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Review Article

Biohydrogen: Opportunities and challenges as an alternative energy resource

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Abstract
As the energy demand is continuously rising with the increase in population, the use of fossil fuels is also increasing at the same rate. These fossil fuels release greenhouse gases (GHG) which are harmful to human health and our environmental health and these fuels are also expected to exhaust in the near future. This eventually has led to an emerging need to shift to a more reliable, sustainable, clean energy source. Biohydrogen as fuel is a potential alternative, as hydrogen has proved to be one such fuel which has the potential to replace fossil fuels. There is a need to produce it in a clean, sustainable way to compete with the fuels that are being used currently. The hydrogen which is produced biologically is known as biohydrogen. Microorganisms also play a huge role in the process of hydrogen generation by virtue of their natural mechanism. Hydrogen can be produced biologically using approaches like biophotolysis (direct and indirect), fermentation (dark and photo) and microbial electrolysis cell (MEC). Among all, dark fermentation seems to be the most efficient when compared to other procedures. The challenges currently being faced with this technology are the yield of hydrogen, the high cost of the reactor and system efficiency. This technology still needs a lot of research and improvement to replace fossil fuels entirely.

Keywords: Algae, Biofuel, Biohydrogen, Fermentation, Future fuel, Microbes

INTRODUCTION

As the population is increasing day by day, it can be acknowledged that more energy consumption will be needed in the future. Presently most of the power generated is from fossil fuels such as coal, petroleum and natural gas. There are various concerns which revolve around using these fossil fuels as an energy source.These natural resources take geological process of about 100 million years for their generation. Coal, oil and natural gas are expected to be exhausted in 20, 40 and 60 years, respectively (Baykara, 2018). These are energy-intensive (Jo *et al.*, 2006), non-renewable and various Green House Gases (GHG) have accumulated in the environment due to their burning, which has already surpassed the dangerous high threshold of 450 ppm CO_2 (Schenk *et al.*, 2008). The Kyoto protocol (1997) by the United Nation Framework Convention on Climate Change (UNFCCC) focused on reducing emission of GHG (Parry *et al.*, 1998) because these GHG eventually leads to impact on our environment such as global warming, climate change, rapidly melting glaciers, rise in sea level and many other negative impacts on human health. The recently held UNFCCC-COP26 meeting in Scotland has emphasized achieving net-zero carbon emission by 2050 (COP26: The Glasgow climate pact, 2021). All these challenges eventually forced our scientists and researchers to explore a clean, renewable, sustainable, cheap energy source that can act as a replacement for fossil fuels (Srivastava *et al*., 2021). A source of energy which could eventually find the balance between economic development and environmental sustainability (Prasad *et al*., 2019). Currently, much effort is being put into searching for one such substitute.

Renewable energy seems to be a source that has positive results in battling all the environmental challenges, making them the next generation fuel (Nath and Das, 2003). Various renewable energy sources like hydro, solar, geothermal, biodiesel, biogas have been explored to provide energy (Kant Bhatia *et al*., 2021). The various criteria which make a fuel choice of interest are high energy emission, eco-friendliness and low cost. A fuel which completes all such requirements is biohydrogen (Jo *et al*., 2006).

The present review discusses the different ways in which hydrogen can be produced biologically, the present state of the technology, its advantages and a few aspects that need to be addressed for further development in this technology.

