# **Algae-Based Biohydrogen: Current Status of Bioprocess Routes, Economical Assessment, and Major Bottlenecks**

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## **1 Introduction**

Hydrogen is a most efficient fuel and has the highest energy density among known fuels (143 GJ/tonne) in terms of energy values as well as from an environmental point of view. It is a zero emission fuel which does not contain carbon, sulphur, or nitrogen and generates water only as a byproduct on combustion. Recently it is being very efficiently used as a vehicle fuel in automobiles and also for electricity generation via fuel cells. Commercially, hydrogen is produced by using fossil fuels such as coal, methane, and other heavy hydrocarbons (Kothari et al. [2008\)](#page-8-0). All these processes of hydrogen production are very expensive and not environmentally friendly. Recently, researchers have sought alternative methods for hydrogen production including photolysis of water and biological methods of hydrogen production (Nayak et al. [2014](#page-8-1)). Biologically produced hydrogen by using microorganisms such as bacteria and algae by photosynthetic and fermentative routes (Monlau et al. [2013](#page-8-2); Julia et al. [2014;](#page-8-3) Kothari et al. [2011;](#page-8-4) Venkata et al. [2007;](#page-9-0) Levin et al. [2004\)](#page-8-5) provides a sustainable approach for society. Biological processes can scale up biohydrogen production by using various microorganisms and making it potentially competitive with chemical processes including thermal gasification, pyrolysis, and reforming among others. Biohydrogen production via a biological route is beneficial because it is neutral regarding  $CO<sub>2</sub>$  emission and free from other greenhouse gases such as carbon monoxide and hydrogen sulphide and it does not require any kind of treatment

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before use in the fuel cell to generate electricity. Yield of biohydrogen production depends on operating cost whereas its rate depends upon its installation cost or reactor cost.

Biophotolysis (direct biophotolysis and indirect biophotolysis), photofermentation, and dark fermentation (Venkata et al. [2009\)](#page-9-1) are the emergent bioprocess routes for the production of biohydrogen. Among these, algae-based bioprocess production routes are projecting more scope in the R&D sector with commercialization. Indeed, algae present several advantages compared to terrestrial plants in virtue of: (1) algae have a higher growth rate than plants and they are more capable in  $CO<sub>2</sub>$  fixation; (2) they can be grown easily in water and wastewater (Venkata et al. [2012\)](#page-9-2); (3) they are rich in carbohydrates and have a lack of lignin (Nayak and Das [2013](#page-8-6)). Besides these, algae is a third-generation biofuel produced from macroalgae, and microalgae are more advantageous than second-generation biofuel produced from nonedible crops because they do not require fertile land for their growth and they have the potential to provide jobs for skilled and unskilled members of society.

There is very modest information available in the literature regarding the journey of lab-scale to large-scale commercial production of biohydrogen with algae. Hence, the present chapter aims to make available considerable research and developmental progress with major bottlenecks through bioprocess routes for algal-biomass–based biohydrogen production with emphasis on the major factors involved.

# **2 Bioprocess Routes for Biohydrogen Production by Algae**

Algae have wide potential for bioenergy generation by their metabolic activity as well as their anaerobic fermentation due to their rapid growth and rich carbohydrate contents. Biohydrogen production through biological process is significant and economically viable by algae because it is less expensive, has an easily available feedstock, and can use

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waste material as a substrate for growth (Venkata et al. [2007](#page-9-0)). In this section, we mainly focus on biophotolysis (BP) routes and dark fermentation (DF) routes for biohydrogen using algal biomass.

## **2.1 Direct and Indirect Biophotolysis**

#### **2.1.1 Direct Biophotolysis**

Direct biophotolysis is the process responsible for algal photosynthetic activities; solar energy is directly converted to hydrogen in the reaction routes of photosynthesis (Eq. ([6.1](#page-1-0))). This natural process is part of its attraction among scientists because it converts available substrate water to oxygen and hydrogen.

$$
2H_2O + light energy \rightarrow 2H_2 + O_2 \tag{6.1}
$$

<span id="page-1-0"></span>This process works at a partial pressure of near one atmosphere of  $O<sub>2</sub>$ . On the other hand, oxygen sensitivity to the hydrogenase enzyme reaction always creates a hindrance in the process (Frigon and Guiot [2010\)](#page-8-7). Monlau et al. ([2013\)](#page-8-2) reported hydrogen production rates on the order of 0.07 mmol/h per litre in their experimental study with direct biophotolysis.

