

Some considerations on fermentative biohydrogen production

Boboescu I. Z.¹ Mirel I.¹ Gherman V.¹

Abstract: Biohydrogen production has been established as a prospective alternative and integral component of green sustainable energy. A challenging problem in making biohydrogen production a more economically feasible is the generation of large quantities of hydrogen gas. Compared with photosynthetic hydrogen production, fermentative hydrogen production has higher hydrogen production efficiency, higher hydrogen production stability, and higher feasibility for industrialization, simpler control requirement and lower operating costs. This method of hydrogen production becomes more economically viable when the process is combined with dark fermentation developed in the fermentation tanks used in high organic load waste water treatment processes.

Keywords: biohydrogen, dark fermentation, photo-fermentation, biophotolysis, anaerobic metabolism

1. INTRODUCTION

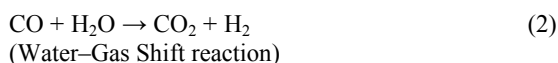
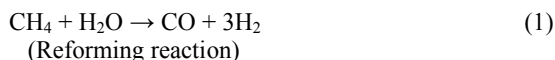
Hydrogen is the simplest element and also the most plentiful gas in the universe. Because hydrogen gas (H₂) is lighter than air and, as a result, it rises in the atmosphere, it is not found by itself on earth but only in compound form with other elements. Hydrogen combined with carbon, forms different compounds such as methane (CH₄), coal, and petroleum [1]. Hydrogen is also found in all growing things-biomass [2, 3]. It has the highest energy content per unit weight of any known fuel (142 kJ/g or 61,000 Btu/lb) and can be transported for domestic/industrial consumption through conventional means [4, 5, 6]. H₂ gas is safer to handle than domestic natural gas and is universally accepted as an environmentally safe, renewable energy resource and an ideal alternative to fossil fuels that does not contribute to the greenhouse effect [7, 8, 9]. Hydrogen satisfies the above requirements because it produces only water, when it is combusted as a fuel or converted to electricity. Its use in fuel cells is inherently more efficient than the combustion currently required for the conversion of other potential fuels to mechanical energy. Hydrogen breakdown generates no pollutants; unlike ethanol for example, whose large scale use is predicted to release large amounts of carcinogenic acetaldehyde with the generation of large amounts of smog. All presently studied biofuels are largely carbon neutral since the carbon released by their combustion is derived,

directly or indirectly, from recently fixed atmospheric CO₂. However, the emitted carbon associated with hydrogen produced by microbial fermentation is released during the production of this biofuel rather than during its utilization, thus potentially permitting easy capture of CO₂ and sequestration. Thus, in this scenario, biological hydrogen production could even be carbon negative [10].

2. HYDROGEN PRODUCTION TECHNOLOGIES

Presently, 40% H₂ is produced from natural gas, 30% from heavy oils and naphtha, 18% from coal, and 4% from electrolysis and about 1% is produced from biomass [11]. Thus, currently H₂ is produced, almost exclusively, by steam reformation of methane or by water electrolysis. Biological production of H₂ (biohydrogen), using microorganisms, is an area of technology development that offers the potential production of renewable H₂ from biomass [12].

Conventional steam methane reforming (SMR) processes use steam at high temperatures (800°C) to react with the methane (CH₄), which forms H₂ and carbon monoxide (CO); see reaction (1), below. The CO further reacts with vaporized water to form carbon dioxide (CO₂), a greenhouse gas, and additional H₂ (reaction (2)).



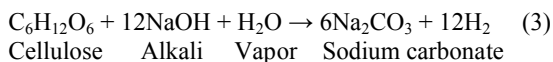
Hydrogen may also be produced by steam or aqueous reforming of various oxygenated hydrocarbons, such as methanol, ethanol, glycerol, or glucose using a wide variety of temperatures and catalysts. The catalytic pathway for the production of H₂ and CO₂ by aqueous-phase reforming of oxygenated hydrocarbons involves cleavage of C–C bonds, as well as C–H and/or O–H bonds to form adsorbed species on the catalyst surface. Oxygenated hydrocarbons can be reformed in the aqueous phase in the presence of a platinum-based catalyst to produce H₂ gas [13, 14]. The conversion takes place at

¹“Politehnica” University of Timisoara, Department of Hydrotechnical Engineering, , George Enescu Street, No. 1/A, RO-300022, Timisoara, Romania, E-mail : boboescu.iulian@yahoo.com

moderate temperatures, around 225–265°C, and at pressures of 27–54 bar, conditions that prevent steam formation and ensure that the reaction sequence takes place in the aqueous phase.

