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Green technology for sustainable biohydrogen production (Waste to energy): A Review

Sharma Mona [1], Smita S Kumar [2, 3], Vivek Kumar [2], Khalida Parveen [4], Neha Saini [1], Bansal Deepak [5], Arivalagan Pugazhendhi [6*]

[1] Department of Environmental Science and Engineering, Guru Jambheshwar University of Science & Technology, Hisar - 125001, Haryana, India

[2] Centre for Rural Development & Technology, Indian Institute of Technology Delhi, Hauz Khas – 110016, Delhi, India

[3] Department of Environmental Studies, J.C. Bose University of Science and Technology, YMCA, Faridabad, 121006, Haryana, India

[4] Department of Environmental Sciences, University of Jammu, J&K, India

[5] JBM Group, Gurugram-122001, Haryana, India

[6] Innovative Green Product Synthesis and Renewable Environment Development Research Group, Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Vietnam

***Corresponding Author Address**

Arivalagan Pugazhendhi

Faculty of Environment and Labour Safety

Ton Duc Thang University

Ho Chi Minh City, Vietnam.

Email: arivalagan.pugazhendhi@tdtu.edu.vn

Abstract

Perceiving and detecting a sustainable source of energy is very critical issue for current modern society. Hydrogen on combustion releases energy and water as a byproduct and has been considered as an environmental pollution free energy carrier. From the last decade, most of the researchers have recommended hydrogen as one of the cleanest fuels and its demand is rising ever since. Hydrogen having the highest energy density is more advantageous than any other fuel. Hydrogen obtained from the fossil fuels produces carbon dioxide as a byproduct and creates environment negative effect. Therefore, biohydrogen production from green algae and cyanobacteria is an attractive option that generates a benign renewable energy carrier. Microalgal feedstocks show a high potential for the generation of fuel such as biohydrogen, bioethanol and biodiesel. This article has reviewed the different methods of biohydrogen production while also trying to find out the most economical and ecofriendly method for its production. A thorough review process has been carried out to study the methods, enzymes involved, factors affecting the rate of hydrogen production, dual nature of algae, challenges and commercialization potential of algal biohydrogen.

Keywords: Biohydrogen; Hydrogenase; Nitrogenase; Fossil fuel; Green algae; Cyanobacteria.

1. Introduction

Energy is one of the substantial factors of a country's economy**.** It is a key derivative of human living standards of any country**.** Since industrial revolution, there has been a major focus on fossil fuels as a source of energy to the world. They are non-renewable resources and take millions of years to be produced again iojiand are not environment friendly. The utilization of fossil fuels is associated with Greenhouse Gas (GHG) emission, mainly consisting of CO_2 , the production of which has increased more than 40% since the industrial

revolution (Moreira and Pires, 2016). The increase in CO_2 emissions is affecting the natural climatic patterns of the earth such as changes in temperature and patterns of precipitation, which are imposing negative impacts on human lives (Costello et al., 2009). For resolving these increasing problems of climatic changes, the use of carbon dioxide neutral fuel systems must be encouraged as suggested in the Copenhagen Climate Conference, 2009 (Christiansen et al., 2018). The increasing population along with pollution and the fast depletion of non-renewable fossil fuels have forced the world to shift to renewable energy sources (Singh and Mahapatra, 2019). The major sources of energy are oil, coal and natural gases. According to the Energy Statistics 2018, the production of coal has increased from 2.9% in 2007-2008 to 3.79% in 2016-2017 whereas its consumption has increased from 2.22% in 2007-2008 to 5.29% in 2016-2017 (Statistics, 2013). The energy demand of the world is likely to be 50% more than the current scenario (Christiansen et al., 2018). Among the renewable sources of energy, the molecular hydrogen shows a huge potential to be used as an alternative source of energy and helps in combating all these problems (Mona and Kaushik, 2015). Hydrogen was first identified by Cavendish when dissolving metals in dilute acids (Cavendish, 1776). Biological hydrogen production was first reported by Gaffron in green microalgae (Gaffron, 1939; Gaffron and Rubin, 1942). Hydrogen can also be utilized in the fuel cell for electricity generation from chemical energy (Rosenbaum et al., 2005). It is also a carbon neutral energy carrier having 2.75 times higher energy yield than the fossil fuels (Singh and Mahapatra, 2019). Further, it has wider applications such as production of fertilizers (ammonia), removal of impurities in oil refineries, methanol production, in pharmaceutical industries, cryogenics, rotor coolants, petroleum recovery, as a fuel in rocket engines, hydrogenating and reducing agent etc. (Shaishav et al., 2013; Singh and Mahapatra, 2019). Hydrogen can be produced by different methods from fossil fuels, coal, biomass gasification, natural gas, and splitting of water or water electrolysis. Since long time, steam reforming of hydrocarbons has

been extensively utilized for hydrogen production along with hydrogen/carbon monoxide gas mixtures (Steinberg, 1999), which is also adverse to the environment. Therefore, the present review article has attempted to compile the information available on the production of hydrogen, role of enzymes involved in production, factors affecting the rate of hydrogen production and their challenges and future perspectives.

2. Biohydrogen from biomass

Biohydrogen can be produced through various feedstocks via thermochemical technologies such as steam reforming of biobased oils, pyrolysis, supercritical gasification of water, simple gasification, gasification of steam (Demirbas, 2001) and biological processes (Fig. 1). These processes are as follows:

2.1 Thermochemical conversion process

The total efficiency of thermal conversion to hydrogen is 52% but the production costs of thermochemical processes are less. The yield of hydrogen from biomass is more than 15% of the dry weight of biomass, which is relatively low (Demirbas, 2001). The energy content and yield of hydrogen and biomass, respectively, are compared in Table 1 (Wang et al., 1999). All the thermochemical procedures of gasification including supercritical water gasification, air gasification or gasification of steam can directly utilize wet biomass without predrying and gives higher efficiency at a low temperature (Yan et al., 2006). However, one of the major disadvantage of the biomass feedstock is its decomposition, which produces char and tar (Swami et al., 2008). Gasification of biomass is a type of pyrolysis which occurs at higher temperatures and produces a gaseous mixture comprising hydrogen of more than 6.0% (Demirbas, 2009). The gas (synthetic in nature) produced by biomass gasification is a mixture of hydrogen, carbon dioxide, carbon monooxide, methane, nitrogen, oxygen, and tar

and the removal of tar is difficult through physical dust removal method (Yoon et al., 2010). Factors such as temperature and the type of the reactor influence the composition of the gas and the products formed (Stevens, 2001).

2.2 Biological process

There is an urgent need to search for a better source of fuel production, which will not only control pollution or another process of climate change but also aids in putting less pressure on consumption of fossil fuel. Therefore, an alternate of energy production is needed. A biological process is an effective approach for hydrogen production as it is environment friendly in nature. Biological production of hydrogen is seen in several species of blue-green algae, bacteria, green algae and also in higher plants (Stevens, 2001). Photoproduction of oxygen and hydrogen, catalyzed by microorganisms occur at an ambient pressure and temperature (Gaffron and Rubin, 1942; Spruit, 1958; Khetkorn et al., 2017). This process leads to the generation of renewable energy from the most plentiful resources such as water and solar energy (Anjana and Kaushik, 2014). Biohydrogen has a huge potential (Table 2) to put back other hydrogen production technologies, which use fossil fuels and have drawn attention all over the globe (Show et al., 2012). These advantages (Table 2) render hydrogen as a potential candidate to reduce the reliance on conventional fossil fuels (El-Sharnouby et al., 2013).

2.2.1 Dark fermentation

For carrying out dark fermentation, a large number of wild and genetically modified strains of anaerobic fermentative bacteria are available (Sen et al., 2008). Several substrates (glucose, sucrose, starch, cellulose) are also available for enhancing the production rate of

hydrogen through the complete conversion of one mole of glucose has 12 moles of hydrogen atom (Hallenbeck and Benemann, 2002):

$$C_6H_{12}O_6 + 6\ H_2O = 12\ H_2 + 6\ CO_2 \quad (1)$$

and produces 4 moles of hydrogen in dark fermentation with organic acids as byproducts (Sen et al., 2008):

$$C_6H_{12}O_6 + 6\ H_2O = 2\ CH_3COOH + 4H_2 + 2CO_2 \quad (2)$$

In dark fermentation, CSTR (Continuously Stirred Tank Bioreactor) and UASB (Upflow Anaerobic Sludge Blanket Reactor) are majorly used along with some other fermentative reactors. The yield was reported (Gavala et al., 2006) to be higher in UASB especially at low retention times under similar reaction conditions. The rate and yield of the dark fermentation process is higher than the others but the problem associated with this process is a low hydrogen concentration (40-60%; v/v). Therefore, the fuel cells cannot use the fermentative effluent gas and a purification step is required (mechanical/chemical process). About 73% hydrogen is formed in a two-step gas membrane separation process. For yield improvement, some pretreatment methods have been reported (Wang et al., 2003). The nature of microflora has direct influence on the hydrogen production yields. Also, the inoculum provided at the proper stage of the dark fermentative process can have a significant increase in hydrogen production yields (Turhal et al., 2019).

The pretreatment steps in hydrogen production method inhibit methanogenic bacterial activity and hence, the hydrogen consumption decreases and the yield increases. Biological hydrogen production processes are more ecofriendly when compared with other thermo and electrochemical processes (Das and Veziroğlu, 2001). The production of hydrogen using anaerobic bacteria has been studied since 1980s (Nandi and Sengupta, 1998). Microbial hydrogen production is a beneficial area of technological development that uses a diversity of renewable resources (Cheong and Hansen, 2006).

