Utilization of biomass for hydrogen fermentation

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13.1   INTRODUCTION

In view of the detrimental effect of fossil fuel utilization on the environment, the need to employ renewable resources for the supply of energy is now globally acknowledged. The production of hydrogen from biomass is one of the options to meet this demand. Besides being derived from a renewable resource, the utilization of hydrogen offers several additional advantages and as a result, hydrogen is often given the epithet "fuel of the future". First of all, utilization of hydrogen as a fuel results in water as the end-product. Secondly, hydrogen is the fuel by choice to feed fuel cells which offer additional advantages such as, very high energy conversion efficiency, low maintenance and low noise. Thirdly, there are many different ways to produce hydrogen from renewable resources (biomass or hydropower, wind, solar or geothermal energy), all contributing to the large-scale introduction of this new fuel.

There are presently several initiatives, all in the Research and Development (R&D) stage, to establish the production of hydrogen from biomass. In general terms, the thermochemical conversion of biomass to "syn" gas is most suited for dry biomass. For wet biomass, research is focussing on supercritical water gasification and biological conversion. Between these two technologies, supercritical water gasification allows the utilization of all biomass components whereas biological conversion is restricted to the fermentable part of the biomass. On the other hand, the gas produced by supercritical water gasification may be mixed with traces of contaminants (CO or higher alkanes) whereas biological conversion is expected to deliver pure hydrogen and CO_2 only.

As stated above, the initiatives are in the R&D stage. Presently there are still many variables, for example, physical and chemical biomass composition, biomass availability, logistics etc., which will have their own specific contribution to the optimization of the hydrogen production from biomass and application chain. It is of prime importance to leave room for exploration of all alternatives and to evaluate and compare research results as soon as these become substantial.

In this chapter the focus is on biological conversion of biomass to hydrogen, with the emphasis on dark hydrogen fermentation, and a case-study is presented with results obtained using potato steam peels as feedstock.

13.2   HYDROGEN PRODUCTION FROM BIOMASS

13.2.1   Energy carriers form biomass by fermentation

There are four options for the biological conversion of biomass to energy carriers: anaerobic digestion to biogas with methane as energy carrier; acetone, butanol and ethanol (ABE) fermentation with butanol as the prime energy carrier; ethanol fermentation and hydrogen fermentation. Table 13.1 shows the amount of energy which is theoretically available in the end-products of an optimal fermentation with glucose as a model substrate (Claassen *et al*. 1999).

All these biological processes have their own advantages and disadvantages and, worldwide, extensive research is in progress to improve the respective drawbacks. Anaerobic digestion is now a well-established technology for conversion of a wide range of different feedstock to biogas (Chapter 7). However, specific conversion rates are rather low and the energy conversion efficiency is the lowest in comparison to other fermentation products (Table 13.1). The ABE fermentation also has the advantage of being suited for a great variety of feedstock. However,

Table 13.1   Comparison of energy yields in products from fermentative conversions with glucose as the substrate.

Fermentation product (mol)	$\Delta G°'$ in product (kJ/mol glucose)
3 methane	−2281
ABE	−2397 (average)
2 ethanol	−2464
12 H_2	−2673

As comparison: $\Delta G°'$ glucose = −2699 kJ/mol.

the inhibition of the fermentation by butanol is quite restrictive in industrial applications (Dürre 1998). At fairly low concentrations, butanol impairs fermentation thus necessitating efficient, and till now costly, product removal and recovery (Maddox *et al.* 1993). The conventional and already industrially applied ethanol fermentation enables high product concentrations (Lynd *et al.* 1996). The drawback here is the current inability of industrial yeasts to convert pentoses to ethanol. This way, the range of feedstock is limited to the fairly expensive feedstock derived from sugary or starchy biomass and therefore it is not surprising that the initiatives to augment the potential of yeasts by genetic modification are extensive and worldwide (Hahn-Hägerdahl *et al.* 1994). The variety of feedstock for fermentative hydrogen seems again quite extensive as reported by de Vrije and Claassen (2003). However, in the case of hydrogen fermentation, the complete conversion of glucose to 12 mol of hydrogen does not occur freely. The thermodynamic characteristics of the envisaged reaction are shown in Table 13.2.

