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MINI-REVIEW

Biofuels from microbes

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Abstract Today, biomass covers about 10% of the world's primary energy demand. Against a backdrop of rising crude oil prices, depletion of resources, political instability in producing countries and environmental challenges, besides efficiency and intelligent use, only biomass has the potential to replace the supply of an energy hungry civilisation. Plant biomass is an abundant and renewable source of energy-rich carbohydrates which can be efficiently converted by microbes into biofuels, of which, only bioethanol is produced on an industrial scale today. Biomethane is produced on a large scale, but is not yet utilised for transportation. Biobutanol is on the agenda of several companies and may be used in the near future as a supplement for gasoline, diesel and kerosene, as well as contributing to the partially biological production of butyl-t-butylether, BTBE as does bioethanol today with ETBE. Biohydrogen, biomethanol and microbially made biodiesel still require further development. This paper reviews microbially made biofuels which have potential to replace our present day fuels, either alone, by blending, or by chemical conversion. It also summarises the history of biofuels and provides insight into the actual production in various countries, reviewing their policies and adaptivity to the energy challenges of foreseeable future.

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Introduction

The Stern-Review on the Economics of Climate Change has publicised the economic necessity to limit global warming (Stern 2006). An accelerated release of fossil entombed CO_2 due to human activity is now generally accepted as a major factor contributing to the green house effect (IPCC 2001). Approximately 28% of the energy available for consumption in the EU25 countries is attributed to transportation, of which, more than 80% is due to road transport (Eurostat 2007). Worldwide, about 27% of primary energy is used for transportation, which is also the fastest growing sector (EIA 2006). Transportation fuels are thus promising targets for a reduction in greenhouse gas emissions. However, only a limited selection of energy-rich chemical compounds, primarily liquid alcohols and esters, can be produced by microbes, while complying with the criteria necessary for modern fuels, suitability for safe storage and high energy efficiency in combustion engines and capacity to power a transportation vehicle. In flight applications where the fuel comprises a significant fraction of the load, the energy density of the fuel also becomes a major factor for consideration.

Biomass fuels have been used throughout man's long history. Most of them were alcohols produced by the fermentation of substances like starch or sugars, others were plant oils. Alongside combustion, they were put to a variety of uses as solvents, greases, cleaners or as basic chemicals for the emerging chemical industry, until a cheaper source was found in fossil oil. Today, with rising prices for crude oil and increasing political instability in oil producing countries, the use of bio-based alcohols as solvents or basic chemicals is again under consideration. The production of chemicals and fuels from locally grown plant material supports political independence through diversification and a decreased dependence on a few essential energy sources, a CO_2 neutral energy production and a surplus of gross national product and economic power being kept in the country. This is especially crucial to remote and economically disadvantaged agricultural areas.

Beginning with a short exploration of the history of biofuels, the state of microbial fermentation to produce biofuels in today's world is reviewed and discussed, giving insight into some prospects which are still in the research stage but have promising prospects for future production.

Biofuels in history

Mankind has, for most of its existence, relied on renewable energy resources like wood, windmills, water wheels and animals such as horses and oxen. The development of new energy resources was a major driving force of the technological revolution. By the beginning of the twentieth century, up to 30% of arable land was still planted with crops to feed horses and oxen used in transportation. Already, early in the nineteenth century, alcohols were repeatedly reported as biofuels. Even the invention of ignition engines was done with biofuels. Nikolaus August Otto developed his prototype of a spark ignition engine in the 1860s using ethanol and was sponsored by the sugar factory of Eugen Langen who was interested in the mass production of ethanol. Deutz Gas Engine Works designed one third of their heavy locomotives to run on pure ethanol in 1902. Safety and cleanness were contributing factors for that. Ethanol was soon recognised as an anti-knocking additive for internal combustion engines and was added to gasoline between 1925 and 1945. The ethanol present in the fuel allowed higher piston compression, increasing engine efficiency.

Henry Ford was engaged in the chemurgy movement to bring farm products into production and energy supply, especially during the "Great Depression" (Finlay 2004). His car company marketed the Model T, the "Tin Lizzy", running on 100% ethanol (Kovarik 1998). In the Midwest (USA), blended gasoline made up 25% of Standard Oil's fuel sales in the 1920s (Giebelhaus 1980; Finlay 2004). In 1940, ethanol production was widely abolished due to the unbeatably low price of gasoline in the USA. However, in WWII, various warring nations used a wide range of biofuels. It was recognised that gasoline additives, such as tetra-ethyl-lead, required for high compressions in ethanol free engines, were environmental and health hazards. Despite these apparent risks, the petro-derived gasoline finally came to dominate the transport market after WWII (Source: http://www.ybiofuels.org/).

Ethanol as a fuel was revived in the 1970s in Brazil (IEA 2004) where one of the largest bioethanol industries is located today. The bioethanol industry in Brazil was criticized as environmentally hazardous, as large land area is being used for monocultures. A similar discussion was sparked in North America and Europe where starch production for biofuel competes over land with the food industry and environmental issues. The sky rocketing price for starch is already hindering the start-up of new bioethanol plants.

Like modern crude oil refinery, the bioindustry for biofuels has a dual purpose in the economy, as it is used as a supply of energy as well as basic chemicals (Zaborsky 1982). The upcoming "biorefinery" revitalizes the old tradition of a careful thrifty economy and intends to make use of all energy and carbon stored in biomass, feeding by-products into secondary conversion process or refining them as fuel.

Different types of biofuels today

Only biodiesel and bioethanol are presently produced as a fuel on an industrial scale (Table 1). Including ETBE partially made with bioethanol, these fuels make up more than 90% of the biofuel market. All biofuels have to exhibit defined chemical and physical properties, meeting the demands of engine application such as stability and predictable combustion at high pressures as well as the demands of transportation such as safety and energy density.

