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Review Article

Hydrogen production from phototrophic microorganisms: Reality and perspectives

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ABSTRACT

Hydrogen is a promising alternative to fossil fuel for a source of clean energy due to its high energy content. Some strains of phototrophic microorganisms are known as important object of scientific research and they are being explored to raise biohydrogen ($BioH_2$) yield. $BioH_2$ is still not commonly used in industrial area because of the low biomass yield and valuable down streaming process. This article deals with the methods of the hydrogen production with the help of two large groups of phototrophic microorganisms – microalgae and cyanobacteria. Microalgal hydrogen is environmentally friendly alternative to conventional fossil fuels. Algal biomass has been considered as an attractive raw source for hydrogen production. Genetic modified strains of cyanobacteria are used as a perspective

Abbreviations: ATP, adenosine triphosphate; $BioH_2$, biohydrogen; Chl, chlorophyll; DF, dark fermentation; Fd, ferredoxin; H_2, hydrogen; H_2ase, hydrogenase; MFC, microbial fuel cell; N_2ase, nitrogenase; O_2, oxygen; PBR, photobioreactor; PSI, photosystem I; PSII, photosystem II; RC, reaction center.

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Photosynthesis
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object for obtaining hydrogen. The modern photobioreactors and outdoor air systems have been used to obtain the biomass used for hydrogen production. At present time a variety of immobilization matrices and methods are being examined for their suitability to make immobilized H_2 producers.

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Contents

Introduction

Hydrogen production from phototrophic microorganisms is commercially appealing due to its potential as an alternative, useful and renewable energy source [1–4], and the microbial fuel cell (MFC) technology has been mentioned as an important object in scientific area for the last 10–15 years [5]. In future hydrogen will become a major fuel that will help us solve the local problems connected with ecology. In addition, the consumption of hydrogen for transport has progressed in several fields, and used in the automobile constructions, including aircraft industry [6]. Thus, hydrogen may be considered as a main biofuel of the future [7,8]. The use of solar light for hydrogen production should be an ideal method for sustainable energy production that uses renewable solar energy [9–11]. Therefore, the production of hydrogen via biological route pools as hydrogen and biomass energy leads to the number of benefits such as reduction of CO_2 emission, waste management and replacement of fossil fuels with sustainable biofuels [12,13]. Production of BioH_2 needs light source to regulate physiological and biochemical processes [14].

At present time, about 80% of total initial energy supply and 66% of electricity light generation are based on natural fossil fuels such as oil, coal and gas [15,16]. The burning of natural fuels, such as petroleum and coal produces carbon dioxide that makes greenhouse gases that cause climate change [8]. Moreover we are running short of fossil fuels supply and they are on the brim of being exhausted someday. Nature has stored solar energy in the form of mineral organic compounds or in fossil fuels such as coal, petroleum, and natural gas through millions of years of biological and non-biological processes [17].

Biomass from algae is used to obtain qualitative gases and drugs. In addition, aqueous algal biomass can be obtained from a natural algal blossoming, which is considered as a substrate for hydrogen production [18]. Hydrogen is seen as an amply clean fuel and environmentally friendly [19,20], renewable energy resource and a potential candidate with the highest energy specific gravity. It has many technical, socioeconomic and environmental benefits to its credence among all other known fuels. Furthermore, it is the only recognized fuel that does not make carbon dioxide as a byproduct when it is used in fuel cells for electricity generation [21]. There are several other methods and many operations for hydrogen production such as electrolysis, photolysis or biohydrogen production [3].

The selection of BioH_2 sources is very important step for hydrogen production [22]. The accumulation rate of the

cyanobacterial and microalgal biomass is faster than that of plant biomass. However, the particular type of growing photobioreactor (PBR) is needed to get the high quantity of the algae biomass [23]. Efficient usage of CO_2 gas is the complementary interesting lineament of the strains of algae and cyanobacteria. Algae is clearly recognized by scientific community as the most promising object for biofuel production and other applications, however deep researches conducted by noted scientists are still needed to operate their potential in wide growing algal systems [24].

There are several alternative fuels that can be considered as environmentally useful fuel, which is produced from mixing up the microscopic microorganisms like cyanobacteria and green algae. Algae cells differ from cyanobacteria in that they have cell walls that serve as the defense from environmental factors [25]. Cyanobacteria do not have a cell wall such as cellulose. Cyanobacterial $BioH_2$ is made by light-dependent reactions held by nitrogen enzymes and sometimes it can be held in dark anaerobic conditions by hydrogen enzymes, whereas in the green algal and cyanobacterial hydrogen is generated photosynthetically [26].

The method of removing nutrients from contaminated water is immobilization of algal and cyanobacteria cells in alginate liquids, which is an economical mechanism, and it shows efficient results. Moreover, microalgae and cyanobacteria immobilization is used as an instrument of creation and research of synthetic mutualisms [27]. Most importantly, cell immobilization method is divided into six different types: affinity immobilization, entrapment in the liquid emulsion, capture behind semi-permeable membrane, covalent coupling adsorption, and entrapment that are used in current works [21].

Economical biohydrogen demands high H_2 production at efficiently operating costs and lower capital [28]. The microalgae require specific bioreactors, which make up a large-scale production of $BioH_2$ [3]. The basic factors influencing new bioreactor project development may be tank depth and agitation [29].

