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Microbiological Methods of Hydrogen Generation

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1. Introduction

As long as any country economy is based on fossil fuels, the prosperity of many nations is in danger. Rapidly growing prices of oil and natural gas can lead to the worldwide economic crisis. Therefore the search for new, clean, cheap and renewable sources of energy and energy carriers is urgently required. Although many different methods are suggested to solve this problem the use of hydrogen as the future energy carrier is necessary. Application of biochemistry in generation of energy is a challenge both for academia and industry. Different types of biomass pyrolysis and/or fermentative processes can partially solve the problems of renewable energy generation. Although other solutions are at the moment much more technologically advanced (e.g. hydropower or wind farms) the future of energetic will belong to the biological systems. Generation of biogas or biohydrogen under anaerobic conditions are the very promising processes, especially at local environment. Different types of agriculture and food industry wastes can serve here as an excellent source of organic carbon in microbiological processes.

It is well known that burning of hydrogen either chemically or electrochemically (e.g. fuel cells) generates large quantities of energy and it is environmentally friendly. Application of biohydrogen in local environment (farms, small communities, etc.) certainly will improve local energy distribution and will lower costs of used energy.

This review paper describes basic principles of fermentative and phofermentative hydrogen generation. Biophotolysis of water, anaerobic dark and photofermentative processes in presence of organic substances, as well as the hybrid systems used in microbiological methods of hydrogen generation are described. The description of the applied microorganisms and enzymes is presented.

2. General

Biophotolysis of water, fermentation and photofermentation of organic substrates are considered to be the best biological methods of hydrogen generation. Reversibility, lack of toxic substances generated in these processes, mild conditions for microbiological reactions, as well as operation at low pressure of these processes are the conditions required for

modern microbiological systems. Moreover, the possibility of application of different waste waters (containing organic carbon) in these processes is an additional benefit.

Fermentation is the process generating basically two gaseous metabolites: hydrogen and carbon dioxide. The volatile fatty acids (VFA) and alcohols represent liquid metabolites of dark fermentation. The low yield of generated hydrogen and high concentration of CO_2 (almost 50%) in gaseous products are the main disadvantages of microbiological hydrogen generation. In contrary, high reaction rate and possibility of biodegradation of many organic substances can be assigned to the benefits of this process.

In photofermentation, the photosynthetic heterotrophoic bacteria under anaerobic conditions and in the absence of nitrogen generate hydrogen in presence of organic compounds. Nitrogenase is the enzyme catalyzing hydrogen generation reaction. Presence of molecular nitrogen or nitrogen compounds directs the reaction route towards ammonia formation. The possibility of application of wide spectrum of light (400-950 nm), lack of methabolism generating molecular oxygen, as well as possibility of use of organic substances originating from wastes are the main advantages of photobiological method of hydrogen generation.

Both fermentation and photofermentation require presence of anaerobic microorganisms and the light in case of photofermentation. Photosynthesis, and in consequence also photofermentation is the series of complex reactions transforming energy of light into chemical energy.

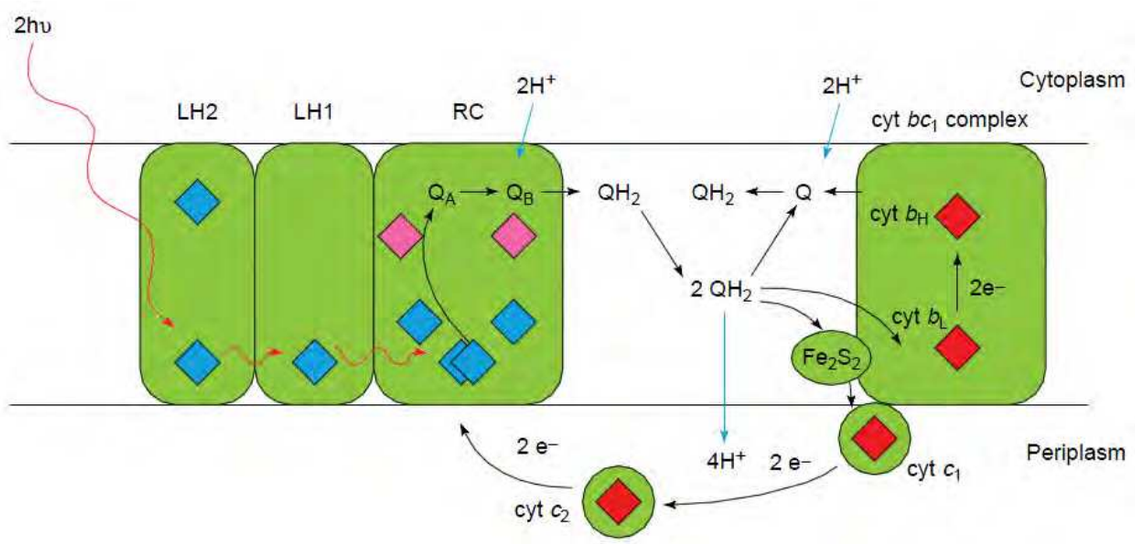


Fig. 1. Scheme of photoinduced cyclic flow of electrons in photosystem of *Rhodobacter sphaeroides* bacteria (Vermeglio, 1999).

The photosynthetic apparatus is localized in invaginations of the cytoplasmic membrane. The photosystem is built of three multimeric (transmembrane) proteins: antennas making the light-harvesting complex (LHC), the reaction centre (RC) and the complex of cytochromes bc_1 (Fig.1) (Vermeglio, 1999). The LHC antennas contain molecules of bacteriochlorophyll and carotenoides. The carotenoides play a double role in LHC systems;

they absorb light from the visible part of the light spectrum in which bacteriochlorophyll is not active and protect the antenna system against damage by singlet oxygen (Isaacs, 1995, Jones, 1997). The majority of the purple bacteria have two different antenna complexes known as LH1 and LH2. The number of LH2 complexes depends on such parameters like light intensity and partial pressure of oxygen, while the number of LH1 complexes is directly correlated with that of the reaction center (RC) to form RC-LH1 center. High ratio of pigment molecules to RC (e.g. 100 molecules of chlorophyll to one RC) increases the area capable of light absorption. Upon absorption of photon by LHC, the reaction centre becomes excited with simultaneous charge separation in a time shorter than 100 picoseconds (ps). The high reaction rate of this process is a consequence of the mutual arrangement of LH1 and RC: one RC is surrounded by a ring of 15-17 LH1 subunits. The closed structure of LH1 complexes in combination with the dense packing of bacteriochlorophyll molecules ensures fast delocalization of the excited state and possibility of energy transfer towards the reaction centre from every point of the ring (Vermeglio, 1999). The reaction centre is an integral part of protein membrane composed of three polypeptides (subunits L, M and H), containing four molecules of bacteriochlorophyll *a* (P_A, P_B, B_A, B_B), two molecules of bacteriofeophityne *a* (H_A, H_B), two molecules of ubichinone(Q_A, Q_B), one molecule of carotenoid (Crt) and one atom of non-heme iron (Fig.2).

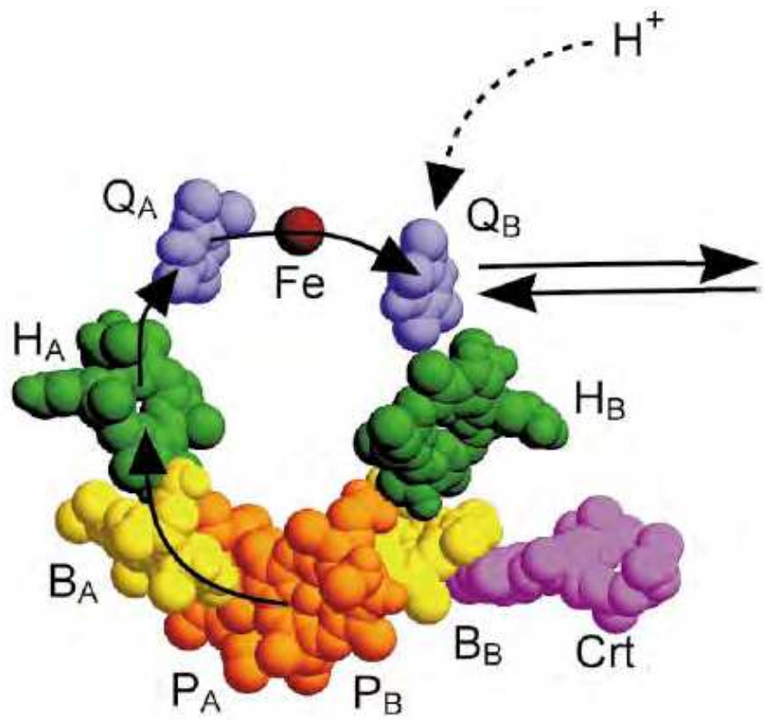


Fig. 2. Reaction center (RC) of photosystem in *Rhodobacter sphaeroides* bacteria (Isaacs, 1995)

All pigments are linked to the heterodimeric protein skeleton of L and M subunits forming five transmembrane protein helixes (Paschenkoa, 2003). The main source of electrons is the "special pair" of the excited bacteriochlorophylls *a* located close to side of the cytoplasmic membrane. The excitation is realized by direct absorption of light by the "special pair" of bacteriochlorophylls absorbing at 870 nm and by energy transfer from other pigment

molecules located at RC or LHC. The transfer of electrons from the special pair to bacteriopheophytin, located in the middle of the dielectric cytoplasmic membrane occurs in 3-4 ps. This reaction is probably intermediated by a transient product of monomeric bacteriochlorophyll B_A. In the next 200 ps the electron is transferred to ubiquinone Q_A (connected with RC) and subsequently to ubiquinone Q_B. The transfer of electron to ubiquinone Q_B is accompanied by its protonation. The full reduction of ubiquinone Q_B requires two subsequent cycles in RC after which electrons finally leave RC with electrostatically neutral doubly reduced ubiquinol QH_2 (Jones, 1997). The two protons required for protonation originate from cytoplasmic space. In the next step ubiquinol is oxidized by the bc_1 cytochrome complex. This complex caused reduction of the $[Fe_2S_2]$ unit which is a part of cytochrome (part of Rieske unit) and releases two protons to periplasmic space. Then the cycle of electron transfer is closed by recombination of cytochrome c_2 by reduction of the special pair of bacteriochlorophylls. The cyclic transfer of electrons is accompanied by transfer of protons from cytoplasm to periplasm leading to the proton gradient between the two sides of cytoplasmic membrane, which is the most important effect of photosynthesis because it stimulates ATP synthesis and reduction of NAD^+ (Vermeglio, 1999). Protons accumulated on the periplasmic space of the membrane return to the cytoplasmic space through the ATP synthase channel, which closes the transfer of protons (Paschenkoa, 2003).

3. Microorganisms

Hydrogen generation in microbiological processes can be realized both by eucariota (green algae), procariota (cyanobacteria) in direct or indirect splitting of water under illumination, as well as in the fermentation and photofermentation reactions in presence of organic substances and numerous strains of bacteria. Due to very low yields of hydrogen obtained in presence of algae and cyanobacteria this paper will concentrate only on fermentative processes.

Dark fermentation process towards hydrogen is performed in presence of organothrophic bacteria. Large variety of microorganisms is involved in these reactions, therefore this paper will focus only on the description of three groups of microorganisms.

The first group belongs to anaerobic, gram-negative, mesophilic bacteria of *Clostridium* and *Bacillus* type. *C. acetobutylicum* (Chin, 2003), *C. butyricum* (Masset, 2010, Cai, 2010) *C. pasterianum, C. bifermentants* (Wang, 2003), *C. beijerinckii* (Skonieczny, 2009), *C.tyrobutyricum* (Jo, 2008), *C. saccharoperbutylacetonicum* (Alalayaha, 2008), *B. lichemiformis* (Kalia, 1994), *B.coagulans* (Kotay, 2007) are the most popular representatives of this group. This strains of bacteria can form spores capable to survive in extreme conditions such as low and high temperature, different pH, irradiation, extreme dry conditions or presence of deadly chemical compounds (eg. NaCl). Bacteria cells under these conditions goes to anabiosis: complete reduction of metabolic processes. The separation of already divided DNA occurs at this stage with simultaneous surrounding by two cytoplasmic membranes. The endospore formed under unfavourable conditions can return to normal activity under appropriate conditions. In this process the external protection of outer coating is destroyed. Appropriate temperature, pH and presence of feed compounds facilitate formation of vegetative cells and their growth (Setlow, 2007). In some cases presence and increase of concentration of specific compounds is the biochemical signal to stop the endospore phase. Presence of alanine, serine, cysteine together with lactic acid accelerate germination *C. botulinum* bacteria (Plowman, 2002).

