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Biofuels

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1.1

Biofuels in General

1.1.1

Introduction

In 1973, over 86% of the world's total primary energy supply came from fossil fuels. While the energy supply has increased since then (from about 6 Gtoe¹ in the 1970s to 12 Gtoe in 2007), the share of fossil fuels remains high. In 2007, still over 81% came from fossil fuels (gas: 20.9%; oil: 34%; coal: 26.5%; IEA, 2009). The European Community (EC) is strongly dependent on fossil fuels for its transport needs and is a net importer of crude oil (EC, 2010). Numerous experts predict that oil production will reach a ceiling by 2020, while the demand will continue to grow, pulled by China and India. Facing this demand calls for finding alternatives in petroleum products. At the same time, concerns are increasing about climate change and the potential economic and political impact of limited oil and gas resources. To address these issues and reduce our dependency on fossil fuels the EC has adopted measures² to encourage the production and use of sustainable biofuels (e.g., achieve 5.75% of biofuels among total fuel in the EC). Interestingly, the agricultural policy in Europe or in the United States is also probably the most important driver for the biofuel production. From 2000 to 2008, biofuel production in the United States has increased 82% even though that market accounts for less

1) Gtoe: Giga tonne oil equivalent = 1000 Mtoe (Mega tonne oil equivalent).

2) See, e.g., the Biofuels Directive (2003/30/EC) http://ec.europa.eu/energy/res/legislation/doc/biofuels/en_final.pdf followed by: the Directive 2009/28/EC on the promotion of the use of energy from renewable sources <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:>

140:0016:0062:EN:PDF; A Strategy for Biofuels http://www.biofuelstp.eu/downloads/An_EU_Strategy_for_Biofuels_2006.pdf; the European Strategic Energy Technology Plan (SET-Plan) http://www.biofuelstp.eu/downloads/SET-PlanCOM_2006_847.pdf; priority is to be given to biofuels research in EC-FP7.

than 5% of total fuel consumption. Nevertheless, current biofuel production is based only on the exploitation of the storage organs of agricultural plants (sugars or oils). Research and development efforts are necessary to diversify the feedstock available, to limit the impact on food markets, and to produce more efficient molecules as biofuels. This new SB application is probably one of the success keys, as suggested by new petroleum investments (Exxon's investment in Craig Venter's synthetic genomics start up, BP invested in Qteros, French Total invested in Gevo, Amyris and Coskata).

Biofuels from biomass (e.g., plant stalks, trunks, stems, leaves) are designed to significantly reduce dependence on imported oil and decrease the environmental impacts of energy use. Biotech research is critical for accelerating the deconstruction of (cellulosic) biomass into sugars that can be converted to biofuels. Wood-chips, grasses, cornstalks, and other ligno-cellulosic biomass are abundant but more difficult to break down into sugars than cereals (corn, wheat, etc.), a principal source of fuel ethanol production today. Cellulosic ethanol is therefore one of the proposed cornerstones for our energy needs. There are, however, other alternatives both in terms of feedstock and end product (e.g., butanol). Butanol is assumed to hold great promise. Aquatic biomass, such as algae, does not compete with arable land for food production. Algae can be used for the production of a variety of products, including biodiesel and hydrogen. In a long-term perspective, producing hydrogen or even electricity directly from solar energy and water by means of artificial photosynthesis would provide an almost unlimited source of energy (Thomassen *et al.*, 2008).

Biotechnology and especially synthetic biology can play a key role in increasing production and promoting the use of sustainable bioenergy through:

- Development of next-generation biofuel feedstocks,
- Advanced sunlight to biomass to bioenergy conversion,
- By considering socio-economic and environmental challenges when designing technological solutions.

Table 1.1 provides an overview of different biofuels and the technology and feedstock needed to produce them.

1.1.2

Economic Potential

The European Union and the United States have already created an artificial market through energy policies that specify the required rate of incorporation of biofuels in petroleum products. They also support this path through important tax rebates. However, with the arrival of the oil production ceiling and thus the increase in oil prices, a real market will be created and these sectors are likely to be profitable. According to the IEA, 45 million barrels per day could be supplied by biofuels in 2030, making up the deficit in petrol production (see Table 1.2).

World ethanol production for transport fuel tripled between 2000 and 2007 from 17 billion to more than 52 billion liters, while biodiesel expanded 11-fold from less than 1 billion to almost 11 billion liters. Altogether, biofuels provided 1.8% of the

Table 1.1 Overview of different generations of biofuels (UNEP, 2009).

Traditional biofuels	Basic technology	Feedstocks
Solid biofuels ^{a)}	Traditional use of dried biomass for energy	Fuel wood, dried manure
First-generation biofuels (conventional biofuels)		
Plant oils ^{b)}	As transport fuel: either adaptation of motors for the use of plant oils; or modification of plant oils to be used in conventional motors	Rapeseed oil, sunflower, other oil plants, waste vegetable oil
	For the generation of electricity and heat in decentralized power or CHP stations	Rapeseed oil, palm oil, jatropha, other oil plants
Biodiesel	Transesterification of oil and fats to provide fatty acid methyl ester (FAME) and use as transport fuel	Europe: rapeseed, sunflower, soya United States: soya, sunflower Canada: soya, rapeseed (canola) South and Central America: soya, palm, jatropha, castor Africa: palm, soya, sunflower, jatropha Asia: palm, soya, rapeseed, sunflower, jatropha
Bioethanol	Fermentation (sugar); hydrolysis and fermentation (starch); use as transport fuel	United States: corn Brazil: sugar cane Other South and Central American countries: sugar cane, cassava Europe: cereals, sugar beets Canada: maize, cereals Asia: sugar cane, cassava Africa: sugar cane, maize
Biogas (CH ₄ , CO ₂ , H ₂)	Fermentation of biomass used either in decentralized systems or via supply into the gas pipeline system (as purified biomethane): (1) To generate electricity and heat in power or CHP stations (2) As transport fuel, either 100% biogas fuel or blending with natural gas used as fuel	Energy crops (e.g., maize, miscanthus, short rotation wood, multiple cropping systems); biodegradable waste materials, including animal sewage

(Continued)

Table 1.1 (Continued)

Traditional biofuels	Basic technology	Feedstocks
Solid biofuels	Densification of biomass by torrefaction or carbonization (charcoal) Residuals and waste for generation of electricity and heat (e.g., industrial wastes in CHP)	Wood, grass cuttings, switchgrass; grains; charcoal, domestic refuse, dried manure
Second-generation biofuels (advanced biofuels)		
Bioethanol	Breakdown of cellulosic biomass in several steps including hydrolysis and finally fermentation to bioethanol	Ligno-cellulosic biomass like stalks of wheat, corn stover and wood; "special energy or biomass" crops (e.g., <i>Miscanthus</i>); sugar cane bagasse
Biodiesel and "designer"-biofuels such as bio-hydrogen, bio-methanol, DMF ^c , bio-DME ^d , mixed alcohols	Gasification of low-moisture biomass (<20% water content) provides "syngas" (with CO, H ₂ , CH ₄ , hydrocarbons) from which liquid fuels and base chemicals are derived	Ligno-cellulosic biomass like wood, straw, secondary raw materials like waste plastics
Third-generation biofuels (advanced biofuels)		
Biodiesel, aviation fuels, bioethanol, biobutanol	Bioreactors for ethanol (production can be linked to sequestering carbon dioxide from power plants); transesterification and pyrolysis for biodiesel; other pyrolysis for biodiesel; other future technologies	Marine macro-algae or micro-algae in ponds or bioreactors

a) Traditional use of biomass included for complete overview.

b) Also known as straight vegetable oil. Plant oil used as direct fuel in transport is common in German agriculture with about 838 000 tonnes, mostly rapeseed oil, in 2007, representing 1.4% of total fuel consumption in transport.

c) 2,5-Dimethylfuran.

d) Dimethyl ether.

Table 1.2 Optimistic scenario of alternative fuel introduction until 2020 in the European Union (Biofuels Platform, 2010).

Year	Biofuels (%)	Natural gas (%)	Hydrogen (%)	Total (%)
2005	2	–	–	2
2010	5.75	2	–	7.75
2015	7	5	2	14
2020	8	10	5	23

Table 1.3 Estimates on future economic indicators in the United States, based on the Renewable Fuel Standard as specified in the US Energy Independence and Security Act of 2007 (BIO-ERA, 2009; EPA, 2010).

	2012	2016	2022	2030
Amount of cellulosic biofuel requirement	1.8 bio l	16 bio l	60 bio l	n/a
Amount of advanced (third-generation) biofuel	7 bio l	27 bio l	79 bio l	n/a
Total renewable fuel requirement	56 bio l	84 bio l	136 bio l	n/a
Direct job creation	29 000	94 000	190 000	400 000
Investments in advanced biofuels processing plants	3.2 bio \$	8.5 bio \$	12.2 bio \$	n/a
Direct economic output from the advanced biofuels industry	5.5 bio \$	17.4 bio \$	37 bio \$	113 bio \$

world's transport fuel. Recent estimates indicate a continued high growth. From 2007 to 2008, the share of ethanol in global gasoline-type fuel use was estimated to increase from 3.7 to 5.4%, and the share of biodiesel in global diesel-type fuel use from 0.9 to 1.5%. Currently, the main suppliers for transport biofuels are the United States, Brazil and the European Union. Production in the United States consists mostly of ethanol from corn starch, in Brazil of ethanol from sugar cane, and in the European Union mostly of biodiesel from rapeseed. Investment into biofuel production capacity probably exceeded \$4 billion worldwide in 2007 and is growing rapidly. Industry, with government support, is also investing heavily in the development of biofuels. The current biofuel production has been stimulated by biofuel subsidies, fuel blending mandates, national interest in energy security, climate change mitigation and rural development programs (see Table 1.3).

International trade in ethanol and biodiesel has been small so far (about three billion liters per year in 2006/07), but is expected to grow rapidly in countries like Brazil, which reached a record-high of about five billion liters of ethanol fuel export in 2008 (Figure 1.1). Predictions forecast that global use of bioethanol and biodiesel will nearly double from 2007 to 2017. Most of this increase will probably be due to biofuel use in the United States, the European Union, Brazil and China. But other countries could also develop towards significant biofuel consumption, among them Indonesia, Australia, Canada, Thailand and the Philippines.

Regarding the global long-term bioenergy potential, estimates depend critically on the underlying assumptions, particularly on the availability of agricultural land for non-food production. Whereas more optimistic assumptions yield a theoretical potential of $200\text{--}400 \times 10^{18}$ J/year or even higher, the most pessimistic scenario estimates 40×10^{18} J/year. More realistic assessments considering environmental

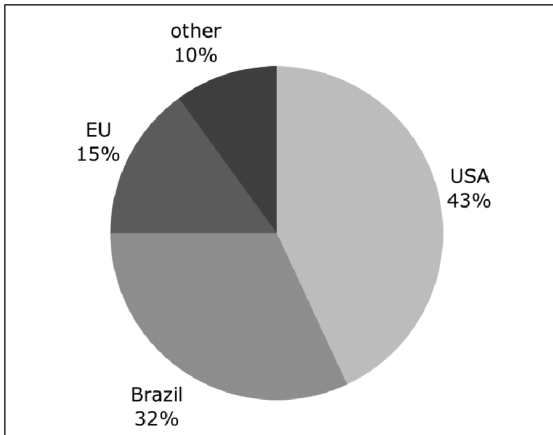


Figure 1.1 Proportion of global production of liquid biofuels in 2007 (Source: UNEP, 2009).

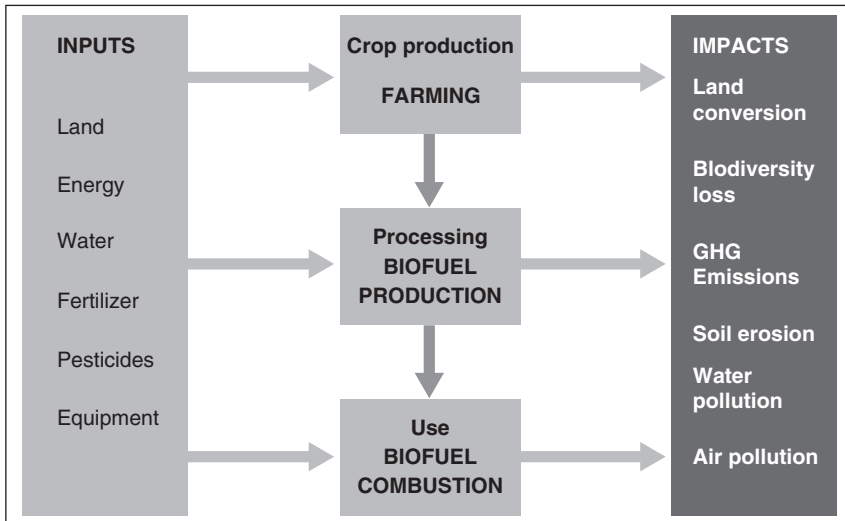


Figure 1.2 Overview of the biofuel life cycle with inputs and relevant environmental impacts.

constraints estimate a sustainable potential of $40\text{--}85 \times 10^{18}$ J/year by 2050. For comparison, current fossil energy use totals 388×10^{18} J. These estimations do not take into account biofuel production from non-agricultural biomass (e.g., bio-photovoltaics).

In 2009, total oil and fuel demand worldwide was 84.9 million barrels/day (mb/d), of which 2 mb/day were biofuels (about 2.3%). Not including the United States and Brazil, the global biofuel supply is only about 0.5 mb/day. Examining the global supply of fuel for 2010 reveals only little growth compared to the previous year (Figure 1.2). Among the key sources of global growth in 2010, however, are biofuels (+210 kb/day) (IEA, 2010).

1.1.3

Environmental Impact

The effects of biofuels on the environment include changes in the emission of greenhouse gases (GHG), the displacement of non-renewable fuels, a positive effect on water and air quality, as well as a beneficial influence on biodiversity, eutrophication and acidification of soils. Currently, biofuel production is mainly related to ethanol and biodiesel. These require an agricultural bio-resource such as sugar cane, corn or rapeseed, as well as a considerable amount of water and fertilisers, not to mention pesticides. Today, most environmental effects are strongly linked to the agricultural practices of the bioenergy crop cultivation.

The extension of cropland for biofuel production is continuing, in particular in tropical countries where natural conditions favor high yields. In Brazil, the planted area of sugar cane comprised nine million hectares (Mha) in 2008 (up 27% since 2007). Currently, the total arable land of Brazil covers about 60 Mha. Also, the total cropping area for soybeans, which is increasingly being used for biodiesel, could potentially be increased from 23 Mha in 2005 to about 100 Mha. Such expansion is most likely on the pastureland and in the savannah (cerrado). For example, palm oil expansion in Southeast Asia is considered as the leading cause of the lost biodiversity of rainforest. Taking an example from Indonesia, plantations of 20 Mha for palm oil trees are planned, while the existing stock has already been at least 6 Mha. Two-thirds of these expanded palm oil cultivations in Indonesia will be grown on lands converted from rainforests, with one-third on previously cultivated or currently fallow land. Of the converted rainforest areas, one-quarter contained peat soil with a high carbon content—resulting in particularly high GHG emissions when drained for oil palms. Based on the 2009 estimate by Bringezu *et al.*, by 2030, a share of 50% from peat soils is expected. If current trends continue, in 2030 the total rainforest area of Indonesia will be reduced by 29% as compared to 2005, and will only cover about 49% of its original area in 1990. Figure 1.3 shows the estimated positive and negative environmental effects of different uses of biomass, including liquid transport fuels (Bringezu *et al.*, 2009).

The Scientific Committee on Problems of the Environment (SCOPE, 2009) conducted a project on the environmental impact of biofuels and recommended—in line with Tilman *et al.* (2009)—that: many of the adverse effects of biofuels on the environment (e.g., loss of biodiversity, eutrophication, acidification of soils, water pollution, production of GHG, in particular nitrous oxide) could be reduced by using best agricultural management practices, if production is kept below sustainable production limits.

In general, biofuels made from organic waste are environmentally more benign than those from energy crops. Using biomass primarily for material purposes, reusing and recycling it, and then recovering its energy content can yield multiple dividends (Figure 1.4).

Low-input cultivation of perennial plants, for example, from short-rotation forestry and grasslands, may be an effective source of cellulosic biomass and provide environmental benefits (reduced pollution and lower greenhouse gas emissions).

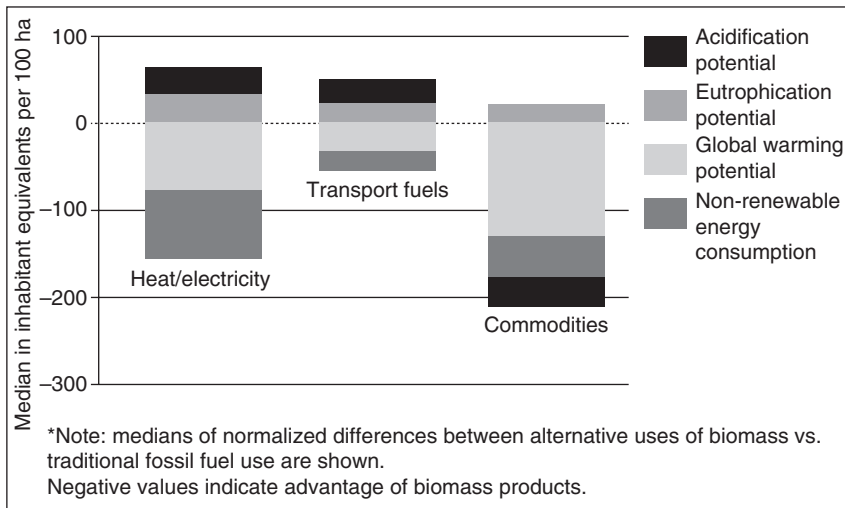


Figure 1.3 Comparative relief of environmental pressure through use of biomass for heat/electricity, transport fuel and material products (Source: UNEP, 2009).

Careful attention to maintaining the long-term productivity of these systems through nutrient additions (particularly potassium) is required.

New liquid hydrocarbon fuels (see Section 1.3) produced from cellulosic biomass are under development and seem likely to offer several advantages over producing ethanol from cellulose in terms of more efficient yields and less environmental impact (Figure 1.5). The economic viability of this technology still needs to be proven, and potential conflicts with traditional wood-based industries should be considered. Note, however, that the aromatics present in lignin provide a good substrate as a traditional fuel, while they are, with our present knowledge, difficult to convert to biofuels.

1.1.3.1 Land Requirements for Projected Biofuel Use

Estimates of land requirements for future biofuels vary widely and depend on the basic assumptions made—mainly the type of feedstock, geographical location, and level of input and yield increase. More conservative trajectories project a moderate increase in biofuel production and use. They have been developed as reference cases under the assumption that no additional policies would be introduced to further stimulate demand. These range between 35 Mha and 166 Mha in 2020. There are various estimates of potentials of biofuel production which calculate cropland requirements between 53 Mha in 2030 and 1668 Mha in 2050. About 118–508 Mha would be required to provide 10% of the global transport fuel demand with first-generation biofuels in 2030 (this would equal 8–36% of current cropland).

