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Genetic improvement of crops for energy generation: comparison of different provision chains with respect to biomass and biofuel production

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1. Introduction

In the next 10 to 15 years a number of future biofuels might potentially come on the market. In this paper we will discuss the supply chains of the most promising biofuels, i.e. Ethanol and ETBE from lignocellulosic (woody) biomass; Fischer-Tropsch diesel from lignocellulosic biomass; HTU diesel. Compared with current biofuels, these new products are expected to show superior performance in terms of cost, environmental impact and socio-economic effects.

All the processes for future biofuels that are currently under development still face technical problems and high costs. Only basical insights generated by research and experimental efforts provide opportunity that improvements can be made at every step of the supply chain. Gains in efficiency and cost reductions can be expected when system adjustments will modernize commercial production.

Table 12.1 shows the potential future biofuel routes which are considered in this report. The fossil fuel component, which is likely to be replaced by the various biofuels, has been included. Most of these petrol and diesel substitutes can either be used as a pure, 100% fuel or blended with

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Table 12.1. Potential future routes included in this report.

Conversion routes not yet commercially available	Fossil fuel counterpart
Bio-ethanol	
From cereal residues	Petrol
From residues containing lignocellulose	Petrol
From cultivated, short-cycle wood	Petrol
Ethyl Tertiary Butyl Ether (ETBE)	
From bio-ethanol	fossil MTBE / petrol
Hydrothermal Upgrading (HTU)	
From residual organic feed with high water content	Diesel
Biomass FT-diesel[1]	
From dry residues	Diesel
From cultivated, short-cycle wood	Diesel

[1] FT stands for the Fischer-Tropsch process, in which syngas is converted into diesel fuel and naphtha (basic petrol), among other products. Syngas is the product of a biomass gasification process

fossil fuels. In some cases engines need to be adapted if the biofuel percentage is higher than 5-15%, or fuels need to be modified to meet the current fuel specifications.

2. Biomass supply

Many common issues can be discussed at a general, i.e. non specific level as all biofuels require biomass and several biofuels use similar or even the same type of biomass. The different types of biomass, used for the production of different biofuels are:

- Residues (from the food, beverage or fodder industry, or organic wastes from households);
- Food crops (like rapeseed, sunflowers, cereals or sugar beet);
- Short-cycle wood and other lignocellulosic biomass (such as poplar, willow, Robinia, eucalyptus, Miscanthus, giant reed, etc.).

The biofuel provision chain faces different processes which need to be made more efficient, i.e. crop production, harvesting and logistics, pretreatment, handling and standardization and biomass processing from crop into fuel.

A major motivation for quality assurance is to fulfill legal limit requirements (e.g. emission limits), or to meet technical demands for the power plant (e.g. avoiding corrosion). To guarantee these basic conditions, chemical and/or physical parameters may be varied theoretically by crop production processes (e.g. modifying the total nitrogen of whole grain crops by Nitrogen fertilization) as well as by harvesting/preparation (e.g. changing wood moisture content through storage).

3. Existing crops

Existing food crops have a number of disadvantages as energy crops. Most of them are annual crops which require large inputs of energy in cultivation and planting each year (Hulsbergen et al. 2001). A great deal of the yield increase, achieved over the past 50 years, results from the improved partition of total biomass into grain to respond to added nitrogen. Since the entire aboveground portion of the plant is used for combustion, partition is of little relevance for an energy crop. A key consideration for the fuel crop system is their "energy balance"(energy in/energy out). The sustainable use of plants as an energy resource requires a substantial net energy gain.

Simulations that take into account all inputs in the plant-based energy generation process tend to show that the net gain currently ranges between negative and a factor two compared with input energy. This is by far insufficient to play a role of importance in resolving future energy demand. The challenge is to focus on the most sensible ways of producing certain types of energy, and to radically reduce the energy input requirements for growing and harvesting biomass, while maximizing energy retention. The ultimate application of this know-how would be the development of an economically competitive, net energy producing system for the energy sector.

In the plants for the future designed by the Strategic Research Agenda promoted by EU, the milestones of the proposal take into account the following points (EU 2005):

- Considering that the worldwide total energy consumption in 2003 was 6.3×10^{17} KJ.
- Assuming that (i) the global energy demand does not increase, (ii) 10% of the current energy demand will be produced by plants growing on 25% of the arable land (in total 1.4×10^{9} ha), (iii) the energy input for crop cultivation is zero, and (iv) the conversion efficiency from the bio-fuel energy to transportation energy, electricity, or heating is 100%, the annual net energy production should be in the order of 1.8×10^{8} KJ/Ha.

- This yield would require an average oil production of 4.5 ton/ha or an average ethanol production of 6.7 ton/ha per year. These production levels can now only be achieved through high yielding oil or sugar crops, such as oil palm (8 ton of oil/ha) or sugar beet (6 ton of ethanol/ha).

In reality, global energy demand is likely to increase. Furthermore, the contribution of plants to the total energy demand may need to exceed significantly the 10% level, while the land available for energy production is likely to be significantly less than 25%. Moreover, a large part of the available land is not suitable for the current high-energy production systems and cultivation, harvesting and conversion of biomass into energy. This combination of factors will lower the net production of energy. These considerations show that the contribution of plants to the world energy production requires a dramatic increase in production capacity. This requires solutions that go beyond traditional crop production methods. Energy production target levels may be as high as 2×10^9 KJ per ha (equivalent to 50 ton of oil or 75 ton of ethanol per ha per year). This target looks very ambitious but is not unrealistic. The challenge is to develop plants producing a high amount of bio-energy under different climatic conditions.

