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MINI-REVIEW

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Utilisation of biomass for the supply of energy carriers

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Abstract Because biomass is a widely available, renewable resource, its utilisation for the production of energy has great potential for reducing CO_2 emissions and thereby preventing global warming. In this mini-review the 'state of the art' of several fermentation processes is discussed, starting with the most advanced process of ethanol production. This is followed by methane production, an established process for waste water purification which is gaining more attention because of the inherent energy production. Subsequently ABE fermentation is discussed and finally the biological production of hydrogen. The last section proposes a new way to assess and compare the different processes by relating their merit to 'work content' values and 'lost work' instead of the combustion values of their products. It is argued that, especially when dealing with energy from biomass, the application of this methodology will provide a uniform valuation for different processes and products. The described fermentation processes enable the supply of pure energy carriers, either gaseous or liquid, from biomass, yet the introduction of these processes is hampered by two major problems. The first is related to technological shortcomings in the mobilisation of fermentable components from the biomass. The second, having a much greater impact, is linked with socio-economics: until full externality costs are attributed to fossil fuels, accounting for their role in pollution and global warming, the competitiveness of the processes described here will hardly stand a chance.

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Resources and conversion technologies

Introduction

Biomass resources can be distinguished as byproducts with no or low profit from agricultural crops or industrial processes and as crops grown solely for the purpose of energy production. Other biomass resources are part of agricultural or industrial waste streams representing negative profit. In Europe and North America, agricultural byproducts include wheat straw, corn stalks and soybean residues whereas large industrial waste streams, in e.g. the United States, originate from the paper-making industry. The major crops presently grown for energy include sugar or starch crops such as sugar cane and corn. In addition, crops containing mainly lignocellulose, e.g. willow and poplar, are getting more attention with respect to their application for energy production.

Conversion technologies for the production of energy from biomass can be classified as biological (fermentation) or thermal (burning, pyrolysis, gasification). The focus of this review is on the biological production of liquid and gaseous energy carriers, i.e. ethanol, methane, acetone with butanol and ethanol (ABE) and hydrogen. These processes have been the subject of previous studies (see below) and can be characterised by the change in Gibbs free energy (Table 1) on the basis of glucose.

At present, the fermentation of sugars to ethanol is the best established process for conversion of biomass to energy. Ethanol production from sugarcane started in Brazil and the United States in the early 1970s. The

Table 1 Gibbs free energy values ($\Delta G'_0$) for product formation from the various fermentation processes with glucose. All calculations are based on stoichiometrical reactions per mol glucose using $\Delta G'_0$ values of formation for all components as given by Thauer et al. (1977). Stoichiometry for ABE is glucose:butanol:acetone:ethanol:acetate:H_2 = 1:0.5:0.3:0.1:0.1:2.6.

Fermentation process	$\Delta G'_0$ kJ/mol glucose
Ethanol	−225.5
Methane	−404.0
ABE	−209.3
H_2 (reaction 2)[a]	+3.2

[a] For reaction 2 see the section on hydrogen production

Brazilian production capacity in 1990 was about 15 billion l ethanol (Wyman and Hinman 1990). In the early 1990s the ethanol capacity in the United States was about 4.5 billion l/year. More than 95% of the United States' ethanol is made from corn produced from 1.3 million ha. For comparison, the estimate of a potential market for ethanol in the European Union is about 3.1 billion l/year, based on an expected petrol demand of about 134 billion l at the end of this century (Ruiz-Altisent 1994).

Supply of biomass

In attempting to assess the present potential biomass supply, assets may be considered in terms of world production of sugar, starch and lignocellulose. Most prevalent cereal produce in Europe and North America includes wheat and corn, while rice is mainly grown in Asia (Table 2).

Sugar and starch crops are produced all over the world but, depending on climatological differences there is a significant change in the type of crops grown in different areas. So sugar cane is produced in South America and Asia whereas sugar beet and potato are typical European crops. Sweet potato and cassava are mainly Asian and African products, respectively. Although these crops are grown primarily for food and feed applications they also produce large amounts for lignocellulosic and starchy side streams. In European countries, for example, 35% of the harvested overground biomass of the wheat crop is straw and 45% is grain. Furthermore, large amounts of starch and lignocellulose are produced as industrial byproducts. In the United States for example these side streams account for about 350 million t/year (US Department of Agriculture 1991). The potential of using lignocellulosic biomass for energy is even more pronounced when one realises that it is the most abundant and renewable organic component in the biosphere. It accounts for approximately 50% of the biomass in the world, which has been estimated at an annual production of 10–50 billion t (Lutzen et al. 1983; Goldstein 1981). Unfortunately, type and availability of lignocellulose in any particular geographic region depend on climatic and environmental factors, cropping practice, culture type and nature of the local technology (Kuhad 1993). As a consequence, general statements regarding regional availabilities are not possible.

Conversion of lignocellulose

Plant cell walls contain three major polymers: cellulose (an insoluble linear unbranched homopolysaccharide consisting of glucose subunits linked via β-1,4 glycosidic linkages), hemicellulose (non-cellulosic polysaccharides including mainly xylans, mannans, glucans) and lignin (an intricate polyphenolic structure). The total complex of these polymers is often referred to as lignocellulose (Coombs 1996). It is built up as cellulose fibres that are partially arranged in a crystalline structure, integrated with hemicellulose and embedded in a matrix of lignin. Therefore, in contrast to glucose and starch, the main problems encountered with the biological conversion of lignocellulose arise from its inaccessible structure. The sugar availability of polymers is low and, generally, hydrolysis of the cellulose and hemicellulose is the rate-limiting step. In addition to this structure-related property, the lignocellulose must be free from contamination with heavy metals, pathogens, parasite eggs, xenobiotics, etc., to allow proper and safe bioprocessing. Finally,

Table 2 Estimated global production of major agricultural crops. ~ only small amounts Source: FAO, Production Year Book, 1996. These figures are on a main product basin, excluding the considerable amounts of crop side products, such as straw, leaves, etc.

Continent	Production of crops (million metric tons)								
	Corn	Rice	Wheat	Soybean	Cane	Beet	Potatoes	Sweet potatoes	Cassava
Africa	44	16	23	~	80	4	8	7	85
Asia	155	513	230	21	505	36	89	124	46
Australia	~	~	~	~	40	~	1	~	~
Europe	60	3	127	1		188	156	~	~
N. and C. America	264	10	96	67	156	25	28	1	1
S. America	48	18	21	39	404	3	12	1	31
Oceania	1	24	~	~	45	~	1	~	~
World Total	576	562	585	130	1192	255	295	234	163

there are the quantitative aspects both of harvesting at low cost in acceptable yields and of transportation and storage.