Biohydrogen

Biohydrogen is the hydrogen that is produced biologically using methods such as bio-photolysis, fermentation (Demirbas, 2009) and Microbial electrolysis cells (MEC). With the current speed at which fossil fuels are being consumed, soon, all our natural sources of energy will be lost. In the present situation, It is observed that about 60% of the power generated in India is still from fossil fuels. After being buried under the ground for about 100 million years, these fuels are formed. Also, the emission of greenhouse gases (CO_2 and CH_4) harmful gases (sulphur dioxide, carbon monoxide, carbon dioxide, nitrogen oxides) and various toxic pollutants (mercury, volatile chemicals, and polycyclic hydrocarbons) (Perera, 2017) during burning of these fuels is one of the major drawback which has consequences like climate change and damage to human health (Shahzad, 2015). The speed at which the population is increasing one decade after another is a sign of the amount of power required in industrialization in the future. The global primary energy demand is expected to increase at a rate of 1.2% annually and ultimately reach 18.9 billion TOE (tons of oil equivalent) by 2040 (Koyama, 2017). Does the question arise whether dependence on fossil fuels only is a wise choice or not?

Hydrogen is the most plentiful element in the universe, being a component of almost 75% matter. However, due to its light weight, the earth's gravity cannot hold on to the gaseous form of hydrogen (Baykara, 2018). Biohydrogen is produced using biological processes like fermentation (Dark and Photo), photolysis (direct and indirect) and microbial electrolysis cells (MEC). Still, most of the H_2 being produced is from fossil fuels and only 4% of hydrogen is being produced through electrolysis (The International Renewable Energy Agency, IRENA,2018). The electrolysis process involves using a large amount of energy, making it a less suitable option.

Hydrogen is considered clean and sustainable only when produced using a carbon neutral and green production route (Dahiya *et al*., 2020). The use of hydrogen in refineries reduces the sulphur content in diesel due to strict sulphur regulation laws, leading to hydrogen production from steam methane reforming (Energy Information Administration, 2016). Developing biohydrogen production on a large scale impacts these sectors too. In comparison, the realistic application in the area of biohydrogen production is still very slow. The low yield and production rate of biohydrogen is one of the limiting factors to large-scale commercialization (Cai *et al*., 2013), Hence forcing people to use fossil fuels to generate hydrogen (Osman *et al*., 2020).Biohydrogen production also depends on the presence of H_2 producing enzyme. Presently three enzymes carry out this reaction: Fe-Hydrogenase, Nitrogenase, NiFe Hydrogenase. The quantities of these enzymes have never been shown to be a limiting factor in hydrogen production (Hallenbeck and Benemann, 2002).

Generation of biohydrogen

Biohydrogen is produced in a way keeping in mind the purpose it is meant to solve (Fig.1). A lot of microbial strains are capable of hydrogen production. Two enzymes, nitrogenase and hydrogenase, mainly catalyse the hydrogen production process in many prokaryotes and some eukaryotes. Both enzymes are involved in the utilization of products of photosynthesis reaction (Kotay and Das, 2008). The basic reaction which is catalyzed by the H_2 producing enzyme is given below as Eq. 1.

$$2H^+ + 2e^- \longleftrightarrow H_2 \qquad \text{(Eq. 1)}$$

Biophotolysis

This hydrogen production method involves using the two most abundant resources available to us - Sunlight and water (Srivastava and Rather, 2021). Photosynthetic algae or cyanobacteria also play a major role during the process. The photolysis of water into H_2 takes place in the presence of sunlight (Show *et al*., 2019). Fe-Fe hydrogenase and nitrogenase act as the main enzyme catalyzing this process.

Direct biophotolysis

In the direct biophotolysis method, solar energy is utilized synergistically with photosynthetic apparatus to convert water into chemical energy. This method involves capturing solar energy by the photosystems present inside the thylakoid membrane of chloroplast. This