The second group of fermentative bacteria active in hydrogen generation belongs to anaerobic gram-negative bacteria. The best activity in biohydrogen generation *via* dark fermentation was found for the following strains: *Enterobacter asburiae* (Jong-Hwan, 2007), *Enterobacter cloacae* (Mandal, 2006), *Enterobacter aerogenes* (Jo, 2008), *Escherichia coli* (Turcot, 2008), *Klebsiella oxytoca* (Wu, 2010) or *Citrobacter Y19* (Oha, 2003). These strains of bacteria can tolerate oxygen in environment. Here, in aerobic condition the oxygen respiration can occurs. The change of metabolic pathway provides method for survival under variable conditions of environment. These bacteria show better biological activity in comparison with those active only in completely anaerobic conditions. However, in aerobic conditions no hydrogen formation is observed. This effect is caused by inhibition of hydrogenase, enzyme catalyzing hydrogen generation.

Thermophilic bacteria operating at 60-85 ^{o}C belongs to the third group of bacteria generating hydrogen in fermentative processes (Zhang, 2003). The following strains of thermophilic bacteria of *Thermoanaerobacterium thermosaccharolyticum* (Thonga, 2008) and hyperthermophilic of *Thermatoga neapolitana* (Mars, 2010, Eriksen, 2008), *Thermococcus kodakaraensis* (Kanai, 2005), or *Clostridium thermocellum* (Lewin, 2006) can generate hydrogen in presence of organic substrates at relatively high temperatures. It was established that thermophilic bacteria are the most effective from all those already described.

Application of *C. saccharolyticus* and *Thermatoga elfii* thermophilic bacteria results in 80 % yield of the theoretical one (theoretically 4 moles of glucose can be transformed into acetic acid with 100% yield) while applying saccharose or glucose (Vardar-Schara,2008), respectively. High yield in hydrogen generation is explained by Guo *et al.* (Guo, 2010) who assumes that high temperature can accelerate hydrolysis of substrates engaged in this process. At the same time Valdes-Vazquez et al. (Valdes-Vazquez, 2005) demonstrates that such results are not surprising, because optimal activity of hydrogenase is 50-70 °C. Unfortunately, the high yield of hydrogen generation with thermophilic bacteria is not equivalent to total amount of generated gas (Hallenbeck, 2009). In this situation the construction of bigger reactors is required what in consequence increase total costs. Moreover, reaction performed at higher temperatures require additional thermal energy supplied to the bioreactor.

Photofermentation in hydrogen generation is the process which requires appropriate strain of bacteria, organic substances (mainly VFA) and light with appropriate intensity. The following strains of bacteria indicate activity in photoproduction of hydrogen: *Rhodobacter sphaeroides* (Koku, 2002), *Rhodobacter capsulatus* (Obeid, 2009), *Rhodovulum sulfidophilum* (Maeda, 2003), *or Rhodopseudomonas palustris* (Chen, 2008). The research of new strains active in photogeneration of hydrogen is performed in numerous laboratories all over the world. These efforts were recently awarded by discovery of activity in *Rheudopseudomanas faecalis* (Ren, 2009).

Rhodobacter sphaeroides belong to the group of bacteria the best recognized in hydrogen generation. These gram-negative bacteria belongs to the purple non-sulfur (PNS) *Proteobacteria* subgroup (Porter, 2008). The morphology is different because the shape of these bacteria as well as their dimensions strongly depends on the medium (see Fig. 3). In medium containing sugars the dimensions are limited to 2.0-2.5 x 2.5-3.0 μm, whereas under other conditions they can vary from 0.7 to 4.0 μm (Garrity, 2005).

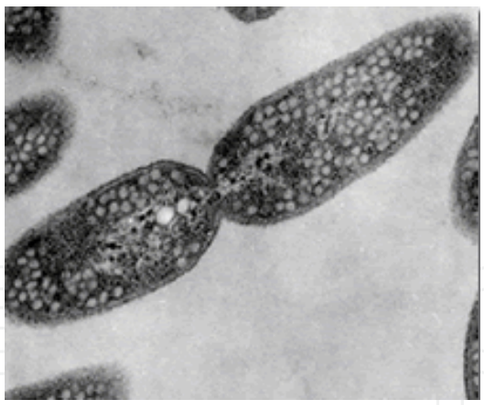


Fig. 3. *Rhodobacter sphaeroides* ATCC 17039 (Garrity, 2005).

Rhodobacter spheroides indicate strong chemotaxis with certain sugars, aminoacids and several organic acids (Packer, 2000). They are also capable to accept molecular nitrogen. Their metabolism is very elastic because they can germinate both in aerobic conditions (with or without light) as well as in anaerobic environment, in presence of light.

Under aerobic conditions this strain is used in purification of animal wastes (Huang, 2001) and biotransformation of toxins present in plant extracts (Yang, 2008). In the absence of oxygen *Rhodobacter spheroids* can be used in synthesis of carotenoides (Chen, 2006) and the most of all in hydrogen generation (Kars, 2010).

4. Enzymes

All biological processes require presence of specific enzymes. Processes of reduction of protons as well as oxidation of hydrogen (see reaction below)require at least presence of three enzymes: iron hydrogenase, nickel-iron hydrogenase and nitrogenase.

$$H_2 \leftrightarrow 2H^+ + 2e^- \quad (1)$$

Hydrogenase exists in a number (~ 40) of prokariota, both aerobic and anaerobic, as well as in certain eukariota, e.g. in photosynthetic algae (Nickolet, 2000). Hydrogenases show different but significant sensitivity towards oxygen and light. Among more than 100 discovered hydrogenases essentially only those containing Fe and Ni atoms in active center are considered as the most attractive:

- [Fe] hydrogenase - containing only Fe atoms is the most sensitive towards oxygen inhibition but almost 100 times more active than [Ni-Fe] hydrogenases
- [Ni-Fe] or [Ni-Fe-Se] - these two types of hydrogenases indicate much higher affinity towards hydrogen than [Fe] hydrogenase (Darensbourg , 2000).

Active centers of both hydrogenases are composed from iron-sulfur clusters coordinated by carbonyl (CO) or cyanide (CN^-) ligands.

Iron hydrogenases are the two directions enzymes because they catalyze both reduction of protons to the molecular hydrogen and the reverse reaction. There are three forms of these enzymes: monomeric – build only from the subunit controlling catalysis, dimeric, trimeric and tetrameric. Active centers located in these enzymes are not uniform either, however, all

of them contain H-cluster (see Fig. 4) (Nicolet, 2000). Applying FTIR, EPR and XRD spectroscopy for analysis of monomeric hydrogenase, isolated from *Clostridium pasterianum* , it was found that H-cluster is composed from two basic units: [4Fe-4S] single group, responsible for electron transport, and the unique arrangement of [2Fe] capable to perform the reverse oxidation reaction of hydrogen. The regular cluster [4Fe-4S] is linked with four cysteine and sulfur atom of one of these forms the bridge bond between [4Fe-4S] and [2Fe]. In this dimeric system, the octahedral iron atoms are linked through two sulfur atoms (see Fig. 5) (Darensbourg, 2000). Moreover, it was found that these atoms are coordinated with five non-protein ligands (CO and CN-1) and water molecule. The bridge sulfur atoms forms additionally the 1,3- propanodithiol structure. The presence of covalent bond between sulfur atoms influence the charge of H-cluster and electric properties (Nicolet, 2000).

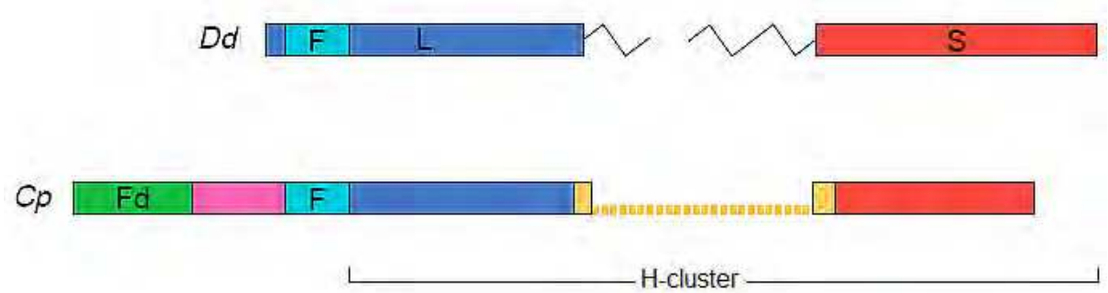


Fig. 4. Scheme of iron hydrogenase in *Desulfovibrio desulfuricans (Dd)* and *Clostridium pasterianum (Cp)*. F – double cluster of [4Fe-4S], L-large subunit of H-cluster, S – small subunit of H cluster, Fd- [2Fe-2S] cluster related to ferredoxin. Pink color represents the unique structure of [4Fe-4S]. In *Dd* hydrogenase large and small subunits are connected via cysteine, whereas in Cp hydrogenase these units are linked with protein chain.

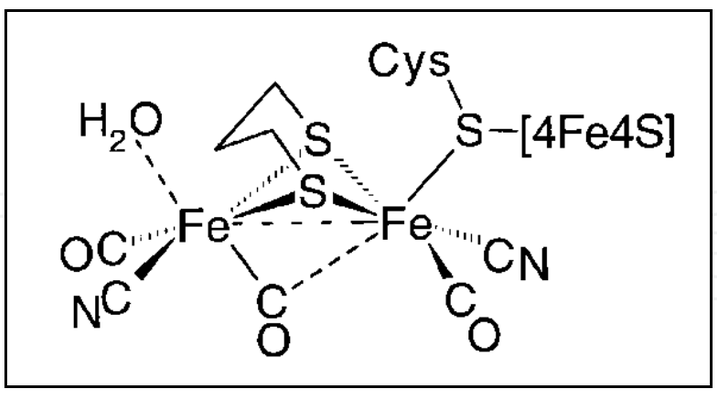


Fig. 5. Scheme of active center of iron hydrogenase (Darensbourg, 2000)

In active center of hydrogenase it is possible to identify such aminoacids as methionine and histidyne (Das, 2006). These two amino acids become attached to active center during formation of channels (for H_2 and H^+) connecting enzyme surface with reaction slit. The comparison of H-clusters in two strains of bacteria *Clostridium pasteurianum (Cp)* and *Desulfovibrio desulfuricans (Dd)* shows that in both cases the [2Fe] group is involved in hydrogen bond formation with lysine. However, when the second iron atom in *Cp* is

engaged with serine, in the case of *Dd,* alanine is involved instead. In the case of fermentative bacteria of the *Clostridium* family in the large unit of monomeric iron hydrogenase it was confirmed a presence of three excessive systems: the [2Fe-2S] structure, rarely existing [4Fe-4S] structure with slit and space constructed from two [4Fe-4S] systems (Vignais, 2006).

Nickel–iron hydrogenase isolated from *Desulfovibrio gigas* and *Desulfofibrio vulgaris* is composed from large subunit α (60 kDa) containing Ni-Fe active center and small subunits β (30 kDa) equipped with three iron-sulfur clusters. These clusters are involved in electron transfer between active centers, donors and acceptors. All these clusters are located in the strait lines in which [3Fe-4S] appears between two [4Fe-4S] structures (Vignais, 2006).

