Replacement of a phase-out technology:

managing sophisticated processes with in-depth insight

by Martin Grolms and Prof. Mani Subramanian

Scientists at The Center for Biocatalysis and Bioprocessing (CBB) at the University of Iowa, USA have been carrying out research on bioprocessing technologies for more than 20 years. For the last three years their research has been facilitated by use of the parallel bioreactors described in this article.

It has been estimated that by the middle or late 21st century the dwindling supply of oil reserves will make extraction increasingly problematic. The effort and costs involved, and more importantly the environmental concerns, are increasing. We are all familiar with the major risks associated with extracting and transporting crude oil. These were recently illustrated by the oil catastrophe in the Gulf of Mexico.

The use of plants, and especially crops, as a source of raw materials is controversial. Critics maintain that it drives up global food prices. However, in light of the damage that results from the extraction and transportation of crude oil, it is generally accepted that the use of plants represents both a viable environmental and economical alternative. The concept of the biorefinery further ensure economic viability. Here the plants are fully recycled, with the different parts of the plant being utilised for different purposes.

Figure 1. DASGIP system for biomass and protein optimisation and reaction engineering.

From biomass to feedstock

Biomass is made up primarily of carbohydrates, lipids and proteins in different ratios. Numerous other compounds such as secondary metabolites can also be found, but mainly in smaller amounts. A variety of products and valuable compounds are derived through biorefinery and biotechnology processes. Among them are food and feed stuffs, basic and fine chemicals, and energy in the form of fuels, heat and even bioplastics.

To prepare the raw materials for bioprocessing, the biomass first has to be crushed, separated and isolated to the extract the useful components. The product range can be expanded by using different extraction methods. In addition to pyrolysis (thermal decomposition using high temperatures), biomass can be transformed via fermentation. Fermentation utilises bacteria, fungi and cell cultures or isolated enzymes to convert the raw materials into a desired product. The initiation and acceleration or modification of chemical reactions using enzymes as catalysts is termed biocatalysis. Enzymes are either isolated or utilised within living cells to catalyse chemical reactions.

The major advantage of using biocatalysis for organic synthesis is selectivity, which is a prerequisite for a high yield. Enzymes frequently react under mild conditions, and only one functional group responds under given reaction conditions. Products derived from biocatalytic processes are therefore often more pure and thus downstream processing is less problematic. By adapting the enzymes to the specific biocatalytic processes, the already enormous potential to significantly increase the yield is further improved.

Optimised processes yield improved cost-efficiencies

The production of first-generation fuels only used a small part of the plant, together with oil, sugar or starch. The use of biorefineries reduces the impact of diverting these food stuffs from the human food chain. To produce second-generation fuels, almost the entire plant is used, including the cellulose, which is difficult to break down. While these fuels are more energy- and resource-efficient, production requires a greater degree of technical complexity. Implementing optimised processes is the only way to ensure that production can effectively compete with production from conventional methods.

Producing ethanol from cellulose using biocatalysis is challenging. Until now, preprocessing has been too expensive and methods to reduce the cost of the saccharifying enzymes have been sought. The efficiency of saccharification of hemicellulose and cellulose, as well as the efficient utilisation of glucose and xylose, had to be improved. Finally, the cost effectiveness of energy production from the co-product and by-product had to be optimised and successfully scaled up.

Fermentation can produce bioplastics, completely new materials with properties that can be precisely defined. Today, polysaccharides such as starch and cellulose are the most common materials used for producing bioplastics. Polylactic acid (PLA) is created through the polymerisation of lactic acid, which in turn is derived by fermenting sugar cane and corn starch with lactic acid bacteria. PLA not only has properties similar to conventional thermoplastic mass polymers, it can also be processed without modifying existing equipment. Polyhydroxybutyrate (PHB) is a fermentation-based biopolymer with properties similar to polypropylene, a petrochemical plastic. Although PHB can be produced from sugar and starch, the synthesis requires other food-related substances such as glycerin and palm oil.

Organic compounds from biomass can also be fermented into ethanol in biorefineries. Ethene is derived through steam cracking, a standard petrochemical process, and can be polymerised to polyethene. As with biofuels, ethanol produced from biomass requires optimal process conditions and fermentation organisms to make production economically viable.

Bioprocessing at the University of Iowa

Scientists at The Center for Biocatalysis and Bioprocessing (CBB) at the University of Iowa have been carrying out research on bioprocessing technologies for more than 20 years. The principal areas of study include fundamental properties of biocatalysts, bioremediation, bioprocessing, discovery of new biocatalysts, novel biocatalyst applications and biosensing technologies. The centre has relied on parallel bioreactors from DASGIP since April 2007 to optimise biocatalysis, and for reaction engineering for biocatalytic processes [Figure 1]. This state-of-the-art bioprocessing laboratory is unsurpassed in a university setting. The CBB laboratory brings together the University of Iowa core fermentation facility and the microbial GLP and GMP pilot plant for conducting industry/university-related research and production. It is a first-rate facility for delivering scale-up solutions and training in biocatalysis and bioprocessing technology.

