Biological methods for obtaining hydrogen

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Introduction

The population increase in the world as well as developing industry, especially in Asia and South America, may, according to the International Energy Agency, lead to the increase of energy consumption by approx. 50% until 2030. Especially arduous and causing considerable increase of air pollution (coal dust, carbon oxides, ozone) in agglomerations is road transport, whose activity in terms of CO_2 emission since the 1970s has risen by approx. 120% [1].

The use of hydrogen as fuel and a universal energy carrier seems an especially interesting method in countering the energy crisis and environmental pollution. Hydrogen is an element commonly occurring on Earth and in the Universe. It may be obtained from water and other substrates, while its combustion product is water. The use of hydrogen as fuel, as long as obtained through renewable processes, will in the long-term perspective allow for not only limiting the emission of gaseous pollution into the atmosphere but it may also become one of the most important energy reservoirs [2].

The properties of hydrogen are decisive when selecting it as an energy carrier. Hydrogen has a considerable combustion heat (~142 kJ/kg) which is approx. three times higher than that for gasoline or liquid gas (47.4 and 48.8 kJ/kg, respectively) and almost four times higher than that for biodiesel (37.0 kJ/kg). During combustion, no toxic gases nor particulates are created. It is widely used in the chemical, food and metallurgical industries as well as in fuel cells. Moreover, the substance may be obtained through many processes which employ renewable and non-renewable energy sources. One problem which has still not been solved is the storage of condensed hydrogen which requires low temperatures (b.p. -252.9°C) as well as its safe use (the combustibility range in a mixture with air equals 4 – 75% volume) [3]. Hydrogen may be obtained through chemical processes, among others (steam reforming, partial oxidation of hydrocarbons), during water electrolysis or in biological processes [4] (Fig. 1).

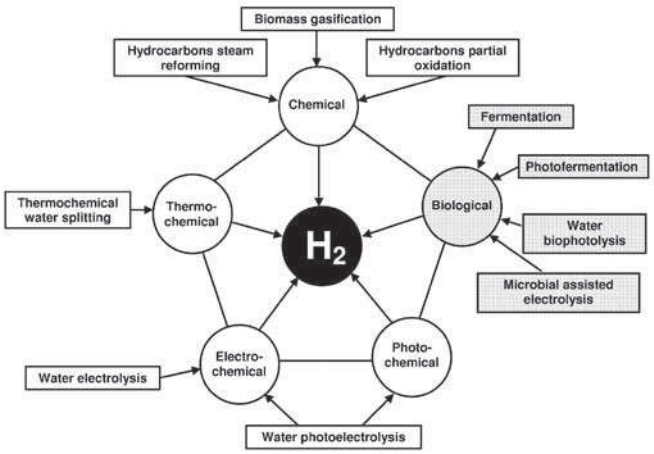

Fig. 1. Methods of obtaining hydrogen

The obtaining and use of hydrogen as fuel is not without sense if the primary source for its obtaining includes raw materials (biomass) or energy coming from renewable sources (wind or solar energy) [5]. During the processes of steam reforming of hydrocarbons or biomass gasification it is necessary to separate hydrogen from reaction byproducts, to use catalysts, heightened pressure and temperature. During water electrolysis hydrogen of high purity is obtained but the process is costly in terms of energy [6]. A solution to this problem may be to employ ways of obtaining hydrogen using bacteria. The process of secreting hydrogen by microorganisms usually occurs at room temperature, in an aqueous environment and under atmospheric pressure. Bioproduction methods for this gas using microorganisms include, among others: direct water photolysis using algae or cyanobacteria, photobiological degradation of organic compounds using heterotrophic purple non-sulfur bacteria, fermentation and bioelectrochemical processes (bioelectrolysis) [7]. It is also possible to employ so-called hybrid methods which combine the aforementioned processes. It is worth noting that the primary source of hydrogen in biological methods includes water in the case of biophotolysis or organic substrates (fermentation, photofermentation) which are waste pollutants [7].

