Postprint of: Łukajtis R., Hołowacz I., Kucharska K., Glinka M., Rybarczyk P., Przyjazny A., Kamiński M.: Hydrogen production from biomass using dark fermentation. RENEWABLE & SUSTAINABLE ENERGY REVIEWS. Vol. 91, (2018), p. 665-694. DOI: 10.1016/j.rser.2018.04.043

Hydrogen production from biomass using dark fermentation

Rafał Łukajtis[1], Iwona Hołowacz[1], Karolina Kucharska[1]*,Marta Glinka[1,2], Piotr Rybarczyk[1], Andrzej Przyjazny[3], Marian Kamiński[1]

[1]Faculty of Chemistry, Department of Chemical and Process Engineering, Gdańsk University of Technology, 11/12 Gabriela Narutowicza Street, 80-233 Gdańsk, Poland

[2]Faculty of Chemistry, Department of Analytical Chemistry, Gdańsk University of Technology, 11/12 Gabriela Narutowicza Street, 80-233 Gdańsk, Poland

[3]Department of Chemistry & Biochemistry, Kettering University, Flint, MI 48504, USA

E-mail address: karkucha@pg.edu.pl

Abstract

Hydrogen applicability in the power, chemical and petrochemical industries is constantly growing. Efficient methods of hydrogen generation from renewable sources, including waste products, are currently being developed, even though hydrogen is mainly produced through steam reforming or thermal cracking of natural gas or petroleum fractions. In paper alternative methods of hydrogen production with a particular emphasis on dark fermentation are discussed. The review compiles essential information on strains of bacteria used in the production of hydrogen from waste products in the agroindustry and from lignocellulosic biomass. The effect of such parameters as kind of raw material, method of processing, temperature, pH, substrate concentration, partial pressure of hydrogen, hydraulic retention time, method of inoculum preparation and the type and operating parameters of a reactor on the yield of dark fermentation is discussed. The review aims at presentation of current state of knowledge on the dark fermentation process utilizing waste materials as substrates. The results of investigations with emphasis on the most important issues regarding operating parameters of dark fermentation are also included.

Highlights

Hydrogen can be produced from biomass by biotechnological methods
Utilization of lignocellulose within dark fermentation requires pretreatment
Optimization of lignocellulosic biomass pretreatment improves fermentation results
Improvement of the efficiency of hydrogen generation requires constant research
Scale-up should result in high-potential strategies for hydrogen technologies

Keywords

biomass; dark fermentation; biohydrogen; lignocellulose; bioreactor

Table of Contents:

1. Introduction

Depletion of fossil fuels, being at present the main energy sources, as well as a negative impact of their combustion products on the environment, have resulted in an increased interest in hydrogen as one of the most promising energy sources in the future. Hydrogen, called the fuel of the future can be converted into energy not only in internal combustion or jet engines, gas power turbines but also in fuel cells. The advantage of hydrogen as a fuel is attributed to its high net calorific value compared with other fuels (Table 1) [1,2]. One kilogram of hydrogen is equivalent to 2.75 kg of gasoline and 6 kg of methanol with respect to its net calorific value [3]. Hydrogen, due to its high energy efficiency, is also competitive with other alternative energy sources such as wind, solar, tidal and geothermal energy [2]. In the age of deterioration of the environment, the fact that hydrogen is an ecologically clean energy source is highly important. Combustion of hydrogen does not yield carbon dioxide, one of the greenhouse gases affecting the climate. A serious limiting factor in the use of hydrogen as an energy source is its explosivity in a mixture with oxygen. In addition, storage of hydrogen fuel is more difficult when compared to other fuels. Due to its low boiling point, hydrogen has to be stored in specially insulated pressure vessels. In spite of this, hydrogen storage is easier than energy storage [3].

Table 1. Comparison of properties of synthetic fuels [2].

Fuel	Relative amount of fuel needed to match weight composition of H_2 [Heat of combustion / Heat of combustion of H_2]	Hydrogen density in fuel [g/dm^3]	Net calorific value [kJ/g]	Storage
Hydrogen	1	70.5	120.0	Very difficult
Methane	2.4	104.1	50.0	Difficult
Methanol	6.0	113.7	20.1	Easy
Ethanol	4.4	104.1	26.8	Easy
Gasoline	2.7	112.1	46.7	Easy

Although hydrogen is being used as a fuel or energy source, at present its main applications are as a starting material in the chemical, refinery and pharmaceutical industries. It is used in the synthesis of ammonia and methanol and for hydrogenation of liquid oils [3]. Among other processes making use of hydrogen, one should mention the synthesis of aniline from nitrobenzene, the synthesis of hexamethylenediamine and the removal of trace amounts of oxygen in corrosion protection [3]. In refinery processes, such as hydrocracking or hydro refining, hydrogen is used to remove sulfur and nitrogen compounds.

Hydrogen does not occur in nature in its elemental form but only in chemical compounds. Thus, despite its abundance on Earth, in order to produce hydrogen it is necessary to convert compounds in which it occurs. At present, the most common sources of hydrogen are nonrenewable fossil fuels, such as crude oil (*ca.* 30%), natural gas (*ca.* 48%), coal (*ca.* 18%), and electrolysis of water (*ca.* 4%) [4]. The leading technologies of hydrogen production using conventional energy sources include steam reforming of natural gas and petroleum, catalytic decomposition of natural gas, partial oxidation of heavy hydrocarbons fraction of petroleum and gasification of coal or coke. These methods are very energy-intensive and require high temperatures (>700 °C). Furthermore, all the processes mentioned above pollute the environment emitting large amounts of oxides of carbon, sulfur and nitrogen as well as ashes containing radioactive substances and heavy metals to the atmosphere [5,6]. Another important process generating hydrogen is electrolysis of water; however, it requires electrical energy coming from coal- or natural gas-fired power plants or from nuclear power plants. The advantage of electrolysis results from lack of emission of carbon dioxide to the atmosphere [4,7].

Novel methods of hydrogen production, such as photocatalytic, plasmochemical, magnetolytic or radiolytic splitting of water as well as high-temperature plasma gasification of hydrocarbons are being developed [8–11]. Decomposition of water yielding hydrogen and oxygen can also take place through mechano-catalytic splitting [12].

The share of individual raw materials in the production of hydrogen is shown in Figure 1. At present 96% of produced hydrogen comes from fossil fuels; therefore, hydrogen cannot

be treated as a product of green chemistry and an ecological energy source.

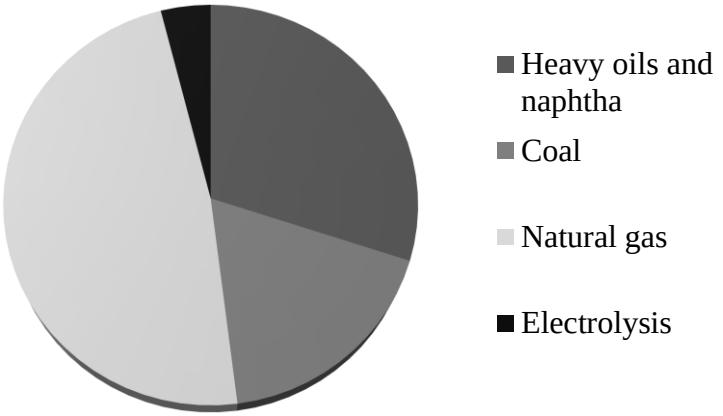

Figure 1. Sources of hydrogen [4,7].

The objectives of the review are to:

- Describe the perspectives on hydrogen production concerning factors affecting dark fermentation that aquire basic understanding and experience about renewable resources for hydrogen generation and associated problems.
- Explain the technological possibilities for hydrogen production and scale-up.
- Identify the areas of additional research and practices to improve the efficiency and the gain of energy associated to the renewable resources management.

1.1. Perspectives on hydrogen production

Despite the broad industrial applications of hydrogen, it is highly probable that hydrogen will soon become a main fuel for transportation services. This is caused by crude oil depletion as well as ecological aspects related to gasoline and diesel fuel usage. The awareness of people and politicians is increasing, regarding the effects of conventional transportation services on environmental pollution. Exhaust fumes emissions *i.e.* nitrogen and carbon oxides, particulate matter or hydrocarbons, especially affect citizens of large urban locations. Beside novel methods of exhaust fumes reduction corresponding to increasingly restrictive regulations, new technological solutions for road transportation are investigated and implemented. Among these technologies, electrical cars gain importance, mainly due to zero local emissions. However, high price, little range, relatively long recharging time and the recycling problem of used batteries limit the popularity of electric cars. Driving electric cars with hydrogen fuel cells seems to be a convenient alternative, mainly due to efficient generation of electric energy [13]. Current technologies enable to offer a similar comfort of usage, comparing to conventional cars with internal combustion engines. Taking into account that there are 1,1 billion of cars globally, road transportation seems to be an important area of future application of hydrogen as a fuel. However, this scenario may be realized providing

that the technologies for safe hydrogen storage and usage as well as hydrogen distribution network are further developed. Globally, an increasing number of countries invests in programs devoted to the development of hydrogen-driven transportation [14]. Moreover, application of hydrogen fuel cells favors the decentralization of electric energy generation for industry, rural and urban areas [15] because the fuel may be used at its generation place. Furthermore, the global increase of hydrogen fuel cells popularity results in an increasing significance of renewable energy sources from biomass of various origin. Hydrogen production from biomass results in a decrease of energetic dependence of countries having limited access to fossil fuels. Biomass to hydrogen conversion may be realized via several processes *i.e.* gasification, pyrolysis, steam catalytic reforming, photofermentation and biophotolysis. Above mentioned methods of biomass to hydrogen conversion are discussed in chapter two and are currently widely investigated [16,17]**.** It is worth notifying that besides fuel generation, biomass may be simultaneously converted to various useful chemical compounds. This concept is realized by so called biorefineries [18–22]. Our paper reviews the current state of hydrogen generation via dark fermentation. The authors are aware of vast applications of dark fermentation process, thus the paper does not cover all the available data. The authors believe that the paper contains valuable set of information as well as it is an updated supplement to previous review papers devoted to hydrogen dark fermentation [23–31].

2. Hydrogen production methods

Production of hydrogen from renewable energy sources, including biomass, is an alternative to traditional methodsof hydrogen generation. Wastes from agri-food industry rich in starch and cellulose (straw, manure, whey, distillery wastewater, molasses), pulp and paper industry wastes, wastes from the production of biofuels (technical grade glycerine) and sewage sludge can all be used in hydrogen production through metabolic processes of microorganisms [32,33]. Crops of rapidly growing plants (energy willow and poplar, silvergrass, switchgrass, grasses) are also used in the production of biomass. Carbon dioxide, being a byproduct in the process of hydrogen generation, can be completely absorbed through photosynthesis on such plantations.

An increasing interest has been put in thermochemical methods of hydrogen production. Biomass gasification is one of above mentioned group of methods. The process is realized in high temperature (600-1000^{o}C) with limited access to oxygen, air and steam so as incomplete combustion takes place. The resulting products are high volumes of gases and little amount of carbon. The process occurs analogously to gasification of coal, resulting in the formation of synthesis gas, containing combustible products, mostly hydrogen, carbon monoxide and methane and other hydrocarbons as acetylene, ethylene as well as noncombustible components: ammonia, carbon dioxide and water vapor [17,34–36] The advantage of gasification is the possibility of its application to various types of biomass. However, the biomass composition affects the composition of final products. A substrate pretreatment is of crucial importance for the gasification [37]. Following parameters are investigated: particle size, temperature, gasifying agent, the catalyst type and concentration

and gasification time [38–42]**.** The lower the size of biomass' particles, the shorter the time to reach the desired temperature. Furthermore, the higher the water content, the higher the hydrogen yield and the lower the calorific value of the hydrogen gas.

The processes of thermochemical conversion of biomass requires large energy input, although it can also be supplied by biomass. Gasification is an efficient thermochemical method with a main disadvantage of the necessity of tar removal from the products.
Another thermochemical method of biomass to hydrogen conversion is pyrolysis. The process is realized at elevated temperature (200-600^{o}C) in non-oxidizing conditions. The pyrolysis process may be microwave assisted in single [43] or two stage mode [39]. The products of pyrolysis include mainly charcoal, wood gas (H_2, CO, CO_2, CH_4. C_2H_6) and bio-oil [15]**.** The efficiency of hydrogen production via pyrolysis depends on the type of catalyst [44–46], temperature, the rate of biomass heating and process time [47].

The highest yields of hydrogen are obtained from fast pyrolysis in high temperature and short reaction time, because for long reaction time charcoal is the main product [48]. Bio-oil is the decomposition product of lignin, cellulose and hemicellulose. It contains mainly phenolic derivatives, carboxylic acids (up to 60-70%) [49] as well as alcohols, hydroxyl acids, aldehydes, ketones and esters [50]. One of the main advantage of pyrolysis is the possibility of its application towards various feedstock materials, while the disadvantage are the high energetic requirements. Following reactors are applied for pyrolysis: ablation, fluidized bed, fluidized bed with circulation and entrained flow reactor [47,51]**.** Fluidized bed reactors are the most popular due to the fastest heating of biomass as compared to other types. Chemical compounds generated in a form of pyrolytic oil may be effectively converted to hydrogen by means of catalytic steam reforming. In paper [52] bio-oil and bio-char were converted to syngas with the application of lanthanum catalyst with hydrogen yield exceeding 80%. The conversion of acetic acid and the applied catalysts are reviewed in [53]. Alcohols may be also converted to hydrogen [54]. Biomass-based methanol and ethanol are converted to hydrogen by catalytic steam reforming [22,55]. Similarly, glycerin may be converted to hydrogen with the application of platinum catalyst with a Mg_3Al-LDH additive.

Alternatively, hydrogen can be produced from biomass by biotechnological methods, using natural capabilities of microorganisms to generate hydrogen as one of the metabolic products. Biological technologies for hydrogen production include direct and indirect biophotolysis and photofermentation as well as the processes taking place in the absence of light, *i.e.* dark fermentation, bioelectrolysis and bioconversion of carbon monoxide [56].

In direct biophotolysis, the substrate for the production of hydrogen is water, which under anaerobic conditions in the presence of light, is converted into hydrogen and oxygen by microalgae, *e.g.* , green algae or cyanobacteria [57–59]. The activity of hydrogenase, the main enzyme responsible for hydrogen synthesis, decreases in the presence of oxygen, because under aerobic conditions the electrons released during splitting of water molecules are not used for the production of hydrogen but for the reduction of carbon dioxide [57]. In the production of hydrogen by cyanobacteria, the hydrogen evolving and oxygen evolving activities are spatially separated [60]. In the case of green algae, several hours of incubation of the algae under anaerobic conditions are required for induction of hydrogenase and

production of hydrogen. It was found that under sulfur deprivation conditions the green algae *Chlamidomonas reinwardtii* in the presence of light produce not oxygen but hydrogen [58].

Indirect biophotolysis is a two-step process in which the reactions of oxygen and hydrogen formation are separated in time. As a result, it is easier to maintain anaerobic conditions during the second step, *i.e.*, during production of hydrogen through hydrogenesis [59]. Nevertheless, the possibility to generate hydrogen using indirect biophotolysis is limited due to low rate of the process and a high cost of photobioreactors. The yield of hydrogen production calculated as the ratio of energy generated in the form of hydrogen to the solar energy used in the process is low in both direct and indirect biophotolysis and it does not exceed 10% [5].

Photofermentation is the process during which organic acids are converted into hydrogen and carbon dioxide by photosynthesis due to the presence of anaerobic bacteria strains: *Rhodobacter*, *Rhodobium*, *Rhodopseudomonas*, and *Rhodospirillum* [61]. Hydrogen is formed as a result of reduction of molecular nitrogen in the presence of nitrogenase which also reduces protons to molecular hydrogen. Oxygen, as a nitrogenase inhibitor, is not formed in these conversions. The yield of hydrogen production is similar to the one obtained in biophotolysis and depends on the kind of microorganisms, kind of medium, design of a photofermenter and intensity of light [62]. The research regarding photofermentation often involves dark fermentation. During dark fermentation, hydrogen and methane are also produced along with volatile fatty acids and alcohols. Such compounds may be the source of electrons and hydrogen to reduce carbon dioxide and carbon source for sulfur-free photosynthetic bacteria. The combination of fermentation and photofermentation in a two-step process results in an increase of the hydrogen yield [27,63].

In the process of bioelectrolysis of water, the energy needed for conversion of organic compounds is supplied in the form of electric energy. Microorganisms support oxidation of substrates at the anode. Protons move across a proton exchange membrane to the cathode where hydrogen is produced [64]. Microbial electrolysis cells (MECs) operate on the same principle [65].

Carbon monoxide coming from thermal gasification of biomass can serve as a substrate for hydrogen production. Bioconversion of carbon monoxide to carbon dioxide with a release of hydrogen takes place in the absence of light in the presence of bacteria *Rubrivivax gelatinosus* [66].

3. Dark fermentation

Dark fermentation is considered to be the most promising method of biohydrogen production through biomass conversion, for which the net energy ratio is equal to 1.9 [64]. For comparison, in the case of steam methane reforming, this ratio is lower and equal to 0.64 [64]. Dark fermentation is also the best understood process among biotechnological methods of hydrogen production.

In dark fermentation, substrates are converted by anaerobic bacteria grown in the dark (Figure 2). Hydrogen is the key substrate in metabolism of many anaerobic microorganisms. Such microorganisms are capable of using energy-rich hydrogen molecules, if they are

available, and utilize the electrons from hydrogen oxidation to produce energy. In the absence of external acceptors of electrons, the organisms have excess of electrons generated in the metabolic processes as a result of reduction of protons yielding hydrogen molecules. The main enzymes regulating the hydrogen metabolism are hydrogenases. The two basic hydrogenases, phylogenetally different, and having different active sites are [FeFe] – hydrogenase and [NiFe]-hydrogenase. These enzymes catalyze the reversible reaction:

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

[FeFe]-hydrogenases are more active in the production of molecular hydrogen than [NiFe]-hydrogenases, which primarily catalyze oxidation of molecular hydrogen. It should be noted that [FeFe] –hydrogenases are usually sensitive to oxygen [32,67].

At present, the mechanism of fermentations via glycolysis, in which metabolic processes result in the production of hydrogen from glucose, is well known (Figure 2.). The first step of this type of fermentation is glycolysis, in which glucose is converted to pyruvate, the key intermediate with the formation of reduced form of nicotinamide adenine dinucleotide (NADH). Under anaerobic conditions pyruvate can be converted to acetyl-CoA in the reaction catalyzed by pyruvate ferredoxin oxidoreductase (PFOR). The products of the reaction, in addition to acetyl-CoA and CO_2, include also reduced ferredoxin, involved in the reduction of [FeFe]-hydrogenases, which subsequently reduce protons yielding hydrogen. Another possible pathway to conversion of pyruvate to acetyl-CoA with the formation of formate is the reaction catalyzed by pyruvate formate lyase (PFL). Formate can be readily converted to hydrogen and carbon dioxide in the presence of [NiFe]-hydrogenases or [FeFe]-hydrogenases. Acetyl coenzyme A can be converted into several organic compounds being fermentation value-added products (ethanol, butanol, acetone, butyric acid or acetic acid) with the simultaneous oxidation of NADH and/or formation of ATP[68,69].

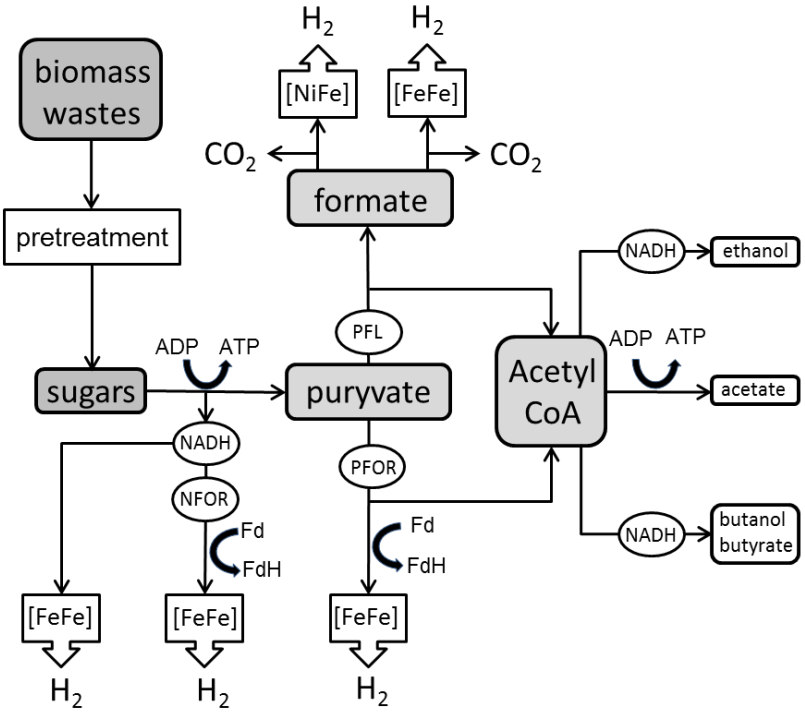

Figure 2. Typical metabolic pathways for conversion of substrate to hydrogen during dark fermentation [13,22,25-27].

Various types of glycolytic fermentations are distinguished, depending on chemical compounds being the main final products. Hydrogen fermentations include butyrate, butanol and mixed-acid (acetic and formic acid) fermentations. The final products in butyrate butanol fermentation include butyric acid, butanol, hydrogen, carbon dioxide and acetic acid as well as other compounds, such as acetone, 2-propanol, ethanol, lactic acid, caproic acid or acetoin [69]. Mixed-acid fermentation yields formic acid, acetic acid, ethanol, hydrogen, carbon dioxide, lactic acid and succinic acid, glycerol, acetoin, and 2,3-butanediol. The butyrate butanol fermentation is carried out by *Clostridium* bacteria. Mixed-acid fermentation is characteristic for *Enterobacter* and *Bacillus* bacteria [69].

The stoichiometric, theoretically maximal, amount of molecular hydrogen per mole of glucose, according to equation 2, equals 12 moles [69]:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2 \quad (2)$$

$$C_6H_{12}O_6 + 6H_2O \rightarrow 2CO_2 + 2CH_3COOH + 4H_2 \quad (3)$$

$$C_6H_{12}O_6 + 6H_2O \rightarrow 2CO_2 + CH_3CH_2CH_2COOH + 2H_2 \quad (4)$$

However, the formation of various final products, such as acetic, propionic and butyric acids as well as methanol, butanol or acetone, lowers the amount of hydrogen produced in fermentation. The formation of acetic acid decreases the amount of molecular hydrogen formed from twelve moles to four moles (Eqn 3). If butyric acid is the final product, one mole of glucose yields only two moles of hydrogen (Eqn 4). In practice, the final product is a mixture of various chemical compounds, which additionally lowers the hydrogen yield to 1-2.5 moles of hydrogen per mole of glucose. The production of hydrogen from biomass is

considered to be economically justified if 60-80% of the energy contained in the substrate is converted to hydrogen [7]. A possible use of organic compounds formed during the production of biohydrogen can make the process economically justified, as some value-added products may be isolated form the fermentation broth.

A number of factors can affect the yield of hydrogen production by fermentation. The design and performance of such a process may be difficult. Thus, numerous studies on the optimization of conditions of dark fermentation have been carried.[68,70–78] in order to obtain the yield close to a theoretical maximum.

4. Raw materials and substrates for dark fermentation

An important factor affecting dark fermentations results is the choice of an appropriate raw material, being the source of organic compounds serving as substrates in the production of hydrogen. This is especially crucial in the production of hydrogen on an industrial scale. Monosaccharides, such as glucose, and disaccharides such as lactose or sucrose are the most preferred sources of carbon for metabolic conversions of microorganisms in dark fermentation. Naturally occurring, renewable sources of sugars are starch as well as cellulose and hemicellulose, present in plants mostly as polymers. The use of raw materials rich in starch, which is readily hydrolyzed to simple carbohydrates, is particularly convenient [69]. The use of sources rich in cellulose and hemicellulose is more complicated. Cellulose, in particular, is resistant to chemical and biological processing due to its compact structure and hydrogen bonds between glucose monomers. Drastic conditions are often required in order to change the chemical structure which can significantly raise the cost of processing. The best starting material is the one with a simple method of initial pretreatment and a high content of readily available carbohydrates [79,80].

4.1. Mono- and disaccharides

Monosaccharides, such as hexoses (*e.g.* , glucose) and pentoses (*e.g.* , xylose) and disaccharides, especially sucrose and lactose are readily biodegradable substrates. They have been used in experiments involving dark fermentation since the 1980s [81]. Because their use in the fermentation processes does not require complicated processing and the results are reproducible, they are currently used to test new designs [82]. The highest yields were obtained for sucrose, *i.e.,* 6 moles of H_2/mol sucrose [83]. Relatively high yield was also obtained for lactose: 3 moles of H_2/mol lactose [83]. The yield of sucrose per mole is similar to the yield obtained for typical hexoses [5].

Most recent trends in the studies on dark fermentation involve technologies capable of continuous production of hydrogen. Simple carbohydrates are not suitable for this type of production since their cost is too high, which makes their use unprofitable on an industrial scale. In addition, simple carbohydrates, such as glucose, are usually used in food products and/or animal feeds and their utilization for the production of biofuels is controversial.

Examples of yields and operating conditions for the production of hydrogen from various simple carbohydrates both in continuous and batch modes are compiled in Table 2.

Table 2. Operating conditions and yields of hydrogen production processes using dark fermentation.

Substrate	Organism	Reactor/ Mode	HRT [h]	pH Temp. [°C]	Hydrogen productivity	Hydrogen yield	% H_2 (in biogas)	References
Glucose 10 g/ dm^3	*Clostridiaceae* and *Flexibacteraceae*,	Membrane Continuous	3.3	pH = 5.5 T=35°C	640 cm^3 H_2/(dm^3·h)	4 mol H_2/mol glucose	60	[84]
Glucose (1%)	*E. cloacae*	Batch	-	pH = 6 T=36°C	447 cm^3 H_2/(dm^3·h)	2.2 mol H_2/mol glucose	-	[83]
Glucose 10 g/dm^3	Mixed culture from compost	Batch	-	pH = 5.5 T=60°C	147 cm^3 H_2/(dm^3·h)	2.1 mol H_2/mol glucose	-	[85]
Glucose 20 g COD/dm^3	*Clostridia sp.*	CSTR Continuous	6	pH = 6 T=28-32°C	7.42 mmol H_2/(gVSS·h)	1.42 mol H_2/mol glucose	43	[86]
Glucose 7 g/dm^3	Mixed culture	CSTR Continuous	6	pH = 5.5 T=36°C	-	2.1 molH_2/mol glucose	64	[87]
Glucose 4.85 g COD/dm^3	Mixed culture	UASB Continuous	26.7	pH = 7.2 T=70°C	11.15 mmol H_2/d	2.46 mol H_2/mol hexose	55	[88]
Sucrose 20 g COD/dm^3	Mixed culture	Immobilized bed Continuous	1	pH = 6.7 T=35°C	1.32 dm^3 H_2/(dm^3·h)	-	34	[89]
Sucrose 10 g/dm^3	*E. cloacae* IIT-BT 08	Batch	-	pH = 6 T=36°C	660 cm^3 H_2/(dm^3·h)	6 mol H_2/mol sucrose	92	[83]
Sucrose 1 g COD/dm^3	Mixed culture	Batch	-	pH = 6 T=26°C	-	1.8 mol H_2/mol sucrose	-	[90]
Sucrose 20 g COD/dm^3	Mixed culture	CSTR Continuous	8	pH = 6.7 T=35°C	0.105 mol H_2/h	3.47molH_2/mol sucrose	42	[91]
Sucrose 25 g/dm^3	Mixed culture	Fermenter Batch	-	pH = 5.5 T=35°C	1504 cm^3 H_2/h	2 mol H_2/mol glucose	-	[92]
Lactose 29 mmol/dm^3	*C. termolacticum*	CSTR Continuous	35.7	pH = 7 T=58°C	2.58 mmol H_2/(dm^3 ·h)	1.5 mol H_2/mol hexose	55	[93]
D-Xylose 10 g/dm^3	*E. cloacae* IIT-BT 08	Batch	-	pH = 6 T=36°C	348 cm^3 H_2/(dm^3·h)	0.95 mol H_2/mol xylose	-	[83]
L-Arabinose 10 g/dm^3	*E. cloacae* IIT-BT 08	Batch	-	pH = 6 T=36°C	360 cm^3 H_2/(dm^3·h)	1.5 mol H_2/mol arabinose	-	[83]

COD – chemical oxygen demand; CSTR – continuously stirred tank reactor; VSS – volatile suspended solids

4.2. Renewable resources

Although substrates rich in simple carbohydrates, sucrose or starch hydrolysates are commonly used in laboratory studies, their utilization on a large scale is unprofitable. Continuous hydrogen production requires renewable resources, preferably of second generation, *i.e.,* rich in lignocellulose or starch waste, which cannot be used as an animal feed [68,94]. Carbohydrate polymers are not suitable substrates for direct usage in the production of hydrogen by dark fermentation due to their resistance to degradation. Substrates of this type are fermented slowly with a low hydrogen yield [32]. To improve the yield of hydrogen, they should be treated to break down long polymer chains to simple carbohydrates or short oligomers. One of the examples confirming this are the studies [95], in which a higher production of hydrogen from corn stover was observed following acid hydrolysis than for the untreated corn stover.

