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Dark hydrogen fermentations

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Abstract

The production of hydrogen is a ubiquitous, natural phenomenon under anoxic or anaerobic conditions. A wide variety of bacteria, in swamps, sewage, hot springs, the rumen of cattle etc. is able to convert organic matter to hydrogen, CO_2 and metabolites like acetic acid, lactate, ethanol and alanine. In general, these bacteria live in the close vicinity of other bacteria which consume these metabolites, including hydrogen, producing their own endproducts like methane and CO_2. In this way, a stable ecosystem is formed where potential feedback inhibition of the hydrogen producers by hydrogen, is annulled by the action of the hydrogen consumers.

In view of the design of a bioprocess for the production of hydrogen from biomass, extreme thermophilic anaerobic bacteria have been selected because of their high yield with respect to hydrogen production. The yield is reported to be approximately 83-100% of the maximal theoretical value of 4 mol hydrogen/mol glucose, in contrast to the strict anaerobic *Clostridia* which produce hydrogen with an approximate yield of 2 mol/mol and the facultative anaerobes which show a H_2 yield of less than 2. Besides optimal H_2 molar yields, high hydrogen production rates are needed. Product formation appeared to be dependent on cell densities. Thermophiles usually grow to low densities and, therefore production rates are expected to be low. High production rates are reported for *Clostridia* and *Enterobacter* of maximal 23 and 58 mmol/L.h, respectively. Hydrogen fermentations by co- and mixed cultures showed production rates of approximately 30-50 mmol/L.h.

5.1 Introduction

This Chapter focusses on the microbial production of hydrogen from biomass by fermentation. In anoxic or anaerobic environments, hydrogen is commonly produced during microbial breakdown of organic compounds. In case organic compounds are the sole carbon and energy source providing metabolic energy, the process is termed 'dark' hydrogen fermentation. When light is required to provide additional energy, the process belongs to the category of photobiological processes (discussed in Chapter 6). In this Chapter, several features of dark hydrogen fermentation are presented and discussed to provide an insight in the state of the art and the presently recognized bottlenecks and R&D challenges associated with the first design of an envisaged production plant for hydrogen from biomass.

An overview of basic hydrogen fermentation processes and hydrogen producing micro-organisms is provided in sections 5.2 and 5.3. In section 5.4 the capacity to utilize various substrates is discussed. The design of a bioprocess for hydrogen from biomass and potential process improvements are dicussed in section 5.5. This section is followed by a description of the economical consequences of this bioprocess in section 5.6. In section 5.7 the current (inter)national developments and programs are summarized. This Chapter is concluded by section 5.8 in which the conclusions and perspectives are presented.

5.2 Physiology of dark hydrogen fermentation

Dark hydrogen production is a ubiquitous phenomenon under anoxic or anaerobic conditions (i.e. no oxygen present as an electron acceptor). A wide variety of bacteria use the reduction of protons to hydrogen to dispose of reducing equivalents which result from primary metabolism. In

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other words: when bacteria grow on organic substrates (heterotrophic growth), these substrates are degraded by oxidation to provide for building blocks and metabolic energy for growth. This oxidation generates electrons which need to be disposed of for maintaining electrical neutrality. In aerobic environments, oxygen is reduced and water is the product. In anaerobic or anoxic environments, other compounds need to act as electron acceptor, e.g. protons, which are reduced to molecular hydrogen (H_2). Other examples of alternative electron acceptors in anaerobic environments are nitrate with nitrogen gas (N_2) as the product or sulfate with dihydrogensulfide (H_2S) as the reduced product. Even organic compounds can act as electron acceptors as e.g. in the microbial production of butanol which is done through the reduction of butyric acid. The capacity to reduce other electron acceptors than oxygen requires the presence of a specific enzyme system in the micro-organisms: hydrogen producing bacteria possess hydrogenase enzymes; nitrate reducing bacteria possess an elaborate set of enzymes catalyzing the stepwise reduction of nitrate to nitrogen etc.

Even though many organic compounds enable the production of hydrogen during dark fermentation, estimations of potential yields are mostly based on hexose conversions. The theoretical yield per mole of glucose is described in the following reaction [1, 2]:

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

$$\Delta G'_0 = -206 \text{ kJ.mol}^{-1}$$

A maximum of 4 moles of H_2 per mole of glucose can be produced concurrently with the production of energy (206 kJ per mole of glucose) which is sufficient to support microbial growth. The remainder of the hydrogen in the hexose is conserved in the byproduct acetate, and under non-ideal circumstances, more reduced products like ethanol, lactate or alanine. The complete oxidation of glucose to H_2 and CO_2 yields a stoichiometry of 12 mole H_2 per mole of glucose but in this case no metabolic energy is obtained. The yield of hydrogen during dark fermentation is severely affected by the partial pressure of the product. At high H_2 partial pressures a metabolic shift to production of more reduced products, like lactate [3] or alanine [4] occurs, thereby decreasing the yield of H_2.

Having established that microbial hydrogen production is a ubiquitous phenomenon, it must be surprising that no hydrogen bubbles are coming out of organic waste piles or the sewer. The underlying reason is the fact that, in natural environments, microbial activity is governed by an ecological niche. This niche is the resultant of many contributing factors including the presence and quality of available organic matter, presence of minerals, temperature, light, pH, salinity, redox potential, synergistic or antagonistic activity of microbial populations etc. The ecological niche governs the activity of certain microbial populations and thus the concentration and variety of the final products, i.e. CO_2, water, hydrogen, nitrate, CH_4 etc. No hydrogen is bubbling out of the sewer since in nature there are numerous other bacteria, which readily consume hydrogen as a source of reducing power. When the aim is to produce hydrogen from organic matter, a specific environment needs to be created in which hydrogen producing microorganisms flourish and others perish (Fig. 1).

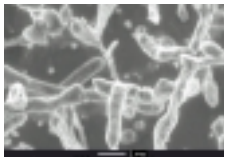
5.3 Hydrogen producing micro-organisms

In reviews by Kosaric and Lyng [5] and Nandi and Sengupta [6] extensive lists of heterotrophic bacteria known to produce hydrogen have been published. An update is listed in this chapter.

5.3.1. Strict anaerobes

5.3.1.1. *Clostridia*

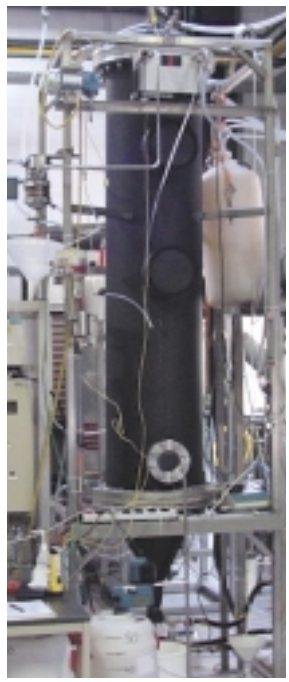
Many anaerobes produce hydrogen from hexoses in acetic acid, butyric acid and acetone-butanol-ethanol fermentations. The highest maximal yield of 4 mole H_2 from 1 mole of glucose is produced in acetic acid fermentations. The production of other, more reduced organic acids and/or alcohols lowers the yield of H_2. For instance the conversion of one mole of glucose into butyrate is accompanied by the production of only 2 moles of H_2. Usually a mixture of products is produced by



A

B1

B2



B3

Figure 1. Growth of Caldicellulosiruptor saccharolyticus (A) on sucrose in flasks (B1) and under controlled conditions in fermentors (B2, B3). Photo of the fermentor (B3) by courtesy of TNO Environment, Energy and Process Innovation, Apeldoorn, The Netherlands.

Clostridia and the available H_2 from glucose is determined by the butyrate/acetate ratio.