4. Which qualities are required for an "ideal" fuel crop?

Briefly, an ideal fuel crop should have a sustained capacity to capture and convert available solar energy into harvestable biomass with maximal efficiency and minimal inputs and environmental impacts. In particular this crop has to provide:

- *Maximum light-use efficiency.* The biomass yield limit is set by the available amount of light, its efficiency of interception and the efficiency with which intercepted light is converted into biomass.
- *Water use efficiency.* Available soil water is a significant limitation to crop production over much of N. America and Europe, and irrigation requires significant inputs of energy whilst placing a demand on diminishing water resources.
- *Nitrogen and nutrient use efficiency.* Nitrogen use efficiency is determined at three levels. First, by maximizing the efficiency of energy transduction into biomass in photosynthesis per unit of nitrogen invested in the photosynthetic apparatus. Secondly, by maximizing the amount of N, and other nutrients, translocated out of the canopy components on their senescence, either into other leaves or storage organs; i.e. efficient internal recycling. Thirdly, by

maximizing the capture of soil nutrients. This property will help to minimize both the quantities of N that need to be applied as fertilizer and the amount lost to drainage water.

- *Cultivation, and disease and pest control*. Cultivation operations including ploughing, planting and chemical applications all represent energy inputs; fuel crops therefore need a form and life cycle that minimizes the need for these operations. Energy efficiency and environmental sustainability will be facilitated by selecting crops resistant/tolerant to biotic and abiotic stress with a minimum need for pesticide, fungicide and herbicide applications.

5. The main barriers for progress

It is of crucial importance to set up an action plan that moves from basic research to the development of crops with novel features and to provide farmers with new commercial varieties and optimized farming practices based on specific monitoring tools.

The main strategies for increasing the biomass production can be grouped into four areas:

1. basic plant science e.g., altering plant metabolic pathways to produce certain carbon molecules with valuable functional properties (engineered metabolic pathways to enhance the yield of specific molecules);
2. development of new varieties for specific end uses;
3. production e.g., lowering unit production costs for consistent-quality raw materials (development, production, and handling of crops);
4. processing e.g., more economically by separating different materials (new separations technologies to better handle heterogeneous plant components).

6. Plant metabolism manipulation and exploitation

To improve plant performance it is necessary to explain how synthesis, accumulation and the function of primary and secondary metabolites are controlled.

Primary compounds (e.g. sugars, cellulose, organic acids) provide plants with much of their nutritional and industrial value. Secondary compounds are unique, often species-specific, providing defense against insects and diseases and making plants such a valuable source of new pharmaceuticals. Only a few pathways that produce primary and secondary compounds have been described; thousands of others remain to be examined. If we can unravel the genetic program of plants for the regulation of the production, storage and use of carbohydrates, lipids and proteins for the food and processing industries, we will be able to i) manipulate the nutritional value and the industrial applications of plant-derived raw, ii) enhance plant defense against pests and parasites, and iii) use plants as bioreactors to produce important plant-derived compounds.

7. Development of elite cultivars for industrial end uses

Different breeding strategies are suitable for different crops. The base of hybrid vigor should be studied in cross-pollinating species, to decipher and exploit underlying mechanisms.

Quantitative trait loci (QTL) analyses has to be performed on specific crops for which the bottleneck productivity may be different.

Once the underlying genes have been identified, their allelic diversity can be exploited. Then, new superior alleles for each of these yield-limiting components could be identified. Advanced breeding strategies would help to combine superior alleles within new crop varieties.

Two parallel strategies should be followed. One uses biotechnological and transgenesis approaches. The other combines the new "Omics" techniques, with traditional breeding approaches, including QTL analysis and marker-assisted breeding. There are potential cross links between these two approaches. Every candidate gene can search for superior alleles within the existing plant material and use a genetic engineering strategy to transfer performing alleles from distantly related species.

7.1 Combining improved photosynthesis and carbon dioxide fixation

Photosynthesis is the primary source of energy for the plant factory and plants have developed specific mechanisms to improve net CO_2 fixation, such as C4 and CAM (Crassulacean Acid Metabolism). The basic cellular processes, involved in the transport of metabolites from source to sink tissues, also needs to be studied. A detailed molecular understanding of these systems and the detection of relatively simple structured variants could result in promising strategies for incorporating such mechanisms in other crop plants with less efficient C fixation or less efficient metabolite production and translocation. This optimization can be achieved by replacing the endogenous sub-optimal alleles with optimized ones. The gene replacement technologies that enable the targeted modification of existing sequences *in planta* currently not available should be developed.

Plant architecture and developmental characteristics play a critical role in crop performances. The efficiency of metabolite translocation into the "sink" tissues to be harvested is a complex and poorly understood factor which deserves specific analysis, being linked to architectural features. An analysis of sink properties should be performed on the main types of sinks (fruits, monocot and dicot seeds, wood forming tissue, tuber and storage roots) to identify mechanisms controlling these processes and ways to optimize them. This will require a crop-by-crop approach, using, when possible, comparative genomics to speed up the discovery of the genes involved and specific performing alleles.

7.2 Development of new high-energy plant biomass production systems with minimum energy input requirements and higher energy preservation

To achieve plant-based energy production systems which produce the maximum net energy, there is a need to minimize energy input. Current high-energy production systems often require a lot of high-energy demanding inputs, such as fertilizers and pesticides. Strategies to be followed may range from improved nutrient uptake, nutrient use efficiency, or pest resistance for existing high-energy crops, to the implementation of new crops that require fewer high-energy inputs.

The second way of improving the net energy balance of plant-based energy production systems is to increase the energy production levels or the more efficient use of produced biomass A classic example of how efficiency can be improved is the utility of plant biomass for biofuels in the biorefining process. Through increasing the accessibility of the cell wall "energy" polymers of cellulose and lignin to hydrolysis, the sugar production cost should decrease and cheaper sugars should become available for bio-ethanol production.

In existing plant production systems, the level of useful energy produced could be improved by a more efficient conversion of the energy input (through faster plant growth and improvement of photosynthetic efficiency) or an increased storage of the high-energy containing compounds (through optimization of plant architecture or sink-source interactions).

In addition, completely new plant-based energy production systems with maximum energy output need to be developed.

7.3 Improvement of factors contributing to yield stability

Climatic fluctuation can impair a variety of processes, such as rooting, fertilization and grain filling, with important consequences for yield. Plants which are able to withstand drought, cold and salt stress would not only stabilize yield potentials but also contribute to reducing the impact of agriculture on the environment. For example, drought-tolerant crops will require less water for their production and this will lead to reduced erosion and soil salination. These adverse environmental conditions also increase susceptibility to pests and pathogens resulting in a higher consumption of agrochemicals and further yield quality losses. This means that there are direct and indirect benefits to improving tolerance to abiotic factors.