Iowa's heavily agriculture-based economy produces more corn and soybeans than any other state in the USA. As a result, Iowa also produces more bioethanol and biodiesel. This has put Iowa in great position for focusing on first and second generation biofuels. Research is also focused on generating basic and fine chemicals from corn stover and other agricultural residues. With this in mind, the CBB laboratory at the University of Iowa is developing technologies for renewables-based production of fine chemicals.

One example is a simplified process for the production of pyruvate using plant

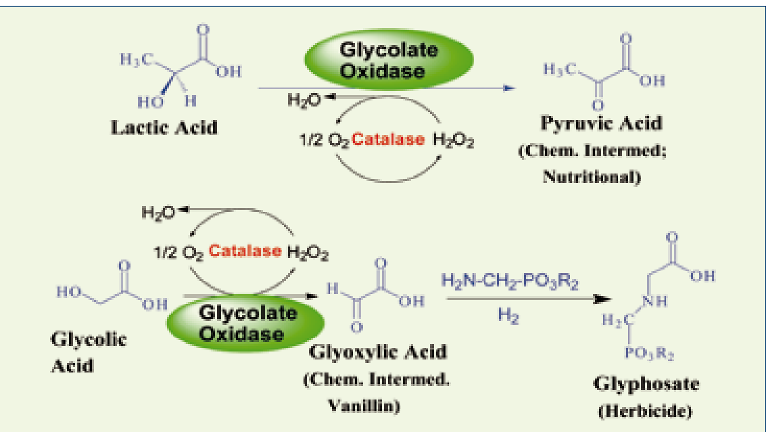

Figure 2. Glycolate oxidase (GO) now serves as a one enzyme one optimised process and several products platform. The process platform has been patented.

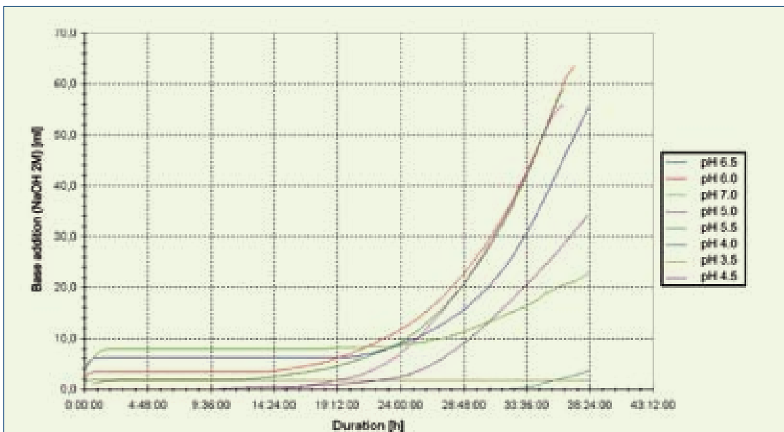

Figure 3. DASGIP parallel bioreactor systems are designed to meet the specific needs of process optimisation. Scientists can compare the efficiency of the key parameters, here different pH values are plotted from eight reactors in parallel.

enzyme glycolate oxidase (GO) cloned in *Pichia pastoris*. Under aerobic conditions, pyruvate can be converted stoichiometrically to lactic acid. The CBB team have reduced the GO-based production of pyruvate from a seven to a two unit operation. Reaction optimisation including enzyme loading, enzyme recycling, substrate loading and other mechanical parameters were carried out in DASGIP parallel bioreactors. This study was further extended for the production of glyoxylate, which in turn can be converted to glyphosate or vanillin [Figure 2].

Technology to meet the specific need

Modern technology enables scientists to develop bioplastics and biofuels that not only represent an environmentally-sustainable, but also an increasingly cost-effective alternative to petrochemical processes. DASGIP parallel bioreactor systems are the tools designed specifically to meet the needs of process optimisation. The DASGIP small scale system is perfect for strain development and screening. Media and conditions can be optimised with limited space and personnel. Each reactor can be completely independently controlled [Figure 3]. With the extended temperature range of 99°C and the excellent control, it is possible to pre-treat biomass for further processing.
The system provides an optimum environment for anaerobic fermentation, including specific measurement and control requirements. Key parameters such as pH, redox potential and gas levels such as methane are continually monitored. All of the complex processes can be completely automated. This makes it possible to screen in bioapplications using the wide variety of cost-effective saccharide enzymes. Scientists can compare the efficiency of different microorganisms under the same conditions or the same microorganisms under different conditions. The high density of information and parallel operation of up to 16 reactors optimises the process and speeds up the selection of the cells and the best process parameters. With minimal effort, laboratory systems can be scaled up to industrial size in one step.

Moreover, the wide selection of centrally-monitored and regulated parameters combined with the large number of parallel reactors makes the DASGIP system an excellent choice for carrying out statistical test planning.

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