Research Journal of Applied Sciences, Engineering and Technology 12(9): 968-982, 2016
DOI:10.19026/rjaset.12.2815
ISSN: 2040-7459; e-ISSN: 2040-7467

Submitted: December 9, 2015 Accepted: February 10, 2016 Published: May 05, 2016

Review Article

Developments in Bio-hydrogen Production from Algae: A Review

N. Saifuddin and P. Priatharsini
Centre for Renewable Energy, Universiti Tenaga Nasional, Jalan IKRAM-UNITEN, 43000 Kajang, Selangor, Malaysia

Abstract: Diversification of biofuel sources has become an important energy issue. Bio-hydrogen production from microalgae has received much attention recently. However, commercial production of microalgae biofuels including bio-hydrogen is still not feasible due to the low biomass concentration and costly downstream processes. It has been reported that exposing some species of algae to environmental stress, e.g., by depriving the algae of sulfur in light, it is possible to produce significant amounts of hydrogen gas. However, this technology is still in its infancy and there is significant potential for technology development and improvement at every level. This review discusses the biological hydrogen production by microalgae (direct bio-photolysis, indirect bio-photolysis, photo fermentation and dark fermentation) and optimization of key parameters to enhance hydrogen production. The effects of different stress reactions on production of the valuable components are described. This knowledge can be used to evaluate the possibilities for producing hydrogen and high value products efficiently in the same process. Further studies of these topics may result in a sustainable process where solar energy can be converted into hydrogen in an integrated manner, where production efficiencies are sufficient for an economic exploitation of algal technology using algal stress reactions.

Keywords: Algal stress reactions, bio-hydrogen, hydrogenase, microalgae, microwave irradiation

INTRODUCTION

Our current energy consumption worldwide is in the proximity of 15 TW, while the energy consumption rate in 2050 has been estimated to be at least 27 TW (Lewis and Nocera, 2006). The majority of this energy is at the moment obtained from fossil fuels and any change requires improved technology for use of alternative energy sources. Fossil fuels are non-renewable energy source and also have seriously negative impacts on the environment. The use of fossil fuels cause excessive global climate change because emissions of greenhouse pollutants and the formation of compounds COx, NOx, SOx, CxHy, ash and other organic compounds that are released into the atmosphere as a result of combustion. The atmospheric concentration of carbon dioxide has been rising extensively since the Industrial Revolution and has now reached dangerous levels not seen in the last 3 million years (Le Quéré *et al*., 2012). Global warming is caused by the emission of greenhouse gases. 72% of the totally emitted greenhouse gases are Carbon Dioxide (CO_2), 18% Methane (CH_4) and 9% Nitrous Oxide (NOx). Carbon dioxide emissions therefore are the most important cause of global warming. CO_2 is inevitably created by burning fuels like fossil oil, natural gas, diesel and petrol. The increase in greenhouse gas emission will result in global warming, climate change, environmental degradation and health problems (Quadrelli and Peterson, 2007; Shaishav *et al*., 2013). The carbon dioxide is released to the atmosphere where it remains for 100 to 200 years. This leads to an increasing concentration of carbon dioxide in our atmosphere which in turn causes the average temperature on Earth to rise. Recent investigations have shown that inconceivable catastrophic changes in the environment will take place if the global temperatures increase by more than 2°C (3.6°F). A warming of 2°C (3.6°F) corresponds to a carbon dioxide (CO_2) concentration of about 450 ppm (parts per million) in the atmosphere (Momirlan and Veziroglu, 2005).

Reducing demand for energy intensive services, improving the efficiency of energy usage and development of renewable energy resources, must all combine to alleviate the crises of fossil fuel depletion, global warming and environmental degradation. It is important to develop an alternative energy sources that are clean, renewable and environmentally friendly for future world's stability (Melis and Happe, 2001). There is no doubt that solar energy is the largest sources of

Corresponding Author: N. Saifuddin, Centre for Renewable Energy, Universiti Tenaga Nasional, Jalan IKRAM-UNITEN, 43000 Kajang, Selangor, Malaysia

Table 1: Comparison of various hydrogen production processes-advantages and disadvantages (Karthic and Shiny, 2012; Das and Veziroglu, 2008)

Process	Advantages	Disadvantages
Solar gasification	Good hydrogen yield	Effective solar collector plates are required.
Thermo-chemical gasification	Higher conversion can be achieved.	Gas conditioning and tar removal is to be done.
Pyrolysis	Gives carbonaceous material with bio-oil, chemicals and minerals	Catalyst deactivation will occur
Supercritical conversion	Sewage sludge can be used easily, difficult by gasification H_2 can be produced directly from water and sunlight.	Selection of supercritical medium
Direct bio-photolysis	Solar conversion energy increased by ten folds as compared to trees, crops.	Requires high intensity of light, low photochemical efficiency and O_2 is inhibitory
Photo-fermentation	A wide spectral energy can be used by photosynthetic bacteria. Can use different organic waste	O_2 is inhibitory on nitrogenase enzyme and light conversion efficiency is low.
Dark fermentation	It can produce H_2 without light. No oxygen limitations and can produce several metabolites as by-products. Various substrates can be used in this anaerobic process.	Relatively lower H_2 yield. At higher H_2 yield, process becomes thermodynamically unfavorable. Product gas mixture contains CO_2 which has to be separated
Indirect bio-photolysis	Can produce relatively higher H_2 yield. By-products (metabolites) can be efficiently converted to H_2. Has the ability to fix N_2 from atmosphere	Requires continuous light source which is difficult for large scale processes. Uptake hydrogenase enzymes are to be removed to stop degradation of H_2. 30% O_2 present in gas mixture

renewable energy that we know of today. The different fields of technology for use of solar radiation include chemical/physical methods like photovoltaic, concentrating solar power, thermovoltaic, photochemical and thermochemical and use of biological approaches such as artificial photosynthesis and bio-photolysis (Rajeshwar *et al*., 2008). Practical use of solar energy requires conversion of the energy into an energy carrier and one of the promising candidates for alternative energy carriers is hydrogen.Hydrogen is seen by many as the fuel of the future because it has a very high energy density, three times that of petrol or diesel and because its use produces only water instead of greenhouse gases and other exhaust pollutants. Furthermore, using petrol and diesel in combustion engines waste at least two thirds of the energy in the fuel, whereas hydrogen can be used in fuel cells, which are about twice as efficient. Hydrogen is one of the most abundant elements in the world that accounts for 75% of the universe mass. It is a colorless, odorless, tasteless and a non-poison gas (Johnston *et al*., 2005). Currently, hydrogen is produced using non-renewable technologies such as steam reformation of natural gas (~50% of global H_2 supply), petroleum refining (~30%) or the gasification of coal (~20%). However, the viability of a future H_2 economy depends entirely upon the development of efficient, large-scale and sustainable H_2 production systems. The development of H_2 technologies has been given high priority in the European Union, the USA, Japan and China. This review intensely discusses the various approaches of photosynthetic hydrogen production from microorganism particularly microalgae. It explores the potential for using various technologies for producing bio-hydrogen from solar energy using algae. Hydrogen produced through the action of living organisms is called bio-hydrogen.

Bio-hydrogen production: Hydrogen holds a promise as a potential clean, renewable and environmental friendly energy source. Currently 95 to 99% of hydrogen are produced from fossil fuel (Shaishav *et al*., 2013; Jo *et al*., 2006). The classical methods of producing hydrogen include steam reforming of natural gases, coal gasification and electrolysis of water (Jo *et al*., 2006). Conventional hydrogen gas production methods are energy intensive processes requiring high temperatures (>840°C) and not environmental friendly (Shaishav *et al*., 2013; Hsia and Chou, 2014). Electrolysis of water, although the cleanest technology for hydrogen gas production, can only be used in areas where electricity is cheap because electricity accounts for 80% of the operating cost of H_2 production (Karthic and Shiny, 2012). Recent reviews on hydrogen indicated that the worldwide need for hydrogen is increasing with a growth rate of nearly 12% per year for the time being and contribution of hydrogen to total energy market will be 8-10% by 2025 (Lemus and Duart, 2010). The advantages and disadvantages of various hydrogen production processes are outlined in Table 1 (Karthic and Shiny, 2012).

Bio-hydrogen is ideal as it can be operated at ambient temperature and pressure with minimal energy consumption and are more environmental friendly. Bio-hydrogen production methods can be broadly categorized into four primary groups (Fig. 1). Brief description of these processes is given in the later section.

