BIOFUELS: USE OF BIOTECHNOLOGY TO MEET ENERGY CHALLENGES

Mrs. S. S. Tanawade*[1], Mr. B. A. Bapat[2] & Dr. Mrs. N. S. Naikwade[3]

1. Lecturer, Dept. of Pharmaceutical Chemistry, Appasaheb Birnale College of Pharmacy, Sangli.
2. PG Student, Dept. of pharmaceutical Chemistry, Appasaheb Birnale College of Pharmacy, Sangli.
3. Vice-Principal, HOD, Dept. of Pharmacology, Appasaheb Birnale College of Pharmacy, Sangli.

Corresponding author*: bapatbhush@gmail.com

This article is available online at www.ssjournals.com

ABSTRACT

Biofuels offer exciting possibilities for reducing the use of conventional energy resources – which are limited & cause immense pollution. Fermentative production of Ethanol & Bio-hydrogen production are the key techniques to obtain biofuels that can satisfy greater portion of energy demand & both are clean, renewable sources of energy. Bio-hydrogen production serves as a tool for production of Hydrogen, (which is ultimate fuel & energy carrier) by the use of micro-organisms. Some photosynthetic bacteria such as *Rhodopseudomonas polustris* generate hydrogen using nitrogenase or hydrogenase enzymes as catalylists, while green algae & cynobacteria use water-splitting photosynthetic processes to generate molecular hydrogen. The limitations like lower efficiency, oxygen sensitivity & ambiguous mechanism of hydrogen formation can be overcome by the use of Biotechnology for designing micro-organisms optimized for hydrogen production. This review comprises of points on the basis of which required goals of hydrogen production can be achieved by the use of Biotechnology.

KEY WORDS: Biofuels, Energy Challenge, Bio-Hydrogen, Biophotolysis of water.

INTRODUCTION

The wide ranges of fuels which are derived from biomass are called as Biofuels. These include solid biomass, liquid fuels and various biogases. Based on biomasses used as precursors these Biofuels are categorized as First generation (biofuels by fermentation of sugars), Second generation (biofuels from non-food crops), Third generation (biofuels from algae) & Fourth generation (biofuels created by processes other than above). Fourth generation technology processes include: pyrolysis, gasification, solar-to-fuel, and genetic manipulation of organisms. [1]

With biotechnological innovations biology can play an important role in producing high energy fuels, Plants and photosynthetic microorganisms are masters at harvesting chemical energy from sunlight, a virtually inexhaustible supply of energy. By harvesting their photosynthetic and other biochemical capabilities, biological systems can be used to satisfy a greater portion of energy demand. [2, 3, 4.]

Use of biotechnology to meet the energy challenge has following advantages:

- Applying biotechnology to build a new bio-energy industry can benefit nation’s energy security, economy and environment in many different ways.

- Consumption of biofuels produces no net CO_2 emissions
- It releases no sulphur and has much lower particulate and toxic emissions than fossil fuels.

Bio-fuels can be classified into two types based on the type of fuel produced

1. Ethanol production by Fermentation
2. Bio-hydrogen production

1. Ethanol production by Fermentation[4, 5.]

It is prepared from biomass obtained from corn grain. Biomass will be a major component of energy technology portfolio and by 2100 biomass usage will be greater than that of all current fossil fuels (oil, natural gas and coal) combined. It will create a new bio-energy industry potentially worth trillions of dollars over 21st century .But it has high production costs, technical difficulties, inefficiencies in biomass conversion and large land is required to grow bio-energy crops.

2. Bio-hydrogen production: [6, 7.]

Hydrogen is a promising energy carrier of future. It can be derived from variety of energy sources and used in fuel cells with high efficiency. Combustion of hydrogen produces only water as by product, making it a nonpolluting carbon -free energy alternative. Some micro-organisms produce hydrogen naturally and biotechnologies based on these microbial systems could lead to development of clean renewable sources of hydrogen.

