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Hydrogen production by photosynthetic bacteria

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Photosynthetic bacteria utilize hydrogen as electron donor for autotrophic CO_2 assimilation. Many of these organisms also evolve hydrogen under dark anaerobic conditions and, in large quantities, anaerobically in the light in the absence of ammonia and molecular nitrogen. Hydrogen photoproduction in photosynthetic bacteria is largely or completely associated with the action of nitrogenase. It is not inhibited by CO, an inhibitor of hydrogenase and is dependent on ATP. The conventional hydrogenase catalyzes the reversible reaction $H_2 \rightleftarrows 2\ H^+ + 2\ e^-$. It seems however that in photosynthetic bacteria this enzyme catalyzes mainly hydrogen uptake in vivo. It has been suggested that a function of hydrogenase is to reutilize the hydrogen which is evolved as a byproduct of the nitrogenase reaction, retaining reducing equivalents for N_2 or CO_2 reduction[1]. In contrast to aerobic bacteria, energy conservation in a Knallgas reaction is not possible for photosynthetic bacteria growing anaerobically in the light[2]. Besides molecular hydrogen, a variety of organic and inorganic electron donors are known in bacterial photosynthesis. Most of them are effective also for hydrogen production in the light.

Hydrogen production and utilization in vivo are catalyzed by different enzymes. A genetic or regulatory linkage between nitrogenase and hydrogenase has been proposed in a study with nif^- mutants of *Rhodopseudomonas acidophila*[2]. It has recently been reported that in *Rhodopseudomonas capsulata* although nitrogenase may influence hydrogenase synthesis by supplying inducers (e.g., H_2), there is no strict correlation between hydrogenase synthesis and nitrogenase synthesis[3].

The exact mechanism of electron transfer in hydrogen metabolism and nitrogen fixation is not resolved so far. The figure shows a possible scheme of electron transport and hydrogen metabolism in the photosynthetic bacterium *Rhodospirillum rubrum*. A light driven electron flow generates ATP. It is assumed that NAD and other substances of negative redoxpotential are reduced in a reversed electron flow utilizing ATP.

When reducing power and energy for N_2 fixation in the cell is produced in excess, nitrogenase evolves hydrogen, which can be recycled probably via the ferredoxin-hydrogenase.

Thermodynamical aspects of hydrogen production in photosynthetic bacteria

Table 1 shows the theoretically possible ways in which glucose may be decomposed to H_2 or CH_4 and CO_2. Hydrogen formation in the dark is not regarded as an efficient process to transfer energy. Only 33% of the combustible energy of organic compounds is conserved in the fermentation products. In contrast to hydrogen, methane formation is more efficient. Approximately 85% of the energy is conserved assuming that 3 moles of methane are formed from 1 mole of glucose. In photosynthetic bacteria complete dissimilation of carbohydrates through a light dependent anaerobic Krebs cycle is thought to operate[4]. The decomposition of 1 mole of glucose yields 12 moles of hydrogen. Almost 100% of the combustible energy of glucose would be conserved. In practice about 70% energy conservation efficiency from lactate is reported[5,6].

Electron donors for hydrogen production

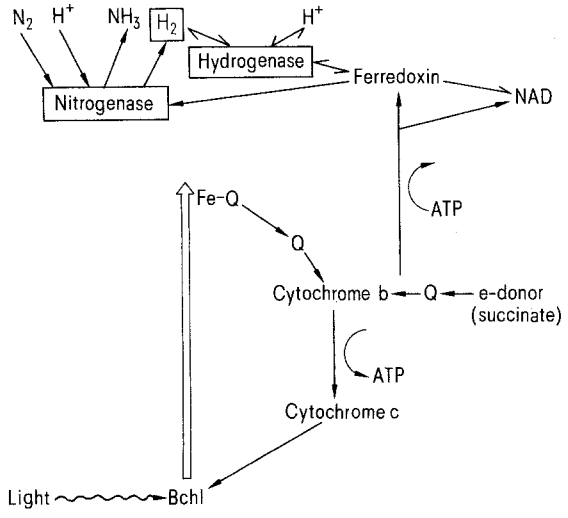
Hydrogen photoproduction has been found in species of all families of photosynthetic bacteria (Rhodospirillaceae, Chromatiaceae and Chlorobiaceae). In general, photosynthetic bacteria utilize a wide spectrum of organic substances such as carbohydrates, lipids, fatty acids and some inorganic sulfur compounds. The substrate specificity for hydrogen production varies from species to species. Some of the common electron donors used for hydrogen production by photosynthetic bacteria are summarized in table 2 (for complete data and references see Kumazawa and Mitsui[7]).

Agricultural byproducts and various wastes have been successfully treated with photosynthetic bacteria to produce biomass and to eliminate waste[8–10]. Prolonged hydrogen evolution from whey or other lactic acid containing wastes have been demonstrated[6]. Hydrogen production is limited by the availabilities of appropriate waste materials.