Transportation and distribution

There are gaseous and liquid energy carriers of microbial origin (Table 1). Fuels that are liquid at atmospheric

Table 1 List of selected biofuels with a potential microbial production route, the status of their use and the engine application

Biofuel	Process	Status	Engine application
Biomethanol	Thermochemical/microbial	Pilot plant	[Pure/blend] MTBE/biodiesel
Bioethanol	Microbial	Industrial	Pure/blend
Biobutanol	Microbial	Pilot plant/industrial (until ca 1990)	Pure/blend
ETBE	Chemical/microbial	Industrial	Blend
Biomethane	Microbial	Industrial	Pure/blend
Biohydrogen	Microbial	Laboratory	Bioethanol (Syngas)/pure
Biodiesel	Physical/chemical (enzymatic)	Industrial (laboratory)	Pure/blend

pressure and at ambient temperature (e.g. alcohols) can be easily stored, distributed, carried and used as an energy source in cars, trucks, trains and planes. Gases of microbial origin have to be liquidised by cooling or compression to reduce specific volume. As this process is energy intensive, gaseous forms of biofuel are primarily used for on-site applications in stationary engines. Gaseous fuels such as hydrogen and methane are difficult to transport and would require for a new distribution infrastructure to be developed.

Liquid biofuels have to remain in a liquid state and pumpable at all temperatures encountered. Further requirements on liquid biofuels are a high heat of combustion value to reduce energy losses and costs during transportation and stability during storage. Some longer chain alcohols like butanol have a heat of combustion sufficiently high to allow for their use in high thrust-to-weight applications such as airplanes (Schwarz and Gapes 2006). For safe and environmentally friendly storage, vapour pressure and ignition temperature are important factors.

The transition from fossil fuels to selected liquid biofuels can be smooth and should use preferably the same, or slightly modified, distribution infrastructure and engine technology in place for fossil fuels.

Engine application

There are, in principle, three strategies to utilise energy from biofuels in engines. The engines have to either be adapted to the existing fuels or the biofuel has to be designed to exhibit, as closely as possible, all important features of traditional fossil fuels. The third strategy is to use biofuels blended with traditional fuels. This often results in an advantageous modification of the fuel's properties, such as an increased octane rating without the use of environmentally harmful additives.

Alcohol-based biofuels like methanol, ethanol and *n*-butanol cannot only extend the supply of gasoline and diesel, or even replace them, but are also good additives to existing fossil fuels as oxigenisers, liquefiers or anti-knocking agents (Schwarz et al. 2007). However, not all energy-rich products of microbial fermentations can be used as fuels because they may promote corrosion or swelling with certain materials or may have other unfavourable characteristics.

Metabolic strategy for production procedures

To extract as much energy content as possible from the biomass during combustion, the transformation into fuels has to reduce the number of oxygen atoms per carbon. This is achieved in a disproportionisation reaction, a balanced redox reaction, during the anaerobic metabolism of microorganisms (Zeikus 1980). During the process, CO_2 is extracted from carbohydrates which have a C/H/O ratio of 1:2:1. Glucose ($C_6H_{12}O_6$), a single sugar molecule, would for example be converted to two molecules CO_2 and two molecules ethanol:

$$C_6H_{12}O_6 \xrightarrow{redox} 2CO_2 + 2C_2H_6O_1$$

Ethanol, with its high (C+H) to O ratio, retains most of the original energy content in combustion. As a cell can produce much less energy from this anaerobic reaction than from oxidative respiration, it has to consume about ten times the amount of substrate to gain the same amount of energy; 2–3 ATP compared to 26–38 ATP in oxidative respiration, depending on the organism. This higher turnover of substrate is an advantage for biotechnology. This anaerobic fermentation also helps to avoid energy intensive aeration during industrial production.

Most fermentation processes work at mesophilic temperatures (25–37°C), but thermophilic processes (45–55°C) seem to be more productive and are increasingly researched and developed. A disadvantage of thermophilic processes is a higher sensitivity of the microorganisms to the toxicity of the solvent products at the higher temperatures. Likewise, a higher sensitivity of the fermentation to media parameters like pH or composition of the substrates was noticed in processes such as thermophilic biomethane fermentations.

Biological systems for fuel production

The following description of biofuels and of their microbial production processes is by no means exhaustive, but will encompass the most interesting existing and possible processes where microbial fermentation from bacteria and yeasts is involved. All microbial fermentation processes require a source of energy to feed the organisms, which has to come from biomass in the form of sugars (Zeikus 1980).

Hydrogen

Hydrogen is regarded as an ideal fuel for future transportation because it can be converted to electric energy in fuel cells or burnt and converted to mechanical energy without obvious production of CO_2 (Malhotra 2007). This has lead to the idea that an economy driven by hydrogen fuel is a viable prospect. However, hydrogen production is usually effected by thermal/chemical means and is energy intensive, so hydrogen produced in such a way cannot be regarded as a renewable primary energy source. In contrast, biological hydrogen production from biomass would provide an energy-saving, cost-effective and pollution-free alternative and should be investigated extensively based on these merits (Das and Verziroglu 2001; Esper et al. 2006).

Hydrogen can be produced biologically by algal and cyanobacterial bio-photolysis of water or by photo-fermentation of organic substrates from photosynthetic bacteria. In addition, it can be produced by "dark" fermentation from organic substances by anaerobic organisms such as acidogenic bacteria. This has the additional appeal of reducing the mass of organic waste (Wu et al. 2005). High hydrogen yields can be achieved by the use of thermophilic microorganisms such as *Caldicellulosiruptor saccharolyticus* or *Thermotoga elfii* (de Vrije et al. 2002; de Vrije and Claassen 2003; Claassen et al. 2004). These fermentations can be operated in liquid phase with immobilised cells or by enabling the formation of self-flocculated granular cells or sludge to prevent washout of the hydrogen-producing cells.

Moreover, H_2 is a common product in anaerobic bacterial fermentations and may be an interesting by-product in future large-scale industrial fermentation. As an example of this, around 40×10^6 m^3 of H_2 and 60×10^6 m^3 of CO_2 were produced annually from the 1960s to the 1980s in a Russian biobutanol plant as by-product, but were not used at the time (Zverlov et al. 2006).