The pathways of fermentation and their end products determine the amount of hydrogen produced from the glucose molecule (Ren et al., 2006) with acetic and butyric acid, which comprise about 80% of the total end products (Tang et al., 2008). 1 mole of glucose produces 4 moles of hydrogen in the acetate type fermentation while 2 moles of hydrogen are generated in the butyrate type fermentation (Wang et al., 2003). There have been various studies on the fermentative hydrogen production from feedstocks such as wastewaters, municipal wastes and leftovers of agricultural and food industries (De Vrije et al., 2010). Nevertheless, this process has a low yield of hydrogen even from the fermentation of the simplest sugars (Zheng et al., 2009). This can be rectified by a two-stage hybrid system wherein the amalgamation of dark and photofermentation occurs. In the hybrid system, glucose or starch is used by anaerobic bacteria via an acetate fermentative pathway and the photosynthetic bacteria convert the resultant acetate into hydrogen in a separate reactor and there is a twofold increase in hydrogen yield when compared with the dark fermentation (Tao et al., 2007).

The economical production of hydrogen is the basic concern of various researchers (Basak and Das, 2007). Several investigations have revealed that the hydrogen production cost is reliant upon the feedstock cost. Hydrogen production from the gasification of biomass involves a higher cost due to lack of technology and problems for extensive market and infrastructure arrangement.

2.2.2 Photofermentation

In this process, the photosynthetic bacteria such as *Rhodopseudomonas capsulate, Rhodobacter sphaerides, R. palusris, Rhodospirillum rubrum,* etc. are used for hydrogen production (Tang et al., 2008). These bacteria use sunlight to convert small organic molecules into biomass releasing carbon dioxide and hydrogen under anaerobic conditions (Basak and

Das, 2007). Purple non-sulfur bacteria are very efficient for hydrogen production because of the following reasons (i) having a good efficiency of substrate conversion, (ii) being anaerobic, they can manage the issue of oxygen sensitivity, which affects the [Fe-Fe] hydrogenase, the *hox EFUYH* [NiFe]-hydrogenase, and nitrogenase enzymes (iii) the ability to utilize both visible and near infrared regions of the spectrum, (iv) the potential to use a variety of substrates (Das and Veziroğlu, 2001). These are gram negative organisms and have been extensively utilized for the physiological and genetic investigations of hydrogen production (Greenbaum, 1979; Madigan et al., 1997). Purple non-sulfur bacteria require less free energy for decomposing organic substrates as compared to the algal hydrogen production, which oxidizes water and produces more than 8.0 kJ/ mol hydrogen from the lactate decomposition (Greenbaum, 1979).

Under nitrogen deficiency conditions, purple nonsulfur (PNS) bacteria produce hydrogen as a byproduct of nitrogenase activity, which is facilitated by sunlight and small organic molecules serving as substrates. The anoxygenic phototrophs perform electron transport and generate proton motive force leading to ATP production by using sunlight. A huge amount of energy is needed to perform nitrogen fixation and hydrogen production reactions (Cheong and Hansen, 2006):

$$N_2 + 8e^- + 8H^+ + 16ATP \rightarrow 2NH_3 + H_2 + 16\ ADP + 16\ Pi \quad (3)$$

The common reaction of photofermentation is as follows:

$$CH_3COOH + 2H_2O + light = 4H_2 + 2CO_2 \quad (4)$$

Factors such as intensity and wavelength of light influence the production of hydrogen in the bacterial system. Light intensity has a direct relationship with the hydrogen production rate until it attains a saturation point, which further depends on the substrates and the microorganisms used (Nakada et al., 1999; Khetkorn et al., 2017). There is a decrease in the photoproduction of hydrogen by 39% if infrared light (750-950 nm wavelengths) is

lacking. Theoretically, 1.6, 4.0 and 2.8 moles of hydrogen are produced from one mole of acetic acid, butyric acid and propionic acid, respectively. Mostly organic acids, which are bulk components of the industrial wastes and the effluents of the dark fermentation process, are used as substrates. Photofermentation have been proven to be a better mechanism for hydrogen production when compared to dark fermentation. A study conducted to produce biohydrogen from corn stover compared three different processes namely dark fermentation, photofermentation and dark-photo co-fermentation for various parameter of substrate consumption, hydrogen production, by-products and energy conversion efficiency. Photofermentation was found to be the most promising method with maximum hydrogen content of 59% and highest energy conversion efficiency of 10% which was higher than the other two methods studied (Zhang et al., 2019).

Photofermentation can be coupled with dark fermentation or used as a wastewater treatment technique. Wastewater treatment using photosynthetic bacteria has given satisfactory results. The concentration of hydrogen in biogas can be as high as 96%. Eroglu et al. (2008) got a maximum production of hydrogen by using olive oil mill wastewater (OMW).

2.2.3 Photobiological process

Photobiological hydrogen production can be fermentation based or photosynthesis based. Photosynthesis based fermentation uses cyanobacteria containing chlorophyll a and other pigments that can utilize solar energy with the help of photosystems (PSII and PSI) to produce sugars and oxygen (Olson and Bernstein, 1982). The PSII (P680) absorbs photons having a wavelength less than 680 nm, produces strong oxidant, which converts water to oxygen, protons and electrons as described in Fig. 2. The energy level of reducing equivalents generated as a result of the oxidation of water is utilized by the photons at PSI

and PSII and the reducing equivalent, NADPH generated from this cycle is further used for the reduction of carbon dioxide in the dark phase of photosynthesis to produce carbohydrates. The reducing power through Fd is transferred to hydrogenase (H_2ase) to produce hydrogen (Yu and Takahashi, 2007).

The electrons pass via a sequence of cytochrome carriers to photosystem I in a non-cyclic manner that forms a Z pattern. The H^+ with a wavelength around 700 nm are absorbed by the pigments in photosystem I, which further reduce the oxidized ferredoxin (Fd) and/or $NADP^+$ into their reduced forms by accepting electrons. ATP is generated through the proton gradient formed as a result of phosphorylation across the thylakoid membrane with the help of ATP synthase. The ATP and NADPH produced are used further to reduce carbon dioxide in the carbon fixation pathways. Hydrogenase and nitrogenase reduce protons to produce molecular hydrogen by using reducing equivalents (ferredoxin) under certain conditions and are represented as $2H^+ + 2Fd^- \rightarrow H_2 + 2Fd$ (Mertens et al., 2004). As suggested by Gaffron and Rubin, *Scenedesmus*, a green microalga produced molecular hydrogen in dark anaerobic conditions with the use of reversible enzyme (Gaffron and Rubin, 1942; Lütz et al., 2006). Benemann and Weare (1974) reported hydrogen evolution in *Anabaena cylindrica,* a nitrogen fixing cyanobacterium in the presence of oxygen when kept in an argon atmosphere (Benemann and Weare, 1974). Nitrogenase reduces nitrogen into ammonia (NH_3) and also produces hydrogen (Masukawa et al., 2002). Both hydrogenase and nitrogenase are sensitive to oxygen and get deactivated even at low partial pressures of oxygen (<2% v/v) (Volbeda et al., 2002; Lee and Greenbaum, 2003). This internal inefficiency of biophotolysis becomes a major hindrance for sustained hydrogen evolution (Ghirardi et al., 2000). There has been an effort to overcome this barrier by modifying the technologies. The biophotolysis (direct and indirect) by green algae and cyanobacteria have also been studied (Benemann, 1997).

Phototrophic microorganisms have diverse physiological and metabolic pathways, which can be utilized to produce hydrogen like photofermentation (Dasgupta et al., 2010).

2.2.3.1 Direct biophotolysis

In this process, solar energy is transformed into chemical energy as:

$$2H_2O + \text{light energy} = 2H_2 + O_2 \quad (5)$$

Microorganisms performing this activity are species of different green algae (photoautotrophic organism) and cyanobacteria. *Chlamydomonas reinharditi* is the most commonly used microalga, besides *Scenedesmus obliquus* and *Chlorella fusca* for hydrogen production. It is an attractive way to produce hydrogen as it uses water and sunlight as an energy source (Sen et al., 2008).

Photosynthetic organisms reduce ferredoxin and $NADP^+$ by extracting electrons and protons from water. $NADP^+$ and ATP are essential energy sources for the metabolic reactions in a cell. In direct photolysis, the light energy absorbed by PSI and PSII reduces water by transporting electrons to ferredoxin (Eroglu and Melis, 2011). The reduced ferredoxin becomes an electron donor and transports electrons to the hydrogenase enzyme, which reversibly catalyzes the conversion of a proton to hydrogen (Melis et al., 2000):

$$2H^+ + 2FD^- \leftrightarrow H_2 + 2FD \quad (6)$$

Like higher plants, green algae with the help of photosystems I and II utilize light energy and perform oxygenic photosynthesis. When there is an oxygen deficiency, the hydrogenase uses e^- from the reduced ferredoxin to reduce H^+ and generates hydrogen.

$$2H_2O + h\nu \rightarrow O_2\uparrow + 4H^+ + \text{Fd (red)} (4e^-) \rightarrow \text{Fd (red)} (4e^-) + 4H^+ \rightarrow \text{Fd (ox)} + 2H_2 \quad (7)$$

Green microalgae have [Fe-Fe] hydrogenase enzyme, which has a relatively high efficiency i.e. 12-14% to convert sunlight to hydrogen (Melis, 2009) and also oxidize water to produce hydrogen (Melis and Happe, 2001; Melis, 2002). The major problem with this

process is the inhibition of all hydrogen related reactions such as enzymatic catalysis, mRNA stability and gene expression and presence of oxygen. The time period of occurrence of the direct biophotolysis is very short and before there is accumulation of oxygen, which inactivates the hydrogen production process. This can be overcome by removing oxygen through inert gas purging in the reaction mixture (Greenbaum, 1982; Greenbaum, 1988) or making cells capable of using their own mitochondrial respiration to consume oxygen than the hydrogen generation can be sustained for many days (Melis et al., 2000; Hemschemeier et al., 2009). The hydrogen production from different processes with their advantages and disadvantages has been discussed in Table 2.