Utilization in dark fermentation of troublesome wastes (organic waste of various origin, cheese whey, olive mill wastewater, glycerol after FAME production, manure) seems to be attractive [5,96–98]. Intensive research on the production of hydrogen on an industrial scale using dark fermentation of this type of raw materials has been carried out recently.

4.2.1. Organic municipal waste

This group includes the organic fraction of readily biodegradable waste originating mainly from households, restaurants, food processing plant residues, *etc.* This waste can contain components rich in polysaccharides, such as starch, cellulose and hemicellulose as well as proteins, lipids and simple carbohydrates. Such a waste is one of the sources releasing methane to the atmosphere. It also pollutes groundwater with wastewater formed during decomposition of organic matter in landfills. The need to limit the negative effect of such a waste stimulates scientists and industry to investigate its possible application as a raw material in dark fermentation [99,100].

Another example of raw material for dark fermentation is sewage sludge. The sludge is rich in simple carbohydrates, disaccharides, and peptides. In order to generate hydrogen as the main product of fermentation of sewage sludge, it is necessary to eliminate bacteria which utilize hydrogen in their metabolic processes (especially methanobacteria). To this end, the raw material is pretreated by heat shock, ultrasonication, acidic or alkaline pretreatment or supplementation with organic compounds [101,102]. Guo *et al*. have studied impacts of sterilization, microwave and ultrasonication pretreatment on hydrogen production using waste sludge [103]. Sterilized sludge was found to have the highest yield (15 cm^3 H_2/g COD), followed by microwave pretreatment (11 cm^3 H_2/g COD), and ultrasonication (4.68 cm^3 H_2/g COD). High temperature treatment (121 ºC) resulted in over a tenfold increase in carbohydrate content [103]. Such a treatment allows eliminating or limiting the activity of the organisms which consume hydrogen in their metabolic processes.

In the case of food waste, the composition of feedstock for fermentation may vary depending on the season which can affect the yields of hydrogen and by-products. Albardii *et*

al. [104] examined the effect of composition of food waste on hydrogen production based on samples containing food waste and slaughterhouse waste in various proportions. The food waste contained primarily pasta, bread, fruit, vegetable, meat and fish while the slaughterhouse waste consisted of skin, fat and meat waste of pork, poultry and beef. The samples differed in moisture as well starch, cellulose, protein, and lipid content. The lowest hydrogen production yield equal to 25 cm^3 H_2/g VS was obtained for the mixture rich in slaughterhouse waste, *i.e.*, rich in proteins and lipids (a total of 48%). For the mixture rich in food waste, *i.e.*, rich in carbohydrates (67%), the yield was over three times higher (85 cm^3 H_2/g VS).

Karlsson *et al*. [105] obtained 16.5 cm^3 H_2 per 1 g of the waste from fermentation of a mixture of wastes (85% slaughterhouse waste, 4% food industry waste, 5% manure). The most efficient process was carried out at 55 °C using a mixed culture of bacteria and a hydraulic retention time of 2 h. Unfortunately, the hydrogen content in the biogas was low (maximally up to 2.5%).

Biohydrogen production from untreated and hydrolyzed potato steam peels by dark fermentation using *Caldicellusiruptor sacharolyticus* and *Thermotoga neapolitana* was also investigated. Two types of hydrolysates were used: one in which the starch was liquefied with α-amylase, and one in which the liquefied starch was further hydrolyzed to glucose by amyloglucosidase. When glucose (*ca.* 10-14 g/L) was added to both hydrolyzed and unhydrolyzed peels, both strains of bacteria produced hydrogen with a relatively high molar yield (2.4-3.4 moles H_2/mol glucose). When the concentration of the substrates was increased, the hydrogen production decreased [77].

Jayalakshmi *et al*. [106] obtained the yield of hydrogen of 72 cm^3 H_2/g VS added during fermentation of kitchen waste (66% food waste, 27% vegetable waste, 0.96% tea waste, 1.09% egg shells, 1.36% packing materials, 3.61% ash) using an inclined plug-flow reactor.

Other examples of conditions and results of dark fermentation using food waste or food production waste are compiled in Table 3.

Table 3. Operating conditions and yields of hydrogen production by dark fermentation using renewable resources.

Substrate	Organism	Reactor/ Mode	Liquid organic products	pH Temp.	Hydrogen productivity	Hydrogen yield	% H_2 in biogas (v/v)	Ref.
Kitchen waste: 66% food waste, 27% vegetable waste, 0.96% tea waste, 1.09% egg shells, 1.36 % packing materials, 3.61% ash	Mixed cultures	Inclined plug-flow reactor Continuous	Butyric acid Acetic acid Propionic acid	pH = 5.5	N.D.	72 cm^3 H_2/g VS	46	[106]
Organic municipal solid waste 110 g TVS/ dm^3/d	Mixed cultures	CSTR Semi-continuous	Butyric acid Acetic acid	pH = 5.0 T = 50 °C	5.7 dm^3 H_2/ dm^3/ d	N.D.	55	[107]
Organic municipal waste mixed with poultry slaughterhouse waste 70.86 g / dm^3	Mesophilic anaerobic sludge	Erlenmeyer flask Continuous	Caproic acid Butyric acid Acetic acid Propionic acid	pH = 6.0 T = 34 °C	N.D.	71.3 cm^3 H_2/g VS	27.5	[108]
Kitchen garbage	Anaerobic digester sludge	CSTR Continuous	Butyric acid Acetic acid Ethanol Lactic acid	pH = 5.0 T = 55 °C	1.7 dm^3H_2/ dm^3/d	66 cm^3 H_2/g VS	7.2	[109]
Synthetic food waste (rice, vegetables, meat 30 g COD/ dm^3	Anaerobic sludge from UASB treating cassava wastewater	Batch Two-stage	Butyric acid Acetic acid Ethanol Propionic acid	pH = 6.0 T = 37 °C	0.9 dm^3 H_2/ dm^3/ d	55 cm^3 H_2/ gVS	49	[110]
Potato steam peels 10 g glucose/ dm^3	Mixed culture	Batch	Acetic acid Lactic acid	pH = 6.9 T = 75 °C	12.5 mmol H_2/ dm^3h	3,8 mol H_2 /mol glucose	N.D.	[77]
Food waste: pasta, bread, fruit, vegetable, fish and meat	Mixed culture from aerobic sludge	Batch	Acetic acid Butyric acid	T = 36 °C	N.D.	70.34 cm^3/gVS	N.D.	[104]
Simulated food waste: fish 5% meat 10%; bread 10%; apple 10%; kiwi 6%; banana 9%; pear 10%; onion 5%; lettuce 5%; carrot 5%; cabbage 10%; potato 15%	Mixed culture from digested sludge	CSTR Continuous	Acetic acid Butyric acid Caproic acid Valeric acid	pH = 5.5 T = 34 °C	0.23 N dm^3 H_2/ dm^3r /d	20.5 dm^3 H_2/kgVS	27.5	[111]
Kitchen waste from several cafeterias 50 g COD/ dm^3	Anaerobic sludge from treatment plant	Batch	Butyric acid Lactic acid Acetic acid	pH = 5.5 T = 55 °C	79 mmol H2/L-medium/d)	N.D.	54.4	[112]
Mixed food waste from residential homes	Anaerobic sludge from digester	Batch	N.D.	pH = 7.5 T = 50 °C	54.2 cm^3 H_2/h	57 cm^3 H_2/gVS	38	[113]
Raw cassava starch	Facultative anaerobic	Batch	Butyric acid	T = 35°C	N.D.	1,44 mol H_2 / mol	61	[114]

	bacteria	Acetic acid Ethanol	glucose

COD – chemical oxygen demand; CSTR – continuously stirred tank reactor; TVS – total volatile solids; VS – volatile solids; N.D. – not defined

4.2.2. Waste of animal origin

Animal waste can be a serious environmental problem. The waste contains primarily manure and leftover feed. It is used mostly on farmlands or to make composts, in which biological processes last. This results in a release of methane and other harmful gases, mostly derivatives of sulfur, nitrogen and phosphorus to the atmosphere. The approach of building closed chamber composting plants, in which methane is produced, and the post-fermentation residue is used as a fertilizer, seems to be more appropriate. To be used in dark fermentation processes in which hydrogen is the main product, the waste has to be pretreated either with high temperature or microwaves, ultrasounds, acids or bases to remove methanogenic bacteria. Another problem is the presence and high content of nitrogen compounds which can be reduced to ammonia during fermentation. Ammonia at a certain concentration inhibits dark fermentation. The hydrogen yield decreases rapidly when the concentration of ammonia exceeds 2 g N/dm^3 [115].

The studies on dark fermentation of manure or compost have been carried out. The possibility of continuous biohydrogen production from liquid swine manure as a substrate for fermentation was investigated [116]. The manure was diluted from a solid content of about 2% to about 0.5% and supplemented with glucose at a concentration of 10 g/dm^3 as a carbon source. Fermentation was carried out in a sequencing batch reactor using the seed sludge from an anaerobic digester. The maximum hydrogen production rate was 0.15 dm^3 $H_2/dm^3/h$ for an hydraulic retention time (HRT) equal to 8 h and the maximum hydrogen yield was 1.63 mol H_2/mol glucose for an HRT equal to 16 h. The hydrogen content in the biogas ranges from 38 to 44% and it depens on HRT.

Conditions and results of dark fermentation of animal waste as well as the main organic metabolites and process parameters (*i.e.* temperature and pH) are compiled in Table 4.

Table 4. Compilation of parameters and results of fermentation to hydrogen of animal waste.

Substrate	Organism	Reactor Mode	Organic metabolites	pH Temp.	Hydrogen productivity	Hydrogen yield	% H_2 (in biogas)	References
Liquid swine manure 13.94 g COD/ dm^3	Mixed cultures from anaerobic digester	ASBR Batch	Acetic acid >>Butyric acid >> Valeric acid > Ethanol > Propionic acid	pH = 5.0 T=37 ^{0}C	0.1 dm^3 H_2 /dm^3/h	1.63 mol H_2 /mol glucose	43%	[116]
Mixture of slaughterhouse waste, food industry residues, hydrolyzing yeast and manure 28.2 g substrate/ dm^3	Mixed cultures	CSTR Continuous	Acetic acid > Butyric acid	pH= 6.8 T=55 ^{0}C	466.8 cm^3/d	16.5 cm^3 H_2/g waste	0.6 %	[105]
Dairy manures 70 g/ dm^3	*Clostridium sp*	CSABR Continuous	Butyric acid >> Acetic acid > Ethanol > Propionic acid > Butanol	pH=5.0 T=36 ^{0}C	N.D.	31.5 cm^3/g TVS	38.6%	[117]
Potato and pumpkin waste, buffalo manure 7.03% TS	Anaerobic sludge	Batch	Acetic acid >> Propionic acid >> Butyric acid > Valeric acid	pH= 7.5 T=35 ^{0}C	N.D.	135.6 cm^3H_2/ VS	N.D.	[118]
Cattle wastewater 1.3 g COD/ dm^3	Sewage sludge	Batch	Butyric acid > Acetic acid >> Ethanol> Propionic acid >	pH= 5.5 T=45 ^{0}C	0.34 dm^3/dm^3h]	12.41 mmol/g COD	32%	[119]

COD – chemical oxygen demand; CSTR – continuously stirred tank reactor; CSABR – continuously stirred anaerobic bioreactor; TVS – total volatile solds; VS – volatile solids; N.D. – not defined; > – more than; >> – much more than

4.2.3. Industrial waste and effluents

Industrial waste and effluents rich in carbohydrates can also be substrates for dark fermentation. These include olive oil mill wastewater [120], palm oil mill effluent [121,122] and wastes from the dairy and brewery industries [75].

Kargi *et al.* [75] obtained hydrogen from cheese whey powder (CWP) solution by thermophilic dark fermentation. Concentrated CWP solution was deactivated by autoclaving at 121 ºC for 15 min which allowed most of the proteins to be precipitated and the solution then filtered. Thus prepared substrate was fermented. The yield of hydrogen was 1.03 mol H_2/mol glucose. Azbar *et al.* [76] produced hydrogen via dark fermentation of cheese whey wastewater rich in lactose using mixed culture under thermophilic (55 °C) and mesophilic (36 °C) conditions. The hydrogen yields were 9.2 mmol H_2 /g COD and 8.1 mmol H_2 /g COD under mesophilic and thermophilic conditions, respectively.

Substrates that do not contain carbohydrates are also being investigated. The use of glycerol, a waste in the production of FAME that is difficult to utilize in large quantities, is also of considerable interest. Nakashimada *et al*. [123] examined the conversion of glycerol to hydrogen via dark fermentation. The hydrogen yield was 6.69 mmol H_2/g glycerol, which was significantly higher than the yields from simple carbohydrates, *i.e.*, glucose (1.97 mmol H_2/g glucose), galactose (1.90 mmol H_2/g galactose) and fructose (2.17 mmol H_2/g fructose). Ito *et al*. [124] investigated hydrogen production from glycerol-containing wastes discharged after biodiesel manufacturing process. For comparison, fermentation of pure glycerol at a concentration of 10 g/l was carried out using *Enterobacter aerogenes*. Both the yield and rate of hydrogen production from the waste were found to be significantly lower for the waste than for pure glycerol: 0.71 mol H_2/mol glycerol and 30 $cm^3/dm^3/h$, and 0.89 mol H_2/mol glycerol and 80 $cm^3/dm^3/h$, respectively (Table 5). The lower yields of hydrogen from the waste were likely caused by a high salt content and the presence of methanol which can inhibit the process. The effect of substrate concentration on the yield and rate of hydrogen production was also examined.

Silva et al. [125] investigated co-fermentation of food waste with glycerin (1, 3 and 5%). Food was originated from students' canteen and was composed of fruits and vegetables (57,4%), grains (13,5%), meat (26,3%; bovine, poultry, fish) and rejected materials (2,8%; bones and wastes not visually identified). The addition of glycerin (3%) resulted in more than twofold increase of hydrogen production compared to that obtained from food waste alone. The hydrogen yield was not further increased when 5% glycerin was added.Zhu *et al*. [126] examined biohydrogen production from purified terephthalic acid processing wastewater by anaerobic fermentation using mixed cultures and a CSTR reactor. A specific hydrogen production rate was 0.073 $dm^3/g\ cm^3$ VSS/d. After reaching stable operation of the reactor (25 days), the main liquid organic products formed during fermentation were acetic acid and ethanol at 476 and 431 mg/dm^3, respectively.

Citric acid wastewater can be used as another substrate for the production of biohydrogen. Continuous biohydrogen production from citric acid wastewater was carried out [127]. Using conditions optimal for mesophilic organisms, a hydrogen yield of 0.84 mol

H_2/mol hexose was obtained. Butyric acid was the dominant product among volatile fatty acids.

Liu et al. [33] applied low-sugar wastewaters from juices production for hydrogen production. The process was realized in CSTR with initial total sugar concentration of 10,39 g/dm^3, leading to hydrogen productivity of 11.39 L/L/d with hydrogen yield of 0.3 mol H_2 per mol of hexose.

The results of hydrogen fermentation of post-production wastes, temperature, main organic metabolites and process parameters, *i.e.*, microorganisms used, temperature and pH, are compiled in Table 5.

Table 5. Compilation of parameters and results of hydrogen fermentation of post-production industrial wastes.

Substrate	Organism	Reactor/ Mode	Organic metabolites	pH Temp.	Hydrogen productivity	Hydrogen yield	H_2 %	Ref.
Cheese whey 20 g/dm^3	Mixed cultures	Batch	Butyric acid> acetic acid> lactic acid>ethanol	pH=5.2 T=35 ^{0}C	2.9 dm^3 H_2/ dm^3/d]	0.78 mol H_2/mol glucose used]	23.8	[128]
Cheese whey wastewater 10 g/dm^3	Mixed cultures (anaerobic bacteria from UASB reactor)	Batch	Acetic acid>> butyric acid>> propionic acid >> heptanoic acid.>valeric acid	pH =4.5 T=55 ^{0}C	1.1 cm^3 H_2/gVSS*h]	3.1 mol H_2/mol lactose	40	[76]
Glycerol-containing waste from biodiesel production Glycerol content 1.7 g/dm^3	*Enterobacter aerogenes HU-101*	Packed Bed Continuous	Ethanol>>acetic acid> 1,3-propanediol>formic acid	pH=6.8 T=37 ^{0}C	63 mmol H_2/l/h	1.12 [mol H_2/mol-glycerol]	N.D.	[124]
Palm oil mill effluent 59 g COD/dm^3	Mixed cultures (isolated from cow dung)	USAB Continuous	N.D.	pH=5.0	73 dm^3/d	N.D.	56	[129]
Byproduct of biodiesel production from sunflower oil 25 g COD/dm^3	Mixed cultures (from anaerobic digester)	UFCB Continuous	Butyric acid> hexanoic acid> 1,3-propanediol> acetic acid>ethanol	pH=6.8 T=35 ^{0}C	107,3 l H_2/kg waste	0.52 mol H_2/mol glycerol	45	[130]
Wastewater from brewery plant 60 g carbohydrates/ dm^3	Mixed cultures (from activated sludge)	Batch	Butyric acid >> acetic acid > valeric acid > ethanol > butanol	pH=5.5 T=35 ^{0}C	-	2 mol H_2/mol hexose	52	[131]
Jatropha curcas – biodiesel industry residue	Mixed cultures (from activated sludge)	CSTR Continuous	Butyric acid > ethanol > acetic acid >> propionic acid > valeric acid	pH=5.5 T=37 ^{0}C	3.65 dm^3/dm^3 d	148 cm^3 H_2/g carbohydrate	58	[132]
Waste glycerol	Mixed cultures (containing immobilized *E. aerogenes ATCC 13048)*	USAB Continuous	Ethanol > 1,3-propanediol > butyric acid = acetic acid = Propionic acid = formic acid > lactic acid	pH=5.5 T=37 ^{0}C	6.2 mmol H_2/ dm^3/h	324 mmol H_2 /mol glycerol	24.2	[133]
Citric acid wastewater 18 g COD/dm^3	Mixed cultures	USAB Continuous	acetic acid>> Propionic acid>	pH=7.0 T=36 ^{0}C	0.72 m3 H_2/m3/ d	0.84 mol H_2/mol hexose	67	[127]
Cassava starch wastewater 25 g COD/dm^3	Mixed cultures (granules and sediment from USAB reactor)	Batch	N.D.	pH=5.5 T=30 ^{0}C	12.87 cm^3 H_2/ dm^3 h]	1.58 mol H_2/mol hexose	-	[134]
Palm oil decanter cake with glycerine	Mixed cultures	Batch and semi-continuous	N.D.	pH=7 T=55^{o}C	0.89 dm^3H_2/dm^{3}d	N.D.	N.D.	[135]

COD – chemical oxygen demand; CSTR – continuously stirred tank reactor; USAB – upflow anaerobic sludge blanket reactor; UFCB – upflow column bioreactor; N.D. – not defined; VSS – volatile suspended solids; > – more than; >> – much more than; = – similar quantity

4.2.4. Agricultural waste and energy plants

This group of residues includes raw materials of plant origin rich in lignocellulose and starch. Waste lignocellulose seems to be particularly attractive as the substrate, being the residue from growing and processing of cereals and plants (wheat, rye, rice, corn, *etc*.) on farms. The waste includes straws, peels, cornstalks, stems, sugarcane bagasse, leaves, and grass unsuitable for a feed. Cultivation of fast-growing energy plants rich in lignocellulose such as energy willow, energy poplar, miscanthus as well as the waste from the wood and paper industry can also provide a valuable raw material for hydrogen production [136].

The annual worldwide yield of lignocellulosic biomass residues is estimated to exceed 220 billion tons which can be used for the production of second generation biofuels, including hydrogen [136,137].

Lignocellulose is composed of three biopolymers bound through covalent bonds: cellulose and hemicellulose (representing up to 70-80% of lignocellulose biomass) which are coated and associated with lignin. Hydrogen bonds are also present within polymers listed above. Depending on the origin of plant material, the content of each of the three polymers is different [136]. Cellulose and hemicellulose are the desired polymers for dark fermentation, since they contain exclusively glucose in case of cellulose and a variety of hexoses and pentoses (*i.e. xylose)* for hemicellulose. In contrast, the presence of lignin is disadvantageous due to its resistance to biodegradation. Only a limited number of species can decompose lignin.

Plant material consisting mostly of lignocellulose is not such a good a substrate to be converted to biofuels as *e.g.* starch. As a result lignin presence, it does not usually undergo rapid or effective biodegradation. At present, two advanced technologies are used for utilization of lignocellulose in dark fermentation. Neither of them is ideal and in addition to significant advantages it also has some limitations.

The first approach, Consolidated Bioprocess (CBP), involves the direct use of comminuted raw materials in a highly integrated, one-step process for the direct conversion of lignocellulosic biomass into biofuels. In this technology, specific bacteria generate enzymatic cocktail, hydrolyze pretreated biomass and in-situ convert the released pentoses and hexoses into biohydrogen [138]. Such an approach lowers the investment costs and the energetic requirements for the pretreatment of raw material.

At present, it is believed that better hydrogen production is achieved by using thermophilic bacteria. At elevated temperatures, conversion of substrates and mass transfer are more rapid, resulting in higher hydrogen yields compared with mesophilic bacteria. However, the volumetric hydrogen production rates are lower than those of mesophilic bacteria as a result of slower proliferation and lower cell densities.

The investigation of conversion of cellulose to hydrogen [139] using mixed bacterial cultures from sewage sludge revealed that the hydrogen production was carried out mostly by microorganisms close to *Thermoanaerobacterium thermosaccharolyticum, Enterobacter cloacae* and *Clostridium* sp. Over the temperature range from 25 to 85 ºC, the activity of bacteria in the inoculum was highest under thermophilic conditions, and specifically at 55 ºC resulting in the highest hydrogen yields: 15.4 mol H_2/kg cellulose and 2.77 mol H_2/mol

hexose used and the hydrogen production was 1381.13 cm^3 H_2/dm^3 medium. The hydrogen yield for mesophilic and hyperthermophilic bacteria is low for these temperature conditions and for 30 °C and 70 °C it is inhibited.

An example of dark fermentation application for substrates rich in lignocellulose is evaluated in [71], where samples of sweet sorghum, sugarcane bagasse, wheat straw, maize leaves and silphium were utilized without pretreatment for hydrogen production by the extreme thermophiles *Caldicellulosiruptor saccharolyticus*. The experimental conditions and results obtained are shown in Table 6.

An often problem observed with this approach is the degree of conversion of the substrate to product. For example, during fermentation of switchgrass only 1.28 g/dm^3 of substrate out of 30 g/dm^3 is converted into product [140].

Dark fermentation requires further comprehensive investigations, especially in the area of finding, isolating and improving strains of bacteria capable of effective direct conversion of lignocellulosic material to hydrogen. The use of genetic engineering [141] to manipulate metabolism of microorganisms can improve yields of products and thus reduce the cost of the entire process. Among others, the genes responsible for the production of organic acids can be removed. Another interesting possibility is the development of strains with efficient multi-enzyme systems, comprising mainly cellulase and xylanase responsible for degradation of hemicellulose and cellulose. It is possible through genetic modifications of cellulosome and xylanosome [142].

Examples of the use of untreated agricultural waste or microcrystalline cellulose for the production of hydrogen under both mesophilic and thermophilic conditions are compiled in Table 6.

Table 6. Compilation of parameters and results of hydrogen fermentation of untreated agricultural waste or microcrystalline cellulose.

Substrate	Organism	Reactor / Mode	Organic metabolites	pH Temp.	Hydrogen productivity	Hydrogen yield	Ref.
Cornstalk 20g/dm^3	*Thermoanaerobacterium thermosaccharolyticum DD32*	Batch	Acetic acid > butyric acid > ethanol > butanol > propionic acid	T= 55 ^{0}C pH=7.5	-	6.38 mmol/g substrate	[143]
Rice straw 1% w/v	*Thermotoga neapolitana (DSM 4359)*	Batch	N.D.	T= 75 ^{0}C pH=7.5	31.77 cm^3 H_2/ dm^{3}d	2.27 mmol H_2/g straw	[144]
Rice straw 90 g/ dm^3	Mixed cultures from sewage sludge	Batch	Acetic acid > butyric acid > ethanol > > propionic acid	T= 55 ^{0}C pH=6.5	744 cm^3 H_2/ dm^3	24.8 cm^3 H_2/g DB	[145]
Wheat straw 5 g/ dm^3	*Thermoanaerobacterium thermosaccharolyticum M18*	Batch	Acetic acid > butyric acid > ethanol > butanol > propionic acid	T= 60 ^{0}C pH = 7.0	0.11 mmol/ dm^{3}h	3.53 mmol/g substrate	[146]
Grass	Mixed cultures	Batch	Acetic acid > propionic acid > butyric acid > ethanol	T= 35 ^{0}C pH=7.0	3.53 cm^3/h	4.39 cm^3/g-S	[147]
Barley hulls 5 g/ dm^3	*Clostridium thermocellum ATCC 27405*	Batch	Acetic acid > formic acid > ethanol	T= 60 ^{0}C	0.09 mmol/ dm^{3}h	1.07 mmol H_2/g substrate	[138,148]
Switchgrass 30 g/ dm^3	*Caldicellulosiruptor saccharolyticus DSM 8903*	Batch	Acetic acid >> succinic acid	T = 65 ^{0}C	0.10 mmol/ dm^{3}h	310 cm^3H_2/g DB	[140]
Cornstalk 15g/ dm^3	*Clostridium sartagoforme FZ11*	Batch	Acetic acid > butyric acid > ethanol > propionic acid > butanol	T= 35 ^{0}C pH=6.5	6.2cm^3H_2/g-stalk h	87.2 cm^3/g-substrate	[149]
Sugarcane bagasse 1%	*Caldicellulosiruptor saccharolyticus*	Batch	N.D.	T=70 ^{0}C	18,21 dm^3 H_2/kg	2.3 mol H_2/mol glucose	[71]
Corn leaves 0.9 %	*Caldicellulosiruptor saccharolyticus*	Batch	N.D.	T=70 ^{0}C	15,33 dm^3 H_2/kg	1.80 mol H_2/mol glucose	[71]
Wheat straw 1%	*Caldicellulosiruptor saccharolyticus*	Batch	N.D.	T=70 ^{0}C	44,68 dm^3 H_2/kg	3.80 mol H_2/mol glucose	[71]
Delignified wood fibers 0.1 g/ dm^3	*Clostridium thermocellum 27405*	Batch	Acetic acid > ethanol > formic acid	T=60 ^{0}C	-	2.32 mol H_2/ mol glucose	[70]
Soybean straw	Mixed cultures	Batch	Butyric acid > valeric acid > propionic acid > acetic acid > ethanol	T= 35 ^{0}C pH = 7.0	-	5.46 cm^3 H_2/g substrate	[150]

DB - ; N.D. – not defined; > – more than; >> – much more than

Utilization of lignocellulose in dark fermentation requires pretreatment prior to fermentation. Two methods are used: Separate Hydrolysis and Fermentation (SHF), where hydrolysis and fermentation are carried out in different tanks and Simultaneous Saccharification and Fermentation (SSF), where both hydrolysis and fermentation are accomplished in the same apparatus [151,152].

Pretreatment involves conversion of structural biopolymers to simple chemical compounds (hexoses, pentoses, carbohydrate oligomers) constituting good substrates for dark fermentation. Pretreatment of raw material usually takes place in two stages. The first stage includes mechanical curing, such as milling and grinding in order to provide increased surface area and porosity and thus reactivity. The next step involves chemical hydrolysis using catalysts: acids (sulfuric, hydrochloric or acetic) [153,154], bases (sodium or calcium hydroxide, ammonia) [155–157] or oxidation (ozone, hydrogen peroxide) [150,158]. An alternative approach is to use microorganisms capable of synthesizing enzymes digesting polysaccharides, mostly fungi [159–163]. Fungal pretreatment of cornstalk by *Phanerochaete chrysosporium* was investigated by Zhao *et al*. [159]. Lignin reduction was up to 34% with holocellulose loss less than 10%. Next, the pretreated cornstalk was subjected to enzymatic hydrolysis. The maximum enzymatic saccharification was found to be 20.3% higher than the control without pretreatment.

