The Application of Biotechnology to Industrial Sustainability

SUSTAINABLE DEVELOPMENT

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FOREWORD

At a meeting in Berlin on 30 May 2000, the Task Force on Biotechnology for Sustainable Industrial Development of the OECD's Working Party on Biotechnology (WPB) was commissioned to prepare a study which has resulted in the present publication. It is the logical extension of the Task Force's previous activities, which culminated in a major report, *Biotechnology for Clean Industrial Products and Processes*, which appeared in 1998.

This publication brings together a wide range of case studies in order to show how companies have implemented biotechnological processes and the means they have used to assess benefits in terms of cost and sustainability. The case studies were analysed to extract key messages, and, to make comparisons easier, they are presented in as uniform a format as possible. The report is intended for two key constituencies, senior managers in industry and government policy makers.

As industrial managers become more aware of what their colleagues have achieved, they may be encouraged to explore the possibilities of biotechnology; government policy makers may use the report as a basis for policy guidelines or for national programmes to underpin the expansion of industrial biotechnology.

This volume was prepared by Dr. Mike Griffiths (OECD consultant), whose efforts on behalf of the Task Force are greatly appreciated. He worked in close collaboration with an editorial team comprising: Dr. Anders Gram (Novozymes A/S, Denmark); Dr. Wiltrud Treffenfeldt (Dow, Germany); Dr. Ulf Lange (BMBF, Germany); Dr. Terry McIntyre (Environment Canada, Canada); Mr. Oliver Wolf (European Commission/JRC/IPTS, Spain). OECD support was provided by Dr. Salomon Wald (Head of Biotechnology Unit) and Dr. Yoshiyasu Yabusaki of the OECD Directorate for Science, Technology and Industry.

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EXECUTIVE SUMMARY

Background

In 1998, the OECD published *Biotechnology for Clean Industrial Products and Processes*. That volume set out many of the challenges for developing techniques to measure environmental friendliness and highlighted the potential contribution of various management tools. However, two major questions remained unanswered:

- Can biotechnology provide a cheaper option than conventional processes?
- Can economic gains and environmental friendliness go hand in hand?

The OECD Task Force on Biotechnology for Sustainable Industrial Development has continued this work, believing that:

- Biotechnology should be on every industrial agenda.
- Significant environmental benefits can be realised.
- Industrial sustainability is a key parameter when deciding on process development.
- There is an urgent need to reconcile economic, environmental and societal requirements in a sustainable development framework.

The present study seeks to answer these questions on the basis of the experience of a number of companies that analysed the potential of biotechnology and decided to adopt or reject a biotechnology process. It is based on a collection of 21 case studies, which are presented in a broadly similar format so that readers can easily compare one application with another. All the available cases have been taken into account, though not all reflect successful application of a new technology. Two major types of biotechnology applications are covered, the use of renewable resources ("biomass") and the use of biosystems (biocatalysts, enzymes) in industrial processes.

A very wide range of industrial sectors is represented: pharmaceuticals, fine chemicals, bulk chemicals, food and feed, textiles, pulp and paper, minerals and energy. The range of countries is also wide: Austria, Canada, Germany, Japan, the Netherlands, the United Kingdom, the United States and South Africa.

The principal audience of the volume is expected to be senior executives and members of company boards and government policy makers. One aim of the volume is to heighten the business community's awareness of biotechnology and the contribution it can make to the "triple bottom line",* by demonstrating what others have achieved and providing a process assessment tool to focus their decision-making process. For policy makers, it seeks to provide a basis for expanding the role of biotechnology and supporting the development of national R&D and technology transfer programmes targeted at sustainable development. The assessment tool provided, the Green Index, has a shortlist of key questions to be answered in any comparison and could be used by government authorities as part of R&D assessment.

* See Shell's recent *Contributing to Sustainable Development – A Management Primer*, available from their library Web site: *www.Shell.com*.

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Findings from case studies

As the case studies make clear, biotechnology does not necessarily always offer the single, best route; sometimes it may be most effectively used as one of a series of tools or integrated into other processes. However, the studies show that the application of biotechnology invariably led to a reduction in either operating costs or capital costs or both. It led to a more sustainable process, a lowered ecological footprint in the widest sense, by reducing some or all energy use, water use, wastewater or greenhouse gas production.

The case studies suggest that decision makers regarded environmental friendliness as secondary to cost considerations, but it is sometimes difficult to separate the two, since the reduction of an input usually means a reduction in cost as well.

Environmental legislation can be a driver for change, and legislative changes may widen the use of biotechnology. Without external pressures, environmental improvements alone are unlikely to lead companies to change their production processes.

At the outset, it was thought that most decisions would be based on analytical processes similar to life cycle assessment. In practice, the decision-making processes were as varied as the companies involved. Therefore, an attempt has been made to document these different processes.

Companies rarely became aware of biotechnology and subsequently adopted it in a systematic way. Biotechnology skills were often acquired by partnering with another company or an academic institute. Once the skills were in place, lead times improved significantly for subsequent developments.

Government policy-makers can tip the balance of risk-taking, for example by developing a sustained, stable legislative base, offering financial incentives for improved sustainability and providing R&D funding for bridging the enabling disciplines.

R&D funding for sustainable development needs to be looked at carefully since, in many cases, it is spread over more than one ministry. A further key role for government is in the field of multidisciplinary education, particularly for engineers.

Conclusion and future directions

This publication takes a number of steps forward in the debate on industrial sustainability. It produces hard evidence on the links between the two roles of biotechnology – environmental friendliness and economic gains. It also gives a more precise picture of how decisions to adopt these new technologies are made by industrial managers. The opportunities and constraints created by policies on industrial sustainability are better understood.

All the case studies point to a future in which the use of renewable resources and the new biotechnological skills, such as functional genomics and pathway engineering, will enable the manufacture of materials, chemicals and fuels in cheaper, more environmentally friendly ways and thereby improve levels of industrial sustainability and quality of life generally.

The next few years will see a number of major plants producing industrial materials and chemicals from renewable sources, as well as the incremental incorporation of bioprocesses into a wider range of industrial manufacturing. Any future publication on this topic should thus have a much wider range of cases on which to base its analysis.

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Chapter 1

BACKGROUND AND AIMS

Introduction

For many years, the OECD has been a focal point for the development of risk assessment procedures and the assessment of biotechnology's potential to contribute to industrial sustainability. In 1998, the OECD's *Biotechnology for Clean Industrial Products and Processes* (BCIPP) identified life cycle assessment (LCA) as the tool with the greatest potential to provide a disciplined, science-based approach to measuring the benefits, environmental or otherwise, of alternative industrial processes. However, although LCA offers great promise, the environmental and social issues peculiar to biotechnology require special consideration. Although ethical issues, risk assessment and the economic aspects of decisions are not strictly part of an LCA, any analytical tool, if it is to be useful, must address these issues. Moreover, although LCAs may be considered helpful, they are used infrequently, are felt to be too complicated and to require data that is difficult to obtain.

An OECD task force which continued the work on sustainable biotechnology has become aware of other comparative analyses in this field not necessarily based on LCA principles. Those assessments in the public domain can be loosely divided into two groups: those undertaken by consultants or academics to examine more closely certain environmental problems, and those undertaken by companies as part of a comparative analysis of process development. Some of these may have led to capital investment or R&D planning decisions; others may have been used to seek approvals or grants from government agencies.

Both groups of assessments were undertaken in ways that suit the needs of their individual authors. No analysis appears to have been made of their more general policy implications, nor have they been brought together as case studies in such a way that decision makers, whether in industry or government, can easily compare the different applications.

Case studies

The task force established a project to bring together as wide a range of these assessments as possible, in order to provide examples of how companies have approached the problem of making choices. Its aim was to examine the data-collecting and decision-making steps employed by the companies when adopting or rejecting biotechnological processes in cases where they have (or have not) replaced more conventional physico-chemical ones. The project's results are presented in this publication.

In all the task force collected 21 examples for which companies were prepared to make sufficient data publicly available to yield a reasonable analysis. While they do not represent a representative sample in a statistical sense, they do cover a broad range of industrial sectors and many OECD countries. The preparation of the cases would not have been possible without considerable assistance from personnel in the companies concerned and their help is greatly appreciated. The companies concerned have approved the case studies, but comments on them and inferences drawn are those of the author(s) alone.

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Table 1 gives a breakdown of the cases by sector and country:

Table 1. **Cases by sector and country**

Industry sector	Pharma-ceuticals	Fine chemicals	Bulk chemicals	Food and feed	Textiles	Pulp and paper	Minerals	Energy
Austria						1		
Canada						2		2
Germany	2			1	1			
Japan		1	1			1		
Netherlands	1			1			1	
South Africa							1	
United Kingdom		1	2					1
United States			1					

In spite of the evidence offered in this report, biotechnology does not inevitably offer the best solution. It may best be used as one of a series of tools and as an integral part of other processes. The comparative analysis recommended here may well reveal strong support for non-biological approaches (see Box 1). BASF, for example, has chosen to make indigo via a chemical synthesis rather than a bioprocess on the basis of a detailed eco-efficiency analysis. Also, ongoing research into inorganic catalysis provides strong competition. It is also the case that choice of a renewable feedstock does not of itself guarantee sustainability. This is particularly true if fossil fuels are used during the manufacturing process (see the annex to Chapter 2 on bioethanol). Also, oil, rather than biomass, may be a more economical source of complex monomers.

Box 1. **The role of alternative technologies**

No single technology can give economic access to a full range of new products and thus attempting to fit a favoured technology to a molecule or customer need is therefore probably ill-judged. This is particularly true of chiral technologies where the pace of development in the international academic community is such that any new technology is rapidly supplanted.

For a new molecule entering the development phase, the need to produce kilo quantities quickly may outweigh economic considerations. It follows that the manufacturing process used at this stage may be modified in the course of optimising against other parameters, such as economic cost.

For example, Avecia Life Science Molecules aims to have a chiral "toolkit" incorporating both biotechnology and physico-chemistry and a range of academic collaborations so as to remain up-to-date. An adjunct to the toolkit is the ability to make rapid evaluations of technical options. In some cases, the optimal development path may include helping customers use elements of the toolkit in their own laboratories.

In a recent development of a chiral intermediate for an US-based pharmaceutical company, three alternative approaches were used: biocatalysis, asymmetric hydrogenation and crystallisation, all of which gave the product of acceptable quality. The enzyme process was used to make tens of kilograms for early supply, but one of the other processes is likely to be chosen as the final manufacturing process.

Source: Avecia, United Kingdom.

The audience

Two distinct audiences, with distinct and separate needs, are addressed here: industrial policy makers (senior management) and government policy makers.

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The case studies are presented in a reasonably uniform format so that both managers and policy makers can easily see how they relate to each other. The analysis draws out the internal processes that lead to a decision and examines the technical and analytical methodologies used. It identifies the key issues and lessons to be drawn from the examples, the decisions for which they were intended, how they met the needs of the originators and how decision makers responded. Not all of the cases are success stories – failures demonstrate some of the obstacles to adoption of new technologies and therefore add to the value of the analysis.

This publication seeks to make company managers aware of what has been done and to show that adoption of biotechnology can have quantitative benefits. Managers are encouraged to look at the cases, to use the analytical tools suggested or develop their own, and to identify the analogies between the cases and their own activities. This should make them more comfortable with the idea of using biotechnology; it should also show how they might compile new case studies both for internal use and in order to demonstrate to the wider public the "sustainable" characteristics of their company.

Biotechnology can be used to increase the sustainability of industrial processes and to encourage a shift in companies' emphasis from end-of-pipe clean-up to inherently clean processes. Several examples show companies moving back up the pipe by, for example, introducing closed loop systems. This is a smaller step than replacing chemical conversion with biocatalysis but still offers a useful lesson.

These case studies make it possible to illustrate analytical techniques that may be of use both to industrial managers and government policy makers. To this end, a simple tool for prior assessment of the environmental impact of two alternative processes is described. It is intended to identify the key sustainability parameters and provides an easy checklist for assembling the facts of the alternatives in comparable form.

This publication shows decision makers in government how forward-looking managers (the "early adopters") have considered risks and advantages before acting. They can then use examples presented to make a wider range of industries aware of the advantages of biotechnology. They may better see which are the key issues that make or break an individual development, learn what they can do to ease the climate for more sustainable processes and be encouraged to design policies that support these decisions. The analyses are intended in part to support guidelines for the development of national programmes and to allow individual countries to derive material relevant to their particular needs. Governments can thus catalyse the spread of biotechnology: as companies see their peers adopting, they will become more confident themselves.

Examples of what can be done by governments are given in Box 2. These examples can be repeated with variations in many other countries.

This publication seeks to assist company decision makers through the individual stages of this process because it is felt that:

- In the first place, biotechnology should be on every industrial agenda.
- Environmental aspects and customer perception issues related to a sustainable choice should be high on the list of parameters.

In addition, it seeks to encourage policy makers to:

- Translate environmental benefits for society into economic benefits for companies by rewarding good or punishing bad environmental performance.
- Establish a clear and stable legal and political environment in which the biotechnological alternative has an equal opportunity to be taken up.
- Educate the general public to understand the risks and benefits of industrial biotechnology.

One limiting factor for confirming the potential of biotechnology is the absence of a scientifically validated technique for measuring its overall long-term sustainability. Joint government-industry action to meet this need is essential to encourage consumer and public confidence in the resultant technologies and, ultimately, to ensure the successful development and industry acceptance of the next generation of bio-based and cleaner industrial products and processes.

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Box 2. **Examples of programmes and initiatives**

United Kingdom. The BIOWISE Programme of the UK Department of Trade and Industry (DTI) aims to support the development of the UK industrial biotechnology sector and to stimulate the use of biotechnology processes to improve the competitiveness of UK manufacturing industry. It estimated that it has identified over 70 000 UK manufacturing companies that could potentially reduce costs and improve profitability by using biotechnology. However, many companies view biotechnology with caution and are unaware of its growing use in manufacturing. On completion of the study, the case studies in this report will be disseminated to UK companies in order to help them address this knowledge gap. Case studies of relevance to the chemical sector will in addition be disseminated to industry via the Specialised Organic Chemicals Sector Association's Emerging Technologies Group.

The United Kingdom's Faraday Partnership initiative is aimed at promoting improved interactions between the science, engineering and technology base and industry. The newly formed Pro-Bio Faraday Partnership seeks to maximise commercial benefits from biotechnology and has identified three core themes: discovering and developing new biocatalysts; developing integrated production processes and designing and modelling new and improved processes.

The DTI proposes to use the case studies and assessment framework report to help advance the research, development, demonstration, assessment and uptake of biotechnology for cleaner products and processes. In addition, the policy implications will be fed into DTI's wider debate on sustainable development.

Belgium **:** The Flemish Institute for Technological Research (Vito) develops and evaluates new industrial technologies for effluent water treatment and decontaminating polluted soils and sludge. In this research domain, Vito provides companies with objective consultancy on the introduction of environmentally friendly production and management techniques and assistance with solving environmental problems. Vito may be a conduit for bringing the case studies to a wider audience in Belgium.

United States: During 1999 and 2000, the US Government articulated a comprehensive "Bioenergy Initiative" to accelerate the development of technologies for using renewable carbon as a feedstock for the production of power, fuel and products. The intent is to create a carbohydrate economy to replace part of the fossil fuels used for these sectors. In 1999, President Clinton signed an executive order, and in 2000 the Sustainable Fuels and Chemicals Act, an integrated policy to stimulate R&D on renewables and biofuels, was signed into law. The Act authorised spending USD 250 million over five years on R&D. It also established a technical advisory committee to provide strategic leadership, advise federal agencies and the congress on the priorities for R&D spending and foster co-operation between the Departments of Agriculture and Energy.

Because a company's performance is no longer judged by financial results alone, it is felt that environmental assessment should be applied to all products and processes, large or small, in companies of all sizes. All stages of a product's or process' life cycle may affect the environment. Consequently, the design of industrial processes must take into consideration everything from choice and quantities of raw materials utilised to reuse of wastes. Environmentally friendly processes will consume less energy and raw materials and markedly reduce or even eliminate wastes. As this publication demonstrates, biotechnology is capable of providing tools that help achieve these goals and, in the process, ensure that industrial sustainability is in fact being achieved.

Sustainable development

In the 1970s and earlier, sustainability was one-dimensional – it was equated with the profit necessary for a company's long-term survival. Later, environmental concerns were added, and, in the 1990s, a third dimension – societal concerns. Hence the "triple bottom line". A valuable description of what is meant by this three-part approach is contained in *Contributing to Sustainable Development – A Management Primer*, recently published by Shell and available on their Web site (*www.Shell.com*).

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More and more companies are adopting the principles of sustainable development in their everyday activities and see that doing so does not generate extra cost but can be an economic advantage (see Box 3). Environmental considerations are thus not being addressed in isolation but are becoming part of a business's economic and social aspects.

Box 3. **Shell's approach to sustainable development**

Many still question the wisdom of striving to integrate the principles of sustainable development into the way we do business. Sustainable development requires us to think about more than just how much money we will make today, but to take a broader view and balance the long term and the short term. We place the emphasis on the balance between the short term and long term, as well as on the integration of the economic, environmental and social aspects of our business. For us sustainable development applies to everyday choices we make like how we dispose of our waste as well as to large regional projects.

Because sustainable development means taking a broader, more integrated approach to our business it opens up exciting business opportunities in emerging markets and new customer groups. Sustainable development is a way of developing and safeguarding our reputation, and it will help us develop our businesses in line with society's needs and expectations.

Shell chairman Sir Mark Moody-Stuart said in a recent speech:

"As you seek to build your business, standing – as it were – on [a] stool, each leg must be in place if you are to build on a sustainable foundation. The truly sustainable development of a society depends on three inseparable factors: the three-legged stool.

"The first leg is the generation of economic wealth, which companies deliver better than anyone else. The second is environmental improvement, where both government and the company have to play their role. The third leg is social equity. Companies have a role to play here, but the main responsibility rests with civil society as a whole, including government. The balance between these three legs is the key.

"Excellent environmental performance is meaningless if no wealth is created. Wealth in a destroyed environment is equally senseless. No matter how wealthy, a society fundamentally lacking in social equity cannot be sustained."

Source: Adapted from *The Shell Report* 2000.

Nevertheless, according to a recent survey by the environmental and engineering consultancy, Entec, industry still lacks a clear understanding of the meaning of sustainable development. From 104 companies surveyed in seven industrial sectors in the United Kingdom, including pharmaceuticals and oil and gas, 45% of directors and chief executives had not heard of sustainable development. Over three-quarters (78%) of respondents thought that pressure for sustainability was coming from regulators, an indication that any moves towards sustainable development are likely to be compliance-driven; 41% felt that the result of sustainable development would be more costs and additional work.

The problem of management education, identified in previous OECD reports, is still one to be faced today. As one interviewee put it, "Sustainability may well be understood at the top levels in big companies – the problem is application and middle management has other objectives. The average manager in a pulp and paper mill, for example, joined at 18-23 with, perhaps, a bachelor's degree, worked in the plant for the whole of his life and is now 53 and only uses his own practical experience gained over the last 30 years. A worry he has is continuity of production process – he doesn't want to report to the board that there have been production problems because of the introduction of new technology."

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Decision making

When an industrial company decides to design and implement a biotechnological process to produce an existing or a novel product, the decision is taken at a crossroads, where many different information streams converge and from which a company may follow one of several alternative routes. The implementation of sustainable biotechnology solutions has been slower than it might have been partly because real-life experience of its application is only slowly acquired by and disseminated among companies. One reason is that the shift to a biotechnology solution appears to the industrial manager to have large economic implications and large associated risks.

A steady stream of innovations is emerging from academia, but these will not necessarily be taken up by industry unless it is clearly demonstrable that they have a cost advantage. Cost reduction can be direct (lower material and/or energy inputs, waste treatment costs, reduced capital expenditure) or indirect (lower risk to the general public, lower obligations in terms of eventual clean-up, contribution to reduced global pollution levels, downstream recycling).

The decision to design and implement one manufacturing process rather than another is always a complex one involving many parameters and is almost always taken on the basis of a less than ideal data set. Environmental benefits alone are not a sufficient incentive for adopting biotechnology. Decisions are much more influenced by economic considerations, company strategy and product quality. In its approach to such a decision, a company needs to decide which parameters to take into consideration: economic (cost of production, investments, etc.), occupational health, regulatory aspects (product approval), environmental, customer perception, company profile and values and many others. It must then gather the facts together, making sure that it has access to comparable data for the alternative processes.

The larger the economic impact, the more complete the required data set is likely to be, simply because a decision with a larger economic impact merits a more thorough analysis, often through conceptual design or exploratory scientific projects to investigate the possibilities and consequences of different alternatives. Although the costs may be assessed reasonably easily, benefits may be more difficult to measure, especially if the company is unfamiliar with the proposed technology and appropriate tools are lacking to allow a reliable assessment of the advantages and disadvantages of the new process.

An essential rationale for the use of biotechnology in industrial processes is that it is thought to bring greater sustainability and lower environmental impacts. However, this raises the joint problems of how to demonstrate that these changes actually occur and how to compare alternative processes while they are still on the drawing board. Ultimately what is required is a framework or methodology, preferably internationally accepted, to evaluate biotechnology and bioprocess technologies with respect to economic and environmental costs and benefits (*i.e.* their contribution to industrial sustainability).

By its very nature, the use of biotechnology, and especially of renewable raw materials, gives rise to a number of specific problems. Factors such as the use of a dedicated crop for manufacture rather than food use and the effect of widespread monoculture on biodiversity need to be considered. Any detailed analysis may need to include production inputs to agriculture such as seeds, fertilisers, pesticides, cultivation, crop storage and farm waste management.

While environmental sustainability is only part of decision making, alongside economic and operating considerations, it is likely to be sufficiently important to be examined on its own. With easier access to positive facts-based stories and with access to a simple “what-if” tool to assess the environmental impact of process alternatives, it becomes easier to demonstrate the viability of the biotechnological option.

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Chapter 2

INDUSTRIAL USES OF BIOTECHNOLOGY

The applications of biotechnology fall conveniently into two distinct groups:

- The replacement of fossil fuel raw materials by renewable (biomass) raw materials.
- The replacement of a conventional, non-biological process by one based on biological systems, such as whole cells or enzymes, used as reagents or catalysts.

Enzymes in this publication are recognisable by the fact that their names invariably end in "ase" (for example, lipase or cellulase). The names of specific micro-organisms are given in italics, *e.g.* B*acillus subtilis*).

Renewable raw materials

Use of renewable resources is very closely bound to the price of the fossil raw materials they might replace and suffers when oil is relatively cheap. Nevertheless, a number of strategic developments, especially those sponsored by the US Department of Energy, are taking place.

For some time, there has been increased interest and very substantial research in the production of chemicals using renewable feedstocks, particularly in the United States. In addition to the environmental attractions of using renewable resources, this has been driven by concerns about the dependence on imported oil. The United States is rich in the supply of renewable agricultural feedstocks, such as corn, which can be used to produce low-cost starch raw materials.

Living plants can be used to manufacture chemicals such as lactic acid, lysine and citric acid on a commercial basis. A novel approach to making plastics is to have the plant either produce the raw materials or, more radically, to make it grow the finished product. In 1999, a team at Monsanto used rape and cress plants to synthesise a biodegradable plastic of a type known as a polyhydroxyalkanoate (PHA) by adding bacterial genes from a bacterium, R*alstonia eutropha*, chosen because it produces high levels of PHAs, into their experimental plants. While bacterial PHAs are too expensive to be commercially viable, those produced in plants should be cheaper. Monsanto has shelved this project, but it is still being pursued by Monsanto's former partners at the University of Durham, England, and the University of Lausanne, Switzerland. In addition, Metabolix (Cambridge, Massachusetts) recently purchased the assets from Onsanto in order to expand its PHA products. BASF has also looked at a related material, polyhydroxybutanoic acid obtained from transgenic canola (rape); although it is competitive with polypropylene on an eco-efficiency basis, the net present value was regarded as too low and the scientific risks in development were seen as too high.

The development of polylactides offers a good example of a new process based on renewable resources. Polylactides are biodegradable plastics with positive properties for packaging applications. They are made by the polymerisation of a lactide that is produced from lactic acid. For many years, lactic acid has been produced by both fermentation and chemical routes. Recently, developments in the fermentation process and particularly in downstream recovery appear to have given the bioprocess an overall economic advantage as well as the environmental benefit of being based on renewable raw materials. Cargill Dow Polymers (CDP) has announced the construction of a plant to produce 140 000 tons a year of polylactide using lactic acid produced from corn by fermentation. The plant is scheduled for completion in late 2001 (see Case Study 9).

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To compete with polyester and other conventional petroleum-based polymers, Cargill Dow is locating its commercial-scale plant next to a low-cost supply of dextrose: Cargill's corn wet-milling complex. Cargill Dow will ferment Cargill's dextrose to pure chiral isomers of lactic acid, a conventional fermentation route impossible with chemical synthesis, and then chemically crack the lactic acid into three chiral isomers of lactide. Finally, the lactides will be combined in various ways to generate a range of polymers.

Relying on dextrose ties bioprocesses to corn wet-mills in North America and, in Europe, to wheat processors, but the ability to use a wider range of sugars is developing rapidly. Cargill Dow is exploring novel processes that would allow the use of feedstocks that are cheaper than dextrose, a capability that would cut the cost of making PLA as well as novel products. Cargill Dow's next plant will not be so limited. The enzyme-converting technology and the ability to adjust fermentations to use a wider variety of sugars have all advanced to the point where corn wet-mills will not be needed.

Processing technology is already available to use sucrose from sugar cane, which costs about USD 0.03/kg compared to USD 0.05-0.06/kg for dextrose. Corn fibre, which corn wet-mills sell locally as animal feed for as little as USD 0.01/kg may be the next major raw material in the United States. Corn fibre consists of a range of five- and six-carbon sugars, but R&D on bioprocesses to ferment these sugars is being developed.

Farm groups in the United States believe PLA to be an important new market, given slumping commodity prices and concerns over the safety of genetically modified foods. Although Cargill Dow Polymer's process uses fermentation, it does not depend on transgenic organisms because many micro-organisms already have the capacity to make lactic acid.

In 1995 the US Department of Commerce approved funding for a USD 30 million five-year research project to develop continuous biocatalytic systems for the production of chemicals from renewable resources. The project consortium, led by Genencor, also included Eastman Chemical Company, Electrosynthesis Company, Microgenomics and Argonne National Laboratory. There are signs that this project is beginning to yield results. Eastman and Genencor have announced plans to commercialise a new process to produce ascorbic acid using a specially engineered organism.

Genencor and Eastman Chemical, which holds a 42.5% stake in Genencor, have developed a one-step fermentation for the ascorbic acid intermediate 2-ketogluconic acid from glucose, which replaces four steps in the conventional synthesis. Two years ago, the firms declared their intention to commercialise the ketogluconic acid bioprocess, and they expect to begin the engineering work next year. Capital costs are estimated to be half of those for the existing process, and low costs might also open up new markets (*e.g.* use of ascorbic acid as a reducing agent). It should be noted however, that during the period of development there has been a significant reduction in the price of ascorbic acid.

Genencor has also been collaborating with DuPont on a bioprocess for the production of 1,3 propanediol (PDO) directly from glucose. The bacterium used as catalyst incorporates genes from two different organisms. Significant progress has been made to improve the productivity of the fermentation and the associated downstream processing operations.

DuPont formed a joint venture last year with Tate and Lyle Citric Acid, a subsidiary of sugar producer Tate and Lyle (London), to demonstrate the feasibility of DuPont's bio-PDO process on a large scale. The firms have already started a pilot plant to produce 90 000 kg/year of bio-PDO at Tate and Lyle's subsidiary, A. E. Staley Manufacturing's corn wet-mill in Decatur, Illinois. The firms plan to begin producing bio-PDO on a commercial scale by 2003. Meanwhile, DuPont is using chemically synthesised PDO to build a market for the PDO-based polyester polytrimethylene terephthalate (PTT), which the company markets as Sorona.

DuPont predicts that lowering the cost of PDO will broaden the commercial appeal of 3GT, a polyester copolymer of PDO and terephthalic acid, and also make PDO an attractive feedstock for polyols used in polyurethane elastomers and synthetic leathers.

ChemSystems reviewed the alternative processes for PDO in late 1998 and concluded that the biological route could compete with petrochemical routes if it was back-integrated to glucose

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production from corn. DuPont says further improvements have taken the process "well beyond the most optimistic case described in that study".

DuPont hopes that bioprocesses will enable it to produce compounds that are currently beyond the reach of industrial chemistry and has a wide range of industrial biotech R&D projects under way.

The company, in addition to internal projects, has a number of other projects as part of a USD 35 million, five-year alliance with the Massachusetts Institute of Technology. DuPont says it is in the process of selecting a follow-up project for large-scale development now that bio-PDO is well on the way to commercialisation. For example, it has engineered another biocatalyst for a different polymer intermediate, dodecandioic acid, which is produced directly from dodecane.

Since the late 1970s, a number of countries have been involved in the manufacture of liquid fuels based on plant raw materials. Production of bioethanol continues on a large scale in Brazil and the United States, with more recent interest in Canada (see the annex this chapter) while a wider range of countries are exploring the potential of biodiesel.

In March 2000, the US Department of Energy announced a tripling of its budget, to USD 13 million in 2001, for its bio-feedstock programme. Companies such as Dow Chemical, DuPont, Great Lakes Chemical, Eastman Chemical and Rohm and Haas are part of the programme. The programme's aim is to increase substantially the number of chemical processes using bio-feedstock and could lead, according to the Department, to a reduction of tens of millions of tons of greenhouse gas emissions.

The Biomass Research and Development Act passed by the US Congress last year allows the US Department of Energy (DOE) to place equal emphasis on biomass as a source of raw sugars for chemicals and on lowering the cost of bioethanol fuel. DOE expects enzyme producers to lead the cost improvements. In particular, cellulase costs must fall tenfold, from USD 0.30-0.40/gallon of ethanol produced to less than USD 0.05/gallon, before biomass conversion becomes profitable for large-scale ethanol production. In 1999, DOE signed three-year contracts with Genencor and Novozymes (USD 17 million and USD 15 million, respectively) to achieve those cost improvements. Like Iogen in Canada, Novozymes and Genencor make cellulase enzymes for textile and pulp processing. Novozymes will try to make currently known cellulases more active but will also search for novel enzymes that could assist the process. The intention is to genetically engineer all of the necessary steps into a single organism.

Crop enhancement may eventually cut the cost of making a wide range of chemical products. Several firms are seeking to make high-value proteins in crops. Prodigene, for example, has developed a corn variety with the genes for avidin, an egg white protein used in medical assays. The company intends to commercialise another protein, a bovine protease inhibitor, aprotinin, used to prevent protein degradation during cell culture. Large-scale production in corn can greatly lower the price, since adding capacity is relatively easy. Prodigene is also working with Genencor to make industrial enzymes in plants. The companies are particularly hopeful about applications in which an enzyme-enriched plant could be added directly to an industrial process, eliminating costly purification steps.

Bioengineering of crop plants will improve the markets for oils and fatty acids. DuPont, Monsanto, and Dow are all marketing vegetable oils enriched in oleic acid. Crop developers hope to manufacture speciality oils for industrial applications, though limited funding for product development and higher-than-expected costs are slowing development.

DuPont is exploring application of its high-oleic soybean oil, which can be chemically epoxidised to form nine-carbon diacids for plasticisers, and has cloned the genes needed to epoxidise fatty acids into the plant. It has also cloned the metabolic machinery to conjugate fatty acids for coatings or hydroxylate them for lubricants.

Monsanto has engineered rapeseed oil for industrial uses, enriching the oil with lauric acid for surfactants, myristate for making soaps and detergents and medium-chain fatty acids for lubricants. However, Monsanto has given these applications low priority in order to concentrate on health and pharmaceutical applications. The spin-off of Monsanto's agro-business, following a planned merger with Pharmacia and Upjohn, could restart the project.

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DuPont believes that production in crop plants is inevitable, because their feedstocks, carbon dioxide and sunlight, are essentially free. At the same time, biotech firms such as Maxygen say there is plenty of room to improve and extend enzymatic catalysis and fermentation.

Bioprocesses

Although enzymes have been used on an industrial scale, in detergents for example, since the 1950s, full acceptance of their role in biocatalysis has been more recent, with the lead coming from the fine chemicals industry. Many of the drawbacks perceived by process engineers, such as low yields and throughput, high dilutions, limited enzyme availability and low enzyme stability, have largely disappeared. It is now accepted that water may be a suitable medium for industrial processes while at the same time enzymes are being modified in such a way that they can be used in the organic media with which chemists are more familiar.

The advantages of bioprocesses are generally thought to be that they operate at lower temperature and pressure, while chemical processes require harsher conditions, and that enzyme catalysts are biodegradable after use but inorganic catalysts are more difficult to dispose of. However, bioprocesses do not always have advantages over their chemical alternatives and it is necessary to determine which process performs better on the basis of a careful examination of the merits and demerits of each.

A wide range of reaction types – oxidations, reductions and carbon-carbon bond formation, for example – can be catalysed using enzymes, and perhaps 10% of all known enzymes are available on an industrial scale. These may be used as free or immobilised whole cells, crude and purified enzyme preparations, bonded to membranes or in cross-linked crystals. Many are based on recombinant organisms.

The potential for discovering new biocatalysts is still largely untapped, since 99% of the microbial world has been neither studied nor harnessed. Recognised through their DNA sequences, members of the Archaeal and Eubacterial domains are expected to provide biocatalysts of much broader utility as this microbial diversity is further understood.

