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Review

Biofuels – challenges & chances: How biofuel development can benefit from advanced process technology

The presented article highlights the process of biofuel production with a special focus on bioethanol. After a short introduction to the "problems" of biofuels – the "first generation" biofuels (in regards to their competition to feed and food production) and the "second generation" biofuels (in regards to the required more complex process technology) – the different steps in the process from natural resources towards the final product are presented and the underlying biotechnological challenges discussed: the pre-treatment of the natural resources followed by the biotechnological processes of hydrolysis and fermentation. Topics such as enzyme screening for efficient or even multi-step hydrolysis as well as microbial strain selection under process conditions and the optimization of the anaerobic fermentative conversion of the saccharides to bioethanol are discussed. Optimizing the production of bioethanol to be competitive with petrochemical fuels is the main challenge for the underlying process development.

Keywords: Biofuels development / Bioreactor technology / Process technology / Small-scale biofuel production

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1 Introduction

This article focuses on how advanced process technology can be beneficial for producing second generation biofuels, i.e. biofuels converted from non-food crops, which is more difficult compared to using food crops.

Since the global prices for crude oil reach one climax after the other, the search for alternative sustainable energy resources is getting more and more in focus bringing back the original fuels the first engines and cars were designed for. Otto and Ford, for example, engineered their inventions to run on bioethanol. The emerging petrochemical industry brought the market for bioethanol down later due to cheaper prices. For the same reason, corn, soy bean, rape seed, sugar cane and beet are becoming again very important today, as their conversion to the so-called first generation biofuels is getting economically more and more interesting.

The concept behind this development is to speed up the natural process of producing fuels from biomass of approx. 1 million years (via crude oil) to a more efficient time scale of 1 to 10 years with the help of optimized biochemical processes focusing on the "artificial" conversion of the biomass carbohydrates. The production of carbohydrates and its fermentative transformation has already led to a new industry with a steady increasing amount of biorefineries.

One negative result of "first generation" biofuel production is and will be the direct competition to the agricultural usage of farming for food and feed. Since in the EU up to 2020 10% of all used oil should result from renewable resources by law, it makes sense to use the produced surplus of all countries in the EU to fulfill this goal. But it is evident from investigations in the UK that even the complete surplus in wheat would result in petrol that could replace only 2.5% of the UK's petroleum consumption. Even if all the cultivable land lying idle for the last years due to European agricultural politics was recultivated, the desired 10% until 2020 would result in a dramatic import of wheat or a direct competition with alimentary products. The impact of this direct competition could be already seen in September 2006 when the prices for corn and wheat nearly doubled within 6 months (the economist 07/07, see also Fig. 1) causing big trouble in the Mexican population.

Another downside of the use of the aforementioned plants for "first generation" biofuel production is next to the disadvantageous growing of monocultures, the overall energy balance, showing even in the most optimistic calculation only a minor energy benefit in respect for the final product.

Therefore the trend leads towards the production of so called "second generation" biofuels, where literally any source

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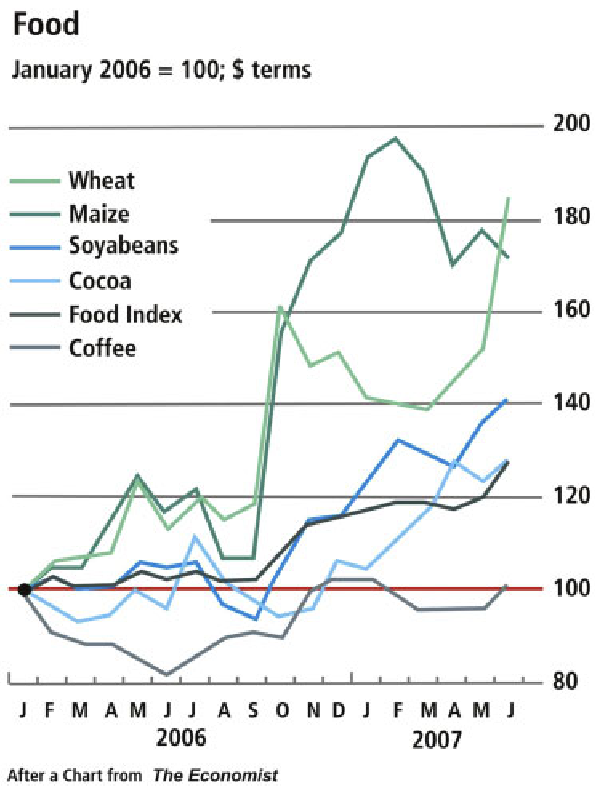

Figure 1. Increase of corn and wheat prices in 2006/2007 (Source: *The Economist*).

of organic carbon is used that is renewed rapidly as part of the carbon cycle. Biomass is all derived from plant materials but can include animal materials.

