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Photobiological Hydrogen Production

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11.1 Introduction

11.1.1 Motivation

Industrial and developing nations are facing an unprecedented combination of economic, environmental, and political challenges. First, they face the formidable challenge to meet ever-expanding energy needs without further impacting the climate and the environment. Second, the continued population growth in developing countries and the emergence of a global economy are creating unprecedented stress on the resources of the Earth. Emerging countries are claiming access to the same standard of living as industrial nations, resulting in large needs for energy sources, fast and reliable transportation systems, and industrial equipment. From the standpoint of international security, energy issues include the potential for conflict over access to remaining supplies of inexpensive fossil fuels, which are often concentrated in politically unstable regions.

Currently, fossil fuels supply more than 81% of the world's energy needs estimated at about 137 PWh/year (1 PW = 10^{15} W) or 493 EJ/year (1 EJ = 10^{18} J) [1]. Oil meets more than 92% of the world transportation energy needs [1]. However, its production is expected to peak between 2000 and 2050 after which its production will enter a terminal decline [2–5]. Simultaneously, the world energy consumption is expected to grow by 50% between 2005 and 2030 [5]. Thus, the end of easily accessible and inexpensive oil is approaching.

Moreover, intensive use of fossil fuels increases concentrations of carbon dioxide (CO₂) in the atmosphere, and their contribution to world climate changes is a topic of worldwide concerns [6]. For example, 71.4% of the electricity consumed in the United States is generated from fossil fuel, especially coal [7], making the United States responsible for about 21% of the world CO₂ emission in 2005 [8]. It is predicted that atmospheric CO₂ levels above 450 ppm will have severe impacts on sea levels, global climate patterns, and survival of many species and organisms [6].

Consequently, the growing energy needs will necessitate much greater reliance on a combination of fossil fuel-free energy sources and on new technologies for capturing and converting CO₂. Hydrogen offers a valuable alternative as an energy carrier for stationary and mobile power generation. It has much larger gravimetric energy content than fossil fuels [9]. In addition, its combustion with oxygen does not produce CO₂ but simply water vapor.

11.1.2 Current Hydrogen Production and Usage

Hydrogen is not a fuel but an energy carrier; as such, it is as clean as the production method. Worldwide, 48% of hydrogen is currently produced by steam reforming, partial oxidation, or autothermal reforming of natural gas, 30% from petroleum refining, and 18% from coal gasification [10]. However, all these thermochemical processes require fossil fuel and produce CO₂. The remaining 4% of hydrogen is produced via water electrolysis [11]. This technology used to be the most common process for hydrogen production, but it now represents a small fraction of the world's production. It is used mainly for producing high-purity hydrogen. Thus, current H₂ production fails to address outstanding issues related to depleting oil reserves, energy security, and global warming.

In 2005, 45% of the US hydrogen production was used in oil refineries and 38% in the ammonia industry [10]. It is also used in rocket propulsion applications [12]. In the future, hydrogen could be used in different energy conversion systems such as (1) internal combustion engines for surface transportation [13], (2) high-pressure H₂/O₂ steam generators for power generation [14–16], and (3) proton exchange membrane (PEM) fuel cells [17]. The demand for hydrogen is expected to increase significantly in the next decades as these technologies become more affordable and reliable.

11.1.3 Sustainable Hydrogen Production Technologies

Several technologies offer the advantage of producing hydrogen in a sustainable manner without either relying on fossil fuels or producing carbon dioxide. They can be listed as follows:

1. *Water electrolysis* can be performed using electricity generated in a sustainable manner, by photovoltaic solar cells, for example. Both photovoltaics and electrolyzers are very expensive and cost remains the major challenge of this technology. Typical efficiency of such a system is less than 8% [10]. Alternatively, wind electrolysis uses electricity generated from wind energy to carry out water electrolysis.
2. *Photoelectrochemical* hydrogen production uses catalysts that absorb solar radiation and generate large current densities on the order of 10–30 mA/cm² [18]. This enables the water-splitting reactions to take place at a significantly lower voltage than conventional electrolysis. Research results for the development of photoelectrochemical water-splitting systems have shown a solar-to-hydrogen efficiency of 12.4% for the lower heating value (LHV) of hydrogen using concentrated sunlight [10]. Catalyst stability and large band gap are the current challenges to be overcome in this technology [10].

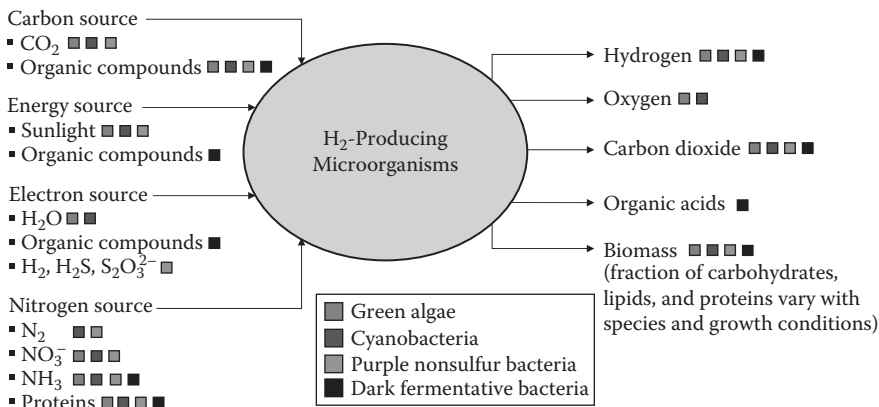


FIGURE 11.1
Schematic of the microalgae/cyanobacteria consuming CO₂ and producing H₂.

- Solar-driven thermochemical hydrogen production* uses solar collectors to concentrate thermal radiation from the sun to produce H₂ from various high-temperature thermochemical cycles. This process is an active area of research [19].
- Biological hydrogen production* by cultivation of microorganisms offers a clean and sustainable alternative to thermochemical or electrolytic hydrogen production technologies with the possible advantage of CO₂ capture. Under certain physiological conditions, some microorganisms can produce H₂. Biological hydrogen production offers several advantages over currently used technologies: (a) it occurs under mild temperatures and pressures; (b) the reaction specificity is typically higher than that of inorganic catalysts; (c) it is tolerant to sulfur gases, thus reducing the cleanup cost of the gas prior to use; and (d) a diverse array of raw materials can serve as feedstock. The major drawbacks of this technology lie in its currently low efficiency and the fact that it requires large surface area and amounts of water.

Like all living organisms, algae or bacteria need (1) an electron source, (2) an energy source, (3) a carbon source, and (4) a nitrogen source to produce biomass. The latter can further be used as a value-added by-product or as animal feed, fertilizer, and raw material for biofuel production [20]. There are four different hydrogen-producing microorganisms, namely, (1) green algae, (2) cyanobacteria, (3) purple nonsulfur bacteria, and (4) dark fermentative bacteria. Figure 11.1 schematically shows these microorganisms as *black boxes* with the different combinations of input and output parameters. More details about photosynthesis and hydrogen production pathways are provided in the next sections.

11.1.4 Solar Radiation

Solar radiation is the most abundant and renewable energy source on Earth. Through photosynthesis, it has provided human beings with food, fuel, heat, and even fossil fuels generated as a result of geologically deposited biomass chemical transformation of over billions of years under extreme pressures and temperatures [9].

Seen from the Earth, the sun is approximately a disk of radius 6.96×10^8 m at an average distance of 1.496×10^{11} m and viewed with a solid angle of 6.8×10^{-5} sr. The sun is often approximated as a blackbody at 5800 K emitting according to Planck's law [21].

The solar constant is defined as the total energy incident per unit surface area at the outer surface of Earth's atmosphere and oriented perpendicular to the sun's rays. It is estimated to be 1367 W/m^2 [21,22].

As the solar radiation travels through the Earth's atmosphere, it is (1) absorbed by atmospheric gases (e.g., CO_2 , H_2O) and (2) scattered by gas molecules and larger aerosol particles, ice crystals, or water droplets. Once it reaches the Earth's surface, most of the ultraviolet (UV) component has been absorbed by oxygen and ozone molecules. Attenuation in the visible is mainly due to Rayleigh scattering by small gas molecules such as oxygen (O_2) and water vapor (H_2O). In the near-infrared (NIR) part of the spectrum, the main absorber is water vapor with contributions from carbon dioxide. Other minor absorbers include nitrous oxide (N_2O), carbon monoxide (CO), and methane (CH_4) [21].

The solar radiation reaching the Earth's atmosphere consists of 6.4% of UV radiation ($\lambda < 380 \text{ nm}$), 48% of visible light ($380 \leq \lambda \leq 780 \text{ nm}$), and 45.6% of infrared radiation ($\lambda > 780 \text{ nm}$) [22]. Overall, the sun delivers $1.73 \times 10^{17} \text{ W}$ or $6.38 \times 10^{19} \text{ Wh/year}$ on the surface of the atmosphere [22]. This should be compared with the 2006 world energy consumption rate of $1.56 \times 10^{13} \text{ W}$ or an annual total energy of $1.37 \times 10^{17} \text{ Wh/year}$ [1].

The American Society for Testing and Materials (ASTM) G173-03 standard [23] provides reference terrestrial solar spectral irradiance distributions for wavelength from 280 to 4000 nm averaged over 1 year and over the 48 contiguous states of the continental United States under atmospheric conditions corresponding to the US standard atmosphere [24]. Figure 11.2 shows

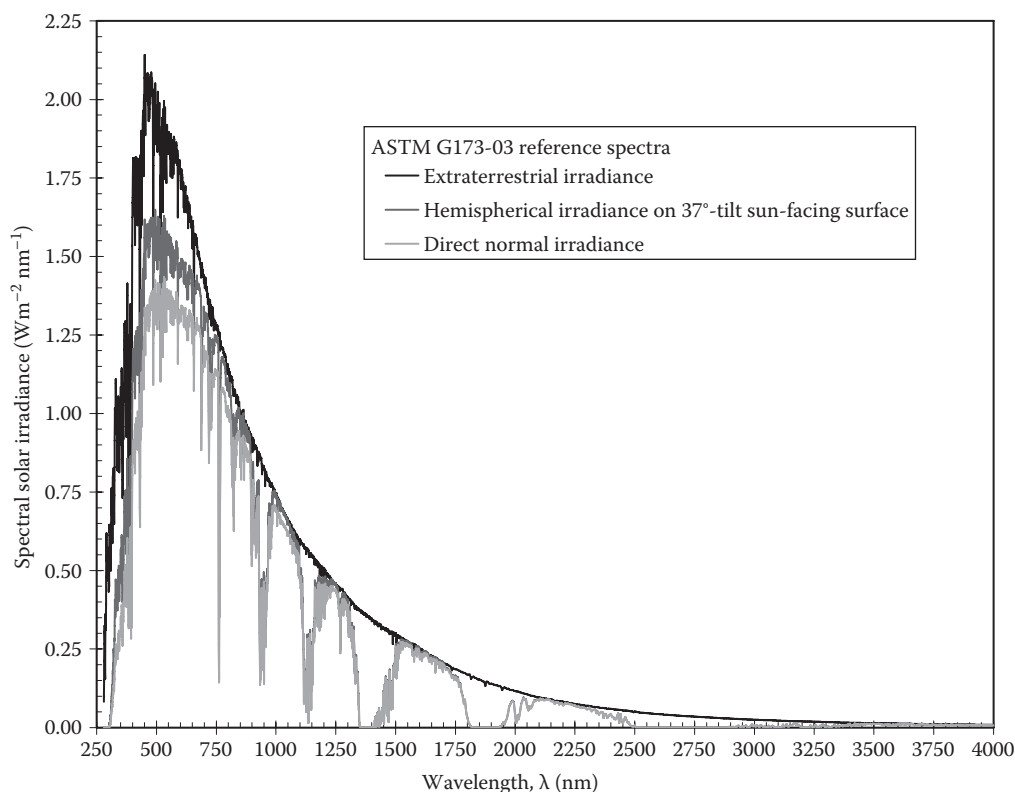


FIGURE 11.2

Averaged daily extraterrestrial solar irradiance and ASTM G173-03 (direct and hemispherical, 37° sun-facing tilted) sea level irradiance in $\text{W/m}^2 \text{ nm}$. (From Gueymard, C. et al., *Sol. Energy*, 73(6), 443, 2002.)

(1) the extraterrestrial spectral irradiance [23], (2) the direct normal spectral irradiance at sea level with an air mass of 1.5, and (3) the hemispherical (or global) spectral irradiance on an inclined plane at sea level, tilted at 37° toward the equator and facing the sun. The data were produced using the Simple Model for Atmospheric Transmission of Sunshine (SMARTS2 version 2.9.2) [25]. Absorption due to atmospheric O_3 , O_2 , CO_2 , and H_2O is apparent in the direct normal irradiance.

Moreover, [Figure 11.3](#) shows the amount of daily solar irradiance in hours incident on an optimally tilted surface during the worst month of the year based on worldwide solar insolation data [26]. The most promising regions for harvesting solar energy are the southwest United States and northern Mexico, the Andes, northern and southern Africa and the Middle East, as well as Australia. Other regions with favorable conditions include southern Europe, southern China, Southeast Asia, Brazil, and most of Africa. Note that many of these regions have limited freshwater resources, and microorganisms should be selected accordingly. Selection criteria to minimize water use include tolerance to wastewater or seawater and to high microorganism concentrations.

11.1.5 Scope of This Chapter

This chapter focuses on photobiological hydrogen production by green algae, cyanobacteria, and purple nonsulfur bacteria. During photobiological hydrogen production, these microorganisms are cultivated in enclosures known as photobioreactors [27]. Due to the multidisciplinary nature of photobiological hydrogen production, this chapter provides the reader with the background on (1) the fundamentals of photosynthesis and photobiological hydrogen production, (2) photobioreactor technologies, and (3) the associated challenges. Finally, economic and environmental considerations along with prospects for this technology are discussed.

11.2 Photosynthesis

Photosynthesis is a series of biochemical reactions converting sunlight into chemical energy [28]. Fixation of CO_2 into organic matter, such as carbohydrates, lipids, and proteins, through photosynthesis also provides food for all living creatures [28]. In other words, photosynthesis is the process to convert solar energy (energy source) and CO_2 (carbon source) into organic material essential for life on Earth.

Photosynthesis involves two types of reactions, namely, (1) light and (2) dark reactions. During light reactions, photons are absorbed by the microorganisms and are used to produce (1) adenosine triphosphate (ATP), the principal energy-carrying molecule in cells, and (2) the electron carrier nicotinamide adenine dinucleotide phosphate (NADPH). These products of the light reaction are then used in the subsequent dark reactions such as CO_2 fixation [28] and H_2 production [29]. The electrons that drive these reactions usually come (1) from reduced sulfur sources such as hydrogen sulfide (H_2S), sulfur (S^0), or thiosulfate ($S_2O_3^{2-}$) in photosynthetic bacteria and (2) from water (H_2O) in plants, algae, and cyanobacteria [30]. When water is used as the electron source, O_2 is produced as a by-product. These processes are known as oxygenic photosynthesis. Those that do not produce O_2 are known as anoxygenic photosynthesis [30]. The reader is referred to Section 11.2.2 for detailed discussion of anoxygenic and oxygenic photosynthesis.

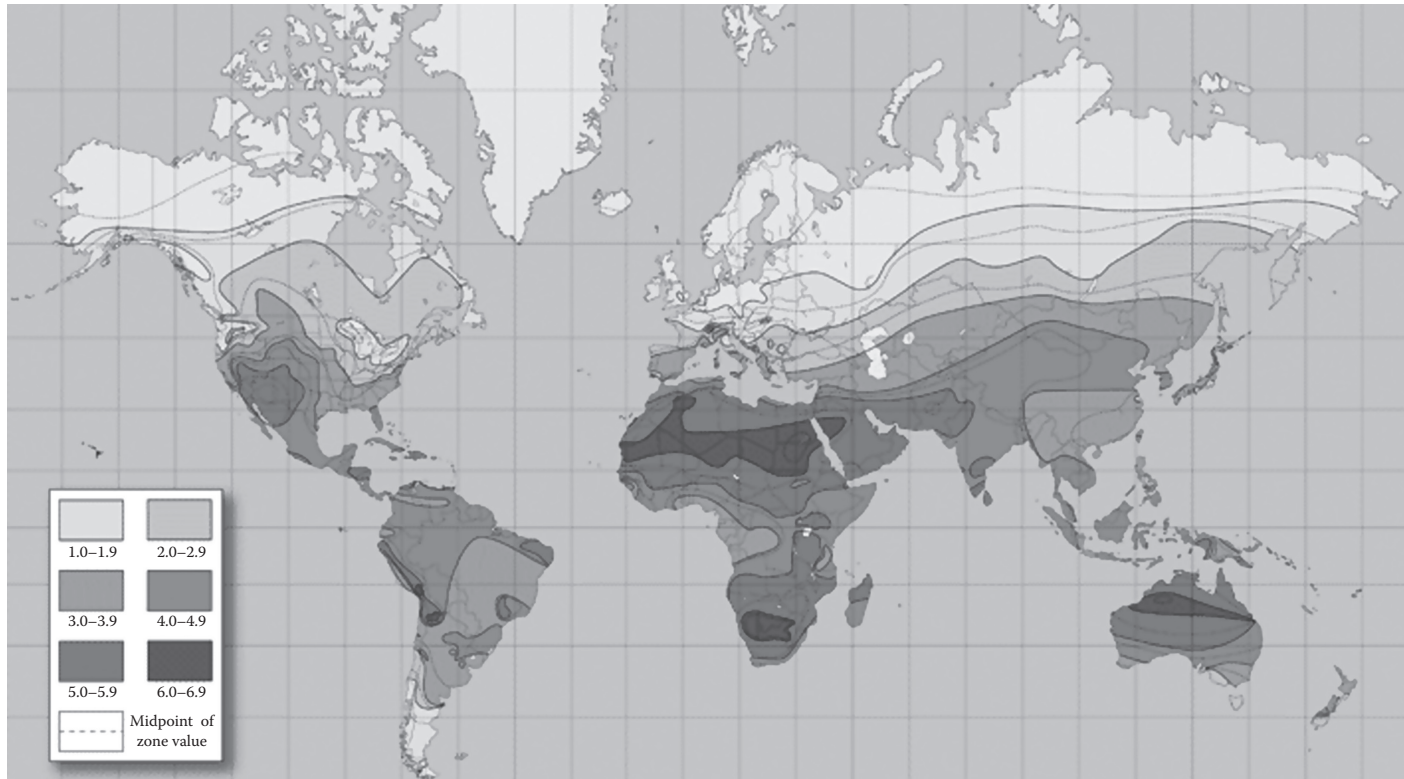


FIGURE 11.3

Average daily local solar irradiance on an optimally tilted surface during the worst month of the year (units are in kWh/m²/day). (Courtesy of SunWize Technologies, Kingston, New York, World insolation map, http://www.sunwize.com/info_center/solar-insolation-map.php, 2008, Used by permission. All rights reserved. [copyright] 2009 SunWize Technologies.)