C. butyricum, C. welchii, C. pasteurianum, C. beijerincki, newly isolated *Clostridium* spp. and mixtures of *Clostridia* have been used in studies dedicated to produce high amounts of hydrogen. Taguchi and collegues isolated various new *Clostridia* strains. A growing culture of *C. beijerincki* AM21B isolated from termites yielded 1.8 to 2.0 mole H_2 on glucose [7]. The strain could also utilize a large number of other carbohydrates, such as xylose, arabinose, galactose, cellobiose, sucrose, and fructose with efficiencies from 15.7 to 19.0 mmol/g of substrate in batch fermentations of 24 h [8]. H_2 was also produced from starch with equal efficiencies, but sustained production was not achieved and production ceased before the exhaustion of carbohydrates in the medium. Another ***Clostridium*** sp., strain no. 2, also isolated from termites, produced H_2 more efficiently from xylose and arabinose (13.7 and 14.6 mmol/g or 2.1 and 2.2 mol/mol) than from glucose (11.1 mmol/g or 2.0 mol/mol) [9]. These results suggest that both *Clostridia* spp. can be used for the production of H_2 from both cellulose and hemicellulose present in plant biomass. The hydrolysis of biomass for the production of a fermentable substrate can either be done during fermentation, in a simultaneous saccharification and fermentation process, or in a separate process preceding fermentation. Using *Clostridium* sp. strain no.2 it was found that the simultaneous saccharification of xylan with a crude xylanase preparation and hydrogen fermentation of the resulting hydrolysate by strain no. 2 could proceed in a single fermentor [10]. However, simultaneous conversion was less efficient than the independent conversion of the hydrolysate. Furthermore, hydrolysis of a cellulose preparation (Avicel) with a commercial cellulase preparation and hydrogen fermentation of the hydrolysate by strain no. 2 could not proceed in one flask because the condi-

tions required for the enzyme activity and the growth of strain no. 2 differed significantly. Another approach is using *Clostridia* strains which are known to produce cellulase or xylanase activity. At present, no strains are available that are able to hydrolyse both glucans and xylans. Taguchi et al. [11] isolated a novel strain, *Clostridium* sp. strain X53 from wild termites, which produced xylanase in a batch culture and converted xylan to hydrogen. In comparison to xylose, the kinetics of hydrogen production from xylan were not significantly different, but the total yield from xylan was lower than from xylose. *Clostridia* have also been used in continuous hydrogen fermentations on glucose [12, 13, 14, 15, 16]. For *Clostridium* sp. strain no. 2 it has been shown that the maximal H_2 production rate and the molar yield were comparable in batch and continuous fermentations. Continuous H_2 production by fermentation of a continuously produced hydrolysate of cellulose in an aqueous two-phase system (polyethylene glycol and dextran) has been studied. The H_2 production rate and the H_2 yield were higher with Avicel hydrolysate compared to glucose. Since the H_2 yield on Avicel is higher than the theoretical maximum the presence of hydrolyzed dextran in the Avicel hydrolysate was suggested [17]. In continuous fermentations maximal H_2 production rates of 20.4 and 21.7 mmol/L.h have been measured [13, 15] at low yields of 1.4 mole H_2 per mole glucose. Higher yields, 2.4 mol/mol were accompanied by lower H_2 production rates of 7 mmol/L.h [15]. On xylose similar results were obtained, maximal H_2 production rates of 21.0 mmol/L.h have been measured at yields of 1.7 H_2 mol/mol xylose. Hydrogen has also been produced from *N*-acetyl-D-glucosamine and chitin waste products. In batch fermentations by the chitinolytic bacterium *C. paraputrificum* M-21 a H_2 yield of 2.2 mol/mol *N*-acetyl-D-glucosamine has been obtained at production rates of 31 mmol/L.h [18, 19]. The ability of *Clostridia* to produce H_2 looks very promising. Hydrogen yields and production rates can still be improved by optimizing process conditions.

5.3.1.2. Rumen bacteria

Other strict anaerobic bacteria producing hydrogen are rumen bacteria. *Ruminococcus albus* has long been known to produce H_2 together with other products like acetate, ethanol, formate and CO_2 from carbohydrates. In a continuous culture a H_2 yield of 2.4 mol/mol glucose was reported by Innotti et al. [20]. Since then production of H_2 by *R. albus* was not studied further.

5.3.1.3. Thermophiles

The hyperthermophile *Pyrococcus furiosus,* an archaebacterium, produces H_2, organic acids and CO_2 from carbohydrates [21, 22, 23]. Hydrogen production efficiencies were not evaluated. From characterization studies of utilized substrates and produced products many extreme- and hyperthermophiles are known to produce hydrogen from carbohydrates (reviewed by [24]). Cellulolytic thermophiles and extreme and hyperthermophilic bacteria producing hydrogen are e.g. species of *Anaerocellum, Caldicellulosiruptor, Clostridium, Dictyoglomus, Fervidobacterium, Spirocheta, Thermotoga and Thermoanaerobacter.* Schröder et al. [25] reported on batch fermentations at 80 °C with *Thermotoga maritima*. A H_2 yield on glucose of 4 mol/mol was obtained which is equal to the maximal theoretical value. However, glucose consumption was low (1.6 mM) and low cell densities (1.4 x 10^8 per mL) were reached. Maximal hydrogen production rates of approximately 10 mmol/L.h were measured. Similar stoichiometries as for *T. maritima* were obtained for two moderate thermophiles, *Acetothermus paucivorans* and *Acetomicrobium flavidum,* grown at 60 °C [26, 27]. Recently, results on growth and hydrogen production by two other extreme thermophiles during sugar fermentation have been published [28]. In cultures of *Caldicellulosiruptor saccharolyticus* grown on sucrose at 70 °C and *Thermotoga elfii* grown on glucose at 65 °C stoichiometries of 3.3 mole H_2 per mole hexose were obtained which is 83% of the theoretical maximum. Maximal hydrogen production rates of 8.4 and 2.7 mmol/L.h, respectively, were measured. These results show that higher hydrogen yields on hexose can be reached by extreme and hyper-themophiles compared to mesophilic facultative and strict anaerobes.

5.3.1.4. Methanogens

Methanogens are characterized by the presence of hydrogenase, which is usually involved in the oxidation of H_2 coupled to CH_4 production and CO_2

reduction. However, under conditions of inhibition of CH_4 formation Bott et al. [29] reported production of H_2 and CO_2 in stoichiometric amounts from CO and H_2O by a strain of *Methanosarcina barkeri,* the so-called water-gas shift reaction.

5.3.2. Facultative anaerobes

Facultative anaerobes are resistant to oxygen. These bacteria have the advantage of rapidly consuming oxygen thereby restoring anaerobic conditions immediately in reactors. Strict anaerobes are very sensitive to oxygen and often do not survive low oxygen concentrations.

5.3.2.1. *Enterobacter*

Enterobacter as well as other members of the *Enterobacteriaceae* can have several beneficial properties favourable for H_2 production. In addition to high growth rates and utilization of a wide range of carbon sources, H_2 production by *Enterobacter* is not inhibited by high H_2 pressures [30]. However, the H_2 yield on glucose is normally lower compared to that of e.g. *Clostridia.* Tanisho et al. [31] isolated strain ***E. aerogenes*** E.82005 from leaves of *Mirabilis jalapa.* Under batch cultivation a hydrogen production rate of 21 mmol/L.h was obtained over a period of 23 h. The H_2 yield was 1.0 mol/mol glucose. In a continuous fermentation hydrogen was produced during 42 days using the same strain and molasses as the substrate. The average H_2 production rate was approximately 17 mmol/L.h. The average H_2 yield on sucrose was 1.5 mole. In contrast to batch fermentations lactate was the major product and butyrate and acetic acid were produced in lower amounts [32]. Although production by *Enterobacter* is not inhibited by high H_2 pressures flushing the culture medium with argon enhanced the H_2 yield to 1.6 mole per mole glucose. It was suggested that the removal of CO_2 was responsible for the yield improvement [33]. Yokoi et al. [34] isolated an aciduric *E. aerogenes,* strain HO-39, which was able to grow and produce hydrogen at low pH of 4.5. In a continuous culture without pH control hydrogen was produced at a rate of approximately 5 mmol/L.h for 26 days with a yield of 0.8 on glucose. To enhance H_2 production rates mutants of *E. aerogenes* and *E. cloacae* were developed. The mutants are blocked in the production of other metabolites, alcohols and organic acids, which normally decreases hydrogen production. In an *E. aerogenes* double mutant which produced lower amounts of ethanol and butanediol but comparable amounts of organic acids the hydrogen production and yield were 2 times higher compared to the wild type [35]. Kumar and Das [36] have isolated *E. cloacae* IIT-BT 08 strain from leaf extracts which was capable to grow and produce hydrogen by using different carbon sources. In batch fermentations the maximum H_2 yield was 2.2 mol/mol glucose and 6.0 and 5.4 on sucrose and cellobiose, respectively. The maximum H_2 production rate measured was 35 mmol/L.h on sucrose as substrate. The same approach as for *E. aerogenes* has been used to develop mutants with enhanced H_2 production [37]. In batch fermentations a double mutant produced less ethanol and butanediol, and lower yields of lactate and butyrate were obtained. The yield of acetate was similar as the wild type strain. The block in formation pathways of alcohols and organic acids was accompanied by a 1.5 times increased H_2 yield on glucose, i.e. 3.4 mole per mole glucose. Continuous fermentations were performed with *E. aerogenes* wild types and a double mutant [38]. Due to self-flocculation, cells were retained in the reactor even at high dilution rates. Maximum production rates of 58 mmol/L.h at a dilution rate of 0.67 h^{-1} were reached for the double mutant, which was nearly 2 times higher compared to the wild type. The molar H_2 yield on glucose was maintained at 1.1. In a packed column with spongy material an *E. aerogenes* wild type strain produced hydrogen on a starch hydrolysate with a yield of 1.5 mol/mol glucose at a dilution rate of 0.1 h^{-1} [39].