Breeding strategies can reduce the impact of a changing environment on yields.

7.3.1 Improve tolerance to water stress and drought

In many countries, including the European Mediterranean areas, water supply is the most limiting factor. Not only the lack of water but also short periods without rain may affect yield and quality. This means that we need to improve the water consumption efficiency and tolerance to water shortages. Different physiological processes contribute to water homeostasis: root morphology and depth, plant architecture, variation in leaf cuticle thickness, stomatal regulation, osmotic adjustment, antioxidant capacity, hormonal regulation, desiccation tolerance (membrane and protein stability), maintenance of photosynthesis, and the timing of events during reproduction (Bray 1997; Nguyen et al. 1997; Edmeades et al. 2001). Biotechnology is focused on the genetic dissection of drought tolerance through the identification of quantitative trait loci (QTL) associated with yield components, secondary morphological traits of interest, and, more recently, physiological parameters. At the same time the potential of functional genomics is pursued, which should provide useful information about the gene regulation level. Understanding the genetic basis of the essential physiological parameters of drought tolerance, together with the data from profiling experiments, should allow the identification of the key pathways involved in drought stress and how they interact. The emergence of molecular genetics and associated technologies represents a very important new breeding tool; the current challenge is to integrate this tool and the information it generates into breeding schemes to further the development of efficient MAS strategies. These researches are being developed in many laboratories, but their complexity remain a challenge. However, some genes suspected of having an impact on the water consumption efficiency have already been identified in plants such as sorghum. Some have already been transferred to such crops as maize.

7.3.2 Improved tolerance to low temperatures and frost

Some spring crops do not produce high yields mostly because their vegetative phase is too short. Their adaptation to cold temperatures and frost needs to be improved if they are bred to produce winter varieties.

Significant progress has been made over the past 15 years in understanding the molecular basis of cold acclimation, initiated largely from the study of induced genes (Thomashow 1999). These studies have led to the identification of the CBF transcription factors that are responsible for activating expression of many of the genes induced during cold acclimation in Arabidopsis (Gilmour et al. 2000). Characterization of the closely related (or in some cases identical) DREB transcription factors led to a similar understanding of gene regulation in response to drought stress and also to an appreciation of the mechanistic links between higher plant responses to cold and drought (Seki et al. 2003). The target genes of the CBF transcription factors (cold-induced genes; CORs) provide some clues to the metabolic processes and cellular changes that are important components of acclimation. For example, COR15a is thought to decrease the rate at which the membranes of the chloroplast inner envelope undergo phase transition at low temperatures (Steponkus et al. 1998). Its activity emphasizes that impairment of membranes is one of the most damaging effects of exposure to freezing, and many cellular processes induced during cold acclimation are associated with membrane stabilization.

Plant breeders need to identify single genes that contribute to freezing conditions tolerance and the ability of plants to acclimate to cold. The transcriptional regulators of COR gene expression have provided some potential candidate genes and specific genes have been identified in monocots and dicots (Martin 2004). The way is open for a further increase of the tolerance of different crops by exploiting biodiversity at the corresponding homeologous loci. At the same time, a better understanding of tolerance to freezing at the cell level will help breeding for this trait. The analysis of tolerance process provides great insight into the development of new winter varieties integrating cold tolerance and high yield.

7.3.3 Improving stress tolerance through homeostasis

Homeostasis (tendency to stability): a self-regulating biological process that maintains the stability and equilibrium of the organism.

When a plant is exposed to pathogens, wounding, drought, cold, physical or chemical shocks, survival mechanisms turn on to reduce damage. The balance between stress and survival signals determines the level of damage suffered by the plant. The reactive oxygen species (ROS), among the key molecules studied in relation to stress, have high reactivity and therefore toxicity (such as superoxide, hydroxyl radicals and hydrogen peroxide). Under stress conditions, the steady state level of ROS usually increases, and it has been hypothesized that ROS might also act as messengers turning on stress-related genes (Ron Mittler et al. 2005). Signal transduction pathways that are activated can lead either to stress acclimation or to cell death depending on the degree of oxidative stress experienced. ROS homeostasis in plant tissues is therefore determined by their relative rates of production and destruction. Most stresses interfere with mitochondrial function, deregulates the physiology of the plant and causes NAD^+ breakdown, ATP overconsumption, and enhanced respiration. These reactions deplete the energy of the plant, cause the production of reactive oxygen species, and consequently induce cell death (De Bloch et al. 2005). The NAD^+ breakdown is caused by the enhanced activity of poly (ADP-ribose) polymerase (PARP), which uses NAD^+ as a substrate to synthesize polymers of ADP-ribose. This poly (ADP ribosilation) is a post-translational modification of nuclear proteins that seems to be initiated by oxidative and other types of DNA damage. Plants with lowered poly(ADP ribosil)ation activity appear tolerant to multiple stresses. The researchers demonstrated that inhibiting PARP activity via chemical inhibitors (nicotinamide) or genetic engineering (RNA constructs of the parp1 and parp2 genes) protects plant from oxidative stress and enhanced tolerance to stress, such as heat and drought (De Block et al. 2005).

It can be concluded that, if the inciting stress is not so extreme, plant responses to stress lead to acclimate and repair damage: the enhanced antioxidant turnover protect against oxidative damage and re-establish homeostasis.

7.3.4 Interactions between plants and biotic agents

Interactions between plant pathogens and their host plant are very specific. Often only one plant species or sometimes even only one cultivar of a plant species becomes infected/attacked. Specific molecules or signals are involved in communication between host plants and their pathogens/pests and eventually determine whether a plant becomes infected/attacked or remains healthy.

Various crop protection methods are being used. They include chemical control, resistance breeding and biological control. For an efficient crop protection pathogens should be detected by the plant before or during early stages of infection. In order to achieve this, sensitive diagnostic tools are being developed to detect particularly viruses and micro-organisms in plants.

