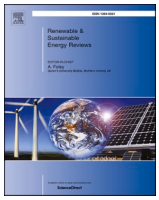
Renewable and Sustainable Energy Reviews 183 (2023) 113389

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

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



# Algal biohydrogen production: Impact of biodiversity and nanomaterials induction

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ARTICLE INFO

*Keywords:*
Bioenergy
Algae cultivation
Biohydrogen
Biodiversity
Nanomaterial

ABSTRACT

Fossil fuels are limited in nature and are not environmentally friendly, thus using them to meet the rising energy needs is insufficient. Another major cause of global warming, which is recognized as one of the greatest hazards to the world, is fossil fuels. Finding alternative energy sources that can counteract the drawbacks of fossil fuels is urgently needed. Due to its low environmental impact and a variety of possible sustainable production methods, biohydrogen is one such alternative energy source that has attracted enormous interest and demand. Due to their wide range of environments, rapid growth, and polyphyletic nature, algae-based biological hydrogen production techniques are gaining significant interest. Nevertheless, the main obstacles to the sustainable and commercial application of the algal biohydrogen generation process are low yield, constrained light penetration, low biomass concentration, and expensive downstream processes. Increased attention to algal diversity may help to overcome the limitation of low algal biomass production and yield while enhancing penetration ability. Additionally, the usage of nanomaterials may speed up the process by altering the entire process' response mechanism. Therefore, this review explores algal diversity as one of the strategies of algal biohydrogen production along with elaboration of the impacts of nanomaterials in different pathways of biohydrogen production, namely dark fermentation, photo-fermentation, direct and indirect biophotolysis. Advances in biohydrogen production employing diversified groups of algae with the application of nanomaterials have been extensively summarized with current update mechanisms and existing roadblocks. As a result, the utilization of nanomaterials as a novel and sustainable catalyst has also been thoroughly described for prospective scaling up of algal biohydrogen production.

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https://doi.org/10.1016/j.rser.2023.113389
Received 29 June 2022; Received in revised form 25 October 2022; Accepted 19 May 2023
Available online 4 July 2023

**Abbreviation full form**

| | |
|---|---|
| BPE | bio-photoelectrolysis |
| NZVI | nano zero valent iron |
| PFR1 | pyruvate: ferredoxin oxidoreductase |
| TCA | tricarboxylic acid cycle |
| ADP | adenosine diphosphate |
| ATP | adenosine triphosphate |
| COD | chemical oxygen demand |
| CoO | cobalt(ii) oxide |
| Cyt $b_6$f | cytochrome $b_6$f complex |
| DF | dark fermentation |
| EPBR | electrochemical photo-bioreactor |
| ESRB | electrochemical sequential batch reactor |
| Fd | ferredoxin |
| $Fe^0NP$ | zero-valent iron nanoparticles |
| $Fe_2O_3$ | ferric oxide |
| $Fe_3O_4$ | magnetite |
| FNR | ferrodoxin $nadp^+$ oxidoreductase |
| MgO | magnesium oxide |
| mRNA | messenger ribonucleic acid |
| MW | microwave |
| NAD | nicotinamide adenine dinucleotide |
| $NAD(P^+)$ | nicotinamide adenine dinucleotide phosphate |
| NADH | nicotinamide adenine dinucleotide (nad) + hydrogen (h) |
| NADP | nicotinamide adenine dinucleotide phosphate |
| NADPH | nicotinamide adenine dinucleotide phosphate |
| $NiFe_2O_4$ | nickel ferrite |
| NiO | nickel oxide |
| NPQR | enzyme plastoquinone reductase |
| NPs | nanoparticles |
| ORP | oxidation–reduction potential |
| PC | plastocyanin |
| PDADMAC | diallyl dimethyl ammonium chloride |
| PFOR | pyruvate ferredoxin oxidoreductase |
| PQ | plastoquinone |
| PSI | plastocyanin-ferredoxin oxidoreductase |
| PSII | plastoquinone oxidoreductase |
| ROS | reactive oxygen species |
| rto | respiratory terminal oxidase |
| SiC | silicon carbide |
| $TiO_2$ | titanium dioxide |
| ZnO | zinc oxide |

## 1. Introduction

Due to rapid urbanization and industrialization, there is a constant need for more energy, which has increased the demand for renewable energy sources to address any potential harmful issues of conventional fossil fuels [1]. Fossil fuels currently account for 82% of all energy consumed worldwide, but due to their short lifespan, they cannot meet global demand [2]. Life-threatening diseases in humans may also result from the extraction of fossil fuels. For instance, black lung disease is a common ailment among coal miners. Furthermore, the continual exposure to hazardous chemicals that natural gas drillers endure is harmful to their health. In particular, burning fossil fuels increases the acidity of the environment. Due to these challenges, the environment has been subjected to unpredictable and severe harm [3,4]. Fossil fuels are also one of the primary causes of global warming, making them one of the greatest hazards to the entire planet. Furthermore, the high cost and unsustainable nature of these fossil fuels pose significant obstacles for modern society and future energy demand. Thus, there is a pressing need to develop an alternative energy source that can make up for the drawbacks of fossil fuels, not the least of which is that they are dangerous and unsustainable [2,5].

Renewable energy sources can be considered the efficient, economical, and environmentally beneficial alternative to traditional energy sources that are based on fossil fuels. There are numerous potential sources of renewable energy, including solar energy, wind turbines, hydropower, and biomass-based derived biofuels [6]. The ability of biofuels to replace fossil fuels is what has drawn the greatest interest among them. The term "biofuel" refers to combustible fuels made from organic materials like plant and animal waste [7]. Unlike other renewable energy sources like solar, wind, hydro, and nuclear energy, biomass may specifically be converted directly into liquid or gaseous fuels to help in meeting transportation fuel needs. While numerous long-term renewable energy sources, including hydrogen, fuel cells, and batteries, are under development, biofuels are one of the few carbon-neutral fuels that are currently highly demanded [8]. Biofuels are highly eco-friendly, burn cleanly, and release substantially less greenhouse gas than fossil fuels. As a result of their ability to produce carbon-neutral carbon dioxide when burned, they are effective replacements for fossil fuels [9]. Carbon-neutral $CO_2$ has zero carbon footprints as it has no residual consequence on the biosphere's carbon concentration [10].

Because biomass is a carbon-rich, renewable, and affordable bio-resource, producing biofuels using it is seen to be a promising strategy to address numerous problems in this context [11]. Biofuels are potential renewable energy sources that can be produced from a variety of organic waste biomass and are considered to be environmentally beneficial. They may be conveniently accessible and inexpensive for a number of applications [12]. There are many possible biofuel choices, including ethanol, biodiesel, methane, and biohydrogen, that are in great demand for commercial use in the energy sector. Additionally, because biofuels degrade naturally, there is less chance that they will contaminate the ground or subterranean water while being transported, stored, or used [6]. In comparison to other energy sources, biofuels may also be employed with existing infrastructure and demand less technological advancement [3]. Because of this, various countries have focused on developing their bioenergy industries and creating cutting-edge regulations for the use of biofuels [13]. First-generation biofuels are those produced from food crops, and include bioalcohols, biodiesel, vegetable oil, bioethers, and biogas. In contrast, second- and third-generation biofuels (such as biohydrogen and biomethanol) are derived from the wastes of non-food crops and microorganisms (like algae), respectively [14].

Due to its advantages of $CO_2$-free burning, inexpensive substrate processing, and the release of water vapour as a byproduct, biohydrogen is thought to be the most promising and clean biofuel option [15]. Additionally, it can be produced using a variety of versatile techniques, including pyrolysis, electrolysis, gasification, and biological mode. The metabolic product is biohydrogen, which is produced by microbial fermentation using readily available, environmentally friendly, and renewable substrates [16]. Additionally, biological ways of producing biohydrogen include a variety of processes such biophotolysis, microbial electrolysis, dark fermentation, and photo fermentation, all of which are practical, viable, and efficient in the context of ecofriendly environment [17]. However, there are a number of problems that prevent its actual viability in the commercial sector, including a lack of an appropriate substrate, poor $H_2$ generation, a lack of process implementation, practicality, and storage [18]. These constraints can now be efficiently overcome by using biological sources for hydrogen production, such as employing waste biomasses, crop leftovers, and microorganisms like bacteria and algae. Contrary to most biological sources, micro and macro-algae have a number of advantages, including a rapid rate of

growth [19], low energy requirements, high-quality water and land, a limited impact on the environment [16]; and the absence of lignin, which eliminates the need for expensive pre-treatment procedures [20].

Algae, both micro and macro, are emerging as a very promising sustainable biomass feedstock for biofuels. Microalgae have a large amount of lipid and carbohydrates, which are used as a key bioresource for the production of biofuel [21]. The main benefit of algae is its higher productivity and its small land requirement need for cultivation [22]. The fact that algae are highly adaptive and diverse should indeed be emphasized. Because of their capacity to thrive in a variety of environments and follow both photoautotrophic and heterotrophic modes, they are also the most robust organisms on Earth [21]. The ability to screen algae for biofuel applications is facilitated by the huge diversity among different organisms. Following the identification of the hydrogen-producing green algae *Scenedesmus* sp., cyanobacterial strain, *cyanobacterial* strain, *Anabaena* cylindrica was also reported to produce hydrogen from water [23]. These findings enlightened the scientific community and led to the characterization of various cyanobacterial as well as algae species for their potential to produce biohydrogen as a biofuel [24]. About 70 species from more than 30 genera have been investigated so far for their capacity to produce biohydrogen [25].

Production of biohydrogen in algae employs two methods: photolysis and fermentation. Additionally, biophotolysis is carried out in two ways, namely direct photolysis and indirect photolysis, whereas fermentation involves two separate modes, namely the, photo-fermentation and dark fermentation [26]. However, the inability to find a potent algal strain, the ineffectiveness of contributing enzymatic reactions with oxygen sensitivity, negative net energy balances, and the low hydrogen yield affected by the integral cell wall of algal biomass are the main obstacles that must be overcome for the commercial use of algal biofuel [27]. The development of effective photo-bioreactors, the use of genetic engineering, the improvement of pretreatment procedures, and the use of nanocatalysts are some of the novel ways aimed to increase the production of fermentative hydrogen [28].

Nanotechnology has a lot of potential, therefore it might be an advanced way to get through the low production issues that occur with using waste biomass to produce biofuel [29]. Nanomaterials have drawn a lot of attention for improving biohydrogen production due to their high surface area to volume ratio and enormous potential to boost enzymatic activity (hydrogenase) [30]. Nanomaterials have the potential to enhance the production of biofuels due to their high catalytic efficiency, high immobilization capacity, and potential to increase enzyme stability [29]. The literature lists a number of metal and metal oxide based nanomaterials, including iron, nickel, gold, $TiO_2$, CoO, $Fe_2O_3$, and NiO, as being effective in enhancing the activity of the enzyme (hydrogenase) and thereby increasing the hydrogen production in the case of third generation biofuels, or biofuels produced from algae [31]. Nanomaterials are internalized by microbial cells as a result of their unique physical and chemical properties, such as size, shape, and surface charge, which also change the pace at which electrons move between microorganisms [32]. They are therefore helpful in the generation of hydrogen due to their increased activity towards the conductivity of pyruvate synthase [33]. Additionally, the presence of nanoparticles enhanced the activation of acetyl-CoA, which stimulates the production of carbohydrates. An improvement to the metabolic pathway speeds up the conversion of $H^+$ into hydrogen *via* NADH, increasing the yields of biohydrogen [34]. Through the research studies, it has been proven that the use of nanomaterials lessens the lag phase of microbial life, including bacteria and algae, and enhances the primary route involved in the production of hydrogen [29,32,35]. Additionally, the production of many enzymes involved in the metabolic activities of the microbial system, such as glutamine synthetase and glutamate dehydrogenase, is enhanced by nanomaterials [36]. Nanoparticles also speed up the metabolic processes of microbial cells, including the formation of proteins and lipids. Due to their potential to enhance metabolic engineering by increasing product yield, nanoparticles have recently gained a great deal of attention [37]. Therefore, technology based on nanomaterials has been widely used to increase the biohydrogen production yields [36].

In light of algal diversity and nanomaterials application, the current review intends to focus recent developments in algal biohydrogen production. The objective is to focus on the typical process technology's advancement from the standpoint of practical application. The review also highlights a detailed analysis of the diversity of algae to identify the strain that is most likely to prevail and their adaptability to the production of algal biohydrogen, as well as any existing obstacles. As a result, the detailed application of nanomaterials as a sustainable catalyst for prospective scaling-up of algal biohydrogen production was also demonstrated [38]. By enhancing the functional stability and strain tolerance of the involved enzymes, the review also investigated the potential of nanomaterial applications in the production of algal biohydrogen [39,40].

## 2. Diversity of algal biomass to produce biological hydrogen

In nearly all ecosystems on Earth, from hot springs to snow-capped mountains and from the deep oceans to freshwater lakes, algae and cyanobacteria are present [38]. Currently, there are 40,000 reported species of microalgae [41], 2698 species of cyanobacteria [42], and 11, 017 species of macroalgae distributed widely [43]. Based on the environmental conditions, their habitat, diversity, morphology, and biochemical compositions can vary together with their action mechanism of ecological adaptability [38]. Fig. 1 depicts diversity of algal biomass imperative to produce biological hydrogen [44]. The advantages of screening algae for various opportunities are provided by the great diversity of organisms. The potential for algae and cyanobacteria to produce hydrogen in a sustainable manner has received much attention [45]. Up till now, the potential for the production of biohydrogen has been investigated in over 70 species from more than 30 genera. Some of these genera include *Chlorella* sp., *Chlamydomonas* sp., *Scenedesmus* sp., *Anabaena* sp., *Spirulina* sp., *Nostoc* sp., *Platymonas* sp., *Coelastrella* sp., *Tetraspora* sp., *and Monoraphidium* sp., *Closterium, Dictyosphaerium, Dimorphococcus, Euastrum, Pandorina, Selenastrum, Staurastrum, Stigeoclonium, Ulothrix*, etc [46–49].

Amongst varied microalgae, *Chlorella* species is considered to be the most promising for a higher hydrogen production yield, which ranges from 6.1 to 31.2 mL $H_2 g^{-1}$ microalgae [50]. The Chlorella genus falls under the family Chlorophyceae. The cells are small, non-motile, sphere-shaped ranging from 2 to 10 μm in diameter. This genus is always in the limelight due to its simple morphology, fast growth rate, and low sensitivity to contamination [50]. Also, Chlorella's cell walls are thick, and glucosamine is a main part of the walls. When they are grown in a bioreactor, the thick wall does not allow any damage to the cells during mixing and pumping. However, this rigid cell wall prevents the biomass from being hydrolyzed effectively, which lowers the availability of nutrients that can be assimilated and, as a result, lowers the amounts of hydrogen production [51]. Some of the species known for their biohydrogen production potential include *Chlorella vulgaris* [46], *Chlorella pyrenoidosa* [52], *Chlorella fusca* [53], *Chlorella lewinii* [54], *Chlorella homosphaera* [55], *Chlorella kessleri* [56], and *Chlorella sorokiniana* [46]. *Chlorella vulgaris* and *Chlorella pyrenoidosa* have been the focus of most research done to examine the potential of the Chlorella genus for the production of biohydrogen. In a recent work, Ruiz-Marin et al. studied the ability of *Chlorella vulgaris* cultivated in urban wastewater to produce biohydrogen through sulphur deprivation under the experimental conditions of 140 $\mu E/m^2/s$ of light intensity, pH 7.5, and 30 °C. The obtained result showed that under purple light, *C. vulgaris* produces 60.4 $mLH_2L^{-1}$ of hydrogen (39.18 mL $H_2$/L/day productivity) [46]. Liu et al. reported *Chlorella vulgaris* HAU-M1, isolated from Henan Agricultural University China, for hydrogen production under various hydrolase and inoculation doses. The results showed that using photo-fermentation, hydrogen generation could reach up to 52.16 mL/g

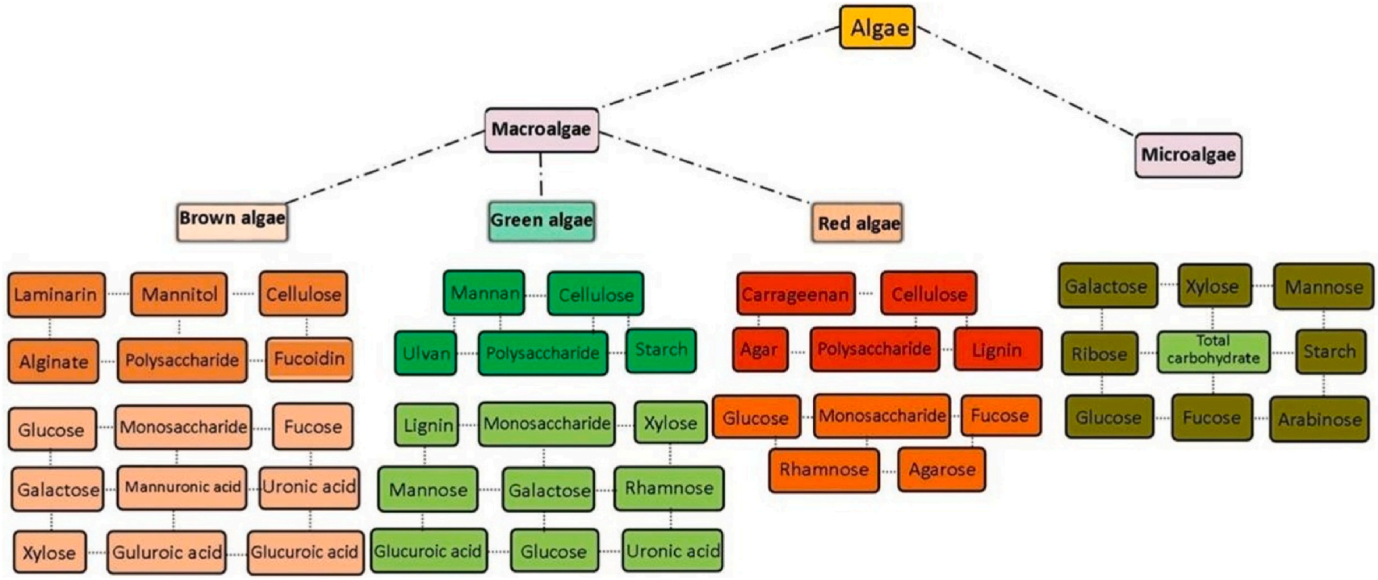


**Fig. 1.** Diversity of algal biomass and carbohydrates present therein to produce biological hydrogen [Adapted with permission from (Ref. 44)].

under the optimal conditions of 10% inoculum and a 3:1 cellulase:protease ratio [57]. Fan et al. investigated the photobiologic production of biohydrogen in *Chlorella pyrenoidosa* FACHB-1222. The results showed that *C. pyrenoidosa* had $O_2$-tolerance, which led to a maximal hydrogen production rate of 0.16 0.04 $mLL^{-1}h^{-1}$ [58]. *Chlorella pyrenoidosa*, on the other hand, was shown to produce 43.2 $mLL^{-1}$ of hydrogen in low-light and anaerobic conditions without the PS II activity being inhibited by sulphur shortage or the addition of carbonyl cyanide m-chlorophenylhydrazone [59]. Li et al. investigated *C. pyrenoidosa* strain IOAC707S for increased photohydrogen generation under nitrogen deficiency in sea water medium [60]. The same research group later studied the underlying mechanism using transcriptomics and proteomics. They found that the PSII photochemical activity and oxygen evolution reduced in the algae under nitrogen starvation because of its complex amendment to formulate for hydrogen production. The activity of the pentose phosphate pathway and glycolysis both increased, indicating more electron transfers from the endogenous substrate for hydrogen photosynthesis. Additionally, reduced activity of the tricarboxylic acid cycle (TCA) lead to declined $CO_2$ levels, which is considered to restrict Calvin cycle activity, and then cause the aggregation of NADPH followed by reduction of the photosynthetic electron transport chain [61]. These circumstances together trigger the hydrogenase activity, which favors hydrogen production.