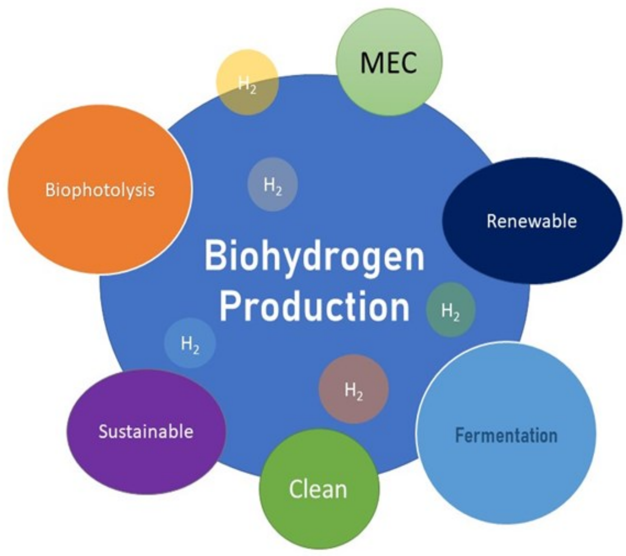

Fig. 1 . *Showing ways of biohydrogen production*

captured energy is transferred in the form of electrons and ultimately leads to the breakdown of water into a hydrogen ion (Eq.2) (Acar *et al*., 2016). The hydrogen ion helps generate a proton gradient, leading to ATP production using ATP synthase machinery (Show *et al*., 2019). It has emerged as a promising technique where solar energy is used to convert readily available substrate water into oxygen and hydrogen. Fe- hydrogenases which are also the most abundant hydrogenase present in the environment (Nagarajan *et al*., 2021) catalyzes the hydrogen production process (Eq. 3). The only problem with this process is the sensitivity of Fe-Hydrogenase to oxygen which results in reversible inactivation(Manish and Banerjee, 2008).

$$2H_2O + \text{light energy} \rightarrow 4H^+ + 4e^- + O_2 \qquad (\text{Eq. }2)$$

$$4H^+ + 4e^- \rightarrow 2H_2 \qquad (\text{Eq. 3})$$

The key requirement for execution of this process is that partial pressure is required around one atmosphere of O_2. Hydrogen has been produced at a 0.7 mmol/h per litrerate in *Anabaena variabilis* (Sveshnikov *et al*., 1997), 1.33 mmol/h in *Anabaena cylindrica* (Weisman and Benemann, 1977).

Indirect biophotolysis

The sensitivity of hydrogenase to oxygen is a major drawback. This problem is resolved in the indirect biophotolysis process by separating the hydrogen production and oxygen evolution into two different stages coupled with the release of CO_2 (Hallenbeck and Benemann, 2002). In the first stage the cell takes up CO_2 to generate cellular substrate and O_2 (Eq. 4). This substrate is later metabolized to produce hydrogen (Eq. 5).

$$12H_2O + 6CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 \qquad (\text{Eq. 4})$$

$$C_6H_{12}O_6 + 12H_2O + \text{light energy} \rightarrow 12H_2 + 6CO_2 \qquad (\text{Eq. 5})$$

A heterocystous Cyanobacteria is a preferable organism that carries out this process (Kumar et al., 2019) as it contains both the enzyme hydrogenase and nitrogenase (Lindberg *et al*., 2012). Nitrogen fixing cyanobacteria like *Oscillatoria, Anabaena, Calothrix, Nostoc* along with non-nitrogen fixing cyanobacteria such as *Synechococcus, Gloeobacter* and *Synechocystis* can be used to produce hydrogen using this method (Sivaramakrishnan *et al*., 2021). Mutant strains of *Anabaena variabilis* have produced hydrogen at the rate of 0.355 mmol h^{-1} per hour (Sveshnikov *et al*., 1997). Indirect bio photolysis is still in a very early stage and needs more practical application.

Fermentation

Fermentation is a prominent mechanism for generating energy using an endogenous electron acceptor. Dark and Photo fermentation are the two methods employed using various strains of bacteria to generate hydrogen (Srivastava *et al*., 2019). The efficiency of the outcome depends on the process used and the end product (Manish and Banerjee, 2008). The fermentation process can be both aerobic and anaerobic. *Clostridium* sp., *Acetanaerobacterium, Pseudomonas* (Cabrol *et al.,* 2017) and *Enterobacter aerogenes* are the common organisms which perform this process (Jaya ingh earachchi *et al.,* 2009).