Production of H₂ by water electrolysis relies on passing an electric current through a conductive electrolyte in water (alkaline or polymeric), which results in splitting water molecules into H₂ and oxygen (O₂). Hydrogen produced by electrolysis of water is of relatively high quality, as no carbon, sulphur, or nitrogenous compounds are generated in the process. Purification costs for fuel cell grade H₂ are thus much less than for SMR. Electrolysis by itself is emission free, but lifecycle emissions are a direct function of the source of the electricity used in the process.

In case of hydrogen production by biomass gasification, traditional gasification reactors generate a complex mixture of gases, called syn-gas, including CO, CO₂, CH₄, and small amounts of longer chain hydrocarbons. A key problem with gasification is how to separate and purify the H₂ from the other gases in the syn-gas. Ishida et al. [15] propose an innovative method for the synthesis of H₂ without CO or CO₂ for fuel cells through the reactions of biomasses, alkali metal hydroxides and water vapor at relatively low temperatures (200-350°C) under atmospheric pressure. The main reaction for this new method can be expressed as:



The reactions of cellulose with sodium hydroxide (NaOH) and water vapor were carried out with a conventional mass controlled gas flow system under atmospheric pressure. The use of nickel (Ni), cobalt (Co), rodium (Rh), or ruthenium (Ru) catalysts supported on aluminum oxide (Al₂O₃) specifically enhanced the formation of H₂. The total yields of H₂ produced through the reactions over these catalysts at 100-500°C were increased from 62% to almost 100%.

Supercritical water partial oxidation is a process that occurs in water at temperatures and pressures above a mixture's critical point [16]. Under these conditions water becomes a fluid with unique properties that can be used to advantage in the destruction of hazardous wastes such as PCBs, or for the production of H₂ [17], although little data for hydrogen via this process are available. The fluid has a density between that of water vapor and liquid at standard conditions, and exhibits high gas-like diffusion rates along with high liquid-like collision rates. Efficient oxidation reactions occur at low temperature (400-650°C) with reduced NOx production.

3. BIOLOGICAL HYDROGEN PRODUCTION

Among various hydrogen production processes, biological method is known to be less energy intensive, for it can be carried out at ambient temperature and pressure [18, 19]. Furthermore, these techniques are well suited for decentralized energy production in small-scale installations in locations

where biomass or wastes are available, thus avoiding energy expenditure and costs for transport [20].

Biohydrogen technologies provide a wide range of approaches to generate hydrogen, including direct and indirect photolysis, photo-fermentation, and dark-fermentation [21, 6].

3.1. BIOPHOTOLYSIS OF WATER USING GREEN ALGAE AND BLUE-GREEN ALGAE (CYANOBACTERIA)

Green algae and blue-green algae split water molecules into hydrogen ion and oxygen via direct and indirect biophotolysis.

3.1.1. DIRECT BIOPHOTOLYSIS

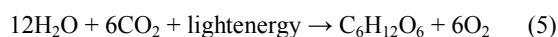
The conversion of water to hydrogen by green algae may be represented by the following general reaction:



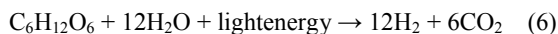
The well-known H₂-producing green algae, *Chlamydomonas reinhardtii*, under anaerobic conditions, can either generate H₂ or use H₂ as an electron donor [22]. The generated hydrogen ions are converted into hydrogen gas in the medium with electrons (donated by reduced ferredoxin) by hydrogenase enzyme present in the cells. This enzyme is very sensitive to O₂. Hydrogenase activity has also been observed in other green algae like *Scenedesmus obliquus* [22], *Chlorococcum littorale* [23] *Platymonas subcordiformis* [23] and *Chlorella fusca* [22]. On the other hand, there are several green algae types that do not have hydrogenase activity such as *Dunaliella salina* and *Chlorella vulgaris* [23].

