Introduction

Today global energy requirements are mostly dependent on fossil fuels (about 80% of the present world energy demand). This will eventually lead to the foreseeable depletion of limited fossil energy resources. Presently, the utilization of fossil fuels are causing global climate change mainly due to the emission of pollutants like COx, NOx, SOx, CxHx, soot, ash, droplets of tars andother organic compounds, which are released into the atmosphere as a result of their combustion [1]. Hydrogen has the highest energy content per unit weight of any known fuel and can be transported for domestic/industrial consumption through conventional means. H2 gas is safer to handle than domestic natural gas. H2 is now universally accepted as an environmentally safe, renewable energy resource and an ideal alternative to fossil fuels that doesn't contribute to the greenhouse effect. The only carbon-free fuel, H2 upon oxidation produces water alone.

Biological Hydrogen Production Methods

Biological hydrogen production methods can be classificated as below:

2.1. Direct biophotolysis

2.2. Indirect biophotolysis

2.3. Photo fermentation

2.4. Dark fermentation

2.5. Two stage process (integration of dark and photo fermentation)

2.6. Biocatalyzed electrolysis

2.1. Direct biophotolysis

This method is similar to the processes found in plants and algal photosynthesis. In this process solar energy is directly converted to hydrogen via photosynthetic reactions (Eq. (1)).

$$2H_2O + \text{'light energy'} \rightarrow 2H_2 + O_2. \quad (1)$$

Algae split water molecules to hydrogen ion and oxygen via photosynthesis. The generated hydrogen ions are converted into hydrogen gas by hydrogenase enzyme. *Chlamydomonas reinhardtii* is one of the well-known hydrogen producing algae [4]. Hydrogenase activity has also been observed in other green algae like *Scenedesmus obliquus*, *Chlorococcum littorale*, *Platymonas subcordiformis* and *Chlorella fusca* [2].

The advantage of this method is that the primary feed is water, which is inexpensive and available almost everywhere (Table 1).

A direct biophotolysis method must perforce operate at a partial pressure of near one atmosphere of O_2, which is a thousand-fold greater than the maximum likely to be tolerated. Thus, the O_2 sensitivity of the hydrogenase enzyme reaction and supporting reductant generating pathway remains the key problem, as it has been for the past 30 years [5].

Hydrogen production by direct photolysis using green algae is currently limited by three parameters: (i) solar conversion effciency of the photosynthetic apparatus; (ii) H_2 synthesis processes (i.e. the need to separate the processes of H_2O oxidation from H_2 synthesis); and (iii) bioreactor design and cost. A number of approaches to improve H_2 production by green algae are currently under investigation. These include genetic engineering of light gathering antennae, optimization of light input into photobioreactors, and improvements to the two-phase H_2 production systems used with green algae [6]. In direct biophotolysis, hydrogen production rates of the order of 0.07 mmol/h per liter has been reported in the literature (Table 2) [7,8].

2.2. Indirect Biophotolysis

In indirect biophotolysis, problems of sensitivity of the hydrogen evolving process are potentially circumvented by separating temporally and/or spatially oxygen evolution and hydrogen evolution. Thus indirect biophotolysis processes involve separation of the H_2 and O_2 evolution reactions into separate stages, coupled through CO_2 fixation/evolution. Cyanobacteria have the unique characteristics of using CO_2 in the air as a carbon source and solar energy as an energy source (Eq. (2)). The cells take up CO_2 first to produce cellular substances, which are subsequently used for hydrogen production (Eq. (3)). The overall mechanism of hydrogen production in cyanobacteria can be represented by the following reactions:

$$12H_2O + 6CO_2 + \text{'light energy'} \rightarrow C_6H_{12}O_6 + 6O_2 \quad (2)$$
$$C_6H_{12}O_6 + 12H_2O + \text{'light energy'} \rightarrow 12H_2 + 6CO_2 \quad (3)$$

Cyanobacteria possess key enzymes (nitrogenase and hydrogenase) that carry out metabolic functions in order to achieve hydrogen generation [9]. Because of the higher rates of H_2 production by *Anabaena* species and strains, these have been subject to intense study [6]. In indirect biophotolysis mutant strains of *A. Variabilis* have demonstrated hydrogen production rate of the order of 0.355 mmol/h per liter [10].