The active center f [Ni-Fe] hydrogenase exhibits the unique location of ligands (see Fig. 6)

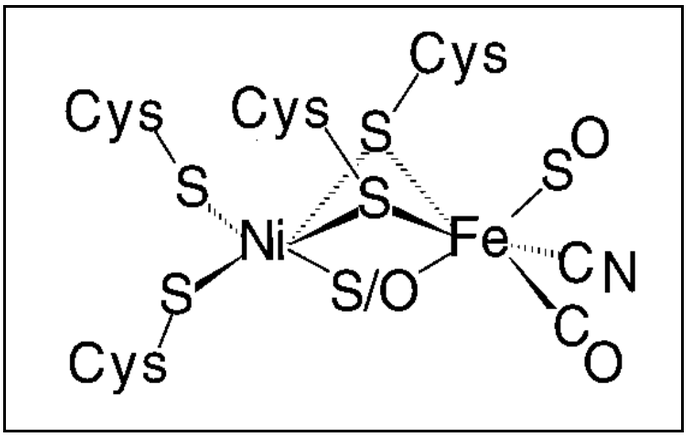


Fig. 6. Scheme of active center of nickel-iron hydrogenase (Darensbourg, 2000).

Here, four molecules of cysteine coordinate one three valent nickel atom. Two of them coordinate simultaneously iron, also located in active center. This kind of arrangement induce formation of sulfur bridges between nickel and iron atoms. Moreover , non-protein ligands such as SO, CO, CN and CO, CN are located in active centers of *D. vulgaris* and *D. gigas*, respectively. Nickel and iron atoms are bonded with monoatomic sulfur (*D. vulgaris)* or oxygen (*D.gigas*) bridges. Generated space is an ideal place for hydrogen reduction with electrons transported by iron-sulfur clusters from the surface of enzyme. The change of nickel valance form III to 0 and the return to basic state together with reconstruction of sulfur (or oxygen) bridge is observed in this catalytic cycle.

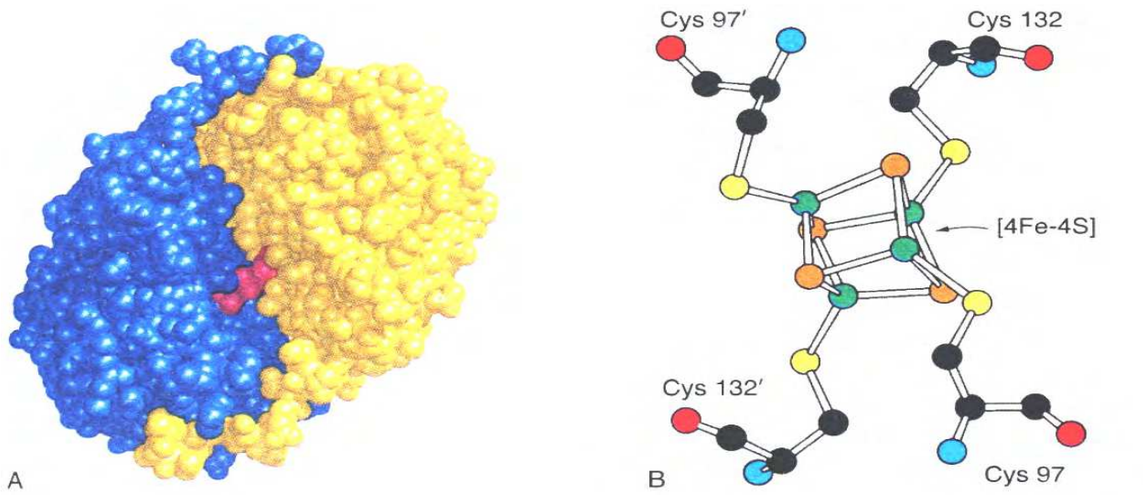
Nitrogenase is considered as the essential part of nitrogen circulation system in the living world. Nitrogen present in the air, needs to be transformed into compounds acceptable by living organisms. The diazotrophic microorganisms, including the PNS bacteria, are able to transform atmospheric nitrogen into NH_3. There three types of nitrogenases built of two separate protein units: dinitrogenase (either Mo-Fe protein, or V-Fe protein, or Fe-Fe protein) and reductase (Fe protein). The main task of reductase is the delivery of electrons of high reductive potential to nitrogenase which uses them to different reduce N_2 to NH_3. Six electrons are involved in this process to reduce the oxidation degree of nitrogen from 0 to 3. The enzyme also transfers two other extra electrons to protons with final formation of one molecule of H_2. Reduction of nitrogen to ammonia is highly energy consuming process

because of the necessity of breaking the stable triple bond in nitrogen molecule and needs 16 ATP molecules per one molecule of nitrogen:

$$N_2 + 8H^+ + 8e + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i \quad (2)$$

Both components, nitrogenase and reductase are iron-sulphur proteins, in which iron is bonded with sulphur both in cysteine and the inorganic sulphide.

Reductase (Figure 7) is a dimer with mass of 30 kDa composed of four iron atoms and four inorganic sulphides (4Fe-4S). The site for ATP/ADP bounding is located on the surface of this subunit. Reductase transfers electrons from the reduced ferredoxin towards dinitrogenase. This process occurs during hydrolysis of ATP with simultaneous dissociation of the complex.



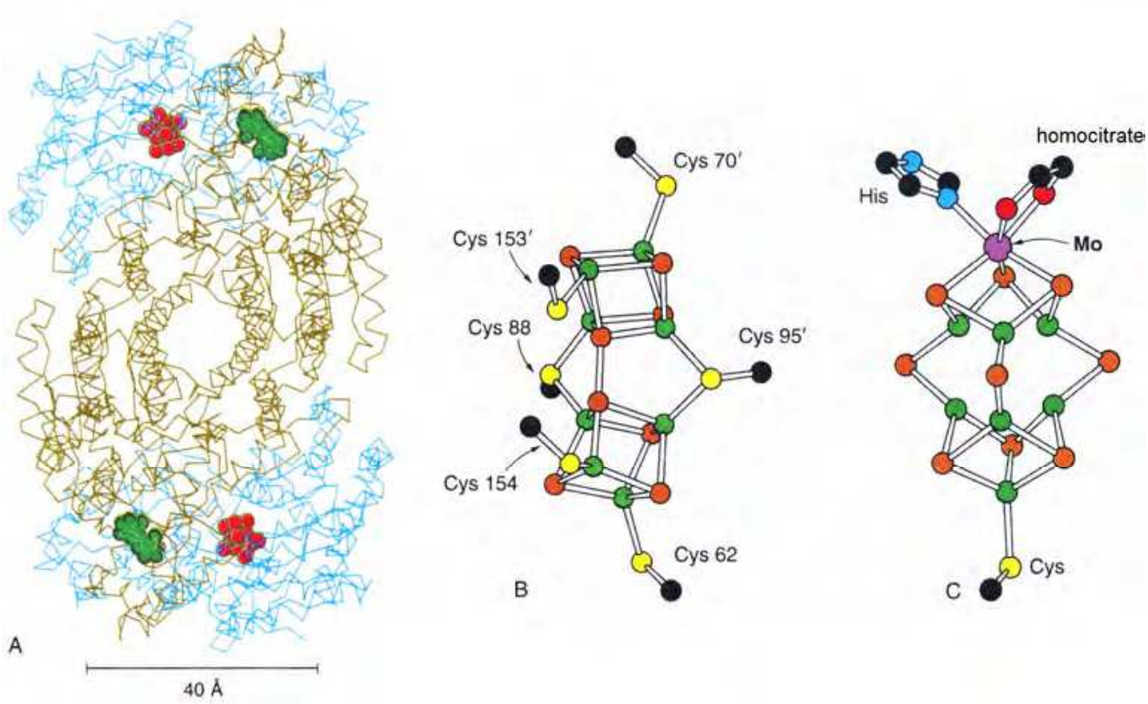
A. Red: ADP molecule obtained during ATP hydrolysis (location at the boundary of two dimers – blue and yellow),
B. [4Fe-4S] cluster located on the boundary of dimers. Green – iron, orange - inorganic sulfur, black – carbon, yellow organic sulfur, blue – nitrogen, red – oxygen,

Fig. 7. Reductase structure- nitrogenase component (Berg, 2002).

Dinitrogenase is a tetramer of the structure $\alpha_2\beta_2$ and molecular weight of 240kDa (Figure 8). At the interface between the α and β subunits there is the P unit through which electrons are able to penetrate. Two cubo-octahedrons of 4Fe-4S are linked *via* sulphur atoms from cysteine residues. The flow electrons is realized from P unit to coenzyme Fe-Mo. This coenzyme is built of two units of M-3Fe-3S linked via sulphur atoms. In one unit M stands for Mo, while in the other one for Fe. Atmospheric nitrogen is transformed in the central part of coenzyme Fe-Mo. Multiple interactions of Fe-N type weaken the triple bond in molecular nitrogen which lowers the activation limit for nitrogen reduction (Berg, 2002). The synthesis of nitrogenase strongly depends on the light access to the medium and its intensity. Catalytic stability of nitrogenase is ensured by alternating light and dark 12-hour periods (day and night sequence) (Meyer, 1978). In the absence of molecular nitrogen and with large quantities of energy provided by ATP (Koku, 2002) nitrogenase catalyses hydrogen generation (see eq.3). Nitrogenase acts as a safety valve regulating cell reduction potential (Kars, 2010).

$$2H^+ + 2e + 4ATP \rightarrow H_2 + 4ADP + 4P_i \quad (3)$$

There are two main inhibitors of nitrogenase during hydrogen photobiogeneration: molecular oxygen and nitrogen. In the presence of molecular nitrogen occurs competitive nitrogen fixation reaction and this stops almost completely hydrogen evolution. Ammonium ions at concentrations higher than 20 μmol are successful but reversible inhibitors of hydrogen generation (Waligórska, 2009) as well. The nitrogen necessary for the cell functioning is usually provided by ethanolamine and glutamate.



A. Blue lines - two chains of α tetramer, yellow lines – β subunits, green – P group, red – Fe-Mo coenzyme.
B. P group composed from two subunits of [4Fe-4S]. Colors description the same as in Figure 7.
C. Mo-Fe coenzyme. Mo bonded with homocitrate of histidine, Fe bonded with cysteine.

Fig. 8. Dinitrogenase construction (Berg, 2002).

However, glutamate can be the source of nitrogen inhibiting hydrogen evolution similarly as non-ammonium compounds. It can when glutamate becomes the source of carbon after the other sources are exhausted (Koku, 2002). In order to avoid such situation a medium with a relatively high ratio of organic carbon to nitrogen should be applied (e.g. malate to glutamate =15/2 (Eroglu, 1999).

5. Substrates and metabolism

The metabolism of carbon during fermentation process towards hydrogen is based on transformation of pyruvate in presence of majority of microorganisms active in this reaction. The first step of dark fermentation is based on glycolysis occurying in cytosol of cell, also known as **Embden-Mayerhof-Parnas** (EMP) pathway (Stryer, 1999). This pathway is

initiated by one molecule of glucose, catalyzed by different enzymes and further transformed into 2 molecules of pyruvate. The energy liberated during oxidation of 3-phosphoglycerol aldehyde is sufficient for phosphorylation of generated acid towards 1,3-bisphosphoglycerol and reduction of NAD^+ to NADH. This reaction is catalyzed by 3-phosphoglycerol dehydrogenase. Transformation of glucose to pyruvate is during glycolysis is accompanied by formation two molecules of ATP and two molecules of NADH.

Glucose is not the only substrate in glycolysis. Simple sugars such as fructose or galactose as well as complex sucrose – saccharose, lactose, maltose, cellobiose or cellulose can be used as the initial substrate for glycolysis. However, the incorporation of these complex sugars into glycolysis pathway require initial hydrolysis to the simple carbohydrates.

Glycerol can be considred as a good substarate for glycolysis. A part of glycerol is oxidized into dihydroxyacetone by glycerol dehydrogenase. Next, dihydroxyacetone is phosphorylated into phosphodihydroxyacetone in the presence of dihydroxyacetone kinase. Thanks to triozophosphate isomerase phosphodihydroxoacetone is transformed into 3-phosphoglycerol aldehyde and further participate in EMP pathway.

