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Biohydrogen production using algae: Potentiality, economics and challenges

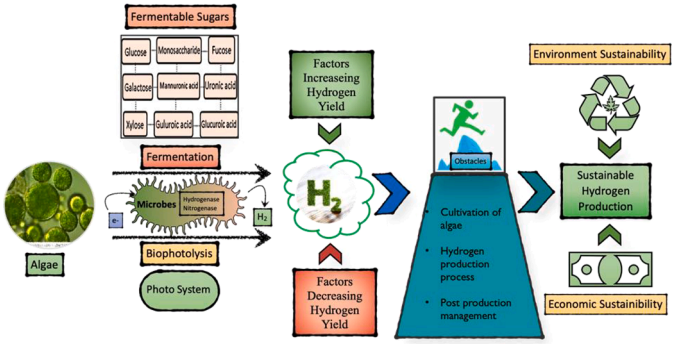
Abhijeet Pathy [a,1], Krishnamoorthy Nageshwari [a,1], Rameshprabu Ramaraj [b], Gaanty Pragas Maniam [c], Natanamurugaraj Govindan [c], Paramasivan Balasubramanian [a,*]

[a] Agricultural & Environmental Biotechnology Group, Department of Biotechnology & Medical Engineering, National Institute of Technology Rourkela, Odisha 769008, India
[b] School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand
[c] Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, 26300, Malaysia

HIGHLIGHTS

- Biohydrogen production potential of algal biomasses was highlighted.
- Factors influencing biohydrogen yield were discussed.
- Economics and challenges of bio-hydrogen production were outlined.
- Emerging trends and future scopes of the biohydrogen process were highlighted.

GRAPHICAL ABSTRACT



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ABSTRACT

The biohydrogen production from algal biomass could ensure hydrogen's sustainability as a fuel option at the industrial level. However, some bottlenecks still need to be overcome to achieve the process's economic feasibility. This review article highlights the potential of algal biomasses for producing hydrogen with a detailed explanation of various mechanisms and enzymes involved in the production processes. Further, it discusses the impact of various experimental parameters on biohydrogen production. This article also analyses the significant challenges confronted during the overall biohydrogen production process and comprehends the recent strategies adopted to enhance hydrogen productivity. Furthermore, it gives a perception of the economic sustenance of the process. Moreover, this review elucidates the future scope of this technology and delineates the approaches to ensure the viability of hydrogen production.

* Corresponding author.
E-mail address: biobala@nitrkl.ac.in (P. Balasubramanian).
[1] Equal Contributions.

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1. Introduction

Fuel used in transporting goods and people is accountable for 33% of total worldwide energy consumption. For instance, the combustion of one-litre diesel leads to the emission of 2.9 kg of greenhouse gases (Ağbulut & Sarıdemir, 2019). In the future scenario, this emission's likeliness is projected to rise substantially due to the increase in economic and social dynamics caused by rapid globalization. Heavy dependency on fossil fuels has severely damaged earth ecosystems causing extreme heatwaves, the meltdown of arctic ice, a rise in sea level, and frequent droughts. Further, fossil fuel is expected to exhaust within a century if it is not substituted by an alternative (Nogueira et al., 2020). So, the search for sustainable, clean, and green energy that could reduce our dependency on fossil fuels is the need of the hour.

Hydrogen is considered the most promising cleaner substitute to fossil fuel. It is the cleanest fuel as its burning results in hydrogen and oxygen combustion, released as water. Hydrogen as fuel emits no carbon into the air, a significant challenge posed by fossil fuel use. Hydrogen fuel potential is quite eminent from its higher specific energy content (142 MJ kg^{-1}), which is much greater than that of methane (56 MJ kg^{-1}), natural gas (54 MJ kg^{-1}), and gasoline (47 MJ kg^{-1}) (Acar & Dincer, 2019). However, most of the current hydrogen production is from fossil fuels and chemical-based processes. So, due to its dependency on non-renewable resources, conventional hydrogen production techniques are not sustainable (Sambusiti et al., 2015).

On the contrary, hydrogen derived from biomasses and photosynthetic microorganisms is considered as a sustainable energy source (Kim et al., 2021). Algae have been used in recent decades for producing hydrogen more sustainably. Algae have several fermentable sugars (Fig. 1) as their constituents which is desirable for hydrogen production. Features such as higher growth rate and absence of lignin (which helps bypass chemical and cost-intensive pre-treatment procedures) strengthen algae's candidature for biohydrogen production in a sustainable and economically viable manner (Behera et al., 2020). However, certain constraints, such as the higher water requirement and a significant initial investment, pose challenges in their cultivation at a larger scale (Anwar et al., 2019; Nageshwari et al., 2021). Research for lowering the infrastructure and working cost of the algal production processes are peaking pace in the last decade (Krishnamoorthy et al., 2021).

The biohydrogen production from algae follows specific distinct mechanisms based on its dependency on light. Biophotolysis and photofermentation utilize light for hydrogen production; contrastingly, dark fermentation occurs in the absence of light (Liu et al., 2022). Each method has its advantages and disadvantages; photolysis has challenges, such as lower biohydrogen yield, whereas fermentation is energy-intensive (Kumar et al., 2021). Hence, overcoming the challenges associated with the light-dependent/independent processes and producing biohydrogen at economically competitive prices has become the research focus. Fig. 2 shows the developments in hydrogen production from algae for the last 15 years since its emergence.

Previous review articles have discussed either regarding microalgae or macroalgae as feedstocks for biohydrogen production. In most cases, the discussion on macroalgae is overshadowed by microalgae due to their credibility and environmental benefits. Also, the focus on economic perspectives of algal biohydrogen generation is very limited. This manuscript not only aims in addressing the above-mentioned concerns by commensuration of all algal biomasses and presenting monetary aspects but also details the mechanisms undergone by the biomasses to produce biohydrogen. In addition, the article emphasizes on the strategies explored to improve production, impact of influential experimental parameters and the practical challenges associated with scaling up of the technology. Although the authors have discussed hydrogen production, the overall process has been attempted only in a few papers. This review paper is intended to cover the recent progress, challenges and research gaps in biohydrogen production from algae. The enzymes essential for the hydrogen production processes, their working mechanisms have been discussed in detail and have been compared with each other. Biohydrogen production is coordinated by multiple sequences of reactions and different parameters (experimental and environmental) that could affect the overall process. Hence, it becomes crucial to understand the effect of various parameters to optimize production. This manuscript has examined and discussed the impact of such influential parameters. In addition, the biohydrogen process's economic aspects have been reviewed to evaluate the viability of hydrogen production from algae on a larger scale. This review also discusses the challenges associated with various steps of biohydrogen production from algae and suggests future scopes for overcoming the bottlenecks.

2. Enzymes involved in algal biohydrogen production

The biohydrogen production in algae occurred after a series of reactions. During these biological and electrochemical reactions, hydrogen is produced and consumed in different steps. The reaction is facilitated by two key enzymes, namely hydrogenase and nitrogenase. These enzymes are vital in defining the net evolution of hydrogen during the complete process.

