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Energy and biofuels from biomass
Hypes, hurdles and hopes

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The world is heading towards a crisis in providing sufficient food, water and fuel for a rapidly expanding population, while also conserving biodiversity and mitigating climate change. Through photosynthesis, plants could provide multiple solutions, helping to secure future supplies of not only food, but also energy, chemicals and materials. These roles have to be balanced to reduce conflict over the limited land resources available for cultivation. In particular, the current use of some food crop products for fuel should be superseded by the use of non-food biomass. To achieve this, the recalcitrance of lignocellulose to break down needs to be overcome and improvements in crop composition and cell walls are required to provide more optimized feedstocks for processing and conversion though biological or thermochemical routes. Research should also focus on improving energy yields of biomass crops on resource-limited land that is less suitable for food-based agriculture. Finally, the exploitation of high-value products is also needed to extract maximum value from biomass through the development of integrated biorefinery systems. In this article, brief highlights are provided of progress already achieved towards some of these goals. Such endeavours provide opportunities for biochemists to join forces with crop geneticists, biotechnologists and engineers to provide more sustainable fuels and chemicals for the future.

The potential of plant biomass as an alternative source of energy and fuel

Photosynthesis is the natural energy transformation where plants use sunlight to concentrate atmospheric carbon over 1000-fold into chemical energy in the form of carbohydrate, the first constituent of dry matter. Plants are primary producers in food chains, and increased agricultural production will be fundamental to achieving future food security. However, crops are also important resources for fuel, fibre and natural products. Even before agriculture began, plant materials were collected for use as a source of heat for cooking and warmth. Today, such traditional use in developing countries comprises the greatest source of renewable energy, constituting two-thirds (48 EJ) of the total energy use of biomass[1]. In the developed world, alternative energies have largely replaced this practice, but increasing concerns over future fuel security and rising greenhouse gas (GHG) emissions has encouraged the industrialized exploitation of plant biomass for bioenergy/biopower (electricity and heat), biofuels (transport fuels) and biomaterials. The future potential of these markets is perceived to be massive[2]. For example, values as high as 200–400 EJ/year were proposed[3] for the potential global contribution of biofuels and bioenergy (current energy use is approximately 500 EJ/year). Although biopower and bioheat are recognized as key components of the future energy mix, alternative renewables also exist (e.g. wind or solar). For liquid transport fuels, however, alternatives are more limited, and transport is also the sector in which emissions are increasing the fastest. The recognition of the vital role that biofuels could play in substituting for fossil fuels became widely promoted, with promises of 'green gold' heralded, and large investments in research and industrial development followed.

Concerns over biomass supply

Since the original hype over biofuels, some concerns have emerged and realism has set in. Vast amounts of biomass will be needed if renewable energy derived from plants is to make a significant contribution to the future energy mixture. How this can be produced without negative effects on food and other ecosystem services has become an increasing cause for concern. A wide variety of biomass can be used for biopower, including wastes and residues from agriculture and forestry, as well as dedicated biomass crops, such as fast-growing trees and grasses. In contrast, biofuel production at cost-effective large industrial scales is currently limited to food crops (and algae) using conventional conversion processes that utilize the easily accessible and convertible portion of the crop as the substrate. Biodiesel is derived by transesterification of lipids (e.g. from algae) or oils of crops such as oilseed rape, oil palm, soya bean and *Jatropha*. Bioethanol is produced by fermentation and distillation of sugars or

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starch (after a hydrolysis step) from grain (e.g. maize or wheat) or sugar crops (e.g. sugar cane or sugar beet). 'First-generation' biofuels derived in this way provide alternative sources of fuel that are renewable, but direct conflicts with food production have arisen. The agricultural practices required to produce the crops are also intensive, particularly requiring high inputs of nitrogen fertilizer, which significantly reduces the energy savings and GHG reductions that can be achieved. In addition to direct conflicts with food production, it has been argued that if the use of land for biofuels results in conversion of uncultivated land for food elsewhere (ILUC or indirect land-use change), loss of considerable carbon stocks in soils and an overall negative GHG balance may result[4].