Photobiological methods for obtaining hydrogen

Biophotolysis of water

Solar radiation is one of the most accessible renewable energy sources. It is estimated that the annual amount of energy reaching the Earth's surface is approx. 5.7×10^{24} J, while the average power of that radiation is approx. 1 kW/m^2. In the case of photobiological processes, solar radiation energy in the presence of microorganisms undergoes direct or indirect conversion into hydrogen [8].

Cyanobacteria and green algae are organisms which may carry out oxygen photosynthesis processes in natural conditions as well hydrogen secretion in anaerobic conditions [9]. Production of hydrogen using these organisms may occur during direct or indirect biophotolysis of water. The first process involves the conversion of water into oxygen and hydrogen. This reaction occurs using a key enzyme called Fe-hydrogenase which reduces protons to molecular hydrogen [10]. Initially, solar radiation energy is captured by PS I and PS II photosystems in the chloroplasts of these microorganisms. During this process secretion of photosynthetic dyes (chlorophylls) occurs which is accompanied by transferring the electron from the water molecule to Fe-hydrogenase using ferredoxin. Next, hydrogenase reduces protons present in the reaction environment to molecular hydrogen [9, 10]. Because of the sensitivity of Fe-hydrogenase to oxygen, this process occurs only in the presence of small amounts of oxygen (0.1% of the general partial pressure of gases) [11]. Indirect biophotolysis of water is a more complex phenomenon which may additionally involve ATP-dependent nitrogenase (in the case of *Anabena cylindrica*). The first stage of this process involves the assimilation of CO_2 in the presence of sunlight (photosynthesis); next, in the presence of sunlight the gathered organic matter is used for secreting hydrogen with the presence of Fe-hydrogenase or nitrogenase [7, 10].

Degradation of organic compounds using solar energy

Photobiological obtaining of hydrogen with the use of purple non-sulfur bacteria has been known for over 60 years [12]. This process, commonly referred to as photofermentation, occurs in anaerobic conditions in the presence of light and organic substrates. Purple non-sulfur bacteria (PNS) from the genus *Rhodobacter* are able to metabolize various organic substrates such as organic acids, monosaccharides or nutrients contained in industrial and agricultural waste [13]. A model example of a microorganism from this genus is the species *Rhodobacter sphaeroides*. This bacterium may photosynthesize in anaerobic conditions as well as feed heterotrophically in aerobic conditions in the absence of light [14]. The key enzyme which takes part in the process of obtaining hydrogen using purple non-sulfur bacteria is nitrogenase [13]. The enzyme, a complex of nitrogenase and nitrogenase reductase, is capable of binding molecular nitrogen according to the following chemical equation [15]:

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

but in the case of a lack of nitrogen it may catalyze the reaction [7]:

$$8H^+ + 8e^- + 16ATP \rightarrow 4H_2 + 16ADP + 16\,P_i$$

The activity of nitrogenase requires the presence of a reduced form of ferredoxin (electron donor) and ATP (adenosine triphosphate) [15]. ATP, a universal energy carrier at the cellular level, is obtained through photosynthesis thanks to the creation of a proton gradient across the cytoplasmic membrane [13]. As seen in the presented equations, the presence of molecular nitrogen acts competitively on the process of hydrogen secretion. The reaction is also inhibited by oxygen, the high ratio of amino acid nitrogen to the content of carbon in the medium [16] and the presence of ammonium ions [17]. A simplified diagram of hydrogen secretion through photosynthesizing purple non-sulfur bacteria is presented in Fig. 2. The diagram includes key elements of the process together with a sequence of events. The photosynthetic apparatus of these bacteria is located in the cell membrane. The system comprises a light-harvesting complex (LHC), a reaction center and a cytochrome complex [14]. At first, the quantum of solar radiation energy reaches the LH2 external light harvesting complex; next, the excitation is transferred to the LH1 complex directly related to the RC reaction center [18, 19]. The reaction center is responsible for transferring protons across the cytoplasmic bacterial cell membrane which leads to the creation of a proton gradient – the main force behind the hydrogen production process [14, 18, 20].