## **2.1.2 Indirect Biophotolysis**

Algal-based biohydrogen production with indirect BP is completed in two stages: the first involves the synthesis of carbohydrate by using a photosynthetic process, and the second stage covers the degradation of stored carbohydrates in anaerobic condition (Azapagic and Stichnothe. [2011](#page-8-8)). Stage 1 and stage 2 are reported as photofermentation and dark fermentation with light and without light, respectively (Tommasi et al. [2012\)](#page-9-3). Cyanobacteria also have the unique feature of using ambient  $CO<sub>2</sub>$  as a carbon source and solar light as an energy source (Eq.  $(6.2)$  $(6.2)$  $(6.2)$ ). The cells take up  $CO<sub>2</sub>$  first to produce cellular substances, which are subsequently used for hydrogen production (Eq. ([6.3](#page-1-2))). The overall mechanism of hydrogen production in cyanobacteria can be represented by the following reactions:

<span id="page-1-1"></span> $12 H<sub>2</sub> O + 6CO<sub>2</sub> + light energy  $\rightarrow C_6 H<sub>12</sub> O_6 + 6O_2$  (6.2)$ 

<span id="page-1-2"></span>
$$
C_6H_{12}O_6 + 12H_2O + \text{light energy } \rightarrow 12H_2 + 6CO_2 \qquad (6.3)
$$

Both algae and cyanobacteria have the capacity to produce biohydrogen but algae are better than cyanobacteria because they require high-energy intensive enzymes and ATP requires nitrogenase for biohydrogen production but in cyanobacteria production of biohydrogen and oxygen both take place at separate times and places known as indirect biophotolysis (Tommasi et al. [2012\)](#page-9-3). Algae produce biohydrogen by a water-splitting process to form hydrogen, but the rate of biohydrogen production is not as high as the  $CO<sub>2</sub>$  reduction. In this process oxygen is also produced, which inhibits the production of biohydrogen, because hydrogenase is highly sensitive to oxygen. Therefore, research work is being done in this field to discover the key component that reduces the production of oxygen out of which sulphur deprivation is best and potassium deficiency has also been found as a biological switch that reduces oxygen production. Here, oxygen is not a problem but solar conversion efficiency is low (Julia et al. [2014\)](#page-8-3). Table [6.1](#page-1-3) shows the result obtained after review of the existing literature based on algal and cyanobacteria biomass available for biohydrogen production by the bioprocess route of direct and indirect biophotolysis.

#### **2.1.3 Factors Affecting Biophotolysis (BP)**

Factors affecting the process are numerous but only a few important ones are discussed here in the subsections.

#### **2.1.3.1 Immobilization**

Microalgae cultivated in the form of immobilized cells would have versatile applications because their  $CO<sub>2</sub>$  capturing rate is high to convert them into organic compounds. The rate of biohydrogen production via immobilized cells is higher than free cells (Brouers and Hall [1986](#page-8-9)).

## **2.1.3.2 pH**

Biohydrogen production is directly related to pH (Table [6.2](#page-1-4)). Hydrogenases and nitrogenase are the biohydrogenproducing enzymes, sensitive to pH because at low pH (less than 5) it reduces the enzymatic activity and also the biohy-

<span id="page-1-3"></span>**Table 6.1** Algae and cyanobacteria biomass for producing biohydrogen cited in the literature

<b>Broad classification</b>	Name of algae.	Reference
Green algae	Chlamydomonas reinhardtii	Julia et al. $(2014)$
	Chlorella sorokiniana	Chader et al. $(2009)$
	Chlorella vulgaris	Rashid et al. $(2011)$
	Chlorella fusca	Das and Veziroglu
	Scenedesmus obliquus	(2008)
	Chlorococcum littorale	
	Platymonas subcordiformis	
Cvanobacteria	Oscillatoria	Pinto et al. $(2002)$
<i>(indirect)</i>	Calothrix	
biophotolysis)	Gloeocapsa	

<span id="page-1-4"></span>**Table 6.2** Algal biohydrogen production with different substrates at optimal pH



drogen production rate. The pH value is also varied for freshwater algae and marine water because the requirement of the pH value for freshwater algae is different to marine water algae because marine algae require low nitrate uptake. The main factor responsible for the change in pH is nitrate uptake along with the fixation of carbon.