The evolution of oxygen during the photosynthetic electron transport chain has always acted as a setback while using green algae for hydrogen production [62]. This is because the evolved oxygen inhibits the hydrogenase enzyme and prevents the formation of hydrogen gas [63]. If this issue can be resolved, chlorella might be one of the most beneficial raw materials for producing biohydrogen [64]. Nevertheless, a few studies also report that tolerance to the oxygen concentration in any microalgae is strain-dependent; therefore, if a high oxygen tolerant strain can be identified, it can serve as a potential feedstock for biohydrogen production. Hwang et al. investigated the effect of oxygen concentration (0–25%) on the hydrogenase activity in two strains of *Chlorella vulgaris; i.e., Chlorella vulgaris* YSL01 and *Chlorella vulgaris* YSL16. They observed that the hydrogenase activity and its mRNA expression continued to stay high till the oxygen concentration reached 21%. Beyond 21% of oxygen, a reduced hydrogenase activity was observed and also the hydrogen production got ceased [65]. Fan et al. investigated the tolerance to high oxygen levels in *Chlorella pyrenoidosa*. This study also reported production of hydrogen under high oxygen concentration (>21%). This tolerance was quite high compared to other reported strains like *Scenedesmus obliquus*, *Chlorellafusca*, *Chlamydomonas reinhardtii* and *Chlorellamoewusi* which had an $O_2I_{50}$ (concentration of added oxygen) values of 1.23%, 0.88%, 0.56% and 0.30%, respectively [58]. Several studies also investigated to explore different methods to prevent the inhibition of hydrogenase enzyme by oxygen that is generated during photosynthesis [39,58,63]. One such study by Xiong et al. reported that salinized *Chlorella pyrenoidosa* strain, treated with poly (diallyl dimethyl ammonium chloride) (PDADMAC), formed aggregates of 100 μm displaying silica nanoparticles on the cell surface. These aggregates of *Chlorella pyrenoidosa* created a balance between the production of electrons during photosynthesis and hydrogenase activity in the core-shell structure of algae clusters, thus creating an interior free of oxygen. The amount of hydrogen produced by *Chlorella pyrenoidosa* using this approach was greatly increased, reaching 1.7 mmol $L^{-1}$. Manipulation at the genetic level has also been attempted to deal with the issue of oxygen-mediated hydrogenase inhibition [66]. Yang et al. studied genetically engineered a *Chlorella* strain to modify a few amino acid residues at the gas tunnel to restrict the entry of oxygen at the hydrogenase enzyme's active site. As a result, it was found that a mutant that carried double mutation produced nearly 30 fold more hydrogen than the wild type during biophoton-catalysis [67].

*Chlamydomonas* is another genus of green microalgae that has extensively been studied for its ability to produce hydrogen. Species like *Chlamydomonas moewusii*, *Chlamydomonas noctigama*, *Chlamydomonas reinherditii,* etc., have been reported to evolve hydrogen during photosynthesis. Compared to *Chlorella*, *Chlamydomonas* cells are oval, motile, and larger in diameter. Among the diverse group of microchlorophyceae, *Chlamydomonas reinherditii* is the most thoroughly studied species. It serves as an ideal organism for understanding the underlying mechanism of biohydrogen production in green microalgae [66,68]. Some studies suggest that *Chlamydomonas reinherditii* is a better feedstock for biohydrogen production than Chlorella [58,69]. Although many studies have been conducted on hydrogen production using green algae, its actual prospect was realized when under sulphur-deprived conditions; a strain of *Chlamydomonas reinhardtii* was found to produce prolonged hydrogen at a conversion efficiency of 0.1%. Since then, many omics approaches, including genomic, transcriptomic, proteomic, and metabolomics have been employed to comprehend the molecular mechanism of hydrogen production under the starved condition in green algae, and find out ways to enhance hydrogen productivity by making alterations to biochemical as well as the molecular level [46]. Xu et al. reported *Chlamydomonas reinhardtii* for biohydrogen production which

includes genomics, transcriptomics, proteomics and metabolomics approach. In this work, key genes for $H_2$ metabolism and $H_2$ buildup, including HydA 1, HydA 2, Sulp, Tla1, Sta 7, and PFL1, were recognized and examined [68]. Another approach increased the ability of *Chlamydomonas reinharditii* to produce hydrogen by 24%, 46%, and 32% when it was co-cultured with bacteria like *Escherichia coli, Pseudomonas stutzeri, and Pseudomonas putida*, respectively. Also, when the same algae was grown with an unknown bacterial consortium, hydrogen production rose to 56% [70]. Nomanbhay et al. reported increased bio-hydrogen yield in *Chlamydomonas reinhardtii* CCAP 11/32A by immobilization (using chitosan) and co-culturing techniques. The results showed that the maximum amount of hydrogen that could be produced was 650,000 ppm, and that the optimal ratio of microalgae to ragi tapai was 1:0.25 [71]. On the genetic level, the hydrogen production capacity of *Chlamydomonas reinherditii* was enhanced by inducing mutations on the D1 protein of PSII. The modifications resulted in lowering the photosynthetic activity, followed by reduced oxygen production. Due to lower oxygen evolution, total hydrogen production by the mutants reached a maximum 290 mL $L^{-1}$, which was approximately 3.5 times more than that of control [72].

Zhang & Vassiliadis reported *Chlamydomonas reinhardtii* for biohydrogen production and their metabolic pathways under non-steady-state photofermentation process. The result obtained illustrated that protein such as NAD(P)H, $NAD(P)^+$ and PQ impact the $H_2$ production rate. Moreover the concentration of protein complexes named as, [fb·PQH]+and [fb·PQ]+also play important role in the process of $H_2$ production [73]. However, despite various advantages, hydrogen production using microalgae still has many unsolved challenges. Very recently, Iqbal et al. reported microalgae to be promising for the biohydrogen production. It was found that *Chlamydomonas reinhardtii*, a microalga, and bacteria can interact to raise the production of biohydrogen. The results showed that a combination of bacteria and algae produced around 50 to 60% more biohydrogen than each of them could produce on their own [74]. This might occur as a consequence of the fact that bacteria use the oxygen that photosynthetic microalgae release and that bacteria can produce $CO_2$, which microalgal cells absorb. It was found that co-culturing *Rhizobium* and *Chlamydomonas reinhardtii* increased the number of algal cells, which improved the microalgal biomass and bioenergy output.

Similar to microalgae, cyanobacteria has seized the spotlight as the cutting-edge feedstock for the production of biohydrogen. Phylum cyanobacteria involved photosynthetic bacteria that sheltered in moist soils and aquatic habitats. They originate in symbiotic relationship with diverse hosts and also arise in extreme environments like salted soils, volcanic ash etc [75]. Cyanobacteria are adaptable to a variety of habitats, including terrestrial, aquatic, and harsh environments such antarctic cold lakes or alkaline soda lakes with a pH range of 9.5–11 [76]. Different types of cyanobacteria such as *Nostoc, Oscillatoria, Spirulina, Microcystis and Anabaena*, spread themselves in different territories such as freshwater, marine, soil crusts, cryoconites [77]. Genera including *Spumigena, Anabaena* spp., *Nodularia* and *Aphanizomenon*, *Trichodesmium* [78] have been also noted to form blooms in different water bodies around the globe. There has been ongoing evidence of bloom formation in the Baltic Sea every summer [79]. Many studies report that cyanobacteria are the potential producers of hydrogen by employing water and solar energy [75–77]. Further, *Anabaena* is a type of cyanobacteria usually identified as blue-green algae that grow naturally in many water bodies. Anabaena cells are bright blue-green and cylindrical, barrel-shaped, or sphere-shaped morphologically; they range in size from 2 to 10 μm in diameter [80]. Genus *Anabaena* exhibits straight, curved, or coiled trichomes that may constrict at the cell walls . They are considered to be heterocystous, filamentous, nitrogen-fixing plankton that can be found in both brackish and freshwater environments [81]. To date, various strains from the *Anabaena* genus have been identified as potential feedstock for different biofuel production, including biohydrogen. Nevertheless, the metabolic mechanisms of its biohydrogen production have not been investigated. Shahkouhi et al. identified diazotrophic filamentous cyanobacterium, *Anabaena variabilis* ATCC 29413 as the potential hydrogen producer, and has been acknowledged as a prominent candidate for biohydrogen production. This strain is a thermotolerant and develops well at around 40 °C. In this study, a regulated two-cell, vegetative and heterocyst integrated metabolic model approach was used to increase the hydrogen production in heterocyst cells [82]. According to the proposed concept, heterocysts with an anoxygenic atmosphere can be used to stimulate other chemical reactions and provide redox cofactors, which are crucial for increasing hydrogen production by about 60% *via* bidirectional hydrogenase. Kossalbayev et al. reported *Anabaena variabilis* A-1 (wild-type cyanobacterium) which was isolated from the rice paddies of Kazakhstan (Kyzylorda and Almaty regions). The obtained result demonstrated enhanced nitrogenase activity, productivity, and a stronger capacity of *Anabaena variabilis* A-1 to produce hydrogen in the dark (8.67 μmol $H_2$ $mg^{-1}$ Chl a $h^{-1}$), which meets the highest yield recorded in the literature [83]. Vargas et al. reported *Anabaena* sp. (UTEX1448) for hydrogen production under the nitrogen-deprived condition. The obtained result exhibited that the maximum hydrogen production under the optimized condition was 9.73 mmol $L^{-1}$ and 43.3 pmol $cell^{-1}$ at pH 7.5 and temperature range between 30 and 40 °C, respectively. In comparison to control, the result obtained showed an increased hydrogen production and productivity *via* cell by 55.2% and 57.6% (67.07 μmol $L^{-1}$ $h^{-1}$), respectively. Additionally, the production rate was 70.5% higher (0.307 $h^{-1}$) than the control [84]. A higher yield in $H_2$ production rate was due to the optimization of biomass during the initial stage of the cultivation, resulted enhanced yield around 18.3% along with advanced heterocyst's formation i.e., 3.4 times greater in nitrogen deprived environments. The overall production was improved by 55.2% as well as yield by 57.6% in comparison to the control culture of *Anabaena* sp.

Among the cyanobacteria, genus *Synechocystis* has also received much consideration as a model photosynthetic cell factory for the production of biohydrogen. *Synechocystis* is a genus of unicellular, freshwater cyanobacteria in the family Merismopediaceae. It can switch between anoxigenic and oxygenic photosynthesis, acquiring electrons from an anoxic $H_2S$ or an oxygenic $H_2O$ source (oxygenic) [85]. Touloupakis et al. elucidated cyanobacterium *Synechocystis* sp. PCC 6803 for hydrogen production through oxygenic photosynthesis in a two-chamber bio-photoelectrolysis (BPE) cell [86]. In this study a wild-type as well as mutant type strain were employed since they lack all three respiratory terminal oxidase activities (rto) under different salt environments. Under the low salt concentration, rto mutant exhibited a less photosynthetic rate compared to wild type. In contrast an increased rate was reported by wild type under the high salt concentration. Mutant rto showed 3 times increase in (Fe $[CN]_6$) reduction rates mutually low and high salt. Highest $H_2$ yields and efficiency factors were observed under the high salt conditions and resulting in a highest $H_2$ production rate of 2.23 mL $H_2$/l/h [86]. Very recently, Kossalbayev et al. reported *Synechocystis* sp. S-1 (cyanobacterial strains) isolated from rice paddies in Kazakhstan for biohydrogen production. The obtained result exhibited highest $H_2$ production rate of 2.35 μmol $H_2$ $mg^{-1}$ Chl a $h^{-1}$ *via* Synechocystissp. S-1 by employing light as a source of energy amongst the 13 isolated cyanobacterial strains [83].

In the series of cyanobacteria, *Spirulina* is also known for its ability to produce hydrogen as a byproduct. *Spirulina* is a rod or disk-shaped, symbiotic and filamentous blue-green microalgae. It has a strong presence in marshes, seas, brackish water, and hot springs. High pH, high salt concentration including high level of solar radiation support increased production of spirulina [85]. Hasnaoui et al. reported enhanced bio-hydrogen production with spirulina utilizing an electrochemical photo-bioreactor (EPBR). The obtained result exhibited 4-fold increased $H_2$ production rate in the presence of applied voltage 0.3 V and ~2.5 mA current as compared to that without the application of voltage/current. This rise in bio-hydrogen production could be attributed to decreased concentration of NADPH. It was noticed that the

electrochemical sequential batch reactor (ESRB) delivered an increased production rate as 2.65 $m^3\ m^{-3}$/d, in contrast to the batch mode, which showed 1.2 $m^3\ m^{-3}$/d [87]. Juantorena et al. reported *Spirulina maxima* 2342 for hydrogen production by using sodium dithionite as a reducing agent. This photosynthetic microorganism was reported for the first time for hydrogen production under optimum experimental conditions such as 0.34 ± 0.02 g of biomass, 150 μE $s^{-1}\ m^{-2}$ of light intensity and reaction times of 19.3 ± 1.2 h. In this work $H_2$ production involved two distinct metabolic methods which known as light-dependent photosynthetic process followed by anaerobiosis/darkness (fermentative). The obtained result showed 21 × $10^{-10}$ mol of $H_2 \bullet h^{-1}$/2.5 mg Chlorophyll *a* along with 19.3 h reaction time [88]. Similarly, Ainas et al. examined *Spirulina platensis* collected from the Sahara region of Tamanrasset (South of Algeria) for hydrogen production. In this study three diverse types of photo-bioreactors namely conical, cylindrical, as well as conical with an excavated base were analyzed for hydrogen production. The obtained result explored that $H_2$ evolution *via* the *S. platensis* was enriched by employing a conical photo-bioreactor having an excavated base. Photo-bioreactor showed unique performance in photobiological hydrogen production due to its illuminated surface of 255 $cm^2$ and also exhibited photolysis phenomena in dense cells cultures [85]. Pansook et al. reported halotolerant alkaliphilic cyanobacterium *Aphanothece halophytica* under nitrogen deprivation for enhanced dark fermentative hydrogen production. In this study $H_2$ production was catalyzed from the catabolism of glycogen under anaerobic dark fermentation by hydrogenase enzyme. The objective of this work was to increase $H_2$ production by *A. halophytica* using several types of inhibitors. The results revealed that simazine effectively supported the maximum $H_2$ production under dark among all different types of inhibitors. When A. halophytica was exposed to 25 M simazine for 2 and 24 h, respectively, it produced the maximum $H_2$ at 58.88 0.22 mol $H_2\ g^{-1}$ dry weight $h^{-1}$ and accumulated the most $H_2$ at 356.21 6.04 mol $g^{-1}$ dry weight. Based on the results of this investigation, simazine has the ability to increase dark fermentative $H_2$ production by A. halophytica [89].