There are three mechanisms for biological hydrogen production.

a. **Nitrogenase mediated hydrogen production:** In the absence of oxygen and presence of light, purple nonsulphur (PNS) photosynthetic bacteria such as *Rhodopseudomonas palustris* and *Rhodobactor sphaeroides* contain nitrogenase enzymes that can generate hydrogen under nitrogen limited conditions. These microbes obtain the electrons they need to reduce protons to molecular hydrogen (H_2) from the breakdown of organic compounds. Certain species of cyanobacteria contain nitrogenase enzymes which are capable of producing hydrogen as a by-product of nitrogen fixation. It can be represented by the simple chemical reaction,

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

This reaction is known to be catalyzed by either nitrogenase or hydrogenase enzymes. [7, 8.]

b. **Fermentative hydrogen production.** The bacterial species such as *E. coli*, *Enterobacter aerogenes*, and *Clostridium butyricum* are known to ferment sugars and produce hydrogen using multi-enzyme systems. These “dark fermentation” reactions do not require light energy, so they constantly produce hydrogen from organic compounds throughout the day and night. As compared to other biological hydrogen-production processes, fermentative bacteria have high evolution rates of hydrogen. However, expensive substrates i. e. sugars are not available in sufficient quantities to produce hydrogen required to meet energy demand. At Industrial level the most common methods for producing hydrogen include - steam reformation of natural gas, coal gasification, and splitting water with electricity typically generated from fossil fuels. These industrial processes release carbon dioxide, other greenhouse gases and pollutants as by-products. Some microorganisms produce hydrogen naturally, and use of biotechnology on these microbial systems could lead to the development of clean, renewable sources of hydrogen. The National Research Council (NRC) noted that “substantial, fundamental research needs to be undertaken before photobiological methods for large-scale hydrogen production are considered.” Although alternative biological hydrogen production-pathways exist, each with its own set of advantages and disadvantages, the following discussion on bio-hydrogen production will focus on the challenges that must be overcome to improve one type of biological hydrogen production known as biophotolysis. [9, 10, 11.]

c. Biophotolysis of water

Biophotolytic Hydrogen: Goals and Impacts [11, 12, 13.]

- Sunlight and seawater are the two resources, can be used to produce the ultimate fuel and energy carrier, hydrogen. Use of hydrogen in fuel cells can produce electricity directly with water as the by-product.
- This energy cycle is carbon free and can be developed as the complement to the electric grid for all energy applications-industrial, transportation, and residential.
- Development of biological photolytic processes to produce hydrogen at high rates and efficiency will enable the hydrogen-economy strategy based on a renewable source.
- Under certain conditions, green algae and cyanobacteria can use water-splitting photosynthetic processes to generate molecular hydrogen (H_2) rather than fix carbon, the normal function of oxygenic photosynthesis. Biophotolysis can produce hydrogen necessary to meet future energy demand. This approach to hydrogen production is promising because

i. The source of electrons to generate hydrogen is water - a clean, renewable, carbon-free substrate available in virtually inexhaustible quantities.
ii. Efficient conversion of solar energy to hydrogen.
iii. Inefficiencies associated with carbon fixation and biomass formation can be overcome by reengineering microbial systems for the direct production of hydrogen from water. [14, 15, 16.]

Theoretically, the maximal energetic efficiency for direct bio-photolysis is about 40% compared with a maximum of about 1% for hydrogen production from biomass. Recognizing the important potential of biophotolysis, NRC has recommended that it is necessary to refocus the bio-based program on more fundamental research on photosynthetic microbial systems to produce hydrogen from water at high rate and efficiency”

Bio-hydrogen research targets

Engineering oxygen-tolerant, efficient Hydrogenases known to tolerate oxygen generally are not very efficient hydrogen producers. During bio-photolytic hydrogen production, oxygen is released from the water-splitting reaction, thus engineering hydrogenases with sufficient activity and oxygen tolerance will be needed. Engineered hydrogenases then could be used in bio-inspired nanostructures that maintain optimal conditions for hydrogen production. Two major types of hydrogenases are defined by their biologically unique metallocenters: Nickel-iron (Ni-Fe) and iron only (Fe). Ni-Fe hydrogenases are found in many bacteria and some cyanobacteria while Fe hydrogenases are found in some bacteria and green algae. In green algae, hydrogenases are bidirectional (capable of catalyzing hydrogen oxidation or proton reduction to produce H_2). [17, 18.]

Although turnover is much higher for Fe hydrogenases, Ni-Fe hydrogenases are more oxygen tolerant. The metallocenters of both Ni-Fe and Fe hydrogenases form complexes with such unusual inorganic cofactors as carbon monoxide (CO) or cyanide (CN). Assembly of an active hydrogenase is not known fully and several genes may be involved in the synthesis of cofactors required for activity. Enzymes with improved function can be engineered by better understanding of hydrogenase assembly. [17, 18.]

Designing microorganisms optimized for hydrogen production

Genetically modified Photosynthetic microbes that produce hydrogen at high rates and efficiency from the biophotolysis of water could be grown in extensive farms of sealed enclosures (photo-bioreactors). Hydrogen would be used in energy applications, with oxygen released as a by-product. [19]

Gaps in Scientific Understanding [20, 21, 22, 23.]