Dark fermentation

It is one of the most efficient approaches due to the amount of biohydrogen production, ambient operating conditions and less reaction time (Srivastava *et al*., 2019). This process allows the production of hydrogen in dark and anaerobic conditions. The basic reaction taking place in this process is given below (Eq. 6).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2 (\text{Eq. }6)$$

The low energy requirement and ability to use a wide variety of substrate (agricultural waste, waste from the pulp/paper industry and food industry waste) makes this process feasible and help in both H_2 production and reduction in waste (Das and Basak, 2021). Hydrogen yield of 4 mol H_2/mol glucose can be achieved by this process. Chemoheterotrophic such as *Clostridium* and *Enterobacter* carry out dark fermentation (Mahidhara *et al*., 2019). The formation of by products is one reason for the reduction of proton to hydrogen, which leads to a drastic reduction in the yield of H_2 (Mazzoli, 2012).

Photo-fermentation

Photo fermentation involves the production of H_2 using light-dependent photosynthetic bacteria which breaks down organic substrate and produce biohydrogen simultaneously, under anoxic condition (Zheng *et al.*, 2022). Photosynthetic bacteria carry out this light-dependent process using nitrogenase only in nitrogen-deficient condition. Fermentative Purple non sulphur bacteria (PNSB) are considered most efficient considering their ability to synthesize biohydrogen using various substrates (Hitam and Jalil, 2020). A theoretical yield of 4 – 10 mol H_2/mol substrate can be achieved using PNSB (Tiang *et al.*, 2020). The reactions taking place in this process have been shown below (Eq. 6 and Eq. 7)

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2 \quad \text{(Eq. 7)}$$

$$2CH_3COOH + 4H_2O \rightarrow 8H_2 + 4CO_2 \quad \text{(Eq. 8)}$$

The above reactions show H_2 production using glucose and acetic acid as substrate. *Rhodobacteria sphaeroides*, *Rhodopseudomona spalustris* and *Rhodobacter capsulatus* are the common photosynthetic hydrogen-producing bacteria (Zhang *et al*., 2021). This process is environmentally friendly, efficient and produces large-scale hydrogen (Hitamand Jalil., 2020). A genetically modified strain of *R. palustris* having the ability to produce 7.5 ml H_2/L of culture has been obtained (Gosse *et al*., 2007). One of the major advantages is the variety of substrates which can be used in H_2 production like butyric acid, propionic acid, lactic acid and malic acid. Wastewater from the olive industry, wine industry and agricultural waste can also act as potential substrates (Pandu and Joseph, 2012).

Microbial electrolysis cells

Microbial electrolysis cell (MEC) is a more recent technology used to produce H_2 using two electrodes, an external voltage and a microbe. Various substrates can be used, including organic waste and different renewable sources (Logan *et al*., 2008). The procedure involves an electrochemically active bacterium which reduces an organic matter to release CO_2, electrons and protons (Eq. 9). The electron gets transferred to the anode while the proton remains in the solution. The electron moves from anode to cathode through a wire connecting both the electrodes. Once the electron reaches the cathode it combines with the free proton, forming a H_2 molecule (Eq. 10) (Kadier *et al*., 2015). Two-chamber MEC and single chamber MEC are being currently used. Two-chamber MEC is formed due to presence of a membrane between both the electrodes. The membranes help to keep H_2 separate and prevent impurity. Single chamber MEC lacks the middle membrane. The presence of methanogens is a disadvantage to this MEC. H_2 yield of 88% (Moles of H_2 yield/Moles of substrate consumed) is seen when using acetate as substrate with an energy input of 1.3 Kwh/m^3 (Cheng and Logan, 2007).