Two quite different approaches to novel enzymes exist, each with its supporters. One is the rational design approach, whereby knowledge of existing protein structures is used to predict and design modified enzymes. The second is forced evolution, in which many mutations and recombinations are made and screened for selected properties. The combination of these techniques, together with detailed sequencing of the genomes of a range of organisms, is giving rise to tailored microbes capable of producing many new and existing products for which only chemical routes have previously been available. Gene shuffling, in which DNA is denatured and then annealed in novel recombinations, can give unexpected results. For example, starting with 26 sources of a protease enzyme, shuffling has given rise to a library of 654 variants, 5% of which are better than the best parent. In another case, shuffling produced a progeny enzyme with properties possessed by *none* of the parents, in this case a heat-stable lipase. In the most exciting example to date, the genes for just two enzymes differing by only nine amino acids were taken, and in the recombinant library produced from these, there were enzymes with activities increased by two orders of magnitude and some entirely novel catalytic activity.

The combination of renewable raw materials *and* a novel process can have important economic advantages (see Box 4).

As most, if not all, novel technologies go through a typical S-curve in their development, it should be appreciated that industrial biotechnology is still near the foot of its growth curve. As chemical products become more diverse, the synthetic trend is shifting from stoichiometric synthesis towards using the complexity of biological systems – moving from biocatalysis and biotransformations to direct fermentation (metabolic pathway engineering) and the industrial applications of "biosynthesis on a chip" and from single synthetic steps to cascade catalysis in which a number of enzymes act in concert, without the need to add and remove protective groups.

In the next few decades, the DNA of all industrially important micro-organisms and plants will be sequenced and their gene structures defined, thereby allowing metabolic pathways to be optimally

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Box 4. **Lysine feed additive**

Midwest Lysine LLC, a joint venture between Cargill and Degussa-Hüls, has built a plant in Blair, Nebraska (United States) to produce 75 000 metric tonnes per year of the amino acid lysine. Based on dextrose as raw material, the lysine will be used as a feed additive to increase the nutritional value of plant proteins.

Lysine has been produced for many years by fermentation, using *Coryne-* or *Brevibacteria*. The conventional product is L-lysine-HCl, which is produced by a multi-step process. When Degussa decided to become a producer, it realised that the "conventional" process would be very expensive, because of the large amounts of waste and bacterial biomass produced as by-products and because of the loss of product during downstream processing.

A new product, Biolys®60, was developed, and a new process was invented and patented by Degussa that reduces the by-products and the wastes almost to zero. Degussa changed raw materials and fermentation process so that the fermentation broth contains lysine and by-products in such a ratio that the product has 60% lysine when dried. Because such a fermentation broth is very difficult to dry, a special technique had to be developed which results in a granulated dust-free product.

In comparison to the conventional process, the new process is very environmentally friendly because no wastes are produced. This is an example of a low-value bulk product which would never have been economical without such savings.

The USD 100 million plant, which employs 70 people, began operations in June 2000.

efficient. Metabolic pathways will be thoroughly understood and fully functional quantitative models will be available. Very low-cost raw materials for bioprocesses will be derived from agricultural and forestry wastes and, to an increasing extent, cultivated feedstock crops. Known biocatalysts will be improved through the application of molecular biology, genome sequencing, metabolic pathway engineering and directed molecular evolution.

The difficulty perceived by the new biotechnology companies lies in persuading chemical engineers of the advantages of the new approach. In practice, this may mean demonstrating a process at large fermentor (small pilot) scale. One company with long-term links to major intermediates producers claims that if it knows a company's ideal process parameters it can provide an enzyme to meet those needs. The idea of adjusting a process around an enzyme tends to put off chemical companies and therefore the enzyme should be optimised to the process. What properties – stability, specificity, activity in solvent, temperature, etc. – are important? It is now possible to search for multiple properties simultaneously.

In parallel with developments in genetic engineering have come improvements in biochemical engineering that have yielded commercial benefits in reactor and fermentor design and operation, improved control techniques and downstream separation. These have resulted in more rapid delivery of products to the marketplace. As the examples in this publication show, it is no longer the case that biotechnological solutions are relevant only to high added-value products such as pharmaceuticals. Bulk chemicals, including polymers, and heavy-duty industrial processes may have a biotechnological component.

The international market for bioproducts and processes is increasing rapidly. Naturally, the lead is coming from the pharmaceuticals sector in which total biopharmaceutical sales reached USD 13 billion in 1998, an increase of 17% over the previous year. Outside the pharmaceuticals sector, the industrial enzyme market is estimated to double in size from 1997 (USD 400 million) to 2004. Currently, bioprocesses account for commercial production of more than 15 million tons a year of chemical products, including organic and amino acids, antibiotics, industrial and food enzymes, fine chemicals, as well as active ingredients for crop protection, pharmaceutical products and fuel ethanol.

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The next generation of bioprocesses will target large volume chemicals and polymers and will compete directly with petroleum-based products. Bioprocesses are becoming competitive with conventional chemical routes, but industry experts believe that further improvements in enzymatic catalysis and fermentation engineering may be required before many companies are prepared to announce world-scale bioprocessing plants. The competitive edge may ultimately come from the development of bioprocesses that use cheap biomass feedstocks such as agricultural wastes, rather than the dextrose that is currently the preferred renewable raw material.

Biotechnology products must compete in economic terms; it is not enough to be environmentally preferable. Cargill Dow's polylactide (PLA) is being brought to market strictly on the basis of price and performance because customers will choose to buy based on value. For example, indigo dye is conventionally produced via a harsh chemical process. Genencor succeeded in modifying the metabolic pathways in E. *coli* to make indigo by giving it a gene from another bacterium to make the enzyme naphthalene dioxygenase. However, by the time bio-indigo was ready to be marketed in 1997, competition from China had eroded the price of indigo by more than 50% and mills were not willing to pay the premium price Genencor needed to justify investment in a commercial-scale operation.

Bioprocessing proponents see a future in which micro-organisms are replaced by purified enzymes, synthetic cells or crop plants. Biotechnology firms are adapting enzymes to reactions with greater volumes and more severe conditions than those involved in the synthesis of fine chemicals. In 1999, Dow Chemical signed a three-year, USD 18 million R&D and licensing deal with biotech firm Diversa to develop novel enzymes for Dow's production processes. The companies have already optimised an enzyme for a dehalogenation step in Dow's alkene oxide process; Dow expects to pilot-test the new enzyme by early 2002.

New participants, including established firms such as Celanese and Chevron, are beginning work on their own bioprocesses through agreements with small specialist companies that have developed tools for metabolic pathway engineering. Celanese, for example, has established a research and royalties agreement with Diversa because the latter has the ability to "genetically engineer the metabolic processes of an entire cell to perform the desired reaction". Chevron Research and Technology has entered into a three-year agreement with Maxygen to develop bioprocesses to replace chemical processes, including the conversion of methane to menthanol, and Hercules has signed with Maxygen to gain access to Maxygen's gene-shuffling catalyst-optimisation technology. Maxygen also has commercial links with Novozymes, DSM, Pfizer and Rio Tinto, while Diversa has similar arrangements with Dow, Aventis, Glaxo and Syngenta.

Diversa recently agreed to work with Novartis to commercialise enzymes for use as animal feed additives and to develop genes that enhance crop plants. It also optimised a heat-tolerant enzyme, discovered in a micro-organism colonising a deep-sea hydrothermal vent, for use by a Halliburton subsidiary (Halliburton Energy Services) to enhance oil field recovery. Diversa is producing the enzyme for incorporation in Halliburton Energy Services's fracturing fluids.

Maxygen is using its gene-shuffling technology, which rapidly generates variants of gene sequences, to help Novozymes optimise industrial enzymes for detergents, food processing and other applications, and to improve antibiotics production for DSM. Maxygen says it will soon be feasible to create an enzyme as required rather than optimising existing enzymes for industrial conditions.

While major companies recognise that products must succeed by competing in economic terms, advances in genomics and genetic engineering, coupled with increasing environmental pressures, mean that the competitive position of bioprocessing will continue to improve. Perhaps even bio-indigo will return to the marketplace.

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Annex

BIOETHANOL

A combination of national security and the need to meet targets agreed under the Kyoto Agreement is driving a third wave of interest in biofuels, particularly bioethanol. Low carbon emissions scenarios reflect the emergence of ethanol as a significant source of fuel both for the transportation and industrial sectors. In the longer term, a zero-emission ethanol fuel could be produced from sustainable agricultural and biomass sources. Cornstarch (United States) and sugarcane (Brazil) are presently the major sources of ethanol, which is either blended with petrol or used on its own.

The United States currently has 58 fuel ethanol plants producing 5.67 billion litres per year. The leading state is Illinois with 2.25 billion litres. By late August 2000, 15 new plants were projected in 12 states with a total capacity of 2.1 billion litres. In the United States, 12% of petrol is blended with corn-derived ethanol.

All major vehicle manufacturers warrant their cars for use of E-10 fuel (10% ethanol + 90% petrol). Many manufacturers are now producing flexible fuel vehicles (FFVs) with engines capable of accepting blends up to 85% ethanol. Over 1.2 million E-85 vehicles (85% ethanol FFVs) were in the US fleet in spring 2001. By 2003, GM predicts it will be building 1 million E-85 vehicles.

The use of cornstarch will always have to compete with alternative food and feed uses, so that most interest is now directed towards the use of cellulose from waste biomass from forest industries or grain production. In the United States, the primary potential raw material is corn stover, while in Canada wheat straw may be the major source.

Corn ethanol plants use coal or natural gas to fuel their distillation process. The CO_2 produced by this combustion has to be taken into account when estimating emissions of greenhouse gases (GHGs) in the transportation sector. The levels of CO_2 emissions fall dramatically when the waste lignin from lignocellulosic raw materials is used as fuel. In its 1997 scenarios, the US DOE made estimates of CO_2 emissions from transportation fuel production (Table 2).

Table 2. **Comparative full cycle CO_2 emissions**

Kg/gallon

Petrol	11.8
Ethanol (corn) – assumes coal-fired boiler	10.2
Ethanol (corn) – assumes natural gas as fuel	7.0
Ethanol (cellulose) – assumes lignin as fuel	0.06

The Government of Canada's Action Plan 2000 on Climate Change reflects the intention to invest CAD 500 million over the next five years. This, together with the CAD 625 million in the 2000 budget, represents a commitment of over CAD 1 billion in specific actions to reduce GHG emissions by 65 megatons a year. The initiatives outlined in the Action Plan will take Canada one-third of the way to achieving the target established in the Kyoto Protocol.

Canada has targeted transportation, which is currently the largest source (25%) of GHGs, as a key sector. Without further action, GHGs from this sector could be 32% above 1990 levels by 2010. Canada's current annual petrol consumption is 25-30 billion litres, 5% of which is E-10. Measures in the Action Plan include increasing Canada's ethanol production from 250 million litres to 1 billion litres, allowing 25% of the total petrol supply to contain 10% ethanol.

The province of Saskatchewan (Canada) estimates that it has enough waste biomass at present, some 22 million tons, to produce 8.7 billion litres of fuel ethanol. However, using hybrid poplars and other agricultural cellulose, this could rise to 50 billion litres, without any reduction of food grain production.

Neither corn-based ethanol nor ethanol from cellulose are economically competitive with petrol. Before the introduction of organisms capable of fermenting multiple sugars, ethanol from biomass was projected to cost USD 1.58/gallon (1980s). In the 1990s, the cost fell to USD 1.16 per gallon. The programme forecasts a fall to USD 0.82

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per gallon in this decade and, as production rises from 1.5 billion gallons per year at present to 6-9 billion gallons, to compete with petrol at USD 0.60 per gallon. According to a US DOE analysis, if the enzymes necessary to convert biomass to ethanol can be bought for less than USD 0.10/gallon of ethanol, the cost of making ethanol could drop as low as USD 0.75/gallon, a figure approaching the production cost of petrol. Genencor, with a one-year, USD 7 million contract from the DOE to develop less expensive enzymes, believe the enzyme cost could be reduced to USD 0.05/ gallon of ethanol.

Iogen, a Canadian company at the forefront of cellulose ethanol production, estimates that their product could be competitive based on a raw material price of CAD 35 per ton, a figure acceptable to Saskatchewan farmers at recent seminars.

Emissions of volatile organic compounds (VOCs) react with nitrogen oxides in sunlight to form ground level ozone, the cause of smog. Because ethanol contains oxygen, it reduces smog and local air pollution. According to the US Environmental Protection Agency (EPA), every 1% increase in oxygenate use decreases toxic emissions by 4.5%.

Chicago has some of the worst levels of air quality in the United States, and strategies for reducing smog in this region have focused largely on VOCs since 1970. The leading approach since 1990 has been the use of reformulated petrol (RFG). By a wide margin, RFG has been the largest single source of emissions reduction in the Chicago area. A number of other US regions have chosen to use RFG with the consequence that, according to the EPA, one-third of all petrol sold in the United States is RFG. RFG contains various compounds containing oxygen (known as oxygenates). In the Chicago area, over 90% of the oxygenate is supplied as ethanol. As well as reducing emissions, RFG oxygenates displace the carcinogen, benzene, found in conventional petrol. Total VOC emissions in metropolitan Chicago fell from about 2 000 tons/day in 1970 to 801 tons/day in 1996. Between 1990 and 1996, RFG contributed 27% of this drop in emissions.

In the 1990s, the US Department of Energy National Biofuels Program focused on developing new, more versatile micro-organisms to extract more ethanol from biomass. The programme's mission is to develop cost-effective, environmentally friendly technologies for production of alternative transportation fuel additives from plant biomass. The goal is to develop technology that can utilise non-food sources of sugars for ethanol production. Additionally, the programme has collected rigorous material and energy balance data to give increased confidence to projected performance and cost figures.

Recent research has focused on cellulase enzymes. Work is also targeted at organisms capable of converting all the sugars in biomass, especially the pentose sugars. Alternative strategies include the use of the E. *coli* workhorse by adding the capability to make ethanol to strains which can metabolise a range of sugars, and the addition of sugar metabolism to yeasts that produce alcohol. The programme is supporting work at the Universities of Wisconsin and Toronto to evaluate both a yeast strain and a recombinant form of the organism *Zymomonas* developed by DOE.

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Chapter 3

ALTERNATIVE TECHNIQUES OF ANALYSIS

Deciding whether or not to adopt a new industrial process, be it based on biotechnology or conventional physics and chemistry, requires a number of important decisions. However, the point at which these decisions are taken is the crossroads where many different pieces of information converge and where a number of alternative routes appear. Steps leading to a decision might include:

- Getting the idea. Can the company make money producing this product the new way?
- Setting the agenda for the decision. Does biotechnology get on the agenda at all?
- Setting the agenda II. Which parameters does the company take into consideration? Economy (cost of producing, investments, etc.), economic risk, occupational health, regulatory (product approval), environmental, customer perception, experience base, company profile and values, etc.
- Getting the facts together. Economics, risk profile, technology base, etc. Does the company have access to comparable and solid data for the alternative processes?
- Looking to the future. Does the company feel confident that it can predict the legal environment and the stakeholder concerns?
- Decision.
- Implementation.

Looking at the whole picture

While environmental considerations are an important subset of the parameters to be considered in any process analysis (see Box 6), they are only that – a subset. Areas such as operating costs or process control are, in principle, as important, although, from the early 1990s, environmental risks began to take on greater importance .

There is a need to see the total picture – only if other parameters are at much the same level can one look at environmental issues. Previous OECD work has shown that there is a steady stream of biotechnological innovation, but this is not necessarily taken up unless it has a clear, demonstrable advantage, which is usually cost-based. Cost reduction can be direct (process capital expenditure or operating costs) or indirect (reduced risk to public and environment, lower clean-up obligations, lower global pollution taxes, etc.).

Techniques for comparing alternative products and processes need to address economic considerations such as capital expenditure and operating costs; supply of raw materials (availability and security); processing considerations, such as the ease of integrating a new process element into an existing operation or onto an existing site; the nature of the marketplace and the activities of the competition. In the marketplace, for example, is there a need for world-scale plants or could the market be better served by smaller modules more conveniently located.

Process Profile Analysis (PPA), as used by DSM, is an example of a technique that may be used at an early stage of process development. It allows for the brainstorming of perhaps ten ideas and the reduction of these to two or three for further consideration (see Box 5). Alternatively, it may be based on an existing process with other possibilities to choose from or on rating one's process a competitor's.

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The technique can equally be used for several alternatives on one site or the same process at different locations. PPA is ordinarily a paper exercise. Whether it is suitable for large volume bulk chemicals is less certain. These may require in-depth analysis because the likely capital expenditure figures will be very large.

Box 5. **Techniques for process analysis**

Many large companies have developed their own set of techniques to analyse new or competitive processes in their early stages of development. DSM in the Netherlands, for example, has a set of four tools they call: Process Profile Analysis (PPA), Technological Assessment, the Cost Curve, and the Experience Curve.

PPA selects five or so key parameters, gives them weightings which may differ for different market sectors and gives each parameter a score from one to ten. Alternatives are likely to have different scores and hence be a better or worse choice.

Technological Assessment separates the fixed and variable costs of alternatives on an equal tonnage basis and makes a direct comparison of different processes leading to the same product. **Cost Curve** does much the same for (different) technologies from competitors. Productivity (yield per given volume) is a very important parameter, especially when comparing biocatalysis with conventional chemistry.

Finally, **Experience Curve**, a progression from conventional "S-curves", plots log constant money prices against log cumulative volumes (inevitably a negative slope straight line) and asks whether any new or existing technology can meet the expected fall in price.

These techniques can equally be used to compare new and old routes to a given product, the same process at different geographic locations or a competitor's technology with one's own.

Source: Professor Alle Bruggink, DSM, Netherlands.

A set of agreed parameters, which are given different weightings for each market sector, should be chosen. These might be, for example: operating costs, capital expenditure, process control, internal risks and external risks. Each can be subdivided. Internal risks, for example, might be waste streams and health risks, while external risks might be availability of key materials, new laws and regulations and patentability of ideas.

BASF has developed a similar process, called eco-efficiency analysis, which is used to compare processes and products. So far, they have conducted over 100 analyses, including 50 in conjunction with their customers. This technique takes into account the views of the end user, Life Cycle Assessment (see below), total costs and environmental burden.

Another company among the case studies has set up a business and technical team in which the commercial group look at costs, quantities and profit, while the technical people look at how to make the product. Possibilities are brainstormed to decide which process to use and how much time and lab effort to spend. In this case, environmental impact is a key element in the analysis. All processes are assessed for effluents using a decision-tree process in which a weighting is given to each effluent which is then incorporated into an overall evaluation of each process's attractiveness. Negative factors do not create a problem if they can be adequately dealt with. Energy consumption is not considered under an environmental heading but rather is an economic factor considered as part of plant occupancy.

All alternatives should have a level playing field, comparing like with like. Thus, alternatives should be imagined as being on the same site, manufacturing the same quantities of product. The problem of missing process data should be addressed and should not be an excuse not to do the analysis. The

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exercise can always be repeated when new information becomes available. Then, together with experienced process engineers, a hypothetical plant should be built for each process.

The result may be that no distinction between the process alternatives is found. This may be a very important conclusion; it shows that the route to the final product is not the deciding factor, so that other socio-economic parameters are more important.

Based on the information collected in the analysis, a trend between price (corrected for inflation) and market volume can be constructed. Extrapolation into the future using the estimated increase in market volume gives a very useful indication of future market prices.

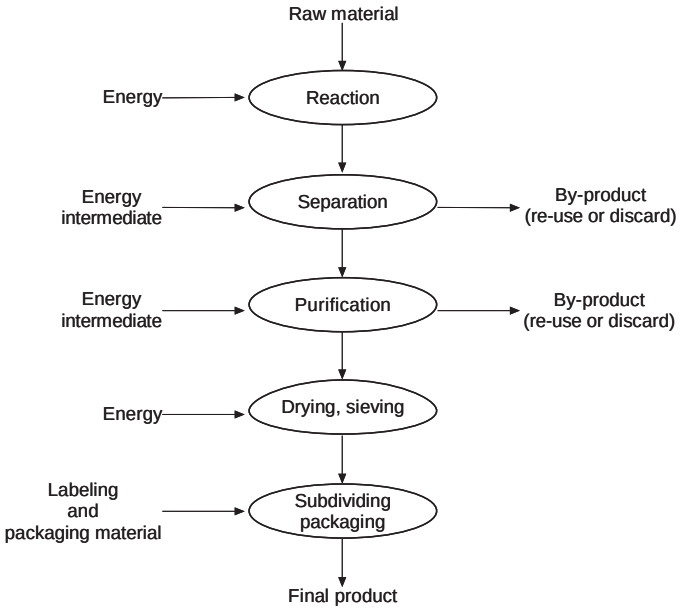
This chapter does not set out to be a text on capital investment. Rather, it concentrates on the impact on the decision-making process of environmental considerations, both local and global, and of environmental legislation. The principles are simple and are relevant to all activities and sizes of company. It is the level of detail that should always be adjusted to ensure fitness for purpose. The goals include comparing alternatives to allow the selection of options with the lowest environmental burden while meeting established standards of safety, quality and cost; and identifying an environmental and economic optimisation of a present process. It should be remembered, however, that assessments always need to be integrated into other management systems – if they are done merely to obtain a permit to proceed, for example, much of the effort and information could be wasted.

Life cycle assessment

In order to evaluate the best process, it is necessary not only to examine individual reactions but also the entire process from raw material supply to final disposal of the product. As shown in the following diagram of a hypothetical bioreactor process, each of the processes requires the treatment of raw material, energy, intermediates, by-products and waste. Thus, one efficient process may affect others adversely, resulting in worsened overall efficiency.

Of all of the methodological approaches to assessment, LCA has been identified as one of the most promising. LCA is a way of evaluating the environmental impact of alternative products and processes

Figure 1. **Bioreactor process**



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in terms of their energy and materials, taking into account the entire life cycle of a product or process from "the cradle to the grave". While this publication is primarily concerned with alternative processes, most LCAs are concerned with products. An LCA includes the production, the use of the product and its disposal. Because it is global and holistic, this type of analysis offers a way to:

- Decide whether a product, process or service in fact reduces the environmental load or merely transfers it upstream to resource suppliers or downstream to treatment or disposal stages.
- Determine where in a process the most severe environmental impact is felt.
- Make quantitative comparisons of alternative process options and competing technologies.

The ideal way of comparing the environmental impact of different processes is to elaborate detailed LCAs for each alternative. At the early stages of process development, however, the level of accuracy and detail required by a traditional LCA may be too high and therefore too costly to generate, and much of the information required may not be available. Instead, a qualitative approach using a relatively short list of parameters can provide valuable feedback.

An LCA can be conducted at three different levels depending on the purpose and application:

- **Conceptual LCA** is the first and simplest level of LCA – it may be regarded as life cycle thinking. At this stage the investigation is used to make assessment of environmental aspects based on a limited and usually qualitative inventory. The results are often presented using qualitative statements or simple scoring systems. The study can be (and is normally) limited with respect to phases as well as parameters. An example of the use that can be made of such a first stage "inventory" can be seen in Cargill Dow's Web site, which shows their approach (see Case Study 9) to their new polylactide biopolymer (*www.cdpoly.com/pdf/30103443_1.pdf*).
- **Simplified LCA**, also called "Screening LCA" or "Streamlined LCA", covers the whole life cycle – as does a detailed LCA – but at a more superficial level. A simplified LCA aims to provide essentially the same results as a detailed LCA, but with a significant reduction in expense and time. To ensure that the overall result gives a true picture of the impact, a quality check of the data is necessary. Roche has carried out a simplified LCA on their product, riboflavin (see Box 6), and BASF AG also reports a similar approach to compare indigo manufacturing and dyeing processes in which the indigo may be synthetic, derived from plants or produced biotechnologically (*www.basf.de/en/umwelt/oekoeffizienz/*).
- **Detailed LCA** comprises a complete LCA from "the cradle to the grave". Nowadays, computer software, such as the SimaPro programme developed in the Netherlands (see Box 7 and Case Studies 15 and 20), is available to ease the task of performing a full LCA. The International Organisation for Standards (ISO) has published a number of documents relating to detailed LCA methodology (ISO 14040-14043 cover principles and framework, goal and scope definition and life cycle inventory analysis, life cycle impact assessment, and life cycle interpretation).

A number of software programmes are available for LCA analysis, each with its own strengths and weaknesses. Such software simplifies the work of analysis, follows correct LCA procedures and correctly interprets the cause and effect chain of any pollutant. They are useful tools for mapping out the overall environmental impacts of production from the cradle to the grave. They can help to reveal the steps in the production process that are crucial for environmental improvement and can illustrate what replacing one product by another really means for the environment. The databases, however, may include data from only one region and may reflect the practices of that region. Other software may have databases with information that is more appropriate to the location of the new process.

It must be kept in mind that the calculations for a complete life cycle are done on the basis of a range of assumptions and different data sources. In particular, alternative manufacturing routes need to be investigated and compared at the same level of detail.

Sometimes, no data on the environmental loading of the process are available. In practice, they are assumed not to exist. This can be important for the results and conclusions of a comparative LCA, if the gaps in one alternative are more important than those in another. However, important gaps may be

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Box 6. **Life cycle analysis of riboflavin manufacture**

Roche has carried out an in-house LCA of the chemical and biological processes for the manufacture of vitamin B_2 (riboflavin) in which glucose, xylidene and the starting materials for barbituric acid are considered as raw materials. The various ecological parameters were chosen and compared with one another on the basis of ISO14040 ff. The results have been documented in such a way as to make a comparison of the eco-efficiency and sustainability of the two processes as comprehensible as possible to a broader public.

Raw materials. The biological process requires 1.5 times as much raw material overall but only a quarter of the non-renewable raw materials.

Water consumption. The biological process requires about double the water but the greater part of this is for cooling and is not purified. The amount of process and waste treatment water in the chemical process is seven times as high.

Energy. Both processes use about the same quantity of energy. The proportion of high-value electricity for stirring, cooling and evaporation is about double in the biological process, but steam and natural gas consumption is reduced and thus the CO_2 emissions from fossil fuel combustion are also reduced.

Emissions to air. Particulates from product formulation are comparable. Solvents are emitted at each stage of the chemical process and in total these are double the ethanol emitted by the biological process. The latter also gives off odours, which are reduced to the necessary level by scrubbing and adsorption. CO_2 emissions from both fermentations (riboflavin vs. ribose) are environmentally neutral since this quantity of CO_2 is used in photosynthesis to make the glucose raw material.

Emissions to water. The chemical process gives rise to three times the emissions of the biological process. The wastewater from the latter contains inorganic salts and residues from easily biodegradable biomass, while the waste from the chemical process also contains organic chemicals.

Solid waste. The solid waste from the biological process is exclusively biomass, which is returned as a nutrient to the soil after composting. The compost contains most of the nitrogen and phosphorus nutrients used in fermentation. The chemical process produces, in addition to a smaller amount of biomass, solid chemical wastes (distillation and filter residues) which are incinerated in an appropriate installation.

Transport. Individual stages of the chemical process are not carried out in the same plant and intermediates are therefore transported from one location to another. This transport gives rise to an extra 130 kg of CO_2 per ton of product from fossil fuel combustion.

Source: Roche, Germany.

Box 7. **LCA software**

SimaPro 4.0 is a software tool developed by PRé Consults B.V. in the Netherlands to simplify the work of an LCA.

Each process is represented by a data sheet, which contains all information received on inputs (raw material input, energy demand, outputs from other processes) and outputs (emissions and products), etc. With this information about each process and a process tree of the life cycle, SimaPro 4.0 is able to draw up an inventory of all the environmental inputs and outputs associated with the product.

For impact assessment, SimaPro 4.0 mainly uses two types of technique. Both are quite similar and are based on the theory of distance to target. In one, the target is derived from real environmental data for Europe, while the other uses policy levels instead of sustainability levels. Policy levels are usually a compromise between political and environmental considerations.

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filled either by additional data gathering or by obtaining the mass and energy balance through process simulation.

A checklist for sustainability

The Green Index (see the annex to this chapter) is a checklist or *aide-mémoire* for industrial managers at the "Conceptual LCA" level that provides a shortlist of key questions to be answered in any comparison. A number of companies, such as Genencor, have developed similar approaches for their own analytical purposes. Possibly the most authoritative version of a conceptual LCA will be contained in ISO documents which are expected to be published shortly.

The Green Index is used as follows:

- Processes to be compared should first be "normalised". In other words, they should be compared on the basis of the same amount (or value) of the product resulting from both processes, made on the same location. When collecting information, it is critically important that the data set chosen should be as comprehensive and reliable as possible. While the accuracy of the data need not be high, all parameters considered relevant must be included.
- For each of the parameters, data are collected for each alternative process and evaluated on an "order of magnitude" scale. If one of the processes is clearly superior (uses less of a non-renewable resource or produces less waste, for example), it should receive a positive score. If a parameter is hard to evaluate because of lack of confidence in, or non-availability of data, then this becomes a sign either to improve the data or to make the judgement that the particular parameter is not relevant. All such decisions should be documented and the process continued until all parameters have been included. The evaluation should be repeated for each alternative process.
- If the total evaluation yields an ambiguous conclusion, if, for example, process A uses less energy but produces more solid waste than process B, this indicates that it is necessary either to do a more thorough analysis (a simplified LCA, for example) or to accept that the change in environmental impact will not be the decisive factor in the choice between the alternatives.
- In more detailed analyses, waste should be weighted. Thus, heavy metal wastes should have a strong negative weighting, followed by solvents (which are recycled as far as possible), by other inorganic wastes and finally biodegradable wastes. Waste safety, for example in terms of toxicity to fish, is important where effluents are discharged to rivers.

The inventory of inputs and outputs should be chosen so as to provide the necessary data for assessment of most potential environmental impacts. ISO has published a preliminary list of potential impacts of a new process or product, but it should be stressed that only certain subsets may be relevant. Moreover, they may only need to be considered at a relatively late stage in the analysis, *i.e.* in relation to a detailed LCA. The ISO preliminary list is as follows:

- Abiotic resources (limited resources).
- Biotic resources (sustainable/non-sustainable use).
- Land use.
- Global warming.
- Stratospheric ozone depletion.
- Photochemical oxidant formation.
- Acidification.
- Eutrophication.
- Ecotoxicological impacts.
- Human toxicological impacts.

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At the conceptual level, is a full risk assessment needed? At the "paper and pencil" stage, it may only be necessary to ask how legislation, current and foreseeable, might apply to alternative processes. At a minimum, any new activity should comply with all local and national legislation. Decision makers should focus on the main features of the system that have environmental implications and adjust the reporting precision to the weakest data to avoid creating a false sense of accuracy.

High temperatures and pressures and the risk of explosion are not necessarily risk factors since they can be translated into investment decisions.

Before moving to a more detailed stage, it is important to realise that the list of environmental impacts may change during the course of analysis. It may also be unmanageable when it has to be applied to every reagent used.

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Annex

THE GREEN INDEX

A CHECKLIST FOR THE SUSTAINABILITY OF BIOTECHNOLOGICAL PROCESSES

Factors affecting sustainability	Scores[1]	
	Process A	Process B
Energy • Energy resource (fossil, renewable, biogas from waste) • Relative amount of energy used in process • Energy efficiency in process (in relation to the amount of product)		
Raw materials • Use of raw materials (abiotic/biotic resources, renewable resources) • Recyclable resources (*e.g.* waste, by-products from other processes) • Unused resources • Relative amount of raw material (in relation to amount of product) (efficiency) • Environmental impact (*e.g.* land use) • Availability		
Waste • Amount of waste, wastewater, waste air (emissions) • Utilisation for other purposes • Biodegradability • Recyclability • Environmental impact (accumulation, odour, acidification, eutrophication, photochemical oxidant formation, stratospheric ozone depletion, global warming)		
Products and by-products • Recyclability • Stability • Biodegradability • Environmental impact (side effects: *e.g.* odour, acidification, eutrophication)		
Process • Process streamlining • Reduction of number of process steps • Reduction of time		

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Factors affecting sustainability	Scores[1]	
	Process A	Process B
Safety • *Safety of product*, by-product, waste, raw materials • Human safety (accumulation, endocrine effects, toxicological impacts, etc.) • Environmental safety (*e.g.* accumulation, spreading/distribution, other negative environmental effects, ecotoxicological impacts) • *Process safety* • Pressure • Temperature • Explosion risk • Non-organic solvent processes • Safety of catalysts, microbes, enzymes		
Total score		

1. Scores can range from 1 (none) to 5 (considerable) or from –2 (much worse) to +2 (much better) relative to another process.

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Chapter 4

LESSONS FROM THE CASE STUDIES

A number of significant general lessons may be drawn from the case studies in this report:

- The first is that the application of biotechnology has invariably led to a process more environmentally friendly than the one it replaces. This appears to be the first time that this has been quantified.
- Another crucial message is that the role of environmental effects tends to be secondary to economic and product quality factors when companies consider adopting a new process. Only

Box 8. **Water re-circulation in the paper industry**

Process water use is a major cost element in many industries, particularly with respect to waste treatment, because of ever tighter discharge limits. While past investment has focused on end-of-pipe treatment, industry is more and more looking to integrated water management and ultimately to closed loop systems. The paper industry in particular is under enormous pressure to reduce organic loads and make better use of available water. The first question to be asked in any paper mill is: Can a closed loop biological system be introduced for water re-circulation?

