



Plant Symbiotic bacteria as the provider of Machines Fuel's hydrogen

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Abstract

Some of symbiotic bacteria as nitrogen-fixing in the roots of Leguminoz plants is producing H_2 which in some of these bacteria, hydrogenase enzymes returns some of this produced hydrogen to the stabilization cycle to produce ATP, but some of these hup-Rhizobium have not mentioned enzyme and so produce H_2 gas. Hydrogen gas as the renewable and important fuel in the industry is produced by several costly ways. This research tries to start some effort in order to producing of Hydrogen by natural symbiotic bacteria and its related laboratorial barriers.



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Introduction

Hydrogen is known as a fuel for hydrogenous machines. Hydrogen is the lightest diatomic gas which is in a gas form in normal temperature and pressure conditions. For use of Hydrogen as fuel, primary energy is required for its extraction from other natural resource and converting it to a suitable form for use in internal combustion engines or fuel cells. Nowadays, hydrogen is produced with the same process which scientists have found two centuries ago by breaking the water's molecule into hydrogen and oxygen (7). High voltage water electrolysis method is normally used to hydrogen produce in hydrogenous machines. In fact hydrogen in the air is as a mixture. The Ministry of Energy of United State of America follows two major programs for the development of hydrogen use: Hydrogen Program of Ministry of Energy and Informational Network of Hydrogen Technologies. Hydrogen is the third abundant energy on the earth which is finding in water and organic compounds primarily (1, 6). Hydrogen is obtained from hydrocarbons or water and used as fuel to generate electricity or to re-combine with oxygen to produce water. Hence, According to the high ability of hydrogen in energy production, many efforts have been made to replace these fuels recently (3). In fact, this amazing element of Mendeleev table with its single-electron capacity layer has also been used in organic and inorganic compounds is still unreachable for modern human who reach to the top of science and knowledge levels (6).

With an attitude to the nature of the agricultural sector, plants with bacterial symbiosis in the way of atmosphere nitrogen-fixing may be producing hydrogen as a side-product. Nitrogen stabilizer such as Rhizobium with creating nodules in the roots of plants leguminuz start to nitrogen-fixing and give it to the plan and taking nutritive materials. There is a symbiosis bacteria such as rhizobium which is called Bacteroides. These Bacteroides are surrounded by the membrane which nowadays is called pre- bacteroides. In pre-bacteroides membrane nitrogen-fixing is catalyzed by an enzymatic complex which is called nitroase. In fact the exchange of carbon and nitrogen take place in the pre-bacteroides's membrane. Naturally these bacteroides revive of molecular nitrogen to ammonia by de-nitrogenase enzyme in which hydrogen is produced (5).



Instead of revival a nitrogen molecule a hydrogen molecule is produced. It should be noted that nitrogen was revived by a ferrous protein which is called ferredoxin in two stages. Hydrogen produce by de-nitrogenase enzyme is the inevitable side-product of nitrogen fixation reactions. If de-nitrogenase enzyme has activity less than its optimum activity, there is not sufficient potential for revival of de-nitrogenase enzyme so a greater number of electrons is conducted to the hydrogen production. It is possible to 25 - 33 percent of ATP and electrons of the de-nitrogenase enzyme are used in hydrogen production. In 1980 it was estimated that over one million tons nitrogen release into the air from the nitrogen -fixing nodules annually (4). But this production of hydrogen in some of scientist's point of view is a waste of resources for some plants because the same energy is used to molecular nitrogen revival. Although all of stabilizers are produce nitrogen but all of them are not necessarily release hydrogen into the atmosphere (8). An oxygen-dependent enzyme which is called absorbing hydrogenase is retrieving some of energy that spent in hydrogen production. It comes to be act by hydrogen oxidation with ATP production. These produced electrons is returned to the revival resource and is used by de-nitrogenase enzyme. Vegetal genetic engineering techniques can be effective in decreasing of hydrogenase (hup) genes expression to more produce of hydrogen's side-product. Regarding to that many famous automakers such as Honda and GM defines their work instructions parallel with increasing of fuel cell production; it is obvious that hydrogen as a mediator still has much potential for fuel storage (2,3). As solar cells are resulting of photosynthesis inspiration, we can also produce hydrogen which plant pay for it cost by symbiotic bacteria inspiration. In fact the plant yield is hydrogen.



Materials and Methods

By cultivating isolated *Japonicum rhizobium beradi* bacteria of bacteroides in soybean root nodules, produced hydrogen can be measured. The bacteria are grown in an environment containing 0.5 g sucrose and 1.5% agar. The cultivation environment was artificial, containing following elements (which were autoclaved to set their pH to be 7.5):

Agar	15.0 g
K ₂ HPO ₄ ...	1.0 g
MgSO ₄ ·7H ₂ O....	0.2 g
NaCl.....	0.2 g
FeSO ₄ ·7H ₂ O...	5.0 mg
Soil extract	100.0mL

In order to creating ability and power of nodulation, rhizobium cultivation takes place in this environment. Then it is transferred to a new environment in which in addition to above mentioned circumstance, Eskuk Morashik's environment also was added to it. The nodulation of rhizobium beradi bacteria is takes place after 64 h, beside of sterile soybean plant which was yield of fiber cultivation in the rooting stage. The nitrogen fixation starts after 2 - 3 days. There were some holes in the door of cultivating container containing a filter in which the rate of gaz production for 20 minutes by gas chromatography has been evaluated.

Results and Discussion

because of absence of hydrogenase gene in hup- bacteria, resulting to increasing of side-product of nitrogen –fixation, lead to increase of H⁺ which has been seen in gas chromatography results. Mixing of Eskuk Morashik's environment and special cultivation environment lead to some problems in pH in which in the majority of cultivations there is no way to increase of soybean plant life for more than 2 weeks. We hope that in the future, further research will be done on pH balance in the mixed environment. Also charcoal was added to the environment for its better growth and making it darker similar to environment's soil. Gas analysis is indicates that three types of gas are produced: 0.4 oxygen, 0.12 Nitrogen and 0.8 Hydrogen which there is also the possibility of genetic engineering to increase the amount of produced gas and also some devices or instruments which are able to separate these three types of produced gas from each others.

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