For achieving durable crop protection it is important to unravel the mechanisms and genes underlying the specific interactions between host plants and their pathogens. Detailed insight in how pathogens recognize and attack their hosts and how host plants defend themselves provide tools for the development of new strategies to protect the crop plants.

Only in a few cases fundamental research has developed new strategies already. Gene expression encoding various viral proteins in plants protect them against several viruses. This kind of resistance is becoming intensively exploited in engineered resistance breeding. In a similar way studies on the molecular basis of gene-for-gene systems led to a better understanding of how pathogens overcome or avoid resistance in plants. Resistance genes against viruses, bacteria, fungi and nematodes show previously unsuspected homologies which might be exploited to create hybrid resistance genes that can be used in molecular natural resistance breeding.

A common defense response occurring in many plants against viruses, bacteria, fungi and even nematodes and endophytic insects is the hypersensitivity response (HR). However, due to high selection pressures uniform crop plants become susceptible to new variants of pathogens. It is important to understand the molecular basis of this adaptation in order to breed more durable resistant plants. Therefore pathogen's avirulence genes and their complementary natural plant resistance genes or gene clusters are isolated and studied in detail.

Also signal transduction pathways initiated by activation of resistance genes after interaction with avirulent pathogens are being studied. They include locally and systemically induced defense response mechanisms and programmed cell death. In addition, resistance mechanisms are studied that are not associated with HR, such as pre-houstorial and non-host resistance and avoidance. Molecular genetic analyses of these resistances proved that the genes, involved in these resistances, are different from those involved in HR. Detailed studies will give further insight into the function of these genes and the evidence of their durability.

7.3.5 Resistance to pests

Pathogens and other biotic factors, such as pests, are the main constraints affecting yield losses in crop plants. Some of them also have an important impact on quality due to the production of toxins. An obvious alternative to agrochemical protection is the exploitation of naturally occurring resistance mechanisms.

Genes that are used frequently for pest resistance are those encoding proteinase inhibitors (PIs). PIs are proteins that form complexes with proteinases and inhibit their proteolytic activity which are widespread in nature. The observation that plant derived proteinase inhibitors inactivate proteinases of animal and microbial origin, while rarely inhibiting endogenous enzymes, is compelling evidence for the current view that they are involved in the protection of plants against pests, and possibly pathogens (Charity et al. 1999). There is evidence that the introduction of genes that code for proteinase inhibitors into plants can delay the development of feeding insect pests (McManus et al. 1999). However, PIs have demonstrated a significant degree of specificity towards insect pests. This makes it very difficult to use a few genes on many insects. In addition to this, it has been found that different strains of the same insect species may show a differential susceptibility to a specific PI (Girard et al. 1998).

Transgenic plants producing environmentally benign *Bacillus thuringensis* (Bt) toxins are also being used more for insect control, but their usefulness will be short-lived if pests adapt quickly (Heckel et al. 1999). The main strategy for delaying insect resistance to transgenic Bt plants is to provide refuges of host plants that do not produce Bt toxins. This potentially delays the development of insect resistance to Bt crops by providing susceptible insects for mating with resistant insects (Liu et al.1999).

Scientists are trying to insert few genes into crops which should generate resistance to as many pests as possible but they have to face several problems. For example, any kind of insect may be susceptible to a particular proteinase inhibitor, which is being inserted into crops. But the species may not be susceptible to many other proteinase inhibitors. Secondly, different strains of the same species (i.e. taken from different areas) may not be susceptible to the same PI.

7.3.6 Improvement of genetic resistance to pathogens and pests

Fundamental aspects of approach are the multidisciplinary investigation of the epidemiology of pathogens and pests, the mechanisms of plant biotic interactions, the influence of crop management (for example, crop rotations versus monocultures), the search for germplasm resistant to pests, and ways of decreasing the spread of epidemics. All these data should be integrated into modelling studies to define optimal practices to minimize the use of sustainable pesticides (i.e. through precision farming). More important changes can be expected from the development of genomics and plant biotechnologies and their use in breeding programs for the selection of plants which are resistant to pests and pathogens without chemical protection.

The resistance genes can either be new superior performing alleles of already known or existing genes, or completely new genes not present within the particular crop species. They have to be introgressed into the corresponding crop varieties via sexual crossing, if possible, or gene transfer. Conventional resistance breeding often suffers from limited access to suitable resistance sources. The development of gene technology has drastically increased the availability of genes conferring resistance, which can be derived from non-related plant species as well as non-plant sources.

The genetic and molecular dissection of resistance and defense mechanisms are progressing rapidly in model plants. The comparison of the genomes of crop and model species belonging to the same botanical families revealed a high degree of conservation in the genome structure. This conserved synteny will facilitate the transfer of information gained in model species to related crops and the identification of genes and quantitative trait loci (QTL) that control resistance to divers biotic stresses. Natural and induced genetic diversity will be exploited in breeding programs to generate germplasm of resistant plants. It is reasonable to expect that a major outcome of plant biotechnology and genomics during the coming two decades will be the construction of plants resistant or tolerant to the various pests and pathogens that threaten them.

8. Converting biomass into energy

The traditional way of converting biomass into energy is simply to burn it to produce heat. Heat can be used directly, for heating, cooking and industrial processes, or indirectly, for the production of electricity. The non combustion methods convert raw biomass into a variety of gaseous, liquid, or solid fuels before using it. The carbohydrates in biomass, which are compounds of oxygen, carbon, and hydrogen, can be broken down into a variety of chemicals, some of which are useful fuels.

Heat production and electricity generation are the most important uses for biomass fuel worldwide. Direct combustion devices are widely distributed with thermal capacities ranging from a few kW in household stoves up to heating plants with several tens of MW. The conversion efficiencies vary from 8 to 18% for simple stoves used traditionally in developing countries, up to 90% and above for modern heating units with high-end technology. Electricity production is based mainly on the conventional steam cycle with efficiencies around 30% and a capacity of several hundreds of kW and above.