Eskicioglu et al. [164] investigated the hydrothermal pretreatment with CO_2 in the temperature range of 26 – 175^{o}C and the pressure ranging from 25 to 102 bar. Following substrate materials were investigated: wheat and rice straw, sorghum, corn stover and fir bark. The obtained hydrogen yield was compared with the hydrogen yield from pure glucose (135 cm^3H_2/g COD added). Following hydrogen yields were identified: 55 (cm^3H_2/g COD added) for sorghum, 52 (cm^3H_2/g COD added) for corn stover, 32 (cm^3H_2/g COD added) for wheat straw and 26 (cm^3H_2/g COD added) for rice straw.

Dark fermentation of rice husk was investigated by Gonzales et al. [165]. The substrate was pre-treated with 5% sulfuric acid at 60^{o}C followed by enzymatic sacharification with varying concentrations of commercially available Cellulast 1.5 L®. The highest obtained hydrogen yield was equal to 473.1 cm^3H_2/g rice husk.

In addition, physicochemical methods (steam-explosion) or physical methods (high temperature, ultrasonication, and microwave irradiation) are also suitable for structural destruction of lignocellulosic materials. Xu *et al*. [166] investigated the yield of hydrogen produced from acetic acid steam-exploded corn straw at different concentrations of acetic acid (Table 7). In addition, after chemical or physicochemical treatment, feedstock can be subjected to enzymatic hydrolysis using cellulases or hemicellulases, during which oligomers present after chemical treatment are hydrolyzed to simple carbohydrates. Usually, such an approach greatly improves the yield of fermentation process when simple organic compounds (pentoses and hexoses) are used as a feedstock. Simple carbohydrates in solution are much more susceptible to digestion by microorganisms than the tough polymeric structures.

Preparation of the feedstock to the fermentation process is complexed and energy intensive. The use of chemical compounds for hydrolysis can impact the environment and application of thermal energy and microwave radiation needed to improve monosugars yield is rather expensive. Furthermore, pretreatment in most cases induces the formation of fermentation inhibitors, such as furfural, 5-hydroxymethylfurfural, phenolic components,

vanillin or fatty acids. Depending on pretreatment conditions, these inhibitors are formed in different amounts and they negatively impact the hydrogen yield during fermentation. Lin *et al*. [167] examined the effect of presence of fermentation inhibitors on hydrogen production yield. Several dark fermentation experiments were carried out using glucose alone and glucose with an addition of four different by-products of hydrolysis of lignocellulose. The hydrogen yields for glucose, glucose with 15 mM of furfural, 5-HMF, syringaldehyde or vanillin were 247 cm^3H_2/g, 242 cm^3 H_2/g, 234.9 cm^3 H_2/g, 215.6 cm^3 H_2/g, and 205.6 cm^3 H_2/g, respectively.

The results demonstrate the inhibitory effect of these compounds on the activity of microorganisms, the phenolic compounds being more toxic. However, at these concentrations the inhibition is not substantial, especially for furfural and 5-HMF. The presence of inhibitors in the mixture also delays the lag phase and the log phase of the bacterial growth cycle during dark fermentation. It is also possible to pretreat a substrate by enzymatic hydrolysis alone using a mixture of isolated enzymes. At present, however, this approach is time-consuming and expensive. Despite using similar conditions of pretreatment for various substrates having similar contents of cellulose, hemicellulose and lignin, different fermentation yields are obtained [155]. Numerous experiments on conditions of processing of lignocellulose from various sources of plant origin have been carried out as demonstrated by a number of research and review papers published. In spite of using various substrates and processing conditions, no universal and efficient method of pretreatment has been found. Several examples of dark fermentation of pretreated substrates along with treatment conditions are compiled in Table 7. Despite great interest of the scientists in utilization of materials rich in lignocellulose to the production of biofuels, attempts to develop an efficient technology of industrial production of biohydrogen have failed up to now.

It would be interesting to find such a pretreatment method and conditions that the kind of substrate used were immaterial. To this end, further research is needed on optimization of pretreatment of lignocellulosic substrates for fermentation and on finding microorganisms resistant to the presence of inhibitors in hydrolysates obtained after chemical pretreatment [168,169].

Table 7. Compilation of parameters and results for hydrogen fermentation of agricultural and energy plants waste subjected to pretreatment.

Substrate	Pretreatment conditioons	Organism	Reactor / Mode	pH Temp.	Hydrogen productivity	Hydrogen yield	Ref.
Pine tree wood 10 g sugar/ dm^3	5% sulfuric acid, T = 121 ^{0}C, t = 60 min	Mixed cultures from sewage sludge digester	Batch	T= 35 ^{0}C pH = 7.0	1629 cm^3 H_2/ dm^3d	0.99 mol/mol substrate	[170]
Rice husk 10 g sugar/ dm^3	5% sulfuric acid, T = 121 ^{0}C, t = 60 min	Mixed cultures from sewage sludge digester	Batch	T= 35 ^{0}C pH = 7.0	1860 cm^3H_2/ dm^3d	1.25 mol/mol substrate	[170]
Soybean straw 40 ml pretreated solution	4% HCl, 30 min, boiled	Mixed cultures	Batch	T= 35 ^{0}C pH = 7.0	-	47.65 cm^3H_2 /g DB	[150]
40 ml pretreated solution	16% H_2O_2, 30 min, boiled	Mixed cultures	Batch	T= 35 ^{0}C pH = 7.0	-	25.30 cm^3 H_2 /g DB	[150]
Cornstalk waste 5 g/ dm^3	0.1 g/1 g $Ca(OH)_2$, 50 °C , 24 h	Mixed cultures enriched from rotted wood crumb	Batch	T= 60 ^{0}C pH = 7.0	-	155.4 cm^3H_2/g TVS	[156]
Wheat straw	White-rot fungal-pretreatment, *Phanerochaete chrysosporium* 30 °C, 21 days	*Clostridium perfringens*	Batch	T= 40 ^{0}C pH = 6.5	-	78.5 cm^3H_2/g	[171]
Corn straw	16% acetic acid, steam-exploded for 10 min, 1.21MPa, cellulase 120 U/g, 50 °C, 10 h	*Ethanoligenes harbinense* B49	Batch	T= 37 ^{0}C pH = 6.5	91 cm^3/g VSS/d	72 cm^3H_2/g	[166]
Cornstalk	Grzyb *Phanerochaete chrysosporium* for 15 days T=29 ^{0}C and enzymatic hydrolysis with cellulase 30 FPA/g substrate, 50 °C, 96 h	*Thermoanaerobacterium. thermosaccharolyticum*	100 ml-vial Batch	T= 60 ^{0}C pH = 7.0	180 cm^3 H_2/ dm^3d	80.3 cm^3H_2/g	[159]
Rice straw	10% NH_4OH (60 min, 121 °C), then filtered solids treated with 1%H_2SO_4 (50 min, 121°C)	*Thermotoga neapolitana*	Batch	T= 75 ^{0}C pH = 7.5	112,38 cm^3H_2 / dm^3h	2.70 mmol H_2/g straw	[96]
Sugarcane bagasse 20 gCOD/dm^3	0.5% H_2SO_4 (60 min, 121 °C, 1.47bar)	*Clostridium butyricum*	Batch	pH = 5.5 T=37 ^{0}C	1611 cm^3H_2/ dm^3 day	1.73 mol H_2/mol total sugar	[172]
Miscanthus	12% NaOH (70 ^{0} C, 4 h) followed by enzymatic hydrolysis (45 ^{0}C, 72 h, pH 4.8)	*Thermotoga elfii* DSM 9442	Batch	T= 65 ^{0}C pH = 5.6	23.99 cm^3H_2/h	82.2 mmol H_2/dm^3	[173]
Cellulose	Untreated	*Thermotoga. neapolitana*	Batch	T= 80 ^{0}C pH = 7.5	122 cm^3H_2 / dm^3 culture	1.59 mol H_2/mol glucose equivalent	[174]
Cellulose	15% NaOH, 80 ^{0}C, 60 min, neutralization	*Thermotoga neapolitana*	Batch	T= 80 ^{0}C pH = 7.5	454 cm^3H_2 / dm^3 culture	1.22 mol H_2/mol glucose equivalent	[174]
Cellulose	10% H_2SO_4, 80 ^{0}C, 120 min, neutralization	*Thermotoga neapolitana*	Batch	T= 80 ^{0}C pH = 7.5	498 cm^3H_2 / dm^3 culture	0.95 mol H_2/mol glucose equivalent	[174]
Cellulose	Ionic liquid 10 % [C_4mim]Cl (10%, 80 ºC, 6 h, EtOH)	*Thermotoga neapolitana*	Batch	T= 80 ^{0}C pH = 7.5	720.2 cm^3H_2 / dm^3 culture	1.22 mol H_2/mol glucose equivalent	[174]
Sweet sorghum bagasse 10 g sugar/ dm^3	12% NaOH, 70 ºC, 4h and enzymatic hydrolysis with cellulase, 45 FPU/g, 50 °C, 24 h, pH=5.0	*Caldicellulosiruptor saccharolyticus*	Batch	pH=6.8 T=72 ^{0}C	10,6 mmol H_2/ dm^3h	2.6 mol H_2/mol hexose	[155]

4.3. Substrate concentration

A parameter important when developing the fermentation process is substrate concentration. The highest hydrogen yields are usually obtained for diluted substrates, that is 10 g/dm^3 or 1% TS. A substantial increase in substrate concentration usually results in a lowered hydrogen yield. However, in order to obtain larger total volumes of hydrogen it is necessary to use higher concentrations [69].

Wu and Chang [175] fermented sucrose and observed the highest hydrogen yield (2.68 mol H_2/mol sucrose) at a concentration of 10 g/dm^3. The highest hydrogen production rate was obtained after increasing the concentration from 10 to 20 g/dm^3. Xing *et al*. [176] also obtained the highest hydrogen yield (1.84 mol/mol glucose and productivity 4.26 mmol/g dry cell/h at a glucose concentration of 10 g/dm^3.

Liu and Shen [177] obtained the maximum hydrogen yield of 194 cm^3 H_2/g- substrate at a starch concentration of 2 g/dm^3. With an increase in starch concentration, the hydrogen yield decreased with the simultaneous increase in specific hydrogen production rate of 237 cm^3/g-VSS/d at 24 g/cm^3. A further increase in starch concentration results in a decrease in both the hydrogen yield and productivity. This could be caused by a higher concentration of volatile fatty acids although the results[178] reveal that the concentrations of volatile fatty acids and alcohols decreasedabove the optimum starch concentration of 20 g/cm^{3}The effect of substrate concentration on hydrogen production from starch was examined. The investigations were carried out for starch concentrations ranging from 15 to 70 g/dm^3. The highest hydrogen yield (2.82 mol H_2/mol glucose) was found to occur at 20 g/dm^3 of starch. Lin *et al*. [215] also investigated hydrogen yield and production rate as a function of substrate concentration and obtained the best results at a concentration of 20 g/dm^3. The substrate was xylose at concentrations ranging from 10 to 100 g/dm^3. At a xylose content of 100 g/dm^3 medium, the hydrogen yield drops almost to zero.

In investigations by Zhang *et al.* [179], the maximum hydrogen yield of 143.79 cm^3 H_2/ g TVS was obtained for a feedstock of pretreated cornstalk waste at a concentration of 15 g biomass per liter. The use of higher concentrations (20, 25, 40 g/ dm^3) resulted in a slight decrease in the yield; *e.g.,* at 40 g/dm^3 the yield was 122.8 cm^3H_2/ g TVS. In contrast, Wu and Chang [121] obtained the highest hydrogen yield and production rate (3.48 mol H_2/mol sucrose and 1.65 dm^3 H_2/ dm^3/h) at a sucrose concentration of 30 g/dm^3, with a decrease in these parameters already at 40 g/dm^3. The substrate concentration also has an important effect on liquid products of fermentation. The effect of substrate concentration in rice winery wastewater was investigated [180] and observed that when the substrate concentration was increased from 14 to 36 g COD/dm^3, the concentrations of acetic and butyric acids both decreased from 37% and 28% to 26% and 20%, respectively, while the concentrations of ethanol and propionic acid increased from 17% and 18%, respectively, to 24% and 30%, respectively.

An impact of initial concentration of organic kitchen waste on both the hydrogen yield and the resulting concentration of organic acids was investigated [181]. Following composition of organic waste was identified: vegetables 44%, fruits 30%, others (including coffee, tea, rice, pasta) 25%. The highest hydrogen yield (76.1 cm^3H_2/g VS) was noted for

initial concentration of 14.3 gVS/dm^3. The highest concentration of volatile fatty acids was obtained for initial concentration of 48.2 VS/dm^3.

Eker et al. [182] investigated an influence of monosugars concentrations on dark fermentation of acidic hydrolysates of waste paper. The highest hydrogen productivity (0.51 cm^{3}H$_2$/g biomass/h) was obtained for initial concentration of the sugars solution of 18.9 g/dm^3. The hydrogen productivity decreases when the concentration of sugars increases above the given value due to increased formation of volatile fatty acids. The inhibition effect on hydrogen production of increased concentration of volatile fatty acids was identified [183].

5. Factors affecting dark fermentation hydrogen yield

Hydrogen can be produced using a number of different processes. Thermochemical processes use heat and chemical reactions to release hydrogen from organic materials such as fossil fuels and biomass. Water (H_2O) can be split into hydrogen (H_2) and oxygen (O_2) using electrolysis or solar energy. Microorganisms such as bacteria and algae can produce hydrogen through biological processes.

An improvement of hydrogen production yield in dark fermentation is the key problem determining the possibility of adaptation of this process to industrial conditions. In addition to previously described effects of kind of substrates, their structure, composition and concentration on the hydrogen production yield, a number of other factors also affect this process, including:

a. Kind of microorganisms used, *i.e.* pure or mixed bacterial cultures, method of preparation of inoculum, composition of a medium.

b. Mode and conditions of dark fermentation process which control metabolism of microorganisms, *i.e.* temperature, initial pH or fixed pH, hydraulic retention time (HRT) and partial pressure of hydrogen.

c. Type of reactor used and its mode of operation (continuous, batch, semi-batch).

Anaerobic or facultative anaerobic bacteria are able to generate biohydrogen via dark fermentation [184]. To estimate the theoretical yields of biohydrogen, the glucose biotransformation reaction is widely accepted as a reference. A limitation of optimization of the fermentation process is thermodynamics of metabolic processes of microorganisms, which in addition to hydrogen also produce other metabolic products during culture growth, such as acetic acid, ethanol, butanol and butyric acid. The composition of fermentation products, including ratio of their concentrations, depends on the kind of microorganism, pH, partial pressure of hydrogen as well as the degree of conversion of a culture medium [185]. The products can be partially metabolized and included in the metabolic pathways of microorganisms but at a certain limiting concentration they become toxic to the organisms producing them, *e.g.* ethanol being toxic to yeast producing it. The main obstacle in obtaining high hydrogen yields during fermentation of organic waste is insufficient degree of decomposition of complex substrates. Furthermore, the need for removal of fermentation products is an important problem in maintaining dark fermentation on an industrial scale at a constant production level, especially in a continuous process.

5.1. Bacteria used in dark fermentation

In fermentation-based systems, microorganisms, such as bacteria, break down organic matter to produce hydrogen. The organic matter can be refined sugars, raw biomass ~~sources~~ such as corn stover, and even wastewater. Because no light is required, these methods are sometimes called the dark fermentation methods. Microorganisms capable of hydrogen

production occur in nature as single strains or mixtures of various species. Depending upon the kind of substrate, in research on dark fermentation both mixed cultures of bacteria originating from sewage sludge, digesters, manure, etc. as well as specially isolated strains of anaerobic and relatively anaerobic bacteria are used. Utilization of mixed cultures does not require aseptic conditions which makes their handling easier. They are also less affected by changes in kind and composition of a substrate. Hydrogen producing bacteria occurring in nature do not exist in isolation. They often live in the presence of other microorganisms, mainly archaea, consuming hydrogen in their metabolic processes. This requires special pretreatment of an inoculum to partly or completely inactivate the microorganisms responsible for hydrogen consumption. The process will be discussed in more details in section 5.b. Bacteria participating in dark fermentation processes are anaerobic and they are classified in two ways. The first classification divides microorganisms according to their sensitivity to oxygen (strict anaerobes and facultative anaerobes). The second classification is based on temperature ranges at which their growth rate and activity are the highest (*e.g.* psychrophiles, mesophiles and thermophiles).

5.1.1. Obligate anaerobes

This group includes microorganisms which require strictly anaerobic conditions. These are anaerobes *Clostridia, Metylotrophs, Methanogenic bacteria, Archaea,* and *Rumen bacteria*) [186]. In practice, the most widely used obligate anaerobes are from a genus *Clostridium,* which includes, among others, the following species: *C. butyricum, C. beijerinckii*, *C. welchii, C. thermolacticum, C. thermocellum, C. paraputrificum C. pasteurianum, C. beijerincki, Clostridium scatologenes, C. acetobutyricum,* and *C. bifermentants.* Clostridia produce hydrogen during the log growth phase. During the stationary phase, the metabolism is shifted towards the production of liquid organic compounds, especially volatile fatty acids [5]. An important characteristic feature is the ability to form non-vegetative forms, so called spores, which allows bacteria to survive difficult conditions, such as temperature extremes, low or high pH or the presence of toxic chemicals in the bacterial environment [186]. As a result of this property, they can be separated from the bacteria that do not form spores by inoculum pretreatment. Clostridia are capable of efficient conversion of a wide variety of carbohydrate substrates [186]. Compared to facultative anaerobes, they produce hydrogen more efficiently. A fermentation characteristic of bacteria from a genus *Clostridium* is butyric and acetic acid fermentation. Metabolism of *Clostridium* bacteria allows formation of the end products which can lower the hydrogen yield from the theoretical maximum of 4 mol/mol glucose when acetic acid is the only organic end product of fermentation (equation (3)) to two moles in the case of butyric acid fermentation (equation (4)). In practice, fermentation of carbohydrates by *Clostridium* bacteria results in formation of a mixture of acetic and butyric acids and depends on substrate concentration. Kim *et al*. [187] observed that lowering the concentration of sucrose used in fermentation from 30 to 10 g COD/L resulted in an increase in concentration of acetic acid in the post-reaction mixture from 5.1% to 40 % with the simultaneous decrease in butyric acid content from 47.1% do 28.9 %. Such an effect should be accompanied by an increase in

concentration of hydrogen produced but this is not the case, especially when using mixed cultures due to utilization by bacteria of a fraction of generated hydrogen to produce acetic acid (equation (5)). The mole ratio of butyric and acetic acid can be an indicator of metabolism of a substrate and the hydrogen production yield.

$$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 2HCO_3^- + 4H_2 + 4H^+ \quad (3)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2COO^- + 2HCO_3^- + 2H_2 + 3H^+ \quad (4)$$

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_3COO^- + 4H_2O \quad (5)$$

It follows from the stoichiometry of equation (4) that the maximum hydrogen yield for a B/A ratio of 1.5 is 2.5 mol H_2/mol glucose. In practice, a monoculture of *Clostridium sp.* can produce from od 1.61 to 2.36 moles of hydrogen per mole of glucose [80].
Even though a wide range of microorganisms (*Methylotrophs, Rumen bacteria, Methanogenic bacteria, Archaea, E. coli, Enterobacter, Citrobacter, Alcaligenes, Bacillus, Clostridium sp., Clostridium butyricum, C. acetobutyricum, C. beijerinckii, C. thermolacticum, C. tyrobutyricum, C. thermocellum,C. paraputrificum, Enterobacter aerogenes, E. cloacae, Caldicellulosiruptor saccharolyticus, Thermoanaerobacterium sp., T. thermosaccharolyticum, Thermotoga sp., T. maritima, T. elfii* [70,188–191] is capable to produce hydrogen via dark fermentation, mixed consortia seem to be a good alternative. Mixed consortia under strictly determined conditions [192,193] allow for a broad choice of feedstock, including a variety of natural sources, anaerobically digested sludge, animal manure, sewage sludge, compost and soil. Another genus of obligate anaerobes is *Ruminococcus albus*. During metabolism of carbohydrates these bacteria produce acetic acid, ethanol, formic acid, hydrogen and carbon dioxide. They are capable of direct hydrolysis of cellulose. One of few examples of utilization of *R. albus* was published by Iannotti *et al.* [194]. The authors fermented glucose (100 mol) in a continuous mode and obtained 237 moles of hydrogen, 74 moles of acetic acid and 65 moles of ethanol. In direct hydrogen fermentation, the microbes produce the hydrogen themselves. These microbes can break down complex molecules through many different pathways, and the by-products of some of the pathways can be combined by enzymes to produce hydrogen.

5.1.2. Facultative anaerobes

Facultative anaerobes are organisms that produce ATP by aerobic respiration if oxygen is present but are capable of switching to fermentation if oxygen is absent. Their resistance to the presence of oxygen makes them easier to work with during dark fermentation. In addition, a high partial pressure in the reactor does not affect the fermentation yield. This group includes *Enterobacteriaceae*, a large family of Gram-negative bacteria which do not form spores [195]. *Enterobacteriaceae* include *Arsenophonus, Branneria, Buchnera, Budvicia, Buttiauxella, Cedecea, Citrobacter, Cronobacter, Dickeya, Edwardsiella, Enterobacter, Erwinia, Escherichia, Ewingella, Hafnia, Klebsiella, Kluyvera, Coserella, Leclercia, Leminorella, Moellerella, Morganella, Obesumbacterium, Pantoea,*

Pectobacterium, Photorhabdus, Plesiomonas, Pragia, Proteus, Providencia, Rahnella, Raoutella, Salmonella, Samsonia, Serratia, Shigella, Sodalis, Tatumella, Thorsellia, Trabulsiella, Wiglesworhtia, Xenorhabdus, Yersinia, and *Yokenella*. *Enterobacteriaceae* are capable of fermentation, including dark fermentation resulting in the production of hydrogen from 2,3-butanediol and organic acids. However, hydrogen and carbon dioxide are formed through decomposition of formic acid which makes the hydrogen production yield lower than in the case of using *Clostridium* [5].

The best known metabolic pathway in this group of microorganisms is glucose metabolism resulting in formation of hydrogen. This pathway was studied for *E. coli.* The maximum theoretical hydrogen yield is 2 moles of H_2 per mole of glucose. The other final products of glucose metabolism are butyric, lactic, and formic acids, as well as ethanol in small amounts. The formation of acetic acid generates ATP [69].

Aerobic bacteria *Bacillus* or *Alcaligenes* can also be used for the production of hydrogen by fermentation; however, at this time there is little interest in this method.

Bacteria from a genus *Bacillus*, similarly to *Clostridium*, are Gram-positive rod-shaped bacteria, able to produce hydrogen by fermentationand a mixture of organic acids in fermentation broth. Most commonly used bacteria in for hydrogen production by dark fermentation include *Bacillus macerans (acetoethylicus), Bacillus cloacae, Bacillus macerans, Bacillus licheniformis,*and *Bacillus polymyxa.*

5.2. Mixed bacterial cultures. Methods of preparation and enrichment of inoculum

It is possible to produce hydrogen from various substrates during dark fermentation using both pure and mixed bacterial cultures. However, the use of a monoculture to produce hydrogen is of research significance, despite higher yields as demonstrated by numerous investigations carried out mainly with Gram-positive *Clostridium* and Gram-negative *Enterobacteriaceae.* Although many investigations have been carried out in a batch mode using glucose or other simple carbohydrates as a substrate and bacterial monocultures, in practice fermentation has to be performed in a continuous mode using mixed cultures on a substrate being organic waste or pretreated lignocellulosic biomass. Under such conditions, sterilization of the substrate is too expensive and energy-consuming. Pure bacterial cultures are sensitive to contamination with other culture, including bacteria consuming hydrogen in their metabolic processes. Utilization of mixed cultures is particularly important in hydrogen production by fermentation on an industrial scale. Mixed bacterial cultures capable of hydrogen production abundantly occur in municipal sewage, composts and organic waste from which they can be isolated. Advantages of this approach include no need for aseptic environment, which is necessary for monocultures, ease of control of the process and a wider variety of available substrates [196,197]. On the downside, mixed cultures in addition to hydrogen-producing microorganisms also contain bacteria consuming hydrogen in their metabolic processes. These are mainly methanogens and homoacetogens as well as bacteria reducing sulfur, producing propionic acid, and reducing nitrates [28,196,198–203]. In order to obtain high hydrogen yields, it is necessary to remove them from the culture or to inactivate

them so as to limit the processes in which hydrogen is the substrate. There are several methods of inoculum pretreatment which can be classified with respect to their implementation as physical or chemical.

Physical methods of inoculum pretreatment include heat-shock, aeration, microwave irradiation, ultrasonication, treatment with infrared, ultraviolet and gamma radiation, freezing and thawing, while chemical methods make use of acids or bases as well as growth inhibitors of microorganisms consuming hydrogen, such as 2-bromoethanesulfonate (BES), 2-bromoethanesulfonic acid (BESA), iodopropane, chloroform, higher fatty acids, especially linoleic acid, or acetylene. These processes aim at elimination of bacteria sensitive to extreme conditions, which do not form endospores, such as hydrogentrophs and especially methanogens [136]. None of the physical methods mentioned above is universal, ensuring a high hydrogen production yield. Inocula originating from different sources require different pretreatment methods, which was the subject of numerous investigations [204–210]. Pretreatment methods also inhibit strains of bacteria not forming spores and producing hydrogen, such as *Enterobacter*, resulting in a lower hydrogen yield compared with inoculum that had not been pretreated [201].

A. The heat-shock method is most common and involves treating the culture with a high temperature. The methods of heat pretreatment of inoculum can be carried out in several ways, *i.e.* by heating, boiling, autoclaving or drying. High temperature, usually 80-121 °C, kills non spore forming bacteria. As a result, spores capable of forming bacteria survive, especially *Clostridium* and *Bacillus*, but also acetic acid bacteria, propionic acid bacteria and lactic acid bacteria. On the other hand, such conditions kill methanogens as well as not forming spores *Enterobacter* or *Citrobacter*, which produce hydrogen. A typical example of using heat-shock as an inoculum pretreatment method is presented in a paper by Wang and Wan [211]. Digested sludge containing microorganisms is heated at 100 °C for 15 min. Thus prepared inoculum wass used in dark fermentation and the maximum hydrogen production potential obtained was 215.4 cm^3/g glucose, which was much higher than that for untreated inoculum (65.6 cm^3/g glucose). The conversion of the substrate to products was also much higher (by 10%). A more complicated method was used by Logan *et al.* [90] who heat-shocked inoculum taken from soils used for tomato plants by drying 1-cm thick sample layers at 104 °C in an aluminum pan for 2 h. Samples were then sieved using a #20 mesh (850 μm) and stored at 4 °C. Thus prepared samples were used as the reactor inoculum for dark fermentation. The hydrogen yield obtained was 0.92 mol H_2/ mol glucose (125 cm^3/g glucose) and the conversion of the substrate amounted to 23%. Another modification of the heat-shock method is described [129], the hydrogen generating microflora was isolated from the cow dung, the pH was adjusted to 5 and after 3 h was subjected to heat treatment at 105 °C for 2 h.

Heat-shock aims at eliminating microorganisms that consume hydrogen, thus enriching inoculum in the strains producing hydrogen which results in a higher yield of dark fermentation. A significant number of the investigations making use of heat-shock results in an increase of hydrogen yield which would indicate a selective enrichment of inoculum in hydrogen-producing bacteria [200,203]. However, not only hydrogen producing bacteria (HPB) are capable of surviving heat-shock. Bacterial strains responsible for the production of volatile fatty acids and those taking part in reduction of sulfates are also able to survive this

type of treatment [212]. Zhu and Béland observed a decrease in the hydrogen production yield with the simultaneous increase in concentration of butyric acid after heat-shocking an inoculum compared to untreated inoculum [213]. A digested sludge was boiled for 20 min, which lowered the fermentation yield from 5.17 to 2.59 mmol H_2/mol sucrose. This pretreatment method resulted in a complete repression of methanogenic activity.