Anaerobic up-flow sludge blanket (UASB) bioreactors have become popular in many industries with a high organic loading of wastewater. These are, however, gradually being overtaken in popularity by a novel variant, the Internal Circulation (IC) bioreactor, designed by Paques in the Netherlands, which has a number of advantages, including better biomass retention and biogas separation, greater tolerance to hard water and smaller reactor volume (and consequent cost) and footprint. Both these reactors currently operate in the mesophilic range (35-45 °C).

The first full-scale plant operating a closed loop system was started up in 1995 at a paper mill in Germany (Zülpich Papier).

Kappa Packaging in the Netherlands has much experience of minimising water consumption for papermaking and has centralised the knowledge gained from a number of mills. Paques, with whom it was collaborating, already had a lot of experience with paper mill effluents and employed a manager with considerable experience from the pulp and paper industry.

Paques proposed new investment to the Kappa management and also made a proposal for a subsidy to a government funding agency, describing the dangers, the advantages and the unknowns. The critical success factor was the manager at Paques with pulp and paper experience. Up to 40% of the cost was covered by a subsidy, and the decision to go ahead was made by Kappa on the basis of the economics.

A closed loop system based on the first thermophilic IC reactor has now been commissioned at Kappa and operates in a temperature range of 50-60 °C. It is currently being optimised and it is hoped this system will establish the advantages of using thermophilic organisms.

The third generation process contemplated for the paper mills is to take the water from the biological treatment and use membrane filtration so that different qualities can be segmented to specific parts of the plant.

Source: Paques, Netherlands.

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Box 9. **A paper mill case study**

A paper factory, which produces approximately 1 000 tons per day of fluting and testliner (the components of corrugated cardboard), discharges wastewater at 10 m^3/ton. The average temperature of the fresh water supply is 10 °C while the effluent emerges at 35 °C. This increase in temperature results in an energy loss of 1 050 MJ/ton. Overall energy consumption of the plant (electricity and steam) is 7 500 MJ/ton. Energy absorbs approximately one-third of the total manufacturing cost.

The water re-circulation and purification process (an IC bioreactor followed by an aerobic reactor) develops a positive energy balance of 200 MJ/ton (as biogas). It is thus possible to save 1 250 MJ/ton (16.7%), equivalent to a cost reduction of 5%.

Additionally, the paper mill can now maintain process water at 55 °C, with a consequent increase of 2% in dry paper solids after pressing. The result is that the speed of the paper machine is increased by 8% with no increase in steam usage.

Source: Paques, Netherlands.

where a regulatory driver exists (in perhaps three of the cases) does the environment become the paramount consideration, for example, the ban on gypsum in the Netherlands or the legal requirement for single enantiomer drugs.

- The case studies clearly show that systematic strategies are rarely applied in industry in order to investigate new biotechnological production pathways. Rather, the impetus to start developing biotechnological production processes seems to be a mixture of motivations that vary from case to case. From the initial conception to the final implementation of the scaled-up process, a variety of inhibiting and favouring factors are apparent (apart from the purely technical problems to be solved).
- A fourth issue, which arises in most of the case studies, is the lack of knowledge of biotechnology, which very often becomes apparent *after* the decision for the uptake of the new technology has been taken. The hard scientists (*i.e.* chemists) grumble that they have to learn the language of the biotechnologists, not the other way round. This often leads to the need to join forces with external research facilities, such as universities or other companies.
- If a company has knowledge about the technology and the economic (and to a lesser degree the environmental) consequences of the technology, the decision process is fairly smooth and the decisions made are reasonable and timely. A company that recognises that it does not have this knowledge in-house and makes a conscious decision to acquire it – through collaboration with other companies or universities – can also ease the decision-making process. A company, however, that tries to make decisions about biotechnology while using the paradigm it has always used for traditional processes fails to realise the problems and advantages of biotechnology. Consequently, the decision process is slow and tortuous and may even lead to an incorrect solution.
- A final point illustrated by many cases is that although there may be long lead times for the introduction of a new technology, development times can be reduced considerably in subsequent cycles. For example, the development time scale for the (S)-CPA process was relatively long but subsequent biotransformations for chiral molecules have benefited from this learning experience and the time scale has been reduced dramatically (Case Study 5).

Origins of new processes

Companies began to look at biotechnological alternatives for a wide variety of reasons. Many have realised that this is a technology that must be embraced and are seeking basic expertise particularly in process development by recruiting from the biotransformations science base.

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One company had no prior experience with biocatalytic processes, but its R&D group leader had long-established contacts with an academic who was active in the area and knew about his research on enzymes (Case Study 8). The environmental effects of the biocatalytic process were of limited importance to the success of this project. The process did lead to environmental improvements compared to other production processes for polyester glues, but the main aim was improved product quality. The sought-after process improvements went hand in hand with economic benefits such as cost reduction and consumer demand for a "natural" product.

Another company began to look at natural products in response to the possibility of an oil shock – guarding against the threat of energy restrictions to their business (Case Study 10). Yet another believes its renewable fuel technology can bring global environmental benefits (Case Study 19).

One company had a philosophy in place to develop new products and processes with minimum environmental impact. This company devotes considerable attention to the development of recycling technologies for (end-use) products (Case Study 3).

Science push was a driving force for Roche to develop a fully biological, one-step process to manufacture riboflavin to replace a largely chemical, multi-step process (Case Study 1). Environmental/regulatory pressures are another driver. The polluting nature of smelting and the resulting high cost to construct and operate clean smelters is favouring hydrometallurgical process options for treatment of base metal sulphide concentrates. This is particularly the case for treatment of ores containing problem elements that are difficult to treat by smelting, such as arsenic or bismuth (Case Study 18).

As long as metal producers are allowed to "store" polluted gypsum, this is by far the cheapest disposal option. However, in the light of changing regulations (the Dutch government has prohibited further storage of residues at the Budel Zink site from 1 July 2000), alternative processes were essential (Case Study 17).

The limited availability of an essential raw material, such as clean water, may be sufficient stimulus (Case Study 11). Where possible, companies use groundwater as the source of their water supply. The advantages are evident: it is bacteriologically safe and can be used without further treatment. However, economic development is leading to increased pressure on the use of groundwater. The groundwater has to be withdrawn from greater depth and as groundwater levels decrease, shortages occur and groundwater quality is deteriorating (salinity is increasing).

An iterative process may be required when a company needs information but is only prepared to spend money at a later stage. Allied Colloid's project analysis was not very sophisticated at the time of the project development and the problem was to nurture something novel at low levels of expenditure. The work was therefore justified a year or two at a time (Case Study 7).

An important hurdle is the question of using genetically modified organisms (GMOs). In a majority of the cases, the degree to which companies have been concerned about their use is closely linked to the confidence they have in national and international regulations. In Germany, for example, there were public and legal restraints against use of GMOs and genetic engineering up to 1993. Since then, clear regulations, based largely on harmonised EC guidelines, have been in place for handling of GMOs in enclosed systems. These have been accompanied by reliable and increasingly quick decisions from the authorities, within weeks or months. In Case Study 1, there was no public discussion of GMOs (although information was made available locally – the company held an open day at the plant and had 10 000 visitors); although DNA cannot be detected in the product, the latter cannot be declared free of gene technology (Case Study 1).

Where the product is a pharmaceutical, registration requires documentation on the manufacturing process. Consequently, the development of a new process, whether it is chemical or biological, whether it uses GMOs or not, requires that the product be re-registered (Case Study 3).

Analysis and data gathering by companies

LCA has been used in very few cases. It is perceived as too complicated and as requiring data that are difficult to obtain. Only the largest companies have undertaken an LCA in house (Case Studies 1

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and 9), and medium-sized companies may call in outside expertise to assist them. For example, members of the Bioprocess Research Group of Dechema e.V. analysed competing techniques for removing residual peroxide in a textile bleaching company (Case Study 12).

One company advises that it is essential to analyse all parameters and never study environment and safety separately. "Greening" in terms of compliance with environmental standards is not to be seen as a separate target as such but as an integral part of a wider industrial strategy that includes economic factors such as cost reduction.

Sometimes the benefits were obvious at an early stage. Although detailed financial analysis came later, it was clear that the bioprocess would be safer, simpler, more environmentally friendly, would have lower concentrations of toxic materials, would have no runaway potential and capital expenditure would be much less and hence the economic scale would be lower (Case Study 7).

Large companies have generally developed their own techniques for analysing the relative merits of alternative processes. Small companies have in the past tended to rely on the intuition of staff members with long experience in their industry. However, they are beginning to see the need for an easily applied kit of tools to systematise the information they have and highlight the information they need.

A barrier to the development of eco-friendly processes is the lack of a systematic search for technologies that favour environmental benefits. Applying analytical tools that identify where effort to reduce environment pollution might best be spent and that draw attention to areas where R&D seems promising could lead to new processes that combine eco-friendliness and (cost) efficiency.

Most initial data gathering was rather haphazard. Since there were few sources of external guidance as to the likely success of relevant bioprocesses, the only way to find out was to do the research. Most novel processes and products will require some R&D.

The Ciba approach to project analysis is an iterative process, with the result that the approval of capital expenditure becomes a much more systematic examination of the economics, rather than being based on decisions of very knowledgeable top management.

Roche, like other large companies, monitors the straight-line graph of log costs against log cumulative production quantity and requires a new process to achieve a significant improvement in this respect to be successful.

In Avecia, a combined business and technical team uses a technique known as New Opportunities Management Process (NOMP) to respond to enquiries for new processes or products. The NOMP process has milestones throughout the development activity and alternatives are all compared against a checklist on the basis of timing, cost and quality.

One company kindly undertook to use the Green Index to compare a new version of its process to manufacture acrylamide with the earlier catalytic process, and the results (see the annex to Case Study 6) demonstrate the improved environmental friendliness of the enzymatic process.

Collaboration with university departments is the best way for small companies to meet their information needs. However, collaboration is a two-way street and must also be viewed from the academic's point of view. Not only is continuity of R&D an important consideration, so also are the funding and the rewards for discovery (Case Study 8). For example, changes in company structure can be problematic for a university partner for two reasons. First, it can make access to the industrial research laboratory more difficult, and second, overseas take-overs can reduce the number of potential co-operation partners in national promotion programmes. If patent rights are inadequately secured, academic researchers may be excluded from future work (Case Study 13).

Decision making and decision makers

Decisions at the start of any innovative biotechnology project are usually taken by high-level management who need to be aware of the possibilities offered by production-integrated techniques. Companies in many sectors that might make use of biotechnology do not do so because they lack this

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awareness. Structural factors, such as a prevalence of small companies in a given sector or the lack of sufficiently detailed data on environmental performance, can slow the uptake of biotechnology.

Questions often raised at an early stage include: "Where can we add most value?" and "Where is the best location to do any development?" In contrast, a comment regularly heard (about biotechnology) is: "This is not our heritage, not what we know". Such doubts require some very convincing arguments.

Changes in the marketplace can provide these arguments. For instance, the pressure exerted on margins for certain antibiotics by companies from Asia and India that focus primarily on market share led DSM to develop more efficient technologies. The driver for the company to remain a manufacturer of antibiotics in spite of high competition and low margins is the attractive size of the market (worldwide sales of antibiotics in 1998 amounted to USD 18.5 billion).

Roche, on the other hand, did not really need a new process, since they had the necessary capacity to meet market demand. They had therefore to demonstrate that a bioprocess had good payback even when their existing chemical plant was closed.

Cost/benefit analysis is such an integral part of project management that many companies repeat it at milestones throughout the project development. Although the costs may be reasonably easily assessed, benefits may be more difficult to measure, especially if the company is unfamiliar with the required technology. A company may not be interested in new processes that improve environmental sustainability even though operating costs may be reduced if they have already invested in new plant, waste minimisation and recycling. Appropriate tools are often missing to allow a reliable assessment of the advantages and disadvantages of the new process. The major advantage of bioprocesses, Roche believes, is that only marginal improvements are possible in well-established and continuously improved chemical processes, while fermentation has a large scope for increases in productivity.

From Lurgi's experience (Case Study 10), the development of a new process has four stages:

- To find a partner to carry out the R&D. This might be an university, a public research institute or another company.
- The R&D itself. This step can be subdivided. After each milestone a go/no-go decision is taken. Internal opposition to innovation can most efficiently be overcome by facts, *i.e.* by achieving set goals or milestones.
- The third step, scaling-up, is usually done in co-operation with the user company. In this case, differing interests could be a problem, as one company (the supplier) is usually interested in selling the process to a number of companies, whereas the other (the user) would prefer to use the technology exclusively.
- Introduction of the product/process. In this stage, it is crucial for the engineering company to have demonstration pilot plants in operation.

Certain considerations make it easier to raise a budget for R&D on a new project. These include short duration from the start of the project to the introduction into the market, low risk, low cost for staff and equipment and a powerful partner during R&D.

Some of the companies believe that a positive factor is a personal promoter or champion inside the company who is dedicated to the project and takes the initiative throughout the whole implementation process. This may not be the optimal procedure, as a decision on the implementation of new technologies should be based on broad consensus among those responsible, rather than depend solely on one person. Only if the decision to implement a new technology is embodied in the responsible management structure is continuous support guaranteed until the full-scale process is operational.

Projects in Allied Colloids tended to be supported by the enthusiasm of senior executives, many of whom were chemists and engineers who had grown up with the company. Following the take-over by Ciba, all the senior management were new to the industry; on the other hand, they had a very detailed system for project analysis (Case Study 7).

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This is not to say that ownership of a new process is not required. In the Baxenden case, personal contact between the two leading people involved not only led to the project but was also one of the success factors.

The case studies offer several examples where failure is due to loss of ownership. In one example, internal pricing structures were such that the profit from sales went to another division so that lower costs from a new process could not be recouped.

The case studies indicate that the ownership of intellectual property rights is an important factor in the initial decision for (or against) the uptake of biotechnological processes. As the patent situation concerning biotechnological processes and products is still in a state of flux, a detailed revision of the relevant regulations might be useful. Companies and researchers have found to their cost that it is essential to file patents (Case Study 13).

A major consideration for a new process is availability of raw materials. These may be a renewable crop, for example, as in the case of bioethanol (Case Study 19), or the vital catalyst for the process, the enzyme. It is unsatisfactory to have a single source of supply. One company required that the enzyme be a commercially available product. The rationale was that the enzyme would have to be used on a large scale in the production process, and that supply had to be guaranteed (Case Study 8).

New biotechnological processes are being developed continuously in academic institutes but they do not necessarily find their way to industry. That the link is still weak is supported by the fact that academics are not always involved at the process development stage. A lack of know-how may only be overcome by co-operation with universities and/or other companies.

In making a decision to invest in a project, especially a collaborative project with a university, the possibility of government funding is a consideration. Often, the economic advantages are not overwhelming and even early adopters may need government stimulus (Case Study 13). In one case in particular, three separate government funding schemes were involved (Case Study 7). One company decided not to ask for external funding, because the procedure would have taken too long, and they felt that confidentiality would not be guaranteed (Case Study 8).

Process technology

The key to success in biotransformation technology is the process of translation of an embryonic laboratory procedure into a cost-effective, reliable and robust plant-scale operation within an acceptable time scale and using appropriate resources. Provided that appropriate plant volumetric and biocatalyst productivities can be achieved and the overall yield target met, cost-effective biotransformation processes are attainable. Attention to costs is a key element in every case (see Box 10).

The increasing complexity of novel chemical products means that conventional chemistry is less and less able to cope, hence the switch to biotechnology. Full fermentation, a complete metabolic pathway in one organism, is the next step. It is conceivably possible to develop a full fermentation for even a bulk product such as caprolactam (a precursor of nylon).

Application of biotechnological processes requires new and extensive expertise in unfamiliar fields in addition to conventional process engineering and the requirements of the market. Alteration of existing operating structures has a technical and cost component – bioprocesses often do not fit into existing process networks which may use by-products from one stage as feedstock for another. On the other hand, biocatalysis can ease the scale-up problem because it is modular: conventional processes have many parameters to optimise but biosynthesis, although very subtle, has a much narrower range of parameters – it rarely uses, for example, a multitude of reagents (Case Study 7).

The development of a biocatalytic process includes some or all of the following elements: screening existing enzymes; strain selection; strain improvement; optimisation of the fermentation process; choice of the form of the enzyme for application (*e.g.* free cells or immobilised, aqueous or

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Box 10. **Propanediol**

On 20 February 2001, DuPont dedicated the world's first continuous polymerisation plant for making its new DuPont Sorona™ 3GT polymer. 1,3-propanediol, or PDO, used as a key ingredient in Sorona™, is currently made using a petroleum-based process. However, DuPont is developing a biotechnology process using sugar from corn or other crops as the raw material for PDO.

In 1998, DuPont and its partner Genencor International succeeded in genetically engineering a micro-organism capable of synthesising PDO from glucose in a single step. The technology involves inserting four genes taken from various species of bacteria and yeast into industrial strains of E. *coli*. The proteins produced from these four genes then subvert E. *coli's* biochemical pathways, feeding raw materials that would normally be destined for other purposes to an enzyme that synthesises PDO.

The following year they achieved a second milestone by developing a second-generation organism capable of much higher yield, rate or product concentration in water. Yields from the process are such that the researchers think they need only double the efficiency of their prototype in order to have a commercial product. The two companies claim to have far exceeded their targets and to be nearing performance suitable for commercialisation. The results demonstrated by the DuPont-Genencor team are beyond the most optimistic case for any bioprocess described in the ChemSystems study of October 1998, which compared all routes to PDO (see below).

The next milestones to meet include refining the micro-organism to achieve the final improvements in biocatalyst performance and starting up pilot plant operations.

Routes to 1,3-propanediol: extract of ChemSystems summary

1,3-propanediol (PDO), or trimethylene glycol (TMG) as it is otherwise known, is currently being used to make the new polyester polytrimethylene terephthalate (PTT).

There are currently two petrochemical routes to the PDO monomer. Shell has developed a process based on ethylene oxide, while Degussa has developed a process based on acrolein. Recently, DuPont acquired the Degussa technology. DuPont has also committed itself to the development of a production process for PDO based on a renewable feedstock. It has strengthened its efforts by forming a strategic collaboration with Genencor. This collaboration has been aimed at producing PDO in a one-step fermentation process from corn sugar, in preference to glycerol, as a feedstock. This could have economic advantages in terms of raw material costs. Acquisition of the Degussa acrolein-based route to PDO allows DuPont to compete with Shell in the short term while they continue to develop a biotech route for the long term.

Source: DuPont, Genencor and ChemSystems Web sites.

solvent); choice of bioreactor type (*e.g.* batch *vs.* continuous, fluidised, membranes, etc.); optimisation of downstream processing and product isolation. Bioreactors generally require less maintenance than chemical reactors but may need more auxiliary equipment.

These stages may all be undertaken in house by a large company, but smaller companies may need to co-operate with specialist suppliers and academic institutions and even large companies benefit from such collaboration. For example, isolation of their first generation organism and development of the production process were undertaken in house by Mitsubishi Rayon (MRC). The second- and third-generation organisms were discovered by staff in Kyoto University and succeeding process development was performed by MRC in co-operation with Kyoto University. The fundamental studies of the fourth generation micro-organism (GMO) were done in co-operation with Kyoto University and the University of Tokyo, and the technology development was done by MRC (Case Study 6).

The presence in whole cells of unwanted enzymes that can interfere with the enzyme of choice or attack raw material or product is an important consideration. While batch growth is less complicated, there are productivity benefits to be derived from continuous technology. For redox reactions, use of whole-cell biotransformations is advantageous to avoid the need for co-factor recycling (Case Study 4).

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DSM developed their own enzymes, and these are regarded as part of the development cost. They were not marketed (too small volume and fear that the customer might try and improve on them). In this context, they see no problems with GMOs since these remain within the process and are not released except as a sterilised biomass. For any given conversion, they expect to be able to develop a new enzyme in three to four months.

Baxenden prefer to buy in their enzyme but see the fact that there is only one producer for the lipase as a potential problem, should Novozymes decide not to sell it anymore. A lipase from another organism could be used, but the enzyme selected functions at higher temperature and is more stable under the conditions used.

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Chapter 5

KEY ISSUES AND CONCLUSIONS

This publication takes a number of steps forward in the debate on industrial sustainability. It produces hard evidence on the links between the two roles of biotechnology – environmental friendliness and economic improvement. It also gives a more precise picture of how decisions to adopt these new technologies are made by industrial managers. We now have a better understanding of the opportunities and constraints created by policies on industrial sustainability.

Why adopt?

There are now many examples of the replacement of chemical routes by biotechnological processes leading to very substantial reductions in the production of emissions and in the use of hazardous raw materials. The specificity, aqueous compatibility and mild operating conditions of biotransformations have resulted in the replacement of existing processes by bioprocesses that produce lower amounts of by-products, generate fewer waste materials and consume less energy. As a consequence, these new processes often have significantly lower environmental impact.

Process innovations which "only" improve environmental performance do not, however, give companies sufficient incentive to modify their operations. They are at most desirable by-products. Only economic advantages convince decision makers in companies to apply ecologically advantageous, innovative processes. Although eco-friendly features were often considerable – and turned out to be more easily achieved than the cost targets for the new process – they did not play a decisive role. The balance of priorities is, of course, affected by the severity of environmental regulations.

Very few of the new processes adopted in the case studies had an improvement in sustainability as the primary driving force, economic competitiveness being by far the most important. Nevertheless, every single example demonstrated an improvement in environmental friendliness. This represents the first hard evidence that a change from a conventional physico-chemical process to a biological one has a very high chance of reducing adverse impacts on the environment. The substitution of a chemical process for a biotechnological one can bring about a reduction in consumption of resources and in environmental pollution without incurring any expensive investments either of a technical or a financial nature.

Drivers for adoption of a new process include:

- Product differentiation (customer demand for a natural product or improved product quality).
- Cost effectiveness (single enantiomer drugs resulting in decreased raw material usage, increased manufacturing capacity and productivity).
- Company strategy and structure (the need for a champion for the new technology who is prepared to give long-term commitment and has a good link to and understanding of the R&D team, the lack of competing internal pressures and a model to calculate the payback/operability requirements).
- A positive investment climate and the availability of government funding to reduce the risk of adopting novel technology. Because biotechnology is still at the foot of its "S-curve", the major advantage of bioprocesses is that they offer a large scope for increases in productivity while only marginal improvements are possible in well established and continuously improved chemical processes. Conventional chemistry is less and less able to cope with the increasing complexity of

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novel chemical products. Environmental benefits do sometimes trigger the development of a new process. The regulatory framework in particular can be a stimulus to process development. The location of the production plant would also appear to be a factor. Germany, for example, has clear regulations on GMOs, which result in quick decisions from regulators. There is also some evidence to support the idea that German and Dutch companies tend to place the environment higher on their agenda and apply stronger legislative pressures. The case studies also illustrate the importance of government support and finance (*e.g.* grants and low interest-rate loans) from, for example, the Dutch government, the German Ministry of Environment and the LINK Biochemical Engineering Programme and Teaching Company Scheme in the United Kingdom.

Cost benefits

Even if a company or industry sector is unable to offer novel products or significantly increased quality, the substitution of a process by one that is economically advantageous can make a considerable contribution towards consolidating or improving the position of a company with regard to its competition. Table 3 shows some of the typical cost and environmental improvements in the cases presented.

Table 3. **Cost and environmental benefits from cases**

Case	Energy	Raw materials	Waste to air	Waste to water	Operating costs
1	Same	–75% (non-renewables)	–50%	–66%	–50%
2			–90%	–33%	–90% (environment-related)
3	Electricity +; steam –		–80%	–80%	Considerable reduction
4	Same				–43%
6	–80%		Down	Down	Down
7		Down	Down	Down	–54% (raw materials)
8	Down		Down		Down
10	–70%			–80%	–40%
11		–50% (groundwater)			–30% (groundwater)
12	–15%	Down (water)		Down	–9%
13	–30-40%	Down		Down	
16		–35% (Cl_2), –65% (ClO_2)		Down	
17		Down (recycle)		Down	
18	Down		Down		Down
21				Down	Increased productivity

While in many cases the enzyme represents a significant cost element, appropriate use, such as immobilisation, can result in a major decrease in production costs. Continuous as opposed to batch-wise operation may mean that the costs of both raw material and labour can be dramatically decreased. Avecia's introduction of a continuous fermentation process for the production of the biocatalyst resulted in a fourfold increase in productivity, and the development of a genetically manipulated organism in 1991 has given a further fivefold increase. This has made a significant impact upon production economics.

Single enantiomer pharmaceuticals and agrichemicals may not only be required by regulatory authorities but also have a positive cost effect in that they decrease raw material use and result in increased manufacturing capacity and productivity.

Some companies have found that there comes a point of diminishing returns when further increases are made in enzyme activity. Although a recombinant strain of an organism may be constructed with yet higher activity, it may not be used in practice because the effect of higher enzyme activity on manufacturing cost seems to be negligible.

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In rapidly changing markets, companies usually do not have time to fully realise productivity potentials because time-to-market is more important than comprehensive process development and optimisation. Mature products, on the other hand, allow for continuous process improvements. Indeed, process optimisation and reduction of operating costs is a requirement in such businesses because competition usually comes in the form of falling product prices. Consequently, the replacement of conventional technologies by biotechnological ones will more probably take place in established businesses, where new processes may lead to a considerable increase in efficiency or product quality. While new versions of conventional process technology are perceived to give only marginal improvements in productivity, biotechnology is seen to hold out the promise of major improvement steps.

Approach of management

Different management philosophies in the companies represented in the case studies have led to different approaches to new process development. For example, some have relied on support from individual champions while others have preferred to work through groups of specialists from widely separated organisations within the company. Successful companies reduce the inevitable uncertainties by stringent project management and repeated reassessments at a number of milestones throughout the project development.

Success is more likely when environmental concerns are part of the philosophy of the company and when an R&D culture is present. However, realising biological processes requires several different skills, which are not necessarily present in a single company. Collaboration between companies proved to be beneficial in many examples. Against this background, the groups of companies in these case studies can be considered as models for alternative collaborative structures.

The case studies clearly demonstrate that although it is advantageous to have in-house biotechnological expertise, even relatively small companies can benefit from biotechnology and that the technical help they need is available from academic sources including, most of the time, local university departments. Where no specialised personnel have existed in house, co-operation with academia has been followed by recruitment of biotechnologists. Networking and sharing of knowledge is vital. In general, when university researchers have been involved, project development has been more successful. Clear IPR rules are however necessary in such collaborations.

Most of the cases represent isolated initiatives – there was no systematic approach to discover and implement new processes. However, these first, sometimes tentative, steps have often led to the creation of a new business sector. Companies have reported benefiting from the learning experience associated with their first attempts at introducing biotechnology, and the time scale for later processes has been reduced dramatically. Time scale has now become possibly the most critical aspect in meeting the needs of the pharmaceutical industry, and what used to be a major constraint to the exploitation of biotechnology in this area has now largely been overcome. The key determining factor now is not the identification of an appropriate enzyme but the speed of scale-up, reflected in the ability to mobilise a multidisciplinary team of biotechnologists, chemists and engineers. The importance of integrated process development is the main lesson.

A number of examples demonstrate that companies, having gained biological skills, are applying them to other products and processes. MRC, for example, is building on its experience to develop the production of other chemicals, such as chiral and non-chiral pharmaceutical intermediates, non-natural amino acids and biodegradable chelating agents. Tanabe Seiyaku has built on its internal experience to develop more and more sophisticated immobilisation technology applied to the biosynthesis of amino acids.

Realisation of the key role played by the engineering aspects of any project and of the need for this to be studied in detail alongside the biology, is an important learning step. The newest research projects consider the cost options by involving engineering at an early stage and identifying where the cost bottlenecks are likely to be. Is, for example, the diminishing return associated with the last small increases of yield on catalyst meaningful?

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Analytical methods

Although few of the companies had undertaken a full LCA on their product or process, most of the largest companies have analytical systems installed, although these may focus more on costs than on sustainability. There are lessons to be learned from the in-house process comparisons developed by the largest companies – DSM's Process Profile Analysis, for example, or the decision milestones used by Ciba and Avecia. It is conceivable that these might be developed into general instruments which could be made available to other companies.

Joint government-industry action to overcome this limiting factor is paramount to encourage both consumer and public confidence in the resultant technologies, and, ultimately, to ensure the successful development and industry acceptance of the next generation of bio-based and cleaner industrial products and processes.

As was pointed out in the earlier OECD report (BCIPP), biotechnology poses unique problems to any process analysis that purports to cover "the cradle to the grave". To investigate how the LCA method can be applied to biotechnology, more case studies need to be done to investigate which methodological issues are important for the applications. When evaluating environmental impacts, the dilemma of how to assess the effects on biodiversity, landscape and land use are often encountered. These are, in practice, very difficult to quantify.

The examples illustrate the need for an easy-to-use tool such as the Green Index illustrated here or ISO's conceptual LCA, as part of the toolkit of any process analyst, particularly in a small company. The Green Index is a first attempt to provide a simple framework for analysing the potential sustainability of a new product and process. Further development will need to take account of sustainability in its full sense – economic, social and environmental – if it is to meet industry's needs.

There does however, seem to be a requirement for knowledge centres or independent consultancies that address the benefits and pitfalls of biotechnology in industry. These could be a primary target for the OECD as part of its goal to promote alternative technology for sustainable industrial development.

Environmental constraints

Where companies can comply with environmental regulations and standards with their established production equipment, and as long as permissible emission thresholds do not constrain the production process, environmentally friendly production scores low in the ranking of customer needs. On the other hand, if environmental concerns are paramount, as in the case of the pollution caused by smelting (with the resulting high cost for constructing and operating clean smelters) or the non-availability of clean process water in the paper and food industries, then sustainability factors override cost criteria. It must be said, however, that in the examples where this has been true, the bioprocess has still been cost-effective.

- Hydrometallurgical process options for treatment of base metal sulphide concentrates, particularly for treatment of ores containing problem elements which are difficult to treat by smelting, are now favoured by companies such as Billiton.
- Reuse of wastewater for process water production in the food industry demands that specific attention be paid to the achievement of guaranteed levels of quality. The hygienic and chemical quality of the water needs to be evaluated and monitored continuously using systems now obligatory for the food industry in the Netherlands.
- Treatment of effluents containing SO_2 or sulphate by conventional techniques leads to the production of large volumes of gypsum and final effluent characteristics that may no longer comply with legislation. Using improved biotechnology, no gypsum is produced and an improvement in water quality results.
- The application, in Canada, of xylanase treatment to reduce chlorine dioxide use is already cost-effective. Enzyme bleaching, by replacing chlorine-containing compounds, can help to produce an improved effluent that is therefore recyclable to the recovery system.

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GMOs have been used in the majority of the case studies and their use has not only increased productivity but has also permitted reactions not otherwise possible. However, all the companies in our cases that use GMOs have been concerned to minimise any problems by addressing them at an early stage and by closely involving the regulatory authorities. One approach is to kill organisms before they leave the plant and ensure that compost made from the waste biomass contains no long strands of DNA. In Roche's case, approval to construct and run their new riboflavin plant took only seven to eight weeks. Major acceptance problems of the product are not expected with final consumers, and there is no obligation to put any GMO label on the product packets. In another case, the most important single factor necessary to bring the new development into full production was an improved regulatory situation concerning permissions. While this example was also in Germany, a clear political situation will be required wherever this type of process is to be developed.

It is by no means claimed that the conclusions reached here will apply in every case and every country. These issues are very complex. However, the advantages of biotechnology shown here seem so overwhelming that it has to be assumed that they are relevant for a much wider range of companies and industry sectors.

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CASE STUDIES

Case Study 1

MANUFACTURE OF RIBOFLAVIN (VITAMIN B_2) (HOFFMANN LA-ROCHE, GERMANY)

Introduction

Vitamin B_2, riboflavin, is a fluorescent yellow compound present in most animal and plant cells. The compound is essential to biochemical redox reactions, and deficiency results in metabolic and skin disorders. It is an important food and feed supplement with steadily growing market demand.

F. Hoffmann-La Roche Ltd (Roche) is one of the leading vitamin producers offering a comprehensive product range, including vitamin B_2. The amounts of vitamin B_2 manufactured by Roche have steadily increased over the past decades. At their plant in Grenzach-Wyhlen, near Basel, Roche manufactures, in addition to pharmaceuticals, vitamins B_1, B_2, B_6, biotin and also vitamin intermediates.

Technical description

Traditionally, riboflavin has been manufactured using a process developed some fifty years ago. This starts with glucose followed by a sequence of six chemical steps. The most recent chemical process involves converting glucose to ribose by fermentation and thereafter reacting the ribose with 3,4-xylidene to form ribityl xylidene. This is further converted to an azo-dye which is reacted with barbituric acid to form riboflavin.

From 1990 on most chemical processes have closed down and have been replaced by fermentation. Competing manufacturers use yeasts (BASF) or fungi (Coors, ADM) in wholly biological processes. The organisms used are not necessarily GMOs but may be classical mutants.

The biological process to manufacture riboflavin is a single step process in which crude riboflavin is produced directly from glucose using a genetically modified strain of B*acillus subtilis*. This process is currently being run alongside the chemical one but will ultimately replace it.

Life cycle assessment

Roche has carried out an in-house LCA of the chemical and biological processes (see Box 6). The main measure of sustainability is the use of renewable raw materials, of which 90%, in the biological process, is glucose. The biological process shows significant improvements with respect to sustainability compared to the chemical synthesis (Table 4):

Table 4. **LCA of chemical and biological processes**

Type of process	Chemical	Biological
Raw materials (%)	100	150
Non-renewable raw materials (%)	100	25
Energy (%)	100	100
Emissions of VOCs (%)	100	50
Emissions to water (%)	100	33

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The amount of energy used in the two processes is about equal. The chemical synthesis uses more steam energy made from fossil fuels but the fermentation requires more electricity. CO_2 emissions from the fermentation processes are environmentally neutral because the glucose is made photosynthetically from CO_2. Emissions to water from the biological process consist only of inorganic salts and residual biomass. Air and water are contaminated to a lesser degree by the biological process.