Whatever production system, the supply of sufficient quantities of plant biomass, with the right composition and at competitive price, is a prerequisite to the future success of bio-energy. More in details, conversion can take place in three ways:

I. Thermochemical. When the plant matter is heated, but not burned, it breaks down into gases, liquids, and solids. These products can then be processed into gas and liquid fuels like methane and alcohol. Thus gasification, pyrolysis and charcoal production are all relevant but only charcoal production is currently widely used. Gasification for electricity production seems to be a quite promising option which might become available on the market in the next few years. An option for the future is Pyrolysis, with the aim of providing a liquid fuel useable in power units.
II. Biochemical. Bacteria, yeasts, and enzymes also break down carbohydrates. Fermentation changes the biomass liquid into alcohol, which is inflammable. A similar process is used to turn corn into ethanol, which is mixed with gasoline to make gasohol. Also, when bacteria break down biomass, methane and carbon dioxide are produced. This methane can be captured, in sewage treatment plants and landfills, for example, and burned for heat and power.
III. Chemical. Biomass oils, like soybean and canola oil, can be chemically converted into liquid fuel similar to diesel fuel, and into gasoline additives. The most important process so far is vegetable oil production from oil seed, and the esterification of this oil fatty acid methyl ester as a substitute for diesel fuel. This technology is used on a large scale across Europe.

Biomass is also used to make gas additives like ETBE and MTBE, which reduce air emissions from cars.

8.1 Bio-ethanol

Nowadays bio-ethanol can be synthesized from a wide variety of biomass, as long as it contains readily fermentable sugars or starch. It can be produced from biomass, grown specifically for bio-ethanol production (such as sugar cane, sugar beet or cereals), or by converting by-products (secondary products) from the sugar and cereal industry. In Brazil, the world's largest bio-ethanol producer, sugar cane is used as feedstock. In Europe, bio-ethanol is normally produced from sugar beet and cereals.

At the same time, a number of companies and research Institutes worldwide are working on the further development of bio-ethanol production processes to enable (ligno-) cellulosic biomass to be used as a feedstock. This would create benefits compared to current technology (IEA 2005):

- Access to a much wider array of potential feedstocks
- Less land use conflicts on food and fodder production
- Greater net GHG (Greenhouse Gas) reduction potential
- More fossil fuel replaced

Below, the options in which ethanol is produced from sugar beet, cereals or wheat by-products are first discussed, followed by an analysis of ethanol from cellulosic biomass.

8.1.1 Ethanol from sugar beet, wheat or residual C-starch

Technology

If sugar beet is used for the production of bio-ethanol, the beets are cultivated and transported to an ethanol plant, where the biomass is broken down by fermentation into sugar beet pulp, the by-product, and a water-ethanol mixture. The latter is converted into pure ethanol via distillation. The beet pulp can be used as animal fodder or fuel.

Ethanol can be produced from wheat grains by milling, hydrolysis, fermentation and distillation. The process is more complex and expensive than with sugar beet. Milling and distilling are the most energy-consuming unit operations. In addition, ethanol can be produced from residual organic biomass, for example from by-products of the wheat and sugar beet industry (Kampman 2005), as long as these contain sufficient amounts of readily convertible sugars (C6 sugars such as glucose).

GHG reduction

The calculations in the General Motors-study (GM et al. 2002) regard sugar beets cultivated in a rotational system. The crop residues are ploughed into the soil. Fertilizer use is approximately 100 kg N/ha/year, while the ethanol yield is estimated about 4.800 liters per ha. Two different reference systems (i.e. replaced crops) are considered: Egyptian clover and rye grass. Ethanol GHG emissions are found to be 41-86% of those associated with petrol production, depending mainly on the use of the by-product.

8.1.2 Ethanol from lignocellulosic biomass

Technology

The ethanol production from lignocellulosic biomass requires an additional pre-treatment of the feedstock, which enables the fermentation of sugars contained in the biomass. The fermentation process itself needs to be adapted, furthermore, new kinds of enzymes being required to convert the C5 sugars into ethanol (in the production of ethanol and alcohol today it is only C6 sugars, the main constituent of the current feedstock, that are converted). The process consists basically of four steps:

- pre-treatment. This stage is necessary to make the material accessible to enzymes mediating enzymatic hydrolysis and to break down hemicellulose into C5 sugars;
- hydrolysis of cellulose;
- fermentation of C5 sugars (xylose) and C6 sugars (glucose);
- distillation.

Today's research is mainly focused on three issues: improvement of the pre-treatment stage, integration of hydrolysis and fermentation in fewer reactors to cut costs, and the improvement of the 5 fermentation process (ECN 2003). The progress and success of these developments will determine the future potential of this route and when these biofuels can first be marketed on significant scale.

Pre-treatment

Pre-treatment is necessary to improve fermentation efficiency. The goals of pre-treatment are:

- to make the material accessible to enzymes for hydrolysis, by reducing its volume and opening up the fibrous material.
- to mobilize the lignin and (hemi)cellulose biopolymers and attain the further break-down of structural components to optimise enzymatic access in the following steps.

Several methods are available for pre-treatment: dilute acid hydrolysis, alkaline pre-treatment, steam explosion, liquid hot water pre-treatment.

Bottlenecks for commercial application

Ethanol production from lignocellulosic biomass has several advantages compared with conventional technology: the use of cellulosic feedstock for fuel production, and therefore a better GHG balance; lower feedstock costs because of higher feedstock yields (in MJ per hectare). The drawback is enzyme technology. Cellulase enzymes are produced commercially, but in low volumes geared to high-value products. The industrial cellulases currently available are not effective enough and too costly for use in large scale production (ECN 2003). Besides, pentose can be converted only in part because there are no fermentation systems available that can use the entire pentose fraction. Different efforts have been made to tackle this problem: genetic modification of backer's yeast; co-culture of different strains of *Zymomonas mobilis*; transfer of pentose-converting genes into ethanol-resistant strains of *E.coli*. Even if some progress is reported in research journal, there is still a lack of experience at the industrial scale.

GHG reduction potential

According to a GM study, GHG emissions of ethanol from wooden biomass crops are 20-30% lower than for ethanol from sugar beet or cereals.

8.2 ETBE

Ethanol can be converted into ETBE (Ethyl Tertiary Butyl Ether), that can be used to replace MTBE, a petrol component. Whereas the MTBE percentage in petrol usually ranges between 1 and 5% (by volume), more ETBE can be added: up to 15%.