5.3.2.2. *E. coli*

E. coli has been shown to be capable of producing H_2 and CO_2 from formate in the absence of oxygen [40, 72]. The catalytic activity, called formate hydrogen lyase, was shown to be a membrane-bound multi-enzyme complex, consisting of a formate dehydrogenase and a hydrogenase [73]. Sustained lysis of formate required blocking of other anaerobic reductases [74]. Production of hydrogen from carbohydrates was also reported [40]. Inconsistency exists on the pathway leading to H_2 production, either via formate or without

formate as an intermediate [40, 41]. The molar H_2 yield on glucose by growing *E. coli* was 0.9 [41, 42] or 1.2 by immobilized cells.

5.3.2.3. *Citrobacter*

A Citrobacter species, *Citrobacter* sp. Y19 isolated from sludge digesters, has been shown to produce hydrogen from CO and H_2O by the water-gas shift reaction under anaerobic conditions [43, 44]. H_2 production was observed in serum-bottles and during continuous operations. In the latter case H_2 production rates of approximately 15 mmol/L.h have been observed. Equimolar amounts of H_2 were produced from the consumed CO, but the conversion efficiency of CO, which was about 20%, was relatively low. This was attributed to short retention times of CO.

5.3.3. Aerobes

5.3.3.1 *Alcaligenes*

A. eutrophus has been shown to grow heterotrophically on gluconate and fructose, and when exposed to anaerobic conditions produced hydrogen [45]. It contains a soluble NAD-reducing hydrogenase [46].

5.3.3.2 *Bacillus*

A hydrogen-producing *B. licheniformis* was isolated from cattle dung [47]. It produced 0.5 mol H_2/mol glucose [48]. Immobilized cells had an average H_2 yield of 1.5 mole per mole glucose and cells were stable during 60 days.

5.3.4. Co- and mixed cultures

Yokoi et al. [49, 50] reported on a co-culture in a continuous fermentation of *Clostridium butyricum* and *Enterobacter aerogenes* in which the higher H_2 yield of the strict anaerobe and the oxygen consumption by the facultative anaerobe were combined. This resulted in fermentations with no need for an expensive reducing agent since the presence of *E. aerogenes* was sufficient to rapidly restore anaerobic conditions in the fermentor upon short oxygen exposures. A continuous fermentation by immobilized mixed cells on porous glass beads and starch as the substrate showed a H_2 production rate of approximately 50 mmol/L.h and a H_2 yield of 2.6 on glucose at dilution rates of 1 h^{-1}.
Microflora for mixed cultures have been isolated from various sources, such as fermented soybean meal or sludges from anaerobic digesters of municipal sewage or organic waste and sludge from kitchen waste water. These microflora often contain unwanted bacteria such as methanogens which consume the produced hydrogen and convert it to methane. Enrichment cultures of the microflora are prepared by forced aeration of the sludge or by heat treatment which inhibits the activity of the hydrogen consumers while the spore forming anaerobic bacteria survive. Additionally, in continuous fermentations higher dilution rates are used to wash out the slow growing methanogens and select for the acid producing bacteria. In industrial applications the use of mixed cultures for hydrogen production from organic wastes might be more advantageous because pure cultures can easily become contaminated with H_2 consuming bacteria. The first reports published on hydrogen production during wastewater treatment showed inhibited methane production but low H_2 yields and lack of stability [51, 52]. Ueno et al. [53] have found that the anaerobic microflora in sludge compost converted cellulose to hydrogen with high efficiency of 2.4 mol/mol hexose in batch experiments at 60 ºC. Furthermore, stable hydrogen production for 190 days from industrial wastewater from a sugar factory by the same microflora in a chemostat culture was reported [54]. Hydrogen yield on hexose was similar as in the batch culture and a H_2 production rate of 1.4 mmol/L.h at a HRT of 3 days was obtained. The maximal removal efficiency of carbohydrates was approximately 97% and also other organic compounds were converted. The hydrogen producers in the thermophilic microflora were identified. Sixty-eight strains were isolated and classified in 9 distinct groups and it was suggested that hydrogen production from cellulose is performed by a consortium of several species of microorganisms [55]. *Thermoanaerobacterium thermosaccharolyticum* was the dominant strain in the enrichment cultures. A patent has been issued [56] claiming a process for stable H_2 production at high efficiency by anaerobic bacteria from sludge compost under anaerobic conditions in combination with treatment of wastewater. Other recent work utilized sewage sludge to convert glucose and sucrose into hydrogen in continuous cultures at 35 ºC [57, 58]. At retention times of 6 to 8 hours a molar H_2 yield of 1.7 and 3.4 on glucose

and sucrose, respectively, was obtained at production rates of approximately 26 to 29 mmol/L.h over a two-week period. Kinetic models were developed to describe and predict the results and based on this it was suggested that product formation was essentially a linear function of biomass concentration. Results on mixed cultures from digester sludge have been reported by Lay [59, 60]. Conditions were varied according to central composite design methodology in order to model and optimize the anaerobic digested sludge converting starch and cellulose to hydrogen. Mizuno et al. [61] improved the H_2 yield on glucose by mixed cultures isolated from fermented soybean meal through sparging the medium in a continuous stirred-tank reactor with N_2. During an 8 week period of continuous operation stable H_2 production rates of approximately 8 mmol/L.h were obtained. Noike and Mizuno [62] reported on hydrogen fermentations of organic waste, such as bean curd manufacturing waste, rice and wheat bran by the same mixed culture in batch reactors. The H_2 yield varied from 1.7 to 2.5 mol/mol hexose and the carbohydrates were used as the main source while soluble protein was hardly degraded.

To summarize, the highest H_2 yields on hexose have been obtained by hydrogen producing extreme thermophilic anaerobic bacteria (Table 1). The yields were approximately 83-100% of the maximal theoretical value of 4 mol/mol. More research is needed to confirm these results and to determine whether this is a general property of thermophilic bacteria. The strict anaerobic *Clostridia* produce hydrogen with higher yields, approximately 2 mol/mol, than facultative anaerobes which show a H_2 yield of less than 2. However, higher molar yields of more than 3 have been obtained in mutants of *Enterobacter* which are blocked in biosynthetic pathways leading to organic acid and alcohol production. In mixed cultures molar H_2 yields of around 2 are obtained, which reflects the dominant presence of *Clostridia* in enriched cultures. Besides optimal H_2 molar yields, high hydrogen production rates are needed. Product formation appears to be dependent on cell density. Thermophiles usually grow to low densities and, therefore production rates are expected to be low. High production rates are reported for *Clostridia* and *Enterobacter* of maximal 23 and 58 mmol/L.h, respectively. Hydrogen fermentations by co- and mixed cultures showed production rates of approximately 30-50 mmol/L.h.

5.4. Feedstocks for dark hydrogen fermentation

In this section two aspects of the feedstock for this type of hydrogen production are discussed. The first concerns the range of organic compounds which can be utilised. The second concerns the quality of the feedstocks which can be used for dark hydrogen fermentation.