The biomass waste material is a high viscosity pumpable liquid which is used for composting. It contains most of the nitrogen and phosphorus and trace metals fed into the process.

Process of innovation

History

Riboflavin was first isolated in 1932. The structure of the molecule was identified in 1933 and two years later it was first synthesised chemically. An improved chemical route to the product on an industrial scale was developed in 1947.

Roche was founded in 1896 and the manufacture of vitamins (vitamin C came first) began in 1934. Early work was mostly chemistry – only B_{12} was made by fermentation. Vitamin B_2 manufacture started in 1942 using chemical processes and this remained the case until 1980 when the chemical step to ribose was replaced with a fermentation process based on glucose.

In the late 1980s, some information appeared in the literature about organisms which might synthesise riboflavin with appropriate yield, and a feasibility study was undertaken to compare the biological with the chemical process on the basis of yield, space yield (productivity) and titre. Goals that the new process had to attain were set.

The development of a new, completely biological process began in 1988. A new strain of micro-organism, based on *Bacillus subtilis*, was created in collaboration with external partners. Metabolic engineering has resulted in one organism capable of carrying out all the reactions from glucose to B_2, a process that took several years. There has been no subsequent genetic engineering, but classical mutagenesis has been applied to increase productivity and reduce use of raw materials.

1995 saw the first decision for a project to demonstrate the feasibility of the fermentation process at industrial scale and to gain experience with the downstream operations (isolation and purification of the product). In 1996 pilot-scale manufacture began in Japan.

The large production plant that will finally replace chemical production was constructed from 1998 to 2000 in Grenzach-Wyhlen. The overall cost was DEM 130 million, and the plant has a capacity of 2 000 tons per year, with the capability of increasing to 3 000 tons/year. Production started in May 2000.

External and internal factors influencing the decision

Roche did not really need a new process since it had all the capacity to meet market demand. It was therefore necessary to demonstrate that a bioprocess had good payback even when the chemical plant was closed.

Although Roche's focus in vitamin synthesis has always been more on chemistry, the culture of biological processing was always incorporated in production processes based on economic considerations. The replacement of the chemical route to ribose by a fermentation process in the early 1980s gave an encouraging example for such a strategy. A broad R&D bio group was set up in support of this approach.

There was no local public discussion of GMOs (although information was made available locally and Roche held an open day at the plant and received 10 000 visitors). DNA cannot be detected in the product, which cannot however be declared free of gene technology. Compost made from the waste biomass contains no long strands of DNA. Approval to construct and run the plant through German GMO legislation took only 7-8 weeks. Major problems of acceptance of the product are not expected from final consumers, and there is no obligation to put any GMO label on the product packets.

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Process comparisons

One benchmark for a new process must be the economics, taking all factors into account, including depreciation and production costs. Roche monitors the straight-line graph of log costs against log cumulative production quantity, and a successful new process should achieve a step improvement in this respect.

If an alternative (for example, bioprocess) is developed, relative competitiveness must improve for the process change to be adopted. An established process always has one advantage – the degree to which capital costs have been written off. Investment in a new plant is a financial burden for any new process.

Another hurdle is the question of using GMOs. The last few years have seen a window for the introduction of products derived from gene technology that may now be closing. Finally, the market will decide on the acceptance of the new product.

While a product change can give rise to improved sustainability in many industries, in the field of performance chemicals (which includes pharmaceuticals and vitamins) the focus is on the active ingredient and the only possible way of increasing sustainability is by improvement in process technology.

Summary and conclusions

Roche have developed a fully biological, one-step process to manufacture riboflavin to replace a largely chemical, multi-step process. The driving force was science push rather than market pull.

The major advantage of bioprocesses, Roche believes, is that only marginal improvements are possible in well-established and continuously improved chemical processes, while fermentation offers a large scope for increases in productivity.

Chemical steps with low yield or which require stereoselectivity are always good targets for a biological alternative. Also, new strain development can be done in relatively small companies.

The project targets were cost reduction in combination with the introduction of sustainable technology.

In developing the new process for riboflavin, a step-function improvement, *i.e.* a cost reduction of 30-50%, was required. In practice, an overall cost reduction of 50% was achieved, largely through the new technology. The capital expenditure is similar to that for an equivalent chemical plant.

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Case Study 2

PRODUCTION OF 7-AMINO-CEPHALOSPORANIC ACID BIOCHEMIE, GERMANY/AUSTRIA)

Introduction

Biochemie is member of the Novartis Group and is based in Kundl/Austria. Novartis was formed by the merger of Ciba-Geigy and Sandoz in 1996. Biochemie is part of Novartis' Generics Sector with an important role in the production of active pharmaceutical ingredients, patent-free pharmaceuticals and biotechnological products.

The company is one of the world's leading producers of antibiotics, *e.g.* penicillins, cephalosporins and erythromycins, with a strategic focus on cephalosporins, hence the investment in 7-amino-cephalosporanic acid (7-ACA), the key intermediate for semi-synthetic cephalosporins. The world market for 7-ACA is approximately 2 000 tons a year.

Technical features of the alternative processes

The chemical and biological routes to 7-ACA are as follows:

- C*hemical route*: Produce the zinc salt or sodium salt of cephalosporin C; treat this with trimethylchlorosilane to protect functional groups; react with phosphorus pentachloride to produce the imide intermediate and hydrolyse the imide to produce 7-ACA.
- B*iotechnological route*: Convert cephalosporin C to keto-adipinyl-7-ACA using the enzyme D-amino acid oxidase. This compound is spontaneously converted to glutaryl-7-ACA, which in turn is converted to 7-ACA by the enzyme glutaryl amidase. Both enzymes have been screened and isolated from naturally occurring micro-organisms, partly by an external research institute. These micro-organisms have then been optimised with respect to high production yields and reduced side activities. The glutaryl amidase gene was isolated and expressed in E. *coli*. In the production process, the enzymes are isolated from a fermentation culture broth, purified and attached to carrier beads to give the final biocatalyst.

Advantages and disadvantages

The chemical process uses toxic and hazardous reagents such as N,N-dimethylaniline, $(CH_3)_3SiCl$ and PCl_5. It also uses chlorinated solvents (CH_2Cl_2), needs inert gas conditions and has expensive waste gas cleaning. The composition of the wastewater prohibits or endangers conventional biological wastewater treatment and thus has to be incinerated. Some processes use zinc salts (with resulting problems of heavy metal disposal). The process uses very low temperature chemistry which gives rise to high energy costs.

The biological process, on the other hand, uses no toxic ingredients. It is an aqueous, room-temperature process, there are no restraints for biological wastewater treatment and no hazardous chemicals or heavy metals. Gas emission is reduced, as are distillation residues. Disposal of liquids by incineration and the amount of wastewater are also reduced but wastewater carbon oxygen demand (COD) is slightly increased. The new biological route together with membrane filtration brings more

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COD to the mycelium fraction, which will be used as fertiliser. In particular, expensive disposal of liquid by incineration is reduced from 31 to 0.3 ton per ton of 7-ACA manufactured.

In summary, the biotechnological route reduces the percentage of process costs used for environmental protection considerably (Table 5).

Table 5. **Comparison of outputs**
Chemical process = 100

	Chemical process	Biological process
Material for incineration	100	0.7
Wastewater	100	90
Solvents, class 1	100	0
Solvents, class 3	100	2.5
Zinc disposal	100	0

Description of the innovation process

History

The reason for the new development was the increased costs of chemical production of 7-ACA owing to a new law that forced Hoechst in Frankfurt to pay additional taxes on wastes to be incinerated. 7-ACA was the basis for the production of the company's own cephalosporin antibiotic specialities. The options that were available were to find a better process or to close the production of 7-ACA.

Internal factors

The company used the opportunity to run a test production of the two enzymes and of 7-ACA in existing pilot plants and was able to show via mass balances, energy balances and marketing studies that biological production would have a chance in economic terms. The breakthrough was made when an effective method for the isolation and immobilisation of the enzymes was found. One enzyme was made by a recombinant E. *coli* and this lowered the production costs. By isolating, purifying and immobilising the enzymes, more stable biocatalysts were made. In consequence, GMOs were not used for the production of 7-ACA, and this made it easier to obtain production permissions.

Although some of the decision makers were sceptical (they were "pure chemists"), Hoechst decided in 1995, after about five years of research, to invest DEM 30 million and to convert the chemical 7-ACA plant into an enzymatic production plant. The production of enzymatic 7-ACA started at the end of 1996.

In November 1998 Biochemie bought from Hoechst the fermentation and downstream processing plants, including the production of 7-ACA. The antibiotic business fitted Biochemie's portfolio better than that of Hoechst, which had changed its policy. Hoechst no longer exists, following a joint venture with Rhone Poulenc to form Aventis.

After a period of process optimisation, Biochemie improved the quality of 7-ACA and was able to produce it economically. Because the Biochemie people from Austria were experienced in developing bioprocesses, they were able to help to optimise the process further, so that it is competitive and gives a comparable quality to the chemical process.

External factors

Up to 1993, there were public and legal restraints in Germany against use of GMOs and genetic engineering. Since then, clear regulations have been in place for handling of GMOs in enclosed

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systems. They are based largely on harmonised EC guidelines. This has been accompanied by reliable and increasingly quick decisions (within weeks or months) from the authorities.

Inside the companies, it was concluded that the use of GMOs for enzyme production could make the process economical and safe and that existing fermentation plants could be used with moderate investment for biologically safe GMOs. This, coupled with the more positive investment climate within Germany for globally oriented companies like Biochemie/Novartis and a favourable infrastructure, led to the decision to invest DEM 15 million in an enzyme production plant and DEM 85 million for a new enzymatic 7-ACA plant to be sited in Frankfurt. The new plant went on stream at the beginning of 2001.

Summary and conclusions

The key elements of the sustainable success of the enzymatic 7-ACA process are:

- An optimised competitive process with forward looking technology.
- A product quality equal to chemically produced 7-ACA.
- Safeguarded employment and a basis for future investments.

The project was sponsored by the German Ministry of Environment, which provided reduced interest rates for part of the investment.

On 22 December 1999, Juergen Trittin, the Environment Minister, stated: "I hope with the successful completion of this project, we will be one step ahead (or further) towards protecting the environment and we will be able to demonstrate that economic and ecological aspects can be harmonised."

The position of the German Biotechnological Industry is clear: reliable and quick decisions from the authorities result in a positive investment climate; too much regulation and bureaucracy hinder environmentally positive industrial developments and do not improve safety features; worldwide harmonisation of regulations in biotechnology would ease the transfer of processes to other sites and countries. One key topic for harmonisation would be better lists and classification of micro-organisms.

The chief reasons for the success of the new development were:

- The know-how in an existing fermentation plant (antibiotics, recombinant insulin) and the procedure for obtaining permission for a production process that uses GMOs.
- A team that is motivated, big enough to have all the manpower needed and short lines of communication and decision.
- Good personal relationships with internal and external decision makers.

The most important single factor necessary to bring the new development into full production was the changed situation concerning permissions. While this is in the main typical for Germany, nevertheless such a clear political situation will be required wherever this type of process is to be developed.

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Case Study 3

BIOTECHNOLOGICAL PRODUCTION OF THE ANTIBIOTIC CEPHALEXIN (DSM, NETHERLANDS)

Introduction

The β-lactam group is a major class of antibiotics that includes cephalosporin C, which is active against gram-negative bacteria resistant to β-lactamase and is much less toxic than penicillin. With respect to its clinical use, cephalosporin C has several disadvantages, however, including relatively low antibacterial activity and a requirement for injection instead of oral application. As a consequence, semi-synthetic antibiotics based on cephalosporin C have been developed with more favourable clinical properties. One of these is cephalexin.

The cephalexin process described here was first introduced at Chemferm (Netherlands). Chemferm was a joint-venture between DSM and Gist-Brocades and was founded in 1992 (all are now part of DSM Life Science Products). The company produced semi-synthetic cephalosporins and expanded into the area of bulk end products such as cephalexin as well as erythromycin salts (antibiotics of the macrolide class).

Gist-Brocades (now DSM Anti-Infectives) is a group of companies that develop and manufacture products for the pharmaceutical and food industries. The group is the world's largest producer of penicillins, and one of the largest for baker's yeast, certain food specialities and feed enzymes. Many of its complex organic products are produced by large-scale fermentation processes.

DSM is a highly integrated international chemicals group. The company's principal products are intermediates and ingredients for the pharmaceutical and food industries, performance materials (*e.g.* engineering plastics, resins and synthetic rubbers), polymers and industrial chemicals.

The developments described here continue in the Life-Science unit at DSM. Biotechnology (fermentation and biocatalysis) and modern organic (catalytic) synthesis are core competencies of this unit.

Technical description

From 1975 to 1985, cephalexin was produced by a ten-step process employing conventional chemistry and generating a waste stream of 30-40 kg per kg of end product (Table 6). In 1985, the waste/product ratio was reduced to 15 after a lengthy process of optimisation and the introduction of recycling. In 1995, Chemferm introduced a six-step process to produce cephalexin from benzaldehyde and penicillin, applying biocatalysis in three reaction steps and generating a waste stream of around 10 kg per kg of end product.

Further process development at DSM has now yielded a one-step technology for producing 7-ADCA (7-aminodeacetoxy cephalosporanic acid, the key raw material for cefalexin and other cephalosporins) by direct fermentation. The six-step process for cefalexin is thus reduced to a four-step process. Further use of modern biotechnology (*i.e.* metabolic pathway engineering) may lead to processes with even fewer steps.

Besides the reduction in quantity of the waste, its toxicity has also significantly decreased. The traditional process required methylene chloride, silylating agents, Dane-salt protected side chains and

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acylating promoters. The new process mainly releases aqueous waste streams containing harmless inorganic salts (Table 6).

Table 6. **Comparison of processes**

Type of process	Conventional	Biocatalysis	Direct fermentation
Waste (kg/kg cephalexin)	50 (1970) to 15 (1995)	10 (1995) to 5 (2000)	2-5
Inorganics (kg/kg)	0.5	0.5	
Organics (non-halogenated) (kg/kg)	1.0	0.2	
Solvents (non-halogenated) (kg/kg)	1.7	0.3	
Solvents (halogenated) (kg/kg)	0.9	0	
Electricity (%)	100	150	
Steam (%)	100	40	
Water (%)	100	300	
Liquid nitrogen (%)	100	0	

Process comparison

Process Profile Analysis (see Box 5) was used at each stage of process development to compare existing with novel process possibilities. Agreed parameters, which are given specific weightings, included: operating costs, capital expenditure, process control, internal risks and external risks. Each of these are subdivided: internal risks, for example, would be waste streams and health risks, while external risks include availability of key materials, new laws and regulations and patents. Waste type is also weighted: heavy metal wastes are heavily weighted, followed by solvents (which are recycled as far as possible), other inorganics and finally biodegradable wastes. High temperatures and pressures and the possibility of explosion are not necessarily risk factors since they can be translated into investment decisions.

While environmental considerations are an important subset of the parameters to be considered in any process analysis, other areas such as operating costs or process control, are in principle equally important although, starting in the early 1990s, the weighting of risks began to increase. In the 1970s and earlier, sustainability was one-dimensional – it was equated with profit – a necessary element for the long-term survival of the company. Later, environmental concerns were added, and in the 1990s a third dimension, societal concerns.

Fixed and variable costs of alternatives are directly compared on an equal tonnage basis. Productivity, yield per given volume, is a very important parameter, especially when comparing biocatalysis with conventional chemistry. This highlights the problems of dilute environments. Considerations of space needed by alternative processes could highlight an energy problem.

Process of innovation

History

Cephalexin was first synthesised in 1967 by Glaxo Research Laboratories and first produced by chemical synthesis at industrial scale by Eli Lilly and Co. in the same year. It is a deacetoxylated derivative of cephaloglycin that is not metabolised *in vivo*. Cephalexin has been used widely and is the most popular orally active antibiotic in the world for treatment of certain infections.

In the early days of antibiotic production, it was not possible to use biocatalysts, because the required enzymes were not available and the reaction would have had to be run in very dilute, and hence uneconomic, conditions. The use of enzymes at low cost in partly aqueous, partly organic media was first described in the late 1970s, an environment that would allow the production of a range of fine

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chemicals. In 1980, the Dutch government funded a research programme for the development of biocatalysis. After a decade, this has led to industrial-scale use of biocatalysis.

DSM introduced biocatalysis because it saw that the use of enzymes might allow reactions that generate less waste (technology push). A second, more pressing, reason was increasing competition from developing countries, principally India in the early 1990s, and later, China. It was decided at DSM to keep up with the competition and to remain the foremost supplier of antibiotics and to develop a leading technology. Accordingly, Gist-Brocades and DSM formed the joint venture Chemferm in 1992. The aim was to maintain coverage of the whole value chain, not just operating a few steps in the production process of the antibiotic but producing the basic materials as an input for the production process and supplying end users of antibiotics.

The development of the biocatalytic process included the following elements: screening existing enzymes; strain selection; strain improvement; optimisation of the fermentation process; choice of form of the enzyme for application (*e.g.* free cells or immobilised, aqueous or solvent); choice of bioreactor type (*e.g.* batch *vs.* continuous, fluidised, membranes, etc.); optimisation of downstream processing and product isolation.

DSM develops its own enzymes, which are regarded as part of the development cost. No problems were perceived with GMOs since these remain within the process and are not released except as a sterilised biomass.

External and internal influencing factors

The pressure exerted on margins for certain antibiotics by companies from Asia and India that focus primarily on market share led to a need to develop more efficient technologies. The driver for DSM to remain a manufacturer of antibiotics in spite of strong competition and low margins is the attractive size of the market (in 1998, worldwide sales of antibiotics in 1998 amounted to USD 18.5 billion).

Despite the promising market perspective, it was difficult to raise funds for R&D in this particular field because of the significantly reduced profit margins. Management was therefore required to define operating cost targets to be met by R&D efforts. The company managed to reduce operating costs considerably by using the more efficient biocatalytic process. Without this, DSM would probably have had to withdraw from the production of these antibiotics. It is expected that the operating costs can be further reduced to compensate for a possible further decline in price. However, in spite of the success in reducing operating costs, current cash flows are still too low to generate the budget required for R&D.

Several problems exist within the process design. In order to make the process cost-efficient, the enzyme had to be recycled. This was achieved by immobilisation. In the later stages of implementation, the engineers faced some problems during scale-up and downstream processing. The overall process yield had to be improved, and an end product of consistent quality had to be achieved. The most crucial steps in the new process were crystallisation and isolation of the desired cephalexin, crystallisation and recovery of excess 7-ADCA and crystallisation and isolation of phenylglycine, which resulted from undesired hydrolysis of both end product and side-chain precursor. The overall process had to be taken into account since a single step could not be optimised in isolation from other parts of the process.

In early stages of the cephalexin project, R&D was constrained by legislation relating to GMOs. Furthermore, the process of registering a pharmaceutical product includes documentation on the manufacturing process. Consequently, the development of a new process, whether it is chemical or biological, uses GMOs or not, requires that the product must be re-registered. In the current stage of development, however, problems related to marketing and in particular, to customer acceptance, are more important than regulations.

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Summary and conclusions

Both DSM and Gist-Brocades had expertise in complementary fields. Gist-Brocades had profound knowledge in microbiology and genetics (strain selection and improvement, over-expression of enzymes, introduction of new enzyme activities to enhance or introduce production of metabolites), biochemistry (identification of bottlenecks in metabolism, for instance by simulation of flows through metabolic pathways, characterisation of enzymes) and fermentation (scale-up, fermentor design, process modelling, development of control systems, etc.). DSM could contribute its experience in biocatalysis and organic chemistry in intermediates for the pharmaceutical industry.

One of DSM's philosophies is to develop new products and processes with minimum environmental impact. Also, as part of its effort in the field of product life cycle management, DSM also devotes considerable attention to the development of recycling technologies for (end-use) products.

When deciding on the introduction of new products and processes, the environmental impact becomes a decisive criterion when higher ranking criteria (operating cost, investment cost, product quality) do not provide a clear choice.

In rapidly changing markets, companies usually do not have time to fully realise productivity potentials because time to market is more important than comprehensive process development and optimisation. Mature products, on the other hand, with stable or slightly growing sales, allow for continuous process improvements. Furthermore, process optimisation and reduction of operating costs are requirements in such businesses because competition usually comes in the form of falling product prices.

Two lessons can be derived from this example:

- The replacement of conventional technologies by the introduction of ecologically friendly biotechnological methods (such as biocatalysis) will more probably take place in established businesses, where new processes may lead to a considerable increase in efficiency or product quality.
- In newly developing, rapidly changing industries, incentives to apply eco-friendly technologies may have a lesser impact. A company has to integrate all relevant aspects (efficiency, ability for scale-up, safety, sustainability, etc.) in the early stages of process development. Otherwise, efforts to optimise will be overtaken by technological change. Often, these aspects conflict with the desire to shorten time to market.

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Case Study 4

BIOPROCESSES FOR THE MANUFACTURE OF AMINO ACIDS (TANABE, JAPAN)

Introduction

Tanabe Seiyaku Company is a major manufacturer of amino acids for the health and food and feed markets. The company is well known throughout the world for its success in developing and industrialising bioprocesses using immobilised enzymes and micro-organisms.

This case study describes three amino acid production processes using immobilised enzymes and micro-organisms, each later one developed on the basis of knowledge gained from previous processes. When comparing them, they should be regarded as a new bioprocess compared to an older one rather than as a bioprocess compared with a conventional chemical process.

Use of immobilised aminoacylase

Utilisation of L-amino acids in medicine, food and animal feed has developed rapidly in recent years, and the economical production of optically active amino acids has been investigated extensively.

Fermentative and chemical synthetic methods have been used, rather than isolation from protein hydrolysates. Chemically synthesised amino acids are optically inactive (racemic) mixtures of the D- and L-isomers. The L-form is the physiologically active one and optical resolution is required to separate it from the D-isomer. While chemical and physicochemical methods exist for these resolutions, the company developed a biological method using an enzyme.

Chemically synthesised acyl-DL-amino acids are asymmetrically hydrolysed by the enzyme aminoacylase to give the L-amino acid and the unhydrolysed acyl-D-amino acid. Both materials are easily separated on the basis of their differing solubilities, and the unconverted material can be racemised and reused.

It was found that an aminoacylase from *Aspergillus oryzae* had broad specificity. It is capable of hydrolysing many acyl amino acids.

From 1953 to 1969, Tanabe employed this enzyme in a batch-wise process to produce several amino acids. However, this process had several disadvantages from an industrial point of view. Because it involved incubating the substrate with the soluble enzyme, isolation of the product required destruction of the enzyme by acid or heat. Thus, although active enzyme might remain in the reaction mixture, it was nevertheless discarded. Additionally, a complicated purification procedure was necessary to remove proteins and coloured materials from the crude enzyme preparation used. Overall, yield of amino acid was low and labour costs high.

To overcome these disadvantages and to improve the enzymatic method, company researchers studied the possibilities of a continuous process using immobilised enzyme in a column reactor. Two factors were particularly important: because the enzyme and possible carriers were expensive, the operational stability of the immobilised enzyme and the ease of regeneration of used columns after long periods of operation had to be maximised.

Various immobilising techniques were examined and one that used ionic binding to DEAE-Sephadex was chosen. Not only did the enzyme thus immobilised have a higher optimum temperature

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and show the highest thermal stability and resistance to organic solvents and protein-denaturing agents, operational stability was also highest and it was easily regenerated. A reactor system was designed for continuous production in which flow rate, pH and temperature are controlled automatically. Since 1969, the company has used this process industrially for the resolution of a number of amino acids, including methionine, tryptophan, phenylalanine and valine. From a 1 000 litre column, production ranges between 6 and 21 tons per month.

The immobilised enzyme is very stable, maintaining over 60% of its activity after continuous operation for 30 days at 50 °C. Regeneration merely requires addition of an equivalent amount of fresh enzyme to the column. The carrier has been shown to retain its capacity to adsorb enzyme and to maintain unchanged other physical properties over five years.

Cost comparison

A continuous process using such a stable immobilised enzyme gives not only high productivity per enzyme unit but also high product yield, because there is no contamination by protein or coloured materials and simpler purification stages are therefore possible. Automatic operation also reduces labour costs and there is a consequent reduction in overall production costs (Table 7).

Table 7. **Relative costs of batch and continuous processing**

Percentages

Cost component	Batch process	Continuous process
Raw materials and substrate	52.0	41.0
Enzyme (aminoacylase)	23.0	3.5
Labour	20.5	6.0
Fuel	4.5	4.5
DEAE-Sephadex	–	2.0
Total	100.0	57.0

In the immobilised enzyme process, overall production costs are lower by more than 40%. Savings on labour and enzyme are the major contributors. Although the carrier is relatively expensive, this does not affect production costs significantly because of its long life.

Use of immobilised E. *coli*

L-aspartic acid is widely used but demand has rapidly increased with its use as a raw material for the synthesis of the artificial sweetener, aspartame. Since 1958, L-aspartic acid has been made by batch fermentation from fumaric acid and ammonia using the enzyme aspartase. Tanabe Seiyaku has manufactured L-aspartic acid on a continuous basis since 1973 using an immobilised form of this enzyme.

As with the aminoacylase reported above, the batch fermentation resulted in active enzyme preparations being discarded. Because the enzyme, which is obtained from E. *coli*, is intracellular, it was originally extracted from macerated organisms and then immobilised by being trapped in polyacrylamide gel. Alternatively, the enzyme can bound to a weakly basic ion exchange resin. This latter system has been used by the Kyowa Hakko Kogyo Company since 1974.

It was thought that if whole cells could be immobilised, the instability of the enzyme could be overcome. Tanabe therefore explored the possibility of entrapping whole cells with various materials and found that the most active preparation again used polyacrylamide gel. In the process, it was found that suspending the immobilised cells in their substrate for 24-48 hours increased their activity tenfold. Once immobilised, the cell column was very stable, with a half-life of 120 days at 37 °C. After acidification

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of the effluent from the column, the L-aspartic acid crystallises out and can be collected by centrifugation or filtration.

This system has been in operation since 1973. Overall costs compared with the batch production are reduced by approximately 40% because of the marked increase in productivity and the reduction in labour costs. Waste treatment costs are also reduced.

While this immobilisation process works well for aspartase, other cells and enzymes can be inactivated by the immobilisation procedure. Other polymers were found to form a suitable lattice for cell entrapment, the most suitable of which was κ-carrageenan obtained by extraction from red algae. Enzyme activity of E. *coli* cells immobilised in κ-carrageenan was much higher than in polyacrylamide, while hardening the gel using glutaraldehyde improved the operational stability up to a half-life of 680 days or almost two years. Overall, the relative productivity was increased fifteen-fold. On this basis, the process was changed to use κ-carrageenan in 1978. Approximately 100 tons per month of L-aspartic acid can be manufactured using a 1 000 litre column.

Novel strains of E. *coli* were developed first by continuous cultivation in a medium having L-aspartic acid as the sole nitrogen source and subsequently by genetic modification whereby a strain that overproduced aspartase was developed. The first step increased specific activity of aspartase six times and the second resulted in a tenfold increase, both over the original E. *coli* strain. Use of the first strain was adopted in 1982.

Use of immobilised E. *coli* and immobilised P*seudomonas dacunhae*

L-alanine has been produced industrially by Tanabe Seiyaku since 1965 by an enzymatic batch reaction using the aspartate β-decarboxylase activity of intact P*seudomonas dacunhae* cells. The immobilisation of these cells, used in a continuous column, has been investigated. The problem was the evolution of CO_2 gas from the decarboxylation reaction which made plug flow of the substrate difficult and caused significant changes in pH.

Because L-aspartic acid has been produced commercially by the company, researchers examined whether there existed the possibility of combining the two organisms into a single reactor to produce L-alanine more efficiently by a two-step enzyme reaction.

The first step was to develop a high-pressure reactor in which the CO_2 does not evolve. This reactor established plug flow for the substrate solution and the pH changes were minimised by the buffering effect of the dissolved CO_2.

Certain side reactions can occur when using whole cells that give the by-products D-alanine and L-malic acid. It was found that a pre-treatment consisting of mild acidification for one hour could eliminate virtually all of these side reactions without reducing the aspartase or decarboxylase activity.

Co-immobilisation of the two micro-organisms was tried in an attempt to improve the overall efficiency but efficiency was in fact reduced, for reasons which are not clear. Consequently, various separate reactor combinations were tried, with the final choice going to an arrangement where pH is adjusted separately for each so that the two enzymes operate at their respective pH optimum. Using a 1 000 litre reactor for immobilised E. *coli* cells and a 2 000 litre pressurised reactor for P. *dacunhae* cells, about 100 tons of L-aspartic acid and 100 tons of L-alanine can be produced per month.

Tanabe commercialised this process in 1982 and believes it to be the first industrial application of sequential enzyme reactions using two kinds of immobilised micro-organisms.

Summary and conclusions

Since the enzyme may represent a significant cost element, immobilisation can result in a more than 40% decrease in production cost, as compared with the previously used batch reaction process, in which the enzyme was disposed of without being recycled. Although the carrier for the immobilisation of the enzyme represents an additional cost, the costs of both raw material and labour can be

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dramatically decreased. Since, at the completion of each batch, the enzyme is discarded, the change to immobilisation results in a major reduction in waste production.

Because the aminoacylase enzyme is not produced by Tanabe itself but purchased from the another company, it was not considered appropriate to consider the utility cost of enzyme production within the boundary of the cost comparison.

This use of aminoacylase was the first industrial application of immobilised enzymes. This enzyme has a very broad specification and has been used not only to manufacture a range of L-amino acids but also the D-isomers which have recently developed into important pharmaceutical intermediates.

In 1973, Tanabe built on its experience with immobilised enzymes by adapting the continuous reaction method to the use of an immobilised micro-organism. The new process did not require either the separation of the enzyme from the disrupted cells or the disposal of waste cells. The improved immobilisation method, applied to the production of L-aspartic acid, resulted in an increase in productivity of more than 15 times. In addition, productivity was further increased by six times by improving the microbial strain used.

Although a recombinant strain of E. *coli* has been constructed with yet higher aspartase activity, it is not used in practice because the effect of higher enzyme activity on the manufacturing cost seems to be negligible, since the percentage of enzyme in the total cost is already very small.

L-alanine was first manufactured by an enzymatic batch reaction process in 1965. This was replaced by the already developed technology based on immobilised micro-organisms in 1982.

Since the reaction used is a decarboxylation that generates carbon dioxide, a pressurised column was designed in order to improve the efficiency of the reactor. In addition, a more efficient system was developed by combining this process with L-aspartic acid production.

Over a more than 30 year period, the Tanabe Seiyaku Company has built on its internal experience to develop more and more sophisticated immobilisation technology applied to the biosynthesis of amino acids.

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Case Study 5

MANUFACTURE OF S-CHLOROPROPIONIC ACID (AVECIA, UNITED KINGDOM)

Introduction

The importance of chirality to the pharmaceutical industry is now well recognised. The principal driving forces for making individual enantiomers rather than racemates has been both product efficacy and regulatory pressures. Cost reduction, through the raw material savings that can come from moving to a single enantiomer, has generally been of lesser importance in this industry.

Chirality has also been of importance in the agrochemical industry but regulatory issues have been less profound and the principal reason for making single enantiomers has been to achieve greater cost-effectiveness in use. However, some companies have recognised the opportunity to gain product differentiation and to lead regulatory change in favour of the more environmentally sound single enantiomeric form. Conversion to a single-isomer product allows up to double the manufacturing capacity of the final product and offers cost savings through reduced raw material use. Coupled with the environmental and potential regulatory benefits, this has given a strong case for change.

(S)-2-Chloropropionic acid [(S)-CPA] was one of the first commercially valuable single enantiomeric materials identified. Although structurally a relatively simple molecule, its importance as a large-volume homochiral building block and the cost sensitivity associated with its major agrochemical end markets have posed both an opportunity and a challenge to business.

One of the earliest phenoxypropionate herbicides to be converted from racemic to single enantiomeric form was the Zeneca product Fusilade. Similar strategies were adopted by BASF in Germany with regard to mecoprop and other older commodity herbicides.

Synthetic approaches to these herbicides not based on (S)-CPA have included microbiological and enzymatic resolution of the final racemate. However, these do not compete economically largely because of the cost implications of yield loss when working with the final active molecule, thus supporting the general rule that resolutions should take place as early as possible to minimise the total quantity of material produced.

Technical description of process

The traditional route to (S)-CPA is as follows: glucose is fermented to R-lactic acid; this is then extracted and recovered, esterified and the ester chlorinated. Esterification is necessary to protect the acid group during chlorination with thionyl chloride.

The biotransformation route starts with racemic CPA which is a commodity chemical. The chosen organism dechlorinates the (R)-enantiomer, producing (S)-lactic acid as reaction product and the (S)-enantiomer carries through unreacted. Following complete hydrolysis of the (R)-enantiomer the reaction mixture is acidified to precipitate biomass which is filtered off. The (S)-CPA is then extracted with solvent, solvent is removed by distillation to yield crude (S)-CPA which can be fractionally distilled to yield higher purity product if necessary. Enzymes that catalyse dehalogenation reactions are widespread in nature. Many such enzymes, with different specificities, can be present in a single species of bacterium. The original wild type *Pseudomonas* organism from which this process was

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developed had both an (S)- and an (R)-specific dehalogenase. The (R)-specific enzyme was inactivated by conventional mutagenesis to produce the first-generation production catalyst.

