THE POTENTIAL OF BIOTECHNOLOGY IN THE SUGARCANE INDUSTRY: ARE YOU READY FOR THE NEXT EVOLUTION?

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Abstract

Sustainability of the sugarcane industry relies heavily on the realisation that sugar, molasses and bagasse can no longer be considered as the final products of a sugar factory. Innovation through biotechnology has the potential to provide a sustainable, competitive edge to the value addition of sugarcane products and processes. Biotechnology can be defined as a combination of technologies which use biological systems, living organisms or derivatives thereof to produce or modify products or processes for specific use. Recent advances in genomics, proteomics and bioinformatics provide access to an enormous information base for facilitating the choice of suitable microorganisms and enzymes for bioconversions. This can shift the resource base for chemical production from fossil feedstocks to renewable raw materials and provides exciting possibilities for the use of industrial biotechnology-based process tools in the sugar industry. This paper explains the fundamentals of biotechnology and highlights the need to introduce these new technologies in a mature industry, for the sake of sustainability.

Keywords: biotechnology, sugarcane, sustainability, value-added products

Introduction

All over the world it is realised that the time has come to face the problems of the past and current uses of fossil resources, and that a major step for the development of a sustainable industrial society will be the shift from our dependence on petroleum to the use of renewable resources. In this respect there is no doubt that biomass from agriculture and forestry will play a key role and lay down the basis for the development of a bio-based economy.

The role of agriculture is changing, and integrated biomass utilisation is a key technology in a sustainable society. Farmland can no longer be seen only as the growth medium for crops for food and feed. It now has a much broader and multi-functional role as a supplier of biomass from energy crops, industrial crops and by-products from food and feed production. The multi-functionality is therefore not only in regard to the use of the land. It is indeed also the case with regards to the complete utilisation of the harvested biomass. Renewable resources can be converted to bio-based products using biological processes (including enzymes and microorganisms) to make products such as fine and bulk chemicals, pharmaceuticals, food and feed, paper and pulp, textiles, energy, materials and polymers. Industrial biotechnology has an important role to play in these developments.

Biotechnology has been driven by economic demand, led by industry, national and international policies, often prompted by public pressure, and advances in science and technology. Modern biotechnology includes an array of tools for gene modification technologies, which have shown considerable potential to enhance human health and wellbeing. Using biotechnology, plant breeders can produce better crops, and these technologies are potentially powerful tools in the quest to alleviate hunger and malnutrition in developing countries. Instead of transferring thousands of genes as traditional crossbreeding does, biotechnology enables the transfer of selected plant genes. In addition, undesirable traits can be targeted and removed. However, the practice of gene modification of food crops is controversial, and has been the subject of considerable public debate. It should be emphasised that the tools of biotechnology are just that: tools, not ends in themselves. As with any tool, they must be assessed within the context in which they are being used. Clearly, biotechnology is more than genetic engineering. Indeed, some of the least controversial aspects of agricultural biotechnology are potentially the most powerful. This paper does not focus on the development of genetically modified crops and related contentious issues. Rather, the aim is to highlight the current trends towards the development of a bio-based economy, the impending role of the sugarcane industry in such an economy and the potential of biotechnology to provide the process tools to achieve this.

Although this paper clearly reflects the views of a biotechnology aficionado, it is well worth discussing the potential of thermochemistry in the sugarcane industry's quest for sustainability. The race between biotechnology and thermochemistry as mainstream contributors towards the establishment of a bio-based economy presents the prospect of exciting discussion in the present time, as well as in the future.

Development of a bio-based economy

With the pace of world economic growth, sustainable socio-economic development will depend on a secure supply of raw material inputs for agriculture, industry, energy and related sectors. Today's heavy reliance on non-renewable resources is increasingly constrained by economic, political and environmental factors. This is accompanied by a heavy reliance on chemical and thermo-chemical processes and, although the current role of biological processes in the global economy is small, it is growing rapidly.