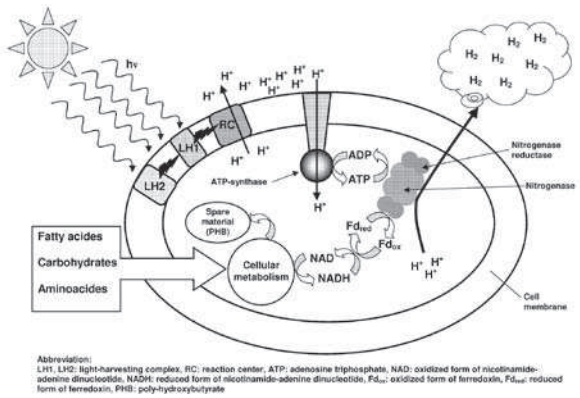

Fig. 2. Schematic presentation of the cellular metabolism of the photosynthesizing purple non-sulfur bacterium

Apart from solar energy, a necessary factor which determines the production of hydrogen is the availability of organic substrates which are the source of so-called reduction power, e.g. in the form of an NADH (nicotinamide adenine dinucleotide) pool, necessary for the reduction of ferredoxin, among other things [13]. As it has been mentioned above, purple non-sulfur bacteria may metabolize a wide range of organic substrates. Table 1 contains examples of organic matter sources which constitute a medium for those microorganisms as well as conditions of carrying out the process.

Table 1

List of selected processes of photobiological obtaining of hydrogen

Microorganism	Carbon source	Process conditions	Process output (maximum)	Literature
Rhodobacter sphaeroides	Malic acid, acetic acid, lactic acid	Process conducted in a flat-plate photobioreactor with a capacity of 6.5 dm^3 at a temperature of 32°C in anaerobic conditions (argon), light source: tungsten filament lamp, light intensity 110 klx	0.01 dm^3/dm^3 of medium/h	[21]
Rhodobacter sphaeroides	Dairy waste	25 cm^3 glass vials, temp. 28 ± 2°C, light source: tungsten mercury lamp, light intensity 5 – 40 klx	3.63 dm^3 H_2/dm^3 of medium	[22]
Rhodobacter sphaeroides	Brewery waste	25 cm^3 glass vials, temp. 28 ± 2°C, light source: tungsten mercury lamp, light intensity: 9 klx, process carried out in day-night type conditions (12h: 12h)	2.24 dm^3 H_2/dm^3 of medium	[23]
Rhodobacter capsulatus	Acetic acid	Process carried out in a pipe-based bioreactor (vol. 80 dm^3) with forced medium circulation	0.31 m^3 H_2/m^3 of medium/h	[24]
Rhodopseudomonas palustris	Glycerol*	Glass vials (vol. 125 cm^3), temp. 30°C, light source: halogen lamp (3 klx)	6 moles H_2/ mole of substrate	[25]

* As a waste product during the production of biodiesel

Fermentation processes

As opposed to anaerobic processes of obtaining hydrogen which occur using solar energy, reactions including the degradation of organic compounds in similar conditions but without photon energy are called dark fermentation. During the aerobic growth of microorganisms, oxygen serves as the acceptor of electrons coming from organic substrates while in the fermentation process the electron may be taken over by various substances, including protons, which results in the production of molecular hydrogen by these microorganisms [7]. In the fermentation process glucose, which is the primary source of carbon, is transformed into pyruvate during the glycolytic pathway. Next, pyruvate is oxidized to acetyl coenzyme A in the presence of coenzyme A and an oxidized form of ferredoxin. Ferredoxin in oxidized form is created during the reduction of protons to molecular hydrogen with the participation of hydrogenase [7]. The diagram of this process is presented in Fig. 3.