The final process for (S)-CPA was established 18 months after invention of the concept. The overall process has a capacity for multi-thousand ton production. The introduction of a continuous fermentation process for the production of the biocatalyst resulted in a fourfold increase in productivity, and the development of a genetically manipulated organism in 1991 has given a further fivefold increase. This has made a significant impact upon production economics and has resulted in even lower-cost, commodity herbicides being switched to single enantiomer products.

Advantages and disadvantages

A number of biological routes to (S)-CPA have been investigated, including the use of esterases and lipases, but all had performance shortcomings. ICI (now Avecia), who invented the process, recognised that the low cost of racemic CPA made resolution approaches potentially economical and that a reaction directly at the chiral centre offered the best chance for high enantioselectivity.

Separation problems can often arise with biotransformation reactions because of the presence of proteins and other biological materials that can interfere with solvent extractions and crystallisation processes. The presence of unwanted enzymes in whole cells that can interfere with the enzyme of choice or that can attack raw material or product is also an important consideration. While batch growth is less complicated, there are productivity benefits to be derived from continuous technology.

Many options needed to be considered for the (S)-CPA process, including isolated enzymes *vs.* whole cells, immobilised vs free cells and batch *vs.* continuous fermentation. Whole cells offer several advantages over isolated enzymes for simplicity and speed of scale-up. Batch fermentation was used at first, but the higher productivity associated with continuous fermentation was the ultimate target. By genetic manipulation, a derivative strain was developed which enabled the use of continuous fermentation culture over many hundreds of hours with much higher enzyme productivity.

The new production organism has a greater than tenfold increase in activity over that of the original mutant. Clones exhibiting much higher dehalogenase activity were also generated but these were less stable in continuous culture and dehalogenase expression rapidly declined. It is felt that there is more scope for increasing productivity but this is not yet financially justified.

The biocatalyst is killed as a result of the processing steps and is disposed of to a waste treatment plant. Off-gases and other wastes are monitored, as is the site, for the presence of GMOs.

History of the innovation process

Staff in the Corporate Laboratory in ICI began exploring the role of enzymes in chemical synthesis in 1978. A second group, ICI Bioproducts, had developed Pruteen (a microbial-based animal feed) and Biopol (a biodegradable plastic) and were looking for new products. The Agrochemicals Division had a racemic herbicide, Fusilade, with sales of the order of hundreds of tons for which they wanted to make the active enantiomer and needed a cheap source of (S)-CPA. One source of this material was via lactic acid, produced by fermentation, but this was already a competitive market. Agrochemicals turned to the ICI Fine Chemicals Manufacturing Organisation (FCMO) which "owned" process chemistry in ICI and whose head was looking for other generic synthesis possibilities.

At first, the manufacturing process used a chemically induced mutant, which was subsequently replaced by a recombinant organism in 1991. At that time, an embryonic genetic engineering group existed within ICI and the cloning of the dehalogenase represented one of the first major projects of this group.

All this work created a commercial product, a team with experience and a demonstration that biocatalysis could work at large scale, and all on a product of not very high value. It became the foundation of a major business activity. The company currently makes 2 000 tons/year at the FCMO site.

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The biocatalyst is made in Billingham and shipped to Huddersfield. At first it was transported as a concentrated cell suspension in water but this proved to have stability problems and was prone to microbial contamination. These problems were overcome by the development of an innovative and patented process for spray-drying the biocatalyst while retaining the bulk of the enzyme activity.

Since 1993, ICI has undergone a number of reorganisations that have resulted in the formation of Avecia, now the home of ICI's former biotechnology-based businesses. The biocatalysis activities are now fully integrated into a business unit servicing the pharmaceuticals market.

In 1992, Merck had a drug with two chiral centres in Phase III clinical trials. The synthesis route to this molecule involved a ketone reduction, which produced the wrong isomer at one centre, which then required epimerising. This was not a very selective reaction. Based on their expertise in biocatalysis, Avecia scientists invented a route to the desired compound using biocatalysis, which gave the desired isomer directly, thus removing the need for an epimerisation.

Within the pharmaceutical business sector of the company there are chemical and biological research groups working together. Asymmetric chemical reactions, diastereoisomeric crystallisations and enzymatic resolutions are all compared on the basis of timing, cost and quality, with a checklist used in the comparison.

A business and technical team responds to all enquiries, using a technique known as New Opportunities Management Process (NOMP). Commercial development looks at costs, quantities and profit, while technical development looks at how to make the product. Possibilities are brainstormed to decide which process to use and how much time and lab effort to spend. The NOMP process has "stage gates": the enquiry, a paper study, prior work leading to development to full scale.

Environmental impact is a key element in any analysis. All processes are assessed for effluents using a decision tree process in which a weighting is given to each effluent which is then incorporated into an overall attractiveness. Negative factors create no problem as long as they can be adequately dealt with. Energy consumption is not considered under an environmental heading but is an economic factor considered as part of plant occupancy. Use of renewable raw materials is generally not a consideration; since the company makes intermediates, the starting material is usually predetermined.

Summary and conclusions

A general rule emerges that resolutions should take place as early as possible to minimise the total quantity of material produced.

The key to success in biotransformation technology is the process of translation of an embryonic laboratory procedure into a cost-effective, reliable and robust plant-scale operation in an acceptable time-scale and using appropriate resources.

Provided that appropriate plant volumetric and biocatalyst productivities can be achieved and the overall yield target met, cost-effective biotransformation processes are attainable.

Many options need to be considered, including isolated enzymes *vs.* whole microbial cells, free *vs.* immobilised enzymes or cells and batch *vs.* continuous reaction for fermentation.

That the development time-scale for the (S)-CPA process was relatively long is not surprising for a novel technology. Subsequent biotransformations developed by Avecia for chiral molecules have benefited from this learning experience and the time scale has been reduced dramatically.

Time-scale has now become possibly the most critical aspect in meeting the needs of the pharmaceutical industry, and what used to be a major constraint to the exploitation of biotechnology in this area has now largely been overcome. The key determining factor now is not the identification of an appropriate enzyme but the speed of scale-up, reflected in the ability to mobilise a multidisciplinary team of biotechnologists, chemists and engineers. The importance of integrated process development is the key lesson.

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To succeed in their chosen markets, Avecia develop partnerships with academic and other external institutions as well as alliances with companies in related business areas.

Avecia believe that there is no single technology that can give economic results in all cases and that force-fitting a favoured technology to a molecule or customer is not the right approach.

In practice, biological and physicochemical resolutions have been used in the manufacture of new products. In one case, a biological route was used for trial quantities but a chemical resolution was used for manufacture. Avecia has even used their own biologically produced (S)-CPA as a resolving agent.

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Case Study 6

ENZYMATIC PRODUCTION OF ACRYLAMIDE (MITSUBISHI RAYON, JAPAN)

Introduction

Acrylamide is a major commodity chemical. It has two functional groups, a vinyl and an amide group, and is consequently used as a starting material for the production of a wide range of chemical derivatives, both monomers and water soluble polymers. Representative applications of these include the manufacture of polymers for paper treatment, flocculants and enhanced oil recovery. The production of acrylamide in 1999 was about 70 000 tons in Japan and exceeded 400 000 tons worldwide (Table 8).

Table 8. **Worldwide acrylamide production capacity**
10^5 tons/year

	Japan	Asia (excl. Japan)	United States	Europe
Catalytic	0.9	0.75	1.35	1.15
Enzymatic (1998)	0.2	0.2	0.1	0.35
Enzymatic (2001, est.)	n.a.	0.5	n.a.	0.45

Acrylamide has been produced from acrylonitrile by two chemical synthetic processes: a sulphuric acid hydrolysis process and a copper-catalysed hydrolysis process. In 1985, Nitto Chemical Industry Co., Ltd. (now Mitsubishi Rayon Co., Ltd. – MRC) started commercial production of acrylamide via a new enzymatic production process (the BA process). Although the starting production capacity of the process was 4 000 tons/year, it had risen to about 20 000 tons/year by 1998 without major changes in the process but by successive innovations in process development and the application of new microbes and genetically engineered enzymes.

This process is now accepted as being low-cost, high-quality and environmentally friendly, and new production facilities are being built worldwide. MRC supports these by licensing its technologies to companies in other countries.

Technical features

Historical overview

Commercial production of acrylamide was first established in 1954 by American Cyanide Co., Ltd. In the classical process, acrylonitrile was hydrolysed by addition of stoichiometric amounts of sulphuric acid in the presence of polymerisation inhibitors to prevent both the starting materials and products from polymerising. This process also produced large amount of ammonium sulphate as a product. In the1970s, Mitsui Toatsu, American Cyanamid, Dow and Mitsubishi Chemical developed the heterogeneous catalytic process that eliminated the need for sulphuric acid. This innovative process had many advantages over the sulphuric acid process and was widely applied in acrylamide

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production. This process has also enabled the production of a high concentration (50%) aquatic solution of acrylamide, with only the treatment of the ion exchange resin for removal of the catalyst and for product concentration.

The development in technologies of polymerisation and polymer applications, however, created a new demand for more highly purified acrylamide monomer and has revealed that the acrylamide produced by the catalytic process, which had been recognised as a high-quality process, nevertheless contained minor by-products that affected the polymerisation reactions.

Therefore, MRC started development of an enzymatic acrylamide production process which was expected to reduce the level of by-products as a result of the high selectivity of the enzymatic reaction.

Isolation of the first-generation organism with the nitrile hydratase enzyme and development of the production process was undertaken solely by MRC. The second- and third-generation organisms, both B-23 and J-1 (see below), were discovered by Prof. Yamada and colleagues in Kyoto University. Succeeding process development was performed by MRC in co-operation with Kyoto University. The fundamental studies of the fourth generation micro-organism (a GMO) were undertaken in co-operation with Kyoto University and University of Tokyo, and the technology development was carried out by MRC.

Pilot-scale development for the first-generation microbe took one and half years for process development and product quality assurance. For the second- and third-generation organisms, about six months of bench-scale tests were enough to ensure the process application and product quality. For the development of the GMO, about seven years were needed to build up the genetic engineering technologies (development of modified genes, vectors and cloning technologies, process improvements, etc.) and the process development.

A number of different parts of the organisation were involved in the early decision-making process. These included technology and quality control staff from the Technology Development Department, a Safety, Environmental Management Group and the Chemicals Division (Technology and Marketing). Since that time, the decision-making organisation has diversified further.

The company relied on product specification and marketing data, information on process economy and process and product safety and guidelines for the industrial application of recombinant DNA technology.

Process characteristics

The two production processes may be described as follows:

- *Catalytic process*: Acrylonitrile is hydrolysed using a copper-based catalyst that has to be periodically reactivated. Hydrolysis is followed by a concentration step in which unused acrylonitrile is recovered. This in turn is followed by a deionisation stage. A range of by-products, including other amides, is produced in the reaction.
- Enzymatic process: The hydrolysis of acrylonitrile uses a recoverable immobilised whole cell catalyst. The old process required decolourisation and concentration steps, but the new process does not. Differences in the reaction conditions of both processes are shown in Table 9.

Table 9. **Comparison of processes**

Reaction process	Catalytic (1971-)	Enzymatic (1985-)
Reaction temperature	343°K	273-288°K
One-pass reaction yield	70-80%	~ 100%
Acrylamide concentration	~ 30%	48-50%
Concentration	Required	Not required
Purification	Catalyst removal	Protein removal

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The BA process consists of four unit processes: microbial enzyme production, immobilisation of microbial enzymes, reaction and removal of catalyst.

Activity was increased by the application of genetic engineering and successive developments in fermentation technology and the microbes for the production of enzymes have been changed three times: from N-774, to B-23 and J-1 and genetically engineered J-1 microbes. These microbes are either R*hodococcus sp*. or P*seudomonas sp*. (Table 10).

Table 10. **Development of new enzymes**

Number	Microbial strain	Acrylamide concentration (%)	MRC capacity (thousands of tons/year)
1	N-774 (old process)	20	4
2	B-23	27	6
3	J-1 (new process)	50	20

In the first production process, the acrylamide concentration in the reaction solution was 20% and required further concentration to make the required 50% aqueous solution. Discovery of the J-1 microbe and succeeding development of application technologies enabled an increase in reaction velocity and selectivity and consequently the elimination of the concentration stage.

Since the company had long produced acrylamide monomers by the chemical process, they had a deep knowledge regarding the toxicity and environmental influences. Consequently, economic aspects played the most important part for the selection of the new process. The previous experience was valuable in the process design and raw materials and product handling.

For microbe selection and gene engineering applications, occupational safety, influence on the environment and governmental guidelines were all considered, and this knowledge was applied to the process improvements.

These technological improvements also enabled the development of a simplified, cost-effective and environmentally friendly process.

Advantages and disadvantages

The essential differences between the BA process and the catalytic process are:

- The production process is simpler and the reaction is carried out at ambient pressure and temperature.
- There is no need for a catalysis recovery and reactivation process.
- Conversion of acrylonitrile is very high and unreacted acrylonitrile is not recovered.
- The 50% acrylamide aqueous solution is achieved in the reactor and further concentration is not required.
- Less energy is consumed.
- Investment in equipment is lower.
- Refrigeration is required in the BA process to remove heat of reaction in order to maintain the low reaction temperature.
- The production cost of the BA process is also lower.
- The high selectivity and low reaction temperature reduces the amount and range of by-products.

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Environmental impact

Energy consumption and carbon dioxide production

Comparative studies have been performed on the environmental impacts of acrylamide production processes on the basis both of energy consumption and carbon dioxide production. These studies concluded that both values of the BA process were lower than those of the conventional process and demonstrated that the BA process is a more environmentally friendly process in these respects (Tables 11 and 12).

Table 11. **Comparison of energy consumption**
MJ/kg acrylamide

	Catalytic	Enzymatic (old)	Enzymatic (new)
Steam	1.6	2.8	0.3
Electric power	0.3	0.5	0.1
Raw materials	3.1	3.1	3.1

Table 12. **Comparison of CO_2 production**
Kg CO_2/kg acrylamide

	Catalytic	Enzymatic (old)	Enzymatic (new)
Steam	1.25	2.0	0.2
Electric power	0.25	0.25	0.1
Raw materials	2.3	2.3	2.3

Products and process safety and treatment of wastes

Both acrylonitrile and acrylamide have neuro-toxic, hepato-toxic and carcinogenic properties. The production process is tightly designed and operates in closed systems, and handling of the compounds is carefully controlled under conditions to avoid both human contact and environmental contamination. All organic wastes are incinerated or sent for wastewater treatment (Table 13).

Material safety data sheets (MSDS) are appended to all acrylamide products to bring the toxic properties to the attention of users.

Table 13. **Comparison of waste production and treatment**

Treatment	Catalytic (1971-)	Enzymatic (1985-)
Reaction process		
Catalyst	+ (copper, etc.)	+ (immobilised enzyme)
Organic waste	Minor	Minor
Inorganic waste	Minor	None
Concentration and purification		
Organic waste	Minor	None
Inorganic waste	Copper	None
Requirements for waste treatment		
Sewage treatment	+	+
Ion exchange resin	+	None
Incineration	None	Enzyme

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Summary and conclusions

Some 15 years have passed since the start-up of the BA process for producing the commodity chemical acrylamide. The process has become a more cost-effective and environmentally friendly one through successive technical improvements.

An earlier version of the Green Index, described in Chapter 3, has been used to compare the BA process with the earlier catalytic process and the results, shown in the annex to this case study, demonstrate the improved environmental friendliness of the enzymatic process.

MRC has licensed the technology for production in foreign countries. In addition, some companies have started their own enzymatic production of acrylamide. In the meanwhile, the company is applying its biological skills to the production of other chemicals such as chiral and non-chiral pharmaceutical intermediates, non-natural amino acids and also biodegradable chelating agents.

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Annex

CHECKLIST FOR SUSTAINABILITY OF ENZYMATIC PROCESSES

Factors affecting sustainability	Score[1]
	Enzymatic process
1. Energy use	
1. Use of fossil fuels?	+2
2. Use of alternative energy?	0
3. Energy efficiency in process?	+2
4. Energy used in transportation?	0
2. Raw material use	
1. Use of fossil resources?	0
2. Use of renewable resources?	0
3. Use of unused resources[2]?	0
4. Use of recyclable resources?	0
3. Renewability of raw material/end product	
1. from a recyclable source?	+1
2. From a renewable source?	0
3. Product is biodegradable?	0
4. Waste production	
1. Waste is biodegradable?	+2
2. Safety? (human, environment)	
2.1. Accumulation	+2
2.2. Risk of endocrine disruption	?
2.3. Other safety aspects	?
3. Waste is recyclable?	0
5. Product and by-product safety	
1. Safety?	
5.1. Accumulation	?
5.2. Risk of endocrine disruption	?
5.3. Other safety aspects	?
2. Efficiency of recyclability?	0
6. Process safety	
1. Risk assessment?[3]	+1
2. Low pressure/temperature reaction?	+2
3. Risk of explosion?	+2
4. Non-organic solvent processes?	0
5. Neutral reaction/acid, alkaline reaction?	0
6. Safety of catalyst, microbes, enzymes?	+2
7. Social and economic aspects	
1. Low energy consuming process	
2. Low consumption of metallic resources	
Total score	**+16**

1. Scores range from –2 (much worse) to +2 (much better) relative to catalytic process.
2. "Unused resources" describes resources that are not used under normal conditions at present. [*e.g.* unused petroleum fractions, unused wood resources (lignin, etc.)].
3. The inventory of inputs and outputs should be adequate to provide the necessary data to assess against the following potential environmental impacts (list is due for ISO approval): abiotic resources (limited resources), biotic resources (sustainable/non-sustainable use), land use, global warming, stratospheric ozone depletion, photochemical oxidant formation, acidification, eutrophication, ecotoxicological impacts, human toxicological impacts.

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Case Study 7

ENZYMATIC SYNTHESIS OF ACRYLIC ACID (CIBA, UNITED KINGDOM)

Introduction

Allied Colloids Ltd (founded in 1935) has, since the 1960s, manufactured a range of polymers, based on acrylamide and acrylic acid, which are used for flocculation as part of water treatment.

In 1982 the company began to look at natural products in response to the possibility of an oil shock – guarding against the threat to the business – their first interest being in xanthan gum. In the mid-1980s, the company back-integrated into acrylic monomers and first considered applying biotechnology to these intermediates.

In March 1998, the company was taken over by Ciba Speciality Chemicals and became Ciba's Water Treatments business unit.

Ciba made their first approach in October 1997, and, from that time, management was in a state of flux until March 1999, by which time virtually all of the original Allied Colloids top management had left and water treatment was absorbed into another division (Additives). This was accompanied by a downturn in the business climate – early 1999 saw a mini-recession in the chemicals industry.

Projects in Allied Colloids tended to be supported via the enthusiasm of senior executives, many of whom were chemists and engineers who had grown up with the company. Following the take-over by Ciba, all the senior management were new to this industry but, on the other hand, they had a very detailed system for project analysis. The Ciba approach to project analysis is an iterative process such that the approval of capital expenditure became a much more systematic economic survey, rather than being based on decisions of very knowledgeable top management.

Following the acquisition, all projects involving significant capital expenditure were re-examined. Because of the global downturn in several industries in mid- to late 1998, particularly in the Asia/Pacific region, the expected rate of growth in consumption of this particular salt of acrylic acid was below original estimates. This resulted in an increase in the estimated payback time for this investment which, when evaluated against other projects competing for capital expenditure, especially those involving safety upgrades, meant that it "missed the cut" under a revised ceiling on total capital expenditure.

Technical description of process

The process which the bio-development sought to replace was the conversion of acrylonitrile (used on site to make acrylamide) to acrylic acid. This process was hazardous and generated substantial quantities of waste material:

Acrylonitrile + H_2SO_4 → (at 100 °C) → acrylamide sulphate → (hydrolysis and steam stripping at 175 °C) → acrylic acid + ammonium sulphate (for disposal).

It was known that dead bacterial cells could convert acrylonitrile to acrylamide at industrial scale and that the reactants and products diffused freely through the cell wall to the enzyme catalyst. It was speculated that this might also be true for a further conversion through to the acid salt.

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The first biological process, developed at laboratory scale for the acid salt preparation, was based on two sequential enzyme catalysts. It had the disadvantage that there existed the possibility of a build-up of the toxic intermediate acrylamide:

Acrylonitrile + H_2O (nitrile hydratase) → acrylamide + H_2O (amidase) → ammonium acrylate.

The process finally used resulted from finding an organism in which one enzyme converted acrylonitrile directly to ammonium acrylate without any build-up of intermediate acrylamide:

Acrylonitrile + $2H_2O$ (nitrilase) → ammonium acrylate.

Risks and benefits

The benefits of the bioprocess include: a simple, single-step, cost-effective, scaleable process; ambient temperature and atmospheric pressure; good-quality product; low acrylonitrile concentration throughout – a much reduced hazard, and very little by-product formed. The in-house acrylic acid route was a multi-step process with high acrylonitrile concentrations, high temperatures and several bar pressures. Comparison between the two therefore strongly favoured the biological route on all aspects. However, in the last year of the project, the benefits of the biologically catalysed route to ammonium acrylate were no longer being drawn from comparison with the in-house acrylic acid process. The justification for the process was from a comparison with the neutralisation of purchased acrylic acid. The neutralisation of the acid is an additional process step that emitted toxic vapours and required diligent monitoring for quality purposes.

The relative consumption of raw materials and services are shown in Table 14.

Table 14. **Relative consumption of raw materials and services**

	Ammonia neutralisation of acrylic acid	Bio-route to ammonium acrylate
Raw materials	0.81 tonnes acrylic acid. 0.19 tonnes ammonia	0.6 tonnes acrylonitrile 0.4 tonnes water Fermentation medium
Cost saving on raw materials		54%
Process operation	Toxic vapour emissions Remote quality monitoring required.	Stoichiometric neutralisation. Simple, at-line process monitoring and control
Utilities	Cooling required	Cooling required
Plant cost	Existing plant	Fermentation and bioconversion plant – combined cost > GBP 2 million

The perceived technical risks were: the need for large scale fermentation know-how (with the possibility of contamination risk); in-house versus toll-manufactured biocatalyst; pilot-scale training and external bioengineering expertise; use of acrylonitrile in a novel process; installation of new plant in an existing monomer plant; control of acrylonitrile concentration (a monitoring system was developed by Huddersfield University), and the integration of batch ammonium acrylate manufacture with the polymer production (manufacturing was heavily involved in plant design).

Process of innovation

Since there were few sources of external guidance as to the likely success of relevant bioprocesses, the only way to find out was to do the research. The first biotechnologist was employed in 1990. Rather than build a laboratory that might turn out to be a white elephant, the company collaborated with the local university (Huddersfield) which had an embryonic interest in biotechnology. The company had little in-house experience of biotechnology at the outset, and the development benefited greatly from governmental support. Company and university made a successful application to the UK government LINK Biochemical Engineering programme, which part-funded a four-year research project. Two members of the company staff were seconded full time to the university over this period.

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While, for the company, acrylamide was the more important monomer – the company is the UK leader making acrylamide from acrylonitrile chemically – the Japanese company Nitto (now Mitsubishi Rayon, see case study 6) had already developed a biotechnological route by 1985. Consequently this would not have been an acceptable project under the pre-competitive research requirements of all LINK programmes. In consequence, the research focused on a route from acrylonitrile to acrylic acid. Also, the conventional acrylamide process had few significant risks associated with it, but there was concern about acrylic acid manufacture both on safety and waste grounds. A runaway industrial incident in 1990 involving hot sulphuric acid, while it did not result in the closure of the existing process, nevertheless increased the importance of finding an alternative. At this time, the existing plant was expensively upgraded. While world-scale plants use a route from propylene to acrylic acid directly, the company had an advantageously priced supply of acrylonitrile, so that it was economical for them to manufacture in this way.

During the LINK project, screening equipment was devised which relied on conductivity to monitor the formation of the acid salt product. Using a PC, steady-state conditions could be maintained which allowed the stability of biocatalysts with different activities to be measured over a range of operating conditions.

The early target was a biocatalyst to convert acrylamide to acrylic acid – the amidase reaction. While product concentrations were uneconomical (owing to product inhibition of the catalyst), this work demonstrated that product quality was excellent and that catalyst activity could be increased through manipulation of culture conditions. Organisms with the two enzymes capable of converting acrylonitrile to acrylic acid were isolated, but the build-up of the intermediate (acrylamide) was a problem.

The breakthrough came at the end of the LINK project with the discovery of a species of R*hodococcus* possessing a nitrilase enzyme that was capable of converting acrylonitrile directly to acrylic acid. The benefit of this catalyst was that it was an excellent scavenger of acrylonitrile. This high scavenging efficiency, even in the presence of high product concentrations, was better than that of any previously reported enzyme.

During the Teaching Company Scheme (TCS), a part-funded government technology transfer scheme, which followed the LINK programme, Allied Colloids staff returned to the company, and Huddersfield University bioprocess technologists worked alongside chemists and engineers on the company's premises. This was very important from a "seeing is believing" viewpoint and was invaluable in getting the process to pilot plant stage. In particular, the development work covered by the scheme addressed reactor design, immobilised vs. free cell processes, biocatalyst disposal, contacting the biocatalyst with acrylonitrile, process optimisation studies and hazard and operability studies.

The TCS was also a very useful learning experience for the university. Maintaining the university as a full development partner throughout meant that several processing route options could be considered simultaneously. This was a major learning curve for all concerned. At the same time, use of a larger-scale version of the screening equipment allowed the biocatalyst's performance to be measured over long periods under a variety of conditions, thus generating information which was important in deriving cost models for the process.

A crucial economic point is that acrylic acid, made by the conventional route, has to be diluted and neutralised with ammonia before it is used in some applications. The biological route effectively adds water to acrylonitrile to make aqueous ammonium acrylate directly. This represents a substantial saving on raw materials.

Owing to the relatively small scale of manufacture required for ammonium acrylate, which is derived from the commodity chemical acrylic acid, the major driver for the project was to minimise the capital cost of the plant to ensure a rapid pay-back. Minimising this was the major reason why the process that was taken to pilot scale was a batch-fed process utilising free cell biocatalyst.

At first, the project team did not appreciate that engineering needs to be done at least in parallel with the biochemistry. For example, their first approach was to immobilise the enzyme, since this is a handy laboratory tool. However, it became clear that using the free organism, despite the biomass waste stream, was the lower cost route.

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By 1997, the company had a small group of staff with a mind-set supportive of biotechnology, the product was being made at 10 kg scale and designs had been prepared for a pilot plant. In 1998, supported by consultancy and advice from a third government scheme (Biotechnology Means Business), the pilot plant was tried on a 600 litre scale and found to be robust, and designs were drawn up for the full-scale plant. Plans were made to locate the bio-plant, including the production of the enzyme (organism) by fermentation, within the existing plant. A biochemical engineering company was ready to collaborate and build a simple fermentation plant on the chemical site which could be run by chemical operatives. However, at this point, following the take-over, the decision was taken to cease manufacture of acrylic acid and all supplies were subsequently bought in.

While the project team wanted to go ahead on a demonstration or "learning" basis – they had suitable partners and an approved government grant – this was been possible. Instead, they have established a strong patent position and have a business plan to license the technology and approach potential licensees.

Summary and conclusions

Allied Colloid's project analysis was not very sophisticated at the time of the project development and the problem was to nurture something novel without putting in a lot of money. There was a need to justify work a year or two at a time. Although financial analysis came later, it was clear at an early stage that the bioprocess would have numerous advantages. It would be safer, simpler, more environmentally friendly, would have lower concentrations of toxic materials, would have no runaway potential and capital expenditure would be much less and hence the economic scale would be lower.

A conclusion from this work concerns the key role played by the engineering aspects of the project and the fact that these need to be studied in detail alongside the biology. The newest research projects consider the cost options by involving engineering at an early stage and identifying where the cost bottlenecks are likely to be. Is, for example, the diminishing return associated with the last small increases of yield on catalyst meaningful?

The factors that finally mitigated against the project were various:

- The polymer derived from acrylic acid was transferred to another selling division and a very advantageous contract was negotiated for an external supply of acrylic acid.
- Internal pricing was such that the profit from sales went to another division and therefore could not be recouped by lower costs from a new process.
- There were difficulties perceived by decision-makers in incorporating a wholly new technology on a chemical site, especially the making of the catalyst by fermentation.
- The big prize is still a new bioprocess for other monomers, such as comparing the chemical and the Japanese biocatalytic process for acrylamide.
- The copper catalyst is difficult to regenerate, polymerisation can occur at the temperature of the reaction, extra purification is needed and the yield is low.
- With the biocatalyst, there is much reduced polymerisation potential, yield is virtually quantitative and downstream processing is simple.

Environmental factors will be less important – the emissions from the recently de-bottlenecked Ciba plant are 50 kg/year benzene and 1 kg/year copper, which are well within regulatory standards. The inherent safety of a bioprocess would mean that, in future, small modules could be constructed economically and operated closer to the point of use.

Key parameters for any new biological catalyst are: capital expenditure, fermentation costs, catalyst lifetime and selectivity. Although a biological process can be inherently safe, risk items can still be regarded as cost items. The major lessons from this project, however, are managerial: first, there is a clear requirement for a project champion at board level throughout the development, and second, any improvement in costs and hence increase in profits must be attributable to those who made the improvements.

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Case Study 8

ENZYME-CATALYSED SYNTHESIS OF POLYESTERS (BAXENDEN, UNITED KINGDOM)

Introduction

Baxenden Chemicals is a UK-based joint venture owned by Crompton Corporation and Croda International plc. The company is at the forefront of polyurethane technology and has the United Kingdom's largest polyurethane dispersions manufacturing facility. In its present form, the company has been established for over 30 years. In addition to polyurethanes, Baxenden manufactures polyesters, acrylic polymers and emulsions and other speciality chemicals.

Discussions between staff of the company and of an academic institution gave rise to the idea that polyesters might be produced using enzymatic catalysis. The company wished to find out whether it would be possible to improve product quality by the use of enzymes and to make the existing production process more efficient, by lowering the reaction temperature from 200 °C to 60 °C. The project aim was to investigate the condensation of diols and diacids to produce polyesters, using lipases in non-aqueous conditions and to create single molecular weight polyesters, which crystallise at an exact temperature.

Before the project started, little work had been done on making ester bonds by taking an ester with a high molecular weight and adding substrate in an iterative condensation process. Another issue of academic interest was the diversity of the mechanism, finding out how many different substrates can be condensed in this way.

Technical features

The conventional chemical process to manufacture polyesters used for adhesives requires an esterification reaction, catalysed normally by titanium or tin, at a temperature of at least 200 °C. In this process no solvent is needed and nitrogen is blown through the reaction mixture to remove the water produced.

In 1990, it was well known that hydrolase enzymes could be used in similar reactions but created oligomeric products or used halogenated substrates. The latter would not be desirable feedstock in industry because of their expense and toxicity.

The biocatalyst used in this project is a lipase from the thermophilic bacterium *Candida antarctica* which is cloned in E. *coli* bacteria for mass production. It is acting contrary to its normal hydrolytic mode of action and forms ester bonds from diols and diacids. It is commercially available, produced only by Novozymes and sold by Novozymes and Boehringer Mannheim.

The enzyme itself has not been modified to make it more suitable for the production process, although, in principle, this might be done later, especially if the process is to be further scaled-up. The product created using the enzyme is not ideal, but it yields a product with only a small range of molecular weights, and is more homogeneous than the earlier product. It sets over a smaller temperature range and is produced at higher efficiency.

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Process selection

Trials carried out by Baxenden and its collaborators gave a clear view of the commercial possibilities of the process. The cost of the enzyme and the operating parameters precluded manufacture of equivalents of conventional low-cost polyesters for applications such as flexible foams. The focus therefore moved to the production of speciality polyesters with superior and novel properties, which could not be produced by conventional processes and for which the technical differentiation between enzymatic and conventional polyesters offered significant benefits. The regular nature of the crystalline matrix was an advantage, since it might yield exceptional adhesive powers, either for the polyester itself or for down-stream polyurethane formulations, such as reactive hot melt adhesives, thermoplastic polyurethanes or polyurethane dispersions. It was also appreciated that the enzymatic polyesterification was capable of producing much higher molecular weight materials than the conventional process. The attainment of high molecular weight polymers hinges on close stoichiometry between the monomers, and volatility of the diol under the high temperature conventional conditions (> 200 °C) is a limiting factor. The enzymatic polyester is produced at substantially lower temperature (60-100 °C), and therefore there is significant improvement in molecular weight. At industrial scale, the reduction of temperature is equivalent to a saving of about 2 000 MW of energy per year.

The commercial value of the project was seen as yielding a low-temperature process more cost-effective than conventional high-temperature production. The cost of the enzyme catalyst hinged on its mode of use. Two options were screened – use of an immobilised enzyme, which could be recycled, and use of a free enzyme. The latter was subsequently ruled out because of doubts over supply. During development work, it was discovered that not only could the process be run solvent-free, a major advantage to industry, but also that high viscosity and high temperature downstream processing would not place limits on enzyme recyclability.

Advantages and disadvantages

The new production process for polymers is more energy-efficient and eliminated the use of organic solvents and inorganic acids. The initial process used a solvent that would have added considerable cost and inconvenience, but this was eliminated during development.

Environmental effects of the biocatalytic process were of limited importance to the success of the project. The process did lead to environmental improvements compared to other production processes for polyester glues, but the main aim was improved product quality. The sought-after process improvements went hand in hand with economic benefits such as cost reduction or consumer demand for a "natural" product.