The analysis of land availability for an aggressive biofuels program is summarized in the following five points (SCOPE, 2009):

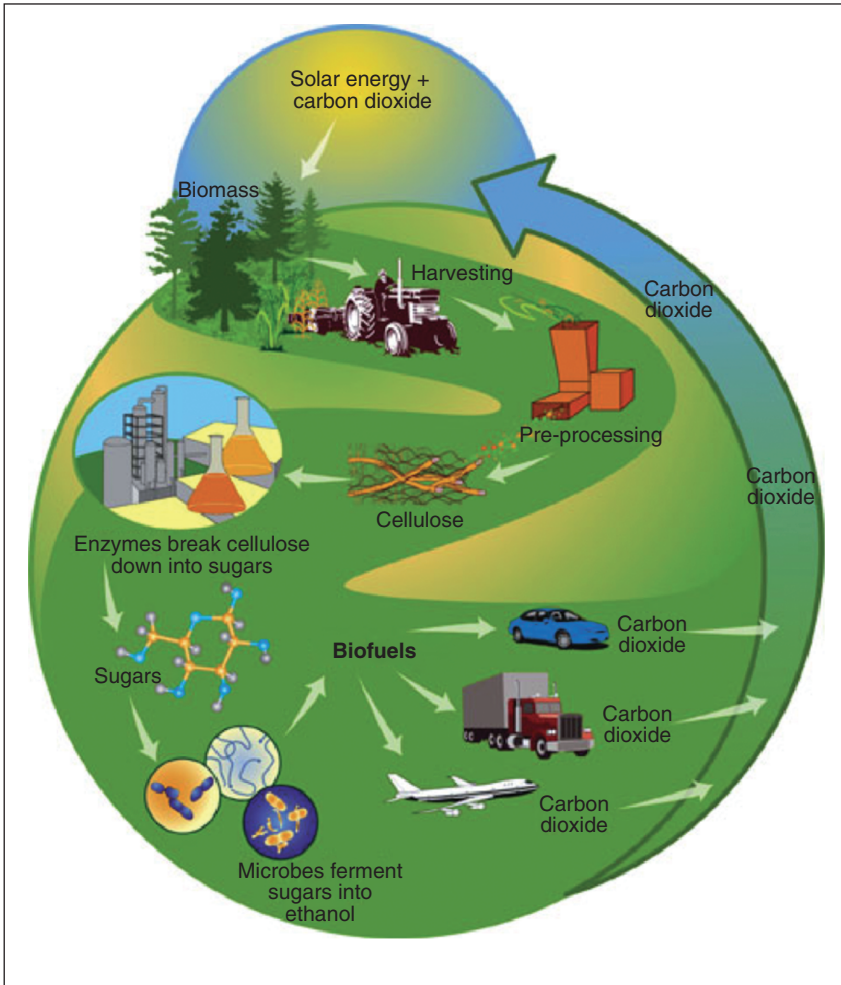


Figure 1.4 Schematic view of the biofuel conversion process (US DOE, 2010).

- 1) Supply of land is tight and a growing population will put increasing pressures on its uses.
- 2) How much land is available, at which yield potential, and in which locations to produce enough biofuels to provide a significant fraction of world energy is the subject of much debate.
- 3) The real pressure points are in the tropics, where new croplands could be developed, where biodiversity values are high, and where much of the population is vulnerable to multiple stresses.

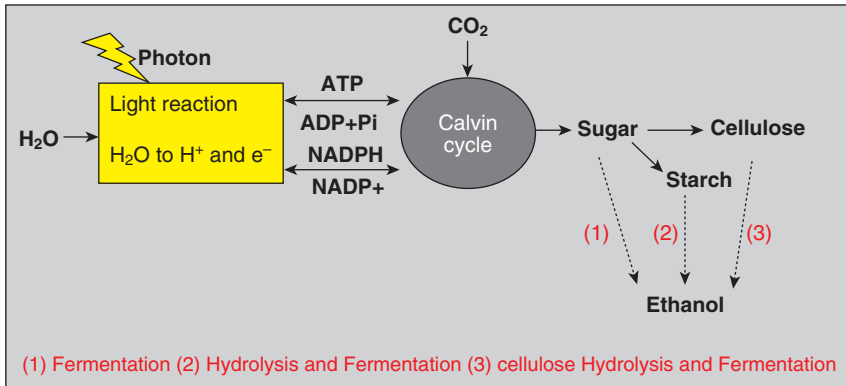


Figure 1.5 Schematic pathway from photosynthesis in plants to biomass to ethanol.

- 4) From an environmental standpoint, there are few areas where biofuels are an acceptable use of land given the alternative uses.
- 5) At the regional and local scales there are opportunities to create acceptable uses of biofuels that have net benefits for society.

1.1.3.2 Other Environmental Concerns

It has been suggested that each biofuel should be evaluated based on its net benefit, that is, a full analysis of its effects on net energy gain, the global food system, greenhouse gas emissions, soil structure and soil fertility, water and air quality and tradeoffs of biodiversity (Tilman *et al.*, 2009). David Tilman has proposed that biofuels should be derived from feedstocks produced from perennial plants grown on degraded lands, crops residues, sustainably harvested wood and forest residues, double crops and mixed cropping systems, as well as municipal and industrial waste. The biofuels derived from those feedstocks will maximize their benefits by not competing with food crops, minimizing impacts on land clearing and offering real GHG reductions.

1.1.3.3 Impact of Legislative Decisions

In the Biofuels Directive (EC, 2003) the EC mentioned a concrete target for the share of biofuels: “Member States should ensure that a minimum proportion of biofuels and other renewable fuels is placed on their markets, and, to that effect, shall set national indicative targets. A reference value for these targets shall be 5.75%, calculated on the basis of energy content, of all petrol and diesel for transport purposes placed on their markets by 31 December 2010”. Setting such a target, although with good intentions, may also cause unintended consequences. Some observers argued that these goals could only be met at an enormous environmental cost, including higher global CO₂ emissions, massive biodiversity loss, and pollution of soil and water in biofuel production countries (Green, 2010). To cope with rising concerns of unwanted

side effects of biofuels, some countries—including the EC—have started to promote criteria for sustainable bioenergy production. The EC decided to adapt their targets for biofuels, and specify under which conditions these targets should be met. The Directive 2009/28/EC on the promotion of the use of energy from renewable sources (EC, 2009) sought to correct the potential incentives for unsustainable developments in order to meet the requirements laid out in the 2003 directive. Confronted with the risk that imports to the EU from biomass- and biodiversity-rich countries like Brazil could actually promote the deforestation of rainforests, the 2009 directive (EC, 2009) formulated clear priorities for the production of biofuels. In its Article 17 on “Sustainability criteria for biofuels and bioliquids” it states for example: “*Biofuels and bioliquids. . . shall not be made from raw material obtained from land with high biodiversity value . . .*”

This example shows that solving one environmental problem (replacing fossil with renewable fuels) may well lead to unintended second-order problems in another area (e.g., deforestation of rainforest to make land for energy crops). This calls for ensuring that the production of biofuels and other forms of bioenergy is carried out in a sustainable manner (See also Section 3.5 on celluloses).

1.1.4

Foreseeable Social and Ethical Aspects

Large-scale production of biofuels and feedstocks will require considerable infrastructure to transport the biomass used upstream of the processes. It may also lead to a further concentration of landholdings and transformation of the rural landscape. In contrast, small-scale production of biofuels can provide local energy security or access and, if managed properly, can have no adverse impacts on food production. If development programmes select small communities for the local production of electricity using biofuels, intra-country inequalities can be reduced. Europe has examples of such small-scale production (such as wood granulates) that are sustainable in both social and environmental terms. These could be adapted for the development of similar programmes using varied feedstocks and management practices in communities in Africa, Asia and Latin America. For example, eco-friendly energy farms have been promoted in Norway and other Scandinavian countries, where small farms produce their own energy (mostly heat and biodiesel) by using biofuels produced locally (Figure 1.6). This model may be applied in local communities in certain developing countries to satisfy local energy needs (SCOPE, 2009).

Producing biofuel feedstocks may promote rural development in some traditional agriculture countries, but the social impacts need to be assessed carefully. For instance, increased demand for palm oil for biodiesel provides increased employment in countries like Indonesia. At the same time, negative social impacts have already been reported. These include poor wages, low labor standards, impact on health and local culture, land grabbing and loss of environmental goods (Tilman *et al.*, 2009). Whether or not second- or third-generation biofuels will bring higher social benefit depends mainly on the economically feasible size of the production

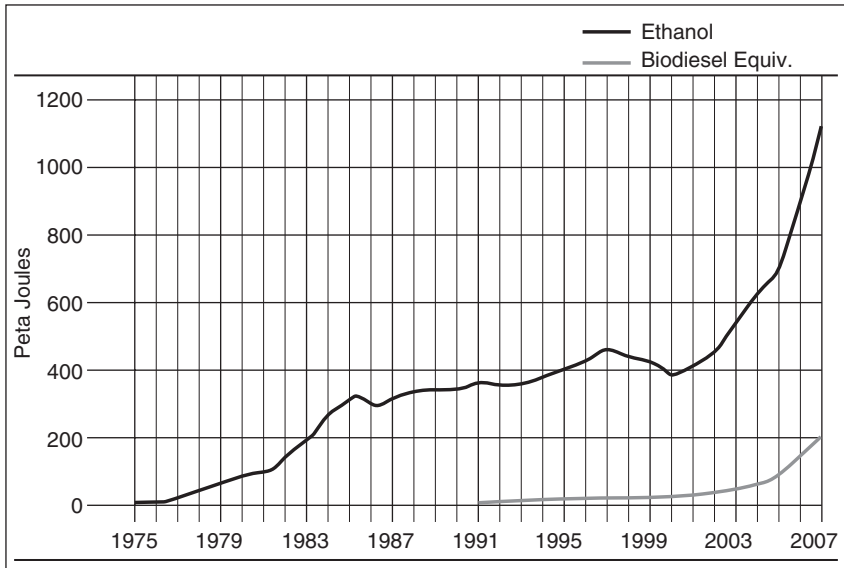


Figure 1.6 Global bioethanol (black line) and biodiesel (gray line) production 1975–2007 (source: UNEP, 2009).

unit. The larger the size of the agricultural field, factory or tank, the higher the probability that only big business will be able to benefit first hand from biofuel production. Future technology developments should consider the design of small- and medium-scale units that are more accessible to SMEs in order to allow for a more socially distributed benefit.

1.1.4.1 How Could the New SB Application Impact Society at Large?

Biofuels and food. The production of biofuels, mainly first-generation (simple sugar- and starch-based) bioethanol, has affected the price of food in the past. Those biofuels that require significant land for their production and/or compete with crops can have this effect. This negative effect is discussed in detail in Section 1.2.

Greenhouse effect. Burning biofuels emits CO₂ and thus theoretically contributes to global warming. This emission is substantially compensated by the succeeding crop, which will absorb atmospheric carbon. Note, however, that transportation, agricultural practices and use of fertilisers will emit additional CO₂. Thus, the net emission of CO₂ is not zero, but it is certainly much lower than that of the fossil fuels.

Socio-ecological impact. The potential side-effects of massive conversion of land surfaces for fuel production include: displacement of traditional cultures, competition over arable land, and indirect contribution to global warming or deser-

tification. This is more a political than a scientific problem because the legal framework and the sustainability of the use of the technology are central. A case by case scenario is described in the following issues.

In general, social interactions may change due to major shifts between traditional farming and biofuel production. Even in those cases where the substrates are not crops (i.e., algal production), competition for space might occur.

Ethical issues. These depend on the biofuel. Factors such as toxicity, flammability or ease of local production may vary considerably. These topics are discussed in the following chapters

Justice of distribution. This is a key point in the ethics of biofuels. It is difficult to forecast the consequences because the future economy might rely on one, several or a mix of biofuels. The kind of biofuel can also have important consequences: some can be either extremely easy to produce *in situ* for local consumption (i.e., vegetable oil for direct use in old cars), relatively easy to produce but difficult to manipulate (hydrogen) or process (algal biodiesel), or show intermediate features (bioethanol). Justice of distribution will likely depend on:

- The nature of the biofuel itself (ease of production and manipulation, security).
- Intellectual property rights, linked to the complexity of the technical system set in place, which might lead to monopolies.
- Political will.

1.2 Ethanol

1.2.1 Introduction

Ethanol fermented directly from starch or sugar, as a typical first-generation biofuel, is the most common liquid biofuel used today (Figure 1.7). (Ligno)cellulosic ethanol, an advanced biofuel, can be produced from a variety of biomass materials. The standard procedure includes:

- A “pretreatment” phase, to make the cellulosic material amenable to hydrolysis.
- Cellulose hydrolysis, to break down the cellulose molecules into sugar molecules, commonly with *Trichoderma reesei* enzymes.
- Separation of the sugar solution from the residual materials (e.g., lignin).
- Microbial fermentation of the sugar solution, usually by the budding yeast *Saccharomyces cerevisiae*.
- Distillation and dehydration to meet biofuel standards (concentration >99.5%).

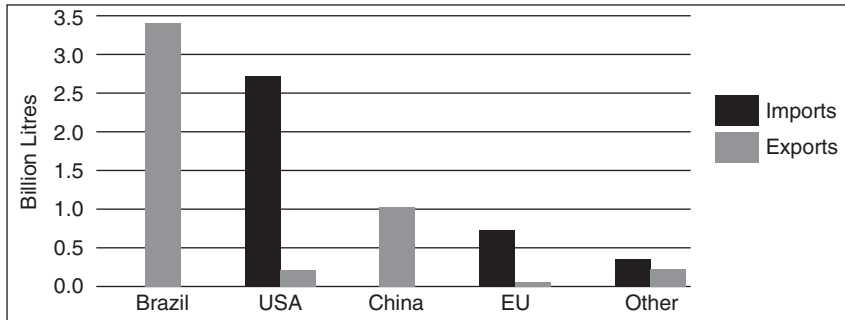


Figure 1.7 International trade in ethanol in 2006 (source: UNEP, 2009).

This conventional process for the production of bioethanol from lignocellulosic biomass is often called separate hydrolysis and fermentation (SHF); modifications of the procedure involving simultaneous saccharification and fermentation (SSF) are also used. In SSF, hydrolytic enzymes and yeast are incubated together under compromised conditions, and ethanol is obtained in one step. The main advantage of this procedure is the lack of end-product inhibition. The main disadvantage is that the compromise conditions are usually suboptimal for both hydrolysis and fermentation. Bioethanol can be produced from biomass such as sugarcane and corn, but lignocellulosic material (i.e., rice straw, wood, paper waste) has an incredible potential for bioethanol production due to the enormous amount of such biomass produced worldwide every year. Assuming that about 33% of the dry weight of the straw is cellulose, and considering that the theoretical ethanol yield is 0.511 g of ethanol per gram of glucose, 1 kg of rice straw could yield 168 g of ethanol. This means that as much as 134 million tonnes of bioethanol would be produced if all the rice straw on earth were converted into biofuel. The efficient transformation of lignocellulosic biomass to ethanol is a challenge for SB, and this approach is expected to have a major influence on the production of bioethanol (see Table 1.4).

1.2.2

Economic Potential

Currently, only the ethanol from sugarcane in Brazil is economically viable. The corn-derived ethanol in the United States and the beet-derived ethanol in France are only feasible because they are supported by government subsidies. For the cellulosic ethanol, the “90-billion gallon biofuel deployment study” by the Sandia National Laboratories and the General Motors R&D center showed that cellulosic ethanol can compete with oil at \$90/bbl based on the following assumptions:

- Average conversion yield of 95 gallons per dry ton of biomass;
- Average conversion plant capital expenditure of \$ 3.50 per installed gallon of nameplate capacity;
- Average farm-gate feedstock cost of \$ 40 per dry ton.

Table 1.4 Evolution of bioethanol production in selected European Union countries.

Country	Annual production (million liters/year)						
	2002	2003	2004	2005	2006	2007	2008
France	114	103	101	144	293	539	950
Germany	0	0	25	165	431	394	581
Spain	222	201	254	303	402	348	346
Poland	63	76	48	64	120	155	200
Hungary	0	0	0	35	34	30	150
Slovakia	0	0	0	0	0	30	94
Austria	0	0	0	0	0	15	89
Sweden	63	65	71	153	140	120	76
Czech Republic	6	0	0	0	15	33	76
United Kingdom	0	0	0	0	0	20	75
Other EU countries	0	0	29	49	173	119	216

These simulations assume technological progress in the conversion technologies in which SB plays a central role. The cost competitiveness of ethanol depends directly on the price of oil and the realization of technological improvements.

A major problem in the large-scale use of ethanol is that it needs to be transported (as in the case of oil). Yet it is an extremely corrosive material, and most of the existing infrastructure used to transport oil would have to be replaced if ethanol played an important role. Transport using trucks, for example, would considerably decrease the interest in its use. Furthermore, its hygroscopy makes it a very awkward fuel for sensitive engines, such as those used in airplanes.

1.2.3

Environmental Impact

Current life cycle assessments (LCA) of biofuels show a wide range of net greenhouse gas savings compared to fossil fuels. This mainly depends on the feedstock and conversion technology, but also on other factors such as methodological assumptions. For ethanol, the highest GHG savings are recorded for sugarcane (70% to more than 100%), whereas corn can save up to 60% but may also cause 5% more GHG emissions (see Table 1.5). (The highest variations are observed for biodiesel from palm oil and soya.) The high savings of the former depend on high yields, those of the latter rely on the credits of byproducts. Negative GHG savings, that is, increased emissions, may result in particular when production takes place on converted natural land and the associated mobilization of carbon stocks is accounted for (Figure 1.8). Besides GHG emissions, other impacts of biofuels, such as eutrophication, are also relevant and contribute to significantly worsened environmental quality in certain regions of the world.

Most of the currently used crops for transport biofuels are also food crops. Global land use for the production of fuel crops recently covered about 2% of global

Table 1.5 Lifecycle GHG thresholds specified in US Energy Independence and Security Act (EISA; % reduction from 2005 baseline; EPA, 2010).

Type of fuel	GHG savings (%)
Renewable fuel	20
Advanced biofuel	50
Biomass-based diesel	50
Cellulosic biofuel	60

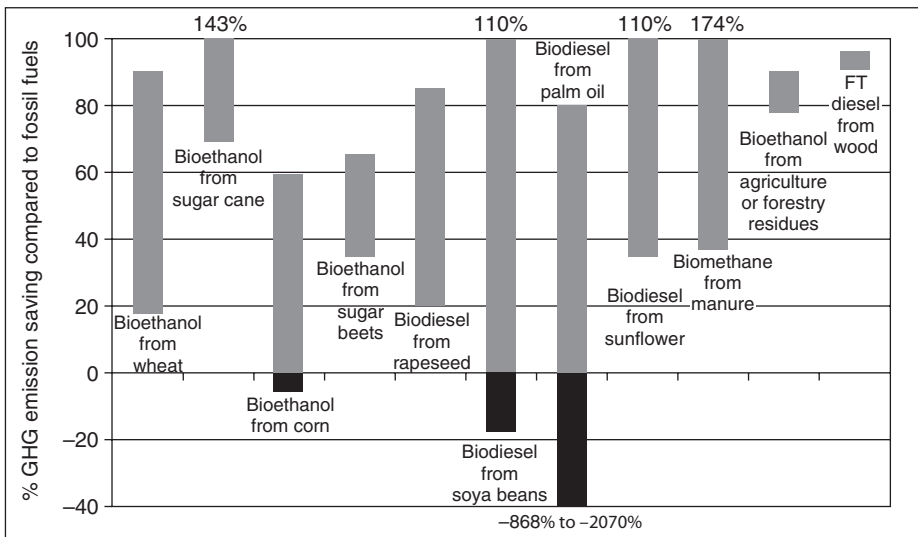


Figure 1.8 Green-house gas (GHG) savings from bioethanol (and biodiesel) compared to fossil fuels (Source: UNEP, 2009).

cropland (about 36 Mha in 2008). This development is driven by volume targets rather than by land use planning. The extension of cropland for biofuel production is continuing, in particular in tropical countries where natural conditions favor high yields. In Brazil, the planted area of sugarcane comprised nine million hectares in 2008 (up 27% since 2007). Currently, the total arable land of Brazil covers about 60 Mha.