The final products of glucose oxidation include organic acids (acetic, propionic), carbon dioxide and hydrogen [7]. Obtaining hydrogen during fermentation is possible with the use of many microorganisms such as from the genus *Clostridium* (*C. butyricum, C. pasteurianum*), genus *Enterobacteriaceae* (*E. coli, E. cloacae*) as well as from thermophilic microorganisms from the genus *Thermoanaerobacterium* (*T. thermosaccharolyticum*) [26]. Bacterial cultures used during the fermentation process of obtaining hydrogen are most often obtained from samples which constitute their natural habitat such as sewage sludge, post-fermentation deposits, industrial equipment elements

[27, 28]. The most desired source of organic matter, necessary for the growth of cells and production of hydrogen, is glucose. This substance may be obtained during the processing of materials commonly recognized as waste. Li et al. [29] used maize straw subjected to hydrolysis with steam (cellulose saccharification) as the source of carbon in the process of obtaining hydrogen using *Clostridium butyricum*. On the other hand, Chen et al. [30] used food waste during the process of obtaining hydrogen together with bacteria contained in sewage waste (activated sludge). Table 2 contains examples of bacterial cultures and carbon sources in processes of fermentation production of hydrogen.

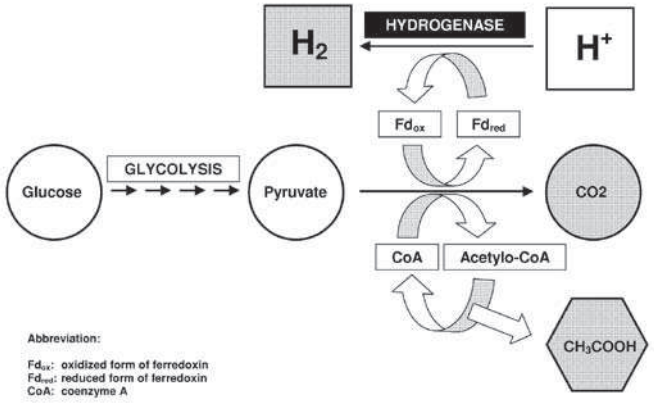

Fig. 3. Mechanism of the fermentation process of obtaining hydrogen

Table 2

Examples of fermentation methods of hydrogen bioproduction

Bacterial culture	Carbon source	Process conditions	Process output (maximum)	Literature
Thermoanaerobacter mathranii A3N (thermophilic bacterium)	Starch, glucose, saccharose, xylose	Process conducted in glass vials with a volume of 125 cm^3, within the temperature range of 40 – 80°C	9.3 molar mass H_2/g d. m. bact. cell/h (in case of saccharose)	[31]
Mixed culture* obtained from active sludge, paper industry installations or manure	Rice straw	Process carried out in a reactor with a vol. of 1.5 dm^3, at a temp. of 55°C (pH 6.5). Rice straw with different degrees of break-up was used as medium (without previous hydrolysis)	0.024 dm^3 H_2/g of straw/h	[32]
Mixed culture**	Solid municipal waste	Production of hydrogen carried out in a reactor with a vol. of 120 cm^3, at a temp. of 37°C using varying proportions of inocula with different composition of bacterial cultures	0.12 dm^3 H_2 dm^3/g of waste	[28]
Mixed culture of soil bacteria***	Glucose	Process carried out in a reactor (10 dm^3) with mechanical agitator, in an atmosphere of inert gas (N_2)	2.8 moles H_2/ mole of substrate	[33]
Bacterial cultures of active sludge, *Rhodobacter sphaeroides*	Waste from the aliphatic industry	Hybrid process. First, fermentation of waste elements using active sludge bacteria; next, photobiological (*R. sphaeroides*) production of hydrogen using fermentation products	29 dm^3 H_2/dm^3 of waste	[34]

*Bacterial cultures containing, among other things: *C. pasteurianum*, *C. stercorarium*, *T. saccharolyticum*; **Bacterial cultures obtained from fermented waste sludge and equipment (silos) for storing soya flour; ***Culture obtained as a result of thermal processing of soil (2 h/100°C), contains mainly bacteria from the genus *Clostridium*

Fermentation may also constitute one of the production stages of hydrogen using a mixed (hybrid) method. In such a case, a complex mixture of organic substances, constituting most often waste products of technological sewage, is subjected to fermentation. Next, the obtained products constitute a medium for microorganisms during the bioproduction of hydrogen using visible radiation energy (photofermentation) [34].