Baxenden sees the fact that there is only one producer for the lipase as a potential problem, should Novozymes decide not to sell it anymore. A lipase from M*ucor miehei* could be used, but the enzyme selected functions at a higher temperature and is more stable under the conditions used.

The unique capability of the low-temperature process is to deliver better characterised and higher molecular weight polyesters. The lack of randomisation in the final product would suggest exceptional properties, and such a material might have potential as an adhesive, either in its own right or in a polyurethane formulation. This proved to be the case, and a range of such polyesters has now been manufactured in small quantities and distributed globally for evaluation.

A key factor in commercialisation is the current high profile of hot-melt adhesives, which have never been produced previously from such simple monomers. Such novel speciality materials would give Baxenden a leading edge over its competitors.

Description of process innovation

Historical overview

The project commenced in 1992 at the initiative of the R&D group leader (Dr. Falmai Binns) at Baxenden Chemicals. Dr. Binns contacted Professor Stanley Roberts at Exeter University, whose

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research concentrated on enzymes in organic synthesis. The company had no prior experience with biocatalytic processes, but the R&D group leader at the company had long-established contacts with Professor Roberts (they had studied together), and knew about his research on enzymes.

The company funded for a year a master's degree student. Baxenden's managing director was involved in the project from the start and his support was crucial for approval of the project by the company board. This was a difficult process in an industry which is normally rather conservative.

The lipase successfully catalysed the reaction in an organic solvent, isopropyl ether, at approximately 60 °C in the laboratory. At this stage, the novelty of the fundamental concept required patenting and to this end the first patent was lodged for the solvent-based process.

The next phase was scale up and supply of a range of consistent samples at kilogram scale for customer evaluation. Little prior art was available at industrial scale, and therefore a classical development programme was conducted in house, in order to simplify the transformation. The molecular sieves originally used to absorb water and drive the polymerisation to completion were found to be unnecessary since the commercial enzyme gave a step-change in efficiency. Then, because the solvent posed major practical problems, the level of dilution was reduced until finally the crucial step was taken to conduct the process solvent-free, a process then entirely novel in the lipase esterification field, which was key in stimulating industrial interest. The next advance came with further attempts to remove the enzyme from what was now a very simple system of the mixed monomers, the diol and adipic acid, with a little water. The immobilised enzyme, Novozyme 435™, was filtered off after the oligomerisation stage, and it was discovered that the oligomer could be pushed to polymer by simple application of a vacuum. Though at first this stage appeared to be enzyme-free, it was later attributed to enzyme leached from the support. It was concluded that the lipase was remarkably robust and was stabilised against temperature inactivation, as well as substantially activated, by the polymeric environment. These novel discoveries required Baxenden to submit a second major patent describing the solvent-free process. The process was then stepped up to 20 litre pilot-plant scale and then progressively to 2 ton scale with great success, using conventional reactors. Studies of both solvent-based and solvent-free systems showed significant differences in mechanisms. Transesterification occurs in solvent-based conditions only, giving a wider dispersity of products during the reaction. This may be due to a difference in active site conformation of the lipase between the two systems. Under solvent-free conditions, step-growth esterification occurs.

A real understanding of the enzymatic polymerisation process was urgently required if the company was to capitalise fully on the discovery. A second student in Professor Roberts' laboratory looked at the basic building blocks of the process and established that the process was one of step-growth of the polymer chain, in contrast to the random chain-transfer nature of the conventional high-temperature process. He also explored a range of diols and acids, including chiral precursors. Comparison of the conventional polyester and its enzymatic mimic using mass spectrometry demonstrated for the first time that there was a sharp difference in the two polyesters on the molecular level. Simply put, the enzymatic material lacked acid-ended components in the polymeric region and so possessed a potentially much more ordered structure, while the conventional counterpart had scrambled components of both acid and hydroxyl-ended type.

Future strategy for the company hinges on evaluations now under way by a number of potential customers worldwide as to the utility of the speciality polyesters. If they prove successful, the company will move to further research into speciality polyesters of a more diverse type to capitalise on their position on the cutting edge of this new technology.

Internal factors relevant to decisions

The availability of reactor technology for the project is important, because the efficiency of enzyme-catalysed reactions depends on reactor size. The results of laboratory tests are not necessarily valid for the same enzyme catalysed reaction at industrial scale (100-1 000 litres). The company itself possessed suitable reactors.

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Nobody in the company had any prior experience with enzymes but a key person working in R&D had an interest and took the initiative to involve the academic partner. Personal contact between the two leading people involved not only led to the project, but was also one of the success factors.

The managing director supported the project from the beginning and actively convinced the board to fund it.

All those involved in the project were eager to learn about new fields of research (polymers, enzymes) and the leader of the R&D group in the company worked in parallel to the students and played an important part in supervising their research. There was sufficient room to negotiate a balance between industrial and academic interests (publications, patents, funding a PhD).

External factors

Once the decision to invest in a collaborative project with the university was taken, the company decided not to ask for external funding, because the procedure would have taken too long and confidentiality would not be guaranteed.

The company required that the enzyme be a commercially available product. The rationale was that the enzyme would have to be used on a large scale in the production process and that supply had to be guaranteed. The existence of only one supplier of the enzyme was problematic.

The issue of ownership of intellectual property rights may have inhibited the project in the beginning. Here, the interests of the company and of the university are clearly contradictory. Eventually, a solution has been negotiated which was satisfactory for both parties.

Co-operation

Researchers stated that communication between them was a crucial success factor and was facilitated by frequent meetings and clear project management. Communication was also facilitated by an exchange of personnel for a few months to allow them to work together on a day-to-day basis.

The partners established a balance between the interests of the company (confidentiality, applicability) and academic interests (research, relationship between research and education). At the beginning of the project, procedures were established for publishing results and for delaying confidential information until it was patented.

Balances gradually emerged in the course of the project. At first, the research was closer to the interests of the company, but later the research became more academically interesting. After proving their usefulness, the academics negotiated a shift in focus of the research.

The success of this project was enhanced by the fact that the work in the university was accompanied by parallel work in the company, and by the fact that the project leader in the company actively supervised the research in the university.

Throughout the collaboration there were joint meetings of the team, which promoted synergy across the board and significantly contributed to maximum efficiency for both the industrial and the academic team. Much of the quantitative analysis of polymer samples was done at Baxenden for the research students by a dedicated group of analytical chemists, who became expert at handling tiny quantities of esoteric molecules.

This is an exceptionally complex interdisciplinary field, highly demanding of skills not natural to either collaborator, so considerable growth in skills and expertise was required, as well as an unusual degree of flexibility. Since the discoveries were of huge commercial impact, there was a pressing need to secure global protection for the inventions through patent protection.

A fundamental facet of this project has been the interweaving of collaboration between industry and academia, which has given the programme tremendous energy.

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Summary and conclusions

The idea of the project originators to produce a near-perfect polymer was realised; the subtlety of the enzymatic mechanism provided the necessary improvement in product quality.

Environmental friendliness was one aim of the project, but it was not of major importance. It is not seen as a selling point by the company. The product developed by Baxenden should result in environmental and health benefits for workers in industries using the adhesives produced by Baxenden rather than traditional products.

The principles of enzymatic polyesterification were unequivocally established as was a fundamental difference in the nature of the enzymatic and non-enzymatic polyesters.

Not all innovative inventions founded in company funded research are subsequently taken up and developed further. The study of the diversity of the reaction mechanism has resulted in a spin-off in the form of optically active chiral polymers, for which the company has patents, but these are not being pursued commercially.

Since the discoveries clearly had commercial significance, there was a pressing need to secure global patent protection for the inventions.

A lack of awareness of the potential of biocatalysts among industrialists in sectors where these are traditionally not used is often mentioned as an important barrier to the dissemination of biocatalysts to these sectors. This study demonstrated that, once this barrier is overcome, biocatalysts can be applied successfully in production. This project was successful even though nobody in the company was an expert on biocatalysis. The R&D group leader and the company managing director both played crucial roles, the latter in convincing the board to support the project.

The company funded the project from its own resources. Factors which played a role in the decision to do this were confidentiality, timetable for the application procedures, scale of the project, who initiated the project and time to market of the research.

A range of high molecular weight polyesters has now been distributed globally for evaluation. At this time, however, the company has not made a decision to manufacture them.

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Case Study 9

POLYMERS FROM RENEWABLE RESOURCES (CARGILL DOW, UNITED STATES)

Introduction

Based in Minnesota, US, Cargill Dow is the first company to offer a family of polymers, derived entirely from annually renewable resources, with the cost and performance necessary to compete with packaging materials and traditional fibres. The company has achieved this breakthrough by applying unique technology to the processing of natural plant sugars to create a proprietary polylactide polymer.

The process for producing NatureWorks™ polylactide (PLA) harnesses carbon stored in plants through the process of photosynthesis to create a PLA polymer that can be used for common consumer items such as clothing, food containers, as well as home and office furnishings. Future applications of the technology could include use in injection blow moulded bottles, foams, emulsions and chemical intermediaries. This development represents a major breakthrough, and several large packaging firms are keen to exploit PLA, not least because of its biodegradability.

Cargill Dow has built a plant in Blair, Nebraska, with a capacity of 140 000 metric tons, to serve the global demand for PLA until capacity is added in Europe and Asia. The Blair plant was online in 2001. Until then, Cargill Dow supplied semi-commercial grades from a smaller facility in Savage, Minnesota.

Technical description

PLAs are compostable and recyclable plastics with valuable properties for packaging applications, which are derived from lactic acid. For many years, lactic acid has been produced by both fermentation and chemical routes. More recently, developments in the fermentation process and particularly in downstream recovery appear to have given the bioprocess an overall economic advantage as well as the environmental benefit of being based on renewable raw materials.

Cargill Dow's process uses fermentation to make two chiral isomers of lactic acid from dextrose, a conventional fermentation route impossible with chemical synthesis, and these are then chemically cracked to form three lactide isomers. Various combinations of the lactides are combined to generate a range of polymers.

The polymer chains come in a number of shapes and lengths, and making useful materials from them requires that the amount of each in the mixture be controlled, as the properties of the resulting material depend on the proportions of its components. The conventional, expensive, way of doing this was to exploit the fact that different PLAs have slightly different solubilities. Recently, however, it has been discovered that they also have different boiling points. That means they can be separated by distillation and Cargill Dow has worked out an inexpensive way to do this.

Relying on dextrose ties bioprocesses to corn wet-mills in North America and, in Europe, to wheat processors, but the ability to use a wider range of sugars is developing rapidly. Cargill Dow is exploring novel processes that would allow the use of cheaper feedstock than dextrose, a capability that would cut the cost of making PLA as well as allow the manufacture of novel products.

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History of the innovation

The PLA project was started by Cargill in 1988 to add value to the starch processed by the company. Dr. Pat Gruber, now Vice President and Chief Technology Officer for Cargill Dow, was the initiator and project champion throughout the life of the project. A small group of scientists and engineers at Cargill invented the key lactic acid to lactide step, lactide purification and lactide polymerisation/devolatilisation technology. This technology remains the key to Cargill Dow's success. Cargill worked with various polymer partners in the period 1989-94, but proceeded for the most part on their own. In 1994, the company built an 8 million-pound/year PLA facility in Savage, Minnesota, to prove the lactic acid to PLA technology at a larger scale and to produce market development quantities of PLA. This plant has operated ever since to further perfect technology but more importantly, to develop the market for PLA. For the past year or so, it has been operating at or above nameplate capacity to seed the market for the PLA which will be made in the Blair plant.

Competing technologies for PLA did exist but were regarded as higher-cost options (because they involved the use of solvents). Cargill also had key technology patents for stabilising the polymer, leaving only very low concentrations of monomer in the final product. This technology is absolutely critical for polymer to have the high-temperature melt processability required by converters.

In early 1995, Cargill realised that it needed a polymer partner with a presence in the polymer market. It was felt that Cargill alone did not have the necessary credibility, and several other PLA producers were regarded as leading the way. Cargill therefore assembled a list of partner attributes and Dow emerged as the best candidate and was approached. A joint evaluation agreement was entered into and the Dow team became believers as the cost of PLA manufacture was verified and the range of PLA properties and applications was appreciated. Customer interest was also very strong, so in late 1997 the 50/50 Cargill Dow joint venture was launched.

The interest of both Dow and Cargill has been critical as the project has moved forward. One partner's confidence in one area would be backed up by the other's confidence in another. It is unlikely either would have had the conviction to move the project forward on its own because of large areas where its corporate expertise was not sufficient.

Environmental benefits and disposal options

PLA is a range of polymers made from annually renewable feedstock. Using renewable raw materials leads to a reduced use of fossil resources and lower carbon dioxide emissions than traditional hydrocarbon based polymers. PLA polymers can be safely disposed of through traditional disposal routes. In addition, PLA products are fully compostable in commercial composting facilities. The environmental benefits of PLA are discussed below, along with processing information.

Reduced fossil fuel use. Conventional hydrocarbon polymers utilise natural reserves of oil and natural gas as their feedstock source. In contrast, the monomer for PLA is derived from annually renewable resources, predominantly corn. One-third of the energy requirement of PLA is derived from these renewable resources, resulting in PLA utilising less fossil fuel than other polymers derived directly from hydrocarbons. In various applications, PLA replaces or competes with PET, polyesters, polystyrene, etc. Depending on the plastic replaced, fossil energy use is reduced by 20-50%.

As with conventional plastics, fossil fuel provides the energy to run the PLA production chain, *e.g*, milling corn to produce starch, fermentation to produce lactic acid, heating to polymerise and fuel for transportation.

Cargill Dow is working on the use of lignocellulosic biomass, such as straws and bagasse or corn stover (residue left in field), for making PLA. This technology promises to reduce the fossil energy use in PLA manufacture by up to 80% or more and will make PLA manufacture a carbon sink if it is combined with the use of wind energy for electricity generation (a planned project).

Carbon dioxide emissions. Carbon dioxide is believed to be a major contributor to global warming. Less carbon dioxide is released into the atmosphere in the production of PLA polymers than in the production of most traditional hydrocarbon-based polymers. The carbon in PLA recycles in the Earth's

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carbon cycle; carbon dioxide is removed from the atmosphere when growing the feedstock crop and is returned when the PLA is degraded. Consequently the use of PLA will have a reduced impact on global warming compared to most hydrocarbon based polymers.

Incineration. PLA polymers incinerate cleanly and with a reduced energy yield (8 400 BTU/lb.) compared to traditional polymers. PLA polymers contain no aromatic groups or chlorine and burn much like paper, cellulose and carbohydrates. Combustion of PLA produces few by-products and 0.01% ash. In areas where capacity is limited, this is an advantage in that the lower heat output permits a higher incinerator facility throughput.

Municipal composting. Composting is a method of waste disposal that allows organic materials to be recycled into a product that can be used as a valuable soil amendment. PLA is made from an annually renewable resource and compost can be used to grow the crops to produce more PLA. Extensive testing, at laboratory and pilot scales according to international standards, and in actual composting facilities, demonstrates that PLA polymers are fully compostable according to ISO, CEN, ASTM and DIN draft regulations. DIN-Certco Compost Certification has been awarded for PLA polymer use in Germany. The compostability of these products is similar to that of products made from paper or cotton.

PLA polymers compost by a two-step process. First, chemical hydrolysis reduces the molecular weight of the polymer, then micro-organisms degrade the fragments and lactic acid into carbon dioxide and water. Heat and water stimulate degradation of PLA polymers.

Post-consumer recycling. In practice, the following conditions need to be met in order to recycle any material: *i*) the material is present in sufficient quantities in a waste stream; *ii*) a disciplined collection system is put into place to collect it; *iii*) the product is clearly marked and physically easy to separate; and *iv*) there are outlets desiring to purchase the recycle feed-stock stream. This infrastructure does not currently exist for PLA. The impact of PLA on existing recycle streams also depends on the above factors and needs to be studied on a case-by-case basis. Because PLA polymers hydrolyse with water to generate lactic acid, it would be straightforward to completely degrade PLA into lactic acid and recover the monomer.

Pre-consumer recycling/regrind. In the processes studied, thermoforming and injection moulding, PLA regrind has been used at the same rate as existing materials. As for traditional polymers, seven passes through the extruder at 100% regrind showed minimal loss in physical properties, as long as the PLA was dried before processing.

Repulpability. Under the agitated water conditions of repulping, PLA easily peels away and releases from paper fibres, permitting fibre recovery. Studies have shown that PLA film remains in much larger pieces than conventional films when repulped under normal conditions of temperature, pH and consistency. This allows PLA to be separated from a repulped paper slurry more easily.

Life Cycle Inventory of PLA polymers

One tool that is helpful in understanding the overall environmental performance of products is their Life Cycle Inventory, or LCI. This technique allows the comparison of the environmental impact of different products, as well as the identification of important areas of improvement within a product system. Cargill Dow has completed a comprehensive LCI of PLA. This “cradle to factory-gate” study includes all the material and energy inputs and outputs associated with growing the corn, through dextrose and lactic acid production to PLA production.

A number of opportunities have already been identified to improve the environmental impact of PLA.

Electricity supply. Much of the polluting gases emitted to the atmosphere as a result of the manufacture of PLA are consequent on the use of electricity drawn from the grid, which is produced mainly from coal. Cargill Dow is pursuing alternative energy sources like wind-power in order to further reduce fossil energy use and reduce air emissions.

Emissions from farming. The farming practices used in growing the crops used as feedstock are not directly controlled by Cargill Dow, but they have an impact on Cargill Dow’s environmental performance. Improvements are being made in reducing farming emissions through the use of variable

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rate fertiliser technology and improved management practices. Cargill Dow is executing a comprehensive study of the agricultural practices around its production facility in Blair, Nebraska. Based on this study, Cargill Dow will carefully monitor and seek to influence agricultural practices that may impact its feedstock.

Development of composting infrastructure. Because composting is not yet widely available, Cargill Dow is supporting the building of cost-effective composting infrastructure.

Raw material production

Some of the issues associated with the use of annually renewable feedstock are not easily addressed by life cycle methodology. Today, PLA is produced from corn, which is the most economical source of dextrose in the United States. The corn that is used by Cargill Dow is grown using typical US farming practices. The feedstocks are harvested from Iowa (95%) and Nebraska (5%). Both locations typically utilise fertilisers and pesticides to improve crop yields. An economical source of fermentable sugars is required to produce PLA. In other parts of the world, crops available locally, such as wheat, rice, sugar beets and cassava, can be used.

Food shortage is primarily an issue of inadequate distribution of available food resources, rather than lack of production. Farmers in the United States and Europe are paid not to plant crops on some land. Only 10% of the annual US corn crop is used directly for food, 50% is used in animal feed, 7% in industrial uses and 33% is exported or stored. Cargill Dow has begun research to investigate the feasibility of using non-food biomass sources as a raw material for PLA. Cargill Dow's ultimate vision is to use agricultural waste materials as a feedstock.

PLA does not contain genetically modified materials, nor does its production require any genetically modified raw materials. However, the corn that currently constitutes the feedstock for PLA could be a mix of genetically modified and conventional corn available in the market. Cargill Dow is actively pursuing development of biomass as its feedstock. This would enable Cargill Dow to affect the agricultural practices used to grow its feedstock. Cargill Dow is committed to using Life Cycle Analysis to continue to reduce the environmental impact of PLA and to identify applications for its products that capitalise on the environmental benefits that they provide. Cargill Dow also intends to collaborate with its customers to carry out life cycle studies of applications and to understand the influence of waste disposal on the environmental impact of its materials.

Summary and conclusions

Cargill Dow's analysis indicates that an improving environmental picture, for example, reduced fossil energy use and greenhouse gas emissions, correlates very strongly with an improved economic picture, both in reduced manufacturing costs and increased market demand.

Cargill Dow has a vision of developing a family of chemical products produced by more sustainable methods. This vision is based on the correlation between the economics and environmental profiles of products such as PLA and others that they have analysed. The company believes there is an advantage to entering markets with environmentally attractive products but this has not yet been quantified and investment decisions are driven solely by the project economics. In other words, the economics and business opportunity have to be in place to realise the environmental benefits.

Sustainability is key to Cargill Dow and reducing fossil energy and carbon emissions are goals that they take very seriously. Time after time, these goals line up with cost reduction goals, as is the case for biomass derived sugars and for electricity from wind.

Use of renewable raw materials will significantly reduce the environmental impact of a process even when fossil fuels are required, for example, to generate electricity.

PLA may be recycled in the same way as conventional polymers, but, since it is biodegradable, it may, in addition, be landfilled or composted.

Life Cycle Inventory (elsewhere called Life Cycle Analysis/Assessment) is a key tool for analysing the reduction of environmental impact.

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Case Study 10

A VEGETABLE OIL DEGUMMING ENZYME (CEREOL, GERMANY)

Introduction

The EnzyMax® process covered in this case study was developed by Lurgi Öl Gas Chemie in Frankfurt. The main business of Lurgi Öl Gas Chemie is to plan, design and build process plant, providing customers with a plant engineering and contracting service in the fields of hydrocarbon technology, petrochemicals and inorganic chemicals, gas technology and renewable resources and fine chemicals. The EnzyMax process was developed in the latter. Lurgi has engineered and built many esterification and hydration plants and also fermentation processes for the production of citric acid, lactic acid and beer. R&D is carried out in a central unit while pilot plants are built directly at a customer's premises.

Cereol Germany, located in Mannheim, is engaged in oilseed crushing, oil refining and oil bottling and packaging. The firm is part of the Eridania Beghin-Say group, which is one of the world leaders in agro-industry. Cereol processes oilseeds to produce crude oils, meals, concentrated proteins and lecithins. The crude oils are refined and sold as edible oils, in bulk or in bottles. Environmental concerns matter for Cereol: when it comes to replacement or expansion investments, eco-friendly technologies are preferred.

During the R&D of the EnzyMax process, Röhm Enzyme GmbH, based in Darmstadt, co-operated with Lurgi, supplying the enzymes and adapting them to the special requirements of the process.

Technical features of the EnzyMax process

Enzymatic oil refining

In general, there are two ways to refine (crude) seed oils: physical and the more conventional chemical refining. The technical difference between these methods is the way and the time of the removal of free fatty acids (FFA) from the crude oil. The advantages of physical refining are higher yield, lower cost and a more environmentally friendly production. For all these reasons, the trend in seed oil refining is towards physical refining.

A prerequisite for physical refining is a low phosphatide content in the oil entering the final deacidification/deodorisation stage. The content of phosphatides is reduced in a degumming step. One way of doing this is enzymatically, in a process based on the hydrolysis of the phosphatide molecule. The enzyme phopholipase A_2 catalyses the splitting of the fatty acid ester and the resulting lysolecithin molecule is water-soluble and can be separated from the oil by centrifugation. The conversion of non-hydratable phospholipids into hydratable phospholipids takes place in an intensive dispersion of the oil with the liquid enzyme solution at a mild temperature of 60 °C and pH 5 (citric acid and caustic soda work as a sodium-citrate buffer). To increase the normally relatively low reaction rates of enzymatic reactions, a battery of continuous stirred tank reactors is applied.

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Advantages of the EnzyMax process

The EnzyMax technique uses considerably reduced amounts of caustic soda, phosphoric and sulphuric acid as well as water (washing and dilution water) and steam than the conventional method (Table 15). The conventional method produces a wastewater stream of about 3 200 kg/hour, which contains sulphate and phosphate, compared to about 400 kg/hour wastewater for the EnzyMax process, a decrease of nearly one order of magnitude. In addition, the amount of sludge is reduced by a factor of about eight.

Table 15. **Consumption figures and costs for conventional and enzymatic refining**

Resource	Conventional method[1]	EnzyMax degumming	Specific cost [USD/unit]	Total cost conventional [USD/ton]	Total cost EnzyMax [USD/ton]
Caustic soda (100%) [kg]	5.3	0.43	0.6	3.18	0.26
Phosphoric acid (75%) [kg]	2.0	–	0.672	1.34	–
Sulphuric acid (96%) [kg]	5.3	–	0.075	0.39	–
Soft water [kg]	127.8	10.76	0.013	1.66	0.14
Steam [kg]	95.5	28	0.09/0.013[2]	1.24	0.36
Cooling water [m^3]	1.5	–	0.09	0.69	–
Electric power [kWh]	7.7	7	0.09	0.69	0.63
Citric acid [kg]	–	1.0	1.87	–	1.87
Enzyme solution [kg]	–	0.014	143.75	–	2.01
Total				9.19	5.27

1. Consumption in units of the resource per metric ton of crude oil.
2. Difference due to different steam pressure.

Although a detailed comparison of the cost of the two methods is difficult because the plants are engineered differently, it can be seen from the table that the enzymatic process is cheaper in terms of operating costs. The price of the enzyme solution has a major impact and this may be reduced further if the production cost for the enzyme can be further decreased by the application of biotechnological methods. Also, the EnzyMax process requires lower capital investment. Integration of the new process into the existing plant design and equipment is simple.

Description of the process of innovation

Historical overview

Lurgi first considered enzymatic degumming of crude oil in 1986 and consulted Röhm about appropriate enzymes for such a purpose. Röhm took four years to screen potential enzymes and it took Lurgi a further five years (1990-95) to design the whole process. The first pilot plants were set up in Mannheim (Germany) in 1995 at Cereol as well as in the Czech Republic and in China.

The technical director of Cereol Germany first heard of the EnzyMax process in 1994, and the whole implementation process took approximately half a year. The technical director acted as a supervisor and promoter of the innovation and was responsible for scientific problems. The operating manager for refining was responsible for the technical implementation of the process.

To achieve the targeted reduction in cost took another two years and the process has been running smoothly since 1997. The enzyme is now supplied by the Danish enzyme producer Novozymes.

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Internal factors

From Lurgi's experience the development of a new process has four stages:

- *Finding a partner to carry out the* R&D. This might be a university, a public research institute or another company.
- *Performing the* R&D. This step can be divided into milestones. After each milestone, a go/no-go decision is taken. Internal opposition to innovation is best overcome by achieving set goals or milestones.
- The third step, *scaling up*. This is usually done in co-operation with the user company. In this stage, differing interests can be a problem, as one partner (the supplier) is usually interested in selling the process to a number of companies, whereas the other (the user) would prefer to use the technology exclusively.
- *Introducing the product/process*. In this stage, it is crucial for the engineering company to have demonstration pilot plants in operation.

In general, each board of directors has to be convinced of the financial potential of a process before R&D can be started. Certain considerations make it easier to raise a budget for R&D on a new project. These include: short duration from the start of the project to the introduction into the market, low risk, low cost for staff and equipment, a powerful partner during R&D and an investor to build a plant as a reference.

In case of the EnzyMax process, the risk was relatively low as there already existed a market for degumming techniques and the aim was to develop a cheaper and better method. Therefore, it was easier to estimate prospective sales generated by the new technology. Also, the R&D costs were relatively low.

At Cereol Germany, opposition occurred due to operation problems after the start of production using the EnzyMax process. The targets concerning emission thresholds were easily achieved after a quarter of a year, but achieving the financial criteria turned out to be more demanding. During this time, the promoters at Cereol had to use their positions as senior managers to push the project because the results were not in favour of the new technology.

Röhm tried to find a way to produce microbially the enzyme used in the EnzyMax process (the phospholipase was a by-product of insulin production where it is extracted from porcine pancreas) but failed and therefore could not supply a large-scale production. Supply is now from Novozymes who in May 1998 received a patent on a novel phospholipase based on an invention in 1996. This phospholipase has improved features and is derived from a fungal strain of the genus H*yphozyma*. This fungal organism has been genetically modified in order to produce the enzyme.

A barrier to the development of eco-friendly processes is the lack of systematic search for technologies favouring environmental benefits. Applying analytical tools to calculate the highest marginal utility for efforts to reduce environmental pollution and to draw attention to areas where R&D seems promising could lead to new processes that combine eco-friendliness and (cost) efficiency.

External factors

Lurgi feels that increasing the dissemination of eco-technologies can only be initiated by the demand side. Engineering companies cannot develop eco-friendly processes that do not offer an additional economic benefit or, worse, are even more costly than conventional techniques. Hence, the EnzyMax process was not developed because of its eco-friendly production features, but specifically because of the considerable reduction in variable cost and high efficiency. Environmental matters are very important if emission thresholds have to be met, but if these are not a constraint then environmentally friendly production scores low on a ranking of customer needs. Besides, emission thresholds for harmful substances in waste water or exhaust gas are not uniform throughout Europe.

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Co-operation

The EnzyMax process was developed in a joint effort by an engineering company, an enzyme producer and a user of the process. This grouping of complementary firms appeared very effective in the development of the new process, which might have been too risky for a single firm. In this case, the companies had already carried out other projects together and thus business contacts had already been established. Universities or public research institutes are considered as co-operation partners in early stages of a project only, largely because of the lack of equipment for large-scale production at these institutions. Doing R&D in research programmes funded by government institutions may have the disadvantage of having to report and to disclose the results.

Summary and conclusions

Realising biocatalytic processes with environmental benefits requires several different skills that are not present in a single company. Against this background, the group of companies in this case study can be considered as a model of how innovations in the food sector may be realised.

The incentive to develop a new production technology was the considerable reduction in variable cost and the high efficiency of the new process compared to conventional processes. Although its eco-friendly production features are considerable – and were more easily achieved than the cost targets for the new process – they did not play a decisive role. The decision whether a new process will be developed or not largely depends on customers' needs and perceived market demand. However, most food companies can comply with environmental regulations and standards with their established production equipment, and as long as permissible emission thresholds do not work as a constraint for the production process, environmentally friendly production scores low on the ranking of customer needs.

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Case Study 11

WATER RECOVERY IN A VEGETABLE-PROCESSING COMPANY (PASFROST, NETHERLANDS)

Introduction

West-Vlaanderen, in Belgium, is a strongly agrarian area, with a large food industry specialising in the handling of fresh vegetables. One of these companies is Pasfrost (located in Passendale), a company producing deep frozen vegetables. The company has grown enormously over the last years and has increased its capacity from 17 000 tons of product per year in 1989 to 55 000 ton/per year in 1999.

Companies like Pasfrost are presently using groundwater as the source for their water supply. The advantages of using groundwater are evident: it is bacteriologically safe and it can be used without any further treatment. However, the economic development in the region is leading to increased pressure on the use of groundwater. This is because:

- The groundwater has to be drawn from a depth of more than 300 meters.
- The local groundwater levels are dropping, leading to shortages.
- The groundwater quality is deteriorating (Table 16 compares some parameters with the guidelines set by the WHO). The increasing salt concentration is particularly noteworthy, and this will lead to a need for additional treatment.

Table 16. **Groundwater quality and guidelines for drinking water quality**

Parameter	Unit	Groundwater	WHO guidelines
pH		8.3	6.5-8.5
Sulphate	mg/l	126	400
Bicarbonate	mg/l	552	
Chloride	mg/l	550	250
Conductivity	mS/cm	2.6	0.25

Technical features

Historical overview

In recent years, companies have looked for alternative water sources. Pasfrost has chosen to opt for partial reuse of treated effluent within their production process. After the execution of a feasibility study at the end of 1998, undertaken jointly with their collaborator, Paques, in the Netherlands, a pilot plant was put into operation in 1999 and, based on the pilot results, a final design was established. In May 2000, the full-scale installation became operational.

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Management responsible for water in the company has taken a number of actions in the past few years:

- Minimising the intake water volume (groundwater) by partial reuse of treated effluent for applications for which drinking water quality is not necessary. In this case, aerobic treatment has been supplemented with a sand-based polishing filtration: In this way, the specific water consumption has been reduced to 3-3.5 m^3/ton of product.
- Introduction of a steam stripping process, which results in decreased salt content in the wastewater.
- Expansion of the aerobic treatment and introduction of the anaerobic pre-treatment. These changes increase the stability of the treatment and result in a stable effluent quality under varying conditions.
- In order to reduce groundwater consumption further, alternative water resources have been investigated. Table 17 shows an evaluation of the quality of different possible sources. The partial re-use of extensively treated wastewater was the choen option. The reasons for this are:
- Drinking water is expensive – prices range from EUR 0.99/m^3 to EUR 1.54/m^3. Furthermore, a rise in price is highly likely in the next few years.
- Drinking water is relatively hard (> 4 mmol/l TH) and requires a softening stage for use in the condensers, resulting in higher chemical consumption for conditioning.
- Surface water is not a realistic option.

Table 17. **Relative advantages of different water sources**

Source	Availability	Present specific price	Development cost price	Environmental impact	Required investments
Groundwater	–	+	–	–	–
Surface water	–		0	0	–
Waste water	+		0	+	–
Drinking water	++	–	–	0	0

Technical features

By reusing extensively treated wastewater, the goal is to replace at least 50% of the present water intake derived from groundwater. By doing so, specific water consumption can be reduced to less than 2 m^3/ton of product. This is achieved by adding a full-scale membrane filtration installation with a net capacity of 20 m^3/hour in a first stage. This will be extended in the second phase to a net capacity of 40 m^3/hour.

Prior to the choice of the system for the extensive post treatment, a feasibility study was executed in order to determine which treatment would be optimal and how the operating costs might compare with buying drinking water. The results of this study have led to a design basis, which was the tested using a pilot installation that consisted of the following components: flocculation filtration, ultra filtration and reverse osmosis.

Different configurations were tested and evaluated. A complicating factor was that the production process was changed at different stages (*e.g.* the introduction of the steam stripping process) and furthermore the wastewater treatment was not yet equipped with an anaerobic pre-treatment.

