Chapter 28 – microalgal biorefineries. In: Jacob-Lopes E, Maroneze MM, Queiroz MI, Zepka LQ, editors. Handbook of microalgae-based processes and products. Academic Press; 2020. p. 771–98. https://doi.org/10.1016/B978-0-12-818536-0.00028-2.

[154] Kamra A, Kaushik A. Enhanced hydrogen production by immobilized cyanobacterium *Lyngbya perelegans* under varying anaerobic conditions. Biomass Bioenergy 2014;63. https://doi.org/10.1016/j.biombioe.2014.01.019.

[155] Abedi S, Astaraei FR, Ghobadian B, Tavakoli O, Jalili H, Chivasa S, et al. Bioenergy production using *Trichormus variabilis* – a review. Biofuels, Bioproducts & Biorefining 2019;13:1365–82.

[156] Cassier-Chauvat C, Veaudor T, Chauvat F. Advances in the function and regulation of hydrogenase in the cyanobacterium *Synechocystis* PCC6803. Int J Mol Sci 2014;15:19938–51.

[157] Hannah S, Shafaat OR, Hideaki O, Wolfgang L. [NiFe] hydrogenases: a common active site for hydrogen metabolism under diverse conditions. BBA – Bioenergetics 2013;1827:986–1002.

[158] Ahuja D, Tatsutani M. Sustainable energy for developing countries. SAPIENS Surveys and Perspectives Integrating Environment and Society; 2009. . [Accessed 6 April 2022].

[159] Nnabuife SG, Ugbeh-Johnson J, Okeke NE, Ogbonnaya C. Present and projected developments in hydrogen production: a technological review. CCS 2022;3:100042. https://doi.org/10.1016/j.ccst.2022.100042.

[160] Projected benefits of federal energy efficiency and renewable energy programs: FY 2006 budget request. https://digital.library.unt.edu/ark:/67531/metadc1406424/. [Accessed 6 April 2022].

[161] Ladage S, Blumenberg M, Franke D, Bahr A, Lutz R, Schmidt S. On the climate benefit of a coal-to-gas shift in Germany's electric power sector. Sci Rep 2021;11:11453. https://doi.org/10.1038/s41598-021-90839-7.

[162] Nikolaidis P, Poullikkas A. A comparative overview of hydrogen production processes. Renew Sustain Energy Rev 2017;67:597–611. https://doi.org/10.1016/j.rser.2016.09.044.

[163] Zhang B, Zhang S-X, Yao R, Wu Y-H, Qiu J-S. Progress and prospects of hydrogen production: opportunities and challenges. J Electron Sci Technol 2021;19:100080. https://doi.org/10.1016/j.jnlest.2021.100080.

[164] 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.

[165] Quinn R, Spreitzer G, Lam CF. Building a sustainable model of human energy in organizations: exploring the critical role of resources. Acad Manag Ann 2012;6:1–60. https://doi.org/10.1080/19416520.2012.676762.