The pretreatment and hydrolysis of lignocellulose can be carried out physically (e.g. steam treatment), chemically (e.g. by acid or alkaline hydrolysis), enzymatically, or by a combination of these methods. Hydrolysis and fermentation steps can be carried out successively, as in the Separate Hydrolysis and Fermentation process, or in a single-stage process in which microbial hydrolysis of the biomass and final fermentation are combined in one process: Simultaneous Saccharification and Fermentation (Vasquez et al. 1993). Although the latter process offers easier operation and fewer equipment requirements than sequential technology, it has some inconveniences including:

1. Different optima of conditions for hydrolysis and fermentation
2. Low substrate to liquid ratios due to the insoluble nature of the substrate
3. Difficult control and optimisation of process parameters

The selection of the most adequate pretreatment and hydrolysis is partially governed by the nature of the subsequent fermentation. However, the major problems in pretreatment and hydrolysis are the great differences in both lignin and hemicellulose composition, which are dependent not only on plant species but also on age, cultivation etc. (Coombs 1996). As a consequence, none of the available pretreatment processes can be used as a generally applicable process.

As a result of the unresolved problems associated with the utilisation of lignocellulose, no large scale lignocellulose-based bioethanol plants have been established yet. During the last decade, only sugar- and starch-based factories have been built in Brazil and the United States. However, this may change in the near future as a result of the worldwide research and development efforts aimed at applying lignocellulose as a fermentation feedstock. These efforts are borne by the insight that improved processing will deliver an abundant and widely available renewable resource with an impressive economic potential. Furthermore, separation and hydrolysis of the various components of biomass will allow total crop use which confers economic as well as environmental benefits.

Ethanol

Introduction

Ethanol has been used as an automotive biofuel ever since the introduction of the combustion engine. Ethanol is used either pure, or mixed with petrol or as the oxygenated fuel additive ethyl tertiary-butyl ether (Wyman and Hinman 1990). In 1935, over 430 million l ethanol were used in Europe alone (Buchanan 1989). However, after 1945 ethanol was replaced by petrol or diesel produced by the petrochemical industry (Amann 1996). The oil crisis in 1973 revived the interest in bioethanol, resulting in the Brazilian National Alcohol Production Program (PROALCOOL) in 1975 and the United States Gasohol Program (Lother and Oetterer 1995).

Microorganisms

The best known microorganisms for the production of ethanol are yeasts. Most are able to convert hexoses, via glycolysis, into pyruvate and subsequently decarboxylate pyruvate to acetaldehyde. To maintain the redox balance, acetaldehyde is further reduced to ethanol. In this way two moles of ATP per mol of hexose are generated.

Yeasts and fungi have an alternative pathway for the disposal of reducing equivalents besides the reduction of acetaldehyde, namely respiration. The regulation of fermentative metabolism on the one hand and respiration on the other is diverse and complex: the main determinants are the concentrations of oxygen and the fermentable carbon source. The baker's or brewer's yeast, *Saccharomyces cerevisiae*, has several distinct advantages over other yeasts:

1. Under excess carbon conditions, its metabolic flux to ethanol is hardly affected by the presence of oxygen (Lagunas 1979).
2. It is able to grow under strict anaerobiosis (Visser 1995).
3. It has a high ethanol tolerance, amounting to 150 g/l ethanol.

Substrates

At present, only cane sugar and corn starch are used as substrates for ethanol production on an industrial scale. The costs for these feedstocks account for 40–70% of the production costs of ethanol (Dale and Linden 1984) and, as they are expensive, the competitive production of bioethanol on the basis of these substrates is not realistic. Therefore, there is a search for alternative feedstock, focussing on the utilisation of lignocellulose (Wyman and Hinman 1990). However, *S. cerevisiae* is not very well equipped for the fermentation of lignocellulose. This organism has a limited substrate spectrum and may ferment only mono- and disaccharides such as glucose, fructose, maltose and sucrose. Furthermore, this yeast has no cellulolytic nor saccharolytic properties and thus lignocellulose must be hydrolysed to release fermentable sugars prior to fermentation. Finally, *S. cerevisiae* is unable to convert the C5-sugars present in hemicellulose and this confers a serious drawback in view of the large quantity of hemicellulose in the lignocellulose complex.

Process technology

The bulk of ethanol produced by yeast is generated in batch processes. The substrate is added to a final concentration of 15–25% solids (w/v). The pH during fermentation is 4–5 to reduce infection risk, and the temperature is 30–35 °C. The substrate efficiency is 85–90% (g ethanol/g substrate) of the theoretical maximum. The remainder is converted to yeast biomass, and by-products such as glycerol and acetate. The biomass produced can be reused several times, although the viability of the yeasts decreases after several transfers. The productivity of batch processes is generally 1–3 g ethanol/l per hour. When fermentation is completed, the broth, containing 80–100 g/l ethanol, is fed into a steam-heated stripping column to remove the ethanol by distillation. The product is a 95% ethanol-5% water mixture (Murtagh 1986). The largest ethanol producing plant is the Archer Daniels plant in Decatur Ill. with an annual capacity of about 1.3 billion l bioethanol using corn as substrate (Lynd et al. 1996).

Process improvements

Technology

The production rate in batch processes is limited by the concentration of both biomass and ethanol, the latter exerting an inhibiting effect on the growth and production rates. Therefore, improvements of the process have been largely targeted at cell retention systems and on-line removal of ethanol. The retention of microbial biomass is achieved by immobilising microorganisms in the carrier material (Lother and Oetterer 1995), by microfiltration membrane modules (Groot et al. 1993) or by fermentation in tower reactors in which the flocculating yeast remains in the reactor.

The ethanol inhibition can be decreased by using continuous cultivation or systems in which a number of reactors is arranged in cascade (Coombs, 1996). Other methods are the continuous removal of ethanol during the process by applying a vacuum to (a part of) the fermentation broth or by membrane pervaporation/perstraction (Groot et al. 1993). The effects of each method on productivity are summarised in Table 3. These technological improvements in the process increase the investment costs drastically and it is still unclear whether the gain from improved productivity can compensate for this.

Table 3 Effect of process operation on the productivity of bioethanol production by *S. cerevisiae* (Coombs 1996)

Process	Productivity (g/l per h)
Batch cultivation	1.8–2.5
Continuous cultivation	6
Continuous cultivation in a cascade of bioreactors	20
Continuous removal of ethanol by means of vacuum	80
Continuous removal of ethanol by membrane separation	100

Microorganisms

Up to now the production of ethanol by *S. cerevisiae* is unchallenged by other yeasts, fungi or bacteria. However, the saccharolytic, cellulolytic and thermophilic properties found in other ethanol-producing microorganisms may result in more efficient ethanol production in the future. Other yeasts such as *Pichia stipitis*, *Candida shehatae* and *Pachysolen tannophilus* are able to ferment C5-sugars. However, the ethanol production rate of these yeasts with glucose as substrate is at least five times lower than observed in *S. cerevisiae* (for a review of xylose fermentation by yeasts, see Hahn-Hägerdal et al. 1994.) Furthermore, these yeasts require oxygen and their tolerance to ethanol is 2–4 times lower than *S. cerevisiae* (Hinamn et al. 1989).