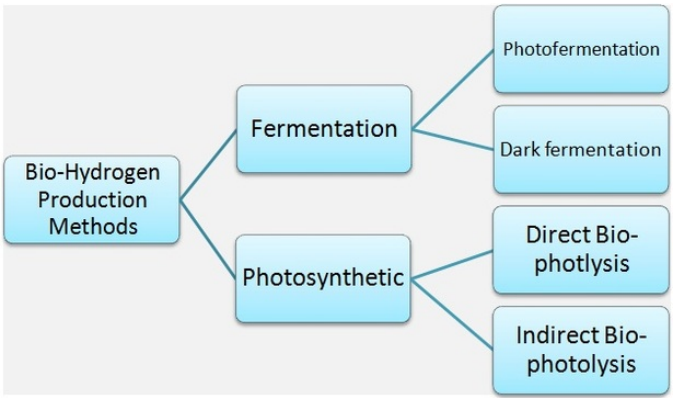

Fig. 1: Bio-hydrogen production methods

Table 2: Microorganisms that has been studied for bio-hydrogen production

Broad classification	Name of Mircoorganism	Reference
Green algae	*Chlamydomonasreinhardii*	Winkler *et al*. (2002)
Cyanobacteria (Blue-green algae)	*Chlamydomonasmoewusii*	Winkler *et al*. (2002)
	Scenedesmus obliquus	Winkler *et al*. (2002)
Heterocytes	*Anabaena variabilis*	Liu *et al*. (2006)
Non-Heterocytes	*Anabaena cylindrical*	Kumazawa and Mitsui (1981)
	Oscillotoria Miami BG7	
Photosynthetic bacteria	*Rhodobactersphaeroides*	Kars *et al*. (2006)
	Rhodobactercapsulates	Öztürk *et al*. (2006)
	Rhodobacterpalustris	Chen *et al*. (2007)
	Rhodospirillumrubnum	Younesi *et al*. (2008)
Fermentative bacteria	*Enterobacter aerogenes*	Fabiano and Perego (2002)
	Enterobacter cloacae IIT-BT 08	Kumar and Das (2000)
	Clostridium butyricum	Fang *et al*. (2006)
	*Citrobacter*spY19	Oh *et al*. (2003)
	Bacillus coagulans	Kotay and Das (2007)
	Clostridium acetobutylicum ATCC 824	Zhang *et al*. (2006)

Bio-hydrogen holds a promise as a potential clean, renewable and environmental friendly energy source. There are three classes of biofuels: -First generation-made from food crops; Second generation-made from non-food crops or wastes; and Third generation-made using microbes. Third generation biofuels have several advantages over 1st and 2nd generation biofuels. Whereas first generation biofuels have caused increases in food prices, third generation biofuels would not. In comparison to second generation biofuels, third generation biofuels could capture sunlight energy 10 times more efficiently, meaning that smaller areas or land are needed to produce enough fuel (Shaishav *et al*., 2013; Momirlan and Veziroglu, 2005). Many types of microbe can convert renewable energy sources into hydrogen. Bio-hydrogen is particularly attractive because of the excellent properties of hydrogen as a fuel and because bio-hydrogen is very easy to collect from the bioreactor (Rupprecht *et al*., 2006). Table 2 summarizes the different microorganisms that have been studied for bio-hydrogen production such as green algae, cyanobacteria (blue-green algae), photosynthetic bacteria and fermentative bacteria. Variety of organisms including the archaea, anaerobic and facultative aerobic bacteria, cyanobacteria and lower eukaryotes (i.e., green algae and protists) produce H_2 which may function singly or as a consortium of similar types or mixed cultures (Chandrasekhar *et al*., 2015).

The major biological processes for bio-hydrogen production are bio-photolysis of water by algae, dark fermentation, photo-fermentation of organic materials and the sequential dark and photo-fermentation processes (Das and Veziroğlu, 2001). Microorganisms are able to convert a diverse number of renewable resources into hydrogen (Levin *et al*., 2004). Microbial hydrogen production through the direct fermentation of organic wastes is one of the potential technologies for producing renewable hydrogen that couples the need for waste reduction and byproduct recovery, simultaneously (Show *et al*., 2012). The biological processes of hydrogen production are fundamentally dependent upon the presence of a hydrogen producing enzyme. These enzymes catalyze the chemical reaction $2H^+ + 2e^- \leftrightarrow H_2$. Three enzymes carrying out this reaction are known; nitrogenase, Fe-hydrogenase and NiFe-hydrogenase (Hallenbeck and Benemann, 2002). Fe-hydrogenase enzyme is used in the bio-photolysis processes whereas photo-fermentation processes utilize nitrogenase. Among various hydrogen production processes, microbial/algal (biological) methods are known to be less energy intensive, for it can be carried

Table 3: Summary of features of hydrogen producing organisms (Adapted from: Dasgupta *et al*., 2010)

Micro-Organism	Light–Harvesting Pigments	Photo system	Source of reducing power
Green Algae	Chlorophyll a, b Carotenoids	PSI and PSII	H_2O and/or organic substrate
Blue-green algae	Chlorophyll a Carotenoids Phycobilisome	PSI and PSII	H_2O and/or organic substrate
Purple sulfur bacteria	Bacterio-chlorophyll a/b Carotenoids	Single photo system similar to PSII	H_2S, S^0, $S_2O_3^{-2}$
Purple non-sulfur bacteria	Bacterio-chlorophyll a/b Carotenoids	Single photosystem similar to PSII	Organic acids
Green sulfur bacteria	Chlorosomes, that containBacterio-chlorophyll a and either Bacterio-chlorophyll c, d, or e.	Single photosystem similar to PSI	H_2S, S^0, $S_2O_3^{-2}$
Green gliding bacteria	Chlorosomes with Bacterio-chlorophyll c/d + Bacterio-chlorophylla.	Single photosystem similar to PSII	-

out at ambient temperature and pressure. The type of light harvesting pigments, photosystems, source of reducing power and enzyme systems involved invarious phototrophic hydrogen production by the organism's aresummarized in Table 3 (Dasgupta *et al*., 2010).

Algae and microalgae: Algae have received a great deal of attention as a novel biomass source for the generation of renewable energy. Algae are both unicellular and multi cellular autotrophic aquatic life forms. The unique feature of algae from the other entire microorganism is that they contain chlorophylls (chlorophyll a and b) which are usually found in higher plants. Chlorophyll is an important feature for photosynthesis which enables algae to absorb energy from light to fuel the manufacture of various biomasses. They are the most robust organism on earth as they are able to grow in a variety of habitats (Shaishav *et al*., 2013). Other components of algae are nucleus, cell wall, chloroplast containing accessory pigments, pyrenoid and adense region containing starch granules on its surface, stigma and flagella (Pelczar *et al*., 2008). Algae are generally divided into two groups, which are macroalgae and microalgae. Both groups of algae do not have roots, stems and leaves. Macroalgae, (or seaweeds) are photoauxotrophic organisms that are able to produce and store organic carbons by utilizing CO_2 and HCO_3 (Chung *et al*., 2011). Macroalgaeare photosynthenic large celled organisms that can be seen without the aid of a microscope. They are classified based on their pigmentations and fall into four basic groups: blue-green algae (Cyanophyta/Cyanobaare often associated with blooms in rivers; green algae (Chlorophyta) such as sea lettuce; the brown algae (Heterokontophyta); and the red algae (Rhodophyta) most diverse group of all. (Sambusiti *et al*., 2015). Macroalgae has low contains of proteins and lipids but have high contents of carbohydrates and water (Sambusiti *et al*., 2015).

Microalgae are small microscopic aquatic photosynthetic unicellular or simple-multicellular microorganism that cannot be seen by the naked eye. They are small free floating organisms and come in different size, shape and color. Microalgae can be grouped into prokaryotic microalgae (cyanobacteria Chloroxybacteria), eukaryotic microalgae (green algaeChlorophyta), red algae (Rhodophyta) and diatoms (Bacillariophta) (Sambusiti *et al*., 2015). They are able to tolerate and adapt to a wide variety of environmental conditions (pH, temperature, light, *etc*.) and can be produced all year round (Uggetti *et al*., 2014). Moreover when cultured at optimal conditions, they are able to double in number within hours, thus permitting a short harvesting cycle (Razeghifard, 2013). Unlike macroalgae, microalgae are mainly composed of proteins (40-60%), carbohydrates (8-30%), lipids (5-60%) and other valuable components (pigments, anti-oxidants, fatty acids and vitamins) (Uggetti *et al*., 2014). Microalgae are the principal producers of oxygen in the world and exhibit enormous potential. Microalgae cultivation is an efficient option for the reduction of CO_2 from gaseous effluent and from the atmosphere (Chisti, 2007). The productivity per unit area of microalgae is high compared to conventional processes for the production of raw materials for biofuels and microalgae represent an important reserve of oil, carbohydrates, proteins and other cellular substances that can be technologically exploited (Chisti, 2007; Gressler *et al*., 2012).

The microalgae biomass can produce biodiesel, bioethanol, biogas, bio-hydrogen and bio-oils (Fig. 2). Microalgae, although having simple structure, have a high photosynthetic efficiency with a growth doubling time as short as 24 h. Moreover, microalgae can be produced all year round. The species abundance and biodiversity of microalgae over a broad spectrum of climates and geographic regions make seasonal and geographical restrictions much less of a concern compared with other lipid feedstocks. The limitations of H_2 production by microalgae are mainly the absence of large scale method, low yield and energy conversion efficiency and inhibition of hydrogenase by the oxygen, by-product of photolysis. Sulfur deprivation is a key to avoid hydrogenase inhibition by oxygen. Under this condition, oxygen evolution is declined below respiration level and an anaerobic atmosphere is formed and hydrogenase may be kept active (Zhu *et al*., 2014; Zhang *et al*., 2014). In depth and important research has been carried out in the field of bio-hydrogen production since the mechanism of hydrogen production by sulfur

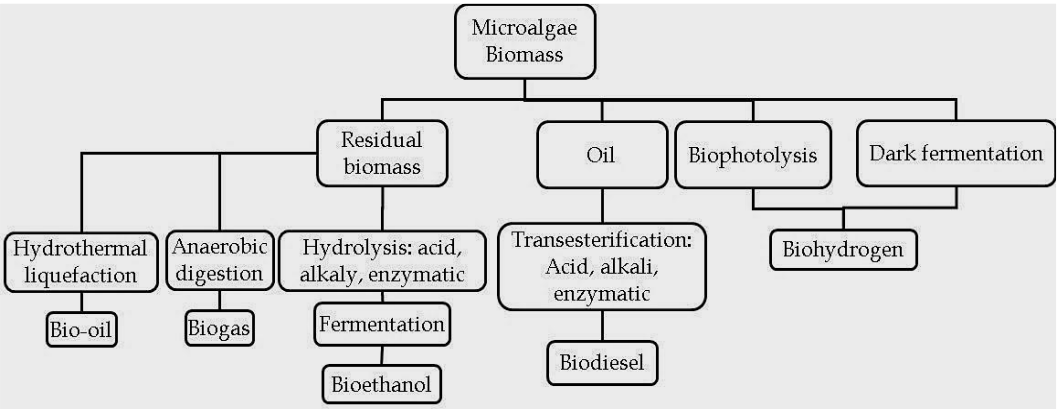

Fig. 2: The major microalgae biomass transformation processes into biofuel

starvation, was discovered (Ghirardi *et al*., 2006; Melis, 2007). Themajority of this research has focused on the model organism *C. reinhardtii*, which is where the process was initially detected.