B. Freezing and thawing is another inoculum pretreatment method playing the same role as heat-shock. It involves freezing inoculum and maintaining it at temperatures between -25 ºC to -10 ºC, followed by thawing and incubating at 20 – 30 ºC.
Even though the method is considered to enrich inoculum mainly in hydrogen producing bacteria, the hydrogen yield reported in a number of studies is not impressive (Table 8). Liu *et al.* [206] observed that *Lactobacillus* sp. is resistant to this type of treatment and since it is known to have an inhibitory effect on HPB, the hydrogen production was lowered by 15% compared with the untreated inoculum.
The freezing-thawing method was also used in paper [214]. Kotay and Das subjected sewage sludge to two freezing-thawing cycles. The inoculum was frozen and stored at -20 °C for 6 hours and then heated to 25 °C and the microorganisms incubated for 6 h. Thus pretreated inoculum was used for dark fermentation resulting in the hydrogen yield of 7 cm^3 H_2/g COD. No methanogenesis was observed in contrast with the fermentation carried out without inoculum pretreatment.

C. Another well studied pretreatment method is aeration. The principle of the method relies on the use of oxygen present in the air to eliminate from mixed cultures the bacteria sensitive to oxygen, including those responsible for hydrogen consumption. It is assumed that methanogens are obligate anaerobes; therefore, oxygen supplied with the air is toxic to them. Inhibition of methanogens results in a higher yield of produced hydrogen [196]. In spite of several studies [211,215] reporting that the method brings about a substantial increase in hydrogen yield, the results of research on inoculum aeration obtained by a number of research groups [149,151,153] are ambiguous. This method is controversial, especially considering the fact that also hydrogen-producing bacteria from the genus *Clostridium* are obligate anaerobes and hence their growth in the presence of oxygen is inhibited [28]. In addition, *Lactobacillus* sp. are resistant to oxygen and their inhibition of the growth of HBP affects the yield of produced hydrogen. One of the examples of lowered hydrogen yield are the results reported by Zhu and Béland [213]. The authors prepared inoculum by 30-min aeration of a sample taken from digested wastewater sludge containing mixed cultures. Following 12-h incubation, the bacteria were transferred to the sample bottle. The hydrogen yield was 4.84 mol H_2/mol sucrose which was worse by 0.33 mol/mol sucrose than the yield of hydrogen obtained for the untreated inoculum.

D. Acid and alkaline treatment of inoculum. The growth of methanogens and methanogenesis processes can be inhibited and an increase in hydrogen production yield accomplished by pretreating inoculum with acids or bases may be achieved. The treatment involves adjustment of pH of inoculum to a value at which microorganisms cannot survive.

For an acidic medium it is a pH in the order of 2 to 4 while for an alkaline medium it ranges from 10 to 12. Such conditions should eliminate microorganisms not forming endospores, especially methanogens, for which the optimum pH ranges from 6.7 to 7.5 [216]. However, both the propionic and acetic acid bacteria are resistant to acidic and basic media. For example, Ren *et al.* [209] observed an increase in concentration of propionic acid during fermentation using an acid-treated inoculum. Optimum conditions for acid and alkaline pretreatment have been studied in a number of papers. For acid pretreatment, sulfuric, hydrochloric or nitric acid at concentrations ranging from 0.1 to 6 M were used most often[92,204,217] whereas in the case of bases it is generally NaOH at a concentration from 1 to 6 M [121,215]. Acid and alkaline pretreatment is used to prepare inoculum and later in a digester to maintain hydrogenic properties of bacteria. The optimum pH depends on the kind of bacteria occurring in a culture. Chaganti *et al.* [204] adjusted pH of anaerobic inoculum from the ethanol manufacturing facility, containing *Archea* as well as mixed cultures of bacteria *Bacteroidaceae, Clostridiaceae, Enterobacteriaceae, Geobacteriaceae,* and *Methylobacteriaceae*, *Baccilaceae,* to 3.0 using 2.0 M HCl, followed by incubation at 37 °C for 24 hours. The hydrogen yield obtained in dark fermentation of glucose containing broth was 1.5 mol H_2/mol glucose which was twice as high as for the untreated inoculum. Furthermore, no methane was found in the biogas in contrast with control (0.4 mol CH_4/mol glucose. Mu *et al*. [92] also used acid- and alkaline-treatment in their investigations on suppression of methanogenesis in mixed cultures and enrichment of hydrogen-producing inoculum. For the acid-treatment, the anaerobic sludge from the soybean-processing wastewater treatment plant was adjusted to pH 3-4 with 0.1 N HCl for 24 h, and then was adjusted back to pH 7 with 0.1 N NaOH. The hydrogen yield for the acid-treated inoculum was 1.3
mol H_2/mol glucose. Cai *et al*. [102] examined the effectiveness of alkaline pretreatment by adjusting the pH of inoculum from the sewage sludge of the aeration tank of a municipal wastewater treatment plant to 12 using 4.0 M NaOH, followed by thermostatting at 25 °C for 24 h. The hydrogen fermentation was carried out at pH 11 using the sewage sludge as the substrate. It was found that dark fermentation using alkaline-treated inoculum provided the highest hydrogen yield (16.59 cm^3 H_2/g substrate), which is a substantial improvement compared to untreated inoculum (9.13 cm^3 H_2/g substrate). In the latter case, a decrease in hydrogen yield was observed after *ca.* 28 h due to hydrogen consumption by bacteria capable of utilizing hydrogen in their metabolic pathways. However, these are not methanogens since methane was absent in the generated biogas.

E. The next inoculum pretreatment method is chemical treatment. Chemical compounds are able to block metabolic pathways of methanogenic bacteria selectively. Compounds such as 2-bromoethanesulfonate (2-BES) or 2-bromoethanesulfonic acid (2-BESA) are believed to be chemical analogs of coenzyme M required for methyl-transfer reactions. The complex methyl-coenzyme M is reduced by its reductase to methane. The inhibitor reacts with the methyl group binding it instead of CoM which prevents the formation of methane. In practice, either inhibition or a substantial reduction of methane formation is observed along with an improvement in the hydrogen production yield [218,219].

However, the results of investigations carried out by various research groups [145,151,153] are not unambiguous. In addition an increase in hydrogen yield was observed in several examples of chemical pretreatment, whereas there are others, which suggest that these compounds are not effective in improving hydrogen production yield as shown in Table 8.,

Pendyala *et al.* [207] investigated inoculum of anaerobic mixed cultures pretreatment with BESA and linoleic acid and found the presence of acetic acid bacteria *Eubacterium aerofaciens,* sulfate reducing bacteria *Desulfuromonas acetoxidans* as well as typical methanogens *Methylophilus methylotrophus* and others consuming hydrogen in the fermentation broth.

Zhu and Béland [213] examined various pretreatment methods, including addition of BESA and inoculum originating from digested anaerobic sludge from a wastewater treatment plant. The BESA pretreatment was carried out by adding the inhibitor to the sludge at a concentration of 10 mmol and maintaining at room temperature for 30 min. This pretreatment method did not improve the hydrogen yield but completely inhibited the activity of methanogenic microorganisms. The hydrogen production yield with sucrose as a substrate was 5.28 mol H_2/mol sucrose [213].

Chloroform is also used for inoculum pretreatment. Its mechanism of action is similar to that of BES and BESA. Chloroform has the ability to limit the inhibiting activity of propionic acid bacteria [215]. Thus, a number of investigations testing the effectiveness of chloroform at various concentrations, usually from 0.005% to 5% over 17-24 h was carried out [210,215,217]. However, also in this case the results are ambiguous. In addition to the results of investigations demonstrating an increase in hydrogen production, there are others reporting lowered yields (Table 8). Some researchers [210,217] suggest that the reason for lowered yields is the side effect of chloroform resulting in inhibition of hydrogen producing bacteria.

Hu *et al*. [217] demonstrated that an effective method of pretreatment of methanogenic granules is the use of chloroform. The authors varied the concentration of chloroform from 0.05% to 2.5%. By using a chloroform concentration of 0.05% it was possible to decrease the amount of methane released during fermentation from 75.76 to 0.04 cm^3 CH_4/g glucose. At the same time, the volume of hydrogen produced was 135.09 cm^3 H_2/g glucose, which is a good result considering the fact that the amount of hydrogen produced for control was 0.42 cm^3 H_2/g glucose. Higher concentrations of the inhibitor have a negative effect on fermentation yield; complete inhibition of hydrogen producing bacteria was observed for 2.5 % (v/v) concentration of chloroform.

Higher fatty acids, such as linoleic, caprylic, caproic, palmitic, lauric, oleic and myristic [157, 141], are used in inoculum pretreatment. Their inhibitory activity involves hindering bacterial growth and activity of methanogens by adsorption of fatty acids onto the cell membrane. Among the fatty acids used for this purpose, linoleic acid was the one studied most. Chaganti *et al*. [204] treated anaerobic inoculum containing Archea and mixed cultures of *Bacteroidaceae, Bacillaceae; Clostridiaceae, Enterobacteriaceae, Geobacteriaceae,* and *Methylobacteriaceae* with linoleic acid at 37 °C for 24 hours. After treatment, a pH was adjusted to 5.5 using 1 M HCI or 1 M NaOH. The

hydrogen production yield using glucose as the substrate was 1.70 mol H_2 /mol glucose, which was higher than in the case of heat shock or acid or alkaline pretreatment.

F. Other methods. Many more inoculum pretreatment methods than those discussed here have been reported. A comprehensive review on this topic was published in 2015 [221]. The other inoculum pretreatment methods included: ultrasounds [222,223], gamma irradiation [224], infrared radiation [225] as well as chemical compounds such as hydrocarbons and their chlorinated derivatives: acetylene, iodopropane, ethylene, cyclopentadiene, cycloheptatriene, benzene, toluene, isoprene, 1-hexyne, ethane, methyl chloride and fluoride [219,226,227], and nitrates [205].

Inoculum pretreatment methods involving application of several procedures in succession, including heat-shock, aeration, chemical, acid and alkaline and ultrasounds, have also been tested [28,218].

Depending on the pretreatment method, inoculum will contain live bacteria with an enhanced ability for lactic acid, acetic acid or ethanol fermentation. However, the treatment of the next generations of bacteria can be a problem. The solution to this problem is likely to use genetic engineering to create mutants with blocked selected metabolic pathways. A promising trend involves molecular biology methods applied to search, selection, isolation and identification of new hydrogen producing bacteria from natural environments.

Biomass pretreatment methods affect the accuracy of the process, as achieving high degrees of solubilization of hemicelluloses and/or lignin unavoidably leads to degradation of the solubilized fragments as a result of the severe conditions they are exposed to. The amount and nature of the formed degradation products, many of which are inhibitory to downstream biocatalytic processes, is directly related to the pretreatment method and conditions.

Table 8. Compilation of parameters describing fermentation to hydrogen using mixed cultures after inoculum pretreatment.

Type of pretreatment/Conditions	Substrate	Organism	Reactor/ Mode	pH/ Temp.	Hydrogen productivity		Hydrogen yield		H_2	Ref.
Untreated		Sludge from a	Batch		4.04	dm^3/	0.20	mol H_2/	12.21 %	[206]
Freezing -25 °C, 24 h	Glucose	beach in		7.2	1.95	dm^3	0.17	mol	21.45 %	
Acid pH = 3, 1 M HCl, 12 h, T = room	20 g/dm^3	Tianjin		37 °C	3.48		0.86	glucose	61.15 %	
Alkaline pH = 12, 1 M NaOH, T = room					2.20		0.11		11.90 %	
Heat-shock T = 100 °C, 30 min					3.04		0.41		32.91 %	
Untreated	POME	Anaerobic	Batch	5.5	1.52	mmol/	0.14	mol H_2/	5 %	
BESA 10 mmol, 30 min	80 g COD/	digested		60 °C	3.56	dm^3h	0.7	mol	22 %	[208]
Acid pH = 3-4, 1 M HCl, 12 h, T = room	dm^3	sludge from a			3.0		0.43	hexose	7 %	
Alkaline pH = 12, 1 M NaOH, 24 h, T = room		biogas reactor			1.74		0.3		15 %	
Heat-shock T=100 °C, 1h					2.6		1.1		40 %	
Untreated	Sucrose	Marine mixed	Batch	7	1.5	mmol/	84	cm^3 H_2/	-	[205]
Freezing -25 °C, 24 h	20 g/dm^3	microflora		35 °C	19.3	dm^3h	850	dm^3		
Acid pH = 3, 1 M HCl, 30 min, T = room					15		659			
Alkaline pH = 10, 1 M NaOH, T = room					9.1		379			
Heat-shock T=100 °C, 30 min					27.5		1225			
Untreated	Glucose	Anaerobic	Batch	8-8.2	N.D.		1.54	Meq /	-	[204]
LA 2000 mg/L, 24 h, T = 37 °C	100 g/dm^3	inoculum		37 °C			3.40	mol		
Acid pH = 3, 2 M HCl, 24 h, T = 37 °C		from ethanol					3	hexose		
Alkaline pH = 11, 3 M NaOH, 24 h, T = 37 °C		manufacturing					2.80			
Heat-shock T=90 °C, 30 min		facility					2.84			
Untreated	Glucose	Digested	Batch	7	45.1	cm^3/h	65.7		-	
Aeration 24 h	1 g/dm^3	sludge from		35 °C	45.7		80.2			[211]
Chloroform 2% 24 h		digester of			3.2		53	cm^3 H_2/g		
Acid pH = 3, 1 M HCl, 24 h		WWTP			86		96.8	glucose		
Alkaline pH = 10, 1 M NaOH, 24 h					94.8		125.9			
Heat-shock T = 100 °C, 15 min					120.4		215.4			
Untreated	POME	Sludge from	Batch	5.5	N.D.		0.12	mmol	-	[121]
Freezing -10 °C, 24 h		anaerobic		35 °C			0.19	H_2/g		
Chloroform 0.1% 24 h, T = room		treatment					0.23	COD		
Acid pH = 3, 6 N HCl, 24 h		plant					0.32			
Alkaline pH = 12, 6 N NaOH, 24 h							0.135			
Heat-shock T = 100 °C, 1 h							0.41			

Untreated	Sucrose	Mixed	Batch	7	4.31	mmol	5.17	mol H_2/		
BESA 10 mmol, 30 min, T = room	10 g/dm^3	cultures from		35 °C	4.4		5.28	mol	-	[213]
Iodopropane 10 mmol, 30 min, T = room		sludge from			4.7		5.64	hexose		
Aeration 30 min		anaerobic			4.03		4.84			
Acid pH = 3, 1 N HCl, 30 min, T = room		digester of			2.58		3.10			
Alkaline pH = 10, 2 N NaOH, 30 min, T = room		WWTP			1.2		1.44			
Heat-shock T=100 °C, 30 min					2.16		2.59			
Untreated	Glucose	Anaerobic	Batch	6.0	N.D.		0.14	mol H_2/	N.D.	[207]
BESA 50 mM	1 g/dm^3	cultures from					1.23	mol		
LA 2000 mg/L		industrial and					1.17	hexose		
Acid pH = 3, 2 N HCl, 24 h,		municipal					1.10			
Alkaline pH = 12, 3 M NaOH, 24 h		WWTP					0.83			
Heat-shock T = 105 °C, 45 min							0.90			
Untreated	Glucose	Activated	Batch	7	2.54	cm^3/h	0.38	mol H_2/	N.D.	[215]
BES 10 mM 24 h, T = room	10 g/dm^3	sludge from		35 °C	2.69		0.33	mol		
Chloroform 1% 24 h, T = room		municipal			9.94		0.61	hexose		
Acid pH = 3, 1 N HCl, 24 h		WWTP			22.26		1.51			
Alkaline pH = 10, 1 M NaOH, 24 h					20.73		1.34			
Heat-shock, T=95 °C, 30 min					21.02		0.9			
Aeration 24 h					15.16		0.7			

BES – 2-bromoethanosulfonate; BESA – 2-bromoethanesulfonic acid ; COD – chemical oxygen demand; LA – linoleic acid; POME – palm oil mill effluent ; WWTP –wastewater treatment plant ; meq – miliequivalent; N.D. – not defined

5.3. Temperature

Fermentation temperature is another important parameter affecting the growth rate of bacteria and the efficiency of conversion of the substrates to hydrogen. Bacteria can be classified into several temperature groups for which the maximum yield of produced hydrogen falls into temperature ranges suitable for the growth conditions of psychrophiles (0 °C-25 °C), mesophiles (25 °C-45 °C), thermophiles (45 °C-65 °C), extreme thermophiles (65 °C-80 °C) and hyperthermophiles (above 80 °C) [228].

Selection of the optimum temperature depends on the kind of bacteria used during fermentation, both for pure cultures and mixtures. The activity of specific enzymes responsible for hydrogen fermentation depends upon the temperature for which it can take an optimum value. A temperature lower or higher than optimal decreases the activity of enzymes. Hence, the optimum fermentation temperature depends on the type of bacteria and the kind of substrate used. Readily biodegradable substrates are converted most efficiently under mesophilic conditions [82]. A lag time is shorter for mesophilic conditions which is due to a smaller population of thermophiles compared with mesophiles in the inoculum [229]. In a study by Pakarinen *et al.* [230] it was found the highest hydrogen yield was obtained for the process carried out at 70 °C but the time of reaching the maximum yield was longer than in the experiments performed at 35 °C and 50 °C. It is believed thermophilic and extreme thermophilic conditions work better with the substrates which undergo hydrolysis during fermentation. Higher temperatures increase the activity of the enzymes responsible for hydrolysis [99,197,231]. It is also believed [28] that the higher yield under thermophilic conditions can be attributed to a lower solubility of gases in water. High concentration of a gas can inhibit bacterial growth. However, using higher temperatures and mixed cultures can result in impoverishment of diversity of bacterial strains and thus a less complete degradation of substrates. This is particularly detrimental during fermentation of wastewater and wastes rich in organic matter of various origin and chemical structures [232]. Another limitation in using high temperatures are economic aspects [233]. The use of thermophilic conditions can be economically justified due to higher hydrogen yields and usage of more complex substrates [234] but extreme thermophilic and hyperthermophilic conditions require supplying considerable quantities of energy which lowers profitability of the process. Azbar *et al*. [76] investigated the effect of mesophilic and thermophilic conditions on the yield of hydrogen and organic acids by fermenting cheese whey wastewater as the substrate. Conversion of the substrate to hydrogen at 55 °C was lower than at 35 °C, being equal to 8.1 mmol H_2/g COD and 9.2 mmol H_2/g COD, respectively. Severalfold higher concentrations of volatile fatty acids were observed under thermophilic conditions. Zhang and Shen [235] reported that in mixed cultures of *Clostridium pasteuranium* the production of hydrogen ceased when the temperature reached 45 °C. A temperature of 35 °C was found to be optimal for the production of hydrogen. On the other hand, Yokoyama *et al*. [236] found that within the range of applied temperatures 37-85 °C there are two maxima of hydrogen production: at 60 °C the hydrogen yield is 392 cm^3 H_2/dm^3 substrate while at 75 °C the yield amounts to 248 cm^3 H_2/ dm^3 substrate. At 85 °C the fermentation process does not occur. The production of methane

was observed at temperatures 37 °C and 50 °C. A few studies on dark fermentation have been reported under psychrophilic conditions (*ca.* 4 °C to 20 °C) [237,238].

Table 9. Compilation of parameters of hydrogen fermentation at optimum temperature.

Substrate	Type of microorganisms	Reactor/Mode	pH	Temperature range [^{o}C]	Optimum temperature [^{o}C]	Hydrogen productivity	Hydrogen yield	H_2 in biogas	Ref.
Cow waste slurry	Mixed cultures	Fermentor/ Batch	6.5	37-75	60	-	743 cm^3H_2/kg-cow dung	41%	[236]
Rice winery wastewater	Mixed cultures from WWTP	USAB	5.5	20-55	55	9.3 dm^3H_2/VSS/d	1.9 mol H_2/mol hexose	-	[180]
Glucose 6.7 g/dm^3	Mixed cultures	Fermentor/ Batch	7	20-55	40	29 m dm^3/h	275.1 cm^3/g glucose	-	[198]
Glucose 1%	*Bacillus coagulans* IIT-BT S1	Fermentor/ Batch	6.0	28-40	37	110 cm^3H_2/ dm^3/h		80%	[239]
Cellulose 5 g/ dm^3	*T.thermosaccharolyticum*	Fermentor/ Batch	7	50-70	55	12.08 mmol/h	2.17 mol H_2/ mol glucose		[96]
Organic waste	Mixed cultures	Semi-continuous	6.4	37-55	55	-	360 cm^3H_2/gVS	58%	[197]
Sucrose 10 g/ dm^3	Mixed cultures	Fermentor/ Batch	6.25	40-80	60	12.12 mmolH_2/ dm^3/h	2.53 mol/mol hexose	-	[240]
Grass silage	Mixed cultures from cow manure	Fermentor/ Batch	6	35, 55, 70	70	-	16 cm^3 H_2/g VS	-	[230]

WWTP – wastewater treatment plant; USAB – upflow anaerobic sludge blanket reactor; VS – volatile solids

5.4. pH

Concentration of hydronium ions plays an important role in dark fermentation processes since it affects the hydrogen production yield. The pH value directly impacts the activity of various strains occurring in a mixed culture, the metabolic pathways of microorganisms as well as their morphology and cell structure [241]. All enzymes participating in metabolic processes of bacteria are active in a certain pH range, reaching maximum activity at the optimum pH value [242]. This includes the enzyme hydrogenase. For mixed bacterial cultures a low pH inhibits methanogenic microorganisms consuming hydrogen. The control of pH and maintaining it at a constant, optimal level is also important during the fermentation process. This is necessary because the production of hydrogen is accompanied by the formation of organic acids (acetic, lactic, butyric and propionic) which lower the pH of the medium thus inhibiting the activity of the enzymes responsible for the production of hydrogen [216]. A low pH (below 5) also limits the ability of bacteria to maintain the intracellular pH at an appropriate level [243]. Despite the importance of maintaining the pH at a constant, optimal level, a number of investigations, especially those carried out in a batch mode, have been performed without pH control. In such cases, researchers examined the effect of initial pH on the hydrogen fermentation yield. Li and Chen [244] investigated the effect of initial pH from 4 to 8 on the hydrogen yield obtained from steam-exploded corn straw in a batch mode. The results indicated that the optimum pH ranged from 7 to 7.5. However, it should be noted that the medium undergoes pH changes during fermentation without pH control which can significantly affect the hydrogen yield and thus the conclusions can be misleading. In batch processes taking place in fermenters, one should distinguish between the initial and operational pH during the production of biohydrogen [232].

It should be kept in mind that the optimum initial and operational pH values depend on the kind of strain or source of mixed bacterial cultures. Moreover, some investigations indicate that the kind of substrate used can also affect the optimum pH although this is not unequivocal [245].

In general, the optimum pH for the fermentative production of hydrogen ranges from 5.0 to 7.0, which corresponds to the pH range that is favorable for bacterial growth [196]. Guo *et al*. [231] reported that the neutral pH is optimal for livestock waste, a pH 6.5-7 is appropriate for crop waste, and a pH from 5 to 6 is best for food waste. Liu and Shen [177] investigated the effect of pH ranging from 4 to 9 on the hydrogen production yield by mixed bacterial cultures using starch as the substrate. The highest hydrogen yields were obtained for a pH of 7 and 8, equal to 103 and 120 ml H_2/g substrate, respectively. At pH=4 the hydrogen fermentation did not take place. Similar observations were reported [246]. The authors obtained a maximum hydrogen yield at pH = 7 using *C. acetobutylicum NCIMBI 13357*. High organic content wastewater frombrewery fermentation allowed to obtain the highest hydrogen yield of 1.41 [mol/mol hexose] for a pH equal to 5.5 [131]. Tapia-Venegas *et al*. [247] used a pH control system during hydrogen fermentation carried out in a continuous mode and obtained the highest hydrogen yield at pH = 5.5 (2.7 mol H_2/mol glucose). Vijayaraghavan and Ahmad [129] established that the optimum pH for the production of

hydrogen by dark fermentation of palm oil mill effluent was 5.0. Won *et al*. [248] used mixed cultures for fermentation of sugar refinery wastewater and observed the maximum hydrogen yield and productivity at a pH 5.5.

pH strongly affects not only the hydrogen production yield but also the kind and concentration of other metabolites, especially liquid organic compounds [249,250]. Fang and Liu [87] investigated the effect of pH on the concentration of by-products of bacterial metabolism. It was found that at low pH values, these are mainly acetic and butyric acids whereas an increase in pH resulted in higher concentrations of ethanol, propionic and lactic acids. A different relationship was observed [180] during investigation of an influence of pH on the yield of hydrogen and other metabolites in a continuous mode using mixed anaerobic cultures and rice winery wastewater as the substrate. The effluent composition was strongly dependent on pH value. At pH = 4 the propionic acid and ethanol contents in the effluent were 40% and 26%, respectively. Concentrations of both substances decreased with an increase in pH to 6.0, accounting for 20% and 13% of the effluent products. At the same time, the percentages of both acetic and butyric acids increased with pH, from 18% and 15%, respectively, at pH 4.0 to 38 and 29 % at pH 6.0. The hydrogen yield reached its maximum, 1.74 mol H_2/mol hexose, at pH 5.5; below and above this value the yield was lower. Interesting results were obtained by Wang *et al*. [251], who carried out fermentation at various pH values using untreated methanogenic sludge or heat-pretreated sludge. For heat-pretreated inoculum, the ethanol-type fermentation was important at a pH less than 3.6, for pH ranging from 4 to 5.6 the main fermentation product is butyric acid and for pH values between 5.6 and 6 the main fermentation product is acetic acid. In the case of untreated inoculum, at pH values below 4, the predominant fermentation product is caproic acid, for a pH ranging from 4.5 to 5.1 the main product is butyric acid and for pH values between 5.6 and 6 it is acetic acid. The maximum hydrogen yield (1.51 and 1.19 mol H_2/mol glucose) and production rate for both untreated and heat-pretreated inoculum were obtained for pH = 4.00. The investigations using a pure culture of *Clostridium butyricum* revealed that at a low pH the main organic fermentation products are ethanol and butyric acid whereas at pH values above 6 the main products are lactic and formic acids. The maximum hydrogen yield was obtained for pH = 5 [252].

During fermentation of glucose by *C. Pasteurianum* at a pH below 5, the main fermentation products were butanol and acetone [253].

5.5. Hydraulic retention time

Production of hydrogen on an industrial scale requires utilization of semi-continuous and continuous processes. Hydraulic retention time (HRT) is an important parameter affecting the hydrogen yield in such processes. Hydraulic retention time is a measure of the average length of time that a substrate remains in a fermentation chamber. HRT has a large impact on the hydrogen productivity during dark fermentation carried out in a continuous or semi-continuous mode. The hydrogen production rate increases over a certain range of HRT values but after exceeding the optimal HRT value the production rate decreases with an increase in HRT [254].

The optimal HRT value depends on the kind of substrate used in dark fermentation and, more specifically, on its biodegradability [82]. During continuous culture growth, the hydraulic retention time is usually gradually refined from long to short intervals to allow the acclimatization of microorganisms to new environments and to prevent the bacteria of interest to be washed away. As a result of the HRT shift, the microbial population dynamically changes, leading to the disappearance of certain species, while others appear [255].Using a short HRT with CSTR reactors can result in a removal of slowly growing methanogens. It also allows the use of smaller reactors, thus reducing the equipment cost [68]. It is possible to adjust HRT in dark fermentation to limit or eliminate the activity of bacteria utilizing hydrogen in their metabolic processes by making use of differences in growth rate of hydrogen producers and consumers [39]. For simple carbohydrates the optimal HRT is usually several hours: 4 hours [254], 2 hours [256] or 12 hours [247]. In order to find the optimal HRT for dark fermentation of the organic fraction of municipal solid waste, Zahedi *et al*. [107] carried out the process in a semi-continuous mode with various HRTs ranging from 6 hours to 10 days. For this kind of substrate, the highest hydrogen production rate of 5.66 $dm^3 H_2/dm^3/d$ was obtained for an HRT = 12 hours. The only liquid products were acetic and butyric acids. For longer hydraulic retention times, the presence of propionic and caproic acids was also observed. Massanet – Nicolau *et al.* [257] found that the optimal HRT for hydrogen production from sewage biosolids was 24 h. Under these conditions, stable hydrogen production has been achieved after three days.