Hydrogen production from microalgae (Table 3) is an attractive and ecofriendly process as it produces hydrogen from water, which is easily available and sunlight as an energy source with no accumulation of carbon dioxide and a solar energy efficiency of more than 80% (Ley and Mauzerall, 1982; Greenbaum, 1988). However, practically, the efficiency is low i.e.11-13% as green algae tends to assemble a large array of light absorbing chlorophyll antenna molecules in their photosystems. At high light intensity, the rate of photon absorption is much higher in the first few layers of chlorophyll antennae molecules than the rate at which the photons are actually used in photosynthesis, which causes the dissipation of the excess photons through fluorescence or heat (Greenbaum, 1988). *Desertifilum* sp. IPPAS B-1220 was found to have 20-fold greater amount of hydrogen produced in the light as compared to dark in a study conducted to find new cyanobacterial strains capable of producing hydrogen and to optimize conditions for improving hydrogen production in light dependent process (Kossalbayev et al., 2020). The species of *Oscillatoria* has been studied to evolve hydrogen via dark biophotolysis (Kossalbayev et al., 2020).

2.2.3.2 Indirect biophotolysis

The normal process of photolysis of water and the production of hydrogen gets hindered when there is sulphur deprivation. D1 protein in photosystem II gets degraded and there is a drop in the activity of PS II and the D1 protein needs sulphur to get repaired. As a result, the breakdown of starch occurs and its electrons are transferred to hydrogenase via the plastoquinone mediated pathway and hydrogen is produced (Kruse and Hankamer, 2010; Esquível et al., 2011).

Moreover, the cyanobacteria are filamentous nitrogen fixing organisms and contains specialized cells called heterocyst. Some of the nitrogen fixing genera are *Nostoc, Anabanea, Calothrix* and *Oscillatoria,* and non-nitrogen fixing genera include *Synechocystis, Synechococcus* and *Gloebacter* (Das and Veziroğlu, 2001). In cyanobacteria, hypoxic nitrogen fixation and oxygenic photosynthetic reactions are separated from each other (Table 3). The anaerobic environment is provided by the heterocysts for nitrogen fixation where hydrogen is also generated, represented as (Sen et al., 2008):

$$N_2 + 8e^- + H^+ + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi \quad (8)$$

Cyanobacteria have the simplest nutritional requirements and are considered as the ideal candidates for the above mentioned process. The commonly used species are *Anabaena, Oscillatoria, Caloyhrix,* and *Gloecapsa.* This is a highly energetic process demanding not only the reducing equivalent but also energy in the form of ATP (16 molecules) per mole N_2 fixed and hydrogen produced (Tamagnini et al., 2002). The energy requirement is met via cyclic photophosphorylation in the thylakoid membranes of heterocyst. Hydrogen is also produced by some non-nitrogen fixing cyanobacteria of the genera viz., *Synechococcus, Synechocystis* and *Gloebacter*. These possess two different kinds of [NiFe] hydrogenases possessing various functions and properties. These are encoded by genes known as *hup* (Tamagnini et al., 2002). These hydrogenase enzymes are co-expressed and co-regulated with nitrogenase, which helps in capturing and recycling the hydrogen produced.

Another group is encoded by *hox FUYH* genes, which is a multi-subunit [NiFe] hydrogenase and has the potential to uptake and produce hydrogen. This enzyme is also used by the non-nitrogen fixing genus to produce hydrogen (Aslan et al., 1999; Tamagnini et al., 2007).

The maximum light conversion efficiency of this process is 16.3%. At low light illumination, a better light conversion efficiency is achieved and it decreases with the increasing light illumination. Practically, the conversion efficiency is 1-2% (Aslan et al., 1999; Lindblad et al., 2002; Troshina et al., 2002). Different species of cyanobacteria possess different kinds of hydrogenase and nitrogenase and different metabolic ways producing hydrogen. Nitrogenase activity of cyanobacterial strains have been focused more for hydrogen production. Few non heterocystic strains have been studied. *Synechocystis* sp. PCC 6803 and *Phormidium corium* B-26 produced the greatest amounts of hydrogen in the dark (Kossalbayev et al., 2020). *Desertifilum* sp. IPPAS B-1220 had less amount of Hydrogen production in the dark. This species contains both hydrogenases and nitrogenase activity. The species of *Anabaena* has been studied to evolve hydrogen via dark and indirect biophotolysis both (Kossalbayev et al., 2020).

3. Enzymes involved

The two main hydrogen producing enzymes are hydrogenases and nitrogenases. Hydrogenase is present in all the three domains of life viz., archaea (methanogens and some extremophiles), eubacteria, and eukaryotes (green algae). Hydrogenase is a metal enzyme that plays a vital role in the metabolism of energy in various microbial communities (Das et al., 2006). Hydrogenase can be categorized into two non-homologous classes based on their metal catalytic subunits – First is the Fe-hydrogenase, which contains only Fe at the active site and the others are [Ni–Fe] Hydrogenase and [Ni–Fe–Se] Hydrogenase, which contain Ni Fe and sometimes, Se (Malki et al., 1995). These two differ in their metal subunits, subunit

composition, molecular weight, electron carrier specificity, sensitivity to oxygen (Fe-Fe is more sensitive), cellular location and their physiological roles (Hallenbeck et al., 1978). Some hydrogenase containing organisms, produce hydrogen along with the anaerobic energy metabolism (Benemann, 1996; Nandi and Sengupta, 1998).

Green algae (Fig. 3), some anaerobes, Protista and fungi are the sources of FeFe-hydrogenases. Hydrogenase gene has been found in a variety of green algal species, including *C. reinhardtii, S. obliquus, C. moewusii and Chlorella fusca.* Only metal iron is present in the FeFe hydrogenases (Ghirardi et al., 2009). They contain an iron-sulfur center, which has carbon monoxide and cyanide on the active site of enzyme (Benemann and Weare, 1974). Fe-Fe hydrogenases have a higher turnover (Dasgupta et al., 2010).

NiFe-hydrogenases (Fig. 4) are not related to the FeFe-hydrogenases phylogenetically and are more predominant than the FeFe-hydrogenases and are found in archaea, bacteria and cyanobacteria. The simplest forms of NiFe-hydrogenases are heterodimeric in nature, along with the complex forms consisting of five subunits of bidirectional hydrogenases, which are found in the cyanobacteria. These hydrogenases contain a catalytic center that is connected to the large subunit known as HoxH, having Fe and Ni atoms attached to the CO and CN ligands with S atoms in cysteine residues in the surrounding protein. The small subunit known as HoxY contains a [4Fe-4S] cluster, which is needed for the transfer of electrons to the large subunit. In the catalytic site, three other subunits known as HoxE, HoxF and HoxU form the diaphorase moiety. Ni-Fe hydrogenases are oxygen tolerant and are typically involved in the hydrogen uptake reactions but can also generate hydrogen (Eroglu and Melis, 2011).

Nitrogenase is found only in prokaryotes and is an irreversible catalyst. Its natural function is to produce nitrogen and some amount of hydrogen with more hydrogen production when nitrogen is deficient (Benemann, 1998). Nitrogenases in cyanobacteria are

spatially separated by heterocyst, which provides oxygen free conditions as they lack photosystem II, which is responsible for producing oxygen and therefore, anaerobic hydrogen production occurs. This is a highly energy consuming process (Dasgupta et al., 2010).

Nitrogenase contains two subunits i.e nitrogenase reductase and dinitrogenase complex. The reductase subunit contains Fe-S protein and is encoded by the nif gene. It is a homodimer of 65 kDa molecular weight and carries electron from the external electron donor to the dinitrogenase complex. Mo-Fe-S protein is present in the dinitrogenase complex, which is a heterotetramer containing α2β2 units and having 230 kDa molecular weight. It reduces dinitrogen (N_2) into two molecules of ammonia in a stepwise manner (Hallenbeck and Benemann, 2002; Allakhverdiev et al., 2010). Simultaneous reduction of proton into molecular hydrogen also occurs. On the basis of metal cofactors in their catalytic sites, nitrogenases are categorized as iron, molybdenum and vanadium types (Hu et al., 2011). The different stoichiometries of production of ammonia and hydrogen by different types of nitrogenases are reported as:

Mo-nitrogenase: $N_2 + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2$ (9)

Fe –nitrogenase: $N + 21H^+ + 21e^- \rightarrow 2NH_3 + 7.5H_2$ (10)

V- nitrogenase: $N + 12H^+ + 12e^- \rightarrow 2NH_3 + 3H_2$ (11)

Some electron transfer steps are endergonic; therefore, ATP is required for the production of ammonia and hydrogen. “Pressurized” hydrogen is produced through this irreversible and unidirectional process (Eroglu and Melis, 2011).