There are known also other anaerobic pathways transforming glucose into pyruvate as e.g. Entner-Daudoroff or phosphate pentose pathway (Schlegel, 2003, Dabrock, 1992, Vardar-Schara, 2008, Chin, 2003).

Entner-Doudoroff pathway goes from glucose to pyruvate and is known also as 2-keto-3-detoxy-6-phosphogluconate. Here, glucose-6-phosphate is transformed with phosphogluconate dehydrogense into 6-phosphogluconate. In the next step, the removal of water from 6-phosphogluconate leads to formation of 2-keto-3-deoxy-6-phosphogluconate. This process is followed by formation of pyruvate and 3-phosphoglycol phosphate. These transformations are analogous to glycolitic pathway already described. One molecule of glucose is transformed into molecules of pyruvate with simultaneous formation of one NADPH (reduced dinucleotide nicotinoamine adenine phosphate and one molecule of ATP (Schlegel, 2003).

Pentophosphate pathway is based on initial phosphorylation of glucose to glucose-6-phosphate with help of hexokinase. Further steps are more complicated. The glucose-6-phosphate dehydrogenaze transfer hydrogen to NAD simultaneously forming of gluconolactone. The phosphate gluconolactone dehydrogenase helps to generate 6-phosphogluconate acid. The last phase is based on decarboxylation of the acid into ribuloso-6-phosphate. The transfer of this compound into riboso-5-phosphate and xylulose-5-phosphate starts a non-oxidative phase. At this stage of reaction the reversible reaction between these compounds occurs with formation of sedoheptulose-7-phosphate and 3-phosphoglycerate aldehyde. Subsequent reactions can generate fructose-6-phosphate an erythrose-4-phosphate. In further reactions erythrose-4-phosphate is transformed into 3-phosphoglycerate aldehyde and fructose-6-phosphate. Thus, one cycle of pentophosphate pathway generates 2 molecules of fructose-6-phosphate, one molecule of 3-phosphateglycerol aldehyde, 3 molecules of CO_2 and 6 molecules of NADPH. The pentophosphate pathway with glycolysis leads finally to the pyruvate formation (Schlegel, 2003).

In the next steps in anaerobic conditions, the oxidative decarboxylation of pyruvate occurs with acetylo-CoA and CO_2 formation. This reaction is catalyzed by pyruvate oxyreductase

and the reduced form of ferredoxin appears as a step in final oxidation catalyzed by hydrogenase. Here, electrons reduce protons to molecular hydrogen. The reduced ferredoxin is also formed in glycolysis as the result of NADH oxidation to NAD (Dabrock, 1992). Carbon dioxide, acetic acid, lactic acid ethanol, butanol and acetone accompany hydrogen formation:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (4)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3COCH_3 + 3CO_2 + 4H_2 \quad (5)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (6)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2\ CH_2OH + 2CO_2 + H_2O \quad (7)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (8)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH \quad (9)$$

These reactions indicate that theoretical yield of hydrogen should 4 moles of hydrogen per one of glucose when acetone or acetic acid are among the products (Vardar-Schara, 2008).

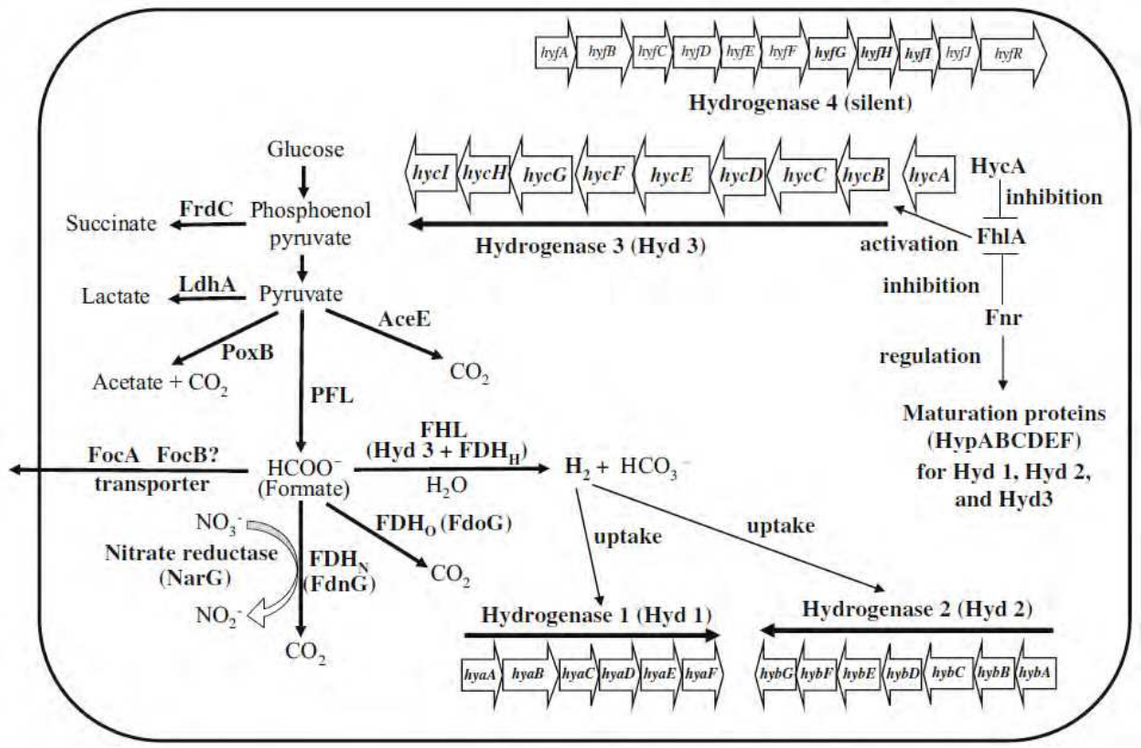


Fig. 9. Scheme of fermentative hydrogen production in *E. coli* (Maeda, 2008).

Cells metabolize glucose into phosphoenolpyruvate, pyruvate, and formate. Phosphoenolpyruvate is converted to succinate by fumarate reductase (FrdC), and pyruvate is converted to either lactate by lactate dehydrogenase (LdhA), to carbon dioxide (CO_2) and acetate by pyruvate oxidase (PoxB), to carbon dioxide by pyruvate dehydrogenase (AceE),

or to formate by pyruvate formate lyase (PFL). Hydrogen is produced from formate by the formate hydrogen lyase (FHL) system consisting of hydrogenase 3 (Hyd 3) and formate dehydrogenase-H (FDHH); the FHL is activated by FhlA that is regulated by Fnr and repressed by HycA. Evolved hydrogen is consumed through the hydrogen uptake activity of hydrogenase 1 (Hyd 1) and hydrogenase 2 (Hyd 2). Formate is exported by FocA and/or FocB and is metabolized by formate dehydrogenase-N (FDHN; FdnG), which is linked with nitrate reductase A (NarG) and formate dehydrogenase-O (FDHO; FdoG). HypABCDEF are maturation proteins for hydrogenases 1, 2, and 3 (Maeda, 2008)

Transformation of pyruvate to acetylo-CoA and formic acid occurs in the presence of puruvate-formate liase with relatively anaerobic microorganisms. Formic acid is then transformed into hydrogen and CO_2 in the presence of formic-hydrogen lyase. Here, 2 molecules of hydrogen from one molecule of glucose can be generated. Similarly as in the case of completely anaerobic bacteria, pyruvate can form lactic acid (reaction 9), whereas acetylo-CoA into ethanol and acetic acid (reactions 8 and 4). These processes can lower the theoretical amounts of generated hydrogen. Additional negative effect comes from the formation of succinic acid. Namely, formate-hydrogen lyase. become active only at low values of pH what in consequence is caused by formation of acids. Thanks to the decomposition of formic acid further fermentation towards other acids can proceed (Dabrock, 1992, Hallenbeck, 2009).

The absence of photosystem II in purple non-sulphur bacteria eliminates the problem of oxygen inhibition in hydrogen generation. However, in order to decompose water molecule and generate an electron in the photobiological process, the PNS bacteria need simple organic and inorganic compounds for photosynthesis. Organic compounds are a source of carbon and electrons. The PNS bacteria can use also CO_2 as a source of carbon after transformation of metabolism into photoautotrophic one. However, if the light intensity is too low to reduce CO_2 then the cell can use H_2 and even H_2S (at low concentrations) as a source of electrons (Kars, 2010). However, CO_2 absorption is the basic metabolic process in the cell developing either in autotrophic or heterotrophic systems. The removal of RuBisCO enzyme *via* genetic modification of PNS bacteria results in the decline of photoheterotrophic development (Akkerman, 2002). Hydrogen generation with PNS bacteria can be realized in the presence of such simple organic molecules as acetate, lactate, malate or glucose. The maximum theoretical yields of conversion of these compounds to photogenerated hydrogen are described by the following equations:

(acetic acid) $$C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 4H_2 \quad (10)$$

(lactic acid) $$C_3H_6O_3 + 3H_2O \rightarrow 3CO_2 + 6H_2 \quad (11)$$

(malic acid) $$C_4H_6O_5 + 3H_2O \rightarrow 4CO_2 + 6H_2 \quad (12)$$

(glucose) $$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2 \quad (13)$$

The theoretical amounts are usually much higher than those observed in experiments. The conversion of lactate and malate occurs easily with relatively high yields, but that of acetate and glucose is much more difficult and gives low yields of hydrogen (Kars, 2010). The discrepancies between theoretical values in hydrogen productivity and those obtained in experiments can be explained by different metabolic pathways of carbon in PNS bacteria (Figure 10, Koku, 2002).

The amount of electrons generated on absorption of organic compounds depends on the source of organic carbon. Even a slight difference in the molecular structure can lead towards completely different metabolic pathway. For example, D- and L-isomers of malate (after conversion into pyruvate) can easily join the TCA cycle. In this way the energy demand for hydrogen generation is met, whereas such a substrate as acetate is used in the other metabolic pathways: e.g. glyoxylate cycle, citramalate cycle, and ethylmalonyl-CoA pathway (Kars, 2010). The excess of electrons generated during assimilation of such substrates as glycerol or butyrate must be accepted during CO_2 photoreduction. Therefore, when the only source of carbon is glycerol, it is not assimilated in significant amounts, which is changed after supplementation of glycerol with malate. Initially, malate is assimilated from the medium and evolution of CO_2 occurs. In the second step of reaction, the evolved CO_2 permits the use of glycerol as a substrate (Pike, 1975).