11.2.1 Photosynthetic Apparatus and Light-Harvesting Pigments

Photosynthesis begins with the absorption of photons by the photosynthetic apparatus. The latter consists of three major parts: (1) the reaction center, (3) the core antenna, and (3) the peripheral antenna. Photochemical charge separation and electron transport take place in the reaction center [28]. The core antenna contains the minimum number of pigments, consisting only of chlorophylls or bacteriochlorophylls, which are necessary for photosynthesis. It is surrounded by the peripheral antenna, which is an assembly of chlorophylls, bacteriochlorophylls, and other accessory pigments such as carotenoids and phycobiliproteins. The peripheral antenna is particularly important in channeling additional photon energy to the reaction center at small light intensities. In algae and cyanobacteria, the photosynthetic apparatus is located on the photosynthetic membrane called thylakoid as shown in transmission electron microscope (TEM) micrographs in Figures 11.8 [31] and 11.9 [32]. In purple bacteria, it is located on vesicular photosynthetic membranes as shown in Figure 11.10 [30,33]. Each of the pigments used in the photosynthesis process is described in detail in the next sections.

11.2.1.1 Chlorophylls and Bacteriochlorophylls

The main pigments necessary for oxygenic photosynthesis are called chlorophylls and those responsible for anoxygenic photosynthesis are called bacteriochlorophylls [30]. Both are molecules containing a magnesium atom at their center. Figure 11.4a shows the structural formula of chlorophyll and bacteriochlorophyll molecules where R1 through R7 are organic chains [30]. The nature of the substituents present in the positions R1 through R7 defines different chlorophylls and bacteriochlorophylls. For example, Figure 11.4b and c shows the structure of chlorophyll *a* and bacteriochlorophyll *a*, respectively.

Moreover, the absorption peak wavelengths of common chlorophyll and bacteriochlorophyll pigments are summarized in Table 11.1. It shows that chlorophylls *a* and *b* have two absorption peaks, one in the blue and one in the red part of the visible spectrum [28]. Chlorophyll *a* absorbs around 430 and 680 nm, while chlorophyll *b* absorbs around 450 and 660 nm. Since they do not absorb green light ($\lambda \approx 520\text{--}570$ nm), they appear green to the human eye. These pigments are also responsible for the green color of plants. On the other

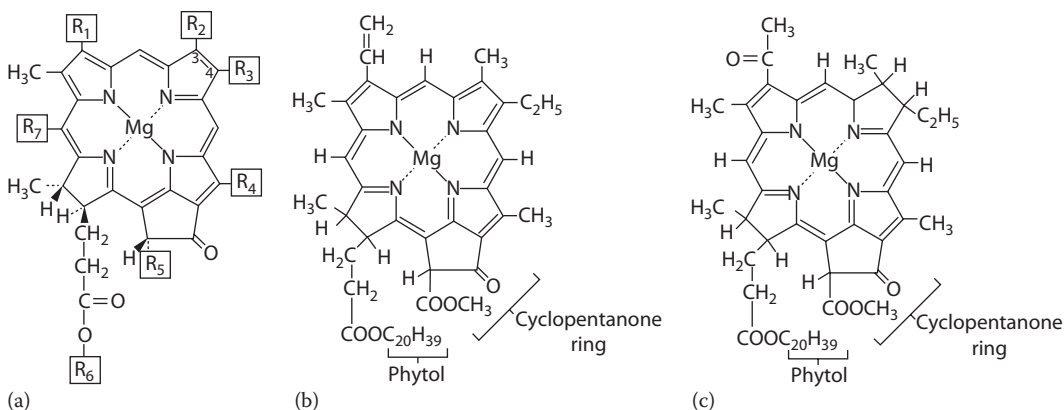


FIGURE 11.4

Structural formulae of (a) general chlorophyll molecule, (b) chlorophyll *a*, and (c) bacteriochlorophyll *a*. (From Madigan, M.T. and Martinko, J.M., *Biology of Microorganisms*, Pearson Prentice Hall, Upper Saddle River, NJ, 2006.)

TABLE 11.1

Common Photosynthetic Pigments in Photosynthetic Microorganisms

Pigment Group	Pigment Name	Absorption Maxima (nm)	Microorganism Type
Chlorophylls	Chl <i>a</i>	430, 680	Cyanobacteria, green algae
	Chl <i>b</i>	450, 660	Green algae
<i>Bacteriochlorophylls</i>	Bchl <i>a</i>	805, 830–890	Purple bacteria
	Bchl <i>b</i>	835–850, 1020–1040	Purple bacteria
	Bchl <i>c</i>	745–755	Green sulfur bacteria
	Bchl <i>c</i> _s	740	Green nonsulfur bacteria
	Bchl <i>d</i>	705–740	Green sulfur bacteria
	Bchl <i>e</i>	719–726	Green sulfur bacteria
	Bchl <i>g</i>	670–788	Heliobacteria
<i>Carotenoids</i>	<i>B</i> -carotene	425, 448, 475	All photosynthetic microbes
	Lutein	421, 445, 474	All photosynthetic microbes
	Violaxanthin	418, 442, 466	All photosynthetic microbes
	Neoxanthin	418, 442, 467	All photosynthetic microbes
	Spheroidene	429, 455, 486	All photosynthetic microbes
<i>Phycobilins</i>	Phycocyanin	620	Cyanobacteria
	Phycoerythrin	550	Cyanobacteria

Sources: Ke, B., *Photosynthesis, Photobiochemistry and Photobiophysics*, Kluwer Academic Publishers, Dordrecht, the Netherlands, 2001; Madigan, M.T. and Martinko, J.M., *Biology of Microorganisms*, Pearson Prentice Hall, Upper Saddle River, NJ, 2006.

hand, bacteriochlorophylls absorb light mainly in the far-infrared to NIR part of the electromagnetic spectrum ($700 \leq \lambda \leq 1000$ nm) [28].

11.2.1.2 Carotenoids

Carotenoids are accessory pigments found in all photosynthetic microorganisms. They absorb mainly the blue part of the spectrum ($400 \leq \lambda \leq 550$ nm) and are responsible for the yellow color of leaves in autumn and orange color of carrots [28]. Carotenoids serve two major functions: (1) shielding the photosynthetic apparatus from photooxidation under large light intensities and (2) increasing the solar light utilization efficiency by expanding the absorption spectrum of the microorganism. They are hydrophobic pigments composed of long hydrocarbon chains and are embedded in the photosynthetic membrane. There are numerous carotenoids [28]. The most common ones are listed in Table 11.1 along with their absorption peak wavelength.

11.2.1.3 Phycobiliproteins

Phycobiliproteins are also accessory pigments that play a role in light harvesting and transferring this energy to the reaction centers. They are found in cyanobacteria and red algae [30]. Two major ones are phycoerythrin absorbing mainly around 550 nm and phycocyanin absorbing strongly at 620 nm [30]. They are essential to the survival of these microorganisms at low light intensities.

Different pigment molecules absorb at different spectral bands of the solar spectrum enabling more efficient utilization of solar energy. They also allow for the coexistence of different photosynthetic microorganisms by sharing different bands of the solar spectrum. Figure 11.5 shows the absorption spectra of chlorophylls *a* and *b*, β -carotenoid,

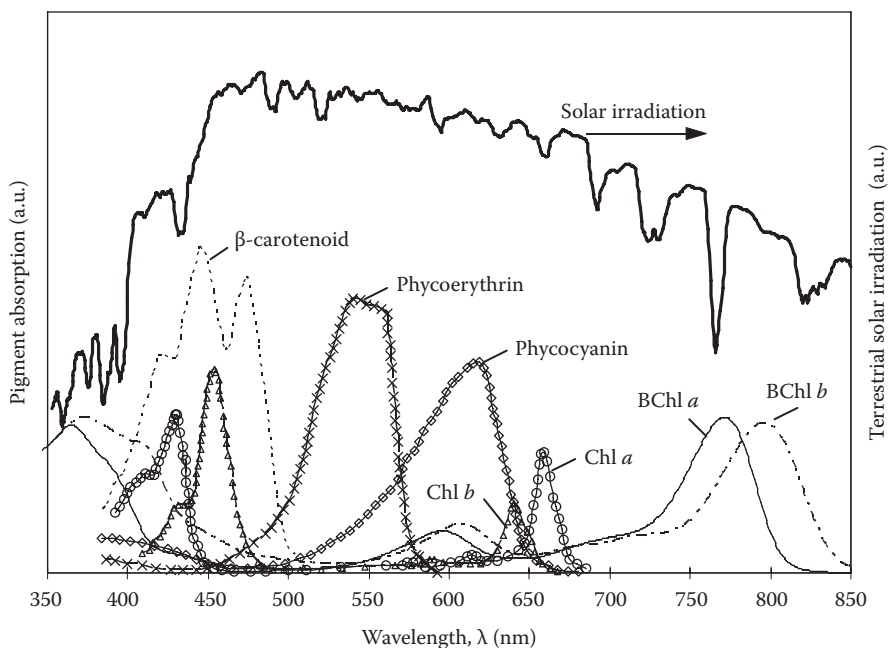


FIGURE 11.5

Absorption spectra of β -carotenoid, phycoerythrin, phycocyanin, chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), bacteriochlorophyll *a* (BChl *a*), and bacteriochlorophyll *b* (BChl *b*) in relation to the terrestrial solar irradiation spectrum. The figure is in arbitrary units and the spectra of different molecules have been scaled arbitrarily for the sake of clarity. (From Ke, B., *Photosynthesis, Photobiochemistry and Photobiophysics*, Kluwer Academic Publishers, Dordrecht, the Netherlands, 2001.)

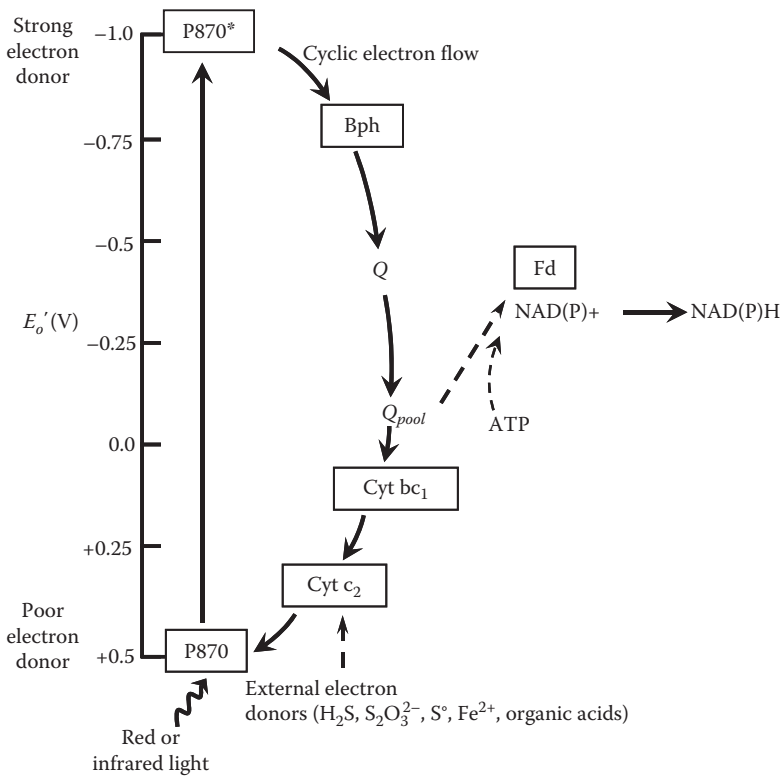
phycoerythrin, and phycocyanin over the spectral region from 400 to 700 nm, known as the photosynthetically active radiation (PAR) [28]. It also shows the profile of solar radiation spectrum (in arbitrary units) indicating that these pigments have evolved to absorb at wavelengths where the solar energy is most abundant.

11.2.2 Anoxygenic and Oxygenic Photosynthesis

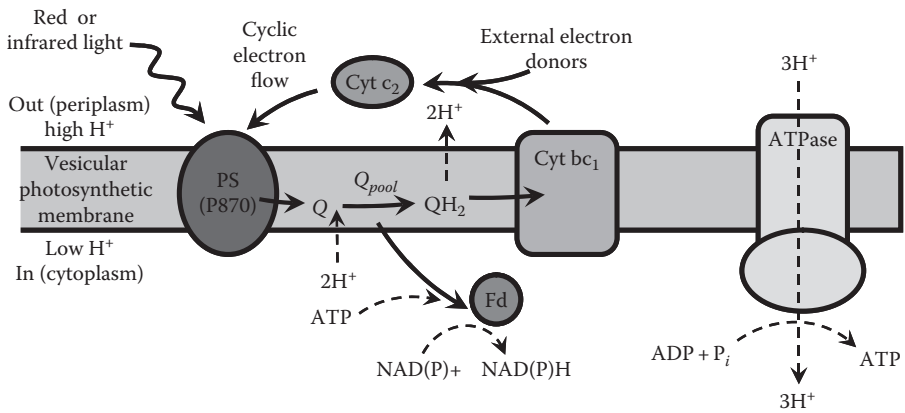
Two types of photosynthetic processes exist depending on whether molecular oxygen is evolved as a by-product [30]. Anoxygenic photosynthesis is mainly conducted by purple and green sulfur and nonsulfur bacteria, whereas oxygenic photosynthesis is conducted by green algae, cyanobacteria, and plants [28,30]. The source of electrons in anoxygenic photosynthesis can be molecular hydrogen, sulfide, or organic acids. However, in oxygenic photosynthesis, the source of electrons is always water [28]. Details of the electron transport in both types of photosynthesis are described in the following sections.

11.2.2.1 Electron Transport in Anoxygenic Photosynthesis

Figure 11.6 shows the electron flow in anoxygenic photosynthesis, conducted by purple bacteria, for example, with respect to the reduction potential E'_o of the molecules expressed in volts [30]. Anoxygenic photosynthesis begins when a photon with wavelength 870 nm is absorbed by the antenna and transferred to the reaction center. The reaction center, known as P870, is a strong electron donor P870* with very low reduction potential. The electrons



(a)



(b)

FIGURE 11.6 Electron flow in anoxygenic photosynthesis in purple bacteria illustrated as (a) redox potentials and (b) membrane schematic. (From Madigan, M.T. and Martinko, J.M., *Biology of Microorganisms*, Pearson Prentice Hall, Upper Saddle River, NJ, 2006.)

from P870* are donated very quickly to bacteriopheophytin *a* (Bph) within the reaction center to prevent electron recombination. These electrons are transported from the reaction center through a series of quinone molecules within the photosynthetic membrane denoted by Q (Figure 11.6). The electron flow in the photosynthetic membrane is also shown schematically in Figure 11.6b. The transport of electrons induces a proton gradient

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across the membrane known as the proton motive force that drives the synthesis of ATP through a reaction known as phosphorylation [30]. With additional energy in the form of ATP, electrons are transferred from quinone pool of the photosynthetic membrane denoted by Q_{pool} either (1) to ferredoxin, which carries electrons to be used in nitrogen fixation and/or hydrogen production, or (2) to $NAD(P)^+$ to be converted to $NAD(P)H$, which carries electrons to biosynthetic reactions such as CO_2 fixation in the Calvin cycle [30,34]. Unused electrons return back to the reaction center via cytochromes Cyt bc_1 and Cyt c_2 , thus forming an electron cycle [30]. The electrons lost during the electron cycle are replaced during photofermentation by the cytochrome Cyt c_2 that oxidizes organic acids such as acetate or reduced compounds such as H_2S [30,34].