Amongst the ethanol-producing bacteria, *Zymomonas mobilis* has emerged following isolation from 'pulque', the fermented juice of agave. This bacterium converts hexoses by the 2-keto-3-deoxy-6-phosphogluconate pathway and splits pyruvate into CO_2 and acetaldehyde. Subsequently, acetaldehyde is reduced to ethanol. In the whole conversion of hexose to ethanol, only 1 mol ATP/mol hexose is formed. This low energetic efficiency is the reason for interest in ethanol production by *Z. mobilis*, because it results in a high flux and yield of ethanol (97% w/w of the theoretical maximum, Lynd et al. 1996). *Z. mobilis* has a fairly high ethanol tolerance, allowing concentrations up to 100 g/l (Lynd et al. 1996). Furthermore, *Z. mobilis* has a higher optimal temperature than *S. cerevisiae* which reduces the cost of cooling during fermentation. One drawback is that the substrate range of *Z. mobilis* is limited as only glucose, fructose and sucrose can be converted into ethanol. This will restrict the application of this bacterium.

Thermophilic, saccharolytic clostridia such as *Clostridium thermohydrosulfuricum*, *C. thermosaccharolyticum* and *C. thermocellum* are able to produce almost 2 mol ethanol/mol hexose. First, glucose is converted to pyruvate by glycolysis. Pyruvate is cleaved into acetyl-CoA and CO_2 and, in order to maintain a neutral redox balance, acetyl-CoA becomes reduced to ethanol after exhaustion of other electron acceptors. Besides hexoses, these bacteria also convert C5-sugars and amino acids to ethanol.

Clostridia are saccharolytic and are thus able to use a wide range of untreated agricultural substrates. *C. thermocellum* grows even on cellulose and hemicellulose and can, therefore, be used for the direct microbial conversion of lignocellulosic materials into ethanol (McMillan 1997). The main disadvantage of clostridia is their low

tolerance to ethanol, i.e. the maximum attainable ethanol concentration by *C. thermocellum* is less than 30 g/l. However, the high growth temperatures make cooling during the fermentation superfluous and create ways for the on-line removal of ethanol by distillation or pervaporation.

Genetic modification

The major disadvantage of *S. cerevisiae* in the production of bioethanol is its inability to utilise C5-sugars. To overcome this drawback, the genes encoding xylose reductase and xylitol dehydrogenase were cloned into *S. cerevisiae*. These enzymes are responsible for the conversion of xylose via xylitol to xylulose which is a C5-sugar that can be fermented by *S. cerevisiae*. After transformation, *S. cerevisiae* was able to produce ethanol from xylose, but the productivity was low and xylitol was formed as byproduct, diverting substrate from ethanol production (Hahn-Hägerdal et al. 1994).

Methane

Introduction

During anaerobic degradation of organic matter in environments in which the availability of inorganic electron acceptors is limiting, organic material serves as both electron donor and electron acceptor, resulting in the production of CO_2 and methane. This process, known as anaerobic digestion, was first discovered in lake bottoms and swamps (Boone 1991). From the end of the last century onwards, anaerobic digestion has been also applied in man-made environments for both energy production and as a cost-effective method for waste stabilisation (Lettinga 1996; Lettinga 1999; Van Lier et al., 1997). The latter refers to the 'dual energy benefit' of anaerobic digestion: no energy is required for stabilising wastes. Quite the contrary, an energy-rich end-product is produced. The positive energy balance on one hand and the increasing energy demand on the other have generated a growing interest in anaerobic digestion. During anaerobic degradation, the chemical energy present in organic compounds is largely conserved as methane. If glucose is fermented in a methanogenic fermentation the Gibbs free energy change under standard conditions ($\Delta G'_0$) is −404 kJ/mol. However, oxidation of the 3 mol methane formed per mol glucose will yield a $\Delta G'_0$ of −760.2 kJ/mol (Thauer et al. 1977). So in total:

$$\text{Glucose} \rightarrow 3\,HCO_3^- + 3\,CH_4 + 3H^+ - 404\ \text{kJ} \quad (1)$$

$$3\,CH_4 + 6\,O_2 \rightarrow 3\,CO_2 + 6\,H_2O - 2280.5\ \text{kJ} \quad (2)$$

From a technological point of view it is important to note that a complete methanogenic conversion occurs by mixed microbial communities yielding methane as the sole reduced organic product.

Microorganisms and substrates

Methane formation is carried out by methanogenic archaea. These microorganisms possess a unique biochemistry which enables them to derive metabolic energy from the methanogenic pathway (Whitman et al. 1992; Thauer 1998). Most of the described species of methanogens are rather specialized. *Methanobrevibacter* spp. are only able to use $H_2 + CO_2$ for growth, whereas *Methanosaeta* spp. only use acetate as their energy substrate. *Methanosarcina* spp. are more versatile; they can use $H_2 + CO_2$, acetate, methanol, methylated amines and pyruvate for growth and methane production (Whitman et al. 1992; Jetten et al. 1992)

As a consequence of the limited range of substrates utilised by methanogens, the anaerobic breakdown of organic matter is carried out by communities of different physiological types of anaerobic bacteria (Stams 1994; Schink 1997; Stams and Oude Elferink 1997). Figure 1 illustrates the different phases of the anaerobic digestion process. Biopolymers like polysaccharides, proteins, nucleic acids and fats are first hydrolysed by extracellular enzymes. The monomers and oligomers which are formed, such as sugars, amino acids, purines, pyrimidines and glycerol are fermented by a wide variety of different types of bacteria. The products that are formed include on the one hand hydrogen, formate and acetate, which in their turn can be converted by methanogens, and on the other hand propionate, butyrate and higher fatty acids. These higher fatty acids have to be anaerobically oxidised to methanogenic substrates prior to further conversion to methane and CO_2, but the $\Delta G'_0$ of this conversion is highly positive. Therefore,

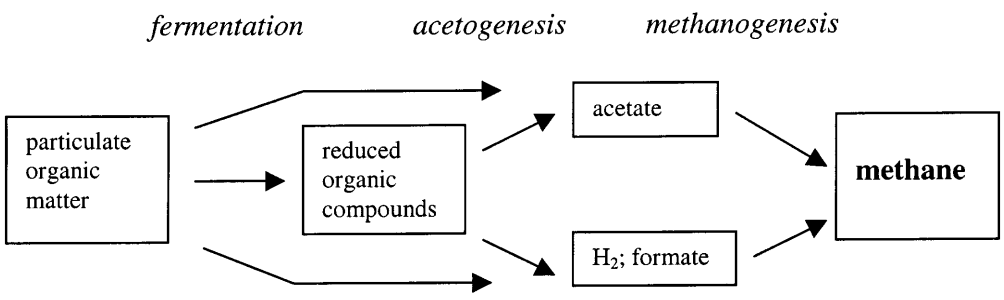

Fig. 1 Phases of the anaerobic digestion process

methanogens are needed in order to efficiently remove hydrogen and acetate. This means that these proton-reducing acetogens are obliged to be dependent on methanogens. *Syntrophobacter* spp. are able to degrade propionate but not butyrate, while *Syntrophomonas* and *Syntrophosporora* are able to degrade butyrate and some higher fatty acids but do not degrade propionate. Recently, *Smithella propionica* was described, a bacterium which oxidises both propionate and butyrate (Liu et al. 1999).