4. Factors affecting biohydrogen production

Numerous factors influence the hydrogen production such as type of the bioreactor used, pH and temperature of the culture media, dissolved oxygen, microalgal strains,

composition of the culture medium, the intensity of light and the type of mixing. These factors are discussed below in detail.

4.1 Type of bioreactors used for the production of biological hydrogen

In mass culture, saturation of light is a very important factor for the performance of photosynthesis, which influences the hydrogen yield and the efficiency of the process (Fig. 2). The incident light and energy conversion efficiency of photosynthesis showed an inverse relationship (Koku et al., 2003; Polle et al., 2003). Therefore, to determine its yield and efficiency, the efficient use of light in photobioreactors is an evaluative parameter (Table 4). The reactors utilized for the production of biomass and hydrogen are categorized into batch, fed-batch and continuous based upon the mode of operation. The photobioreactors are majorly categorized into these following types:

(i) Open system, consisting of raceway ponds, lakes, etc.

(ii) Closed system, consisting of tubular, pyramidal, conical, flat plate, fermenter etc.

Open system

In an open pond system, algae are cultivated where the natural conditions for their growth are maintained in a shallow pond having a depth of 1 foot. The raceway configuration is designed in which a paddle wheel provides circulation, which assists in the mixing of nutrients for the growth of algal cells (Fig. 5). The raceways are simply made by digging the earth and are bordered with plastic or made with concrete so that the liquid doesn't seep into the ground. This system is a continuous mode where the nutrients such as nitrogen, phosphorus, and other inorganic salts are added in front of the paddle wheel. The algal broth is circulated from behind the paddle wheel (Fig. 5). As it is an open air system, it faces challenges such as water loss due to evaporation. The uptake of carbon dioxide is not

efficiently done by the growing microalgae; thus, limiting the biomass production (Chisti, 2007). The contamination due to unwanted species limits the productivity of the system. The maintenance of optimum culture conditions is also difficult in open ponds and the cost of recovery of the biomass from dilute culture is also high.

Closed system

The amount of biomass produced has been found to be three times more in the closed systems than in the open systems (Borowitzka, 1996; Carvalho et al., 2006), which also reduces the harvesting costs. Vertical tubular reactors (VTR) are a type of closed reactors and these include bubble column and airlift reactors. VTR consists of transparent vertical tubes, which are made of polyethylene or glass where agitation is attained with the help of bubbles produced at the bottom. In this type of reactor, CO_2 supply and O_2 removal is very necessary. This system has a low contamination risk, high transparency, efficient biomass productivity, high area to volume ratio and reduced material costs. There is difficulty in scaling up, gas holdup, gas transfer at the top regions of the reactor temperature control.

Another type of closed reactors includes horizontal tubular reactors (HTR), which are designed in such a way to face sunlight and have high light conversion efficiency. The gas is supplied into the tube connection through the gas exchange unit. Temperature control is the major limitation of HTR. Photobleaching occurs as a result of oxygen buildup and the efficiency is reduced (Miron et al., 1999). Near horizontal tubular reactors (NHTR) contains parallel tubes, which are tilted at an angle of 5° from the surface and connected at the top and bottom ends (Tredici et al., 1998). In NHTR, the elevation is helpful in reducing the holdup of gas and O_2. Helical tubular reactors are made of coiled tubes containing plastic (flexible in nature) with 3-Dimensional helical frameworks. These reactors also have heat and gas exchangers. The α-shaped reactor is a form of tubular photobioreactor where the algal

physiology and the direction of sunlight are the criteria of their design (Lee et al., 1995). Flat plate reactors and alveolar panels have a high A/V ratio with a minimum thickness (Carvalho et al., 2006). They have an open area for gas transfer, which reduces the requirement of the degassing unit. A flat panel reactor (V-shaped) because of its engineering features has a very low shear stress and a high rate of mixing (Iqbal et al., 1993). Alveolar panel bioreactors consist of alveoli made with transparent PVC or polycarbonate. Oxygen buildup is one of the major disadvantages of this alveolar panel system. The internal or external exchange systems of gas have also been recommended for alveolar panels for hydrogen production (Ugwu et al., 2008).

4.2 Temperature and pH

Temperature and pH are the crucial factors influencing the fermentation process. Considering temperature, dark fermentative processes can be categorized as ambient having the temperature range of 15–27 °C, mesophilic with the range of 30–45 °C, thermophilic with the range of 50–60 °C, and extremely thermophilic with temperature higher than 60 °C temp (Yokoyama et al., 2009). Hydrogen production at high temperature is thermodynamically favorable (Chandrasekhar et al., 2015). pH regulates various metabolic pathways. Pyruvates formed as fermentative metabolites determine the yield of hydrogen (Antonopoulou et al., 2008). The pH-dependent fermentations are affected by the pH dependent activity of the microorganisms. For the production of hydrogen in the dark process, the suitable pH range is 5.5–6.0, which also prevents methanogenesis and solventogenesis (Chandrasekhar et al., 2015).

4.3 Nutrients

To increase the hydrogen production, the availability of nutrients is also important, which is required for the microbial growth (Mona el al., 2011). Phosphorus has a major role in energy production in the form of ATP (adenosine triphosphate) and act as a buffer and an alternative to carbonate. For any fermentative process, the availability of a suitable nutrient is necessary for the proper functioning of enzymes, which are consistently related with cellular transport and essential for the growth and metabolism of microbes.

4.4 Hydraulic Retention Time (HRT)

For the selection of microorganisms, the hydraulic retention time is a necessary feature so that microbes can withstand the mechanical dilution caused as a result of continuous volumetric circulation. When the time of fermentation exceeds, it causes a metabolic shift from acidogenesis to methanogenesis, which is not favorable for the production of hydrogen. Several factors such as the type of microorganisms used, type and composition of substrate used, rate of organic load, and the system redox condition, influence HRT.

4.5 Partial pressure of hydrogen

The biopathways (Fig. 6) generating hydrogen are highly vulnerable to the partial pressure of hydrogen. Hydrogenase regulates the process of dark fermentation as it transfers electron from an intracellular electron carrier to H^+ and feedback inhibition is involved. When dissolved hydrogen increases in the medium, the reduction of Fd oxidized is more favored than the oxidation of Fd reduced. There are some key challenges in the processes, which limit hydrogen production.

Dark fermentation

The process has a low efficiency of substrate conversion, thermodynamic limitations, low yield of hydrogen, and a separation is required for the gaseous mixture (Hydrogen and Carbon dioxide).

Photofermentation

The process is regulated by circadian cycles as they require an external light source and this limits the hydrogen yield because of the less conversion efficiency of light.

Biophotolysis (Direct)

Customized photobioreactors are required for O_2 generation and low yield of hydrogen is caused by less conversion efficiency of light.

Biophotolysis (Indirect)

An eternal light source is required, low light conversion efficiency limits the lower hydrogen yield. The effective design of hydrogen producing bioreactors can help to overcome challenges along with the selection of an appropriate feedstock and process modifications (Chandrasekhar et al., 2015).

4.5 Microorganism strains

Hydrogen production is dependent on the type of strains used for its production and the environmental factors that contribute to induce metabolic pathways to generate hydrogen. Most of the green algae have genetic codes to produce hydrogen and not all have this potential and is a characteristic of particular taxonomic groups. Mainly, genera such as *Chlamydomonas, Scenedesmus, Chlorella, Tetraspora* and others have the potential to produce hydrogen. Some new strains are studied for hydrogen production potential such as

Ulothrix, Dimorphococcus, Closterium, Dictyosphaerium etc. *C. reinhardtii* is the widely used and studied strain for hydrogen production and has given satisfactory results. *Chlorella* is becoming a new potential candidate for hydrogen production by bypassing the weaknesses of *C. reinhardtii.* Various strains of *Chlorella* used include *C. vulgaris* var. *vulgaris, C. salina, C. fusca, C. Sorokiniana, C. pyrenoidosa* etc. These species cam accumulate large amounts of carbohydrates when are in nutrient deficient conditions and show a good hydrogen production rate along with other values added products such as vitamins, carotenoids, anti-freeze proteins, glycerol, unsaturated fatty acids, mycosporine like amino acids etc. Correct knowledge of taxonomic strains is advisable for choosing right strains for further work and to avoid misleading data (Jiménez-Llanos et al, 2020).

5. The dual potential of microalgae

Biohydrogen production technology unlike various other technologies shows the potential of using renewable sources of energy such as biomass, food wastes (Ferchichi et al., 2005; Karlsson et al., 2008; Chong et al., 2009), agricultural residues (Hawkes et al., 2002), microalgal biomass (Nagarajan et al., 2020) and byproducts of different industrial processes such as refining sugar (Ren and Wang, 1994), distilling alcohol (Sasikala et al., 1992), olive processing (Eroğlu et al., 2004), cheese production (Davila-Vazquez et al., 2009) and tofu production (Yu et al., 2002). The leftover biomass from the photobioreactor has been utilized for dual benefits such as bioremediation of wastewater from the textile industries, energy production and saving the cost of waste disposal (Kaushik et al., 2011; Mona et al., 2011; Mona et al., 2013).

