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BIOLOGICAL PHOTOHYDROGEN PRODUCTION BY CYANOBACTERIA : FUTURE PROSPECTS AS A FUEL

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ABSTRACT

Biological hydrogen production by cultivation of photosynthetic microorganisms offers an alternative clean, sustainable and conventional source of energy. Cyanobacteria produce molecular hydrogen by the activity of nitrogenases or hydrogenases. Cyanobacteria have evolved several strategies to protect the nitrogenases from the inhibitory effect of oxygen, which include spatial and temporal separation of nitrogen fixing and oxygen evolving processes and developing physical barriers like heterocysts to diffusion of oxygen. Reversible hydrogenases catalyze both uptake and evolution of hydrogen. Oxygen and nitrogen free atmosphere have been reported to dramatically increase the activity of reversible hydrogenase. In view of the requirement of oxygen free environment for both nitrogenases and hydrogenases involved in cyanobacterial hydrogen production, future research needs to be focused on exploration of novel species from diverse habitats with greater hydrogen evolving capability, development of mutants that lack hydrogen oxidizing enzyme, uptake hydrogenase and optimization of conditions in the algal bioreactors. Synergy between basic and applied aspects needs to be developed to make cyanobacterial hydrogen production sustainable and feasible alternative fuel in future.

Key Words : Hydrogenases, Hydrogen production, Heterocysts, Reversible hydrogenases, Nitrogenuse

INTRODUCTION

Energy is one of the most important inputs in every sector of country's economy and the standard of living of a country is generally related directly to its per capita energy consumption. Now a days we are facing energy crisis all over the world due to rapid depletion of limited fossil fuels. This has prompted prospecting of various non-conventional energy sources. Molecular hydrogen which is a clean, efficient and renewable energy resource is one such fuel which can overcome these problems. Photobiological hydrogen production by microorganisms involves generation of renewable energy from nature's most plentiful resources like solar energy and water[1]. Among the microorganisms, cyanobacteria are of special interest which promises both oxygenic photosynthesis and hydrogen production[2,3]. The present paper highlights the photobiological conversion of water to molecular hydrogen by cyanobacteria, its potential and prospects.

Cyanobacterial Hydrogen Production

Cyanobacteria or blue green algae are phototrophic prokaryotes in which hydrogen is produced by a light dependent reaction catalysed by nitrogenase or, in dark anaerobic conditions by a hydrogenase[4]. Photosynthetic electron transport system in the cyanobacteria provides the energy for generating reduced ferredoxin for the hydrogenases or nitrogenases to liberate hydrogen.

Filamentous cyanobacteria have three enzymes directly or indirectly involved in hydrogen metabolism viz.

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(a) Reversible hydrogenase, which is capable of evolving as well as taking up hydrogen
(b) Uptake hydrogenase, that oxidise hydrogen evolved during nitrogen fixation and
(c) Nitrogenase which produces hydrogen during fixation of molecular nitrogen[5].
Nitrogenase is present in heterocystous forms of filamentous cyanobacteria.

$$N_2 + 8H^+ + 8e^- + 16ATP \longrightarrow 2NH_3 + H_2 + 16\ ADP + 16\ Pi$$

There are three types of dinitrogenases are known. Type one contains molybdenum (Mo), the second type contains vanadium (V) and third type contains iron (Fe). Hydrogenases catalyze the oxidation of hydrogen to protons and the reduction of protons to hydrogen[6].