Biohydrogen production

One of the most widespread elements in the nature is hydrogen [30]. However, hydrogen molecules accumulate in water and fossil fuels. Approximately 55 million tons of hydrogen are produced each year, whilst the utilization rises up by about 6% each year, and its rise could reach 10%. The production of hydrogen is a subject of the interest of many global industrial companies. Nowadays several global industrial companies have great interest in producing hydrogen to make profit, as 1 kg H_2 costs about 1,25 USD. So hydrogen can be affordable by using solar energy or by water electrolysis where cheap electricity is generated. In terms of energy, this manufacturing continues from the equation that 1 kg of hydrogen is the equivalent of energy of 3,8 L of gasoline [8,31,32].

Mechanisms of biohydrogen production

Biophotolysis is one of the promising concepts for clean hydrogen manufacture among various biological protocols through the facts that eventually water is the sole necessary substrate and hydrogen obtaining is not related to metabolic carbon pathways [33](Fig. 1).

Currently there are four methods of biological hydrogen production and appropriate methods are utilized for each type of microorganisms. In any case, there are some advantages and disadvantages (Table 1).

Direct biophotolysis

Direct biophotolysis is similar to the photosynthesis process, which occurs in plants and algae cells [34]. This method is a biological and chemical process, which can produce $BioH_2$ straight from water using microalgae photosynthesis system in order to transform solar energy into chemical energy in form of molecular hydrogen, the reaction is present below [35–37]:

$$2H_2O + \text{solar energy} \rightarrow 2H_2 + O_2$$

The green algae producing biohydrogen under anaerobic conditions, for example, *Chlamydomonas reinhardtii*, can either generate H_2 or use H_2 as an electron donor [38]. Decreased ferredoxin (Fd) acts as the electron donor in the process called biophotolysis of water for hydrogen generation by hydrogenase (H_2ase) enzyme. By the end of this process, hydrogen gas is converted from water and fuel protons [39].

The H_2ase enzyme receives the electrons from Fd to produce hydrogen as shown in Fig. 2.

There are two photosynthesis processes: photosystem I (PSI) producing a reductant for CO_2 reduction and photosystem II (PSII) splitting water and evolving oxygen molecules. Two photons from water can be yielded during the biophotolysis operation, either hydrogen formation with the presence of H_2ase or CO_2 reduction by PSI. In all green plants, due to the lack of H_2ase, only CO_2 reduction can take place. During this operation, water electrons are generated when PSII uptakes light energy from solar system. Then the electrons are moved to the Fd component using the solar energy [40].

As H_2ase enzyme is sensitive to oxygen molecules, it is necessary to maintain the oxygen content at 0,1% level so that hydrogen production can be maintained. Green algae *Chlamydomonas reinhardtii* showed similar operation that can exhaust oxygen molecules during the oxidative breathing. However, due to the enormous amount of substrate being respired and consumed during this process it shows low efficiency. Lately it was established that mutants clipped from microalgae and cyanobacteria have good O_2 production ability and therefore higher hydrogen production [43].

Cyanobacteria and microalgae can utilize light to carry out photosynthesis as they have chlorophyll (Chl) and the photosynthetic systems: PSII and PSI, respectively [21].

Indirect photolysis

Microalgae and cyanobacteria produce hydrogen from stored glycogen and starch in case of indirect biophotolysis. This process has two steps. Firstly, the synthesis of carbohydrates goes under the light. Secondly, the hydrogen is made from carbohydrates through photofermentation [23].

Hydrogen production via indirect biophotolysis by algae could be carried out if photon conversion could be improved

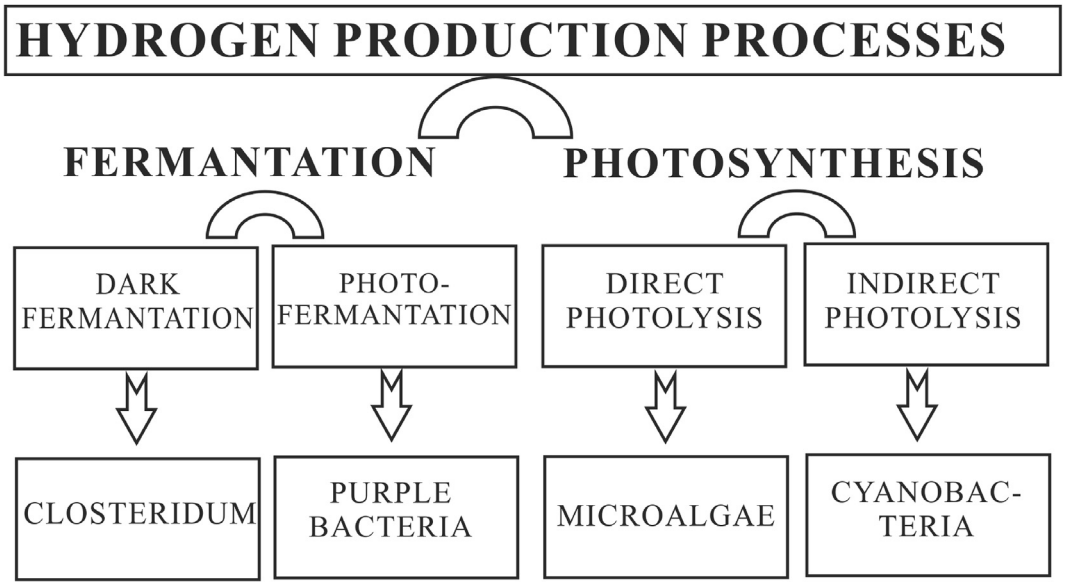

Fig. 1 – Hydrogen production methods. Modified from Ref. [21].

for large-scale applications. The improvement of photosynthesis efficiency is too difficult to achieve for conventional crop plants [36] (Fig. 3).