The complete oxidation of glucose to 12 mol of hydrogen is hampered by unfavourable thermodynamics ($\Delta G^{\circ\prime} > 0$). This means that this reaction will not occur under standard conditions (Claassen *et al.* 1999). Furthermore, no metabolic energy becomes available by performing this reaction. In order to establish the production of 12 mol of hydrogen from 1 mol of glucose, two consecutive fermentations need to be joined to make one bioprocess (Figure 13.1). The reactions which form the basis of this bioprocess are shown in Table 13.2. The first fermentation enables the conversion of biomass to hydrogen and organic acids. Since many thermophilic bacteria, growing at 70–80°C, oxidize glucose to acetate as the lowest reductive state, the highest conversion efficiency with respect to hydrogen production is obtained using these bacteria (van Niel *et al.* 2002; de Vrije and Claassen 2003). In contrast, during anaerobic mesophilic fermentation, mixtures of acids and/or alcohols are produced and the hydrogen yield is lower (Table 13.3).

The conversion of the end-product of the thermophilic fermentation is hampered by unfavourable thermodynamics (Table 13.2). However, phototrophic purple, non-sulphur bacteria are able to overcome this barrier by employing energy from light during the utilization of acetate, which, like lactate, is a prime carbon source for these bacteria. Thus, complete conversion of hexoses, pentoses, oligosaccharides or starch, is established by coupling a thermophilic heterotrophic fermentation to a consecutive, photo-heterotrophic fermentation.

Table 13.2   Hydrogen production from glucose or acetic acid.

$C_6H_{12}O_6 + 6H_2O \longrightarrow 6CO_2 + 12H_2$	$\Delta G^{\circ\prime} = +3.2\,kJ$
$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CO_2 + 2CH_3COOH + 4H_2$	$\Delta G^{\circ\prime} = -206\,kJ$

(hyper)thermophilic
bacteria h?

$CH_3COOH + 2H_2O \longrightarrow 2CO_2 + 4\,H_2$ $\Delta G^{\circ\prime} = +104\,kJ$
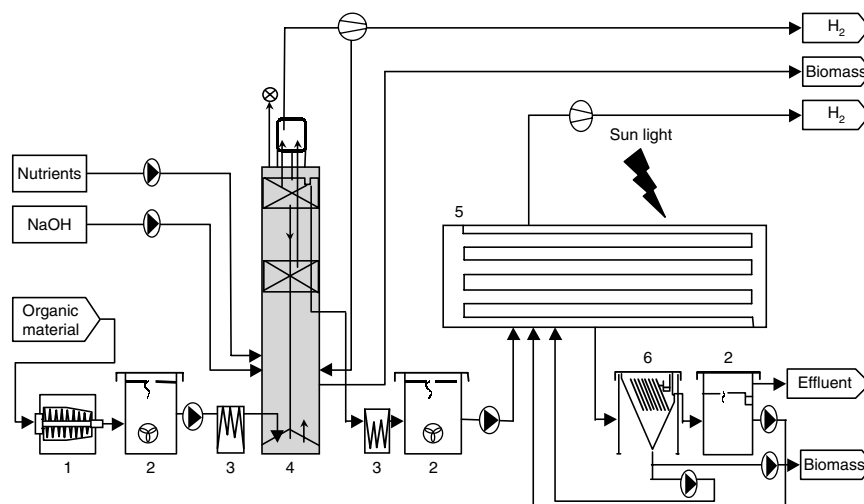
purple, non-sulphur
bacteria

Figure 13.1   Simplified flow sheet of a bioprocess for hydrogen production. The first reactor is for thermophilic heterotrophic fermentation; the second reactor is for photo-heterotrophic fermentation; 1: extruder; 2: tank; 3: heat exchanger; 4: thermoreactor; 5: photoreactor; 6: tilted plate settler.

Table 13.3   Hydrogen production by heterotrophic bacteria.