$$\text{Anode} - C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 8e^- + 8H^+ \quad \text{(Eq. 9)}$$

$$\text{Cathode} - 8H^+ + 8e^- \rightarrow 4H_2 \quad \text{(Eq. 10)}$$

MEC are also considered to be used as a platform for energy-efficient waste water treatment (Sim *et al.*, 2018). Various studies shows that bacteria which transfers electron to cathode belong to *Pseudomonas* and *Shewanella* species (Liu *et al*., 2008). The key asset of this method compared to others are mild operation, several substrates and high order of energy recovery (Cui *et al*., 2021). The material used as anode can be carbon paper, carbon cloth, graphite granules and graphite brushes. While for cathode (where H_2 evolution takes place) platinum catalyzed electrodes are suitable.

Relevance of biohydrogen production

The main cause of the shift to biohydrogen is the continuous damage done to the environment by fossil fuels for the past several centuries. The worldwide impact on the climatic condition triggered the focus on biohydrogen. The various advantages of biohydrogen are -

Less energy is utilized during production and less harm is done to the environment. It comes under green hydrogen and green chemistry as biohydrogen can be produced using the waste and byproducts of the food industry, agricultural waste, beverage industry, dairy waste, municipal waste and even the waste from kitchen act as rich source of carbohydrate and can be used to produce H_2 (Das and Basak, 2021).

There is no requirement like fertile fields which were necessary for the production of first and second-generation biofuels. The first-generation biofuel (sugar cane, grains, and vegetable oils) and second-generation biofuels (produced from wheat straw, woody biomass, agricultural and forestry residue) needs land area to be produced(Mohr and Raman, 2013). While biohydrogen can use waste effluent and algal biomass as substrates making it third-generation biofuel (Singh and Rathore, 2017).

The only byproduct which is formed due to the combustion of biohydrogen is water (there is no gaseous molecule like in the case of many fossil fuel) (Brentner*et al*., 2010). This water can be used for various other purposes.

Currently, hydrogen is created by energy intensive techniques such as partial oxidation, reforming, water electrolysis and coal gasification (Baykara, 2018). These procedures cause some environmental damage. On the other hand, biohydrogen employs a renewable substrate for manufacturing, is cost-effective, and does not harm the environment.

Direct hydrogen-burning can provide energy to run vehicles as hydrogen generates three times more energy than gasoline (Nagarajan *et al*., 2020). Hydrogen has

Table 1. Showing, microbes for biohydrogen production, pathway, yield and their substrates

S. No	Name of the microorganism	Product	Pathway	Yield	Substrate	Reference
1.	*C. reinhardtii*	H_2	Biophotolysis	40.2 ml/Kg	Water	Hoshino *et al.* (2013) & Uyar *et al* (2009)
2.	*Chlorella* sp.	H_2	Biophotolysis	38.0 ml/L	Water	Batyrova *et al.* (2015
3	*Nostoc*	H_2	Photo Bioreactor	6.2 ml/L/h	Water	M. Nyberg *et al.* (2015)
4	*Lyngbya*sp.	H_2	Biophotolysis	17.1 µmol H_2/g	Benzoate	Shi and Yu (2016)
5.	*Clostridium beijerinckii*	H_2	Dark fermentation	1,117 ml/L	Sorghum rusk	Saratale *et al.* (2015)
6.	*Clostridium butyricum*	H_2	Dark fermentation	1.73 mol H_2/ mol glucose	Sugar bagasse	Pattra *et al.* (2016)
7.	*Enterobacter asburiae*	H_2	Dark Fermentation	21.9 mmol L/ h	Formate	Shin *et al.* (2010)
8.	*E. coli*	H_2	Dark fermentation	0.75 ± 0.03 mmol H_2/L	Brewery spent grain	Poladyan *et al.* (2018)
9.	*Trichoderma asperellum*	H_2	Dark fermentation	402.01 mL	Sweet sorghum	Shanmugam *et al.* (2020)
10.	*Rhodobactersphaeroides*	H_2	Photo Fermentation	22 ml H_2/L/h	Propionate	Uyar *et al.* (2009)
11.	*Spirulina platensis*	H_2	Photo Fermentation	1.92 ± 0.20 mmolH2/ mol	Wet Biomass	Pandey *et al.* (2021)
12.	*Clostridium thermocellum*	H_2	Dark Fermentation	109.6 mL/g	Sugar bagasse	Tian *et al.* (2015)
13.	*Rhodopseudomonas palustris*	H_2	Photo Fermentation	9.1 ml H_2/L/ h	Lactate	Barbosa *et al.* (2001)
14.	*Rhodobiummarinum*	H_2	Photo Fermentation	13.6 mmol H_2/L	Malate	Ike *et al.* (1999)
15.	*Rhodopseudomonassphaeroides*	H_2	Photo Fermentation	8.35 mol H_2/ mol	Hexose	Kim and Kim (2013)