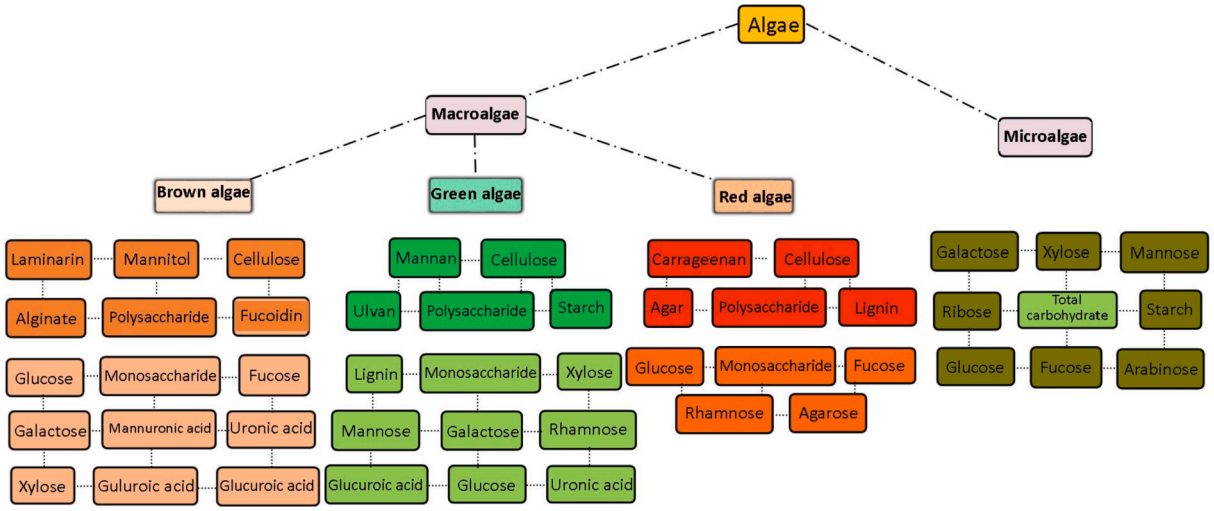

Fig. 1. Carbohydrates present in different types of algae.

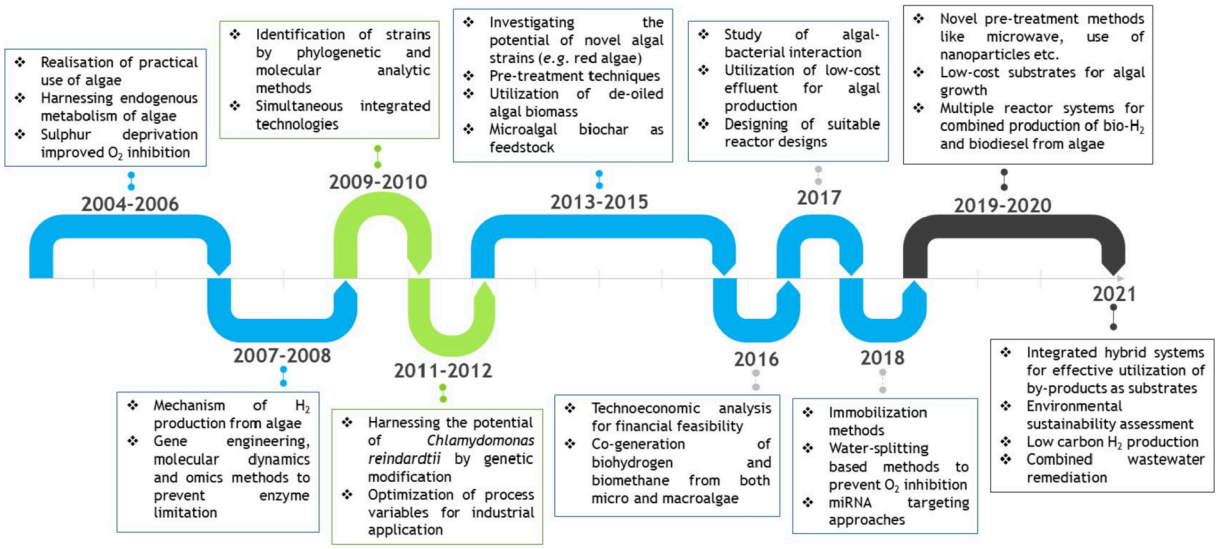

Fig. 2. Timeline indicating development of algal biohydrogen production.

2.1. Hydrogenase

Hydrogenase is a metalloenzyme; depending on the metal present in the active site, it could be broadly classified into [NiFe], [FeFe], and [Fe] types (Singh & Das, 2018). Among them, [Fe] type is found only in the archaea, which has been excluded from the discussion. [FeFe] hydrogenase mainly catalyzes the reduction of H^+ ions and produces hydrogen, though it could also catalyze the oxidation of H_2. Contrastingly, [NiFe] hydrogenase catalyzes hydrogen consumption reactions. The consumed H_2 is oxidized to H^+, which helps recover the energy lost during N_2 fixation (Yan et al., 2021). The active sites of [FeFe] hydrogenase have a protein subunit linked with peptides that allow the interactions. The modular domain has additional clusters that transfer electrons to the catalytic H-clusters. The unique structure of [FeFe] hydrogenase is due to the existence of H-clusters, making them 100 times more effective than other H_2 producing enzymes (Birrell et al., 2021).

The [FeFe] hydrogenase is highly sensitive to oxygen and thus gets inhibited when exposed to aerobic conditions. During bio-photolysis (discussed in the next section), the generated O_2 impedes hydrogenase activity and reduces the hydrogen production efficiency. However, the [NiFe] hydrogenase's active site of the dinuclear thiolate bridged the Ni-Fe complex, showing a better O_2 tolerance than the former (Sun et al., 2019). The eukaryotic organisms only have [FeFe] hydrogenase; for instance, the stroma in the chloroplast of green algae contains this enzyme. On contrary, [NiFe] hydrogenase is found in prokaryotes such as cyanobacteria, bacteria, and archaea (Yan et al., 2021).

2.2. Nitrogenase

Nitrogenase is found in photosynthetic bacteria and archaea, it reduces the molecular nitrogen (N_2) to a readily usable form of ammonium ion. This is an ATP-consuming process, essentially serving two purposes; first, it fulfils the nitrogen requirement for the microbes; second, it maintains the atmosphere's nitrogen cycle. The reduction process is conjugated with hydrogen ions reduction to hydrogen. So during the nitrogen fixation process catalyzed by nitrogenase, hydrogen is produced as a by-product (Burén and Rubio, 2018). Like hydrogenase, nitrogenase is also a metalloenzyme, in which different metal co-factors aid the catalytic site. Depending on the metal present in the active area, nitrogenase enzyme is classified as molybdenum, vanadium, or iron nitrogenases (Einsle & Rees, 2020). All three participate in the nitrogen fixation reaction and can produce hydrogen; however, molybdenum nitrogenase is the most studied enzyme related to hydrogen production. The nitrogenase is comprised of two subunits of protein: (i) Mo-Fe-S protein (which is the larger dinitrogenase coded by *nif*K and *nif*D genes), and (ii) Fe-S protein (dinitrogenase reductase coded by *nif*H gene). The average weight of Mo-Fe-S protein and Fe-S protein subunits are 210 kDa and 65 kDa respectively (Einsle & Rees, 2020). The dinitrogenase working depends on both subunits; the electron coming from an external electron donor (flavodoxin or ferredoxin) enters the reductase protein and then gets transferred to the dinitrogenase protein. This electron subsequently reduces the proton into H_2. For one electron transferred into the metal cluster complex during each cycle, 2 ATP is hydrolyzed. The nitrogenase turnover is 6.4 s^{-1}, which is considered very slow and results in low efficiency (Lunprom et al., 2019).

3. The mechanisms involved in algal biohydrogen production

Biological production of hydrogen employing algae involves two main approaches: (1) Light-independent process, where the algal intracellular macromolecules assist as substrate for fermentation and (2) light-dependent process entailing photolysis.