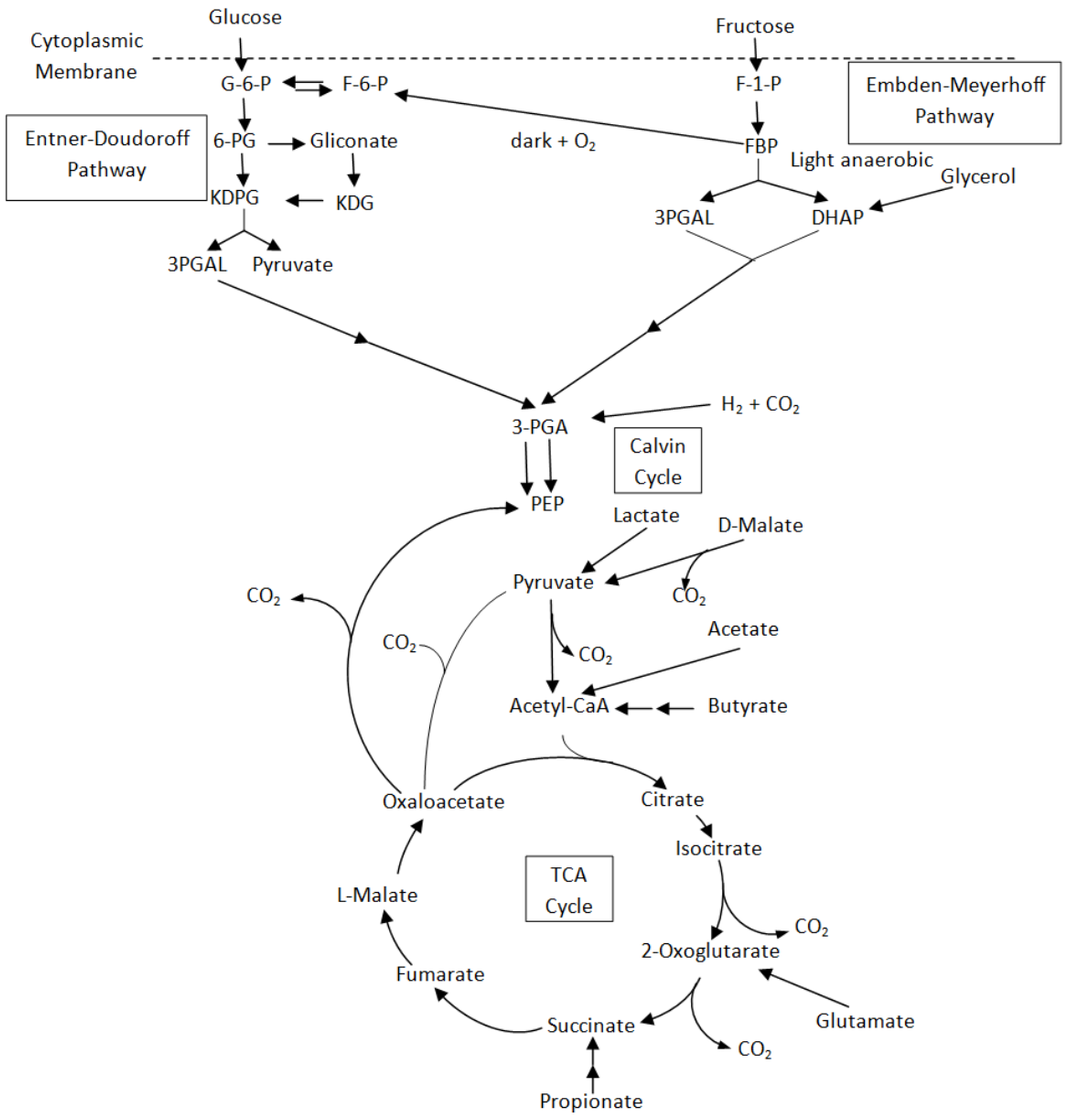


Fig. 10. Simplified scheme of carbon metabolism in *Rhodobacter sphaeroides* bacteria (Kotay, 2008).

Although the large variety of substrates can be used by photosynthetic bacteria, only a few fulfill the requirements for fast reaction rate and high yield of photogenerated H_2. In general, the preferred substrates as anions of organic acids, whereas carbohydrates do not meet the above criteria (Koku, 2002).

6. Microbiological methods of hydrogen generation

Biological methods of hydrogen generation from water in presence of microalgae and cyanobacteria are known since seventies of XX century. This process can performed in direct and undirect biophotolysis.

Direct biophotolysis

Cells of certain algae (eg. *Chlamydomonas reinhardtii, Chlorealla fusca*) or cyanobacteria are capable to split water into molecular hydrogen and oxygen under illumination.

$$2H_2O + \text{light energy} \rightarrow 2H_2 + O_2 \quad (14)$$

This process require absolutely anaerobic conditions. Light energy with wavelength lower than 680 nm is absorbed by photosystem II (PSII) and generate stream of electrons and protons originating from water. Other photosystem (PSI) is induced with light wavelength lower than 700 nm. This allows for transportation of electrons from PSII to PSI *via* chain of reductors called cytochrome *bf*. Electrons from PSI system are transferred *via* ferrodoxine to hydrogenase (algae or cyanobacteria) or nitrogenase (cyanobacteria) and these enzymes reduce protons to molecular hydrogen. In direct biophotolysis neither CO_2 nor liqid metabolites are observed. Hydrogenase is very sensitive to oxygen and irreversibly inhibits its activity, therefore constant removal of oxygen is required (Das, 2008). Recent studies concentrate on elimination of sensitivity of algae towards oxygen (Benemann, 1997).

Indirect biophotolysis

This process can be performed with certain cyanobacteria (e.g. *Anabeana variabilis*) in two steps:

$$12H_2O + 6CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 \quad (15)$$

$$C_6H_{12}O_6 + 12H_2O + \text{light energy} \rightarrow 12H_2 + 6CO_2 \quad (16)$$

Different periods of oxygen and hydrogen generation allows to eliminate the inhibiting effect of oxygen on enzymes. Similarly, as in direct biophotolysis, photons activate PSI and PSII. In the presence of RuBisCO enzyme the CO_2 adsorption occurs, what in consequence of photosynthetic reactions generate glucose and oxygen . In the second step in presence of hydrogense and nitrogenase (Kars, 2009) the decomposition of organic compound occurs.

This type of metabolism in industry is difficult to perform because of periodicity of process. The hydrogen yields generated either by direct or indirect photolysis are unfortunately very low in comparison with other fermentative methods (Das, 2008).

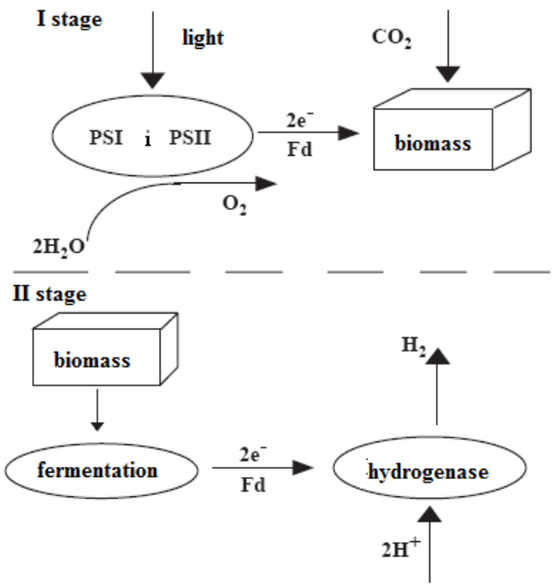


Fig. 11. Scheme of indirect photolysis of water (Maness, 2001)

Photofermentation

This process is based on decomposition of organic compounds to hydrogen in the absence of both oxygen and nitrogen but in presence of photosynthetic bacteria under illumination. Scheme on Fig. 12 shows the process of photofermentation catalyzed by nitrogenase.

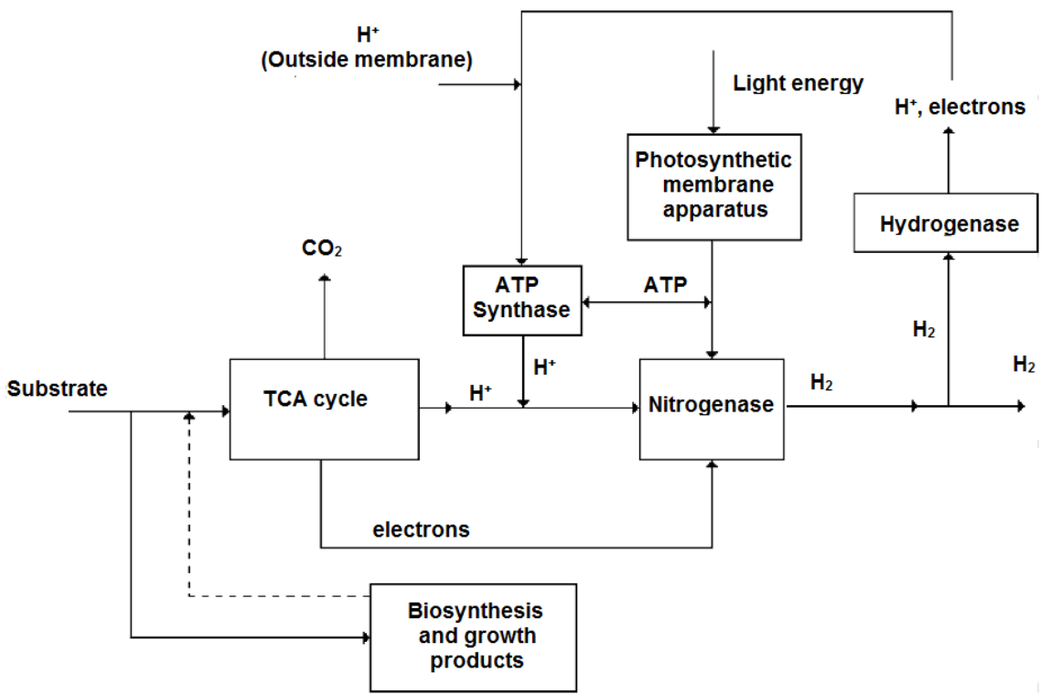


Fig. 12. Scheme of hydrogen generation in photofermentation process (Koku, 2002)

Organic substrate is oxidized to CO_2 in the cycle of tricarboxylic acids (TCA). Generated in this process electrons are transferred to nitrogenase *via* many carriers (e.g. NAD and ferredoxin). Nitrogenase reduce protons to molecular hydrogen. The photosynthetic apparatus acts simultaneously with TCA cycle transforming light into chemical energy. Here, the ATP with protons and electrons are directed towards nitrogenase. Photosynthetic bacteria contain the reverse hydrogenase enzyme which oxidize hydrogen back to protons. The final amount of photogenerated hydrogen is the difference between hydrogen formed in presence of nitrogenase and hydrogen consumed by reversed hydrogenase. The main advantage of this process rely on the high yield of hydrogen while transforming organic compounds to H_2 and CO_2.

Dark fermentation

Dark fermentation can occur in the absence of light. Anaerobic microorganisms are generating hydrogen while transforming biodegradable substances under oxygen free conditions. Unfortunately hydrogen is not the only gaseous product of this process. Carbon dioxide, methane, hydrogen sulfide can be found among generated gases, as well as liquid metabolites such as simple volatile fatty acids (VFA) and simple alcohols. In the presence of hydrogenase an organic compound is transformed in glycolysis (17) process into pyruvate. Next, it is oxidized to acetylo-Co-A with the reduction of ferredoxin (18). In the third step ferrodoxine is oxidized and evolved electrons are directed to protons and formation of molecular hydrogen (19).

$$\text{Glucose} \rightarrow \text{pyruvate} \tag{17}$$

$$\text{Pyruvate} + \text{CoA} + 2\text{Fd(ox)} \rightarrow \text{acetylo-C-A} + 2\,\text{Fd(red)} + CO_2 \tag{18}$$

$$2\text{Fd (red)} \rightarrow 2\text{Fd(ox)} + H_2 \tag{19}$$

Theoretically, one mole of glucose should generate 4 moles of hydrogen and acetic acid in dark fermentation process. In practice, this yield is lower (2.5 – 2.7 moles of H_2). Final amount of generated hydrogen depends on many factors including type and concentration of the substrate, pH value, hydraulic time of retention, substrate to inoculum ratio, Fe ions concentration etc. The relatively high rate of hydrogen production is the important factor influencing possible industrial applications.

Hybrid systems

One-step hybrid system

Application of hybrid systems allows the use of apparently useless and difficult to operate substrates in the photofermentation process. These compounds (e.g. saccharides) are decomposed in dark fermentation process into simple organic acids (e.g. acetic or butyric) which further undergo photofermentation by PNS bacteria. In one-step hybrid systems both types of bacteria grow in one pot. The amount of generated hydrogen comes from two processes occurring almost simultaneously.

Synchronization of activity of both types of bacteria cultures is the main parameter in hybrid system. The rate of reaction is usually much higher in dark fermentation than in photofermentation. As a result of this discrepancy, the excessive accumulation of VFA and

alcohols is observed. High concentration of VFA in the medium leads to the substrate inhibition (Kargi, 2010) as well as to the lowering of pH value (Liu, 2010) which consequently decreases the hydrogen yield or completely stops hydrogen generation. In hybrid systems photofermentation is the rate limiting step and slows down the overall reaction rate (Ozmihci, 2010). The unfavorable effect caused by the difference in the reactions rates can be counteracted by appropriate choice of concentrations of different strains of bacteria. The optimum concentration ratios can vary from 1:3.9 (Argun, 2010) even to 1:600 (Liu, 2010) depending on the strains of bacteria and types of substrates. The use of hardly soluble substrates such as e.g. starch leads towards formation of suspensions and flocculation of bacteria cells and further to limited accessibility of organic carbon to the bacteria and decrease in the yield of microbiologically generated hydrogen (Argun, 2009).