The fixed carbon source during dark periods of cells growth is the main advantage of the method of hydrogen generation by green algae. These green algae can also be portrayed as dark respiration assisted dark fermentation (DF) [39,40].

The production of hydrogen from indirect biophotolysis with cyanobacteria can be observed via the following reactions:

$C_6H_{12}O_{16} + 6H_2O \rightarrow 6CO_2 + 12H_2$

Dark fermentation

Dark fermentative biohydrogen production provides a cost-effective and environmentally friendly process [45]. This is

Table 1 – Hydrogen production comparison by direct and indirect biophotolysis. Modified from Refs. [3,21,35,41,42].

Process	Formula of processes	Advantages	Disadvantages
Direct biophotolysis	$2H_2O + Fd_{(ox)}+light \rightarrow O_2+4H + Fd_{(red)}$ ($4e^-$); $4H^+ + Fd_{(red)}$ ($4e^-$) $\rightarrow 2H_2+Fd_{(ox)}$	High theoretical efficiency There is no requirement of adding the substrate nutrients Water is the substrate and solar energy is the Source of energy It is not necessary to produce ATP	Oxygen evolved in vicinity of oxygen-Sensitive hydrogenase Sensitivity of hydrogenases enzymes to O_2 Inhibition by O_2 Low light conversion efficiencies
Indirect biophotolysis	$N_2+8H^+ + Fd$ (red) (8e_)+16ATP $\rightarrow$ $2NH_3+H_2+Fd_{(ox)}+16ADP+16P_i$	Can produce H_2 from H_2O Simple mechanism and inexpensive Microorganisms grow in environments containing simple minerals	High energy costs Need lighting Need for ATP High energy costs
Photo fermentation	$CH_3COOH+2H_2O$ + light $\rightarrow 4H_2+2CO_2$ $N_2+8H^+ + 8e\_+16ATP \rightarrow 2NH_3+H_2+16ADP+16P_i$	It has no activity for O_2 evolution Ability to use a long light spectrum Ability to consume organic substrates derived from waste Ability to use a wide spectrum of light	Low conversion efficiency of solar energy Requires anaerobic photobioreactors with Large area exposed to sunlight Light is necessary
Dark fermentation	Pyruvate + CoA $\rightarrow$ acetyl-CoA + formate $C_6H_{12}O_6+6H_2O \rightarrow 12H_2+6CO_2$	Requires no illumination Does not depend on O_2 (anaerobic process) It produces by-products with organic acids having commercial value Wide variety of carbon sources as substrate	Produces biogas containing H_2 and CO_2, and also CH_4, H_2S and CO; The residue of the fermentation should be treated to prevent environmental pollution.

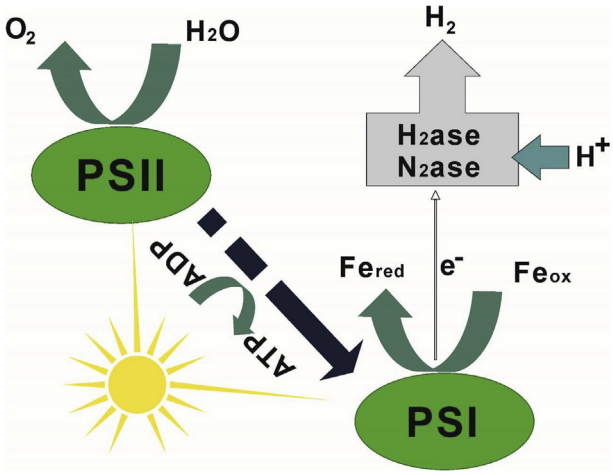

Fig. 2 – Direct biophotolysis of green algae or cyanobacteria. Modified from Refs. [35,40].

an aggregate process revealed by bacterial diverse groups, implying a series of biochemical reactions using several steps similar to anaerobic transition. Dark fermentation is used primarily with anaerobic bacteria, although some algae are also used, on carbohydrate rich substrates grown without the need of light energy [46] (Fig. 4).

Biological DF for hydrogen gas production is very appealing because it becomes renewable and carbon neutral with the help of this process [47–49]. However, different toxic or overwhelming compounds can significantly limit sustainable process and widespread acceptance of the biotechnology area, and these days, the DF process has not been widely spread due to the low productivity of H_2 production [50,51].

The microbe metabolism in DF system consists of several chemical components such as excess substrate, micronutrients, macronutrients, metal ions, high temperature, acidic pH, organic acids, rival microbes, and substrate toxic substances. The possible prohibiting compounds and suppression mechanisms are introduced and engineering prospects on the control of braking are provided [40,52].

Light independent hydrogen production by dark-fermentation ordinarily operates at a high rate. DF hydrogen is produced from various wastes as the carbon source and ends off predominantly in the obtaining of acetic and butyric acid along with other volatile fatty acids together with hydrogen as [42,52,53]:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 2H_2 + 6CO_2$$

Photofermentation

Photofermentation is the fermentative transformation of organic substrate into hydrogen demonstrated by a varied group of photosynthetic microorganisms. Photofermentation is represented as one of the most efficient modes without high risk for $BioH_2$ production [54,55]. In this paper, extensive modeling and simulator of bio-hydrogen production are represented via photofermentation method [56,57]. It is recognized that electrons are clipped from water through photochemical oxidation by PSII and are moved to the [Fe]-hydrogenase leading to the photosynthetic hydrogen production in direct biophotolysis process [36] (see Fig. 5).