With respect to the range of potential substrates which can be utilised by the broad range of hydrogen producing bacteria it can be stated that, at present, it is vast and open for further exploration. From a thermodynamic point of view, the conversion of carbohydrates to hydrogen and organic acids is preferred because it yields the highest amount of hydrogen per mole of substrate. These carbohydrates can be monosaccharides but may also be polymers such as starch, cellulose or xylan, as discussed in section 3. Besides carbohydrates (Fig. 2) also formate and peptides have, until now, been studied as substrates for dark hydrogen production.
From our own work with extreme thermophilic bacteria, it has recently become clear that amino acids can also be oxidised to hydrogen by certain strains. It is not clear whether specific amino acids, entering bacterial metabolism at the level of pyruvate are selected or whether this phenomenon is more general. Furthermore, we have observed that growth of hydrogen producers on certain saccharides is not always associated with reduction of protons to hydrogen but limited to reduction of other electron acceptors which were, allegedly, used for analytical reasons, e.g. like thiosulfate being reduced to the easily detected H_2S in the study of Ravot [63].
Finally, to add to the confusion, there is the fact that there have not (yet) been many studies on dark hydrogen production. This, together with the large number of hydrogen producing microbial species, allows the suggestion that most carbohydrates are a suitable feedstock for dark hydrogen fermentation. Proteins, peptides and amino acids are probably less suitable for dark

TABLE 1. Hydrogen yields and production rates by microorganisms as reported in the literature.

microorganism	conditions				substrate	H_2 yield mol/mol monosaccharide	H_2 production rate (maximal) mmol/h.L	source
	culture	*D*, h^{-1}	pH	T, °C				
Strict anaerobes								
Clostridia								
Clostridium sp. no 2	batch		6.0	36	glucose	2.0	23.9	[9]
	batch		6.0	36	xylose	2.1	21.7	
C. paraputrificum M-21	batch		uncontrolled	37	GlcNAc[1]	2.5	31.0	[18, 19]
C. butyricum LMG1213tl	continuous	0.222	5.8	36	glucose	1.5	21.7	[13]
Clostridium sp. no 2	continuous	0.18	6.0	36	glucose	2.4	7.1	[15]
	continuous	1.16	6.0	36	glucose	1.4	20.4	
	continuous	0.96	6.0	36	xylose	1.7	21.0	
Thermophiles								
Thermotoga maritima	batch		uncontrolled	80	glucose	4.0	10	[25]
Thermotoga elfii	batch		7.4	65	glucose	3.3	2.7	[28]
Caldicellulosiruptor saccharolyticus	batch		7.0	70	sucrose	3.3	8.4	[28]
Facultative anaerobes								
Enterobacter								
E. aerogenes E.82005	batch		6.0	38	glucose	1.0	21	[30, 31]
E. cloacae IIT-BT 08 wt	batch		uncontrolled	36	glucose	2.2		[36, 37]
	batch		uncontrolled	36	sucrose	3.0	35	
E. cloacae IIT-BT 08 m DM_{11}	batch		uncontrolled	36	glucose	3.4		
E. aerogenes E.82005	continuous	0.32	6.0	38	molasses	0.7	20	[9]
E. aerogenes HU-101 wt	continuous	0.67	uncontrolled	37	glucose	0.6	31	[38]
E. aerogenes HU-101 m AY-2	continuous	0.67	uncontrolled	37	glucose	1.1	58	
Co-culture								
C. butyricum IFO13949 + *E. aerogenes* HO-39	continuous	1.0	5.2	36	starch	2.6	53	[49, 50]
		HRT, h						
Mixed cultures from:								
- sludge compost	continuous	12	6.8	60	waste water sugar factory	2.5	8.3	[54]
- sewage sludge	continuous	6	5.7	35	glucose	1.7	29.6	[57]
	continuous	8	6.7	35	sucrose	1.7	26.2	[58]
- fermented soybean meal	continuous	8.5	6.0	35	glucose	1.4	8	[61]

[1]GlcNAc=*N*-acetyl-D-glucosamine

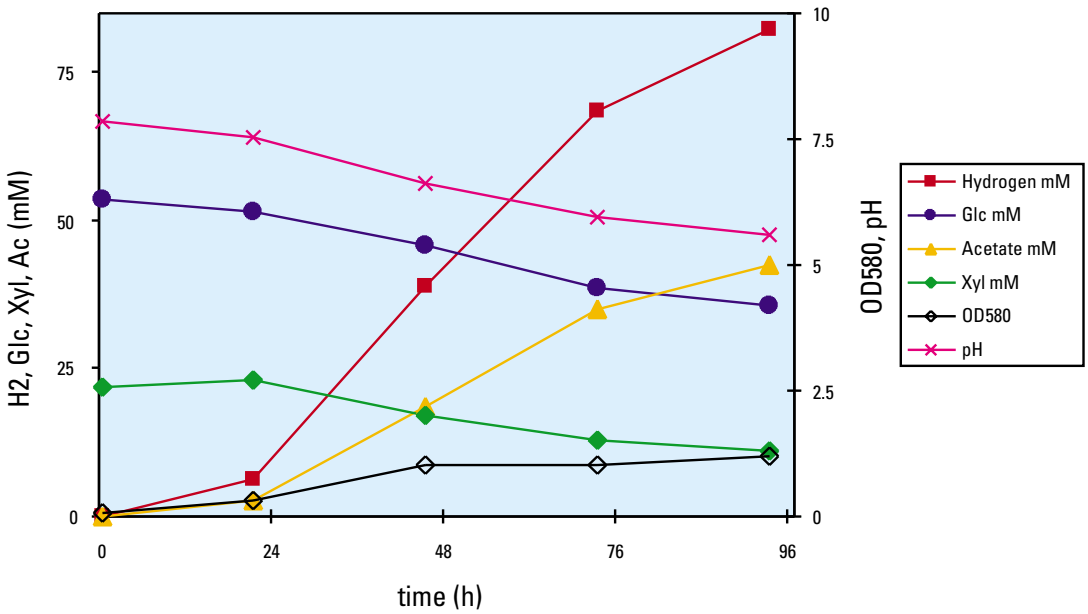


Figure 2. Simultaneous consumption of glucose and xylose during growth of Thermotoga elfii on Miscanthus hydrolysate at 65 ºC. (from: de Vrije et al., 2002 [75])

hydrogen production whereas biopolymers like lipids will be unsuited.

This great potential of dark hydrogen fermentation, i.e. the vast range of potential organic substrates, has also been recognized by other workers in the field. Noike and Mizuno [62] and Yu et al. [78] refer to several forms of organic waste streams ranging from solid wastes like rice straw to waste water from a sugar factory and a rice winery, which have been successfully used for dark hydrogen production. Besides the organic substrates, CO in syngas has been used as feedstock for biological H_2 production. Syngas or fuel gas is a mixture of (mainly) CO and H_2, which can be produced cheaply and on a large scale by thermochemical gasification of coal or wood. A wide range of anaerobic micro-organisms are capable of CO oxidation with concomitant H_2 production in a biological variant of the water-gas shift reaction [29, 77]. These organisms could serve as a biological alternative for chemical catalysts to remove CO from H_2 rich gases, and produce H_2 linked to CO oxydation. CO uptake has been shown to occur at very high rates. The process could be used for fuel gas conditioning and upgrading, both by CO removal and H_2 production. A technological challenge is to enhance CO mass transfer which is the rate limiting step in the process.

At this point the second aspect of the feedstock, i.e. the quality, enters the discussion. Even though there have been reports on dark hydrogen fermentation using solid organic waste, this phenomenon has also been denied. Besides this contrast which is probably due to different species being involved, there is the even more basic discussion concerning the configuration of the feedstock on the molecular level. As stated above and shown in section 3 several hydrogen producers are able to convert biopolymers like starch, cellulose and xylan to hydrogen and organic acids (Fig. 3).

This is very convenient, because pretreatment of biomass is then only, eventually, needed from the process technological point of view e.g. for improving rheological properties. However, apart from the more easily degradable feedstocks such as starch and cellulose, the main components of future feedstocks will, most probably, to a large extent be derived from lignocellulosic raw materials. Lignocellulose is a biopolymer consisting of