Microbiological hydrogen production is not yet developed into an economically viable technology, and hydrogen production is lagging behind expectations. Biological production from renewable biomass, which would make it a sustainable primary energy source, still needs more research and development. Hydrogen (and also alcohols) can be used in fuel cells to create electric power for transportation. BMW claims that the technology of hydrogen utilisation in combustion engines is mature, while other companies focus on the use of fuel cells. Although cars with fuel cells are technically feasible, VW announced that hydrogen cars will not play a prominent role in mass production until 2020. Initial systems are established on a local basis to test components during all stages from production to utilisation.

Methane/biogas

Biogas plants produce methane gas sustainably along with carbon dioxide from plant biomass, which may come from organic household or industrial waste or from specially grown energy plants (Yadvika et al. 2004). The advantage of the biogas process is the option to use the polysaccharide constituents of plant material to produce energy, such as electrical power and heat, in relatively easy-to-manage and small industrial units. Alternatively, the gas can be compressed after purification and enrichment and then fed to the gas grid or used as a fuel in combustion engines or cars. Its greatest advantage is the environmentally friendly aspect of the technology, which includes the potential for complete recycling of minerals, nutrients (phosphate etc.) and fibre material (for humification) which come from the fields and return to the soil, playing a functional role by sustaining the soil's vitality for future plantation. The technology is currently mature, but there is plenty of room for optimisation, which will result in large high-tech production plants with integrated utilisation of by-products (biorefinery; Fig. 1).

Substrate can be cow manure which is also useful for inoculation, manure from other farm animals such as pigs, chickens and horses, fat from slaughter waste or frying oil,

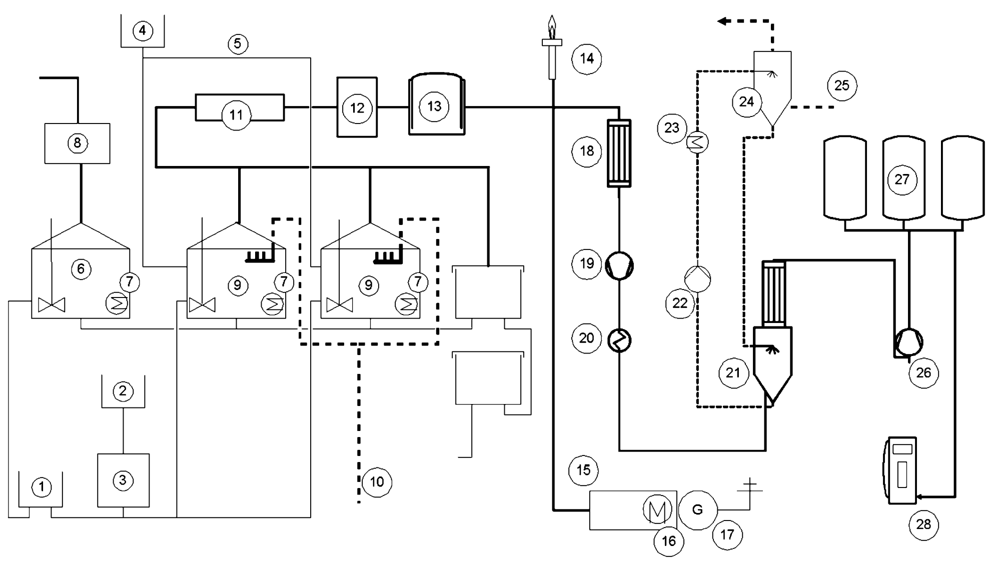

Fig. 1 Schematic representation of a two-stage biogas plant with production of electric power/heat and compressed biogas as fuel: *1* slurry storage, *2* waste storage, *3* sanitisation plant, *4* solid substrate storage, *5* solid substrate feed, *6* hydrolysis stage, *7* heating, *8* biofilter, *9* methanogenesis stage, *10* air for desulfurisation, *11* condenser, *12* desulfurisation (microbial/chemical), *13* gas holder, *14* emergency flare, *15* biogas engine, *16* heat recovery for plant heating, *17* generator, *18* activated carbon filter, *19* compressor, *20* chiller, *21* absorption column, *22* pump, *23* heating, *24* desorption column, *25* air, *26* compressor, *27* compressed biogas storage, *28* gas station

organic household or garden waste, municipal solid waste and rotten foodstuff. Even organic waste from hospitals containing paper and cotton, municipal sewage sludge, waste from agriculture or food production, organic-rich industrial waste water etc. can be used as consumable substrate. Often, energy crops such as maize (whole plant including the corn), clover, grass, young poplar and willow are especially grown for biogas production and added purely or in mixture. To ensure a homogeneous substrate quality throughout the year, the green plant material is usually stored as silage, preferably by a process favouring homofermentative lactobacilli to minimise carbon loss (Gassen 2005).

Biogas formation from plant fibres is generally a three-stage process involving a different set of anaerobic and facultatively anaerobic microorganisms in each stage:

1. hydrolysis of polysaccharides (starch, cellulose, hemicellulose etc.), proteins and fats into oligosaccharides and sugars, fatty acids and glycerol. This is followed by acidogenesis, the fermentation of these products into mainly acetic, propionic and butyric acid, carbon dioxide and hydrogen, alcohols and other minor compounds;
2. acetogenesis: the production of acetic acid and carbon dioxide. Due to the long generation time of these bacteria this seems to be the limiting process step;
3. methanogenesis with up to 70% (*v*/*v*) CH_4 and 30% CO_2 and the by-products NH_3 and H_2S by slow-growing archaea, which are sensitive to acidification, ammonia accumulation, low amounts of oxygen and other factors.

The bacterial community engaged in these three stages may be similar to those in cows rumen (Einspanier et al. 2004) or wastewater treatment plants (Ariesyady et al. 2007). However, their composition varies depending on the substrate, the type of fermenter and the process (e.g. mesophilic or thermophilic; O'Sullivan et al. 2005; Syutsubo et al. 2005; Klocke et al. 2007; Cirne et al. 2007). Some bacteria involved have been isolated and characterised, but comprehensive studies on the biological system in pure plant biomass fermenting plants are still widely missing, especially on the hydrolytic and the thermophilic processes.