The goals for the full-scale design were: a robust design, with a high process reliability; minimising the operational costs; no unnecessary pumping (lowering the energy consumption); decrease of chemicals use; high membrane life times; optimisation of the biological treatment in order to achieve a good and constant effluent quality; a safe design with emphasis on the reliability; and guaranteed quality.

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Description of the installation

The treatment consists of an anaerobic pre-treatment (reactor volume 5 000 m^3, load 30 tons COD/day, specific load 6 kg COD/m^3/day) for the degradation of the bulk of oxygen-consuming organic components, followed by an aerobic activated sludge plant for the degradation of the remaining oxygen consuming components. After sedimentation, the effluent is treated in two serial steps by means of a proprietary sand filtration, with a capacity of 100 m^3/h maximum (Table 18).

Table 18. **Typical water quality data**

Parameter	Wastewater	After sedimentation[2]	Filtrate polishing	Sterile water	Ground water[3]	Process water[1]
COD (mg/l O_2)	12.000	114		0	0	
pH	8.5	8.3	8.2	5.5	8.3	7
Turbidity (NTU)		16	4.3	0		0
Total phosphorus (mg/l)		34	34	< 0.5	< 0.5	< 0.5
Conductivity (mS/cm)	4.3	4.3	4.2	0.15	2.6	1.4
Nitrogen (NH_4^+) (mg/l)		< 2	< 2	< 2	0.17	
Iron (mg/l)				0.03	0.23	0.1
TH (mmol/l)		1.0	1.0	<0.3	0.3	< 0.3
Bicarbonate (g/l)		2.9	2.9	<0.3	< 0.1	< 0.2
CFU (i/ml)				0	0	0
E. *coli* (i/ml)				0	0	0

1. Mixture groundwater/sterile water: 50%/50%.
2. Data 26 June 2000.
3. Data 10 March 2000.

COD = chemical oxygen demand; NTU = Nephelometric Turbidity Unit; mS = milliSieverts; CFU = Colony Forming Unit.

In the first filtration step (roughing filtration), variations in water quality from the sedimentation tank are balanced, while in the second step, using flocculation filtration, the remaining suspended solids are extensively removed. The filtrate is now ready for further treatment in the membrane filtration installation, and it can be reused for low-grade applications within the factory (cleaning of machines, cooling water).

The ultrafiltration (UF) is fed with 40 m^3/hour filtrate. Before the water is fed into the ultrafiltration plant, the pH can be corrected and/or additional flocculation chemicals can be applied. The ultrafiltration membranes are hollow fibre membranes, and are operated at low pressure (0.5-1.0 bar). Cleaning takes place periodically in counter-current flow mode and chemical cleaning is applied on a regular basis. The ultrafiltration membranes retain bacteria, macromolecules, viruses and proteins effectively; the permeate does not contain any suspended solids and has a low SDI (standard density index).

After the ultrafiltration, the water is fed to a two-step reverse osmosis installation (spirally wound membranes). In this two-step mode, the retentate of the first step is boosted up to the second step. The fixed recovery of the reverse osmosis is about 70% and the net production is 20 m^3/hour. The operating pressure is 8-10 bar. The reverse osmosis permeate has a transmission of 100%, is almost completely free of salts and is bacteriologically safe. UV radiation is nevertheless implemented for extra security.

The produced water is then mixed with groundwater and pumped into the production process. Table 18 gives typical water quality data at the different stages of the treatment scheme. The produced waste streams (wash water from the sand filters) and UF backwash water (rinsing liquid from the ultrafiltration) is recycled to the biological treatment. Only the reverse osmosis concentrate is discharged.

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Operational costs

In Table 19, the operating costs are summarised for the extensive post-treatment. Because the infrastructure has already been designed for a net production capacity of 40 m^3/hour, while the first phase only produces 20 m^3/hour, a correction for the annual fixed costs of the plant is made. The total figure needs to be compared with the present price of drinking water (EUR 0.99-1.54/m^3).

Table 19. **Operating costs for process water production**

Item	Specific costs per m^3 product (EUR)
Annual fixed costs	0.49
Annual fixed membranes	0.14
Chemicals	0.21
Energy	0.09
Maintenance/operation	0.10
Total	1.03

In the second phase, the specific costs will drop below the present level of the cost of drinking water. If the decreased amount of volume discharged is also taken into account, the present cost level is already below that of drinking water.

Summary and conclusions

Reuse of wastewater for process water production in the food industry demands that specific attention be paid to the achievement of guaranteed levels of quality. The hygienic and chemical quality of the water should be evaluated and monitored continuously. In this respect, the treatment scheme will be part of the HACCP (Hazard Analysis Critical Control Point) system, which is obligatory as a preventive management system for the food industry. As a part of the quality insurance system the following items should be addressed:

- Inventory of the risk assessments.
- Definition of control measurements.
- Definition of a monitoring system.
- Periodical inspection, evaluation and reports.

Pasfrost has put a quality assurance scheme in place.

The installation of a water re-circulation scheme, designed and built by Paques, has resulted in a major reduction in groundwater withdrawal at a cost at, or below, the level of the only feasible alternative, drinking water. There is potential for continued improvement, reducing the use of groundwater still further.

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Case Study 12

REMOVAL OF BLEACH RESIDUES IN TEXTILE FINISHING (WINDEL, GERMANY)

Introduction

Many processing changes, for example the substitution of a chemical process for a biotechnological one, can bring about an impressive reduction in consumption of resources and environmental pollution without incurring expensive investments either of a technical or a financial nature. This goes hand in hand with a drop in costs and an increase in competitiveness. This is demonstrated in this case by an analysis of one process step in textile finishing.

The textile finishing industry is characterised by high consumption of energy and resources and time-consuming production processes. For these reasons, production-integrated biotechnological processes could make a considerable contribution to conserving energy and water, reducing emissions and to shortening the processes and consequently the throughput time.

The textile finishing industry differs from other branches in that it is generally unable to offer unrivalled products or new or significantly increased quality. However, the substitution of a process by one that is economically advantageous can make a considerable contribution towards consolidating or improving the position of a company with regard to its competition.

Windel Textil GmbH and Co. of Bielefeld in Germany, the company with whose co-operation the analysis was made, has been involved with textile bleaching for over 125 years, has a turnover of over DEM 70 million and some 400 employees. It adopted the enzyme process described below directly from a laboratory scale exercise in the space of one year.

Technical features of the process

Hydrogen peroxide is generally used for bleaching textiles. To achieve high quality in the ensuing dyeing process, it is necessary to remove bleach residues as completely as possible from the textile.

In conventional processes, residual peroxide is removed by repeatedly rinsing (at least twice) the textile in hot water. This method is not only energy- and water-intensive but cannot guarantee the complete removal of residual hydrogen peroxide that is required.

For this reason, an enzymatic process was developed that may now be considered as established and that has been extensively, but by no means exhaustively, applied in the textile finishing industry. With this new, biotechnological, process only one high-temperature rinse is necessary (at 80-95 °C, depending on the type of fabric) after the oxidative bleaching. The enzyme catalase is added to the next rinse and allowed to react for approximately 15 minutes at 30-40 °C. In the example studied, the compound "KAPPAZYM AP-Neu" (Kapp Chemie GmbH) was applied. The enzyme degrades residual peroxide into water and oxygen. Then the necessary consecutive steps can be started. The results are of significantly higher quality compared with the conventional process. The optimal application temperature range of the enzyme is 20-60 °C; its optimal pH value is 5-10, and the application time is 10-15 minutes.

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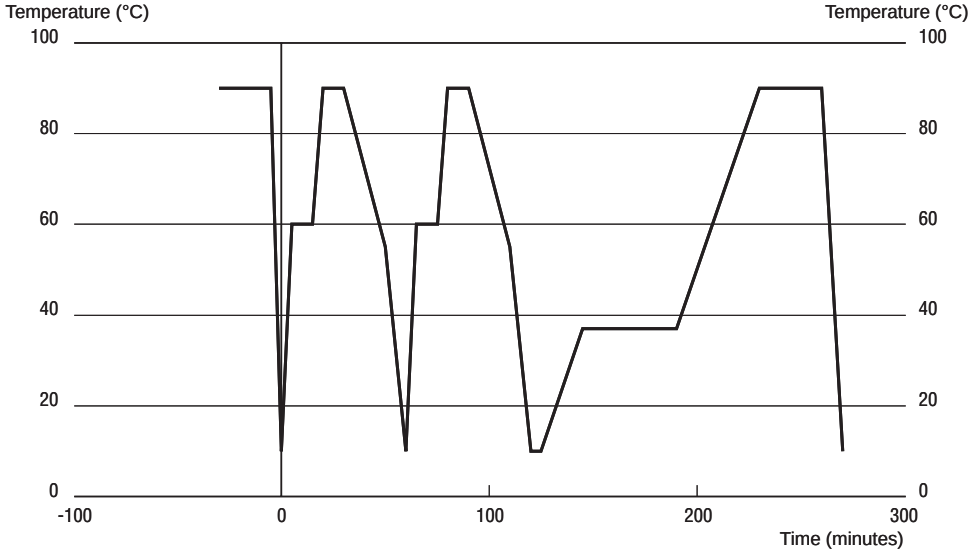
Figure 2 shows the time from the end of the oxidative bleaching step. The two high temperature washes (one of which is omitted when the enzyme is used) take two hours and the enzyme is added at 150 minutes.

Figure 2. **Process diagram**



Description of analysis

Members of the Bioprocess Research Group of DECHEMA e.V analysed the two competing techniques for removing residual peroxide. In order to make a comparison under equal conditions, the boundaries within which the analysis was undertaken had to be identified. The chosen boundaries were the two points up to which and from which the operating processes are the same, independent of the process substitution.

For the two processes concerned, this means the step after the bleach has been drained off and the rinsing liquid introduced into the dyeing apparatus. In each case, it ends with the introduction of chemicals which trigger off the reductive bleaching of synthetic textile components. In the diagram this spans 0-180 minutes.

In order to carry out the analysis several assumptions had to be made.

Number of bleaching processes per year

On account of the technical specifications of the two processes, it was not necessary to make any changes to the textile finishing plant that required investments. Only the programmes for the machine control system were modified. A comparison between the old and the new process of breaking down residual peroxide could, therefore, be carried out for a single bleaching process. Comparability of the economic results over a longer time-span was optimised by basing the analysis on a year's production.

For this purpose, the total production orders for several months were examined and the number of bleaching processes of the type under study determined. For this the three preceding months, May-July 1998 inclusive, were selected as, according to the management, they were representative. The

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number of processes determined was then projected for a complete business year (12 months) (Table 20).

Table 20. **Total number of bleaching processes with the Kappazym enzyme**

	Month			Quarter total	Year total[1]
	May	June	July		
Beam dyeing machine	63	101	117	281	1 124
Jet dyeing machine	31	27	16	74	296

1. Calculated by extrapolating the quarterly value.

Selection of the production plant

Windel Textil uses two different types of plant for peroxide bleaching: beam dyeing machines and jet dyeing machines.

The beam dyeing machine derives its name from the fact that the textile roll is wrapped around beam-shaped metal cylinders made of perforated steel. The cylinder with the material is then inserted into the machine. With the jet dyeing machine, the textile is transported through the dyeing liquid by the action of jets. Thus the mechanical impact on the fabric is minimised.

In the period under study, May to July 1998, 355 bleaching processes were carried out; 281, approximately 80% of the total, were carried out on the beam dyeing machines, the remaining 20% (74 processes) on the jet dyeing machines (Table 20).

As the beam dyeing machines at the company under study were of different sizes, it was important to establish an "average" machine. Approximately 33% of all bleaching processes on beam dyeing machines ran on one machine with a filling capacity of 5 800 litres of liquid, which can be regarded as of average size. As a further 50% of the processes were almost equally divided among two other machines whose filling capacities are equidistant from the 5 800 litres (2 600 litres and 8 500 litres), this first machine was chosen for the analysis based on the beam dyeing machinery.

This problem does not arise in the case of the jet dyeing machines, as the two machines have the same size and filling capacity. One was selected as approximately 75% of all the processes were carried out on this machine during the period under study.

Furthermore, the average quantity of textiles per process had to be determined, as this determines the use of process water. The jet dyeing machine was found to take an average of 157 kg material per run, the beam dyeing machine 226 kg per run (Table 21).

All assumptions made were discussed on the spot with the machine operators who confirmed their validity.

Table 21. **Material load (kg) per machine type and unit of time**

	Month			Quarter total	Year total[1]
	May	June	July		
Beam dyeing machine	13 011.2	22 731.5	27 898.0	63 640.7	254 562.8
Jet dyeing machine	5 407.8	4 225.3	1 980.5	11 613.6	46 454.4

1. Calculated by extrapolating the quarterly value.

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Once this ideal process had been established, an economic analysis was made of the two processes. This was based on fixed and proportional costs from the current cost accounting, the current purchase prices of chemicals and operating supplies and the current local prices for water and energy.

Besides hydrogen peroxide a range of other chemicals is used in bleaching blended fabrics, *e.g.* stabilisers, common salt and fabric-protective agents. Steam is required to heat the water, cooling water to cool it. The term process water means water that comes into direct contact with the textiles.

This research was supported by the Deutsche Bundesstiftung Umwelt (German Foundation for the Environment).

Results

For data protection reasons, no absolute figures can be given, but the costs of the new process are shown in Table 22, in relation to the traditional process.

Table 22. **Savings according to type of machine**

	Beam dyeing machine			Jet dyeing machine		
	Savings	Percentage of total costs	Weighted savings	Savings	Percentage of total costs	Weighted savings
Costs for:						
Chemicals	+7.18%	34.33%	+2.47%	+10.73%	17.11%	+1.84%
Steam	–14.36%	4.81%	–0.69%	–9.30%	3.67%	–0.34%
Cooling water	–16.66%	9.99%	–1.66%	–19.48%	12.73%	–2.48%
Process water	–19.97%	20.06%	–4.01%	–19.97%	14.96%	–2.99%
Other finishing costs	–9.77%	30.82%	–3.01%	–8.96%	51.54%	–4.63%
Total		100.00%	–6.90%		100.00%	–8.60%

The enzymatic process can achieve savings both with the beam dyeing and the jet dyeing machines. Costs for chemicals rise by 7% and 11%, respectively, due to the additional cost of the enzyme. In all other areas, the costs drop, some by up to 20%. Moreover it should be borne in mind that the individual cost factors represent different percentages of total costs. As one rinsing cycle is omitted completely, the reduction in process water costs is particularly striking. The enzyme application reduces the bleaching process by one hour. Thus, costs such as those for labour, machinery and electricity, among others, are also reduced. These costs are included in "other finishing costs" and contribute substantially to the savings. The enzymatic process finally turns out to be between 6% and 8% cheaper than the traditional process.

The approximate savings in energy, water and time resulting from the use of the enzyme process are shown in Table 23.

Table 23. **Savings of energy, water and time with the enzyme process**

	Beam dyeing machine	Jet dyeing machine
Energy	14%	9%
Water	18%	17%
Time	10%	9%

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Summary and conclusions

Windel now use the enzyme process as standard. The substitution of the conventional multiple rinsing to remove residual hydrogen peroxide in cotton bleaching by enzymatic treatment with catalase has the following advantages:

- Reduced consumption of natural resources owing to the water savings (for rinsing and also for cooling throughout the process) and by process energy savings in the form of steam.
- Reduced environmental pollution by the above-mentioned drop in consumption of resources and by the reduced discharge of industrial wastewater.
- Significant savings for the company by using this process step; depending on the type of machine used and the type of textile processed, reductions of between 6 and 8% per annum were achieved and documented.

The results of this cost analysis show that even modest, production-integrated biotechnological process changes can significantly reduce resource consumption and environmental pollution without causing expensive technical and financial investments. At the same time such a process-induced cost reduction increases the competitiveness of a company.

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Case Study 13

ENZYMATIC PULP BLEACHING PROCESS (LEYKAM, AUSTRIA)

Introduction

From 1991 to 1994, Professor Kurt Messner, head of the Mycology Department of the Institute of Biochemical Technology and Microbiology of the Technical University of Vienna, was responsible for a biopulping project in co-operation with the Austrian company Leykam Mürztaler, as part of an Austrian promotion programme. The Department of Mycology focused on the development of environmentally friendly processes based on wood-degrading micro-organisms and the elucidation of the enzymatic processes involved. It was financed primarily by research grants from national funding bodies, the EU and private companies.

Leykam Mürztaler Papier und Zellstoff AG has been a pulp and paper producer since the 18th century. Before this project, Leykam Mürztaler was an independent public limited company but in 1988 the majority of its shares were sold to the Dutch paper company Koninklijke Nederlandse Papierfabriken NV (KNP). Because of financial difficulties Leykam-Mürztaler merged with the paper activities of KNP in 1994 and in spring 1998, KNP Leykam was taken over by the South-African company Sappi and is now called Sappi Gratkorn. Leykam was a member of an international biopulping consortium, comprised mainly of US companies, in which there is a regular exchange of experience on biopulping.

At the time of the project, the research department played a relatively important role at Leykam. Leykam was considered to be very progressive and to be a leader in environmental protection. The main emphasis of its R&D was on chlorine-free bleach, modern bleaching sequences and biological wastewater processing plants. In 1996, KNP Leykam Gratkorn GmbH was awarded the "Austrian Eco-Audit Prize 1996" for overall environmental activities. Leykam's head of research was very interested in biotechnology and his team had a high level of training in biotechnology.

At the time of the merger the innovation project was terminated and all research now takes place in the central research department of Sappi, situated in South Africa. The staff of the Leykam research department has been cut.

The innovation goal: biopulping

The aim of the innovation project considered in this case study was the development of a biotechnological process known as biopulping, in which white rot fungi, which selectively degrade lignin, are used to improve pulp production.

In pulp production, two main groups of methods can be distinguished: chemical pulp production and mechanical pulp production. Together, these methods have a 60% share of the world market, of which 42% is produced chemically. In the chemical process, wood chips are boiled in a chemical solution at high temperatures and pressures. The resulting pulp is of high quality but yield is only 45-55%. In chemical pulping the sulphide and sulphate processes predominate – approximately 13 times more sulphate pulp than sulphide pulp is produced worldwide.

In mechanical pulp production machines are used instead of chemicals to transform the raw material into pulp. With pulp yields of 88% to 98%, these processes are much more effective than

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chemical processes in terms of quantity, but the fibres are damaged by the machines, thus yielding a lower pulp quality. The pulp is also less suitable for bleaching. Thus its range of possible applications is narrower than for chemically produced pulp.

The biopulping method

Biopulping describes a pre-process in which white rot fungi, which selectively degrade lignin, are used to break down wood cell wall structure. Wood chips are sterilised by a steam treatment lasting 30 to 60 seconds to kill off so-called "bio control" fungi which would otherwise hinder the growth of the biopulping fungi. The wood chips are then injected with the biopulping fungus and a growth medium. Over a two-week incubation period air is supplied from below to provide optimal growth conditions for the fungus.

When the next step is mechanical, the environmental benefit of biopulping is a reduction of energy input by 30-40% as compared to conventional methods. When chemical treatment is used, 30% more lignin can be removed or the boiling time can be correspondingly reduced. Moreover, the remaining lignin is already broken down ready for bleaching. However, there is a darkening of the resultant pulp which has to be compensated by the use of additional chemicals.

Biopulping yields pulp of a higher quality, enabling savings in terms of raw materials, chemicals used as processing aids and energy. A further advantage from the environmental viewpoint is the lower wastewater toxicity.

The innovation process

The project, which received financial support from the Austrian government, was initiated in 1991 and continued for three years before being broken off. The new parent company has never taken up the project and is believed to be working on a similar activity in South Africa. The initiative came from academia and while the head of research in Leykam was in favour of the project he nevertheless had difficulties convincing the board about the project. However, due to the previous biotechnological training of the members of Leykam's project team, there was already a certain degree of internal competence in the firm.

In the laboratory phase, the development team was highly successful in screening for biopulping fungi. The results of the innovation in this area were subsequently confirmed by an American developer who first introduced functioning biopulping methods in industrial sulphide pulp production. No breakthroughs were achieved in the area of pre-sterilisation of the wood chips.

The decisive phase for the project proved to be the transition from laboratory to industrial scale. First, problems arose due to the lack of support from the process engineering sector. Andritz AG, of Graz, Austria, one of the biggest producers of pulping machines, was sceptical from the beginning, less in relation to the technical realisation of the project but rather in the form of a negative attitude of the engineers to the whole area of biotechnology. It was at this decisive phase that the innovation project was discontinued by KNP the new parent company of Leykam.

Because the project results were patented for Austria only and not worldwide, it was possible for the American developer (a member of the biopulping consortium), to develop the work further. His biopulping method is now being used in sulphide pulp production and he now holds the decisive patents in this field.

Favourable and unfavourable factors

Functioning biopulping processes on an industrial scale have been developed in the area of sulphide pulp production. However, despite successful market entry these processes are only used to a limited extent. Table 24 gives an overview of the favourable and unfavourable characteristics which are responsible for this.

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Table 24. **Characteristics of biopulping that favour or impede market success**

Characteristics of biopulping that favour market success	Characteristics of biopulping that impede market success
Cost reduction through savings in energy and chemicals	The incubation period of several weeks raises costs of capital tie-up.
No basic changes to existing process steps but only an additional process step necessary	No suitable biopulping method has been developed for the two most important areas of use: sulphate pulp production and mechanical pulp production.

The main condition favouring the biopulping project itself proved to be the positive attitude of the head of research, the strategic orientation of research and development of Leykam in the fields of environmental protection and biotechnology and the training in biotechnology of the Leykam research staff.

Another positive element was the firm's inclusion in the international biopulping consortium, whose regular sessions were a source of important information and made possible a comparison of the company's own research efforts with internationally leading groups. A third factor was the funding of the project provided by the Austrian state.

The main negative factors were Leykam's economic difficulties and its subsequent take-over by the Dutch KNP and the South African enterprise Sappi. Such changes are problematic for a university partner for two reasons. First, it makes access to industrial research laboratories more difficult, and second, overseas take-overs can reduce the number of potential co-operation partners in national promotion programmes.

Another negative aspect was the resistance both from the Leykam board and from the industrial plant manufacturers which impeded the transition from the laboratory phase to the pilot phase. Finally, the inadequate securing of patent rights for the interim results has made it more difficult to take up work on the project again.

Summary and conclusions

There has been a general trend towards increasing market concentration in the pulp and paper industry. Companies have tried to increase volume of sales and productivity by mergers and acquisitions, transformations which were linked with extensive job losses.

The Austrian company Leykam Mürztaler was a typical example. Because of financial problems it was taken over by two larger pulp and paper companies. Before the mergers, Leykam had been a rather progressive company: it was a leader in environmental protection, and R&D played an important role. The emphasis of its R&D was on environmental protection and biotechnology and the research on the biopulping process was internationally competitive.

Following the abandonment of the biopulping project, the process has been successfully developed to industrial scale in the United States, but has not yet been introduced in Europe. The comparative success of biopulping in the United States can be attributed, among other things, to the existence of the biopulping consortium.

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Case Study 14

USE OF XYLANASE AS A PULP BRIGHTENER (DOMTAR, CANADA)

Introduction

Domtar Incorporated is the largest producer of speciality and fine papers in Canada with annual sales of CAD 4 billion, mostly to the United States. Domtar traces its origins to the turn of the century in a coal tar distillation plant in Sydney, Nova Scotia. Incorporated in 1929 as Dominion Tar and Chemical Company, it relocated in that year to Montreal, Quebec.

The 1950s marked the beginnings of the Corporation's expansion into the pulp and paper and construction materials businesses. In 1961, a complex multiple-merger resulted in the formation of one of Canada's first conglomerates. The name "Domtar" was officially adopted in 1965. By 1984 the company had 20 000 employees and had businesses in wood preserving, coal tar products, particle board resins and speciality chemicals.

Other initiatives included construction during the late 1980s of a world-class communication papers mill at Windsor, Quebec. Domtar's current focus on its integrated forest products, fine papers and packaging businesses has led, in the 1990s, to the divestiture of its salt and other chemical operations, wood preserving, newsprint and groundwood specialities, and construction materials divisions.

In 1996, the company sold its gypsum division and its decorative panels division leaving a company of 9 000 employees solely active in pulp and paper, operating four bleach kraft mills. There have been subsequent acquisitions of pulp and paper-related businesses.

Environmental issues

A major development occurred around 1985 that affected the chemical pulp industry worldwide. In its "7-Tier" national dioxin monitoring study, a link was made by the US EPA between the use of chlorine in pulp bleaching and the formation of chlorinated dioxins and furans. Not long afterwards, the industry's effluents were characterised for the presence of chlorinated organic by-products by the so-called "total organic chlorine" test (TOCl), subsequently revised as the "absorbable organic halides" test, or AOX. This surrogate parameter measures the amount of chlorine in wastewater that is organically bound. According to some estimates, approximately 90-95% of the atomic chlorine is converted into inorganic chloride (*e.g.* NaCl) and around 5-10% into AOX. Current environmental regulations worldwide require bleach chemical mills to have non-detectable concentrations of dioxins/furans and low levels of AOX in its effluents.

The Domtar central corporate R&D centre was very active from the mid-1980s to late-1990s, investigating the most economical solutions for substantially reducing organochlorine by-product formation at its bleaching mills. Working in conjunction with the Pulp and Paper Research Institute of Canada (PAPRICAN) and chemical suppliers, the R&D centre developed and helped implement site-specific cost-effective technologies at Domtar mills to help them become dioxin-free and comply with the new AOX regulations.

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Pulping and bleaching

Pulp digestion (cooking) reduces softwood lignin from about 30% to about 4-5% and hardwood lignin from 16-18% to 2-3%. Wood chips (wood/liquid – 1:4) are cooked at 160-170 °C in 18-20% alkali that includes sodium sulphide. A kraft mill is virtually energy self-sufficient from the dissolved organics from the cooking stage. The alkali and sulphide are regenerated from the cooking liquor.

Cooking is followed by bleaching which is a multistage process (anything from 3-6 stages involving bleaching and washing steps) which removes the lignin incrementally to yield a bright, strong, clean pulp. Traditionally, the bleaching agent has been elemental chlorine.

The solution to decreasing AOX in wastewater is either to reduce lignin in the pulp before bleaching or to change the bleaching chemistry, moving from elemental chlorine (Cl_2) to more oxygenated bleaching chemicals such as ozone (O_3), hydrogen peroxide (H_2O_2), oxygen (O_2) or chlorine dioxide (ClO_2). Peroxide is effective but cannot be used as a total replacement because it weakens the pulp; ClO_2 is most commonly used, in a process referred to as ECF (elemental chlorine-free). The Scandinavians elected to reduce pollution at source by opting for oxygen de-lignification (*i.e.* reduce lignin).

The first uses of biotechnology came in 1986 with a role for enzymes in de-lignification and brightening (see Annex A to this case study). Enzymes have a major potential for bulk de-lignification, by cutting the lignin/hemicellulose bonds, but this is still at the R&D stage. De-resinators, which remove extractives such as pitch, address a quality issue associated with mechanical pulping (primarily for newsprint in which Domtar has no interest) rather than chemical (kraft) pulping. These use fungal enzymes which have very low activity in cold climates.

Brightening enzymes are hemicellulases (*e.g.* xylanases) and oxidative enzymes such as laccases. The former have reached the point of commercialisation but the latter, because they require expensive co-factors, are not yet economic.

Most development work has been done on xylanases. Originally these were very expensive and very fragile, needing carefully controlled conditions of pH and temperature. Additionally they contained cellulases which are not desirable because they break up the cellulose polymer chains reducing yield and pulp strength. Iogen, the major supplier of xylanase in Canada (see Annex B to this case study), have brought the cost down and produced a high-purity product which is more robust.

Domtar's corporate research centre was restructured in 1999 to redirect technical resources at the production facilities where the current drivers have become process optimisation, cost-reduction and product development. Domtar now conducts pre-commercial R&D via membership in PAPRICAN, contractual R&D at specialised independent labs and funding of postgraduate projects at university pulp and paper centres. For example, a master's level project on laccases was funded at the University of Toronto which has now been completed.

Pressures for change

Originally the driver for the use of xylanase was the need to reduce chlorine at mills where there was insufficient ClO_2 to achieve dioxin-free and/or ECF status. This bleaching chemical is made on the mill site by reducing sodium chlorate with, for example, methanol, or from brine. In the early days of xylanase use, the mills absorbed the high cost of the enzyme to avoid having to build larger ClO_2 generators.

Iogen has now reduced the cost of xylanase to the point where they are promoting it on its ability to reduce operating costs alone – they give a guarantee to that effect.

The advantage of xylanase is that it does not need any investment in equipment – no pumps, washing, etc. It is applied in-line ahead of the bleaching sequence. Xylanase is not a de-lignifier – there is speculation that by breaking bonds it somehow "activates" the lignin and consequently treated pulp requires some 10-15% less bleaching chemicals.

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Domtar conducted in-house testing of xylanase in the 1980s, looking at enzymes from three or four suppliers. However, no further developments occurred because the products were too expensive. Domtar is currently trialing xylanase again at a market pulp mill that is experiencing a very small shortage of ClO_2 to become full-time ECF (see Annex A to this case study).

Process history

Domtar (excluding its subsidiaries) has four bleached kraft mills, two cook hardwoods, one softwood and one a mixture of the two (in dedicated fibre lines). All mills comply with current regulations. Interestingly, one hardwood pulp responds to xylanase but the other, the newer one, does not. This may have something to do with the cooking process.

Currently, the 100% softwood mill has a small shortage of ClO_2 to become full-time ECF. The mill has taken H_2O_2 as far as it can but still has a gap in bleach supply which makes it an ideal candidate for applying xylanase. This mill has an integrated ClO_2 generator (sodium chlorate, the precursor to ClO_2, is made on site) that is next to impossible to scale-up short of building a twin facility.

Between the ordering and the start-up of the generator, the Quebec government introduced more stringent regulations on AOX to come into force on 1 January 2001. The new regulations were such that the AOX limit fell from 2 kg/ton to 0.8 kg/ton of pulp and the only way to achieve this would be to use 100% ClO_2. Additionally, since two-thirds of the mill's products are shipped to the United States, the plant must meet US EPA Cluster Rules AOX limits effective April 2001.

Instead of spending major capital, Domtar's senior management challenged the mill to come up with a creative solution at little or no cost. This was achieved after 17 months of intensive trials at the mill involving corporate and mill technical personnel, with a lot of enthusiastic support from the shift operators. Only a month before the deadline, having further optimised the use of hydrogen peroxide and revised brightness targets in most stages, the mill was able to achieve AOX compliance with a 50% margin of safety and a bleaching cost that was significantly lower than 17 months previously. Once stable with a reliable baseline, the latest xylanase trial was initiated in February 2001 and ran until end April 2001. There have been some positive signs, with bleach chemical charges being reduced.

Summary and conclusions

This case history describes one situation where there is a strong strategic fit for xylanase enzymes. Overall, if a company is too proactive with respect to potential environmental restrictions it will become un-competitive. This has been the case in other companies where de-inking enzymes have been used. While there are a few local regulations requiring zero discharge, *i.e.* closed circuit operation, this has not materialised as a general regulation in Canada.

Potentially the most far-reaching biotechnological development in this industrial sector involves PAPRICAN, an industry body which conducts consortium research and has a programme for mapping the genome of the aspen – the tree chosen as a fast-growing species for Canada. The idea is eventually to breed a fast-growing, low lignin source of hardwood.

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Annex A

STATUS OF PULPING ENZYMES
(excludes enzymes used for de-inking and drainage improvement, etc.)

Softwoods contain 25-28% lignin while hardwoods contain 16-18% lignin (bulk lignin). Initial "cooking" reduces this to 4-5% and 2-3% respectively (residual lignin).

De-lignifier (work on bulk lignin)	Deresinators	Brighteners (work on residual lignin)
Tremendous potential	Reduce pitch-related problems	Hemicellulases (*e.g.* Xylanase)
Simplify process	More an issue with mechanical pulping	Used ahead of bleaching
Enhance yield	"Digest" extractives such as resins	Yield a brighter pulp
Reduce cost	and fatty acids	Mode of action uncertain
Improve quality	One fungal treatment commercialised	Oxidative enzymes (*e.g.* laccases)
Customise for specific lignins and products	(Clariant?)	Currently too expensive
Still at R&D stage	Trials with some success	
White rot fungi showing promise	Current status unknown	
Reaction too slow	Limited in northern locations because of cold winters	

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Annex B

IOGEN'S XYLANASE BUSINESS
(For background on Iogen, see Case Study 19)

Background

In 1986, Liisa Viikari in Finland, reported the possible role of xylanase as a brightening agent, reducing chlorine use in bleaching. A number of enzyme companies responded, including Novozymes and Genencor, and the first trials were carried out in Finland in 1991.

Also in 1986, dioxins were identified in milk cartons in Montreal. Chlorine had been the bleach of choice for a century and the best candidates to replace it were chlorine dioxide or H_2O_2. The latter can only be used to a limited extent because too much weakens the pulp.

In 1989 Iogen entered the enzyme business with cellulase for clarifying apple juice.

The company was aware of the functionality of xylanase, and *Trichoderma*, the organism used to manufacture their cellulase, also made large quantities of xylanase.

In May 1991 Iogen conducted their first xylanase trial and had their first Canadian customer in 1992 – a mill at risk of closure should their levels of AOX not be reduced. Iogen now has 11 customer mills and the business is growing (one mill at a time).

Since measurement of immediate performance is difficult – the results of the enzyme action are only seen at the end of the bleaching process – xylanase is a different sort of business from that of conventional enzymes. It requires high levels of service and monitoring, in Iogen's case by way of installing computer monitoring at the mill linked on-line to Iogen's computer in Ottawa.