The main advantage of one-step hybrid systems is the high rate and much higher yield of hydrogen produced in comparison to those obtained in the process of dark fermentation performed by one culture only. Further increase in the yield of hydrogen generated by hybrid systems can be achieved by application of two-step systems (Argun, Kapdan 2009).

Two-steps hybrid systems

The yield of hydrogen generation in the photofermentation process can be lowered by low access of light, inappropriate concentration of the medium, substrate inhibition, presence of ammonium ions or other contaminants (Ozmihci, 2010). Because a much greater number of parameters influence the yield of hydrogen in photofermentation than in dark fermentation, the former process should be performed in an independent photobioreactor. Application of two-step hybrid systems allows the use of wastes containing inhibitors of photofermentation process (e. g. ammonium ions) (Azbar, 2010). These inhibitors are neutral for bacteria engaged in the dark fermentation.

Moreover, separation of these processes into two-step hybrid systems extends the list of organic substrates as it permits the use of highly thermophilic bacteria operating in temperatures higher than 70 °C (Ozgur, 2010). The natural organic substrates and wastes that can be used in two-step hybrid system. One mole of glucose theoretically generates four moles of hydrogen in dark fermentation, whereas acetic acid is the only side product (Antgenent, 2004). In practice, dark fermentation of liquid wastes generates much lower amounts of hydrogen (2.5-2.7 mole H_2 per mole of glucose in waste) (Ueno, 1998, Yokoi, 2001, Yokoi, 2002). The hybrid systems are much more efficient. These results suggest that further development of two-step hybrid system can lead towards effective, economically feasible commercial applications.

7. Modifications

Genetic engineering is one of the methods for improvement of activity in hydrogen generation by microorganisms. Although the yields of generated hydrogen can be performed by optimization of the reaction conditions, genetic modifications seems to be the appropriate solution at the moment. The main idea of modification rely on implantation of other genes into the bacteria strains containing hydrogenase.

The *E. coli* are very frequently use in genetic modifications due to the well recognized metabolism of these bacteria. The *E. coli* are producing hydrogen as the result of

decomposition of formic acid in presence of formate-hydrogen liaze (FHL) representing the set of enzymes localized in the inner cell membrane. Hydrogenase 3 coded as *hycA* and formate dehydrogenase known as *fdhF* are the main components of the FHL. The presence of *hycA* gene limits the synthesis of *fhlA*, responsible for better activity of FHL towards hydrogen. Therefore the removal of hycA increases the *fhlA* gene expression and in consequence hydrogen production by 5-10%. (Hallenback, 2009). The research of the FHL genes expression were performed by Bisaillon *et al*. and other authors (Bisaillon, 2006, Turcot, 2008, Penfold, 2003) and they found almost two times higher rate of hydrogen generation for modified strain of E. coli HD701. Genes responsible for nickel-iron hydrogenases (hydrogenase I and II) coded by *hya* and *hyb* operons were found in the *E. coli* genom as well. It was found that elimination of these enzymes by genetic modification can result with almost 35% higher production of hydrogen (Hallenback, 2009, Bisaillon, 2006, Turcot, 2008). Other profits originating from genetic engineering are related to deactivation of enzymes responsible for transformations of glucose into lactic, succinic and fumaric acids. The removal of *ldhA* (lactic acid) and *frdBC* (succinic and fumaric acids) genes results in increase of hydrogen formation. The 1.4 fold higher amount of hydrogen were found by Yoshida *et al.* (Yoshida, 2006) in this situation. The new mutant strain of SR 15 can produce 1.82 mol H_2/mol glucose what is close to the theoretical value (2 mol H_2/mol glucose). Studies performed by *Maeda et al.* (Maeda, 2007) showed that bacteria BW2513 with seven modified genes (*hyaB, hybC, hycA,fdoG, frdC*, *ldhA and aceE*) generate 4.6 fold more hydrogen than wild-type strain.

The nitrogenase and uptake hydrogenase play an important role in the photofermentation process of hydrogen generation by PNS bacteria. The engineering of the mutants free of uptake hydrogenase is the basic task of gene modifications. Genes coding hydrogenase (*hup*) can be modified by resistance gene insertion into the *hup* genes or by deletion of *hup* genes (Kars, 2009, Kars, 2008, Kim, 2006). Appropriately modified *Rhodobacter spheroids* can generate hydrogen also in the absence of light (Kim, 2008).

Production of polyhydroxybutyrate (PHB) accompany hydrogen generation by PNS bacteria what applies the excess of reducing equivalents in other metabolic pathway. The PHB is the storage material stored in cytoplasm. This compound is formed in the environment rich in carbon compounds but lean in nitrogen (Kemavongse, 2007). The PHB is unwanted competition product accompanying hydrogen generation. The removal of genes responsible for formation of PHB syntase effectively stops generation of the polymer (Kim, 2006). Low activity in PHB formation not always results in an increase of hydrogen yield. Whereas in presence of lactate, malate or malate the amount of photogenerated hydrogen is not influenced by PHB (Hustede, 1993) the presence of acetate can increase photofermentation towards hydrogen. However, the importance of PHB as biodegradable polymer significantly increased in recent years. Therefore, simultaneous photogeneration of hydrogen and PHB gained economic dimension (Yigit, 1999).

There are genetic modifications influencing changes in the amount of LHC (light harvesting complexes). The reduction of pigment present in LHC diminish the self-shadow effect and therefore better access of light into deeper located cells. The decrease of amount of LH1 (Vasilyeva, 1999) complexes with maximum of absorption at 875 nm or those with absorption maximum at 800 and 850 nm (LH2) (Kim, 2006) can increase the amount of photo generated hydrogen. Genetic manipulations cannot lead to total elimination of the pigments (Kim, 2006).

The negative influence of ammonium ions on nitrogenase is well recognized. Therefore, genetic modifications of nonsensitive to NH_4^+ ions should be the subject for considerations. Among many methods reducing the role of ammonium ions in photofermentation is blockage of Calvin cycle via mutation of genes coding the RuBisCO enzyme. This way the excess electron stream is directed to nitrogenase even in the presence of NH_4^+ ions. Another modification can be achieved by disruption of proteins transporting NH_4^+ ions through cytoplasmic membrane. Strains of this type (e.g. *Rhodobacter capsulatus*) loose their ability to regulate nitrogenase in presence of ammonium ions. (Qian, 1996). Such modifications allow to perform photofermentation even in the presence of molecular nitrogen. Although the amount of generated hydrogen is lower than in nitrogen free atmosphere but economically much more favorable (Yakunin, 2002).

Genetic modifications can be very effective but also troublesome and very expensive. Therefore other methods of process improvement are under investigations. Optimum value of pH equals 7. Photofermentation with *Rhodobacter sphaeroides* starting at pH=6.8 and ending at pH=7.5 results in significant drop of activity (7 times) but PHB concentration is tripled (Jamil, 2009).

Photofermentative bacteria belongs to mesophilic microorganisms and operate between 30 and 35 ^oC. Therefore, any critical temperatures act against high yield of hydrogen. For example *Rhodobacter capsulatus* operating at temperatures varying from 15-40 ^oC produce 50% less hydrogen than the same bacteria kept at constant temperature of 30 ^oC (Özgüra, 2010).

The access of photobacteria to the light with appropriate length and intensity play a crucial role m hydrogen photogeneration. Better access of light induce better phosphorylation and in consequence more effective synthesis of ATP and better yield of photofermentation (Kars, 2010).

Although the PNS bacteria absorb light in wide spectrum 400-950 nm the range of 750-950 nm is the most important (Eroglu, 2009, Ko, 2002). The light intensity is as well important as their wavelength. For *Rhodobacter sphaeroides* the amount of generated hydrogen grows linearly from 270 W/m^2 (4klx) to 600 W/m^2 (~ 10 klx). Below 270 W/m^2 no activity of bacteria is observed (Miyake, 1999, Uyar, 2007).

Application of illumination with wavelength longer than 900 nm results in overheating of the system. This require additional cooling systems because of decrease the amount of generated hydrogen. An application of appropriate filters cutting the unwanted range of spectrum seems to be the only solution in this situation (Ko, 2002). Considering natural irradiation one should remember about day-night periodicity. It was found, however, that amount of generated hydrogen is even higher under periodic irradiation than under the continuous one (Eroglu, 2010, Koku, 2003). The day-night illumination induces better activity of nitrogenase what results from better adjustment of PNS bacteria to live in natural conditions (Meyer, 1978).

The presence of organic compounds, also those containing nitrogen (except NH_4^+ ions) is the key issue for the photofermentation. However, presence of macro and microelements at appropriate concentration can influence the hydrogen productivity. Iron belongs to the most important ones. This element exists mainly as the cofactor of proteins engaged in metabolism. Process of photofermentation, strictly related to the transport of electrons.

There are many electrons carriers such as cytochromes (proteins containing Fe) or ferredoxin. Moreover, the main enzyme in photofermentation - nitrogenase contains 24 atoms of iron in each molecule. The presence of iron ions in medium containing PNS bacteria is one of the very important factors influencing hydrogen productivity. At concentrations of Fe^{2+} ions lower than 2.4 mg/l there is no hydrogen in products. At concentrations higher than 3.2 mg/l the gradual decrease of evolved hydrogen is observed. It was assumed that non physiological coagulation of the cells can occurs (Zhu, 2007). Molybdenum is the second microelement playing an important role in photofermentaive hydrogeneration. The optimal concentration of molybdenum is 16.5 µmol/l (Kars, 2006).

The substrate yield in hydrogen production can be significantly improved by adding other strains of bacteria into the liquid medium. Improvement in photofermentation was achieved by adding halofilic archeons of *Halobacterium salinarum* type. The integral membrane protein - bacteriorhodopsin as the pump for the light excited electrons. The H^+ ions are pumped out from cytoplasm outside the cell . The proton gradient is then engaged in ATP synthesis by *Rhodobacter sphaeroides* and this way increasing hydrogen generation. In this case, it is advised to use strains of PNS bacteria tolerating high concentrations of salts (Zabut, 2006) because of the high activity of bacteriorodopsyne in aqueous solution with high ionic strength.

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9. References

Akkerman, I.; Janssen, M.; Rocha, J.; Wijffels, RH. (2002). Photobiological hydrogen production: photochemical efficiency and bioreactor design, *Int J Hydrogen Energy*, vol.27, pp.1195-1208.

Alalayaha, WM.; Kalila, MS.; Kadhuma, AAH.; Jahima, JM.; Alaujb, NM. (2008). Hydrogen production using *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564), *Int J Hydrogen Energy*, vol.33, pp.7392-96.

Antgenent, LT.; Karmi, K.; Al-Dahhan, MH.; Wernn, BA.; Domiguez-Espinoza, R. (2004). Production of bioenergy and biochemicals from industrial and agricultural wastewater, *Trends In Biotechnol,* vol.22, pp.477-485.

Argun, H.; Kargi, F. (2010). Bio-hydrogen production from Grodnu wheat starch by continuous combined fermentation using annular-hybrid bioreactor, *Int J Hydrogen Energy*, vol.35, pp.6170-6178.

Argun, H.; Kargi, F. (2009). Effects of the substrate and cell concentration on bio-hydrogen production from ground wheat by combined dark and photo-fermentation, *Int J Hydrogen Energy*, vol.34, pp.6181-6188.

Argun, H.; Kargi, F.; Kapdan, IK. (2009). Hydrogen production by combined dark and light fermentation of ground wheat solution, *Int J Hydrogen Energy*, vol.34, pp.4305-4311.

Azbar, N.; Tuba, F.; Dokoz, C. (2010). The effect of dilution and L-malic acid addition on bio-hydrogen production with *Rhodopseudomonas palustris* from effluent of an acidogenic anaerobic reactor, *Int J Hydrogen Energy*, vol.35, pp.5028-5033.