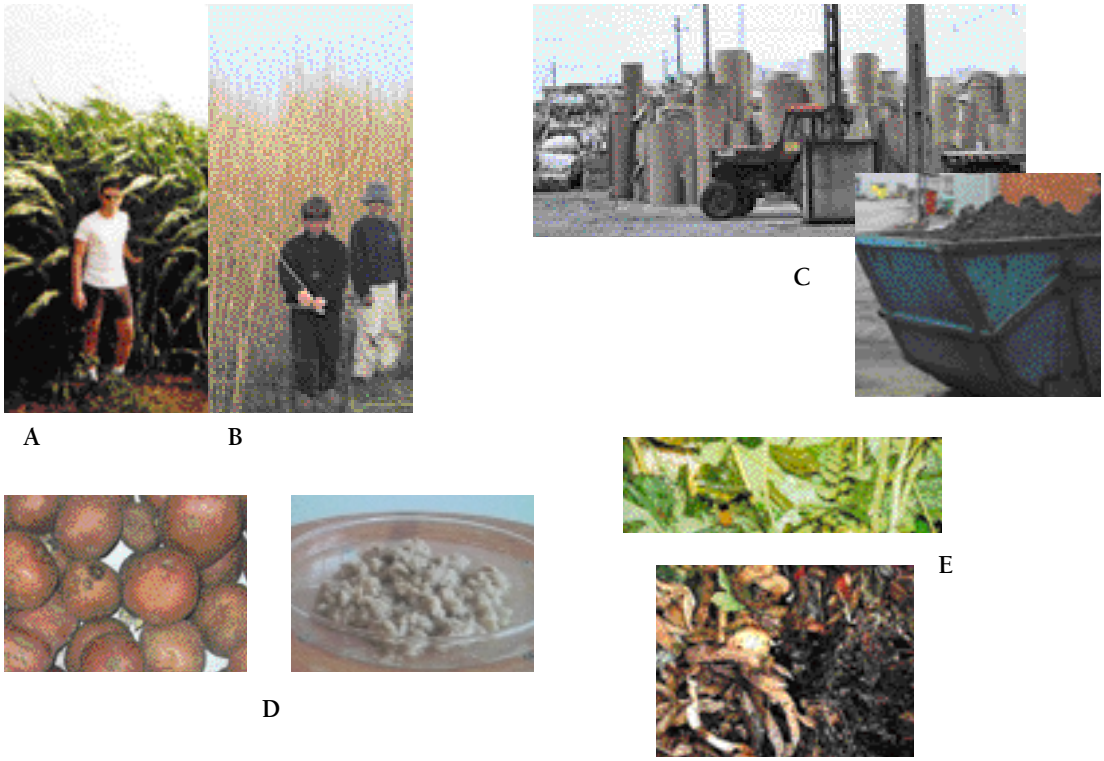


Figure 3. Feedstocks which have been successfully used for hydrogen production by extreme thermophiles (A. Sweet Sorghum, B. Miscanthus, C. paper sludge, D. potato steam peels, E. domestic organic waste).

tightly bound lignin, cellulose and hemicellulose. Whereas cellulose and hemicellulose can be feedstocks for hydrogen fermentation, lignin is not degraded under anaerobic conditions. Moreover, lignin strongly hampers the utilisation of cellulose and hemicellulose because a) the bonding in lignocellulose resists mobilisation and b) chemically degraded lignin is often inhibitory to microbial growth.

These parameters need to be studied in view of producing cheap feedstocks for dark hydrogen fermentation from lignocellulosic biomass residues

Figure 4. Chopped Miscanthus before (Left) and after extrusion in combination with a sodium hydroxide treatment (Right). The pretreatment removed 77% of the lignin.

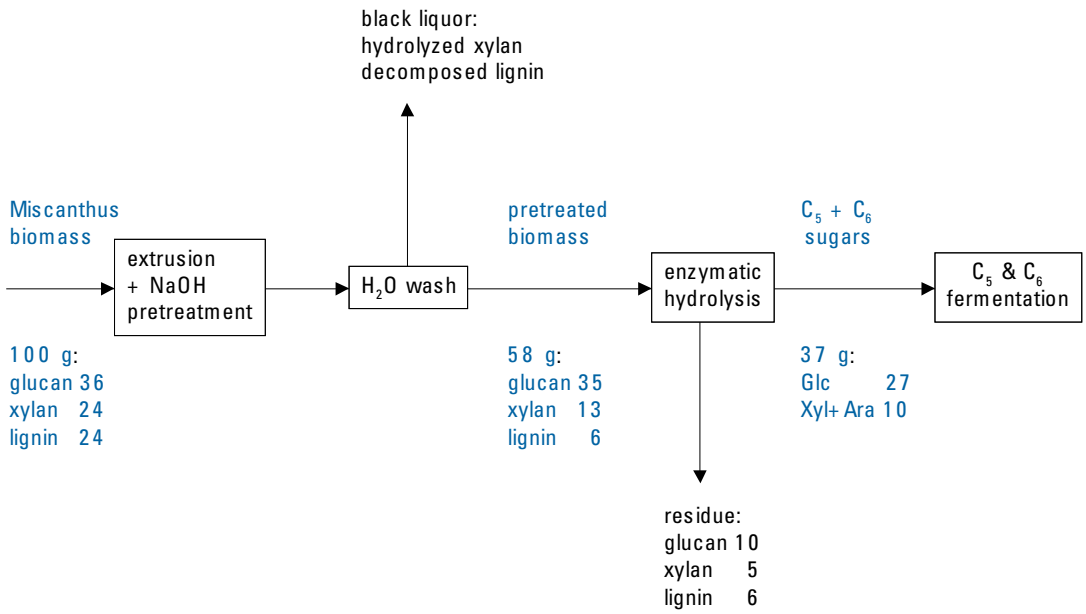


Figure 5. Flowsheet of pretreatment (extrusion combined with sodium hydroxide) and enzymatic hydrolysis of Miscanthus. Pretreatment and enzymatic hydrolysis results in fermentable sugars and (non)fermentable sidestreams. (from: de Vrije et al., 2002 [75])

and/or energy crops. It seems obvious that producing cheap feedstocks will require the development of cost effective pretreatment methods with a low energy demand (Fig. 4).

This constraint in dark hydrogen fermentation is also studied in our own hydrogen projects which focus on the whole chain of events for hydrogen production from biomass. The approach for successful pretreatment is to use a combination of physical and (bio)chemical methods for mobilisation of saccharides for fermentation, while simultaneously preserving the nonfermentable components for further valorisation, e.g. lignin as a biofuel (Fig. 5).

5.5. Bioprocesses for hydrogen from biomass

5.5.1. Current process development

The physiology of dark hydrogen fermentation explains, thisfar, that hydrogen production is a common microbial asset and that for obtaining this hydrogen as the final product, only a specific environment needs to be created in which hydrogen producing bacteria flourish and others perish. However, as outlined above, dark hydrogen fermentation is an incomplete oxidation. This means that organic matter is not completely oxidised to CO_2 but to intermediate compounds, like acetic acid or lactate. Further oxidation of these products in the dark, to hydrogen and CO_2, is thermodynamically very unfavourable. Thus, dark hydrogen fermentation delivers, besides very pure hydrogen, other, reduced carbonaceous products which need to be utilised for making a sound balance when considering energy production from organic matter. As can be read in the chapters dealing with methane production (Chapter 4) and photobiological hydrogen production (Chapter 6) these intermediate products can be further metabolised to methane or converted to hydrogen in the presence of light, respectively.
Our approach is to counter one of the physiological drawbacks of dark hydrogen fermentation, i.e. the incomplete oxidation, by coupling the process to a subsequent fermentation (Fig. 6).
In this way the chemical energy present in the initial organic matter remains preserved as much as possible. This also applies to situations where the yield of hydrogen is lower than theoretically

expected. Theoretically, 1 mole of glucose is converted to 4 moles of hydrogen, 2 moles of acetic acid and 2 moles of CO_2. As can be read in section 3 of this chapter, there are several microbial conversions where the yield is less than 4 moles of hydrogen. Other workers in the field [64] strongly adhere to attaining this theoretical maximum. Even though our present projects aim at reaching a yield of 4 moles of hydrogen in the dark fermentation, it is our conviction that the whole chain should be considered here. The substrate range of phototrophic bacteria in a subsequent fermentation is fairly wide. This means that a lower hydrogen production during dark fermentation is compensated by a higher hydrogen production in e.g. a consecutive photobiological fermentation. Thus, in the end, the same amount of hydrogen will be produced. When the dark hydrogen fermentation is followed by a methane fermentation, there may be less room for negotiation, although acetic acid is amongst the favorite substrates for methane production (Fig. 7).

Several approaches can be considered to increase hydrogen yields in the dark fermentation. The optimisation of methane and photobiological fermentations, is discussed in Chapters 3 and 5, respectively.

Metabolic engineering of hydrogen producing micro-organisms to minimise production of other more reduced products by blocking their biosynthetic pathways will provide higher yields [35, 37, 38]. Additionally, higher hydrogen yields will most probably be achieved by limiting cell growth through nutrient limitations, thereby enhancing catabolic processes. High cell densities are needed to maximize hydrogen production rates. Therefore, major improvements are expected in systems with biomass retention, e.g. by immobilized cells [79], under nutrient limitations operating in a continuous mode.