Micro-organism(s)	T (°C)	Substrate	mol H_2/mol monosaccharide	Reference
Thermotoga elfii	65	Glucose	3.3	van Niel *et al.* 2002
Caldicellulosiruptor saccharolyticus	70	Glucose, sucrose	2.7–3.3	van Niel *et al.* 2003
Clostridium spp.	36	Glucose	1.4–2.4	Taguchi *et al.* 1995
Enterobacter aerogenes	38	Glucose	0.6–1.0	Rachman *et al.* 1998
Mixed population in sewage sludge	35	Glucose, sucrose	1.7	Lin and Chang 1999; Chen *et al.* 2001

13.2.2   Important parameters in hydrogen production

As stated above, the highest yield in H_2 from glucose is obtained when the sub-strate is oxidized to 2 mol of acetate and 2 mol of CO_2 (Table 13.2). However, this equation has to be modified with biomass production since the oxidation of glu-cose to hydrogen is growth related during heterotrophic growth. As a result, the observed hydrogen production will amount to 75–80% of the maximum theoretical efficiency, which is usually observed in thermophilic cultures (Table 13.4). Similarly in photo-heterotrophic cultures, part of the carbon source is used for biomass production and again the equation in Table 13.2 has to be modified to account for biomass synthesis. Presently, the observed ranges of conversion efficiency in photo-heterotrophic cultures are wide. This is partially due to the contribution of

Table 13.4   Typical fermentation parameters obtained during heterotrophic and photo-heterotrophic hydrogen fermentation.

	Enzyme for H_2 production	H_2 production efficiency (% of theoretical maximum)	Light energy conversion efficiency (%)	H_2 production rate (mmol/g DW h)	Critical H_2 in gas phase (kPa)
Caldicellulosiruptor saccharolyticus	Hydrogenase	74–80	n.a.	29	20–56
Rhodopseudomonas sp.; *Rhodobacter capsulatus**	Nitrogenase	26–87	1–2	0.8–1.6	≥90

* Light limitation; n.a.: not applicable.

the photochemical efficiency, which is an important parameter during photo-heterotrophic growth as discussed by Akkerman *et al*. (2003).

In thermophilic bacteria, during heterotrophic growth electroneutrality is maintained by reducing protons to hydrogen through the action of the hydrogenase enzyme. Unfortunately, hydrogenase is inhibited by its own product, hydrogen. This leads to a critical hydrogen concentration in the gas phase that is dependent on the growth phase, indicated by the range of critical hydrogen concentrations shown in Table 13.4. As a result, hydrogen must be removed as soon as it is formed. This confers a great challenge for bioreactor design because, in general terms, high volumetric productivity is important for favourable cost balances. After evaluating several alternatives, amongst others adsorption to palladium slurries, the application of a trickle bed reactor has been shown to offer a great potential with respect to cost effectiveness (van Groenestijn *et al*. 2002). In this case, gas is the continuous phase and bacteria are growing in a thin biofilm allowing a maximal gas–liquid interface.

Purple non-sulphur bacteria employ the nitrogenase enzyme for hydrogen production instead of the hydrogenase enzyme (Akkerman *et al*. 2003). As a result, the fermentation is not inhibited by hydrogen and high hydrogen concentrations are allowed. This way, high volumetric productivities in dense cultures can be envisaged to improve the specific hydrogen production rate this far established (Table 13.4). However, high density cultures will conflict with sufficient light penetration so again, there is a severe demand on elaborate bioreactor design. Although high conversion efficiencies with respect to the carbon source have been achieved, light energy conversion efficiency and hydrogen production rate are parameters that necessitate further research and development (Table 13.4).

13.2.3   Perspectives

Several feedstocks have been investigated with respect to their potential application for the production of hydrogen and organic acids by thermophilic bacteria. Table 13.5 shows the occurrence and composition of potential feedstock throughout Europe. Generally, the feedstock that confers a competitive edge has high sugar or starch and high moisture content because of high conversion efficiencies.

Table 13.5   Production and composition of biomass for hydrogen production (w/w %).

Raw material	Production, dry weight	Starch	Cellulose	Hemi-cellulose, Pectin	Sucrose	Extractives	Lignin	Ash
Miscanthus	15–25 ton/ha, NL	–	38	24	–	7	25	2
Sorghum bicolor	30–40 ton/ha, GR	–	18	10	61	–	7	2
Paper sludge	8.5 Mton/ha, EU	–	36	4	–	–	18	35
Potato steam peels	0.6 Mton/ha, NL	51	n.d.	n.d.	–	28	n.d.	8
Domestic organic waste	1.6 Mton/ha, NL	–	24	20	–	13	12	16

Extractives are soluble sugars, protein, organic acids, lipids; n.d.: not determined; –: not applicable.