Microalgae have gained attention in the past decade due to their potential of hydrogen production by utilizing sunlight as an energy source. Anthropogenic as well as natural activities release organic and inorganic substances in water bodies, thereby, making the water

unfit for daily activities, especially, drinking. The primary and secondary treatment of water can remove nearly about 90% of biological materials and suspended solids. Some species of cyanobacteria can remove dyes and metals from the solution through biosorption (Kiran et al., 2007; Khalaf, 2008). Cyanobacterial species, *Nostoc linckia*, was isolated from the textile industry and studied for the production of hydrogen and removal of dye from wastewater (Mona et al., 2011). Therefore, microalgae show dual potential by helping to curb the atmospheric pollution by sequestering atmospheric carbon dioxide and by reducing the pressure on the fossil fuels by producing a clean energy source, hydrogen, and further, the leftover biomass can be utilized for various other purposes.

6. Feasibility of hydrogen utilization

According to the current scenario, the world's energy need will increase almost upto 60% more in 2035 than today's need with China and India requiring about 35% of the total world's energy (Khatib, 2011). The multifaceted approach should be the basis of reduction in ecological footprints such as solar energy, wind, nuclear and biohydrogen along with the fossil fuels (Pacala and Socolow, 2004; Patil et al., 2008; Sudhakar et al., 2011). Fossil fuels are limited resources and their continuous use is not sustainable (Srivastava and Prasad, 2000) while the emissions of greenhouse gases such as CO_2 are increased by their combustion. Hydrogen gas can be appreciated as an energy carrier for future demands with the advantage of carbon negative approach and also producing huge amount of energy per unit weight when combusted and being an inexhaustible source of energy. Biohydrogen production has numerous benefits when compared with various other conventional sources of hydrogen generation (Dutta et al., 2005). The estimated initial production cost for a microalgal based biophotolysis system in open ponds (140 ha) and photobioreactors (14 ha) is supposed to generate more than 1.0 million GJ/yr of the plant capacity (around 90%), with

respect to the total estimated cost of capital for the system for more than USD 40 million and around more than USD 10 million annual cost of operation. The total estimated cost of production of hydrogen was USD 10/GJ and this capital cost was almost 90% of the total cost at an annual capital charge of 25% (Akkerman et al., 2002). Hydrogen production by utilizing algal biomass of various kinds is a technological advancement as well as a challenge. Hydrogen has been considered as one of the safest, ecofriendly and pollution free fuels and its demand is increasing from the past decade (Sathyaprakasan and Kannan, 2015). As there are no emissions of carbon dioxide and hydrogen sulfide, it is very efficient to be utilized for electricity generation by feeding in fuel cells (Macaskie et al., 2005). There should be an association of scientists and engineers working in the field of biohydrogen and fuel cell so that the applicability of this fuel would be promising and the bioreactors are able to generate a sufficient amount of hydrogen such that the fuel cells could run continuously for 24 h.

The production cost for the generation of biohydrogen is determined by the bioreactor design and the production system selected. The estimated cost for 300 kg/d standalone system of 110,000 m^2 area of pond, 10 cm depth of pond is shown in Table 5 (Amos, 2004). Therefore, it is strongly suggested that cost-effective and rigorous R & D technologies should be used for the production of hydrogen. As H_2 can be fed directly in fuel cells for generation of electricity at ambient temperaturehence, most developed countries are planning to create so-called 'Hydrogen Highways' with the aim of encouraging the use of vehicles powered by H_2 fuel cells (Sargent and Kelly, 2013). The costs of different materials utilized for the manufacturing of the reactors are clear: PVC ($5.56), Glass ($51.61), Plexiglass ($15.47), and blue tarp ($0.46) (Sathyaprakasan and Kannan, 2015). As reported by certain studies, the total estimated cost of labor for a closed plant is more than $15,000/ hectare and after assuming the maintenance cost (10%)/ hectare, the labor cost will be around $26,000/ hectare

and the total cost is $100,000. The bioreactors with a large area (around 2-million-hectare) would have a corresponding cost of labor more than $50 billion (Sathyaprakasan and Kannan, 2015).

7. Recent advancement in biohydrogen production- Microbial Electrolysis Cell

The renewable and versatile technique of microbial electrolysis cell (MEC) is a newly developing technique and uses wastewater for the evolution of biohydrogen. The structure of MEC includes a cathode chamber and an anode chamber, which are partitioned by an ion exchange membrane. Similar to the microbial fuel cell (MFC), the MEC anode utilizes electrogenic bacteria but the cathode has an anaerobic environment different from the MFCs, which have an aerobic environment at the cathode. These are the bio-electrochemical cells where the reduction of organic acids takes place at the anode and the production of hydrogen occurs at the cathode. The electrogenic bacteria at the anode release electrons, which get combined with protons and evolve hydrogen in an anaerobic environment. The external power supply has to be provided to transfer the electrons to the opposite cathode. The microbial community can be of a different kind and can get affected by the occurrence of other competitive microbes found in the wastewater. The acetogenic and electrogenic bacteria could compete for the substrate, which leads to the loss of hydrogen yield. Similarly, the methanogens utilize carbon dioxide and hydrogen to produce methane; this proposes limitation to the hydrogen production. The use of inhibitors such as 2-bromomethanesulfonate (BES) and lumazine, has been suggested to overcome this limitation. The cathode material should be selected carefully as it is the main site for hydrogen evolution. The anode should be conductive and non-corrosive. Parameters such as pH, temperature, potential applied and the substrate should be monitored for better yield. The anode should have a higher pH whereas the cathode requires a lower pH. Temperature

changes affect the growth of various microbial species. A minimum of 0.2 V potential should be applied, and the evolution of hydrogen increases with the increase in the applied voltage. Conventional direct current supply, MFCs or dye sensitized solar cells are used to supply power to the MECs. Apart from hydrogen production, MECs have other value-added applications such as production of biohythane (Methane+ Hydrogen), which can be used as an alternative fuel to produce ethanol by the microbial reduction of electrons, recovery of ammonia and desalination of water, etc. MECs are emerging renewable and sustainable sources for the production of biohydrogen but the technology has not reached commercial practices and needs to be worked upon to make it more efficient and usable (Varanasi et al, 2019). Unlike MECs, Electrochemical Photo-Bioreactor (EPBR) are also used to generate biohydrogen using double compartmentalization. Protons are generated on the anode and the hydrogen is produced on the cathode using small voltage. Both the systems eliminates oxygen in the cathode chamber and produces hydrogen according to the reaction -

$$2H^+ + 2e^- \rightarrow H_2 \quad (12)$$

In a study by Hasnaoui et al. (2020) on hydrogen production using EPBR, it was found that hydrogen was also produced in the anode chamber by *Spirulina* strain due to its potential to produce hydrogen under dark and light phases and can be called as biohydrogen while the electrochemical hydrogen production occurred in the cathode chamber using voltage applied. Voltage applied has effect on hydrogen production rate in both the chambers. The anodic hydrogen increased linearly with increasing voltage while in the cathodic chamber hydrogen production took place at voltage greater than 0.2 V. EPBR has been considered as an efficient system for hydrogen production at less energy requirement. It has also been considered better than previous works on MECs due to the production of hydrogen at low applied voltage (Hasnaoui et al., 2020).

8. Challenges and Improvements

The delicacy of hydrogenase for oxygen sensitivity is the key challenge to utilize hydrogen as a source of energy. To combat this issue, a practical approach needs to be developed for establishing algal hydrogen production system. Bioengineered oxygen tolerant algal species can be seen as a feasible approach. Oxygen separating methods can be developed to enhance hydrogen production (Show et al, 2019a). For the separation of hydrogen and oxygen in the biohydrogen systems, spatial and temporal techniques have been developed. In the spatial systems, open microalgal ponds are used where atmospheric carbon is fixed into carbohydrates and oxygen. The resultant carbohydrates are fed into the second stage of the process where they are converted into organic acids and hydrogen and the algae consumed are recycled back into the first stage open ponds. In the temporal systems, the photosynthesis and oxygen evolution are suppressed through sulphur deprivation, which enhances the hydrogenase activity (Show et al, 2019b). The main problem arising because of indirect biophotolysis is time limitation and practical use. The process comes to an end after few days as algae need to go back to photosynthesis. The process cannot maintain a stable hydrogen production (Show et al, 2019a; Show et al, 2019b). Improvements are needed in this area. Algae-bacterial co-culture can be helpful in improving hydrogen production yield in indirect photolysis. A single-cell green alga, *Chlamydomonas reinhardtii* and the bacterium *Pseudomonas* sp. strain D, were used in the co-culture experiment. Oxygen produced as a result of photosynthesis was rapidly consumed by bacterial respiration, thereby, maintaining an anaerobic environment and enhancing the hydrogenase activity. In addition, the algal and bacterial relationship maintained the protein composition and stability of chlorophyll, which helped improving hydrogen production. *Chlorella* and *Scenedesmus* also formed a syntrophic relationship with the *Pseudomonas* sp. strain D (Ban et al., 2018). Dark fermentative process has become an attractive method for sustainable hydrogen production. It produces a mixture

of gases, which needs to be purified before practical hydrogen utilization. Moreover, it is yet in competition with the hydrogen produced from the fossil fuels in terms of conversion efficiency, cost and process reliability (Show et al., 2019b). If the organic acids produced can be re-used, the low productivity of biohydrogen can be improved. Microbial fuel cells can also be incorporated into the dark fermentative system for the conversion of organic acids generated into biohydrogen (Kumar et al, 2018; Show et al, 2019a). The pretreatment of the substrates used in dark fermentation makes the process more efficient. The chemical and physicochemical pretreatment techniques have been considered to be most efficient. The bioreactor design should be taken into consideration for the better performance of the system. Anaerobic Sequencing Batch Reactor (ASRB) is considered to show the best performance among the available reactor designs (Kumar et al, 2018). Consolidated bioprocessing (CBP) is the integration of hydrolysis and fermentation of biomass to the desired product. This lowers the energy demand and the requirement of equipment's in the process. Genera namely, *Caldicellulosiruptor, Clostridium, E. coli* and *Thermoanaerobacter,* show the potential as CBP microbes for biohydrogen production (Kumar et al., 2018). Further, the production rate and low yield of hydrogen pose major challenges for its practicability. Bioengineering and genetic manipulation of the strains used can be helpful. A gene knockout of the competing pathways of hydrogen production and an increased homologous expression of the enzymes involved, have been achieved (Show et al., 2019a). Biorefinery concept (Fig. 7) can also be exploited to reduce the production costs. It can integrate and generate various fuel and non-fuel value added products from the leftover biomass (Kumar et al., 2018). More research in biohydrogen production is required at the basic and advanced stages of its production to make it more understandable and apply at large scales.