an energy content of 122MJ/Kg, gasoline has about 44MJ/Kg, methane has approx. 50MJ/Kg and ethanol about 26.8 MJ/Kg respectively (Kumar and lin, 2014) (Fig. 2).

Current status of biohydrogen generation

At present stage H_2 is being produced mainly through fossil fuels using steam reforming process. More than 1 billion m^3 hydrogen is produced per day of which 48% is produced from natural gas, 30% using oil, 18% from coal and remaining 4% from water splitting using electrolysis (Fig. 3) (Chandrasekhar *et al*., 2015). Biohydrogen has various valuable benefits but still this technology is in its developmental stage. Research in various sectors is going on like different substrates are currently being tested for their H_2 producing efficiency.

Research on Biomass from different sources is going on to check for a better renewable source, like using industrial waste water (Usman *et al*., 2019), lignocellulosic waste (Singh *et al*., 2021), food waste (Kuang *et al*., 2020), kitchen waste (Srivastava *et al*., 2021) and using different methods for hydrogen production using these biomasses. Wastewater from an industry rich in organic matter is also used to produce H_2 gas. Methods like dark fermentation and photo fermentation are being used for efficient H_2 production. The carbon-rich nature of organic substrate is the main reason for their use

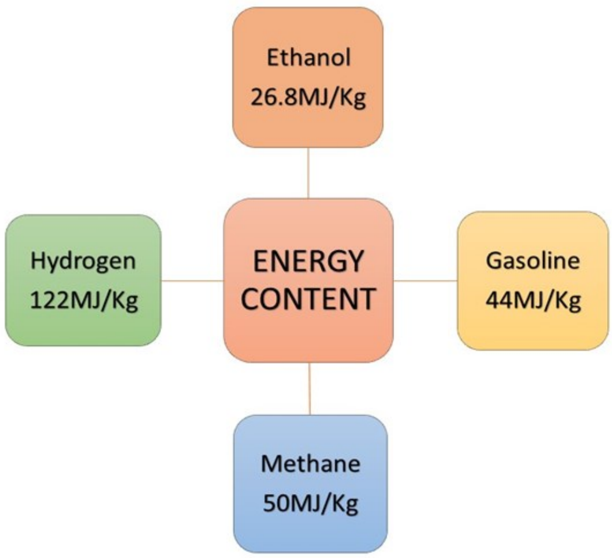

Fig. 2. *Energy content of different fuels*

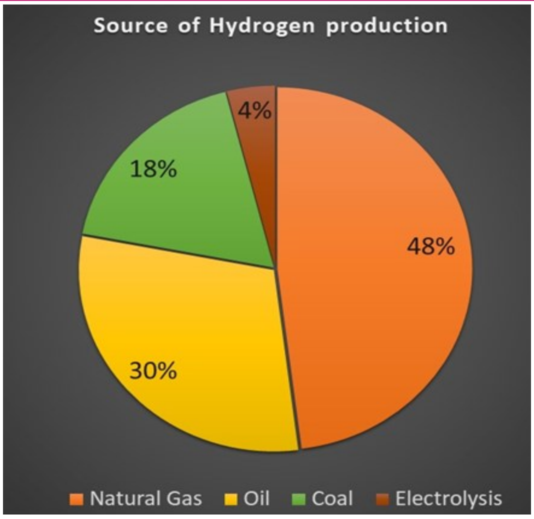

Fig. 3. *Current sources of hydrogen production*

(Chandrasekhar *et al*., 2015). These renewable substrates contain a lot of biodegradable biomass, which can maintain the balance between energy applied and the product recovered (Angenent *et al*., 2004).

Various genetically engineered and mutant strains are being developed like strains of *E. coli*, *Bacillus subtilis*, *Rhodovulum sulfidophilum* (Cai and Wang, 2013), *Pantoea agglomerans* (Liu and Wang, 2013), *Enterobacter aerogenes* (Song *et al*., 2020),*Chlamydomonas reinhardtii* (Hoshino *et al*., 2013), *Clostridium* sp. (Cai *et al*., 2013), *Rhodobacter* M 19 and *Enterobacter aerogenes* (Veeramalini *et al*., 2019) and thermophilic microorganisms (Pawar and Niel, 2013).

Limitations of biohydrogen

Biohydrogen is without a doubt one of the most suitable fuels which can replace fossil fuels, but since it is relatively new compared to other sources of energy, there are certain aspects which need to be focused on:

The yield of hydrogen is still low from the biological methods. Dark fermentation is said to be the best method for hydrogen production. A maximum hydrogen yield of 4 mol H_2 per mole of glucose is practically achievable (Osman *et al*., 2020). The theoretical yield of 12 moles is not achieved due to the formation of various byproducts.