Apart from microalgae and cyanobacteria, macroalgae show a wide possibility for biohydrogen production owing to their property of higher carbohydrate content [90]. Macroalgae, also called seaweeds and are classified into three leading groups listed as: Chlorophyta (green algae), Phaeophyceae (brown algae), and Rhodophyta (red algae). Among 11, 017 species of macroalgae, 1901 are reported green algae, 7083 are described as red algae, and 2033 are termed as brown algae [91]. Macroalgae as a sustainable biofuel feedstock rank higher compared to other known extra-terrestrial biomasses. They require less energy and water for their growth, absorb a significant amount of carbon dioxide from the surrounding, and contain an insignificant amount of lignin, making them easily hydrolyzed for fermentation. Despite its abundance and worthy biochemical profile, only a few macroalgae have been screened for their potential to produce Biohydrogen [92]. Jung et al. studied eight marine macroalgae strains, namely red (*Porphyra tennera*) green (*Codium fragile*), and brown (Laminaria japonica) for the prospect of biohydrogen production. Out of these eight isolates, Laminaria japonica owned the maximum potential of hydrogen production, i.e., 69.1 mL $H_2$/g $COD_{added}$. Its efficiency was further enhanced to 109.6 mL $H_2$/g $COD_{added}$, by means of thermal pretreatment at 170 °C for 20 min [93]. In another study, Laminaria japonica macroalga was pre-processed with gamma rays (**γ**) to break down the algal biomass cell. The disintegration of cell structure releases dissolved organic compounds, such as protein, and polysaccharides, making them accessible for dark fermentation, which elevated amount of hydrogen produced [94]. The study demonstrated that gamma rays (**γ**) between 10 and 30 kGy successfully disintegrated the macroalga cells. However, the intensity of 20 kGy was optimized as the suitable intensity for maximum hydrogen production, which caused an increase by 71.4%. Another macroalga, *Gelidium amansii,* was reported as a promising macroalgal species to produce biohydrogen. Sulfuric acid hydrolyzed *Gelidium amansii* biomass under mesophilic conditions, at continuous mode could produce hydrogen at a rate of 2.7 $L\ L^{-1}d^{-1}$ and reached a value 1.3 mol/mol substrate hexose at 24 h hydraulic retention time, using immobilized cells as microbial catalyst [95]. The same group also used a similar strategy to reduce the inhibition of 5-hydroxymethylfurfural during the hydrogen production process. They fed a fixed-bed continuous bioreactor, partly packed with hybrid immobilized beads, with acid-treated *Gelidium amansii* hydrolysate comprising 1.5 g 5-$HMFL^{-1}$ as well as 15-g hexose . Using this strategy, they could achieve 2.3 mol/mol of hydrogen yield *via* hexose as substrate at 6 h hydraulic retention time with a hydrogen production rate 20 $LL^{-1}d^{-1}$ [20]. Similarly, Kumar et al. studied, *Chaetomorpha antennina,* marine macroalgae cumulated from southern shoreline of Tirunelveli, Tamilnadu, India for hydrogen production by employing ADS surfactant (ammonium dodecyl sulfate) of 0.0035 g/g TS dosages assisted with microwave disintegration. The obtained result exemplified enhanced $H_2$ generation of 74.5 mL/g COD [96].

The aforementioned studies highlight the dominance of algae throughout a range of habitats and how they have demonstrated a functional role in a range of environmental circumstances. Therefore, it is vital to take into account algae's significant contribution to the hydrogen production process.

## 3. Algae as a biohydrogen feedstock

Algae, as a feedstock to produce biohydrogen production holds a great promise in context to the future implementation of a sustainable and renewable energy source and represent the third generation biofuel. Some of the green algae and cyanobacterial classes which are identified to be potential candidates for biohydrogen production include *Chlamydomonas reinhardtii*, *Chlorella fusca*, *Scenedesmus obliquus*, *and Platymonas subcordiformis, Spirulina pirulina, Anabaena cylindrical* etc [97–99]. Algae, including cyanobacteria, chlorophyceae, and other eukaryotic algae, possess photosynthetic pigments that fix the sun's light energy into energy-rich carbohydrate molecules. Chemically, algal biomass comprises 20–30% carbohydrates, 40–60% proteins, and 10–20% lipids [100]. This composition ratio may however vary among different strains depending on their genotypic makeup, surrounding environmental conditions, and also due to the influence of some biotic factors [101]. Algae isolates that accumulate more carbohydrates, compared to lipids and proteins, are of greater interest for biohydrogen production. The characteristic morphology of algae and its ability to function in both photoautotrophic and heterotrophic modes make acclimatization and energy conversion more efficient for them [102]. They are therefore considered to be one of the most dynamic groups of organisms thriving in all the ecosystems on the earth. Their growth and carbon-capturing capabilities are also much higher than that of terrestrial plants [97]. On average, the growth rate of algae is two-fold higher than that of the land plants, and also, they can capture ten times more carbon than the latter one. The above-mentioned properties display algae as a prominent and potential future source of biohydrogen.

However, there are a few bottlenecks associated with the technology which need to be addressed before the technology can be taken to the commercial level. One such bottleneck includes the hydrolysis of algal biomass for the purpose of fermentation. The presence of the integral cell wall structure in algae cells limits the approachability of the intracellular biomolecule to different hydrolytic enzymes, which results in the overall process compromise in terms of biohydrogen yield [103, 104]. Therefore, it is important to have an effective pretreatment technique for hydrolyzing algal biomass. Pre-treating the algal biomass would promote depolymerization of carbohydrates in the cell wall, which in turn would facilitate the release of the organic biomolecules from the algal cell making them available for degradation [104,105]. Easy and larger availability of these molecules would help the fermenting microbes to degrade the compounds faster and with more efficiency, and thereby offer improved biohydrogen production. Physical, chemical, and biological, as well as a combination of various treatments have been applied to resolve this shortcoming. The most widely used

pretreatment methods to increase carbohydrate hydrolysis involve ultrasonic milling, steam explosion, microwave, chemical oxidation, and enzymatic hydrolysis [106]. In a study, Wieczorek et al. illustrated hydrogen production using enzymatically treated and non-treated biomass of *Chlorella vulgaris*. They observed a seven-fold increase in hydrogen evolution in the case of the cells treated with Omozuka R-10 and macerozyme R-10 compared to the control condition [107]. Roy et al. in a study used both physical and chemical treatment to enhance the yield of hydrogen using microalgae *Chlorella sorokiniana*. Through this study, they could recorded cumulative hydrogen 1.93 $dm^3m^{-3}$ and a hydrogen yield of 958 $dm^3kg^{-1}$ volatile suspended solid or 2.68 mol/mol of hexose by treating the cells with 200 $dm^3m^{-3}$ HCl-heat [108].

The necessity of bioreactors in the production of biohydrogen from algae is another issue . In order to fulfill the increasing fuel demand with the growing technological advancements, large bioreactors are required to culture the microalgae cells. However, because these bioreactors are costly and their use raises production costs, the resulting biohydrogen may not compete with other fuels on the market. Therefore, attempts are being made to design low-cost bioreactors and also optimize the culture conditions to make the process economically viable [109,110]. One such approach includes the integration of two biological systems (e.g. consortia of algae-bacteria). The advantage of co-culturing algae and bacteria is that the overall yield of hydrogen can be increased, and thereby the cost of production can be reduced . To further explain this idea, it should be mentioned that oxygen is released by algae as a result of the production of hydrogen. This oxygen forms the inhibitory loop and hinders the functioning of the hydrogenase enzyme, which is responsible for hydrogen evolution. However, when algae cells are co-cultured with bacterial cells, the bacterial cells utilize oxygen to carry out their metabolism. In this way, both organisms release hydrogen steadily without disturbing each other [111]. Also, the metabolites released by algae cells during their growth are used as a substrate by the bacteria for hydrogen production. Thus, together both these organisms yield higher amount of hydrogen compared to the amount they can produce alone [112]. To further improve the technology of biohydrogen production with the use of genetically engineered strain, nanotechnology, and/or biochemical manipulations, attempts are being made to decipher the underlying metabolic mechanism in algae [113].

### 3.1. Process overview of biohydrogen production by algae

Biohydrogen is produced using algae *via* two separate processes: biophotolysis and fermentation. In the case of biophotolysis, some of the photosynthetic algae, like green algae and cyanobacteria, use photonic energy to release molecular hydrogen with the help of enzyme hydrogenase or nitrogenase [114]. However, in the case of fermentation, hydrogen is produced under anaerobic conditions. Here the algae are used directly as biohydrogen producers or as a substrate to be utilized by a specific group of bacteria to release hydrogen [115]. The different processes of biohydrogen production through algae are represented in Fig. 2 [116].

#### 3.1.1. Biophotolysis

The word biophotolysis has been derived from three Greek words bios = life + photo = light + lysis = decomposition. The process is exclusive within photoautotrophic organisms, of which green and blue-green algae display maximum proclivity [119]. The process of biophotolysis, first functioned in preliminary photosynthetic bacteria and with time evolved in microalgae as well. In both green algae and cyanobacteria, the thylakoid membrane serves as the reaction site, within which the two protein complexes, i.e., PSI (plastocyanin-ferredoxin oxidoreductase) and PSII (plastoquinone oxidoreductase) co-operate to accomplish the process [120]. As the name implies, biophotolysis is a light-driven mechanism in which light energy splits water molecules into protons and molecular oxygen. This process generates electrons that move through a series of protein complexes residing in the thylakoid membrane, reducing nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) [121]. The center from which the electron is transported to the hydrogenase/nitrogenase enzyme is ferredoxin, which acts as the terminal electron acceptor in this electron transport chain. The enzymes hydrogenase/nitrogenase receives these electrons and generates molecular hydrogen. The process of hydrogen production diverges from photosynthesis in the fact that for hydrogen production, the process needs to be carried out under anaerobic conditions [122]. The advantage this process offers is that it does not necessitates the use of any organic form of carbon, making the process economically promising. It primarily depends on the photosynthetic efficiency of microalgae, which is ten times higher than terrestrial plants. Additionally, the approach is eco-friendly and avoids harmful emissions of any sort [41]. Nevertheless, one setback that limits the scope of this method is the inhibitory nature of the oxygen released as a by-product during the photolysis of water. This oxygen inhibits the entire hydrogenase enzyme system and impedes hydrogen production [123]. Apart from this, algae require very high-intensity light to carry out the process, which adds to the production cost [124]. Furthermore, the process of biophotolysis is classified into two types: direct biophotolysis and indirect biophotolysis.

*3.1.1.1. Direct biophotolysis.* The light-capturing pigments in algae and cyanobacteria offer them advantage of accomplishing the process of direct biophotolysis. The process of direct biophotolysis has been described abundantly in chlorophytes. However, only a few studies reported the use of cyanobacteria for biohydrogen production using direct-biophotolysis [125]. The process starts with the capturing of light energy which is used for splitting water molecules inside the cells. This step of photolysis is facilitated by the PSII present in the chloroplast under anaerobic conditions. Diving deep into the biochemistry, for both green algae and cyanobacteria, the photosynthetic pigments are associated with two protein complexes, i.e., PSI (plastocyanin-ferredoxin oxidoreductase) and PSII (plastoquinone oxidoreductase). The antenna pigments capture the excitation energy from the photon source and transfers it to chlorophyll reaction center [121]. The chlorophyll reaction center is the one where the primary charge separation and formation of strong oxidants and reductants take place. Due to charge separation strongly oxidizing cation radical $P_{680}$*, which further catalyzes the water oxidation through a series of redox active components including Mn tetramer complex $Mn_4O_4Ca$ of the oxygen evolving complex of PSII. The electrons released are then relocated *via* a unidirectional electron transport chain from PSII to PSI and finally to iron-sulphur protein, ferredoxin (Fd), which is the terminal electron acceptor [125]. This transport involves various electron transporters, including plastoquinone (PQ), cytochrome $b_6$f complex (Cyt $b_6$f), and plastocyanin (PC). Depending on the cell's requirement, ferredoxin undergoes two fates. In the case of photosynthesis, the electrons are transferred from Fd to NADP to form NADPH by the enzyme ferredoxin-NADP-reductase. This NADPH, along with ATP, powers the Calvin-Benson Cycle. In the case of hydrogen production, ferredoxin donates the electrons to the hydrogen-producing enzymes hydrogenase/nitrogenase [121]. The electron transportation chain *via* the PQ pool forms a proton gradient in the thylakoid membrane's luminal side and generates a proton motive force to run the ATP-synthase to produce ATP molecules.

$$2H_2O \xrightarrow{\text{Sunlight}} 4H^+ + 4e^- + O_2 \left(\Delta G^o = 749 \text{ KJ mol}^{-1}\right) \quad (1)$$

$$4H^+ + 4e^- + Fe_{red} \xrightarrow{\text{Hydrogenase}} 2H_2 + Fe_{ox} \quad (2)$$

*3.1.1.2. Indirect biophotolysis.* Indirect biophotolysis differs from direct biophotolysis mainly on the ground of proton and electron sources. In case of indirect biophotolysis, the protons and electrons are not generated through the splitting of water; instead, they are obtained from

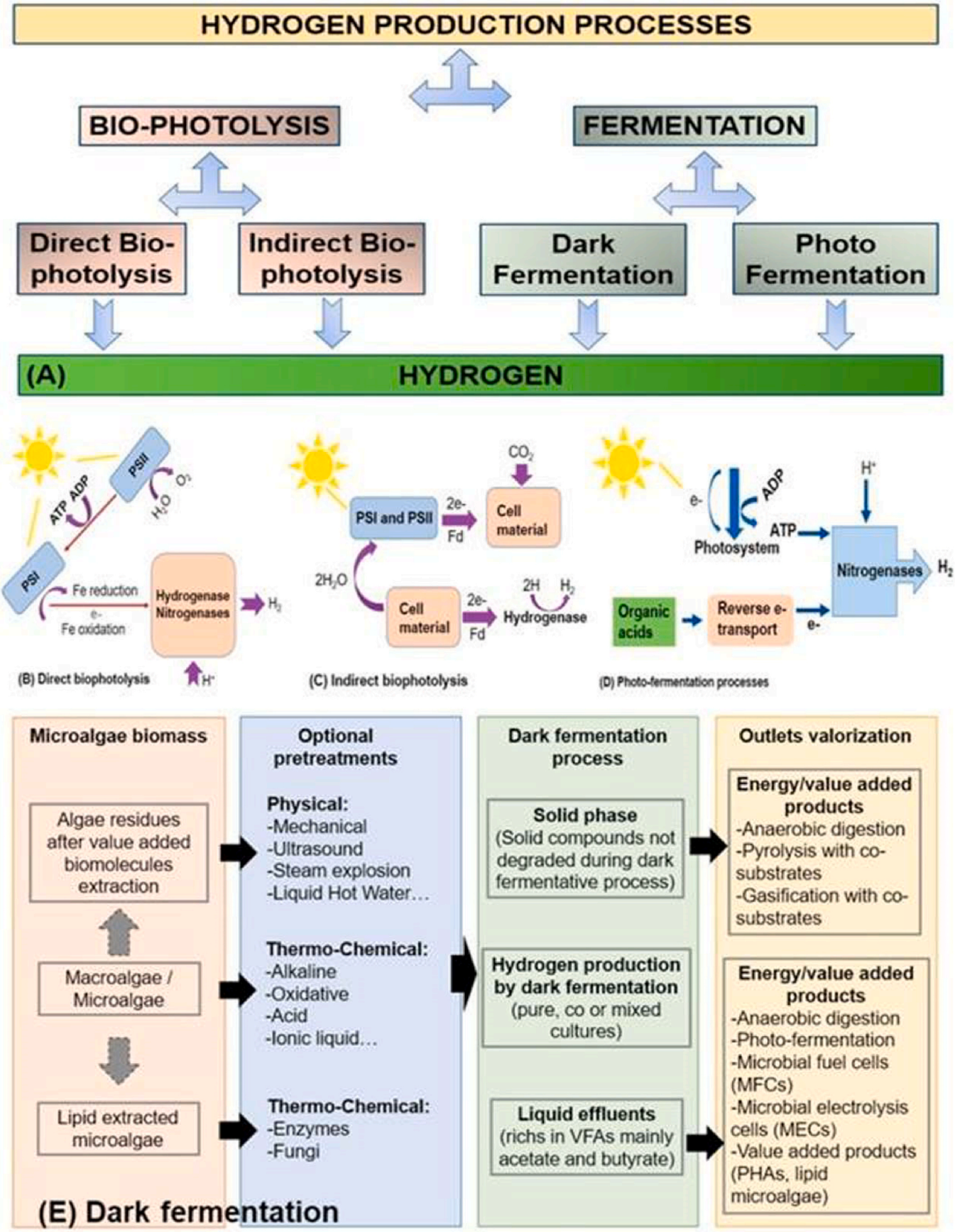


**Fig. 2.** Hydrogen production processes algae (A), direct bio-photolysis (B), indirect bio-photolysis (C), photo-fermentation (D), and dark fermentation processes (E). [Adapted with permission from (Ref. 116); also, credit to Ref. 97, Ref. 117, and Ref. 118].

hexose sugar that was fixed through Calvin-Benson Cycle. Here the NADPH is generated *via* the process of glycolysis [97]. This NADPH directly reduces the PQ pool with the help of NADP/H-plastoquinone oxidoreductase (NPQR). It also reduces ferredoxin, which like in the case of direct biophotolysis, transfers it to hydrogen evolving enzymes for hydrogen production. Indirect biophotolysis comes with the advantage of protecting the nitrogenase enzyme from being inhibited by oxygen [121]. The biochemical mechanism of indirect biophotolysis is divided into two phases: (i) aerobic phase, in which the water and light energy is utilized to form carbohydrates (ii) anaerobic phase in which shortage of oxygen causes the electron transport chain to disfunction, and therefore the low potential electrons are released *via* catabolism of the stored carbohydrate.

$$12H_2O + 6CO_2 + \xrightarrow{\text{Sunlight}} C_6H_{12}O_6 + 6O_2 + 6H_2O \quad (3)$$

$$C_6H_120_6 + 12H_2O + Fe_{red} \xrightarrow{\text{Hydrogenase}} 12H_2 + 6CO_2 + 6H_2O \quad (4)$$

Among both the processes mentioned above, indirect biophotolysis holds the upper hand over direct biophotolysis [125]. This is because, firstly, indirect biophotolysis results in a much better yield of hydrogen when compared to direct biophotolysis. Cheonh et al. mentioned that through indirect biophotolysis, a biohydrogen synthesis rate of 0.355 mmol $H_2$/h for a 337 $m^3$ bioreactor was achieved, whereas through direct photolysis, synthesis rate of only 0.07 mmol $H_2$/h could be obtained that too in a 1707 $m^3$ bioreactor. Secondly, the oxygen evolved from photolysis does not interrupt the production process and can be separated easily from the hydrogen produced [126]. Thirdly, the by-products of indirect biophotolysis can be converted in to hydrogen, which makes the process economically more efficient when compared to direct biophotolysis [127]. The cost of biohydrogen produced *via* indirect biophotolysis is lower compared to direct biophotolysis which is one of the most significant factors. Based on studies reported to date, hydrogen production *via* direct biophotolysis costs between \$2.13 $kg^{-1}$ to \$7.54 $kg^{-1}$, whereas that of indirect biophotolysis reaches around \$1.4 $kg^{-1}$ [23]. Nevertheless, a high input of ATP and a continuous requirement of light remains a question for the economic viability of the indirect biophotolysis approach [23]. Also, the efficiency of indirect biophotolysis is lower as compared to direct biophotolysis due to the involvement of multiple steps [103].