3.1. Dark fermentation

Hydrogen is known to be biologically produced by fermentation, with microorganisms' ability to utilize accessible nutrients such as carbohydrates and proteins. Dark fermentation (DF) is the most utilized strategy for bio-H_2 production due to its simplicity, high production rate, versatility of using a range of substrates and ability scale-up (Srivastava et al., 2020). The dark fermentative microbes assimilate the sugar-rich compounds (glucose, galactose, mannose etc.) and convert them into hydrogen. Hydrogen production by microorganisms is a natural way of venting out the excess electrons coupled with fatty acids or alcohols production. Broadly, there are two common pathways for H_2 production through DF (Bhatia et al., 2021). The detailed mechanism of dark fermentation can be found in Sambusiti et al. (2015). The efficiency of DF largely relies on the microbial inoculum, substrate type used as they directly influence the end product of fermentation. For example, if lignocellulosic biomasses are used, inclusion of pre-treatment technologies for better assimilation of the substrate becomes necessary. Similarly, the process also depends on the anaerobic strain, rate of substrate disintegrations and its consumption by the organisms, thereby affecting the H_2 production (Bhatia et al., 2021; Soares et al., 2020). DF requires a precise balancing of operational factors such as pH, specific organic loading rate and temperature. While dealing with fermentation

processes, the role of bioreactor configuration and operation becomes indispensable. DF, as a core technology, depends on the aspect of biorefinery. While dealing with fermentation processes, the role of bioreactor configuration and operation becomes indispensable. The batch mode of DF is highly reported in the literature due to its flexibility and simplicity. However, a continuous way is more reliable in terms of economic feasibility and industrial applications. They can relatively handle and process large amounts of substrates. However, this is a generic statement provided from a point of view that most of the pilot-scale reactors (up to 10,000 L) developed for biohydrogen production are based on dark fermentation with substrate concentration up to 75 g L^{-1}. It is value addition of DF that the pathways associated contribute to the simultaneous production of bio-H_2 and profitable by-products such as acetic acid, lactic acid, butyric acids etc, which in turn can be used as substrates for other fermentation processes or purified and commercialized. The fermentative system can also be coupled for treating residual liquid streams. In addition, it can also be coupled with other hydrogen production technologies for improving H_2 yield (Kumar et al., 2020).

3.2. Photo-Fermentation

In photo-fermentation, the VFAs and organic acid compounds formed as a result of DF are utilized as natural substrates by a group of photo-fermentative microorganisms in light. The organic wastes are broken down into CO_2 and H_2 using ATP molecules. The photo fermentation can result in higher hydrogen (theoretical yield of 8 mol H_2 mol^{-1} glucose) and this process is not affected by the negative feedback inhibition of nitrogenase enzyme in the presence of O_2 as the organisms that undergo photo-fermentation lack PS II (which is sensitive to O_2) (Kapdan et al., 2009). These organisms are flexible in switching between photosynthesis and photo-fermentation in the presence of carbon-rich substrate and nitrogen-deprived conditions, respectively. However, this method's bottleneck resides in the long duration required for the process completion (Bundschuh & Chen, 2014).

3.3. Bio-photolysis

Like photosynthesis, bio-photolysis is a photon-driven process frequent among algae and cyanobacteria for hydrogen production. It differs from photosynthesis by directing the reductants produced by splitting water for hydrogen evolution without entering the Calvin cycle or Pentose phosphate pathway. Photolysis is also reported in algal species such as *Chlorella vulgaris, Chlorella sorokiniana, Platymonas subcordiformis,* and *Platymonas helgolandica* (Ban et al., 2018). The photochemical oxidation occurs at the thylakoid membrane of algae and cyanobacteria, where the light-absorbing pigments are arranged as two functional arrays, photosystems (PS) I & II. Under anaerobic conditions, or when the system absorbs very high energy, certain microorganisms tend to direct the excess electrons to hydrogenase, converting the H^+ ions to H_2 gas. It has also been reported that the electrons and protons generated in the process can be recombined to form H_2 (98% purity) using the chloroplast hydrogenase enzyme). Bio-photolysis is further categorized as direct bio-photolysis and indirect bio-photolysis. In direct bio-photolysis, hydrogen is liberated by decomposing water molecules due to light absorbed by PS II. In the case of indirect photolysis, the light energy gets converted to chemical energy, leading to biomass production. Subsequently, the cellulosic substrate (carbohydrates) in the biomass is hydrolyzed by hydrogenase and nitrogenase enzymes in the presence of H_2O and light with subsequent liberation of H_2. The overall process from algal biomass treatment to hydrogen production has been summed up in Fig. 3.

4. Factors influencing algal biohydrogen production

Besides the type of organism chosen for H_2 production, the operational conditions such as light intensity, pH, temperature, substrate availability and conversion efficiency can significantly affect the performance of bio-H_2 synthesis. The influence of various parameters on hydrogen yield has been shown in Fig. 4.

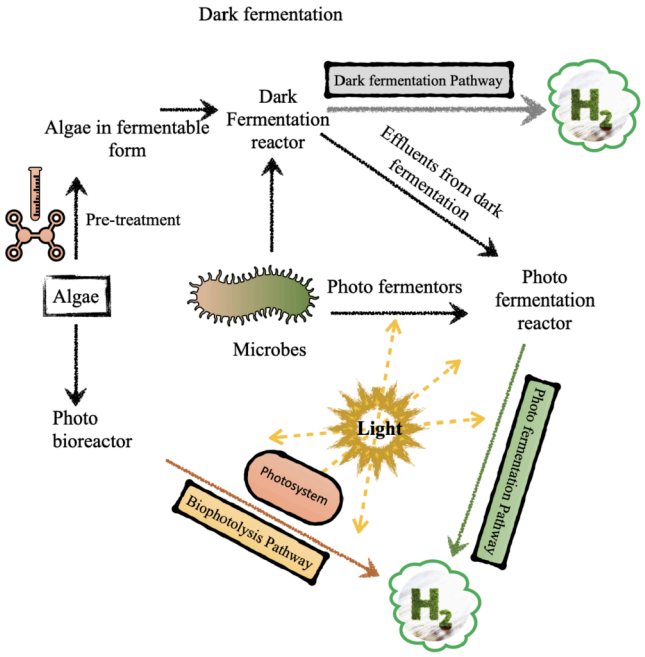

Fig. 3. Illustration representing the overall algal biohydrogen production process.

4.1. Light

A sufficient light supply is necessary for algal growth and the consequent photosynthetic generation of hydrogen (Kumar et al., 2018a; Kumar et al., 2018b; Kumar et al., 2018c). For this purpose, the reactors used should be designed to expose the organisms to optimum light conditions. For example, algal photobioreactors can be constructed to control algal response to light for enhanced photon conversion efficiency. Sometimes, due to the light saturation effect, the light conversion efficiency is low at higher intensities. This inefficiency is because of the high rate of photon absorption by chlorophyll molecules present on algal surface exceeding the photosynthesis rate. As a survival strategy, algae accumulate large quantities of chlorophyll molecules for photon absorption. High absorption can lead to the emission of excess photons as heat. This can also cause photo-inhibition in the upper layers of algae due to the high photon absorption rate. Other critical issues associated with optical limitations, such as light saturation of photosynthesis and chlorophyll antenna size, should also be addressed in the account of mass culture conditions (Degrenne et al., 2010).

4.2. pH

pH plays a crucial role in the fermentation reactions of bio-H_2 production. It affects the specific activity of enzymes involved, hydrolysis of complex substrates, microbes' diversity, and their metabolic pathways. The optimum pH range is said to be between 4.5 and 8.0 (Vargas et al., 2020). This broad range can be due to the variety of substrates and microorganisms utilized. For instance, the use of glucose (simple sugar) required an ideal pH of 6 for high hydrogen yields, whereas, for food waste (complex), high H_2 yields were recorded at a pH of 8.0. Fewtimes, the pH declines below 4.5 due to the accumulation of VFAs, which can shift the metabolic pathways towards solventogenesis, thereby decreasing the H_2 production (Srivastava et al., 2020).