## **2.1.3.3 Carbon Source**

The carbon source is one of the important factors for the cultivation of microalgae. During the process of photosynthesis these microalgae use carbon and store it in the form of starch and glycogen but the storage of this starch and glycogen is limited as a result of which biohydrogen production is also limited, thereby requiring an exogenic source of carbon (organic carbon such as glucose, fructose, malt extract, etc. in wastewater). The role of the carbon source in the cultivation of microalgae is not well understood but some research work has been done on the effect of the carbon source on microalgae in anaerobic conditions.

A cyanobacteria and green algae *Microsystis aeruginosa*, *Chlorella vulgaris*, respectively, were used on substrates including malt extract, glucose, and sucrose, and maximum biohydrogen production was on the malt extract: 1300 ml/l. *Anabaena* species strain CH<sub>3</sub> was cultivated by using fructose, galactose, sucrose, and glucose as a feeding material and it was found that the most suitable substrate for biohydrogen production was fructose and sucrose that produced 0.0016 mol and 0.001 mol of biohydrogen production, respectively (Table [6.3](#page-2-0)).

#### **2.1.3.4 Light**

The most suitable light frequency that provides energy for algal growth is in the 400 to 700 nm wavelength. In a temperate climate algal biomass production is much lower than in a tropical climate because of variation in solar radiation. Sutherland et al. ([2013\)](#page-9-6) have investigated that in summer algae biomass production increased about 250 % because of the presence of three times more solar radiation in summer than in winter.

<span id="page-2-0"></span>**Table 6.3** Yield of algal-based biohydrogen with different substrates as carbon source

Microbial sp.	Substrate used	Biohydrogen <b>Production Rate</b>	Reference
Microsystis aeruginosa (cyanobacteria)	Malt extract	$1300$ ml/L	Song et al. (2011)
Chlorella vulgaris (green algae)	Malt extract	$1300$ ml/L	Song et al. (2011)
Anabaena sp. Strain CH3	Fructose	$0.0016$ mol/200 ppm	Chen et al.
	Sucrose	$0.001$ mol/200 ppm	(2008)

#### **2.2 Dark Fermentation (DF)**

Dark fermentation is a simple process manifested by anaerobic bacteria with the capacity to produce biohydrogen by using organic acid and waste material as a substrate. This process mainly involves two pathways: acetate and butyrate. There are two common pathways in the production of hydrogen by dark  $H_2$  fermentation (Kothari et al. [2012](#page-8-13)): one producing acetate and the second butyrate. Theoretically, the hydrolytic fermentation of 1 mol of glucose yields 4 and 2 mol of  $H_2$  through acetate and butyrate pathways, respectively (Angenent et al. [2004\)](#page-7-0):

1. 
$$
C_6H_{12}O_6 + 2H_2O = 4H_2 + 2CO_2 + 2CH_3COOH
$$
 (6.4)

(Hydrogen fermentation to acetate pathways)

2. 
$$
C_6H_{12}O_6 = 2H_2 + CH_3CH_2CH_2COOH + 2CO_2
$$
 (6.5)

(Hydrogen fermentation to butyrate pathways)

#### **2.2.1 Factors Affecting DF**

## **2.2.1.1 Substrate**

Microalgae and cyanobacteria have recently been more emphasised for bioenergy production. The algal biomass is rich in carbohydrates (starch/glycogen/cellulose) and does not contain lignin as does other biomass. Thus, it is easier to obtain monosaccharides from algal biomasses than other lignocellulose material. Some species of cyanobacteria such as *Anabaena* sp., *Synechocystis* PCC6803, *Synechococus*, and *Spirulina* sp. can accumulate contents up to 20–30 % of dry weight (Cao et al. [2010\)](#page-8-14). However, some cyanobacteria store carbohydrate in the cytoplasm and in their cell walls in the form of polysaccharides. These sugars need to be converted to monomers by the application of some pretreatment; chemical (acids and alkaline) and enzymatic hydrolysis are common pretreatment methods.