Ottaviano *et al.* [258] used an AFBR reactor to continuously produce hydrogen under thermophilic conditions (55°C) from cheese whey powder solution. The HRT was changed from 8 to 0,5 h at a fixed substrate concentration. The hydrogen production rate (HPR) increased with decreasing of HRT and the maximum hydrogen production rate obtained was 4.1 ± 0.2 $dm^3 H_2/h/ dm^3$ for the HRT of 0,5 h. The maximum hydrogen yield was of $3.67 \pm 0,59$ mol H_2/mol lactose for HRT of 4h. Liu *et al.* [33] reported increase of HPR from 2.25 ± 0.58 $dm^3 H_2/d/ dm^3$ to 11.39 ± 1.39 $dm^3 H_2/d/ dm^3$ with the decrease of HRT from 4 to 1 h. In the process, beverage wastewater was used as a substrate. Veeravalli *et al.* [259] studied the influence of HRT on the hydrogen production from a steam exploded switchgrass liquor. A hydrogen yield increased to from 2.56 ± 0.10 mol H_2/mol hexose with decreasing of HRT from 12 to 6h. An increase of hydrogen yield from 0.03 to 0.06 mmol H2/g COD with the reduction of HRT from 8 to 4h was observed during hydrogen production from cassava processing wastewater [260]. Further reduction of HRT to 2 h caused decrease of the hydrogen yield. The authors explain it by the acclimation of methanogenic archea and stabilizing of methane production. It wasrevealed [180] that an increase in HRT from 2 to 24 h resulted in an increase in hydrogen yield from 1.74 to 2.14 mol H_2/ mol glucose which was due to conversion of a larger amount of the substrate into hydrogen. However, this was accompanied by a drop in hydrogen production rate from 8.02 $dm^3 H_2/g$ VSS/d for 2 h to 1.40 $dm^3 H_2/g$ VSS/d for 24 h.

Hydraulic retention time also affects the concentration of hydrogen in the biogas produced[261]. A change in HRT from 16 to 20 hours resulted in an increase in hydrogen concentration in the biogas by 1.8% (from 35.8% to 37.6%).

The key H_2 producing bacteria such as *Clostridium* sp. tend to produce in addition to H_2 also VFA (acetic and butyric acid) in the exponential growth phase and pass to generate alcohol during the steady-state phase [29].An increase in hydrogen production rate with a decrease in HRT can also be accompanied by a drop in yield of such metabolites as volatile organic compounds from *ca.* 8000 mg/ dm^3 VFA for HRT = 2 days to below 3000 mg/ dm^3 for HRT = 0.5 day [111]. It is preferable to establish HRT so that useful microorganisms can be maintained in the exponential phase.

Sweet sorghum was fermented in a CSTR and obtained a maximum production rate (2550 ml H_2/d) for an HRT equal to 6 h; on the other hand, the highest yield (10.4 dm^3 H_2/kg sweet sorghum) was achieved for an HRT=12 h [262]. Chen and Lin [263] reported that a decrease in HRT from 13.3 to 3 h caused an increase in the production rate of hydrogen from sucrose from 4.9 liters per day to 26.9 liters per day. HRT below 3 h had a negative effect on hydrogen yield.

It is generally understood that short HRT inhibits hydrogen activity, because the rate of proliferation of the participating microorganisms is significantly lower than that of H_2 producing bacteria. This is why at short HRTs growth kinetic control should be carried out in such a way that undesirable bacteria such as propionic acid and methane producers cannot compete with the dilution effect [29].

Table 10. Compilation of parameters describing hydrogen fermentation for optimal HRT values.

Substrate	Microorganisms	pH temp, °C	HRT range, h	Optimal HRT, h	Liquid organic products	Hydrogen productivity	Hydrogen yield	H_2 in biogas, %	Ref.
Sucrose 20 g COD/ dm^3	Mixed cultures from municipal sewage sludge	5.5	2-12	4	Butyric acid>>acetic acid>>ethanol>propionic acid	41.1 dm^3/ dm^3d	4.70 molH_2/mol sucrose	41.1	[254]
Sucrose 20 g COD/ dm^3	Mixed cultures from sludge from WWTP	6.7 35	0.5-4	0.5	Butyric acid >> acetic acid >>ethanol>propionic acid>>valeric acid	9.31 dm^3/h dm^3	3.91 mol H_2/mol sucrose	41.7	[264]
Sucrose 20 g COD/ dm^3	Mixed cultures from sludge from WWTP	5.8 35	1-6	2	Butyric acid >> acetic acid >propionic acid>>ethanol	0.511 dm^3/h dm^3	1.47 mol H_2/mol sucrose	28	[265]
Sucrose 20 g/ dm^3	Mixed cultures from sludge from WWTP	6.7 40	0.5-4	0.5	Butyric acid >> acetic acid > propionic acid>ethanol> valeric acid	7.66 ± 0.56	3.15 ± 0.08	40.1	[233]
Glucose 10 g/ dm^3	Mixed cultures from sludge from WWTP	4.4 35	2-12	2	Acetic acid >butyric acid>lactic acid>ethanol	19.05 mmol H_2/ dm^3h	0.55 mmol H_2/mol glucose	29.4	[266]
Glucose g/ dm^3	Mixed cultures from granular sludge from WWTP	5.5 37	6-14	12	Acetic acid >butyric acid>>ethanol	5.8 mmol/dm^3 h	2.7 mol H_2/ mol glucose	-	[247]
Sugar refinery wastewater	Mixed cultures Sewage sludge	5.5 31	10-30	10	Ethanol>> acetic acid >propionic acid>butyric acid	2.11 dm^3 H_2/ dm^3d	0.95 mol H_2/mol sucrose	71.8	[248]
Food wastes	Mixed cultures	5.5 34	0.5-2 d	0.5 d	Acetic acid >butyric acid> propionic acid=valeric acid=caproic acid	0.60 N dm^3 H_2/ dm^3r d	13.13 (N dm^3 H_2/kg VS added)	30.4	[111]
Sucrose 20 g/ dm^3	Mixed cultures from sludge from WWTP	6.7 40	0.5-4	0.5	Butyric acid >> acetic acid > propionic acid>ethanol> valeric acid	7.66 ± 0.56	3.15 ± 0.08	40.1	[233]
Sweet sorghum	Mixed cultures	5.3 35	4-24	6	Butyric acid >> acetic acid >lactic acid> ethanol> propionic acid	2550 cm^3/d	7.6 (dm^3 H_2/kg sweet sorghum	39.2	[262]

Cassava processing wastewater and glucose mixture	Mixed cultures from sludge from SP and Sw	4.5 30	2-10	6	butyric acid>methanol>ethanol>>acetic acid > lactic acid	2.9 dm^3/ dm^3d	1.0 mmol H_2/g COD	10	[260]
Switchgrass steam exploded liquor	Mixed cultures from brewery WT	- 37	6-12	6	butyric acid> acetic acid > propionic acid>ethanol>	6.3±0.5 dm^3/ dm^3d	2.56±0.10 mol/ mol hexose	20	[259]
Washing wastewater of beverage	Mixed cultures WWTP	5.5 40	1-24	1	butyric acid> acetic acid> propionic acid> ethanol> Lactic acid>formic acid>	11.39±1.39 dm^3/ dm^3d	0.30±0.06 mol/ mol hexose	30.18	[33]
Cheese wey powder	Mixed cultures from sugarcane stillage T	- 55	0.5-8	0.5	butyric acid>> acetic acid>>propionic acid> formic acid>ethanol	4.1±0.2 dm^3/ dm^3h	3.67±0.59 mol/ mol lactose	51.3	[258]
Sewage biosolids	Mixed cultures	5.5 35	18-48	12	butyric acid> acetic acid>propionic acid>	-	27.0 dm^3 H_2/kg VS added	-	[257]

COD – chemical oxygen demand; WWTP – wastewater treatment plant;

5.6. Partial pressure of hydrogen

The partial pressure of hydrogen is the next important parameter affecting hydrogen production. During production of hydrogen the enzyme hydrogenase oxidizes and reduces ferredoxin in a reversible process. Depending on thermodynamics of the reaction, an increase in partial pressure of hydrogen can favor the reduction of ferredoxin which inhibits further conversion of the substrate to hydrogen. When the partial pressure of hydrogen increases, the synthesis of hydrogen decreases while the concentrations of other products of metabolism such as lactic acid, ethanol, acetone, butanol and others increase [69,82]. Thus, it is essential to remove hydrogen as it is formed in order to maintain its high and constant production rate. At present, there are several methods of lowering hydrogen pressure in a reactor and, consequently, the concentration of hydrogen dissolved in a liquid medium. The simplest method involves stirring the medium during fermentation. Chou *et al*. [267] tested various stirring speeds in the process carried out in a 100 L reactor. An increase in stirring speed from 20 rpm to 100 rpm was found to improve the hydrogen production rate from 35 to 214 (cm^3 H_2/g TVS/d). Lowering the partial pressure of hydrogen can also be accomplished by sparging the fermentation mixture with an inert gas, usually nitrogen. Mizunio *et al*. [268] used nitrogen sparging to enhance hydrogen production from glucose, obtaining an increase in hydrogen yield from 0.85 to 1.43 mol H_2/ mol glucose and production rate from 1.434 to 3.126 cm^3/min/g biomass]. At the same time, the percentage of hydrogen in the biogas was lowered from 53.4% to 5.3%. Massanet – Nicolau *et al.* [257] used nitrogen to sparge the bioreactor for hydrogen production from sewage biosolids and found that the hydrogen production was sustained for a period of more than 12 days with a yield of 27.0 dm^3 H_2/kg VS. Kim et al. [269] used nitrogen and carbon dioxide to sparge the reactor at various flow rates (100, 200, 300 and 400 cm^3/min). The use of carbon dioxide was found to be more effective than nitrogen. The best performance was obtained by CO_2 sparged at 300 cm^3/min, resulting in the highest hydrogen yield of 1.68 mol H_2/mol $hexose_{consumed}$ compared to nitrogen sparging which yielded 0.95 mol H_2/mol $hexose_{consumed}$. The use of a sparging gas had an effect on the content of other metabolites formed during the fermentation process. For both nitrogen and CO_2 the concentration of butyric acid increased while the concentrations of acetic and propionic acids decreased, although the effect was more pronounced for CO_2. Veeravalli et al. [259] showed that a combination of nitrogen sparging with linoleic of acid treatment was more beneficial in increasing the hydrogen yield whith comparing to a single factor. The results showed that nitrogen sparging together with pretreating the culture increased the hydrogen yield to maximum of 2.56 ± 0.10 mol H_2/ mol hexose when the reactor was operated at HRT equal 6h.

Even though the use of a sparging gas improves the hydrogen yield, the final product is diluted and the cost of its recovery can be substantial. Therefore, an improvement of hydrogen yield may not compensate the cost of hydrogen recovery and the cost of sparging the reactor. Removal of the gaseous phase from the reactor is an effective method of lowering the partial pressure of hydrogen. Lee *et al.* [270] lowered the concentration of dissolved hydrogen by using a vacuum pump. The authors investigated the effect of changes in

hydrogen pressure on the yield and production rate of hydrogen as well as the changes in composition of liquid organic metabolites. A significant improvement in hydrogen production rate (from 0.348 to 0.376 mol $H_2/dm^3/h$) was found upon lowering the hydrogen pressure from 760 to 380 mmHg. The change in pressure also affected the concentrations of metabolites formed. The concentration of butyric acid increased by 9% with the simultaneous decrease in concentrations of acetic and propionic acids by 4.1 and 4.2 %, respectively. Mandal *et al*. [271] lowered the partial pressure of hydrogen over the fermentation medium from 760 mm Hg to 380 mm Hg and observed an increase in the hydrogen yield from 1.9 to 3.9 mol H_2/mol glucose. The maximum production rate of hydrogen was found at a lowered pressure and amounted to 0.017 mmol $H_2/h/dm^3$. An alternative solution is the removal of hydrogen by using active membranes highly selective to hydrogen. Examples of application of this type of solution are provided in papers by Teplyakov *et al*. [272] and Nielsen *et al*. [273]. These authors investigated reactors equipped with membrane systems using polyvinyltrimethylsilane [203] and palladium-silver membranes [204]. A disadvantage of using membranes is the formation and growth of biofilms containing methanogenic microorganisms [231]. Despite the availability of a variety of techniques for lowering the partial pressure of hydrogen, further research is needed to develop an effective and inexpensive method of hydrogen pressure control.

6. Reactors used in dark fermentation

The fundamental classification of reactors used for fermentation is based on the mode of implementation of the process, *i.e.* batch, semi-continuous and continuous. Batch reactors are the simplest reactors used primarily in the laboratory for preliminary investigations on the selection of optimum conditions for dark fermentation [69]. The reactors have a simple and inexpensive design; fermentation parameters, especially temperature and pH, can be controlled in a convenient way.

However, the production of hydrogen on an industrial scale requires the use of continuous reactors, due to higher expectable process efficiencies. The continuous reactors usually start in batch mode to ensure the proper inoculum preparation and its pretreatment. The successful transition to continuous mode operation is highly influenced by the start – up strategy [255]. The most commonly used bioreactor configurations are continuous stirred-tank reactors (CSTR), upflow anaerobic sludge blanket reactor (UASB), anaerobic fluidized bed reactor (AFBR) and membrane bioreactor (MBR).

6.1. Continuously stirred-tank reactor

The reactors most commonly used for continuous fermentation are continuously stirred-tank reactors (CSTR). The reactors are cylindrical in shape and are equipped with a mechanical stirring system. The feedstock is fed to the reactor and the effluent removed from it in a continuous or sequential mode. Dimensions of this type of reactors are much smaller than those of conventional fermenters used for the production of methane due to short

hydraulic retention times [232]. The CSTR reactors are commonly used owing to their simple design, easy adjustment of operating conditions, stirring that facilitates homogeneous conditions of the medium and provides a good contact of microorganisms with substrates [68,274]. Stirring also provides an effective control of conditions (pH, temperature) inside the reactor. Continuous stirring improves the effectiveness of removal of hydrogen from the reaction mixture which lowers its partial pressure and raises the hydrogen production yield. In contrast to typical fermenters, in CSTRs bacteria are better suspended in a liquid medium. However, the reactor also has a number of drawbacks. The main parameters during the CSTR operation are HRT and organic loading rate. The concentration of biomass in the reaction mixture is limited as a result of its retention time which is identical to HRT. This has an undisputed effect on the hydrogen production rate [196]. During its operation there is a problem with biomass removal and the bacteria are incapable of activity at high dilutions [69]. HRT must be less than the maximum growth rate of microorganisms used [274]. The hydrogen production rates in CSTR reactors are not high when using short HRTs. A diagram showing the general idea of a CSTR reactor is shown in Figure 3 [268].

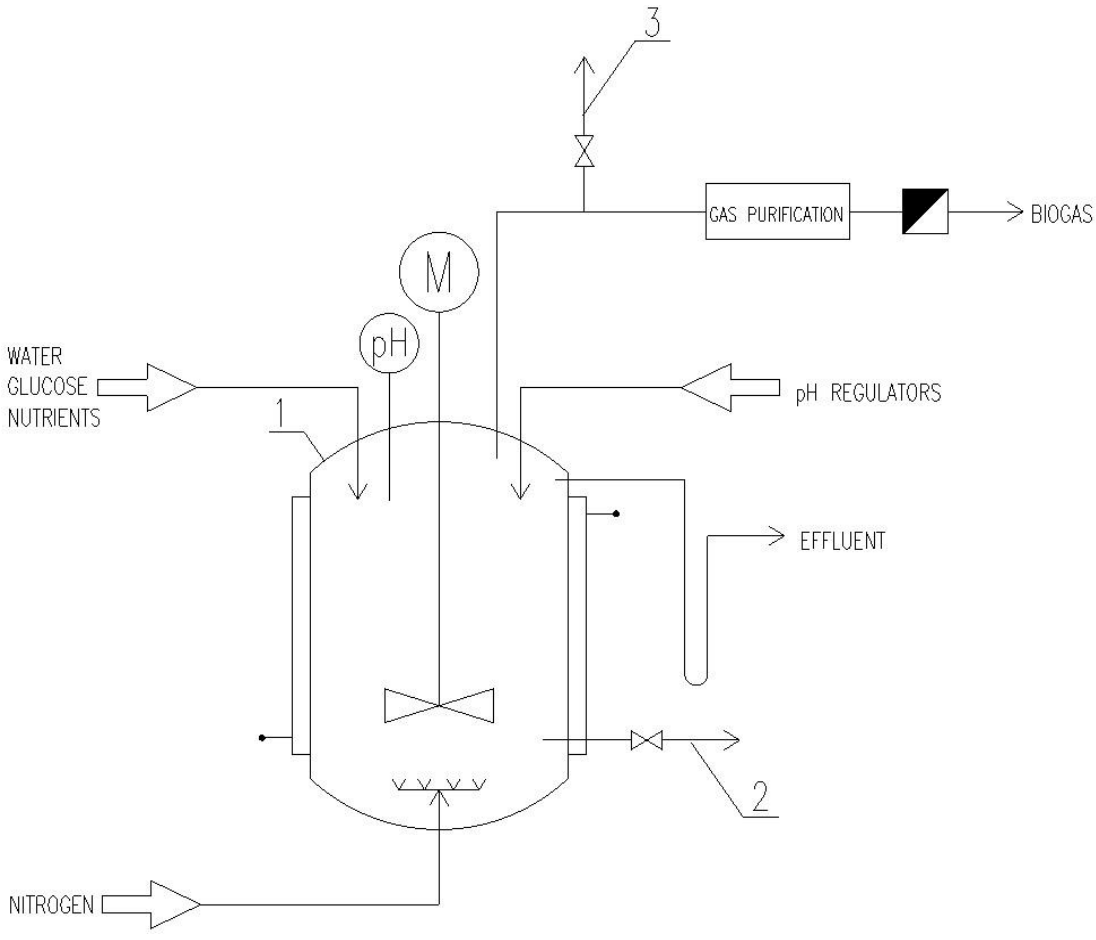

Figure 3. General idea of continuously stirred tank reactor [26,27,134,202,208].

In a paper by Mizuno *et al.* [268] a continuously stirred tank reactor (CSTR), similar to (1) in figure 3, with a working volume of 2.3 dm^3 was used. The reactor was equipped with a mechanical stirrer, a pH control system and a water jacket. Fermentation was carried out at

35 ^{o}C at pH = 6.0. Liquid and gaseous samples, collected through sampling ports, eg. (2) and (3) in figure 3 were analyzed. Krishnan et al. [275] investigated the two – stage process for the hydrogen and methane production using palm oil mill effluent as a substrate in a similar bioreactor.

6.2 Membrane bioreactor

A membrane bioreactor (MBR) is a typical reactor used to carry out fermentation processes; however, it is equipped with a membrane or a membrane system. The membranes can be placed outside the reactor (side-stream MBR) using the cross-flow (Figure 4) or can be immersed. The latter design is advantageous as it has lower operating costs and the membranes are smaller. The use of a membrane bioreactor eliminates the major drawback of a CSTR reactor which is the elution of biomass. The membrane retains microorganisms inside the reactor which allows maintaining the biomass at a constant, high concentration [196]. The presence of the membrane facilitates selection of an optimal HRT independent of retention time of activated sludge which allows a better control of process parameters [84]. An increase in retention time improves HRT of biomass which consequently improves the rate of conversion of substrates but has a negative effect on the hydrogen production rate. The main disadvantages of membrane bioreactors include fouling of membranes and high operating costs of the side-stream membrane systems. The general idea of membrane bioreactors for the production of hydrogen by dark fermentation is shown in Figure 4. This type of bioreactor was used by Oh *et al* [84], Palazzi *et al*. [276], Wu *et al*. [277], Wu *et al*. [256], Kumar *et al*. [278]. A ceramic-aluminum tubular membrane module packed with a 2-L volume of biomass was used.

A feedstock to the bioreactor (1) was delivered by means of a pump (2), maintaining a constant liquid level in the reactor, corresponding to a working volume of 1 dm^3. In order to adjust the solids retention time, solids were wasted from the reactor intermittently using a timer and a pump. A nutrient solution was fed by a nutrient-dosing pump (3). An alumina membrane was used in the membrane module (4). During the experiments by Oh *et al.* [84], three different membrane pore sizes: 0.2, 0.5 and 0.8 μm were used, and the membrane surface area was 0.0055 m^2. A constant permeate flow was maintained by a circulation loop (5). Periodic backpulsing (every 10 to 30 s) was used to limit membrane fouling. It was achieved by injecting nitrogen gas in short pulses (0.5-1 s) into the permeate side of the membrane module. The pressure drop across the membrane was measured using manometers $P_{G.}$

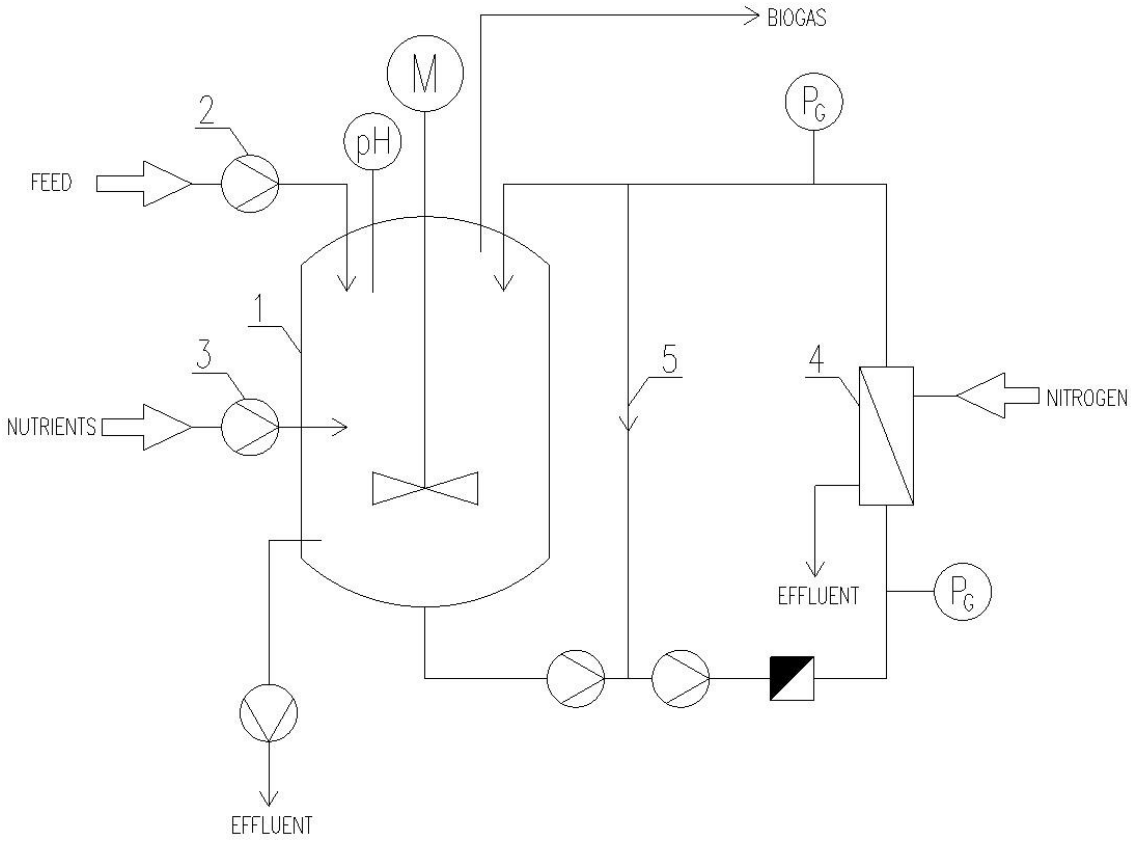

Figure 4. General idea of membrane bioreactor [42,194,209-211].

6.3. Packed - bed bioreactors

An alternative to CSTRs are packed-bed reactors. In contrast with CSTRs they can be used for conversion of biomass with a high content of organics. They are also characterized by short HRTs without the risk of elution of biomass. The reactor bed can have the form of granules, biofilm or biomass particles bonded to a gel [96]. Typical packed-bed reactors include upflow anaerobic sludge blanket (UASB) reactors, anaerobic fluidized-bed reactors (AFBR) and expanded granular sludge bed (EGSB) reactors.

In order to optimize the yield of fermentation, various materials with a variety of shapes are tested for immobilization of microorganisms on their surface.

Palazzi *et al*. [276] used a continuous packed column for dark fermentation of starch by *Enterobacter aerogenes*, in which the bacteria were immobilized on fragments of spongy structures having dimensions of 5x5x2 mm and glass granules of 7 mm in diameter. This was to lower a stress reaction of the organisms to stirring and to limit the removal of microbial biomass from the reactor. The highest hydrogen yield obtained in this way was 3.02 mmol H_2/mmol glucose.

Wu *et al*. [277] used a packed-bed reactor filled with immobilized-cell beads for dark fermentation of glucose, fructose and sucrose. The maximum hydrogen yield was 0.70 mol H_2/mol glucose.

Wu *et al*. [256] used for fermentation immobilized sewage sludge in a three-phase fluidized bed reactor. The sludge was immobilized with a modified alginate gel entrapment

method. The cells immobilized in this way protect the bacteria from excessive elution. The maximal hydrogen yield obtained during dark fermentation was 2.67 mol H_2/mol sucrose.

Kumar *et al*. [278] examined the effect of shape of lignocellulosic solid matrices with immobilized bacteria *Enterobacter cloacae* IIT-BT 08 on the hydrogen production rate. Out of the three bed configurations studied, tubular, tapered and rhomboid, the rhomboid bioreactor was found to be most effective. The gas hold-up was reduced by 67% using the rhomboid bioreactor as compared to tubular one. At a glucose concentration equal to 1%, the maximum hydrogen production rate achieved was 75.6 mmol/ dm^3 h, which was 2.1 times higher than that of batch system.

6.3.1. Upflow anaerobic sludge blanket reactor

The UASB reactor usually has an elongated shape with a three-phase separator in the upper part of the reactor, in which hydrogen producing granules (HPG) are quickly formed and deposited at the bottom resulting in a thick layer of biomass [279].

The granules are formed and grow during fermentation process through aggregation of activated sludge. Their diameter varies from 0.2 to 2 mm. The advantage of granulated form of microorganisms is their higher retention in the reactor and higher resistance to toxic conditions. The closeness of microorganisms in granules results in a greater conversion of substrates into hydrogen. Bubbles of gas formed during fermentation move upward, causing turbulence in the medium and hence mixing; therefore, mechanical stirring is not used. The feedstock is pumped to the bottom of the reactor. A disadvantage of UASB is a long initiation time when using granulated activated sludge. These reactors can be used for a wide variety of substrates, especially organic wastewater of various origin. Hydrogen production in this type of reactors is highly effective and reproducible. However, the use of UASBs is limited by the long time (even several months) needed for HPG to form [232]. The advantages of UASB reactors include high effectiveness of hydrogen production, short HRT and stable operating conditions. According to Lee *et al*. [264], the disadvantage of a UASB reactor is its sensitivity to channeling effects which give rise to poor substrate-biomass contact. The general idea of a UASB reactor is shown in Figure 5.

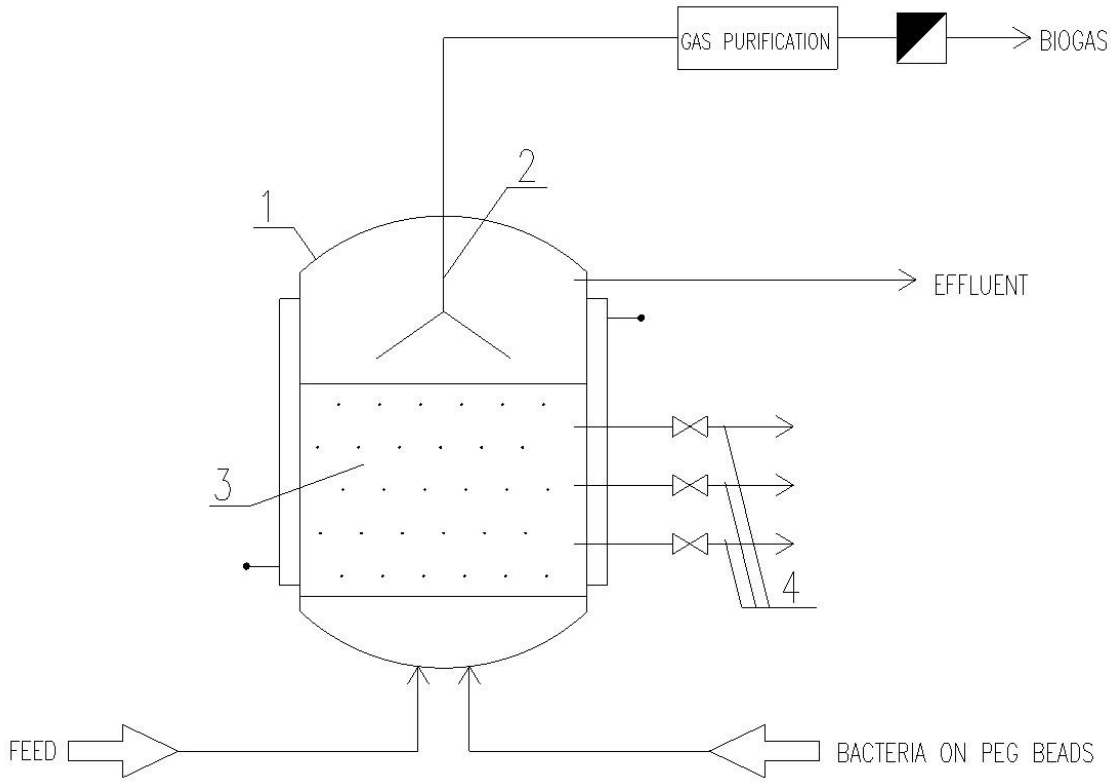

Figure 5. General idea of flow anaerobic sludge blanket reactor [171,198,213].