ETBE can be produced using any of the previously discussed ethanol variants, regardless of the feedstock or conversion process used. This means two things:

- Nowadays ETBE can be produced from ethanol, using cereals or sugar beet (or secondary products yielded as by-products of the food and fodder industry) as a feedstock.
- The environmental and cost performance of ETBE and its potential availability will benefit from any improvements in the ethanol production process (in particular, the expected development of technology enabling conversion of (ligno)cellulosic biomass).

ETBE, compared to ethanol, can be blended with petrol without changing the vapor pressure (in case of low-percentage ethanol blends). In EU countries like France, Spain and Italy, ETBE has already been added to petrol as an alternative to MTBE.

8.3 Biodiesel

Two sources of diesel substitute, vegetable oil-based biodiesel and synthetic diesel from a lignocellulosic biomass-based Fischer-Tropsch process, are assumed to replace diesel.

All the stages of the vegetable oil-based biodiesel chain can use proven commercial technologies and several European countries have an established biodiesel industry, e.g. Germany, Austria and France (based on rapeseed and recovered vegetable oils).

Significant improvements are expected to affect rapeseed yield and the reduction of energy needs for oil extraction and esterification.

8.3.1 Biomass Fischer-Tropsch diesel

Technology

Fischer-Tropsch (FT) hydrocarbons can be produced by gasification of biomass, followed by downstream gasification. The biomass FT plant comprises: biomass pre-treatment (chipping, drying), gasification (resulting in syngas), gas cleaning and conditioning, FT-reactor, hydro-craker. As with the ethanol production process, different configurations are possible. Most configurations produce electricity and heat as by-products. Overall process efficiencies vary according to the plant design from 40% up to 60-65% (Ecofys 2003).

The Fischer-Tropsch technology is one of the options available for utilizing cellulosic biomass for fuel production. As discussed earlier, this reduces the amount of land needed for biomass production compared with current biofuels. It also leads to cost reductions and thus to more cost effective GHG reduction.

Bottlenecks for commercial application

Several technical limitations still stand in the way of commercial application (Hamelinck 2004). The most critical step is the cleaning of the syngas (mixture of CO and H2). According to Hamelinck it is not clear whether the strict cleaning requirements for biomass FT synthesis can be achieved (some impurities need to be removed down to levels of less than 10 ppb by volume).

8.3.2 HTU diesel

The Hydro Thermal Upgrading process is based on depolymerisation and deoxigenation of biomass by means of hydrolysis and decomposition. The process converts biomass into a “biocrude” using highly pressurized water (100-200 bar) at 300-360°C. This biocrude is non-miscible with water and has a relatively high energy content. For application, two routes are possible:

- Power generation;
- Diesel fuel production by catalytic hydrodeoxygenation (HDO).

Croezen and Kampman (2005) analyzed their potential.

A comparative advantage of the HTU process is that it can process biomass streams with a otherwise limited applications potential that are therefore low-priced.

Whereas most biomass conversion techniques require dry biomass, the HTU process can process wet biomass.

8.3.3 Greenhouse gas (GHG) reduction potential

The General Motors study (GM et al. 2002) provides a comprehensive review of the various steps in the rapeseed biodiesel chain. The study presumes that rapeseed is grown on set-aside land in a rotational system with a rye grass reference system (i.e. the crop that would otherwise have been grown on the land). The GM-study assumes a fertilizer use between about 100 and 145 kg per ha and a crop yield of about 3 tons per ha. N_2O emissions are calculated according to the IPCC method, with average assumptions. Crop residues are ploughed in, as is usual in the case of rape crop. The by-product glycerine will normally replace glycerine produced conventionally in the chemical industry. Using these assumptions and ranges, the GM-study estimated the biodiesel GHG emissions to be 21-73% of diesel emissions. Most of these emissions occur during cultivation of the rapeseed. These results are based on average emission factors for nitrous oxide. In reality, however, greenhouse gas emissions are highly variable, differing per soil type, climate and fertilizer input. They are consequently of major influence on total GHG emissions.

9. Potential future biofuel processes

In the next 10 to 15 years a number of future biofuels may be put on the market. The most promising biofuels are:

- Ethanol and ETBE from lignocellulosic (woody) biomass.
- Fischer-Tropsch diesel from lignocellulosic biomass.
- HTU diesel.

All these potential future biofuels are still under development, with conversion processes not yet fully operational on any substantial scale. Compared with current biofuels, these new products are expected to show superior performance in terms of cost, environmental impact and socio-economic effects. This superior performance derives from new processes being able to convert alternative types of biomass feedstock.

10. Technical, economical and environmental limitations

The potential future biofuels cited above are still in the research and development phase and are not yet available on the market due to technical limitations. Even when these technical problems have been solved, economical limitations could hinder large-scale application: significant investments are still required for developing these new biofuel technologies. At least until these biofuels are marketed on a large scale or the costs of other fuels increase significantly, they are likely to remain more expensive than conventional fossil fuels. Market access will then be dependent on government incentives. In the long term, however, costs are predicted to fall, so that eventually all of the future biofuels discussed here will be able to compete with their fossil counterparts as well as with current biofuels. The realization of this cost reduction will depend on:

- technological developments (resulting in a specific process design, conversion efficiency, etc.);
- biomass prices (which, in turn, depend on competition with other potential users of the biomass or land, such as food or energy sector);
- conversion process operating costs (e.g. cost of enzymes for producing lignocellulosic ethanol and ETBE);
- fossil fuel prices;
- government incentives and policies that promote the development and market introduction of these fuels.

Despite these uncertainties, cost estimates for future biofuels can be found in literature. In Figure 12.1 (Hamelinck 2004) they are compared with existing biofuels, petrol and diesel costs.

We can see that cost estimates are quite similar for all the future biofuels considered. Estimated production costs for HTU diesel are comparable to current diesel production costs if wet organic residues with a negative market value are applied as a feedstock.

11. Potential for net greenhouse gas reduction

Figure 12.2 (Hamelinck 2004) compares the Greenhouse gas emissions of the various biofuels with their fossil counterparts analyzed in this paper.