11.2.2.2 Electron Transport in Oxygenic Photosynthesis

In contrast, during oxygenic photosynthesis conducted by algae and cyanobacteria, electron transport is not cyclic but follows the *Z-scheme* shown in Figure 11.7 [30].

In this scheme, two distinct but interconnected photochemical reactions known as photosystem I (PSI) and photosystem II (PSII) function cooperatively. Oxygenic photosynthesis begins when photons with wavelength around 680 nm are absorbed and transferred to the reaction center known as P680 located in PSII. This converts P680 to a strong reductant that can oxidize water to liberate electrons and protons and evolve molecular O_2 according to $2H_2O \rightarrow 4e^- + 4H^+ + O_2$. Electrons from the reduced P680* are quickly transferred to pheophytin (Ph) within the reaction center to prevent electron recombination. Subsequent electron transfer in the photosynthetic membrane from the reaction center drives the proton motive force responsible for the generation of ATP. The electrons reaching the cytochrome Cyt bf are transported to P700 of PSI with plastocyanin (PC). Absorbing light energy at about 700 nm, P700 is reduced to P700*, which has a very low reduction potential. The electrons are quickly donated to a special chlorophyll *a* molecule (Chl a_0) within the reaction center. These electrons are then donated to $NAD(P)^+$ to synthesize $NAD(P)H$ through a cascade of quinone molecules (Q), nonheme iron–sulfur protein (FeS), ferredoxin (Fd), and flavoprotein (Fp) as shown in Figure 11.7. Since the electrons generated from water splitting are not returned back to P680, this form of ATP generation is known as noncyclic phosphorylation. However, if sufficient reducing power is present in the cells, a cyclic phosphorylation can also take place around PSI as shown in Figure 11.7 [35,36]. In both oxygenic and anoxygenic photosynthesis, ATP and $NAD(P)H$ produced are used by the microorganisms as their energy and electron carriers in order to fix CO_2 .

11.3 Microbiology of Photobiological Hydrogen Production

11.3.1 Hydrogen-Producing Microorganisms

There are various methods for biological hydrogen production depending on the type of microorganism used in the process. Thus, it is necessary to classify the different hydrogen-producing microorganisms and understand their metabolism. On the most basic premise, microorganisms can be divided into two major groups known as prokaryotes and eukaryotes. Unlike prokaryotes, eukaryotes have a nucleus where the genetic material is stored and other membrane-enclosed organelles [30]. Members of *bacteria* such

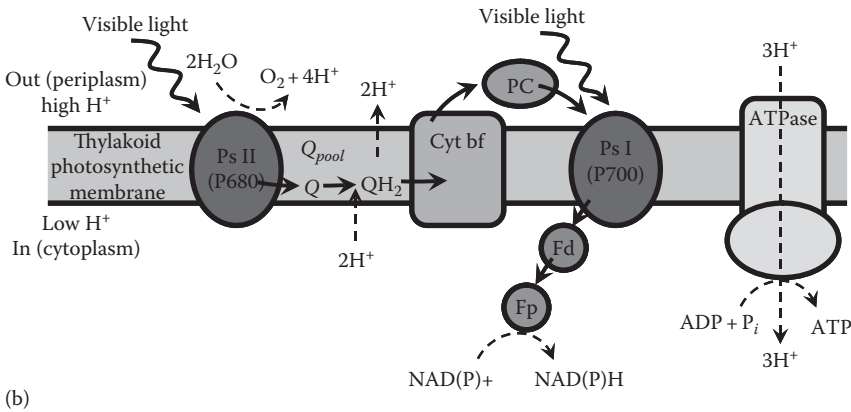
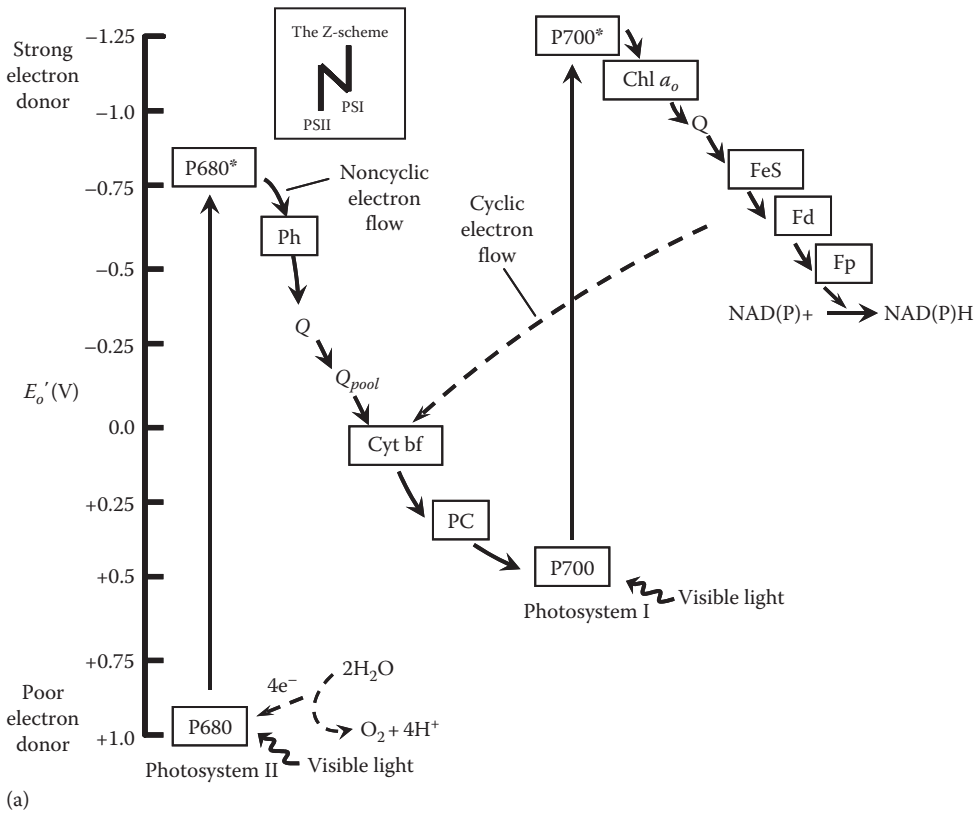


FIGURE 11.7 Electron flow in oxygenic photosynthesis in cyanobacteria and green algae illustrated as (a) redox potentials and (b) membrane schematic. (From Madigan, M.T. and Martinko, J.M., *Biology of Microorganisms*, Pearson Prentice Hall, Upper Saddle River, NJ, 2006.)

as cyanobacteria, purple nonsulfur bacteria, and fermentative bacteria are prokaryotes. Algae, on the other hand, are eukaryotes.

Microorganisms that can use solar radiation as their energy source and CO₂ as their sole carbon source are known as photoautotrophs. Cyanobacteria, algae, and purple nonsulfur bacteria are capable of a photoautotrophic life style. Among these, cyanobacteria

and algae use water as their electron source and conduct oxygenic photosynthesis (see Section 11.2.2.2). Purple nonsulfur bacteria use molecular hydrogen, sulfide, or organic acids as their electron source and conduct anoxygenic photosynthesis (see Section 11.2.2.1). Some of these photoautotrophs can also live as photoheterotrophs, that is, they can utilize light as their energy source and organic compounds as both their carbon and electron sources. Organic compounds include (1) organic acids (e.g., acetic acid, amino acids, lactic acid, citric acid, butyric acids) and (2) carbohydrates (sugars) such as monosaccharides (e.g., glucose, sucrose, fructose, $(\text{CH}_2\text{O})_n$) and polysaccharides (e.g., starch, cellulose, $(\text{C}_6\text{H}_{10}\text{O}_5)_n$).

On the other hand, some microorganisms use chemical compounds as their energy source and are known as chemolithotrophs. Among these, those using organic compounds as their energy, carbon, and electron sources are known as chemoorganotrophs. This is the case of dark fermentative bacteria. Finally, prokaryotes, like most cyanobacteria and all purple nonsulfur bacteria, are capable of using molecular nitrogen as their nitrogen source. They achieve this through nitrogen fixation that uses special enzymes called nitrogenase and requires energy in the form of ATP. On the other hand, eukaryotes cannot fix molecular nitrogen and require sources of nitrogen in the form of ammonia, nitrates, or proteins (e.g., albumin, glutamate, yeast extract) [30,37].

11.3.1.1 Green Algae

Green algae are eukaryotic organisms that contain chlorophylls and conduct oxygenic photosynthesis [38,39]. They live in freshwater and most of them have cellulose cell walls. They can produce hydrogen through direct and indirect biophotolysis as well as photofermentation (Section 11.3.2.2). All these processes require anaerobic conditions, that is, the absence of oxygen from the algae environment. Examples of green algae capable of photobiological hydrogen production include (1) freshwater species such as *Chlamydomonas reinhardtii* [40], *Chlamydomonas moewusii* [41], and *Scenedesmus obliquus* [42] as well as (2) saltwater species such as *Chlorococcum littorale* [43], *Scenedesmus obliquus* [44], and *Chlorella fusca* [45]. Figure 11.8 depicts the TEM micrograph of the green algae *Chlamydomonas reinhardtii* [31]. It shows the location of the nucleus; the chloroplast, where photosynthetic pigments are located; and the pyrenoid, where CO_2 fixation takes place.

11.3.1.2 Cyanobacteria

Cyanobacteria, also known as blue-green algae, are photoautotrophic prokaryotes that are capable of conducting oxygenic photosynthesis [30]. These microorganisms are the first organisms that could evolve oxygen and are responsible for converting Earth's atmosphere from anoxic (oxygen lacking) to oxic (oxygen containing) [28]. There exist unicellular and filamentous forms and their size can range from 0.5 to 40 μm in diameter depending on the strain [30]. Most species are capable of fixing atmospheric nitrogen using the nitrogenase enzyme and play an important role in the global nitrogen cycle [30]. Some filamentous forms have evolved to contain the nitrogenase enzyme in special cells called heterocysts. Heterocysts protect nitrogenase from oxygen inhibition.

Just like green algae, cyanobacteria can produce hydrogen through direct and indirect biophotolysis as well as photofermentation (Section 11.3.2.2). In addition to anaerobic conditions, nitrogen-fixing cyanobacteria also require the absence of nitrogen sources (N_2 , NO_3^- , or NH_4) in order to produce H_2 . Examples of cyanobacteria capable of photobiological

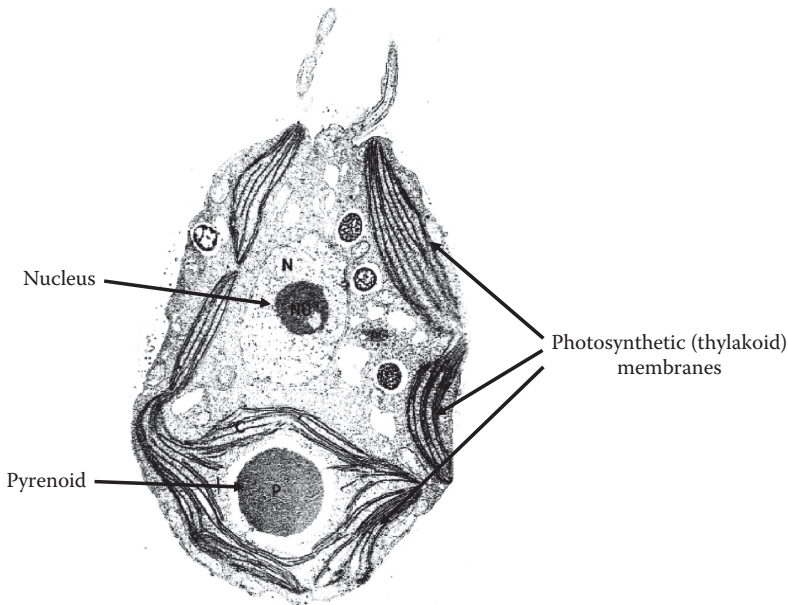


FIGURE 11.8

TEM micrograph of *Chlamydomonas reinhardtii*. A typical cell is ellipsoidal with major and minor diameters equal to about 9 and 8 μm , respectively. (From Harris, E.H., *The Chlamydomonas Sourcebook*, Vol. 1, Academic Press, San Diego, CA, 1989. With permission; Berberoğlu, H. et al., *Int. J. Hydrogen Energy*, 33, 6467, 2008. With permission.)

hydrogen production include (1) freshwater species such as *Anabaena variabilis* [46], *Anabaena azollae* [46], and *Nostoc punctiforme* [47] as well as (2) saltwater species such as *Oscillatoria* Miami BG7 [48] and *Cyanothece* 7822 [49]. Figure 11.9 presents the TEM micrograph of the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413 [32]. It shows the location of the thylakoid membrane where the photosynthetic apparatus is located. Note the absence of nucleus and organelles.

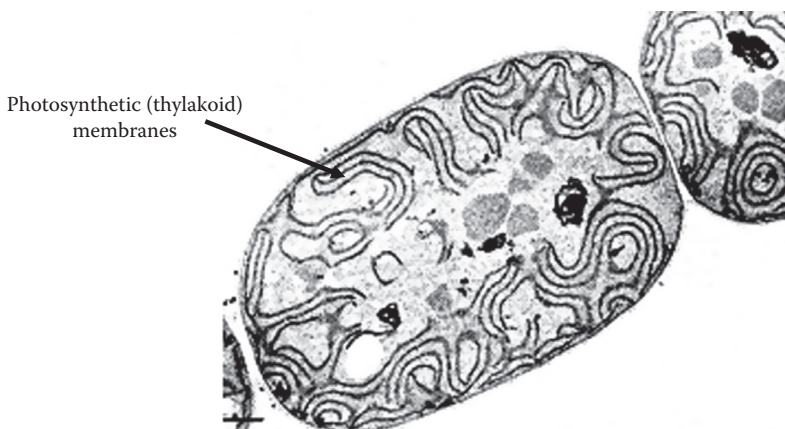


FIGURE 11.9

TEM micrograph of *Anabaena variabilis* ATCC 29413. Typical cell is 5 μm wide. (From Lang, N.J. et al., *J. Bacteriol.*, 169(2), 920, 1987. With permission.)

11.3.1.3 Purple Nonsulfur Bacteria

Purple nonsulfur bacteria are prokaryotes that conduct anoxygenic photosynthesis, that is, they do not produce oxygen. In general, purple nonsulfur bacteria survive by photoheterotrophy using light as their energy source and organic compounds as their carbon source. Organic compounds include fatty, organic, or amino acids; sugars; alcohols; and aromatic compounds [30]. Purple nonsulfur bacteria contain bacteriochlorophylls and carotenoids and have a brown/dark red color, hence their name. The photosynthetic apparatus of purple nonsulfur bacteria is located on an intracytoplasmic photosynthetic membrane [30]. Some species can also grow in the dark conducting fermentation and anaerobic respiration, while others can grow photoautotrophically fixing CO_2 using H_2 or H_2S as their electron source [30]. All species have the nitrogenase enzyme and can fix molecular nitrogen.

Purple nonsulfur bacteria produce hydrogen by photofermentation, which requires removal of both oxygen and nitrogen from the environment (Section 11.3.2.2). Examples of purple nonsulfur bacteria capable of producing hydrogen include *Rhodobacter sphaeroides* [37,41] and *Rhodospirillum rubrum* [50]. Figure 11.10 illustrates the TEM micrograph of the purple nonsulfur bacterium *Rhodobacter sphaeroides* during cell division [33]. It shows the location of the intracytoplasmic membranes where the photosynthetic apparatus is located.

11.3.1.4 Dark Fermentative Bacteria

Dark fermentative bacteria are chemoorganotrophs deriving their energy, carbon, and electrons from the degradation of organic compounds including carbohydrates, amino acids, cellulose, purines, and alcohols [30]. Their size is typical of bacteria, that is, 0.5–1.5 μm . They live in soil and organic nutrient-rich waters. Some grow best at temperatures ranging from 25°C to 40°C and are known as mesophiles, and others grow best at even higher temperatures ranging from 40°C to 80°C and are known as thermophiles. Examples of hydrogen-producing mesophiles and thermophiles are *Enterobacter cloacae* IIT BT-08 [51] and *Clostridium butyricum* [52], respectively.

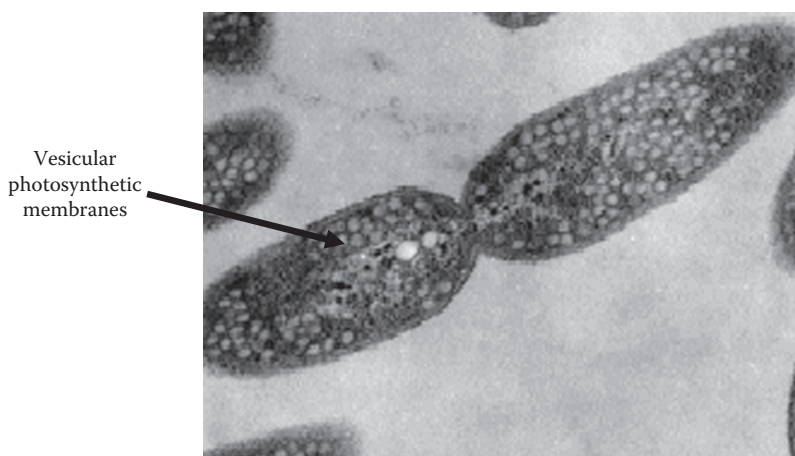


FIGURE 11.10

TEM micrograph of *Rhodobacter sphaeroides*. A typical cell is about 1.5 μm long. (From Department of Energy, *Rhodobacter sphaeroides*, Joint Genome Institute, Walnut Creek, CA, <http://genome.jgi-psf.org/finished-microbes/rhosp/rhosp.home.html>, accessed on April 19, 2008.)