Traditional farm biogas plants are run as a single- or two-stage process at around 37°C with an uncontrolled secondary fermentation in large storage tanks. Due to different optimal conditions specific for the hydrolytic and the methanogenic bacteria, two-stage processes are increasingly applied, particularly in large industrial biogas plants. Most biogas fermentation tanks are run as liquid fermenters. The biogas fermentation tank may contain more than 12% (*w*/*v*) dry mass (so-called dry fermentation) or less (liquid fermentation).

The German Renewable Energy Act (EEG) and a similar law in Austria has spurred the construction of 3,500 (G) +600 (A) biogas plants with an average electrical power output of 500 kW (and even more heat) in combined heat and power plants. Larger plants of 5 MW electrical power output are still rare, but will be constructed in increasing numbers. Their size is limited by land intensive production and the transportation costs for bulky substrate. The large quantity of energy crops required for biogas production provoked discussions among environmentalists, especially in Germany, about the issues of monoculture and resulting soil deprivation. However, proper crop rotation and the recycling of material, such as waste fibre materials, minerals and nutrients, can minimise these effects.

Further development of biogas technology is expected to increase production efficiency. Presently, only up to a maximum of about 70% of the organic matter in biomass is converted to CH_4 and CO_2. In order for this to increase, the hydrolysis stage must be enhanced. The separation of the processes for hydrolysis and for acetogenesis/methanogenesis allows for the application of different optimised conditions in the two stages, such as pH and temperature adjustment. Aside from the traditional mesophilic processes, thermophilic processes are being used more frequently to speed up the reactions and especially to optimise biomass hydrolysis. However, whereas in many industrial biogas plants the separation of the hydrolysis stage has already been carried out, most agricultural biogas plants use the single-stage technology. Therefore, several plant components laid out in Fig. 1 may be absent.

Dried and desulfurised biogas is usually fuelled without CO_2 separation into stationary block heat and power plants connected to the biogas plants. Utilisation of the excess heat is rarely possible because farms are usually located far away from residential or industrial areas where it could be used for domestic heating or manufacturing processes. This is not a problem if biogas is compressed like compressed natural gas stored in high-pressure cylinders and used in the engines of urban co-generation plants. In addition, direct use in car combustion engines is possible (Fig. 1).

Ethanol

Bioethanol fermentation is by far the largest scale microbial process. State of the art industrial ethanol production uses sugar cane molasses or enzymatically hydrolysed starch (from corn or other grains) and batch fermentation with yeast *Saccharomyces cerevisiae* to create ethanol. Byproducts of this process are CO_2 and low amounts of methanol, glycerol etc. Ethanol does not need to be rectified to high purity if it is to be used as a fuel. The azeotrop of 95.57 wt % ethanol with 4.43 wt% water can be used in ignition cars. This is known as AEHC in Brazil (álcool etílico hidratado combustível). However, higher water content causes problems in ethanol–gasoline mixtures partly because it leads to phase separation between the water and gasoline.

Yeast fermentation of glucose syrups to ethanol has been well progressed in recent years. Inhibitor sensitivity, product tolerance, ethanol yield and specific ethanol productivity have been improved in modern industrial strains to the degree that up to 20% (*v*/*v*) of ethanol are produced in present-day industrial yeast fermentation vessels from starch-derived glucose.

The future will see a technical process using lignocellulosic hydrolysates (Gray et al. 2006). However, as the enzymatic hydrolysis reaction of cellulose is about two orders of magnitude slower than the average ethanol fermentation rate with yeast, there is a theoretical gap in simultaneous saccharification of cellulosic biomass and ethanol fermentation (SSF). This must be addressed if total biomass is to be fermented, not only glucose syrups, for example from starch (Hahn-Hägerdahl et al. 2007). This method would considerably widen the substrates available for use in ethanol production. Improvement of substrate plants are reviewed in Torney et al. (2007). Substrate consists of glucose (hexose) and xylose (pentose) in mass proportions depending on the type of plant material. However, the fermentation of pentose sugar with industrial yeast strains is a difficult task and still under development (Hahn-Hägerdahl et al. 2007), although some pilot plants are already running.

The fermentation of all sugars in cellulosic biomass is a task easily performed by most bacteria, although there may be problems with catabolite repression. Hence, bacterial ethanol fermentation is still discussed. *Zymomonas mobilis* is considered the work horse of bacterial ethanol fermentation and the source of the enzymes for metabolic engineering of other bacteria towards ethanol production (Alterthum and Ingram 1989). *Z. mobilis* is restricted to the substrates sucrose, glucose and fructose which it catabolises through the Entner–Doudoroff pathway. It also, however, produces considerable amounts of the by-products sorbitol, acetoin, glycerol and acetic acid as well as the extracellular polymer levan. This bacterium was used for continuous ethanol fermentation in a fluidised bed reactor (Weuster-Botz 1993).

On a different microbiological platform, L. O. Ingram developed two strains of Enterobacteriaceae to circumvent the inherent problems with yeast and *Z. mobilis* (Ingram and Doran 1995). A new bioethanol plant, located in the bay of Osaka, Japan, will be in operation later in 2007 using a combination of mechanical pretreatment and mild acid hydrolysis of waste wood to yield a pentose syrup which is to be fermented with an advanced recombinant *Escherichia coli* B strain, LY165 (Moniruzzaman et al. 1996). This strain is devoid of the genes responsible for side product formation and contains the pyruvate decarboxylase and alcohol dehydrogenase from *Z. mobilis* (Ohta et al. 1991).

The residual slurry from the mild acid hydrolysis is, after neutralisation, fermented in an SSF process in the presence of industrial cellulases to hydrolyse the cellulose to glucose with a derivative of *Klebsiella oxytoca* strain M5A1, which was similarly engineered to contain the *Z. mobilis* ethanol producing pathway (strain P2). M5A1 also harbours a natural cellobiose uptake system and two additional cellulase genes from *Erwinia chrysanthemi* which reduces the amount of added fungal cellulase dramatically (Brooks and Ingram 1995; Zhou et al. 2001). Although bacterial fermentations do not yield such high ethanol concentrations as yeast fermentations, with *E. coli* LY165, up to 9% (*v*/*v*) of ethanol could be reached (L. Ingram, personal communication; Golias et al. 2002).