Hydrogenases in algae: Hydrogen production in green algae is catalyzed by FeFe hydrogenases, which are small, bidirectional enzymes with high activities and very high sensitivity against oxygen (Vignais, 2008). FeFe hydrogenases can be found in both bacteria and algae and parts of the enzyme are very similar between the two groups. While the so called H-cluster part of the enzymes where the active site is located is very similar, the major differenceis the presence of an F-cluster part of the enzyme in bacterial FeFe hydrogenases. This F-cluster is the electron donor to the active site, while in hydrogenases which do not contain the F-cluster, the active site receives electrons directly from ferredoxin. Most algal hydrogenases do not have an F-cluster.

Exposure to oxygen leads to a complete and irreversible inactivation of algal FeFe hydrogenase by destruction of the (4Fe-4S) domain of the active site H-duster (Erbes *et al*., 1979; Stripp and Happe, 2009). This sensitivity against oxygen represents a challenge when the goal is to produce hydrogen from solar energy using the photosynthetic apparatus. Oxygen sensitivity of algal hydrogenases is an important topic which is being explored from many angles. The sulfur deprivation approach, which is common method employed to enhance hydrogen production leads to anaerobic conditions in the culture by a partial inactivation of the oxygen producing PSII, thereby providing an environment for efficient hydrogen production. While promising for the production of clean and sustainable bio-hydrogen, these processes requires improvement to be economically viable.

Processes of bio-hydrogen production: Table 4 gives a brief description of these processes. Although there are striking advantages, the low production rates, low substrate conversion efficiencies and accumulation of acid-rich intermediate metabolites from the acidogenic process are practical hindrances that must be overcome for the successful biological production of H_2. To overcome these limitations, many research projects on the biological production of H_2 are in progress and numerous novel approaches are being studied to address some of the existing problems and to overcome these problems by increasing the efficiency of the process.

Strategies to enhance the bio-hydrogen production: Molecular hydrogen has the potential to become the fuel of the future, but only if it is produced by a sustainable process. Hydrogen production from water photolysis under sunlight would be the cleanest energy conversion process; however, this process is hindered by low hydrogen productivity. New knowledge and technical innovations in hydrogen enzymes, electron carriers, biomaterials and nanotechnology may be able to overcome the intrinsic incompatibility of simultaneous hydrogen and oxygen evolution and splits water into separated gas streams. For a technically feasible hydrogen production with the help of algae, its efficiency must be increased by a factor of about 70 compared to the natural process.

Using green algae as a means of producing bio-hydrogen is a very good alternative as an attractive future energy carrier due to its conversion to energy yielding only pure water and it has the capability of eliminating all the problems that fossil fuels create (Show *et al*., 2012). However, the hydrogen yielded by biological processes is far too low compared to hydrogen produced by current chemical systems (Srirangan *et al*., 2011). Even though substantial progress is continuously being made, there are still many unknown aspects regarding hydrogen production mechanisms and how the efficiency can be improved. A fundamental understanding of this topic at every level is still needed in order to obtain a sustainable system in

Table 4: Summary of different processes of bio-hydrogen production

Process	Description	Reference
Direct biophotolysis	• Biophotolysis is the action of light on biological systems that result in dissociation of water into molecular hydrogen and oxygen; $H_2O \rightarrow H_2 + ½ O_2$.	Johnston *et al*. (2005)
	• The solar energy is directly converted to hydrogen. $2H_2O$+'light energy'$\rightarrow 2H_2+O_2$.	Akkerman *et al*. (2002)
	• Cyanobacteria or green microalgae, can use light to carry out photosynthesis (they possess chlorophyll a and the photosynthetic systems: PSII and PSI). The pigments in PSII (P680) absorb the photons with a wavelength shorter than 680 nm, generating a strong oxidant capable of splitting water into protons (H^+), electrons (e^-) and O_2.	Ghirardi *et al*. (2000, 2006)
	• The electrons reduce the ferredoxin (Fd) and/or nicotinamide adenine dinucleotide phosphate ($NADP^+$) into their reduced forms.	Hallenbeck and Benemann (2002)
	• Under special conditions, the reduced ferredoxin can also be used by hydrogenase or nitrogenase to reduce protons for evolution of molecular hydrogen ($2H^+ + 2Fd^- \rightarrow H_2 + 2Fd$).	Azwar *et al*. (2014)
	• Disadvantage: The enzyme hydrogenase is very sensitive to oxygen (O_2), hence when a certain amount of O_2 are present it will inhibit hydrogenase activity and stops it from producing hydrogen	
	• Advantage: Solar conversion in cyanobacteria or green microalgae is 10 fold more than compared with trees or crops.	
Indirect biophotolysis	• Indirect biophotolysis avoids the inhabitation of hydrogenase by separating the hydrogen production process from the oxygen production process into two stages.	Prince and Kheshgi (2005)
	• At first it involves the splitting of water molecules by sunlight to produce protons and oxygen and at the same time carbon dioxide fixation occurs to produce storage carbohydrate, followed by the production of hydrogen gas by hydrogenase: $12H_2O+6CO_2$+'light energy'$\rightarrow C_6H_{12}O_6+6O_2$	Karthic and Shiny (2012)
	• $C_6H_{12}O_6+12H_2O$+'light energy'$\rightarrow 12H_2+6CO_2$	Momirlan and Veziroglu (2005)
	• Example: Blue-green algae (cynobacteria)	Mathews and Wang (2009)
	• Cyanobacteria that produces hydrogen can either be nitrogen fixing (ex : non-marine Anabaena sp) or non-nitrogen fixing organism (ex : Synechoccussp)	Das and Veziroglu (2008)
	• Advantage: H_2 evolution is separated from O_2 evolution.	
	• Disadvantage: Significant ATP requirement of nitrogenase	
Dark fermentation	• Involves the production of hydrogen in a dark environment without the presence of sunlight, water and oxygen.	Lin and Jo (2003)
	• Fermentative/hydrolytic microorganisms hydrolyzes complex organic polymers to monomers which are further converted to a mixture of lower molecular weight organic acids and alcohols by necessary H_2 producing acidogenic bacteria.	Das and Veziroglu (2008)
	• Anaerobes utilizes glucose as substrate to produce pyruvate and NADH through glycolysis. Oxidation of NADH by Ferredoxin reduction and NADH-ferredoxin reductase are able to produce additional hydrogen. Pyruvate is then oxidized to acetyl-CoA which is then furthered converted to acetyl phosphate resulting in the production of ATP and excretion acetate from which hydrogen can be derived	Vardar-Schara *et al*. (2008)
	• Advantages: Uses a variety of carbon sources. Can produce hydrogen without light. Produces valuable by-products eg. Butyric acid, lactic acid and acetic acid. There is no oxygen limitation problem.	Nath and Das (2011)
		Hallenbeck and Benemann (2002)
Photo-fermentation	• It is a fermentative conversion of organic substrates into hydrogen and carbon dioxide by using sunlight as the source of energy.	Manish and Banerjee (2008)
	• Under anaerobic conditions these bacteria are able to use simple organic acids as electrons donors which are transported to nitrogenase enzyme by ferredoxin using energy in the form of ATP. In the absence of nitrogen, nitrogenase enzyme reduces proton into hydrogen gas using extra energy in the form of ATP.	Akkerman *et al*. (2002)
	• $CH_3COOH+2H_2O$+light$\rightarrow 4H_2+2CO_2$	Azwar *et al*. (2014)
	• Using light as the energy source, the organic acid substrates is oxidized using the tricarboxylic acid cycle (TCA), producing electrons, protons and carbon dioxide. The produced electrons are then delivered to cytochrome c and are shuttled through a number of electron-transport-chain using NAD/NADH before being delivered to ferredoxin. At the same time protons are pumped through the membranes forming proton gradient, which then drives ATP production by ATP synthase. The ATP produced are used to drive the activity of nitrogenase enzyme to catalyze the production of hydrogen gas from protons.	Mathews and Wang (2009)
	• Example: Purple non sulfur bacteria (PNS)	Kim and Kim (2011)
	• Advantage: Helps in removal of environmental pollutants. Use of industrial waste. Use of organic acids produced from dark fermentation.	
	• Disadvantage: Need N_2limit condition. Need pretreatment of industrial effluent as it may be toxic.	

the future. Therefore optimization of the physiological assay and growth parameters are required in order to enhance the hydrogen production from biological processes. Table 5 gives an account of some of the parameters along with the details of each of the process involve in optimizing the hydrogen production.

