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Editorial

A new era for lignocellulosics utilization through biotechnology

The 20th century was characterized, amongst other things, by a worldwide shaping of society brought about by the industrial use of fossil-carbon and -energy resources. However, the main drawback to this way-of-life and development model is that such resources are not renewable (in the short term) and present consumption of the remaining limited stocks is so intense that their depletion now seems imminent. Furthermore, the increase of environmental, economic and political concerns linked to Man's dependence on fossil fuels is making the problems that need to be solved even more complex. This situation imposes worldwide global and coordinated actions, adapted to the local specificities of each country and society.

Mankind is thus facing the major challenge of how to maintain existing levels of activity, comfort and security given the expected shortages in the energy pools, which currently drive the system. Another essential consideration is how to facilitate the access and/or sustain the progress of emerging and developing countries.

All these considerations are influencing current R&D policies to develop sustainable energy production and find new alternatives to chemical feedstocks. One viable alternative pathway, among the various route maps being proposed and developed for such purposes, is the biological conversion of biomass.

This approach is based on:

(i) natural bioconversion of the energy of sunlight into carbon- derived compounds through photosynthesis; and;
(ii) man-induced transformation of this renewable biomass into easy-to-use biofuels and key molecules.

This technologically-induced acceleration of the long natural conversion of bio- and phyto-mass to fossil fuels can only be achieved in successive complex biological and (bio)chemical steps. Some of these steps, however, have not yet been fully mastered and this is impacting the cost-effectiveness of the solutions proposed. Nevertheless, promising and exciting perspectives in this area are being opened up by a mandatory integrated approach combining science, technology, economy and sociology (vide infra).

It was in this highly complex, fast moving and competitive context that the first symposium on Biotechnology applied to Lignocelluloses was organized in Reims, 28 March–1 April 2010, to gather together world leaders in the field of lignocellulose bioconversion and pulping processes. The excellent lectures delivered during the meeting highlighted the rapid progress that has been made in this area and the ever-increasing contribution of "omics" techniques and biotechnology tools. Thus, a new generation of tailor-made enzymes and microbial strains for the sustainable production of value-added products is being obtained, using renewable plant polymers as feedstocks. This is a really exciting time for "Biotechnology applied to Biomass exploitation" and it is not unrealistic to predict that the challenges of mastering new versions of the slow natural and biological processes will be partially solved in the short term.

The following is a synopsis of the main themes tackled during this outstanding meeting.

1. Biomass pretreatment and enzymatic conversion of polysaccharides

Significant challenges in enzymic deconstruction are posed by the chemical and physical complexity of the plant cell wall, which limits exploitation of this resource in the biofuel and bioprocessing sector. The main components of the wall cellulose, hemicellulose and lignins are tightly associated and their degradation by different biocatalysts requires facilitated contact between the substrates and enzymes. In particular, the presence of lignins in plant cell walls impedes the breakdown of the wall polysaccharides to simple sugars.

Pretreatments are therefore necessary to facilitate access of the degrading enzymes to the corresponding substrates. These include various procedures such as kraft pulping, used in the pulp industry, hydrothermolysis, organosolvolysis, and ball milling. However, the development of efficient biomass treatments still requires a better understanding of how plant polymers interact. For example, ester-linked hydroxycinnamic derivatives, such as ferulic and p coumaric acids, can link polysaccharide

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chains and also polysaccharides and lignin polymers. These chemical bonds represent an additional target for lignocellulosic biomass deconstruction.

A wide range of fast-growing cellulolytic fungi has been used for the industrial production of cellulose-degrading enzymes. Structure function relationships of cellulases facilitated by genomic studies, a wide range of "omics" techniques, the impact of induced mutations and in-depth studies of favourable induction conditions have provided useful information for strain improvement. The objective is to obtain more robust enzyme blends with improved ability to enzymatically degrade the partially disrupted biomass. More generally, the cost of the enzymes needed for hydrolysis will have to be significantly reduced in order for the process to be economically viable.