An upflow anaerobic sludge blanket (UASB) reactor, marked as (1) in the figure 5 with a working volume of 5 dm^3, equipped with a water jacket and a three-phase separator (2) was used [280]. Fermentation was carried out at 37 ^oC. The feedstock to the reactor was palm oil mill effluent (POME) and bacteria immobilized in a PEG polymer. The reactor was loaded with an appropriate amount of PEG-immobilized cells (3) to obtain a final solution of 4-16 % w/v. For the first 6 hours of fermentation the reactor was operated in batch mode before being switched to continuous mode. Liquid samples were collected during the reactor operation using sampling ports (4) located at various heights of the reactor.

Krishnan *et al* [275] investigated the two – stage process for the hydrogen and methane production. The first stage was realized in UASB reactor for hydrogen production, the second stage was accomplished with the CSTR reactor for methane production. The well – mixed slurry fluid from the mixing tank was fed into the UASB reactor in 24 h cycles, including 30 min of filling and 22,5 h of reaction time. The effective total volume of UASB reactor was 5 dm^3. The feedstock to the reactor was palm oil mill effluent (POME) and heat – treated anaerobic sludge. The dark fermentation was carried out at 55 ^oC and a pH of 5,5. The effluent from hydrogen production was mixed with untreated anaerobic digestion sludge and flowed to CSTR reactor. The maximum hydrogen production rate from palm oil mill in UASB reactor was reached at 2-days HRT under thermophilic condition. The biogas was composed mainly of H_2 and CO_2, with concentrations of 55% and 41-47%, respectively.

The two identical units of UASB reactors with the working volume of 24 dm3 was used to hydrogen production [281]. The cassava wastewater was fed continuously to the bottom of each UASB reactors at different flow rate. The operating conditions were: 37 ^oC, pH of 5,5, a recycle ratio of the effluent flow rate to feed flow rate was fixed at 1:1, COD

loading rate was from 10 to 30 kg/m^3/d. For each COD loading, it took about 10 d to reach a steady state conditions. The effluent from the hydrogen UASB reactors was used as a feed to two UASB methane producing reactors. For the hydrogen production stage, maximum hydrogen production was achieved at a COD loading rate of 25 kg/m3/d and the produced gas contained 36,4% hydrogen and 63,6% carbon dioxide without methane.

6.4. Anaerobic fluidized bed reactor

Reactors of this type combine features of two reactors: CSTR and immobilized-bed reactors. Microorganisms are deposited on a solid surface nas granules or a biofilm. The advantage of an AFBR is a good mass and heat transfer. A gas is fed from the bottom of the reactor to generate a fluidized bed. This affects catalytic activity of the system and thus the degree of degradation of substrates. Since microorganisms are deposited on a solid surface, there is a lower probability of biomass being washed off compared to a UASB reactor. A disadvantage of a AFBR is a higher energy consumption needed to maintain the bed in a fluidized state [282]. Figure 6 depicts an AFBR used by Zhang *et al*. [282].

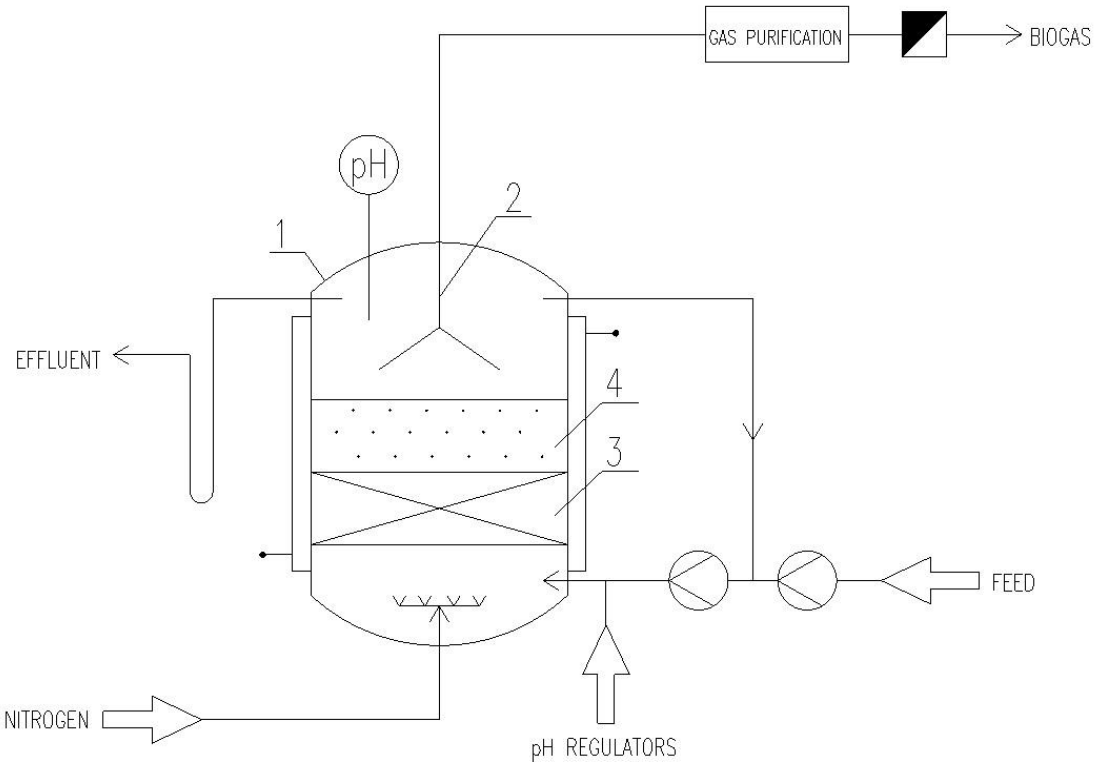

Figure 6. General idea of anaerobic fluidized bed reactor [214-216].

An anaerobic fluidized bed reactor (AFBR) was used [282]. The main element of the system is a bioreactor, similar to (1) in the figure 6 with a three-phase separator (2) installed in the upper part. Glass beads of 5 mm in diameter made a bed (3) located at the reactor bottom to serve as influent distributor. Granular activated carbon was used as the support

medium for bacteria (4). The pH was controlled at 4.0 and the reactor was operated at a temperature of 37 ^{o}C by a heating blanket. Mixed bacterial cultures were used in fermentation [242]. The system was fed with synthetic wastewater containing glucose and other nutrients.

Two AFBR reactors were used to hydrogen production from cassava processing wastewater [259]. The AFBR1 reactor was inoculated with poultry slaughterhouse wastewater (SP) and the AFBR2 reactor was inoculated with swine wastewater (SW). Both types of sludge were thermally pretreated. Prior to be used in the feed, the cassava processing wastewater was acidified and heat pretreated. The recirculation pump was set at 1.3 times the minimum fluidization velocity of the polystyrene particles (0.74 cm/s). The reactors were operated for 142 days. For the first 48 h, the reactors were operated in batch mode to begin the hydrogen production. Then, they were operated in a continuous mode. For the AFBR1 the maximum hydrogen yield was 0.6 mmol H_2/g COD and 1.0 mmol H_2/g COD for the AFBR2.

Ottaviano et al. [258] used two AFBR reactors to continuously produce hydrogen under thermophilic conditions (55°C) from cheese whey. The AFBR1 reactor was operated in a fixed CW feed concentration decreasing the HRT from 8 to 0.5 h. The AFBR2 reactor was operated in a fixed HRT increasing CW feed concentration from 2800 ± 100 to 14600 ± 300 mg lactose/dm^3. The total volume of each reactor was 1.98 dm^3. The support material for the immobilization of biomass were expanded clay particles. The recycle flow rate was 76.58 dm^3/h, bed expansion 30%, the superficial velocity was 1.30 times higher than minimum fluidization velocity. After the batch operation for adaptation of the inoculum to the CW, the reactors were switched to continuous mode. For AFBR1, the maximum hydrogen production was reached for the HRT of 0.5 h. For AFBR2, the maximum hydrogen production was reached at the concentration of 6800 ± 300 mg lactose/dm^3.

The apparatus used for dark fermentation can also be configured as multistage reactor systems. The most common systems are two-stage ones, in which hydrolysis and fermentation take place separately. Fermentation alone can also be carried out in reactors connected in series [283]. Such a solution allows a greater degree of conversion of the feedstock and improved yield of the process but at the expense of a longer total retention time and higher operating costs of the system. As an example, Han and Shin [284] used sequential batch technique in specially designed reactors to produce hydrogen from food waste. The leaching-bed reactors connected in series operated in batch mode and were rotated. The hydrogen yield in such a multistage system was 0.31 m^3/kg VS added. Biohydrogen and methane production from cheese whey in a two-stage anaerobic process has also been investigated [285]. Another approach to hydrogen production is a combination of dark fermentation and photofermentation of organic acids formed in the first stage of dark fermentation.

7. Summary

The possibility of hydrogen production from renewable resources and from post-production wastes, especially from the agricultural industry, is being extensively investigated.

Other approaches, including gasification, pyrolysis, electrolysis of water, magnetolysis, radiolysis, plasmolysis or biophotolysis of water are also being pursued. Waste biomass can be converted to hydrogen by microbiological processes. Especially, dark fermentation is recognized as the most promising method due to its advantages such as relatively low energy demand, the usability of wide range of biomass as a feedstocks and the possibility to integrate with other processes *e.g.* methane production processes or membrane – based processes. There is a considerable interest in the production of hydrogen from waste lignocellulosic biomass as demonstrated by a number of research and review papers. This research has to be continued in order to develop efficient technologies for hydrogen production. The limitation step of hydrogen production by dark fermentation from biomass waste is the low degree of conversion of the substrate to product.

Fast growing trees, such as energy willow or poplar, could be used to produce biohydrogen by dark fermentation after pretreatment. The fermentation process is affected by a number of parameters, including: temperature, pH, hydraulic retention time for continuous processes, partial pressure of hydrogen, kind of microorganisms used and in the case of mixed cultures also the method of inoculum pretreatment. As demonstrated, the composition of a growth medium and optimum concentration of biomass in the feedstock are also important. Bioreactor configuration and operation mode are also significant technological indicators and were discussed in the present paper. Regardless the type of the fermenter, dark fermentation should be conducted in continuous mode rather than in batch system, with special attention paid to the conditions of the start – up strategy.

It is crucial to continue additional research and improve the efficiency of hydrogen generation. The possible gain of energy is the main driving force for amelioration concerning the greater reliability and sustainability. Continuous analysis of diversified hydrogen generation approaches may help to identify the deficiencies that hinder infrastructure evolution. Therefore constant tracking of the supporting systems for scale-up and analysis of feedback effects should result in high-potential strategies for widespread deployment of hydrogen technologies.

Acknowledgements

This work was carried out within the framework of the project „Investigation of basic hydrolysis of lignocellulosic biomass and conversion of the products to biogases", supported financially by the National Science Center through the grant UMO-2014/13/B/ST8/04258

The authors would like to express their thanks to dr hab. inż. Hubert Ciesliński, Department of Microbiology, Faculty of Chemistry, Gdańsk University of Technology, for his help and valuable comments.

Declarations of interest:
None

Bibliography

[1] Graboski MS, McCormick RL. Combustion of fat and vegetable oil derived fuels in diesel engines. Prog Energy Combust Sci 1998;24:125–64. doi:10.1016/S0360-1285(97)00034-8.

[2] Zajic JE, Kosaric N, Brosseau JD. Microbial production of hydrogen. Adv. Biochem. Eng., Berlin, Heidelberg: Springer Berlin Heidelberg; 1978, p. 57–109. doi:10.1007/BFb0048091.

[3] Grimes CA, Varghese OK, Ranjan S. Light, Water, Hydrogen. The solar generation of hydrogen by water photoelectrolysis. New York: Springer Science+Business Media, LLC; 2008. doi:10.1007/978-0-387-68238-9.

[4] Sinha P, Pandey A. An evaluative report and challenges for fermentative biohydrogen production. Int J Hydrogen Energy 2011;36:7460–78. doi:10.1016/j.ijhydene.2011.03.077.

[5] Kapdan IK, Kargi F. Bio-hydrogen production from waste materials. Enzyme Microb Technol 2006;38:569–82. doi:10.1016/j.enzmictec.2005.09.015.

[6] Momirlan M, Veziroglu T. Current status of hydrogen energy. Renew Sustain Energy Rev 2002;6:141–79. doi:10.1016/S1364-0321(02)00004-7.

[7] Logan BE. Extracting hydrogen and electricity from renewable resources. Environ Sci Technol 2004;38:4–8.

[8] Kato H, Kudo A. Photocatalytic water splitting into H2 and O2 over various tantalate photocatalysts. Catal Today 2003;78:561–9. doi:10.1016/S0920-5861(02)00355-3.

[9] Givotov VK, Fridman AA, Krotov MF, Krasheninnikov EG, Patrushev BI, Rusanov VD, et al. Plasmochemical methods of hydrogen production. Int J Hydrogen Energy 1981;6:441–9. doi:10.1016/0360-3199(81)90076-8.

[10] Ghoroghchian J, Bockris JO. Use of a homopolar generator in hydrogen production from water. Int J Hydrogen Energy 1985;10:101–12. doi:10.1016/0360-3199(85)90042-4.

[11] Crumière F, Vandenborre J, Essehli R, Blain G, Barbet J, Fattahi M. LET effects on the hydrogen production induced by the radiolysis of pure water. Radiat Phys Chem 2013;82:74–9. doi:10.1016/j.radphyschem.2012.07.010.

[12] Ohta T. A note on the gas-evolution of mechano-catalytic water-splitting system. vol. 26. 2001. doi:10.1016/S0360-3199(00)00110-5.

[13] Reith JH, Wijffels RH, Barten H. Bio-methane and bio-hydrogen: status and perspectives of biological methane and hydrogen production. Hague: Dutch Biological Hydrogen Foundation - NOVEM; 2003.

[14] Pudukudy M, Yaakob Z, Mohammad M, Narayanan B, Sopian K. Renewable hydrogen economy in Asia - Opportunities and challenges: An overview. Renew Sustain Energy Rev 2014;30:743–57. doi:10.1016/j.rser.2013.11.015.

[15] Production of hydrogen by catalytic steam reforming of oxygenated model compounds on Ni-modified supported catalysts. Simulation and experimental study. Int J Hydrogen Energy 2015;40:11217–27. doi:10.1016/J.IJHYDENE.2015.05.167.

[16] Shahbaz M, yusup S, Inayat A, Patrick DO, Ammar M. The influence of catalysts in biomass steam gasification and catalytic potential of coal bottom ash in biomass steam gasification: A review. Renew Sustain Energy Rev 2017;73:468–76. doi:10.1016/j.rser.2017.01.153.

[17] Hydrogen production from renewable and sustainable energy resources: Promising green energy carrier for clean development. Renew Sustain Energy Rev 2016;57:850–66. doi:10.1016/J.RSER.2015.12.112.

[18] Yang L, Seshan K, Li Y. A review on thermal chemical reactions of lignin model compounds. Catal Today 2016. doi:10.1016/j.cattod.2016.11.030.

[19] Sarma SJ, Pachapur V, Brar SK, Le Bihan Y, Buelna G. Hydrogen biorefinery: Potential utilization of the liquid waste from fermentative hydrogen production. Renew Sustain Energy Rev 2015;50:942–51. doi:10.1016/j.rser.2015.04.191.
[20] Yan K, Jarvis C, Gu J, Yan Y. Production and catalytic transformation of levulinic acid: A platform for speciality chemicals and fuels. Renew Sustain Energy Rev 2015;51:986–97. doi:10.1016/j.rser.2015.07.021.
[21] Bardhan SK, Gupta S, Gorman ME, Haider MA. Biorenewable chemicals: Feedstocks, technologies and the conflict with food production. Renew Sustain Energy Rev 2015;51:506–20. doi:10.1016/j.rser.2015.06.013.
[22] Yan K, Liu Y, Lu Y, Chai J, Sun L. Catalytic application of layered double hydroxide-derived catalysts for the conversion of biomass-derived molecules. Catal Sci Technol 2017;7:1622–45. doi:10.1039/C7CY00274B.
[23] Sen B, Aravind J, Kanmani P, Lay CH. State of the art and future concept of food waste fermentation to bioenergy. Renew Sustain Energy Rev 2016;53:547–57. doi:10.1016/j.rser.2015.08.065.
[24] Kothari R, Singh DP, Tyagi V V., Tyagi SK. Fermentative hydrogen production - An alternative clean energy source. Renew Sustain Energy Rev 2012;16:2337–46. doi:10.1016/j.rser.2012.01.002.
[25] Kumar G, Bakonyi P, Kobayashi T, Xu KQ, Sivagurunathan P, Kim SH, et al. Enhancement of biofuel production via microbial augmentation: The case of dark fermentative hydrogen. Renew Sustain Energy Rev 2016;57:879–91. doi:10.1016/j.rser.2015.12.107.
[26] Sivagurunathan P, Kumar G, Mudhoo A, Rene ER, Saratale GD, Kobayashi T, et al. Fermentative hydrogen production using lignocellulose biomass: An overview of pre-treatment methods, inhibitor effects and detoxification experiences. Renew Sustain Energy Rev 2017;77:28–42. doi:10.1016/j.rser.2017.03.091.
[27] Trchounian K, Sawers RG, Trchounian A. Improving biohydrogen productivity by microbial dark- and photo-fermentations: Novel data and future approaches. Renew Sustain Energy Rev 2017;80:1201–16. doi:10.1016/j.rser.2017.05.149.
[28] Wong YM, Wu TY, Juan JC. A review of sustainable hydrogen production using seed sludge via dark fermentation. Renew Sustain Energy Rev 2014;34:471–82. doi:10.1016/j.rser.2014.03.008.
[29] Sivagurunathan P, Kumar G, Bakonyi P, Kim SH, Kobayashi T, Xu KQ, et al. A critical review on issues and overcoming strategies for the enhancement of dark fermentative hydrogen production in continuous systems. Int J Hydrogen Energy 2016. doi:10.1016/j.ijhydene.2015.12.081.
[30] Kumar G, Sivagurunathan P, Sen B, Mudhoo A, Davila-Vazquez G, Wang G, et al. Research and development perspectives of lignocellulose-based biohydrogen production. Int Biodeterior Biodegradation 2017;119:225–38. doi:10.1016/j.ibiod.2016.10.030.
[31] Rezania S, Din MFM, Taib SM, Sohaili J, Chelliapan S, Kamyab H, et al. Review on fermentative biohydrogen production from water hyacinth, wheat straw and rice straw with focus on recent perspectives. Int J Hydrogen Energy 2017;42:20955–69. doi:10.1016/j.ijhydene.2017.07.007.
[32] Hallenbeck PC. Fermentative hydrogen production: Principles, progress, and prognosis. Int J Hydrogen Energy 2009;34:7379–89. doi:10.1016/j.ijhydene.2008.12.080.
[33] Liu CM, Zheng JL, Wu SY, Chu CY. Fermentative hydrogen production potential from washing wastewater of beverage production process. Int J Hydrogen Energy 2016. doi:10.1016/j.ijhydene.2015.08.079.

[34] García CA, Betancourt R, Cardona CA. Stand-alone and biorefinery pathways to produce hydrogen through gasification and dark fermentation using Pinus Patula. J Environ Manage 2015;203:695–703. doi:10.1016/j.jenvman.2016.04.001.
[35] Jia S, Ning S, Ying H, Sun Y, Xu W, Yin H. High quality syngas production from catalytic gasification of woodchip char. Energy Convers Manag 2017;151:457–64. doi:10.1016/j.enconman.2017.09.008.
[36] Tijmensen MJA, Faaij APC, Hamelinck CN, Van Hardeveld MRM. Exploration of the possibilities for production of Fischer Tropsch liquids and power via biomass gasification. Biomass and Bioenergy 2002;23:129–52. doi:10.1016/S0961-9534(02)00037-5.
[37] Effects of biochemical composition on hydrogen production by biomass gasification. Int J Hydrogen Energy 2017;42:19723–32. doi:10.1016/J.IJHYDENE.2017.06.174.
[38] Hydrogen and syngas production by catalytic biomass gasification. Energy Convers Manag 2017;135:270–3. doi:10.1016/J.ENCONMAN.2016.12.056.
[39] Hydrogen production via catalytic pyrolysis of biomass in a two-stage fixed bed reactor system. Int J Hydrogen Energy 2014;39:13128–35. doi:10.1016/J.IJHYDENE.2014.06.158.
[40] Hydrogen production through hydrothermal gasification of industrial wastewaters using transition metal oxide catalysts. J Supercrit Fluids 2016;114:32–45. doi:10.1016/J.SUPFLU.2016.03.028.
[41] Hydrogen rich gas production from catalytic gasification of biomass. Renew Energy 2016;85:1290–300. doi:10.1016/J.RENENE.2015.07.082.
[42] Hydrothermal gasification of Cladophora glomerata macroalgae over its hydrochar as a catalyst for hydrogen-rich gas production. Bioresour Technol 2016;222:232–41. doi:10.1016/J.BIORTECH.2016.09.082.
[43] Hossain MA, Ganesan P, Laxmi J, Chinna K. Optimization of process parameters for microwave pyrolysis of oil palm fiber (OPF) for hydrogen and biochar production. Energy Convers Manag 2016;133:349–62.
[44] Moud PH, Kantarelis E, Andersson KJ, Engvall K. Biomass pyrolysis gas conditioning over an iron-based catalyst for mild deoxygenation and hydrogen production. Fuel 2018;211:149–58. doi:10.1016/j.fuel.2017.09.062.
[45] Production of syngas from pyrolysis of biomass using Fe/CaO catalysts: Effect of operating conditions on the process. J Anal Appl Pyrolysis 2017;125:1–8. doi:10.1016/J.JAAP.2017.05.007.
[46] Pyrolysis/reforming of rice husks with a Ni–dolomite catalyst: Influence of process conditions on syngas and hydrogen yield. J Energy Inst 2016;89:657–67. doi:10.1016/J.JOEI.2015.05.006.
[47] Principles and practice of biomass fast pyrolysis processes for liquids. J Anal Appl Pyrolysis 1999;51:3–22. doi:10.1016/S0165-2370(99)00005-4.
[48] Elliott DC, Beckman D, Bridgwater A V., Diebold JP, Gevert SB, Solantausta Y. Developments in direct thermochemical liquefaction of biomass: 1983-1990. Energy & Fuels 1991;5:399–410. doi:10.1021/ef00027a008.
[49] Bu Q, Lei H, Ren S, Wang L, Holladay J, Zhang Q, et al. Phenol and phenolics from lignocellulosic biomass by catalytic microwave pyrolysis. Bioresour Technol 2011;102:7004–7. doi:10.1016/j.biortech.2011.04.025.
[50] Nabgan W, Tuan Abdullah TA, Mat R, Nabgan B, Gambo Y, Ibrahim M, et al. Renewable hydrogen production from bio-oil derivative via catalytic steam reforming: An overview. Renew Sustain Energy Rev 2017;79:347–57. doi:10.1016/j.rser.2017.05.069.
[51] Hosseini SE, Wahid MA, Ganjehkaviri A. An overview of renewable hydrogen

production from thermochemical process of oil palm solid waste in Malaysia. Energy Convers Manag 2015;94:415–29. doi:10.1016/j.enconman.2015.02.012.
[52] Yao J, Liu J, Hofbauer H, Chen G, Yan B, Shan R, et al. Biomass to hydrogen-rich syngas via steam gasification of bio-oil/biochar slurry over LaCo1-xCuxO3 perovskite-type catalysts. Energy Convers Manag 2016;117:343–50. doi:10.1016/j.enconman.2016.03.043.
[53] Chen G, Tao J, Liu C, Yan B, Li W, Li X. Hydrogen production via acetic acid steam reforming: A critical review on catalysts. Renew Sustain Energy Rev 2017;79:1091–8. doi:10.1016/j.rser.2017.05.107.
[54] Ma Y, Guan G, Phanthong P, Li X, Cao J, Hao X, et al. Steam reforming of methanol for hydrogen production over nanostructured wire-like molybdenum carbide catalyst. Int J Hydrogen Energy 2014;39:18803–11. doi:10.1016/j.ijhydene.2014.09.062.
[55] Sharma YC, Kumar A, Prasad R, Upadhyay SN. Ethanol steam reforming for hydrogen production: Latest and effective catalyst modification strategies to minimize carbonaceous deactivation. Renew Sustain Energy Rev 2017;74:89–103. doi:10.1016/j.rser.2017.02.049.
[56] Nath K, Muthukumar M, Kumar A, Das D. Kinetics of two-stage fermentation process for the production of hydrogen. Int J Hydrogen Energy 2008;33:1195–203. doi:10.1016/j.ijhydene.2007.12.011.
[57] Winkler M, Hemschemeier A, Gotor C, Melis A, Happe T. [Fe]-hydrogenases in green algae: Photo-fermentation and hydrogen evolution under sulfur deprivation. Int J Hydrogen Energy 2002;27:1431–9. doi:10.1016/S0360-3199(02)00095-2.
[58] Melis A, Zhang L, Forestier M, Ghirardi ML, Seibert M. Sustained Photobiological Hydrogen Gas Production upon Reversible Inactivation of Oxygen Evolution in the Green Alga Chlamydomonas reinhardtii 1. Plant Physiol 2000;122:127–35.
[59] Tamagnini P, Axelsson R, Lindberg P, Oxelfelt F, Wünschiers R, Lindblad P. Hydrogenases and hydrogen metabolism of cyanobacteria. Microbiol Mol Biol Rev 2002;66:1–20. doi:10.1128/MMBR.66.1.1-20.2002.
[60] Hallenbeck PC, Benemann JR. Biological hydrogen production; fundamentals and limiting processes. Int J Hydrogen Energy 2002;27:1185–93. doi:10.1016/S0360-3199(02)00131-3.
[61] He D, Bultel Y, Magnin JP, Roux C, Willison JC. Hydrogen photosynthesis by Rhodobacter capsulatus and its coupling to a PEM fuel cell. J Power Sources 2005;141:19–23. doi:10.1016/j.jpowsour.2004.09.002.
[62] Chen JS, Toth J, Kasap M. Nitrogen-fixation genes and nitrogenase activity in Clostridium acetobutylicum and Clostridium beijerinckii. J Ind Microbiol Biotechnol 2001;27:281–6. doi:10.1038/sj.jim.7000083.
[63] Zagrodnik R, Łaniecki M. Hydrogen production from starch by co-culture of Clostridium acetobutylicum and Rhodobacter sphaeroides in one step hybrid dark- and photofermentation in repeated fed-batch reactor. Bioresour Technol 2017;224:298–306. doi:10.1016/j.biortech.2016.10.060.
[64] Manish S, Banerjee R. Comparison of biohydrogen production processes. Int J Hydrogen Energy 2008;33:279–86. doi:10.1016/j.ijhydene.2007.07.026.
[65] Logan BE, Call D, Cheng S, Hamelers HVM, Sleutels THJA, Jeremiasse AW, et al. Microbial Electrolysis Cells for High Yield Hydrogen Gas Production from Organic Matter. Environ Sci Technol 2008;42:8630–40. doi:10.1021/es801553z.
[66] Maness PC, Huang J, Smolinski S, Tek V, Vanzin G. Energy generation from the CO oxidation-hydrogen production pathway in Rubrivivax gelatinosus. Appl Environ Microbiol 2005;71:2870–4. doi:10.1128/AEM.71.6.2870-2874.2005.
[67] Mishra J, Khurana S, Kumar N, Ghosh AK, Das D. Molecular cloning,

characterization, and overexpression of a novel [Fe]-hydrogenase isolated from a high rate of hydrogen producing Enterobacter cloacae IIT-BT 08. Biochem Biophys Res Commun 2004;324:679–85. doi:10.1016/j.bbrc.2004.09.108.
[68] Hawkes FR, Hussy I, Kyazze G, Dinsdale R, Hawkes DL. Continuous dark fermentative hydrogen production by mesophilic microflora: Principles and progress. Int J Hydrogen Energy 2007;32:172–84. doi:10.1016/j.ijhydene.2006.08.014.
[69] Hallenbeck PC. Microbial technologies in advanced biofuels production. New York: Springer Science+Business Media, LLC; 2012. doi:10.1007/978-1-4614-1208-3.
[70] Levin DB, Islam R, Cicek N, Sparling R. Hydrogen production by Clostridium thermocellum 27405 from cellulosic biomass substrates. Int J Hydrogen Energy 2006;31:1496–503. doi:10.1016/j.ijhydene.2006.06.015.
[71] Ivanova G, Rákhely G, Kovács KL. Thermophilic biohydrogen production from energy plants by Caldicellulosiruptor saccharolyticus and comparison with related studies. Int J Hydrogen Energy 2009;34:3659–70. doi:10.1016/j.ijhydene.2009.02.082.
[72] Oztekin R, Kapdan IK, Kargi F, Argun H. Optimization of media composition for hydrogen gas production from hydrolyzed wheat starch by dark fermentation. Int J Hydrogen Energy 2008;33:4083–90. doi:10.1016/j.ijhydene.2008.05.052.
[73] Argun H, Kargi F, Kapdan IK, Oztekin R. Batch dark fermentation of powdered wheat starch to hydrogen gas: Effects of the initial substrate and biomass concentrations. Int J Hydrogen Energy 2008;33:6109–15. doi:10.1016/j.ijhydene.2008.08.004.
[74] Kargi F, Pamukoglu MY. Dark fermentation of ground wheat starch for bio-hydrogen production by fed-batch operation. Int J Hydrogen Energy 2009;34:2940–6. doi:10.1016/j.ijhydene.2008.12.101.
[75] Kargi F, Eren NS, Ozmihci S. Hydrogen gas production from cheese whey powder (CWP) solution by thermophilic dark fermentation. Int J Hydrogen Energy 2012;37:2260–6. doi:10.1016/j.ijhydene.2011.11.018.
[76] Azbar N, Dokgöz FT, Keskin T, Eltem R, Korkmaz KS, Gezgin Y, et al. Comparative evaluation of bio-hydrogen production from cheese whey wastewater under thermophilic and mesophilic anaerobic conditions. Int J Green Energy 2009;6:192–200. doi:10.1080/15435070902785027.
[77] Mars AE, Veuskens T, Budde MAW, Doeveren PFNM, Lips SJ, Bakker RR, et al. Biohydrogen production from untreated and hydrolyzed potato steam peels by the extreme thermophiles Caldicellulosiruptor saccharolyticus and Thermotoga neapolitana. Int J Hydrogen Energy 2010;35:7730–7. doi:10.1016/j.ijhydene.2010.05.063.
[78] Gómez X, Cuetos MJ, Prieto JI, Morán A. Bio-hydrogen production from waste fermentation: Mixing and static conditions. Renew Energy 2009;34:970–5. doi:10.1016/j.renene.2008.08.011.
[79] Bartacek J, Zabranska J, Lens PNL. Developments and constraints in fermentative hydrogen production. Biofuels, Bioprod Biorefining 2007;1:201–14. doi:10.1002/bbb.17.
[80] Hawkes FR, Dinsdale R, Hawkes DL, Hussy I. Sustainable fermentative hydrogen production: Challenges for process optimisation. Int J Hydrogen Energy 2002;27:1339–47. doi:10.1016/S0360-3199(02)00090-3.
[81] Yokoi H, Ohkawara T, Hirose J, Hayashi S, Takasaki Y. Characteristics of hydrogen production by aciduric Enterobacter aerogenes strain HO-39. J Ferment Bioeng 1995;80:571–4. doi:10.1016/0922-338X(96)87733-6.
[82] Ghimire A, Frunzo L, Pirozzi F, Trably E, Escudie R, Lens PNL, et al. A review on dark fermentative biohydrogen production from organic biomass: Process parameters and use of by-products. Appl Energy 2015;144:73–95.