TABLE 11.2Energy, Carbon, Electron, and Nitrogen Sources of H₂-Producing Microorganisms

Microorganism	Green Algae	Cyanobacteria	Purple Nonsulfur Bacteria	Dark Fermentative Bacteria
Energy source	Light (oxygenic)	Light (oxygenic)	Light (anoxygenic)	Organic matter
Carbon source	CO ₂ or organic matter	CO ₂	Organic matter	Organic matter
Electron source	H ₂ O	H ₂ O	Organic matter	Organic matter
Nitrogen source	Ammonia or NO ₃ ⁻	NH ₄ , NO ₃ ⁻ , N ₂ , or proteins	NH ₄ , NO ₃ ⁻ , N ₂ , or proteins	NH ₄ , NO ₃ ⁻ , or proteins
Photosynthetic pigments	Chl <i>a</i> , Chl <i>b</i> , carotenoids	Chl <i>a</i> , carotenoids, phycobilins	Bchl <i>a</i> , Bchl <i>b</i> , carotenoids	None
H ₂ -producing enzyme	[Fe-Fe]-hydrogenase	[NiFe]-hydrogenase and/or nitrogenase	Nitrogenase	[NiFe]-hydrogenase
Products	H ₂ , O ₂ , carbohydrates	H ₂ , O ₂ , CO ₂ , carbohydrates	H ₂ , organic acids	H ₂ , organic acids

Table 11.2 summarizes the energy, carbon, electron, and nitrogen sources for each of the microorganisms described earlier along with the enzymes responsible for H₂ production and the by-products of this process in these microorganisms. The table also lists the pigments present in these microorganisms that are responsible for absorbing and utilizing solar radiation. These enzymes and pigments are presented in detail in the next section.

11.3.2 Enzymatic Pathways

Photobiological hydrogen production takes place when the electrons generated during (1) the light reactions, (2) the degradation of carbohydrates, or (3) respiration are directed to specific enzymes. There are two types of enzymes that catalyze the production of hydrogen in microorganisms, namely, nitrogenase and hydrogenase [53]. Table 11.3 summarizes the advantages and disadvantages of each enzyme group.

11.3.2.1 Enzyme Systems

11.3.2.1.1 Nitrogenase

Nitrogenase is found in prokaryotes such as most photosynthetic bacteria and some cyanobacteria [30]. It is not present in eukaryotes such as green algae [53]. The main role of nitrogenase is to reduce molecular nitrogen to ammonia during fixation of nitrogen dissolved

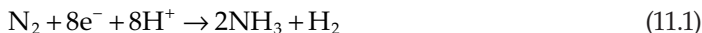
TABLE 11.3

Advantages and Disadvantages of Nitrogenase and Hydrogenase Enzymes in Producing Hydrogen

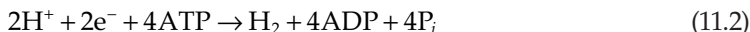
Enzyme	Microorganisms	Advantages	Disadvantages
Nitrogenase	Most photosynthetic bacteria and some cyanobacteria	Robust H ₂ production Able to generate H ₂ under large H ₂ partial pressure	Low efficiency (16%) Small turnover rate Requires 2 ATP/electron Sensitive to O ₂
Hydrogenase	Cyanobacteria, green algae, and purple nonsulfur bacteria	Does not require ATP High efficiency (41%) Large turnover rate (very active)	Unable to generate H ₂ under large H ₂ partial pressure Very sensitive to O ₂

Source: Prince, R.C. and Kleshgi, H.S., *Crit. Rev. Microbiol.*, 31(1), 19, 2005.

in the liquid phase [41] that provides the nitrogen source needed by the microorganism to produce biomass. This primary reaction catalyzed by nitrogenase is given by [53]



In this reaction, H_2 is produced at low rates as a by-product of nitrogen fixation. In the absence of N_2 , nitrogenase catalyzes the irreversible production of H_2 provided that reductants, that is, electrons, and ATP are present via



where ADP and P_i are adenosine diphosphate and inorganic phosphate, respectively. Since the cell energy carrier ATP is used by nitrogenase, this hydrogen production route is energy intensive. The electrons for nitrogenase are donated either by ferredoxin (Fd) or by flavo-protein (Fp) flavodoxin from the photosynthetic electron flow shown in [Figures 11.6 and 11.7](#) [53]. This enables nitrogenase to evolve H_2 even at a partial pressure of H_2 larger than 50 atm making the process robust [53]. However, nitrogenase-based H_2 production suffers from (1) a small turnover rate of less than 10 s^{-1} , that is, nitrogenase can catalyze less than 10 reactions per second, and (2) low quantum efficiency, defined as the ratio of the number of moles of H_2 produced to the number of photons absorbed by the photosystems. Thus, both the rate of H_2 production and the solar-to- H_2 energy conversion efficiency are low.

Nitrogenase enzymes have an organometallic reaction center. The efficiency of H_2 production by the nitrogenase enzyme varies depending on the type of transition metal located at the reaction center [53]. The most common type of nitrogenase enzyme uses molybdenum at its reaction center, but vanadium and iron can also be found [53].

11.3.2.1.2 Hydrogenase

There are two types of bidirectional (or reversible) hydrogenase enzymes, namely, (1) [Fe-Fe]-hydrogenase and (2) [FeNi]-hydrogenase, also called uptake hydrogenase. The iron [Fe-Fe]-hydrogenase is present in green algae [53]. It is a very active bidirectional enzyme with a large turnover rate of 10^6 s^{-1} [53]. It receives electrons from ferredoxin (Fd in [Figures 11.6 and 11.7](#)) and does not require energy (ATP) to produce H_2 . Thus, [Fe-Fe]-hydrogenase has better quantum efficiency than nitrogenase. The bidirectional [Fe-Fe]-hydrogenase catalyzes both the production and consumption of hydrogen through the reaction



The rate at which [Fe-Fe]-hydrogenase can catalyze the production of hydrogen decreases significantly with increasing partial pressure of H_2 .

Finally, [NiFe]-hydrogenase is the commonly known uptake hydrogenase that is found in nitrogen-fixing microorganisms [54]. It is present only in nitrogen-fixing microorganisms such as cyanobacteria and purple nonsulfur bacteria [29]. It catalyzes both H_2 evolution and uptake. In purified form, it has been shown to evolve hydrogen at a low turnover rate of 98 s^{-1} . It also enables microorganisms to consume back H_2 produced as a by-product of nitrogen fixation (Equation 11.1) and, thus, recover some energy.

Both [Ni-Fe]- and [Fe-Fe]-hydrogenases are very sensitive to the presence of O_2 . In particular, the [Fe-Fe]-hydrogenase is irreversibly inhibited by O_2 , whereas the [NiFe]-hydrogenase is reversibly affected (see Section 11.6.1.2) [53].

11.3.2.2 Pathways for Biological Hydrogen Production

Biological processes resulting in hydrogen production can be grouped in four categories, namely, (1) direct and (2) indirect biophotolysis, (3) photofermentation, and (4) dark fermentation.

11.3.2.2.1 Direct Biophotolysis

In this mechanism, H_2 is produced by diverting the electrons generated from water splitting from the Calvin cycle to the bidirectional hydrogenase enzyme according to Equation 11.3 [41]. The energy source is the sunlight in the spectral range from 400 to 700 nm. This mechanism is theoretically the most energy efficient for H_2 production with a theoretical maximum of 40.1% [53]. However, the oxygen produced during water splitting irreversibly inhibits the functioning of the [Fe-Fe]-hydrogenase and makes the process impractical for industrial applications [54]. Green algae such as *Chlamydomonas reinhardtii*, *Chlamydomonas moewusii*, *Scenedesmus obliquus*, and *Chlorococcum littorale* are capable of producing H_2 via direct biophotolysis [41].

11.3.2.2.2 Indirect Biophotolysis

The source of electrons in indirect biophotolysis is also water. However, in this mechanism, the electrons are first used to reduce CO_2 into organic compounds during photosynthesis where O_2 is generated. Then, the electrons are recovered from the degradation of the organic compounds and used in generating H_2 through the action of nitrogenase [54]. Thus, no O_2 is generated during H_2 production. The maximum possible light-to- H_2 energy conversion efficiency of indirect biophotolysis is only 16.3% [53] due to the facts that (1) multiple steps are involved in converting solar energy to H_2 and (2) the use of nitrogenase enzyme requires ATP. Cyanobacteria such as *Anabaena variabilis*, *Anabaena azollae*, *Nostoc muscorum* IAM M-14, and *Oscillatoria limosa* are capable of indirect biophotolysis [49]. The nitrogenase enzyme also gets inhibited by O_2 ; however, cyanobacteria have evolved in many ways to circumvent this problem [55]. For example, *A. variabilis* has evolved to contain the nitrogenase enzyme in special O_2 protective cells called heterocysts as illustrated in Figure 11.11.

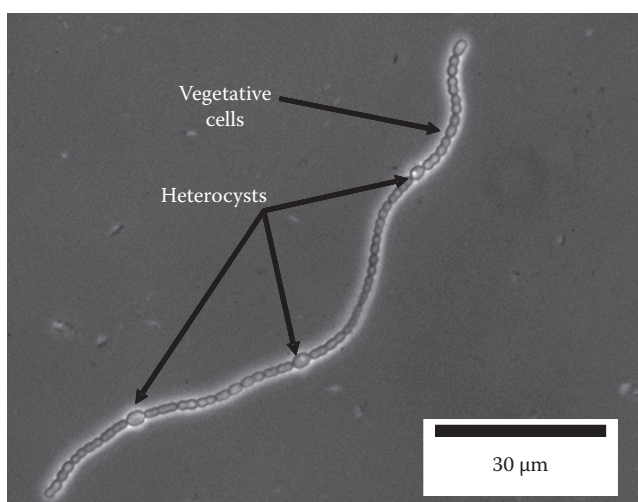


FIGURE 11.11

Micrograph of *Anabaena variabilis* ATCC 29413-U. (From Berberoğlu, H. and Pilon, L., *Int. J. Hydrogen Energy*, 32(18), 4772, 2007.)

11.3.2.2.3 Photofermentation

This mechanism is similar to indirect biophotolysis with the distinction that the organic compounds used are produced outside the cells via the photosynthesis of other organisms, for example, plants. These extracellular organic materials, such as organic acids, carbohydrates, starch, and cellulose [37], are used as the electron source, and sunlight is used as energy source to produce H_2 by nitrogenase enzyme [41]. Due to the fact that the cells do not need to carry out photosynthesis, no O_2 is generated and all the solar energy can be used to produce H_2 . Thus, this mechanism is viewed as the most promising microbial system to produce H_2 [41]. The major advantages of this route are (1) the absence of O_2 evolution that inhibits the H_2 -producing enzymes and (2) the ability to consume a wide variety of organic substrates found in wastewaters. Due to their ability to harvest a wider spectrum of light, from 300 to 1000 nm, purple nonsulfur bacteria such as *Rhodobacter sphaeroides*, pictured in Figure 11.12, *Rhodospirillum rubrum*, and *Rhodopseudomonas sphaeroides* hold promise as photofermentative H_2 producers.

11.3.2.2.4 Dark Fermentation

In dark fermentation, anaerobic bacteria use the organic substances (e.g., glucose, hexose monophosphate, and pyruvate [41]) as both their energy and electron sources. These bacteria mainly use the [NiFe]-hydrogenase enzyme to produce H_2 . Due to the absence of O_2 in the environment and the use of hydrogenase, they can produce H_2 at a higher rate without inhibition. Moreover, H_2 production is continuous throughout the day and night because these microorganisms do not depend on sunlight as their energy source. The hydrogen production is accompanied by CO_2 production as well. Examples of fermentative hydrogen producers include *Enterobacter cloacae* IIT BT-08, *Enterobacter aerogenes*, *Clostridium butyricum*, and *Clostridium acetobutylicum* [41]. This process falls outside the scope of this chapter and will not be discussed further.

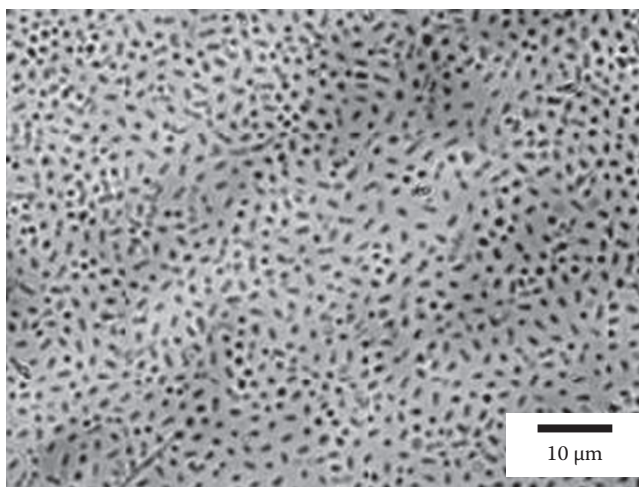


FIGURE 11.12
Micrograph of *Rhodobacter sphaeroides* ATCC 494119.

11.3.2.2.5 Quantum Efficiency of Different Photobiological H₂ Production Pathways

In order to compare the different photobiological pathways, Prince and Kleshgi [53] defined the maximum theoretical quantum efficiency of H₂ production based on monochromatic illumination at 680 nm as

$$\eta_{th} = \frac{n_{H_2} \Delta H_{H_2}}{n_{p,680} hc / \lambda} \quad (11.4)$$

where

n_{H_2} is the number of moles of produced H₂

ΔH_{H_2} is the enthalpy of formation of hydrogen equal to 285.83 kJ/mol at standard state (25°C and 0.1 MPa) [56]

Light input energy is computed as the product of the number of moles of photons needed at 680 nm denoted by $n_{p,680}$ and the energy of photons at 680 nm that is equal to $hc/\lambda = 176$ kJ/mol. In their definition, photons at 680 nm are used as this wavelength corresponds to the absorption peak of the reaction center in PSII. Note that the absorption peak of PSI is at 700 nm and that of purple bacteria is at 790 and 850 nm. The minimum number of photons needed is evaluated by considering the electron and ATP requirement of the reactions and assuming that two photons are needed per electron and two ATPs are needed per electron [53]. Finally, note that this definition of efficiency does not apply to process efficiency of a photobioreactor.

11.3.3 Performance Assessment and Units

Photobiological hydrogen production and/or carbon dioxide mitigation are very interdisciplinary topics involving researchers from many different disciplines including microbiologists and plant biologists as well as engineers. Owing to its diversity, it lacks standards in units to report experimental conditions and results and to enable direct comparisons between studies and microorganisms. This section aims to inform the reader on the different units found in the literature.

11.3.3.1 Microorganism Concentration

The microorganism concentration is usually reported in kilogram of dry cell per cubic meter of the liquid medium denoted by kg dry cell/m³ or simply kg/m³. This requires sampling a known volume of the cell suspension, drying it using an oven overnight, and weighing it using a high-precision analytical balance. Another method is to report the number of cells per cubic meter of liquid medium. This also requires sampling a known volume of cell suspension and counting the cells in that volume as observed under an optical microscope. This technique may be challenging if the microorganisms are (1) very small (<1 μm) such as purple nonsulfur bacteria, (2) in high concentration, and/or (3) having a morphology that is complex such as filamentous cyanobacteria (see [Figure 11.11](#)). Finally, some researchers perform chlorophyll *a* extraction and report the chlorophyll *a* concentration, denoted by mg Chl *a*/m³, as a measure of the microorganism concentration. Chlorophyll *a* is chosen as it is present in all plants, algae, and cyanobacteria that photosynthesize. The results reported in mg Chl *a*/m³ make comparisons difficult with other results as the chlorophyll *a* content

per microorganism depends on the species and growth conditions. Moreover, different microorganisms can have different pigment concentrations or different pigments altogether. Then, reporting the chlorophyll *a* concentration can make results difficult to compare.

Thus, it is recommended that the microorganism concentration be reported in kg dry cell/m³ or in number density (#/m³). Rapid measurements of microorganism concentrations can be performed by measuring the optical density (OD) of microorganism suspension at one or more wavelengths using a UV-visible spectrophotometer. Then, convenient calibration curves can be developed to relate the OD to the dry cell weight or the number density.

11.3.3.2 Hydrogen Production Rate

The hydrogen production rate is reported either as the total mass or volume of hydrogen produced per unit time by the photobioreactor, expressed in kg/h, mmol/h, or m³/h, or as the specific rate per kg dry cell or per milligram of Chl *a*, denoted by kg/kg dry cell/h or kg/mg Chl *a*/h. When reported in volumetric units, the pressure and temperature of the sample must be specified. Unfortunately, many volumetric production rates reported in the literature lack this detail making the results impossible to compare with other studies.