Concerning cellulose and glucans hydrolysis by carbohydrate-degrading enzymes, recent studies have revealed the complexity and intricacies of substrate recognition and catalysis. Carbohydrate binding modules play a central role in the optimization of catalysis through their ability to bind to specific plant structural polysaccharides and/or their possible disruptive effects on the plant cell wall network. New combinations of catalytic sites and carbohydrate binding modules are thus being explored to improve the efficiency of biocatalysts.

This symposium also provided information on new enzymes and highlighted the ongoing need to discover novel lignocellulolytic enzymes. For these purposes, scientists are investigating the biodiversity of fungal and bacterial enzymes involved in wood degradation, including white rot and brown rot fungi.

The complete degradation of all components of plant cell walls, by fungi and microorganisms, involves a large set of enzymes acting in concert. Data collected on diverse microorganisms, which use specific strategies to degrade the components of lignocellulosics, seem to suggest that to optimize biomass conversion, we need in future to look at the activities expressed by microbial communities rather than by single isolates. The aim in this much more complex approach will be to reproduce the context of efficient natural conditions and will involve engineering the different members of the microbial consortium in complementary ways.

Actually, in view of their optimal pH, t° and stability, enzymes are not naturally suited to industrial processes. They must be able to function in viscous media with high solid concentrations and in the presence of reaction products and various inhibitors. The improvement of enzymes in relation to these different characteristics is vital to identify high performance biocatalysts adapted to the multiple demands of industrial processes.

2. Lignins: degradation, bioconversion, functionalisation of fibres

Lignin is highly recalcitrant to degradation and, as such, greatly hinders the efficiency of biomass conversions. Peroxidases and laccases are the major enzymes involved in lignin degradation. However, the enzymes isolated from microorganisms in natural ecosystems do not work optimally under industrial conditions. Increased knowledge of the sequences in basidiomycete peroxidases (at least, lignin peroxidase, manganese peroxidase, and versatile peroxidase) has contributed to a better understanding of the structure/function relationships of these biocatalysts. In this context, peroxidase engineering is being investigated to modify their catalytic and operational properties and develop tailor-made biocatalysts for industrial applications. Moreover, new strains with high enzymatic activities are being selected by pursuing high throughput screening of large collections of fungal strains from different sources.

Ligninolytic enzymes, in addition to their use in the delignification of plant biomass for bioethanol production, are widely considered to have potential for industrial applications such as the biodegradation of environmental pollutants (e.g. polycyclic aromatic hydrocarbons, textile dyes...) stain bleaching, biobleaching and biopulping of wood chips, bioconversion of lignins in high added-value products. Indeed the chemical nature of lignins, which include aromatic and aliphatic moieties, makes them an interesting alternative source of aromatic chemicals. Laccases, for example, are being extensively investigated in combination with redox mediators for the potential development of new lignin-based products.

Apart from their potential use in delignification and the release of high value aromatic derivatives, laccases are becoming important in the pulp industry where the aim is to reduce energy consumption and improve pulp quality. Energy savings of up to 30% have been observed in some studies. Other approaches envisage the upgrading of lignocelluloses and derived fibres by functionalisation methods (for example, using laccases to catalyse the formation of phenoxy radicals which can further bond with functional chemical groups). These methods may confer to lignin-containing materials completely new properties. For example, it is possible to obtain antimicrobial paper by coupling low molecular weight phenols to unbleached pulp through fibre functionalisation.

For these different reasons, laccases represent important commercial and economic markets. To improve the performances of these biocatalysts, various programs are then dealing with different molecular approaches, such as mutations and molecular evolution techniques with the final goal of producing recombinant optimized proteins. Different strategies for improving their activities or their stability (immobilization...) are actively being investigated. Whatever the enzymes, all procedures likely to increase efficiency and applicable under industrial conditions, are being systematically explored, such as the use of additives and surfactant polymers.