According to thermodynamic calculations, the fatty acid oxidation can only proceed within very narrow ranches of the hydrogen partial pressure, typically 1–100 Pa (Cord Ruwisch et al. 1988; Stams 1994), implying that high conversion rates are only possible if hydrogen-producing acetogens and hydrogen-consuming methanogens are in close proximity to each other. Therefore, the biomass is highly aggregated in bioreactors. Disruption of the structure results in a severe drop of the specific methanogenic activity.

Under moderate conditions about 70% of the methane is formed by cleavage of acetate, while about 30% of the methane is derived from $H_2 + CO_2$ or formate (Gujer and Zehnder 1983). At higher temperature (60–70 °C) most methane is formed from $H_2 + CO_2$, because at higher temperatures acetate is anaerobically oxidised to $H_2 + CO_2$ (Zinder 1994). There are indications that at low temperatures (<15 °C) the relative contribution of acetate cleavage to total methanogenesis is substantially higher than 70%.

Process applications

Anaerobic digestion of sewage sludge, manure and other kinds of organic waste material has already been applied for a long time. Farm-scale digesters were developed in countries like India and China for biogas generation from livestock manure. The energy produced is used for cooking and lighting on the farms. At present the technology is strongly supported by government aid programmes to combat deforestation in ecologically sensitive regions and to produce bioenergy.

The production of bioenergy is also of interest in Western countries where environmental concerns and depletion of resources are leading the further development of anaerobic digestion technology. A classic example is the ordinary landfill where municipal solid waste is stored and slowly mineralised under anaerobic conditions. Different full scale projects have been realised, producing 1000–2000 m^3 biogas/h. The energy content of the biogas is 20–25 MJ/m^3 with a methane content of 55–70% (by volume) and a CO_2 content of 30–45% (IEA 1996).

The Danish Government is using a favourable tariff system to subsidise centralised biogas plants for the treatment of manure, industrial waste and municipal solid waste. The stabilised residue is distributed to farmers linked to the centralised digester, for soil conditioning and fertiliser. The annual potential for biogas production from biomass resources presently available in Denmark is estimated to be of the order of 25–30 PJ (700–800 million m^3 methane/year). Animal manure comprises about 85% of this potential (Tafdrup, 1994). While aerobic composting requires 20–35 kW h_e per ton of waste input, anaerobic digestion produces about 100–200 m^3 biogas per ton, which is equivalent to a production of 75–150 kW h_e per ton. In addition to methane and CO_2 odorous gases like H_2S are also collected with the biogas (generally 200–4000 ppm by volume). The latter reveals an additional, important environmental advantage of controlled stabilisation of organic wastes under anaerobic conditions: reduced emissions of methane, CO_2 and odorous gases into the air.

By introducing source separation, the energy content of the incoming municipal solid waste gradually increases, making the system more compact. Anaerobic digestion for municipal solid waste treatment is performed in several types of reactor systems, which can roughly be divided into continuous flow systems and batch-type systems. The latter is considered to be more economic (Jumelet and van der Knijff, 1991).

Advantages and disadvantages

The advantages of anaerobic waste treatment are obvious. Organic waste is dealt with in a sustainable and controlled manner, additionally yielding methane which can be used for various purpose. Speece (1996) has listed advantages and disadvantages of the anaerobic wastewater treatment technology in comparison with conventional aerobic methods. Although anaerobic waste (water) treatment was developed for environmental engineering purposes, it may significantly contribute to meeting the energy demands of industry and replace energy from fossil resources. For instance, an alcohol distillery can derive up to 50% of the industrial energy demand by using its own wastewater, reducing organic pollution to the environment by 80%.

Important disadvantages are that anaerobic treatment is associated with sulphide and odour formation and that nitrification is not possible. Thus, aerobic post treatment is essential. Efficient methods to deal with sulphide and odour problems have been developed recently (Buisman et al. 1990). Sulphide can be converted in a micro-aerobic process to elementary sulphur, and the recently observed anaerobic ammonium oxidation can be implemented in existing anaerobic treatment technologies (Jetten et al. 1998). Also, the anaerobic process is relatively easily disturbed and methanogenic bacteria are fairly susceptible to toxic compounds. However, once immobilised in biofilms or granules, this susceptibility of methanogens is largely annulled.

Process improvements

Anaerobic digestion is a relatively cheap and plain type of technology that can be implemented on any scale and at any place (Lettinga 1996). Technologically, there are not many obstacles to couple waste treatment with energy production. Problems are more in the field of public perception of the implementation of anaerobic treatment technology in existing processes. Nonetheless, it is obvious that methanogenic treatment of organic waste and wastewater is a way to preserve fossil resources by avoiding the use of electricity in environmental technologies and in this way energy is produced from biomass.

Acetone, butanol and ethanol (ABE)

Introduction

During the first part of this century the anaerobic production of ABE by solventogenic clostridia was the second largest biotechnological process in the world. This fermentation was initially aimed at the production of acetone for the war industry and later at the production of butanol for the lacquer industry (Jones and Woods 1986). After World War II, petroleum-based production of solvents replaced the biological processes and, as a result, almost all the industrial-scale fermentation facilities have been closed. Currently the ABE fermentation process is operated commercially only in China (Dürre 1998).

The oil crisis in the 1970s revived interest in ABE fermentation because of the potential utilisation of agricultural resources for the production of chemicals or fuels instead of fossil fuels. The three major factors that hamper the economic viability of ABE fermentation are; (1) the high cost of the substrates (e.g. molasses), (2) the low product concentration (about 20 g/l due to solvent toxicity) and (3) the high product recovery costs (distillation has been used). Since the 1980s there has been considerable research on this process in order to make it economically viable again, generating extensive knowledge on the physiology and genetics of the solventogenic clostridia (Woods 1995; Girbal and Soucaille 1998) and leading to the development and improvement of product recovery techniques (Maddox et al. 1993; Dürre 1998).