#### **2.2.1.2 Inoculums**

The anaerobic fermentation of algal biomass is mostly done by an anaerobic consortium taken from wastewater treatment (Table [6.4](#page-3-0)). There are various types of pure strains also used, such as species of *Clostridium* and *Enterobacter*. A mixed fermentative culture is more common for biohydrogen production as it is simple to operate and does not require sterile conditions as do pure strains. The mixed culture inoculums are mostly taken from soil and anaerobic sludge of wastewater treatment plants. These inoculums are mainly characterised by the bacteria belonging to the genus *Clostridia* and *Bacillus*.

	Carbohydrate contents			
Feedstock	(% of dried biomass)	Bacteria/inoculums	$H_2$ yield	References
Chlorella vulgaris	57.0	Clostridium butyricum	$85.3$ ml/g-TVS	Liu et al. $(2012)$
Chlamydomonas reinhardtii	11.8	Clostridium butyricum + Rhodobacter sphaeroides	$128.3$ ml/g-TVS	Kim et al. $(2006)$
Arthrospira platensis	44.4	Mixed culture	$354.7$ ml/g-TVS	Cheng et al. $(2012)$
C. Pyrenoidosa sp.	NA	Anaerobic digested sludge,	$6.1$ ml/g-TS	Sun et al. (2011)
Chlamydomonas Reinhardtii	NA	C. butyricum NCIB 9576	$40 \text{ ml/g-TS}$	Kim et al. $(2006)$
Nannochloropsis sp.	NA	Enterobacter aerogens ATCC13048	$48 \text{ ml/g-TS}$	Nobre et al. $(2013)$

<span id="page-3-0"></span>**Table 6.4** Effects of inoculum on biohydrogen production from various algal biomass in dark fermentation process

## **2.2.1.3 Temperature**

The process of hydrogen production is highly affected by temperature changes as a small increase or decrease in temperature might alter the substrate utilization process, hydrogen yield, or formation of liquid products as well as microbial community of the system (d'Ippolito et al. [2010](#page-8-16); Hafez et al. [2012\)](#page-8-17). Most of the studies of biohydrogen production are done under mesophilic conditions as they are preferable from economic and technical points of view to thermophilic bacteria and they exhibit high yield under stable conditions (Zhang et al. [2003;](#page-9-8) Munro et al. [2009](#page-8-18)). However, the mesophilic biohydrogen production process also favours the growth of nonhydrogen-producing microbes.

### **2.2.1.4 pH**

pH has a profound effect on the fermentative hydrogen production process due to its major role in determination of the acidic and alkaline condition of the system, in the limitation of the growth of bacteria, and regulation of solvent production. Solvent generated at the end of fermentation decreases the pH by acid accumulation. The optimum pH for hydrogen production is found between 5.5 and 6.5 avoiding the solvantogenic phase (Khanal et al. [2004\)](#page-8-19).

# **2.3 Factor Affecting Both BP and DF Bioprocess Routes**

## **2.3.1 Reactors**

There are various types of bioreactors used for algal biomass production for production of biohydrogen in particular. Details of some important bioreactors, different in structural designs (Fig. [6.1](#page-4-0)) are as follows.

#### **2.3.1.1 Tubular Airlift and Bubble Column**

This reactor having vertical transparent tubes made up of glass or polyethylene to get adequate light penetration and  $CO<sub>2</sub>$  supply is allowed through bobbing. As we know, fabrication of a vertical tubular bioreactor is cheap but it is not versatile. It does not provide high culture volume and efficient gas transfer because a bioreactor should possess a high area–volume ratio and due to lack of these things its photosynthetic efficiency also decreases (Martnez-Jeronimo and Espinosa-Chavez [1994](#page-8-20)). Another drawback is that it has a large angle size in comparison to sunlight therefore most of the sunlight would be reflected back, making it a disadvantage in terms of biomass productivity.