doi:10.1016/j.apenergy.2015.01.045.
[83] Kumar N, Das D. Enhancement of hydrogen production by Enterobacter cloacae IIT-BT 08. Process Biochem 2000;35:589–93. doi:10.1016/S0032-9592(99)00109-0.
[84] Oh S-E, Iyer P, Bruns MA, Logan BE. Biological hydrogen production using a membrane bioreactor. Biotechnol Bioeng 2004;87:119–27. doi:10.1002/bit.20127.
[85] Morimoto M, Atsuko M, Atif AAY, Ngan MA, Fakhru'l-Razi A, Iyuke SE, et al. Biological production of hydrogen from glucose by natural anaerobic microflora. Int J Hydrogen Energy 2004;29:709–13. doi:10.1016/j.ijhydene.2003.09.009.
[86] Lin C, Chang R-C. Fermentative hydrogen production at ambient temperature. Int J Hydrogen Energy 2004;29:715–20. doi:10.1016/j.ijhydene.2003.09.002.
[87] Fang HHP, Liu H. Effect of pH on hydrogen production from glucose by a mixed culture. Bioresour Technol 2002;82:87–93. doi:10.1016/S0960-8524(01)00110-9.
[88] Kotsopoulos TA, Zeng RJ, Angelidaki I. Biohydrogen production in granular up-flow anaerobic sludge blanket (UASB) reactors with mixed cultures under hyper-thermophilic temperature (70 degrees C). Biotechnol Bioeng 2006;94:296–302. doi:10.1002/bit.20844.
[89] Chang JS, Lee KS, Lin PJ. Biohydrogen production with fixed-bed bioreactors. Int J Hydrogen Energy 2002;27:1167–74. doi:10.1016/S0360-3199(02)00130-1.
[90] Logan BE, Oh SE, Kim IS, Van Ginkel S. Biological hydrogen production measured in batch anaerobic respirometers. Environ Sci Technol 2002;36:2530–5. doi:10.1021/es015783i.
[91] Chen CC, Lin CY, Chang JS. Kinetics of hydrogen production with continuous anaerobic cultures utilizing sucrose as the limiting substrate. Appl Microbiol Biotechnol 2001;57:56–64. doi:10.1007/s002530100747.
[92] Mu Y, Yu HQ, Wang G. Evaluation of three methods for enriching H2-producing cultures from anaerobic sludge. Enzyme Microb Technol 2007;40:947–53. doi:10.1016/j.enzmictec.2006.07.033.
[93] Collet C, Adler N, Schwitzguébel JP, Péringer P. Hydrogen production by Clostridium thermolacticum during continuous fermentation of lactose. Int J Hydrogen Energy 2004;29:1479–85. doi:10.1016/j.ijhydene.2004.02.009.
[94] Ren N, Guo W, Liu B, Cao G, Ding J. Biological hydrogen production by dark fermentation: Challenges and prospects towards scaled-up production. Curr Opin Biotechnol 2011;22:365–70. doi:10.1016/j.copbio.2011.04.022.
[95] Zhang SC, Lai QH, Lu Y, Liu ZD, Wang TM, Zhang C, et al. Enhanced biohydrogen production from corn stover by the combination of Clostridium cellulolyticum and hydrogen fermentation bacteria. J Biosci Bioeng 2016;122:482–7. doi:10.1016/j.jbiosc.2016.03.014.
[96] Show KY, Lee DJ, Tay JH, Lin CY, Chang JS. Biohydrogen production: Current perspectives and the way forward. Int J Hydrogen Energy 2012;37:15616–31. doi:10.1016/j.ijhydene.2012.04.109.
[97] Chookaew T, Prasertsan P, Ren ZJ. Two-stage conversion of crude glycerol to energy using dark fermentation linked with microbial fuel cell or microbial electrolysis cell. N Biotechnol 2014;31:179–84. doi:10.1016/j.nbt.2013.12.004.
[98] Varrone C, Rosa S, Fiocchetti F, Giussani B, Izzo G, Massini G, et al. Enrichment of activated sludge for enhanced hydrogen production from crude glycerol. Int J Hydrogen Energy 2013;38:1319–31. doi:10.1016/j.ijhydene.2012.11.069.
[99] Shin HS, Youn JH, Kim SH. Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis. Int J Hydrogen Energy 2004;29:1355–63. doi:10.1016/j.ijhydene.2003.09.011.
[100] Kim S-H, Han S-K, Shin H-S. Feasibility of biohydrogen production by anaerobic co-

digestion of food waste and sewage sludge. Int J Hydrogen Energy 2004;29:1607–16. doi:10.1016/j.ijhydene.2004.02.018.
[101] Wang CC, Chang CW, Chu CP, Lee DJ, Chang B V., Liao CS. Producing hydrogen from wastewater sludge by Clostridium bifermentans. J Biotechnol 2003;102:83–92. doi:10.1016/S0168-1656(03)00007-5.
[102] Cai M, Liu J, Wei Y. Enhanced biohydrogen production from sewage sludge with alkaline pretreatment. Environ Sci Technol 2004;38:3195–202. doi:10.1021/es0349204.
[103] Guo L, Li XM, Bo X, Yang Q, Zeng GM, Liao D xiang, et al. Impacts of sterilization, microwave and ultrasonication pretreatment on hydrogen producing using waste sludge. Bioresour Technol 2008;99:3651–8. doi:10.1016/j.biortech.2007.07.026.
[104] Alibardi L, Cossu R. Composition variability of the organic fraction of municipal solid waste and effects on hydrogen and methane production potentials. Waste Manag 2015;36:147–55. doi:10.1016/j.wasman.2014.11.019.
[105] Karlsson A, Vallin L, Ejlertsson J. Effects of temperature, hydraulic retention time and hydrogen extraction rate on hydrogen production from the fermentation of food industry residues and manure. Int J Hydrogen Energy 2008;33:953–62. doi:10.1016/j.ijhydene.2007.10.055.
[106] Jayalakshmi S, Joseph K, Sukumaran V. Bio hydrogen generation from kitchen waste in an inclined plug flow reactor. Int J Hydrogen Energy 2009;34:8854–8. doi:10.1016/j.ijhydene.2009.08.048.
[107] Zahedi S, Sales D, Romero LI, Solera R. Hydrogen production from the organic fraction of municipal solid waste in anaerobic thermophilic acidogenesis: Influence of organic loading rate and microbial content of the solid waste. Bioresour Technol 2013;129:85–91. doi:10.1016/j.biortech.2012.11.003.
[108] Gómez X, Moran A, Cuetos MJ, Sanchez ME. The production of hydrogen by dark fermentation of municipal solid wastes and slaughterhouse waste: A two-phase process. J Power Sources 2006;157:727–32. doi:10.1016/j.jpowsour.2006.01.006.
[109] Chu CF, Xu KQ, Li YY, Inamori Y. Hydrogen and methane potential based on the nature of food waste materials in a two-stage thermophilic fermentation process. Int J Hydrogen Energy 2012;37:10611–8. doi:10.1016/j.ijhydene.2012.04.048.
[110] Nathao C, Sirisukpoka U, Pisutpaisal N. Production of hydrogen and methane by one and two stage fermentation of food waste. Int J Hydrogen Energy 2013;38:15764–9. doi:10.1016/j.ijhydene.2013.05.047.
[111] Redondas V, Gómez X, García S, Pevida C, Rubiera F, Morán A, et al. Hydrogen production from food wastes and gas post-treatment by CO2 adsorption. Waste Manag 2012;32:60–6. doi:10.1016/j.wasman.2011.09.003.
[112] Mohd Yasin NH, Rahman NA, Man HC, Mohd Yusoff MZ, Hassan MA. Microbial characterization of hydrogen-producing bacteria in fermented food waste at different pH values. Int J Hydrogen Energy 2011;36:9571–80. doi:10.1016/j.ijhydene.2011.05.048.
[113] Pan J, Zhang R, El-Mashad HM, Sun H, Ying Y. Effect of food to microorganism ratio on biohydrogen production from food waste via anaerobic fermentation. Int J Hydrogen Energy 2008;33:6968–75. doi:10.1016/j.ijhydene.2008.07.130.
[114] Wang S, Ma Z, Zhang T, Bao M, Su H. Optimization and modeling of biohydrogen production by mixed bacterial cultures from raw cassava starch. Front Chem Sci Eng 2017;11:100–6. doi:10.1007/s11705-017-1617-3.
[115] Strik DPBTB, Domnanovich AM, Holubar P. A pH-based control of ammonia in biogas during anaerobic digestion of artificial pig manure and maize silage. Process Biochem 2006;41:1235–8. doi:10.1016/j.procbio.2005.12.008.

[116] Wu X, Zhu J, Dong C, Miller C, Li Y, Wang L, et al. Continuous biohydrogen production from liquid swine manure supplemented with glucose using an anaerobic sequencing batch reactor. Int J Hydrogen Energy 2009;34:6636–45. doi:10.1016/j.ijhydene.2009.06.058.
[117] Xing Y, Li Z, Fan Y, Hou H. Biohydrogen production from dairy manures with acidification pretreatment by anaerobic fermentation. Environ Sci Pollut Res 2010;17:392–9. doi:10.1007/s11356-009-0187-4.
[118] Ghimire A, Frunzo L, Pontoni L, d'Antonio G, Lens PNL, Esposito G, et al. Dark fermentation of complex waste biomass for biohydrogen production by pretreated thermophilic anaerobic digestate. J Environ Manage 2015;152:43–8. doi:10.1016/j.jenvman.2014.12.049.
[119] Tang G-L, Huang J, Sun Z-J, Tang Q-Q, Yan C-H, Liu G-Q. Biohydrogen production from cattle wastewater by enriched anaerobic mixed consortia: influence of fermentation temperature and pH. J Biosci Bioeng 2008. doi:10.1263/jbb.106.80.
[120] Ntaikou I, Kourmentza C, Koutrouli EC, Stamatelatou K, Zampraka A, Kornaros M, et al. Exploitation of olive oil mill wastewater for combined biohydrogen and biopolymers production. Bioresour Technol 2009;100:3724–30. doi:10.1016/j.biortech.2008.12.001.
[121] Mohammadi P, Ibrahim S, Mohamad Annuar MS, Law S. Effects of different pretreatment methods on anaerobic mixed microflora for hydrogen production and COD reduction from palm oil mill effluent. J Clean Prod 2011;19:1654–8. doi:10.1016/j.jclepro.2011.05.009.
[122] O-Thong S, Prasertsan P, Intrasungkha N, Dhamwichukorn S, Birkeland NK. Improvement of biohydrogen production and treatment efficiency on palm oil mill effluent with nutrient supplementation at thermophilic condition using an anaerobic sequencing batch reactor. Enzyme Microb Technol 2007;41:583–90. doi:10.1016/j.enzmictec.2007.05.002.
[123] Nakashimada Y, Rachman MA, Kakizono T, Nishio N. Hydrogen production of Enterobacter aerogenes altered by extracellular and intracellular redox states. Int J Hydrogen Energy 2002;27:1399–405. doi:10.1016/S0360-3199(02)00128-3.
[124] Ito T, Nakashimada Y, Senba K, Matsui T, Nishio N. Hydrogen and ethanol production from glycerol-containing wastes discharged after biodiesel manufacturing process. J Biosci Bioeng 2005;100:260–5. doi:Doi 10.1263/Jbb.100.260.
[125] Silva FMS, Oliveira LB, Mahler CF, Bassin JP. Hydrogen production through anaerobic co-digestion of food waste and crude glycerol at mesophilic conditions. Int J Hydrogen Energy 2017;42:22720–9. doi:10.1016/j.ijhydene.2017.07.159.
[126] Zhu G-F, Wu P, Wei Q-S, Lin J, Gao Y-L, Liu H-N. Biohydrogen production from purified terephthalic acid (PTA) processing wastewater by anaerobic fermentation using mixed microbial communities. Int J Hydrogen Energy 2010;35:8350–6. doi:10.1016/j.ijhydene.2009.12.003.
[127] Yang H, Shao P, Lu T, Shen J, Wang D, Xu Z, et al. Continuous bio-hydrogen production from citric acid wastewater via facultative anaerobic bacteria. Int J Hydrogen Energy 2006;31:1306–13. doi:10.1016/j.ijhydene.2005.11.018.
[128] Venetsaneas N, Antonopoulou G, Stamatelatou K, Kornaros M, Lyberatos G. Using cheese whey for hydrogen and methane generation in a two-stage continuous process with alternative pH controlling approaches. Bioresour Technol 2009;100:3713–7. doi:10.1016/j.biortech.2009.01.025.
[129] Vijayaraghavan K, Ahmad D. Biohydrogen generation from palm oil mill effluent using anaerobic contact filter. Int J Hydrogen Energy 2006;31:1284–91. doi:10.1016/j.ijhydene.2005.12.002.

[130] Dounavis AS, Ntaikou I, Lyberatos G. Production of biohydrogen from crude glycerol in an upflow column bioreactor. Bioresour Technol 2015;198:701–8. doi:10.1016/j.biortech.2015.09.072.
[131] Chu C-Y, Tung L, Lin C-Y. Effect of substrate concentration and pH on biohydrogen production kinetics from food industry wastewater by mixed culture. Int J Hydrogen Energy 2013;38:15849–55. doi:10.1016/j.ijhydene.2013.07.088.
[132] Kumar G, Sen B, Sivagurunathan P, Lin CY. High rate hydrogen fermentation of cello-lignin fraction in de-oiled jatropha waste using hybrid immobilized cell system. Fuel 2016;182:131–40. doi:10.1016/j.fuel.2016.05.088.
[133] Reungsang A, Sittijunda S, O-Thong S. Bio-hydrogen production from glycerol by immobilized Enterobacter aerogenes ATCC 13048 on heat-treated UASB granules as affected by organic loading rate. Int J Hydrogen Energy 2013;38:6970–9. doi:10.1016/j.ijhydene.2013.03.082.
[134] Sittijunda S, Pattra S. Evaluation of different pretreatment methods to prepare an inoculum for bio-hydrogen production from cassava starch wastewater. KKU Res J 2016;21:81–92.
[135] Pierucci S, Klemeš JJ, Piazza L, Bakalis S, Kanchanasuta S, Kittipongpattana K, et al. Improvement of Biohydrogen Fermentation by Co-digestion of Crude Glycerol with Palm Oil Decanter Cake. Chem Eng Trans 2017;57. doi:10.3303/CET1757328.
[136] Kumar G, Bakonyi P, Periyasamy S, Kim SH, Nemestóthy N, Bélafi-Bakó K. Lignocellulose biohydrogen: Practical challenges and recent progress. Renew Sustain Energy Rev 2015;44:728–37. doi:10.1016/j.rser.2015.01.042.
[137] Sharma A, Arya SK. Hydrogen from algal biomass: A review of production process. Biotechnol Reports 2017;15:63–9. doi:10.1016/j.btre.2017.06.001.
[138] Ren NQ, Zhao L, Chen C, Guo WQ, Cao GL. A review on bioconversion of lignocellulosic biomass to H2: Key challenges and new insights. Bioresour Technol 2016;215:92–9. doi:10.1016/j.biortech.2016.03.124.
[139] Jiang H, Gadow SI, Tanaka Y, Cheng J, Li YY. Improved cellulose conversion to bio-hydrogen with thermophilic bacteria and characterization of microbial community in continuous bioreactor. Biomass and Bioenergy 2015;75:57–64. doi:10.1016/j.biombioe.2015.02.010.
[140] Talluri S, Raj SM, Christopher LP. Consolidated bioprocessing of untreated switchgrass to hydrogen by the extreme thermophile Caldicellulosiruptor saccharolyticus DSM 8903. Bioresour Technol 2013;139:272–9. doi:10.1016/j.biortech.2013.04.005.
[141] Cha M, Chung D, Elkins JG, Guss AM, Westpheling J. Metabolic engineering of Caldicellulosiruptor bescii yields increased hydrogen production from lignocellulosic biomass. Biotechnol Biofuels 2013;6:85. doi:10.1186/1754-6834-6-85.
[142] Thomas L, Joseph A, Gottumukkala LD. Xylanase and cellulase systems of Clostridium sp.: An insight on molecular approaches for strain improvement. Bioresour Technol 2014;158:343–50. doi:10.1016/j.biortech.2014.01.140.
[143] Sheng T, Gao L, Zhao L, Liu W, Wang A. Direct hydrogen production from lignocellulose by the newly isolated Thermoanaerobacterium thermosaccharolyticum strain DD32. RSC Adv 2015;5:99781–8. doi:10.1039/C5RA20000H.
[144] Nguyen TAD, Kim KR, Kim MS, Sim SJ. Thermophilic hydrogen fermentation from Korean rice straw by Thermotoga neapolitana. Int J Hydrogen Energy 2010;35:13392–8. doi:10.1016/j.ijhydene.2009.11.112.
[145] Chen CC, Chuang YS, Lin CY, Lay CH, Sen B. Thermophilic dark fermentation of untreated rice straw using mixed cultures for hydrogen production. Int J Hydrogen Energy 2012;37:15540–6. doi:10.1016/j.ijhydene.2012.01.036.

[146] Cao G-L, Zhao L, Wang A-J, Wang Z-Y, Ren N-Q. Single-step bioconversion of lignocellulose to hydrogen using novel moderately thermophilic bacteria. Biotechnol Biofuels 2014;7:82. doi:10.1186/1754-6834-7-82.
[147] Cui M, Shen J. Effects of acid and alkaline pretreatments on the biohydrogen production from grass by anaerobic dark fermentation. Int J Hydrogen Energy 2012;37:1120–4. doi:10.1016/j.ijhydene.2011.02.078.
[148] Magnusson L, Islam R, Sparling R, Levin D, Cicek N. Direct hydrogen production from cellulosic waste materials with a single-step dark fermentation process. Int J Hydrogen Energy 2008;33:5398–403. doi:10.1016/j.ijhydene.2008.06.018.
[149] Zhang JN, Li YH, Zheng HQ, Fan YT, Hou HW. Direct degradation of cellulosic biomass to bio-hydrogen from a newly isolated strain Clostridium sartagoforme FZ11. Bioresour Technol 2015;192:60–7. doi:10.1016/j.biortech.2015.05.034.
[150] Han H, Wei L, Liu B, Yang H, Shen J. Optimization of biohydrogen production from soybean straw using anaerobic mixed bacteria. Int J Hydrogen Energy 2012;37:13200–8. doi:10.1016/j.ijhydene.2012.03.073.
[151] Kogo T, Yoshida Y, Koganei K, Matsumoto H, Watanabe T, Ogihara J, et al. Production of rice straw hydrolysis enzymes by the fungi Trichoderma reesei and Humicola insolens using rice straw as a carbon source. Bioresour Technol 2017;233:67–73. doi:10.1016/j.biortech.2017.01.075.
[152] Cheng N, Koda K, Tamai Y, Yamamoto Y, Takasuka TE, Uraki Y. Optimization of simultaneous saccharification and fermentation conditions with amphipathic lignin derivatives for concentrated bioethanol production. Bioresour Technol 2017;232:126–32. doi:10.1016/j.biortech.2017.02.018.
[153] Monlau F, Aemig Q, Trably E, Hamelin J, Steyer JP, Carrere H. Specific inhibition of biohydrogen-producing Clostridium sp. after dilute-acid pretreatment of sunflower stalks. Int J Hydrogen Energy 2013;38:12273–82. doi:10.1016/j.ijhydene.2013.07.018.
[154] Guo P, Mochidzuki K, Cheng W, Zhou M, Gao H, Zheng D, et al. Effects of different pretreatment strategies on corn stalk acidogenic fermentation using a microbial consortium. Bioresour Technol 2011;102:7526–31. doi:10.1016/j.biortech.2011.04.083.
[155] Panagiotopoulos IA, Bakker RR, De Vrije T, Koukios EG, Claassen PAM. Pretreatment of sweet sorghum bagasse for hydrogen production by Caldicellulosiruptor saccharolyticus. Int J Hydrogen Energy 2010;35:7738–47. doi:10.1016/j.ijhydene.2010.05.075.
[156] Cao GL, Guo WQ, Wang AJ, Zhao L, Xu CJ, Zhao QL, et al. Enhanced cellulosic hydrogen production from lime-treated cornstalk wastes using thermophilic anaerobic microflora. Int J Hydrogen Energy 2012;37:13161–6. doi:10.1016/j.ijhydene.2012.03.137.
[157] Feng X, Wang H, Wang Y, Wang X, Huang J. Biohydrogen production from apple pomace by anaerobic fermentation with river sludge. Int J Hydrogen Energy 2010;35:3058–64. doi:10.1016/j.ijhydene.2009.07.015.
[158] Wu J, Ein-Mozaffari F, Upreti S. Effect of ozone pretreatment on hydrogen production from barley straw. Bioresour Technol 2013;144:344–9. doi:10.1016/j.biortech.2013.07.001.
[159] Zhao L, Cao GL, Wang AJ, Ren HY, Dong D, Liu ZN, et al. Fungal pretreatment of cornstalk with Phanerochaete chrysosporium for enhancing enzymatic saccharification and hydrogen production. Bioresour Technol 2012;114:365–9. doi:10.1016/j.biortech.2012.03.076.
[160] Zhao L, Cao GL, Wang AJ, Guo WQ, Ren HY, Ren NQ. Simultaneous saccharification and fermentation of fungal pretreated cornstalk for hydrogen

production using Thermoanaerobacterium thermosaccharolyticum W16. Bioresour Technol 2013;145:103–7. doi:10.1016/j.biortech.2013.01.144.
[161] Saritha M, Arora A, Lata. Biological Pretreatment of Lignocellulosic Substrates for Enhanced Delignification and Enzymatic Digestibility. Indian J Microbiol 2012;52:122–30. doi:10.1007/s12088-011-0199-x.
[162] Zheng Y, Zhao J, Xu F, Li Y. Pretreatment of lignocellulosic biomass for enhanced biogas production. Prog Energy Combust Sci 2014;42:35–53. doi:10.1016/j.pecs.2014.01.001.
[163] Agbor VB, Cicek N, Sparling R, Berlin A, Levin DB. Biomass pretreatment: Fundamentals toward application. Biotechnol Adv 2011;29:675–85. doi:10.1016/j.biotechadv.2011.05.005.
[164] Eskicioglu C, Monlau F, Barakat A, Ferrer I, Kaparaju P, Trably E, et al. Assessment of hydrothermal pretreatment of various lignocellulosic biomass with CO2 catalyst for enhanced methane and hydrogen production. Water Res 2017;120:32–42. doi:10.1016/j.watres.2017.04.068.
[165] Gonzales RR, Kim S-H. Dark fermentative hydrogen production following the sequential dilute acid pretreatment and enzymatic saccharification of rice husk. Int J Hydrogen Energy 2017:1–7. doi:10.1016/j.ijhydene.2017.08.185.
[166] Xu J, Deshusses MA. Fermentation of swine wastewater-derived duckweed for biohydrogen production. Int J Hydrogen Energy 2015;40:7028–36. doi:10.1016/j.ijhydene.2015.03.166.
[167] Lin R, Cheng J, Ding L, Song W, Zhou J, Cen K. Inhibitory effects of furan derivatives and phenolic compounds on dark hydrogen fermentation. Bioresour Technol 2015;196:250–5. doi:10.1016/j.biortech.2015.07.097.
[168] Kumar AK, Sharma S. Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. Bioresour Bioprocess 2017;4:7. doi:10.1186/s40643-017-0137-9.
[169] Khan MI, Lee MG, Shin JH, Kim JD. Pretreatment optimization of the biomass of Microcystis aeruginosa for efficient bioethanol production. AMB Express 2017;7:19. doi:10.1186/s13568-016-0320-y.
[170] Gonzales RR, Sivagurunathan P, Parthiban A, Kim SH. Optimization of substrate concentration of dilute acid hydrolyzate of lignocellulosic biomass in batch hydrogen production. Int Biodeterior Biodegrad 2016;113:22–7. doi:10.1016/j.ibiod.2016.04.016.
[171] Zhi Z, Wang H. White-rot fungal pretreatment of wheat straw with Phanerochaete chrysosporium for biohydrogen production: Simultaneous saccharification and fermentation. Bioprocess Biosyst Eng 2014;37:1447–58. doi:10.1007/s00449-013-1117-x.
[172] Pattra S, Sangyoka S, Boonmee M, Reungsang A. Bio-hydrogen production from the fermentation of sugarcane bagasse hydrolysate by Clostridium butyricum. Int J Hydrogen Energy 2008;33:5256–65. doi:10.1016/j.ijhydene.2008.05.008.
[173] De Vrije T, De Haas G, Tan GB, Keijsers ERP, Claassen PAM. Pretreatment of Miscanthus for hydrogen production by Thermotoga elfii. Int J Hydrogen Energy 2002;27:1381–90. doi:10.1016/S0360-3199(02)00124-6.
[174] Nguyen TAD, Han SJ, Kim JP, Kim MS, Oh YK, Sim SJ. Hydrogen production by the hyperthermophilic eubacterium, Thermotoga neapolitana, using cellulose pretreated by ionic liquid. Int J Hydrogen Energy 2008;33:5161–8. doi:10.1016/j.ijhydene.2008.05.019.
[175] Wu KJ, Chang JS. Batch and continuous fermentative production of hydrogen with anaerobic sludge entrapped in a composite polymeric matrix. Process Biochem