The effluents from dark fermentation reaction increases the biological oxygen demand of the water bodies where they are released (this is due to the presence of organic matter in the effluent). There is a need to develop technology to treat this effluent, as it could further lead to eutrophication (Turon *et al*., 2015).

Superior strains of microbes need to be developed which could increase hydrogen yield. There have been some attempts, a mutant species of *Chlamydomonas* has shown 8 folds increase in H_2 production under sulphur deficiency and high light intensity conditions (Kosourov *et al*., 2011). The better understanding of the mechanism in the microbes is needed, which could eventually lead to the production of efficient strain.

High operation cost in developing these genetically improved strains and eventually using them at a large scale is an issue. The environmental issue followed by using genetically engineered strains should also be kept in mind (Boboescu *et al*., 2016).

Storage and transportation are costly when compared to gasoline and other energy sources. The low storage density of hydrogen (one-tenth of gasoline) makes it a hefty task. High storage pressure makes it expensive and also have safety issues. (Dunn, 2002).

The cost of a large-size bioreactor is a limiting factor as different fuel cells need bioreactors of different sizes to achieve a satisfactory level of hydrogen yield. More research is needed for optimization of bioreactor design keeping the total cost into consideration. (Levin *et al*., 2004).

The Bioreactor used for biohydrogen generation has issues related to its own operation, like its start-up, biomass washout, temperature issues, pH related issues and issues related to substrate pre-treatment (Banu J *et al*., 2021).

Conclusion

With the speed at which natural resources are utilized, they will soon face exhaustion. The time for transition to a clean, sustainable energy source has arrived. Biohydrogen is a promising substitute for fossil fuels, as it is already known that hydrogen is the future fuel. Much literature work has been done in this field in the past decade, but the practical application has been limited. The practical implementation of this process should be increased. There are many advantages of biohydrogen over fossil fuels apart from a few exceptions. The low hydrogen yield is one of the major drawbacks of this technology. There is a need to focus on developing genetically modified microbes strains and a better pilot-scale system that would ultimately launch this technology at the commercial level.

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Conflict of interest

The authors declare that they have no conflict of interest.

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