- Understanding bio-photolysis well enough to model hydrogenase structure and function, regulatory and metabolic networks, and eventually entire organisms will stimulate the kind of biotechnological innovation needed to engineer the ideal organism to use in hydrogen bioreactors or the ideal enzyme-catalyst to use in bio-inspired nanostructures for hydrogen production. But for understanding this one has to investigate a greater range of hydrogen-producing enzymes and organisms, mechanisms of hydrogenase assembly, oxygen sensitivity of hydrogenase, electron-transfer rate limitations, and regulatory and metabolic processes that influence hydrogen production.
- Factors that can impact the partitioning of electrons between hydrogenase and competing pathways – a) the buildup of a pH gradient across the photosynthetic membrane and b) variations in the concentrations of critical electron-transport carriers. Understanding mechanism of electron fluxes in an organism will aid the development of mechanisms for directing more electrons towards proton reduction and hydrogen production. [14]
- A vast majority of organisms that contain hydrogenases have not been identified and probably cannot be cultured in the laboratory using current procedures. Studying hydrogenase enzymes involved in non bio-photolytic pathways could provide structural or functional insights to guide the engineering of bio-photolytic systems.
- A thorough examination of hydrogen metabolism in green algae and different strains of cyanobacteria will provide new information about how hydrogen-production pathways are controlled. By understanding mechanism of regulation of hydrogen-production, we will be able to determine which metabolic pathways contribute, how eliminating hydrogen-consuming reactions affects hydrogen metabolism and other cellular processes, and how organisms can be adapted to increase hydrogen yields.
- The bidirectional Fe hydrogenases that catalyze the hydrogen-evolution reaction in bio-photolytic systems are highly sensitive to oxygen, a product of the water-splitting reaction in the first step of the photosynthetic pathway. Oxygen sensitivity causes isolation of hydrogenase

from cells and its subsequent analysis is a challenge which has to be met by new technologies.

Scientific and technological capabilities required to achieve goals

Key capabilities needed to address many of the gaps in current understanding of bio-photolytic hydrogen production include developing microbial hosts to produce hydrogenase enzymes, screening large numbers of enzymes for desired functionalities, large-scale molecular profiling to provide a global-view of hydrogen production, in vivo visualization of hydrogenase structure and activity, modeling of regulatory and metabolic networks, and metabolic engineering (see Table). Roadmap for development of biophotolyic hydrogen technologies and specific needs include the following: [23, 24.]

- **Microbial hosts to produce hydrogenases from many different organisms -** Thousands of enzymes from many different organisms need to be produced and analyzed. Other requirements include - methods for producing eukaryotic enzymes in prokaryotic systems, designing host organisms that can provide the intracellular environment required for proper protein assembly and folding, and screening the proteins produced from these host organisms.
- **Methods for producing large numbers of enzymes to screen for desired hydrogenase properties -** With so much variability among natural hydrogenases and engineered variants, developing high-throughput capabilities for producing large numbers (perhaps hundreds of thousands to millions) of enzymes to screen for O_2 tolerance, H_2-production activity, spectroscopic examination, and structural analysis could accelerate the discovery of enzymes best suited for biotechnological applications.
- **Molecular profiling to understand cellular activity during hydrogen production -** Improvements in computational capabilities and large-scale molecular profiling techniques (transcriptomics, proteomics, metabolomics, and measurements of metal abundance) are needed to obtain a global view of microbial hydrogen production. Systems-level analyses could guide experimental investigations by defining gene regulatory networks controlling the expression of genes involved in hydrogen production or cofactor synthesis and identify pathways activated or deactivated during hydrogen production for multiple organisms under varying conditions. [20]
- **Methods to perform in vivo visualization and characterization of molecular machines -** Though information about crystal structures of some hydrogenases is known, this provides only snapshots of enzyme structure. The need is to design advanced techniques for visualizing the different stages of hydrogenase assembly or monitoring hydrogenase activity in living cells that will enable building predictive models that can be used to engineer hydrogenases optimized for biotechnological applications. [22, 23.]
- **Techniques for system-level studies to model and simulate regulatory and metabolic networks -** Studying hydrogenase function taking into account network maintained by living cells is essential to understanding how this process is influenced by different pathways and environmental conditions. Traditional in vitro biochemical methods do not provide sufficient information to understand enzymatic activities in living cells. Tools for monitoring hydrogenase activity in vivo and integrating diverse sets of experimental data are needed to build in

silico models of a bio-photolytic organism under H_2-producing conditions.