The bio-hydrogen market: Research and development of biological hydrogen production have expanded significantly in the past decade. The International Energy Agency has commented that bio-hydrogen provides a high market potential in the future (Maniatis, 2003). Although no commercial scale renewable bio-

Table 5: Details of various strategies to enhance hydrogen production from microalgae

Parameters	Description
Two stage photosynthesis: Separation of oxygen and hydrogen production process through Sulphur deprivation	• In direct bio-photolysis oxygen and hydrogen are co-evolved together. The hydrogenase enzyme is extremely sensitive to oxygen and a slightest amount of oxygen present will completely inhibit the activity of hydrogenase (Srirangan *et al*., 2011). To circumvent this problem a two stage bio-photolysis process was develop to allow the temporal separation of oxygenic photosynthesis and photo-biological hydrogen production (Melis *et al*., 2000). • The two-stage bio-photolysis is done by sulphur deprivation. In the first stage the algal cells are grown in sulphur rich medium leading to vigorous cell growth and high rate of photosynthesis. In the presence of sulphur, green algae is able to reduce sulphur to sulphide and incorporate it into cysteine which is the central intermediate form of most sulphur compound, hence sulphur plays a key role in the growth of the algae cells (Ghirardi *et al*., 2000; Jo *et al*., 2006). Once sufficient amount of growth is obtained the algal cells are then transferred to a medium deprived of sulphur. Upon sulphur deprivation oxygen production notably declined due to defective Photosystem II repair cycle. This is because the biosynthesis of D1(reaction centre protein) which is an essential protein in the Photosystem II reaction centre, was damaged due to the inability of chloroplast to synthesize pertinent amount of sulphurous amino acids, cysteine and methionine that needs to be frequently replaced (Srirangan *et al*., 2011; Kothari, 2013). This results in anaerobiosis and with illumination by light, hydrogenase enzyme is activated leading to active production of hydrogen gas for several days (Srirangan *et al*., 2011; Melis and Happe, 2001; Zhang *et al*., 2002; White and Melis, 2006; Ghirardi *et al*., 2000; Melis, 2002; Melis *et al*., 2000). • Thus, in the presence of sulphur the green algae undergoes normal photosynthesis of water oxidation, oxygen evolution and biomass accumulation in order for the cells to grow. In the absence of sulphur the green algae turns into hydrogen production mode. This process is reversible hence is enables the cells to cycle between oxygen production and hydrogen production mode.
Solar Conversion Efficiency Avoids wastage of photon absorption by truncating the light harvesting chlorophyll (Chl) antenna size of Photo-system II and I	• Light utilization efficiency by algae is one of the most important factors in the hydrogen production, however, the solar conversion efficiency of algae cells currently is below 1% (Hallenbeck and Benemann, 2002; Allakhverdiev *et al*., 2010), which is not high enough to compete with the current petrochemical methods (Mussgnug *et al*., 2007). • The low solar conversion energy by green algae is due to their genetic tendency to assemble large arrays of light absorbing chlorophyll (Chl) antenna in their photosystems (Melis, 2002). At high solar intensities the rate of photon absorptions by the chlorophyll antenna in the chloroplast far exceeds the rate of photosynthesis which eventually results in dissipation and loss of excess photons via non-photochemical photons by fluorescence or heat (Melis, 2002). Hence 95% of the absorbed photons will be wasted resulting in low solar conversion efficiency and cellular productivity. Moreover, the algae cells at the surface of the mass culture are subjected to severe photo inhibition because of over absorption by the algae cells at the surface of the mass culture. The light is unable to penetrate efficiently into the mass culture resulting in an unequal and sub-optimal distribution of photon absorption (Zhang *et al*., 2002). It has also been noted that if culture is maintained in culture bottles, due to excessive chlorophyll content, light cannot pass efficiently through two or three layer of algal culture. Thus, inner layers of cells masked away from light due to cells at the exposed surface of culture bottle. • One of the strategies to overcome the low solar conversion energy of green algae is by truncating the light harvesting chlorophyll antenna size of Photosystem II and Photosystem I (Polle *et al*., 2002; Srirangan *et al*., 2011). • A lot of experiments have been done to show that a smaller chlorophyll antenna size improves the solar conversion efficiency in green algae (*C. reinherditii*) (Beckmann *et al*., 2009; Polle *et al*., 2002, 2000, 2003; Mussgnug *et al*., 2007). A smaller chlorophyll antenna will avoid over absorption and wasteful dissipation of excitation energy, as well as diminish photo-inhibition of photosynthesis on the surface of mass culture (Melis, 2002). Hence truncated chlorophyll antenna size will result in greater photosynthetic productivity and photo-biological hydrogen production as well as improved solar utilization efficiency in mass culture (Melis, 2002; Eroglu and Melis, 2011). • Wahal and Viamajala (2010) reported that the minimum amount of chlorophyll molecules required for Photosystem II is 37 and for Photosystem I is 95. It is also believed that a smaller chlorophyll antenna size of the photosystems (PS II and PS I) could solve the problem of fully pigmented chlorophyll antenna (Melis, 2002).
pH and temperature Optimum pH and temperature for enhanced hydrogen production	• The pH is also one of the factors that influence hydrogen production as it may affect the metabolism pathway thus affecting the hydrogen production rate (Manish and Banerjee, 2008). • Hydrogen production rate is dependent on the internal pH of the cells as the pH determines the concentration of protons (Kothari, 2013). Moreover the hydrogenase enzyme which is responsible for hydrogen production is inhibited by pH shift, either to the acidic or alkaline side as pH has a direct effect on the catalytic function of the hydrogenase enzyme (Kosourov *et al*., 2003). • Different types of microalgae strains have different optimal pH activity for hydrogen production; most microalgae species favors neutral pH.The optimum pH for increased hydrogen yield depends largely on the type of microorganism and substrates used (Nath *et al*., 2006). • Jeberlin Prabina and Kumar (2010) demonstrated that *Anabaena*-TE 1, *Fischerella*-TE 1 and *Nostoc*-TE 1 showed higher hydrogen evolution at pH 7.5, a lower pH of 5.5, 6.5 and higher pH of 9.5 has reduced hydrogen evolution. Moreover they also showed that hydrogen production decreases more at acidic than at alkaline pH as low pH results in lower level of ATP in the cell (Ferchichi *et al*., 2005). Kosourov *et al*. (2003), on the other hand showed that *C. reinhardtii* has a maximum hydrogen production at pH 7.7 and lower hydrogen production at pH 6.5 or pH 8.2. At the optimal pH of 7.7 the rate of hydrogen evolution increased and decline slowly compared to all the other pHs. Guan *et al*. (2004), showed that the optimal pH for maximum hydrogen production by *P. subcordiformis*is at pH 8 and the lowest is at pH 5 and pH 11. Low pH results in decrease in hydrogen production due to the increase in the formation of acidic metabolites which in turn destroys the cell's ability to maintain internal pH, resulting in lower intracellular level of ATP (Nath and Das, 2011). • Temperature regulates the cellular, morphological and physiological responses of microalgae where at higher temperatures the metabolic rates of microalgae increases (Kumar *et al*., 2010). Maximum growth rate of microorganism and substrate utilization during hydrogen production are also affected by temperature (Nath *et al*., 2006). Higher temperatures beyond the optimal temperature leads to thermal deactivation resulting to inactivation of enzymes responsible for controlling metabolic pathways in the hydrogen production process (Nath and Das, 2011).