9. Conclusion

Considering the fact that hydrogen is a carbon negative fuel, it appears to be an energy carrier for the future and thus proves to be environment friendly. The total energy generated per mass during combustion is very large, which can be easily transformed into electricity. Photobiological hydrogen production technology is far advantageous as compared to any other conventional method of hydrogen production such as autothermal reforming, steam reforming and partial oxidation. The most tempting feature of biohydrogen generation is its initial source, which is naturally available i.e. water. This research is in its developing phase and there is scope of more study to be done to generate sufficient hydrogen and make it practically usable. All the individual nations should have access for hydrogen production so that monopolies over fuel can be reduced. More research is needed for hydrogen production by microorganisms before this commodity can be effectively utilized.

Hence, there is a need to tackle the limitations faced during the biological hydrogen production process. To address the current world energy problems, it is believed that this is the right time for the implementation of advanced technologies, which are carbon negative and are sustainable and renewable in nature.

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Figures Legends

Figure 1. Conversion of biomass into bioenergy using different wates.

Figure 2. Schematic mechanisms of photosynthesis and biophotolysis shown by photoautotrophic microbes (Yu and Takashashi, 2007)

Figure 3. Structure of the [Fe-Fe]-active site (Ghirardi et al., 2009)

Figure 4. The structure of the [NiFe]-active site (Ghirardi et al., 2009)

Figure 5. Schematic representation of an open pond system for algal culturing (Borowitzka and Moheimani, 2013).

Figure 6. Light-dependent different electron transport pathways for hydrogen production. Showing the oxygenic hydrogen production in green algae through hydrogenase. Specially showing that, how blue-green algae (N_2 fixing) produced hydrogen through nitrogenase, driving electrons from photosynthetically produced reserve carbon source. O_2 evolution separated from H_2 evolution either by heterocyst or by temporal separation. Showing an oxygenic hydrogen production in photosynthetic bacteria through nitrogenase. Purple bacteria. Green bacteria

Figure 7. Biorefinery Approach (Koutra et al., 2020)