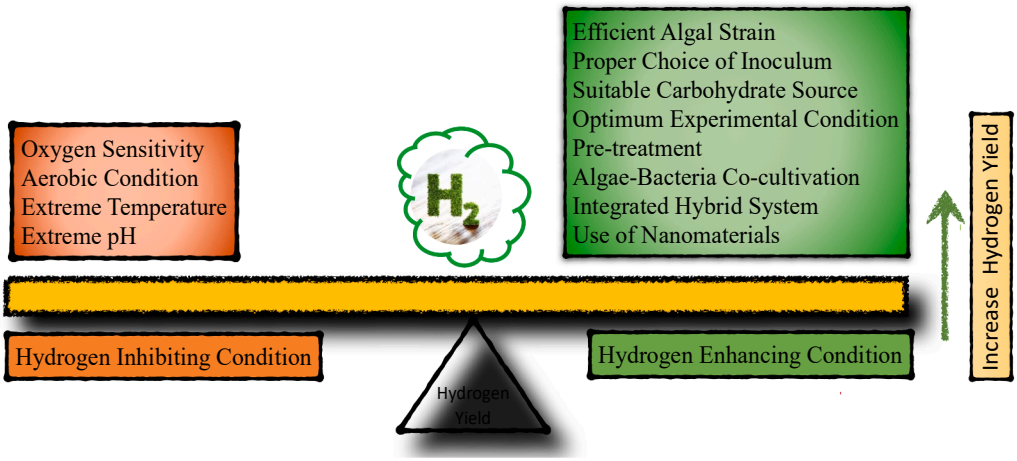

Fig. 4. Factors affecting hydrogen yield from algal biomass.

4.3. Temperature

Temperature can be the most influential parameter concerning the diversity and composition of microbe, an organic acid produced and its subsequent metabolic pathway involved in fermentation. A significant difference among the bacterial communities in the temperature between 37 and 55 °C is observed; overall, the temperature ranges investigated for hydrogen production from algae includes mesophilic (35 °C), thermophilic (>50 °C) and hyperthermophilic (>65 °C) (Vargas et al., 2020). The highest H_2 production can be placed in the order mesophilic > hyperthermophilic > thermophilic. The organisms varied from *Clostridium* to *Thermoanaerobacterium* at mesophilic to thermophilic conditions, respectively. However, the temperature regime did not substantially influence the hydrogen yield but affected the metabolic pathways. The distribution of predominant metabolites formed also varied between thermophilic and mesophilic. Butyrate was formed under mesophilic conditions and acetate under thermophilic conditions (Kumar et al., 2018a; Kumar et al., 2018b; Kumar et al., 2018c).

4.4. Carbon source

Carbohydrate is the critical component that contributes to the fermentative production of H_2. Many mono and polysaccharides have been reported as substrates intended for bio-H_2 production. The process efficiency might change based on carbon source used (Bundschuh & Chen, 2014). Galactose is a carbohydrate component commonly present in macroalgae. In some strains like *Gracilaria verrucosa, Gelidium sesquipedale* and *Gelidium amansii* the galactose composition varies from 20 to 30% on a mass basis. However, it requires more enzymes for consumption than glucose; hence, glucose is highly preferred. However, galactose has opted in conditions where acetic acid and butyric acids can be acquired as by-products (Maurya et al., 2016a; Maurya et al., 2016b). Sometimes, a combination of sources has also been proven beneficial. In a study by Fan et al. (2016), the mixture of 60% glucose and 40% peptone enhanced H_2 production more than with glucose alone. Though peptone did not directly contribute to this effect, ammonia released by its breakdown neutralized the organic acids, thereby preventing acidification of the system. The list of various other carbon sources presents in algae that can be employed for H_2 production has been shown in Fig. 1.

4.5. Availability of macro and micronutrients

Besides the carbon (substrate) and energy requirements, microorganisms need macronutrients (nitrogen, phosphorus etc.) and micronutrients (iron, zinc etc.) essential for their growth. This is why nutrient composition directly affects the efficiency of fermentation (Kumar et al., 2018a; Kumar et al., 2018b; Kumar et al., 2018c). Algae are rich in almost all the vital nutrients, making them a worthy feedstock for bio-H_2 production (Srivastava et al., 2020). However, other intrinsic parameters such as carbohydrate/protein ratio, chemical composition and heavy metal concentrations are also critical for fermentation. It has been found that monosaccharides yield H_2 production 18 times greater than that produced from proteins (Kumar et al., 2020). However, proteins are needed to maintain the C/N ratio for the growth of fermentative bacteria. The optimum concentration of protein is still unclear. However, the ideal range of C/N reported is between 10 and 90.

Conversely, high protein concentration in algal biomass increases ammonium and ammonia levels, resulted in decline of the internal pH of H_2 generating bacteria culture, thereby rising the energy demand for maintenance of cell and the inhibition of specific enzymes (nitrogenases) required for bio-H_2 production. Additionally, high ammonium levels tend to inhibit the photosynthetic potential of bacteria. Hence, the removal of ammonium using zeolite is recommended.

Considering micronutrients, some metal ions are suspected of assisting enzyme and co-enzyme activation and cell growth. While dealing with algae, its ability to accumulate heavy metals also has to be considered. Hence the metal ions can be categorized as light (magnesium, calcium, sodium etc.) and heavy (iron, nickel, zinc, copper, lead, chromium, cadmium etc.). Depending on their concentration, these metals can have either a favourable or unfavorable impact on the biochemical pathways involved in H_2 production. Nevertheless, iron and nickel are the active core elements of [FeFe]-hydrogenase and [NiFe]-hydrogenase enzymes that catalyze the oxidative production of H_2. Among these, iron is the most studied, because it involved biosynthesis of proteins and enzymes, and bacterial growth critical for DF, other metals, namely magnesium and zinc are engaged in the transportation across cell membranes or might serve as co-factors for different enzymes. However, the accumulation of certain ions beyond the limit might alter the system's salinity and fermentation efficiency. For example, the build-up of Na^+ concentration beyond two mg L^{-1} was shown to decrease the bio-H_2 levels drastically (Kumar et al., 2016b). The impact of several biotic and abiotic experimental parameters on biohydrogen yield and their generalized correlations have been summarised in Table 1.

5. Economic assessment of the algal biohydrogen production processes

The predominantly used technologies for commercializing hydrogen production are autothermal reforming (ATR), partial oxidation (POX) and steam reforming of methane (SMR). However, around 90% of total

Table 1
Effect of experimental parameters and conditions on algal biohydrogen yield.