### **2.3.1.2 Helical Tubular Bioreactor**

This bioreactor possesses a flexible tubular pipe with a coiled framework along with a heat exchanger and gas exchange tower. Due to its coiled conical shape structure it receives maximum solar radiation for algal growth. In HTR the areato-volume ratio is high therefore it is possible to gate 6.6 % photosynthetic efficiency and have volumetric productivity of 0.9 g L<sup>-1</sup> d<sup>-1</sup> (Tredici and Rodolfi [2004](#page-9-9)).

#### **2.3.1.3 Flat Plate Bioreactor**

Such type of reactor is fabricated mainly to achieve maximum solar radiation therefore it is made by using narrow panels to achieve the maximum area-to-volume ratio. The main advantage of such a reactor is that it possesses an open unit of gas transfer which is also important due to restriction in oxygen buildup, which plays an inhibitory role in biohydrogen production; the main drawback is that its open unit may increase the chances of contamination. It is also beneficial due to its high productivity and uniform light distribution. This system can also be oriented towards the sunlight to achieve maximum radiation. A flat plate solar bioreactor has

<span id="page-4-0"></span>**Fig. 6.1** Different types of photobioreactors (Dasgupta et al. [2010\)](#page-8-26)



Tubular column

bioreactor



Airlift column

bioreactor



Helical tubular bioreactor





Flat plate bioreactor

Fermentor bioreactor

been made to produce 10 ml hydrogen  $L^{-1}$  h<sup>-1</sup> with 6.5 L capacity and at 30° temperature (Eroglu et al. [2008](#page-8-25)).

#### **2.3.1.4 Fermentor Type of Bioreactor**

The main advantage of this bioreactor is control of parameters such as sunlight, flow rate, and mixing but the main drawback is that it does not do well in receiving solar radiation. It is not applicable at the industrial level (Pohl et al. [1988](#page-9-11))

Commercial algal production is low worldwide. Probably 6000 t/year in terms of dry biomass are produced autotrophically in the presence of sunlight and  $CO<sub>2</sub>$ . Today at the global level, there is no adequate and meaningful amount of algalbased biohydrogen being produced. Biohydrogen production using algal biomass is significant in terms of negative emission of carbon also because when bioenergy sources used atmospheric carbon for its growth at the same time it also removed atmospheric carbon, therefore it is also positive in the sense of carbon sequestration.

# **3 Economic Stresses on Bioprocess Routes**

Economic feasibility of any system depends on the various parameters such as (1) algal biomass required; design of reactor; capital costs; operating costs including power, labour, and water; and general supplies for resulting biomass and energy balance outputs. In the case of biohydrogen production through algal biomass, capital costs were estimated based on vendor quotes, and prior literature studies on standard energy estimates (Tapie and Bernard [1988](#page-9-12)). Similarly, other factors responsible for economic stress may be fixed operation cost (labour, maintenance, insurance, tests), indirect capital cost, internal rate of return, plant's life duration, and so on. In spite of this, algal biomass production rate with the type of culture/strain is also an important factor in observing the economic status of any bioprocess route. After an extensive literature survey on concerned bioprocess routes for the last 10 years, various researchers discuss the pros and cons in respect of economic inputs, and are listed in Table [6.5](#page-5-0).

It has been reported that efficiency of biohydrogen production by using solar energy is very low, that is, less than 1.5 %. Research work has been done to enhance the efficiency by applying some nutrient stress such as sulphur and potassium to suppress the production of oxygen, yet it increases only 10 %. In the photosynthesis process water is used as the substrate thus the operating cost is very low and it requires only its maintenance cost but production of biohydrogen is very low, therefore the reactor should be large and the installation cost is very high. Zaborsky ([1999\)](#page-9-13) has reported that a reactor having 10 % light conversion efficiency would cost about \$50/m<sup>2</sup> for a single stage but will cost \$100/m2 for a two-stage system; he has also suggested

<span id="page-5-0"></span>Table 6.5 Economic stresses and feasibilities of algae-based bioprocess routes for biohydrogen as per research accomplished in the last 10 years