It is recommended that hydrogen production be reported in kg of H₂/h as this rate does not depend on the temperature and pressure of the measurement conditions. Similarly, the specific hydrogen production rates can be reported in kg of H₂/h per kg dry cell or in kg of H₂/h per unit volume of the photobioreactor.

11.3.3.3 Illumination

Light irradiance G_{in} is reported (1) in total luminous flux expressed in lux (1 lux = 1 cd · sr/m²), (2) in photon flux expressed in μmol/m²/s, or (3) in energy flux expressed in W/m². The total luminous flux, also known as illuminance, is a photometric unit that measures light accounting for the human eye sensitivity. The energy emitted by the source is wavelength weighted by the luminosity function that describes the average sensitivity of the human eye to light at different wavelengths between 400 and 700 nm. Different light sources with different emission spectra could have the same illuminance. Thus, illuminance is not recommended for reporting illumination.

On the other hand, photon flux refers to the number of moles of photons (6.02×10^{23} photons/mol) incident on a unit surface area per unit time in the PAR, that is, from 400 to 700 nm. This is a more appropriate unit for reporting the incident energy in photosynthetic systems. It is measured with a quantum sensor that is calibrated to measure the photon flux in the PAR. However, both illuminance and photon flux are valid only in the spectral range from 400 to 700 nm and cannot be used to quantify energy in the NIR part of the spectrum. Thus, in experiments using microorganisms that absorb in NIR such as purple nonsulfur bacteria, illumination reported in illuminance or photon flux cannot be used. Instead, the energy flux should be recorded with a pyranometer having the sensitivity from about 300 to 2800 nm and reported in W/m² μm. Moreover, it is recommended that the spectral sensitivity of the detector and the emission spectrum of the light source be also reported for clarity and reproducibility of the experiments. For benchtop experiments using artificial light, it is useful to report not only the total but also the spectral irradiance.

11.3.3.4 Light-to-Hydrogen Energy Conversion Efficiency

Light-to-hydrogen energy conversion efficiency of photobioreactors is defined as the ratio of (1) the energy that would be released from the reaction of the produced hydrogen with oxygen to produce water and (2) the energy input to the system as light, that is [57],

$$\eta_{\text{H}_2} = \frac{[\Delta G_o + RT \ln(P_{\text{H}_2}/P_o)]R_{\text{H}_2}}{G_{in}A_s} \quad (11.5)$$

where

R_{H_2} is the rate of production of H_2 in mol/s

A_s is the illumination area in m^2

ΔG_o is the standard-state free energy of the formation of H_2 from the water-splitting reaction, equal to 236,337 J/mol at 303 K and 1 atm

The term $RT \ln(P_{\text{H}_2}/P_o)$ is the correction factor for ΔG_o when H_2 production takes place at H_2 partial pressure P_{H_2} instead of the standard pressure P_o of 1 atm. The term G_{in} is the power input to the system as light, that is, irradiance in W/m^2 . In reporting the light energy conversion efficiency, it is important to report the spectral range over which G_{in} is measured. Indeed, the efficiency computed using G_{in} defined over the PAR is about 2.22 times larger than that obtained with G_{in} computed over the entire solar spectrum.

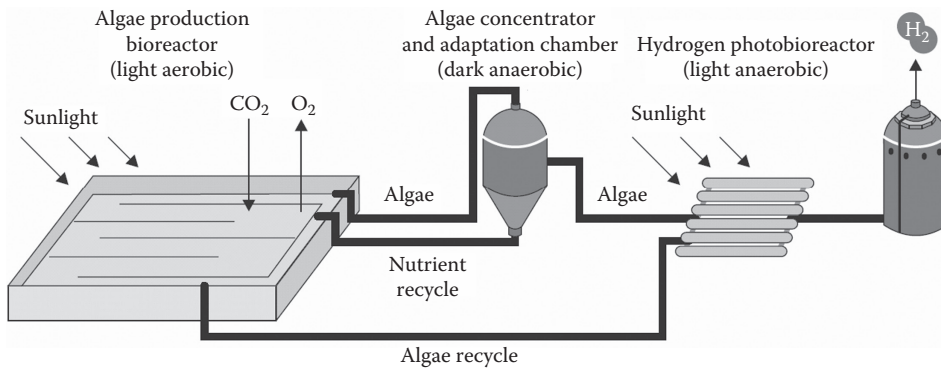
11.4 Photobioreactor Systems

Photobiological hydrogen production by direct and indirect biophotolysis and by photofermentation typically consists of a first stage when microorganisms are grown by photosynthesis in the presence of air and CO_2 . It is followed by a second stage when hydrogen is produced at constant microorganism concentration in the absence of CO_2 , O_2 , and N_2 . During the growth phase, cyanobacteria fix CO_2 and nitrogen from the atmosphere to grow and produce photosynthates. In the H_2 production phase, they utilize the photosynthates to produce H_2 . Alternatively, green algae *C. reinhardtii* are grown in a medium containing acetate. Then, the microorganisms are transferred into a sulfur-deprived medium where anoxia is induced by algae respiration resulting in H_2 production under relative high light irradiance, as first proposed by Melis et al. [40].

For economic reasons, the growth phase should be performed in open ponds [58]. In the hydrogen production phase, open systems will not be appropriate as the method of collection of hydrogen will pose serious difficulties. Similarly, closed indoor systems are not economically feasible since using artificial lighting defeats the purpose of solar energy utilization. Figure 11.13 schematically illustrates the typical process flow envisioned for photobiological hydrogen production at industrial scale [59]. This section presents the different types of photobioreactors used for the hydrogen production phase with emphasis on closed outdoor photobioreactors. It also discusses performances and modeling of mass and light transfer in photobioreactors.

11.4.1 Photobioreactor Types

Photobioreactors have been used for a wide range of applications including the production of pharmaceuticals, food additives for humans, feed for animals, and cosmetic chemicals

**FIGURE 11.13**

Typical process flow for industrial-scale photobiological hydrogen production. (From Riis, T. et al., Hydrogen production and storage—R&D priorities and gaps, International Energy Agency—Hydrogen Co-Ordination Group—Hydrogen Implementing Agreement, 2006, www.iea.org/Textbase/papers/2006/hydrogen.pdf; Courtesy of the International Energy Agency, Paris, France.)

using photosynthetic microorganisms [60]. They also found applications in environmental engineering such as wastewater treatment, heavy metal removal, and CO₂ mitigation [60]. More recently, they have been considered for hydrogen production [61–65].

On the most basic premises, photobioreactors can be grouped into three main categories, namely, (1) open cultivation systems, (2) closed outdoor systems, and (3) closed indoor systems [66]. The open systems are constructed in natural or artificial ponds and utilize sunlight [66]. Closed outdoor photobioreactors consist of specially designed light transparent containers usually in the form of tubes or flat plates and also utilize sunlight [66]. Closed indoor systems, on the other hand, use artificial lighting such as fluorescent lights and light-emitting diodes (LEDs). Their construction is usually a light transparent adaptation of the conventional fermenter systems including stirred-tank bioreactors and vertical cylindrical columns. The advantages and disadvantages of closed and open systems are summarized in Table 11.4.

TABLE 11.4

Comparison of Photobioreactors

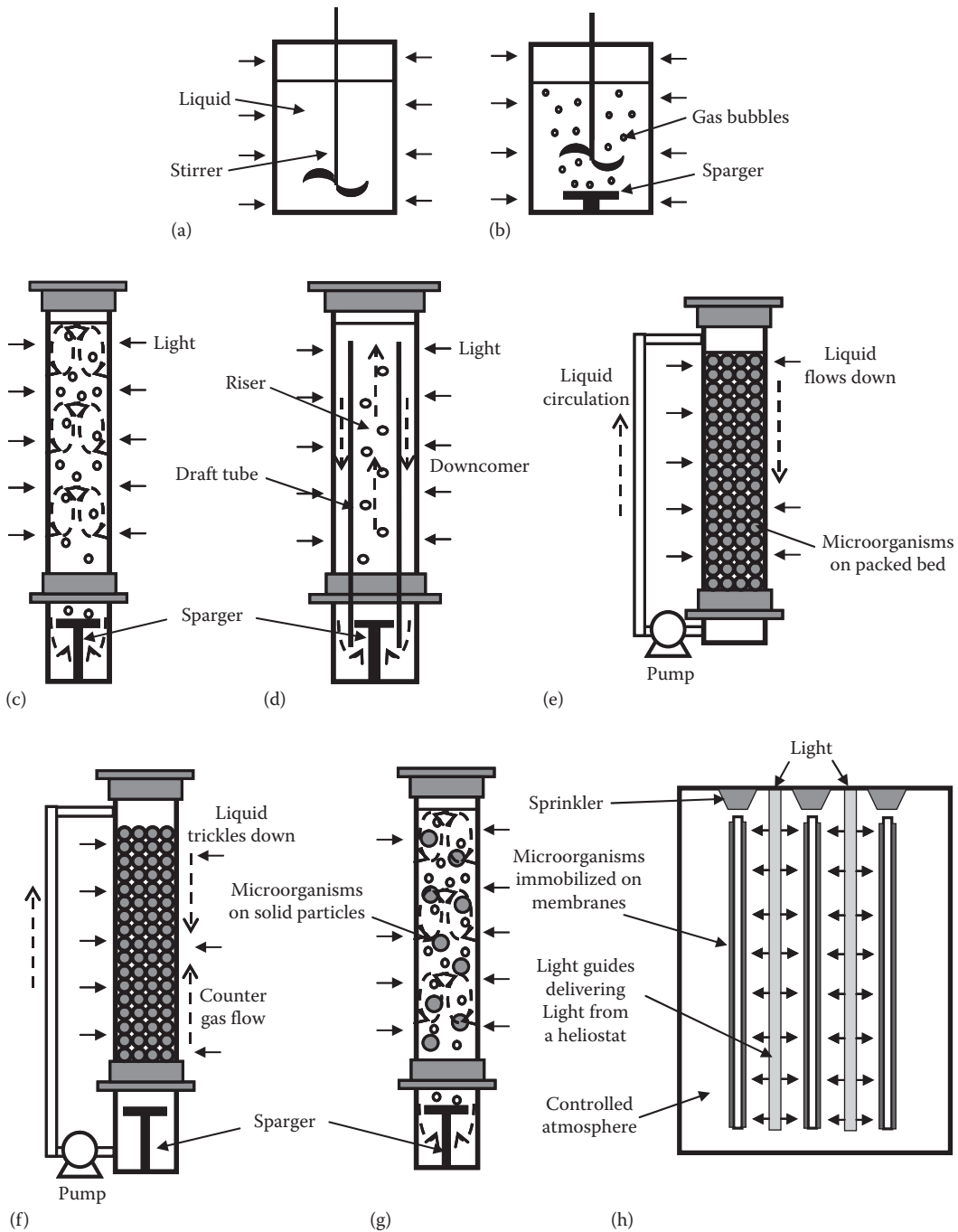
Type	Advantages	Disadvantages
Open Systems	Inexpensive to build and operate Uses sunlight	Small cell densities (0.1–0.2 g/L) Large space requirements Difficult to maintain monoculture Large water and CO ₂ losses
Closed Outdoor	Improved control Limited water losses Uses sunlight Large cell densities (2–8 g/L) Easy to maintain monoculture	Relatively high installation costs Susceptible to ambient temperature variations Difficult to scale up Thermal management challenges
Closed Indoor	Large cell densities (2–8 g/L) Total control over physiological conditions	Relatively high installation and operation costs Inefficient

Sources: Pulz, O., *Appl. Microbiol. Biotechnol.*, 57(3), 287, 2001; Suh, I.S. and Lee, C.G., *Biotechnol. Bioprocess Eng.*, 8(6), 313, 2003.

11.4.2 Closed Outdoor Photobioreactor Designs and Performances

Most common types of closed photobioreactors are vertical or horizontal tubular, helical, and inclined or horizontal thin panel types [66]. Figure 11.14 shows some of these photobioreactor types that can be listed as follows:

- *Stirred-tank photobioreactors* are mechanically stirred photobioreactors to enhance mass transfer as shown in Figure 11.14a [67]. They are mostly suited for reactor volumes between 0.2 and 3 L. Examples include torus photobioreactors [68]. These reactors are often used for research purposes as they enable the control and uniformity of the growth conditions including hydrodynamics conditions, light exposure, concentrations, and pH. This permits full analysis of the system based on mass and energy conservation principles [69–71]. The drawback of these reactors is that very high stirring rates (>600 rpm) might be required to avoid the reactor to become mass transfer limited. Thus, continuously stirring the photobioreactor increases the operating costs, makes scale-up difficult, and decreases the reliability of the system.
- *Sparged- and stirred-tank photobioreactors*. To effectively stir the photobioreactor, gas can be sparged into the photobioreactor as shown in Figure 11.14b [67]. Sparging consists of injecting gas into the liquid phase through a porous medium called sparger or diffuser. This creates bubbles that could be further broken up by mechanical stirring to increase the interfacial area between the liquid and gas and enhances mass transfer. These photobioreactors require lower stirring rates than nonsparged ones and can accommodate liquid volumes greater than 500,000 L [67].
- *Bubble-column photobioreactors* use only sparging for agitation and aeration purposes as shown in Figure 11.14c [67]. They have a high liquid height to base width ratio to increase the bubble residence time and consequently the interfacial area available for mass transfer. Compared with stirred-tank photobioreactors, bubble columns provide less shear on the microorganisms and thus are more suitable for cultivation of plant cells [67].
- *Airlift photobioreactors* are very similar to bubble-column reactors except that they house a draft tube to regulate the flow of bubbles in the reactor as shown in Figure 11.14d [67]. This draft tube provides better heat and mass transfer efficiencies as well as more uniform shear levels. Excessive foaming and cell damage due to bubble bursting are among the drawbacks of airlift photobioreactors. Bubble-column and airlift designs include cylindrical or flat-plate types that can be oriented vertically upright or tilted at an angle [34,72].
- *Packed-bed photobioreactors*. The volume of the reactor is packed with small particles that provide a high surface area substrate on which microorganisms can grow as shown in Figure 11.14e [67]. The packed bed is completely filled with nutrient medium that is constantly circulated. Packed-bed photobioreactors suffer from clogging that inhibits effective mass transfer and limited light transfer to the microorganisms.
- *Trickle-bed photobioreactors* are very similar to packed-bed photobioreactors. However, the reactor liquid does not completely submerge the packing where the microorganisms are immobilized but, instead, trickles on the particles' surface as illustrated in Figure 11.14f [67]. This offers the advantage of minimizing water use. Usually, a gas flow counter to the liquid flow is also provided in trickle-bed photobioreactors for enhanced aeration. One drawback of these reactors is that

**FIGURE 11.14**

Typical photobioreactor designs: (a) stirred-tank, (b) sparged- and stirred-tank, (c) bubble-column, (d) airlift, (e) packed-bed, (f) trickle-bed, (g) fluidized-bed, and (h) membrane photobioreactors. (After Bayless, D.J. et al., *J. Environ. Eng. Manage.*, 16(4), 209, 2006.)

they have relatively low gas transfer per unit volume compared with sparged systems and have limited light transfer.

- *Fluidized-bed photobioreactors.* Microorganisms are immobilized within and/or on lightweight solid particles that are free to move with the fluid and are circulated within the photobioreactor as depicted in Figure 11.14g [67]. This can achieve large mass transfer rates at high cell concentrations.
- *Membrane photobioreactors.* Microorganisms are immobilized on membranes that are constantly being wet by a drip or a sprinkler system as shown in Figure 11.14h [73,74]. Light is collected by a heliostat unit and delivered to the microorganism via lightguides. These photobioreactors minimize the use of water, deliver controlled irradiance to the microorganisms, and can achieve large growth rates. However, they are expensive to build and operate.

Although most of these photobioreactor types were designed for fermenters, their adaptations to photobiological technologies have been discussed in the literature and need not be repeated here [66,75,76]. Despite recent advances, the performances of photobioreactors remain far from theoretical maxima even for benchtop systems [76].

11.4.3 Microorganism and Photobioreactor Performances

Table 11.5 provides a sample of selected studies conducted in laboratories reporting hydrogen production rates by a wide variety of microorganisms. The performance of various microorganisms and growth conditions and photobioreactors has been compared and discussed in Refs. [34,77,78].

Instead of providing an exhaustive review of past studies in this rapidly evolving field, the reader is referred to Ref. [79] for consulting and contributing to the latest experimental results. This webpage makes use of the cyberinfrastructure to develop a virtual community focusing on photobiological CO₂ fixation and H₂ production. This resource should benefit this community in several ways. First, it provides a platform to share recent advances, experimental tips and data, database of bacterial properties, medium, as well as teaching material. Unlike textbooks, this resource can be regularly updated to reflect new advances so its content is less likely to become outdated. The content of the wiki is not dictated by a few experts but is entirely editable by the readers. Consequently, peer review is built into the publishing process and remains an active and continuous component throughout the life of the repository. Finally, it will bridge currently distinct communities in microbiology and plant biology on the one hand and engineering on the other.

11.4.4 Simulating Photobioreactors

In order to design, scale up, optimize, and compare the various photobioreactor designs, it is essential to develop experimentally validated simulation tools that account for light transfer, hydrodynamic conditions, and microorganism growth or H₂ production, along with mass conservation for nutrients and gas species. This section briefly reviews efforts in this area. For the sake of brevity, only selected studies are discussed.