Developing countries have a vast agricultural resource base for alternatives for bioenergy and industrial biotechnology; however, in many of these countries, biomass is generally used inefficiently, with very few high value-added product markets. Bio-based renewable resources can provide raw materials for many new and growing industries, while also stimulating rural development, job creation and greenhouse gas reduction. A greater reliance on bio-based resources and biological processes is an inevitable part of an overall sustainability transition, and the main problem for technical innovation and policy development is thus how to positively implement the nature and pace of such changes. In assessing the options and strategies of a bio-based economy, economic, environmental and social issues need to be addressed to ensure that sustainable development objectives can be met.

One of the strategies proposed for the development of a bio-based economy is the concept of biorefining, which has received major attention over the past few years and is currently a topic of considerable interest for government policy makers and commercial entities worldwide. Although the term biorefining is now being used broadly to describe processes that convert biological materials to multiple useful products, the United States National Renewable Energy Laboratory has applied the narrower definition of a biorefinery 'as a facility that integrates biomass conversion processes and equipment to produce fuel, power and chemicals from biomass' (http://www.nrel.gov/biomass/biorefinery.html). It has to be clarified here that biorefining is not the use of biotechnology *per se*, but rather the integration of multiple technologies to process the biological material (biomass).

Developing the biomass resource base for industrial sector applications requires a reliable supply of feedstock and processing technologies that are adapted to these feedstocks. Quantity, quality and availability of feedstock are relevant. Depending on the feedstock available in different countries, biomass of different origins have been suggested as raw materials, and include corn (Gaspar *et al*., 2005), wheat (Koutinas *et al*., 2004), sugarcane (Pye, 2005; Edye *et al*., 2006), rape, cotton, sorgo, cassava (Enze, 2006) and lignocellulose (Pan *et al*., 2006). Numerous biorefinery technologies for processing lignocellulosic materials are presently in development around the world. In order to achieve efficient conversion of the raw material, a mixture of mechanical, biocatalytic and chemical treatments are expected to be combined. These range from the relatively simple processes of gasification and pyrolysis, through to steam explosion and ammonia fibre explosion (AFEX) processes, combined with acid or enzymatic hydrolysis of the cellulose and hemicellulose to produce fermentable sugars, to more sophisticated technologies such as organosolv and supercritical fluid extraction.

Multi-product refineries are the likely long-term end point for the industry, much like today's petroleum refining and corn wet milling industries. In the short-term, however, industries may well add one new product or process at a time in order to avoid undue risk, just as petroleum refining and corn wet milling developed incrementally over time, evolving from single to multi-product industries.

There are several advantages for current bio-processing industries to reinvent themselves as biorefineries through the production and marketing of multiple products. These advantages include (Pye, 2005):

- The enhanced revenue from a single facility that utilises its infrastructure and raw material resource to the maximum possible extent, and the additional revenues from high value co-products that could reduce the selling price of the primary product.
- A lower financial risk, since the profitability of the facility will be less dependent on fluctuations in the selling price of, and market for, a single commodity product.
- A reduction in waste generation and the lower disposal and treatment costs, since more of the raw material is converted to saleable products.
- The opportunity for more efficient operations through greater process integration and utilities optimisation.
- A possible degree of operating flexibility through changes in the ratio of the different products that can be made in response to changes in markets and raw materials.

The key to successful development of biorefineries will ultimately depend on the progress in three areas: (i) low energy milling of bio-feedstocks to its components, (ii) efficient bioconversion of mixed sugars to products and (iii) the utilisation of by-products. These improvements will require integration of biomass conversion technologies with all major areas of industrial biotechnology such as novel enzymes and microorganisms, functional genomics, pathway engineering, protein engineering, biomaterial development, bioprocess design, product development and applications.

Role of the sugarcane industry

Sugarcane has one of the widest potential product portfolios of any industrial crop and has long been prominent from an energy-industry perspective, due to the ready availability of fibrous bagasse at the factory site. Although sugarcane has been used primarily to produce sugar and molasses, the co-products obtainable from field, intermediate and final streams of cane sugar production can make a valuable contribution towards the sustainability of the industry.