Figure. 1

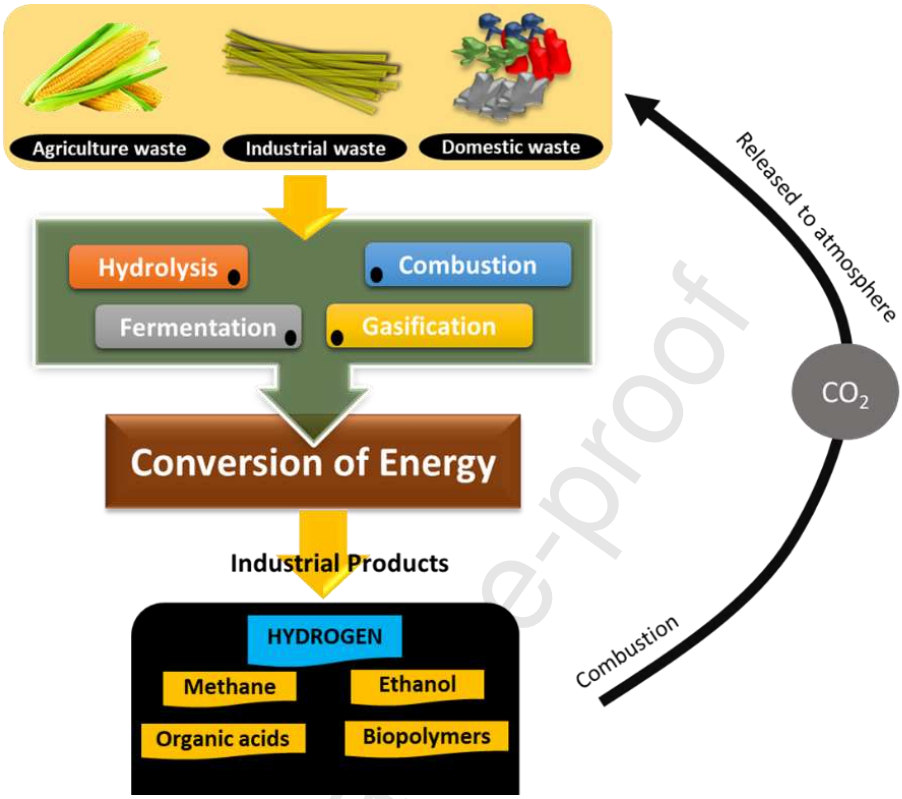


Figure. 2

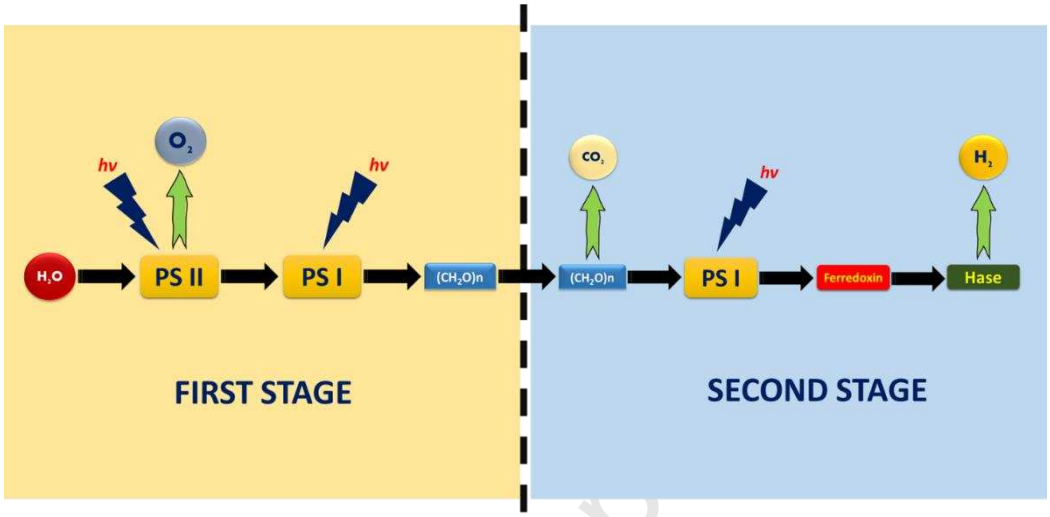


Figure. 3

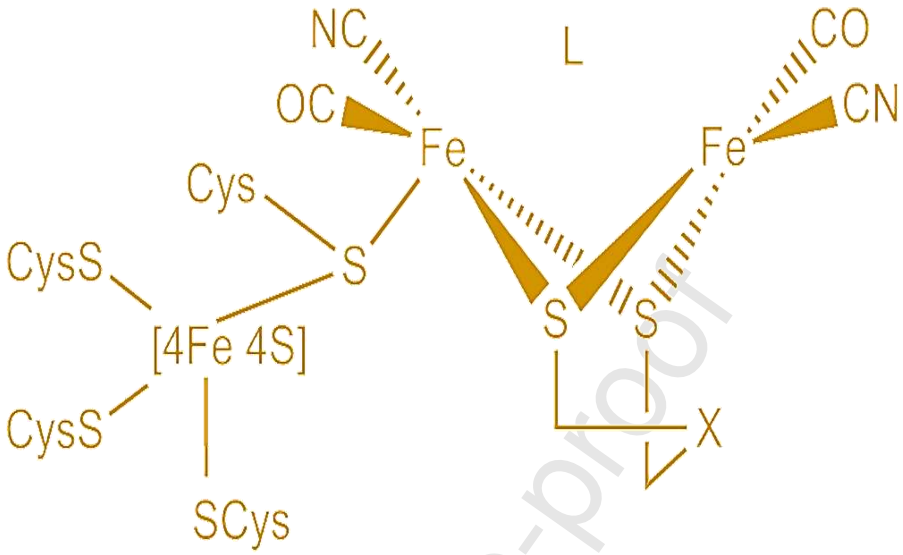


Figure. 4

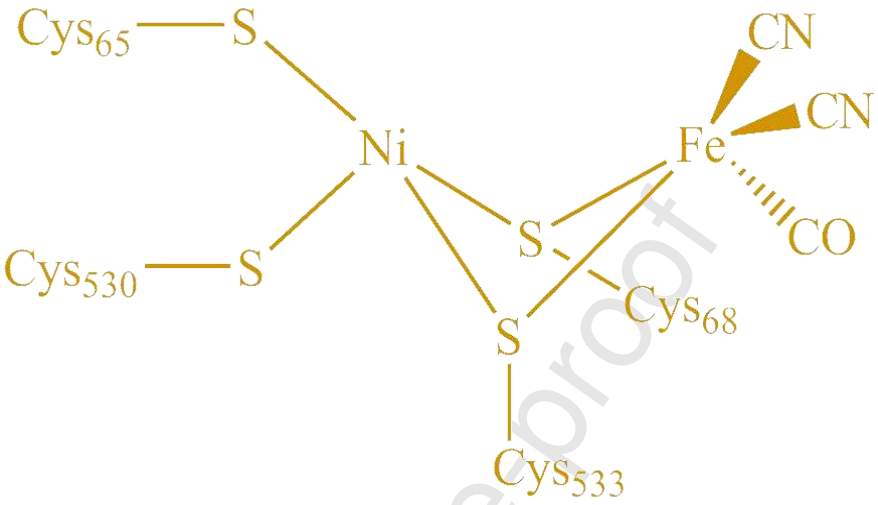


Figure. 5

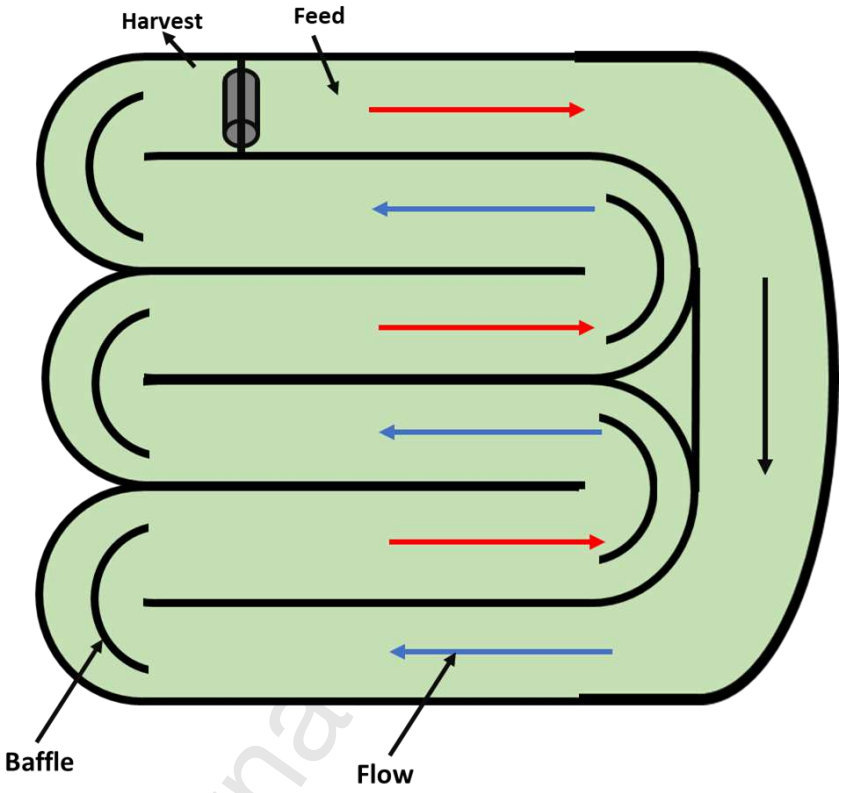


Figure. 6

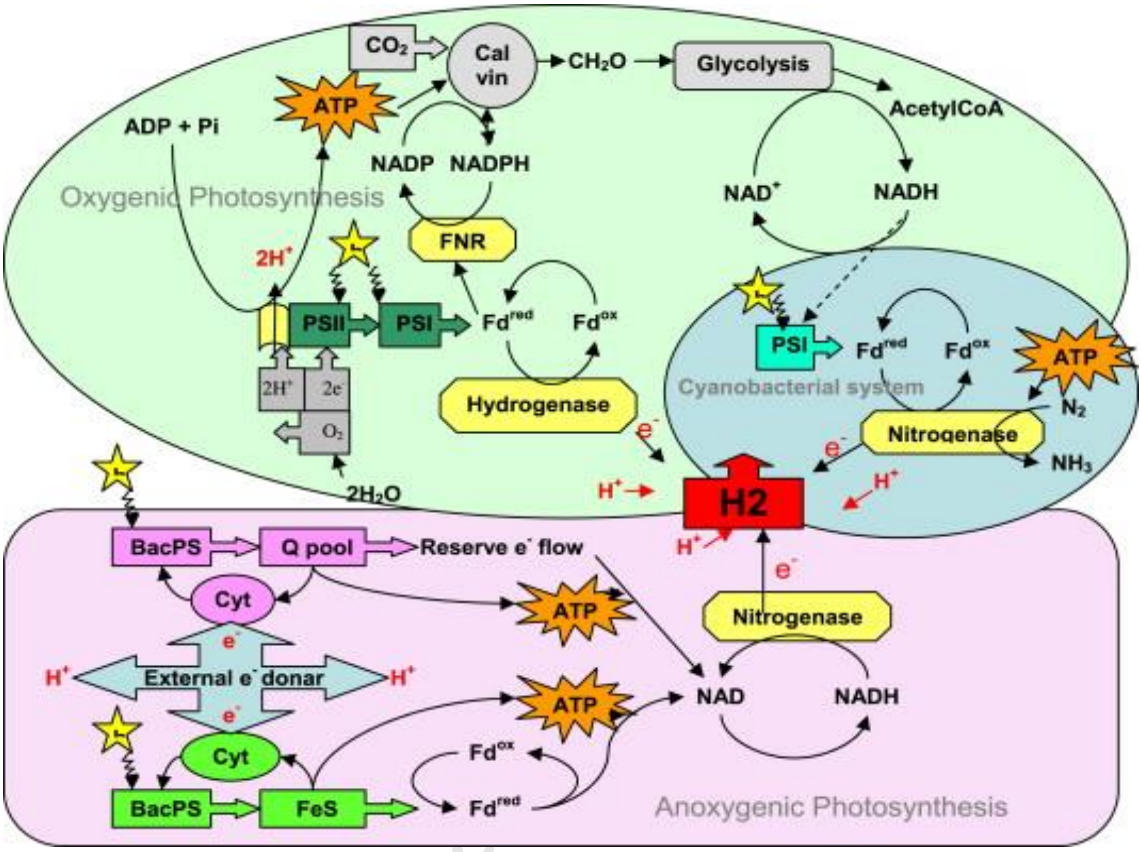


Figure. 7

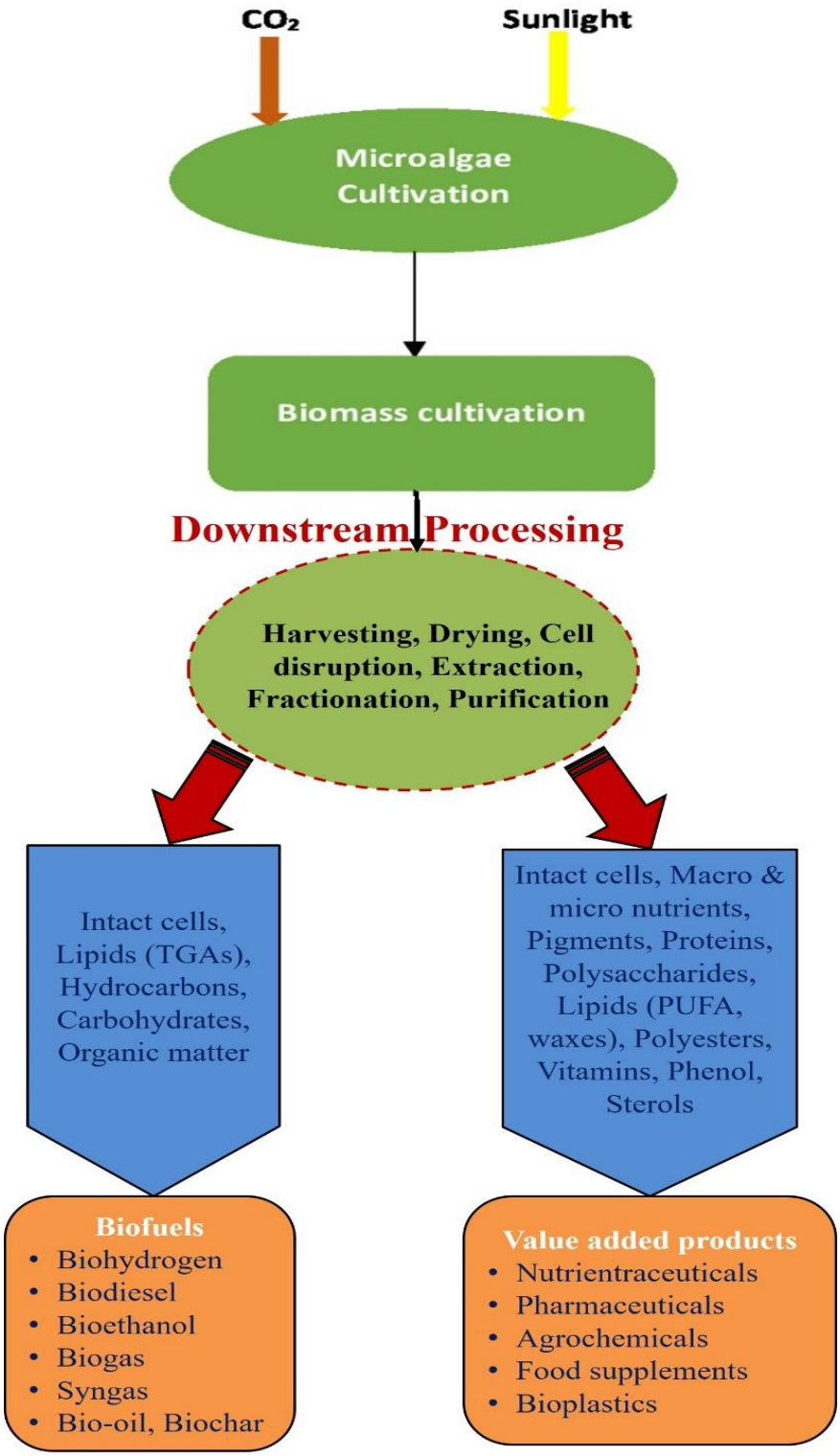

CO_2
Sunlight
Microalgae Cultivation
Biomass cultivation
Downstream Processing
Harvesting, Drying, Cell disruption, Extraction, Fractionation, Purification
Intact cells, Lipids (TGAs), Hydrocarbons, Carbohydrates, Organic matter
Intact cells, Macro & micro nutrients, Pigments, Proteins, Polysaccharides, Lipids (PUFA, waxes), Polyesters, Vitamins, Phenol, Sterols
Biofuels
• Biohydrogen
• Biodiesel
• Bioethanol
• Biogas
• Syngas
• Bio-oil, Biochar
Value added products
• Nutrientraceuticals
• Pharmaceuticals
• Agrochemicals
• Food supplements
• Bioplastics

Table 1: Different biohydrogen production processes and their yield (Wang et al., 1999)

Processes	Hydrogen yield (wt %)	Hydrogen yield (wt %) /biomass energy content
Pyrolysis + catalytic reforming	12.6	12.6
Gasification + shift reaction	11.5	83
Biomass +steam + except heat (theoretical maximum)	17.1	124

Table 2: Different algal biohydrogen production processes with their limitations and advantages

Process	Reactions	Advantages	Major limitations	Approaches to overcome
Direct Biophotolysis	$2H_2O$ + light = $2H_2$+ O_2	Can produce hydrogen directly from water and sunlight; Solar conversion energy increased by 10 folds as compared to trees	Sensitivity of hydrogenase for O_2; Light conversion efficiency is low	Use of O_2 absorbers. b) Use of O_2 tolerant hydrogenase; Genetic manipulation of light gathering antenna; Optimization of light input into photobioreactor
Indirect Biophtolysis	$6H_2O$+$6CO_2$+light = $C_6H_{12}O_6$+$6O_2$	Produced by both microalgae and Cyanobacteria; Can produce hydrogen from water. Cyanobacteria have the ability to fix N_2 from atmosphere	enzyme inhibition by O_2; Overall low production rate.	To achieve O_2 tolerant hydrogenase activity by classical; Mutagenesis Genetic modification to increase levels of bidirectional hydrogenase activity
Photo-fermentation	CH_3COOH+$2H_2O$+ light = $4H_2$+ $2CO_2$	Produced by both Purple bacteria and microalgae; A wide spectral light energy can be used by these bacteria	O_2 is a strong inhibitor of hydrogenase	(a) Use of O_2 absorbers b) Use of O_2 tolerant hydrogenase.

Table 3: Rate of hydrogen production by various strains of cyanobacteria and green algae

Microorganisms	Light illumination ($\mu E/m^2/s$)	Rate (mL/h)	Light conversion efficiency (%)
Anabaena PCC7 120	456	14.9	0.042