It is difficult to calculate GHGs from biofuels because the production systems are inherently complex and the methods used to quantify savings are subjective. Several factors affect emissions, among them emissions embodied in biomass production, the use of electricity in the conversion process, indirect land-use change and fertilizer replacement (Figure 1.9). Indirect emissions from biofuels should also be taken into account; this occurs when biofuel production on agricultural land displaces agricultural production. This type of land-use changes will increase net GHG emissions (Melillo *et al.*, 2009).

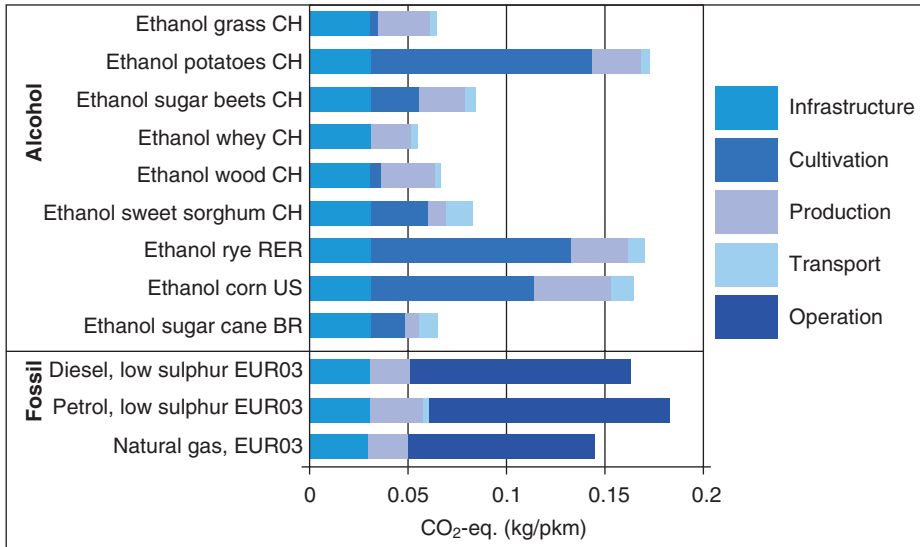


Figure 1.9 Comparison between the greenhouse gas emissions of bioethanol for transport and fossil fuels, differentiated by phase. Ethanol production clearly does not

automatically lead to a reduction in GHG emissions. (The unit is kilogram CO₂ equivalent per person kilometer; Zha, 2007; UNEP, 2009).

Table 1.6 Companies using synthetic biology to produce biofuel.

Companies	Products
Amyris ^{a)}	Renewable diesel
Dyadic International	Ethanol, enzymes
GEVO	Butanol, isobutanol, chemicals
Joule Biot	Ethanol, chemicals
LS9	Renewable gasoline, diesel, jet fuel, chemicals
Metabolic Explorer	Butanol, 1,3.propanediol
OPX Biot	Renewable acrylic
Solazyme	Green crude, biodiesel, algal oil, others
Synthetic Genomics	Algal oil, biodiesel, chemicals
Vendezyne	Ethanol

a) Amyris left the biofuel sector for lack of positive results in early 2012.

The carbon neutral hydrocarbons (CNHCs) have been brought into play to define those biofuels compatible with the existing transportation infrastructures and therefore capable of a gradual deployment with minimum supply disruptions (Zeman and Keith, 2008). Ethanol is seen by many as such a CNHC, although its real environmental benefits could be rather limited compared to alternative next-generation biofuels (see Table 1.6).

Because of the environmental (and social) impact of big agribusiness, many researchers have recommended that instead of cultivating energy crops for ethanol, a more environmentally friendly biomass to biofuel conversion can be achieved by using approaches (Tilman *et al.*, 2009) such as:

- Perennial plants grown on degraded lands abandoned by agricultural use;
- Crop residues;
- Sustainably harvested wood and forest residues;
- Double crops and mixed cropping systems;
- Municipal and industrial wastes.

1.2.4

Foreseeable Social and Ethical Aspects

Current large-scale (ethanol) first-generation biofuel production in Brazil (and to a lesser extent other countries) has done little to improve the economic situation of the average fuel consumer or promote sustainable rural development to the benefit of small-holder farmers or the urban poor (Rodrigues and Moraes, 2007; Sawyer, 2008; SCOPE, 2009). Little evidence and only few arguments suggest that future large-scale biofuel production will have a positive social effect (ETC, 2008). Indeed, the small body of research on the socio-economic impacts of biofuels suggests that an expansion of the areas under feedstock cultivation for biofuels will benefit only large land owners, speculators and urban elites in developing countries. Small-scale farmers and the poor in developing countries will be negatively impacted. In contrast, very small-scale biofuel production for local consumption shows some promise in the provision of energy security and improvements in human well-being and equity (EEA, 2006).

An ongoing debate, especially during the food crisis in 2008, was about the ramifications of cultivating energy crops *instead* of food crops. The following quote shows the relevance of the problem:

“And what I’ve said is top priority is making sure people are able to get enough to eat. If it turns out we need to make changes in our ethanol policy to help people get something to eat, that has got to be the step we take . . . We have rising food prices around the United States. In other countries, we’re seeing riots because of the lack of food supply, so this is something we’re going to have to deal with.”

Barack Obama (D-IL) on Meet the Press, Sunday, May 4, 2008

The successful application of SB to bioethanol production might, as for biofuels in general, create a new business with the corresponding social classes (producers, traders, sellers, consumers, etc). The nature of the social interactions is not expected to differ from that of any other new and successful energy-related business.

If SB-based strategies succeed, the volume of ethanol produced each year might dramatically increase. For example, the calculations mentioned above on straw-

based bioethanol show that a huge volume corresponding to eightfold the world-wide production of bioethanol in 2008 might be achieved. And this scenario considers only this particular lignocellulosic biomass. The implementation of procedures to efficiently produce bioethanol from molasses might also be a reality in the near future (Hatano *et al.*, 2009). If these predictions are met, the impact on society will be deep. Although the societal consequences of a dramatic turnover in the use and availability of biofuels are difficult to forecast, at least these major issues arise:

Stability of ethanol as a major biofuel: the price factor. The price of the ethanol might suffer from unpredictable variations linked to the market economy. This would be the case if bioethanol production increased in such a way that a significant substitution of fossil fuels occurs. It would also be the case in a scenario in which bioethanol dominates the biofuels market. The impact of these variations would affect both producing countries (see last issue) and consumer countries. It seems reasonable to forecast that, in a future economy based on biofuels, a parallelism with the situation of oil can occur. The price of a barrel of oil is difficult to forecast over the long term. Likewise, ethanol might be subject to price fluctuations that would affect its competitiveness with other fuels.

Price of food. The effect of massive bioethanol production associated with advances in the SB approaches designed to take advantage of lignocellulosic biomass should not directly affect food prices because byproducts rather than grains or vegetables would be used to produce ethanol. Nonetheless, food prices might well rise in scenarios in which significant portions of the surface dedicated to basic crops were to be transformed into ethanol-producing varieties. It is important to highlight that, rather than being a scientific issue, the use of a novel technology is the responsibility of policy makers. This calls for a legal framework that fosters the use of agricultural lignocellulosic byproducts such as prune rests or straw, and that strongly regulates the transformation of food-producing crops.

Greenhouse effect. The impact of a bioethanol-based economy on reigning in global warming is expected to be high. Indeed, the conversion of lignocellulosic biomass into biofuels represents huge CO₂ savings: although CO₂ is released when ethanol is burned, this emission, unlike that of fossil fuels, is of recent atmospheric origin and it is fixed again by the following harvest. The result: the net CO₂ emission of bioethanol is close to zero. Nonetheless, a regulatory framework limiting the artificial extension of the crops for ethanol production is envisaged. This is because certain cultures that yield more lignocellulosic biomass, such as paddy rice, are net greenhouse gas producers: paddy culturing is in fact thought to be responsible for global methane emissions of as much as 28.2 Tg/year.

1.2.4.1 Could the Application Change Social Interactions?

The successful application of SB to bioethanol production might, as for biofuels in general, create a new business with the corresponding social classes (producers, traders, sellers, consumers, etc). The nature of the social interactions is not expected to differ from that of any other new and successful energy-related business.

Ethical issues of applying SB to improve ethanol production for biofuel use involve:

- i) Ethics of the technology;
- ii) Ethics of the product of the technology;
- iii) Ethics of the use of the product (bioethanol).

Regarding (i), no major differences are evident compared to standard biotechnology such as transgenic crops or GM microorganisms expressing enzymes for the textile industry. On (ii), ethanol itself is a well-known chemical compound with a rather low toxicity and very well-characterized effects. The inflammability of ethanol and its transportation might, by contrast, be a source of conflict of interest and security hazards. These, along with the ecological or economic issues described here, correspond to the application (iii) of the technology rather than the technology itself.

1.2.4.2 Producing Countries, Rich Countries?

If the current scenario of first-generation bioethanol is maintained in the future, then countries such as Brazil or the United States will probably monopolize the first-generation bioethanol production as they do now. However, significant advances in processing lignocellulosic biomass might change the world distribution of producers and consumers. Indeed, lignocellulosic biomass is present evenly wherever vegetables grow, that is, worldwide. Nonetheless, tropical and sub-tropical regions along with certain moderate climate areas have a larger production potential because of the climate. The expectation therefore is that the production of lignocellulosic bioethanol would be mainly based in countries with the ability to produce grain, such as wheat, rice and maize. South-Eastern Asia, North America, and to a lesser extent, Europe would then become the major producing regions (see Table 1.7; Domínguez-Escribà and Porcar, 2010).

Access to Bioethanol Depending on the type of technology developed to transform biomass into bioethanol, the required steps might be performed in large, confined structures. In a contrasting scenario, local producers would be involved. If the latter was the case, the farmers of remote areas would be autonomous in terms of fuel production and use. Alternatively, if the technology involved requires (or is anyhow monopolized by) large biochemical enterprises, the availability and thus the price of the final product will be controlled by the manufacturers. Beyond the limitations of the technology (enzymatic reactions might be difficult to control by local producers, for example), political will is a key point to ensure fair access to bioethanol.

Table 1.7 Consumption of vehicle fuels in Europe (EU-27; Eurostat, 2005^a).

Country	Consumption of vehicle fuels in Europe (PJ)				Objectives 2010
	Gasoline	Diesel	Biofuels	Total	Biofuels
Belgium	2 410	7 298	0	9 846	665
Germany	31 379	29 522	2 673	63 720	4 570
Spain	9 928	27 351	357	37 718	2 571
France	14 322	35 254	597	50 479	3 447
Italy	18 397	26 539	224	47 470	3 256
Netherlands	5 603	7 370	–	13 624	1 022
Poland	5 392	6 313	76	14 507	947
Sweden	5 281	3 539	289	9 110	680
United Kingdom	25 615	22 898	111	48 845	3 655
EU-27	148 144	203 828	4 413	364 494	25 603

a) See: epp.eurostat.ec.europa.eu

1.3

Non-ethanol Fuels

1.3.1

Introduction

Ethanol is considered by many researchers and engineers as a sub-optimal biofuel due to technical challenges (corrosive and extremely hygroscopic: it tends spontaneously to be associated to 4% water). Ethanol is not the best energy molecule and poses many problems of transport and incorporation in gasoline. In addition, as the European vehicle market is very focused on diesel engines, this molecule represents only a moderate interest there. Furthermore, the power energy of this molecule is insufficient to provide the energy needed to fly an airplane.

In terms of energy yield, the fact that ethanol is already a partially oxidized derivative of carbon does not make it a fuel of choice. Nonetheless, ethanol is not the only biofuel available; a number of different chemical substrates have been investigated as alternative liquid transportation fuels, for example, fatty acids, fatty alcohols, biodiesel, biobutanol, biopropanol, acetone, methanol. For comparison, Figure 1.10 shows the energy densities of a variety of fuel substrates.

Biodiesel is commonly produced by the transesterification of vegetable and animal oils. It is composed of fatty acid methyl and ethyl esters. Based on the current techniques and lifecycle accounting, biodiesels from soybean yield more net energy gain (93%) than ethanol from corn grains (25%) (Hill *et al.*, 2006). However, the problem of availability of traditional agricultural resources and the European diesel demand led some companies to develop alternative production pathways: companies like Amyris and LS9 have used SB technologies to

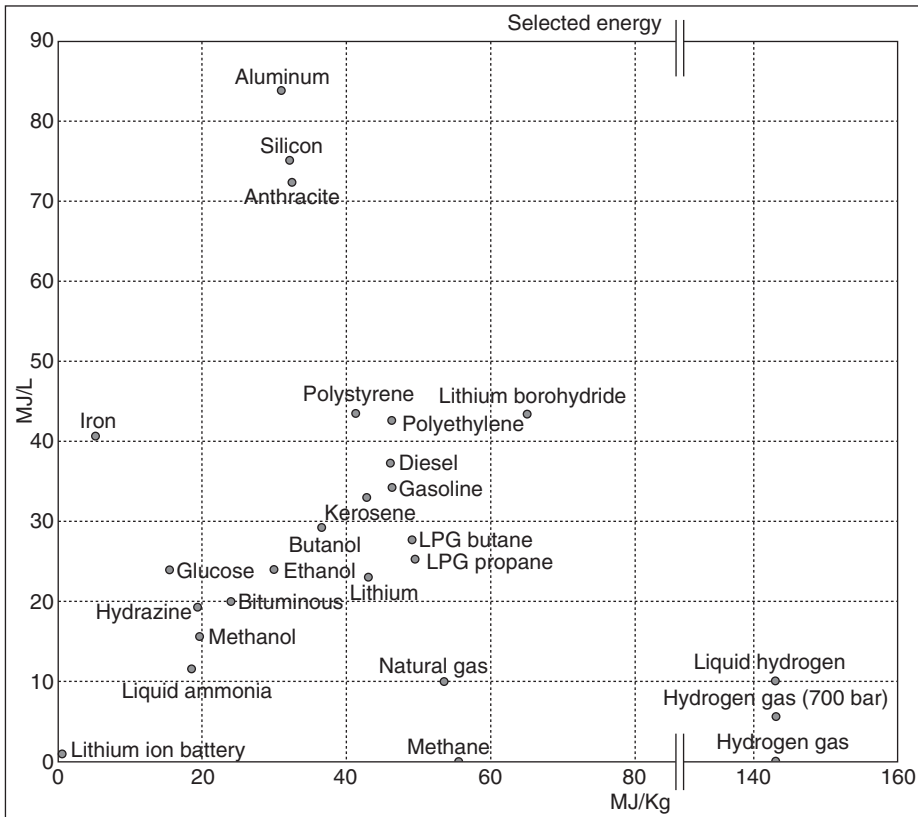


Figure 1.10 Energy densities for selected fuel substrates.

develop the new pathway of sugar to diesel. Amyris, based on the work of Jay Keasling on the isoprenoid pathway, wanted to create so-called truly “No Compromise® fuels”. Amyris wrote: “these renewable fuels don’t demand sacrifice in performance or penalty in price, and offer a superior environmental profile by reducing lifecycle (GHG) emissions of 80% or more compared to petroleum fuels. They are also delivered to consumers using existing petroleum distribution infrastructure and work in today’s engines.” Amyris stopped its biofuel R&D in early 2012.

Fatty acids are the raw material for producing biodiesel (renewable diesel) and other products. Fatty acids react like any other carboxylic acid, which means they can undergo esterification and acid-base re-actions. Reduction of fatty acids yields **fatty alcohols**, which can be used as biofuels (Lestari *et al.*, 2009; Steen *et al.*, 2010). To yield a better fuel, biodiesel could be produced using bio-ethanol for the trans-esterification to produce ethyl esters, which are less viscous (Kleinov *et al.*, 2007). A possible longer-term route to biodiesel, currently under active investigation, involves the direct production of fatty acid ethyl esters from ligno-

cellulose by engineered organisms such as Actinomycetes and also via production of wax esters in plants (Kalscheuer, Stolting, and Steinbüchel, 2006; Steen *et al.*, 2010). The future of this pathway is linked to two key challenges: cost and scale. Currently, this is not competitive with ethanol production. Nevertheless, the producers claim that costs will decrease by commercialization and point out that gasoline is far more lucrative than ethanol.

Butanol. Third-generation biofuels include alcohols like **bio-propanol** or **bio-butanol**, which due to current lack of production experience are usually not considered to be relevant as fuels on the market before 2050 (OECD/IEA, 2008). Increased investment could accelerate their development. The same feedstocks as for first-generation ethanol can be used, but requires more sophisticated technology. Propanol can be derived from chemical processing such as dehydration followed by hydrogenation. As a transport fuel, butanol has properties closer to gasoline than bioethanol (UNEP, 2009). Butanol is generally considered a better alternative fuel than ethanol. As a “higher” (4-carbon) alcohol, it more closely resembles the hydrocarbons in gasoline (usually 4–12 carbon atoms) and in diesel (usually 9–23 carbon atoms).

Compared with ethanol, butanol has higher energy density, can be transported by the current infrastructure of pipelines, and can be used directly in **conventional petrol engines**. Butanol has been produced along with acetone and ethanol in the Acetone butanol ethanol (ABE) fermentation by *Clostridium acetobutylicum*, with the drawback of low yields and **low product concentrations** due to the toxicity of butanol to the fermentative strains (Lee *et al.*, 2008). To enhance the yield, the butanol production pathway has been engineered in *Escherichia coli* (Figure 1.11; Atsumi and Liao, 2008a, 2008b). An even higher productivity was achieved by introducing the isobutanol pathway while also overexpressing a key enzyme in the photosynthetic pathway in *Synechococcus elongatus* (Atsumi, Higashide, and Liao, 2009). Alcohols with longer carbon chains would be even better (e.g., octanol). It is clear, however, that alkanes would always be preferred choices whenever possible. The future of this pathway is linked to two key challenges: (i) demonstrating that there is a process with which the butanol can be commercially and economically produced at scale; and (ii) market adoption.

Acetone. Compared with other alcohols, acetone, one of the co-products from the ABE fermentation, can be recovered easily by direct exhaust from the gas stream due to its high volatility. It can be produced in engineered *E. coli* (Bermejo, Welker, and Papoutsakis, 1998). It can be reduced to isopropanol, another potential biofuel or chemical feedstock, from strains co-expressing a secondary alcohol dehydrogenase (Hanai, Atsumi, and Jiao, 2007).

Methanol can be produced from a wide range of biomass feedstocks via a thermochemical route similar to the Fisher–Tropsch process for BtL, a process creating synthetic fuels made from biomass through a thermochemical route. It can be blended in petrol at 10–20%. Methanol can be converted to dimethylether

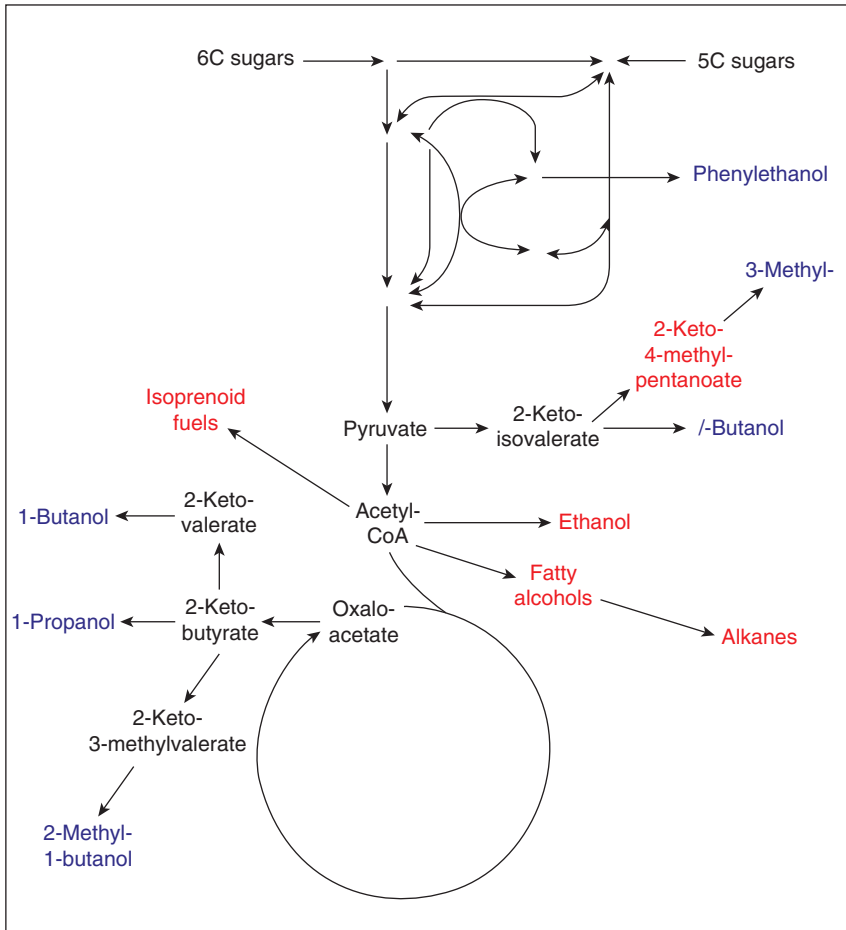


Figure 1.11 Metabolic engineering routes in *E. coli* to deliver next-generation biofuels (Source: Keesling and Chou, 2009).