Historically, mature bio-processing industries such as cane sugar mills have focused mainly on the production of a single primary product with only limited attention being given to opportunities for improved revenues through the production and upgrading of potentially valuable co-products. For sugar mills, molasses has generally been recognised as a low value, marketable by-product of raw sugar production, and the vast quantities of bagasse that are also produced by the mill are viewed almost without exception as a low value solid fuel suitable only for the provision of power and steam. In some parts of the world, mills with more efficient boilers will burn bagasse simply as a means of disposal, since the amount of bagasse frequently exceeds the needs of the mill for fuel. Conversely, one has to concede and acknowledge that many cane sugar producing countries have been successesful in developing co-products and processes. Examples of these include the ethanol produced by Brazil, energy production from the combustion of bagasse in Mauritius and Hawaii, paper and pulp industries in South Africa, Asia and the Middle East and the production of furfural and ensuing chemicals by South Africa's Illovo Sugar. These successes should act as an inspiration and driving force for future developments towards a bio-based economy in the cane sugar industry.

The transition to a multi-product industry will have a positive effect on the aggregated value of the sugarcane industry especially for developing countries, by allowing them not only to cope with the increasing prices of non-renewable fuels, but also to contribute to lower greenhouse gas emissions and to substitute actual products that are currently being made by petrochemical industries. In addition, it will enable the sugarcane industry to support itself during the cyclical variations of sugar prices. Diverting sugar and molasses from traditional food and feed markets into the production of renewable chemicals, such as glycerol, succinic acid, ethanol and aconitic acid, could become a major new opportunity for the industry (Kampen, 2002). In addition, when processed by a biorefinery technology, bagasse can create a range of products that have a combined value at least in the order of magnitude more than its fuel value (Pye, 2005).

A sugarcane based biorefinery for the production of renewable chemicals could potentially be a very attractive commercial proposition. Physically it could be an expansion or modification of an existing sugar mill, thus making use of the mill's existing infrastructure, labour force and utilities, and reducing capital and operating costs for the biorefinery. Although such a mill would continue to extract juice from the cane, this could be used either directly as a fermentation feedstock for renewable chemicals production, or converted to raw sugar, depending on the market conditions for each product at that time. Where raw sugar is produced, there would be an on-site use for molasses as a fermentation feedstock in the manufacturing of renewable chemicals, or fuel ethanol.

The major technical change that would be required for the existing mill would be the use of the bagasse as a source of chemicals rather than as a boiler fuel. This diversion of bagasse to the biorefinery would require the provision of an outside source of fuel, which could be other crop residues, deliberately-grown high fibre cane or wood waste. For such a biorefinery, in which the amount of available bagasse might be close to the amount of sugar produced, a very substantial portion of total mill revenues would come from products obtained from the biorefining of the bagasse. Depending on the process technology employed, revenues from products derived from bagasse could easily exceed those from raw sugar. Clearly, bagasse could have more value as a biorefinery feedstock than it does in its current use as a solid fuel. It is unfortunate that sugar mills today may be burning the highest value portion of their raw material! This may change the economic relationship between the grower and the mill, since growers are generally paid on the basis of the sugar content of their cane. For a biorefinery, a remuneration model will have to be designed to ensure fair compensation for all parties involved.

For many industries such as the cane sugar industry, the transition from a single-product operating mode to a biorefinery mode will not come without difficulties. The mill operations will necessarily become more complex and more technically sophisticated, and management of the business will become more difficult, especially as it relates to the marketing of a product mix that spans a range from commodities to highly specialised products. However, the current changes in the world economic profile are a driving need for agricultural industries to explore these opportunities to maximise revenue from their raw material, and previously mentioned successes with the developments of co-products and processes in the cane sugar industry should reinforce the industry's confidence in this regard.