Influencing factor	Effect on biohydrogen production	Illustration and remark	References
Reaction Temperature	The reaction generally takes place in three different sets of temperatures such as mesophilic, thermophilic and hyperthermophilic; however, in most cases the temperature is kept in a mesophilic range (30–40 °C)	Temperature impacts the hydrogen production process by effecting the metabolic pathway and enzymic activities	Vargas et al., (2020)
pH	The optimum pH range for H_2 production has been reported to be between 4.5 and 8.0	pH affects the hydrolysis of complex substrates and activity of enzyme. Depending on the substrate, the optimum pH for hydrogen production varies	Vargas et al., (2020)
Algal strain and their oxygen tolerance capability	More the oxygen tolerance capability, more the potential of algae to produce hydrogen	For instance, among five algal biomasses (*Chlamydomonas moewusii, Chlorella fusca, Chlorella pyrenoidosa, Chlamydomonas reinhardtii and Scenedesmus obliquus*), *C. pyrenoidosa* was found to be more tolerable to oxygen and hence produce the highest hydrogen yield	Fan et al., (2016)
Carbohydrate concentration	The percentage of carbohydrates plays a vital role in dark fermentation due to its direct correlation with the fermentation rate	A higher proportion of fermentable sugars such as glucose and galactose potentially increase the chance of higher production of hydrogen	(Kumar et al., 2020)
Microbes (based on the fermentation process)	Hydrogen production through different processes involves process-specific bacteria	*Clostridium butyricum, C. beijerincki* and other species of Clostridium are essentially used for dark fermentation, whereas *Rhodopseudomonas palustris* is primarily reported to aid photo fermentation process	(Kumar et al., 2016a)
Microbes used	Among the mesophilic and thermophilic microbes, thermophilic inoculum shows the higher hydrogen production capability	Mesophilic microbes show certain amount of methanogenic activity; however, in case of thermophilic inoculum no methanogenic activity was reported resulting in higher hydrogen productivity	(Kumar et al., 2016b)
Light intensity	In case of biophotolysis, hydrogen production efficiency increases with the increase in light intensity; yet, at the higher light intensity it may lead to photoinhibition and a decrease in hydrogen production capability	Increase in light intensity increases the cell and chlorophyll number. Moreover, it also helps in achieving the sulphur deprivation state rapidly leading to reduction in lag time and increase in biohydrogen productivity increase. However, when the light intensity is too high, it damages the PS II system and reduces hydrogen production	Liu et al., (2019)
Algal growth condition	Based on the growth of algae in different mediums, the biochemical properties might vary and that adds up for a change in biohydrogen production	With the increment in swine manure concentration, the hydrogen production increased. Yet at high concentration, the productivity decreased	Kumar et al., (2018b)
Sulphur deprivation	Sulphur deprived algal biomass produce a comparatively larger amount of hydrogen as that of non-deprived ones	Depriving cells off sulphur partially inactivates the PS II activity, making the culture anaerobic and ceasing biomass formation. This makes the cells to produce hydrogen to get rid of the excessive energy which could have damage the cells	Skjånes et al., (2008)
Other chemical deprivation	In addition to sulphur. nitrogen and potassium deprivations have been reported	Potassium deprivation have a positive impact on hydrogen production, but nitrogen deprivation leads to decrement of hydrogen production	Papazi et al., (2014); Vargas et al. (2018)
Acid concentration	Acid treatment at lower concentrations enhances the hydrogen yield; however, the yield decreases at higher concentration	When the biomass was treated with a range of acid concentrations (0.5–5 %), the optimum hydrogen yield was observed at 2 %	Cheng et al., (2019b); Fakhimi et al., 2019
Microwave treatment temperature	With an increase in temperature the biohydrogen yield increases; however, at higher temperatures, the yield lowers	With increase in temperature, the cell disintegration increases and more carbohydrates will be available for degradation. But at very the high temperatures, polysaccharide to protein ratio decreases, thus decreasing the hydrogen production	Yin et al., (2019); Kumar et al., (2022)
Type of acid	The most commonly reported acids are HCl, H_2SO_4, HNO_3, and H_3PO_4. Among them H_2SO_4 has showed to produce more hydrogen	Acid treatment leads to enhanced production of volatile fatty acids such as butyrate and acetate. The fermentative hydrogen production mediated through the butyrate mediated pathway was observed to produce more hydrogen. H_2SO_4 treatment results in maximum production of butyrate and hence generates the highest hydrogen	Cheng et al., (2019a); Cheng et al. (2019b))
Biomass concentration variation	With the increase in biomass concentration, hydrogen production increases; however, at higher biomass concentration, the hydrogen yield decreases	Biomass is used as the substrate, which provide the requisite amount of nutrient required for the process, however at very high concentration, inhibitory substances like furfural are formed that negatively affect the hydrogen production	Giang et al., (2019)
Inoculum percentage variation	When the inoculum was varied at lower concentration (0–6%), the inoculum concentration of microbes directly correlated with hydrogen yield. With subsequent increase in inoculum concentration, the hydrogen decreased	Higher inoculum loading ratio leads to the formation of large number of metabolites. These metabolites induce stress and creates an unfavourable condition for biohydrogen production	Sivagurunathan et al., (2018)
Age of algal biomass	Microalgal biomass harvested in their exponential growth phase produces higher hydrogen than that of stationary phase harvested one	Cells present in exponential phase are more viable and metabolically active, resulting in higher hydrogen productivity than that of biomasses present in stationary phase	Vargas et al., (2020)
Effect of ammonia	The presence of ammonium in the growth medium of algae can reduce or enhance the hydrogen production	Ammonium ions can serve as electron acceptor and hence could activate the nitrogenase activity. This can lead to increased hydrogen production. On the other hand, few researchers have also reported that ammonium ion can be redirected to synthesize amino acids instead of hydrogen	Vargas et al., (2020)
Addition of chemical	Chemical such as Oxysorb (100 mM sodium ascorbate and 5 ppm cupric sulfate) remove oxygen produced during the process, thus improve the hydrogen yield	Oxysorb added in non-cytotoxic concentration (100 mM) observed to increase the hydrogen yield by 2–5.5-fold as compared to sulphur deprived cultures	Khosravitabar & Hippler, (2019)
Use of nanomaterial			Srivastava et al., (2020)

(*continued on next page*)

Table 1 (*continued*)

Influencing factor	Effect on biohydrogen production	Illustration and remark	References
	Nanomaterial addition could significantly improve the hydrogen yield. Their catalytic activities facilitate many essential processes of hydrogen production	Nanomaterials at higher concentrations can inhibit hydrogen production and might cause toxicity	
Gene manipulation	Gene manipulations are mainly intended to increase the oxygen tolerance ability of the enzyme, and consequently increase in hydrogen production	Gene manipulation can be performed by random mutagenesis under oxygen stressed environment, or by modifying the genes responsible for hydrogenase oxygen sensitivity	Khan & Fu, (2020)
Sparging of gas	Sparging of gas ensuresthe anaerobic condition required for the process and thus can increase the hydrogen production efficiency	Sparging inert gas can lower the partial pressure of O_2, helping in maintaining the activity of hydrogenase. However, the purging of gas could increase the hydrogen production cost	(Lam et al., 2019)
PSII inhibitor	PS II inhibitors namely DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), can increase the hydrogen production	The PS II inhibitors block the electron flow between PS II and PS I, hence induce the generation of molecular H_2. It also blocks the generation of O_2 by PS II	Ban et al., (2018)
Algal bacterial interaction	The interaction allows starch, protein, and chlorophyll accumulation that is essential for hydrogen production	They exhibit co-metabolism. For instance, the oxygen produced from algae is consumed by bacteria, providing the necessary oxygen-deficient environment	Ban et al., (2018)

hydrogen generation is through the SMR technique. It could avail hydrogen at the cost of USD 7/GJ (El-Emam and Özcan, 2019). Unfortunately, the intermittent processes result in the emission of CO_2, which makes the process carbon positive, thus jeopardizing environmental sustainability. To make the process carbon neutral, catalytic conversion techniques such as Fischer-Tropsch (FT) synthesis are generally implemented, increasing the production cost by 30–40% (Sun et al., 2017). Alternatively, to avoid/minimize greenhouse emissions, the fossil-based biomass could be replaced by carbon–neutral biomass. However, the cost and the requisite conversion routes nearly double the production cost, making the process less economically alluring. Apart from SMR, water electrolysis has been implemented on a larger scale for hydrogen production. Although the process is feasible from an environmental perspective, converting high-grade electric energy to low-grade chemical energy is not economically sustainable.

Asides from hydrogen production at an industrial scale, hydrogen generation at a smaller scale have attracted more interest. In the case of small-scale decentralized hydrogen production, transportation costs could be reduced significantly, and the potential of utilizing onsite non-usable biomass has made the process more economical.