Microorganisms and process biochemistry

Since the beginning of the ABE process, many solvent-producing clostridial strains have been described. In the past, most of these strains have been classified as *Clostridium acetobutylicum*. Currently, the solventogenic clostridia are classified into four groups of species based on biochemical and genetic differences (Woods 1995). The best known groups are *C. acetobutylicum* and *C. beijerinckii*, all growing at 30–37 °C. During the exponential growth of *C. acetobutylicum* at pH values greater than about 5.6 (depending on the strain), the major fermentation products are acetic and butyric acid, CO_2 and hydrogen. As acids accumulate in batch culture, growth becomes linear and gradually stops. When the pH drops to 4.5–5.0, a shift in fermentation occurs and solvent formation is triggered. Solventogenesis requires the induction of a new set of enzymes catalysing the formation of acetone, butanol and ethanol from glucose and reassimilated acids. The shift to solventogenesis is induced by high intracellular concentrations of acids, low pH, a growth limiting factor (such as phosphate or sulphate depletion) and high concentrations of glucose and nitrogen compounds (Woods 1995). Interestingly, solventogenic clostridia, depending on the strain and fermentation conditions, can produce other alternative solvents such as propanol, isopropanol and 1,2 propanediol (Rogers and Gottschalk 1993).

Substrates

Formerly, ABE fermentation was operated as a batch process followed by distillation to recover the products. Sugars (molasses) or starch (corn, wheat, potatoes) were used as substrates. In this process the price of the substrate accounts for up to 60% of the cost, dramatically affecting the economic viability of ABE fermentation. This and the ability of saccharolytic clostridia to utilise a wide range of carbohydrates have stimulated research into the use of alternative, cheaper substrates. A number of different compounds have been checked as possible substrates for ABE fermentation (Table 4). Byproducts from the dairy industry (i.e. whey) and agricultural by-products seem to be the most interesting substrates.

Table 4 Novel substrates for ABE fermentation by *Clostridium* spp

Substrates	Main carbon components	Organism	Reference
Non-cellulosic			
Apple pomace	Fructose, glucose, sucrose	*C. beijerinckii*	(Voget et al. 1985)
Jerusalem artichokes	Glucose	*C. beijerinckii*	(Marchal et al. 1985)
Whey	Lactose	*C. acetobutylicum*	(Maddox et al. 1993)
Low-grade potatoes	Starch, glucose	*C. beijerinckii*	(Nimcevic et al. 1998)
Lignocellulosic			
Wood hydrolysate	Glucose, mannose	*C. acetobutylicum*	(Maddox and Murray 1983)
Domestic organic waste hydrolysate	Glucose, xylose	*C. acetobutylicum*	(Claassen et al. 1998)
Peat	Glucose, xylose	*C. beijerinckii*	(Forsberg et al. 1986)
Palm oil effluent	Lipids, glucose, xylose, starch	*C. aurantibutyricum*	(Sombrutai et al. 1996)

However, even though most solventogenic clostridial strains are able to utilise all the sugars present in lignocellulose hydrolysates and can degrade some polymeric substrates, such as starch or xylan, they are not able to grow on cellulose as sole carbon and energy source (Jones and Woods 1986; Mitchell 1998).

Process improvements

Technology

Much effort has been devoted to the production of cheap fermentable feedstocks or a combination of processes in which hydrolysis of polymers is intimately linked to the production of solvents. Many studies have shown that acid and/or enzymatic hydrolysates of cellulosic material from a variety of biomass sources are potential feedstocks for ABE fermentation (Jones and Woods 1986; Claassen et al. 1998). Often, the cellulosic material has to be pretreated in order to make it more accessible to chemical or enzymatic hydrolysis; the most common pretreatment is steam explosion (Maddox and Murray 1983) but extrusion has been also used (Claassen et al. 1998). Yu et al. (1984) have investigated the utilisation of cellulose and hemicellulose in acid hydrolysed, steam exploded wood. The production of 9 g/l, with nearly theoretical product yields (0.26 g butanol/g sugar), indicates that the bioconversion of carbohydrates from wood cellulose and hemicellulose is feasible.

Another approach to enable the utilisation of biomass is the application of several forms of Simultaneous Saccharification and Fermentation systems:

1. Co-culture systems. The direct conversion of cellulose and solka floc to solvents by co-cultures of *C. acetobutylicum* with the cellulolytic organisms *C. cellulolyticum* or *C. thermocellum*, respectively has been shown. In both cases, the production of solvents was very poor, possibly due to the fact that the concentrations of sugars and butyric acid in the medium were too low to induce the solventogenic phase. To increase the level of butyric acid in the medium, co-culture of *C. acetobutylium* and *C. beijerinckii* with butyric acid-producing strains of *C. butyricum* and *C. pasteurianum* have been tested, but the amount of solvents produced was no higher that in the monocultures (Jones and Woods 1986).
2. Addition of cellulases to the culture medium. Several strains of *C. acetobutylicum* and *C. beijerinckii* are able to grow and produce solvents from crystalline cellulose when a cellulase preparation from the fungus *Trichoderma reesei* is present in the medium. Fermentation of alkali-pretreated wheat straw by *C. acetobutylicum* in a medium supplemented with cellulase from *T. reesei* resulted in 17.3 g solvents/l and solvent yields of 18.3 g ABE/100 g pretreated wheat straw after 36 h fermentation. These results may represent an economic improvement with respect to separate hydrolysis and fermentation of these substrates.

Genetic modification

Developments in the genetics of several clostridia have provided molecular techniques for the genetic manipulation of these organisms (Young 1993; Blaschek and White 1995; Dürre 1998). The genome of *C. acetobutylicum* ATCC 824 has been sequenced by Genome Therapeutics Genome Sequence Center (USA) as a component of the Department of Energy Microbial Genome Project (USA). Raw data have been made publically available via the internet (http://www.cric.com/genesequences/clostridium/clospage.html). Attempts have been made to increase the cellulolytic activity of *C. beijerinckii* by transformation with cellulase (endoglucanase) genes from *C. cellulolyticum* and *C. cellulovorans* (Minton et al. 1993; Kim et al. 1994). However, no cellulolytic recombinant strains have been constructed so far.

Another genetic approach to improve ABE fermentation is the development of strains with increased solvent production. *C. acetobutylicum* ATCC 824 has been transformed with a plasmid containing endogenous genes involved in acetone formation. The transformed strain was stable and showed a significant increase in solvent production. However, a control strain transformed only with the vector also showed increased solvent production, albeit to a lesser extent than the strain containing the extra genes (Mermelstein et al. 1992). Other reports show modification in solvent production in genetically manipulated strains (Green and Bennett 1998; Nair et al. 1999). Recently, the pilot-scale production of butanol by *C. beijerinckii* NCIMB 8052 parent and by butanol hyper-producing mutant strain BA101 in an inexpensive glucose/corn steep water medium has been described (Parekh et al. 1999). In a 200 l batch fermentation, *C. Beijerinckii* NCIMB 8052 produced 6.2 g/l acetone and 12.6 g/l butanol, whereas *C. beijerinckii* BA101 produced 5.5 g/l acetone and 17.8 g/l butanol. These experiments indicate that solvent production can be manipulated at the genetic level.