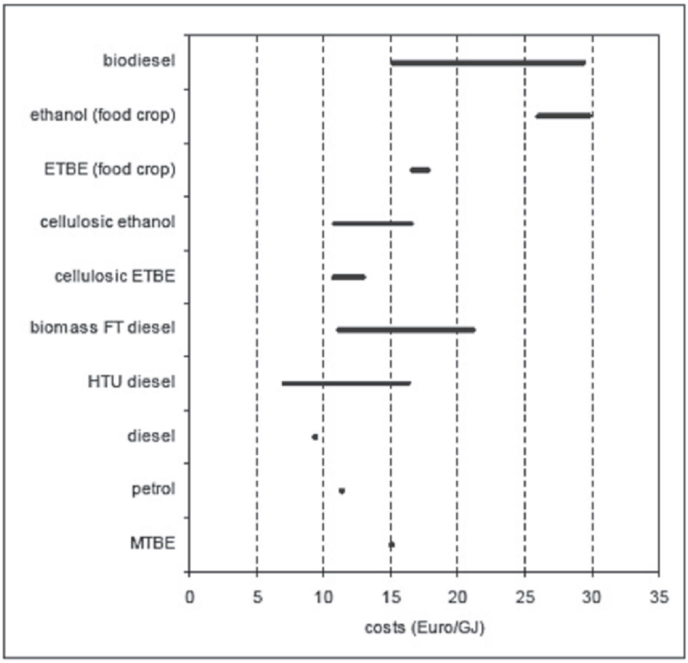

Fig. 12.1. Cost estimates of the various biofuels, compared with the average cost of diesel and petrol in 2002-2004.

Cellulosic ethanol, biomass FT diesel and HTU diesel are expected to produce much higher GHG reductions than currently available biodiesel and ethanol from wheat or sugar beet. Total GHG reductions of over 90% are in fact expected from biomass FT and HTU diesel. Converting ethanol into ETBE has clear advantages from the perspectives of biofuels quality control, but reduces GHG reduction potential significantly because it is produced only partly from ethanol and partly from fossil isobutylene.

The GHG reductions of the various biofuels were found to depend strongly on the emissions associated with biomass feedstock. The superior performance of the observed future biofuels is mainly due to their potential for using lignocellulosic biomass as feedstock.

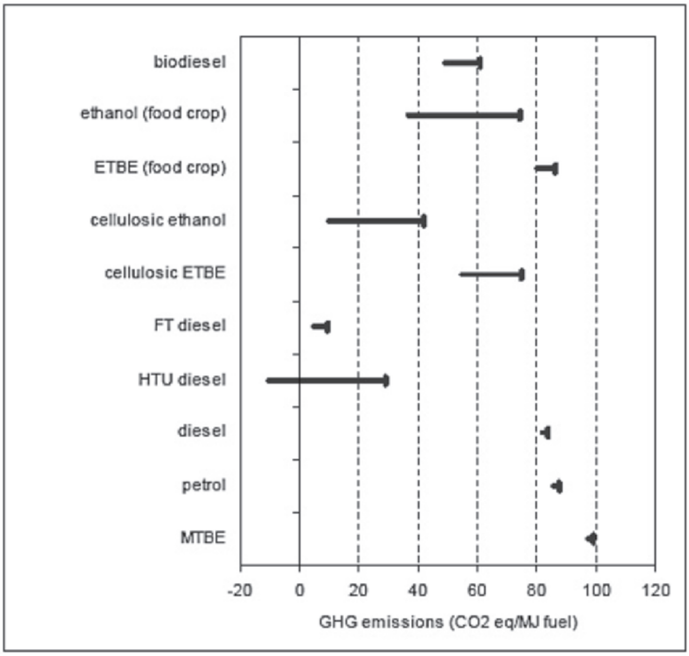

Fig. 12.2. Overview of the GHG emissions of each of the biofuels analyzed, compared with those of diesel, petrol and MTBE (CO2 eq/MJ fuel).

12. A case study

The experiments which were carried out in clayey soil in Bologna (Italy) in the three-years period 2002-2004, compared 7 species, 2 of which annual (*Cannabis sativa* and *Sorghum bicolor*), 2 perennial herbaceous (*Miscanthus sinensis* and *Arundo donax*) and 3 woody species ("*Populus* x *canadensis*", *Salix alba* and *Robinia pseudoacacia*) managed with the method of Short Rotation Forestry (SRF) (Di Candilo et al. 2004a,b).

The woody species were planted at a density of 0.9 plants m^{-2}, with a distance of 1.80 m between the rows and 0.60 m along the rows, whereas the herbaceous perennial were planted with a 0.60 x 0.60 m layout. Hemp and sorghum were sown with distances of 20 and 50 cm between the rows for a theoretical density of 100 and 20 plants m^{-2}, respectively.

Harvesting occurred annually for hemp (first ten days of August), sorghum (first ten days of October), Miscanthus and Arundo (early February). Instead, biomass from woody crops was harvested twice. The first time at the end of the second year (on two-year old trees) and the second at the end of the third year (on one year regrowth).

The parameters assessed were: plant density, plant height, stem diameter, fresh biomass and dry matter production. On the latter were detected net calorific value, ashes, silica and low-melting salt contents.

The thermo-electric conversion of biomass was performed at the Dister power generating plant at Faenza (Ravenna, Italy) which has a capacity of 13.5 Mw. The heating cycle involved the production of steam at 47 bar and 430°C, its subsequent expansion in a turbine up to a counter-pressure of 3.3 bar, stabilization of the steam at 165°C and its distribution to the technological services. The power station is normally supplied with natural gas, biogas, wood-cellulose compounds, such as fruit stones, or spent marc. The boiler can burn three fuels at the same time and fuel changeover can take place without switching off.

Results

Plant emergence for annual species (hemp and sorghum) was very satisfying.

The rooting of cuttings and rhizomes for perennial species was satisfying as well. The crops showed vigorous growth, except for August 2003 when the woody species, especially willow, due to particularly high temperatures and heavy drought, showed an early halt in vegetative growth, immediately restored after the first rain in September.