(DME) by catalytic dehydration. Above -25°C or below 5 bar, DME is a gas. Hence its use as a transport fuel is similar to that of liquefied petroleum gas (LPG). It cannot be blended with standard diesel fuels. DME can also be created directly from syngas. The BioDME project³⁾ aims to demonstrate production of environmentally optimized synthetic biofuel from lignocellulosic biomass at an industrial scale. The Supermethanol project on reforming crude glycerine in supercritical water to produce methanol for re-use in biodiesel plants (FP7-212180)⁴⁾ aims to produce methanol from crude glycerine, and then re-use the

3) <http://www.biodme.eu/about-dme>

4) <http://www.supermethanol.eu/>

methanol in the biodiesel plant. This will improve the energy balance, carbon performance, sustainability and overall economics of biodiesel production. Glycerine is a co-product of the biodiesel process. The work expands on expertise generated by the consortium on reforming glycerine in supercritical water, and then producing a synthesis gas suitable for direct once-through methanol synthesis (glycerine to methanol; GtM). Producers will be less dependent on the methanol spot price: there is a (partial) security of methanol supply, and their byproduct is used as a green, sustainable feedstock.

Isobutene. The French company Global Bioenergies developed a totally artificial metabolic pathway to produce the gaseous (predecessor) biofuel isobutene.⁵⁾ The metabolic pathway developed by Global Bioenergies makes it possible to transform renewable resources, such as sugar cane, beet or cereals, into isobutene, a synthon that can easily be converted into fuels and several polymers.

The large-scale establishment of cheap chemical processes has allowed the transformation of isobutene into diverse fuels: gasoline, kerosene, diesel and ETBE. These products have been used for decades because of their high energy density and the ease with which they can be handled and stored. These products are fungible in the petrochemical industry of today, unlike ethanol, which would require a complete change in infrastructure if its use were to be generalized. This production process is a biological analog of the Fischer–Tropsch process but does not require a high-temperature step and is therefore better as regards both energy and environment (Global Bioenergies, 2010)⁶⁾.

Other futuristic biometabolites. Archaea produce methane. Ethylene is produced at a low level by plants and some microorganisms (*Pseudomonas* sp.). It is therefore not more far-fetched than some of the directions proposed previously to consider improving, via SB, the synthesis of alkanes and alkenes by living organisms. It will be important to invest in the analysis and reconstruction of metabolic pathways in the very near future.

1.3.2

Economic Potential

Although large volumes of bioethanol have been produced in the past years and further increases have been projected for the future, its long-term feasibility has been questioned because of its low energy density, its miscibility with water, its corrosive properties, and necessary engine modifications. Other biofuels may have a promising advantage over bioethanol, such as biodiesel and butanol, both of which can be handled in the existing petrol infrastructure and transportation vehicles. Biodiesel has been considered as the primary renewable alternative to diesel, which is globally in the greatest demand and has a growth rate three times

5) <http://www.global-bioenergies.com/index.php?lang=en>

6) www.global-bioenergies.com

that of the gasoline market (Steen *et al.*, 2010). The US Defense Advanced Research Project agency spent \$2 million in 2009 for **bio-butanol** production research (DARPA, 2010). Glycerine is a major byproduct of biodiesel production. The rapid increase in biodiesel production capacity in Europe has been associated with a correspondingly rapid increase in the amount of (crude) glycerine. Since 2004, glycerine production has exceeded consumption, and the mismatch is increasing. As a result of increasing biodiesel production, and a lack of viable market outlets for glycerine, the price of crude glycerine plummeted in the ten years from the mid-1990s. In 2006, the price stood at less than 100€/t (5.8€/GJ), which is only slightly higher than its energy value. At this price level it is too costly for small- and medium-scale biodiesel producers to refine their own crude glycerine. These producers are therefore desperately seeking new glycerine applications. Thus, the leading stakeholders in the European biodiesel sector are urgently seeking to identify new (crude) glycerine applications. The supermethanol project, funded by EC-FP7, aims to identify a viable alternative application by investigating whether (crude) glycerine can be reformed in supercritical water for syngas production and conversion into methanol.

Third-generation biofuels include alcohols like **bio-propanol** or **bio-butanol**, which due to current lack of production experience are usually not considered to be relevant as fuels on the market before 2050 (OECD/IEA, 2008). Increased investment, however, could accelerate their development. The same feedstocks as for first-generation ethanol can be used, albeit using more sophisticated technology. Butamax (Dupont+BP) anticipates that its first commercial plant will be operational by 2013 because its fuel already conforms to European Union standards.

Propanol can be derived from chemical processing such as dehydration followed by hydrogenation.

As a transport fuel, butanol has properties closer to gasoline than bioethanol (UNEP, 2009).

1.3.3

Environmental Impact

The environmental impact of biodiesel has been studied by Hill *et al.* (2006), revealing major advantages over corn-derived bioethanol regarding environmental benefits. By one estimate, biodiesel can reduce GHGs emission by 41% compared with diesel, reduces several major air pollutants, and only minimally impacts human and environmental health through nitrogen, phosphorus and pesticide release. One caveat needs to be mentioned here: biomass often presupposed fertilization using nitrogen, and one underestimated GHG pollutant derived from inappropriate use of nitrogen, namely nitrous oxide, has an enormous and rapidly increasing greenhouse effect. The same estimate points out that corn-based bioethanol provides smaller benefits through a 12% reduction in GHGs. The positive effects of biodiesel on air pollution have also been shown in many studies (Hill *et al.*, 2006). For the non-ethanol biofuels derived from non-food feedstocks (such as lignocellulose), the impacts are comparable to those of bioethanol with

regard to greenhouse gas emissions, land use, water consumption, air pollution, and biodiversity. Although biodiesel can be used in conventional engines without major modification, the chemical composition of biodiesel differs distinctly from conventional diesel. Current production of biodiesel comprises a mixture of methyl esters, which is made from the “transesterification” of plant oils such as from rapeseed, soybean or palm oil, using methanol, usually derived from fossil fuels. The process reduces the viscosity of the oil, improves its consistency and miscibility with diesel, and also improves other properties such as its viscosity when cold (AMEC, 2007). As the chemical composition of the oils from each plant species are slightly different, the properties of the final product also differ, and blends of the various oils may be needed to produce a standard. A better fuel could be produced using bioethanol for the transesterification. This would yield ethyl esters that are less viscous, particularly when cold (Kleinov *et al.*, 2007). Owing to minor levels of contaminants and the performance of different processes, there is still some variability in quality. This is more pronounced when waste oils and fats are used as the raw material. Here, the fatty acid content needs to be completely neutralized and either removed or converted to ensure complete reaction and a clean product. Biodiesel is currently limited to 5% in diesel in Europe due to concerns over engine warranties, materials, cold weather performance, and compatibility.

Another problem that needs consideration is the way the initial ligno-cellulosic material meant to be transformed into biofuels is made available: what are the techniques permitting its transport or its cracking. As long as agriculturally derived biomass is used, the situation parallels that of bio-ethanol.

1.3.4

Foreseeable Social and Ethical Aspects

Non-ethanol biofuels are a mixture of very different compounds that can impact society very differently. An evaluation of the impact of such biofuels must first deal with the certainty with which biofuel will predominate in the future. Lacking this knowledge, a case by case estimation of the societal impact can be made:

Bioalcohols. Biobutanol can be used directly as a car fuel instead of gasoline. Its use would therefore imply important savings in terms of delivery and for the automobile industry in particular (no engine modifications required). Additionally, it is less corrosive than ethanol. This as well as other non-ethanol bioalcohols, if produced at a global consumption scale, would imply important savings and recycling of resources and technical material associated with petrol.

Biodiesel. Past experience with the vast use of biodiesel, particularly in city buses and trucks, and the ease of use in standard engines (as in the case of biobutanol) points to a low societal impact of a future large-scale biofuels industry based on biodiesel. The expected substitutions, however, are not gasoline by diesel, but gasoline by bioethanol and diesel by biodiesel. In all cases, the societal impact would be low.

Biogases (biogas and syngas). Gas biofuels can be produced from organic wastes, even locally. Their general use would imply deep societal changes if a local rather than a ready to use delivered fuel economy was set in place.

Solid biomass. As in biogases, solid biomass management might have an important impact because of local production. Additionally, coupling waste processing, such as home garbage or agricultural waste, with energetic production would amount to a turnover in current energy policy and imply obvious societal changes. These include the creation and destruction of jobs linked to waste management, and the transformation, design and trade of the required equipment, and so on.

1.3.4.1 Impact on Social Interaction

The successful application of SB to non-ethanol production (Khalil and Collins, 2010) might help create new businesses including substrate management (organic and agricultural waste, sawdust, etc), promote the development and trade of the required techniques and equipment, and establish a link between both areas. Another issue is the conversion of infrastructures such as pipelines; the specifications of such pipelines will depend on the type of biofuel being transported due to major differences in their corrosive properties.

Ethical issues. Compared to bioethanol, there are no major differences in terms of ethics between non-ethanol and ethanol biofuels. In some cases, the ease of local production for local consumption has to be taken into account (see next point)

Justice of distribution. Two very different scenarios arise: one with massive production of bioalcohols or biogas and ulterior transportation, and another in which large factories are complemented with local production for local use. This would particularly benefit exotic (in the sense of distant) farmers, who might use their agricultural waste as fuel by burning it, use their animal feces for biogas production or produce biodiesel from vegetable oil. The success of direct use of edible oil in old diesel cars (i.e., Mercedes) suggests that taking advantage of the energetic potential of common waste as biofuels does not necessarily require complex reactions. Most of these home-made biofuels might well challenge petrol in terms of dependency. Although these local strategies might be difficult to spread, they would certainly arise in developing countries. Such strategies imply a shift from less sustainable biofuels based on grain, such as corn, towards sustainable feedstock and production practices (Solomon, 2010).

1.4 Algae-based Fuels

1.4.1 Introduction

Algae fuel, also called oilgae, is a biofuel from algae and addressed as a **third-generation biofuel** (UNEP, 2009). **Microalgae contain lipids and fatty acids** as membrane components, storage products, metabolites and sources of energy. **Algal oils possess characteristics similar to those of animal and vegetable oils** and can thus be considered as potential substitutes for the products of fossil oil. Algae are feedstocks from aquatic cultivation for production of triglycerides (from algal oil) to produce biodiesel. The processing technology is basically the same as for biodiesel from second-generation feedstocks. While many microalgae species are capable of producing **high amounts of lipids** (lipid contents exceed those of most terrestrial plants), **higher lipid concentrations** can be obtained in a **nitrogen-limited environment**.

The concept of using algae to make fuel was first discussed more than 50 years ago, but a concerted effort began with the oil crisis in the 1970s (Hu *et al.*, 2008). The US Department of Energy (DOE) from 1978 to 1996 devoted \$25 million to algal fuels research in its aquatic species program at the National Renewable Energy Lab (NREL) in Golden, Colorado. The program yielded important advances that set the stage for algal biofuel research today (Waltz, 2009). The first genetic transformation of microalgae came in 1994, and scientists a few years later successfully isolated and characterized the first algal genes that express enzymes thought to enhance oil production. From 1990 to 2000, the Japanese government funded algae research through an initiative at the Research Institute of Innovative Technology for the Earth (Kyoto). The program focused on carbon dioxide fixation and improving algal growth with concentrated mirrors that collect light. These approaches yielded some successes, and many are still the focus of scientists today, but none have proven to be economical on a large scale. The DOE program closed in 1996, in part because algal systems could not compete with the cheap crude oil of the late 1990s. Since the mid-1990s, however, the tools for genetic engineering have improved, and scientists are increasingly applying them to algae with fuel applications in mind. Much of the work is focused on identifying the genes involved in lipid synthesis and how those genes are regulated. The idea is to manipulate those genes so that the organisms' metabolic pathways are tricked into producing storage lipids, even when the algae are not under stressful conditions (which is when they start overproducing oil and lipids; Waltz, 2009).

Microalgae can produce high yields of oil that can be refined into transport fuels such as diesel and jet fuel. The advantages of microalgae biofuels over conventional agricultural biofuels are that they can (Spolaore *et al.*, 2006; Waltz, 2009; Carbon Trust, 2010):

- Produce higher yields of oil per hectare of land;
- Do not require arable land or freshwater to grow and thus do not compete with food crops;
- Produce a higher-quality fuel product;
- Can produce non-fuel high-value products (e.g., biopolymers, proteins, animal feed).

If sustainable and profitable processes can be developed, the potential benefits of these technologies for the common good appear compelling and include the production on nonarable land of biodiesel, methane, butanol, ethanol, aviation fuel, and hydrogen using waste or saline water as well as CO₂ from industrial or atmospheric sources (Beer *et al.*, 2009).

Many microalgae are promising for the production of an enormous variety of compounds, including biofuels (see Figure 1.12). To cultivate these algae and their products, monocultures have to be maintained, typically requiring enclosed photobioreactors. A photobioreactor can be described as an enclosed, illuminated culture vessel designed for controlled biomass production of phototrophic liquid cell suspension cultures (Tredici, 1999). Photobioreactors, despite their costs, have several major advantages over open pond systems.

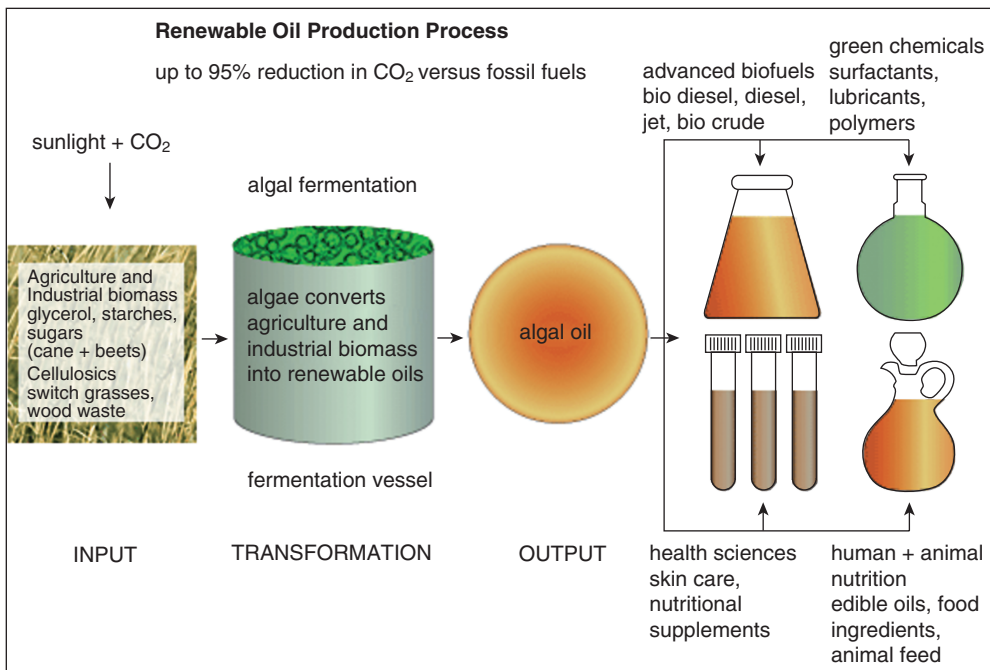


Figure 1.12 Schematic description of the use of microalgae for the production of biomaterials including biofuels. (Source: Solazyme, 2010).

Table 1.8 Productivity of algal strains reported in some outdoor photobioreactors (Ugwu, Aoyagi, and Uchiyama, 2008).

Photobioreactors	Photosynthetic strain	Daily productivity (g/l)
Airlift tubular	<i>Porphyridium cruentum</i>	1.5
Airlift tubular	<i>Phaeodactylum tricornutum</i>	1.2–1.9
Inclined tubular	<i>Chlorella sorokiniana</i>	1.47
Undular row tubular	<i>Arthrospira platensis</i>	2.7
Outdoor helical tubular	<i>Phaeodactylum tricornutum</i>	1.4
Parallel tubular (AGM)	<i>Haematococcus pluvialis</i>	0.05
Bubble column	<i>Haematococcus pluvialis</i>	0.06
Flat plate	<i>Nannochloropsis</i> sp.	0.27

They can:

- Prevent or minimize contamination, permitting axenic algal cultivation consisting of only one species of microalgae;
- Offer better control over biocultural conditions (pH, light, carbon dioxide, temperature);
- Prevent water evaporation;
- Lower carbon dioxide losses due to gassing;
- Permit higher cell concentrations.

However, certain requirements of photobioreactors—cooling, mixing, control of oxygen accumulation and biofouling—make these systems more expensive to build and operate than ponds. New, cheaper, innovative systems are being designed, and waste streams are used to make the production of microalgae commercially attractive. (See Table 1.8 for details on the productivity of different photobioreactors). Although algae are commercially cultivated in developed countries, most of these regions are characterized by seasonal variations in temperatures and solar light energy over the year. Tropical developing countries might be better potential cultivation sites for the commercial production of algal products (Ugwu, Aoyagi, and Uchiyama, 2008).

1.4.2

Economic Potential

Algae have long been thought to be a rich and ubiquitous source of renewable fuel, but thus far have failed to be economically competitive with other more conventional sources of energy. The question is, what are the major limitations for economic competitiveness and how can they be overcome?

In the United Kingdom the Carbon Trust (2008) launched the Algae Biofuels Challenge in 2008, a research and development investment strategy that could help make low-cost algae biofuels a commercial reality by 2020. The total program

cost is estimated to be within £20–30m. The two phases of this initiative are: (i) address fundamental research and development challenges and (ii) a pilot-scale demonstration of algal oil production.

Several studies have demonstrated that the energy yield of microalgae is significantly higher than that of energy crops (see Table 1.9). Depending on the achievable photosynthetic conversion efficiency, microalgae systems could reach even higher oil biomass yields, as seen in Table 1.10.

A detailed economic analysis of microalgae production was carried out by Stephens *et al.* (2010). Two models were used, the base case and the projected case. The base case is intended to represent an emerging scenario from the industry and involves the following assumptions: (i) production of microalgal biomass using 500 ha of microalgal production systems, (ii) the extraction of oil, (iii) the co-production and extraction of a high-value product (HVP; e.g., β -carotene at 0.1%

Table 1.9 Potential oil yields of different crops per year (Waltz, 2009).

Crop	Annual yield (liters of oil/ha)
Soybeans	402
Sunflower	804
Canola	1 600
Jatropha	2 002
Palm oil	5 996
Microalgae ^{a)}	56 124

a) With future technology.