Pentose sugars released from the hemicellulose in lignocellulosic biomass present a problem. The maize plant (without the corn) contains about 24% (of dry mass) xylose and 2% arabinose (in comparison with 45% glucose) which cannot be neglected if the yield of solvents and complete substrate utilisation are an issue. These values for single sugars vary, but general hardwoods and agricultural raw material contain a larger proportion of D-xylose and L-arabinose, while softwood is less rich in pentoses (Hayn et al. 1993). Many of the industrial yeast strains lack the xylose utilisation pathway consisting of xylose reductase and xylitol dehydrogenase. The state of metabolic engineering to yield pentose-fermenting yeast strains is reviewed in Hahn-Hägerdahl et al. (2007). These strains will play an important role in the efficient conversion of plant biomass to ethanol and are already being used in pilot projects. Further research will concentrate on osmotolerance, inhibitor sensitivity and ethanol yield of new yeast strains.

Cheese whey has also been used as a substrate for ethanol fermentation. Several industrial processes were run worldwide using *Streptococcus fragilis* as in the Dansk Gaerings industry process and *Kluyveromyces fragilis* in the Milbrew process, whereas *K. fragilis* is used in most commercial plants (Pesta et al. 2006; Siso 1996). Whey permeate was fermented for decades in New Zealand (Gapes and Gapes, 2007). The Canbery process currently in operation in Ireland uses ultrafiltrated whey with yeast fermentation. In future, cheese whey substrate will gain importance because of increasing cheese production and problems during disposal resulting from the high organic matter content.

Ethanol fermentation from cellulosic biomass has been proposed using cellulolytic clostridia (Zeikus 1980; Lynd et al. 2002; Demain et al. 2005). The thermophilic bacterium *Clostridium thermocellum* will readily hydrolyse cellulosic biomass and will degrade hemicellulose as well as cellulose, but uses only the cellodextrins (not the glucose) derived from cellulose (Lynd et al. 2002). However, the strains are sensitive to higher ethanol concentrations and produce a range of less favourable by-products (Tailliez et al. 1989). Metabolic engineering of *C. thermocellum* seems to be feasible now, and strain development for ethanol production is a promising target.

The production of bioethanol by fermentation of Syngas (a CO/H_2 mixture from gasified biomass) is conceivable. A number of bacteria forming organic compounds like acetate, ethanol and butanol have been isolated including *Clostridium* ssp., *Moorella* ssp. *Carboxydocella* ssp. An industrial process for the fermentation of Syngas to ethanol based on *C. ljungdahlii* has already been developed. During this process, municipal waste is gasified and cooled down to fermentation temperature (while using the waste heat to produce electricity) and is blown into the fermenter. The ethanol produced by the organisms is then separated by distillation. The process has already been run at pilot plant scale. A commercial plant is expected (Henstra et al. 2007; http://www.brienergy.com).

Biological ethanol fermentation from molasses and starch is basically a mature technology. The utilisation of non-food substrates such as cellulose-containing waste material is in the pilot stage. A lot of experience with the use of bioethanol in engines currently exists in the field. Present-day spark ignition engines have to be modified for mixtures of more than 5% ethanol to gasoline, especially for the use of E85 or E100. However, the technology exists and is installed in so-called flex fuel vehicles (FFV). Most car companies are now offering FFVs, e.g. as “Totalflex” (VW) or “Flexpower” (Chevrolet/Opel/GM). Ford, Saab and Volvo were the likely pioneers of this technology. In Brasil, 3 million FFVs have been sold to date, and in Sweden, 15,000 FFVs were sold in 2005 alone. Bioethanol can be run in diesel engines, but additives are necessary to prevent phase separation (Lapuerta et al. 2007).

Biodiesel

Biodiesel is a monoalkyl ester of fatty acids from vegetable oil and is presently produced by catalytical transesterification with petrochemically derived methanol, also called alcoholysis (e.g. rape seed oil methyl ester, RME; Ma and Hanna 1999). Instead of using vegetable oil, microalgae could be grown in photobioreactors for the production of a suitable oil. Because of their high oil productivity, the specific demand of land area needed is strongly reduced by this concept in contrast to oil from plants (Yusuf 2007). Although bacterial processes are presently not involved in its production, biodiesel is mentioned here due to its potential for 100% biological production in the future. As a first step, alcohols from microbial fermentations such as ethanol, propanol and butanol can be used instead of methanol (Kildiran et al. 1996). Even a mixture of alcohols characteristic of acetone–butanol fermentation could be used (Nimcevic et al. 2000). Eventually, enzymatic transesterification can be utilised (see Microdiesel below).

At present, the microbiology of biodiesel degradation is a concern because of bacterial oxidation etc. during storage as well as the unavoidable water content leading to corrosion problems. Another problem is the high energy input in RME production which diminishes the overall energy gain (see Table 4).

The glycerol produced during transesterification creates a deposit problem in some areas. It could be fermented, e.g. to 1,3-propanediol and possibly to other products by metabolically engineered bacteria (Cheng et al. 2007) or to methane in biogas plants where it can be added in low concentrations as co-substrate.

A potential future fuel completely produced by bacteria is Microdiesel (Kalscheuer et al. 2006). *E. coli* cells were metabolically engineered by introducing the pyruvate decarboxylase and alcohol dehydrogenase genes *pdc* and *adhB*, respectively, from *Zymomonas mobilis* for abundant ethanol production. The gene *atfA* for an unspecific acyltransferase from *Acinetobacter baylyi* was introduced to esterify ethanol with the acyl moieties of CoA thioesters of fatty acids. If the cells are grown aerobically in the presence of glucose as an energy and carbon source and of oleic acid, ethyl oleate was the major product. However, de novo synthesised fatty acids were not used by the acyltransferase, which made the external addition of fatty acids necessary. This indicates that considerable further development is needed (O’Connell 2006). However, a new concept of the microbiological production of biodiesel has been shown with these experiments.

Conversion of plant oil to biodiesel is a mature technology. However, microbial contribution to the production process is close to zero at present. Inclusion of biologically fermented ethanol and butanol will not pose technical problems. The use of enzymes or biological systems in transesterification is to be developed.