Research challenges

The challenge ahead, to reduce the problems associated with first generation biofuels, is to develop efficient means of exploiting lignocellulose (the cell wall component of biomass) from non-food biomass crops and crop residues (such as straw). Lignocellulose is mostly composed of cellulose, hemicellulose, lignin and pectin, of which the (1-4)-β-linked glucose cellulose polymer is the most abundant polysaccharide on Earth[5]. However, the cell wall is designed to provide the plant with strength and resistance to degradation. Polymeric linkages between the lignin, cellulose and hemicelluloses prevent access by cellulose-degrading enzymes, limiting the amount of sugars available for conversion. An initial pre-treatment step is required in which the cross-linkages are removed to enable access by the enzymes. Different methods exist, including heat and acid treatment and steam explosion. These are costly to perform and also result in the production of toxic compounds which can inhibit the hydrolytic enzymes in the conversion process. Biological routes to conversion use fermentation once the sugars are released whereas thermochemical routes are based on production of a syngas, which is converted into biodiesel (and any other products) through processes such as Fischer–Tropsch.

Leaving aside the technological challenges of cost-effectively producing biofuels from lignocellulose, concerns remain over whether there is sufficient land to grow lignocellulosic crops for fuel without conflicting with food production and/or causing indirect land use change. Central to these debates is increasing the amount of energy produced from land so that more fuel can be generated from less land requirement. Research efforts therefore need to focus on optimizing the energy yield of biomass crops, improving biomass and cell wall composition and structure, identifying enzymes that are better able to digest lignocellulose and improving the efficiency of the pre-treatment, processing and conversion steps. Among the research initiatives worldwide addressing these goals is the UK BBSRC Sustainable Bioenergy Centre (BSBEC), described more fully in the 'Biofuels for the Future' article elsewhere in this issue. The BSBEC-BioMASS programme (http://www.bsbec.bbsrc.ac.uk/), within this, led by Rothamsted Research, is focusing on short-rotation coppice (SRC) willow and *Miscanthus*, two commercially grown perennial crops that can achieve high biomass yields with minimal inputs[6] (Figure 1). *Miscanthus* is grown from rhizomes and is harvested annually. SRC willows (*Salix* spp.) are established from cuttings and grown in SRC cycles of 3–4 years.

Figure 1. *Miscanthus* (top) and willow (bottom) are among the perennial crops commercially grown for biomass. *Miscanthus* (*Miscanthus* × *giganteus*) is a Asian rhizomatous grass that is established from rhizomes (it is a sterile triploid) and is harvested annually. Willows are dioecious catkin-bearing temperate trees and are grown in SRC cycles. They are established as cuttings, cut back after 1 year to stimulate the coppiced habit, and then harvested on a 2–3-year cycle.

Figure 2. SRC willow varieties vary in the number of stems that re-grow after coppicing – from many thin stems (left) to fewer thicker stems (right). Research in BSBEC-BioMASS is relating compositional variation and saccharification potential to stem characteristics in both willow and *Miscanthus* for use in genetic improvement programmes.

Exploiting natural variation in glucose release

Rothamsted Research maintains the National Willow Collection, a highly diverse germplasm of some 1300 different genotypes. This rich repository is crucial for the Rothamsted willow genetic improvement programme which is breeding willows for the biomass industry. The breeding effort is underpinned by a genetic mapping and genomics programme, including the establishment of several large mapping families which have been used to map quantitative trait loci (QTLs) for important biomass traits. Willow is already used as a feedstock for heat and power and in gasification (to make biodiesel), but, until recently, its potential for biological conversion into ethanol was not yet known. To assess the potential of willows to release glucose, in a collaboration with Imperial College London, saccharification tests were performed on a range of willow genotypes and wide variation in enzyme-derived glucose (EDG) yields was revealed. A limited comparison with *Miscanthus* grass revealed willow to be at least as good in releasing glucose. To identify QTLs associated with enzymatic saccharification yield, stem segments were tested from 138 progeny of the K8 willow mapping population, which is planted on three sites. Four EDG QTLs were mapped on to chromosomes V, X, XI and XVI. These results indicate that opportunities exist to improve willows for second-generation biofuels by breeding for increased enzymatic saccharification[7]. In BSBEC-BioMASS, saccharification yields are now being compared with stem traits (Figure 2), biomass composition and cell wall structure, and the results integrated in process-based crop models.