Behera et al. (2019) performed a techno-economical analysis of an algal production plant with a capacity of 4565 tonnes (wet mass based on an operation of 330 days). They calculated the total capital expenditure to be 819 million USD and the operational cost to be 440 million USD. The 64% of the total capital cost was attributed to equipment purchase, installation, and construction occupancy. In particular, the highest cost is associated with the belt filter, reactor, clarifier, and mixing tank. Similarly, in operational cost, 38% is related to maintenance costs, property taxes, and other overhead charges, whereas raw material cost accounts for 36% of the total operational cost. Algae promise higher biomass and hydrogen yield than terrestrial biomasses. So, choosing algal biomass for biohydrogen production is more economically advantageous. The biohydrogen production cost from algae could be further reduced with proper research and development in selecting the appropriate strain, cheap and effective pretreatment process, optimizing the process conditions during the algal growth, implementing innovative techniques for efficient harvest action, and maximizing the yield by providing a specific environment for hydrogen production.

For instance, the currently adopted pre-treatment techniques are still at emerging stage. They need to be upgraded further to maximize sugar recovery without creating any secondary pollution. Unlike other biomasses, algae have high lipid content that could be extracted and processed to produce biodiesel. Bryant et al. (2012) estimated the de-oiled microalgae biomass (DMB) value around 100–225 USD ton^{-1}. It is crucial to note that DMB remains the only by-product after oil extraction from the algal biomasses. The biodiesel market is predicted to grow at a 16.43% CAGR and is anticipated to reach a valuation of USD 47.9 billion by 2025 (Chandel et al., 2020). Thus, this process (Utilising the remnant biomass in other applications such as hydrogen *production*) yields an additional credit of USD 0.95–2.43 per gallon of biodiesel. It has been estimated that while producing 1 gallon of algal biodiesel, around 10 kg of DMB is produced (This estimation assumes that algal biomass consists of 25% of lipid and the rest is DMB). Consequently, there is a reduction in the DMB price from 100 to 225 to 28.5 USD ton^{-1} (Maurya et al., 2016a; Maurya et al., 2016b). These circular biorefinery approaches ensure the cheap availability of biomass, improving the profitability of biohydrogen production.

Capital expenditure and operating costs are two pivotal factors determining the profitability of biohydrogen production from algae. Based on the process, the cost breakup might vary. However, the cost of land (required for the open pond algal culture), photobioreactor setup (along with the installation charge), expenditure for handling the gas produced during the process (some gas might inhibit the process), energy requirement, maintenance cost, and workforce requirement for process operation in the production facility are the primary considerations for an economically sustainable operation (Fig. 5). Although many researchers have highlighted the biohydrogen production potential of algae, very few works have been reported in which the economic assessment of the process was discussed (Table 2). It could be observed that operating costs are higher compared to capital expenditure for fermentative processes. The availability of substrates in the fermentation process could be a significant contributing factor to higher operational costs. Sathyaprakasan & Kannan (2015) compared the theoretical estimated cost for biohydrogen for all four methods (direct/indirect

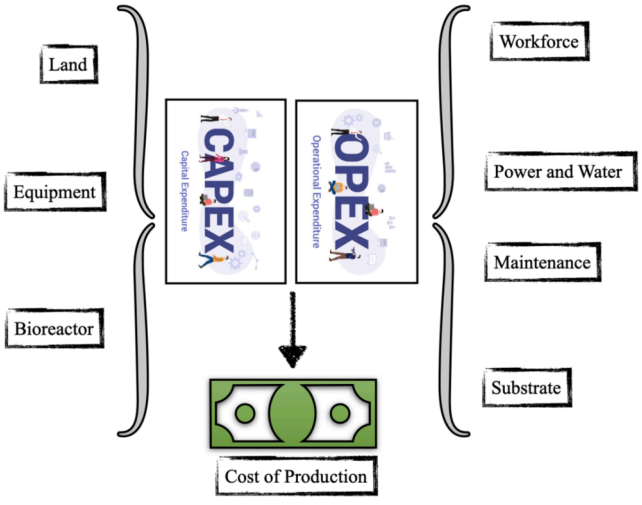

Fig. 5. Expenditures in algal biohydrogen production process.

Table 2
Economic analysis of algal biohydrogen production.

Mechanism of biohydrogen production	Biomass used	Expenditure breakdown		Cost of production (USD kg^{-1} H_2)	Scale/amount of production	References
		Capital cost (%)	Non-capital cost (%)			
Direct photolysis		82	18	1342		
Indirect photolysis	Algae	80	20	2	Estimated theoretically for large scale production	Sathyaprakasan & Kannan, (2015)
Photo fermentation		14	86	3.7		
Dark fermentation		2	98	18.7		
Photolysis	Microalgae	~ 78	~ 22	0.57–13.53	Large scale	Amos (2004)
Indirect photolysis	Algae	78	22	10 GJ^{-1}	Large scale	Benemann (1997)
Direct photolysis	Algae	50/m^{-2}	–	2.13	Estimated for large scale production	Nikolaidis & Poullikkas (2017)
Indirect photolysis	Algae	135/m^{-2}	–	1.42		
Photolysis	Green algae	–	–	2.80	Large scale	Melis & Happe (2001)
Photolysis	Algae	80	20	15 GJ^{-1}	Large scale	Tredici and Zittelli (1998)
Dark fermentation	Food waste	74	26	3.2	Large scale (Estimated)	Yun et al., (2018)
Fermentation	Macroalgae	35	65	22.8	Batch reactor(Estimated)	Mthethwa et al., (2018)
Direct photolysis	Algae	–	–	10–20 GJ^{-1}	Large scale (Estimated)	(Akkerman et al., 2002)

(USD: United States Dollar; GJ: Gigajoules).

photolysis, photo and dark fermentations). The hydrogen production cost for direct photolysis was incredibly higher than other methods. The labour cost, culture production cost, and general supply cost for indirect photolysis were>500 times as compared to other methods. This is because direct photolysis has a large land requirement, and hence the capital and operating cost is much higher. Whereas for indirect photolysis, the hydrogen production rate is higher, and hence the cost of production is more reasonable. Though dark and photo fermentation do not require a large land area but the cost of glucose substrate (that will be use in the process) is significantly higher.

Thus, the indirect photolysis process could produce hydrogen at a lower price than the fermentative processes. In between the photo and dark fermentation, photo fermentation is more economical. However, it is essential to note that majority of them are operational expenditures. This indicates that these prices can be brought down with the advancement of research and development. It is highly crucial to note that majority of the economic assessments were based on optimistic assumptions, and only significant factors have been considered, which makes it a little presumptive. While dealing with the process's overall economics, smaller elements could add up and might result in a diversion from the anticipated economic outcome. Hence, a margin of safety needs to be there before setting up a large-scale production facility. The reported economic assessments are a bit old, so the inflation rates need to be adjusted after looking into the numbers. Moreover, in the last two decades, a significant level of changes (the land price or use of advanced equipment, or better transport and storage facility) have been taken place in this sector, consequently affecting the cost of hydrogen production. Therefore, to properly understand the current market potential of algal biohydrogen, it becomes imperative to perform the latest economic assessment of the overall production process. The analysis should also consider the integrated hybrid systems (combination of sequential or simultaneous processes) to make the hydrogen production from algae economically more competitive in the market. For example, the hybrid systems integrating DF and photofermentation can yield 12 mol H_2 mol^{-1} hexose in theory. It was stated that the bio-H_2 potential increased from 96.6 to 337 mL g^{-1} of total solids when this combined process was used (Ren et al., 2019).