2007;42:279–84. doi:10.1016/j.procbio.2006.07.021.
[176] Xing D, Ren N, Wang A, Li Q, Feng Y, Ma F. Continuous hydrogen production of auto-aggregative Ethanoligenens harbinense YUAN-3 under non-sterile condition. Int J Hydrogen Energy 2008;33:1489–95. doi:10.1016/j.ijhydene.2007.09.038.
[177] Liu GGZ, Shen JJQ. Effects of culture and medium conditions on hydrogen production from starch using anaerobic bacteria. J Biosci Bioeng 2004;98:251–256. doi:10.1016/S1389-1723(04)00277-4.
[178] Akutsu Y, Li Y-Y, Harada H, Yu H-Q. Effects of temperature and substrate concentration on biological hydrogen production from starch. Int J Hydrogen Energy 2009;34:2558–66. doi:10.1016/j.ijhydene.2009.01.048.
[179] Zhang ML, Fan YT, Xing Y, Pan CM, Zhang GS, Lay JJ. Enhanced biohydrogen production from cornstalk wastes with acidification pretreatment by mixed anaerobic cultures. Biomass and Bioenergy 2007;31:250–4. doi:10.1016/j.biombioe.2006.08.004.
[180] Yu H, Zhu Z, Hu W, Zhang H. Hydrogen production from rice winery wastewater in an upflow anaerobic reactor by using mixed anaerobic cultures. Int J Hydrogen Energy 2002;27:1359–65. doi:10.1016/S0360-3199(02)00073-3.
[181] Slezak R, Grzelak J, Krzystek L, Ledakowicz S. The effect of initial organic load of the kitchen waste on the production of VFA and H 2 in dark fermentation. Waste Manag 2017;68:610–7. doi:10.1016/j.wasman.2017.06.024.
[182] Eker S, Sarp M. Hydrogen gas production from waste paper by dark fermentation: Effects of initial substrate and biomass concentrations. Int J Hydrogen Energy 2017;42:2562–8. doi:10.1016/j.ijhydene.2016.04.020.
[183] Liu Y, Chen T, Yang M, Wang C, Huo W, Yan D, et al. Analysis of mixtures of fatty acids and fatty alcohols in fermentation broth. J Chromatogr A 2014;1323:66–72. doi:10.1016/j.chroma.2013.10.094.
[184] Nandi R, Sengupta S, Zajic J, Kosaric N, Brosseau J, Nandi R, et al. Microbial production of hydrogen: an overview. Crit Rev Microbiol 1998;24:61–84. doi:10.1080/10408419891294181.
[185] Hallenbeck PC. Fundamentals of the fermentative production of hydrogen. Water Sci Technol 2005;52:21–9.
[186] Nandi R, Sengupta S. Microbial production of hydrogen: an overview. Crit Rev Microbiol 1998;24:61–84. doi:10.1080/10408419891294181.
[187] Kim SH, Han SK, Shin HS. Effect of substrate concentration on hydrogen production and 16S rDNA-based analysis of the microbial community in a continuous fermenter. Process Biochem 2006;41:199–207. doi:10.1016/j.procbio.2005.06.013.
[188] Evvyernie D, Yamazaki S, Morimoto K, Karita S, Kimura T, Sakka K, et al. Identification and characterization of Clostridium paraputrificum M-21, a chitinolytic, mesophilic and hydrogen-producing bacterium. J Biosci Bioeng 2000. doi:10.1016/S1389-1723(00)80063-8.
[189] Tanisho S, Ishiwata Y. Continuous hydrogen production from molasses by the bacterium Enterobacter aerogenes. Int J Hydrogen Energy 1994. doi:10.1016/0360-3199(94)90197-X.
[190] Yokoi H, Saitsu A, Uchida H, Hirose J, Hayashi S, Takasaki Y. Microbial hydrogen production from sweet potato starch residue. J Biosci Bioeng 2001. doi:10.1016/S1389-1723(01)80112-2.
[191] Van Niel EWJ, Budde MAW, De Haas G, Van der Wal FJ, Claassen PAM, Stams AJM. Distinctive properties of high hydrogen producing extreme thermophiles, Caldicellulosiruptor saccharolyticus and Thermotoga elfii. Int J Hydrogen Energy 2002;27:1391–8. doi:10.1016/S0360-3199(02)00115-5.
[192] Sparling R. Hydrogen production from inhibited anaerobic composters. Int J Hydrogen

Energy 1997. doi:10.1016/S0360-3199(96)00137-1.
[193] Lay JJ, Lee YJ, Noike T. Feasibility of biological hydrogen production from organic fraction of municipal solid waste. Water Res 1999. doi:Doi 10.1016/S0043-1354(98)00483-7.
[194] Iannotti EL, Kafkewit D, Wolin MJ, Bryant MP. Glucose Fermentation Products of Ruminococcus-Albus Grown in Continuous Culture with Vibrio-Succinogenes - Changes Caused by Interspecies Transfer of H2. J Bacteriol 1973;114:1231–40.
[195] Brosseau JD, Zajic JE. Hydrogen-gas production with Citrobacter intermedim and Clostridium pasteurianum. J Chem Technol Biotechnol 2007;32:496–502. doi:10.1002/jctb.5030320310.
[196] Li C, Fang HHP. Fermentative Hydrogen Production From Wastewater and Solid Wastes by Mixed Cultures. Crit Rev Environ Sci Technol 2007;37:1–39. doi:10.1080/10643380600729071.
[197] Valdez-Vazquez I, Ríos-Leal E, Esparza-García F, Cecchi F, Poggi-Varaldo HM. Semi-continuous solid substrate anaerobic reactors for H2 production from organic waste: Mesophilic versus thermophilic regime. Int J Hydrogen Energy 2005;30:1383–91. doi:10.1016/j.ijhydene.2004.09.016.
[198] Wang J, Wan W. Effect of temperature on fermentative hydrogen production by mixed cultures. Int J Hydrogen Energy 2008;33:5392–7. doi:10.1016/j.ijhydene.2008.07.010.
[199] Singh L, Wahid ZA. Methods for enhancing bio-hydrogen production from biological process: A review. J Ind Eng Chem 2015;21:70–80. doi:10.1016/j.jiec.2014.05.035.
[200] Valdez-Vazquez I, Poggi-Varaldo HM. Hydrogen production by fermentative consortia. Renew Sustain Energy Rev 2009;13:1000–13. doi:10.1016/j.rser.2008.03.003.
[201] Kraemer JT, Bagley DM. Improving the yield from fermentative hydrogen production. Biotechnol Lett 2007;29:685–95. doi:10.1007/s10529-006-9299-9.
[202] Pandu K, Joseph S. Comparisons and Limitations of Biohydrogen Production Processes: a Review. Int J Adv Eng Technol 2012;2:2231–1963.
[203] Yasin NHM, Mumtaz T, Hassan MA, Abd Rahman N. Food waste and food processing waste for biohydrogen production: A review. J Environ Manage 2013;130:375–85. doi:10.1016/j.jenvman.2013.09.009.
[204] Chaganti SR, Kim DH, Lalman JA. Dark fermentative hydrogen production by mixed anaerobic cultures: Effect of inoculum treatment methods on hydrogen yield. Renew Energy 2012;48:117–21. doi:10.1016/j.renene.2012.04.015.
[205] Cai JL, Wang GC, Li YC, Zhu DL, Pan GH. Enrichment and hydrogen production by marine anaerobic hydrogen-producing microflora. Chinese Sci Bull 2009;54:2656–61. doi:10.1007/s11434-009-0399-5.
[206] Liu H, Wang G, Zhu D, Pan G. Enrichment of the hydrogen-producing microbial community from marine intertidal sludge by different pretreatment methods. Int J Hydrogen Energy 2009;34:9696–701. doi:10.1016/j.ijhydene.2009.10.025.
[207] Pendyala B, Chaganti SR, Lalman JA, Shanmugam SR, Heath DD, Lau PCK. Pretreating mixed anaerobic communities from different sources: Correlating the hydrogen yield with hydrogenase activity and microbial diversity. Int J Hydrogen Energy 2012;37:12175–86. doi:10.1016/j.ijhydene.2012.05.105.
[208] O-Thong S, Prasertsan P, Birkeland NK. Evaluation of methods for preparing hydrogen-producing seed inocula under thermophilic condition by process performance and microbial community analysis. Bioresour Technol 2009;100:909–18. doi:10.1016/j.biortech.2008.07.036.
[209] Ren NQ, Guo WQ, Wang XJ, Xiang WS, Liu BF, Wang XZ, et al. Effects of different pretreatment methods on fermentation types and dominant bacteria for hydrogen

production. Int J Hydrogen Energy 2008;33:4318–24. doi:10.1016/j.ijhydene.2008.06.003.
[210] Argun H, Kargi F. Effects of sludge pre-treatment method on bio-hydrogen production by dark fermentation of waste ground wheat. Int J Hydrogen Energy 2009;34:8543–8. doi:10.1016/j.ijhydene.2009.08.049.
[211] Wang JL, Wan W. Comparison of different pretreatment methods for enriching hydrogen-producing bacteria from digested sludge. Int J Hydrogen Energy 2008;33:2934–41. doi:10.1016/j.ijhydene.2008.03.048.
[212] Madigan MT, Martinko J, Parker J. Brock. Biology of microorganisms. Upper Saddle River NJ: Prentice Hall; 2000.
[213] Zhu H, Béland M. Evaluation of alternative methods of preparing hydrogen producing seeds from digested wastewater sludge. Int J Hydrogen Energy 2006;31:1980–8. doi:10.1016/j.ijhydene.2006.01.019.
[214] Kotay SM, Das D. Novel dark fermentation involving bioaugmentation with constructed bacterial consortium for enhanced biohydrogen production from pretreated sewage sludge. Int J Hydrogen Energy 2009;34:7489–96. doi:10.1016/j.ijhydene.2009.05.109.
[215] Chang S, Li JZ, Liu F. Evaluation of different pretreatment methods for preparing hydrogen-producing seed inocula from waste activated sludge. Renew Energy 2011;36:1517–22. doi:10.1016/j.renene.2010.11.023.
[216] Deublein D, Steinhauser A. Biogas from Waste and Renevable Resources: an Introduction. Weinheim: Wiley-VCH; 2008.
[217] Hu B, Chen S. Pretreatment of methanogenic granules for immobilized hydrogen fermentation. Int J Hydrogen Energy 2007;32:3266–73. doi:10.1016/j.ijhydene.2007.03.005.
[218] Venkata Mohan S, Lalit Babu V, Sarma PN. Effect of various pretreatment methods on anaerobic mixed microflora to enhance biohydrogen production utilizing dairy wastewater as substrate. Bioresour Technol 2008;99:59–67. doi:10.1016/j.biortech.2006.12.004.
[219] Liu H, Wang J, Wang A, Chen J. Chemical inhibitors of methanogenesis and putative applications. Appl Microbiol Biotechnol 2011;89:1333–40. doi:10.1007/s00253-010-3066-5.
[220] Saady NMC, Chaganti SR, Lalman JA, Veeravalli SS, Shanmugam SR, Heath DD. Assessing the impact of palmitic, myristic and lauric acids on hydrogen production from glucose fermentation by mixed anaerobic granular cultures. Int J Hydrogen Energy 2012;37:18761–72. doi:10.1016/j.ijhydene.2012.09.116.
[221] Bundhoo MAZ, Mohee R, Hassan MA. Effects of pre-treatment technologies on dark fermentative biohydrogen production: A review. J Environ Manage 2015;157:20–48. doi:10.1016/j.jenvman.2015.04.006.
[222] Guo Y, Kim S, Sung S, Lee P. Effect of ultrasonic treatment of digestion sludge on bio-hydrogen production from sucrose by anaerobic fermentation. Int J Hydrogen Energy 2010;35:3450–5. doi:10.1016/j.ijhydene.2010.01.090.
[223] Wang H, Fang M, Fang Z, Bu H. Effects of sludge pretreatments and organic acids on hydrogen production by anaerobic fermentation. Bioresour Technol 2010;101:8731–5. doi:10.1016/j.biortech.2010.06.131.
[224] Kim JH, Lee JW, Kim JH, Seo JH, Han SB, Chung HJ, et al. Effect of gamma irradiation on Listeria ivanovii inoculated to iceberg lettuce stored at cold temperature. Food Control 2006;17:397–401. doi:10.1016/j.foodcont.2005.01.008.
[225] Song ZX, Dai Y, Fan QL, Li XH, Fan YT, Hou HW. Effects of pretreatment method of natural bacteria source on microbial community and bio-hydrogen production by dark

fermentation. Int J Hydrogen Energy 2012;37:5631–6. doi:10.1016/j.ijhydene.2012.01.010.
[226] Chan ASK, Parkin TB. Evaluation of potential inhibitors of methanogenesis and methane oxidation in a landfill cover soil. Soil Biol Biochem 2000;32:1581–90. doi:10.1016/S0038-0717(00)00071-7.
[227] Chidthaisong A, Conrad R. Pattern of non-methanogenic and methanogenic degradation of cellulose in anoxic rice field soil. FEMS Microbiol Ecol 2000;31:87–94. doi:doi:10.1111/j.1574-6941.2000.tb00674.x.
[228] Levin DB, Pitt L, Love M. Biohydrogen production: Prospects and limitations to practical application. Int J Hydrogen Energy 2004;29:173–85. doi:10.1016/S0360-3199(03)00094-6.
[229] Zhang T, Liu H, Fang HHP. Biohydrogen production from starch in wastewater under thermophilic condition. J Environ Manage 2003;69:149–56. doi:10.1016/S0301-4797(03)00141-5.
[230] Pakarinen O, Lehtomäki A, Rintala J. Batch dark fermentative hydrogen production from grass silage: The effect of inoculum, pH, temperature and VS ratio. Int J Hydrogen Energy 2008;33:594–601. doi:10.1016/j.ijhydene.2007.10.008.
[231] Guo XM, Trably E, Latrille E, Carrre H, Steyer JP. Hydrogen production from agricultural waste by dark fermentation: A review. Int J Hydrogen Energy 2010;35:10660–73. doi:10.1016/j.ijhydene.2010.03.008.
[232] Jung KW, Kim DH, Kim SH, Shin HS. Bioreactor design for continuous dark fermentative hydrogen production. Bioresour Technol 2011;102:8612–20. doi:10.1016/j.biortech.2011.03.056.
[233] Lee KS, Lin PJ, Chang JS. Temperature effects on biohydrogen production in a granular sludge bed induced by activated carbon carriers. Int J Hydrogen Energy 2006;31:465–72. doi:10.1016/j.ijhydene.2005.04.024.
[234] Foglia D, Wukovits W, Friedl A, Vrije T De, Pieternel a M. Fermentative Hydrogen Production : Influence of Application of Mesophilic and Thermophilic Bacteria on Mass and Energy Balances. Energy 2006;2:815–20.
[235] Zhang Y, Shen J. Effect of temperature and iron concentration on the growth and hydrogen production of mixed bacteria. Int J Hydrogen Energy 2006;31:441–6. doi:10.1016/j.ijhydene.2005.05.006.
[236] Yokoyama H, Waki M, Moriya N, Yasuda T, Tanaka Y, Haga K. Effect of fermentation temperature on hydrogen production from cow waste slurry by using anaerobic microflora within the slurry. Appl Microbiol Biotechnol 2007;74:474–83. doi:10.1007/s00253-006-0647-4.
[237] Dębowski M, Korzeniewska E, Filipkowska Z, Zieliński M, Kwiatkowski R. Possibility of hydrogen production during cheese whey fermentation process by different strains of psychrophilic bacteria. Int J Hydrogen Energy 2014;39:1972–8. doi:10.1016/j.ijhydene.2013.11.082.
[238] Lu L, Ren NQ, Zhao X, Wang HA, Wu D, Xing DF. Hydrogen production, methanogen inhibition and microbial community structures in psychrophilic single-chamber microbial electrolysis cells. Energy Environ Sci 2011;4:1329–36. doi:10.1039/c0ee00588f.
[239] Kotay SM, Das D. Microbial hydrogen production with Bacillus coagulans IIT-BT S1 isolated from anaerobic sewage sludge. Bioresour Technol 2007;98:1183–90. doi:10.1016/j.biortech.2006.05.009.
[240] O-Thong S, Prasertsan P, Karakashev D, Angelidaki I. Thermophilic fermentative hydrogen production by the newly isolated Thermoanaerobacterium thermosaccharolyticum PSU-2. Int J Hydrogen Energy 2008;33:1204–14.

doi:10.1016/j.ijhydene.2007.12.015.
[241] Lin CY, Lay CH, Sen B, Chu CY, Kumar G, Chen CC, et al. Fermentative hydrogen production from wastewaters: A review and prognosis. Int J Hydrogen Energy 2012;37:15632–42. doi:10.1016/j.ijhydene.2012.02.072.
[242] Jiunn-Jyi L, Yu-You L, Noike T. Influences of pH and moisture content on the methane production in high-solids sludge digestion. Water Res 1997. doi:10.1016/S0043-1354(96)00413-7.
[243] Bowles LK, Ellefson WL. Effects of butanol on Clostridium acetobutylicum. Appl Environ Microbiol 1985;50:1165–70.
[244] Li D, Chen H. Biological hydrogen production from steam-exploded straw by simultaneous saccharification and fermentation. Int J Hydrogen Energy 2007;32:1742–8. doi:10.1016/j.ijhydene.2006.12.011.
[245] Wang J, Wan W. Factors influencing fermentative hydrogen production: A review. Int J Hydrogen Energy 2009;34:799–811. doi:10.1016/j.ijhydene.2008.11.015.
[246] Alshiyab H, Kalil MS, Hamid AA, Yusoff W. Effect of some environmental parameters on hydrogen production using C. acetobutylicum. Pakistan J Biol Sci 2008;11:2073–82.
[247] Tapia-Venegas E, Ramirez JE, Donoso-Bravo A, Jorquera L, Steyer JP, Ruiz-Filippi G. Bio-hydrogen production during acidogenic fermentation in a multistage stirred tank reactor. Int J Hydrogen Energy 2013;38:2185–90. doi:10.1016/j.ijhydene.2012.11.077.
[248] Won SG, Baldwin SA, Lau AK, Rezadehbashi M. Optimal operational conditions for biohydrogen production from sugar refinery wastewater in an ASBR. Int J Hydrogen Energy 2013;38:13895–906. doi:10.1016/j.ijhydene.2013.08.071.
[249] Ginkel S V, Sung S, Lay JJ. Biohydrogen production as a function of pH and substrate concentration. Environ Sci Technol 2001;35:4726–30. doi:10.1021/es001979r.
[250] Temudo MF, Kleerebezem R, van Loosdrecht M. Influence of the pH on (open) mixed culture fermentation of glucose: a chemostat study. Biotechnol Bioeng 2007;98:69–79. doi:10.1002/bit.21412.
[251] Wang Y, Mu Y, Yu HQ. Comparative performance of two upflow anaerobic biohydrogen-producing reactors seeded with different sludges. Int J Hydrogen Energy 2007;32:1086–94. doi:10.1016/j.ijhydene.2006.07.016.
[252] Masset J, Hiligsmann S, Hamilton C. Effect of pH on glucose and starch fermentation in batch and sequenced-batch mode with a recently isolated strain of hydrogen-production Clostridium butyricum CWBI 1009. Int J Hydrogen Energy 2010;35:3371–8.
[253] Dabrock B, Bahl H, Gottschalk G. Parameters Affecting Solvent Production by Clostridium pasteurianum. Appl Environ Microbiol 1992;58:1233–9. doi:0099-2240/92/041233-07$02.00/0.
[254] Chen CC, Chen HP, Wu JH, Lin CY. Fermentative hydrogen production at high sulfate concentration. Int J Hydrogen Energy 2008;33:1573–8. doi:10.1016/j.ijhydene.2007.09.042.
[255] Bakonyi P, Nemestóthy N, Simon V, Bélafi-Bakó K. Review on the start-up experiences of continuous fermentative hydrogen producing bioreactors. Renew Sustain Energy Rev 2014. doi:10.1016/j.rser.2014.08.014.
[256] Wu SY, Lin CN, Chang JS. Hydrogen production with immobilized sewage sludge in three phase fluidized bed bioreactors. Biotechnol Prog 2003;19:828–32. doi:10.1021/bp0201354.
[257] Massanet-Nicolau J, Guwy A, Dinsdale R, Premier G, Esteves S. Production of hydrogen from sewage biosolids in a continuously fed bioreactor: Effect of hydraulic retention time and sparging. Int J Hydrogen Energy 2010.

doi:10.1016/j.ijhydene.2009.10.076.
[258] Ottaviano LM, Ramos LR, Botta LS, Am??ncio Varesche MB, Silva EL. Continuous thermophilic hydrogen production from cheese whey powder solution in an anaerobic fluidized bed reactor: Effect of hydraulic retention time and initial substrate concentration. Int J Hydrogen Energy 2017. doi:10.1016/j.ijhydene.2016.11.168.
[259] Veeravalli SS, Chaganti SR, Lalman JA, Heath DD. Fermentative H2 production using a switchgrass steam exploded liquor fed to mixed anaerobic cultures: Effect of hydraulic retention time, linoleic acid and nitrogen sparging. Int J Hydrogen Energy 2014. doi:10.1016/j.ijhydene.2014.04.114.
[260] Rosa PRF, Santos SC, Sakamoto IK, Varesche MBA, Silva EL. Hydrogen production from cheese whey with ethanol-type fermentation: Effect of hydraulic retention time on the microbial community composition. Bioresour Technol 2014. doi:10.1016/j.biortech.2014.03.020.
[261] Zhu J, Li Y, Wu X, Miller C, Chen P, Ruan R. Swine manure fermentation for hydrogen production. Bioresour Technol 2009;100:5472–7. doi:10.1016/j.biortech.2008.11.045.
[262] Antonopoulou G, Gavala HN, Skiadas I V., Angelopoulos K, Lyberatos G. Biofuels generation from sweet sorghum: Fermentative hydrogen production and anaerobic digestion of the remaining biomass. Bioresour Technol 2008;99:110–9. doi:10.1016/j.biortech.2006.11.048.
[263] Chen C., Lin C. Using sucrose as a substrate in an anaerobic hydrogen-producing reactor. Adv Environ Res 2003;7:695–9. doi:10.1016/S1093-0191(02)00035-7.
[264] Lee KS, Lo YC, Lin PJ, Chang JS. Improving biohydrogen production in a carrier-induced granular sludge bed by altering physical configuration and agitation pattern of the bioreactor. Int J Hydrogen Energy 2006;31:1648–57. doi:10.1016/j.ijhydene.2005.12.020.
[265] Wu T, Zhu G, Jha AK, Zou R, Liu L, Huang X, et al. Hydrogen production with effluent from an anaerobic baffled reactor (ABR) using a singlechamber microbial electrolysis cell (MEC). Int J Hydrogen Energy 2013;38:11117–23. doi:10.1016/j.ijhydene.2013.03.029.
[266] Gavala HN, Skiadas I V., Ahring BK. Biological hydrogen production in suspended and attached growth anaerobic reactor systems. Int J Hydrogen Energy 2006;31:1164–75. doi:10.1016/j.ijhydene.2005.09.009.
[267] Chou CH, Wang CW, Huang CC, Lay JJ. Pilot study of the influence of stirring and pH on anaerobes converting high-solid organic wastes to hydrogen. Int J Hydrogen Energy 2008;33:1550–8. doi:10.1016/j.ijhydene.2007.09.031.
[268] Mizuno O, Dinsdale R, Hawkes FR, Hawkes DL, Noike T. Enhancement of hydrogen production from glucose by nitrogen gas sparging. Bioresour Technol 2000;73:59–65. doi:10.1016/S0960-8524(99)00130-3.
[269] Kim DH, Han SK, Kim SH, Shin HS. Effect of gas sparging on continuous fermentative hydrogen production. Int J Hydrogen Energy 2006;31:2158–69. doi:10.1016/j.ijhydene.2006.02.012.
[270] Lee K, Tseng T, Liu Y, Hsiao Y. Enhancing the performance of dark fermentative hydrogen production using a reduced pressure fermentation strategy. Int J Hydrog … 2012;37:15556–62. doi:10.1016/j.ijhydene.2012.04.039.
[271] Mandal B, Nath K, Das D. Improvement of biohydrogen production under decreased partial pressure of H2 by Enterobacter cloacae. Biotechnol Lett 2006;28:831–5. doi:10.1007/s10529-006-9008-8.
[272] Teplyakov V V., Gassanova LG, Sostina EG, Slepova E V., Modigell M, Netrusov AI. Lab-scale bioreactor integrated with active membrane system for hydrogen production:

Experience and prospects. Int J Hydrogen Energy 2002;27:1149–55. doi:10.1016/S0360-3199(02)00093-9.
[273] Nielsen a T, Amandusson H, Bjorklund R, Dannetun H, Ejlertsson J, Ekedahl LG, et al. Hydrogen production from organic waste. Int J Hydrogen Energy 2001;26:547–50. doi:10.1016/S0360-3199(00)00125-7.
[274] Balachandar G, Khanna N, Das D. Biohydrogen Production from Organic Wastes by Dark Fermentation. 1st ed. Elsevier B.V.; 2013. doi:10.1016/B978-0-444-59555-3.00006-4.
[275] Krishnan S, Singh L, Sakinah M, Thakur S, Nasrul M, Otieno A, et al. An investigation of two-stage thermophilic and mesophilic fermentation process for the production of hydrogen and methane from palm oil mill effluent. Environ Prog Sustain Energy 2017. doi:10.1002/ep.12537.
[276] Palazzi E, Fabiano B, Perego P. Process development of continuous hydrogen production by Enterobacter aerogenes in a packed column reactor. Bioprocess Eng 2000;22:0205–13. doi:10.1007/s004490050721.
[277] Wu KJ, Chang CF, Chang JS. Simultaneous production of biohydrogen and bioethanol with fluidized-bed and packed-bed bioreactors containing immobilized anaerobic sludge. Process Biochem 2007;42:1165–71. doi:10.1016/j.procbio.2007.05.012.
[278] Kumar N, Das D. Continuous hydrogen production by immobilized Enterobacter cloacae IIT-BT 08 using lignocellulosic materials as solid matrices. Enzyme Microb Technol 2001;29:280–7. doi:10.1016/S0141-0229(01)00394-5.
[279] Jung KW, Kim DH, Shin HS. Continuous fermentative hydrogen production from coffee drink manufacturing wastewater by applying UASB reactor. Int J Hydrogen Energy 2010;35:13370–8. doi:10.1016/j.ijhydene.2009.11.120.
[280] Singh L, Wahid ZA, Siddiqui MF, Ahmad A, Rahim MHA, Sakinah M. Application of immobilized upflow anaerobic sludge blanket reactor using Clostridium LS2 for enhanced biohydrogen production and treatment efficiency of palm oil mill effluent. Int J Hydrogen Energy 2013;38:2221–9. doi:10.1016/j.ijhydene.2012.12.004.
[281] Intanoo P, Chaimongkol P, Chavadej S. Hydrogen and methane production from cassava wastewater using two-stage upflow anaerobic sludge blanket reactors (UASB) with an emphasis on maximum hydrogen production. Int J Hydrogen Energy 2016. doi:10.1016/j.ijhydene.2015.10.125.
[282] Zhang ZP, Tay JH, Show KY, Yan R, Tee Liang D, Lee DJ, et al. Biohydrogen production in a granular activated carbon anaerobic fluidized bed reactor. Int J Hydrogen Energy 2007;32:185–91. doi:10.1016/j.ijhydene.2006.08.017.
[283] Lin CY, Cheng CH. Fermentative hydrogen production from xylose using anaerobic mixed microflora. Int J Hydrogen Energy 2006. doi:10.1016/j.ijhydene.2005.08.010.
[284] Han S-K, Shin H-S. Performance of an innovative two-stage process converting food waste to hydrogen and methane. J Air Waste Manage Assoc 2004;54:242–9. doi:10.1080/10473289.2004.10470895.
[285] Antonopoulou G, Stamatelatou K, Venetsaneas N, Kornaros M, Lyberatos G. Biohydrogen and methane production from cheese whey in a two-stage anaerobic process. Ind Eng Chem Res 2008;47:5227–33. doi:10.1021/ie071622x.