### 3.1.2. Fermentation

The term "fermentation" refers to the process of producing energy from the oxidation of organic waste materials using a variety of different microorganisms. Fermentation process is divided into two types depending on the use of light energy to carry out the process [16]. As the name implies, light-dependent fermentation process is called photofermentation, whereas, the light-independent process is called dark fermentation [128]. Photofermentation occurs in case of purple sulphur and purple non-sulphur bacteria. On contrary, dark fermentation has been found successfully in algae, bacteria as well as in some fugal strain.

*3.1.2.1. Photofermentation.* Anoxygenic photosynthetic purple sulphur and purple non-sulphur bacteria are well known for their ability to produce hydrogen in large amount from endogenous organic substrate. Like algae, they too utilize photonic energy to carry photosynthesis [129]. The photosynthetic machinery of photosynthetic sulphur bacteria is located in the cell membrane. The electron transfer in these organisms takes place in cyclic mode. Also, the reductant for carbon fixation is extracted from organic and inorganic compounds in sulphur and non-sulphur bacteria, respectively [130]. These bacterial species possess both hydrogenase and nitrogenase enzymes, however, for photo-mediated hydrogen production nitrogenase is the preferred enzyme. As photosynthesis in these bacteria does not evolve oxygen, the activity of the enzyme remains unhindered. This process however is not energy efficient as for every one molecule of hydrogen released, four ATP molecules are invested [131].

$$CH_3COOH + 2H_2O + Fe_{red} \xrightarrow{\text{Light,PNS and [Fe−Fe]−Hydrogenase}} 12H_2 + 6CO_2 + 6H_2O \quad (5)$$

*3.1.2.2. Dark fermentation.* Dark fermentation is a photon energy independent process. This method uses bacterial species to break down the polymeric compounds like carbohydrates, proteins, and lipids of the algae cells to release hydrogen [125]. Herein, macroalgae is preferred over microalgae due to their high carbohydrate content. The carbohydrate content of macroalgae varies among 25–60% in Rhodophyta (red macroalgae), 30–60% in Chlorophyta (green macroalgae), and 30–50% in Phaeophyta (brown macroalgae) [132]. The carbohydrates from algae are extracted as fermentable sugars and utilized as a substrate to produce biohydrogen. Theoretically, for every glucose molecule metabolized to pyruvate, 2 mol of hydrogen can be produced during the subsequent regeneration of the NADH.

$$\text{Pyruvate} + \text{CoA} \rightarrow \text{Acetyl} - \text{CoAH} + \text{Formate} \quad (6)$$

$$C_6H_120_6 + 6H_2O + Fe_{red} \xrightarrow{\text{Fermenatative bacteria}} 12H_2 + 6CO_2 + 6H_2O \quad (7)$$

Margareta et al. suggested dark fermentation as a sustainable and promising method for biohydrogen production in macroalga, *Ulva* sp., using the bacterium *Clostridium butyricum*. Under optimized conditions, in a continuous fermentation mode, they obtained the highest hydrogen productivity of 782.45 $mL^{-1}L^{-1}h^{-1}$ and a yield 1.52 mol $H_2$/mol hexose [133]. Apart from this, brown macroalga *Laminaria* sp. and red macroalga *Gelidium* have been reported as potential macroalgal candidates for biohydrogen production [95,134]. Cyanobacteria, *Anabaena* PCC 7120 was also tested for its capability to produce hydrogen by the process of thermophilic dark fermentation using high temperature resistant mixed microflora [135]. The study reported the production of 1600 $mLL^{-1}$ of hydrogen upon using amylase pretreated biomass. Another study reported microchlorophyceae, Spirogyra, as a potential feedstock for hydrogen production. Two-step acid pretreated crude biomass of Spirogyra gave a daily equivalent of 10.4 $LH_2L^{-1}$ of the biomass by employing *Clostridium butyricum* [136]. Dark fermentation is considered a budding mechanism for producing biohydrogen using algae and cyanobacteria. The anaerobic conditions in the dark fermentation process constitute a promising condition for supporting the activity of catalyst for [NiFe] hydrogenase as well as for [FeFe] hydrogenase, resulting in a comparatively higher hydrogen evolution [137]. Additionally, the method displays future scope to be economical with few optimizations, as there is no investment in terms of light energy, and the process also gives valued by-products such as butyric acid, lactic acid, and acetic acid. Nevertheless, dark fermentation is accompanied by a few shortcomings, including lower yield and the additional cost of separating hydrogen from carbon dioxide. The results of the investigations on $H_2$ production using algae are summarized in Table 1.

### 3.1.3. Mechanism of hydrogen production in green algae and cyanobacteria

*3.1.3.1. Mechanism involved in green algae.* The mechanism of hydrogen evolution in green algae is dependent on the fermentative pathway, which comprises of two light-dependent and one light-independent pathway. [119,120]. These pathways are operated in the presence of two classes of unidirectional hydrogenases, i.e., either [Fe] hydrogenases or [FeFe] hydrogenases [137–152]. In case of the first light-dependent pathways, electrons are generated by the photolysis of water molecules at PSII and are transferred through the electron transport chain to Fd. Once the Fd gets reduced, it donates the electrons to the hydrogenase enzyme, which then catalyzes the evolution of hydrogen following the mechanism indicated in equation-8:

**Table 1**
Numerous studies on algae involved in different modes of $H_2$ production and the corresponding cumulative $H_2$ yields under the optimized conditions.

| Sr. No. | Name of the Algae | Methods of $H_2$ production | pH/Temperature | $H_2$ yield/cumulative $H_2$ | Ref. |
|---|---|---|---|---|---|
| 1 | *Echeumaspinosum* | – | 37 °C Temperature | 21.58 ± 1.59 L/L-d | [138] |
| 2 | *Chlamydomonas reinhardtii and Chlorella sorokiniana* | Photoproduction | – | 108 ± 4 μmol $L^{-1}$ and 88 ± 7 μmol $L^{-1}$ respectively | [139] |
| 3 | *Dunaliella primolecta* | Dark fermentation | 85 °C Temperature | 192.35 mL/g VS | [140] |
| 4 | *Ulva reticulate* | Dark fermentation | pH 7.5, temp 37 °C | 92.5 mL $H_2$/g COD | [96] |
| 5 | *Chaetomorpha antennina* | Dark fermentation | pH 6.8, Temp 35 °C | 74.5 mL/g COD | [96] |
| 6 | *Chlorella vulgaris* | Dark fermentation | pH 5.5Temp, 35 °C | 190.90 mL $H_2$/g-VS. | [141] |
| 7 | *Microcystis aeruginosa* | Dark fermentation | pH > 7 | 35 mL/g (dw) | [142] |
| 8 | *Padinate trastromatica* | Dark fermentation | pH 6.5 | 78 ± 2.9 mL/0.05 g (VS) | [143] |
| 9 | *Tetraspora* sp. CU2551 | Photobiological method | pH 6.5 | 35.1 μmol/mgdw | [144] |
| 10 | *Chlamydomonas reinhardtii* | Photoproduction | pH 6.5–7.0, temp. 24 °C | 200 mmol $m^{-2}$ | [145] |
| 11 | *Scenedesmus* sp. and *Chlorella* sp. | Dark fermentation | pH 7.2, temp 37 °C | 37.7 ± 0.4 mL/g (VS) | [146] |
| 12 | *Chlorella vulgaris MSU-AGM 14* | Dark fermentation | pH 7.66, temp 37 °C | 12.6 mL H2/g volatile solids | [147] |
| 13 | *Chlorella sorokiniana* | Dark fermentation | pH, 6.5Temp 60 °C | 958 $dm^3$/kg | [108] |
| 14 | *Anabaena PCC 7120* | Dark fermentation | pH 6.5 and Temperature 60 °C | 1926 mL/L | [135] |
| 15 | *Chlorella vulgaris* | Dark fermentation | pH 7.0 | 43.1 mL $H_2$/g dw | [148] |
| 16 | *Arthrospira (Spirulina) platensis* | Hetero-fermentation | pH 6.5, temp 35 °C | 92.0 mL $H_2$/g-dw | [149] |
| 17 | *Chaetomorpha*sp. GAF 99 | Dark fermentation | – temp 23.2 °C | 30 mL | [150] |
| 18 | *Dunaliella tertiolecta and Chlorella vulgaris* | Photoproduction | pH 7.66, temp 32 °C | 12.6 mL $H_2$/g (VS) and 10.8 mL $H_2$/g (VS) respectively | [151] |

$$2H^+ + 2e^- \rightarrow H_2 \quad (8)$$

In another light-dependent pathway, electrons are generated through the breakdown of the cell's endogenously reserved compounds such as carbohydrates or lipids. When these stored carbohydrates are catabolized, they produce NAD(P)H molecules. These molecules of NAD(P)H generated are further oxidized using the enzyme plastoquinone reductase (NPQR), causing the release of electrons, along with protons and NAD ($P^+$) [73]. These electrons released are then introduced into the electron transport chain, which thereafter reduces Fd, and subsequently activates hydrogenase and generates hydrogen using the same reaction as shown in equation 1.

Green algae follow a light-independent pathway when it is grown in dark anoxic conditions. Under such conditions, endogenously stored starch is catabolically utilized to maintain an alga's metabolism [118]. Here, the electrons for reducing the Fd are derived from pyruvate, and the step is facilitated by Pyruvate: ferredoxin oxidoreductase (PFR1). As the process takes place under dark and anoxic conditions, fermentation products like format, acetate, ethanol, and conceivably hydrogen are produced [153]. Furthermore, numerous studies are still being undertaken globally to look into the genetic basis of hydrogen synthesis. Genes like hydrogenase A, sulfate permease [154,155], pyruvate formate lyase [156], isoamylase [157], etc. were identified to be associated with the process of hydrogen production in algae. In their report, Xu et al. suggested that to understand the underlying mechanism effectively, more intense studies should be deployed using the omics approach to uncover the pathway components regulating the mechanism [68].

*3.1.3.2. Mechanism involved in cyanobacteria.* The hydrogen production mechanism in cyanobacteria depends mainly on the enzymes hydrogenase and nitrogenase. Hydrogenase is presented as two different classes, i.e., (i) uptake [NiFe] hydrogenases and (ii) bidirectional/reversible [NiFe] hydrogenases. All nitrogen-fixing cyanobacteria studied to date possess uptake hydrogenase, whereas bidirectional hydrogenase exists in nitrogen fixer as well as in non-nitrogen fixer strains of cyanobacteria [158]. Nitrogenase ([MoFe] nitrogenase), on the other hand, is found exclusively in nitrogen-fixing cyanobacteria. Cyanobacteria use two different sources as electron donors, i.e., water and stored organic compounds (glycogen, a product of the Calvin Cycle). In one of the pathways for hydrogen production, the enzyme bidirectional [NiFe] hydrogenase accepts these electrons at the level of NPQR. If the electrons are not diverted to NPQR, they are then directed to Fd [154]. In another condition, some of the electrons from Fd are retransmitted to NPQR *via* cyclic electron flow. Under dark and oxygen deficient conditions, the photosynthetic electron transport chain becomes inactive, and NAD(P)H is generated through the catabolism of endogenous stored glycogen. This glycogen is further oxidized by the enzyme NPQR to generate electrons which will be essential for producing hydrogen [117]. The bidirectional [NiFe] hydrogenase is a multi-meric enzyme that contains four to five distinct subunits. The presence of these subunits may, however, vary among different species. Bidirectional [NiFe] hydrogenase is a product of the hoxYH gene and is brought to maturity with the help of the hyp group of proteins [159]. It is located in the cytoplasmic membrane, and being bi-directional, it is capable of producing as well as up-taking hydrogen. Net productivity of hydrogen by bidirectional [NiFe] hydrogenase is better than the other two catalyzing enzymes [160]. Fig. 3 illustrates the bio-hydrogen generation pathways using algal biomass under different modes [125].

The second enzyme, uptake [NiFe] hydrogenase, is the product of hupSL gene and has the capacity to oxidize hydrogen. This hydrogenase is located in the heterocyst of filamentous cyanobacteria, where they are found to be embedded in the thylakoid membrane [161]. In cyanobacteria with uptake hydrogenase, the process of oxyhydrogenation is accomplished by this hydrogenase, which shuttles the electron from hydrogen to reduce oxygen in the process of respiration. Being unidirectional, it is not involved in the production of hydrogen [122]. Therefore, the net hydrogen production in cyanobacterial strains with only uptake hydrogenase lands at zero. The unidirectional reaction catalyzed by the enzyme uptake hydrogenase is shown below in equation-9:

$$H_2 \rightarrow 2H^+ + 2e^- \quad (9)$$

The nitrogenase enzyme is located in the heterocyst of filamentous cyanobacteria. This enzyme plays a major role in fixing atmospheric nitrogen into ammonia and nitrogen [162]. Under nitrogen starved conditions, these nitrogen-fixing species of cyanobacteria produce hydrogen gas

$$16ATP + 16H_2O + N_2 + 10H^+ + 8e^- \rightarrow 16ADP + 16Pi + 2NH_4^+ + H_2 \quad (10)$$

as a by-product of the fixation process. The reaction catalyzed by nitrogenase is shown in equation-10:

The electrons and the ATP molecules required by the nitrogenase enzyme are fetched either from the photosynthetic electron transport

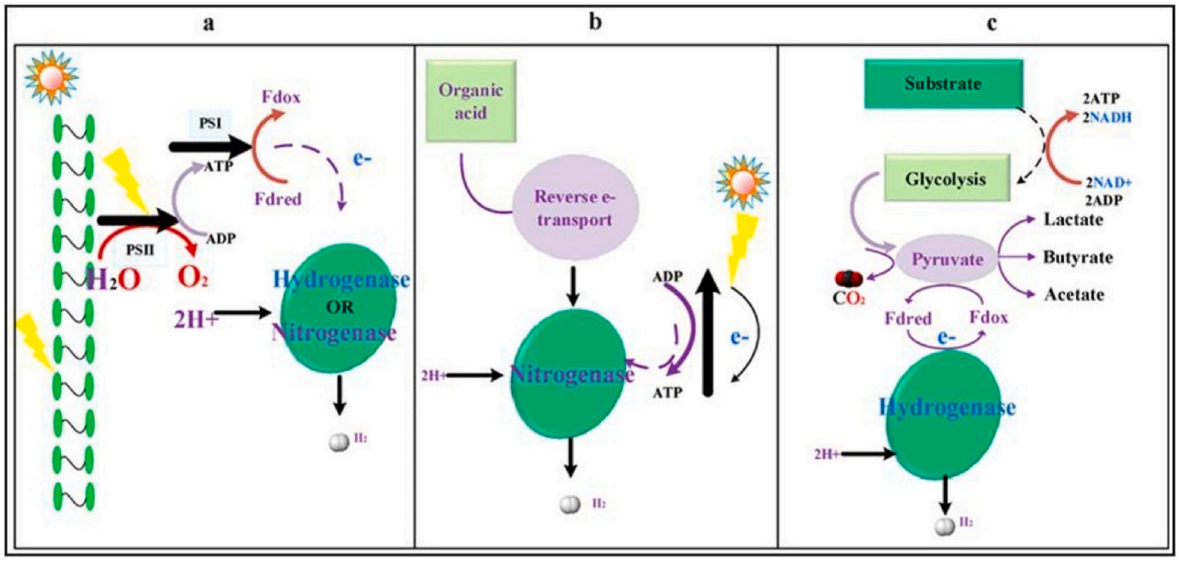


**Fig. 3.** Conceptual illustration of the bio-hydrogen generation pathways: (a) biophotolysis, (b) photofermentation. (c) DF (dark fermentation), PSI represents photosynthesis system 1, PSII is photosynthesis system 2, Fdox is the oxidized Ferredoxin, and Fdred is the reduced Ferredoxin [adapted from [Ref. 125 open access CCBY].

chain or from the breakdown of stored carbohydrates. Nitrogenase consists of two subunits: (i) dinitrogenase and (ii) dinitrogenase reductase . Dinitrogenase is a heterotetramer and functions by cleaving nitrogen atoms. It is a translated product of the nif D and nif K genes. In contrast, dinitrogenase reductase is a homodimer that acts as a mediator during the electron transportation from ferrodoxin/flavodoxin to dinitrogenase [163]. For this reason, the electron obtained from oxidations are initially transmitted to NPQR or FNR (ferrodoxin $NADP^+$ Oxidoreductase) and then forwarded to the electron transportation chain at PQ. The enzyme, FNR, is also capable of unswerving the transportation of electrons to nitrogenase. Apart from this, the reducing power that is spent on the production of hydrogen during the process of nitrogen fixation may be recovered using the uptake [NiFe] hydrogenase which facilitates the consumption of hydrogen [164]. These electrons that were gained during the uptake of hydrogen by the hydrogenase are recycled and directed back into the electron transport chain. In addition to this, the enzyme cytochrome *c* oxidase can also utilize these electrons to reduce molecular oxygen into water molecules. The electrons can also be redirected to nitrogenase past the PSI and Fd (heterocyst-specific) [162]. This hydrogen production process is energy-intensive, as for every electron to be transferred; the pathway utilizes two molecules of ATP. One major drawback associated with nitrogenase enzyme is its sensitivity to oxygen. Under oxygen stress condition, proteolysis of the subunits of the enzyme takes place, as a result of which nitrogenase synthesis gets hampered and further leads to the lack of respiratory substrates and reductants that are must for the fixation and assimilation of nitrogen [164]. As hydrogen is the byproduct of the nitrogen fixation process, suppression of the fixation process also hampers the hydrogen evolution [164].