The main process of microbial hydrogen production is defined by the pyruvate anaerobic metabolism and degradation of pyruvate is catalyzed by one of two enzyme systems shown in Eqs.:

$$\text{Pyruvate} + \text{CoA} \rightarrow \text{acetyl} - \text{CoA} + \text{formate}$$

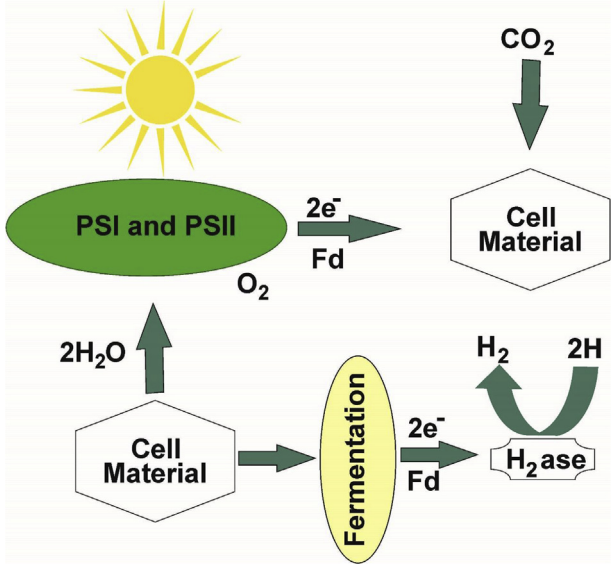

Fig. 3 – Indirect biophotolysis of hydrogen production. Modified from Refs. [12,40,44].

Biomass for biohydrogen production

Today it is definitely recognized that $BioH_2$ can be obtained from biomass of renewable resources without using energy of fossil fuels [58]. The biomass is a renewable energy source that is derived from living or recently living organisms [59]. Cyanobacterial, algal, and plant biomass is produced due to atmospheric or water-dissolved CO_2 fixation during the process of photosynthesis [49,60]. Biomass has been considered as a major power source for hydrogen supplying, and it can be productively converted to energy via a series of biological and chemical methods.

A promising source of $BioH_2$ is conversion of algal biomass, which is abundant, clean and renewable. Unlike other well-developed biofuels such as bioethanol and biodiesel [44], the production of hydrogen from algal biomass is still in the early stage of development. There are various technologies for algal hydrogen production, and several laboratories and scale systems that have demonstrated a good potential for full-scale implementation [21,44]. Many algal species show potential to produce hydrogen under suitable conditions. Cyanobacterial and microalgal species that inhabit in fresh or saline water are able to transform carbon dioxide, water and sunlight into biomass through photosynthesis. The growth rate and oil content of microalgae are faster and higher than that of macroalgae. Besides, they have less complex structures than that of macroalgae cells, and

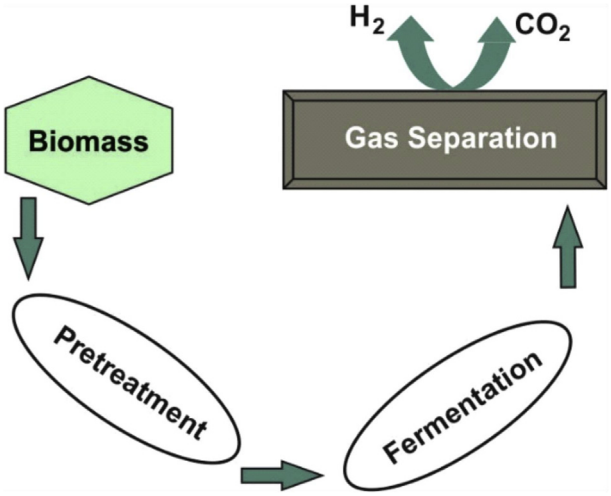

Fig. 4 – Hydrogen production by dark fermentation. Modified from Refs. [40,44].

the growing rate and oil of microalgae are higher than that of macroalgae. Moreover, they have less complex structures than that of macroalgae. Different forms of biofuels can be obtained from microalgal biomass through different pathways as shown in Fig. 6.

So far as the structure of algal biomass changes quickly into its species, the studies are summed up and listed in accordance with the algae and cyanobacteria strains. It seems that microalgae components are more useful substrate than that of microalgae biomass. The microalgae biomass has simpler structure than that of microalgae, thus, simpler pretreatments are required. Besides, microalgae can be cultivated in different conditions and more easily obtained [62].

Strains and growth conditions

These days some green algae and cyanobacterial species are still being studied the genetic researches show that some of them can produce biohydrogen under convenient condition.