Most diesel cars are now licensed to use a biodiesel–diesel blend of up to 5% (*v*/*v*). The conversion of a conventional diesel engine for pure biodiesel use is offered by many companies and costs in Germany up to 1,500€ per car. The modified engine, however, requires more frequent engine oil changes. Whether microbially produced biodiesel will run without problems and which engine modifications will have to be installed are yet to be shown.

n-Butanol and acetone

Acetone was produced up to WWI from wood. The supply of wood became insufficient at the start of the war because acetone demand increased in line with the manufacture of cordite, a cartridge and shell propellant in which acetone was an essential ingredient. The Russian chemist C. Weizmann, later Israeli President, developed the ABE fermentation process at Manchester University. He identified a producing bacterium which was later known as *C. acetobutylicum*. Facilities were built in the UK and in France using maize starch as a substrate, while rice starch was used at facilities in India (Jones and Woods 1986). In this process, acetone is produced together with butanol and ethanol (ABE 3:6:1). Butanol had no value at the time (Schwarz et al. 2007).

The ABE fermentation was the largest scale bioindustry ever run second to ethanol fermentation. By 1927, butanol was increasingly used for the production of the lacquer solvent butylacetate and for the synthetic rubber industry. Japan, and possibly other combatants, used butanol as an aviation fuel during WWII when they exhausted their fossil fuel supply. Japan's converted sugar refineries on the island of Formosa were therefore a strategic target for allied bombers.

Acetone and butanol production from fossil fuel became competitive in the early 1960s, eventually forcing the ABE fermentation industry out of production except in the USSR and in South Africa. The South African commercial ABE fermentation industry continued into the 1980s. During the 1960s and 1970s, more than 100,000 tonnes per annum of biobutanol were produced by fermentation in the USSR. Substrates included hydrolysates of lignocellulosics from agricultural waste materials (Zverlov et al. 2006). The process was constantly improved and run in continual mode, generally unknown to producers in the West. As the USSR disintegrated in the early 1990s, production stopped.

Despite the lack of a viable biobutanol economy, research and development in universities of Europe, South Africa, New Zealand and the USA continued. A continuous fermentation pilot plant operating in Austria in the 1990s introduced new technologies and proved economical feasibility with agricultural waste potatoes (Nimcevic and Gapes 2000; Gapes 2000). The Austrian plant helped bridge the skill gap between the termination of US, USSR and South African production and the recently reported renewal of production (Schwarz et al. 2007). However, the traditional clostridial fermentation of butanol and acetone is suffering from the difficulties of switching the acidogenic fermentation stage to the solventogenic stage, and thus, a discontinuous production mode, from common phage infections, the rising substrate costs and the effort required for downstream processing.

Often mis-described as a "new" fuel, biobutanol has been in almost continuous production since 1916, and most of the time as a solvent as well as a basic chemical. Today, new uses for butanol are emerging, e.g. as a diesel and kerosene replacement, as silage preserver, biocide and C4 compound for chemical industry. *n*-Butanol has many advantageous characteristics which make it a superior gasoline replacement (Schwarz and Gapes 2006).

There is much interest in bacterial butanol fermentation, especially after the announcement by BP and DuPont to finance development of a modernised production plant supported by research and development. This could include the introduction by recombinant gene technology of the butanol metabolic pathway from one of the solvent producing clostridia to another bacterium which is more tolerant of the products. The production of the byproducts could be reduced or terminated, and the process could greatly benefit from new and cheaper substrates derived by hydrolysis from lignocellulosic biomass, as well as from advanced sterile fermentation and downstream processing technology. A biogas plant connected to the plant would enhance energy conservation considerably.

It was demonstrated in June 2006 that *n*-butanol can be used either 100% in unmodified 4-cycle ignition engines or blended up to at least 30% (70% diesel) in a diesel compression engine or to 20% (80% kerosene) in a jet turbine engine (Schwarz et al. 2006). However, biobutanol production has only recently started again after decades of inactivity. The production of biobutanol from lignocellulosic biomass is promising and is on the agenda for a number of companies.

Others

Methyl-*tert*-butylether (MTBE) is produced by acid catalysis from isobuten and methanol, both coming from petrochemical sources. As much as 15% (*v*/*v*) is added to gasoline as anti-knocking agent, but became discredited due to its carcinogenicity and ground water contamination ability. Recently, some production sites were converted to produce the ethyl ether instead (ETBE) which introduces biologically produced ethanol into the fuel additive (e.g. at Degussa). The use of microbially produced *n*-butanol instead of ethanol is possible and would increase the mass percentage of biologically produced sustainable fuel. The resulting BTBE has favourable characteristics.

Methanol was widely used as fuel in Germany during WWII (Demirbas 2007). It can be used as pure fuel, as a blend or in the form of MTBE. The traditional process produced renewable methanol by the thermochemical conversion of wood (wood alcohol). A biomass-to-biomethanol process using bacteria is based on sugar beet pulp as substrate (Atlantic Biomass Conversions). Dry sugar beet pulp contains about 60% (*w*/*w*) pectin. The methylated carboxyl groups of galacturonic acid in pectin are de-esterified by a pectin methyl esterase (PME) to yield methanol. Therefore, the well-studied PME of *Erwinia chrysanthemi* B374 was transferred into *E. coli* to achieve a higher temperature tolerance. Methanol conversion from sugar beet pulp was studied as was the separation of the methanol. An industrial process was designed but not yet realised (Wang 2006; Anonymous 2004).

Future biofuels will be produced by a combination of chemical–physical treatments and biological fermentation, e.g. chemical ETBE synthesis from bioethanol or the acid pretreatment of lignocellulosics followed by enzymatic hydrolysis and fermentation. The mass production of industrial ethanol has come under public scrutiny and criticism, as it has become evident that increased demand for starch substrate derived from corn has pushed up prices (Odling-Smee 2007). Alternative substrates such as lignocellulosics have yet to be exploited (Zaldivar et al. 2001; Lynd et al. 2002; Demain et al. 2005). Development is ongoing, and many companies and publicly funded institutions are involved in the development of feasible processes.