This is the case with potato steam peels and the juice of sweet sorghum, which is obtained after pressing the sucrose-rich stalks of the plants. However, in line with the search for cheap biomass for energy production, also lignocellulosic biomass, derived from energy crops or agro-industrial waste streams, as feedstock for hydrogen production has been applied (Claassen *et al*. 2002). When using *Miscanthus*, the residue of sweet sorghum stalks, paper sludge or domestic organic waste, pre-treatment and hydrolysis is required to mobilize the sugars in the (hemi)cellulose. This far, the industrial application for converting lignocellulosic biomass to fermentable feedstock, is hampered by either high environmental burden or high cost for environmental friendly procedures such as enzymatic hydrolysis. This problem is shared with other initiatives for biofuel production, such as ethanol. Progress in this respect has been recently achieved but further decrease in pre-treatment and hydrolysis costs is still required (www.novozym.com).

Thermophilic bacteria offer the advantage of the ability to metabolise hexoses and pentoses simultaneously, producing hydrogen from both substrates (de Vrije *et al*. 2002) but in anaerobic systems lignin remains untouched. Since several initiatives for hydrogen production from biomass are currently being researched, an obvious development would be to make an alliance with a thermochemical method to convert the non-fermentable biomass to hydrogen. This way, the moist fermentable part of the biomass would be substrate for fermentative conversion to hydrogen whereas the drier part can be transported to large-scale installations for thermochemical conversion to hydrogen.

13.3 HYDROGEN PRODUCTION FROM POTATO STEAM PEELS

Potato steam peels form a highly viscose slurry obtained as a by-product in the potato processing industry. The current use of this by-product is as component of

Table 13.6   Concentration of substrates and products after thermophilic fermentation by *Caldicellulosiruptor saccharolyticus* and subsequent photo-heterotrophic fermentation by *Rhodobacter capsulatus*, of potato steam peels hydrolysate.

mM	Glucose	H_2	Acetate	Lactate	CO_2
Start	63	0	7	16	0
End of thermophilic fermentation	18	131	75	22	67
End of photo-fermentation	0	280	0	0	n.d.

n.d.: not determined.

wet feed in the fodder industry. Because of the low N over C balance, mixing of potato steam peels with other wet by-products from, for example, the food industry is needed to achieve a nutritious feed. Due to several international developments in the feed industry as well as the energy sector, there is a current interest to convert this by-product to biofuel.

The main component in potato steam peels is starch (Table 13.5). Even though thermophilic bacteria are able to convert starch to hydrogen, liquefaction is desirable in view of adequate rheological properties. Besides, separation of the liquid hydrolysate and the solid residue results in a secondary by-product that is enriched in protein and possesses improved properties for processing to fodder.

13.3.1   Proof of principle

Potato steam peels hydrolysate, with glucose as its main carbohydrate component, is suited for hydrogen fermentation by *Caldicellulosiruptor saccharolyticus*. In Table 13.6 results of an experiment are shown of which the purpose was to demonstrate the complete bioprocess, that is the combination of a thermophilic heterotrophic and a photo-heterotrophic fermentation. As a result, ammonium ions were omitted from the substrate mixture for the thermophilic fermentation and this has led to the incomplete and relatively slow utilization of the substrate. Consumption and production of substrate and products, respectively, at the end of the batch fermentation in a submerged culture are shown. Hydrogen was continuously removed by stripping with nitrogen gas. The concentration of hydrogen is presented as cumulative hydrogen and was calculated from on-line measurements in the gas phase where the partial concentration was maximally 1.5%. The effluent of the thermophilic fermentation was transferred to a cylindrical photobioreactor and inoculated with *Rhodobacter capsulatus*. Hydrogen production was fairly slow but very efficient with respect to acetate conversion as 87% of the substrate was used for hydrogen production.