As another potential improvement the production of hydrogen at high temperatures by extreme and hyper-thermophiles could be considered [65]. At increased temperatures hydrogen production

5.5.2. Process optimisation

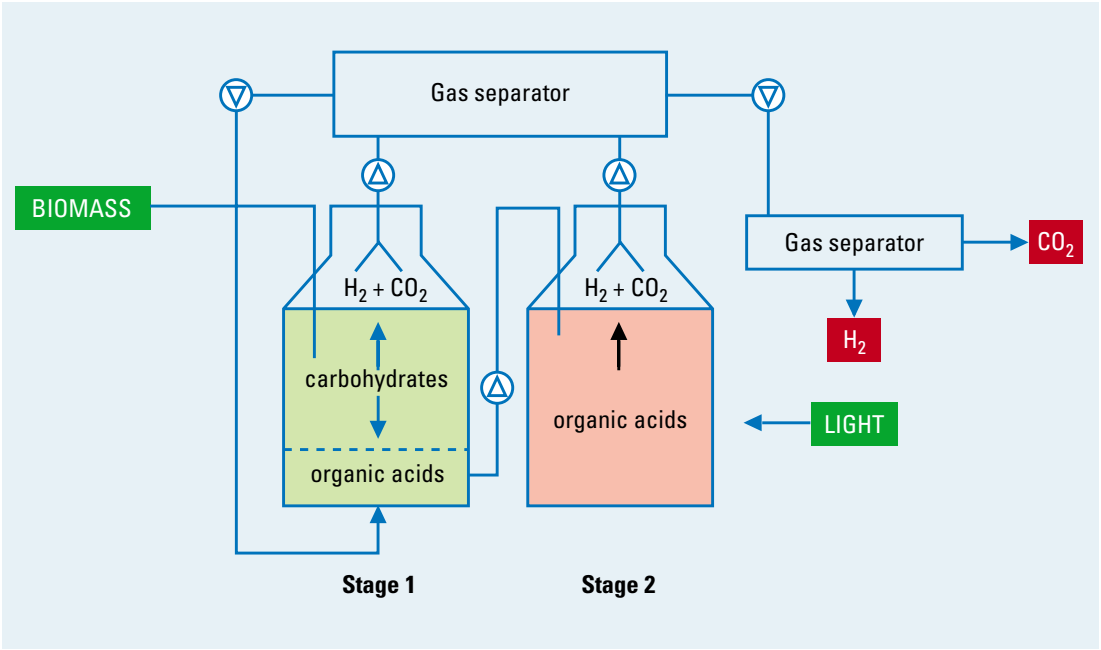


Figure 6. Outline of the bioprocess for production of hydrogen from biomass in a 2 stage fermentation. Stage 1 is for heterotrophic fermentation of carbohydrates to hydrogen, carbon dioxide and organic acids. In stage 2 the photoheterotrophic fermentation of organic acids to hydrogen and carbon dioxide takes place.

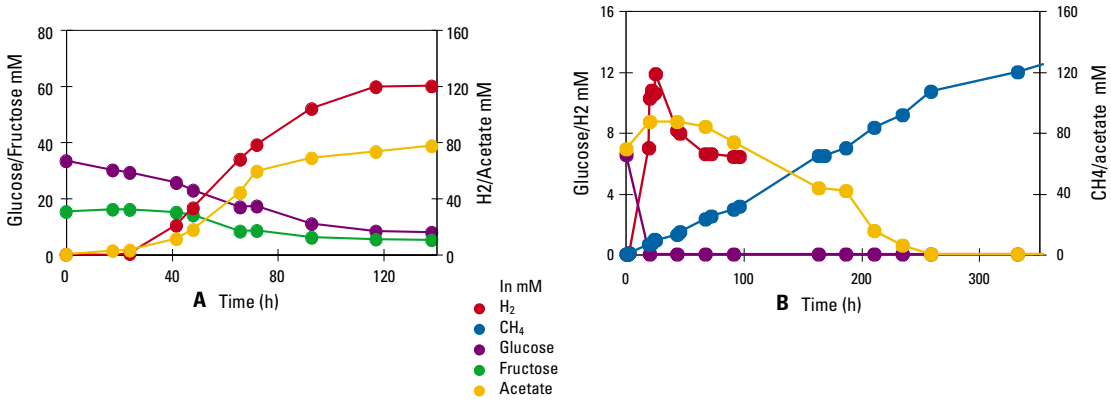


Figure 7. Results from a bioprocess for production of hydrogen and methane from domestic organic waste in a 2 stage bioprocess. In stage 1 hydrogen and acetic acid are produced during growth of Thermotoga elfii on domestic organic waste hydrolysate (A). In stage 2 the methanogenic fermentation of acetic acid to methane takes place (B). (from: Claassen et al., 2002 [76])

becomes more exergonic [66]. *Pyrococcus furiosus* hydrogenase showed a dramatic increase in H_2 evolution activity above 80° C which may be partly due to a decreased affinity of the enzyme for H_2 [67]. Therefore, extreme- and hyper-thermophiles show a better resistance to high hydrogen partial pressures [68] which otherwise cause a metabolic shift to production of more reduced lactate or alanine instead of acetate. This could be one of the reasons that extreme- and hyper-thermophiles produce hydrogen with an efficiency of almost the theoretical maximum. Another advantage of fermentations at extreme temperatures is that the process is less sensitive to contaminations by e.g. hydrogen consumers, thus establishing a specific environment enabling maximum evolution of hydrogen. In spite of the higher tolerance for hydrogen in thermophiles, this product may still impose feed-back inhibition. Therefore, the design of a highly efficient hydrogen removal step is needed to further augment productivity [80, 81].

5.6. Economics for hydrogen from biomass

Here data are presented which were collected during a Kiem-EET study (Nov 1998–Feb 2000) performed by ATO (co-ordinator), WU-Laboratory for Microbiology, WU-Department Agrotechnology and Food Sciences, TNO-MEP and Paques Biosystems [69] and financially supported by the Dutch Ministries of Economic Affairs (EZ), Education, Culture and Science (OCenW), and Housing, Spatial Planning and the Environment (VROM) via the Economy, Ecology, Technology Programme (EET) and the Ministry of Agriculture, Nature Management and Fisheries.
As stated above, dark hydrogen fermentation is an incomplete oxidation, yielding not only hydrogen and CO_2, but also organic acids like acetic acid. For an economically sound process, the reduced carbonaceous compounds need to be converted too; either in a photo-bioreactor to H_2 and CO_2 or in a methane reactor to CH_4 and CO_2. If the dark hydrogen fermentation is not followed by further conversion, the H_2 yield will not warrant economic feasibility. Therefore, the costs of hydrogen production were estimated from a first design of a complete bioprocess for hydrogen from biomass, consisting of an extruder for preparing fermentable feedstock, a thermo-bioreactor (95 m^3) for

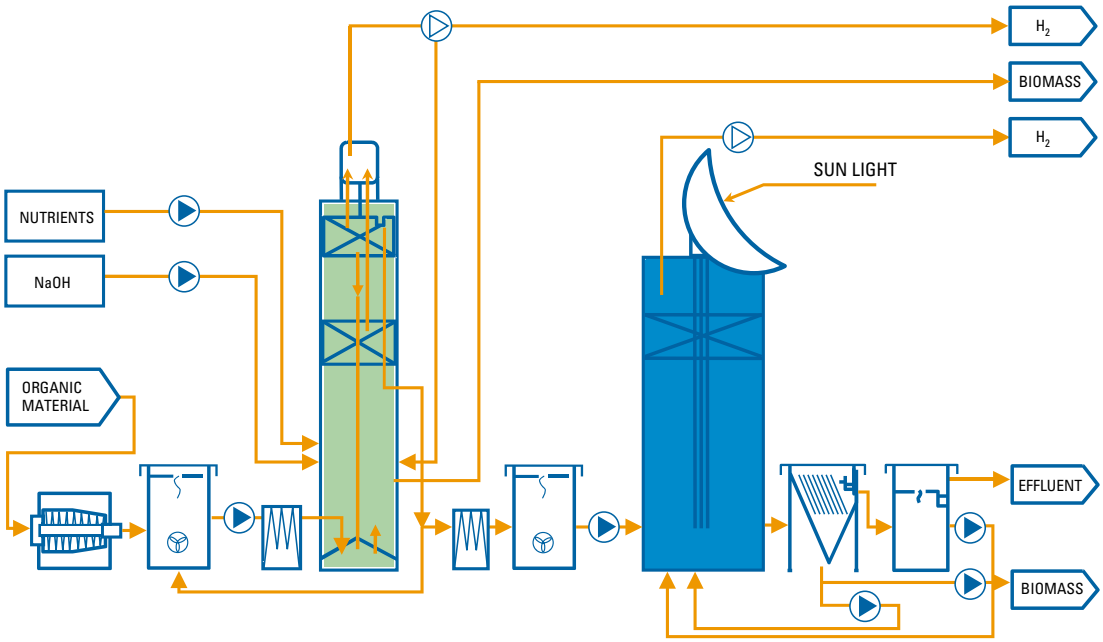


Figure 8. First design of a bioprocess for the conversion of biomass to hydrogen in a thermo-bioreactor, followed by a photo-bioreactor (adapted by ATO from the conceptual design by Paques Biosystems B.V.).

dark hydrogen fermentation and a photobioreactor (300 m^3) equipped with a sunlight collector, for the conversion of acetic acid to hydrogen and CO_2 (Fig. 8).