Hydrogen

Introduction

Interest in hydrogen has been renewed in the last decade, especially in Japan and Germany and to some extent in the United States, for the purpose of replacing the utilisation of fossil fuels in the energy and chemical industries. The major advantage of energy from hydrogen is the lack of polluting emissions since the utilisation of hydrogen, either via combustion or via fuel cells, results in pure water. In contrast to the other energy carriers discussed in this review, hydrogen from biomass can be directly produced by gasification or fermentation. The current status and prospects of the biological production of hydrogen from biomass will be summarised here. For further insight the reader is referred to the extensive reviews covering the

significant progress that is being made for all possible biological processes and their economic feasibility (Benemann 1996), or the biological hydrogen production rates obtained in an extensive amount of studies since the 1970s (Nandi and Sengupta 1998).

Microorganisms produce hydrogen from organic compounds either through utilisation of the chemical energy of these substrates only (heterotrophic fermentation) or by additional utilisation of light energy (photoheterotrophic fermentation; Benemann 1996). In both cases, the production of hydrogen is intimately linked with the respective energy metabolism. Hydrogen evolves as the final product of reductant disposal from hydrogenase or nitrogenase activity. The primary electron donor for both enzymes is ferredoxin, which receives electrons in its turn from the reduced products of glycolysis, i.e. NADH or NADPH (Glass et al. 1977; Bryant and Adams 1989).

The two processes have their advantages and disadvantages, but in general the yield of hydrogen from either process is still below 20% (Benemann 1996). As yet the technical feasibility of the fermentations has not been demonstrated and therefore no biohydrogen production processes are currently operative on a commercial basis.

Microorganisms and process biochemistry

Photoheterotrophic fermentation

The majority of the studies on biohydrogen production from organic waste over the past two decades have focussed on the use of photosynthetic bacteria. Best results were obtained with species of the genera *Rhodopseudomonas* and *Rhodobacter*. Successful hydrogen formation from organic waste (yoghurt wastes and whey) was demonstrated for the first time by Zürrer and Bachofen (1979), with the photosynthetic bacterium *Rhodospirillum rubrum*. The maximum production rate obtained was 20 ml H_2/h per g cells, which is the order of magnitude seen in many studies with photofermenting organisms.

Since most of the energy in photofermentation is derived from the organic substrate, a relatively small light-energy input is required for hydrogen production. Hence a smaller photobioreactor is necessary as compared to a photoautotrophic hydrogen production process. However, the efficiency of the photofermentation remains low because the photosystem of the cell becomes light saturated at low light intensities (6.5–20 klx; Sasikala et al. 1993), which controls the maximum hydrogen evolution rate.

In general, factors determining optimal hydrogen production, such as pH, temperature, light intensity and wavelength, concentration of electron donor, age of culture, cell density and nutritional history of the cells, may differ from optimal factors for growth (Sasikala et al. 1993). Therefore, separation of microbial growth and product formation have often improved hydrogen production yields (Hillmer and Gest 1977; Sasikala et al. 1993). For instance, best hydrogen production was obtained by growing photosynthetic bacteria under dark aerobic conditions and subsequent exposure to light under anaerobic non-growth conditions (Mao et al. 1986).

Another unique type of hydrogen production can be accomplished with photosynthetic bacteria performing the shift of CO and H_2O into hydrogen and CO_2 in darkness (Uffen, 1976). This CO was generated from thermally gasified wood chips (Weaver et al. 1998). Apparently, the CO-linked hydrogenase is most suited for practical applications and oxygen-resistant enzymes have been identified (Weaver et al. 1998). The enzyme mediates hydrogen production from CO at rates up to 3 mmol/min per g cell dry weight.

Heterotrophic fermentation

Heterotrophic fermentations aimed at the production of hydrogen can be carried out by a wide variety of microorganisms such as strict anaerobes (clostridia, ruminococci, and archaea), facultative anaerobes (*Escherichia coli* and *Enterobacter aerogenes*) and aerobes (e.g. *Alcaligenes eutrophus* and *Bacillus licheniformis*) when kept under anoxic conditions (Nandi and Sengupta 1998). Production rates up to 11 H_2/h per g cells can be accomplished (calculated from a study with a *Clostridium* strain on cellobiose, Taguchi et al. 1996). Many types of organic compounds, ranging from polymers to monomeric carbohydrates, fats and amino acids are known to be sources for hydrogen. Here, the focus is on the conversion of glucose for the estimation of potential yields. The yield per mol of glucose has been found to be maximally 4 mol hydrogen as is described in the reaction (Solomon et al. 1995; Kengen et al. 1996):

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

The $\Delta G'_0$ of this reaction is −206 kJ/mole, which is sufficient to allow microbial growth.

Because of a positive ΔG under standard conditions ($\Delta G'_0 = +3.2$ kJ/mole), essentially no energy is obtained from the complete conversion of glucose to hydrogen and CO_2 (Thauer 1976):

$$C_6H_{12}O_6 + 12\,H_2O \rightarrow 12\,H_2 + 6\,HCO_3^- + 6\,H^+ \quad (4)$$

The remainder of the potential hydrogen is contained in byproducts like acetate and, to a lesser extent, ethanol and lactate. The conversion of acetate to hydrogen according to:

$$CH_3COOH + 2\,H_2O \rightarrow 4\,H_2 + 2\,CO_2 \quad (5)$$

is thermodynamically unfavourable at moderate temperatures ($\Delta G'_0 = +104.6$ kJ mole) and is strongly determined by the hydrogen partial pressure. At hydrogen

partial pressures of 10–100 Pa, acetate oxidation becomes thermodynamically feasible at temperatures of 40 °C and higher (Lee and Zinder 1988b).

With respect to the whole process of thermophilic heterotrophic fermentation, the partial hydrogen pressure in the gas phase should be kept low (<2 kPa), otherwise the growth rate may decline (Kelly 1988) and, depending on the strain, the product formation may shift from acetate to lactate (Janssen and Morgan 1992) or to alanine (Kengen and Stams 1994), thus decreasing the final yield of hydrogen per mol of glucose. At concentrations higher than 2 kPa, hydrogen may even inhibit growth (Schröder et al. 1994; Schäfer and Schönheit 1991). However, a shift of the metabolism in favour of hydrogen production may be established by optimising process conditions, in particular by limiting nutrient and product concentrations (e.g. hydrogen partial pressure below 50 Pa; Lee and Zinder 1988b) and by increasing the temperature. The increase in temperature is aimed at a better performance of the hydrogenases of the hyperthermophiles, because the affinity for hydrogen decreases (Adams 1990) and the thermodynamic equilibrium of hydrogen formation from e.g. acetate is favoured at higher temperatures (Lee and Zinder 1988b). Indeed, a bacterium that oxidised acetate anaerobically to hydrogen and CO_2 at a temperature of 60 °C has been isolated and described (Lee and Zinder 1988a). Since then, similar organisms have been demonstrated in thermal activated sludges (Ahring et al. 1995; Van Lier 1996) and in Islandic hot springs at 70 °C (Ahring 1992), proving that the ΔG at this temperatures is negative enough to allow growth.