The achieved yield of hydrogen from glucose and organic acids in this two-stage bioprocess amounted to 47%, which is quite promising as compared to the 69% being the maximum achievable yield. This maximum achievable yield is derived from two separate fermentations that operate at 80% conversion efficiency. In the first fermentation one third of the hydrogen is produced, in the second the remaining two thirds. As a result, the total achievable conversion efficiency of the bioprocess becomes 69%.

Table 13.7   Techno-economic evaluation of a conceptual design
for biological hydrogen production from potato steam peels.

	€/h
Depreciation, maintenance, insurances and overhead	114.94
Personnel	10.00
Potato steam peels	39.37
Enzymes for hydrolysis	0.02
Caustic	6.27
Electricity	7.33
Total cost	177.93

13.3.2   Economic evaluation of a conceptual design

On the basis of results obtained and improvements that are deemed feasible on the short to medium term, the production costs of hydrogen in an industrial plant have been calculated. The assumed conditions in the conceptual design are a capacity of 17 and 40 kg hydrogen/h in the thermophilic and photo-heterotrophic fermentation, respectively, amounting to 57 kg hydrogen/h in total. The required volume of the trickle bed reactor used for the thermophilic fermentation is 450 m^3. This reactor is run at 70°C and a reduced pressure of 0.5 bar. The main dimension of the tubular photobioreactor is its surface area which amounts to 12 ha in total. The photobioreactor operates at 35°C and 2.5 bar. The dry off gas from the thermophilic fermentation contains 50% hydrogen whereas the off gas from the photobioreactor contains >85% hydrogen.

Most apparatus required for the industrial plant (reactor vessels, compressors, heat exchangers, etc.) are commercially available with the exception of the tubular photobioreactor. The cost of the available apparatus has been derived from the handbook of the Dutch Association of Cost Engineers and using a Lang factor of 4. The cost of the photobioreactor has been estimated on the basis of an experimental installation (400 m^2) employed for cultivation of other phototrophic micro-organisms. For operation of the industrial plant continuous operation of 8000 h per year was assumed with two operators working on an 8 h/day shift.

Potato steam peels were used as biomass, to be acquired at a cost which is presently in competition with the amount paid by the fodder industry in the Netherlands.

Table 13.7 shows a preliminary estimate of the operating cost of the plant in €/h. The total production cost of hydrogen amounted to €3.10/kg which is approximately three times the amount currently paid for hydrogen produced from fossil fuels in large-scale installations.

13.4   CHALLENGES FOR BIOLOGICAL HYDROGEN PRODUCTION

Biological hydrogen is aimed at providing a clean biofuel for use in fuel cells of small-scale installations. As such it meets all the societal demands for clean

environment, sustainable energy production, independence of foreign countries and development of rural communities (see www.biohydrogen.nl). Notwithstanding, even though it seems realistic that a cleaner environment will need to be paid for, decrease in hydrogen production cost is the main challenge. Since the presented bioprocess is still in the early stages of development, there appears to be sufficient room for optimization of all process units such as, reactor design, and increase of system efficiency.

The development of sustainable hydrogen production systems is associated with the development of fuel cells. Pure hydrogen is the feed by choice for proton exchange membrane (PEM) fuel cells with an operating temperature of around 90°C. On the other hand, molten carbonate fuel cells (MCFC) or solid oxide fuel cells (SOFC) that operate at much higher temperatures (600–900°C), enable the application of methane as feed. Presently, no fuel cells have reached the market yet with competitive prices. It is still obscure which fuel cell will fulfil best the future demands of cost-effective sustainability in the automotive sector or the stationary grid.

In spite of the uncertainties described above, there is one great, globally acknowledged, certainty with respect to the need for sustainability to decrease emissions as described in the Kyoto protocol. As such, it is of prime importance to further develop and meet the challenges inherent to the introduction of new energy carriers such as hydrogen, which enable the most efficient conversion of renewable resources.

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REFERENCES

Akkerman, I., Janssen, M., Rocha, J.M.S., Reith, J.H. and Wijffels, R.H. (2003) Photobiological hydrogen production: photochemical efficiency and bioreactor design. In: *Bio-methane and Bio-hydrogen* (eds. J.H. Reith, R.H. Wijffels and H. Barten), pp. 152–155. Dutch Biological Hydrogen Foundation, Smiet Offset, The Hague.