Table 5: Continue

	• The optimum temperature for higher hydrogen production varies considerably with different microalgae species. Studies by Jeberlin Prabina and Kumar (2010) showed that the optimum temperature for higher hydrogen production in *Anabaena*-TE 1, *Fischerella*-TE 1 and *Nostoc*-TE 1 (cyanobacteria isolates) is at 27°C.
Immobilization Entrapment of microbial cells on various polymer matrices for enhanced hydrogen production	• Cell immobilization is defined as the physical localization of a viable microbial cell on a certain material in a way that limits the free migration of the cells while still retaining their catalytic activities for repeated and continuous use (Kilonzo and Bergougnou, 2012). Immobilization involves the attachment or entrapment of cells onto a particular polymer matrices and the type of polymer matrices that can be used are polyacrylamide gel, agarose gel, alginate, chitosan, porous glass, polyurethane and so on. • Immobilization of cells on solid matrices for greater hydrogen production has been reported to be more advantages than free floating cell suspension as immobilized cells occupies less space, requires small volume of growth medium, easier to handle and can be used repeatedly for product generation (Eroglu and Melis, 2011). Moreover bound cells can shift more readily between the oxygenic photosynthesis (growth phase) and hydrogen production phase, which are controlled by shifting the cells between sulfur containing and sulfur free culture media (Eroglu and Melis, 2011; Hahn *et al*., 2007). Cell immobilization also provides robustness against cell washout under hydraulic shock loadings (Keskin *et al*., 2012). • Laurinavichene *et al*. (2006) used immobilized *C.reinhardtii* on Al-borosilicate porous glass sheets and saw an increased on the hydrogen production rate from 2.5 up to 4.3 mL/L/h. Kosourov and Seibert (2009) used alginate beads to immobilize *C. reinhardtii* and revealed higher cell densities and hydrogen production rates (12.5l mol/mg/Chl/h). They also reported that the alginate polymer helped to boost the hypoxic environment within the cells promoting hydrogen production conditions. • Hence, the rate of hydrogen production can be greatly enhanced by using immobilized algae cells compared to free floating algae cells.
Light intensity and Wavelength Optimum light intensity for enhanced hydrogen production	• Light intensity is also an important factor for bio-hydrogen production especially for photosynthetic microorganism (green algae and photosynthetic bacteria) and different strains requires different light intensities for enhanced hydrogen production (Kothari, 2013). • Microalgae cultures pre-grown under low light intensities and exposed to high light intensities during sulfur deprivation produced higher hydrogen production as these cultures are able to transition more rapidly to anaerobiosis. During the pre-growth phase with low light intensities, microalgae have higher chlorophyll content, decreased hydrogen evolution and CO_2 fixation capacities per chlorophyll, compared to being grown under high light intensities. The reason for accelerated an aerobiosis conditions and increase in hydrogen production is because when pre-grown under low light intensities the damage to PSII D1 protein takes place and its repair rate decreases under sulphur deprivation hence they experience additional photo inhibition when placed under high light intensities and sulphur deprived condition (Tsygankov *et al*., 2006). • Jeberlin Prabina and Kumar (2010) reported that *Anabaena*–TE1, *Fischerella*-TE1 and *Nostoc*-TE1 (cyanobacteria) produced maximum hydrogen at 3500 lux and a higher light intensity than 3500 lux decreases the hydrogen production in all three cyanobacteria's. For sulphur deprived *C. reinhardtii*, Kim *et al*. (2006) reported that the maximum production of hydrogen was produced at 200 $\mu E\ m^{-2}\ S^{-1}$ of light intensity with a maximum hydrogen volume of 2.01 mL $H_2\ g^{-1}$ cell h^{-1}. Also observed was that the initiation time for hydrogen production decreased from 62 to 22 hours with increasing light intensity. • The hydrogen production phase was accelerated by high light intensity resulting in prolonged production time as higher light intensities quickly induced sulphur deprived conditions so that the culture becomes more anaerobic resulting in hydrogenase enzyme to be activated sooner (Kim *et al*., 2006). With increasing light intensity the cell number and chlorophyll concentration increases, as when light is absorbed by chlorophyll antenna more electron are released which will then combine with proton to produce hydrogen (Kim *et al*., 2006). • However hydrogen production decreased at 300 $\mu E\ m^{-2}\ S^{-1}$ because the cell number and chlorophyll concentration decreased sharply due to rapid destruction of Photosystem II at very high light intensity (Kim *et al*., 2006). • Microalgae and cyanobacteria contain pigments (chlorophylls, carotenoids and phycobilins) which each has different light absorption range (Gutierrez-Wing *et al*., 2014). Chlorophylls, the most plentiful found pigments in microalgae has two major absorptions ranges which are blue light (450-475 nm) and red light (630-675 nm) (Gutierrez-Wing *et al*., 2014). • Carotenoids have an absorption range of 400 to 500 nm and phycobilins have an absorption range of 500 to 650 nm (Gutierrez-Wing *et al*., 2014). • Although the primary photosynthetic pigment for all microalgae and cyanobacteria is chlorophyll "a", different species responds to different distinct wavelength (Gutierrez-Wing *et al*., 2014). • Experiments conducted have found that microalgae growth and bio-products are obtained more efficiently using red light. Uyar *et al*. (2007), reported that using *Rodobacter sphaeropids* highest yield of hydrogen was produced using red and infrared light (Uyar *et al*., 2007). The red light spectrum improves the bio-hydrogen production as the photons in that spectrum provides the energy that matches the energy needed by the chlorophyll to reach its first excited stage hence greater photosynthetic activity will be obtained (Carvalho *et al*., 2006).
Photo-period Cycling the growth and hydrogen production phase of microalgae through dark : light cycle	• The light and dark photo-periods are two phases of photosynthesis. The light phase is used as the storage phase and the dark phase is used as the catabolism phase, depleting oxygen leading to an increase in the hydrogenase activity to produce hydrogen in the dark. Studies have shown that the light and dark cycles help to increase the hydrogen production yield. According to Jeberlin Prabina and Kumar (2010), 16 hours of darkness followed by 8 hours of light gave the best hydrogen production yield by *Fischerella*-TE1, while 24 hours of light without a dark period gave minimum hydrogen yield. Moreover those two photoperiods had no oxygen co-production.Hence the best photo-period would be 16 hours dark: 8 hours light as *Fischerella*-TE1 will not be able to survive with only 24 hours of darkness and no exposure to light (Jeberlin Prabina and Kumar, 2010). • Koku *et al*. (2003), also reported that even when little or no hydrogen was produced during the dark period, the total amount of hydrogen gas produced in the cycle (14 h light:10 h dark) yielded more hydrogen than a continuously illuminated reactor. The reason why the overall amount of hydrogen produced in the cycle culture is significantly higher could be due to the high cell densities on the cycle cultures or due to the beneficial effect of illumination cycles on nitrogenase (Lazaro *et al*., 2015).

Table 5: Continue

	• A contradicting report on the other hand by Oncel and Vardar Sukan (2011) stated that when *C. reindhartii* were used, the hydrogen production declined in the light/dark cycles that when compared to continuously illuminated cultures.
Carbon and nitrogen Consideration of the best source of carbon and nitrogen as well as amount for increase hydrogen production	• Microalgae require carbon dioxide for photosynthesis. The level of hydrogen production by microalgae using carbon dioxide as the sole carbon source is low (Rashid *et al*., 2009). The microalgae are also able to store carbon in the form of starch during photosynthesis and can use it during anaerobic condition, however, the amount of starch that they can accumulate are low hence only low level of hydrogen is produced (Rashid *et al*., 2013). • Exogenous carbon sources can be added in order to significantly increase the hydrogen production (Rashid *et al*., 2011). Exogenous carbon sources either organic or inorganic carbon can be used and the selection of type of carbon sources is important as the hydrogen yields varies with the type of carbon sources and algal strain (Rashid *et al*., 2013). • Burrows *et al*. (2008) used concentration of bicarbonate as a carbon source and saw an increase (2 fold) in the hydrogen produced by *Synechocytis sp*. Jeberlin Prabina and Kumar (2010) reported that *Anabaena* TE1, *Fischerella* TE1 and *Nostoc* TE1 recorded maximum amount of hydrogen produced when 0.3 % carbon dioxide in gas phase with 50% argon was used, however above 0.3 % the hydrogen production starts to decline which may be due to inhabitation of nitrogenase. • Glucose can also be used as an exogenous carbon source and Rashid *et al*. (2009) reported that when 30 mM of glucose were used the hydrogen production increased till all the glucose had been consumed. • Nitrogen is also an important element for microalgae as it is directly associated with the primary metabolism of microalgae (Kumar *et al*., 2010). Nitrogen is essential for long term hydrogen production as they are important for nitrogen fixation and cell metabolism, however nitrogen inhibits some nitrogenase mediated hydrogen production in some cyanobacteria (Jeberlin Prabina and Kumar, 2010). Limitations of nitrogen supply to microalgae are known to alter its photosynthetic metabolism and direct it more towards the release of excess energy and reducing power in the form of hydrogen (Koku *et al*., 2003). • The source of nitrogen is also an important consideration. When nitrate it used as nitrogen source in cyanobacteria, it requires reducing equivalents to be reduce to ammonia, hence elimination of nitrate from the growth media increases the reductant flow to hydrogenase for hydrogen production (Gutthann *et al*., 2007). Troshina *et al*. (2002) reported that when non-nitrogen fixing cynobacterium *G. alpicola* was grown in limited nitrate, they observed an increase in the rate of hydrogen production and specific hydrogenase activity. • Addition of ammonium instead of nitrate results in an increase in hydrogen production as the electrons are not directed towards the reduction of nitrate to ammonium hence all electrons are directed towards hydrogenase for hydrogen production (Burrows *et al*., 2008).
Co-culturing Culturing of microalgae with bacteria to increase hydrogen production yield	• A mix culture of green algae and photosynthetic bacteria is able to enhance the overall hydrogen production rates, as photosynthetic bacteria are able to evolve hydrogen in the dark and light by utilizing the fermentation products of green algae. Integration of photosynthetic hydrogen production by microalgae that uses visible region of light spectrum (400-700nm) with hydrogen producing photosynthetic bacteria using near infrared region (700-950 nm) are able to improve the solar energy utilization and widening the range of solar spectrum to include the wavelengths from 400 to 950 nm (Eroglu and Melis, 2011). Moreover co-culturing holds a promise of metabolic integration where microalgae generate organic carbon from CO_2 and H_2O while photosyntheticbacteria generate organic nitrogen via nitrogenase, when both are producing hydrogen (Eroglu and Melis, 2011). • In the mixed culture, the fermentation products of the *C. reinhardtii* accumulated during the first 6 hours of dark, hereafter the formate concentration starts to decrease as the *R. rubrum* evolves hydrogen by using the formate formed by *C.reinhardtii* hence the hydrogen evolution by mixed culture increased four times compared to *C. reinhardtii* alone (Eroglu and Melis, 2011). • Wu *et al*. (2012) co-cultured *B. japonicum* a nitrogen-fixing bacterium species with *C. reinhardtii* strain- 849, which is a cell wall deficient mutant algal strain and another *C. reinhardtii* transgenic algal strain (transgenic 1ba strain). Their findings showed that the hydrogen production in the co-culture increase 14.2 times higher for transgenic 1ba strain and 5.5 times higher for strain-849 compared to the hydrogen production level of the green algae alone. It was noticed that the oxygen content of both the co-cultures decreased more quickly than the single algae culture (Wu *et al*., 2012). • The rapid decrease of oxygen content is due to the rapid respiration rate, promoting anaerobic conditions in the co-cultured which might lead to lower consumptions of aerobic respiration metabolism, higher Fe-hydrogenase activity leading to higher hydrogen production (Wu *et al*., 2012). Hence co-culturing of photosynthetic bacteria with green algae is able to promote the enhancement of the green algae hydrogen production.
Microwave irradiation Enhances hydrogen production by affecting the growth and enzyme activity of microalgae	• Microwaves (MW) are non-ionizing radiation and part of the electromagnetic spectrum with frequencies ranging from 300 MHz to 300 GHz corresponding to wavelength range of 1 mm to 1 m (Mishra *et al*., 2013). • Microwave irradiation produces thermal and also non-thermal effects. When microwave irradiation is subjected to microalgae and bacteria, the thermal effect among others can cause the whole organism or major portions of them participate in heat transfer process (Mishra *et al*., 2013). The effect is generated from vibrational energy due to penetration of electromagnetic waves into biological materials heating up intra- and extra- cellular fluids by transfer of vibrational energy (Mishra *et al*., 2013). • For the non-thermal effect, it is postulated that there is a direct stabilizing interaction of microwave with specific (polar) molecules in reaction medium with no rise in temperature (Herrero *et al*., 2008). The effect of the MW on the cell are influenced by multiple factors, such as MW frequency, duration of exposure, pulsed or continuous MW treatments and the medium/matrix in which the cells are embedded during MW exposure (Banik *et al*., 2003; Herrero *et al*., 2008). Different microwave frequencies in continuous waves and modulated modes produced significantly different physiological effects (Banik *et al*., 2003; Herrero *et al*., 2008).