Table 1.10 Practical and theoretical yield maxima for microalgal biomass and oil production (Waltz, 2009).

Photosynthetic conversion efficiency	Annual biomass energy	Biomass energy	Oil	Daily biomass production	Annual biomass yield	Annual oil yield	Annual residual biomass
(%)	(GJ/ha)	(MJ/kg)	(%)	(g/m ²)	(T/ha)	(l/ha)	(T/ha)
2.1	1677	22.98	25	20	73	19837	55
6.5	5220	22.98	25	62.2	227	61400	170
6.5	5220	27.95	50	51.2	187	100943	93
8	6424	22.98	25	76.7	280	75570	210
8	6424	27.95	50	63	230	124237	115
10	8030	22.98	25	95.9	350	94462	262
10	8030	27.95	50	78.6	287	155297	143

of biomass, \$ 600/kg), and (iv) the sale of the remaining biomass as feedstock (e.g., soymeal or fishmeal substitute). In contrast, the projected case is intended to represent the microalgal biofuel industry at maturity and no longer incorporates the co-production of HVPs. The internal rate of return (IRR)⁷ has to be above 15% in order for the investment to be profitable, which can be met in the base case but can only be achieved in the projected case (fuel production only) when the oil price exceeds \$ 100 per barrel. Economic feasibility for the microalgae industry seems to be restricted to large industry because approximately 200 ha are needed to be profitable (not including government taxes and other regulatory mechanisms to favor new and renewable energies; see Figure 1.13). Considerable synergies also exist between microalgae biofuel production and a wide range of other industries, including human and animal food production, veterinary applications, agrochemicals, seed suppliers, biotech, water treatment, coal seam gas, material supplies and engineering, fuel refiners and distributors, bio-polymers, pharmaceutical and cosmetic industries, as well as coal-fired power stations (CO₂ capture) and transport industries, such as aviation. Sound opportunities therefore exist for the development of a rapidly expanding sustainable industry base (see Table 1.11) whose productivity is independent of soil fertility and less dependent on water purity. Thus, these technologies can conceivably be scaled to supply a substantial fraction of oil demand without increasing pressure on water resources while potentially contributing to food production.

For the algae-fuel companies the goal is a fuel production at \$50–60 per barrel within three to five years. The Defense Advanced Research Projects Agency, which is developing new jet fuel technology for use by the military, is targeting \$ 1 per gallon algal oil, or \$ 1 per gallon finished cost of jet fuel at a capacity of 189 million liters per

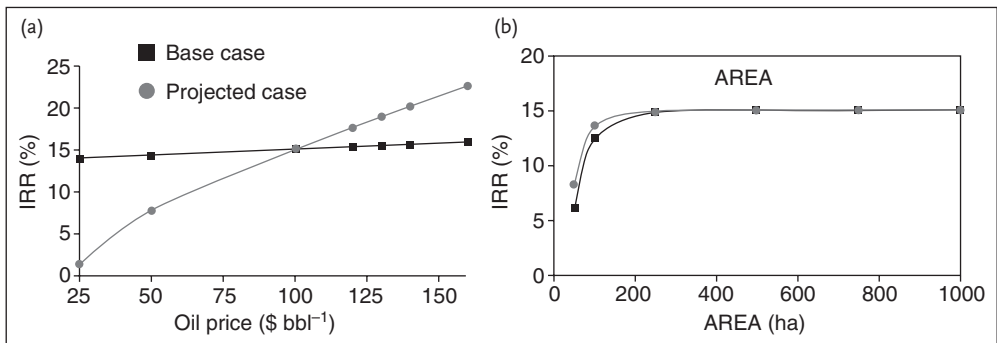


Figure 1.13 Results of a calculation estimating the economic feasibility of microalgae systems. Two cases were calculated, a base case and a projected case. (a) Internal rate of return depending on

current oil price. (b) Internal rate of return depending on size of algae factory. The internal rate of return needs to be higher than 15% to be profitable (Waltz, 2009).

7) The IRR of an investment is the interest rate at which the costs of the investment lead to the benefits of the investment.

Table 1.11 Overview of companies that work on biofuel production from algae, applying different genetic techniques (source: Waltz, 2009).

Company	Technology investment	Investors
Aurora Biofuels	Overexpressing genes, such as carboxylic acetylcoenzyme A, to improve triglyceride synthesis	US\$25 million; Gabriel Venture Partners, Noventi, Oak Investment Partners
Algenol	Metabolically enhancing cyanobacteria to directly synthesize ethanol; expressing enzymes pyruvate decarboxylase and alcohol dehydrogenase II	US\$70 million; firm's founders; license with Biofields (Mexico) for undisclosed fee and royalties
Sapphire Energy	Producing a "crude-like oil" that can be refined into gasoline or jet fuel	US\$100 million in 2008; ARCH Venture Partners, Wellcome Trust, Cascades Investments Gates
Solarvest BioEnergy	Hydrogen production in a single cycle from algae; plastid engineering through introduction of promoters that target proteins involved in photosynthesis, hydrogenases and proteins involved in their regulation	Publicly traded on the TS X Venture Exchange
Solazyme	Use of antisense and RNA interference to regulate light-harvesting genes, chlorophyll biosynthesis genes and signaling, together with synthetic genes containing unnatural codons to maximize triglyceride production through fermentation. Business plan is to target nutraceuticals market, then biofuels	US\$70 million; Roda Group, Harris & Harris Group, Lightspeed Venture Partners, Chevron Technology Ventures
Synthetic Genomics	Modifying genes to create new secretion pathways through the outer membranes of algae so they expel the oil, making harvesting easier. Patent describes genome assembly technology in which cassettes of algal genes involved in triglyceride productions are cloned and rebooted into appropriate recipient prokaryotic cells	Valued at US\$300 million Investors include founders, Exxon Mobile, BP, Biotechnology, and others

year (mlpy) or 50 million gallons per year (mgpy) by the end of 2011. However, the current price per gallon for algae fuel is significantly higher, and significant hurdles need to be overcome before algae-fuel becomes economically viable.

Solazyme is the most mature company; it has developed products that met several United States and European standards for biodiesel, renewable diesel and jet fuel, and they envision commercialization within five years. Their fuels have

been tested in unmodified engines and meet US Department of Defense specifications. For companies using phototrophic algae, commercialization is not expected for another 10 years.

Two main biological factors govern the cost of algal technologies. The first is the quick selection of the best algal strains among the 40 000 species of microalgae known. The second is engineering the metabolic pathways that control oil production in algae organisms. The genetic modifications can be used to increase the algae's photosynthetic efficiency, growth rate and oil content, and to identify and control the biochemical triggers that cause the organism to accumulate oil. The potential of genetic engineering technology has led some industry experts to conclude that it is a certainty. Several companies, including Algenol, Solazyme, Seambiotic, Sapphire Energy, and Hawaii-based Kuehnle AgroSystems, are working on strains of genetically improved algae.

1.4.3

Environmental Impact

Algae-based biofuel has a high potential to replace a significant proportion of petroleum used in transportation, which will reduce consumption on tremendous amount of fossil carbon every year globally. Some reports conclude that microalgae are the only source of renewable biodiesel that can meet global demand for transportation fuel (Chisti, 2008).

Initial forecasts suggest that algae-based biofuels could supplement 70 billion liters of fossil-derived fuels used worldwide annually in transportation by 2030. This would equate to an annual carbon saving of over 160 million tonnes of CO₂ globally and a market value of over €17 (£15) billion. If successful, algae could deliver six to 10 times more energy per hectare than conventional cropland biofuels, while reducing carbon emissions by up to 80% relative to fossil fuels. Also, unlike traditional biofuels, algae can be grown on non-arable land using seawater or wastewater. Many species of algae thrive in seawater, water from saline aquifers, or even wastewater from treatment plants. It is believed that the production of algae-based biofuel will not compete for the scarce arable land and can remove nutrients and contaminants from waterways. Therefore, using algae as a biofuel feedstock avoids many of the negative environmental and ecological impacts associated with first-generation biofuels (Carbon Trust, 2010). Algal biofuels offer environmental benefits by reducing anthropogenic pollutant release to the environment and by requiring fewer water subsidies (Smith *et al.*, 2009). Moreover, the algae production unit can potentially be **coupled to an industry that emits CO₂**, thus also **limiting CO₂ emission**. At full scale, algae ponds are estimated to be capable of consuming approximately 80 tonnes of CO₂ per acre per year. For heavy carbon emitters such as steel and coal-fired power plants, cement factories and manufacturing facilities, PetroAlgae offers a cost-effective process to meet increasingly stringent global carbon standards. Also, with micro-crops able to absorb approximately twice their weight in CO₂, PetroAlgae sees carbon management as another potentially profitable enterprise for the future.

1.4.4

Foreseeable Social and Ethical Aspects

Although the assumption that all “land-grown” biofuels adversely affect the supply of food and other crop products is not always true (particularly in case of lignocellulosic bioethanol), it is a fact that aquatic biofuel cultures should not compete with crops. Note, however, that large artificial or semi-artificial aquatic extensions used for culturing algae might indeed compete with another type of human food: fish and seafood from aquaculture. Thus, the societal impact of a hypothetically large algae-based biofuel industry will depend on the interaction between the industry with aquaculture and agriculture (see next issue), and will also be influenced by its ecological effects, such as transformation of wetlands into biofuel factories (see Figure 1.14).

1.4.4.1 Could the Application Change Social Interactions?

Culturing algae is very different from agriculture. Should SB approaches boost the already high lipid production rates of green algae such as to promote their use as biofuel producers, a series of social interaction changes might occur. One scenario

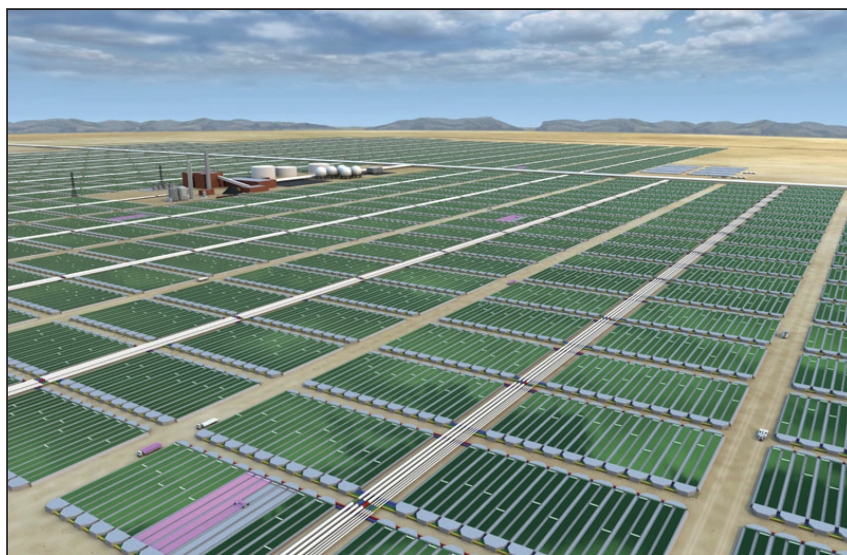


Figure 1.14 High light requirements might require vast surfaces to be transformed for algal culturing (Source: Dasolar Energy, 2010).¹²⁾

12) See: <http://www.dasolar.com/alternative-energy/biofuels>

envisages that farmers on suitable lands (mainly coastal areas but also those living near wetlands and lakes) will to shift to green algae culturing. This would have important social effects in terms of the industrial structure of the respective societies. Moreover, the local- versus monopoly-based economy represents two non-exclusive future possibilities, as in the case of other biofuels. In the case of green algae, however, local production would be easier than local transformation of algae to biodiesel.

Ethical issues. Optimization of the surface to volume ratio in order to allow proper illumination of algal cultures might require vast surfaces for growing the algae. Note that the conflict between algal culturing, agriculture and aquaculture is not merely a technological issue but should be regulated with a suitable legal framework.

Justice of distribution. Local production is indeed possible, but local transformation might be poor in developing countries. Another expectation is that the technical requirements to efficiently illuminate algal cultures will not be met in developing countries, implying a lower productivity even under tropical climates. Although difficult to forecast, a reasonable scenario is that both local and specialized production coexist, although with a predominant role of the latter in terms of production and, particularly, processing.

1.5

Hydrogen Production

1.5.1

Introduction

The need to seek alternative sources of energy has become a question of major relevance and serious social concern because fossil fuels have limited reserves and adverse environmental effects. Thus, several biofuels such as biodiesel or ethanol have been proposed as suitable candidates to partially substitute oil, although their production potentials are currently limited. Massive efforts are currently underway to enlarge the spectrum of efficiently usable biomass from cellulose. Nonetheless, other alternatives have to be found in the medium and long term. Currently, hydrogen is considered to be a most promising candidate as a future energy source due to its marked reduction to CO₂ emission and extraordinary energy density (142 MJ/kg for H₂ vs 42 MJ/kg for oil).

At the present time, steam reforming of natural gas is the best established system to produce hydrogen on the industrial scale; however, it is based on non-renewable energy sources and generates substantial sulfur and CO₂ emissions. In fact, most of the H₂-producing processes based on fossil fuels release approximately twice as many moles of CO₂ for each mole of hydrogen. In order to produce hydrogen from renewable and clean sources of energy, several alternatives are

Table 1.12 Overview of different hydrogen production processes, including photosynthetic biohydrogen.

Hydrogen production process	Basic technical principle	Positive aspect	Challenge
Coal gasification	Coal is exposed to hot steam and breaks down into a mixture of gases including hydrogen	Technology is available now	Needs high temperature (>700°C) and energy, CO ₂ pollution
Thermochemical	At high temperature various chemical reactions can split hydrogen and oxygen	Heat could be generated by concentrating sunlight	Need to find the most efficient among dozens of candidate reactions
Photo-electrochemical	A single electrode absorbs solar energy and splits water by electrolysis	More efficient than electrolysis of water using power from solar cells	Need to find materials that work well, but do not corrode
Biomass-fermentation	Some bacteria can metabolize cellulose and produce hydrogen	Cellulose is available in large quantities in crop waste	Bacterial metabolism must be re-engineered to make process more efficient
Biohydrogen farming	Cultures of algae/cyanobacteria produce hydrogen using sunlight	The most environmental friendly form of hydrogen production	Photosynthesis must be re-engineered to produce more hydrogen rather than sugars

under study. Among them, biological hydrogen production is considered a promising alternative provided that several limitations are overcome. Hydrogen is being promoted as a potential transport fuel, fulfilling the energy needs of buildings and portable electronics (instead of batteries). Free hydrogen does not occur naturally in large quantities and must thus be generated by various processes (Table 1.12). In contrast to carbon or carbohydrates, hydrogen is only an energy carrier (like electricity), but not a primary energy source. Photosynthetic biofuels entail the direct application of photosynthesis to generate biofuels. In this process, a single organism acts both as catalyst and processor, synthesizing and secreting ready to use fuels. Diverting the natural flow of photosynthesis in autotrophic organisms can generate hydrogen and hydrocarbon gas, instead of the normally produced oxygen. The characteristic of this approach is product generation directly from photosynthesis, and spontaneous product separation from the organism, bypassing the need to harvest and process the respective biomass (Melis, 2007).^{8),9)} Modification of photosynthesis in green microalgae may enable the generation of these biofuels as clean, renewable and economically viable commodities (Melis and

8) <http://epmb.berkeley.edu/facPage/dispFP.php?I=25>

9) This holds also true for gaseous hydrocarbon fuels such as isobutene, as demonstrated, e.g., by the company Global Bioenergies.

Happe, 2002). However, specific biological problems associated with a sustained, high-yield photosynthetic production of these biofuels remain to be addressed.

Currently, the following types of H₂ photoproduction processes are known (for more details see Table 1.12; Tamagnini *et al.*, 2002; Schütz *et al.*, 2004; Ghirardi *et al.*, 2009):

- 1) Oxygenic photosynthesis:
 - Green algal [FeFe]-hydrogenases;
 - Cyanobacterial [NiFe]-hydrogenases;
 - Cyanobacterial nitrogenases.
- 2) Non-oxygenic photosynthesis:
 - Bacterial nitrogenases.

Current **objectives of H₂ photoproduction** are (Pulz, 2001; Richmond, 2004; Stripp *et al.*, 2009):

Maximize the solar to chemical conversion efficiency of photosynthesis under mass culture conditions;

Improve the continuity and yield of the green microalgal hydrogen and hydrocarbon production;

Develop advanced tubular photobioreactors for biofuel production;

Enable hydrogen production in the presence of oxygen (engineering O₂-tolerant hydrogenase).

Algae such as *Chlamydomonas reinhardtii* will switch to the photosynthesis pathway under murky conditions, without the full glare of the sun. When sunlight is scant, the arrays of chlorophyll and other pigments will be actively organized into structures termed “antenna complexes” that are remarkably efficient at absorbing sunlight. In an hydrogen farm with plenty of sun, such extensive antenna complexes would not be necessary, but in contrast, would prevent sunlight from reaching the cells located in the center. Scientists are now attempting to engineer algae that contain less chlorophyll. The antenna complexes of normal *C. reinhardtii* cells contain a total of 470 chlorophyll molecules, but they should still be able to photosynthesize if these were stripped down to just 132 chlorophylls—scientists have calculated that this would increase a hydrogen farm’s productivity by a factor of four. Unfortunately, that alga does not yet exist. In creating a strain that has these properties, researchers are going through the laborious process of making thousands of mutant *C. reinhardtii*. This involves disrupting their genes by inserting marker sequences of DNA into the cells, which become randomly incorporated into the genome. So far, several promising mutants have been identified (Hemschemeier and Happe, 2005; Tetali, Mitra, and Melis, 2007; Surzycki *et al.*, 2007).

The **ideal algal hydrogen production system** would meet the following criteria:

- It would have no cell wastage—the cells would naturally maintain the same cell density without a net increase in cell mass, and new cells would obtain nutrients through cryptic growth off dead cells.

- The pond depth would be just enough to maximize light adsorption, but no deeper.
- The cells would have a reduced antenna complex size so that they would adsorb only as many photons as they could convert to hydrogen and no more. This would allow the additional photons to pass deeper into the algal solution and be adsorbed by cells further down in the liquid. All the incident photons would thereby be absorbed and converted to hydrogen.
- All electrons passing through photosynthetic system II (PS II) would be used for hydrogen production, with no side reactions.
- The cells would produce hydrogen at the maximum rate at which they could process electrons, without any concerns about oxygen production and inhibition.
- The cells would be contained in a cheap, durable translucent reactor material that fully transmitted all required wavelengths, would have a low hydrogen permeation rate to contain the hydrogen, and would not allow algae cells to attach to the inner surface and block the sunlight.

1.5.2

Economic Potential

The hydrogen economy is defined as an economic system depending on hydrogen-based energy. Hydrogen could be used as an alternative fuel system to hydrocarbons or batteries and therefore has an immense economic potential. The utility of a hydrogen economy depends on a number of issues, including use, availability and costs of fossil fuels, climate change, efficiency of the hydrogen production, and policies for sustainable energy generation. The proposed replacement of the traditional fossil fuel economy by a hydrogen economy would, however, require a massive change in infrastructure, as the pipelines, tanks and motors of these two systems are not compatible (Rifkin, 2002; Garman *et al.*, 2003; Kammen *et al.*, 2003; NAE, 2004). The feasibility of an hydrogen economy depends also on the price of hydrogen production; see Tables 1.13 and 1.14 for details.

1.5.2.1 Cost Comparison with Gasoline for Transport Fuels

In a recent test a Toyota Highlander Hybrid drove 110km with 1 kg hydrogen under real-world testing conditions.¹⁰⁾ Compared to internal combustion engines in cars, the estimated price (including taxes) of gasoline in Europe is 1.2€/l. Used in rather fuel-efficient cars (100km per 6l), the cost for 110km is 7.92€, which means that at current costs (not taking into account the different costs of cars with fuel cells), hydrogen would have to be cheaper than 7.92€ to be more cost-effective than gasoline in cars (Table 1.15).