3. The fermentation step in the production of bioalcohols

Diverse strains of microorganisms able to convert sugars into alcohols are already available, but the general trend is to envisage engineering them to increase the yield of ethanol and tolerance to ethanol, acetate and inhibitors. In addition to *Saccharomyces cerevisiae*, organisms such as *Thermoanaerobacterium saccharolyticum* and *Clostridium thermocellum* are being significantly improved for practical applications. These laboratory-scale studies may rapidly have a commercial outcome.

Heavy investments are also being focused on improving yeast strains for the overall conversion of lignocellulosic hydrolysates, which may contain hexoses and pentoses. Pentoses can represent up to 40% of the total sugars in lignocellulosic materials, and their microbial conversion presents a major challenge. Despite the complexity of the fermentation pathways of xylose and arabinose, some groups have engineered industrial yeast strains normally unable to use pentoses, to ferment xylose and arabinose, together with glucose, in plant biomass hydrolysates. Other studies have dealt with the characterization of natural xylose-fermenting yeasts such as *Candida shehatae* or *Pichia stipitis* at the laboratory scale.

Current research projects are also dealing with engineering *S. cerevisiae* to produce butanol instead of ethanol since the four carbon alcohol butanol shows superior properties, in comparison to ethanol, as a potential biofuel. Some results have already been obtained with a laboratory strain and should be extended to commercial strains, although these are more difficult to handle due to instability, or more difficult genetic transformation.

4. Biorefinery pilot plants

A general trend in the exploitation of different sources of plant biomass is based on the biorefinery concept i.e. a more complete utilization of lignocellulosic resources through sequential or complementary use of the different sub-fractions of the plant material to produce fuels and chemicals. Hemicelluloses, for example, can be used to provide high value products from xylooligosaccharides (xylitol and furfural) and the resulting lignocellulosic residues are used to produce ethanol (from cellulose) and solid biofuel (from lignins).

Biorefining processes based on a whole plant approach should rapidly become a reality, providing that the economic aspects and environmental benefits also match up.

Different pilot plants for the production of lignocellulosic bioethanol are already functional such as the Inbicon process in Denmark (http://www.inbicon.com/) or are just starting up like the Futurol project in France (http://projet-futurol.com/). The Inbicon process converts wheat straw and other soft lignocellulosic biomasses (corn stovers, rice straw...) into three final products: ethanol for transportation, C5 molasses for animal feed and lignin as a solid biofuel to replace coal in power and heat regeneration. In 2009, a commercial demonstration plant able to convert 4 tons of wheat straw per hour with an annual bioethanol production of 35 000 tons, was inaugurated. The yield of final products from 1 ton of pretreated wheat straw was 435 kg of solid biofuel, 370 kg of C5 molasses and 145 kg of ethanol. No chemicals are involved in the process, which only uses steam, enzymes and yeast.

Other players, such as the Mascoma Corporation (founded in 2005; http://www.mascoma.com/), are exploiting the concept of consolidated Bioprocessing (CBP), using an organism that simultaneously releases sugars and produces ethanol from pretreated lignocellulosic biomass.

Significant improvement of the economic viability of processes, beyond their technical feasibility, is still possible particularly in the area of pentose fermentation. At the present time this economic viability is rarely achieved. However, techniques are continually being improved as the decision makers need to anticipate the unavoidable increases in fossil fuel prices, fears about the increased negative effects of climatic changes and the expected cost-decrease of mature biotechnologies. These motivations are also consistent with the willingness of several countries to limit their energy dependencies on fossil resources.

5. Quality and availability of the upstream resource

Regular availability of the quantity and quality of the plant resource is a key factor in the success of biomass-based industrial projects and constitutes a major challenge given the considerable variations in plant growth over time and space. The securing of lignocellulosic feedstocks is therefore essential and should comply with the demands of sustainable agriculture.