Table 5: Continue

• Non-thermal physiological effects of continuous waves and modulated microwaves MW irradiation is currently a rapidly growing area of research. Research by Asadi *et al*. (2011) showed that when *Phormidium spp. Kutzing* ISC31 (a cyanobacterium) was treated with a frequency of 2450 MHz by combining five different frequencies intensities (180, 360, 540, 720 and 900 W/cm^2) and three pretreatments (10, 20 and 30 s), the content of the chlorophyll a decreased with increase in intensity and exposure time. • In MW irradiation, the treatment time rather than temperature is the key factor to obtain high hydrogen production. This is because regardless of the temperature if the microorganism is irradiated for too long the hydrogen production decreases as the cell will lyse drastically due to harsh ambient conditions causing inhibition of hydrogen production (Bakonyi *et al*., 2014). • Recently, Hsia and Chou (2014) studied ultrasonic effect on bio-hydrogen production based on four changeable parameters (frequency, intensity, duration and starch concentration). They reported that the optimal hydrogen production rate occurred with ultrasonic energy 4 joules, exposure for 15 minutes followed by no exposure for 15 minutes, transduces 0.5MHz and starch concentration 30 g/L. • The research on the effects of microwave irradiation on microalgae to enhance hydrogen production is still in the early stages. More work is required in this area to better understand the MW effects.

hydrogen production facilities are currently in operation, a few pilot scale systems have been demonstrated successfully. The industrial scale production of bio-hydrogen still faces a number of technical and economic barriers. The future of the hydrogen economy depends on the availability of a low cost and environmentally friendly source of hydrogen and appropriate technological knowhow. In their review on patents for hydrogen and fuel cells, Lai *et al*. (2011) stated that Taiwan pays more attention to the bio-hydrogen field, while America and Japan pay more attentions to hydrogen application and the development of fuel cells. Based on their analysis, it is estimated that bio-hydrogen production technology is currently in the initial developmental stage. In another study by Olivo *et al*. (2011) on patent analysis for advanced bio-hydrogen technology development and commercialization, indicated that China was the biggest patent contributor worldwide in terms of hydrogen production while Japan was identified as a huge patent contributor in terms of methods aimed at rear-end product application of hydrogen. A study by Lee and Chiu (2012), revealed that China will become the largest bio-hydrogen market with the highest total output multiplier by 2050.

CONCLUSION

In conclusion, the fossil fuel reserves shortage towards the 21st century due to increasing energy demand and increasing greenhouse gas emission makes it important to develop alternative energy carriers that are renewable, clean and environmentally friendly. Hydrogen holds an increasing role as a future fuel and renewable source of energy however the current classical methods of producing hydrogen are energy intensive, costly and are not environmentally friendly. Major technical challenge in achieving practical applications of bio-hydrogen would be lowering the cost of production, delivery, storage, conversion and practical applications. Bio-hydrogen production employing renewable biomass may be a potential answer to overcome some of the economic constraints to fulfil many of our energy needs. Other challenge of the bio-hydrogen production includes unstable hydrogen production possibly attributed to the metabolic shift of hydrogen producing organisms.

Bio-hydrogen production from biological processes using microalgae holds an alternative to hydrogen production from classical methods as it offers promising advantages such as hydrogen can be produced from renewable sources and eliminates environmental pollutions. However bio-hydrogen yield from microalgae are relatively low to compete with the classical methods of hydrogen production. Hence for it to be commercially competitive, sustained hydrogen production and improvement on the yields of hydrogen has to be achieved. In order to achieve that, further research and development on the optimization of key parameters for enhanced hydrogen production has to be done. The optimization of key experimental factors, genetic modification and metabolic engineering of microalgae are the ultimate approaches to make hydrogen production cost-effective and sustainable. Bio-hydrogenyields and production rates must at least surpass considerably the present achievements for realistic applications. Technological breakthrough must be sought after to extract most of hydrogen from various substrates. Investigation addressing this challenge should be one of focuses of future research.

ACKNOWLEDGMENT

The authors would like to thank the Malaysian Ministry of Education through the Fundamental Research Grant Scheme (FRGS) (20150203FRGS) for the financial grant to carry out this research. We also would like to thank University Tenaga Nasional for the research facilities.

REFERENCES

Akkerman, I., M. Janssen, J. Rocha and R.H. Wijffels, 2002. Photobiological hydrogen production: photochemical efficiency and bioreactor design. Int. J. Hydrogen Energ., 27(11-12): 1195-1208.

Allakhverdiev, S.I., V. Thavasi, V.D. Kreslavski, S.K. Zharmukhamedov, V.V. Klimov, S. Ramakrishna, D.A. Los, M. Mimuro, H. Nishihara and R. Carpentier, 2010. Photosynthetic hydrogen production. J. Photoch. Photobio. C, 11(2-3): 101-113.

Asadi, A., R.A. Khavari-Nejad, N. Soltani, F. Najafi and A. Molaie-Rad, 2011. Physiological variability in cyanobacterium Phormidium sp Kutzing ISC31 (Oscillatoriales) as response to varied microwave intensities. Afr. J. Agric. Res., 6(7): 1673-1681.

Azwar, M.Y., M.A. Hussain and A.K. Abdul-Wahab, 2014. Development of biohydrogen production by photobiological, fermentation and electrochemical processes: A review. Renew. Sust. Energ. Rev., 31: 158-173.

Bakonyi, P., B. Borza, K. Orlovits, V. Simon, N. Nemestothy and K. Bélafi-Bakó, 2014. Fermentative hydrogen production by conventionally and unconventionally heat pretreated seed cultures: A comparative assessment. Int. J. Hydrogen Energ., 39(11): 5589-5596.

Banik, S., S. Bandopadhyay and S. Ganguly, 2003. Bioeffects of microwave--a brief review. Bioresource Technol., 87(2): 155-159.

Beckmann, J., F. Lehr, G. Finazzi, B. Hankamer, C. Posten, L. Wobbe and O. Kruse, 2009. Improvement of light to biomass conversion by de-regulation of light-harvesting protein translation in *Chlamydomonas reinhardtii*. J. Biotechnol., 142(1): 70-77.

Burrows, E.H., F.W.R. Chaplen and R.L. Ely, 2008. Optimization of media nutrient composition for increased photofermentative hydrogen production by *Synechocystis sp*. PCC 6803. Int. J. Hydrogen Energ., 33(21): 6092-6099.

Carvalho, A.P., L.A. Meireles and F.X. Malcata, 2006. Microalgal reactors: A review of enclosed system designs and performances. Biotechnol. Progr., 22(6): 1490-1506.

Chandrasekhar, K., Y.J. Lee and D.W. Lee, 2015. Biohydrogen production: Strategies to improve process efficiency through microbial routes. Int. J. Mol. Sci., 16(4): 8266-8293.

Chen, C.Y., W.B. Lu, J.F. Wu and J.S. Chang, 2007. Enhancing phototrophic hydrogen production of *Rhodopseudomonas palustris* via statistical experimental design. Int. J. Hydrogen Energ., 32(8): 940-949.

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv., 25(3): 294-306.

Chung, I.K., J. Beardall, S. Mehta, D. Sahoo and S. Stojkovic, 2011. Using marine macroalgae for carbon sequestration: A critical appraisal. J. Appl. Phycol., 23(5): 877-886.

Das, D. and T.N. Veziroğlu, 2001. Hydrogen production by biological processes: A survey of literature. Int. J. Hydrogen Energ., 26(1): 13-28.

Das, D. and T.N. Veziroglu, 2008. Advances in biological hydrogen production processes. Int. J. Hydrogen Energ., 33(21): 6046-6057.

Dasgupta, C.N., J.J. Gilberta, P. Lindblad, T. Heidorn, S.A. Borgvang, K. Skjanes and D. Das, 2010. Recent trends on the development of photobiological processes and photobioreactors for the improvement of hydrogen production. Int. J. Hydrogen Energ., 35(19): 10218-10238.

Erbes, D.L., D. King and M. Gibbs, 1979. Inactivation of hydrogenase in cell-free extracts and whole cells of *Chlamydomonas reinhardi* by oxygen. Plant Physiol., 63(6): 1138-1142.

Eroglu, E. and A. Melis, 2011. Photobiological hydrogen production: Recent advances and state of the art. Bioresource Technol., 102(18): 8403-8413.

Fabiano, B. and P. Perego, 2002. Thermodynamic study and optimization of hydrogen production by *Enterobacter aerogenes*. Int. J. Hydrogen Energ., 27(2): 149-156.

Fang, H.H.P., H. Zhu and T. Zhang, 2006. Phototrophic hydrogen production from glucose by pure and co-cultures of *Clostridium butyricum* and *Rhodobacter sphaeroides*. Int. J. Hydrogen Energ., 31(15): 2223-2230.

Ferchichi, M., E. Crabbe, G.H. Gil, W. Hintz and A. Almadidy, 2005. Influence of initial pH on hydrogen production from cheese whey. J. Biotechnol., 120(4): 402-409.

Ghirardi, M.L., L. Zhang, J.W. Lee, T. Flynn, M. Seibert, E. Greenbaum and A. Melis, 2000. Microalgae: A green source of renewable H(2). Trends Biotechnol., 18(12): 506-511.

Ghirardi, M.L., P. King, S. Kosourov, M. Forestier, L. Zhang and M. Seibert, 2006. Development of Algal Systems for Hydrogen Photoproduction: Addressing the Hydrogenase Oxygen-Sensitivity Problem. In: Collings, A.F. and C. Critchley (Eds.), Artificial Photosynthesis: From Basic Biology to Industrial Application. Wiley-VCH Verlag GmbH and Co., Weinheim, pp: 213-227.