3.1.2. INDIRECT BIOPHOTOLYSIS

The general reaction for hydrogen formation from water by cyanobacteria can be represented by following reactions:



And



Cyanobacteria are also known as blue-green algae, cyanophyceae or cyanophytes. It is a large and diverse group of photoautotrophic microorganism. Cyanobacteria contain photosynthetic pigments, such as chl a, carotenoids and phycobiliproteins, and can perform oxygenic photosynthesis. Hydrogen is produced both by hydrogenase and nitrogenase enzymes. The nutritional requirements of cyanobacteria are simple: air (N₂ and O₂), water, mineral salts and light. Hydrogen producing cyanobacteria may be either nitrogen fixing or non-nitrogen fixing. The examples of nitrogen fixing organisms are non-marine *Anabaena sp.*, marine cyanobacteria *Calothrix sp.*, *Oscillatoria sp.* Non-nitrogen fixing organisms are *Synechococcus sp.*, *Gloeobacter sp.* and *Anabaena sp.* They are found

suitable for higher hydrogen evolution as compared to other cyanobacteria species [21, 22, 23, 24].

In recent years, some species of *Anabaena* genus had received more attention regarding hydrogen production [23, 25]. The growth conditions for *Anabaena* are simple which include nitrogen free media, illumination, CO₂ and N₂. Nitrogenase plays important role for the hydrogen generation. Activity of the nitrogenase is inhibited by oxygen. Hydrogen production takes place under anaerobic conditions. Some cultures require CO₂ during hydrogen evolution phase, although CO₂ is reported to give some inhibition effects on photo-production of H₂. Lower CO₂ concentrations (4–18% w/v) have been reported to increase cell density during growth phase, resulting in higher hydrogen evolution in the later stage. Simple sugars have been found suitable for hydrogen production. Recently more emphasis has been given to increase hydrogenase activity and bidirectional hydrogenase deficient mutants of *Anabaena sp.* to increase the rate of hydrogen production. However, at the present time the rate of hydrogen production by *Anabaena sp.* is considerably lower than that obtained by dark or photo-fermentations [21, 22, 23, 24, 25].

Even though photosynthetic hydrogen production is a theoretically perfect process with transforming solar energy into hydrogen by photosynthetic bacteria, applying it to practice is difficult due to the low utilization efficiency of light and difficulties in designing the reactors for hydrogen production [8, 9, 18, 19, 26, 27, 28].

3.2. PHOTODECOMPOSITION OF ORGANIC COMPOUNDS BY PHOTOSYNTHETIC BACTERIA

H₂ production by purple non-sulfur bacteria is mainly due to the presence of nitrogenase under nitrogen-deficient conditions using light energy and reduced compounds (organic acids). The reaction is as follows:



Photosynthetic bacteria have long been studied for their capacity to produce significant amounts of hydrogen [29]. The advantage of their use is in the versatile metabolic capabilities of these organisms and the lack of Photosystem II (PSII), which automatically eliminates the difficulties associated with O₂ inhibition of H₂ production. Phototrophic bacteria require organic or inorganic electron source to drive their photosynthesis. They can utilize a wide range of cheap compounds. These photoheterotrophic bacteria have been found suitable to convert light energy into H₂ using organic wastes as substrate [29, 30, 31] in batch processes [32], continuous cultures [33], or immobilized whole cell system using different solid matrices like carrageenan [34], agar gel [35], porous glass [31], and polyurethane foam [30].

3.3. FERMENTATIVE HYDROGEN PRODUCTION FROM ORGANIC COMPOUNDS

Dark hydrogen fermentation is a ubiquitous phenomenon under anoxic conditions (i.e., no oxygen

present as an electron acceptor) [36]. When bacteria grow on organic substrates (heterotrophic growth), these substrates are degraded by oxidation to provide building blocks and metabolic energy for growth. This oxidation generates electrons which need to be disposed of to maintain electrical neutrality. In oxic environments, oxygen is reduced and water is the product. In anoxic environments, other compounds, e.g., protons, which are reduced to molecular hydrogen (H₂), need to act as electron acceptor [27, 21]. In the hydrogen fermentation process, glucose is initially converted to pyruvate by the glycolytic pathways. This is oxidized to acetyl-CoA, which can be converted to acetyl phosphate and results in the generation of ATP and the excretion of acetate (Fig. 1).