Bioelectrolysis

The process of bioelectrochemical production of hydrogen combines classic electrolysis with metabolic capabilities of microorganisms. Applying external potential to the clamps of the bioelectrolyzer (*Microbial Electrolysis Cell* – MEC) leads to the oxidation of organic compounds at the surface of the anode covered with bacterial biofilm and to the secretion of molecular hydrogen from the surface of the cathode (most often platinum) connected with the reduction of protons [35]. The diagram of such device is presented in Fig. 4.

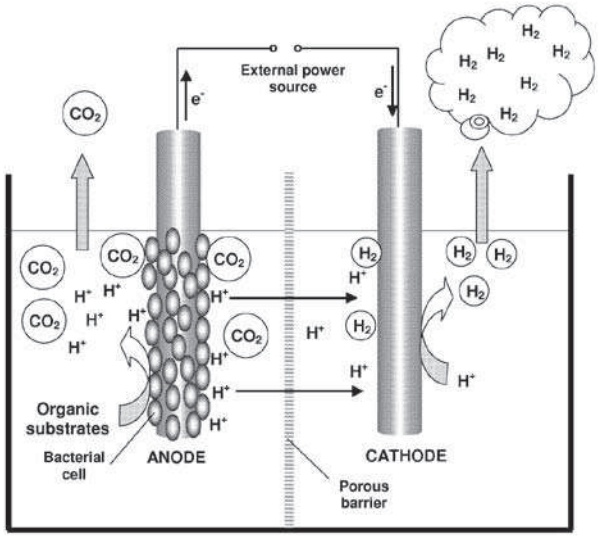

Fig. 4. Construction and functioning mechanism of the bioelectrolyzer

Bioelectrochemical production of hydrogen may take place using fermentation products or organic pollutants contained in waste [36]. Processes are known of an electrolytic obtaining of hydrogen using microorganisms from the following genera: *Geobacter, Pseudomonas, Clostridium, Desulfuromonas* or *Klebsiella*. The direct benefit resulting from using an anode covered with bacterial biofilm is the lowering of its potential. The potential reduction is related to the concentration gradient of substrates and products inside the bacterial biofilm, intracellular transport of electrons, resulting from cellular metabolism and extracellular transport of electrons to the surface of the anode [35]. This phenomenon allows for lowering the voltage necessary for carrying out the electrolysis process. The biological system containing an acetate anion with a concentration of 0.8 g/dm^3 allows for obtaining a bioanode potential equal to approx. –0.300 V, while the value of the potential, necessary for reducing protons, equals –0.414 V, which means that applying external voltage which is suitable suitably higher than the difference of potentials for specific half-cells (with a value of 0.114 V) will start the electrolysis process [37]. In practice, the voltage applied to the clamps of the bioelectrolyzer must be slightly higher, approximately 0.6 – 1.2 V [35]; however, it is lower (economic benefit of the process) than the one required during electrolysis of pure water. In the case of an electrochemical water breakdown the reversible (balance) potential of the process equals 1.2 V; however, because of the overpotential of electrodes or electrical resistance of the aqueous environment, voltage necessary for the occurrence of electrolysis is considerably higher [38].

Summary

Due to the environmental pollution resulting from the combustion of fossil fuels as well as the increasing consumption of energy by human beings, biological methods for obtaining hydrogen are becoming increasingly significant in the development of a sustainable energy system. The production processes of hydrogen using microorganisms allow for the use of potentially useless products of humans' activities (sewage, industrial waste) as well as create the possibility of transforming and storing widely available energy (solar energy). The dynamic development of science in the field of practical use of photosynthesizing bacteria and microorganisms that ferment organic matter give hope for the quick and widespread application of cost-effective energy production systems.

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