Benemann, JR. (1997). Feasibility analysis of photobio;ogical hydrogen production, , *Int J Hydrogen Energy*, vol.22, pp.979-987.

Berg, JM.; Tymoczko, JL.; Stryer, L. (2002). Biochemystry, New York: WH Freeman.

Bisaillon, A.; Turcot, J.; Hallenbech, PC. (2006). The effect of nutrient limitation on hydrogen production by batch cultures of *Escherichia coli, Int J Hydrogen Energy*, vol.31, pp.104-1508.

Cai, G.; Jin, B.; Saint, C.; Monis, P. (2010). Metabolic flux analysis of hydrogen production network by *Clostridium butyricum* W5: Effect of pH and glucose concentration, *Int J Hydrogen Energy*, vol.35, pp.6681-90.

Chen, ChY.; Lu, WB.; Liu, CH.; Chang, JS. (2008). Improved phototrophic H_2 production with *Rhodopseudomonas palustris* WP3-5 using acetate and butyrate as dual carbon substrates, *Bioresource Technology*, vol.99, pp.3609-3616.

Chen, D.; Han, Y.; Gu, Z. (2006). Application of statistical methodology to the optimization of fermentative medium for carotenoids production by *Rhodobacter sphaeroides*, *Process Biochemistry*, vol.41, pp.1773-1778.

Chin, HL.; Chen, CP.; Chou, CP. (2003) Fed batch operation using *Clostridium acetobutylicum* suspension culture as biocatalyst for enhancing hydrogen production, *Biotechnology Progress*, vol.19, pp.383-388.

Dabrock, B.; Bahl, H.; Gottschalk, G. (1992). Parameters affecting solvent production by *Clostridium pasteurianum*, *Applied and Environmental Microbiology*, vol.58, pp.1233-1239.

Darensbourg, MY.; Lyon, EJ.; Smee, JJ. (2000). The bio-organometallic chemistry of active site iron in hydrogenases, *Coordination Chemistry Review*, vol.206-207, pp.533-561.

Das, D.; Dutta, T.; Nath, K.; Kotay, Das, AS.; Veziroglu, TN. (2006). Role of Fe-hydrogenase in biological hydrogen production. *Current Science*, vol.90, pp.1627-1637.

Das, D.; Veziroglu, TN. (2008). Advances in biological hydrogen production processes, *Int J Hydrogen Energy*, vol.33, pp.6046-6057.

Eriksen, NT.; Nielsen, TM.; Iversen, N. (2008). Hydrogen production in anaerobic and microaerobic *Thermotoga neapolitana*, *Biotechnology Letters*, vol.30, pp.103-109.

Eroglu, I.; Aslan, K.; Gunduz, U.; Yucel, M.; Turker, L. (1999). Substrate consumption rates for hydrogen production by *Rhodobacter sphaeroides* in a column photobioreactor, *Journal of Biotechnology*, vol.70, pp.103-113.

Eroglu, E.; Eroglu, I.; Gunduz, U.; Yucel, M. (2009). Treatment of olive mill wastewater by different physicochemical methods and utilization of their liquid effluents for biological hydrogen production, *Biomass and Bioenergy,* vol.33, pp.701-705.

Eroglu, E.; Gunduz, U.; Yucel, M.; Eroglu, I. (2010). Photosynthetic bacterial growth and productivity under continuous illumination or diurnal cycles with olive Mill wastewater as feedstock, *Int J Hydrogen Energy*, vol.35, pp.5293-5300.

Garrity, GM.; Brenner, DJ.; Noel, NR.; Staley, JR. (2005) Bergey's Manual of Systematic Bacteriology, Volume Two:The Proteobacteria, Part A 166, Springer-Verlag

Guo, XM.; Trably, E.; Latrille, E.; Carrere, H.; Steyer, JP. (2010). Hydrogen production from agricultural waste by dark fermentation:A review, *Int J Hydrogen Energy*, vol.35, pp.10660-10673.

Hallenbeck, PC. (2009). Fermentative hydrogen production: Principles, progress, and prognosis, *Int J Hydrogen Energy*, vol.34, pp.7379-7389.

Hallenbeck, PC.; Ggosh, D. Advances in fermentative biohydrogen production: the way forward?, *Trends in Biotechnology*, vol.27, pp.287-297.

Huang, JS.; Wu, CS.; Jih, CG.; Chen, CT. (2001). Effect of addition of *Rhodobacter sp*. To activated-sludge reactors treating piggery wastewater, *Water Research*, vol.35, pp.3867-3875.

Hustede, E.; Steinbuchel, A.; Schlegel, HG. (1993). Relationship between photoproduction of hydrogen and the accumulation of PHB in non-sulphur purple bacteria. *Appl Microbiol Biotechnol,* vol.39, pp.87-93.

Isaacs, NW.; Cogdell, RJ.; Freer, AA.; Prince, SM. (1995) Light-harvesting mechanisms in purple photosynthetic bacteria, *Current Opinion in Structural Biology*, vol.5, pp. 794-797.

Jamil, Z.; Suffian, M.; Annuar, M.; Ibrahim, S.; Vikineswary, S. (2009). Optimization of phototrophic hydrogen production by *Rhodopseudomonas palustris* PBUM001 via statistical experimental design, *Int J Hydrogen Energy*, vol.34, pp.7502-7512.

Jo, HJ.; Lee, DS.; Park, D.; Choe, WS.; Park, JM. (2008). Optimization of key process variables for enhanced hydrogen production by *Enterobacter aerogenes* using statistical methods, *Bioresoure Technology*, vol.99, pp.2061-66.

Jones, MR. (1997) Photosynthesis:A new twist to biological solar power, *Current Biology*, vol.7, pp.541-543.

Jong-Hwan, S.; Yoon, H.; Eun, J.; Ahn, K.; Mi-Sun, K.; Sang, JS.; Hyun, PT. (2007) Fermentative hydrogen production by the newly isolated *Enterobacter asburiae* SNU-1, , *Int J Hydrogen Energy*, vol.32, pp.192-199.

Kalia, VC.; Jain, SR.; Kumar, A.; Joshi, AP. (1994). Fermentation of biowaste to H_2 by *Bacillus licheniformis*, *World J Microbiol Biotechnol*, vol.10(2), pp.224-7.

Kanai, T.; Imanaka, H.; Nakajima, A.; Uwamori, K.; Omori, Y.; Fukui, T.; Atomi, H.; Imanaka, T. (2005). Continuous hydrogen production by the hyperthermophilic archaeon, *Thermococcus kodakaraensis* KOD1, *Journal of Biotechnology*, vol.116, pp. 271-282.

Kargi, F.; Ozmihci, S. (2010). Effects of dark/light bacteria ratio on bio-hydrogen production by combined fed-batch fermentation of ground wheat starch, *Biomass and Bioenergy*, vol.34, pp.869-874.

Kars, G.; Gunduz, U. (2010). Towards a super H_2 producer: Improvements in photofermentative biohydrogen production by genetic manipulations, *Int J Hydrogen Energy*, vol.35, pp.6646-6656.

Kars, G.; Gunduz, U.; Yucel, M.; Rakhely, G.; Kovacs, KL.; Eroglu, I. (2009). Evaluation of hydrogen production by *Rhodobacter sphaeroides* O.U.001 and its hupSL deficient mutant Rusing acetate and malate as carbon sources, *Int J Hydrogen Energy*, vol.34, pp.2184-2190.

Kars, G.; Gunduz, U.; Rakhely, G.; Yucel, M.; Eroglu, I.; Kovacs, KL. (2008). Improved hydrogen production by uptake hydrogenase deficient mutant strain of *Rhodobacter sphaeroides* O.U.001, *Int J Hydrogen Energy*, vol.35, pp.3056-3060.

Kars, G.; Gunduz, U.; Yucel, M.; Turker, L.; Eroglu, I. (2006). Hydrogen production and transcriptional analysis of *nif*D, *nif*K and *hup*S genes in *Rhodobacter sphaeroides* O.U.001 grown in media with different concentrations of molybdenum and iron, *Int J Hydrogen Energy*, vol.31, pp.1536-1544.

Kemavongse, K.; Prasertsan, P.; Upaichit, A.; Methacanon, P. (2007). Effect of co substrate on production of poly-β-hydroxybutyrate (PHB) and copolymer PHBV from newly identified mutant *Rhodobacter sphaeroides* U7 cultivated under aerobic-dark condition, *Songklanakarin Journal of Science and Technology*, vol.29(4), pp.1101-13.

Kim, MS.; Baek, JS.; Lee, JK. (2006). Comparison of H2 accumulation by *Rhodobacter sphaeroides* KD131 and its uptake hydrogenase and PHB synthase deficient mutant, *Int J Hydrogen Energy*, vol.31, pp.121-127.

Kim, EJ.; Kim, MS.; Lee, JK. (2008). Hydrogen evolution under photoheterotrophic and dark fermentative conditions by recombinant *Rhodobacter sphaeroides* containing the genes for fermentative pyruvate metabolism of *Rhodospirillum rubrum*, *Int J Hydrogen Energy*, vol.32, pp.5131-5136.

Kim, EJ.; Kim, JS.; Kim, MS.; Lee JK. (2006). Effect of changes in the level of light harvesting complexes of *Rhodobacter sphaeroides* on the photoheterotrophic production of hydrogen, *Int J Hydrogen Energy*, vol.31, pp.531-538.

Ko, IB.; Noike, T. (2002). Use of blue optical filters for suppression of growth of algae in hydrogen producing non-axenic cultures of *Rhodobacter sphaeroides* RV, *Int J Hydrogen Energy*, vol.27, pp.1297-1302.

Koku, H.; Eroglu, I.; Gunduz, U.; Yucel, M.; Turker, L. (2002). Aspects of the metabolism of hydrogen production by *Rhodobacter sphaeroides*, *Int J Hydrogen Energy*, vol.27, pp.1315-1329.

Koku, H.; Eroglu, I.; Gunduz, U.; Yucel, M.; Turker, L. (2003). Kinetics of biological hydrogen production by the photosynthetic bacterium *Rhodobacter sphaeroides* O.U. 001, *Int J Hydrogen Energy*, vol.28, pp.381-388.

Kotay, SM.; Das, D. (2007). Microbial hydrogen production with *Bacillus coagulans* IIT-BT S1 isolated from anaerobic sewage sludge, *Bioresoource Technology*, vol.8, pp. 1183-90.

Kotay, SM.; Das, D. (2008). Biohydrogen as a renewable energy resource – Prospects and potentials, *Int J Hydrogen Energy*, vol.33, pp.258-263.

Levin, DB.; Islam, R.; Cicek, N.; Sparling, R. (2006). Hydrogen production by *Clostridium thermocellum* 27405 from cellulosic biomass substrate, *Int J Hydrogen Energy*, vol.31, pp.1496-1503.

Liu, BF.; Ren, NQ.; Tang, J.; Ding, J.; Liu, WZ.; Xu, JF.; Cao, GL.; Guo, WQ.; Xie, GJ. (2010). Bio-hydrogen production by mixed culture of photo-and dark-fermentation bacteria, *Int J Hydrogen Energy*, vol.35, pp.2858-2862

Maeda, I.; Miyasaka, H.; Umeda, F.; Kawase, M.; Yagi, K. (2003). Maximization of hydrogen production ability In high-density suspension of *Rhodovulum sulfidopilum* Celle

Rising intracellular poly(3-hydroxybutyrate) as sole substrate, *Biotechnology and Bioengineering*, vol.81, pp.474-481.

Maeda, T.; Sanchez-Torrez, V.; Wood, K. (2008). Metabolic engineering to enhance bacterial hydrogen production, *Microbiol Biotechnology*, vol.1, pp.30-39.

Maeda, T.; Sanchez-Torrez, V.; Wood, K. (2007). Enhanced hydrogen production from glucose by metabolically engineered *Escherichia coli, Applied Microbiological Biotechnology*, vol.77, pp.879-890.

Maness, PC.; Weaver, PF. (2001). Evidence for three distinct hydrogenase activities in *Rhodospirillum rubrum. Applied Microbiology and Biotechnology*, vol.57, pp.751-756.