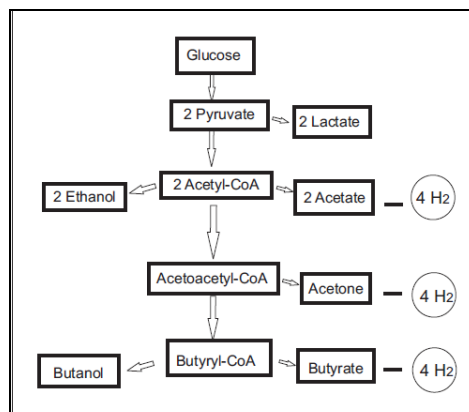
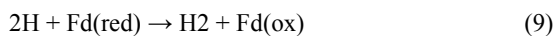
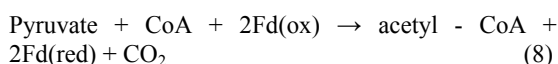
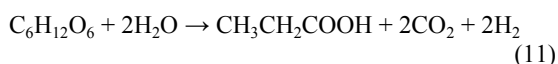
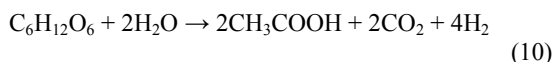


Fig. 1. The metabolic pathways representing hydrogen production fermentation in strictly anaerobic *Clostridium acetobutylicum*; adapted from [46]

Pyruvate oxidation to acetyl-CoA requires ferredoxin (Fd) reduction. Reduced Fd is oxidized by hydrogenase which generates Fd(ox) and releases electrons to produce molecular hydrogen [37, 38]. The overall reaction of the process can be described as follows:



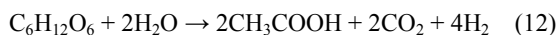
Anaerobic fermentation enables the mass production of hydrogen via relatively simple processes from a wide spectrum of potentially utilizable substrates, including refuse and waste products [39]. Moreover, fermentative hydrogen production generally proceeds at a higher rate and does not rely on the availability of light sources. Carbohydrates, mainly glucose, are the preferred carbon sources for fermentation processes, which predominantly give rise to acetic and butyric acids together with hydrogen gas [38], as follows:



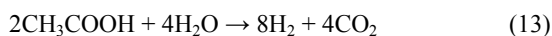
The end-products of glucose fermentation by anaerobic and facultative anaerobic chemoheterotrophs, e.g., clostridia and enteric bacteria, are produced through pyruvate. Facultative anaerobic bacteria give 2 mol of hydrogen per mol of glucose, whereas strictly anaerobic bacteria give four. Facultative anaerobes are less sensitive to oxygen, and are sometimes able to recover hydrogen production activity after accidental oxygen damage to them by rapidly depleting oxygen present in the broth. As a consequence, a facultative anaerobe is considered a better microorganism than a strict anaerobe to carry out fermentative hydrogen production process [40]. A fermentative hydrogen production process can be conducted by using either pure cultures or mixed cultures. However, in a fermentative hydrogen production process using mixed cultures, the hydrogen produced by hydrogen-producing bacteria can be consumed by hydrogen-consuming bacteria. Thus, in order to harness hydrogen from a fermentative hydrogen production process, the seed sludge often needs a pretreatment to suppress as much hydrogen consuming bacterial activity as possible while still preserving the activity of the hydrogen-producing bacteria [41]. One of the main constraints of fermentative biohydrogenation process is the lower yield of hydrogen, maximally 4 mol/mol glucose, compared with other processes (Table 1).

carbohydrate (or organic wastes) produces intermediates, such as low molecular weight organic acids, which are then converted into hydrogen by photosynthetic bacteria in the second step in a photobioreactor. The overall reactions of the process can be represented as:

(1) Stage I. Dark fermentation (facultative anaerobes):



(2) Stage II. Photo-fermentation (photosynthetic bacteria):



So, theoretically it is evident that using glucose as the sole substrate in dark anaerobic fermentation, where acetic acid is the predominant metabolite product, a total of 12 mol hydrogen could be expected in a combined process from one mol of glucose. In recent years, a number of studies concerning the combination of purple non-sulfur (PNS) photosynthetic bacteria and anaerobic bacteria for the efficient conversion of wastewater, sewage sludge and glucose into hydrogen had been made [44, 45, 38].

Process	Feedstocks	Conversion efficiencies	Co-products
Electrolysis	H ₂ O + electricity	50-60%	only H ₂
Steam methane reforming	Methane, glycerol, alcohols, polyols, sugars, organic acids	70-85%	CO, CO ₂ , C ₁₀ -C ₂₂ carbon chains
Aqueous reforming	Glycerol, alcohols, polyols, sugars, organic acids	35-100%	CO, CO ₂ , alkanes, alcohols, polyols, organic acids
Biomass gasification	Lignocellulosic biomass	35-50%	CO, CO ₂ , CH ₄
Partial oxidation	Glycerol, alcohols, polyols, sugars, organic acids	60-75%	NO and NO ₂
Biophotolysis	H ₂ O + sunlight	0,5%	only H ₂
photo-fermentation	Organic acids + sunlight	0,1%	CO ₂
dark fermentation	Biomass	60-80%	CO ₂

This yield is too low to be economically viable as an alternative to existing chemical or electrochemical processes of hydrogen generation [42]. Therefore, the ultimate goal, and challenge, for fermentative hydrogen research and development focuses essentially on attaining higher yields of hydrogen.

Comparison of the rates of H₂ production by various biohydrogen systems, however, suggests that dark-fermentation systems offer an excellent potential for practical application and integration with emerging hydrogen and fuel cell technologies [21, 4].

3.4. HYBRID SYSTEM USING FERMENTATIVE AND PHOTOSYNTHETIC BACTERIA

The light independent bacteria could provide an integrated system for maximizing the hydrogen yield [43]. In such a system, the anaerobic fermentation of

4. CHARACTERISTICS OF THE BIOHYDROGEN PRODUCTION METHODS

The discussed biohydrogen production methods are characterized by some fundamental quantitative and qualitative characteristics. The advantages and disadvantages of different biohydrogen production methods are summarized in table 2.

Compared with photosynthetic hydrogen production, fermentative hydrogen production has higher hydrogen production efficiency, higher hydrogen production stability, higher feasibility for industrialization, simpler control requirement and lower operating costs. Thus fermentative hydrogen production is more feasible and widely used. In addition, it is of great significance to produce hydrogen from organic wastes by fermentative hydrogen production, because it plays the dual role of

Process	Advantages	Disadvantages
Direct biophotolysis	Can produce H ₂ directly from water and sunlight; Solar conversion energy increased by ten folds as compared to trees, crops, etc.	Requires high intensity of light; O ₂ can be dangerous for the system; Lower photochemical efficiency;
Indirect biophotolysis	low nutrient input requirements; Cyanobacteria can produce H ₂ from water; Has the ability to fix N ₂ from atmosphere.	Uptake hydrogenase enzymes are to be removed to stop degradation of H ₂ ; About 30% O ₂ present in gas mixture.
Photo-fermentation	A wide spectral light energy can be used by these bacteria; Can use different organic wastes.	O ₂ has an inhibitory effect on nitrogenase; Light conversion efficiency is very low, only 1–5%.
Dark fermentation	It can produce H ₂ all day long without light; A variety of carbon sources can be used as substrates; It produces valuable metabolites such as butyric, lactic and acetic acids as by products; It is anaerobic process, so there is no O ₂ limitation problem.	O ₂ is a strong inhibitor of hydrogenase; Relatively lower achievable yields of H ₂ ; As yields increase H ₂ fermentation becomes thermodynamically unfavorable; Product gas mixture contains CO ₂ which has to be separated.

waste reduction and energy production. However, the reported H₂ production rates, stabilities and efficiency of these processes are far below commercialization.