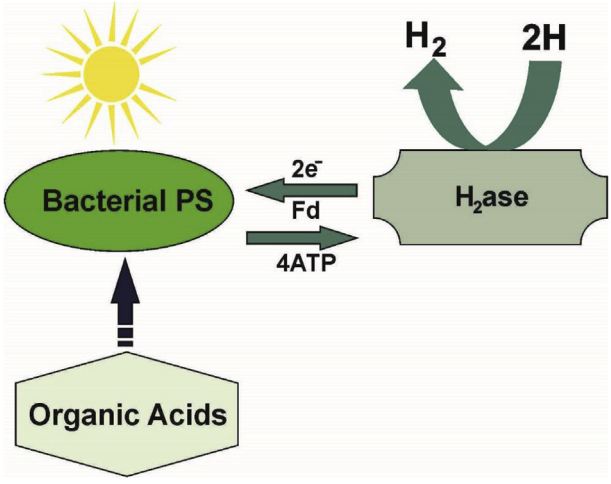

Fig. 5 – Photofermentation schematics. Modified from Refs. [35,40,42].

In addition, most studies for improving the hydrogen production from microorganisms are based on genetic improvement of the strains [63].

Table 2 explains numerous microorganisms involved in hydrogen production. Lately progresses in bioenergy have been really developing thanks to ongoing researches with phototrophic organisms.

Some types of microorganisms can produce $BioH_2$ under suitable conditions [72]. Microalgae, cyanobacteria (heterocyst-forming cyanobacteria, filamentous non-heterocystous cyanobacteria, and unicellular cyanobacteria) and purple bacteria can produce $BioH_2$ from biomass.

Microalgae and cyanobacterial species produce the $BioH_2$ with the help of H_2ase and nitrogenase enzymes. Cyanobacteria is prokaryote that has oxygenic photosynthetic activity like eukaryotic green algae. They have only Chl *a*, Chl *b* is absent, and the major antennas are pigment-protein complexes called phycobilins [73].

Purple bacteria composes a group of Gram-negative pink to purplish-brown bacteria that comprises type RCII, which cannot use H_2O as the e^- donor on the contrary organisms like cyanobacteria, algae and land plants contain PSII (see Table 2).

Culture conditions

The process of hydrogen obtaining occurs under the suitable condition of several factors such as pH, nutrients, temperature, and substrate concentration. Hi-tech photobioreactors used in manufacturing of the $BioH_2$ provide these factors. Culture condition is the main factor to obtain productive $BioH_2$ using the algal and cyanobacterial species used importantly in scientific areas by researchers. Lately several interesting articles have been published that microbiological objects were cultivated in the different cultural conditions and the variations influenced by $BioH_2$ yields [66]. Culture condition is the most impacted factor for BioH2 production. In fermentation process, nitrogen, phosphate and other inorganic trace minerals are necessary supplements for hydrogen production. Lin et al. studied influence of trace metals on *C. pasteurianum* [74], and according to Yokoi et al. the organic nitrogen seems to be more favorable for hydrogen evolution compared with inorganic one [75].

pH effect

Various factors affect the hydrogen production, and one of them is pH that has strong effect on hydrogen production. According to the latest studies, the optimum pH value was between 5 and 7 [76]. pH effect is one of the major chemical parameter to $BioH_2$ production that is associated with any chemical reactions. It governs the efficiency of enzymatic machinery of the microorganisms and it plays a magic role in oxidation-reduction potential of the cells [77,78].

Indicator of pH is one of the magic factors of the hydrogen production and it plays considerable role in the metabolism of cells [79]. Range of pH varies between 5 and 6 and it is the ideal ranges for $BioH_2$ producing, but previous researches show that optimal pH indicator is 5,2–5,4 [71]. In any case immobilized cyanobacterium *Synechocystis* sp. PCC 6803 produces supreme hydrogen, with an initial pH of 7,4 [66]. The study of Troshina et al. shows indirect biophotolysis with cyanobacterium *Gloeocapsa alpicola* [80]. The research shows

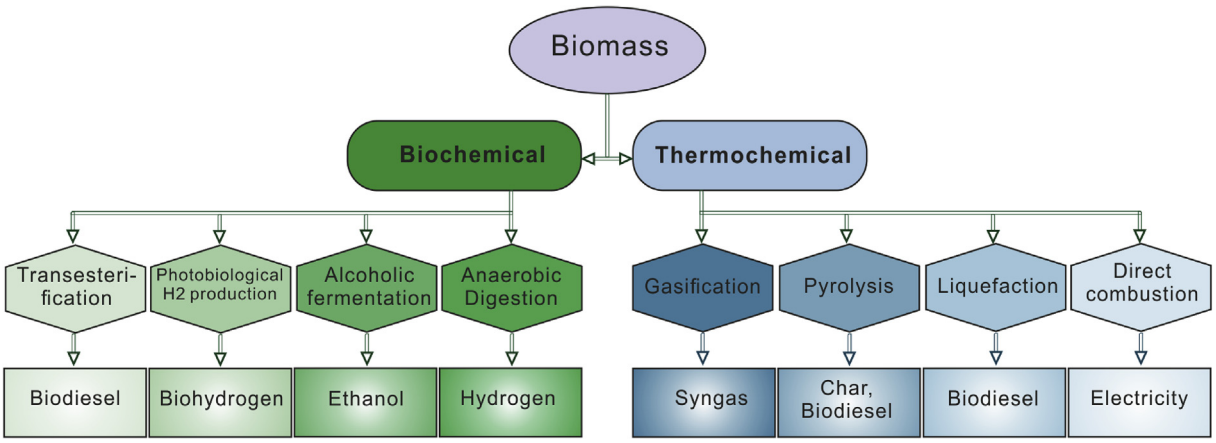

Fig. 6 – Major pathways for conversion of biomass to biofuel and derived products. Modified from Ref. [61].

that $BioH_2$ is optimized by preserving the pH value between 6,8 and 8,3. Fang et al. showed the effect of pH on biohydrogen production from glucose in a CSTR over the range of 4,0–7,0 and proved the optimum hydrogen yield occurring at a pH of 5,5 [81].