Process improvements

The development of hydrogen-producing photofermentations with high yields still requires a considerable amount of research. A major improvement would be to increase the level of light saturation of the photosystems to enhance the efficiency of light energy conversion. Overall, selection of suitable microorganisms and optimisation of process conditions are required for fast and efficient production of hydrogen. In heterotrophic fermentation, the liquid-to-gas mass transfer of hydrogen should be improved by developing efficient gas-liquid separation installations in order to avoid process limitations, as hydrogen, being a poorly soluble gas, can be easily overconcentrated to as much as 80 times the value of thermodynamic equilibrium (Pauss et al. 1990). In both cases, i.e. photoheterotrophic fermentation and heterotrophic fermentation, there is room for the option of genetic modification to redirect metabolism to generate the reducing power for hydrogen production (Benemann 1996). Finally, bioreactors for optimal performance especially of photofermentations or combinations of different fermentations could be designed, such as the combination of a heterotrophic fermentation performing e.g. reaction (3) and a photofermentation process carrying out reaction (5) enabling the total conversion of 1 mol glucose into 12 mol hydrogen.

As compared to the other fermentations discussed, the biological production of hydrogen as an energy carrier is still in its infancy. Therefore, it is too early to predict which of the possibilities mentioned above will ultimately be successful, or how they would appear in practice as small-scale or large-scale production processes. It is expected that in the near future it will be evident whether higher efficiencies of hydrogen production from organic compounds (including cellulose) can be obtained with hyperthermophiles, which feature prominently. At present, yields of 10–20% have been reported, but economic feasibility will only be achieved when yields of 60–80% can be realised (Benemann 1996). Besides the production of hydrogen, the application of hyperthermophiles has other specific properties on offer which make the heterotrophic process outstanding. The extreme temperatures create a selection pressure for hydrogen producers preventing other processes which compete for the subtrates or consume hydrogen. Accordingly, this process is less sensitive to contamination. Furthermore, hyperthermophiles excrete a variety of exoenzymes that convert biopolymers such as starch, cellulose, hemicellulose, xylan and peptides, thus simplifying the degradation of biomass.

Assessment of bioenergy

Introduction

From the above it may be clear that when dealing with bioenergy, i.e. energy derived from biomass, all sorts of combinations of renewable resources, processes and products are possible. Firstly, energy from agricultural resources will never occur as a single product from conversion processes. Instead, it will appear in a 'basket of product streams'. Besides bioenergy, such a 'basket' may contain food products, feed products or non-food products. Secondly, as has been shown in the previous sections, different conversion technologies are becoming available to extract energy or energy carriers from the biomass. Finally, the energy carriers can have more than one application, e.g. as an automotive fuel or as a fuel for the production of electricity.

The relationship between the various possibilities is shown in Fig. 2. The complex matrix makes it difficult to compare products from different 'baskets' that result from different processes or different resources as described in the previous sections. For instance, in the case of fermentation of wheat by yeast, the 'basket' consists of ethanol, CO_2, specific proteins, fibrous material and, when wheat is used for the production of ABE, the 'basket' will contain acetone, butanol, ethanol, CO_2, hydrogen' proteins and fibrous material. The protein fractions obtained in the two processes have different

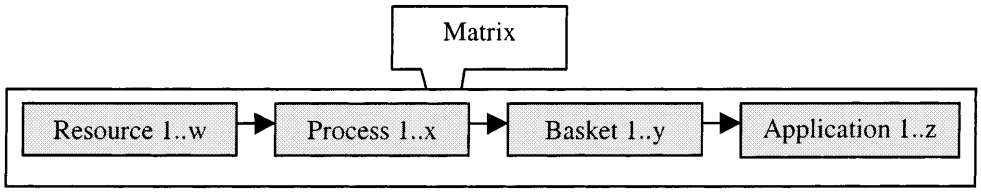

Fig. 2 The complex matrix of resources, processes, 'baskets' and applications

compositions, which is also true for the fibre fraction, the mineral fraction and the other fractions. The classical 'energy related' methods compare energy from different resources and different processes on the basis of energy obtained from combustion. Table 5 shows the energy obtained after combustion of the energy carriers produced in the fermentation processes described with glucose as feedstock.

It will be clear that this method is not adequate to determine which process is best since it refers to 'pure' compounds. Even though an extrapolation could be performed to estimate costs related to downstream processing, handling, storage etc. of the energy carriers, other applications and non-energy products in the 'basket' remain neglected (Wright and Hughes 1993; Sonesson 1993; Poitrat 1994; IEA/AFIS 1998). Therefore, production processes are only described partially by the classical 'energy related' methods. This emphasises the necessity to define a new methodology for a valid comparison of all processes aimed at the production of energy from biomass. The application of the concepts of 'work content value' and 'lost work' is introduced below as a new methodology for the valuation and comparison of such energy production processes.

Concepts of 'work content value' and 'lost work'

The methodology is based on thermodynamics and comprises two related concepts, i.e. the concepts of 'work content value' and 'lost work'. The concept of 'work content value' was formerly described as 'available work' (Çengel and Bowles 1994) or 'energy' (Kotas 1995; Szargut et al. 1988). The concept of 'work content value' is defined as the work potential of an amount of substance in relation to the equilibrium environment in which the work content is zero. The equilibrium environment is defined as a reservoir of substances in the atmosphere, the hydrosphere and the lithosphere, all in equilibrium with each other. The concept of 'lost work' is defined as the loss of 'work content value' due to irreversible changes during processing.

In Fig. 3 the 'energy related' methods and the method based on the 'work content value' are compared with respect to reference situation and applicability. The wide applicability of the concept of 'work content value' allows the uniform valuation of all products in the 'basket'. This may be used e.g. in industrial processes where some product streams are fixed at zero or negative prices because of the lack of an actual outlet even though the outlet for bioenergy is recognised. As a result, a process becomes based on wrong or uncertain cost prices (De Vries 1998).

Furthermore, the uniform valuation delivers a tool for the allocation of ingoing streams in processes to outgoing product streams in the 'basket'. In this way, separate products from different processes or different resources can now be compared e.g. with respect to CO_2 emission as a life cycle aspect. In short, the application of the concepts 'work content value' and 'lost work' produces secure data on several aspects, including:

1. the comparison of different resources in terms of process efficiencies to obtain different product streams
2. the comparison of different processes in terms of efficiency by analysis of 'lost work'
3. the allocation of external inputs in processes to product streams and per unit of a product stream
4. a stable and consistent analysis of life cycle environmental aspects related to product streams

When these concepts are applied for valuation and comparison of energy production from wheat grain, it becomes clear that fermentation shows relatively small losses of 'work content value'. On the other hand, electricity production processes such as the Rankine steam cycle process, utilising comparable agricultural resources, have much higher losses and are less efficient in terms of the product streams obtained (De Vries 1998, 1999). These results differ markedly from energy balance calculations that are based on 'energy related' methodologies (Fig. 3).