The estimate is based on the use of extreme thermophilic bacteria for the dark hydrogen fermentation since only these have been shown, until now, to achieve the theoretical production of 4 moles of hydrogen per mole of glucose consumed. The conversion efficiency in the thermo-bioreactor was assumed to be 80%. The same efficiency was assumed for the consecutive photo-bioreactor where acetate from the effluent of the thermo-bioreactor was converted to hydrogen at a ratio of 4 moles hydrogen per mole of acetate.
Hydrogen produced in the thermo-bioreactor was recovered using gas stripping and this hydrogen was further purified to specifications for fuel cell application using pressure swing adsorption.
The size of the plant was set at a production capacity of 425 Nm^3 H_2/hour (39 kg H_2/hour), aiming at relatively small scale systems, fed by locally produced feedstock. As feedstock a common lignocellulosic substrate was chosen consisting of 65% (w/w dry matter) (hemi)cellulose of which 65% becomes available for fermentation after pretreatment in the extruder. On this basis it was calculated that 1000 kg biomass (dry weight)/hour is required to produce 425 m^3 H_2/hour.
The contribution of the investment costs and energy demand of the separate steps to the production costs of hydrogen from biomass is shown in Tables 2 and 3. As the current costs of biomass range from low to even negative, being very volatile in view of future demands, no value was included for the acquisition of the feedstock. The same accounts for the mobilisation of fermentable substrates from the feedstock. This is a potential cost factor which is dependent on intrinsic feedstock properties and applied pretreatment and hydrolysis schemes and therefore impossible to consider under a common denominator.
The data shown in Tables 2 and 3 result in an estimated overall cost of EURO 2.74/kg H_2, equiva-

TABLE 2. *First estimate of investment costs for equipment required for a hydrogen from lignocellulosic biomass production process for production of 425 m^3 H_2 /h or 312 tonne H_2 /year, and the contribution to the H_2 production costs.*

Item	Investment costs (EURO)	Annual capital costs (EURO)	Costs/kg H_2 (EURO)
Extruder	1,045,455	156,818	0.50
Bioreactors, pumps etc.	1,295,455	194,318	0.62
Sunlight collector	811,064	121,660	0.39
Equipment for H_2 recovery from thermo-bioreactor	403,182	60,509	0.20
Equipment for H_2 recovery from photobioreactor	196,803	29,520	0.10
Total:	3,751,959	562,825	1.81

TABLE 3. *First estimate of energy consumption and costs for a hydrogen from lignocellulosic biomass production process for production of 425 m^3 H_2 /h or 312 tonne H_2 /year. Assumed electricity costs 0.068 EURO / kWh.*

Item	Energy consumption (GJ/h)	Energy costs/year (EURO)	Costs/kg H_2 (EURO)
Extruder	0.547	82,879	0.26
Bioreactors	0.842	127,576	0.41
Recovery of H_2 from thermo-bioreactor	0.225	34,030	0.11
Recovery of H_2 from photobioreactor	0.281	42,545	0.14
H_2 Purification	0.010	1,515	0.01
Total:	1.90	288,545	0.93

lent to 0.25 EURO/Nm^3 H_2 or 19.2 EURO/GJ (based on upper combustion value). This final cost estimation is based on acquisition of biomass at zero value, zero hydrolysis costs and excludes personnel costs and costs for civil works, all potential cost factors. On the other hand, process units have been considered separately, thus precluding the opportunity to couple technical devices and energy requiring and energy yielding process units. For comparative purposes, present production costs for hydrogen produced in small scale production plants based on alternative technologies are presented in Table 4. Except for the technologies where natural gas or conventional electricity is used for the production of hydrogen, these production methods are without net CO_2 emission.

In the bioprocess for hydrogen from biomass, CO_2 is one of the products. However, since this CO_2 is derived from biomass, no net emission occurs and thus this process is considered as CO_2 neutral. Besides CO_2, formed in a ratio of 1 : 2 (CO_2 : H_2 v/v), no other volatile products are expected from the dark fermentation. So, besides being CO_2 neutral, another advantage of this bioprocess is the production of pure product streams.

Besides the final cost of the produced hydrogen, the energy balance of this bioprocess has been considered. The production of 425 m^3 H_2/h is equivalent to an energy production of 5.4 GJ/h, based on the upper combustion value of 12.74 MJ/Nm^3. The electricity requirement in this design is estimated at 1.9 GJe/h. These observations show the weak points in the first design which are now being studied in new projects.

First of all the yield in terms of hydrogen is addressed. The yield is inherently related to the mobilisation of fermentable feedstock which can

TABLE 4. *Comparison of H_2 production costs and net CO_2 emissions in small-scale production plants with capacities in the range 100 – 1000 Nm^3 H_2 /h.*

Technology[1)]	Production costs (EURO/Nm^3 H_2)	CO_2-emission (kg/Nm^3 H_2)
Steam-reforming of natural gas	0.32	0.8
Electrolysis with conventional electricity	0.23	1.8
Electrolysis with CO_2-lean electricity [2)]	0.27-0.36	0
2-stage bioprocess for hydrogen from biomass (this estimate)	0.25	0
Steam-reforming of bio-methane	0.32	0
Electrolysis with electricity from wind turbines	0.25	0
Electrolysis with electricity from photovoltaic cells	2.95	0

[1)] *Data for alternative technologies were provided by TNO Environment, Energy and Process Innovation (personal communication).*
[2)] *Here CO_2 is sequestered in e.g. aquifers, rendering this process CO_2 neutral.*

TABLE 5. *Estimated energy potential of a bioprocess for hydrogen from biomass in The Netherlands.*

		Units
Available biomass for biohydrogen production[1)]	2,650	ktonne d.w./year
Total bio-H_2 production (39 kg H_2 /tonne biomass)[2)]	103	ktonne / year
Total energy content of Bio-H_2 produced[3)]	14.7	PJ
Potential number of Bio-H_2 plants [2)]	330	
Total electricity production [4)]	7.4	PJe / year
Electricity production per bio-H_2 plant	22,294	GJe / year
Electricity consumption per household; 3380 kWh/yr[5)]	12.2	GJe / year
Number of households per Bio-H_2 plant	1830	
Total number of households with Bio-H_2 electricity	600,000	

[1)] *50 % of total available biomass (5.3 Mtonne/a according to Faaij et al. [71])*
[2)] *For a plant producing 39 kg H_2 /hour from 1 tonne (d.w.) of biomass.*
[3)] *Based on upper combustion value: 142.7 GJ/tonne H_2*
[4)] *Fuel cell with 50% conversion efficiency*
[5)] *Ref: http://www.energie.nl/*
[6)] *Total number of households is 6.86 million Ref: http://www.energie.nl/*

be converted to hydrogen. Besides increasing the efficiency of mobilisation, e.g. from 65% to 85%, also other feedstocks besides lignocellulosic feedstocks, e.g. energy crops (Sweet Sorghum) or starchy wastes (potato steam peels) are now under consideration. Secondly, the energy requirement of the bioprocess is addressed. By a new reactor design the energy demand has become significantly decreased [81]. A new, but certainly necessary strategy, is the utilisation of the residual biomass for the production of energy in a non-fermentative way. It seems feasible to generate sufficient energy from the residues to prevent the addition of external electricity from the grid.

An indication of the energy potential of the bioprocess for The Netherlands is provided in Table 5. The estimate is based on data provided by Faaij et al (1997) [71] for the total biomass availability (5.3 Mtonne/yr) in The Netherlands. It is assumed that 50% of the total amount of biomass (2,650

ktonne/y) is available for biohydrogen production (103 ktonne H_2/y) and that the energy requi rement of the bioprocess is fully covered by thermochemical conversion of residual non-fermented biomass. It is further assumed that the produced hydrogen is converted to electricity in fuel cells at 50% efficiency. As the total number of households is 6.86 million [2000; ref http://www.energie.nl/], the coverage with respect to electricity demand is 9% of all households. If the biomass availability increases, e.g. due to a decreased demand in the animal feed industry, the coverage increases accordingly. Finally, fuel cells generate heat besides electricity. This aspect has not yet been introduced in the calculations presented here because at present its quantification is unsure.