Biofuels in different countries and regions

Some biofuels are presently on the edge of economic viability, but commercial introduction needs further subsidation until viability is reached (Gapes 2000). Most countries, recognising the hydrocarbon reduction benefits, allow tax exemptions on biofuels (Gapes and Gapes 2007).

Europe

The European Union intends that biofuels will account for 5.75% (energy value) of automotive fuels by 2010 and 10% by 2020. To achieve this goal, a tax exemption of up to 100 % on biofuels was implemented by an EU directive in 2003. The development of biofuels production in the EU, Germany and Spain is shown in Fig. 2 and the future goals for some industrialised countries in Table 2.

The European country most dedicated to ethanol utilisation is Sweden, which has focused on ethanol production from crops, sugar cane and wood waste. FFVs are on the market since 2001. The fuel E85 for these cars is sold at 140 public gas stations around the country. Already, in 2005, Sweden achieved its 2009 target (Table 2).

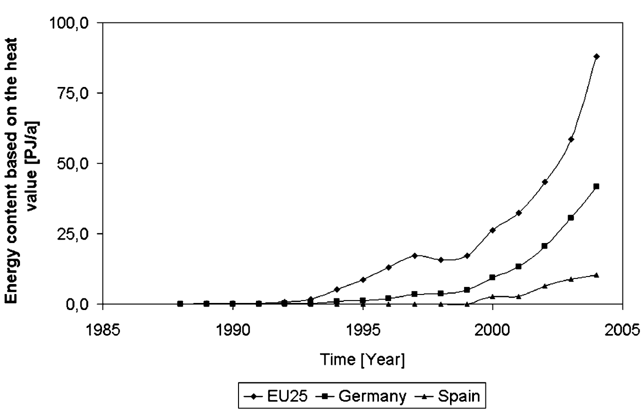

Fig. 2 Increase in production of biofuels for transportation in EU25 countries, Germany and Spain

Table 2 The future goals for the implementation of biofuels in various countries (total production)

Percentage of biofuels	2007	2008	2009	2010	2015	2020
Australia				1.00		
Austria	4.30	5.75				
Canada				3.50		
China	3.00					10.00
France		5.75		7.00		
Germany			6.25	6.75	8.00	
Japan	3.00	10.00				
Spain				9.00–12.00		
Sweden			5.00			100.00
UK						5.00

Numbers are percent (energy) of fuel consumption.

The largest single ethanol production facility in Europe is located in Zeiz (Germany) where wheat, barley and triticale starch are fermented and 260,000 m^3/annum ethanol is produced in addition to DDGS (sold as "Protigrain®"). An extension for sugar beet fermentation is under construction, which will increase total production to 360,000 m^3/annum.

In Germany alone, 2.5 MT of biodiesel have been produced in 2005 from rapeseed oil grown on 1.3 million hectares of land area and are sold on 1,900 gas stations.

Germany allowed tax exemption on biofuels, triggering a high increase in biodiesel production. Recently, the tax exemption, an effective instrument for promoting biofuels, has been replaced in Germany by a law forcing the addition of biofuels to conventional fuels. A new law starts taxation on biodiesel and vegetable oil (as fuel) from 2006 on which will gradually increase up to 0.45 € per litre in 2012. This should be compared with the taxation of 0.4857 €/l on fossil diesel fuel. The tax exemption for bioethanol still applies because the market is not yet regarded as mature.

Tax exemption was not implemented in the UK, which allows other incentives for biofuels. Consequently, the goal for biofuels implementation in the UK for 2020 is comparably low (Table 2). In contrast, Spain and France have made endeavours to promote biofuels and produce bioethanol as well as biodiesel.

Australia

The performance of the biofuels market in Australia is poor. Less than 0.1% of the gasoline market was substituted with bioethanol in 2005. Biodiesel is sold to an even lesser extent. The reasons for this were the absence of a consumer demand for biofuels because of high prices and poor consumer confidence in biofuels. Current endeavours by government and industries aim to reach a 1.0% implementation of biofuels in 2010.

Africa

The biofuel industry in Africa is marginal, whereas the production potential is enormous. According to information from the operating company, a bioethanol plant is presently being built in South Africa (Bothaville).

North and South America

In Brazil, a mixture of ethanol and gasoline is sold as Gasohol or "gasoline type C" which contains 21–23% EtOH. Fifteen billion litres of bioethanol have been produced together with bagasse and vinasse (dried distillers grains and solubles) as feed for cattle. However, bagasse from sugar cane has a low nutrition value and is generally used in biogas production to produce additional energy to run the plants. Brazil also plans to achieve a production of 2 billion litres of biodiesel per year by 2020.

The USA has surpassed Brazilian production in June 2006 with an installed annual capacity of 18 billion litres bioethanol production and an additional 8 billion litres planned for the near future. The Energy Tax Act (1978) was the primary motive for tax exemptions on biofuels in the USA. In 2005, the Bush administration announced a strategy to increase biofuel production from 14.8 to 27.8 billion litres within 10 years.

Only small volumes of biofuel are sold in Canada. The government began an investment programme to achieve 3.5% biofuel use by 2010. Specifically, it may support the construction of a large-scale plant for the production of cellulosic ethanol in the coming years.

Asia

Because it lacks agricultural acreage, Japan must import its biofuels. However, a demonstration plant producing ethanol from waste wood is under construction. A bilateral cooperation agreement to import bioethanol from Brazil is to be implemented. China, India and Thailand are emerging as significant fuel ethanol producers. In China, the average ethanol plant size is already 0.38×10^3 m^3/annum, the largest plant producing 1.1×10^3 m^3/annum. Biodiesel production is on the agenda of some countries.

Global microbial biofuel production

Biodiesel and bioethanol are the only biofuels produced worldwide in substantial amounts, bioethanol the only fuel by microbial fermentation. About 48.7×10^6 m^3/annum of bioethanol were produced worldwide in 2005, of which, 72.6% was produced in Brazil and the USA. The USA multiplied its output in the past decade and is now the world's most potent ethanol producer. Table 3 lists the ten most important producers of bioethanol, who combined, are responsible for over 91% of the world's production.