Temperature effect

The suitable temperature is main parameter that determines the optimum metabolic pathways of hydrogen synthesis as well as the inhibition of the hydrogen consuming processes [82]. Anaerobic processes are influenced strongly by temperature and there is considerable disagreement about the suitable and unsuitable temperature for biological hydrogen production. One of the basic factors of H_2 production is temperature, and it plays an important role in increasing its ability to produce hydrogen. Similar to the influence of pH, temperature also controls metabolism through mediating the enzymatic reactions. Every enzyme has an optimal temperature range at which high activity is observed [77].

Minnan et al. studied the hydrogen production capability of a culture under varying temperatures from 25 °C to 35–36 °C and they improved the hydrogen production activity [83,84].

Cell immobilization

Algae cell immobilization with alginate hydrogels is a soft operation, and a transparent and permeable material is produced permitting a rise of cell thickness. It can also help to protect from natural harmful factors and contamination occurs in the outside area [66]. Besides, immobilization methods are easier to scale-up and demand little area for PBRs construction compared to the use of algae cultures [85]. *Synechocystis* sp. PCC 6803 strain was studied by Touloupakiset et al. and showed successful immobilization of vital cells in the calcium alginate gels. There were only two steps to get high hydrogen production in the immobilized alginate gels [66]. Li et al. studied the effect of immobilization on growth and organics removal of chlorella by fracturing flow back fluids treatment, and this research proved the indication that immobilization could improve growth and organics removal of chlorella for processing fracturing blowback fluids [86]. Rouillon et al. [87] showed good examples of immobilization techniques as the co-reticulation in an albumin-glutaraldehyde crosslinked matrix [88].

Table 2 – Hydrogen producing microorganisms.

Microorganisms	Type of strains	Mode of Operation	References
Calothrix sp. 336/3	wild-type	Anaerobic fermentation	[16]
Anabaena sp. PCC 7120	Δhup Lmutant	Photo fermentation	
Chlamydomonas reinhardtii CC-124	sulfur-deprived	Anaerobic fermentation	
Chlamydomonas reinhardtii Stm6	wild type	Photo fermentation	
Scenedesmus obliquus	wild type	Photo fermentation	[64]
Chlorella vulgaris	wild type	Dark fermentation	[65]
Synechocystis sp. PCC 6803	immobilized	Anaerobic fermentation	[66]
Spirulina platensis	wild-type	Anaerobic fermentation	[26]
Chlamydomonas MGA 161	wild-type	Dark-fermentation	[67]
Chlamydomonas reinhardtii	sulfur-deprived	Dark fermentation	
Anabaena sp.	nitrogen deprivation	Anaerobic fermentation	[68]
Anabaena siamensis TISIR 8012	wild type	Anaerobicfermentation	[69]
NostocPCC 7120,	hup Wmutant	Anaerobic fermentation	[70]
Clostridium butyricumCWBI10	strictly anaerobic strain	Anaerobic fermentation	[71]
Clostridium pasteurianum (MTCC116)	wild type	Dark fermentation	[13]

Photobioreactors

Main stages of biomass development from phototrophic organisms as an investigation to the competitive product is the cheap price of PBRs and good cultivation systems [32,89,90].

It is very important to consider some practical aspects of microbial H_2 production processes, specifically the design and operation of the bioreactors, which must both contain the microbial culture and capture the H_2 as it is generated. The entire bioreactor system must be considered, including front H_2 production.

Economic hydrogen production requires high H_2 production efficiencies at low capital and operating costs. Large-scale production of $BioH_2$ mediated by microalgae requires specific bioreactors [3]. The old methods of the $BioH_2$ production are represented with high-price, and it is necessary to spend enormous amount of costs on achieving certain results. Thus, hi-tech PBRs, which correspond to all demand, are very necessary. There are different types of PBRs and they differ from each other appropriately in which places they are used for. The design of the PBRs is changed to corresponding strains characteristics [21,91] (see Table 3).

It took several decades to make PBRs that are capable to produce $BioH_2$ with algae and cyanobacterial strains, all PBRs abundant in entrance light that is obtained from sunlight. Continuous stirred tank reactor, fixed-bed bioreactor, membrane bioreactor, multi-stage bioreactors, hybrid bioreactors, flat panel photobioreactors are widely used PBRs [92] (see Fig. 7).

The major obstacles of creating the PBRs are the light penetration into completely deeper sides of liquids and attendance of the characteristic of observed algae species used for $BioH_2$ in the PBRs. The light penetration into the deepest sides of PBRs is a big problem in several hydrogen research areas. In this case, obtaining $BioH_2$ by sunlight is not enough to produce H_2. Mixing, the size of medium area, temperature controller and gas exchange are very important things for producing the biohydrogen in PBRs. All hydrogen PBRs are divided into three types of PBRs: vertical column reactor, tubular type and flat panel PBRs. Other types of PBRs are distributed from these three main photobioreactors [3,71].