S. No.	Description/Highlights	References
1.	Hybrid fermentation that incorporates hydrogen and methenation production both, provides an economically promising and applicable bioprocess route for alternative energy resources.	Arni et al. (2010)
$\overline{2}$ .	Researchers' work based on urban wastewater treatment in combination with biohydrogen production using microalgae provides the possibility of biofuel with nutrient removal and improves the economic profitability of the whole system for bioenergy prospects.	Batista et al. (2015)
3.	Wastewater treatment and bioenergy generation via algae is an integrated suitable approach, but for the production of economically viable and sustainable algae-based biofuel research work is needed to be done from cultivation of algae to conversion of biomass into energy.	Abbas et al. $(2015)$
4.	Technoeconomic assessment and life-cycle assessment are the most important tools through which one can easily understand the current status of algae and technologies related to the production of biofuel/biohydrogen, potential and conversion efficiency, and major R&D challenges required in the field of algae technologies.	Jason and Ryan (2015)
5.	For efficient algae culture and biomass harvesting, cost-effective technologies are needed.	Hallenbeck and Benemann (2002)
6.	The authors concluded that at present, biohydrogen productions via biological process routes are not economically viable in comparison to other fuel alternatives. Various technological and engineering challenges have to be solved preceding economic barriers. Economic analyses stated that major R&D challenges are concerned with development of cost-effective photobioreactors and improvement in photosynthetic efficiency.	Show et al. (2012)
7.	The bioprocess route for biohydrogen production requires less energy in comparison to chemical and electrochemical processes. It may be possible to produce biohydrogen in places where biomass is easily available in the form of waste and transport would be possible at low cost as well as low energy expenditure.	Olga and Pavel (2012)
8.	In the case of biological hydrogen production major challenges are low yield of biohydrogen and its production rate, making it not economically viable; these gaps can be bridged by the use of suitable algae species, with the improvement in bioreactor design and improvement in genetic and molecular engineering technologies.	Show et al. (2012)
9.	Researchers provide an inverse relationship between the bioprocess route and economic viability for sustainable energy production, that is, biohydrogen production through dark fermentation is economically viable but has a low yield. Photofermentation is efficient but not economically viable.	Song et al. (2011)
10.	Algal-based biofuel costs about $\epsilon$ 50 per litre which is far from an economical point of view.	Ahrens and Sander (2010)
11.	Economic possibilities with biofuel, an important energy source, reduces the dependency on fossil fuel and shows economical vulnerability for new era.	Demirbas and Ayhan (2009)
12.	By using activated sludge for biohydrogen production, a significant amount of biomass may be produced that may compete economically over fossil fuel and provide a better energy supply in the twenty-first century.	Ren et al. (2007)
13.	It had been predicted that biohydrogen production via indirect biophotolysis would have capital cost 2.4 \$/gj/year	Resnick $(2004)$
14.	Compared with photobiological hydrogen production, fermentative hydrogen is three times more in per unit cost of energy generation and conversion efficiency in both cases is the same, almost 10 %.	Nath and Das $(2003)$

that for this process the cost of a tubular bioreactor would be \$50/m<sup>2</sup> and the project cost of biohydrogen production would be \$15/gj. It has also been reported (Amos [2008](#page-7-1)) that a pond type of bioreactor having an area  $110,000$  m<sup>2</sup> using unicellular green algae would have a reactor cost of \$10/m<sup>2</sup>. It has also been reported that biohydrogen production by using cyanobacteria would incur a cost of \$25/m<sup>2</sup> (Block and Melody [1992\)](#page-8-27).

Hence, economic stress for these discussed bioprocess routes (direct and indirect biophotolysis and dark fermentation) can be overcome through (1) biological and engineering improvement opportunities; (2) for significant cost reduction potential in capital and operating cost, research should be more focused on two parameters, lipid content and algal growth; and (3) optimizing nutrient stress conditions and  $CO<sub>2</sub>$  requirements to reduce the capital cost by utilizing suitable wastewater (urban and industrial) as a substrate.

# **4 Major Bottlenecks in Bioprocess Routes**

There are certain shortcomings associated with algae-based bioprocess routes of biohydrogen production, which are obstacles and affect biohydrogen production, therefore these conditions should try to be minimized.