The price alone, however, is not the only requirement for a viable hydrogen transportation system to emerge. The US National Academy of Engineering (2004)

10) See <http://multivu.prnewswire.com/mnr/toyota/39419>

Table 1.13 Characteristics of the three major biological H₂ photoproduction processes.

	Green algae and cyanobacteria (hydrogenase-based)	Cyanobacteria (nitrogenase-based)	Purple bacteria (non-oxygenic, nitrogenase-based)
Light absorption spectra	400–700 nm	400–700 nm	400–600 nm and 800–1010 nm
Photons/H ₂ generated	4	15	15
Estimated maximum light conversion efficiency (EMLCE)	10–13%	6%	6%
Electron donor	Water	Water	Organic acids

Table 1.14 Estimated cost of hydrogen per kilogram in a variety of scenarios.^{a)}

Source	Hydrogen selling price (\$/kg)
Norwegian hydrogen gas station in 2008	6.28
Hydrogen from natural gas (produced via steam reforming at fueling station)	4.00–5.00
Hydrogen from natural gas (produced via steam reforming off-site and delivered by truck)	6.00–8.00
Hydrogen from wind (via electrolysis)	8.00–10.00
Hydrogen from nuclear (via electrolysis)	7.50–9.50
Hydrogen from nuclear (via thermochemical cycles—assuming the technology works on a large scale)	6.50–8.50
Hydrogen from solar (via electrolysis)	10.0–12.00
Hydrogen from solar (via thermochemical cycles—assuming the technology works on a large scale)	7.50–9.50
US DOE future pricing goal	2.00–3.00

a) <http://www.reuters.com/article/idUSTRE54A42Z20090511?pageNumber=1&virtualBrandChannel=0>; <http://www.h2carblogger.com/?p=461>; http://www1.eere.energy.gov/hydrogenandfuelcells/news_cost_goal.html; http://www.microbemagazine.org/index.php?option=com_content&view=article&id=309:photobiological-hydrogen-productionprospects-and-challenges&catid=132:featured&Itemid=196

summarized four major challenges that would have to be solved for a hydrogen economy to be possible. They concluded that it is necessary:

- To develop and introduce cost-effective, durable, safe, and environmentally desirable fuel cell systems and hydrogen storage systems.
- To develop the infrastructure to provide hydrogen for vehicle users.¹¹⁾

11) See <http://www.fuelcells.org/info/charts/h2fuelingstations.pdf> for a list of worldwide hydrogen fueling stations.

Table 1.15 Capital costs, operating costs and projected hydrogen selling prices for different algal hydrogen system configurations (e.g., 300 kg/day hydrogen output, \$10/m² reactor cost; PSA: pressure swing adsorption purification; Amos, 2004).

System design	Capital cost (\$)	Annual operating cost (\$)	Minimum hydrogen selling price (\$/kg)
300 kg/day, \$ 100/m ² , PSA, high-pressure storage	22.2 mio	614 000	439.00
300 kg/day, \$ 10/m ² , PSA, high-pressure storage	5.2 mio	119 000	13.53
600 kg/day, \$ 10/m ² , PSA, high-pressure storage	9.1 mio	214 000	11.96
300 kg/day, \$ 10/m ² , PSA, pipeline delivery	3.2 mio	131 000	5.92
300 kg/day, \$ 10/m ² , high-pressure storage	5.0 mio	115 000	12.93
300 kg/day, \$ 10/m ² , pipeline delivery	2.9 mio	127 000	5.52
300 kg/day, \$ 10/m ² , ponds only, no compression	1.9 mio	101 000	3.68
300 kg/day, \$ 1/m ² , ponds only, no compression	0.2 mio	51 000	0.57
300 kg/day, \$ 1/m ² , PSA, high-pressure storage	3.5 mio	70 000	8.97
300 kg/day, \$ 1/m ² , PSA, pipeline delivery	1.5 mio	81 000	2.83

- To reduce sharply the costs of hydrogen production from renewable energy sources, over a timeframe of decades.
- To capture and store (“sequester”) the carbon dioxide byproduct of hydrogen production (from fossil fuels such as coal).

Clearly, cheap hydrogen production from renewable energy sources is one important goal and a necessary requirement, but by far not the only one. Carbon dioxide sequestration will also play a role when using fossil fuel for hydrogen production (see also Section 6.3), at least as a temporary energy source to pave the way for a sustainable hydrogen economy. Other challenges such as more efficient fuel cells, storage systems and a proper infrastructure are also key requirements; they cannot be solved by synthetic biology, but require other engineering fields. The hydrogen economy is a complex scientific, technological and political goal: synthetic biology could contribute, but will not be able to solve all the problems

by itself. Interdependencies between different science and engineering fields and political decisions will determine whether the hydrogen economy will take off or not.

On 24 March 2009, Sapporo Breweries Ltd announced that the company would start proof production experiments of biohydrogen using agricultural produce like sugarcane. This is a joint project with the Brazilian oil company Petrobras (Rio de Janeiro) and Ergostech Co. (São Paulo), specializing in research and consulting on renewable energy. This proof experiment to produce hydrogen from cellulose-type biomass is the first venture in the world. In fact, Sapporo Breweries Ltd has formerly succeeded in developing a pilot plant for the production of hydrogen-methane in a two-stage fermentation process utilizing waste bread as raw material. This achievement is based on the company's original technology and know-how as regards fermentation as well as processing plant design which have been gained in brewing beer. They plan to install and operate a pilot plant with 1 m³ capacity at the experimental laboratory of Ergostech by mid-September 2009. In 2010, they intend to conduct a continuous fermentation experiment, utilizing waste from vegetables and crops. They also plan to install a pre-commercial production plant to enable a proof production experiment in 2013 and later. They intend to spend \$2.5 million for the project in order to realize, within 10 years, a production cost of 40 Yen/m³, which would be equal to and competitive with the cost of crude oil and natural gas.

1.5.3

Environmental Impact

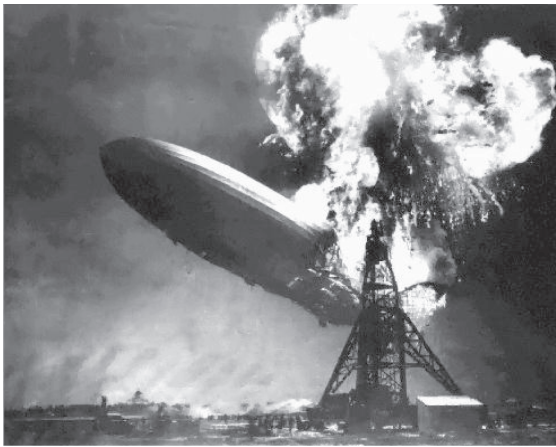
Safety issues: Hydrogen ignites easily upon contact with oxygen (air) and a flame/spark. Hydrogen has a wide flammability range (4–74% in air) and the energy required to ignite hydrogen (0.02 mJ) can be very low. At low concentrations (below 10%), however, the energy required to ignite hydrogen is higher—similar to the energy required to ignite natural gas and gasoline in their respective flammability ranges—making hydrogen realistically more difficult to ignite near the lower flammability limit. Nonetheless, if conditions exist where the hydrogen concentration increases toward the stoichiometric (most easily ignited) mixture of 29% hydrogen (in air), the ignition energy drops to about one-fifteenth of that required to ignite natural gas (or one-tenth for gasoline; see Table 1.16; Hydrogen Association, 2010).

Hydrogen is lighter than air and diffuses rapidly. This rapid diffusivity means that, when released, hydrogen dilutes quickly into a non-flammable concentration. Hydrogen rises two times faster than helium and six times faster than natural gas. Therefore, unless an enclosed room contains the rising gas, the laws of physics prevent hydrogen from lingering near a leak. Care has to be taken in enclosed spaces such as buildings, tunnels, underground parking lots and so on (Hydrogen Association, 2010; see Figure 1.15).

The odorless, colorless and tasteless of hydrogen gas make it difficult to detect any possible leak. Therefore, hydrogen sensors need to be used to detect possible leaks. By comparison, natural gas is also odorless, colorless and tasteless, but

Table 1.16 Relevant safety data for three major fuels (source: Hydrogen Association, 2010).

	Hydrogen	Gasoline vapor	Natural gas
Flammability limits (in air; %)	4–74	1.4–7.6	5.3–15.0
Explosion limits (in air; %)	18.3–59.0	1.1–3.3	5.7–14.0
Ignition energy (mJ)	0.02	0.20	0.29
Flame temperature in air (°C)	2045	2197	1875
Stoichiometric mixture (most easily ignited in air; %)	29	2	9

**Figure 1.15** The famous Hindenburg disaster in 1937. Hydrogen explodes—the main reason why today’s zeppelins are filled with helium. (Source: Shere, 1937).¹³⁾

industry adds a sulfur-containing odorant, called mercaptan, to make it detectable by people. Currently, all known odorants contaminate fuel cells and create complications for food applications. Researchers are investigating other methods that might be used for hydrogen detection, among them tracers and advanced sensors.

Asphyxiation: With the exception of oxygen, any gas can cause asphyxiation. In most scenarios, hydrogen’s buoyancy and diffusivity make it unlikely to be confined and potentially cause asphyxiation (Hydrogen Association, 2010).

Toxicity/poison: Hydrogen is non-toxic and non-poisonous. It will not contaminate groundwater (it is a gas under normal atmospheric conditions), nor will a release of hydrogen contribute to atmospheric pollution. Hydrogen does not create “fumes” and is not a greenhouse gas (Hydrogen Association, 2010).

13) See: <http://iconicphotos.wordpress.com/2009/07/25/hindenberg-disaster/>

1.5.3.1 Environmental Concerns

Hydrogen can be used as an additive in internal combustion engines (ICE); an ICE running on hydrogen may produce nitrous oxides and other pollutants, for example, nitric acid (HNO_3) and hydrogen cyanide gas (HCN). As a transportation fuel, however, hydrogen is mainly used in fuel cells, not internal combustion engines, thus avoiding the burning of hydrogen in the presence of nitrogen.

Concerns have also been raised over possible problems related to hydrogen gas leakage and subsequent effects on the atmosphere. Molecular hydrogen leaks slowly from most containment vessels and it has been hypothesized that if significant amounts of hydrogen gas (H_2) escape, hydrogen gas may, because of ultraviolet radiation, form free radicals (H^\bullet) in the stratosphere (Schultz *et al.*, 2003; Tromp *et al.*, 2003). These free radicals would then be able to act as catalysts for ozone depletion. A large enough increase in stratospheric hydrogen from leaked H_2 could exacerbate the depletion process. However, the estimations behind those proposed effects of these leakage problems may not be correct. Tromp *et al.* (2003) note that the amount of hydrogen that leaks today is much lower (by a factor of 10 to 100) than the estimated 10–20% figure conjectured by some researchers. A more realistic value is only about 0.1%, which is less than the natural gas leak rate of 0.7%. Tromp *et al.* (2003) conclude that the effect of hydrogen leakages on the atmosphere and the ozone layer (which we hope, based on current trends, will be largely repaired once the hydrogen economy is in full swing by 2050) will be negligible.

A positive effect of the hydrogen economy and its use in transport (cars) will be the near elimination of controllable urban air pollution by the end of the century (HTAC, 2010). Eliminating current vehicle exhaust will save thousands of lives and would hardly affect tropospheric water vapor concentrations (Jacobson, Colella, and Golden, 2005). Some researchers, however, note that the widespread use of fuel cell cars will reduce urban pollution but will create effects on the microclimate due to the increased water vapor that fuel cells emit (Pielke *et al.*, 2005). Pielke *et al.* conclude that “In the case of hydrogen cars, the cure may indeed be better than the disease, but we should make sure before taking our medicine.”

1.5.4

Foreseeable Social and Ethical Aspects

Hydrogen is perhaps one of the alternative biofuels that might have a deeper societal impact. There are many ways to produce hydrogen, and hydrogen is often considered an “energetic carrier” because it can be produced from water (with electricity) and then distributed for energetic purposes. This is one of the bases of the “economy of hydrogen” theory by Jeremy Rifkin. If, however, we consider only biomass-obtained hydrogen, then a real biofuel instead of an energetic carrier arises. The ease of transportation of this biomass-obtained hydrogen and the ease of *in situ* conversion are important advantages. A main concern is the explosive nature of the gas. Irrespective of its origin, the combustion of hydrogen

yields only heat and water. No global warming emissions or toxic pollutants are formed (Zeman and Keith, 2008). Thus, the non-carbonic nature of hydrogen makes this the most environmentally friendly of the biofuels. Accordingly, a real “hydrogen economy” would be a turnover of the current carbon-based fuel economy.

1.5.4.1 Could the Application Change Social Interactions? If Yes, in Which Way?

Important changes in terms of social interactions are expected if SB approaches help achieve a complete development of the hydrogen economy. New jobs and businesses related to the technology and especially to the distribution of hydrogen are expected to be created. It is important to note that security issues due to the explosion risk may also require novel control structures, jobs and even terrorist risk assessment.

Two main **ethical issues** mark the difference between hydrogen and carbon-based biofuels. The former is the absence of environmental impact in terms of greenhouse effect when hydrogen is burned [only applies to biomass hydrogen because non-biomass hydrogen production (i.e., electricity-based) does indirectly produce CO₂]. The latter is the improvement in the justice of distribution of hydrogen as an energetic source (see below).

Justice of distribution. If hydrogen was locally produced, it could be used as a fuel without many technical difficulties. Alternatively, if the production of hydrogen was centralized, it could be relatively easily transported through pipelines. One uncertainty is whether developing countries would be able to afford the construction of such structures and the price of the delivery.

Among the major challenges for the hydrogen economy, the US DOE (2010) lists public acceptance. This is because the hydrogen economy will be a revolutionary change from the world we know today. The necessary steps to foster hydrogen’s acceptance as a fuel are: education of the general public, training personnel in the handling and maintenance of hydrogen system components, adoption of codes and standards, and development of certified procedures and training manuals for fuel cells and safety.

1.6

Microbial Fuel Cells and Bio-photovoltaics

1.6.1

Introduction

A microbial fuel cell (MFC) is a device that uses the ability of microorganisms to produce electrical power by converting chemical to electrical energy.

Different MFC technologies have been tested in the laboratory at a fast pace, and power densities have reached over 1 kW/m³ (reactor volume) and 6.9 W/m² (anode area) under optimal conditions (Logan, 2009; see Figure 1.16). The real

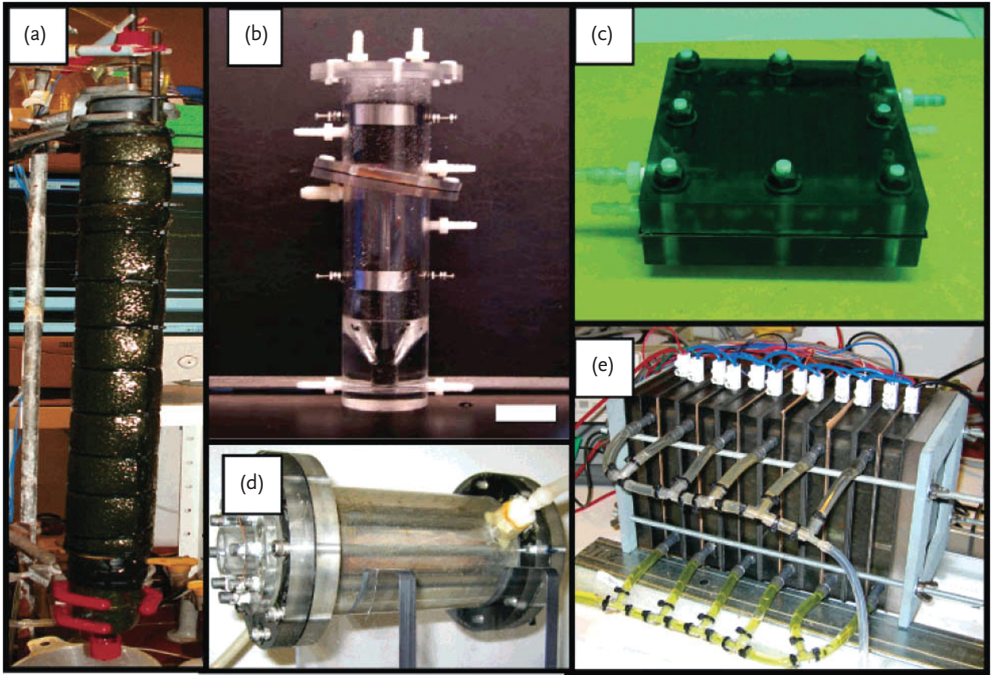


Figure 1.16 MFCs used for continuous operation: (a) upflow, tubular type MFC with inner graphite bed anode and outer cathode; (b) upflow, tubular type MFC with anode below and cathode above; (c) flat plate design; (d) single-chamber system with an

inner concentric air cathode surrounded by a chamber containing graphite rods as anode; (e) stacked MFC, in which six separate MFCs are joined in one reactor block (Source: Logan *et al.*, 2006).

challenge, however, is to convert these technologies developed in the laboratory into commercial applications to engineer systems for bioenergy production at larger scales. Recent advances in the global performance of MFCs are the discovery of new types of electrodes and a better knowledge about the role of membranes, separators and nanowires (Reguera *et al.*, 2005; see Figure 1.17). Commercialization of MFCs could start within only a few years (Table 1.17).

MFCs were originally designed to produce electricity (Lovley, 2006), but applications for other purposes also exist. For example, additional voltage added to the potential generated by the bacteria allows the production of methane, hydrogen and hydrogen peroxide. Additionally, membranes can be used in MFCs in such a way that water is desalinated, while electrical power production is kept (Cao *et al.*, 2009).

Since over 100 000 TW of solar energy falls on the Earth every year, solar energy is by far the most abundant primary energy source. The current use of energy on Earth is estimated at 10 TW per year and will double to 20 TW by 2050. The present

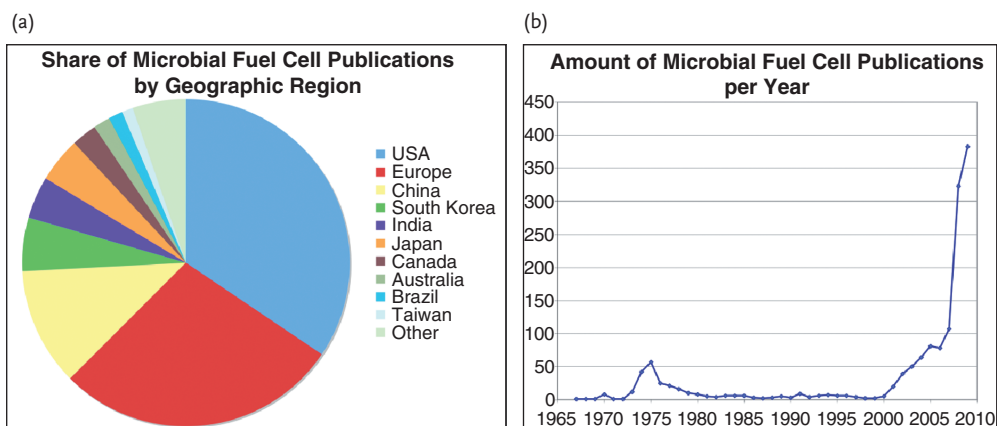


Figure 1.17 (a) In recent years there was a dramatic increase in in the scientific literature about MFCs; (b) the United States published most MFC articles, followed by Europe and China (GoPubMed, 2010).

techniques to convert solar energy directly to electrical energy, namely photovoltaic cells based on semi-conductors, are less effective and expensive. They contain rare elements, making their producibility also depending on uncertain market conditions. Nature possesses highly effective mechanisms for harvesting solar energy. These could be the basis for a new, effective, cheap and renewable photovoltaic system.