Role of thermochemistry

Thermochemistry is the study of the heat produced or consumed in chemical reactions, and thermochemical conversion (or chemical processes induced by heat) is currently considered by some as the most suitable conversion process for the major biomass feedstocks. Although the title of this paper emphasises the potential of biotechnology in the sugarcane industry, the role of thermochemistry in biomass conversion cannot be ignored.

To alleviate dependence on fossil fuels, biomass (which is unique in providing the only renewable source of fixed carbon) could be used as feedstock for the thermochemical processes similar to those used in petroleum refineries. The concept of a biorefinery is quintessentially a modern petroleum refinery modified to accept and process a different feedstock via some of the same unit operations along with some novel unit operations. The optimal biorefinery may not consist of only one type of conversion, but may rely on a fully integrated system that employs fermentation, gasification/pyrolysis and even digestion (Orts *et al*., 2008).

Pyrolysis (high-temperature heating in the absence of air or oxygen) produces pyrolysis oils (fuel liquids) that can be used as is (e.g. as boiler fuel), or refined for higher quality uses such as engine fuels, chemicals, adhesives and other products. Pyrolysis also produces fuel gases, and the solid residue contains most of the inorganic portion of the feedstock as well as large amounts of solid carbon or char.

Gasification (high-temperature conversion in the presence of limited oxygen) produces a synthesis gas from biomass feedstocks consisting largely of carbon monoxide and hydrogen. It can be used directly as a fuel for heat recovery or can be further processed by Fischer-

Tropsch synthesis to methanol, dimethyl ether, mixed alcohols and/or a mixture of hydrocarbons. Gasification processes also produce a solid residue as a char, ash or slag. The key is in the choice of catalysts that perform the reformation reactions, as well as the choice of feedstocks.

Although the thermochemical conversion of bio-based feedstocks could play a vital role in the development of a bio-based industry, considerable research into the thermochemistry of biomass and subsequent reactor designs are necessary to fully optimise the potential of this technology.

Role of biotechnology

Biotechnology is recognised globally as one of the key enabling technologies of the 21st century. Confidence in this view stems from the promise it holds for achieving industrial sustainability by the optimal use of renewable resources, amelioration of global warming and the introduction of clean or cleaner industrial products and processes. These driving forces have catalysed the development of biotechnology as a means of generating new markets, resolving long-standing and emerging problems, and gaining cost and efficiency improvements in industrial processing. Industrial biotechnology has an important role to play in development of a sustainable bio-based economy. The past decade has seen intense activity in the area of developing new biological products. During this period, biotechnology has gone from being a peripheral consideration to a central element in the business strategies of many companies in the chemical process industry. Its versatility is so great that industries that have not previously used biological systems in their operations are now exploring such options (Bull *et al*, 1998, 2000; ten Kate, 1999).

Biotechnology is a broad term that applies to all practical uses of living organisms, from microorganisms used in the fermentation of beer to the most sophisticated application of gene therapy. The application of biotechnology is a knowledge intensive business and requires a basic understanding of life forms and their complexity, as well as proper application in processes and/or product development. Successful application of biotechnology integrates multiple scientific disciplines including microbiology, biochemistry, genetics, molecular biology, chemistry and chemical engineering.

To address the challenges presented by bioconversion of feedstocks for the production of fuels, and bulk and fine chemicals, new or improved biocatalysts have to be developed and new or improved and tailored biotechnological processes have to be designed, developed and assessed. Key to this success is the cost- and eco-efficient production of the desired compounds by developing (a) the best biocatalyst for a specific function or process, (b) the most favourable possible environment for the catalyst to perform and (c) an optimal strategy for the recovery, purification and further chemical conversion of the desired products from the fermentation process.