4. CONCLUSION

Biohydrogen production has been established as a prospective alternative and integral component of green sustainable energy. A challenging problem in establishing biohydrogen as a source of energy from the renewable substrate and environmentally friendly immobilized solid matrices is the generation of large quantities of hydrogen gas.

The major bottlenecks of the biohydrogen production processes for the commercialization are:

- development of mixed microbial consortia or metagenomic approaches may be used to develop efficient microbial strains for the better utilization of industrial wastewater, which has different carbon content;
- improvement of H₂ yield of the processes using cheaper raw materials;
- in two-stage processes, the major bottleneck lies on the photo-fermentation process. Improvement of these processes surely will improve overall hydrogen yield as well as economy of the process.

Acknowledgement

This work was partially supported by the strategic grant POSDRU 107/1.5/S/77265, inside POSDRU Romania 2007-2013 co-financed by the European Social Fund – Investing in People.

REFERENCES

[1] *I. Mirel, E. Man, D. Chivoreanu, A. Carabeț*, Biogas Installations in Romania, Buletinul științific al Universității “Politehnica” din Timișoara. Facultatea de Hidrotehnică, 1996, Fasc. 1, pp. 33-38

[2] EIA. Hydrogen. Available from: <http://www.eia.doe.gov> [accessed 20.03.2011]

[3] *R.C., Saxena, D., Seal, S., Kumar, H.B., Goyal*, Thermochemical routes for hydrogen rich gas from biomass: a review, Renewable and Sustainable Energy Reviews, 2008, Fasc. 12, pp. 1909–1927

[4] *I. Mirel*, The optimization of the plants exploitation for producing and using biogas, Buletinul științific al UP Timișoara, seria Mecanică, Tom 52 (66), 2007, Fasc. 4, pp. 32-38

[5] *I. Mirel*, Biogazul, resursă neconvențională de energie, Cercetări în domeniul energiilor neconvenționale în zona de Vest a României, Zilele Academice Timișene, 24-25 mai 2007

[6] *I. Mirel, E. Pantea, A. Pop, V., Gherman*, Consideratii privind obtinerea biohidrogenului din ape uzate cu incarcare organica mare, Revista AGIR Anul XII, 2007, Fasc. 3-4, pp. 12-16

[7] *I. Mirel, A. Nacu*, Considerations on wastewater with high level of organic matter treatment, Universitatea Bacău, Studii și cercetări științifice, Biologie Serie nouă, 1999, Fasc. 4, pp. 327-330

[8] *F.R., Hawkes, R., Dinsdale, D.L., Hawkes, I., Hussy*, Sustainable fermentative hydrogen production: challenges for process optimization, International Journal of Hydrogen Energy, 2002, Fasc. 27, pp. 1339–1347

[9] *I.K., Kapdan, F., Kargi*, Bio-hydrogen production from waste materials, Enzyme and Microbial Technology, 2006, Fasc. 38, pp. 569–582

[10] *I. Mirel, V. Ghergheș, D. Popescu, C. Ghergheș, E. Pantea*, Installation to produce and utilize biogas in countryside, MICROCAD 2004 International Scientific Conference. Section A: Environmental and Earth Sciences University of Miskolc – Ungaria, 2004, pp. 131-136

[11] *D., Das*, Advances in biohydrogen production processes: An approach towards commercialization, International journal of hydrogen energy, 2009, Fasc. 34, pp. 7349 – 7357

[12] *I. Mirel, Z. Creț, M. Mitrașcă*, Used waters with high organic charge, Analele Universității din Oradea, Tom V part II, 2000, Fascicolul Protecția mediului, pp. 7-14

[13] *R.D., Cortright, R.R., Davda, J.A., Dumesic*, Hydrogen from catalytic reforming of biomass-derived hydrocarbons in liquid water, Nature, 2002, Fasc. 418, pp. 964–967