One of the parameters which can be considered to evaluate the success of a pilot-scale study is a return on investment (ROI). The ROI can be calculated using Eq. (17).

$$ROI = \left(\frac{AF}{FCI}\right)*100 \quad (17)$$

where AF is the annual profit (difference between annual revenue and annual profit) and FCI is the fixed capital cost. Fixed capital cost is the one-time investment such as procuring the land, and buying and installing the equipment. An ROI above 20% is considered as profitable for scaling up the process. Han et al. (2016a) have analyzed the profitability of hydrogen production from food waste via dark fermentation; they estimated the ROI of a pilot-scale plant for a life span of 15 years. The AF (after tax) was 146,473 USD and the FCI was 547,504, this gives rise to an ROI of 26% with a payback period of 5 years. Han et al. (2016b) the ROI of a continuously mixed reactor with a life span of 10 years. They observed the ROI varies with the reactor volume, for instance, when the reactor volume was increased from 10 to 50 m^3 the ROI increased from −37% to 47%. This indicates the larger reactor volume could provide a more attractive ROI. Thus, the equipment cost and reactor volume can have a significant impact on the profitability of the process.

Recently, Nikolaidis and Poullikkas (2017) estimated that biohydrogen from algal biomass can be produced at the cost of 2.13 USD and 1.42 USD by using direct and indirect photolysis pathways which is quite encouraging from an economic perspective. However, Sathyaprakasan and Kannan (2015) estimated that the cost for direct photolysis could be much higher than this. Similarly, Mthethwa et al. (2018) estimated the hydrogen production cost through dark fermentation is coming out to be 22.8 USD. However, Yun et al. (2018) reported that by utilizing food waste, the hydrogen production cost through dark fermentation could be brought down to 3.2 USD. With the present scenario, the average algal biohydrogen production cost lies between 10 and 20USD/GJ (Show et al., 2019), which is at par or better than conventionally implemented hydrogen production techniques. However, it is still more costly than other fuel alternatives. So to make the process economically acceptable at an industrial scale, the economic barriers need to be tackled meaningfully, for which certain technical and engineering challenges need to be addressed.

6. Challenges associated with biohydrogen production: The research gap

The biologically produced hydrogen from algal biomass is a sustainable source, and in terms of energetic value, the hydrogen is considered superior to that of other conventional and available fuels. However, hydrogen from algae still has to overcome a number of bottlenecks before being accepted on the industrial scale. The overall process consists of several sub-processes (starting from selecting an algal strain to the purification of produced hydrogen) with bottlenecks. Some of the significant and common challenges have been outlined in this section.

6.1. Challenges with algal biomasses

Several researchers have investigated the utilization of wastewater for algae growth; the microalgae can remediate the water pollutants

during its growth. This makes the process more sustainable and economical. Moreover, algae can also be grown in wastewater, lessening their dependency on freshwater. Both macroalgae and microalgae have been grown using various wastewater such as municipal effluent, industrial effluent, and animal wastewater (Whangchai et al., 2021; Arashiro et al., 2020). It was observed that algal biomass biochemical composition is dependent on its growing conditions, especially on the nutrient content of the media (Choudhary et al., 2020). For instance, algae grown in brewery effluents have higher carbohydrate content, similarly, Zhu et al. (2013) noticed that use of piggery wastewater helps in increasing the carbohydrate content of the algae, however, when the concentration of the wastewater increased significantly then the growth condition favours algal growth having higher protein content. However, industrial effluents were observed to contain contaminants such as heavy metals, hydrocarbons, organic acids, ammonia, and urea, which could impact the algal growth (Hodaifa et al., 2009). Hence, careful consideration should be given while choosing the wastewater for growing the algae.

The water footprint for the overall process can be calculated by estimating the water required for algal cultivation, harvesting, evaporation, cooling of algal biomass, and then hydrogen production from the biomasses. The water footprint for hydrogen production comes out to be 76.77 L per MJ of hydrogen (Cui et al., 2021). It was estimated that 99% of the water footprint is contributed by biomass cultivation, and the rest of 1% is attributed to transportation and hydrogen production; however, the estimation is for lignocellulosic biomass. Junior et al. (2018) estimated that around 3726 kg of water is required to produce 1 kg of algae and to produce bioethanol from it, but by using recycled water, the water requirement can be brought down to 3.7–1612 kg. However, the recent surge in algal growth in wastewater for simultaneous treatment and nutrient recovery can potentially replace freshwater utilization. More studies need to be done to evaluate the water footprint associated with algal biohydrogen.

Storage of algal biomasses is a problem. Though drying the biomass helps store the algae for a longer duration, it is energy-intensive and costly. Ensiling could be a cheaper alternative where the algae are stored in a wet anaerobic condition in the presence of organic acids. However, it is not effective for long-term storage, as it was observed in days of ensiling, the carbohydrate composition of the algae decreased by 15% (Wahlen et al., 2019). Hence, more research should develop cheaper alternatives to store alage for a longer duration.

Another major challenge lies in the pre-treatment of algal biomasses. To enhance the isolation of sugar, a range of treatment methods has been adopted. During these, a significant amount of chemicals are used. Even though there is an increase the profitability, it makes the process chemical-intensive (Nagarajan et al., 2020). The environmental assessment of chemicals' fate and management needs to be performed to increase the process's sustainability.

Many photobioreactors are available for small and medium-scale production; however, attention is required to build large-scale production reactors. This will help increase productivity and thus would add economic value to the process. Apart from this, the availability of cheaper carbon sources is crucial for the process's economic feasibility.

The benefits that come with the introduction of genetic engineering in enhancing biohydrogen production are undeniable. It has helped alleviate several challenges. However, the potential risks associated with genetically modified organisms (GMOs) and their acceptability is a significant bottleneck for utilizing them on a commercial scale. One such problem associated with GMOs is the risk of horizontal gene transfer. Moreover, the currently implemented genetic modifications aim to disrupt endogenous genes instead of introducing a gene that could add new activities to the algae. The risk of horizontal gene transfer could be addressed by eliminating the plasmids containing the antibiotics markers and chromosomal integration (Datsenko and Wanner, 2000). The problem associated with genetically modified organisms needs to be resolved by balancing productivity and ethical issues.

6.2. Challenges with microbial cell culture

Sugar fermentation by appropriate hydrogen-producing organisms with high productivity is crucial. In general, mesophilic culture is preferred owing to its lesser requirements of heating and energy consumption. However, more research on mesophilic organisms is needed to evaluate and isolate the strain with more yield and production rates.

Another challenge associated with hydrogen-producing microbes is the requirement of immobilization. Much emphasis is required while selecting support material. The characteristics of the material (having a highly porous structure and surface area, high biomass holding capacity, lesser toxicity) need to be evaluated against the cost; a proper evaluation would increase hydrogen productivity without compromising the process's economic aspects. Although immobilization is intended to increase productivity, effective immobilization techniques such as entrapment and encapsulation often face the challenge of mass transfer resistance (Sigurdardóttir et al., 2018). This decreases the substrate's diffusibility to the cells and might lessen the overall productivity of the process. Hence, optimizing the immobilization method's operating parameters or supporting particle and substrate type is highly essential.

6.3. Challenges with the core hydrogen-producing processes

Generally, during the dark fermentation of algae, the fermentation of sugar most often results in the synthesis of inhibitors. Consequently, the formation of inhibitors affects hydrogen production and reduces the process's efficiency (Elbeshbishy et al., 2017). Hence for increasing the hydrogen yield, the formation of inhibitors needs to be eliminated or at least needs to be reduced with the proper optimization of process and experimental parameters. The other way is to remove the formed inhibitors immediately from the process environment.