11.4.4.1 Simulating Light Transfer

As light penetrates in the photobioreactor, it is absorbed by the microorganisms or by the medium and scattered by microorganisms and, possibly, by gas bubbles. These scatterers are much larger than visible wavelengths and therefore scattering is strongly

TABLE 11.5

Maximum Reported Hydrogen Production Rates for Various Types of Microorganisms

	Strain Name	Maximum Reported H ₂ Production Rate	References
Freshwater Green Algae	<i>Chlamydomonas moerousii</i>	460 mmol/g chl a/h	[136]
	<i>Chlamydomonas reinhardtii</i>	200 mmol/g chl a/h	[136]
	<i>Lobochlamys segnis</i>	96 mmol/g chl a/h	[136]
	<i>Chlamydomonas noctigama</i>	31 mmol/g chl a/h	[136]
Marine Green Algae	<i>Scenedesmus vacuolatus</i>	155 mmol/g chl a/h	[136]
	<i>Scenedesmus obliquus</i>	150 mmol/g chl a/h	[44]
	<i>Chlorococcum littorale</i>	52 mmol/g chl a/h	[43]
Freshwater Cyanobacteria	<i>Anabaena variabilis</i> PK 84	167.6 mmol/g chl a/h	[46]
	<i>Anabaena variabilis</i> PK 17R	59.18 mmol/g chl a/h	[46]
	<i>Anabaena variabilis</i> ATCC 29413	45.16 mmol/g chl a/h	[46]
	<i>Anabaena azollae</i>	38.5 mmol/g chl a/h	[46]
	<i>Anabaena</i> CA	2.14 mol/kg/h	[49]
	<i>Gloeobacter</i> PCC 7421	1.38 mmol/g chl a/h	[77]
	<i>Anabaena cylindrica</i>	1.3 mol/kg/h	[77]
	<i>Nostoc muscorum</i> IAM M-14	0.6 mmol/g chl a/h	[77]
	<i>Synechococcus</i> PCC 602	0.66 mmol/g chl a/h	[77]
Marine Cyanobacteria	<i>Cyanothece</i> 7822	0.92 mmol/g chl a/h	[137]
	<i>Oscillatoria limosa</i>	0.83 mmol/g chl a/h	[49]
	<i>Oscillatoria</i> Miami BG7	0.3 mol/kg/h	[49]
Purple Nonsulfur Bacteria	<i>Rhodospseudomonas sphaeroides</i>	133 mol/kg/h	[41]
	<i>Rhodobacter sphaeroides</i>	5.9 mol/kg/h	[138]
	<i>Rhodospirillum rubrum</i>	2.5 mol/kg/h	[139,140]
Dark Fermentative Bacteria	<i>Enterobacter cloacae</i> IIT BT-08	211.63 mol/kg/h	[51]
	<i>Enterobacter aerogenes</i>	17 mol/kg/h	[41]
	<i>Citrobacter intermedius</i>	11.5 mol/kg/h	[141]
	<i>Clostridium butyricum</i>	7.3 mol/kg/h	[142]

forward [80,81]. Light transfer through the photobioreactor is governed by the radiative transfer equation (RTE) that expresses an energy balance in a unit solid angle $d\Omega$, about the direction \hat{s} at location \hat{r} . The steady-state RTE in a well-mixed photobioreactor containing microorganisms and bubbles is expressed as [82]

$$\begin{aligned} \hat{s} \cdot \nabla I_{\lambda}(\hat{r}, \hat{s}) = & -\kappa_{eff,\lambda} I_{\lambda}(\hat{r}, \hat{s}) - \sigma_{eff,\lambda} I_{\lambda}(\hat{r}, \hat{s}) + \frac{\sigma_{X,\lambda}}{4\pi} \int_{4\pi} I_{\lambda}(\hat{r}, \hat{s}_i) \Phi_{X,\lambda}(\hat{s}_i, \hat{s}) d\Omega_i \\ & + \frac{\sigma_{B,\lambda}}{4\pi} \int_{4\pi} I_{\lambda}(\hat{r}, \hat{s}_i) \Phi_{B,\lambda}(\hat{s}_i, \hat{s}) d\Omega_i \end{aligned} \quad (11.6)$$

where

$I_{\lambda}(\hat{r}, \hat{s})$ is the radiation intensity at wavelength λ in direction \hat{s} at location \hat{r}

$\kappa_{eff,\lambda}$ and $\sigma_{eff,\lambda}$ are the effective spectral absorption and scattering coefficients, respectively

The coefficients $\sigma_{X,\lambda}$ and $\sigma_{B,\lambda}$ are the spectral scattering coefficients of the microorganisms and the bubbles, respectively

The scattering phase functions of microorganisms and bubbles are denoted by $\Phi_{X,\lambda}(\hat{s}_i, \hat{s})$ and $\Phi_{B,\lambda}(\hat{s}_i, \hat{s})$, respectively

They describe the probability that radiation traveling in the solid angle $d\Omega_i$ around the direction \hat{s}_i will be scattered into the solid angle $d\Omega$ around the direction \hat{s} . They equal unity when scattering is isotropic. The effective absorption coefficient $\kappa_{eff,\lambda}$ accounts for the absorption by the liquid phase and by the microorganisms at wavelength λ . It can be written in terms of the bubble void fraction f_B and of the microorganism concentration X (in kg/m^3),

$$\kappa_{eff,\lambda} = \kappa_{L,\lambda}(1 - f_B - Xv_X) + A_{abs,\lambda}X \tag{11.7}$$

where v_X is the specific volume of microorganisms. The absorption coefficient of the liquid phase $\kappa_{L,\lambda}$ is expressed in m^{-1} , and the mass absorption cross section of microorganisms $A_{abs,\lambda}$ is expressed in m^2/kg . The term $\kappa_{X,\lambda} = A_{abs,\lambda}X$ corresponds to the absorption coefficient of microorganisms. Finally, the term Xv_X represents the volume fraction of the photobioreactor occupied by microorganisms. Assuming independent scattering, the effective scattering coefficient of the composite medium $\sigma_{eff,\lambda}$ can be expressed as the sum of the scattering coefficients of the microorganisms $\sigma_{X,\lambda}$ and of the bubbles $\sigma_{B,\lambda}$ as

$$\sigma_{eff,\lambda} = \sigma_{X,\lambda} + \sigma_{B,\lambda} = S_{sca,\lambda}X + \frac{A_i}{4}Q_{sca,B}(a,\lambda) \tag{11.8}$$

where

$S_{sca,\lambda}$ is the mass scattering cross section of microorganisms expressed in m^2/kg

$Q_{sca,B}(a,\lambda)$ is the scattering efficiency factor of monodisperse bubbles of radius a at wavelength λ obtained from the Mie theory [83]

The interfacial area concentration A_i is defined as the total surface area of bubbles per unit volume and expressed as $A_i = 3f_B/a$. Note that a similar approach can be used to model (1) mixed cultures, (2) scattering by beads in packed beds, and/or (3) polydispersed bubbles [84], for example.

Beer–Lambert’s law provides the solution of the 1D steady-state RTE accounting for both absorption and out-scattering but ignoring in-scattering. It physically corresponds to cases when photons experience at most one scattering event as they travel through the reactor, that is, single scattering prevails. It gives the local spectral irradiance $G_\lambda(z)$ within the photobioreactor as

$$G_\lambda(z) = \int_0^{4\pi} I_\lambda(z, \hat{s}) d\Omega = G_{\lambda,in} \exp(-\beta_{eff,\lambda} z) \tag{11.9}$$

where

$G_{\lambda,in}$ is the spectral irradiance incident on the photobioreactor

z is the distance from the front surface

$\beta_{eff,\lambda}$ is the effective extinction coefficient of the suspension at wavelength λ defined

$$\text{as } \beta_{eff,\lambda} = \kappa_{eff,\lambda} + \sigma_{eff,\lambda}$$

Beer–Lambert’s law has been used extensively to predict the local irradiance within photobioreactors [85,86].

Moreover, Cornet et al. [87–89] solved the RTE using the Schuster–Schwarzschild two-flux approximation to model light transfer in filamentous cyanobacterium *Spirulina platensis* cultures. This approach consists of solving a pair of coupled ordinary differential equations obtained by integrating the RTE over two complementary hemispheres. It can account for in-scattering terms as well as anisotropic scattering [84]. It can also provide an analytical solution for $G_\lambda(z)$ albeit more complex than Beer–Lambert’s law [68,69,90]. Finally, most of the aforementioned studies did not account for the spectral dependency of the radiation characteristics and/or for the presence of bubbles. More recently, Berberoğlu and Pilon [82] simulated light transfer in a bubble sparged photobioreactor accounting for absorption and anisotropic scattering by bubbles and microorganisms. Spectral variations of radiation characteristics over the spectral range from 400 to 700 nm were accounted for using the box model [82]. Genetically engineered microorganisms with reduced pigment content were also considered. The authors established that (1) Beer–Lambert’s law cannot be applied to predict the irradiance inside the photobioreactor, that is, multiple scattering must be accounted for, (2) isotropic scattering can be assumed for wild-strain microorganisms for all practical purposes in the absence of bubbles, (3) anisotropic scattering by the bubbles must be accounted for particularly as the interfacial area concentration increases, (4) for microorganisms with reduced pigment concentration, their anisotropic scattering should be considered.

In order to simulate light transfer in photobioreactors and use any of the aforementioned light transfer models, the spectral radiative characteristics, namely, κ_λ , σ_λ , and $\Phi_\lambda(\hat{s}_i, \hat{s})$ of the microorganisms and/or the bubbles are required. They can be determined either through experimental measurements [80,81] or theoretically by using the Mie theory [69]. Theoretical predictions often assume that the scatterers have relatively simple shapes (e.g., spherical) and ignore their heterogeneous nature by assuming that the complex index of refraction is uniform. Pottier et al. [69] acknowledged that for complex microorganism shapes (e.g., cylinders and spheroids), advanced numerical tools are required to predict radiative characteristics. Alternatively, experimental measurements account for the actual shape and morphology and size distribution of the microorganisms. A comprehensive review of the experimental techniques for measuring the radiation characteristics has been reported by Agrawal and Mengüç [91] and need not be repeated. Pilon and Berberoğlu [80,81] experimentally measured the radiation characteristics of H_2 -producing microorganisms, namely, (1) purple nonsulfur bacteria *R. sphaeroides* [80], (2) cyanobacteria *A. variabilis* [80], and (3) green algae *Chlamydomonas reinhardtii* strain CC125 and its truncated chlorophyll antenna transformants *tla1*, *tlaX*, and *tla1-CW*⁺ [81]. The absorption and scattering cross sections of all strains studied were obtained over the spectral range from 300 to 1300 nm along with their scattering phase function at 632.8 nm. The latter can be assumed to be independent of wavelength in the PAR [92,93]. It was established that *R. sphaeroides* absorbs mainly in two distinct spectral regions from 300 to 600 nm and from 750 to 900 nm. The major absorption peaks can be observed around 370, 480, 790, and 850 nm and can be attributed to the presence of bacteriochlorophyll *b* and carotenoids in the antenna complexes B850 and the reaction center complex [30,94]. Moreover, *A. variabilis* and the wild strain *C. reinhardtii* CC125 absorb mainly in the spectral region from 300 to 700 nm with absorption peaks at 435 and 676 nm corresponding to in vivo absorption peaks of chlorophyll *a*. *A. variabilis* also absorbs at 621 nm corresponding to absorption by the pigment phycocyanin [30], while *C. reinhardtii* has additional absorption peaks at 475 and 650 nm corresponding to absorption by chlorophyll *b*. The genetically engineered strains of *C. reinhardtii* were shown to have less chlorophyll pigments than the wild strain and thus smaller absorption cross sections as illustrated in Figure 11.15. In particular, the mutant *tlaX* features a significant reduction in chlorophyll *b* concentration. For all mutants, however, the reduction in the absorption

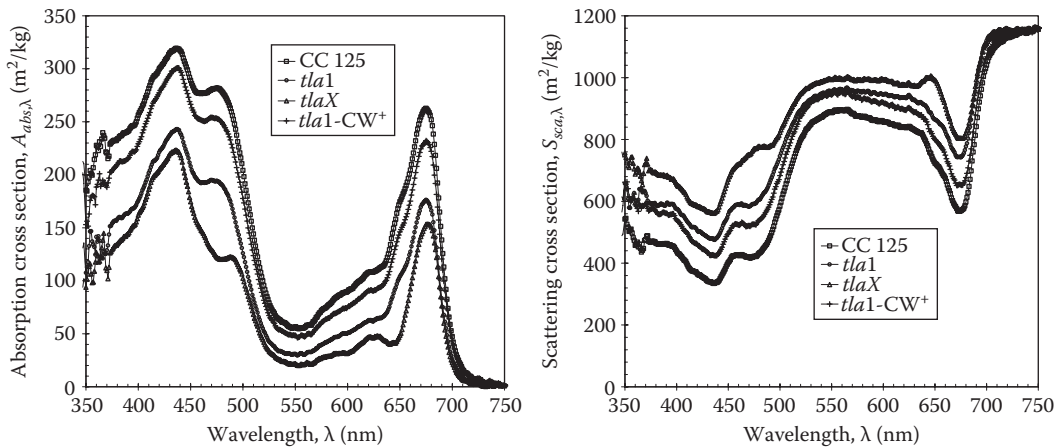


FIGURE 11.15

Absorption and scattering cross sections of the green algae *C. reinhardtii* CC 125 and its truncated chlorophyll antenna transformants *tla1*, *tlaX*, and *tla1-CW+*. (From Berberoğlu, H. et al., *Int. J. Hydrogen Energy*, 33(22), 6467, 2008.)

cross section is accompanied by an increase in scattering cross section [81] (see Figure 11.15). Although scattering becomes the dominant phenomenon contributing to the overall extinction of light, it is mainly in the forward direction so light penetrates within the reactor.

11.4.4.2 Simulating Hydrodynamic Conditions

Hydrodynamic conditions in a photobioreactor affect the shear stress and the amount of light to which individual microorganisms are subjected. Both affect the system's productivity [90]. Simulations of hydrodynamic conditions consist of, first, solving mass and momentum (or Navier–Stokes) conservation equations for a specific reactor geometry to find the local fluid velocity within the photobioreactors. The Lagrangian approach is used to identify the trajectory of individual microorganisms as they are carried by the medium [85]. The instantaneous light flux received by a microorganism can, then, be determined as a function of time [85], and the frequency of light–dark cycles can be determined. These cycles are known to have a strong effect on the microorganism growth, and the cycle frequency should optimally range between 1 Hz and 1 kHz, which is difficult to achieve at industrial scale [76]. Finally, the average light energy received by a microorganism can be computed and used to estimate their growth or hydrogen production rate based on kinetic models.

11.4.4.2.1 Photosynthetic Growth Kinetics

During the growth phase, the time rate of change of microorganism concentration X can be modeled as [95]

$$\frac{dX}{dt} = \mu X \quad (11.10)$$

where μ is the specific growth rate expressed in s^{-1} and function of the average available irradiance denoted by G_{av} . The specific growth rate has been modeled using the modified Monod model taking into account light saturation and inhibition as [67]

$$\mu = \mu_{max} \left(\frac{G_{av}}{K_{s,G} + G_{av} + (G_{av}^2/K_{i,G})} \right) \quad (11.11)$$

where μ_{max} is the maximum specific growth rate while the coefficients $K_{s,G}$ and $K_{i,G}$ are the light half-saturation and inhibition constants, respectively. Similar models can be formulated to account for saturation and inhibition due to limited or excessive carbon dioxide concentrations or excessive microorganism concentrations, for example. The average available irradiance G_{av} can be estimated by averaging the local irradiance over the depth of the culture L as

$$G_{av} = \frac{1}{L} \int_0^L G(z) dz = \frac{1}{L} \int_0^L \int_0^\infty G_\lambda(z) d\lambda dz \quad (11.12)$$

where $G_\lambda(z)$ is estimated by (1) solving the RTE, (2) using approximate solutions such as Beer–Lambert’s law (Equation 11.9), or (3) averaging the light energy received by microorganisms as predicted by hydrodynamics simulations. Fouchard et al. [71] identified μ_{max} , $K_{s,G}$, and $K_{i,G}$ for *C. reinhardtii* in TAP medium without acetate to be 0.2274 h^{-1} , $81.38 \text{ } \mu\text{mol photon/m}^2/\text{s}$, and $2500 \text{ } \mu\text{mol photon/m}^2/\text{s}$, respectively.

11.4.4.2.2 Photobiological H_2 Evolution Kinetics

Similarly, the specific production rate π_{H_2} has been modeled with a modified Michaelis–Menten-type equation given by [95]

$$\pi_{H_2}(z) = \pi_{H_2,max} \frac{G_{av}(z)}{K_{s,H_2} + G_{av}(z) + (G_{av}^2(z)/K_{i,H_2})} \quad (11.13)$$

where $\pi_{H_2,max}$ is the maximum specific hydrogen production rate expressed in $\text{kg } H_2/\text{kg dry cell/h}$. The parameters K_{s,H_2} and K_{i,H_2} account for the saturation and the inhibition of hydrogen production due to excessive irradiation or limited light irradiance.