In one application, Peter *et al.* (2006) proposed to separate the processes of oxygen evolution and hydrogen production in a semi-biological photovoltaic device using intact photosynthetic cells. Here, protein complexes are intrinsically more stable and have mechanisms for self-repair. The device will be composed of two chambers, or half-cells, with oxygen evolution confined to one chamber and hydrogen production to the other. In addition, the approach can be used to produce a DC electrical current, in a manner analogous to standard silicon-based photovoltaic panels.

Another type of a bio-photovoltaic conversion device is being investigated by Yutaka Amao and Tasuku Komori (Department of Applied Chemistry, Oita University, Japan). It is based on dye-sensitized solar cells (DSSC) using the visible light sensitization of chlorine- e_6 (Chl- e_6). The latter is derived from chlorophyll from *Spirulina* adsorbed on a nanocrystalline TiO_2 film.

Developing an alternative technique, the Department of Chemical Engineering and Biotechnology of the University of Cambridge is working on a bio-photovoltaic device which exploits the photosynthetic apparatus of biological material such as cyanobacteria or algae. The idea is, to convert the solar energy into electrical energy and then use this electrical energy to drive a current or create a potential difference to drive a chemical reaction. These few examples show that extensive research is going on in this key field and that there are numerous interesting approaches.

Table 1.17 Potential microbial fuel cells and applications (Davis and Higson, 2007).

Microbial fuel cell	Fuel	Application
<i>In vivo</i> power supply	Blood glucose and oxygen	Pacemaker, glucose sensor, prosthetic valve actuator power supply
Transcutaneous power socket	Blood glucose and oxygen	Variety of low-power electronic devices
Waste remediation	Process residues	Remediation of process wastes with power recovery
Waste remediation	Urine	Use of wastes for power generation in remote areas
Portable power cell	Alcohol	Portable power supply for mobile telephones or other consumer electronics. Instant recharge times, lifetime on the order of one month between replacements. No precious metals required, therefore readily recyclable or disposable
Biosensors	Target molecule	Can act as a specific biosensor (if enzyme-based) or a non-specific one if microbe based. The latter has a potentially indefinite lifetime
Static power generation	Cellulosic materials	Potentially lignocellulosic materials (e.g., corn stalks, wood) could be broken down and used to directly generate power sustainably
Static power generation	Sewage	Sewage-digesting bacteria have been demonstrated to be capable of generating electricity, and the biological oxygen demand of the fuel itself could help maintain a system in an anaerobic state, but the power levels of for a practical system have not yet been demonstrated
Static power generation	Marine sediment	The biofuel cell demonstrated by Tender <i>et al.</i> (2002) is almost immediately applicable to provide long-term power to remote marine electronics
Mobile power generation	Organic materials	Start and forget gastrobot ^{a)} (energy autonomous robots) operations, if suitable food-locating behaviors can be programmed

a) Gastrobot literally means “robot with a stomach”: they are machines that power themselves by digesting food.

MFCs can be classified according to their electron transfer scheme and main bacterial species (Bullen *et al.*, 2007; Lovley, 2008):

- Indirect electron transfer through the interaction of reduced metabolic products with the anode;
- Enhanced electron transfer with artificial mediators: *Escherichia coli*, *Pseudomonas*, *Proteus*, and *Bacillus* species;

- Microorganisms that produce their own mediators: *Shewanella oneidensis*, *Geothrix fermentans* and *Pseudomonas* species (*Pseudomonas aeruginosa*);
- Direct electron transfer to electrodes: *Shewanella putrefaciens*, *Aeromonas hydrophila*;
- Oxidation of organic matter with electricigens (benthic unattended generator): *Geobacter sulfurreducens*, *G. metallireducens*, *G. psychrophilus*, *Desulfuromonas acetoxidans*, *Geopsychrobacter electrodiphilus*, *Rhodoferax ferrireducens*.

1.6.2

Economic Potential

The economic benefits of electricity from MFC can be considered in two sectors: the automotive sector and special applications (fine mechanics, medical technology, telecommunications, IT, etc.). An application in the automotive sector is conceivable. This would create an enormous turnover because this is a global key industry. Special applications in other fields also bear the potential for billion-dollar-scale turnovers because of the diversity of such applications (e.g., a notebook battery working with alcohol which is converted to water and discarded; see, e.g., Debabov, 2008).

1.6.3

Environmental Impact

Due to global environmental concerns and energy insecurity, there is a need to develop cost-effective wastewater treatment processes and sustainable clean energy sources, preferably without the use of fossil fuel. A MFC has a great potential to solve this problem by generating direct electricity during the oxidation of organic matter. MFCs have recently received increased attention as a means to produce “green” energy from organic wastewater or synthetically prepared carbohydrate substrates (Ghangrekar and Shinde, 2008). Over the medium term, MFCs could become an interesting tool to generate electricity from waste (waters; see Figure 1.18). So far, experiments have been carried out with the following substrates (fuels; Ren, Steinberg, and Regan, 2008; Wang, Feng, and Lee, 2008; Pant *et al.*, 2010):

- Acetate;
- Glucose;
- Lignocellulosic biomass;
- Synthetic or chemical wastewater;
- Brewery wastewater;
- Animal wastewater;
- Starch processing wastewater;
- Dye wastewater;
- Landfill leachates;

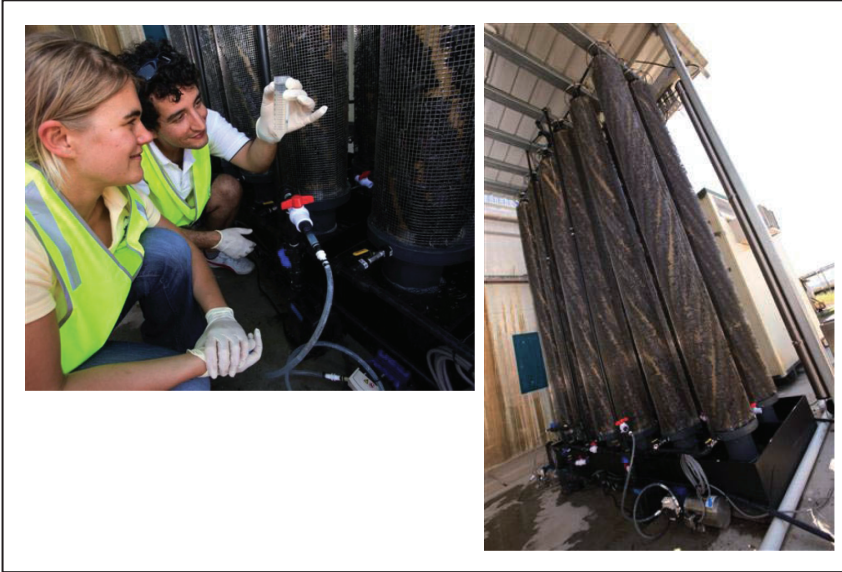


Figure 1.18 MFC pilot plant for brewery wastewater (see: www.microbialfuelcell.org).

- Cellulose and chitin;
- Inorganic and other substrates.

Tested substrates have grown in complexity and content strength (higher organic loading rate). The output of these systems (electric current and electric power) is still some way from large-scale applications. It is believed that more innovations are needed to achieve more technological advancements in terms of materials, costs and substrates will make these systems to be commercially competitive. A promising aspect, however, is that a variety of new substrates (see list above) can serve as substrates under the MFC set up. These include wastewater from molasses-based distilleries rich in organic matter and produced in large volumes, wastewater from a large number of biorefineries, wastewater from the pharmaceutical industry with recalcitrant pollutants, or waste plant biomass (agriculture residue) which is currently being burned. Economically, the integration of MFCs with existing separation, conversion and treatment technologies is probably the best option (Pant *et al.*, 2010). One clear benefit of MFCs is that they can be used as bioremediation tools and electricity generation tools at the same time, working as a direct waste to energy conversion system. Although the obtained power output is still relatively low, the technology is improving rapidly and eventually could be useful to reduce the cost of small sewage and industrial wastewater treatment plants (Ghangrekar and Shinde, 2008). Although waste can fuel MFCs, natural environments can do the same job. For example, MFCs deployed in natural waters can produce enough energy to operate (bio)sensors requiring low power. Note, however, that all these microbial fuel cells produce a maximum cell potential

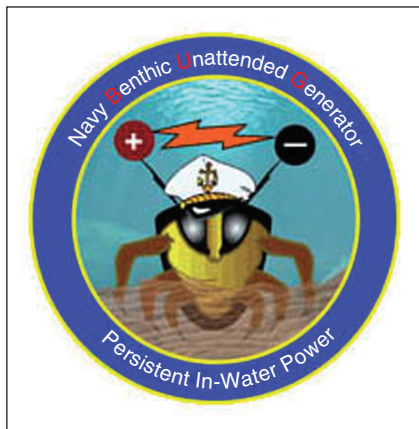


Figure 1.19 Seal of the United States Navy's benthic unattended generator (BUG).

around 800 mV, which currently limits their use to power electronic devices (Donovan *et al.*, 2008). The **benthic unattended generator** or BUG, developed by the US Naval Research Laboratory's Center for Bio/Molecular Science and Engineering, is a weather buoy that operates in the Potomac River; see Figure 1.19). The buoy is unique in that it is solely powered by a set of microbial fuel cells. These BUGs consist of electrodes imbedded in sediment in the bottom of the river that are electrically connected to electrodes in the overlying water. The buoy monitors air temperature and pressure, relative humidity, water temperature, and performance indicators of the BUGs, and sends data by a radio transmitter (also BUG-powered) to a receiver. Organic matter deposited in many fresh- and saltwater environments constitutes a practically inexhaustible fuel. Furthermore, substantial oxygen present in overlying water constitutes a practically inexhaustible oxidant. BUGs electrochemically react with this fuel and oxygen to generate electrical power that persists indefinitely (as long as the fuel cell lasts). BUGs are being developed to persistently power a wide range of remotely deployed marine instruments. BUG research is partly funded by the Office of Naval Research (ONR) and the Defense Advanced Research Projects Agency (DARPA, 2010). A similar system can also be used in rice paddies. Researchers have developed a microbial fuel cell that allows them to generate up to 330 watts of power per hectare of farmed rice paddies. Taking advantage of a process called rhizodeposition, the MFC can capture some of the energy produced in rice paddies, possibly before it is released in the form of methane. As rice paddies cover well over one million square kilometers of land and are responsible for approximately 10–20% of the world's methane emissions, a method that simultaneously utilizes rice plants to make electricity and reduce methane emissions would have a very positive environmental impact (De Schampelaire *et al.*, 2008).

The entire system—the microbial fuel cell, sensor, and telemetry systems—may last up to 7.5 years. If needed, this can be doubled or tripled by merely changing the specifications, for example, increasing the numbers of anodes (Shantaram

et al., 2005). The advantage to using an MFC as opposed to a normal battery is that it uses a renewable form of energy and would not need to be recharged like a standard battery would. Moreover, they could operate well in mild conditions, 20–40 °C, at a pH of around 7.

1.6.4

Foreseeable Social and Ethical Aspects

One of the most important features of this technology is the possibility of coupling several energetic procedures in the same place. For example, waste treatment, electric power generation, hydrogen production and water desalinization could be obtained at the same time and with the same equipment. This feature makes biophotovoltaic applications unique, and their massive use would certainly have a deep societal impact by grouping two of the most important factors that hamper human development: energy and waste treatment. As with the other biofuels, new businesses and jobs would arise. Another expected high impact on social interactions would come from the rearrangement of treatment, biofuel and electric power fields into a single technology. Of course, this would only be the case if a significant contribution to these processes was linked to microbial cell fuel-based strategies. Even a relatively reduced spread of the technique, however, would have an impact because of the grouping effect.

Ethical issues. MFC does not have particular ethical issues, except those related to the societal effects of grouping waste treatment and energy policies and those of distribution. Another type of ethical issues could appear when higher order animals would be used to generate electricity, e.g., a dog with MFC that uses the blood glucose and oxygen to power a GPS receiver on its necklace, or cetaceans (dolphins) using a similar system to power electronic sensors searching for enemy submarines.

Justice of distribution. The state of the art of bio-photovoltaic devices consists of laboratory-scale modules that work but produce low voltage/intensities. Assuming a successful development of more productive systems, local production with very simple equipment (see Figure 1.20) would be possible. The outcome of these devices, electrical power, would be ready to use. This aspect, along with the fact that the byproducts of cell fuels can be used as biofuels and the possibility of coupling MFC to water treatment, makes biophotovoltaics one of the most promising technologies in terms of justice of distribution.

1.7

Recommendations for Biofuels

We are convinced that synthetic biology can help to produce state of the art and next-generation biofuels. Current efforts are mainly targeted towards an improved production of **bio-ethanol** from agricultural products, although we see significant problems with this approach because ethanol exhibits certain technical problems

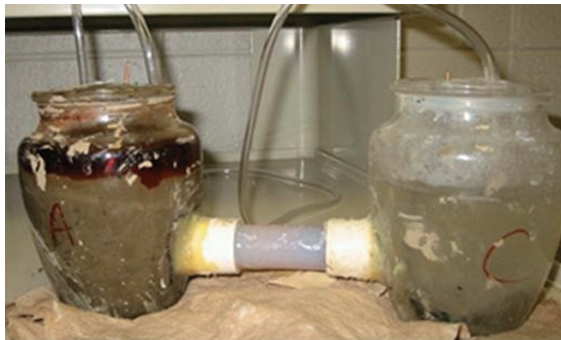


Figure 1.20 Few MFCs are commercially available, but the internet is full of examples of home-made prototypes (source: Makezine, 2006).¹⁴⁾

(mixes with water, limited use in existing engines). Other non-ethanol biofuels such as **bio-butanol** or **biodiesel** are much better suited to replace petroleum-based gasoline because their chemical properties resemble it much closer. Synthetic biology could help to overcome current impasses in the production of butanol and other non-ethanol fuels, namely poor fermentation yield and toxicity to butanol-producing microorganisms. One problem facing most biofuels produced from plant material are limitations on the use of hemi- and lignocellulosic material. Any improvement in that area would definitely increase the economic feasibility of biofuel production. Another important issue will arise should synthetic biology be able to help solve the above-mentioned technical problems: more and more agricultural land will be devoted to plant energy crops instead of food crops. In order to avoid this competition for food, we suggest also using non-food-competing biological resources such as perennial plants grown on degraded lands abandoned for agricultural use, crop residues, sustainably harvested wood and forest residues, double crops and mixed cropping systems, as well as municipal and industrial wastes.

In addition to agriculturally based ethanol, biodiesel and butanol, another option is **algae-based biofuels** and **biohydrogen**. Current concepts foresee a significant advantage of algae-based biofuels over agriculture-based biofuels because of a higher yield per area and the independence from arable land and clean water. Initial calculations predict, however, that future algae production systems will be economically feasible only if the price for one barrel of oil remains consistently above \$70 and if the production systems entail an area of at least 200ha. The capital costs of such large production facilities will probably exclude SMEs and favor “big oil” (or big energy companies). Nonetheless, algae production systems could be a highly promising avenue of future fuel production once major obstacles are resolved dealing with algae genomics, metabolism and harvesting. Although **biohydrogen** has been praised as an extremely promising fuel by many scientists, our assessment is more cautious. Hydrogen is only useful as fuel if large changes in

14) See: http://blog.makezine.com/archive/2006/05/how_to_make_microbial_fue.html

infrastructure take place (distribution and storage system, new fuel cell engines). This points to a more distant future beyond 2050, also termed the hydrogen economy. Although synthetic biology could contribute to improve the yield of hydrogen-producing cyanobacteria, the actual impact of hydrogen in society and economy depends much more on other areas such as infrastructure. Finally, we analyzed the prospects of **microbial fuel cells** (MFCs) as energy converters. Although we see MFCs as extremely promising and a sector in which synthetic biology could make a significant contribution, such fuel cells will most likely be applied in certain niche markets and areas of application, rather than large-scale deployment, due to the limited energy production.

References

1. BIOFUELS

1.1. BIOFUELS IN GENERAL

- Biofuels Platform (2010) The situation in the EU: background and objectives, <http://www.biofuels-platform.ch/en/infos/eu-background.php> (accessed 28 May 2010).
- Bringezu, S., Schütz, H., O'Brien, M., Kauppi, L., Howarth, R.W., and McNeely, J. (2009) Towards sustainable production and use of resources: assessing biofuels. United Nations Environment Programme.
- EC (2003) Biofuels directive 2003/30/EC, http://ec.europa.eu/energy/res/legislation/doc/biofuels/en_final.pdf (accessed 28 May 2010).
- EC (2009) Directive 2009/28/EC on the promotion of the use of energy from renewable sources, <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:140:0016:0062:EN:PDF> (accessed 28 May 2010).
- EC (2010) Market observatory. EU: crude oil imports, http://ec.europa.eu/energy/observatory/oil/import_export_en.htm (accessed 28 May 2010).
- Green, C. (2010) Biofuels directive review and progress report: public consultation, <http://ec.europa.eu/energy/res/legislation/doc/biofuels/contributions/citizens/green.pdf> (accessed 28 May 2010).
- IEA (2009) Key World Energy Statistics, http://www.iea.org/textbase/nppdf/free/2009/key_stats_2009.pdf (accessed 28 May 2010).
- IEA (2010) Oil market report released, <http://omrpublic.iea.org/omrarchive/15jan10full.pdf> (accessed 28 May 2010).
- SCOPE (2009) Biofuels: environmental consequences and interactions with changing land use. SCOPE, Gummingsbach. <http://cip.cornell.edu/biofuels/> (accessed 28 May 2010).
- Tilman, D., Socolow, R., Foley, J.A., Hill, J., Larson, E., Lynd, L., Pacala, S., Reilly, J., Searchinger, T., Somerville, C., and Williams, R. (2009) Beneficial biofuels: the food, energy, and environment trilemma. *Science*, **325**, 270–271.
- Thomassen, D.G., Simmons, K., Fernandez-Guiterrez, M., and Lex, M. (2008) EC-US taskforce on biotechnology research: workshop on biotechnology for sustainable bioenergy, http://ec.europa.eu/research/biotechnology/ec-us/docs/us-ec_bioenergy_workshop_proceedings_en.pdf (accessed 28 May 2010).
- UNEP (2009) Assessing biofuels, http://www.unep.fr/scp/rpanel/pdf/Assessing_Biofuels_Full_Report.pdf (accessed 28 May 2010).

1.2. ETHANOL

- BIO-ERA (2009) U.S. economic impact of advanced biofuels production: perspectives to 2030, <http://bio.org/ind/EconomicImpactAdvancedBiofuels.pdf> (accessed 28 May 2010).