To overcome this shortcoming the cyanobacterial cells develop specific mechanism which make use of compartmentalization to carry out the process of oxygen evolution in one cell and that of hydrogen evolution in other cells (i.e., heterocyst) [165]. To elaborate this, photosynthetic electrons transportation in cyanobacteria occurs in the presence of sunlight, water and carbon dioxide. In the presence of a photon source, PSII complex in the cyanobacterial facilitates the breakdown of water molecule and releases oxygen [87]. This process is followed by synthesis of carbohydrate through Calvin cycle. The carbohydrate produced is then transferred to the heterocyst cell where glycolysis facilitates the release of energy rich ATP and NADPH molecules [121]. Heterocyst in cyanobacteria lacks PSII and as a result of which even though light and water is available photolysis of water does not take place. Therefore, here the electrons required for the electron transport chain is derived from the carbohydrate produced during Calvin cycle [121]. The electron transport chain in heterocyst starts with PSI and ends at ferredoxin. Electron from the ferredoxin complex is fed to the nitrogenase enzyme, present only in heterocyst, and which further accomplishes the process of nitrogen fixation with evolution of hydrogen [166].

The efficiency of biohydrogen production using cyanobacteria has been investigated in many studies. Rather et al. isolated a cyanobacterial strain (Cyanothece sp. *ATCC 51142*) from the subtidal sands of the texas gulf coast in order to study and understand its potency in producing hydrogen. They found that the strain *Cyanothece* 51,142 had an inherent property to produce hydrogen aerobically and was also flexible to be grown phototrophically and mixotropically. Both hydrogenase and nitrogenase enzymes known for producing biohydrogen, were present in the isolate *Cyanothece 51,142* [167]. Pansook et al. illustrated the mechanism of hydrogen production in halotolerant cyanobacterial species, *Aphanothece halophytica*. Under dark conditions, *A. halophytica* displayed a significant rise in hydrogen production compared to cells grown in the presence of light [89].

## 4. Role of nanomaterial to improve algal biohydrogen production

Low production rate and yield are considered to be two main causes towards the sustainable biological hydrogen production. Herein, incomplete reaction, low efficiency of participating enzymes, partial conversion of substrate as well as high acidic pH of the fermentation medium are the leading issues. To resolve these issues, utilizations of nanocatalysts has been proposed as one of the potential alternate along with various other sustainable approaches such as pretreatments, optimized bioprocessing & operations, co-fermentation and genetic engineering [168,169]. Nanomaterials are well known for their unique physicochemical properties such as high surface area to volume ratio, and quantum confinement. Since co-factors play essential roles in the management of the biohydrogen production process, which is an enzyme-mediated process, and because co-factors are typically metals by nature, nanocatalysts derived from these metals have the potential to enhance the entire $H_2$ production process by altering the molecular functions of enzymes [170]. Enhancing the biological hydrogen ($H_2$)

production process employing nanomaterials in the form of nano-catalysts may result in improved production technology and is a potential strategy. Nanomaterials such as metallic, metal oxides, carbon nanotubes, nanofibers, and nanocomposites have been reported to accelerate algal based biohydrogen production process [171–174]. When producing biohydrogen, nanocatalysts may modulate the intracellular participating enzymes of microorganisms as well as their metabolic activity. In addition to improving enzymatic efficiency, nanomaterials may change the production and activity of the enzymes, speeding up the process of producing hydrogen [172–174].

### 4.1. Mechanism of nanomaterials induction in biohydrogen production

Nanomaterials are well known for their potential use in accelerating biohydrogen generation because of their impact on metalloenzymes (cofactor), intracellular electron transport, and microbial growth rate [175]. Nanomaterials are able to easily interact with microbial cell walls, internalize, and consequently dramatically alter the microbial metabolism because of their extremely small size, large surface area, presence of surface charge and strong penetrating efficiency [176]. Reactive oxygen species (ROS) can be tailored once nanomaterials are entered the microbial cell, providing a more favourable environment for fermentative microorganisms involved in the synthesis of biohydrogen, including the induction of biohydrogen-producing enzymes [171]. Through binding to the active sites of the enzymes, nanomaterials act as a catalyst and are anticipated to activate the function of hydrogenase and nitrogenase, two crucial enzymes in the production of hydrogen [177]. In order to function effectively and sustain their structural integrity, hydrogenase and nitrogenase enzymes need the metallic co-factors iron and nickel in their active sites [177]. In this scenario, nanomaterials function as a cofactor to increase hydrogenase enzyme efficiency (sensitive to oxygen and impeding large-scale application) and speed up the process of $H_2$ generation [174]. Fig. 4 explores a general mechanism of improved biohydrogen production in presence of nanomaterials [174].

Because $Fe^{2+}$ is a crucial component of the hydrogenase active core, it has been reported in the literature that $Fe^{2+}$ could increase hydrogenase activity [171–178]. Similarly, Elreedy et al. reported the enhanced activity of dehydrogenase enzyme involved in $H_2$ production *via* the application of Ni NPs. This led to a maximum $H_2$ yield of 130 6.0 mL/L and a 30% improvement over the control at doses of 20 mg/L NiO NPs [179]. In a similar study, Pandey et al. investigated the impact of $TiO_2$ NPs on the hydrogenase enzyme. The obtained results demonstrated 1.54 times greater $H_2$ generation when 60 g/mL of $TiO_2$ nanoparticles were employed [180]. Another important enzyme in hydrogen fermentation is pyruvate ferredoxin oxidoreductase (PFOR), which catalyzes the conversion of pyruvate to reduced ferredoxin and acetyl-CoA. Given that this enzyme's active core contains three (4Fe–4S) clusters, the application of nanomaterials like $Fe^0$ NPs may promote PFOR activity [181]. Nanomaterials also enhance the rate of electrons transfer at microbial interfaces because of its characteristic to bind to the active site of the enzymes. As a result of their high interfacial area and quantum size, ferredoxin oxidoreductase functions more speedily. Thus, increase in $H_2$ production during the DF process is primarily due to the ferredoxin oxidoreductase's enhanced activity [174]. As per the findings of previous studies, two pathways have been discussed for the production of hydrogen *via* glucose fermentation [109,174,182]. First one is being formate $H_2$ production in which formate is broken down *via* the protein formate hydrogen lyase. While in the second case, reduced NADH dependant hydrogen production method has been discussed [109,177]. In the latter case, the formation of $H_2$ is aided *via* hydrogenase enzyme, where the re-oxidation of NAD is observed. Further, nanomaterials supplementation accelerates the production of acetate and delays the concentration of $C_2H_5OH$ in DF [177].

The enhanced photocatalysis induced by nanomaterials is also believed to be responsible for improved photo-fermentation of organic

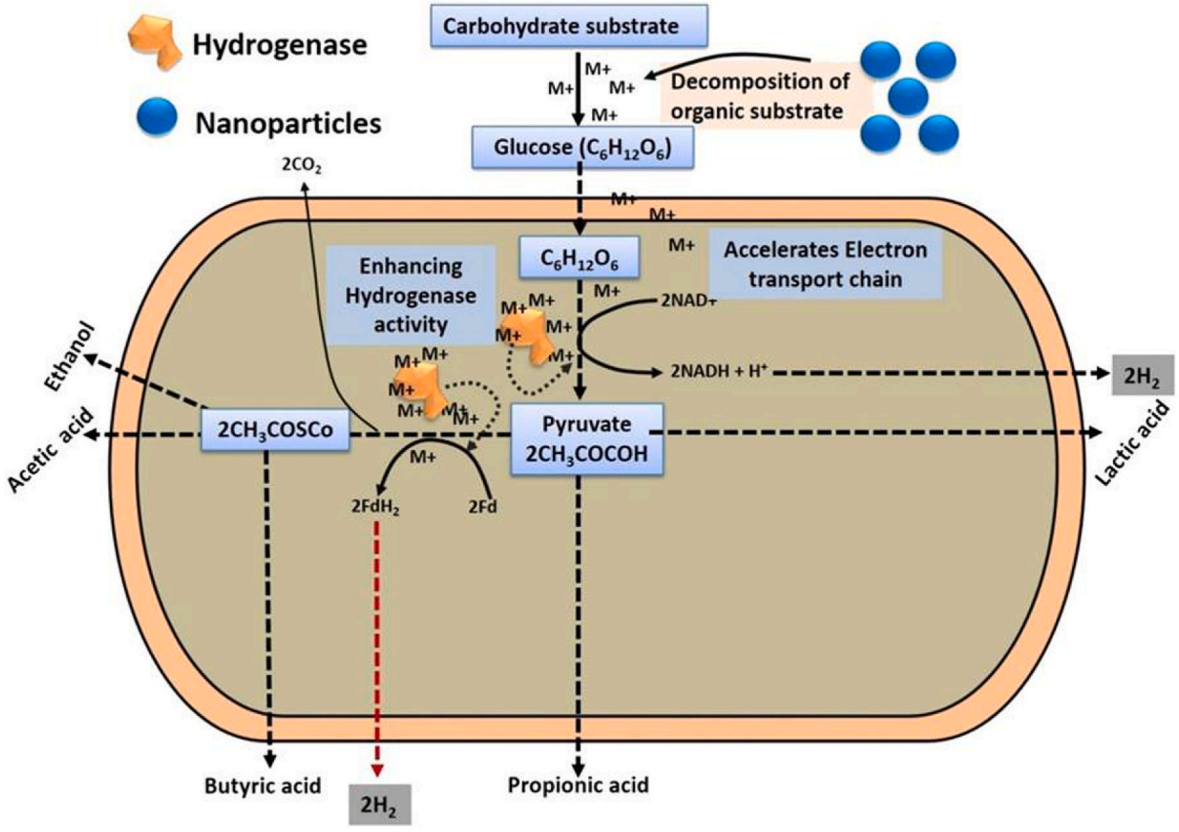


**Fig. 4.** General mechanism of nanomaterials mediated improved biohydrogen production using (adapted with permission from Ref. [174]).

compounds, which results in higher hydrogen production. By increasing the transfer of e−between the nanomaterial and the e-acceptor and by attaching to the active site of the nitrogenase enzyme, which catalyzes the biohydrogen generation, nanomaterials have the potential to increase the yield of photo-fermentative biohydrogen production [39]. In addition photofermentation rely on the ability of the enzyme system to metabolize light into energy is also a factor in biohydrogen production. The photofermentation ability of bacteria to convert light into energy can be accelerated by nanomaterials like $TiO_2$, ZnO, and SiC, which also supply the energy needed to generate biohydrogen [183]. When $TiO_2$ nanoparticles dosage was at its optimum, *Rhodobacter sphaeroides* NMBL-02 mediated photofermentation showed a 1.54 fold increase in $H_2$ production [180]. Liu et al. also investigated the effects of $TiO_2$, ZnO, and SiC nanoparticle addition on the increased photo-fermentative hydrogen production by *Rhodopseudomonas* sp. strain A7. It was revealed that *Rhodopseudomonas* sp. strain A7 could only produce 1860 mL-$H_2$/L of cumulative hydrogen in the absence of nanomaterials. However, all three of the nanoparticles showed a considerable improvement in cumulative hydrogen. $TiO_2$ (300 mg/L), ZnO (100 mg/L), and SiC (200 mg/L) supplements could increase the total hydrogen to around 2174, 1919, and 2272 mL-$H_2$/L, respectively. Consequently, incorporating nanoparticles into the process is a possible way to increase the production of photo-fermentative hydrogen [183]. Because of the presence of more reactive sites on the surface of nanomaterials, the adsorption effectiveness is also increased, which enhances photocatalysis. Additionally, the accelerated electron transfer induced by these nanoparticles to the enzyme system increased the productivity and production of biohydrogen [177]. Furthermore, nanomaterials that increase the thermostability and pH stability of nitrogenase enzyme generated by microorganisms make it easier to immobilize it. This greatly supports bacterial growth under a variety of pH and temperature conditions [174].

In addition to the fermentative process, nanoparticles in the case of bio-photolysis boost the light absorption through photosystems found in microalgae and cyanobacteria to decompose water into hydrogen. Nanoparticles also have the ability to attach to the active sites of the enzymes hydrogenase and nitrogenase, which allows them to accelerate the rate of reaction and increase biohydrogen yield [180]. The reusability of enzymes is also facilitated by the use of nanomaterials. As a result, the process for producing biohydrogen is more efficient and sustainable since enzymes are more capable of keeping their activity even after repeated cycles [171,173]. However, previous research reports indicate that, rather than increasing hydrogen production, an excess of nanomaterials has a negative impact on hydrogen production [177,184]. This suggests that high concentration nanomaterials can reduce the activities of enzymes (hydrogenase) as well as their metabolic pathways that ultimately affect the hydrogen generation. Therefore, $H_2$ production is found to depend considerably on the nature of nanomaterials as well as their concentration. In this context, Yin and wang illustrated an enhanced production of biohydrogen using macroalgae (*Saccharina japonica*) as a substrate and by employing $Fe^0$NP (zero-valent iron nanoparticles) in dark fermentation. The obtained result showed 6.5 times enhanced hydrogen production compared to the control experiment [171]. This enhancement in $H_2$ production was supposed to be because of favourable condition for dark fermentation due to the application of Fe0 NPs. Herein, $Fe^0$ NPs is a type of reducing agent, and when it react with oxidants present in the broth it resulted reduced oxidation–reduction potential (ORP) and producing more favourable condition for hydrogen production [171]. Furthermore, $Fe^0$ NPs plays important part in gene expression and for enzyme production which is essential in hydrogen generation. When $Fe^0$NPs supplemented into the fermentation medium, corrosion effect may occur *via* the formation of ferrous ions ($Fe^{2+}$) as well as ferric ions ($Fe^{3+}$). Further, $Fe^{2+}$ could stimulate the gene expression of enzyme such as hydrogenases and dehydrogenase [171,181]. Moreover $Fe^{2+}$ also assists as critical proteins employed in hydrogen-producing metabolism, such as ferredoxins and various hydrogenases. Besides, iron also eliminates the sulphides, which act as an inhibitor for hydrogen production in dark fermentation. However, on increasing the $Fe^0$ NPs dose beyond the optimum, it decreased both cumulative hydrogen production and hydrogen yield, indicating the decrease in pH induced by the accumulation of acetate with surplus $Fe^0$ NPs [184]. In addition, Zaidi et al. reported improved hydrogen yield using green algae *Enteromorpha* by employing combined microwave (MW) pretreatment and iron oxide nanoparticles ($Fe_3O_4$ NPs). It was noticed that the combined treatment could provide biogas amounts and hydrogen (% v/v) of 328 mL and 51.5%, respectively. In this study, it was illustrated that the cell wall of *Enteromorpha* composed of cellulose and hemicellulose exists in the internal layer gets dissolved by the application of $Fe_3O_4$ NPs and improves the hydrolysis stage as well as the lag time to produce a high amount of biohydrogen [169]. Similarlly Zaidi et al. studied the impact of metal (NiCo) and metal-oxide ($Fe_3O_4$ and MgO) nanoparticles on biogas as well as hydrogen production employing green microalgae *Enteromorpha*. The obtained result showed that Ni-NPs give rise to improve biohydrogen i. e, 51.42% followed by $Fe_3O_4$ NPs, 44.61% as compared to the control. According to the findings, applying NPs causes higher cell wall disruption and the breakdown of glycoproteins, carbohydrates, and cellulose when compared to a control, which boosts the production of biohydrogen [169]. Srivastava et al. reported nickel ferrite nanoparticles ($NiFe_2O_4$ NPs) induced enhanced production of cellulase enzyme by employing cyanobacteria *Lyngbya limnetica* as substrate [173]. The results showed that crude cellulase stabilized with $NiFe_2O_4$ NPs had improved enzymatic hydrolysis, indicating that nanomaterials have a major impact on enzyme enhancement and consequently increase the rate of hydrogen production [173]. Zhao et al. used the co-digestion of dry lake algae to study the effect of nano zero valent iron (NZVI) on dark fermentative biohydrogen generation. The obtained results exhibited that 10 mg. $gTS^{-1}$ addition of NZVI enhanced the co-digestion process, with the net hydrogen yield augmented by 29.20%–40.04 mL.$gVS^{-1}$, in comparison to the control. However, by further increasing the dosages of NZVI from 20 to 40 mg. $gTS^{-1}$, the cumulative yield of hydrogen was reduced to 14.77 and 5.37 mL.$gVS^{-1}$ respectively [170]. When NZVI was administered in doses of 10 mg. $gTS^{-1}$, both protein and carbohydrate breakdown rates reached their maximum levels. The results showed that adding NPs NZVI up to the optimum dosages boosted the microbial activity and metabolic activity of the dehydrogenase enzyme in the anaerobic reaction system, increasing hydrogen yield [170]. However, NPs that are added in excess of the optimum dosage are hazardous to microorganisms and stop their growth [170,185]. According to the findings of the studies stated above, nanomaterials are highly capable of supporting cutting-edge methods for producing biohydrogen. In this approach, the application of NPs might considerably result in increased enzyme activity as well as its dependability for boosting the generation of biohydrogen in a specific manner. Some of the studies and their results on the use of nanomaterials to enhance algal biohydrogen production are listed in Table 2.