2.3. Photo fermentation

H_2 production by purple non-sulfur bacteria is mainly due to the presence of nitrogenase under nitrogen-deficient conditions using light energy and reduced compounds (organic acids). The reaction is as follows (Eq. (4)) [2]:

$$CH_3COOH + 2H_2O + \text{'light energy'} \rightarrow 4H_2 + 2CO_2$$

Photosynthetic bacteria have long been studied for their capacity to produce significant amounts of hydrogen. The advantage of this method are that oxygen does not inhibit the process [2]. These photoheterotrophic bacteria have been found suitable to convert light energy into H2 using organic wastes as substrate [11,12,13] in batch processes [14], continuous cultures [15], or immobilized whole cell system using different solid matrices like carrageenan [16], agar gel [17], porous glass [11], and polyurethane foam [12].

2.4. Dark fermentation

Hydrogen can be produced by anaerobic bacteria, grown in the dark on carbohydrate-rich substrates. Bacteria known to produce hydrogen include species of *Enterobacter*, *Bacillus*, and *Clostridium* [6]. Carbohydrates, mainly glucose, are the preferred carbon sources for fermentation processes, which predominantly give rise to acetic and butyric acids together with hydrogen gas [25]. Theoretically bioconversion of 1 mol of glucose yields 12 mol of hydrogen gas (H_2). According to reaction stoichiometry, bioconversion of 1 mol of glucose into acetate yields 4 mol H_2/mol glucose (Eq. (6)), but only 2 molH_2/mol glucose is formed when butyrate is the end product (Eq. (7)) [4]. Currently fermentative processes produce 2.4 to 3.2 moles of hydrogen per mole glucose [26].

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \tag{6}$$
$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3CH_2COOH + 2CO_2 + 2H_2 \tag{7}$$

While direct and indirect photolysis systems produce pure H2, dark fermentation processes produce a mixed biogas containing primarily H2 and carbon dioxide (CO2), but which may also contain lesser amounts of methane (CH4), CO, and/or hydrogen sulfide (H2S). Dark-fermentation proves to be superior over photo-fermentation as this requires no light and the energy produced is relatively higher, due to the fermentation of sugar and carbohydrates. The process is initiated by the hydrolysis of organic polymers to monomers, thereafter acetogenic conversion of monomers to organic acids, alcohols, and release of hydrogen. Although biohydrogen production by dark fermentation is promising and advantageous over photo-fermentation [27]. However, the requirement of organic biomass as a feedstock makes this process quite expensive [28].

Table 1. Advantages and disadvantages of different hydrogen production processes [2].

Process	Advantages	Disadvantages
Direct biophotolysis	Can produce H_2 directly from water and sunlight Solar conversion energy increased by ten folds as compared to trees, crops	Requires high intensity of light O_2 can be dangerous for the system Lower photochemical efficiency
Indirect biophotolysis	Cyanobacteria can produce H_2 from water Has the ability to fix N_2 from atmosphere	Uptake hydrogenase enzymes are to be removed to stop degradation of H_2 About 30% O_2 present in gas mixture
Photo-fermentation	A wide spectral light energy can be used by these bacteria Can use different organic wastes	O_2 has an inhibitory effect on nitrogenase Light conversion efficiency is very low, only 1–5%
Dark fermentation	It can produce H_2 all day long without light A variety of carbon sources can be used as substrates It produces valuable metabolites such as butyric, lactic and acetic acids as by products It is anaerobic process, so there is no O_2 limitation problem	O_2 is a strong inhibitor of hydrogenase Relatively lower achievable yields of H_2 As yields increase H_2 fermentation becomes thermodynamically unfavorable Product gas mixture contains CO_2 which has to be separated