5.7. International status of development

The potential of biological hydrogen production is recognized worldwide. At the recent international conference Biohydrogen 2002, with 150 participating researchers from around the world, the status and progress in fundamental microbiological/biochemical research and technological R&D in the field of both photobiological and dark fermentative hydrogen production was reviewed [82]. Biohydrogen 2002 shows that the international attention and R&D efforts in the field of dark fermentative hydrogen production from biowastes and wastewater are rapidly increasing. At present, The Netherlands is leading in research on application of thermophilic bacteria for hydrogen production in projects supported by national governmental organisations as well as the European Union. The targeted feedstocks include biowastes (potato-processing residues, organic fraction of municipal solid wastes, paper sludge) as well as energy crops such as Miscanthus and Sweet Sorghum. In some countries R&D focuses primarily on mesophilic H_2 fermentations. In Japan H_2 fermentation R&D has included feedstocks such as bean manufacturing waste ('okara'), rice bran, wheat bran, apple and potato peels, palm oil mill effluent and tofu waste water. Additional R&D takes place in China (rice winery wastewater) and in Hungary (in co-operation with The Netherlands) on H_2 fermentation of paper sludge hydrolysate. A relatively new focus in the field is the development of combined two-stage H_2 and CH_4 fermentation systems e.g in The Netherlands and Japan. A major technical prerequisite for efficient H_2 fermentations is the maintenance of low H_2 partial pressures through continuous removal of H_2 from the fermentation broth. At the Biohydrogen 2002 conference several recent developments were presented including the use of membranes, which indicates that this obstacle can be overcome with continued development [82]. The development of dark hydrogen fermentations also benefits from the rapid progress in the field of fundamental hydrogenase research that includes the recent elucidation of the structure of the catalytic sites and basic physiological research.

In order to accelerate the technological development and to generate critical mass for the development of a hydrogen based economy international knowledge exchange and co-operation are required. The IEA Hydrogen Program supports collaborative activities for the advancement of hydrogen technologies in various tasks focusing on hydrogen production and storage technologies and infrastructural integration [83]. Work on dark hydrogen fermentations is included in Task 15, 'Photobiological hydrogen production' that has recently been extended. The objectives of the European COST Action 841 'Biological and Biochemical Diversity of Hydrogen Metabolism' are to enhance the understanding of the basic molecular and physiological aspects of biological hydrogen metabolism as an indispensable basis for fundamental and applied research [84]. The European Union further supports the European Thematic Network on Hydrogen: HyNet [85].

In the USA the activities for the transition to a hydrogen economy are combined in the 'Hydrogen, Fuel Cells & Infrastructure Technologies Program' supported by the Department of Energy [86]. In November 2002 the 'National Hydrogen Energy Roadmap' was presented in which biological hydrogen production is seen as one of the options for renewable hydrogen production on the longer term.

Increasingly, expert groups of various disciplines throughout Europe, Canada, Asia and, to a smaller extent, the USA are focussing on the biological production and application of hydrogen, as well as the societal impacts of implementation. The

insight that hydrogen needs to be produced from renewable sources in the future is recognised at the level of the European Commission and described in reports like 'Future Needs and Challenges for Non-Nuclear Energy Research in the European Union' (2002). The future role of hydrogen as a clean fuel for fuel cells producing near-zero emissions and as an intermediate energy carrier for storage and transport of renewable energy is increasingly recognized in Europe. The EU will therefore intensify the R&D in the field of hydrogen and related technologies. Several Expression of Interests related to biological hydrogen production and supported by more than 30 workers in the field have been submitted to the European Commission in June 2002. This may form the basis for continuation and expansion of bio-hydrogen development in Europe. Finally, the combination of fermentation processes with the use of product gas in fuel cells is particularly relevant for the future application of bio-hydrogen. This topic is being explored in 'BFCNet': 'Network on Biomass Fermentation Towards Usage in Fuel Cells' [87]. The objectives of BFCNet include joint research and demonstration and the development of standards on EU level.

It is clear from the above that biological hydrogen production through fermentation of biowastes is receiving increased attention. Furthermore, the potential of (renewable) hydrogen is increasingly recognized internationally, which provides a stimulus for the further development of biological hydrogen production.

5.8. Conclusions and perspectives for further development

5.8.1. Brief conclusions

- Dark hydrogen fermentation is a natural phenomenon but is, in natural environments often obscured due to rapid consumption of hydrogen by other species.
- The capacity to ferment organic compounds to hydrogen is widespread amongst micro-organisms. There are great differences in yields and production rates.
- Dark hydrogen fermentation can be done with almost all carbohydrates. The production of fermentable feedstocks from lignocellulosic material requires further investigation and technology development.
- The realization of a bioprocess for hydrogen requires two consecutive steps for complete utilisation of the chemical energy in the substrate. In the first step hydrogen is produced by dark hydrogen fermentation. In the second step the effluent is converted to either hydrogen or methane. Solutions for obtaining high yields and high production rates in the dark fermentation phase are envisaged.
- For an economically feasible hydrogen from biomass production process, a two stage fermentation is required for complete conversion of sugars to hydrogen.

A tentative cost estimate for hydrogen from biomass in a first design shows a cost for hydrogen which is favourable as compared to other sustainable hydrogen production processes. The energy demand of the bioprocess should be covered by utilisation of the non-fermentable residual biomass.

5.8.2. R&D challenges and perspectives

The mobilisation of fermentable feedstocks from biomass is an important R&D issue for every fermentative biofuel process, including the hydrogen from biomass process described here. This technological obstacle can be tackled by further development and optimisation of pretreatment techniques, aiming at an increase in efficiency of the current 65% to 85%. Improved mobilisation of fermentable substrate will increase the yield of hydrogen from biomass and hence the overall energy efficiency of the bioprocess. Here it is important to realise that some pretreatment techniques are more suitable for pretreatment of a specific biomass type than others. When different biomass types are considered, e.g. steam explosion or other techniques may be more adequate. In general, pretreatment is followed by, presently very expensive or environmentally undesirable, (bio)chemical hydrolysis. Even though many hydrogen producing micro-organisms are able to directly convert (hemi)cellulose to hydrogen, this conversion may be rate-limiting or impair proper process performance. Therefore, this topic has to be considered too in further optimisation.

In addition, it is very important that new techniques are developed for the utilisation of non-fer-

mentable biomass fractions, such as lignin, for energy production (Supercritical Water Gasification) or other product (composites) applications. For the designed 2-stage bioprocess, the volumetric H_2 productivity in the thermophilic fermentation step must be increased at least 10-fold in order to meet the production capacity of 425 m^3 H_2 /h. This can be done by conventional methods such as optimisation of culture conditions, biomass retention, or selection of highly productive species or strains from the broad range of available hydrogen producing micro-organisms.

The productivity of the photobiological fermentation step should be increased by at least a factor 15 through optimization of sunlight conversion efficiency. In this case the main improvement has to come from technological improvements of sunlight collection and light transfer systems, and photobioreactor development. The reader is referred to Chapter 5 'Photobiological hydrogen production' for a discussion of these topics. To support the profitable utilisation of the effluent of the thermo-bioreactor, the conversion to methane should be considered here as well. It is obvious that during the night, sunlight is lacking. Instead of storing the effluent, methane production might act as a substitute. When this option turns out favourable, the advantages of a partial photofermentation supplemented with a methane fermentation have to be weighed against a complete replacement of the photofermentation step. This issue needs to be evaluated together with the progress in the field of the application of methane in fuel cells and the utilisation of H_2/CH_4 mixtures as new energy carriers.

At the current stage of development, the estimated investment costs of the bioprocess and, especially, the energy requirement are substantial. This is mostly due to the fact that this bioprocess has been the first designed hydrogen from biomass process. Several components in the design have already been abandoned to improve process technology and reduce energy consumption. Also, the utilisation of the residual biomass has not been considered thusfar. It has become clear that application of the residue for energy production will significantly contribute to a sound energy balance. Furthermore, the biomass pretreatment, the two consecutive fermentations, and the H_2 recovery have been considered separately. It is expected that process integration of the separate units will enable substantial reduction of both the investment costs and the energy consumption. Finally, other products from the bioprocess such as new, thermostable proteins from the dark fermentation, new secondary metabolites (including vitamins) from the photobiological fermentation, and even clean carbon dioxide produced on site, may find their own application and thus contribute to making the bioprocess even more economically viable.

5.9. References

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