Nogi et al. [96] measured the specific hydrogen production rate π_{H_2} of the purple non-sulfur bacteria *Rhodospseudomonas rubra* as a function of incident irradiance. The authors reported the absorption spectrum, the hydrogen production rate as a function of spectral incident radiation, and the specific hydrogen production rate as a function of usable radiation. The parameters $\pi_{H_2,max}$, K_{s,H_2} , and K_{i,H_2} were estimated by least-squares fitting of the experimental data reported over the usable incident radiation range from 0 to 80 W/m^2 [96]. The values of $\pi_{H_2,max}$, K_{s,H_2} , and K_{i,H_2} were found to be $1.3 \times 10^{-3} \text{ kg } H_2/\text{kg dry cell/h}$, 25 W/m^2 , and 120 W/m^2 , respectively. Figure 11.16 compares the prediction of Equation 11.13 for π_{H_2} with data reported by Nogi et al. [96].

11.4.4.2.3 Mass Conservation Equations

Mass conservation principles should be satisfied by all gas and nutrient species such as dissolved oxygen, hydrogen, starch, and/or sulfur for green algae. For a well-mixed photobioreactor, the concentrations are assumed to be uniform throughout the reactor and their time rate of change is expressed as

$$\frac{dC_i}{dt} = r_i - k_L a (C_i - C_{i,eq}) \quad (11.14)$$

where

r_i is the net production rate of species “*i*”

$k_L a$ is the specific gas–liquid mass transfer coefficient

$C_{i,eq}$ is the equilibrium concentration between the gas and the liquid phases

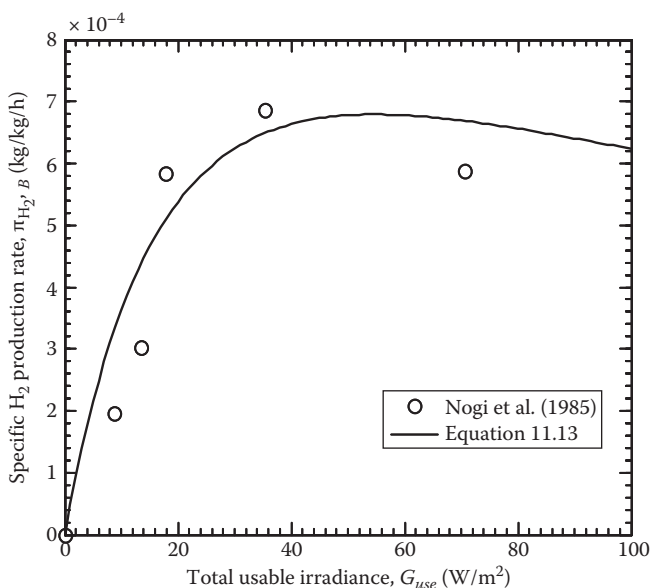


FIGURE 11.16

Experimental data and the modified Michaelis–Menten model (Equation 11.13) for the specific hydrogen production rate of *R. rubra* as a function of the usable incident radiation G_{use} with parameters $\pi_{H_2, max} = 1.3 \times 10^{-3}$ kg H₂/kg dry cell/h, $K_c = 25$ W/m², and $K_l = 120$ W/m². (From Nogi, Y. et al., *Agric. Biol. Chem.*, 49, 3538, 1985.)

For oxygen, for example, r_{O_2} accounts for O₂ generated during photosynthesis and consumed by respiration. It is often assumed to be proportional to the microorganism growth rate, that is, $r_{O_2} = Y_{O_2} \mu X$, where Y_{O_2} is the yield coefficient of O₂ conversion. The reader is referred to Ref. [71] for an illustration of modeling of photobiological H₂ production by *C. reinhardtii* accounting for light transfer, algal growth, and extracellular and intracellular sulfur, starch, and oxygen consumption and/or production along with an estimate of the associated model parameters.

11.5 Technical Challenges and Limitations

Current photobiological hydrogen production suffers from low solar-to-hydrogen energy conversion efficiency that is typically less than 1% [97] under outdoor conditions. In addition, scale-up and economic viability remain major challenges mainly due to issues related to (1) light transfer limitation, (2) mass transfer and hydrodynamics limitations, (3) thermal management, (4) contamination and maintenance of monoculture, and (5) the photobioreactor cost.

11.5.1 Light Transfer

Light transfer in photobioreactors is one of the main barriers to the technology [98,99]. Indeed, photosynthetic microorganisms require an optimum irradiance to achieve the most efficient photosynthesis and H₂ production. This optimum depends on the particular

microorganism but, in general, is about 100 W/m^2 for naturally occurring strains [100]. Thus, photobioreactors can suffer from the following:

- *Light inhibition.* Excessive irradiance inhibits the microbial photosynthesis and H_2 production through a process called photooxidative damage [101,102]. In outdoor systems, where this technology is meant to be deployed, solar irradiance can reach as high as 1000 W/m^2 [103]. For more efficient use of sunlight, it has to be redistributed uniformly throughout the photobioreactor.
- *Limited light penetration.* Due to light absorption by the microorganisms and the medium and scattering by both the microorganisms and gas bubbles, the local irradiance within the photobioreactor may decrease below the required levels for photosynthesis and/or H_2 production [87–89,97]. This, in turn, limits the productivity and scale-up of the system.

Ways to address these light transfer challenges are discussed in Section 11.6.

11.5.2 Mass Transfer and Hydrodynamics

Hydrogen and possibly oxygen produced by the microorganisms have inhibitory effects on photobiological hydrogen production [55,103]. Therefore, these gas species must be efficiently removed from the photobioreactor for sustained H_2 production. The issues related to mass transfer and hydrodynamics are the following:

- *By-product buildup.* Algae and cyanobacteria produce O_2 as a by-product of photosynthesis. Excessive O_2 concentrations result in inhibition of nitrogenase and hydrogenase [97,101]. Similarly, excessive buildup of H_2 decreases the production rate in hydrogenase-based systems [101,104]. Methods such as applying partial vacuum [61] and sparging with an inert gas [105] have been suggested to remove H_2 and O_2 . However, these techniques have been considered economically unfeasible at industrial scale [106]. Currently, this challenge is being addressed by microbiologists where O_2 -tolerant enzymes are being isolated/developed and expressed in selected microorganisms [106]. Alternatively, the inhibition of O_2 production by sulfur deprivation of green algae *C. reinhardtii* has been demonstrated [40].
- *Low interfacial area concentration.* Mass transfer from gas to liquid phase requires large gas–liquid interfacial area. As the reactor is scaled up, the surface area available for gas transfer per unit volume of the reactor decreases in nonsparged reactors. This makes CO_2 transfer a limiting factor, during the growth phase, for achieving large cell densities in scaled-up systems having volumes larger than 50 L [55,66]. It also limits the removal of O_2 and H_2 during the H_2 production phase and may result in by-product buildup and reduce the overall efficiency.
- *Sedimentation of microorganisms and nutrients.* This causes limitations on the availability of nutrients and light to the settled microorganisms [27,66]. The photobioreactor can be stirred or sparged with bubbles to keep microorganisms in suspension in addition to alleviating the mass transfer limitations. However, unfavorably high shear can be detrimental to microorganisms during liquid flow and/or bubble collapse [66,107]. In addition, stirring is prohibitively expensive for large-scale systems.

11.5.3 Nutrient Composition

The concentration of both macro- and micronutrients has significant effect on the CO₂ consumption and H₂ production by the microorganisms [55,101,105]. However, the effects of different nutrient concentrations and different media on photobiological hydrogen production and the associated pathways are not known precisely. The optimization of nutrient composition could contribute to increasing the efficiency of the photobiological system. For example, Berberoğlu et al. [108] reported a factor 5.5 increase in hydrogen production rate by *Anabaena variabilis* ATCC 29413 using Allen–Arnon medium compared with BG-11 and BG-11_o media under otherwise identical conditions (light, concentration, pH, temperature). Moreover, the heterocyst frequency was 5%, 4%, and 9% for *A. variabilis* grown in BG-11, BG-11_o, and Allen–Arnon media, respectively. The authors also reported larger specific hydrogen production rates, efficiencies, and cyanobacteria concentrations achieved using Allen–Arnon medium. This was attributed to higher concentrations of magnesium, calcium, sodium, and potassium in the medium. Finally, the presence of vanadium in Allen–Arnon medium could have induced the transcription of vanadium-based nitrogenase that is capable of evolving more hydrogen than the molybdenum-based one. Further research is needed in this area.

11.5.4 Thermal Management

Economic and practical difficulties are faced in maintaining an optimum reactor temperature over night and day and over the season cycle [58,66]. Active temperature control has been considered in pilot systems but this adds cost and decreases the economic viability of the technology [58]. A practical solution for this problem can be the choice of favorable geographic locations or marine systems for which the ocean can act as a temperature bath whose temperature varies slightly over the course of the year. Alternatively, infrared solar radiation that would otherwise heat up the photobioreactor could be filtered before delivering only usable light to the photobioreactor.

11.5.5 Sterility and Monoculture

It is essential that the photobioreactors do not get contaminated by other microorganisms that could compete for light and nutrients, thus, adversely affecting the performance of the system [53]. This becomes a major challenge for large-scale systems. Alternatively, in order to overcome contamination, commercial algae growers have been using strains that survive in harsh environments such as high salinity and/or low pH media where most other microorganisms cannot live [58].

11.5.6 Freshwater Consumption

Many of the algal and cyanobacterial strains considered for CO₂ mitigation and H₂ production grow in freshwater. Once scaled up, such a system may require large quantities of freshwater competing with the resources used for domestic and agricultural needs. Several approaches for overcoming the need for large quantities of freshwater are (1) using trickle-bed, fluidized-bed, and membrane photobioreactors (Figure 11.14) minimizing the water usage [73,74], (2) using saltwater species where water from the oceans can be utilized [109], and (3) using wastewater where more value can also be added to the process through wastewater treatment [37].

11.6 Prospects

To date, the aforementioned limitations still remain challenges to the commercial realization of the technology. Several strategies are being pursued to increase the efficiency of photobiological hydrogen production. First, with the advent of genetic engineering, microorganisms can be engineered to have the desired pigment concentrations, optimum enzymatic pathways and electron transport, as well as reduced O₂ inhibitions. Second, processes and photobioreactors can be designed to increase efficiency by achieving optimum light delivery, maximizing sunlight utilization, and providing ideal conditions for growth and H₂ production. Third, the construction, operation, and maintenance costs of the photobioreactor systems should also be reduced.

11.6.1 Bioengineering of Microorganisms

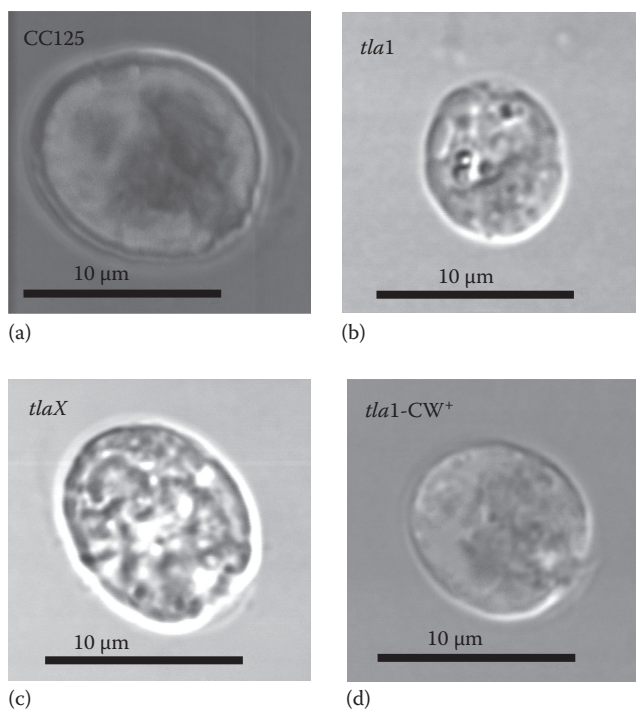
As previously discussed, there are several intrinsic limitations to hydrogen production by the enzymes. These issues are being addressed by microbiologists and genetic engineers. Efforts include (1) truncating the light-harvesting antenna, (2) developing O₂-tolerant enzymes, (3) eliminating the expression of uptake hydrogenase, and (4) inserting proton channels in thylakoid membranes. Each of these approaches is briefly described in detail in the following sections.

11.6.1.1 Truncating the Light-Harvesting Antenna

Microorganisms that are found in nature are not always subjected to optimum illumination. Therefore, as a survival mechanism, they have adapted to produce relatively large amounts of pigments. This maximizes the probability of capturing and utilizing photons at low light intensities. However, when these microorganisms are mass cultured in photobioreactors, they absorb more photons than they can utilize and waste the light energy as heat and fluorescence [102]. In addition, light does not penetrate deep into the photobioreactor. Thus, the quantum efficiency of photobiological hydrogen production decreases. Moreover, high intensities can catalyze the formation of harmful oxides that can damage the photosynthetic apparatus, a process known as photooxidation [28]. Therefore, it is desirable to decrease the chlorophyll antenna size down to the size of the core antenna [100].

Melis et al. [102,110] physiologically reduced the pigment content of the green algae *Dunaliella salina* from 1×10^9 chlorophyll molecules per cell (Chl/cell) to 0.15×10^9 Chl/cell. More recently, Polle et al. [100] genetically engineered the green algae *Chlamydomonas reinhardtii* to have a truncated light-harvesting chlorophyll antenna size. The authors reported that the microorganisms with less pigments had higher quantum yield, photosynthesis rate, and light saturation irradiance [100].

Figure 11.17 shows the *in vivo* differential interference contrast (DIC) and chlorophyll fluorescence micrographs of green algae *C. reinhardtii* CC125 and its truncated chlorophyll antenna transformants *tla1*, *tlaX*, and *tla1-CW⁺* [81]. The images were obtained using a Zeiss 510 confocal scanning laser microscope in the transmission and epifluorescence mode simultaneously as reported by Chen and Melis [111]. The excitation was provided by a helium–neon laser at 543 nm, while the chlorophyll fluorescence emission was detected in the red region with a longpass filter with a cutoff wavelength of 560 nm placed in front of the detector. It illustrates the size and shape of each strain as well as the location of the

**FIGURE 11.17**

DIC and fluorescence micrographs of (a) CC125, (b) *tla1*, (c) *tlaX*, and (d) *tla1*-CW⁺. The scale bars correspond to 10 µm. (From Berberoğlu, H. et al., *Int. J. Hydrogen Energy*, 33(22), 6467, 2008.)

chlorophyll pigments that fluoresce in red [31]. The strong red fluorescence observed in the wild strain CC125 qualitatively shows that it has the largest concentration of chlorophyll while *tlaX* has the least.

11.6.1.2 Oxygen-Tolerant Enzymes

As previously discussed, there have been numerous approaches in overcoming the O₂ inhibition of hydrogen production such as applying partial vacuum [61], sparging with an inert gas [105], and sulfur deprivation [40]. Developing O₂-tolerant enzymes concerns mainly green algae and cyanobacteria as they produce O₂ as a result of oxidation of water during oxygenic photosynthesis. Moreover, it depends on the type of enzyme used by these microorganisms. Green algae use bidirectional [Fe-Fe]-hydrogenase, whereas cyanobacteria use either bidirectional [NiFe]-hydrogenase or nitrogenase.

[Fe-Fe]-hydrogenase in green algae: Hydrogen production by green algae is due to bidirectional [Fe-Fe]-hydrogenase [53]. However, the functioning of this enzyme is irreversibly inhibited by micromolar concentrations of O₂ [106]. Inhibition takes place when O₂ molecules bind to the catalytic site of the enzyme. Based on the structural modeling of the algal hydrogenase, Forestier et al. [112] suggested that inhibition takes place due to the presence of a large gas channel leading to the catalytic center. Figure 11.18 shows the structural model of the *C. reinhardtii* [Fe-Fe]-hydrogenase [112]. The channel enables the formed H₂ molecule to escape. However, due to its large size, it can also let O₂ diffuse to the catalytic center and

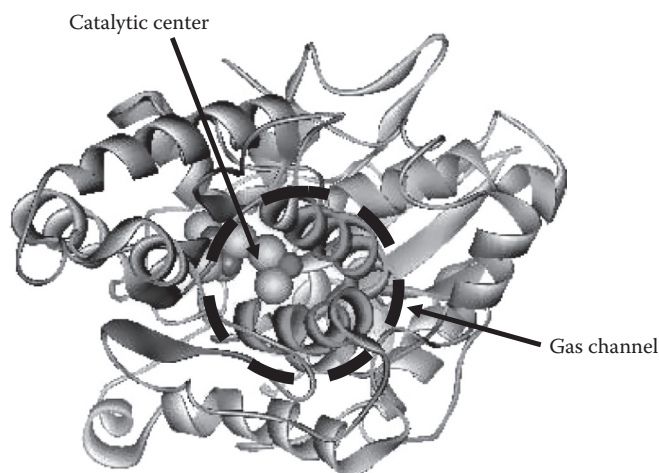


FIGURE 11.18

The structural model of the *C. reinhardtii*'s [Fe-Fe]-hydrogenase enzyme. (From Forestier, M. et al., *Eur. J. Biochem.*, 270(13), 2750, 2003.)

inactivate the enzyme. Strategies to engineer a novel hydrogenase with a steric restriction to O₂ diffusion and express it in algae are being pursued [112]. This will allow the photobiological hydrogen production to take place in air making the process more efficient and less expensive.