Mandal, B.; Nath, K.; Das, D. (2006). Improvement of hydrogen production under decreased partial pressure of H_2 by *Enterobacter cloacae*, *Biotechnology Letters*, vol.28, pp. 831-835.

Masset, J.; Hiligsmann, S.; Hamilton, C.; Beckers, L.; Franck, F.; Thonart, P. (2010). Effect of pH on glucose and starch fermentation in batch and sequenced-batch mode with a recently isolated strain of hydrogen-producing *Clostridium butyricum* CWBI1009, *Int J Hydrogen Energy*, vol.35, pp.3371-3378.

Meyer, J.; Kelley, BC.; Vignais, PM. (1978). Effect of light on nitrogenase function and synthesis in *Rhodopseudomonas capsulate*, *Journal of Bacterology*, vol.136, pp.201-208.

Miyake, J.; Wakayama, T. (1999). Simulation of the daily sunlight illumination pattern for bacterial photo-hydrogen production. *Journal of Bioscience and Bioenginering,* vol.88(6), pp.659-663.

Nicolet, Y.; Lemon, BJ.; Fontecilla-Camos, JC.; Peters, JW. (2000). A novel FeS cluster in Fe-only hydrogenases, *Trends in Biochem Sciences*, vol.25, pp.138

Obeid, J.; Magnin, JP.; Flaus, JM.; Adrot, O.; Willison, JC.; Zlatev, R. (2009). Modelling of hydrogen production in batch cultures of the photosynthetic bacterium *Rhodobacter capsulatus*, *Int J Hydrogen Energy*, vol.34, pp.180-185.

Oha, YK.; Seola, EH.; Kimb, JR.; Parka, S. (2003). Fermentative biohydrogen production by a new chemoheterotrophic bacterium *Citobacter sp*. Y19, *Int J Hydrogen Energy*, vol.28, pp.1353-1359.

Ozgur, E.; Mars, AE.; Peksel, B.; Louwerse, A.; Yucel, M.; Gunduz, U.; Pieternel, AM. (2010). Biohydrogen production from beet molasses by sequential dark and photofermentation, *Int J Hydrogen Energy*, vol.35, pp.511-517.

Ozmihci, S.; Kargi, F. (2010). Effects of starch loading rate on performance of combined fed-batch fermentation of ground wheat for bio-hydrogen production, *Int J Hydrogen Energy*, vol.35, pp.1106-1111.

Ozmihci, S.; Kargi, F. (2010) Bio-hydrogen production by photo-fermentation of dark fermentation effluent with intermittent feeding and effluent removal, *Int J Hydrogen Energy*, vol.35, pp.6674-6680.

Özgüra, E.; Uyard, B.; Ozturkb, Y.; Yucel, M.; Gunduz, U.; Eroglu, I. (2010). Biohydrogen production by *Rhodobacter capsulatus* on acetate at fluctuating temperatures, Resources, *Conservation and Recycling,* vol.54, pp.310-314.

Packer, HL.; Armitage, JP. (2000). Inverted behavioral responses in wild-type *Rhodobacter sphaeroides* to temporal stimuli, *FEMS Microbiology Letters*, vol.185, pp.299-304.

Paschenkoa, VZ.; Gorokhova, VV.; Knoxa, PP.; Krasilnikova, PM.; Redlinb, H.; Rengerc, G.; Rubina, Ab. (2003). Energetic and mechanisms of light efficiency of charge separation and electron transfer processes in *Rhodobacter sphaeroides* reaction center, *Bioelectochemistry*, vol.61, pp.73-84.

Penfold, WW.; Forster, CF.; Macaskie, LE. (2003). Increased hydrogen production by Escherichia coli strain HD701 in comparison with the wild-type parent strain MC4100, *Enzyme and Microbial Technology*, vol.33, pp.185-189.

Pike, L.; Sojka, GA. (1975). Glycerol dissimilation in *Rhodopseudomonas sphaeroides*, *Journal of Bacteriology*, vol.124, pp.1101-1105.

Plowman, J.; Peck, MW. (2002). Use of a newel methods characterize the response of spores of non-proteolytic *Clostridium botulinum* types B,E and F to a wide range of germinates and conditions, *Journal of AppliedMicrobiology*, vol.92, pp.681-694

Porter, SL.; Wadhams, GH.; Armitage, JP. (2008). *Rhodobacter sphaeroides*: complexity in chemo tactic signaling, *Trends in Microbiology*, vol.16, pp.251-260.

Qian, Y.; Tabita, FR. (1996). A Global Signal Transduction System Regulates Aerobic and Anaerobic CO2 Fixation in *Rhodobacter sphaeroides*, *Journal of Bacteriology*, vol.178(1), pp.12-18.

Ren, NQ.; Liu, BF.; Ding, J.; Xie, GJ. (2009). Hydrogen production with *R. Faecalis* RLD-53 isolated from freshwater pond sludge, *Bioresource Technology*, vol.100, pp.484-487.

Schlegel, G. (2003) , PWN, Warszawa

Setlow, P. (2007). I will survive: DNA protection in bacterial spores, *Trends in Microbiology*, vol.15, pp.172-180.

Skonieczny, MT.; Yargeau, V. (2009) Biohydrogen production from wastewater by *Clostridium Beijerinckii*: Effect of pH and substrate concentration, *Int J Hydrogen Energy*, vol.34, pp.3288-3294.

Stryer, L. (1999). Biochemistry PWN, Warszawa

O-Thonga, S.; Prasertsanb, P.; Karakasheva, D.; Angelidakia. (2008). Thermophilic fermentative hydrogen production by the newly isolated *Termoanaerobacterium thermosaccharolyticum* PSU-, *Int J Hydrogen Energy*, vol.33, pp.1204-1214.

Turcot, J.; Bisaillon, A.; Hallenbeck, PC. (2008). Hydrogen production by continuous cultures of Escherichia coli under different nutrient regimes, *Int J Hydrogen Energy*, vol.33, pp.1465-1470.

Ueno. Y.; Otauka, S.; Morimoto, M. (1998). Hydrogen production from industrial wastewater by anaerobic microflora in hemostat culture, *J Ferment Bioeng*, vol.82, pp.194-197.

Uyar, B.; Eroglu, I.; Yucel, M.; Gunduz, U.; Turker, L. (2007). Effect of light intensity, wavelength and illumination protocol on hydrogen production in photobioreactors, *Int J Hydrogen Energy*, vol.32, pp.4670-4677.

Valdez-Vazquez, Rios-Leal, E.; Esparza-Garcia, F.; Cecchi, F.; Poggi-Varaldo, HN. (2005). Semi-continous solid substrate anaerobic reactors for H_2 production from organic waste: Mesophilic versus thermophilic regime, *Int J Hydrogen Energy*, vol.30, pp.1383-1391.

Vardar-Schara, G.; Maeda, T.; Wood, TK. (2008). Metabolically engineered bacteria for producing hydrogen via fermentation, Microbial Biotechnology, vol.1, pp. 107-125.

Vasilyeva, l.; Miyake, M.; Khatipov, E.; Wakayama, M.; Sekine, M.; Hara, M.; Nakada, E.; Asada, Y.; Miyake, J. (1999). Enhanced Hydrogen Production by a Mutant of *Rhodobacter sphaeroides* Having an Altered Light-Harvesting System, *Journal of Bioscience and Bioengineering,* vol.87(5), pp.619-624.

Vermeglio, A.; Joliot, P. (1999). The photosynthetic apparatus *of Rhodobacter shaeroides*, *Trends in Microbiology*, vol.7(11), pp.435-440

Vignais, PM.; Billoud, B.; Meyer, J. (2001). Classification and phylogeny of hydrogenases, *FEMS Microbiology Reviews*, vol.25, pp.455-501.

Waligórska, M.; Seifert, K.; Górecki, K.; Moritz, M.; Łaniecki, M. (2009). Kinetic model of hydrogen generation by *Rhodobacter sphaeroides* in the presence of NH_4^+ ions, *Journal of Applied Microbiology*, vol.107, pp.1308-1318.

Wang, CC.; Chang, CW.; Chu, CP.; Lee, DJ.; Chang, BV.; Liao, CS. (2003). Producing hydrogen from wastewater sludge by *Clostridium bifermentans*, *Journal of Biotechnology*, vol.102, pp.83-92.

Wu, X.; Li, Q.; Dieudonne, M.; Cong, Y.; Zhou, J.; Long, M. (2010). Enhanced H_2 gas production from bagasse using adhE inactivated *Klebsiella oxytoca* HP1 by sequebtial dark-photo fermentation, *Bioresource Technology*, vol.101, pp.9605-9611.

Yakunin, AF.; Hallenbeck, PC. (2002). AmtB Is Necessary for NH4-Induced Nitrogenase Switch-Off and ADP-Ribosylation in *Rhodobacter capsulatus*, *Journal of Bacteriology*, vol.184, pp.4081-4088.

Yang, G.; Zhang, Z.; Bai, H.; Gong, J.; Wang, Y.; Li, B.; Li, J. (2008). Biotransformation of β-Amyrin acetate by *Rhodobacter sphaeroides*, *Journal of Bioscience and Bioengineering*, vol.105, pp.558-561.

Yokoi, H.; Saitsu, A.; Uchida, H.; Hirose, J.; Hayashi, S.; Takasaki, Y. (2001). Microbial Hydrogen Production from Sweet Potato Starch Residue, *J Bioscien Bioeng,* vol.91, pp.58-63.

Yokoi, H.; Maki, R.; Hirose, J.; Hayashi, S. (2002). Microbial production of hydrogen from starch-manufacturing wastes, *Biomass Bioenerg,* vol.22, pp.389-395.

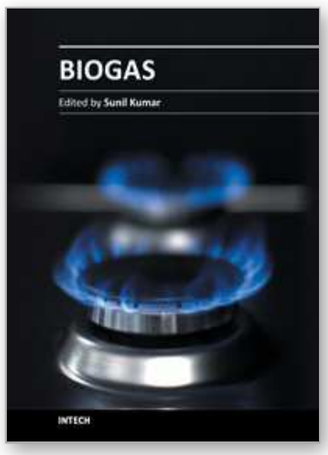
Yoshida, A.; Nishimura, T.; Kawaguchi, H.; Inui, M.; Yukawa, H. (2006). Enhanced hydrogen production from glucose using ldh- and *frd*-inactivated Escherichia coli strains, *Applied Microbiological Biotechnology,* vol.73, pp.67-72.

Yigit, D.; Gunduz, U.; Turker, L.; Yucel, M.; Eroglu, I. (1999). Identification of by-products in hydrogen producing bacteria; *Rhodobacter sphaeroides* O.U. 001 grown in the waste water of a sugar refinery, *Journal of Biotechnology,* vol.70, pp.125-131.

Zabut, B.; El-Kahlout, K.; Yucel, M.; Gunduz, U.; Turker, L.; Eroglu, I. (2006). Hydrogen gas production by combined systems of *Rhodobacter sphaeroides* O.U.001 and *Halobacterium salinarum* in a photobioreactor, *Int J Hydrogen Energy*, vol.31, pp. 1553-1562.

Zhang, T.; Liu, H.; Fang H. (2003). Biohydrogen production from starch in wastewater under thermophilic condition, *Journal of Environmental Mangament* , vol.69, pp.149

Zhu, H.;. Fang, HP.; Zhang, T.;. Beaudette, LA. (2007). Effect of ferrous ion on photo heterotrophic hydrogen production by *Rhodobacter sphaeroides*, *Int J Hydrogen Energy*, vol.32, pp.4112-4118.



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This book contains research on the chemistry of each step of biogas generation, along with engineering principles and practices, feasibility of biogas production in processing technologies, especially anaerobic digestion of waste and gas production system, its modeling, kinetics along with other associated aspects, utilization and purification of biogas, economy and energy issues, pipe design for biogas energy, microbiological aspects, phyto-fermentation, biogas plant constructions, assessment of ecological potential, biogas generation from sludge, rheological characterization, etc.

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