Innovative and improved biocatalysts

Finding new microbial strains or novel enzyme activities is imperative. Unfortunately, the standard microbiological cultivation and screening methods cannot deliver the wealth of novel strains and enzyme activities needed in an appropriate time frame. A common estimate among microbiologists is that the vast majority (99%) of prokaryotes (single-celled organisms that lack a nucleus, such as bacteria) have never been investigated in the laboratory and that their characteristics and potential are therefore unknown. Whether these organisms are 'unculturable' or whether they simply do not grow on the media and culture conditions used, this enormous untapped reservoir of potentially new enzyme activities could not be used to date. Metagenomic gene discovery is the process of extracting genes directly from nucleic acids gathered in the environment bypassing tedious microbiological cultivation and product search (Cowan *et al*., 2005).

While the metagenomic approach is searching for novel enzymes in the natural reservoir, directed evolution is a different approach to reach the same goal. With directed evolution, an existing and known enzyme is subjected to repeated cycles of variations and screening to finally select an enzyme protein with the desired functions. This is a fairly well established technology, and there are numerous companies worldwide who offer directed evolution, often with propriety and specific patent-protected techniques. Directed evolution combines techniques such as different engineering methods to create genetic diversity, high-throughput screening and advanced analytical and computational methods. In contrast to the classical and linear enzyme improvement, directed evolution is a branched process which allows the fast creation of novel enzyme variants for industrial application. As the functional recombinant expression in a suitable microorganism is a prerequisite, this shows also how important it is to have a choice of several microbial host and expression systems.

High-throughput experimental techniques and parallel fermentation techniques generate large amounts of data at various levels, which are routinely logged and stored. The development in bioinformatics will allow the study of complex biological systems by a combination of high-throughput experimental techniques with *in silico* (computers in conjunction with informatics) model and experimental design. The integration of wet and *in silico* experimentation can be used as a new approach to strain improvement. The sequencing of genomes and corresponding computer programs to search homologies are also a prerequisite, for example, for the abovementioned metagenomics. Systems biology is the attempt not only to integrate and analyse a variety of biochemical information, but also to actually predict a phenotype based on models which are fed with data from unrelated data sets (Mack, 2004).

Innovative fermentation technology and engineering

Fermentation processes are commonly used today for the production of numerous products. However, due to the growing demands in very competitive markets, high efficiency fermentation processes need to be developed to increase product yields, to facilitate the scaling-up of processes and to develop and intensify novel bioprocesses for the production of bioproducts.

A current technological challenge for improved fermentation processes is a low volumetric productivity due to the harsh fermentation conditions of some processes for microorganisms (pH, temperature, substrate concentration). There is also the necessity to move from laboratory to large-scale bioreactors, and the capital investment that will be required to develop and implement a novel or improved bioprocess, which will account for a significant part of the total production costs.

The improvement and intensification of current fermentation technologies will depend greatly on the development of new generation bioreactors with alternative novel reactor concepts to allow more intensified production and create optimal environmental conditions for the production of certain metabolites by microorganisms. In addition, micro-bioreactors based on realistic full scale production conditions are necessary as screening tools to shorten process development time.

Innovative down-stream processing
Down-stream processing relates to the methods used to isolate fermentation products during the fermentation processes and includes methods to convert fermentation products to interesting chemicals without prior purification of the fermentation product from the broth. Innovative down-stream processes require the development of combined technologies which will enable parallel production of different products and the development of bioprocesses and technologies that will allow energy generation from waste products. Chemical engineering principles play a vital role here in terms of designing and operation of the separation systems. Improvements in down-stream processing will benefit the overall efficiency and process cost and will ensure that biotechnology based processes are competitive with the conventional chemical methods.

Production of bio-based chemicals

There is no question that currently one major driver for the commercialisation of biorefineries is the rapidly rising demand for fuel ethanol and other 'sugar platform' chemicals. Shifting our society's dependence from petroleum to renewable biomass does not mean that the chemical industry will lose importance. Biorefineries will not be able to directly provide every required material, but they can be used to deliver the feedstock for a sustainable chemical industry. Today's task is the development of useful building-block chemicals that can be produced from biomass and subsequently be converted to several high-value chemicals and materials. Building-block chemicals are molecules with multiple functional groups that can be transformed into new families of useful molecules.