## 5. Future prospect

Complete dependency on fossil fuels is insufficient to meet the rising demand for energy and to prevent severe environmental effects including environmental contamination. There is an urgent need to develop sustainable technology which can be implemented commercially at a low cost. Biohydrogen production from algal wastes is one promising approach towards the development of economic biofuels on a practical scale. The primary benefit of producing biohydrogen is that no harmful byproducts are created, and as a result, demand for this form of energy is constantly on the rise. The biggest barrier to the economic and commercial viability of this field, however, is a number of significant production yield and rate concerns. Since biohydrogen production is an enzyme-mediated process, the efficiency of the enzymes hydrogenase and nitrogenase is the main determinant of the process. Algal diversity

**Table 2**
Studies on nanomaterials and its optimum dosages used to improve the algal biohydrogen production along with corresponding cumulative $H_2$ yields under the optimized conditions.

| Sr. No. | Name of algae | Type of $H_2$ Production | Nanomaterials/Concentration | $H_2$ yield/cumulative $H_2$ | Ref. |
|---|---|---|---|---|---|
| **1.** | *green algae (Enteromorpha)* | Dark fermentation | $Fe_3O_4$ NPs | - | [169] |
| **2.** | *Taihu Lake algae* | Dark fermentation | Nano zero valent iron (NZVI)/10 mg.gTS$^{-1}$ | 40.04 mL.gVS$^{-1}$ | [170] |
| **3.** | *Saccharina japonica* | Dark fermentation | $Fe^0$ NPs/200 mg/L | 20.25 mL $H_2$/g | [171] |
| **4.** | *Lyngbya limnetica* | Dark fermentation | $NiFe_2O_4$ NPs/1.5% | 1820 mL/L | [173] |
| **5.** | *Chlamydomonas reinhardtii* CC124 | Photofermentation | Silica NPs/60 mg/L | 3121.5 ± 178.9 mL | [186] |

may provide various hydrogenase and nitrogenase enzyme-producing genes capable of withstanding higher temperatures and pH tolerance. Thus, research investigations based on in-depth molecular analysis may provide an opportunity to develop various stress-tolerant hydrogenases and nitrogenases. Furthermore, the effectiveness of enzymes that produce hydrogen can be improved by the introduction of nanomaterials. However, thorough and in-depth investigations dealing with the interaction of nanomaterial with enzyme systems at the biochemical and molecular levels are needed in order to develop this method on an economic scale. Additionally, the characteristics of using nanomaterials as nanocatalysts may improve the process mechanism and its economy, according to a number of literatures. However, influence of nanomaterials on the structure and dynamics of microbes has to be given more attention. Deep understanding on the technical obstacles related to commercial uses of biohydrogen production is also crucial. Reusing the nanomaterials that are used at each stage of the entire process is another problem with this technique. To lower the overall cost, it is essential to employ a green synthesis approach for the production of nanomaterials. Furthermore, an understanding of algal cell wall organization is crucial to improve enzyme amalgam for degradation of algal cell wall. More investigations are needed to explore the sustainable integrated fermentation refinery to expand the production efficiency in order to increase the maximum $H_2$ yield. Subsequently, advanced familiarity of the algal physiology in the process of anoxic hydrogen production is vital, and focused research is required in order to practically understand this phenomenon. Also, the transformation due to technological advancements in the upcoming future could ease the problems related to the commercial PBR design in addition to production of cost-effective photobiological hydrogen.

## 6. Conclusions

This review emphasizes and gives a general overview of the diversified groups of algae and the mechanism of biohydrogen production. Additionally, it emphasizes how nanoparticles affect different biohydrogen production processes. Based on the information available in the aforementioned literatures, it was concluded that algae are exceedingly varied, have the capacity to spread across a number of habitats, and are polyphyletic in nature. These properties allow them to contribute significantly to the production of biohydrogen, despite a few limitations. Nanomaterials are crucial in enhancing this process in a sustainable manner, which will help to address these limits. Employing nanomaterials is intended to obtain a high yield of biohydrogen on a practical scale. Nevertheless, this strategy is still in its infancy and needs to be further investigated in order to close the present gap between its practical uses and economic sustainability. Continuous investigation of algal strains with desirable cellular composition, their lower production costs, and the use of nanomaterials could help meet a considerable demand for energy in the form of biohydrogen as a clean energy source in the future.

## Credit author statement

Tripti Singh: writing-original draft; Anisha Sehgal: writing-original draft; Rajeev Singh: writing-original draft; Shalini Sharma: reviewed & editing; Dan Bahadur Pal: reviewed & editing; Hanaa M. Tashkandi: writing-reviewed & editing; Rajaa Raddadi: writing-reviewed & editing; Steve Harakeh: writing-reviewed & editing; Shafiul Haque: writing-reviewed & editing; Manish Srivastava: conceptualization, writing-reviewed & editing; Ashraf Aly Hassan: Revision, writing-reviewed & editing; Neha Srivastava: conceptualization, writing-reviewed & editing; Vijai Kumar Gupta: Conceptualization, writing-reviewed & editing.

## 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.

## Data availability

The authors do not have permission to share data.

## Acknowledgement

Author N.S. thankfully acknowledges DST, New Delhi, India for providing Woman Scientist-B Fellowship (DST-WOS-B/ER/2021) and Department of Chemical Engineering and Technology, IIT (BHU) Varanasi.

## References

[1] Charles Rajesh Kumar J, Majid MA. Renewable energy for sustainable development in India: current status, future prospects, challenges, employment, and investment opportunities. Energy Sustain Soc 2020;10. https://doi.org/10.1186/s13705-019-0232-1.
[2] Perera F. Pollution from fossil-fuel combustion is the leading environmental threat to global pediatric health and equity: solutions exist. Int J Environ Res Publ Health 2018;15. https://doi.org/10.3390/ijerph15010016.
[3] Gielen D, Boshell F, Saygin D, Bazilian MD, Wagner N, Gorini R. The role of renewable energy in the global energy transformation. Energy Strategy Rev 2019;24:38–50. https://doi.org/10.1016/j.esr.2019.01.006.
[4] Khan S, Nisar A, Wu B, Zhu QL, Wang YW, Hu GQ, et al. Bioenergy production in Pakistan: potential, progress, and prospect. Sci Total Environ 2022;814. https://doi.org/10.1016/j.scitotenv.2021.152872.
[5] Kumar M. Social, economic, and environmental impacts of renewable energy resources. Wind Sol Hybrid Renew Energy Syst; 2020. https://doi.org/10.5772/intechopen.89494.
[6] Beschkov V. Biogas, biodiesel and bioethanol as multifunctional renewable fuels and raw materials. Front Bioenergy Biofuels 2017. https://doi.org/10.5772/65734.
[7] Chozhavendhan S, Rajamehala M, Karthigadevi G, Praveenkumar R, Bharathiraja B. A review on feedstock, pretreatment methods, influencing factors, production and purification processes of bio-hydrogen production. Case Stud Chem Environ Eng 2020;2. https://doi.org/10.1016/j.cscee.2020.100038.
[8] Dincer I, Bicer Y. Chapter 6- Integration of renewable energy systems for multigeneration. 2020. p. 287–402.
[9] Singh L, Wahid ZA. Methods for enhancing bio-hydrogen production from biological process: a review. J Ind Eng Chem 2015;21:70–80. https://doi.org/10.1016/j.jiec.2014.05.035.
[10] Fankhauser S, Smith SM, Allen M. The meaning of net zero and how to get it right. Nat Clim Change 2022;12:15–21. https://doi.org/10.1038/s41558-021-01245-w.
[11] Malode SJ, Prabhu KK, Mascarenhas RJ, Shetti NP, Aminabhavi TM. Recent advances and viability in biofuel production. Energy Convers Manag X 2021;10. https://doi.org/10.1016/j.ecmx.2020.100070.
[12] Mahapatra S, Kumar D, Singh B, Sachan PK. Biofuels and their sources of production: a review on cleaner sustainable alternative against conventional fuel, in the framework of the food and energy nexus. Energy Nexus 2021;4:100036.

[13] Khan N, Sudhakar K, Mamat R. Role of Biofuels in Energy Transition, Green Economy and Carbon Neutrality. Sustainability 2021;13(22):12374. https://doi.org/10.3390/su132212374.

[14] Ambaye TG, Vaccari M, Bonilla-Petriciolet A, Prasad S, van Hullebusch ED, Rtimi S. Emerging technologies for biofuel production: a critical review on recent progress, challenges and perspectives. J Environ Manag 2021:290. https://doi.org/10.1016/j.jenvman.2021.112627.

[15] Saravanan A, Senthil Kumar P, Khoo KS, Show P-L, Femina Carolin C, Fetcia Jackulin C, Chang J-S. Biohydrogen from organic wastes as a clean and environment-friendly energy source: production pathways, feedstock types, and future prospects. Bioresour Technol 2021;342:126021. https://doi.org/10.1016/j.biortech.2021.126021.

[16] Osman AI, Deka TJ, Baruah DC, Rooney DW. Critical challenges in biohydrogen production processes from the organic feedstocks. Biomass Convers Biorefinery 2020. https://doi.org/10.1007/s13399-020-00965-x.

[17] Ghavam S, Vahdati M, Wilson IAG, Styring P. Sustainable ammonia production processes. Front Energy Res 2021;9. https://doi.org/10.3389/fenrg.2021.580808.

[18] Lui J, Chen WH, Tsang DCW, You S. A critical review on the principles, applications, and challenges of waste-to-hydrogen technologies. Renew Sustain Energy Rev 2020;134. https://doi.org/10.1016/j.rser.2020.110365.

[19] Sharma A, Arya SK. Hydrogen from algal biomass: a review of production process. Biotechnol Reports 2017;15:63–9. https://doi.org/10.1016/j.btre.2017.06.001.

[20] Anburajan P, Yoon J-J, Kumar G, Park J-H, Kim S-H. Evaluation of process performance on biohydrogen production in continuous fixed bed reactor (C-FBR) using acid algae hydrolysate (AAH) as feedstock. Int. J. Hydrog. Energy 2019;44:2164–9. https://doi.org/10.1016/j.ijhydene.2018.09.098.

[21] Wang K, Khoo KS, Chew KW, Selvarajoo A, Chen W-H, Chang J-S, Show PL. Microalgae: the future supply house of biohydrogen and biogas. Front Energy Res 2021;9. https://doi.org/10.3389/fenrg.2021.660399.

[22] Bošnjaković M, Sinaga N. The perspective of large-scale production of algae biodiesel. Appl Sci 2020;10:1–26. https://doi.org/10.3390/app10228181.

[23] Mona S, Kumar SS, Kumar V, Parveen K, Saini N, Deepak B, et al. Green technology for sustainable biohydrogen production (waste to energy): a review. Sci. Total Environ. 2020;728:138481. https://doi.org/10.1016/j.scitotenv.2020.138481.

[24] Sarkar D, Shimizu K. An overview on biofuel and biochemical production by photosynthetic microorganisms with understanding of the metabolism and by metabolic engineering together with efficient cultivation and downstream processing. Bioresour Bioprocess 2015;2:17. https://doi.org/10.1186/s40643-015-0045-9.

[25] Arimbrathodi SP, Javed MA, Hamouda MA, Aly Hassan A, Ahmed ME. Bio$H_2$ Production Using Microalgae: Highlights on Recent Advancements from a Bibliometric Analysis. Water 2023;15:185. https://doi.org/10.3390/w15010185.

[26] Nagarajan D, Lee DJ, Kondo A, Chang JS. Recent insights into biohydrogen production by microalgae – from biophotolysis to dark fermentation. Bioresour Technol 2017;227:373–87. https://doi.org/10.1016/j.biortech.2016.12.104.

[27] Arora K, Kumar P, Bose D, Li X, Kulshrestha S. Potential applications of algae in biochemical and bioenergy sector. 3 Biotech 2021;11. https://doi.org/10.1007/s13205-021-02825-5.

[28] Pachapur VL, Kutty P, Pachapur P, Brar SK, Le Bihan Y, Galvez-Cloutier R, et al. Seed pretreatment for increased hydrogen production using mixed-culture systems with advantages over pure-culture systems. Energies 2019;12. https://doi.org/10.3390/en12030530.

[29] Thiruvengadam M, Rajakumar G, Chung IM. Nanotechnology: current uses and future applications in the food industry. 3 Biotech 2018;8. https://doi.org/10.1007/s13205-018-1104-7.

[30] Patel SKS, Lee JK, Kalia VC. Nanoparticles in biological hydrogen production: an overview. Indian J Microbiol 2018;58:8–18. https://doi.org/10.1007/s12088-017-0678-9.

[31] Zhanga J, Li W, Yang J, Li Z, Zhang J, Zang L. Cobalt ferrate nanoparticles improved dark fermentation for hydrogen evolution. J Clean Prod 2021;316:128275.

[32] Brandelli A. The interaction of nanostructured antimicrobials with biological systems: cellular uptake, trafficking and potential toxicity. Food Sci Hum Wellness 2020;9:8–20.

[33] Wang S, Tang H, Peng F, et al. Metabolite-based mutualism enhances hydrogen production in a two-species microbial consortium. Commun Biol 2019;2:82. https://doi.org/10.1038/s42003-019-0331-8.

[34] Silva RM, Abreu AA, Salvador AF, Alves MM, Neves IC, Pereira MA. Zeolite addition to improve biohydrogen production from dark fermentation of C5/C6-sugars and Sargassum sp. biomass. Sci Rep 2021;11. https://doi.org/10.1038/s41598-021-95615-1.

[35] Chen H, Simoska O, Lim K, Grattieri M, Yuan M, Dong F, et al. Fundamentals, applications, and future directions of bioelectrocatalysis. Chem Rev 2020;120:12903–93. https://doi.org/10.1021/acs.chemrev.0c00472.

[36] Grzechowiak M, Sliwiak J, Jaskolski M, Ruszkowski M. Structural studies of glutamate dehydrogenase (isoform 1) from Arabidopsis thaliana, an important enzyme at the branch-point between carbon and nitrogen metabolism. Front Plant Sci 2020;11. https://doi.org/10.3389/fpls.2020.00754.

[37] Liu Y, Benitez MG, Chen J, Harrison E, Khusnutdinova AN, Mahadevan R. Opportunities and challenges for microbial synthesis of fatty acid-derived chemicals (FACs). Front Bioeng Biotechnol 2021;9. https://doi.org/10.3389/fbioe.2021.613322.

[38] Limongi AR, Viviano E, De Luca M, Radice RP, Bianco G, Martelli G. Biohydrogen from microalgae: production and applications. Appl Sci 2021;11:1616. https://doi.org/10.3390/app11041616.

[39] Srivastava N, Srivastava M, Mishra PK, Kausar MA, Saeed M, Gupta VK, et al. Advances in nanomaterials induced biohydrogen production using waste biomass. Bioresour Technol 2020;307. https://doi.org/10.1016/j.biortech.2020.123094.

[40] Gross R, Hanna R, Gambhir A, Heptonstall P, Speirs J. How long does innovation and commercialisation in the energy sectors take? Historical case studies of the timescale from invention to widespread commercialisation in energy supply and end use technology. Energy Pol 2018;123:682–99. https://doi.org/10.1016/j.enpol.2018.08.061.

[41] Khan MI, Shin JH, Kim JD. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microb Cell Factories 2018;17. https://doi.org/10.1186/s12934-018-0879-x.

[42] Nabout JC, da Silva Rocha B, Carneiro FM, Sant'Anna CL. How many species of Cyanobacteria are there? Using a discovery curve to predict the species number. Biodivers Conserv 2013;22:2907–18. https://doi.org/10.1007/s10531-013-0561-x.

[43] Stiger-Pouvreau V, Zubia M. Macroalgal diversity for sustainable biotechnological development in French tropical overseas territories. Bot Mar 2020. https://doi.org/10.1515/bot-2019-0032.

[44] Pathy A, Nageshwari K, Ramaraj R, Maniam GP, Govindan N, Balasubramanian P. Biohydrogen production using algae: Potentiality, Economics and Challenges. Bioresource Technology 2022;360:127514. https://doi.org/10.1016/j.biortech.2022.127514.

[45] Jackson DD. On odors and tastes of surface waters, with special reference to Anabaena, a microscopical organism found in certain water supplies of Massachusetts. 1896. p. 410–20. Report of the Massachusetts State Board of Health.

[46] Ruiz-Marin A, Canedo-López Y, Chávez-Fuentes P. Biohydrogen production by Chlorella vulgaris and Scenedesmus obliquus immobilized cultivated in artificial wastewater under different light quality. AMB Express 2020:10. https://doi.org/10.1186/s13568-020-01129-w.

[47] Dolganyuk V, Belova D, Babich O, Prosekov A, Ivanova S, Katserov D, et al. Microalgae: A promising source of valuable bioproducts. Biomolecules 2020;10:1–24. https://doi.org/10.3390/biom10081153.

[48] Bagul SY, Chakdar H, Pandiyan K, Das K. Conservation and Application of Microalgae for Biofuel Production. In: Sharma S, Varma A, editors. Microbial Resource Conservation. Soil Biology, 54. Cham: Springer; 2018. https://doi.org/10.1007/978-3-319-96971-8_12.

[49] Singh R, Parihar P, Singh M, Bajguz A, Kumar J, Singh S, et al. Uncovering potential applications of cyanobacteria and algal metabolites in biology, agriculture and medicine: Current status and future prospects. Front Microbiol 2017;8. https://doi.org/10.3389/fmicb.2017.00515.

[50] Shengnan Li, Chang H, Zhang S, Ho S-H. Production of sustainable biofuels from microalgae with CO2 bio-sequestration and life cycle assessment. Environ. Res 2023;227:115730. https://doi.org/10.1016/j.envres.2023.115730.

[51] Giang TT, Lunprom S, Liao Q, Reungsang A, Salakkam A. Improvement of hydrogen production from *Chlorella* sp. biomass by acid-thermal pretreatment. PeerJ 2019;7:e6637. https://doi.org/10.7717/peerj.6637.

[52] Jiménez-Llanos J, Ramírez-Carmona M, Rendón-Castrillón L, Ocampo-López C. Sustainable biohydrogen production by Chlorella sp. microalgae: A review. Int J Hydrogen Energy 2020;45:8310–28. https://doi.org/10.1016/j.ijhydene.2020.01.059.

[53] Arrojo MÁ, Regaldo L, Calvo Orquín J, Figueroa FL, Abdala Díaz RT. Potential of the microalgae Chlorella fusca (Trebouxiophyceae, Chlorophyta) for biomass production and urban wastewater phycoremediation. AMB Express 2022;12. https://doi.org/10.1186/s13568-022-01384-z.

[54] Touloupakis E, Faraloni C, Benavides AMS, Torzillo G. Recent achievements in microalgal photobiological hydrogen production. Energies 2021;14. https://doi.org/10.3390/en14217170.

[55] Dębowski M, Zieliński M, Świca I, Kazimierowicz J. Algae Biomass as a Potential Source of Liquid Fuels. Phycology 2021;1:105–18. https://doi.org/10.3390/phycology1020008.