In the biorefinery context, sustainable agriculture means the combined management of actions dealing with soils, waste and global environmental issues. Particular emphasis is being focused on soils as they are at the very beginning of the production chain and must therefore be preserved from fertility-depletion. Organic matter degradation, soil microbial activity and the recycling of mineral C,N,P,S elements need to be understood in order to make appropriate decisions about cultivation sequences and intensity. Again, the diversity of the production ecosystems (forestry, grassland, agricultural) is greatly complexifying the efficient management of lignocellulosic resources.

Other socioeconomic aspects have also to be considered. These include the net energy balance, the greenhouse gas intensity generated by biomass transformation, the preservation of biodiversity and landscapes and the social acceptance of new lignocellulosic species (i.e. no competition with the food chain).

Another research field concerns direct improvement of the resource, which is envisaged through the production of more suitable plant materials (with low lignin content, or tailored architecture for example), whether by conventional breeding, molecular marker-assisted breeding, or genetic engineering strategies. In the latter case, targeting of the different key enzymes in the lignin biosynthetic pathway has been successfully exploited but a trade-off between low lignin content and a viable plant is clearly needed. Cinnamyl alcohol dehydrogenase down-regulated plants which do not contain fewer lignins, but instead a more easily extractable modified lignin (aldehyde-rich) seems to provide some of the best compromise at the present time.

6. Future prospects

High performance enzymes and engineered microorganisms with optimized biosynthetic pathways are the prerequisites to new alternatives for renewable energy and chemicals from lignocellulosic biomass. However these strategies will only be successful if their production costs can be made to compete with or even out-compete current fuel and petrochemicals production costs.

Recent advances in "omics" techniques, metagenomics in particular, and the potentialities of synthetic biology offer new opportunities for the identification of high performance enzymes. Metagenomics, for example, is being used to investigate new genes/enzymes in the digestive flora of termites, which are able to efficiently break down lignocellulose. However these powerful tools for new enzyme discovery require careful and efficient downstream screens. Additional approaches include site-directed mutagenesis, "in vitro" enzyme evolution and random engineering strategies to produce more efficient enzymes. The next step after the discovery of more efficient biocatalysts will be their adaptation to the constraints of industrial processes.

Other solutions may be provided by synthetic biology which, in the future, will allow the design and building of novel networks and pathways to re-engineer organisms for practical applications. The main problems will concern identification of the most convenient and efficient biosynthetic pathways and the choice of appropriate host organism. For biofuel production for instance, some microorganisms have evolved to be proficient in converting lignocellulosic materials to diverse biofuels. Unfortunately the corresponding enzymatic activities exist in pathways that are highly regulated according to the evolved needs of the specific microorganism and which may not be suitable in their native state for production on an industrial scale. One of the main challenges will then be to determine how to improve production in a convenient industrial model host such as *E. coli* or *S. cerevisiae*. Once these steps have been completed it will still be necessary to optimize the expression of each of the components, using particularly strong and inducible promoters. The number of applications involving synthetic biology should then increase considerably in the future because of their huge potential in lignocellulose conversion processes.

As a conclusion to this symposium, it is apparent from the great variety of scientific and technological approaches presented (although not exhaustive) that the industrial and environmental feasibility of lignocellulose bioconversion requires a global and integrated approach involving combinatorial strategies (i.e. microbial communities) and careful improvement of each component of the long and complex chains involving several individual steps. In fine, it seems that there will not be one winning technology, but instead several combined pathways, taking into account the complexity of Nature and including that of Man's behaviour and capabilities!

The second edition of Lignobiotech, to be held in Japan, 14–17 October 2012 (http://www.congre.co.jp/lignobiotech-2/), will therefore provide an interesting "rendez-vous" for following progress in the fascinating paradigms of lignocellulose deconstruction.

Disclosure of interest

The author has not supplied their declaration of conflict of interest.

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