[NiFe]-hydrogenase in Cyanobacteria: Another approach for overcoming O₂ inhibition is to identify naturally occurring oxygen-tolerant [NiFe]-hydrogenases in photosynthetic bacteria (e.g., *Rubrivivax gelatinosus* and *Thiocapsa roseopersicina*) and expressing them in selected cyanobacteria (e.g., *Synechocystis* sp. PCC6803) [113,114].

Nitrogenase in Cyanobacteria: The thermophilic filamentous actinobacteria *Streptomyces thermoautotrophicus* synthesize a unique nitrogenase that is both structurally and functionally different from the classic [Mo]-nitrogenase. This unusual nitrogenase is reported to be completely insensitive to O₂ and consumes half the amount of ATP consumed by the classic [Mo]-nitrogenase [30]. However, hydrogen production by this enzyme has not been demonstrated. The expression of such a nitrogenase in cyanobacteria through genetic engineering can prove beneficial for more robust and cost-effective photobiological H₂ production.

11.6.1.3 Eliminating the Expression of Uptake Hydrogenase

Uptake hydrogenase is found in cyanobacteria that produce hydrogen using nitrogenase. It catalyzes the consumption of H₂ in the presence of O₂ to recover energy and produce water [53]. This decreases the net H₂ production rate by cyanobacteria. To address this issue, several researchers have proposed to improve the cyanobacterial H₂ production by eliminating the expression of uptake hydrogenase [46,115,116]. Sveshnikov et al. [46] reported that *Anabaena variabilis* mutant PK84 lacking the uptake hydrogenase had 3–4.3 times larger hydrogen production rates compared with the wild strain. In an independent study, Tsygankov et al. [117] reported that at a dissolved O₂ concentration of 315 μM in the medium, the net hydrogen production rate of the wild strain *A. variabilis* was only 7%

of the control experiment with no dissolved oxygen in the medium. On the other hand, under the same conditions, the mutant PK84 showed 75% of the hydrogen production rate of the same control experiment. Note that the equilibrium concentration of dissolved O_2 in water exposed to air at 1 atm and 25°C is about 250 μM .

11.6.1.4 Inserting Proton Channels in Photosynthetic (Thylakoid) Membranes

The rate of photobiological H_2 production from water is slowed down by inefficient electron coupling to ferredoxin due to the large proton gradient across the algal photosynthetic (thylakoid) membrane [113,118]. Lee [118] proposed that genetic insertion of proton channels in the photosynthetic membrane can decrease the proton gradient and overcome this limitation. Moreover, this will aid in preventing the electrons from participating in the Calvin cycle and in diverting the electron flow to hydrogenases, thus improving the rate of H_2 production.

11.6.2 Process Optimization

Several process optimizations have been considered to further develop photobiological hydrogen production technologies including (1) symbiotic or mixed cultures of different types of microorganisms, (2) controlled and optimum light delivery, and (3) cell immobilization.

11.6.2.1 Symbiotic and Mixed Cultures

To date, the majority of research efforts have concentrated on cultivating single species of microorganisms for photobiological hydrogen production. Among these, cyanobacteria and green algae that utilize solar energy in the spectral range from 400 to 700 nm to produce hydrogen have been studied extensively [119]. On the other hand, purple nonsulfur bacteria have also been identified as potential hydrogen producers that mainly use solar energy in the NIR part of the spectrum from 700 to 900 nm [96]. Note that only about 45% of the total solar radiation is emitted between 400 and 700 nm and an additional 20% is emitted between 700 and 900 nm [50].

Thus, mixed cultivation of green algae and purple bacteria has the potential to achieve higher solar-to-hydrogen energy conversion efficiencies than single cultures by using solar radiation in the spectral range from 400 to 900 nm where 65% of the total solar radiation is concentrated. Such a mixed culture has been demonstrated by Melis and Melnicki [50] where the green algae *C. reinhardtii* were cocultured with the purple bacteria *Rhodospirillum rubrum*. The authors suggested that once the photosynthesis to respiration (P/R) ratio of the green algae is reduced to 1, such a coculture could be used for more efficient photobiological hydrogen production. Currently, the wild-strain algae have a P/R of about 4 [50]. Unfortunately, the purple bacteria also absorb light in the visible part of the spectrum due to the presence of bacteriochlorophyll *b* and carotenoids [80]. Consequently, the two species may compete for light during both the growth and the hydrogen production phases.

Recently, Berberoğlu and Pilon [120] reported a numerical study aiming to maximize the solar-to-hydrogen energy conversion efficiency of a mixed culture containing the green algae *Chlamydomonas reinhardtii* and the purple nonsulfur bacteria *Rhodobacter sphaeroides*. The authors used the radiation characteristics measured experimentally [80,81] as input parameters for calculating the local spectral incident radiation within a flat-panel photobioreactor. Their results show that for monocultures, the solar-to- H_2 energy conversion

efficiency depends only on the optical thickness of the system. The maximum solar energy conversion efficiency of monocultures of *C. reinhardtii* and *R. sphaeroides*, considering the entire solar spectrum, was found to be 0.061% and 0.054%, respectively, corresponding to optical thicknesses of 200 and 16, respectively. Using mixed cultures, a total conversion efficiency of about 0.075% could be achieved corresponding to an increase of about 23% with respect to that of a monoculture of *C. reinhardtii*. The choice of microorganism concentrations for maximum solar energy conversion efficiency in mixed cultures was nontrivial and requires careful radiation transfer analysis coupled with H_2 production kinetics.

Another strategy is to grow symbiotic cultures such as combining purple nonsulfur bacteria and anaerobic fermentative bacteria. For example, Miyake et al. [121] used symbiotic cultures of the anaerobic fermentative bacteria *Clostridium butyricum* and the purple nonsulfur bacteria *R. sphaeroides* to produce H_2 . In this symbiotic culture, the anaerobic bacteria converted sugars to H_2 and organic acids, whereas the purple nonsulfur bacteria converted the organic acids to H_2 . Overall, their symbiotic system produced 7 mol of H_2 per mole of glucose.

11.6.2.2 Advanced Light Delivery Systems

The saturation irradiance of a photosynthetic apparatus is on the order of 5000–6000 lux [108]. This corresponds to about one-tenth of the total solar irradiance where the rest of the energy is wasted as heat and fluorescence. Thus, light can be delivered to a 10 times larger surface area using solar collectors and lightguides to enhance the solar energy utilization efficiency. To do so, cost-effective light delivery technologies need to be developed and integrated into the design of future photobioreactors.

System engineers are designing novel photobioreactors that collect and deliver solar light in a controlled manner within the photobioreactor [34,73,109,122–124]. These systems usually involve a heliostat comprised of either Fresnel lenses [109] or reflective dishes [34,73] that concentrate the solar radiation to be distributed via fiber optics or lightguides. The lightguides are made of glass or acrylic and can deliver sunlight deep into the photobioreactors by total internal reflection. At desired locations, the lightguides have rough surfaces and light *leaks* out providing the desired irradiance. In some elaborate designs, LEDs are also incorporated into the lightguide delivery system to provide artificial light to the microorganisms at night [74]. Lightguides and fiber optics have been used to increase the light irradiance in the center of photobioreactors where it is typically the smallest [124]. However, this technology is judged too costly to be adopted at industrial scale [98].

Alternatively, Kondo et al. [98] proposed the simultaneous culture of the purple nonsulfur bacteria *Rhodobacter sphaeroides* RV and its reduced pigment mutant MTP4 in two separate but stacked plate-type photobioreactors. MTP4 produces H_2 more efficiently under large irradiance, while *R. sphaeroides* RV is more efficient under low irradiance. The authors showed that two stacked flat-plate photobioreactors with MTP4 in the front reactor facing the light source and *R. sphaeroides* RV in the rear reactor produced more H_2 than any other configuration. The front reactor acted as an absorption filter to the second.

11.6.2.3 Immobilized Cell Photobioreactors

In order to achieve high H_2 production rates, Markov et al. [61] immobilized *A. variabilis* on hollow fibers. They operated the photobioreactor in two stages alternating between (1) growth and (2) H_2 production phases. The authors reported a CO_2 consumption rate of 7000 mmol/kg dry cell/h and an H_2 production rate of 830 mmol H_2 /kg dry cell/h.

Moreover, Bagai and Madamwar [125] immobilized a mixed culture of the nonheterocystous cyanobacteria *Phormidium valderianum*, the halophilic bacteria *Halobacterium halobium*, and the hydrogenase containing *Escherichia coli* in polyvinyl alcohol (PVA) alginate beads for prolonged production of H₂. The authors demonstrated H₂ production by the mixed culture for over 4 months.

More recently, Laurinavichene et al. [126] immobilized *C. reinhardtii* on glass mesh cloth in an enclosed photobioreactor of a total volume of 160 mL. The immobilized system enabled easy switch between sulfur-containing and sulfur-depleted media during growth and H₂ production stages, respectively. The authors reported a maximum hydrogen yield of 380 mL over 23 days with a maximum H₂ production rate of 45 mL/day. The immobilized cell system prolonged the H₂ production up to 4 weeks compared with suspended cell systems investigated by Kosourov et al. [127].

11.7 Economic and Environmental Considerations

Photobiological hydrogen production aims to produce hydrogen in an environmentally friendly and sustainable manner. Thus, its environmental impacts must be discussed in terms of toxicity, water usage, and lifecycle analysis. Economic considerations are also essential to assess the feasibility of the technology and ensure it provides a viable and competitive alternative to fossil fuel or other H₂ production technologies.

11.7.1 Economic Analysis

The US Department of Energy (DOE) set a hydrogen cost goal of \$2.00–\$3.00 (2005 US dollars) per kilogram of delivered and untaxed H₂ by 2015 [128]. For comparison with gasoline cost, note that the energy contained in 1 kg of H₂ is equivalent to that contained in 1 gal of gasoline. The average 2002 price for compressed hydrogen gas produced from fossil fuel and delivered in tube trailers was \$11.0/kg of H₂. The price of pipeline-delivered compressed-H₂ merchant hydrogen ranged from \$0.8 to \$3.4/kg of H₂ in 2003 [129]. Prices for commercial hydrogen have risen steadily in recent years primarily due to growing demand in the refinery sector and increase in oil and natural gas prices. Similarly, the price of gasoline has increased significantly and may make hydrogen more competitive if produced from renewable energy. For example, the cost of H₂ produced by wind electrolysis was \$5.90/kg of H₂ in 2006 [10].

Economic analysis of photobiological hydrogen production considers (1) the construction and maintenance costs of the photobioreactor; (2) the operating cost including labor, power, and water supplies for mixing, periodic cleaning, and powering compressors, for example; (3) the purification of hydrogen gas and its compression for transportation or storage; (4) land purchase; and (5) daily solar irradiance of the site. It was estimated that to achieve a 10% return on investment, the photobioreactor cost should be less than \$165/m² of footprint, for a system having 10% light-to-H₂ energy conversion [57]. Moreover, to be economically viable, the system should achieve conversion efficiencies larger than 10% [57]. Note that theoretically, H₂-producing microorganisms have a maximum light-to-H₂ efficiency ranging between 16% and 41% depending on the metabolic pathway used [53].

Photobioreactor cost is a major contributing factor to the cost of photobiological H₂ production and the most important parameter to the economic feasibility of the

technology [57,130]. For example, it was estimated that the price of a photobioreactor could be in excess of \$100/m² if glass or plexiglass were used as transparent windows. It could be reduced to about \$1/m² if low-density polyethylene (LDPE) films were used [130]. Amos [130] estimated the selling price of H₂ at \$ 2.04/kg for hydrogen delivered via a pipeline and produced by *C. reinhardtii* at a concentration of 0.2 g/L in a 10 cm deep photobioreactor and accounting for a 15% return on investment. However, the price can rise up to \$13.53/kg for purified and compressed H₂ at 2 MPa for a photobioreactor cost of \$10/m². In this case, the compression and storage cost alone contributed \$2.25/kg. The author acknowledged that the algal hydrogen production rate was the maximum rate biologically possible that is much larger than that achieved in practice even in benchtop photobioreactors. Finally, cost associated with energy consumption for mixing and gas injection was stated not to exceed 2 W/m² [76].

The annual average solar irradiance (over 24 h and over 365 days) at favorable locations such as the southwestern United States is about 210 W/m² [131]. In addition, fuel cells running on hydrogen have fuel efficiency of about 30%–50%, whereas internal combustion engines using gasoline are only about 30% efficient. Thus, a hydrogen refueling station providing similar service to public will have to supply about one-third the fuel supplied by a gasoline station. A light-to-hydrogen conversion efficiency of 10% would represent a production rate of 5.45 kg of H₂/m²/year. To put this in perspective, it would require a surface area of 54,000 m² or 14 ac to supply the equivalent of 800,000 gal of gasoline sold every year by one of the 168,000 gasoline service stations in the United States [53]. Further improving the efficiency of this technology through photobioreactor design and genetic engineering would reduce the footprint requirement.

Moreover, to achieve sustainable energy production, the total energy used to build the system should be much lesser than that produced by the system during its entire lifetime. Thus, the service lifetime and the energy cost of materials used for building the photobioreactors should be considered in addition to their financial cost. Burgess and Fernandez-Velasco [132] defined the so-called net energy ratio (NER) as the ratio of the higher heating value of the produced hydrogen to the total primary energy input into the construction of the system. The authors reported that for tubular photobioreactors, LDPE film and glass have significantly higher NER than rigid polymers such as polymethyl methacrylate (acrylic) [132]. Similar lifecycle analysis should be performed for other novel photobioreactor designs to assess their sustainability.

In brief, photobiological hydrogen production is at a very early stage of development. It currently does not constitute an economically viable hydrogen production method and needs additional basic and applied research to approach practical efficiency and production rates. Thorough economic analysis has to be performed for various organisms and photobioreactor design in order to assess the viability in the short run and the sustainability in the long run of photobiological hydrogen production. More realistic economic analysis will require operation, maintenance, and field data from pilot photobioreactor systems taking into account the seasonal performance variations.

11.7.2 Environmental Impacts

Some strains of cyanobacteria are known to produce toxins that are harmful to human and animal health such as anatoxins, microcystins, or saxitoxins [133]. Most algae species are harmless to animals and humans. These toxins can cause acute or chronic illnesses such as gastroenteritis, adverse respiratory effects, skin irritations, allergic responses,

and liver damage [134]. In addition to producing toxins, freshwater cyanobacteria can cause nuisance effects such as excessive accumulations of foams and scums and discoloration of water [135].

Moreover, current photobiological hydrogen production would require large amounts of water if the benchtop processes were scaled up for industrial production. This would create large demand for freshwater that would compete with domestic and agriculture uses both of which are scarce in regions with high solar irradiance. One may argue, however, that the system would be closed and water would simply need to be supplied to compensate for evaporation and separation needs. Thus, water consumption for H₂ production is likely to be much lesser than that of agricultural crops. In addition, photobioreactors designed to be constructed on land are likely to compete with land use for humans, forests, or agricultural use.

Alternatively, wastewater or seawater in combination with cyanobacteria and marine algae could be used to reduce demand on freshwater and land space. Marine green algae *Chlorococcum littorale* and marine cyanobacteria *Oscillatoria* sp. as well as *Miami BG7*, *Anabaena cylindrical* B-629, and *Calothrix scopulorum* 1410/5 are known hydrogen producers [77]. Marine-based systems benefit from (1) thermal regulation of the ocean preventing the overheating of the photobioreactors under direct sunlight and (2) agitation of the reactor fluid that could be achieved by the ocean waves. Some of the major issues concerning the development of marine photobioreactors include (1) contingency engineering for possible microorganism leakage into the ocean, (2) durability of the materials used in marine environment, and (3) contamination and damage to the photobioreactors by the marine animals and microorganisms. Thus, research should be directed to address these issues for developing cost-effective marine photobioreactors.

Finally, public perception and potential fear associated with the use and possible release of genetically modified microorganisms in the environment will need to be addressed. Public reaction is expected to vary from one region of the world to another. However, it may constitute a major obstacle in some countries. Past experiences with genetically modified crops (e.g., corn or soybean) can constitute a valuable reference and provide useful lessons.

11.8 Conclusion

This chapter presented the current state of knowledge in photobiological H₂ production as well as CO₂ fixation. It provided the reader with a basic background in the microbiology of photosynthesis and photobiological H₂ production. Then, photobioreactor designs, operations, performances, and simulation tools were reviewed. The challenges associated with the technology were discussed followed by strategies to overcome the biological barriers and to optimize the process. Finally, economic analysis and potential environmental impacts were presented. In brief, photobiological hydrogen production is at a very early stage of development and requires additional basic and applied research efforts. However, progresses from genetic engineering to innovative photobioreactor designs with advanced light delivery and reduced water consumption are promising. If successful, this technology can offer a long-term solution for sustainable hydrogen production. It can also alleviate concerns over energy security with the advantage of capturing CO₂.

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