[56] Manoyan J, Gabrielyan L, Kozel N, Trchounian A. Regulation of biohydrogen production by protonophores in novel green microalgae Parachlorella kessleri. J Photochem Photobiol B Biol 2019;199. https://doi.org/10.1016/j.jphotobiol.2019.111597.

[57] Liu H, Zhang H, Zhang Z, Xia C, Li Y, Lu C, Zhang Q. Optimization of hydrogen production performance of Chlorella vulgaris under different hydrolase and inoculation amount. Journal of Cleaner Production 2021;310:127293. https://doi.org/10.1016/j.jclepro.2021.127293.

[58] 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.

[59] Wang H, Fan X, Zhang Y, Yang D. Sustained photo-hydrogen production by Chlorella pyrenoidosa without sulfur depletion. Biotechnol Lett 2011;33:1345–50.

[60] Li L, Zhang L, Liu J. The enhancement of hydrogen photoproduction in marine Chlorella pyrenoidosa under nitrogen deprivation. International Journal of Hydrogen Energy 2015;40(43):14784–9. https://doi.org/10.1016/j.ijhydene.2015.09.022.

[61] Li L, Zhang L, Liu J. Proteomic analysis of hydrogen production in Chlorella pyrenoidosa under nitrogen deprivation. Algal Res 2021;53. https://doi.org/10.1016/j.algal.2020.102143.
[62] Milrad Y, Schweitzer S, Feldman Y, Yacoby I. Green algal hydrogenase activity is outcompeted by carbon fixation before inactivation by oxygen takes place. Plant Physiol 2018;177:918–26. https://doi.org/10.1104/pp.18.00229.
[63] Pow T, Krasna AI. Photoproduction of hydrogen from water in hydrogenase-containing algae. Arch Biochem Biophys 1979;194:413–21. https://doi.org/10.1016/0003-9861(79)90635-0.
[64] Liu H, Ru G, Zhang Z, Li Y, Xia C, Lu C, Zhang Q. Experimental study on optimization of initial pH for photo-fermentation bio-hydrogen under different enzymatic hydrolysis of chlorella vulgaris. Bioresour Technol 2021;338:125571. https://doi.org/10.1016/j.biortech.2021.125571.
[65] Hwang JH, Kim HC, Choi JA, Abou-Shanab RA, Dempsey BA, Regan JM, Kim JR, Song H, Nam IH, Kim SN, Lee W, Park D, Kim Y, Choi J, Ji MK, Jung W, Jeon BH. Photoautotrophic hydrogen production by eukaryotic microalgae under aerobic conditions. Nat Commun 2014;5:3234. https://doi.org/10.1038/ncomms4234.
[66] Xiong W, Zhao X, Zhu G, Shao C, Li Y, Ma W, et al. Silicification-Induced Cell Aggregation for the Sustainable Production of H2 under Aerobic Conditions. Angewandte Chemie 2015:54. https://doi.org/10.1002/anie.201504634.
[67] Yang D-W, Syn J-W, Hsieh C-H, Huang C-C, Chien L-F. Genetically engineered hydrogenases promote biophotocatalysis-mediated H2 production in the green alga Chlorella sp. DT. International Journal of Hydrogen Energy 2019;44(Issue 5):2533–45. https://doi.org/10.1016/j.ijhydene.2018.11.088.
[68] Xu L, Fan J, Wang Q. Omics application of bio-hydrogen production through green alga chlamydomonas reinhardtii. Front Bioeng Biotechnol 2019;7. https://doi.org/10.3389/fbioe.2019.00201.
[69] Batyrova K, Hallenbeck PC. Hydrogen production by a Chlamydomonas reinhardtii strain with inducible expression of photosystem II. Int J Mol Sci 2017;18. https://doi.org/10.3390/ijms18030647.
[70] Fakhimi N, Tavakoli O. Improving hydrogen production using co-cultivation of bacteria with Chlamydomonas reinhardtii microalga. Mater Sci Energy Technol 2019;2:1–7. https://doi.org/10.1016/j.mset.2018.09.003.
[71] Nomanbhay S, Purvunathan P, Yin OM. Enhancement of Bio-Hydrogen Production in Chlamydomonas Reinhardtii by Immobilization and Co-Culturing. Int J Bio-Science Bio-Technology 2017;9:35–52. https://doi.org/10.14257/ijbsbt.2017.9.1.03.
[72] Oncel SS, Kose A, Faraloni C, Imamoglu E, Elibol M, Torzillo G, Vardar Sukan F. Biohydrogen production using mutant strains of Chlamydomonas reinhardtii: The effects of light intensity and illumination patterns. Biochemical Engineering Journal 2014;92:47–52. https://doi.org/10.1016/j.bej.2014.06.019. ISSN 1369-703X.
[73] Zhang D, Vassiliadis VS. Chlamydomonas reinhardtii Metabolic Pathway Analysis for Biohydrogen Production under Non-Steady-State Operation. Ind Eng Chem Res 2015;54:10593–605. https://doi.org/10.1021/acs.iecr.5b02034.
[74] Iqbal K, Saxena A, Pande P, Tiwari A, Chandra Joshi N, Varma A, et al. Microalgae-bacterial granular consortium: Striding towards sustainable production of biohydrogen coupled with wastewater treatment. Bioresour Technol 2022:354. https://doi.org/10.1016/j.biortech.2022.127203.
[75] Pushkareva E, Pessi IS, Namsaraev Z, Mano MJ, Elster J, Wilmotte A. Cyanobacteria inhabiting biological soil crusts of a polar desert: Sør Rondane Mountains, Antarctica. Syst Appl Microbiol 2018;41:363–73. https://doi.org/10.1016/j.syapm.2018.01.006.
[76] Gärtner G, Stoyneva-Gärtner M, Uzunov B. Algal toxic compounds and their aeroterrestrial, airborne and other extremophilic producers with attention to soil and plant contamination: A review. Toxins 2021;13(5):322. https://doi.org/10.3390/toxins13050322.
[77] Cirés S, Casero MC, Quesada A. Toxicity at the edge of life: A review on cyanobacterial toxins from extreme environments. Mar Drugs 2017;15. https://doi.org/10.3390/md15070233.
[78] Teikari JE, Fewer DP, Shrestha R, Hou S, Leikoski N, Mäkelä M, et al. Strains of the toxic and bloom-forming Nodularia spumigena (cyanobacteria) can degrade methylphosphonate and release methane. ISME J 2018;12:1619–30. https://doi.org/10.1038/s41396-018-0056-6.
[79] Janssen F, Neumann T, Schmidt M. Inter-annual variability in cyanobacteria blooms in the Baltic Sea controlled by wintertime hydrographic conditions. Mar Ecol Prog Ser 2004;275:59–68. https://doi.org/10.3354/meps275059.
[80] Ölberg J. Cyanobacterial blooms in the Baltic Sea. HELCOM Balt Sea Environ Fact. Sheets 2018;45:1–7.
[81] Wang Y, Gao Y, Li C, Gao H, Zhang CC, 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.
[82] Shahkouhi AM, Motamedian E. Reconstruction of a regulated two-cell metabolic model to study biohydrogen production in a diazotrophic cyanobacterium Anabaena variabilis ATCC 29413. PLoS One 2020;15. https://doi.org/10.1371/journal.pone.0227977.
[83] Kossalbayev BD, Kakimova AB, Bolatkhan K, Zayadan BK, Sandybayeva SK, Bozieva AM, et al. Biohydrogen production by novel cyanobacterial strains isolated from rice paddies in Kazakhstan. Int. J. Hydrog. Energy 2022;47:16440–53.
[84] Vargas SR, Santos PV dos, Zaiat M, Calijuri M do C. 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.
[85] Mahfoud A, Selma H, Rabah B, et al. Hydrogen production with the cyanobacterium Spirulina platensis. Int J Hydrogen Energy 2017;42:4902–7. https://doi.org/10.1016/j.ijhydene.2016.12.056.
[86] Touloupakis E, Cicchi B, Benavides AMS, Torzillo G. Effect of high pH on growth of Synechocystis sp. PCC 6803 cultures and their contamination by golden algae (Poterioochromonas sp.). Appl Microbiol Biotechnol 2016;100:1333–41. https://doi.org/10.1007/s00253-015-7024-0.
[87] Hasnaoui S, Pauss A, Abdi N, Grib H, Mameri N. Enhancement of bio-hydrogen generation by spirulina via an electrochemical photo-bioreactor (EPBR). Int J Hydrogen Energy 2020;45:6231–42. https://doi.org/10.1016/j.ijhydene.2019.12.144.
[88] Juantorena AU, Santoyo E, Lastres O, Hernández G, Bustos A, Gamboa SA, Sebastian PJ. Gas Chromatography as an Analytical Monitoring Technique for Hydrogen Production from Spirulina maxima 2342. Green sustain. chem 2016;6:78. https://doi.org/10.4236/gsc.2016.62007.
[89] Pansook S, Incharoensakdi A, Phunpruch S. Simazine Enhances Dark Fermentative H2 Production by Unicellular Halotolerant Cyanobacterium Aphanothece halophytica. Front Bioeng Biotechnol 2022;10. https://doi.org/10.3389/fbioe.2022.904101.
[90] M DK V GS, Pugazhendi A, Bajhaiya AK, Gugulothu P J RB. Biofuel production from Macroalgae: present scenario and future scope. Bioengineered 2021;12:9216–38. https://doi.org/10.1080/21655979.2021.1996019.
[91] Ismail MM, Alotaibi BS, EL-Sheekh MM. Therapeutic uses of red macroalgae. Molecules 2020;25. https://doi.org/10.3390/molecules25194411.
[92] Yin Y, Wang J. Pretreatment of macroalgal Laminaria japonica by combined microwave-acid method for biohydrogen production. Bioresour Technol 2018;268:52–9. https://doi.org/10.1016/j.biortech.2018.07.126.
[93] Jung K-W, Kim D-H, Shin H-S. Fermentative hydrogen production from Laminaria japonica and optimization of thermal pretreatment conditions. Bioresou Technol 2011;102:2745–50. https://doi.org/10.1016/j.biortech.2010.11.042.
[94] Chen Y, Yin Y, Wang J. Biohydrogen production using macroalgal biomass of Laminaria japonica pretreated by gamma irradiation as substrate. Fuel 2022;309:122179.
[95] Kumar G, Sivagurunathan P, Anburajan P, Pugazhendhi A, Saratale GD, Choi CS, Kim SH. Continuous biogenic hydrogen production from dilute acid pretreated algal hydrolysate using hybrid immobilized mixed consortia. International Journal of Hydrogen Energy 2018;43:11452–9.
[96] Kumar D, Eswari AP, Park JH, Adishkumar S, Banu JR. Biohydrogen Generation From Macroalgal Biomass, Chaetomorpha antennina Through Surfactant Aided Microwave Disintegration. Front Energy Res 2019;7. https://doi.org/10.3389/fenrg.2019.00078.
[97] Anwar M, Lou S, Chen L, Li H, Hu Z. Recent advancement and strategy on bio-hydrogen production from photosynthetic microalgae. Bioresour Technol 2019;292:121972. https://doi.org/10.1016/j.biortech.2019.121972.
[98] Lackner M. 3rd-generation biofuels: Bacteria and algae as sustainable producers and converters. Handb Clim Chang Mitig Adapt Second 2016;4:3173–210. https://doi.org/10.1007/978-3-319-14409-2_90.
[99] Nagarajan D, Chang JS, Lee DJ. Pretreatment of microalgal biomass for efficient biohydrogen production – Recent insights and future perspectives. Bioresour Technol 2020;302. https://doi.org/10.1016/j.biortech.2020.122871.
[100] Goria K, Singh HR, Singh A, Kothari R, Tyagi VV. Insights into biohydrogen production from algal biomass: Challenges, recent advancements and future directions. Int. J. Hydrog. Energy 2023. https://doi.org/10.1016/j.ijhydene.2023.03.174.
[101] Juneja A, Ceballos RM, Murthy GS. Effects of Environmental Factors and Nutrient Availability on the Biochemical Composition of Algae for Biofuels Production: A Review. Energies 2013;6(9):4607–38. https://doi.org/10.3390/en6094607.
[102] Ghosh A, Khanra S, Mondal M, Halder G, Tiwari ON, Saini S, Bhowmick TK, Gayen K. Progress toward isolation of strains and genetically engineered strains of microalgae for production of biofuel and other value added chemicals: A review. Energy Conversion and Management 2016 Apr 1;113:104–18.
[103] Sharma A, Arya SK. Hydrogen from algal biomass: A review of production process. Biotechnol Reports 2017;15:63–9. https://doi.org/10.1016/j.btre.2017.06.001.
[104] D'Hondt E, Martín-Juárez J, Bolado S, Kasperoviciene J, Koreiviene J, Sulcius S, Elst K, Bastiaens L. 6 - Cell disruption technologies. In: Cristina Gonzalez-Fernandez, Raúl Muñoz. Woodhead Publishing Series in Energy, Microalgae-Based Biofuels and Bioproducts. Woodhead Publishing; 2017. p. 133–54. https://doi.org/10.1016/B978-0-08-101023-5.00006-6. 9780081010235.
[105] Nitsos C, Filali R, Taidi B, Lemaire J. Current and novel approaches to downstream processing of microalgae: A review. Biotechnol Adv 2020:45. https://doi.org/10.1016/j.biotechadv.2020.107650.
[106] Onumaegbu C, Mooney J, Alaswad A, Olabi AG. Pre-treatment methods for production of biofuel from microalgae biomass. Renewable Sustainable Energy Rev 2018;93:16–26. https://doi.org/10.1016/j.rser.2018.04.015.
[107] Wieczorek N, Kucuker MA, Kuchta K. Fermentative hydrogen and methane production from microalgal biomass (Chlorella vulgaris) in a two-stage combined process. Appl Energy 2014;132:108–17. https://doi.org/10.1016/j.apenergy.2014.07.003.
[108] Roy S, Kumar K, Ghosh S, Das D. Thermophilic biohydrogen production using pre-treated algal biomass as substrate. Biomass and Bioenerg 2014;61:157–66. https://doi.org/10.1016/j.biombioe.2013.12.006.
[109] Ahmed SF, Rafa N, Mofijur M, Badruddin IA, Inayat A, Ali MS, Farrok O, Yunus Khan TM. Biohydrogen production from biomass sources: metabolic pathways and economic analysis. Front. Energy Res. 2021;9:753878. https://doi:10.3389/fenrg.2021.753878.

[110] Qyyum MA, Ismail S, Ni SQ, Ihsanullah I, Ahmad R, Khan A, et al. Harvesting biohydrogen from industrial wastewater: Production potential, pilot-scale bioreactors, commercialization status, techno-economics, and policy analysis. J Clean Prod 2022;340:130809. https://doi.org/10.1016/j.jclepro.2022.130809.

[111] Fakhimi N, Gonzalez-Ballester D, Fernández E, Galván A, Dubini A. Algae-Bacteria Consortia as a Strategy to Enhance H2 Production. Cells 2020;9:1353. https://doi.org/10.3390/cells9061353.

[112] Azbar N, Levin DB. Prospects for biohydrogen production. State Art Prog Prod Biohydrogen 2012;250–7:250–7. https://doi.org/10.2174/978160805224011201010250.

[113] Hemschemeier A, Fouchard S, Cournac L, Peltier G, Happe T. Hydrogen production by Chlamydomonas reinhardtii: An elaborate interplay of electron sources and sinks. Planta 2008;227:397–407. https://doi.org/10.1007/s00425-007-0626-8.

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

[115] Wang J, Yin Y. Fermentative hydrogen production using pretreated microalgal biomass as feedstock. Microb Cell Fact 2018;17. https://doi.org/10.1186/s12934-018-0871-5.

[116] Li S, Li F, Zhu X, Liao Q, Chang J-S, Ho S-H. Biohydrogen production from microalgae for environmental sustainability. Chemosphere 2022;291:132717. https://doi.org/10.1016/j.chemosphere.2021.132717.

[117] Azwar MY, Hussain MA, Abdul-Wahab AK. Development of biohydrogen production by photobiological, fermentation and electrochemical processes: A review. Renewable Sustainable Energy Rev 2014;31:158–73. https://doi.org/10.1016/j.rser.2013.11.022.

[118] Sambusiti C, Bellucci M, Zabaniotou A, Beneduce L, Monlau F. Algae as promising feedstocks for fermentative biohydrogen production according to a biorefinery approach: A comprehensive review. Renewable Sustainable Energy Rev 2015;44:20–36. https://doi.org/10.1016/j.rser.2014.12.013.

[119] Chandrasekhar K, Lee YJ, Lee DW. Biohydrogen production: Strategies to improve process efficiency through microbial routes. Int J Mol Sci 2015;16:8266–93. https://doi.org/10.3390/ijms16048266.

[120] Kuppam C, Pandit S, Kadier A, Dasagrandhi C, Velpuri J. Biohydrogen production: Integrated approaches to improve the process efficiency. Microb Appl 2017:189–210. https://doi.org/10.1007/978-3-319-52666-9_9.

[121] Madavi TB, Chauhan S, Jha M, Choi KY, Pamidimarri SDVN. Biohydrogen Machinery: Recent Insights, Genetic Fabrication, and Future Prospects. Chem Eng Technol 2021;44:179–90. https://doi.org/10.1002/ceat.202000527.

[122] 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.

[123] Ghirardi ML. Implementation of photobiological $H_2$ production: The $O_2$ sensitivity of hydrogenases. Photosynth Res 2015;125:383–93. https://doi.org/10.1007/s11120-015-0158-1.

[124] Metsoviti MN, Papapolymerou G, Karapanagiotidis IT, Katsoulas N. Effect of light intensity and quality on growth rate and composition of Chlorella vulgaris. Plants 2020;9. https://doi.org/10.3390/plants9010031.

[125] Sun Y, He J, Yang G, Sun G, Sage V. A review of the enhancement of bio-hydrogen generation by chemicals addition. Catalysts 2019;9:353. https://doi.org/10.3390/catal9040353.