- [14] E., Chornet, S., Czernik, Renewable fuels: harnessing hydrogen, *Nature*, 2002, Fasc. 418, pp. 928–929
- [15] M., Ishida, S., Takenaka, I., Yamanaka, K., Otsuka, Production of CO_x-free hydrogen from biomass and NaOH mixture: effect of catalysts, *Energy and Fuels*, 2006, Fasc. 20, pp. 748–753
- [16] D.T., Hong, M.H., Spritzer, Supercritical water partial oxidation, *Proceedings of the U.S. DOE Hydrogen Program Review*, NREL, 2002
- [17] Y., Lu, H., Jin, L., Guo, X., Zhang, C., Cao, X., Guo, Hydrogen production by partial oxidation gasification of biomass in supercritical water with fluidization bed reactor, *Journal of Xi'an Jiotong University*, 2009, Fasc. 43, pp. 5–9
- [18] J.T., Kraemer, D.M., Bagley, Improving the yield from fermentative hydrogen production, *Biotechnology Letters*, 2007, Fasc. 29, pp. 685–695
- [19] N., Nishio, Y., Nakashimada, High rate production of hydrogen/methane from various substrates and wastes, *Advances in Biochemical Engineering and Biotechnology*, 2004, Fasc. 90, pp. 63–87
- [20] I., Mirel, E., Man, D., Chivoreanu, A., Carabeț, Instalații de biogaz pentru cvartale de locuințe, *Analele Universității din Oradea, Fasciculul Construcții și instalații hidroedilitare, Tom I*, 1998, pp. 3-10
- [21] D.B., Levin, L., Pitt, M., Love, Biohydrogen production: prospects and limitations to practical application, *International Journal of Hydrogen Energy*, 2004, Fasc. 29, pp. 173–185
- [22] M., Winkler, A., Hemseheier, C., Gotor, A., Melis, T., Happe, [Fe]-hydrogenase in green algae: photo-fermentation and hydrogen evolution under sulfur deprivation, *International Journal of Hydrogen Energy*, 2002, Fasc. 27, pp. 1431–1439
- [23] R., Nandi, S., Sengupta, Microbial production of an overview, *Critical Reviews in Microbiology*, 1998, Fasc. 24, pp. 61–84
- [24] F.A.L., Pinto, O., Troshina, P., Lindblad, A brief look at three decades of research on cyanobacterial hydrogen evolution, *International Journal of Hydrogen Energy*, 2002, Fasc. 27, pp. 1209–1215
- [25] J., Liu, V.E., Bukutin, A.A., Tsygankov, Light energy conversion into H₂ by *Anabaena variabilis* mutant PK84 dense culture exposed in nitrogen limitations, *International Journal of Hydrogen Energy*, 2006, Fasc. 31, pp. 1591–1596
- [26] D., Das, T.N., Veziroglu, Advances in biological hydrogen production processes, *International Journal of Hydrogen Energy*, 2008; Fasc. 33, pp. 6046–6057
- [27] D., Das, T.N., Veziroglu, Hydrogen production by biological processes: a survey of literature, *International Journal of Hydrogen Energy*, 2001, Fasc. 26, pp. 13–28
- [28] F.R., Hawkes, I., Hussy, G., Kyazze, R., Dinsdale, D.L., Hawkes, Continuous dark fermentative hydrogen production by mesophilic microflora: principles and progress, *International Journal of Hydrogen Energy*, 2007, Fasc. 32, pp. 172–184
- [29] J.R., Bolton, Solar photoproduction of hydrogen, *Solar Energy*, 1996, Fasc. 57, pp. 37–50
- [30] A.S., Fedorov, A.A., Tsygankov, K.K., Rao, D.O., Hall, Hydrogen photoproduction by *Rhodobacter sphaeroides* immobilised on polyurethane foam, *Biotechnology Letters*, 1998, Fasc. 20, pp. 1007–1009
- [31] A.A., Tsygankov, Y., Hirata, M., Miyake, Y., Asada, J., Miyake, Photobioreactor with photosynthetic bacteria immobilized on porous glass for hydrogen photoproduction, *Journal of Fermentation and Bioengineering*, 1994, Fasc. 77, pp. 575–578