Table 3 Bioethanol production in various countries (data from RFA 2007)

	2004 (10^6 m^3)	2005 (10^6 m^3)	2006 (10^6 m^3)
Brazil	15.09	15.99	16.99
USA	13.37	16.13	18.37
China	3.65	3.80	3.85
India	1.75	1.70	1.90
France	0.83	0.91	0.95
Russia	0.75	0.75	0.65
South Africa	0.42	0.39	0.39
UK	0.40	0.35	0.28
Saudi Arabia	0.30	0.12	0.20
Spain	0.30	0.35	0.46
Thailand	0.28	0.30	0.35
Germany	0.27	0.43	0.76
Others	3.34	4.75	3.55
Total	40.75	45.97	48.70

Ranking by production in 2004

An example of the rapid development of the bioethanol industry is the USA (Fig. 3). The general tendency to build larger plants can be observed in all countries allowing tax exemptions for biofuels. It is an undisputed fact that biofuels are presently on the edge of economic viability, and their introduction requires subsidisation to make the industry capable of sustaining itself economically.

A rapidly expanding market for biofuels can be predicted for the near future. The demand of the EU25 countries for fuel ethanol is estimated to reach 11.4×10^6 m^3/annum in 2010, causing a large gap between actual production and estimated demand based on 5% displacement of conventional fuel (on energy basis; IEA 2004).

However, the strong demand for biofuel substrates causes competition with food production. Its early effects

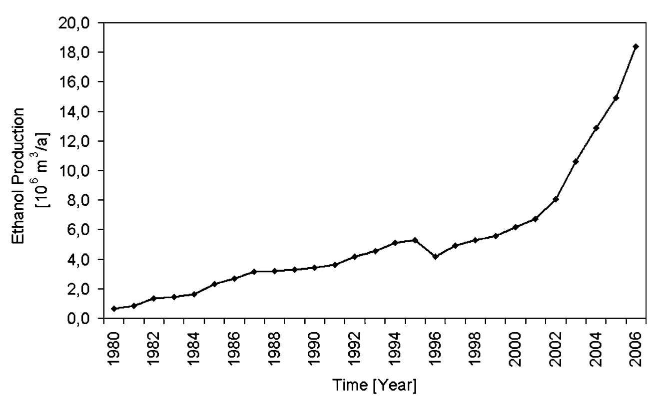

Fig. 3 Development of bioethanol production in the USA (RFA 2007)

Table 4 Yields of biofuel per hectare

	Country	Crop	GJ fuel/ (ha annum)
Plant oil		Rapeseed	50.8
Biodiesel	US	Soybean	16.3
	EU	Sunflower/Barley	32.5/35.8
	Germany	Rapeseed	50.4
	Tanzania	Palm Oil	186.0
Bioethanol	USA	Corn	66.0
	EU	Wheat	52.9
	EU	Sugar Beet	116.3
	Brazil/India/ Tanzania	Sugar Cane	137.5/112.1/ 173.0
	Germany	Wheat	54.1
	New Zealand	Corn Stover	36.2
	–	Switchgrass	84.6
	–	Corn Stover	23.2–46.5
	–	Miscanthus	154.4
	–	Switchgrass	65.5–160.7
	–	Poplar	78.2–126.9
Biomethane	Germany	Wheat	71.9
	Germany	Corn silage	163.4
	Germany	Grassland crop	29.9
	Germany	energy crops/ residues	217.3
BTL-Fuel	–	Energy crops	115.3–139.7

The energy yield from microbially produced biofuels based on hectare and year in different countries and on biofuels yields per tonne on crop input. For comparison, some non-microbially produced renewable biofuels are included. Projected data for bioethanol production from projected lignocellulosic substrates are added (calculation on data of IEA 2004, GTZ 2005, FNR 2007, Sanderson 2006, Judd 2003, Malhotra 2007 and own data).

have been observed in Mexico, causing a starch price too high for people to pay. To minimise such negative effects, a high yield of biofuels must be targeted. In Table 4, different yields of biofuels production per hectare are compared. These data are based on real figures available for common crops with realised industrial processes and prognosis for new crops and technologies. The figures are based on biofuel yields per tonne on crop input. To evaluate total greenhouse-gas savings or other environmental consequences, a detailed accounting of life cycles and net energy balances of the biofuels have to be determined (e.g. made in Hill et al. 2006). However, different studies yield in strongly differing results (Sanderson 2006; FNR 2007). Anyhow, it can be seen that depending on the country of production, an average fermentative process can be more acreage effective than biodiesel production (FNR 2007; GTZ 2005; Hill et al. 2006; Judd 2003; RFA 2007; Sanderson 2006).

However, for all these processes, substrate production methods which do not interfere with the production of food must be developed, and further research needs are numerous (Table 5).

Table 5 Further development needs for biofuel development

Needs for development
Breeding of climate adapted energy plants for each area
Utilisation of cellulose and hemicellulose (whole plant) as substrate
Simultaneous fermentation of pentoses and hexoses
New strains or enzymes for biofuel fermentation, e.g. strains with higher fermentation temperature and better product tolerance
Improvement in energy balance including all relevant factors: substrate, growth, refinery, product separation, waste management and disposal, distribution etc.; effect on CO_2 balance
Standardisation of new types of biofuels and fuel mixes and adaptation to present day engines
Development of new engines for the optimised use of biofuels

Conclusion

Biofuels produced from renewable biomass are the sustainable energy resource with the greatest potential for CO_2 neutral production. They can easily be implemented gradually to supplement fossil fuels by processes such as blending.

Biofuels can be produced from biomass by thermochemical means (e.g. BTL) or by fermentation with microbes. Both seem to be about equally land-efficient. Production maturity will be reached more rapidly by new and (at least partially) microbially produced biofuels.

Biotechnology for future biofuels will include microbial as well as chemical/technical production methods and often a mixture of both. Bioethanol blending to gasoline is only the beginning of these technologies.

Biofuel production will have to include the biomass of entire plants to reach higher yield. This will also reduce competition with food production and nature conservation.

Microbial biofuels have great development potential in process steps such as pretreatment, fermentation, substrate separation, energy coupling and others.

Biological research will need to contribute to an improved biofuel production by breeding of energy plants, enzymatic hydrolysis, specialised fermentation strains and waste treatment.

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