[126] Cheonh PYY, Kansedo J, Lau JSY, Tan YH. Renewable Biomass Wastes for Biohydrogen Production. Compr Renew Energy 2022:273–98. https://doi.org/10.1016/b978-0-12-819727-1.00091-1.

[127] Khan MAH, Bonifacio S, Clowes J, Foulds A, Holland R, Matthews JC, Percival CJ, Shallcross DE. Investigation of Biofuel as a Potential Renewable Energy Source. Atmosphere 2021;12(10):1289. https://doi.org/10.3390/atmos12101289.

[128] Das SR, Basak N. Molecular biohydrogen production by dark and photo fermentation from wastes containing starch: recent advancement and future perspective. Bioprocess and biosystems engineering 2021;44:1–25. https://doi.org/10.1007/s00449-020-02422-5.

[129] Hanada S. Anoxygenic photosynthesis —A photochemical reaction that does not contribute to oxygen reproduction. Microbes Environ 2016;31:1–3. https://doi.org/10.1264/jsme2.ME3101rh.

[130] George DM, Vincent AS, Mackey HR. An overview of anoxygenic phototrophic bacteria and their applications in environmental biotechnology for sustainable Resource recovery. Biotechnol Reports 2020;28. https://doi.org/10.1016/j.btre.2020.e00563.

[131] Basak N, Das D. The prospect of purple non-sulfur (PNS) photosynthetic bacteria for hydrogen production: The present state of the art. World J Microbiol Biotechnol 2007;23:31–42. https://doi.org/10.1007/s11274-006-9190-9.

[132] Chen H, Zhou D, Luo G, Zhang S, Chen J. Macroalgae for biofuels production: Progress and perspectives. Renew Sustain Energy Rev 2015;47:427–37. https://doi.org/10.1016/j.rser.2015.03.086.

[133] Margareta W, Nagarajan D, Chang JS, Lee DJ. Dark fermentative hydrogen production using macroalgae (Ulva sp.) as the renewable feedstock. Appl Energy 2020:262. https://doi.org/10.1016/j.apenergy.2020.114574.

[134] Shobana S, Kumar G, Bakonyi P, Saratale GD, Al-Muhtaseb AH, Nemestóthy N, et al. A review on the biomass pretreatment and inhibitor removal methods as key-steps towards efficient macroalgae-based biohydrogen production. Bioresource Technology 2017. http://doi.org/10.1016/j.biortech.2017.05.172.

[135] Nayak BK, Roy S, Das D. Biohydrogen production from algal biomass (Anabaena sp. PCC 7120) cultivated in airlift photobioreactor. Int J Hydrogen Energy 2014;39:7553–60. https://doi.org/10.1016/j.ijhydene.2013.07.120.

[136] Ortigueira J, Pinto T, Gouveia L, Moura P. Production and storage of biohydrogen during sequential batch fermentation of Spirogyra hydrolyzate by Clostridium butyricum. Energy 2015;88:528–36. https://doi.org/10.1016/j.energy.2015.05.070.

[137] Saifuddin N, Priatharsini P. Developments in Bio-hydrogen Production from Algae: A Review. Res J Appl Sci Eng Technol 2016;12:968–82. https://doi.org/10.19026/rjaset.12.2815.

[138] Sim YB, Jung JH, Baik JH, Park JH, Kumar G, Rajesh Banu J, et al. Dynamic membrane bioreactor for high rate continuous biohydrogen production from algal biomass. Bioresour Technol 2021;340:125562. https://doi.org/10.1016/j.biortech.2021.125562.

[139] Hwang JH, Lee WH. Continuous photosynthetic biohydrogen production from acetate-rich wastewater: Influence of light intensity. Int J Hydrogen Energy 2021;46:21812–21. https://doi.org/10.1016/j.ijhydene.2021.04.052.

[140] Chen S, Qu D, Xiang X, Miao X. Biohydrogen production with lipid-extracted Dunaliella biomass and a new strain of hyper-thermophilic archaeon Thermococcus eurythermalis A501. Int J Hydrogen Energy 2020;45:12721–30.

[141] Stanislaus MS, Zhang N, Yuan Y, Zheng H, Zhao C, Hu X, Zhu Q, Yang Y. Improvement of biohydrogen production by optimization of pretreatment method and substrate to inoculum ratio from microalgal biomass and digested sludge. Renew Energy 2018;127:670–7. https://doi.org/10.1016/j.renene.2018.05.022.

[142] Xu L, Zhou M, Ju H, Zhang Z, Zhang J, Sun C. Enterobacter aerogenes metabolites enhance Microcystis aeruginosa biomass recovery for sustainable bioflocculant and biohydrogen production. Sci Total Environ 2018;634:488–96. https://doi.org/10.1016/j.scitotenv.2018.03.327.

[143] Radha M, Murugesan AG. Enhanced dark fermentative biohydrogen production from marine macroalgae Padina tetrastromatica by different pretreatment processes. Biofuel Res J 2017;4:551–8. https://doi.org/10.18331/BRJ2017.4.1.5.

[144] Maneeruttanarungroj C, Phunpruch S. Effect of pH on Biohydrogen Production in Green Alga Tetraspora sp. CU2551. Energy Procedia 2017;138:1085–92. https://doi.org/10.1016/j.egypro.2017.10.122.

[145] Antal TK, Kukarskikh GP, Volgusheva AA, Krendeleva TE, Tyystjärvi E, Rubin AB. Hydrogen photoproduction by immobilized S-deprived Chlamydomonas reinhardtii: Effect of light intensity and spectrum, and initial medium pH. Algal Res 2016;17:38–45. https://doi.org/10.1016/j.algal.2016.04.009.

[146] Kumar G, Sivagurunathan P, Thi NBD, Zhen G, Kobayashi T, Kim SH, Xu K. Evaluation of different pretreatments on organic matter solubilization and hydrogen fermentation of mixed microalgae consortia. Int J Hydrogen Energy 2016;41:21628–40. https://doi.org/10.1016/j.ijhydene.2016.05.195.

[147] Lakshmikandan M, Murugesan AG. Chlorella vulgaris MSU-AGM 14, a fresh water microalgal strain - growth and photobiological hydrogen production in acid hydrolysate of seaweed Valoniopsis pachynema. Int J Hydrogen Energy 2016;41:13986–92. https://doi.org/10.1016/j.ijhydene.2016.06.237.

[148] Yun YM, Kim DH, Oh YK, Shin HS, Jung KW. Application of a novel enzymatic pretreatment using crude hydrolytic extracellular enzyme solution to microalgal biomass for dark fermentative hydrogen production. Bioresour Technol 2014;159:365–72. https://doi.org/10.1016/j.biortech.2014.02.129.

[149] Cheng J, Xia A, Song W, Su H, Zhou J, Cen K. Comparison between heterofermentation and autofermentation in hydrogen production from Arthrospira (Spirulina) platensis wet biomass. Int J Hydrogen Energy 2012;37:6536–44. https://doi.org/10.1016/j.ijhydene.2012.01.025.

[150] Saeed FFM, Ibrahim MA. Hydrogen production by green alga GAF99 in sea water bioreactor: 1. isolation of alga and evaluation of environmental conditions. Biotechnology 2012;11(5):280–5. https://doi.org/10.3923/biotech.2012.280.285.

[151] Lakaniemi AM, Hulatt CJ, Thomas DN, Tuovinen OH, Puhakka JA. Biogenic hydrogen and methane production from Chlorella vulgaris and Dunaliella tertiolecta biomass. Biotechnol Biofuels 2011;4. https://doi.org/10.1186/1754-6834-4-34.

[152] Xuan J, He L, Wen W, Feng Y. Hydrogenase and Nitrogenase: Key Catalysts in Biohydrogen Production. Molecules 2023;28(3):1392. https://doi.org/10.3390/molecules28031392.

[153] Jimenez-Llanos J, Ramirez-Carmona M, Rendón-Castrillón L, Ocampo-López C. Sustainable biohydrogen production by Chlorella sp. microalgae: A review. Int. J. Hydrog. Energy 2020;45:8310–28. https://doi.org/10.1016/j.ijhydene.2020.01.059.

[154] Maneeruttanarungroj C, Lindblad P, Incharoensakdi A. Sulfate permease (SulP) and hydrogenase (HydA) in the green alga Tetraspora sp. CU2551: dependence of gene expression on sulfur status in the medium. Int. J. Hydrog Energy 2012;37(20):15105–16. https://doi.org/10.1016/j.ijhydene.2012.07.109.

[155] Walker ME, Zhang J, Sumby KM, Lee A, Houlès A, Li S, Jiranek V. Sulfate transport mutants affect hydrogen sulfide and sulfite production during alcoholic fermentation. Yeast 2021;38:367–81. https://doi.org/10.1002/yea.3553.

[156] Zhang Q, You S, Li Y, Qu X, Jiang H. Enhanced biohydrogen production from cotton stalk hydrolysate of Enterobacter cloacae WL1318 by overexpression of the formate hydrogen lyase activator gene. Biotechnol biofuels 2020;13. https://doi.org/10.1186/s13068-020-01733-9.

[157] Xu L, Li D, Wang Q, Wu S. Improved hydrogen production and biomass through the co-cultivation of Chlamydomonas reinhardtii and Bradyrhizobium japonicum. Int J Hydrog Energy 2016;41(22):9276–83. https://doi.org/10.1016/j.ijhydene.2016.04.009.

[158] 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. http://10.1590/1678-4685-GMB-2016-0050.

[159] Eckert C, Boehm M, Carrieri D, Yu J, Dubini A, Nixon PJ, et al. Genetic analysis of the Hox hydrogenase in the cyanobacterium Synechocystis sp. PCC 6803 reveals subunit roles in association, assembly, maturation, and function. J Biol Chem 2012;287:43502–15. https://doi.org/10.1074/jbc.M112.392407.
[160] Gutekunst, Kirstin, Schulz. The Physiology of the Bidirectional NiFe-hydrogenase in Cyanobacteria and the Role of Hydrogen Throughout the Evolution of Life. In: Microalgal Hydrogen Production. London: Royal Society of Chemistry; 2018. p. 107–38. https://doi.org/10.1039/9781849737128-00107.
[161] Zhang X, Sherman DM, Sherman LA. The uptake hydrogenase in the unicellular diazotrophic cyanobacterium Cyanothece sp. Strain PCC 7822 protects nitrogenase from oxygen toxicity. J. Bacteriol. 2014;196:840–9. http://10.1128/JB.01248-13.
[162] Magnuson A. Heterocyst Thylakoid Bioenergetics. Life (Basel), 9; 2019. p. 13. http://10.3390/life9010013.
[163] Herrero A, Flores E. Genetic responses to carbon and nitrogen availability in Anabaena. Environ Microbiol 2019;21:1–17. http://10.1111/1462-2920.14370.
[164] Dutta D, De D, Chaudhuri S, Bhattacharya SK. Hydrogen production by Cyanobacteria. Microb Cell Fact 2005;4:36. http://10.1186/1475-2859-4-36.
[165] Sadvakasova AK, Kossalbayev BD, Token AI, Bauenova MO, Wang J, Zayadan BK, Balouch H, Alwasel S, Leong YK, Chang JS, Allakhverdiev SI. Influence of Mo and Fe on Photosynthetic and Nitrogenase Activities of Nitrogen-Fixing Cyanobacteria under Nitrogen Starvation. Cells 2022;11(5):904. http://10.3390/cells11050904.
[166] Mishra AK, Kaushik MS, Tiwari DN. In: Mishra AK, Tiwari DN, Rai AN, editors. Chapter 8 - Nitrogenase and Hydrogenase: Enzymes for Nitrogen Fixation and Hydrogen Production in Cyanobacteria. Cyanobacteria: Academic Press; 2019. p. 173–91. https://doi.org/10.1016/B978-0-12-814667-5.00008-8. 9780128146675.
[167] Feng X, Bandyopadhyay A, Berla B, Page L, Wu B, Pakrasi HB, et al. Mixotrophic and photoheterotrophic metabolism in Cyanothece sp. ATCC 51142 under continuous light. Microbiology 2010;156(8):2566–74. https://doi.org/10.1099/mic.0.038232-0.
[168] Haque S, Singh R, Pal DB, Faidah H, Ashgar SS, Areeshi MY, et al. Thermophilic biohydrogen production strategy using agro industrial wastes: Current update, challenges, and sustainable solutions. Chemosphere 2022;207:136120. https://doi.org/10.1016/j.chemosphere.2022.136120.
[169] Zaidi AA, Feng R, Malik A, Khan SZ, Shi Y, Bhutta AJ, Shah AH. Combining microwave pretreatment with iron oxide nanoparticles enhanced biogas and hydrogen yield from green algae. Processes 2019;7:24. https://doi.org/10.3390/pr7010024.
[170] Zhao M, Liu Z, Xu J, Liu H, Dai X, Gu S, et al. Dosing effect of nano zero valent iron (NZVI) on the dark hydrogen fermentation performance via lake algae and food waste co-digestion. Energy Reports 2020;6:3192–9. https://doi.org/10.1016/j.egyr.2020.11.200.
[171] Yin Y, Wang J. Enhanced biohydrogen production from macroalgae by zero-valent iron nanoparticles: Insights into microbial and metabolites distribution. Bioresour Technol 2019;282:110–7. https://doi.org/10.1016/j.biortech.2019.02.128.
[172] Zaidi AA, RuiZhe F, Shi Y, Khan SZ, Mushtaq K. Nanoparticles augmentation on biogas yield from microalgal biomass anaerobic digestion. Int J Hydrogen Energy 2018;43:14202–13. https://doi.org/10.1016/j.ijhydene.2018.05.132.
[173] Srivastava N, Hussain A, Kushwaha D, Haque S, Mishra PK, Gupta VK, et al. Nickel ferrite nanoparticles induced improved fungal cellulase production using residual algal biomass and subsequent hydrogen production following dark fermentation. Fuel 2021;304:121391. https://doi.org/10.1016/j.fuel.2021.121391.
[174] Kumar G, Mathimani T, Rene ER, Pugazhendhi A. Application of nanotechnology in dark fermentation for enhanced biohydrogen production using inorganic nanoparticles. Int J Hydrogen Energy 2019;44:13106–13. https://doi.org/10.1016/j.ijhydene.2019.03.131.
[175] Shanmugam S, Hari A, Pandey A, Mathimani T, Felix LO, Pugazhendhi A. Comprehensive review on the application of inorganic and organic nanoparticles for enhancing biohydrogen production. Fuel 2020:270. https://doi.org/10.1016/j.fuel.2020.117453.
[176] Sánchez-López E, Gomes D, Esteruelas G, Bonilla L, Lopez-Machado AL, Galindo R, Cano A, Espina M, Ettcheto M, Camins A, Silva AM, Durazzo A, Santini A, Garcia ML, Souto EB. Metal-Based Nanoparticles as Antimicrobial Agents: An Overview. Nanomaterials (Basel) 2020 ;10:292. https://doi.org/10.3390/nano10020292.
[177] Li S, Li F, Zhu X, Liao Q, Chang JS, Ho SH. Biohydrogen production from microalgae for environmental sustainability. Chemosphere 2022:291. https://doi.org/10.1016/j.chemosphere.2021.132717.
[178] Sekoai PT, Ouma CNM, du Preez SP, Modisha P, Engelbrecht N, Bessarabov DG, et al. Application of nanoparticles in biofuels: An overview. Fuel 2019;237:380–97. https://doi.org/10.1016/j.fuel.2018.10.030.
[179] Elreedy A, Fujii M, Koyama M, Nakasaki K, Tawfik A. Enhanced fermentative hydrogen production from industrial wastewater using mixed culture bacteria incorporated with iron, nickel, and zinc-based nanoparticles. Water Res 2019;151:349–61. https://doi.org/10.1016/j.watres.2018.12.043.
[180] Pandey A, Gupta K, Pandey A. Effect of nanosized TiO2 on photofermentation by Rhodobacter sphaeroides NMBL-02. Biomass and Bioenergy 2015;72:273–9. https://doi.org/10.1016/j.biombioe.2014.10.021.
[181] Wu H, Wang C, Chen P, He AY, Xing FX, Kong XP, et al. Effects of pH and ferrous iron on the coproduction of butanol and hydrogen by Clostridium beijerinckii IB4. Int J Hydrogen Energy 2017;42:6547–55. https://doi.org/10.1016/j.ijhydene.2017.02.094.
[182] Mishra P, Krishnan S, Rana S, Singh L, Sakinah M, Ab Wahid Z. Outlook of fermentative hydrogen production techniques: An overview of dark, photo and integrated dark-photo fermentative approach to biomass. Energy Strateg Rev 2019;24:27–37. https://doi.org/10.1016/j.esr.2019.01.001.
[183] Liu B, Jin Y, Wang Z, Xing D, Ma C, Ding J, et al. Enhanced photo-fermentative hydrogen production of Rhodopseudomonas sp. nov. strain A7 by the addition of TiO2, ZnO and SiC nanoparticles. Int J Hydrogen Energy 2017;42:18279–87. https://doi.org/10.1016/j.ijhydene.2017.04.147.
[184] Yang G, Wang J. Improving mechanisms of biohydrogen production from grass using zero-valent iron nanoparticles. Bioresour Technol 2018;266:413–20. https://doi.org/10.1016/j.biortech.2018.07.004.
[185] Rambabu K, Bharath G, Banat F, Hai A, Show PL. Phong Nguyen TH. Ferric oxide/date seed activated carbon nanocomposites mediated dark fermentation of date fruit wastes for enriched biohydrogen production. Int J Hydrogen Energy 2021;46:16631–43. https://doi.org/10.1016/j.ijhydene.2020.06.108.
[186] Giannelli L, Torzillo G. Hydrogen production with the microalga Chlamydomonas reinhardtii grown in a compact tubular photobioreactor immersed in a scattering light nanoparticle suspension. Int J Hydrogen Energy 2012;37:16951–61. https://doi.org/10.1016/j.ijhydene.2012.08.103.