World Journal of Microbiology & Biotechnology **16**: 757–767, 2000.

Review

Cyanobacterial hydrogen production

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Received 20 June 2000; accepted 23 October 2000

Keywords: Cyanobacteria, hydrogen, hydrogenase, nitrogenase

Summary

With the global attention and research now being focussed on looking for an alternative to fossil fuel, hydrogen is the hope of future. Cyanobacteria are highly promising microorganisms for biological photohydrogen production. The review highlights the advancement in the biology of cyanobacterial hydrogen production in recent years. It discusses the enzymes involved in hydrogen production, viz. hydrogenases and nitrogenases, various strategies developed by cyanobacteria to limit nitrogenase inactivation by atmospheric and photosynthetic O_2, different biochemical and physicochemical parameters influencing the commercial cyanobacterial hydrogen production and the methods opted by different researchers for eliminating them to obtain maximum and sustained hydrogen production. Integrating the existing knowledge, techniques and expertise available, much future improvement and progress can be made in the field.

Introduction

With the rapid industrialization taking place in the last two decades, we have exploited, damaged and neglected the very environment which forms the very basis of our existence. Massive energy and material consumption have disrupted the delicate balance of the environment.

Present energy requirements are based on utilization of fossil fuels. Fuels such as oil, natural gas and coal supply more than 90% of the world's energy needs (Kalia 1995). Due to the escalated use of these fossil fuels, according to 'Geological survey of United States', 80% of the coal reserve will be utilized roughly within 300 years from 2000 AD and oil production will last less than a century. Also associated with the use of fossil fuels are problems of environmental pollution, global warming, acid rain and other multiplicative effects. In view of this crisis, more efforts are being focussed on development of clean and sustainable energy resources; which are renewable, non polluting and capable of meeting the global need.

Of the alternatives available, molecular hydrogen [H_2] can supplement and substitute fossil fuels. It is the most abundant element in the universe and has maximum energy per unit weight (122 KJ g^{-1}). On a weight basis, it is calculated that the heating value of H_2 is 141.65 MJ kg^{-1} which is the highest amongst known fuels (Ali & Basit 1993). It is easy to collect, therefore can be used during peak periods of demand, easy to transport and can be stored as gas-metal hydride or as liquid. It has greater energy conversion efficiency than petroleum. Hydrogen when used as a fuel does not pollute the environment, because its only conversion by-product is water. Besides its use as a fuel, hydrogen is useful in industrial applications like iron ore reduction and ammonia production (fertilizer). It has successfully been tested in aviation and automobiles (Ogden & Williams 1989).

Hydrogen can be produced in a number of ways:

1. Production from fossil fuels and biomass: coal gasification, steam reforming, partial oxidation of oil. Although these processes involve non-renewable sources and expensive techniques, these are still practised due to the abundant availability of low cost coal and oil.

2. Production from water through non-biological methods: thermal and thermochemical processes, electrolysis and photolysis. Owing to the heavy utilization of fossil and non-fossil fuels and other problems the possibility of these methods of reaching a commercial scale is very meagre.

3. Biological hydrogen production:

   a. *Fermentative hydrogen production*: Several members of the Enterobacteriaceae family generate reducing equivalents (e^-) during the degradation of organic carbon and cells evolve H_2 to dispose of these excess reductants by the action of the enzyme hydrogenase (Vos *et al.* 1983). However the disadvantages associated with these methods are the strict requirement of anaerobic conditions, energy sources and generation of CO_2 gas.

b. *Photobiological hydrogen production*: Harvesting solar energy is one of the approaches to develop clean and renewable energy resources. Solar energy is considered to be our largest and ultimate non-fossil, non-nuclear energy resource. In photobiological energy conversion photosynthetic bacteria, cyanobacteria, and green algae act under solar radiation to convert H_2O, reduced sulphur compounds and organic compounds into hydrogen. The present review is limited to photobiological conversion of water to H_2 using cyanobacteria. Cyanobacteria are oxygen-evolving, photosynthetic prokaryotes that can grow in air (N_2 and CO_2 as N and C source), water (electrons and reductant source) and simple mineral salts with light as the energy source.