Anabaena variabills	32 W/m^2	7.73 l/kg/h	1.32
Anabaena variabills	353	20	1.0
Gloeocapsa alpicola	165	25	-
Synechococcus sp.	25 W/m^2	-	2.6
Chlorella reinharditi	110	2.5	0.125
Chlorella reinharditi	100	2.8-2.9	-
Chlorella reinharditi	-	2.5	-

Table 4: Types of photo-bioreactor with their optimal features (Dasgupta et al., 2010)

Type of photo Bioreactor	S/V ratio	Agitation system	Gas exchange	Advantages	Disadvantages
Reactors Vertical tubular	Small	Airlift, bubble column	Open gas exchange at headspace	Good mixing, efficient CO_2 supply and O_2 removal	Scale up is limited, major light is reflected due to angle
Horizontal tubular	Large	Recirculation with diaphragm/mechanical pumps	Injection into feed, and dedicated degassing units	Adequate angle towards sunlight	High shear due to pumps, risks of O_2 buildup, biofouling, separate gas exchange unit required
Helical tubular	Large	Centrifugal pumps	Injection into feed, and dedicated degassing units	High S/V, easy scale up by increasing the number of units	O_2 buildup, separate gas exchange, pumps exert more shear, cell debris accumulate inside

α shaped reactor	Large (high S/V)	airlift	Injection in the vertical units and degassed at top	High unidirectional flow rate with low air flow rate	Foam formation due to high cell density
Flat panel bubbled at bottom	medium	Bubbling at bottom or from sides, circulation	Bubbling	Open gas transfer avoids O_2 buildup	Agitation system can dilute hydrogen formed
V-shaped panel	Medium	Bubbling	Bubbling	Very high mixing rate, low shear	Agitation system can dilute hydrogen formed
Flat panel pivoted at center	Medium	Pulsating motion	Degasser	Good mixing, low shear	Scale up is difficult
Floating type bioreactor	Medium	Sea saw motion	Degasser	Low energy for operation, good agitation, can be installed on lakes and sea floor	
Torus shaped reactor	Medium	Marine Impeller	CO_2 inlet after impeller, outlet at top	Good mixing conditions owing to shape avoiding dead zones	

Table 5: Algal Hydrogen Production Capital Costs (Adopted from Amos, 2004)

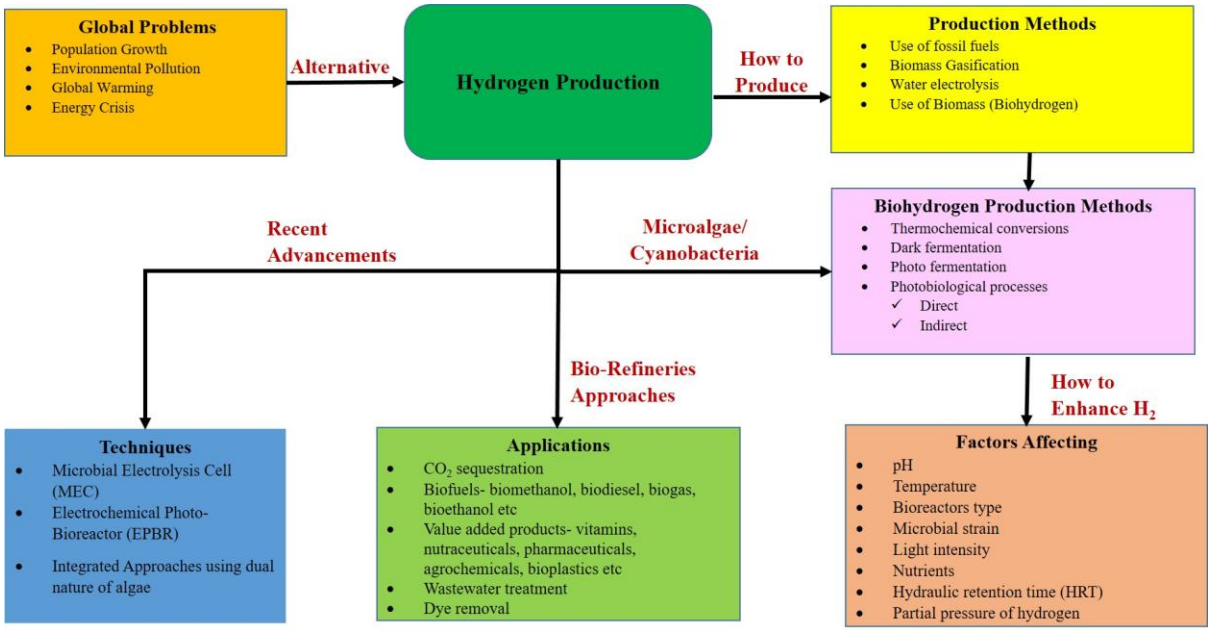
Item	Cost ($)
Algal Ponds	1,100,000
Pressure Swing Adsorption (PSA) Unit	121,000
Pressure Swing Adsorption (PSA) Compressor	359,000
Storage Compressor	578,000
High-Pressure Storage	913,000
Equipment Cost	3,071,000
Engineering & Construction	1,423,000
Contractor Fees & Contingency	674,000
Total Investment	5,168,000

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract



Highlights

- Microalgae is a third-generation biofuel feedstock.
- Hydrogenases and nitrogenases produce biohydrogen in microalgae.
- Biohydrogen biorefinery can be useful for reducing cost for its production.
- Challenges and improvements of hydrogen production have also been discussed.