Main advantages of vertical PBRs are fast circulation of water and sufficient entry of light into the deepest area of suspension. In addition, water jacket is covered to maintain vital of these PBR, and temperature system is controlled by circulated water. Clear vertical columns filled with medium are aerated from the bottom [93]. It is quite easy to maintain the conditions of growth in such PBRs [23].

Algae and cyanobacterial cells in the tubular type PBRs are moved by air bridge device and maintenance of CO_2 occurs with air system. Each tube of the equipment can be taken out easier and cleaned fully clear. The flat plate PBR system is controlled by a created control system that can monitor and control pH, temperature, optical density, and amount of produced hydrogen. However, this kind of PBRs is used only in laboratories and small size factories. Advantages: the big area of illumination and as a result, maximal photosynthetic activity of organisms. Here, the small layer of medium flows via

Table 3 – Comparison of advantages and disadvantages of different culture systems. Adapted from Refs. [3,23].

PBRs or culture system	Advantages	Disadvantages
Open-air systems	Economical Easy to clean up Easy maintenance Utilization of non-agricultural land Low energy inputs	Little control of culture conditions Poor mixing, light and CO_2 utilization Difficult to grow algal cultures for long time Only one culture can be used Problem with fast growing High contamination
Tubular photobioreactors	Relatively cheap Large illumination surface area Suitable for outdoor cultures Good biomass productivities Can monitor all the indicators	Some degree of wall growth Require large land space Photo inhibition
Flat photobioreactors	Relatively cheap Easy to clean up Large illumination surface area Low power consumption Good productivities of biomass Good light path	Difficult scale-up Difficult temperature control Biomass of culture sticks on the wall Hydrodynamic stress to some algal strains
Column photobioreactors	Low energy consumption Readily tempered High mass transfer Good mixing Low shear stress High photosynthetic efficiency	Small illumination surface area Sophisticated construction materials Shear stress to algal cultures Expensive
Stirred tank photobioreactors	Good mixing Good productivities of biomass Large illumination surface area Easy to clean up Easy maintenance Controlled with computer system High photosynthetic efficiency. Can monitor all the indicators	Small illumination surface area Used only for laboratory scales

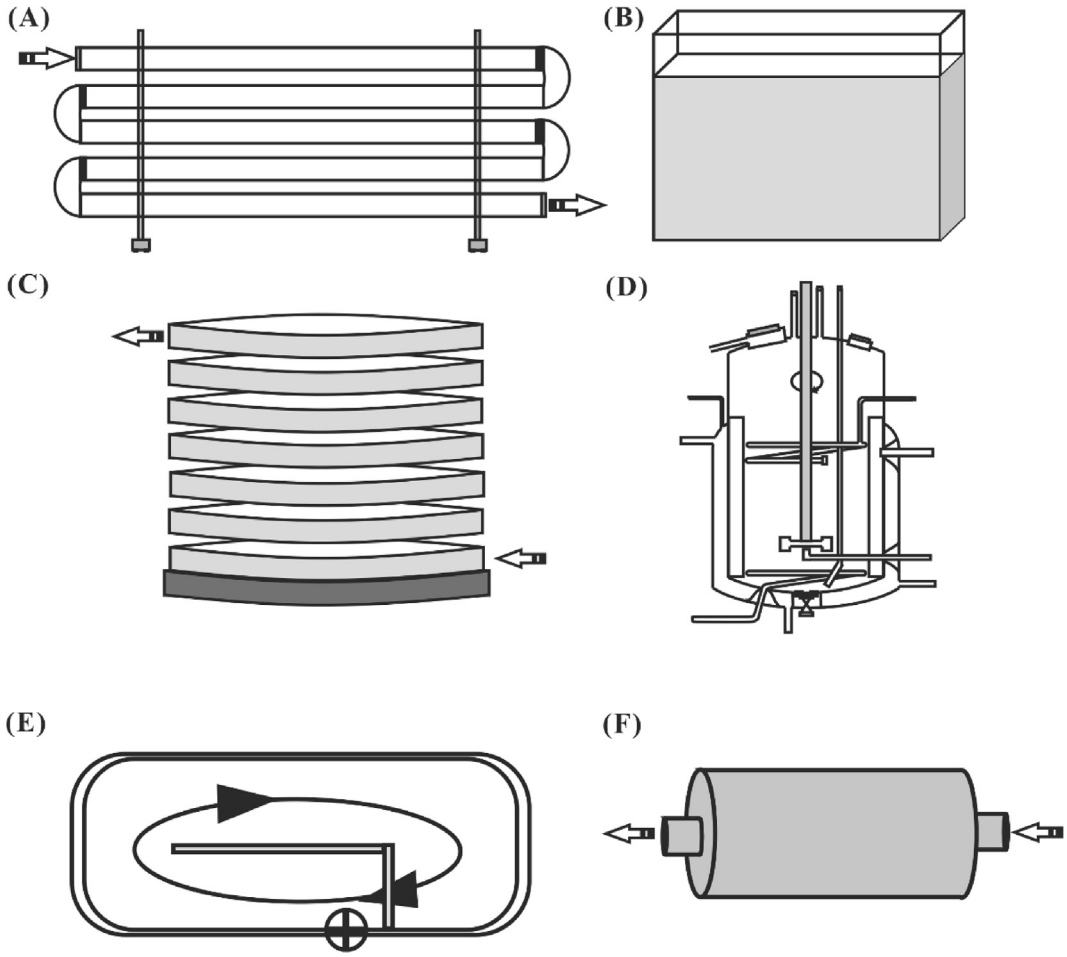

Fig. 7 – Schematic representation of the different PBRs for biomass and BioH$_2$ production. Fence tubular (A), vertical flat panel (B), helical tubular (C), stirred tank (D), open-air systems (E), column PBRs (F). Modified from Ref. [3].

the flat clear exterior [23]. In addition, high photosynthetic efficiencies and effectual control of gas tension can be reached in flat-plate PBRs and it is considered more economical compared to other type of bioreactors. Anyway, privation rises to maintain the particular culture temperature and appropriate mixing system during hydrogen production [3].