Gressler, P., R. Schneider, V. Corbellini, T. Bjerk, M. Souza, A. Zappe and E.A. Lobo, 2012. Microalgas: Aplicações em biorremediação e energia. In English: Microalgae: Aplications in bioremediation and energy. Cad. Pesquisa Sér. Biol., 24(1): 48-67.

Guan, Y., M. Deng, X. Yu and W. Zhang, 2004. Two-stage photo-biological production of hydrogen by marine green alga *Platymonas subcordiformis*. Biochem. Eng. J., 19(1): 69-73.

Gutierrez-Wing, M.T., A. Silaban, J. Barnett and K.A. Rusch, 2014. Light irradiance and spectral distribution effects on microalgal bioreactors. Eng. Life Sci., 14(6): 574-580.

Gutthann, F., M. Egert, A. Marques and J. Appel, 2007. Inhibition of respiration and nitrate assimilation enhances photohydrogen evolution under low oxygen concentrations in *Synechocystis* sp. PCC 6803. Biochim. Biophys. Acta, 1767(2): 161-169.

Hahn, J.J., M.L. Ghirardi and W.A. Jacoby, 2007. Immobilized algal cells used for hydrogen production. Biochem. Eng. J., 37(1): 75-79.

Hallenbeck, P.C. and J.R. Benemann, 2002. Biological hydrogen production; fundamentals and limiting processes. Int. J. Hydrogen Energ., 27(11-12): 1185-1193.

Herrero, M.A., J.M. Kremsner and C.O. Kappe, 2008. Nonthermal microwave effects revisited: On the importance of internal temperature monitoring and agitation in microwave chemistry. J. Org. Chem., 73(1): 36-47.

Hsia, S.Y. and Y.T. Chou, 2014. Optimization of biohydrogen production with biomechatronics. J. Nanomater., 2014: 1-11.

Jeberlin Prabina, B. and K. Kumar, 2010. Studies on the optimization of cultural conditions for maximum hydrogen production by selected cyanobacteria. ARPN J. Agric. Biol. Sci., 5(5): 22-31.

Jo, J.H., D.S. Lee and J.M. Park, 2006. Modeling and optimization of photosynthetic hydrogen gas production by green alga *Chlamydomonas reinhardtii* in sulfur-deprived circumstance. Biotechnol. Progr., 22(2): 431-437.

Johnston, B., M.C. Mayo and A. Khare, 2005. Hydrogen: The energy source for the 21st century. Technovation, 25(6): 569-585.

Kars, G., U. Gündüz, M. Yücel, L. Türker and I. Eroglu, 2006. Hydrogen production and transcriptional analysis of nifD, nifK and hupS genes in *Rhodobacter sphaeroides* O.U.001 grown in media with different concentrations of molybdenum and iron. Int. J. Hydrogen Energ., 31(11): 1536-1544.

Karthic, P. and J. Shiny, 2012. Comparisons and limitations of biohydrogen production processes. Res. J. Biotechnol., 7(2): 59-71.

Keskin, T., L. Giusti and N. Azbar, 2012. Continuous biohydrogen production in immobilized biofilm system versus suspended cell culture. Int. J. Hydrogen Energ., 37(2): 1418-1424.

Kilonzo, P. and M. Bergougnou, 2012. Surface modifications for controlled and optimized cell immobilization by adsorption: Applications in fibrous bed bioreactors containing recombinant cells. J. Microbial Biochem. Technol., S8(1): 1-9.

Kim, D.H. and M.S. Kim, 2011. Hydrogenases for biological hydrogen production. Bioresource Technol., 102(18): 8423-8431.

Kim, J.P., C.D. Kang, T.H. Park, M.S. Kim and S.J. Sim, 2006. Enhanced hydrogen production by controlling light intensity in sulfur-deprived *Chlamydomonas reinhardtii* culture. Int. J. Hydrogen Energ., 31(11): 1585-1590.

Koku, H., I. Eroğlu, U. Gündüz, M. Yücel and L. Türker, 2003. Kinetics of biological hydrogen production by the photosynthetic bacterium *Rhodobacter sphaeroides* O.U. 001. Int. J. Hydrogen Energ., 28(4): 381-388.

Kosourov, S., M. Seibert and M.L. Ghirardi, 2003. Effects of extracellular pH on the metabolic pathways in sulfur-deprived, H_2-producing C*hlamydomonas reinhardtii* cultures. Plant Cell Physiol., 44(2): 146-155.

Kosourov, S.N. and M. Seibert, 2009. Hydrogen photoproduction by nutrient-deprived C*hlamydomonas reinhardtii* cells immobilized within thin alginate films under aerobic and anaerobic conditions. Biotechnol. Bioeng., 102(1): 50-58.

Kotay, S.M. and D. Das, 2007. Microbial hydrogen production with *Bacillus coagulans* IIT-BT S1 isolated from anaerobic sewage sludge. Bioresource Technol., 98(6): 1183-1190.

Kothari, A., 2013. Improving cyanobacterial hydrogen production through bioprospecting of natural microbial communities. Ph.D. Thesis, Department of Molecular and Cellular Biology, Arizona State University, pp: 268.

Kumar, A., S. Ergas, X. Yuan, A. Sahu, Q. Zhang, J. Dewulf, F. Xavier Malcata and H. van Langenhove, 2010. Enhanced CO2 fixation and biofuel production via microalgae: Recent developments and future directions. Trends Biotechnol., 28(7): 371-380.

Kumar, N. and D. Das, 2000. Enhancement of hydrogen production by *Enterobacter cloacae* IIT-BT 08. Process Biochem., 35(6): 589-593.

Kumazawa, S. and A. Mitsui, 1981. Characterization and optimization of hydrogen photoproduction by a saltwater blue-green alga, *Oscillatoria sp*. Miami BG7. I. Enhancement through limiting the supply of nitrogen nutrients. Int. J. Hydrogen Energ., 6(4): 339-348.

Lai, W.H., H.Y. Chen, F.Y. Chang, C.C. Wu, C.Y. Lin and S.R. Huang, 2011. Market and patent analysis of commercializing biohydrogen technology. Int. J. Hydrogen Energ., 36: 14049-14058.

Laurinavichene, T.V., A.S. Fedorov, M.L. Ghirardi, M. Seibert and A.A. Tsygankov, 2006. Demonstration of sustained hydrogen photoproduction by immobilized, sulfur-deprived C*hlamydomonas reinhardtii* cells. Int. J. Hydrogen Energ., 31(5): 659-667.

Lazaro, C.Z., M.B.A. Varesche and E.L. Silva, 2015. Effect of inoculum concentration, pH, light intensity and lighting regime on hydrogen production by phototrophic microbial consortium. Renew. Energ., 75: 1-7.

Lee, D.H. and L.H. Chiu, 2012. Development of a biohydrogen economy in the United States, China, Japan, and India: With discussion of a chicken-and-egg debate. Int. J. Hydrogen Energ., 37(20): 15736-15745.

Lemus, R.G. and J.M.M. Duart, 2010. Updated hydrogen production costs and parities for conventional and renewable technologies. Int. J. Hydrogen Energ., 35(9): 3929-3936.

Le Quéré, C., R.J. Andres, T. Boden, T. Conway, R.A. Houghton, J.I. House, G. Marland, G.P. Peters, G. van der Werf, A. Ahlström, R.M. Andrew, L. Bopp, J.G. Canadell, P. Ciais, S.C. Doney, C. Enright, P. Friedlingstein, C. Huntingford, A.K. Jain, C. Jourdain, E. Kato, R.F. Keeling, K. Klein Goldewijk, S. Levis, P. Levy, M. Lomas, B. Poulter, M.R. Raupach, J. Schwinger, S. Sitch, B.D. Stocker, N. Viovy, S. Zaehle and N. Zeng, 2012. The global carbon budget 1959-2011. Earth Syst. Sci. Data Discuss., 5(2): 1107-1157.

Levin, D.B., L. Pitt and M. Love, 2004. Biohydrogen production: Prospects and limitations to practical application. Int. J. Hydrogen Energ., 29(2): 173-185.

Lewis, N.S. and D.G. Nocera, 2006. Powering the planet: Chemical challenges in solar energy utilization. P. Natl. Acad. Sci. USA, 103(43): 15729-15735.

Lin, C.Y. and C.H. Jo, 2003. Hydrogen production from sucrose using an anaerobic sequencing batch reactor process. J. Chem. Technol. Biot., 78(6): 678-684.

Liu, J., V.E. Bukatin and A.A. Tsygankov, 2006. Light energy conversion into H_2 by *Anabaena variabilis* mutant PK84 dense cultures exposed to nitrogen limitations. Int. J. Hydrogen Energ., 31(11): 1591-1596.

Maniatis, K., 2003. Pathways for the Production of Bio-hydrogen: Opportunities and Challenges. In: Towards Hydrogen. IEA, Paris.

Manish, S. and R. Banerjee, 2008. Comparison of biohydrogen production processes. Int. J. Hydrogen Energ., 33(1): 279-286.

Mathews, J. and G. Wang, 2009. Metabolic pathway engineering for enhanced biohydrogen production. Int. J. Hydrogen Energ., 34(17): 7404-7416.

Melis, A., 2002. Green alga hydrogen production: Progress, challenges and prospects. Int. J. Hydrogen Energ., 27(11-12): 1217-1228.

Melis, A., 2007. Photosynthetic H2 metabolism in C*hlamydomonas reinhardtii* (unicellular green algae). Planta, 226(5): 1075-1086.

Melis, A. and T. Happe, 2001. Hydrogen production. Green algae as a source of energy. Plant Physiol., 127(3): 740-748.