Table 5 Gibbs free energy values ($\Delta G'_0$) for the combustion of energy carriers from different fermentation processes. All calculations are based on stoichiometrical reactions per mol glucose using $\Delta G'_0$ values of formation for all components as given by Thauer et al. (1977). For comparison, the $\Delta G'_0$ value for the direct combustion of glucose is given. Stoichiometry for ABE is glucose:butanol:acetone:ethanol:acetate:H_2 = 1:0.5:0.3:0.1:0.1:2.6. For reactions 3, 4, 5 see the section on hydrogen production

Fermentation process	$\Delta G'_0$ (combustion) in kJ
Ethanol	−2463.6
Methane	−2280.5
ABE (−H_2)	−1780.0
ABE (+H_2)	−2396.7
H_2 (reaction 3)	−890.9
H_2 (reaction 5)	−1781.8
H_2 (reaction 4)	−2672.7
Glucose direct combustion	−2698.6

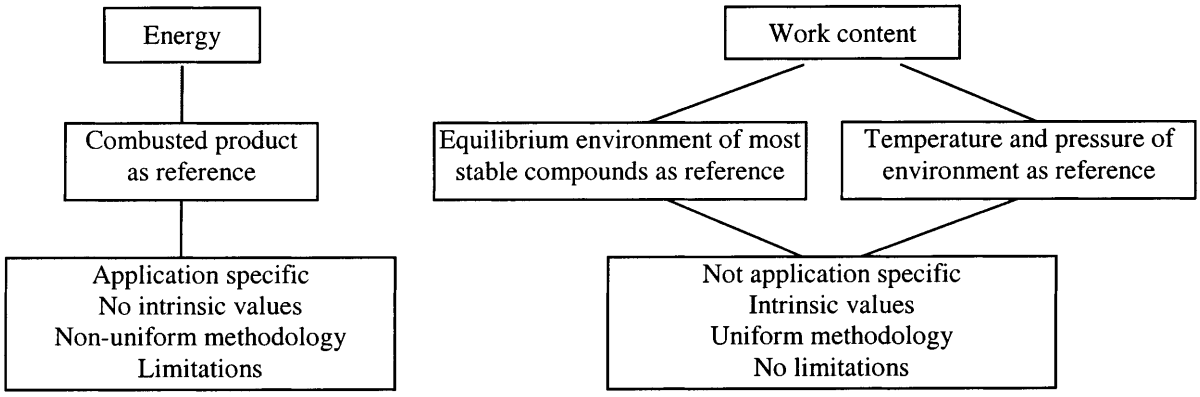

Fig. 3 General differences between 'energy related' methods and the method based on the concept of 'work content value'

Requirements and applications

Application of the methodology to determine 'work content value' requires a thorough description of the biomass in a detailed chemical way up to the level of chemical groups. The complex composition of biomass will initially be a bottleneck because agricultural science has not focussed enough on the acquisition of data in this specific field. Besides, process descriptions have to be executed on the basis of detailed process parameters to allow analysis in terms of 'lost work' and to allocate ingoing streams to separate main products in the 'basket'.

At present, the application of the thermodynamic concepts is hampered by the lack of this detailed information in most literature on bioenergy. This is unfortunate because the established processes for bioethanol and methane production might already benefit from such analyses, facilitating their socio-economic acceptance. Thus, an adequate methodology is of prime importance since the methodology used to analyse bioenergy options influences the final results and may affect decision making with respect to bioenergy. Furthermore, process analysis in terms of 'lost work' will provide clear indications as to the directions in which research and development can be most effectively implemented.

Future perspectives

Even though energy saving programmes will be installed in the near future, the global demand for energy is expected to keep increasing. To reduce the CO_2 emission, the most sensible solution to meet the increased energy demand is the utilisation of renewable instead of fossil resources. From the several options in development, such as the utilisation of solar, wind, or geothermal energy, the utilisation of biomass seems closest at hand. By applying the biological processes discussed here, which offer different options dependent on the nature of the available feedstock, local technology and infrastructure, biomass is able to supply gaseous or liquid fuels (Table 6). However, the competitiveness of these fermentations, compared to the production processes of similar energy carriers from fossil resources, is low mainly due to the present extremely low price of oil. This problem will only be solved when the intrinsic environmental burden from fossil fuels becomes realistically assessed and subsequently subjected to payment e.g. through eco-taxation.

From that point on, there will be only the competition between biological conversion and other biomass conversion technologies such as combustion, gasification, pyrolysis, or hydrothermal upgrading. As is true for all of these conversion technologies, the experiences with the few existing plants combined with further research and development will eventually indicate which process has the optimal mix of features with respect to regional products, technological development, infrastructure etc. In short: the present state of research and development is not far enough advanced to make definite choices yet.

In contrast to the total crop use in thermal conversion technologies, which result in heat and/or synthesis gas, pyrolysis oil or a fraction called biocrude (because of its similarity to crude oil), fermentation processes produce

Table 6 Current 'state of the art' of potential biotechnological processes for energy production

Energy carrier	Substrates currently applied	Pretreatment of biomass required	Process	Product recovery	Technology of process
Ethanol	Hexoses	Yes	Established	Fairly easy	Moderate
Methane	Biomass	No	Established	Easy	Low
ABE	Starch, lignocellulosic hydrolysates	Starch:no Biomass:yes	Awaiting improvement	Awaiting improvement	Moderate
Hydrogen	Under study	Under study	Development	Easy	Under study

well defined compounds which are energy carriers, feedstock for chemical industry or may be utilised as such, plus valuable byproducts. These valuable byproducts have not yet been studied in detail but it is easy to understand that after anaerobic fermentation of lignocellulose, lignin plus the proteinaceous bacterial biomass will be found in the byproducts. Besides these major byproducts, there may be other much smaller fractions like amino acids and vitamins, the recovery of which will add to the total economic feasibility, or profitability of a biological process. Sometimes, fermentation even offers the possibility of favouring one product at the cost of another or vice versa, dependent on process parameters. All these features form in fact the quintessence of the application of fermentation for a balanced conversion of biomass. The willingness of mankind to pay high prices for energy in the future is a great uncertainty. It seems more realistic to extract all components from biomass in order to produce a multifunctional 'basket' of products each with their own, maybe marginal, return on investment. This scenario requires an increase of research into the steps preceding fermentation, i.e. biomass pretreatment and hydrolysis, and the immediate commencement of an investigation of the byproducts obtained after recovery of the primary products.

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