## **4.1 R&D: In Growing Stage**

## **4.1.1 Suitable Substrate: Demand in Search**

Waste materials from the ecosystems that are suitable as a substrate for biohydrogen production are also a challenge because the complex nature of organic compounds sometimes adversely affects biodegradability. Simple sugars such as glucose, maltose, lactose, and sucrose can be easily degraded and suitable for biohydrogen production. Agricultural and food industry waste are highly rich in starch, cellulose, and also in terms of carbohydrate. It is easy to produce biohydrogen from waste containing starch or carbohydrate because it can easily hydrolyse to glucose and maltose to form organic acid and then biohydrogen gas whereas using agricultural waste containing cellulose and hemicelluloses always possesses the problem of pretreatment. First, it has to go through the process of delignification because lignin content and the efficiency of hydrolysis are inversely proportional to each other. There are some industrial wastes which, like dairy, tannery, olive mill, and brewery wastewater are potential applicants for biohydrogen but the main challenge in using these wastes is that they require pretreatment to remove undesirable substances, then convert to organic acid, and then biohydrogen production. In a wastewater treatment plant, a huge amount of waste sludge is generated which is also rich in carbohydrate and protein content so this sludge can also be used as raw material for biohydrogen production; however, it also has toxic substances and complex organic compounds which cannot be easily degraded due to their complex nature. Therefore they also require pretreatment which is cost effective and not economically viable thus the use of wastewater as a raw material for algal biomass is also challenging when producing biohydrogen (Kothari et al. [2010,](#page-8-34) [2012;](#page-8-13) Bhaskar et al. [2008](#page-8-35)).

## **4.1.2 Optimization of Parameters: Challenge from Lab Scale to Pilot Scale**

At lab scale there are various parameters which play an important role in biohydrogen production such as pH, temperature, nutrient ratio, and substrate (Krupp and Widmann [2009](#page-8-36)) among others, and these parameters can be maintained easily at lab scale as a result of which biomass productivity would enhanced. When doing this at industrial scale it is quite difficult to maintain these parameters and the cost factor is also prominent and cannot be ignored. There are various factors which affect the production of biohydrogen when high-level large-scale cultivation of algae requires additional fertilizers such as phosphorus and nitrogen and these fertilizers from the dry algal biomass cannot be ignored as they

may have an adverse impact on biohydrogen production; therefore some technologies should be developed for nutrient recycling (Ferreira et al. [2013\)](#page-8-37). The use of excess fertilizers can also cause nutrient pollution or eutrophication as a result of which the structure and function of the ecosystem of concern may change. By the process of leaching, if these nutrients leach to a nearby water body they could have an adverse effect on aquatic flora and fauna. Under controlled conditions, algae cultivation requires inputs of fossil fuel in the form of electricity and drying algae to form dry biomass natural gas is also required. Algae are also temperature sensitive, therefore maintenance of temperature also requires use of fossil fuel so we have to develop such a technology and system designed to minimize the use of energy and enhance biomass productivity (Slade and Bauen [2013\)](#page-9-17). It is important to know that algae also produce some toxic substances including polypeptide ammonia and polysaccharide. At the end of the process by-products are sometimes used as manure so these toxins can have an adverse impact in the food chain of the ecosystem, therefore care should be taken in the selection of algae species.

## **4.2 Road to Commercialization**

Biohydrogen production as a third-generation fuel is very new. Most of the work is being performed on a lab scale by using different micro- and macroalgae and bacteria but its industrial application is not as high as it should be. Although it has been reported that for the growth of algae, pure culture medium was being used, the scenario has now changed and there is a shift from pure culture medium to food and industrial waste as a substrate, which is easily and cheaply available and a renewable source for energy generation. In biohydrogen production, rate and yield are two important parameters that should always be in consideration. Scientific research efforts have focused on microalgae that are already commercially significant with the greatest prospects for highly efficient energy production coming from species such as *Chlorella*, *Spirulina*, *Dunaliella*, and *Haematococcus* (Bruton et al. [2009\)](#page-8-38). These algae are already used in commercial nonfuel operations, where they are used to make a variety of high-value products for use in human and animal nutrition, aquaculture, and cosmetics (Spolaore et al. [2006](#page-9-18)).