Melis, A., L. Zhang, M. Forestier, M.L. Ghirardi and M. Seibert, 2000. Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii*. Plant Physiol., 122(1): 127-136.

Mishra, T., P. Kushwah, K. Dholiya and V. Kothari, 2013. Effect of low power microwave radiation on microorganisms and other life forms. AMWT, 1(1): 4.

Momirlan, M. and T.N. Veziroglu, 2005. The properties of hydrogen as fuel tomorrow in sustainable energy system for a cleaner planet. Int. J. Hydrogen Energ., 30(7): 795-802.

Mussgnug, J.H., S. Thomas-Hall, J. Rupprecht, A. Foo, V. Klassen, A. McDowall, P.M. Schenk, O. Kruse and B. Hankamer, 2007. Engineering photosynthetic light capture: Impacts on improved solar energy to biomass conversion. Plant Biotechnol. J., 5(6): 802-814.

Nath, K., A. Kumar and D. Das, 2006. Effect of some environmental parameters on fermentative hydrogen production by *Enterobacter cloacae* DM11. Can. J. Microbiol., 52(6): 525-532.

Nath, K. and D. Das, 2011. Modeling and optimization of fermentative hydrogen production. Bioresource Technol., 102(18): 8569-8581.

Oh, Y.K., E.H. Seol, J.R. Kim and S. Park, 2003. Fermentative biohydrogen production by a new chemoheterotrophic bacterium *Citrobacter* sp. Y19. Int. J. Hydrogen Energ., 28(12): 1353-1359.

Olivo, C., I. Lebedeva, C.Y. Chu, C.Y. Lin and S.Y. Wu, 2011. A patent analysis on advanced biohydrogen technology development and commercialisation: Scope and competitiveness. Int. J. Hydrogen Energ., 36: 14103-14110.

Oncel, S. and F. Vardar Sukan, 2011. Effect of light intensity and the light: Dark cycles on the long term hydrogen production of *Chlamydomonas reinhardtii* by batch cultures. Biomass Bioenerg., 35(3): 1066-1074.

Öztürk, Y., M. Yücel, F. Daldal, S. Mandaci, U. Gündüz, L. Türker and I. Eroğlu, 2006. Hydrogen production by using *Rhodobacter capsulatus* mutants with genetically modified electron transfer chains. Int. J. Hydrogen Energ., 31(11): 1545-1552.

Pelczar, Jr. M.J., E.C.S. Chan and N.R. Krieg, 2008. Microbiology Concepts and Application. 2nd Edn., Pearson Makron Books.

Polle, J.E.W., J.R. Benemann, A. Tanaka and A. Melis, 2000. Photosynthetic apparatus organization and function in the wild type and a chlorophyll b-less mutant of *Chlamydomonas reinhardtii*. Dependence on carbon source. Planta, 211(3): 335-344.

Polle, J.E.W., S. Kanakagiri, E. Jin, T. Masuda and A. Melis, 2002. Truncated chlorophyll antenna size of the photosystems—a practical method to improve microalgal productivity and hydrogen production in mass culture. Int. J. Hydrogen Energ., 27(11-12): 1257-1264.

Polle, J.E., S.D. Kanakagiri and A. Melis, 2003. Tla1, a DNA insertional transformant of the green alga *Chlamydomonas reinhardtii* with a truncated light-harvesting chlorophyll antenna size. Planta, 217(1): 49-59.

Prince, R.C. and H.S. Kheshgi, 2005. The photobiological production of hydrogen: Potential efficiency and effectiveness as a renewable fuel. Crit. Rev. Microbiol., 31(1): 19-31.

Quadrelli, R. and S. Peterson, 2007. The energy–climate challenge: Recent trends in CO_2 emissions from fuel combustion. Energ. Policy, 35(11): 5938-5952.

Rajeshwar, K., R. McConnell, K. Harrison and S. Licht, 2008. Renewable Energy and the Hydrogen Economy. In: Rajeshwar, K., R. McConnell and S. Lich (Eds.), Solar Hydrogen Generation: Toward a Renewable Energy Future. Springer, New York, pp: 1-18.

Rashid, N., K. Lee and Q. Mahmood, 2011. Bio-hydrogen production by *Chlorella vulgaris* under diverse photoperiods. Bioresource Technol., 102(2): 2101-2104.

Rashid, N., M.S.U. Rehman, S. Memon, Z. Ur Rahman, K. Lee and J.I. Han, 2013. Current status, barriers and developments in biohydrogen production by microalgae. Renew. Sust. Energ. Rev., 22: 571-579.

Rashid, N., W. Song, J. Park, H.F. Jin and K. Lee, 2009. Characteristics of hydrogen production by immobilized cyanobacterium *Microcystis aeruginosa* through cycles of photosynthesis and anaerobic incubation. J. Ind. Eng. Chem., 15(4): 498-503.

Razeghifard, R., 2013. Algal biofuels. Photosynth. Res., 117(1-3): 207-219.

Rupprecht, J., B. Hankamer, J.H. Mussgnug, G. Ananyev, C. Dismukes and O. Kruse, 2006. Perspectives and advances of biological H2 production in microorganisms. Appl. Microbiol. Biot., 72(3): 442-449.

Sambusiti, C., M. Bellucci, A. Zabaniotou, L. Beneduce and F. Monlau, 2015. Algae as promising feedstocks for fermentative biohydrogen production according to a biorefinery approach: A comprehensive review. Renew. Sust. Energ. Rev., 44: 20-36.

Shaishav, S., R.N. Singh and T. Satyendra, 2013. Biohydrogen from algae: Fuel of the future. Int. Res. J. Environ. Sci., 2(4): 44-47.

Show, K.Y., D.J. Lee, J.H. Tay, C.Y. Lin and J.S. Chang, 2012. Biohydrogen production: Current perspectives and the way forward. Int. J. Hydrogen Energ., 37(20): 15616-15631.

Srirangan, K., M.E. Pyne and C. Perry Chou, 2011. Biochemical and genetic engineering strategies to enhance hydrogen production in photosynthetic algae and cyanobacteria. Bioresource Technol., 102(18): 8589-8604.

Stripp, S.T. and T. Happe, 2009. How algae produce hydrogen--news from the photosynthetic hydrogenase. Dalton T., 45: 9960-9969.

Troshina, O., L. Serebryakova, M. Sheremetieva and P. Lindblad, 2002. Production of H2 by the unicellular cyanobacterium *Gloeocapsa alpicola* CALU 743 during fermentation. Int. J. Hydrogen Energ., 27(11-12): 1283-1289.

Tsygankov, A.A., S.N. Kosourov, I.V. Tolstygina, M.L. Ghirardi and M. Seibert, 2006. Hydrogen production by sulfur-deprived *Chlamydomonas reinhardtii* under photoautotrophic conditions. Int. J. Hydrogen Energ., 31(11): 1574-1584.

Uggetti, E., B. Sialve, E. Trably and J.P. Steyer, 2014. Integrating microalgae production with anaerobic digestion: A biorefinery approach. Biofuel. Bioprod. Bior., 8(4): 516-529.

Uyar, B., I. Eroglu, M. Yücel, U. Gündüz and L. Türker, 2007. Effect of light intensity, wavelength and illumination protocol on hydrogen production in photobioreactors. Int. J. Hydrogen Energ., 32(18): 4670-4677.

Vardar-Schara, G., T. Maeda and T.K. Wood, 2008. Metabolically engineered bacteria for producing hydrogen via fermentation. Microb. Biotechnol., 1(2): 107-125.

Vignais, P.M., 2008. Hydrogenases and H(+)-reduction in primary energy conservation. Results Probl. Cell Differ., 45: 223-252.

Wahal, S. and S. Viamajala, 2010. Maximizing algal growth in batch reactors using sequential change in light intensity. Appl. Biochem. Biotech., 161(1-8): 511-522.

White, A.L. and A. Melis, 2006. Biochemistry of hydrogen metabolism in *Chlamydomonas reinhardtii* wild type and a Rubisco-less mutant. Int. J. Hydrogen Energ., 31(4): 455-464.

Winkler, M., A. Hemschemeier, C. Gotor, A. Melis and T. Happe, 2002. [Fe]-hydrogenases in green algae: Photo-fermentation and hydrogen evolution under sulfur deprivation. Int. J. Hydrogen Energ., 27(11-12): 1431-1439.

Wu, S., X. Li, J. Yu and Q. Wang, 2012. Increased hydrogen production in co-culture of *Chlamydomonas reinhardtii* and *Bradyrhizobium japonicum*. Bioresource Technol., 123: 184-188.

Younesi, H., G. Najafpour, K.S. Ku Ismail, A.R. Mohamed and A.H. Kamaruddin, 2008. Biohydrogen production in a continuous stirred tank bioreactor from synthesis gas by anaerobic photosynthetic bacterium: *Rhodopirillum rubrum*. Bioresource Technol., 99(7): 2612-2619.

Zhang, H., M.A. Bruns and B.E. Logan, 2006. Biological hydrogen production by *Clostridium acetobutylicum* in an unsaturated flow reactor. Water Res., 40(4): 728-734.

Zhang, L., M. He and J. Liu, 2014. The enhancement mechanism of hydrogen photoproduction in *Chlorella protothecoides* under nitrogen limitation and sulfur deprivation. Int. J. Hydrogen Energ., 39(17): 8969-8976.

Zhang, L., T. Happe and A. Melis, 2002. Biochemical and morphological characterization of sulfur-deprived and H_2-producing *Chlamydomonas reinhardtii* (green alga). Planta, 214(4): 552-561.

Zhu, L.D., E. Hiltunen, E. Antila, J.J. Zhong, Z.H. Yuan and Z.M. Wang, 2014. Microalgal biofuels: Flexible bioenergies for sustainable development. Renew. Sust. Energ. Rev., 30: 1035-1046.