One of the particular concerns associated with the dark fermentation of macroalgae is carbohydrates such as alginate, agar, carrageenan, etc. The monomers of these polymers have a high potential to copolymerize, making the fermentation process difficult for the anaerobic microbes, ultimately resulting in reduced hydrogen production (Shobana et al., 2017a; Shobana et al., 2017b). To reduce the polymerization of the fermentable sugars, saccharification of the existing carbohydrates could be an appropriate solution. Optimizing saccharification protocols could help avoid the re-polymerization and enhance efficient sugar recovery (as the monomeric form of the carbohydrates can be fermented easily).

While hydrogen production from the photolysis procedure has several potential advantages, too much oxygen liability of hydrogenases is a major hindrance to uncapping the benefits. The hydrogen production by bio-photolysis ceases within a few days by the inhibition of hydrogenase by oxygen; this jeopardizes hydrogen production's sustainability (Winkler et al., 2021). Many techniques achieve practical approaches to address the challenge related to hydrogenase oxygen sensitivity. One of the most commonly used procedures is to deprive the algae of sulphur; this ensures the prolonged hydrogen production in a light-dependent manner. Apart from this, genetically modified algae could be engineered oxygen tolerant; so that hydrogen production would not get affected by the formation of oxygen amidst the process. Finally, novel methods can be designed to separate the formed oxygen immediately from the process environment. If more research and development would be implemented to overcome the challenges posed by the hydrogenase oxygen sensitivity, the productivity of the light-dependent hydrogen production process could be increased significantly, which would add economic value to bio-photolysis.

The commercial implementation of light-dependent hydrogen production lags behind other processes due to its poor productivity. The utilization of fermentative by-products such as acetic acid can increase the energy productivity of the biohydrogen process and add economic value to bio-photolysis. The organic acids could be converted to methane and other alkanes to incorporate suitable units in the system (Betts et al., 2018).

The recent advancement in hydrogen production is the endogenous substrate catabolism. Although the process has been established, the detailed mechanism is yet to be understood. Due to the limited availability of information, the process is yet to be optimized; hence, the hydrogen production by endogenous substrate catabolism is not sustainable (Show et al., 2019). However, the prospect of endogenous substrate catabolism is essential, and its potential could be entirely tapped with the proper advancement of molecular bioengineering.

6.4. Post-production management and other challenges

The hydrogen production process consists of a series of processes and reactions. Hence, it is evident that several by-products will be produced during the overall process. Some products get reutilized or converted to other value-added products. However, certain organic compounds/residues also exist that leave out the process as waste. To make the biohydrogen production process entirely environment-friendly, safe disposal of these products or converting them to environmentally acceptable form is needed (Argun et al., 2017). Safe and efficient techniques need to be developed to process and utilize the left-over organic residues.

Hydrogen accumulation in the headspace of the reactor results in partial pressure of the hydrogen and could affect the reactor performance. The partial pressure is generally reduced by purging inert gas for ensuring safety. However, in lowering partial pressure, the purity of the hydrogen should not be compromised, as the purging of inert gas reduces hydrogen purity by 30–60% (Neves et al., 2009). Hence, by designing a suitable membrane, simultaneous production and separation of hydrogen can occur without compromising safety and purity.

Nanoparticles (NPs) are known to have an excellent capability of catalyzing activity and have the potential of greatly enhancing the catalytic effects of key enzymes associated with the saccharification of biomasses and biohydrogen production. Thus, the application of nanoparticles could increase the hydrogen yield significantly. However, the process's efficacy depends on the careful manipulation of parameters related to the incorporation of NPs. The main challenge associated with NP's application in biohydrogen production is toxicity. Depending on the type of nanoparticles and the used concentration, it could adversely impact organisms' growth and affect fermentation. For example, copper and palladium NPs inhibit microbes' growth and are reported to reduce biohydrogen productivity (Shanmugam et al., 2020). Therefore, the toxicity of NPs should be analyzed for a better understanding of their impact on microorganisms. Further attention should be given to their biocompatibilities and easy separation from the process mixture. So, an appropriate choice of NPs and an optimum concentration could benefit the microbes and increase biohydrogen productivity.

The techno-economic aspect of the overall bio-hydrogen production process is one of the least explored domains in the majority of the studies. To make biohydrogen production from algae plausible at an industrial scale, the economic aspects need to be focused.

7. Future scope

By focusing on the following research aspects, the cost of operation and production could be reduced without compromising the process's sustainability.

Availability of cheaper substrate (here algal biomass) is one of the critical needs for hydrogen production. To reduce the cost, wastewater can be utilized for growing algal biomass; moreover, emphasis on fast-growing algal strain could also be the right step in increasing the production efficiency.

For overcoming the challenges of dark fermentation, algal biomass having high carbohydrate content should be screened, and the fermentation possibility of sugar present in it needs to be researched.

More effort towards molecular-level research is needed for identifying and understanding the chromosomal gene associated with substrate utilization, its response to various nutrient limitations, and its role in hydrogen production.

The utilization of recombinant algal strain has shown some promising results towards alleviating the oxygen sensitivity issue of hydrogenase; hence, more research on recombinant technology could lessen the cost associated with algal strain and help eliminate the stigma towards the utilization of genetically mutated strains.

The optimum design of the bioreactor is highly essential for increasing process efficiency. The biochemical need for algae and easy manipulation of experimental process parameters must be considered while designing the bioreactor (Jung et al., 2022).

The COD-rich effluent from DF can also be utilized as a substrate for microbial fuel cells (MFCs) and microbial electrolysis cells (MECs). MFCs have exoelectrogens that can oxidize the organic matter at the anode terminal and reduce protons at the cathode terminal to generate electricity. In MECs, an external voltage supply is used to mobilize electrons across terminals to produce H_2 by proton reduction at the cathode. These integrated systems also achieve a high bio-H_2 production rate and yield (Wu et al., 2021; Nguyen et al., 2020).

The selection of appropriate catalysts could significantly improve biohydrogen production. The use of nanoparticles in catalyzing various reactions in the hydrogen production process has been emerging, thus, research needs to be focused on developing highly efficient, environmentally sustainable, and cost-effective catalysts.

The individual processes (bio-photolysis, dark and photo fermentation) were observed to mark low efficiency in hydrogen production. Therefore, to increase efficiency, many integrated approaches have been developed. More research needs to be done to simplify and optimize the overall integrated processes.

Total time consumed in the overall hydrogen production process plays an essential role in its economy. Hence, research on this aspect needs to be done to reduce the lag time in the process.

Many studies are done on a laboratory scale, but to understand the challenges associated with commercializing biohydrogen, more studies need to be carried out on a larger scale. Instead of growing in laboratory normal light illumination, the culture needs to be developed in solar irradiation. Bioreactors need to be designed to cope and withstand the impact of weather variation.

All the suggestions are intended to select appropriate algal strains and optimize the experimental conditions to simplify the process and reduce the cost of biohydrogen production.

8. Conclusion

Hydrogen could potentially be a clean and renewable substitute for the currently utilized fossil fuels. Although hydrogen from algae promises a clean energy source, the overall production cost is still expensive compared to other available fuel options. Recent researchers are focused on developing various techniques for improving efficiency and reducing the cost of hydrogen production from algae. However, there are several bottlenecks associated with the overall hydrogen production process which need immediate attention for achieving a sustainable and economically viable biohydrogen production from algae.

CRediT authorship contribution statement

Abhijeet Pathy: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Krishnamoorthy Nageshwari:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Rameshprabu Ramaraj:** Conceptualization, Writing – review & editing. **Gaanty Pragas Maniam:** Conceptualization, Writing – review & editing. **Natanamurugaraj Govindan:** Conceptualization, Writing – review & editing. **Paramasivan Balasubramanian:** Conceptualization, Investigation, Funding acquisition, Writing – review & 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

No data was used for the research described in the article.

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