#### **4.2.1 Reactors**

One major challenge in biohydrogen production is reactor design at the commercial level because it is has a direct relation to algal biomass production. There are various types of bioreactors but designing a suitable bioreactor with relation to its efficiency is a tedious task. The most important parameters when designing a bioreactor are light penetration, mixing, and flow, which depend upon area-tovolume ratio. In order to get a high area and volume ratio several bioreactors of various shape and size have been designed which have given successful responses. The flatplate bioreactor, tubular bioreactor, and fermentor type of bioreactor are designed to get high light penetration and based on the principle of high area-to-volume ratio for proper mixing, light penetration, and flow rate (Owende and Brennan [2010;](#page-9-19) Yeow et al. [2011](#page-9-20)).

Cultivation of algae at the commercial level is not feasible although it is technologically feasible at the lab-scale level. Commercialization of algal cultivation for biohydrogen production is too far from being realised (Richmond [1987](#page-9-21)). For the successful commercialization of algae-based biohydrogen production it is always a big obstacle to discover the best and most suitable fast-growing algae strain with high photosynthetic efficiency and high oil content. For commercialization of algae as a fuel two important things are that there should be an easy algae culture harvesting system and use of a photobioreactor should be economically viable (Davis et al. [2011\)](#page-8-39). Supporting the infrastructure, maintenance, and operational costs for algae culture and biohydrogen production for its commercialization is very important. Today freshwater demand has increased and it is also required for agricultural crops therefore the freshwater requirement for algal growth would add pressure in areas where water is scarce. Algae cultivation for biohydrogen production in an open pond system is not suitable because the adjustment of parameters for optimum growth is not easy task. It is more suitable in a closed type of bioreactor but here we cannot enhance the production rate of biohydrogen. A life-cycle assessment report has shown that algae cultivation in an open pond system for biohydrogen production is not environmentally suitable in comparison to normal crop plants (Clarens et al. [2010\)](#page-8-40) (Table [6.6](#page-7-4)).

<span id="page-7-4"></span>**Table 6.6** Status of major technologies and gaps [http://www.intech.](http://www.intech.unu.edu/events/workshops/hfc05/chopra_ppt.pdf) [unu.edu/events/workshops/hfc05/chopra\\_ppt.pdf](http://www.intech.unu.edu/events/workshops/hfc05/chopra_ppt.pdf)

Technology	International status	National status
Coal gasification	Commercially available	Efforts underway to set up pilot plant
Biological rout for hydrogen production	In precommercial stage	Demonstration plant set up
Metal hydrides for hydrogen storage	Hydrides with $1.5-2.0$ $wt\%$ storage capacity for ambient conditions developed	Hydrides with 2.42 $wt\%$ storage capacity for ambient condition developed

# **5 Environmental Benefits of Biohydrogen Economy**

For sustainable economic development in the world a biohydrogen economy with energy and environmental aspects provides a clean solution (Kothari et al. [2010](#page-8-34), [2012](#page-8-13); Panwar et al. [2012\)](#page-9-22). These solutions are reviewed in the available literature and given in highlights below:

- Waste material generated by the combustion of hydrogen is water.
- It helps in the eradication of greenhouse gases.
- Elimination of fossil fuel pollution.
- Elimination of economy dependency.
- Biohydrogen production routes are commonly done at ambient temperature and pressure, therefore less energy is used in bioprocess routes.
- This is an ecofriendly method of bioenergy production and use of a renewable source of energy makes it significant because it is inexhaustible.

## **6 Conclusions**

Technologies related to algae cultivation and bioprocess routes for biohydrogen production are commercially viable and give us a positive source of energy for our society. It is an integrated approach through which one can produce biohydrogen as energy, and at the same time it can also be used for wastewater treatment. Use of algal-based biohydrogen as an energy source is more significant than a conventional source of energy because it does not produce any kind of greenhouse gases and by the combustion of biohydrogen it produces only water vapour which is not harmful to our environment. Hence the economic analysis of biohydrogen production by algae shows that it is a most feasible feedstock for future energy production and in addition to a lack of lignin content and being rich in carbohydrate content make algae a promising feedstock for future energy production.

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