The enzymes of hydrogen production

Hydrogen catalyzed by biological methods occurs by the two enzymes as H_2ase and N_2ase [94].

Hydrogenases

[FeFe]-hydrogenases catalyze the uptake and release of molecular hydrogen at a unique iron-sulfur cofactor. The absence of electrochemical over potential in the H_2 release reaction makes [FeFe]-hydrogenases a prime example of efficient biocatalysis [39], and H_2ases are also sensitive to oxygen like N_2ases [95].

Life of H_2ase enzymes is short in low oxygen concentration. Only a modified H_2ase with high hydrogen producing sensitivity will allow for the commercial hydrogen production [95].

H_2ases are enzymes that catalyze the production and consumption of hydrogen. H_2ases in the microorganism cells were detected as early as 1930s, but their molecular structures were only known about twenty years ago [96]. Green microalgae and cyanobacterial H_2ases are varied group of enzymes. There are three different groups of H_2ase, they are [FeFe]-hydrogenase, [NiFe]-hydrogenase, and [Fe]-hydrogenase [3,39,97,98].

H_2ase catalyzes the following reaction [73,99]:

$$2H^+ + 2e^- \leftrightarrow H_2$$

Either the [FeFe]-hydrogenase can catalyze the production of H_2 or proton freed from hydrogen. It is encrypted by *hydA* gene in the nucleus, also localized inside the chloroplasts after time enzyme maturation [73,100]. Found only in eukaryotes the [FeFe]-hydrogenases are nearly related to a protein. These days this protein is being implicated as having a significant role in cytoplasmic sulfur cluster biosynthesis or repair, and shows to bear a resemblance to [FeFe]-hydrogenase that lacks a 2Fe subcluster. Homology models of Narf et al. offer the existence of an open cavity adjoining to the [4Fe–4S] cubane that could accommodate the 2Fe subcluster [80,97,100].

The [NiFe]-hydrogenase generates the enormous amount of H_2ases. Cyanobacteria contains [NiFe]-hydrogenases necessary to the bidirectional processes and absorption H_2ase enzymes [73]. The [NiFe]-hydrogenase consists of a large (~34 kDa) and a small (~64 kDa) subunit. The [NiFe] acting area, which is supposed to connect and break up the H_2 molecule, is mounted in the center of the large subunit, which also is maintained with a hydrogen canals, through which gaseous H_2 passes into the active center [100]. The small subunit comprises 3 [FeS] clusters. The electrons are moved from the [NiFe] area via the [FeS] clusters to the distal [FeS] cluster where they are transmitted to the electron acceptor [96,99].

The new known enzyme [Fe] homodimer was found in some methanogenic archaea, and it still is being studied. The [Fe]-hydrogenase is suitable in both structure and activity. The acting area of [Fe]-hydrogenase comprises only one Fe area accommodated by one cysteine sulfur, two *cis*-oriented CO, and a bidentate guanylyl pyridinol ligand [96,97,99].

Nitrogenase

Nitrogenase (N_2ase) plays a crucial role in global nitrogen cycle on the planet, and supplied in a group of microorganisms called diazotrophs, N_2ase is capable of catalyzing the reduction of atmospheric dinitrogen (N_2) into bioavailable ammonia (NH_3) in a nucleotide-dependent process. The capacity of N_2ase to interrupt the inert N≡N ternary relations under encircling conditions not only enables the producing of an ample supply of nitrogen with a biological operation, but also makes N_2ase a charming subject from the prospective of chemical energy, and N_2ase has remained a topic of strong research for last ten years [101].

N_2ase was found in some microorganisms including bacteria and archaeal life domains, and it catalyzes whole biological-chemical N_2 fixation, to be more precise 60% of the fixed N is up taken from N_2 into the global biogeochemical nitrogen cycle [102].

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i$$

Three homologous N_2ases, namely, the molybdenum (Mo), vanadium (V) and iron (Fe)-only N_2ases, have been identified [3,97,101–104].

The last studies show Mo-nitrogenase, which has the ability to reduce a number of small molecules with dual and ternary bonds [105]. The V-nitrogenase is also capable to reduce CO_2 to CH_4, C_2 and C_3 hydrocarbons. In these latter days, both in vivo and in vitro studies of the Fe-nitrogenase indicate that this enzyme shows the highest reduction of CO2 to CH_4 of the three N_2ases [105–107].

Conclusion

The advancements in research and development show that biohydrogen production from algal biofuel can be used as a clean energy for the future. BioH_2 as a renewable energy will give significant economical complicities, and BioH_2 has been used in several industrial fields as an energy source for several decades. To change modern success in BioH_2 studies to the next point of achievement, the available scientific and well-modified technological equipment should be invented. In this case, existent problems of BioH_2 production can be solved by genetic researches.

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