These resources are cheap and most of them need to be treated anyway for sustainable reasons. So why not take advantage of it?

However, today they are known for being less easy to utilize and therefore rather a cost-intensive source. To become a serious alternative for energy production, some issues have to be solved – and process technology can help to do this.

2 Pre-treatment and hydrolysis

Raw materials for "second generation" bioethanol like needles and spears cannot be converted directly to the desired product as the di- and monosaccharides are bound in the form of cellulose, hemicellulose and pectin which are themselves bound into the plant's scaffold. The first process step to crack those matrices and liquefy the raw material is called pre-treatment. Different techniques like hydrothermal cracking or steam explosion are under investigation to gain the optimal result. Those methods involve high temperatures and sometimes high pressures, and so their outcome has to be analyzed carefully regarding their effect on the subsequent hydrolysis and fermentation. Poisonous by-products might inhibit a later required enzymatic reaction.

In a next step, the mixture of different substrates within these resources needs to be transformed into biofuels to

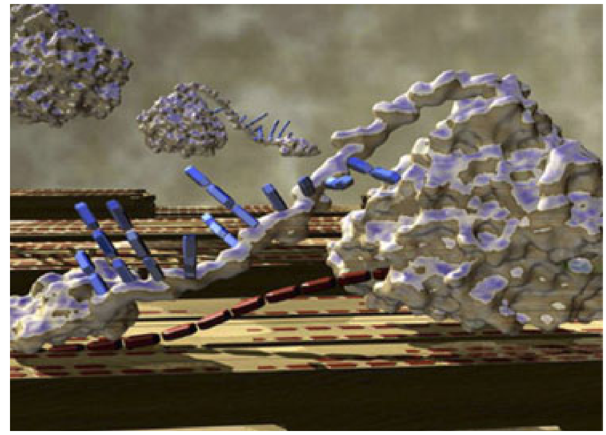

Figure 2. Illustration of an enzymatic interaction with cellulose (Source: *Laboratory for Industrial Microbiology and Biocatalysis, Faculty of Bioscience Engineering, Ghent University*).

achieve an efficient energy yield. The usable contents are first of all cellulose, hemicellulose, lignin and pectin containing chains of different carbohydrates in different ratios. To gain the optimum amount of biofuel, all these pentoses and hexoses need to be released from the crude mix. The so-called hydrolysis is the first step in the biotechnological processing. This process involves enzymes breaking down the chains of carbohydrates into monosaccharides and disaccharides that can be metabolized directly by different microbial systems (see Fig. 2).

One of the keys is to find either enzymes with a high affinity towards a variety of different raw materials or to construct them via biotechnological techniques such as directed evolution. An extensive screening for the capabilities of existing enzymes, the determination of the characteristics of newly found or designed enzymes needs to be carried out. Furthermore, the optimum process for the enzymatic reaction has to be defined. To evaluate the right conditions with the right enzymatic tools even side effects like inhibitions (by lignin or pectin) have to be taken into account. After a first screening with high throughput methods it is therefore necessary to include process parameters to the further selection of effective enzymes of strains: physical parameters such as pH, temperature and dissolved oxygen (or its absence) in the case of whole cells. Especially the first two parameters are well known to have a tremendous impact on the enzyme activities and therefore need to be carefully controlled.

3 Strain selection

As soon as the raw material is transformed to monosaccharides and disaccharides, the main transformation into bioethanol can take place. The most common saccharides are the C6 sugars glucose and mannose, and the C5 saccharides galactose, xylose and arabinose [1]. While hexoses are a main substrate for a great number of microorganisms such as *S. cerevisiae* *E.coli* and *Z. mobilis*, pentoses serve much more seldom as a source for their metabolism. While *E.coli* can metabolize

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pentoses it is not able to convert it to methanol while vice versa *Z. mobilis* or *S. cerevisiae* ferment the alcohol but are usually unable to use C5 as a substrate.

Therefore again, the screening for suitable strains is the main task on the way to an effective biofuel process. Research from Sassner et al. [2] has shown that the additional C5 conversion can drop the production costs of bioethanol by 20%. Once more, controlled and reproducible process parameters are of great use, as the fermentation of different microorganisms under the same conditions ensures finding the producing system with the best productivity. As the whole production process has to compete with existing petrochemical fuel generation, it is also of interest to find the most economical organism. For example, some organisms work at high temperatures or just within a small range of pH levels, which results in higher input costs and are therefore inefficient.

An efficient and thus profitable process on the other hand favors only the strains of interest and inhibits those causing contamination.

Precisely controlled low pH regimes, high substrate and product concentrations and strictly anaerobic conditions lead to the desired process and also ensure reproducible processes in large reactors. These conditions exclude many other strains that could interfere with the desired biofuel production and thus could decrease the product yield. The list of suitable microbial systems is therefore shortened by the unconventional fermentation conditions.