- Domínguez-Escribà, L., and Porcar, M. (2010) Rice straw management: the big waste. *Biofuels Bioprod. Bioref.*, **4**, 154–159.
- EEA (2006) How much bioenergy can Europe produce without harming the environment? EEA Report No 7. Office for official publications of the European communities, Luxembourg.
- EPA (2010) EPA finalizes regulations for the national renewable fuel standard program for 2010 and beyond, <http://www.epa.gov/OMS/renewablefuels/420f10007.pdf> (accessed 28 May 2010).
- ETC (2008) Commodifying nature's last straw?: extreme genet eng and the post-petroleum sugar econ, <http://www.etcgroup.org/en/node/703> (accessed 28 May 2010).
- Hatano, K., Kikuchi, S., Nakamura, Y., Sakamoto, H., Takigami, M., and Kojima, Y. (2009) Novel strategy using an adsorbent-column chromatography for effective ethanol production from sugarcane or sugar beet molasses. *Bioresour. Technol.*, **100** (20), 4697–4703.
- Melillo, J.M., Reilly, J.M., Kicklighter, D.W., Gurgel, A.C., Cronin, T.W., Paltsev, S., Felzer, B.S., Wang, X., Sokolov, A.P., and Schlosser, C.A. (2009) Indirect emissions from biofuels: how important? *Science*, **326** (5958), 1397–1399.
- Rodrigues, L.P., and de Moraes, M.A.F.D. (2007) Estrutura de mercado da indústria de refino de açúcar na região centro-sul do Brasil. *Rev. Econ. Sociol. Rural*, **45**, 93–118.
- Sawyer, D. (2008) Climate change, biofuels and eco-social impacts in the Brazilian amazon and Cerrado. *Philos. Trans. R. Soc. B Biol. Sci.*, **363**, 1747–1752.
- SCOPE (2009) Biofuels: environm consequences and interactions with changing land use. SCOPE, Gummingsbach. <http://cip.cornell.edu/biofuels/> (accessed 28 May 2010).
- Tilman, D., Socolow, R., Foley, J.A., Hill, J., Larson, E., Lynd, L., Pacala, S., Reilly, J., Searchinger, T., Somerville, C., and Williams, R. (2009) Beneficial biofuels: the food, energy, and environ trilemma. *Science*, **325**, 270–271.
- UNEP (2009) Assessing biofuels, http://www.unep.fr/scp/rpanel/pdf/Assessing_Biofuels_Full_Report.pdf (accessed 28 May 2010).
- Zah, R. (2007) Umweltauswirkungen von Biotreibstoffen. Presentation at the Energy Science Colloquium Zurich. <http://www.esc.ethz.ch/news/colloquia/2007/PresentationZah.pdf> (accessed 28 July 2010).
- Zeman, F.S., and Keith, D.W. (2008) Carbon neutral hydrocarbons. *Philos. Transact. A Math. Phys. Eng. Sci.*, **366** (1882), 3901–3918.

Interesting link:

- A list of cellulytic bacteria: <http://www.wzw.tum.de/mbiotech/cellmo.htm> (accessed 28 May 2010)
- Cellulosic ethanol. http://en.wikipedia.org/wiki/Cellulosic_ethanol (accessed 28 May 2010)
- Food before fuel. <http://www.foodbeforefuel.org/facts> (accessed 28 May 2010)
- Range fuels: biomass to energy. www.rangefuels.com (accessed 28 May 2010)
- Renewable Fuel Standard Program <http://www.epa.gov/otaq/fuels/renewablefuels/index.htm> (accessed 28 May 2010)

1.3. NON-ETHANOL FUELS

- AMEC (2007) AMEC to Design First Commercial Scale Biodiesel Production Plant in Canada for Biox Corporation in Hamilton, Ontario, <http://www.amec.com/page.aspx?pointerid=c267400018044310a1c5ce27a03f0847> (accessed 28 August 2010).
- Atsumi, S., and Liao, J.C. (2008a) Directed evolution of *Methanococcus jannaschii* citramalate synthase for biosynthesis of 1-propanol and 1-butanol by *Escherichia coli*. *Appl. Environ. Microbiol.*, **74** (24), 7802–7808.
- Atsumi, S., and Liao, J.C. (2008b) Metabolic engineering for advanced biofuels production from *Escherichia coli*. *Curr. Opin. Biotechnol.*, **19** (5), 414–419.
- Atsumi, S., Higashide, W., and Liao, J.C. (2009) Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. *Nat. Biotechnol.*, **27** (12), 1177–1180.
- Bermejo, L.L., Welker, N.E., and Papoutsakis, E.T. (1998) Expression of

- clostridium acetobutylicum ATCC 824 genes in *Escherichia coli* for acetone production and acetate detoxification. *Appl. Environ. Microbiol.*, **64** (3), 1079–1085.
- DARPA (2010) Justification book volume 1: research, development, test & evaluation, defense-wide–0400, <http://www.darpa.mil/Docs/FY2011PresBudget28Jan10%20Final.pdf> (accessed 28 May 2010).
- Eurostat (2005) Consumption of vehicle fuels in the EU-27, epp.eurostat.ec.europa.eu (accessed 28 May 2010).
- Global Bioenergies (2010) www.global-bioenergies.com (accessed 28 May 2010).
- Hanaï, T., Atsumi, S., and Jiao, J.C. (2007) Engineered synthetic pathway for isopropanol production in *Escherichia coli*. *Appl. Environ. Microbiol.*, **73** (24), 7814–7818.
- Hill, J., Nelson, E., Tilman, D., Polasky, S., and Tiffany, D. (2006) Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *Proc. Natl. Acad. Sci. U.S.A.*, **103** (30), 11206–11210.
- Kalscheuer, R., Stolting, T., and Steinbüchel, A. (2006) Microdiesel: *Escherichia coli* engineered for fuel production. *Microbiology*, **152** (9), 2529–2536.
- Keasling, J.S., and Chou, H. (2009) Metabolic engineering delivers next-generation biofuels. *Nat. Biotechnol.*, **26**, 298–299.
- Khalil, A.S., and Collins, J.J. (2010) Synthetic biology: applications come of age. *Nat. Rev. Genet.*, **11** (5), 367–379.
- Kleinov, A., Paligov, J., Vrbov, M., Mikulec, J., and Cvengro, J. (2007) Cold flow properties of fatty esters. *Process Saf. Environ. Prot.*, **85** (B5), 390–395.
- Lee, S.Y., Park, J.H., Jang, S.H., Nielsen, L.K., Kim, J., and Jung, K.S. (2008) Fermentative butanol production by clostridia. *Biotechnol. Bioeng.*, **101** (2), 209–228.
- Lestari, S., Mäki-Arvela, P., Beltramini, J., Lu, G.Q., and Murzin, D.Y. (2009) Transforming triglycerides and fatty acids into biofuels. *ChemSusChem*, **2** (12), 1109–1119.
- OECD/IEA (2008) Energy technology perspectives. Scenarios and strategies to 2050. Paris. <http://www.iea.org/techno/etp> (accessed 28 August 2010).
- Steen, E.J., Kang, Y., Bokinsky, G., Hu, Z., Schirmer, A., McClure, A., Del Cardayre, S.B., and Keasling, J.D. (2010) Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. *Nature*, **463** (7280), 559–562.
- Solomon, B.D. (2010) Biofuels and sustainability. *Ann. N. Y. Acad. Sci.*, **1185**, 119–134.
- UNEP (2009) Assessing biofuels, http://www.unep.fr/scp/rpanel/pdf/Assessing_Biofuels_Full_Report.pdf (accessed 28 May 2010).

1.4. ALGAE-BASED FUELS

- Beer, L.L., Boyd, E.S., Peters, J.W., and Posewitz, M.C. (2009) Engineering algae for biohydrogen and biofuel production. *Curr. Opin. Biotechnol.*, **20** (3), 264–271.
- Carbon Trust (2008) Green oil by 2020, <http://www.carbontrust.co.uk/news/news/archive/2008/Pages/algae-biofuels-challenge.aspx> (accessed 28 May 2010).
- Carbon Trust (2010) Algae Biofuels Challenge, <http://www.carbontrust.co.uk/emerging-technologies/current-focus-areas/algae-biofuels-challenge/pages/algae-biofuels-challenge.aspx> (accessed 28 May 2010).
- Chisti, Y. (2008) Biodiesel from microalgae beats bioethanol. *Trends Biotechnol.*, **26** (3), 126–131.
- Dasolar Energy (2010) <http://www.dasolar.com/alternative-energy/biofuels> (accessed 28 May 2010).
- Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., and Darzins, A. (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J.*, **54**, 621–639.
- Pulz, O. (2001) Photobioreactors: production systems for phototrophic microorganisms. *Appl. Microbiol. Biotechnol.*, **57**, 287–293.
- Richmond, A. (2004) *Handbook of Microalgal Culture*, Blackwell Science, Oxford.
- Smith, V.H., Sturm, B.S., deNoyelles, F.J., and Billings, S.A. (2009) The ecology of algal biodiesel production. *Trends Ecol. Evol.*, **25** (5), 301–309.

- Solazyme (2010) Technology, <http://www.solazyme.com/content/technology> (accessed 2 September 2010).
- Spolaore, P., Joannis-Cassan, C., Duran, E., and Isambert, A. (2006) Commercial applications of microalgae. *J. Biosci. Bioeng.*, **102**, 87–96.
- Stephens, E., Ross, I.L., King, Z., Mussgnug, J.H., Kruse, O., Posten, C., Borowitzka, M.A., and Hankamer, B. (2010) An economic and technical evaluation of microalgal biofuels. *Nat. Biotechnol.*, **28** (2), 126–128.
- Tredici, M.R. (1999) Photobioreactors, in *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation* (ed. M.C. Flickinger, S.W. Drew), John Wiley & Sons, Inc., New York, pp. 395–419.
- Ugwu, C.U., Aoyagi, H., and Uchiyama, H. (2008) Photobioreactors for mass cultivation of algae. *Bioresour. Technol.*, **99**, 4021–4028.
- UNEP (2009) Assessing biofuels, http://www.unep.fr/scp/rpanel/pdf/Assessing_Biofuels_Full_Report.pdf (accessed 28 May 2010).
- Waltz, E. (2009) Biotech's green gold? *Nat. Biotechnol.*, **27** (1), 15–18.

Interesting links:

- Algal biomass organization annual report. <http://www.algalbiomass.org/> (accessed 28 May 2010)
- European algae biomass association. <http://eaba-association.eu/legislation.php> (accessed 28 May 2010)
- OriginOil. <http://www.originoil.com/> (accessed 28 May 2010)

1.5. HYDROGEN PRODUCTION

- Amos, W.A. (2004) Updated cost analysis of photobiological hydrogen production from *Chlamydomonas reinhardtii* green algae. Milestone completion report. <http://www.nrel.gov/docs/fy04osti/35593.pdf> (accessed 28 May 2010).
- Garman, D., Eiler, J.E., Tromp, T.K., Shia, R.L., Allen, M., Yung, Y.L., Keith, D.K., and Farrell, A.E. (2003) The Bush administration and hydrogen. *Science*, **302** (5649), 1331.
- Ghirardi, M.L., Dubini, A., Yu, J., and Maness, P.C. (2009) Photobiological hydrogen-producing systems. *Chem. Soc. Rev.*, **38**, 52–61.
- Hemschemeier, A., and Happe, T. (2005) The exceptional photofermentative hydrogen metabolism of the green alga *Chlamydomonas reinhardtii*. *Biochem. Soc. Trans.*, **33**, 39–41. (accessed 28 May 2010).
- HTAC (2010) 2009 HTAC annual report: the state of hydrogen and fuel cell commercialization and tech development, http://www.hydrogen.energy.gov/pdfs/2009_htac_annual_report.pdf (accessed 28 May 2010).
- Hydrogen Association (2010) Hydrogen safety, http://www.hydrogenassociation.org/general/factSheet_safety.pdf (accessed 28 May 2010).
- Jacobson, M.Z., Colella, W.G., and Golden, D.M. (2005) Cleaning the air and improving health with hydrogen fuel-cell vehicles. *Science*, **308**, 1901–1905.
- Kammen, D.E., Lipman, T.E., Lovins, A.B., Lehman, P.A., Eiler, J.M., Tromp, J.K., Shia, R.L., Allen, M., and Yung, Y.L. (2003) Assessing the future hydrogen economy. *Science*, **302** (5643), 226b.
- Melis, A. (2007) Photosynthetic H₂ metabolism in *Chlamydomonas reinhardtii* (unicellular green algae). *Planta*, **226**, 1075–1086.
- Melis, A., and Happe, T. (2002) Hydrogen production: green algae as a source of energy. *Plant Physiol.*, **127**, 740–748.
- NAE (2004) The hydrogen economy: opportunities, costs, barriers, and R&D needs, http://www.nap.edu/openbook.php?record_id=10922&page=R1 (accessed 28 May 2010).
- Pielke, R.A., Jr., Klein, R., Maricle, G., and Chase, T. (2005) Hydrogen cars and water vapor. *Science*, **302** (5631), 1329.
- Rifkin, J. (2002) *The Hydrogen Economy*, Tarcher/Putnam, New York.
- Schultz, M.G., Diehl, T., Brasseur, G.P., and Zittel, W. (2003) Air pollution and climate-forcing impacts of a global hydrogen economy. *Science*, **302**, 624–627.
- Schütz, K., Happe, T., Troshina, O., Lindblad, P., Leitão, E., Oliveira, P., and Tamagnini, P. (2004) Cyanobacterial H₂ production: a comparative analysis. *Planta*, **218**, 350–359.

- Shere (1937) Hindenberg disaster, <http://iconicphotos.wordpress.com/2009/07/25/hindenberg-disaster/> (accessed 28 May 2010).
- Stripp, S.T., Goldet, G., Brandmayr, C., Sanganas, O., Vincent, K.A., Haumann, M., Armstrong, F.A., and Happe, T. (2009) How oxygen attacks [FeFe] hydrogenases from photosynthetic organisms. *Proc. Natl. Acad. Sci. U.S.A.*, **106**, 17331–17336.
- Surzycycki, R., Cournac, L., Peltier, G., and Rochaix, J.D. (2007) Potential for hydrogen production with inducible chloroplast gene expression in *Chlamydomonas*. *Proc. Natl. Acad. Sci. U.S.A.*, **104** (44), 17548–17553.
- Tamagnini, P., Axelsson, R., Lindberg, P., Oxelfelt, F., Wünschiers, R., and Lindblad, P. (2002) Hydrogenases and hydrogen metabolism of cyanobacteria. *Microbiol. Mol. Biol. Rev.*, **66**, 1–20.
- Tetali, S.D., Mitra, M., and Melis, A. (2007) Development of the light-harvesting chlorophyll antenna in the green alga *Chlamydomonas reinhardtii* is regulated by the novel *tla1* gene. *Planta*, **225** (4), 813–829.
- Tromp, T.K., Shia, R.L., Allen, M., Eiler, J.M., and Yung, Y.L. (2003) Potential environmental impact of a hydrogen economy on the stratosphere. *Science*, **300** (5626), 1740–1742.
- US DOE (2010) Fuel cell technology validation, http://www1.eere.energy.gov/hydrogenandfuelcells/tech_validation (accessed 28 May 2010).
- Zeman, F.S., and Keith, D.W. (2008) Carbon neutral hydrocarbons. *Philos. Transact. A Math. Phys. Eng. Sci.*, **366** (1882), 3901–3918.
- Links to Hydrogen Safety pages:**
- Hydrogen and fuel cell safety: www.HydrogenSafety.info (accessed 28 May 2010)
- Hydrogen Safety Bibliographic Database: www.hydrogen.energy.gov (accessed 28 May 2010)
- Hydrogen Safety for First Responders: <http://www.hydrogen.energy.gov/firstresponders.html> (accessed 28 May 2010)
- Reporting Lessons Learned: www.h2incidents.org (accessed 28 May 2010)
- Sharing Best Practices: www.h2bestpractices.org (accessed 28 May 2010)
- Other interesting links:**
- Anastasios Melis <http://epmb.berkeley.edu/facPage/dispFP.php?I=25> (accessed 28 May 2010)
- Biological hydrogen production http://en.wikipedia.org/wiki/Biological_hydrogen_production (accessed 28 May 2010)
- Engineered Modular Bacterial Photoproduction of Hydrogen <http://biomodularh2.epigenomique.genopole.fr/index.php/Public/Impact> (accessed 28 May 2010)
- Growing hydrogen for the cars of tomorrow http://www.science.org.au/nova/newscientist/111ns_002.htm (accessed 28 May 2010)
- 1.6. MICROBIAL FUEL CELL (MFC) AND BIO-PHOTOVOLTAIC**
- Bullen, R.A., Arnot, T.C., Lakeman, J.B., and Walsh, F.C. (2007) Biofuel cells and their development. *Biosens. Bioelectron.*, **21** (11), 2015–2045.
- Cao, X., Huang, X., Liang, P., Xiao, K., Zhou, Y., Zhang, X., and Logan, B.E. (2009) A new method for water desalination using microbial desalination cells. *Environ. Sci. Technol.*, **43** (18), 7148–7152.
- DARPA (2010) Benthic unattended generator, <http://www.nrl.navy.mil/code6900/bug/> (accessed 28 May 2010).
- Davis, F., and Higson, S.P.J. (2007) Biofuel cells—Recent advances and applications. *Biosens. Bioelectron.*, **22** (7), 1224–1235.
- De Schampelaire, L., Van den Bossche, L., Son Dang, H., Höfte, M., Boon, N., Rabaey, K., and Verstraete, W. (2008) Microbial fuel cells generating electricity from rhizodeposits of rice plants. *Environ. Sci. Technol.*, **42** (8), 3053–3058.
- Debabov, V.G. (2008) Electricity from microorganisms. *Mikrobiologiya*, **77** (2), 149–157. (in Russian).

- Donovan, C., Dewan, A., Heo, D., and Beyenal, F. (2008) Batteryless, wireless sensor powered by a sediment microbial fuel cell. *Environ. Sci. Technol.*, **42** (22), 8591–8596.
- Ghangrekar, M.M., and Shinde, V.B. (2008) Simultaneous sewage treatment and electricity generation in membrane-less microbial fuel cell. *Water Sci. Technol.*, **58**, 37–43.
- GoPubMed (2010) Pubmed metasearch website www.gopubmed.org (accessed 28 August 2010).
- Logan, B. (2009) Scaling up microbial fuel cells and other bioelectrochemical systems. *Appl. Microbiol. Biotechnol.*, **85** (6), 1665–1671.
- Logan, B., Aelterman, P., Hamelers, B., Rozendal, R., Schroder, U., Keller, J., Freguia, S., Verstraete, W., and Rabaey, K. (2006) Microbial fuel cells: methodology and technology. *Environ. Sci. Technol.*, **40** (17), 5181–5192.
- Lovley, D.R. (2006) Bug juice: harvesting electricity with microorganisms. *Nat. Rev. Microbiol.*, **4**, 467–508.
- Lovley, D.R. (2008) The microbe electric: conversion of organic matter to electricity. *Curr. Opin. Biotechnol.*, **19**, 1–8.
- Makezine (2006) How to: make microbial fuel cells, http://blog.makezine.com/archive/2006/05/how_to_make_microbial_fue.html (accessed 28 May 2010).
- Pant, P., Van Bogaert, G., Diels, L., and Vanbroekhoven, K. (2010) A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. *Bioresour. Technol.*, **101** (6), 1533–1543.
- Peter, L.M., Walker, A.B., Boschloo, G., and Hagfeldt, A. (2006) Interpretation of apparent activation energies for electron transport in dye-sensitized nanocrystalline solar cells. *J. Phys. Chem. B*, **110** (28), 13694–13699.
- Reguera, G., McCarthy, K.D., Mehta, T., Nicoll, J.S., Tuominen, M.T., and Lovley, D.R. (2005) Extracellular electron transfer via microbial nanowires. *Nature*, **435**, 1098–1101.
- Ren, Z., Steinberg, L.M., and Regan, J.M. (2008) Electricity production and microbial biofilm characterization in cellulose-fed microbial fuel cells. *Water Sci. Technol.*, **58** (3), 617–622.
- Shantaram, A., Beyenal, H., Raajan, R., Veluchamy, A., and Lewandowski, Z. (2005) Wireless sensors powered by microbial fuel cells. *Environ. Sci. Technol.*, **39** (13), 5037–5042.
- Tender, L.M., Reimers, C.E., and Stecher, H.A. (2002) Harnessing microbially generated power on the seafloor. *Nat. Biotechnol.*, **20** (8), 821–825.
- Wang, X., Feng, Y.J., and Lee, H. (2008) Electricity production from beer brewery wastewater using single chamber microbial fuel cell. *Water Sci. Technol.*, **57** (7), 1117–1121.

Interesting links:

Microbial fuel cells. <http://www.microbialfuelcell.org/> (accessed 28 May 2010)