The US Department of Energy has published a list of top value chemical building blocks, i.e. platform chemicals that can be derived from biomass by biological or chemical conversion and subsequently be converted to a number of high-value bio-based chemicals or materials (Werpy and Petersen, 2004). The 12 top value building blocks are listed in Table 1. Each building block can be converted to numerous high-value chemicals or materials and the potential industrial applications are immense. All building blocks listed can be produced from biomass (cellulose, hemicellulose, starch or vegetable oils) either by fermentation or by *in vitro* enzymatic conversions via the intermediate sugars: glucose, fructose, xylose, arabinose, lactose and sucrose, respectively. Organic acids are becoming increasingly important in this respect. Of the 12 sugar-derived building-block chemicals identified by the US Department of Energy, nine are organic acids.

Most of these building block chemicals are new in the sense that they are not currently used in chemical processes because of their high costs. Their low-cost availability is an obvious prerequisite for the chemical industry for these substances to be considered as starting materials. Nevertheless, it is anticipated that as soon as such new building block chemicals can be provided at a sufficiently low price, their market potential might rise instantly and significantly.

Table 1. Prioritised sugar-derived building blocks as identified by the US Department of Energy (Werpy and Petersen, 2004).

Building blocks	Direct use or uses of derivatives
1,4 diacids (succininc, fumaric and malic)	Green solvents, fibres, water soluble polymers
2,5-furan dicarboxylic acid	Furanoic polyesters (bottles, films, containers), polyamides (new nylons)
3-hydroxypropionic acid	Sorona fibre, contact lenses, diapers (super adsorbent polymers)
Aspartic acid	Amino analogues of C4 1,4 dicarboxylic acids, sweetener intermediates
Glucaric acid	Solvents, nylons of different properties
Glutamic acid	Monomers for polyesters and polyamides
Itaconic acid	Solvents, polymers (butanediol, gamma-butyrolactone, tetradydrofuran), nitrile latex
Levulinic acid	Fuel oxygenates, solvents, polycarbonate synthesis
3-hydroxybutyrolactone	High value pharmaceutical compounds, solvents, amino analogues to lycra fibres
Glycerol	Personal/oral care products, pharmaceuticals, foods/beverages, polyether polyols, antifreeze
Sorbitol	Polyethylene isosorbide, terephthalates (bottles), antifreeze, polylactic acid, water soluble polymers
Xylitol/arabitol	Non-nutritive sweeteners, anhydrosugars, unsaturated polyester resins, antifreeze

Conclusions

History records that the crude oil refining industry has gone through the exact evolutionary process which we are faced with today. In the earliest days of the industry, crude oil refining was undertaken mostly to produce a lamp oil that was an alternative to a progressively short supply of whale oil. Later it was found that a low value by-product of lamp oil production, gasoline, could replace ethanol as fuel for automobiles. Considering the structure of the oil industry today, it is hard to believe that originally the primary product of crude oil refineries was lamp oil, and a relatively worthless product was gasoline (Pye, 2005). Will the same concept be true for the sugarcane industry, decades from now?

Clearly, the potential of biotechnology to improve the quality of our lives and the quality of our environment is considerable. It could bring huge advances in health, nutrition and remediation of the environment, to name but a few. In the realisation of these benefits we will have to be sensible and selective, avoiding those technologies which challenge our ethical values (such as human cloning) and focusing instead on those which can provide significant advances with minimum risk. In the process, it will be important to continuously engage with and inform public understanding of the work of biotechnologists, in order to avoid misunderstanding and to ensure public support.

Biotechnology poses a number of unique challenges for politicians, scientists, policy makers and members of the public; sustainable progress will be possible only with the active collaboration of all these role players. There exists a real opportunity to discover new ways to produce novel and quality products within the context of sustainability issues that are beginning to pervade recent industrial thinking. In this context, issues such as producing biomaterials from renewable resources using biotechnology are at the forefront and present an invaluable opportunity for the sugarcane industry to ensure sustainability.

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