The biggest natural process of conversion of light energy to chemical energy is through photosynthesis. Cyanobacteria posses photosynthetic systems having pigment molecules that capture solar quanta in the visible portion of the electromagnetic spectrum and channel this excitation energy into one of the two specialized reaction centres (PS-II) producing molecular O_2 by splitting water (Luque *et al*. 1994).

The other (PS-I) generates reducing equivalents which eventually reduce atmospheric CO_2 to organic compounds. The precise molecular distribution of the fixed carbon compounds, as well as local physiological growth conditions, will vary from species to species. However, all the reducing equivalents are ultimately derived from water. This is to say, the photosynthetic process is capable of synthesizing energy-rich biomolecules from H_2O, salts, CO_2 and sunlight.

Enzymes involved in hydrogen metabolism

The biological capacity to take up or evolve molecular hydrogen probably occurs only in the microbial system of the biosphere. These phenomena are especially more prevalent among photosynthetic microorganisms. Most photosynthetic bacteria, cyanobacteria and eucaryotic algae carry out both hydrogen consumption and evolution functions (Kosaric & Lyng 1988; Miyamoto *et al*. 1990).

Two general classes of enzymes, hydrogenase and nitrogenase, that catalyse hydrogen metabolism, are closely associated with the final H_2-evolving act in photosynthetic microorganisms (Benemann 1997). However, the primary functions of the enzymes are quite distinct. Nitrogenase is normally operative in biological nitrogen fixation whereas hydrogenase normally catalyses hydrogen uptake or hydrogen consuming reactions.

The major differences between the hydrogenase- and nitrogenase-mediated reactions is in the H_2 evolution reaction itself. H_2 evolution by nitrogenase requires considerable ATP for H_2 evolution in addition to reductants. The quanta required for H_2 evolution by nitrogenase would double those required for CO_2 fixation with an overall minimum quantum requirement of approx. 9–10 quanta/H_2. In principle, hydrogenase requires less than 1 quantum/H_2 and thus, should exhibit three times the efficiency of any nitrogenase-mediated system (Benemann 1994).

Hydrogenases

The term hydrogenase refers not to a single enzyme but a class of enzymes. Hydrogenases are enzymes that catalyse the oxidation of hydrogen to protons and the reduction of protons to hydrogen.

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

This property makes them very simple enzymatic entities that could be used under a variety of environmental conditions either to get rid of excess electrons or to gather energy through the oxidation of hydrogen by different substrates. Hydrogenases are very diverse in their relative molecular mass, co-factor composition and spectroscopic properties (Krasna 1979). Hydrogenases in microorganisms have either a Fe-S centres, nickel/Fe-S centres (selanocysteine or non selanocysteine) or are metal-free. Cyanobacteria mostly have nickel/Fe–S–(selanocysteine) hydrogenases (Appel & Schulz 1998; Schlegel & Schneider 1985).

Uptake hydrogenase

Uptake hydrogenase is located at the cytoplasmic face of the cell membrane or thylakoid membrane, where it utilizes hydrogen evolved by nitrogenase. There is a considerable loss of energy through the production of hydrogen during nitrogen fixation. Some of this energy can be regained through the action of uptake hydrogenase. This enzyme splits the hydrogen and feeds the electrons back into the electron-transport chain. The reduction of a substrate with a relatively high redox potential like cytochrome through this hydrogenase seems to be a wasteful process. But since nitrogen-fixing cells maintain a highly reducing environment, it seems necessary to use part of the reductive power of hydrogen and saving reducing equivalents. The whole process is ultimately light-driven (Figure 1).

Hydrogen-utilizing uptake hydrogenase has several functions: (1) it serves as one of the mechanisms to protect oxygen-sensitive nitrogenase (Robson & Postgate 1980), (2) it generates ATP in the hydrogen-dependent respiratory oxygen uptake ('Knallgas' or oxyhydrogen reaction) and (3) it provides additional reducing equivalents to photosystem–I. Uptake hydrogenase has been found in all heterocystous cyanobacteria and some non-heterocystous cyanobacteria (Peschek 1979).

The structural genes encoding cyanobacterial uptake hydrogenases have been sequenced and characterized in only a few strains (Axelsson *et al*. 1999). The large subunit of the enzyme is encoded by *hup*L genes and small subunit is encoded by *hup*S genes. In the organisms studied so far, there is a high degree of homology in the gene sequence of *hup*SL (Tamagnini *et al*. 1997). But