The potential for modifying cell walls

Improved understanding of plant cell walls could lead to routes for modifying composition in ways that enhance conversion efficiencies. Despite being the subject of much research effort, the genetic and biochemical regulation of cellulose biosynthesis is still not well understood, and the dynamic complexity of cell walls presents considerable challenges to modification attempts[5]. Cell wall structure also differs between trees and grasses, with the former generally containing higher amounts of lignin and the latter more abundant phenolic acids such as ferulic and p-coumaric acids. Several authors have reviewed ways of improving bioethanol production by modifying cell wall composition (e.g. [5,8,9]). These include the development of specialized cellulases/cellulose cocktails, heterologous expression of cellulases in native and engineered microbes and engineering of cellulosomes, macromolecular structures which are anchored to the outside of microbes containing hydrolytic enzymes (for a review, see [8]). Transgenic plants have also been engineered to express enhanced levels of cell-wall-targeted glycoside hydrolases, cellulose-binding molecules or other cell-wall-modifying proteins (for a review, see [5]). These latter authors also propose a novel approach in which non-crystalline soluble polysaccharides are introduced to the cell wall[5]. Strategies based on modifying lignin include down-regulation and up-regulation of genes in the lignin biosynthesis pathway (e.g. reviewed in [9]). For example, reduction of cinnamyl alcohol dehydrogenase (CAD) gene expression and overexpression of ferulate 5-hydroxylase (F5H) resulted in improved processibility of poplar biomass. More recently, improved extractability of polysaccharides from lignocellulose has been achieved through manipulation of the hemicellulose component. Hemicelluloses include glucomannan, xyloglucan, mixed-linkage glucan and xylan. Improved extractability of xylan from the cell wall was demonstrated in *gux1 gux2* double mutants of *Arabidopsis*[10]. In these mutants, the activity of the xylan glucuronyltransferase was shown to be lacking, affecting the substitution of glucoronic acid and 4-O-methylglucuronic acid branches in xylan synthesis. No changes were found in the xylan backbone, and, although the stems were weakened, the plants grew to normal size. Related research is also being pursued further within BSBEC in the Cell Wall Sugars programme led by the University of Cambridge.

Increasing the value of biomass

Supply and demand studies indicate that the use of biomass as a resource for energy and fuel is likely to be more limited by biomass supply than by energy and fuel demand. In contrast, chemical production requires lower quantities of biomass to satisfy demand. The value of the chemical industry is comparable with the value of the fuel industry, and an economic opportunity thus exists to add value to biomass by extracting not only fuels, but also chemicals[11]. This potential is encapsulated in the concept of the biorefinery, in which conversion processes are integrated to produce a range of fuels, power, materials and chemicals from biomass, ideally with minimal or even no waste (for a recent review, see[11]). The overall GHG reductions that can be achieved through biofuel chains and land requirement calculations are also affected by whether other materials are co-produced or generated as by-products. For example, it has been shown that life cycle models that omitted co-production of oil-seed meals (a co-product of oil-seed diesel) or dried distillers grains (a co-product of wheat ethanol) overstated cropland conversion from US and EU mandates by about 27%[12]. It has thus become increasingly apparent that the future lies not so much in biofuels, but in integrated biorefining. In this way, more value is extracted from fuel chains and there is simultaneous improvement of the economic, energy and GHG balances.

Conclusions

To develop efficient and sustainable ways of utilizing non-food biomass from plants as renewable feedstock for future energy and fuels, further improvements in the extractability of sugars from lignocellulose are required to provide more optimized feedstocks and integrated biorefinery systems are needed to extract maximum value from biomass through co-production of high-value chemicals as well as fuels. ■

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