At this time, only yeasts such as *S. cerevisiae* (or bacteria such as *Clostridia*) are known for being able to stand such drastic environments. But the common yeast strains are not capable of using broad ranges of pentoses as substrates. To engineer these strains, researchers like van Maris et al. [3] have considered a number of pathways for a successful process: a cross-membrane transporter for the C5 saccharides has to be found, the enzymes need to be coupled to the glycolytic pathway, while the inner cell redox balance has to be closed. During the screening for these desirable functions, the researchers fall back on the broad set of wild type strains. In the next step, the selected strain needs to be engineered to utilize the broad range of hexoses and pentoses while inhibitions from lignin or other unfavorable substances in the fermentation broth have to be taken into account.

4 Process development

Having identified the most efficient enzyme for hydrolysis and a matching, productive and cost effective pair of "organism and ethanol", the process has to be developed by optimizing the production conditions in terms of costs, productivity and time. An essential part of this task is the monitoring and control of process parameters such as pH, redox potential/dissolved oxygen and temperature.

Especially the anaerobic conditions enclose different aspects: the widely used dissolved oxygen probe is not suitable to measure the necessary traces of oxygen in the medium. In this case, the redox sensor is the adequate tool to measure the chemically available oxygen in situ. As *S. cerevisiae* produces ethanol, only at a redox potential in between $-0.25\,V$ and $0.05\,V$, the product yield then needs to be optimized in this range. An additional redox control can only be reproducible in combination with a pH control as it is highly dependent on the acidity of the medium.

Simply stripping oxygen with sparging of nitrogen does not lead to the desired redox values as only a minimum of $-0.05\,V$ can be reached. The precise addition of reducing agents and/or oxidants will control the redox in the desirable lower levels.

5 Discussion

All the above-mentioned screening and process development steps require multiple experiments to closely examine the capabilities of enzymes, strains and processes. Process Technology effectively controlling physical parameters enables the researcher to compare the performance of different enzymes and producing organisms. Sequential experiments will consume a lot of time, as the result of a parameter variation will be clear only after the last experiment. Therefore, a parallel set-up could accelerate process development: Multiple fermenters will consequentially lead to higher throughput in media and energy, and the personnel workload rises as well. So the most useful system would be small and manageable in set-up and maintenance, while offering the same control strategies and precision as the later production plants to receive a reproducible and scalable process (as presented in Fig. 3).

Furthermore, integrating the different steps of pre-treatment, enzymatic break down of the raw carbon source, seed stage and alcohol production into one automated process and one set-up can save a lot of time and thus money. This could be achieved by integration of different vessels for each step in one control system, which ensures the modification of the fermentations conditions in these vessels to

Figure 3. The whole biofuel development procedure can be represented on a small scale by integrating the multi-step processes into one bench top bioreactor system (Source: *DASGIP AG*).

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meet specific monitoring and control requirements in the different steps.

Biofuels will remain one of the most exciting areas of today's biotech research for the next years. One reason is: It not only has to be environmentally friendly, but it also needs to become an economically reasonable contribution to today's energy generation.

Conflict of interest statement

The authors have declared no conflict of interest.

References

[1] K. Öhgren, Fuel ethanol production from corn stover: Optimization of steam pretreatment and improvement of simultaneous saccharification and fermentation, PhD Thesis, Department of Chemical Engineering, Lund University, 2006.

[2] P. Sassner, C.-G. Mårtensson, M. G. Galbe, Zacchi, Steam pretreatment of H2SO4-impregnated Salix for the production of bioethanol, *Bioresource Technol.* **2008**, *99*, 137–145.

[3] A. J. A. van Maris, D. A. Abbott, E. Bellissimi, J. van den Brink, M. Kuyper, M. A. H. Luttik *et al.*, Alcoholic fermentation of carbon sources in biomass hydrolysates by Saccharomyces cerevisiae: current status, *Antonie von Leeuwenhoek* **2006**, *90*, 391–418.

[4] J. Sridhar, M. A. Eiteman, Influence of redox potential on product distribution in Clostridium thermosuccinogenes, *Appl. Biochem. Biotechnol.* **1999**, *82*, 91–101.

[5] S. K. Darhod, Redox potential as a better substitute for dissolved oxygen in fermentation process control, *Biotech. Bioeng.* **1982**, *14*, 2123–2125.

[6] B. Hahn-Hägerdal, M. F. Gorwa-Grauslund, Biotechnology applied in the traditional process industry, *Proc. Illmac Basel* **2007**.

[7] W. Soetaert, White biotechnology: A key technology for building the biobased economy, *Proc. Illmac Basel* **2007**.

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