Hydrogen production rates and biomass potential

Hydrogen production rates reported by different researchers vary considerably. They do not always represent maximum rates that could be achieved under optimal conditions, and they are difficult to compare due to different experimental conditions. Hillmer and Gest[11] reported a rate of 130 ml of hydrogen per h and l culture with *Rhodopseudomonas capsulata* using lactate or pyruvate as electron donor. With *Rhodospirillum rubrum* a rate of 160 ml of hydrogen per h and l continuous culture was achieved (lactate as electron donor). The dilution rate was 0.05 h^{-1} and about 0.12 g of bacterial dry substance was produced per h and l culture[12].

Cells of photosynthetic bacteria are composed of about 65% protein, containing large quantities of essential amino acids and vitamins[13]. Mass culture of *Rhodopseudomonas capsulata* and their single cell protein values are reported and discussed referring to their effect on laying-hens upon mixing the bacterial



Scheme for photosynthetic electron flow and hydrogenase-nitrogenase catalyzed hydrogen metabolism in *Rhodospirillum rubrum.*

Table 1. Combustible energy of glucose compared to the combustible energy of the formed methane or hydrogen

$Glucose + 2\ H_2O \rightarrow 2\ acetate^- + 2\ H^+ + 2\ CO_2 + 4\ H_2$ $4\ H_2 + 2\ O_2 \rightarrow 4\ H_2O$	$\Delta\ G^{o\prime} = -227$ kcal/mole
$Glucose + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$	$\Delta\ G^{o\prime} = -686$ kcal/mole
$Glucose \rightarrow 3\ CO_2 + 3\ CH_4$ $3\ CH_4 + 6\ O_2 \rightarrow 3\ CO_2 + 6\ H_2O$	$\Delta\ G^{o\prime} = -586$ kcal/mole
$Glucose + 6\ H_2O \rightarrow 6\ CO_2 + 12\ H_2$ $12\ H_2 + 6\ O_2 \rightarrow 12\ H_2O$	$\Delta\ G^{o\prime} = -680$ kcal/mole

Table 2. Electron donors used for hydrogen production by photosynthetic bacteria

Rhodospirillaceae	Chromatiaceae	Chlorobiaceae
Acetate	Acetate	Citrate
Butyrate	Fumarate	Formate
Formate	Malate	Glucose
Fructose	Oxalacetate	α-Ketoglutarate
Fumarate	Pyruvate	Lactate
Glucose	Succinate	Mannitol
α-Ketoglutarate	Sulfide	Pyruvate
Lactate	Thiosulfate	Xylose
Malate		
Oxalacetate		
Propionate		
Pyruvate		
Succinate		
Sucrose		
Thiosulfate		

biomass in their feed[14]. Since phototrophic bacteria are able to grow in continuous culture using molecular nitrogen as sole nitrogen source[15], cultures can be grown on media essentially lacking bound nitrogen.

Inhibition of nitrogenase

As mentioned above, photoproduction of hydrogen is catalyzed by nitrogenase. In photosynthetic bacteria the enzyme is quickly inhibited by low concentrations of ammonium salts. This efficient inhibition poses severe problems when waste material containing varying amounts of nitrogen compounds is used as a hydrogen donor. It has been shown however that the glutamate analog L-methionine-DL-sulphoximine (MSO) relieves the repression exerted by exogenous ammonia on nitrogenase activity. Nitrogen fixation or acetylene reduction was shown to operate in the presence of ammonia upon the addition of MSO[16,17]. The inhibition of the photoproduction of hydrogen by ammonia is also released by MSO[18]. Genetically altered bacteria could also be of interest for the photoproduction of hydrogen. Glutamine auxotroph mutants of *Rhodopseudomonas capsulata* have been described, which synthesize nitrogenase and produce hydrogen in the presence of exogenous ammonia[19].

Immobilized cells

In resting cells of *Rhodospirillum rubrum* the rate of photoevolution of hydrogen decreases slowly[6]. As with other organisms it should be possible to prolong the active period by immobilizing the cells on polymer lattices. In many cases the use of immobilized enzymes and cells results in increased production[20]. Hydrogen production by immobilized whole cells of different origin *(Rhodospirillum[21], Clostridium[22], Anabaena[23])* has been reported, but so far no systematic survey is available, which compares different techniques, yield, stability etc. This technique may improve solar energy conversation into hydrogen.

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Part 2 of this review, covering: Conversion of biomass to fuel and chemical raw material; The formation of methane from biomass-ecology, biochemistry and applications; and Future systems, will appear in the February issue of Experientia.

The review will be published in its entirety as a special supplementary issue of Experientia. Copies will be available early this spring.