$$H_2 \rightleftharpoons 2H_2^+ + 2e^{-6}$$

Hydrogenases are very diverse in their relative molecular mass, cofactor composition and spectroscopic properties[2]. Cyanobacteria mostly have Ni/FeS (Selanocysteine) hydrogenases[6]. Uptake hydrogenase, encoded by *hupSL*, is located at the cytoplasmic face of thylakoid membrane, where it utilizes the hydrogen evolved by nitrogenase, thus regaining some of the reductants and energy lost during N_2 fixation. Thus, in *Anabaena variabilis* for example, *hupSL* is transcribed under conditions of nitrogen depletion through the action of the cyanobacterial global nitrogen regulator NtcA[7]. This enzyme has been found in all heterocystous cyanobacteria and some non - heterocystous cyanobacteria[8]. Uptake hydrogenase performs several functions like serving as one of the mechanisms to protect oxygen sensitive nitrogenase[9], generating ATP in hydrogen dependent respiratory oxygen uptake and providing additional reducing equivalents to photosystem I .
Nitrogenases are highly sensitive to oxygen and hydrogen production catalyzed by the nitrogenase / hydrogenases can only function under anaerobic conditions because of its extreme sensitivity to oxygen[10]. Some cyanobacteria have solved this problem by developing specialized thick walled cells known as heterocysts which maintain low oxygen tension inside, thereby facilitating nitrogenase activity, which produces hydrogen during N_2 fixation. A wide range of nitrogenase activity has been reported in cyanobacteria. **Table 1.**
Reversible hydrogenase is located in the cytoplasmic membrane. It is capable of catalyzing both hydrogen uptake and production, but it is postulated that this enzyme operates generally in the direction of hydrogen uptake in vivo[11]. Its role in cyanobacterial metabolism is still unclear. It has been suggested that this enzyme acts as a mediator in the release of excess of reducing power in anaerobic conditions. However, hydrogenase activity can also potentially serve to correctly poise the photosynthetic apparatus for action upon illumination. Indeed, dark adapted cells often show a transient burst of hydrogen upon reillumination[8].
Hydrogen production has been examined in various cyanobacteria including heterocystous as well as non-heterocystous. Light intensity, oxygen, uptake hydrogenase and gas phase composition are the key factors influencing hydrogen evolution. Hydrogen evolution shows a wide range under different growth conditions for different cyanobacterial strains. **Table 2.**
Potential of hydrogen production of Nostoc *linckia* IAM M-30 was found to be 0.17 mol. mg chl $a^{-1}h^{-1}$ in the presence of air[12]. However, hydrogen production by *Anabaena variabilis* PK84 was observed as 0.11 mol. mg chl $a^{-1}h^{-1}$ in aerobic conditions with 2% CO_2 in outdoor conditions[14].
Although there are considerable studies providing evidence for cyanobacterial hydrogen production, but there are several problems that restrict the development of cyanobacterial hydrogen as potential source of hydrogen. Hydrogen production by cyanobacteria suffers from the major drawbacks like (a) Hydrogen production rates and solar energy conversion efficiencies of some strains of cyanobacteria are very low (b) Limited number of strains of cyanobacteria has been isolated for efficient hydrogen production[15].
Hence, future research should be concentrated on the screening of cyanobacterial strains from diverse habitats having high nitrogenase activity and also

Table 1 : Nitrogenase activity of various cyanobacterial species

Name of the species	Nitrogenase activity as C_2H_4 formed (nmol. mg dry wt.$^{-1}$ h^{-1})
Anabaena sp. Strain CA[16]	0.17
Anabaena sp. Strain N9AR[16]	0.13
Phormidium valderianum BDU 20041[17]	2.2
Nostoc calcicola[18]	9.021
Anabaena cylindrica[18]	5.579
Anabaena oryzae[18]	5.076
Nostoc commune	6.3469

Table 2 : Cyanobacterial hydrogen production under different growth conditions

Name of the species	H_2 evolution (mol.mg chl a^{-1}h^{-1})	Growth conditions	H_2 evolution assay conditions
Nostoc muscorum IAMM-14 [12]	0.60	Air; 20 E/m^2/s	Air; 60 E/m^2/s
Nostoc linckia IAM M-30[12]	0.17	Air; 20 E/m^2/s	Air; 60 E/m^2/s
Anabaena sp. PCC 7120[12]	2.6	Air; 20 E/m^2/s	Ar; 60 E/m^2/s
Nostoc commune IAM M-13[12]	0.25	Air; 20 E/m^2/s	Ar; 60 E/m^2/s
Anabaena variabilis PK84[14]	0.11	Air and 2% CO_2; outdoor conditions	Air + CO_2(2%); outdoor condition (about 400 W/m^2)
Anabaena variabilis AVM13[19]	68	Air and 1% CO_2; 100 E/m^2/s	
Gloeocapsa alpicola CALU 743[15]	0.58 mol/mg protein	Sulphur free, 4% CO_2; 25 mol photons/m^2/s	Same as growth condition

efforts should be made to tackle the problem of oxygen sensitivity of these enzymes. For this various kinds of oxygen scavengers can be used.

For optimizing hydrogen production in the system, rate of electron transport from H_2O oxidizing complex to hydrogenase under anaerobic

conditions can be manipulated partially by altering pH and sulphur in the medium[16]. Development of a database for hydrogen evolving microorganisms is already underway and efforts are now focused on genetics and enzymology of the hydrogen producing system.

CONCLUSION

Above study concludes that future research should be focused on screening of more indigenous strains possessing high rates of hydrogen evolution. Also, optimization of culture conditions for hydrogen evolution conditions like light intensity, CO_2 content, temperature, pH or micronutrient content for photobioreactors would help improve production of hydrogen. Hence, linking of basic and applied research in this field is needed for making hydrogen production a feasible alternative to our fuel based energy in the future [19-20].

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