- **Metabolic engineering** - Metabolic engineering involves genetically modifying microorganisms to target and manipulate enzymatic, regulatory, or transport pathways that impact a particular microbial process such as hydrogen production. Models could guide metabolic engineering, for example, by identifying control points for manipulating the flow of electrons to hydrogenase or by predicting how cellular activity and hydrogen yields may be impacted by a variety of conditions like – a) the elimination of a particular metabolic pathway or b) the buildup of a pH gradient across the photosynthetic membrane.

REFERENCES

1) Demirbas, A. 2009. "Political, economic and environmental impacts of biofuels: A review". Applied Energy, **86**, S108–S117.
2) Herrera, S. June 2004. "Industrial Biotechnology— A Chance at Redemption," Nat. Biotechnology. **22**(6), 671–75.
3) Abraham, S. 2004. "The Bush Administration's Approach to Climate Change," Science **305**, 616–17.
4) Keller, M., and K. Zengler. Feb. 2004. "Tapping into Microbial Diversity," Nat. Rev. Microbiol. **2**, 141–50.
5) Demain, A., et al. 2005. "Cellulase, Clostridia, and Ethanol," Microbiol. Mol. Biol. Rev. **69**, 124–54.
6) Hess, W. R. 2004. "Genome Analysis of Marine Photosynthetic Microbes and Their Global Role," Curr. Opin. Biotechnol. **15**(3), 191–98.
7) Falciatore, A., and C. Bowler. 2002. "Revealing the Molecular Secrets of Marine Diatoms," Annu. Rev. Plant Biol. **53**, 109–30.
8) Elowitz, M. B., et al. 2002. "Stochastic Gene Expression in a Single Cell," Science **297**, 1183–86.
9) Ellis, D. I., et al. 2004. "From Genomes to Systems: A Report on the 2nd Conference of the Consortium for Post-Genome Science (CPGS) 'Genomes to Systems,' Manchester, U.K., 1–3 September 2004," Genome Biol. **5**, 354.
10) Croal, L. R., et al. 2004. "The Genetics of Geochemistry," Annu. Rev. Genet. **38**, 175–202.
11) Fuhrman, J. 2003. "Genome Sequences from the Sea," Nature **424**, 1001–2.
12) Nealson, K. H. 2005. "Hydrogen and Energy Flow as 'Sensed' by Molecular Genetics," Proc. Natl. Acad. Sci. **102**(11), 3889–90.
13) Tringe, S. G., et al. 2005. "Comparative Metagenomics of Microbial Communities," Science **308**, 554–57.
14) Kitano, H. 2002. "Systems Biology: A Brief Overview," Science **295**, 1662–64.
15) Melis, A., and T. Happe. 2001. "Hydrogen Production. Green Algae as a Source of Energy," Plant Physiol. **127**, 740–48.
16) Falkowski, P., et al. 2000. "The Global Carbon Cycle: A Test of Our Knowledge of Earth as a System," Science **290**, 291–96.
17) Luengo, J. M., et al. 2003. "Bioplastics from Microorganisms," Curr. Opin. Microbiol. **6**, 251–60.
18) Zengler et al., 2002. "Cultivating the Uncultured," Proc. Natl. Acad. Sci. USA **99**(24), 15681–86.

19) Frazier, M. E., et al. 2003a. “Realizing the Potential of the Genome Revolution: The Genomes to Life Program,” Science **300**, 290–93.

20) Gaietta, G., et al. 2002. “Multicolor and Electron Microscopic Imaging of Connexin Trafficking,” Science **296**(5567), 503–7.

21) Meyer, J. 2004. “Miraculous Catch of Iron-Sulfur Protein Sequences in the Sargasso Sea,” FEBS. Lett. **570**, 1–6.

22) Brady, S. F., and J. Clardy. 2000. “Long-Chain N-Acyl Amino Acid Antibiotics Isolated from Heterologously Expressed Environmental DNA,” J. Am. Chem. Soc. **122**(51), 12903–4.

23) Brady, S. F., C. J. Chao, and J. Clardy. 2002. “New Natural Product Families from an Environmental DNA (cDNA) Gene Cluster,” J. Am. Chem. Soc. **124**, 9968–69.

24) Aebersold, R. and J. D. Watts. 2002. “The Need for National Centers for Proteomics,” Nat. Biotechnol. **20**, 65.