- [32] H., Zurrer, R., Bachofen, Hydrogen production by the photosynthetic bacterium, *Rhodospirillum rubrum*, *Applied and Environmental Microbiology*, 1979, Fasc. 37, pp. 789–793
- [33] E., Fascetti, O., Todini, *Rhodobacter sphaeroides* RV cultivation and hydrogen production in a one- and two-stage chemostat, *Applied Microbiology and Biotechnology*, 1995, Fasc. 22, pp. 300–305
- [34] N., Francou, P.M., Vignais, Hydrogen production by *Rhodopseudomonas capsulata* cells entrapped in carrageenan beads, *Biotechnology Letters*, 1984, Fasc. 6, pp. 639–644
- [35] M., Vincenzini, R., Materassi, C., Sili, G., Florenzano, Hydrogen production by immobilized cells III: prolonged and stable H₂ photoevolution by *Rhodopseudomonas palustris* in light-darkcycles, *International Journal of Hydrogen Energy*, 1986, Fasc. 11, pp. 623–626
- [36] I., Mirel, D., Popescu, Gh., Bănățean, M., Varga, Biogas – how to produce and utilize in industrial equipment, *Lucr. Simpozionului Internațional “Biogazul”- oportunități și soluții*, 2000, pp. 35-40
- [37] K., Nath, D., Das, Improvement of fermentative hydrogen production – various approaches, *Applied Microbiology and Biotechnology*, 2004, Fasc. 65, pp. 520–529
- [38] K., Nath, D., Das, Hydrogen production by *Rhodobacter sphaeroides* strain O.U. 001 using spent media of *Enterobacter cloacae* strain DM11, *Applied Microbiology and Biotechnology*, 2005, Fasc. 68, pp. 533–541
- [39] I., Mirel, E., Man, D., Popescu, K., Barabas, Biomass Sources of Energy for Rural House-Keeping, *Scientific Bulletin of the „Politehnica” University of Timisoara, Transactions on Mechanics, Tom 51(65)*, 2006, Fasc. 2, pp. 347-352
- [40] Y-K., Oh, E-H., Seol, E., Yeol, S., Park, Fermentative hydrogen production by a new chemolithotrophic bacterium *Rhodopseudomonas palustris* P4, *International Journal of Hydrogen Energy*, 2002, Fasc. 27, pp. 1373–1379
- [41] J.L., Wang, W., Wan, Effect of Fe²⁺ concentrations on fermentative hydrogen production by mixed cultures, *International Journal of Hydrogen Energy*, 2008, Fasc. 33, pp. 1215–1220
- [42] P.C., Hallenbeck, J.R., Benemann, Biological hydrogen production: fundamentals and limiting processes, *International Journal of Hydrogen Energy*, 2002, Fasc. 27, pp. 1185–1193
- [43] H., Yokoi, S., Mori, J., Hirose, S., Hayashi, Y., Takasaki, H₂ production from starch by a mixed culture of *Clostridium butyricum* and *Rhodobacter sp.* M-19, *Biotechnology Letters*, 1998, Fasc. 20, pp. 890–895
- [44] C.M., Lee, P.C., Chen, C.C., Wang, Y.C., Tung, Photohydrogen production using purple non-sulfur bacteria with hydrogen fermentation reactor effluent, *International Journal of Hydrogen Energy*, 2002, Fasc. 27, pp. 1308–1314
- [45] M.S., Kim, T.J., Lee, Y.S., Yoon, I.G., Lee, K.W., Moon, Hydrogen production from food processing wastewater and sewage sludge by anaerobic dark fermentation combined with photofermentation, In: J., Miyake, T., Matsunaga, A.S., Pietro, editors, *Biohydrogen II*. Amsterdam: Elsevier, 2001, pp. 263–272
- [46] H-L., Chin, Z-S., Chen, C.P., Chou, Fedbatch operation using *Clostridium acetobutylicum* suspension culture as biocatalyst for enhancing hydrogen production, *Biotechnology Progress*, 2003, Fasc. 19, pp. 383–388
- [47] J.D., Holladay, J., Hu, D.L., King, Y., Wng, An overview of hydrogen production technologies, *Catalysis Today*, 2009, Fasc. 139, pp. 244–260