The mean annual production of fresh biomass was very high for sorghum and Arundo (93.7 and 78.4 t ha^{-1}). Other species showed more modest production levels, between 47.3 t ha^{-1} for hemp and 23.0 t ha^{-1} for Robinia (Figure 12.3).

Arundo provided the highest average annual yield of dry matter (35.4 t ha^{-1}). High yields were also obtained with sorghum and miscanthus (29.7 and 23.3 t ha^{-1}). Hemp, poplar and robinia supplied similar yields, between 15.6 and 13.4 t ha^{-1}, significantly lower than miscanthus.

Willow was found at the bottom of the list due to its sensibility to water stress; in fact, a previous study (Lindroth and Bath 1999) shows that water deficiency is often a growth-limiting factor in willow cultivation.

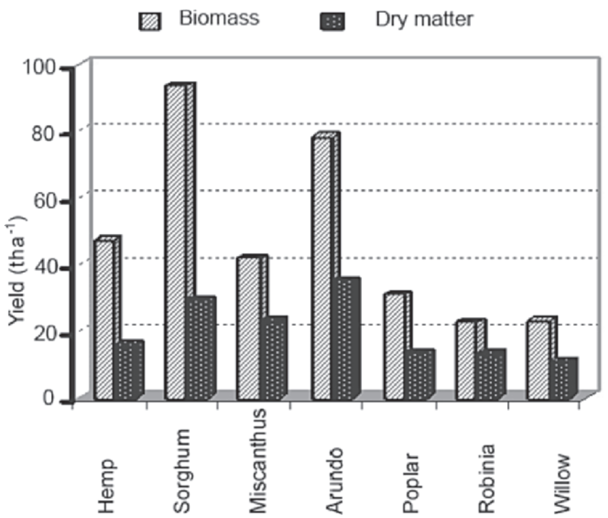

Fig. 12.3. Average annual production of the species compared.

Table 12.2 shows the Net Calorific Value (N.C.V.) and the content of ashes in the dry matter. Robinia, hemp and poplar show the highest values for the first parameter.

Table 12.2. Net Calorific Value and ash contents of biomasses.

Species	N.C.V. (MJ kg^{-1} d.m.)	Ashes (% d.m.)
Hemp	16.4	2.5
Sorghum	14.1	4.5
Miscanthus	14.6	2.6
Arundo	14.6	5.1
Poplar	15.9	2.5
Robinia	16.4	2.5
Willow	15.1	2.6
Means	15.3	3.2

Ash contents in the dry matter were also low in hemp, robinia and poplar. Arundo and sorghum obtained the highest content (5.1 and 4.5%).

Considering biomass production and the net calorific value of the same biomass at the time of combustion in the boiler, the corresponding values in m^3 of natural gas were calculated as well as the equivalent in tons of oil required per hectare (Table 12.3). The biomass provided by arundo and sorghum was equivalent, after energy conversion, to 14,966 and 12,126 m^3 ha^{-1} of natural gas and to 12.35 and 10.00 t ha^{-1} of oil. The values obtained for SRF were much lower.

Table 12.3. Biomass performance at the combustion in the boiler and energy equivalent in natural gas and crude oil.

Species	Biomass (t ha^{-1})	Moisture (%)	N.C.V. (mj kg^{-1})	CH4 Equiv. (m^3 ha^{-1})	Oil Equiv. (t ha^{-1})
Hemp	18.4	15.1	3.83	7,408	6.11
Sorghum	38.1	22.1	10.85	12,126	10.00
Miscanthus	27.7	16.0	12.16	9,850	8.13
Arundo	44.8	21.0	11.40	14,966	12.35
Poplar	28.2	51.1	7.46	6,354	5.24
Robinia	22.3	40.0	9.59	6,363	5.25
Willow	16.6	35.0	9.66	4,754	3.92
Means	28.0	28.6	10.71	8,832	7.29

Research achievements

- Arundo, sorghum and miscanthus seem to be the most suitable species for the investigated site. Arundo proved to be very rustic, with a strong resistance to pests and lodging, and a good adaptation to dry conditions. These characteristics are fundamental for the good adaptation of species to different pedo-climatic conditions, as well as for a stable yield over time.
- The production levels of SRF were quite modest, compared with the greater pest control requirements (at least for poplar and willow) and water (in the case of willow). For these crops the biennial harvest provided better results than annual harvests.
- The most important factors for the assessment of the species performance are the production of dry matter per unit of surface area and the net calorific value of the dry matter which indicate the total amount of calories that can be obtained from the biomass in the boiler.

13. Coordinated approach and economic sustainability

In order to increase the biomass production we need to develop plants able to tackle biotic and abiotic stress and to provide high performance even in poor areas. The challenge is to modify plat metabolic pathways to enhance the yield of specific molecules.

The future utilization of renewable resources requires a multi-disciplinary, cross-industry approach. Already exciting opportunities exist for research in areas such as processing, logistic, harvesting and new sources of renewable energy. This means that progress in single isolated technical areas will not be sufficient. It will be much more important to have interrelated research projects conducted in a parallel and coordinated manner. The outcome should produce improved fit and flow through the chain, and avoid progress in one area that results in a "surprise" at another point in the system. For example, a scientist may discover a entirely new polymer with functional potential to be the source for an advanced biodegradable plastic replacement. However, the value of this research result is limited until: the appropriate gene is found; it is expressed in the correct viable metabolic pathways; the optimum crop type is grown with enough yield for cost-effective sourcing; a process is created to separate the component polymer; and a method is developed to utilize the material in the manufacturing of the novel product (Zhu et al. 2004).

The sustainability of biofuels also depends on economic aspects. In many cases, the costs for the use of plant-based materials is rather high, and not competitive with fossil fuels. However, cost-competition contains very complex interactions among factors like: product value, material costs, volume of throughput, degree of processing required, and performance of the biofuel generated. Thus, strategies for the future will not be successful if they are based on cost reduction alone.

The most important economic driver is not cost per se, but the difference between the obtained price and manufacturing costs. The obtained price covers aspects such as product utility, performance, and consumer preference and demand. Manufacturing costs cover raw material costs, supply consistency, process required, waste handling costs and investment.

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