Chen C-C., Lin C-Y. and Chang J-S. (2001) Kinetics of hydrogen production with continuous anaerobic cultures utilizing sucrose as the limiting substrate. *Appl. Microbiol. Biotechnol.* **57,** 56–64.

Claassen, P.A.M., van Lier, J.B., Lopez Contreras, A.M., van Niel, E.W.J., Sijtsma, L., Stams, A.J.M., de Vries S.S. and Weusthuis R.A. (1999) Utilisation of biomass for the supply of energy carriers. *Appl. Microbiol. Biotechnol.* **52,** 741–755.

Claassen, P.A.M., Budde, M.A.W., van der Wal, F.J., Kádár, Zs., van Noorden, G.E. and de Vrije, T. (2002) Biological hydrogen production from biomass by thermophilic bacteria. *Proceedings of 12th European Conference and Technology Exhibition on Biomass for Energy, Industry and Climate Protection*, Amsterdam, 17–21, pp. 529–532.

de Vrije, T. and Claassen, P.A.M. (2003) Dark hydrogen fermentations. In: *Bio-methane and Bio-hydrogen* (eds. J.H. Reith, R.H. Wijffels and H. Barten), pp. 150–152. Dutch Biological Hydrogen Foundation, Smiet offset, The Hague.

de Vrije, T., de Haas, G.G., Tan, G.B., Keijsers, E.R.P. and Claassen, P.A.M. (2002) Pretreatment of Miscanthus for hydrogen production by *Thermotoga elfii*. *Int. J. Hydrogen Energ.* **27,** 1381–1390.

Dürre, P. (1998) New insights and novel developments in clostridial acetone/butanol/ethanol/isopropanol fermentation. *Appl. Microbiol. Biotechnol.* **49,** 639–945.

Hahn-Hägerdahl, B., Jeppson, H., Skoog K. and Prior B.A. (1994) Biochemistry and physiology of xylose fermentation by yeasts. *Enzyme Microbiol. Technol.* **19,** 933–943.

Lin, C-Y. and Chang R-C. (1999) Hydrogen production during the anaerobic acidogenic conversion of glucose. *J. Chem. Technol. Biotechnol.* **74,** 498–500.

Lynd, L.R., Elander R.T. and Wyman C.E. (1996) Likely features and costs of mature biomass ethanol technology. *Appl. Biochem. Biotechnol.* **57/58,** 741–761.

Maddox, I.S., Qureshi, N. and Gutierrez N.A. (1993) Utilization of whey by clostridia and process technology. In: *The clostridia and biotechnology* (ed. D.R. Woods) pp. 343–370. Butterworth-Heinemann, Stoneham.

Rachman, M.A., Nakashimada, Y., Kakizono, T. and Nishio, N. (1998) Hydrogen production with high yield and high evolution rate by self-flocculated cells of *Enterobacter aerogenes* in a packed-bed reactor. *Appl. Microbiol. Biotechnol.* **49,** 450–454.

Taguchi, F., Mizukami, N., Saito-Taki, T. and Hasegawa K. (1995) Hydrogen production from continuous fermentation of xylose during growth of *Clostridium* sp. strain no.2. *Can. J. Microbiol.* **41,** 536–540.

van Groenestijn, J.W., Hazewinkel, J.H.O., Nienoord, M. and Bussmann, P.J.T. (2002) Energy aspects of biological hydrogen production in high rate bioreactors operated in the thermophilic temperature range. *Int. J. Hydrogen Energy* **27,** 1141–1147.

van Niel, E.W.J., Budde, M.A.W., de Haas, G.G., van der Wal, F.J., Claassen, P.A.M. and Stams, A.J.M. (2002) Distinctive properties of high hydrogen producing extreme thermophiles *Caldicellulosiruptor saccharolyticus* and *Thermotoga elfii*. *Int. J. Hydrogen Energ.* **27,** 1391–1398.

van Niel, E.W.J., Claassen, P.A.M. and Stams A.J.M. (2003) Substrate and product inhibition of hydrogen production by the extreme thermophile, *Caldicellulosiruptor saccharolyticus*. *Biotechnol. Bioeng.* **81,** 255–262.