Xylanase development

Xylanase was originally a by-product of cellulase but mills require that there be no cellulase present. At first Iogen improved xylanase yield by adjusting pH and temperature and by induction – feeding the organisms xylan. Genetic engineering has since been used to increase the copy number of the gene and install a stronger promoter. Most recently, the company has explored protein engineering in collaboration with the Canadian National Research Council. So far they have achieved a shift in pH optimum from pH 6 to pH 8.5 and an increase in optimum temperature from 50 °C to 65 °C.

Xylanase is the most commercial of pulp and paper enzymes. Biopulping or de-inking enzymes have not really taken off although the University of Wisconsin has a huge effort in the former. The pulp and paper sector is a competitive commodity business with very low margins. Enzymes cannot command the prices they do in biostoning, for example.

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Case Study 15

A LIFE CYCLE ASSESSMENT ON ENZYME BLEACHING OF WOOD PULP (ICPET, CANADA)

The following is a very abbreviated version of a study prepared by Gloria Z. Fu of the National Research Council of Canada's Institute for Chemical Process and Environmental Technology in April 2000 (Draft Working Document of the National Research Council of Canada).

Introduction

Cellulose and hemicellulose are inherently white and do not contribute to colour. It is generally agreed that chromophoric groups on the lignin are principally responsible for colour, and that part of the lignin's phenolic groups can also be oxidised to quinine-like substances that are known to absorb light. A bleaching treatment is normally required to eliminate residual lignin and other chromophores.

In traditional bleaching processes, molecular chlorine (Cl_2) was used to remove the last traces of lignin from the pulp. However, over the past ten years, Canadian producers have changed to ECF (elemental chlorine-free) technology. ECF, by definition, does not permit the use of Cl_2 in bleaching but does permit the use of chlorine dioxide (ClO_2). The use of ClO_2 can lead to the formation of Cl_2 but the peak of Cl_2 concentration during the ECF bleaching process is almost a full order of magnitude lower than the peak Cl_2 concentration in the traditional chlorine bleaching sequence. In 1998, 76% of Canadian bleached kraft production used the ECF process.

There is continuous demand for technical innovation in the pulp and paper industry, be it for a more cost-efficient paper-making process or because of environmental concerns. In kraft pulping, bleaching is still one of the most expensive operations in the pulp mill, and therefore a prime target for cost reduction. ECF bleaching improved greatly the quality of the mill discharges but has further increased operating costs.

The biological bleaching process uses enzymes to supplement chemicals in the process to extract lignin. In Canada, about 10% of bleached kraft pulp is now manufactured with xylanase treatment to reduce chlorine dioxide use. This application is already cost-effective. Enzyme bleaching, by replacing chlorine-containing compounds, can help to produce an improved effluent that is therefore recyclable to the recovery system.

Of possible enzyme candidates in pulp bleaching processes, laccase is probably the most interesting one for the paper and pulp industry. Laccases are a group of oxidoreductase enzymes, able to oxidise a wide range of phenolic and amine compounds. Laccase can be isolated from different micro-organism strains and extracted by different methods. For instance, laccase from B*otrytis cinerea* has been shown to be as potent as many widely studied white-rot fungal laccases in the de-lignification of kraft pulps and in the oxidation of lignin model compounds. The B*otrytis* laccase has the advantage of being constitutive and extracellular. The laccase-NHA mediator system – a combination of the enzyme and a chemical redox-co-factor – is presently close to commercialisation.

Objective of the study

The purpose of the study was to use LCA methodology to assess the environmental impacts of the emerging biotechnology-based process, laccase-NHA pulp bleaching and to compare this with an existing widely-used, technology-based, process, ECF bleaching processes.

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The study also attempted to find out those parts of the process which consume the most energy or material resources or which give the largest emissions, and thus to identify the opportunities and barriers for improvements. The commercial software SimaPro 4.0, developed in the Netherlands, was used as the tool to perform the LCA study. The results are presented in ten impact categories, including energy consumption and greenhouse gas emissions.

The boundaries of the system investigated are those of the bleach plant only. The study covers all the processes within the bleach plant, the production of all the inputs, such as chemicals, energy and fuels, and all the emissions generated by the plant.

The laccase-NHA system does not completely replace chlorine dioxide in the whole bleaching process, but only replaces it in the first stage of the five-stage ECF bleaching sequence.

The ECF bleaching process usually involves two components, a chlorine dioxide stage and an alkaline extraction stage with a number of washing steps between. In the chlorine dioxide stage, pulp and ClO_2 react together for 2-4 hours at pH 3.5-4 and a consistency of 12-15%. Formation of chlorinated organic compounds is minimal in this stage. The gases from this step are treated in a scrubber with alkali (NaOH) and sodium sulphide (Na_2S), to neutralise both residual chlorine and chlorine dioxide. In the alkaline extraction stage, NaOH dissolves not only some of the resins but also some of the hemicelluloses in the pulp.

Enzymatic de-lignification (laccase-mediator treatment) is carried out at a 10% consistency in a pressurised vessel at 50 °C, pH 4.5, adjusted with sulphuric acid, and in the presence of O_2. The incubation time is two hours. Mediator is consumed in the reaction, and its reduction product is formed together with other unidentified products.

The mode of laccase/mediator action during de-lignification is a fast initial attack on phenolic sub-units, followed by slower oxidation of non-phenolic units to give structures susceptible to alkaline degradation. The efficiency of de-lignification depends on reaction conditions, such as pH, temperature, oxygen pressure, etc. The pH decreases continuously during the course of the reaction because organic acids are liberated from the lignin.

The primary data (direct chemical inputs and emissions in the bleaching processes) were provided by paper industry personnel. The data reflect the average technology in Canada rather than any specific mill.

Energy sources in this study are taken from the average for the Canadian pulp and paper industry in 1995. For a typical mill, about 55.7% of its energy needs come from self-generated power using renewable sources such as wood wastes and spent pulping liquor. The kraft pulp sector self-generates more energy than the industry average. Canadian kraft mills are typically 60-80% energy self-sufficient. The form of energy supply in pulp mills is some steam and mostly electricity.

All transportation involved, except transportation of mediator, is assumed to be road transportation using diesel trucks. Mediator is assumed to be purchased from Germany and transported by air cargo and then by diesel truck from the airport to the mill.

The secondary data, such as the production of the inputs, were collected mainly from individuals, literature, and recent environmental reports of major companies in related fields. If those attempts were not successful, the data would then be taken from the SimaPro 4.0 database, using average western European data.

In this study, the average raw material consumption, emissions and energy demand associated with enzyme manufacturing were taken from environmental data on Novozymes' Franklinton, United States, site, where the full-scale enzyme production took place.

Due to lack of sources, the following gaps exist in the data:

- No environmental charge is associated with mediator manufacturing.
- No emissions are associated with cryogenic oxygen production.
- No raw material consumption is associated with H_2O_2 manufacturing.

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- No raw material consumption or energy demand is associated with electricity generation from wood wastes. In other words, the environmental charge associated with production of wood wastes was not allocated to energy production.

For electricity generated by spent pulping liquor, no emissions related to the evaporator and no energy consumption related to the recovery furnace are allocated to energy production. Depending on what type of recovery furnace is used in the mill, the possible air emissions from recovery furnace include VOCs, HCl, PAHs, H_2SO_4 and trace metals. Only the VOCs from the recovery furnace were allocated 100% into energy production.

Results and discussion

The results are presented in terms of ten impact categories: energy demand, greenhouse gas emissions, acidification, eutrophication, winter smog, summer smog, ozone depleting substances, heavy metals, carcinogenic substances and solid wastes.

The highest energy consumption in the enzyme-bleaching process relates to the consumption of chlorine dioxide. This is perhaps due to the consumption of relatively large amounts of chlorine dioxide in the process and/or the fact that chlorine dioxide manufacturing itself is quite energy-intensive.

The highest greenhouse gas emissions also derive from the consumption of chlorine dioxide. This is mostly due to the energy needed for producing sufficient $NaClO_3$ and chlorine dioxide for the bleaching process.

Comparison of enzyme bleaching and ECF bleaching process

Table 25 compares the enzyme bleaching process and the traditional ECF bleaching process in terms of the impact categories. The figures indicate that the enzyme bleaching process gives less ozone-depleting substances, less acidification, less heavy metals, less carcinogenic substances, less winter smog and less solid wastes, and consumes less energy. However, it gives more greenhouse gas emissions and more summer smog emissions. These are mainly due to the international flight for mediator transportation from Germany to Canada. Enzyme bleaching also results in more eutrophication than ECF bleaching. Enzyme manufacturing, as well as feedstock cultivation, are the source of these emissions.

Table 25. **Comparison of processes by environmental impact category**

Environmental impact categories	Enzyme bleaching (%)	ECF bleaching (%)
Greenhouse gases	100	92
Ozone	85	100
Acidification	92	100
Eutrophication	100	98
Heavy metals	88	100
Carcinogens	84	100
Winter smog	87	100
Summer smog	100	89
Energy consumption	97	100
Solid waste	87	100

Conclusions

The following conclusions may be drawn from the study:

- Replacing ECF bleaching by enzyme bleaching will save energy, discharge less ozone depleting substances, less acidification, less heavy metals, less carcinogenic substances, less winter smog and less solid wastes. It produces somewhat higher eutrophication.

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- When the mediator is assumed to be transported from Germany by air, the enzyme-bleaching process will give more greenhouse gas emissions and summer smog emissions than ECF bleaching process. It will however, based on a sensitivity analysis, give less greenhouse gas emissions and summer smog emissions, if the mediator can be purchased locally.
- Breaking down the emissions in terms of resource consumption, the main polluters in the enzyme bleaching process, except for the eutrophication impact, are chlorine dioxide consumption, energy consumption, NaOH consumption and transportation (Table 26).
- Table 26 also shows that the biggest polluter in the bleaching process is the consumption of chlorine dioxide. This means that virtually anything that can be done to replace or reduce chlorine dioxide consumption may benefit the environment. Anything that can improve the energy efficiency of the whole bleaching process or the chemical manufacturing can also benefit the environment.
- The laccase bleaching stage consumes least energy and gives the least environmental impact.

Table 26. **The rating of emissions by resource consumption**

	ClO_2	Energy used in bleach plant	NaOH	Transportation
Energy consumption	1	2	3	3
Greenhouse gases	1	2	3	4
Ozone depletion	1	2	–	–
Acidification	1	2	2	3
Winter smog	1	3	2	4
Summer smog	1	3	4	2
Heavy metals	1	2	3	–
Carcinogenic	1	2	–	–
Solid wastes	1	–	2	–

Note: 1 = largest emission; 4 = least emission; – = almost no emission.

It must be kept in mind that the results are obtained on the basis of certain assumptions and a number of different data sources. Further study will be required to investigate the sensitivity of the assumptions, to identify those assumptions that are critical to the final conclusion, and to check data quality where possible.

Sometimes, no data on the environmental loading of the process are available. They are in practice assumed not to exist. This can be important for the results and conclusions of a comparative LCA, if the gaps of one alternative are more important than those of the other. The important gaps then may be filled either by additional data gathering or by obtaining the mass and energy balance through process simulation.

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Case Study 16

ON-SITE PRODUCTION OF XYLANASE (OJI PAPER, JAPAN)

Introduction

The history of Oji Paper goes hand in hand with the history of Japan's modern papermaking industry, which began in 1873, in Oji-mura. In that year, Mr. Ei-ichi Shibusawa launched a papermaking company which, 20 years later, was renamed Oji Paper in honour of the place of its founding. In 1933, Oji Paper merged with Fuji Paper and Karafuto Industries, developing into a corporation that produced 80% of Japan's Western-style paper.

Following World War II, the company was divided into three components: Tomakomai Paper, Jujo Paper, and Honshu Paper. Tomakomai began as a one-plant operation, but upon its expansion into Kasugai, Aichi Prefecture in 1952, the company was renamed Oji Paper Industries, and in 1960, it became Oji Paper. Eventually, the company merged with Kita Nippon Paper, Nippon Pulp Industries, and Toyo Pulp. In 1993, Oji Paper merged with Kanzaki Paper to become New Oji Paper, and furthermore, in 1996, New Oji Paper and Honshu Paper merged to become Oji Paper. Today, Oji Paper has become one of the largest and most influential paper manufacturers in the world with some 12 000 employees.

Since Viikari *et al.* reported enzyme bleaching by xylanase in 1986, xylanase treatment has attracted attention in the pulp and paper industry. Enzymatic treatment has already been introduced in many mills in Europe, North America, South Africa and Oceania. However, enzyme bleaching has not become popular in Japan, because the oxygen de-lignification technology has been preferred instead.

Oji Paper bleaches hardwood kraft pulp at their Yonago mill. The production capacity of this mill is 1 300 tons of hardwood kraft pulp and 300 tons of softwood kraft pulp per day. In 1998, as a step to modernise the Yonago mill, changes were introduced in paper machines, coaters, kraft pulp fibre lines and the wastewater treatment system to increase production capacity. At the same time, the use of an enzyme bleaching system in the bleaching sequence was studied as a way to reduce the environmental burden.

A search had been conducted for an effective xylanase to be applied to the pulp bleaching process and various xylanase-producing micro-organisms were isolated and tested. A new bacterial species, which produces a thermostable xylanase, was discovered and the possibility of industrial-scale production of xylanase using this micro-organism was examined. Based on these findings, a process for on-site production of the enzyme and for enzyme bleaching was developed which was aimed to contribute to the reduction of the environmental load.

Process innovation

The enzyme

A micro-organism, isolated from Japanese soil by repeated screening, was selected for use in this process. This micro-organism, *Bacillus* sp.S-2113, produces two extracellular xylanases, XP1 and XP2 when cultivated in the presence of xylan and a suitable nitrogen source. Their properties are shown in Table 27.

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Table 27. **Properties of two xylanases**

Property	XP1	XP2
Optimal pH range	5-8	5-8
Stable pH range	3-9	4.5-9
Optimal temperature (°C)	50-80	60-90

These xylanases are characterised by high activity over a wide pH range. Also, both were found to have high temperature optima – the reaction occurred at temperatures up to 80 °C. Both enzymes showed that the activity did not decline substantially when they were kept at room temperature. Therefore the enzyme can be stored at room temperature without addition of preservatives. Moreover, no cellulolytic activity was detected in cultures of this micro-organism, which is, of course, very advantageous in this application.

Enzyme production

The mill-scale production of the enzyme has been successfully achieved starting from a flask scale experiment in the laboratory. As a result of searching for a low cost substrate, hard wood kraft pulp was found to induce the xylanase production, which could reduce the enzyme production cost drastically. In addition, when pulp was used as an inducer, the same or even higher enzyme productivity was achieved in comparison with the use of birch xylan. After bleaching, the micro-organism is sterilised by the bleaching chemicals used in the subsequent steps of the bleaching process. The pulp used as an inducer can be returned to the fibre line as raw material. Furthermore, because the quantity of enzyme used is relatively low, there are no adverse influences on the pulp quality. This operation has been working well for two years since its start-up.

Enzyme production facilities that include fermentation tanks to cultivate the micro-organism were installed near the bleach plant. After oxygen de-lignification, the hardwood kraft pulp is washed and its pH is adjusted to near neutral before the enzyme is added. The entire xylanase-containing culture, including the bacterial cells and the culture medium, is then added to the pulp. The conditions for enzyme bleaching are as follows: pulp consistency: 10%, temperature: 50-70 °C, reaction time: two hours or longer. After the enzyme treatment, pulp is washed and transported to the subsequent chemical bleaching process. Utilities such as steam, electricity, air, water, acid and alkali could all be supplied by the existing facilities at the mill. The wastewater from the enzyme production facilities is treated by the mill's existing effluent treatment system without any additional special facilities.

Experience with the enzyme production operation

More than two years have passed since this process started (in October 1998). The operation has been quite stable as indicated in Figure 3, which presents the relative productivity of the enzyme (as a percentage) during 1998-99 operation. These productivities were higher than those in the laboratory experiments.

Based on the actual enzyme bleaching operation over two years confirmed the following advantages of the enzyme treatment. Quantities of chlorine and chlorine dioxide were reduced by 35% and 65%, respectively. Additionally, under the mill operating conditions used, a 40% reduction of AOX (absorbable organic halides) has been achieved, although no reduction of COD (chemical oxygen demand) has been detected.

Cost benefits

This enzyme bleaching process has made cost reductions possible mainly through the reduced usage of expensive chlorine dioxide, with no additional investment in wastewater treatment facilities.

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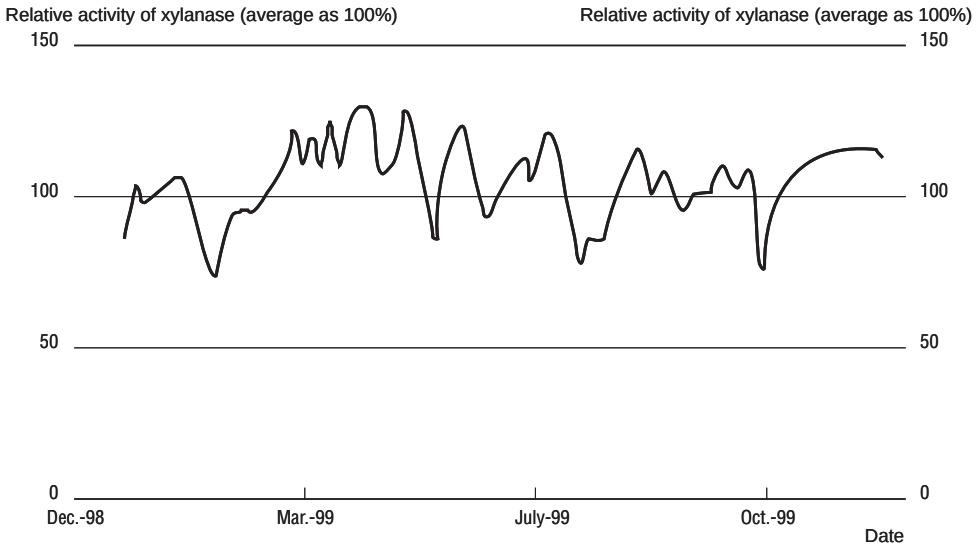
Figure 3. **Enzyme production operation**



A further cost reduction was achieved by manufacturing the enzyme at the bleaching site rather than purchasing it from a supplier. The large decrease in the cost of the enzyme production was one of the major criteria for the decision by Oji Paper to adopt this particular process at Yonago mill. The micro-organism that produces the enzyme, xylanase, can be cultivated in a medium containing corn steep liquor with the addition of inorganic salts. These nutrients are waste materials and very cheap.

An enzyme is more expensive when it is purchased from a supplier than it is produced in-house because the supplier needs to carry out a series of procedures, such as extraction of the enzyme from the micro-organism, its concentration and storage, before the enzyme is delivered to a user. Moreover, the supplier needs additional facilities for the treatment of the culture broth and cell debris in order to decrease biological oxygen demand (BOD). However, these procedures are not needed for in-house production and consumption.

The enzyme can be applied without any purification, because the micro-organism does not make any cellulolytic enzymes, which would adversely affect pulp quality. In addition, kraft pulp was found to act both as a substrate for the micro-organism and an inducer of the enzyme. The entire culture, containing the micro-organism, the enzyme and the kraft pulp, can be added to the pulp after oxygen bleaching without separation. The enzyme helps bleach the pulp. Kraft pulp is used as a pulp source and the micro-organisms are sterilised in the subsequent bleaching process.

Summary and conclusions

An application of enzyme to the bleaching process was introduced to reduce the amount of organic chlorine in wastewater from the bleaching and to make the pulp bleaching process environmentally friendly.

The particular feature of this xylanase application is that the enzyme itself is produced in the mill, not purchased from a supplier. This unique factor has enabled the bleaching costs to be reduced, not only through lower cost of enzyme production but also because of the following factors:

- Reduction of expensive chlorine dioxide.
- Omission of separation and/or purification of the enzyme because of the direct addition of enzyme-containing culture to the pulp bleaching line.
- Use of kraft pulp to culture the micro-organism that produces the enzyme.

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Case Study 17

A GYPSUM-FREE ZINC REFINERY (BUDEL ZINK, NETHERLANDS)

Introduction

Electrochemical and metal-finishing industries produce wastewater streams contaminated with heavy metals. These streams often also contain high levels of sulphate and sulphuric acid. Traditionally, metals in process streams were precipitated as hydroxides by neutralising with caustic soda and the sulphate removed by precipitation as gypsum using milk of lime or limestone.

As long as metal producers are allowed to "store" polluted gypsum, this is by far the cheapest option. However, in the light of changing regulations, Paques in the Netherlands has developed the closed loop principle in which metals are recovered and recycled while excess sulphur is of sufficient purity to be sold as a final product or for conversion to sulphuric acid. Full-scale operations have been in place in various locations for nearly a decade.

These second-generation processes precipitate the metals as sulphides, the advantages being a more flexible pH range and much lower concentrations left in the effluent. Choice of pH can result in individual metals being selectively precipitated. A plant operated by Philips Semiconductors B.V., which has been running since 1997, recovers tin, copper, nickel, manganese, chromium, lead and iron. Sulphate removal by gypsum precipitation leaves sulphate concentrations in effluent water of 2-3 g/l. Biological treatment, reducing the sulphate to sulphide or sulphur, on the other hand, reduces the effluent concentration to 50-200 mg/l.

The use of sulphide is often rejected for reasons of safety and cost. However, Paques has shown that it is far cheaper and safer to produce sulphide on site rather than buy in NaSH or H_2S (the advantages are no transport or on-site storage). The biological process, using sulphur and an electron donor such as ethanol, yields a lower concentration of sulphide which, paradoxically, gives a metal sulphide precipitate that is more crystalline and can thus be dried to 70% rather than the 30% previously – a better product quality.

Budel Zink B.V., a Pasminco Ltd. owned company, has operated a zinc refinery at Budel-Dorplein in the Netherlands since 1973. Over 200 000 tons of zinc are produced annually. The conventional roast-leach-electrowin process produces various wastewater streams containing sulphate and zinc. Until mid-2000 these streams were treated conventionally by neutralisation with milk of lime resulting in the production of gypsum.

The Dutch government indicated that they would prohibit further storage of residues at the Budel Zink site as from 1 July 2000. For this reason, alternative wastewater treatment processes were studied over several years in order to arrive at a process in which storage of gypsum is avoided and in which an effluent can be produced that complies with Dutch legislation.

The envisaged difficulties with producing a constant high quality of clean gypsum for marketing purposes led to a decision not to pursue this alternative and a route where no gypsum is produced was chosen using Paques' high-rate biological sulphate reduction technology. Using this Thiopaq® technology, zinc and sulphate are converted into a zinc sulphide product, which can be recycled to the refinery.

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Process description

Within the Budel Zink process, various wastewater streams are produced, mainly containing sulphate and zinc. A distinction can be made between streams with low and high sulphate concentrations.

The streams with a relatively low sulphate level (< 1 g/l) can be treated in the existing biological wastewater plant, the SRB (sulphate-reducing bacteria) installation. This plant was also designed by Paques, commissioned in 1992 and was destined for purification of the groundwater underneath the Budel Zink terrain. In the SRB plant, sulphate is reduced to sulphide, thereby precipitating metal ions, which are present in the groundwater, as metal sulphides. These metal sulphides are recycled to the main zinc process and no gypsum is produced. The SRB plant was extended in 1999 in order to allow the treatment of both groundwater and low-sulphate wastewater streams.

Two streams are identified as high-sulphate wastewater:

- Wash tower acid (scrubber discharge from the roaster acid plant). Typically this is about 25 m^3/hour containing 10 g/l H_2SO_4, 0.5 g/l HF, 1 g/l HCl and 0.5 g/l Zn.
- Magnesium bleed. This bleed is necessary to prevent accumulation of magnesium in the electrolyte. Typically 0.5 m^3/hour of purified solution and/or spent electrolyte has to be bled from the circuit in order to control the magnesium concentration. The magnesium bleed contains up to 300 g/l of sulphate.

These two streams cannot be treated in the SRB, since the maximum sulphate-loading capacity would be exceeded and the fluoride would not satisfactorily be removed.

In the period 1995-99, various processes were studied in order to purify these streams in a way where they can be discharged directly or after a final treatment in the SRB. Extensive test work was carried out on laboratory and pilot-plant scale and, based on the results of this work, the choice was made for Paques' biological sulphate reducing technology.

The biological process route consists of the following steps:

- Neutralisation of wash tower acid.
- Fluoride removal by precipitation as CaF_2 in a Crystalactor®.
- Mixing with magnesium bleed.
- Biological conversion of $ZnSO_4$ to ZnS, using hydrogen as an electron donor.
- Precipitation and separation of the produced ZnS.
- De-watering of the produced ZnS.
- Treatment of the bioreactor effluent in the existing SRB installation where the excess sulphide is converted into elemental sulphur.

Fluoride removal

The fluoride content of the neutralised wash tower acid is precipitated as CaF_2 in a Crystalactor® according to the reaction:

$$CaCl_2 + 2NaF \rightarrow CaF_2 \text{ (pellets)} + 2NaCl$$

The Crystalactor® is a fluidised-bed reactor in which sand is used as seeding material for the CaF_2 crystallisation. The product consists of pellets (approximately 1 mm) with a sand core and a layer of crystallised CaF_2. The pellets are automatically discharged and fresh seed material is added on the basis of the pressure in the column. After atmospheric drying, almost water-free pellets are obtained.

In order to obtain pure CaF_2 pellets, it is planned to crush part of the sand-seeded product into smaller particles (approximately 0.2 mm) and use these as seeding material.

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Biological sulphate reduction

In the bioreactor, sulphate-reducing bacteria convert the aqueous $ZnSO_4$ into solid ZnS using hydrogen as electron donor. The hydrogen is produced on-site in a reformer unit in which natural gas is catalytically converted with steam into a mixture of H_2 and CO_2. The installed reformer unit produces approximately 500 m^3/hour H_2 and approximately 1 ton/hour of export steam using 200 m^3/hour of natural gas. The product gas mixture contains approximately 80-vol % H_2 and is fed directly into the bioreactor where the CO_2 functions as a carbon source for the bacteria. In order to enable excellent mixing characteristics without the disadvantages of high shear forces, a gas lift loop reactor is used for the bioreactor.

Operational experience

The design, engineering and construction of the full-scale plant was completed in July 1999, and mechanical and software tests were completed in August 1999. Since October 1999, the installation has been in operation successfully, treating the wash tower acid and the magnesium bleed of the electrolysis circuit.

During the first six months of operation, up to 30 m^3/hour of wash tower acid was treated together with up to 0.5 m^3/hour of magnesium bleed to produce up to 8.5 tons a day of ZnS. Sulphate is reduced to levels as low as 50 ppm and fluoride is reduced from an original level of 100-250 ppm to as low as 20-40 ppm (design level: 50 ppm).

Environmental impact

Treatment of the wash tower acid with the conventional neutralisation process led to the production of large volumes of gypsum and effluent characteristics that did not comply with legislation. With the successful implementation of the Thiopaq® technology, using a high-rate sulphate-reduction bioreactor, no gypsum is produced and water quality has been improved. In addition, calcium fluoride and valuable zinc sulphide are produced. Zinc sulphide is recycled to the roaster feed and gypsum production of 18 tons a day is eliminated.

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Case Study 18

COPPER BIOLEACHING TECHNOLOGY (BILLITON, SOUTH AFRICA)

Introduction

Copper smelters are inherently heavily polluting, with 50% of global smelter capacities operating with a sulphur dioxide recovery of less than 85%. While emissions from best-practice smelter operations, based on a smelter producing 200 000 tons per year of copper, are quoted as 1 200 tons per year of SO_2, in worst-practice operations, SO_2 emissions are from 148 000 to 364 000 tons per year. Hydrometallurgical processing, which involves transferring the metal constituents of an ore into solution, is generally considered to be more environmentally acceptable than smelting. This is particularly true for the treatment of ore resources containing problem elements such as arsenic.

I*n situ* leaching of copper has been recorded since the first century AD. On an industrial scale, heap leaching of copper was carried out in Spain in the 1750s. However, not until the 1940s and 1950s has the role played by micro-organisms been understood and only more recently still have the sulphur-oxidising archaea been discovered.

The low pH, metal-rich, inorganic mineral environment in which bioleaching reactions occur is populated by a group of bacteria which are highly adapted to growth under these conditions. Most of the commercial, mineral-processing bioreactors are operated with mesophilic bacteria at about 40 °C. Approaching this temperature, however, activity can be exceeded by that of moderate thermophiles such as T*hiobacillus caldus* and S*ulfobacillus* species, which grow optimally at about 45 °C. Between 50 °C and 55 °C, growth becomes progressively restricted whereas that of the S*ulfolobus*-like and M*etallosphaera*-like archaea increases, with some strains active to at least 85 °C. All these organisms are obligatory autotrophic organisms, which obtain carbon by the fixation of carbon dioxide and obtain their energy from the oxidation of either ferrous iron or reduced inorganic sulphur compounds. Ferrous iron and reduced sulphur compounds serve as the electron donor and oxygen serves as the preferred electron acceptor.

Bacteria currently used in commercial biomining or bioleaching operations are ubiquitous in nature. Archaea, although less widespread, are found in nature in hot sulphur springs and volcanic thermal vents. Both groups used in commercial operations are the same as those found in nature, except that in some cases they have been selected for rapid growth on the ore or concentrate concerned. Biomining micro-organisms are very sensitive to organic matter. They are unable to grow on plants, insects or animals, including humans and are thus non-pathogenic.

Technical features

Currently, there are some nine main hydrometallurgical technology alternatives, including the bioleaching process described here. Apart from high-pressure leaching and bioleaching, most processes produce elemental sulphur as a reaction product and require the presence of high chloride concentrations to treat chalcopyrite concentrates. (The low-pressure leach operations generally require ultrafine grinding to achieve satisfactory copper dissolution). General concerns relating to the hydrometallurgical process routes include high power costs, corrosion and maintenance.

Key factors motivating development of hydrometallurgical process routes include:

- Environmental: limiting sulphur dioxide emissions.

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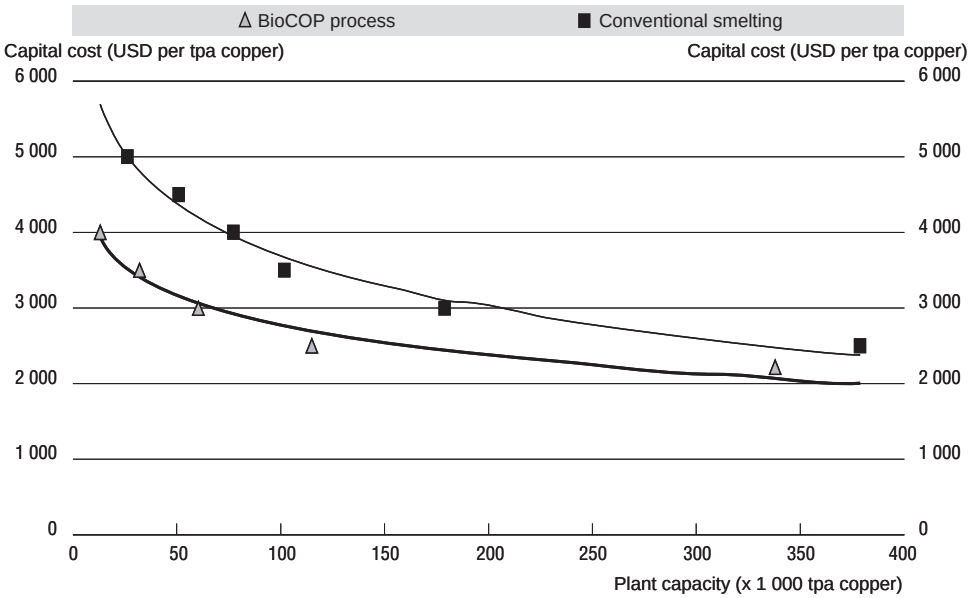
- Ability to treat low-grade ores and concentrates containing problem elements.
- High transport costs for shipment of concentrates to a remote smelter.
- Proven success of hydrometallurgy in treatment of gold, zinc and nickel/cobalt sulphide concentrates.
- Success of heap leach operations for copper recovery.

Based on comparative costs for achieving equally low environmental emissions, bioleaching is competitive with smelting for treatment of sulphide concentrates. For example, for the treatment of copper sulphide concentrates, bioleaching shows lower operating and capital costs for plants producing less than 150 000 tons per year of copper. Figures 4 and 5 compare capital and operating costs.

Figure 4. **Comparison of capital costs for smelting and bioleaching**



1. tpa: tons per year.
2. x 1 000 = kilo tons.

Copper production from other hydrometallurgical routes (including bioleaching) is expected to grow significantly in the future (Figure 6). Smelter technology has nevertheless improved significantly in the past 20 years, achieving significant reductions in SO_2 emissions and largely eliminating arsenic emissions (also due to restricted feedstock selection). Because 80% of the world's copper production is currently derived from concentrate smelting, smelters will remain the major producers of copper metal in the medium term.

Use of thermophiles represents a major breakthrough in the bioleaching of base metal sulphide concentrates, particularly because of their ability to leach chalcopyrite (copper iron sulphide, $CuFeS_2$). Other benefits are still under assessment, but will include a significant shift in economic performance compared to mesophiles. However, stirred-tank bioleaching still requires the fine grinding of ores to produce a flotation feed and concentrate. The bioleaching, grinding and flotation steps are all, relatively speaking, capital-intensive.

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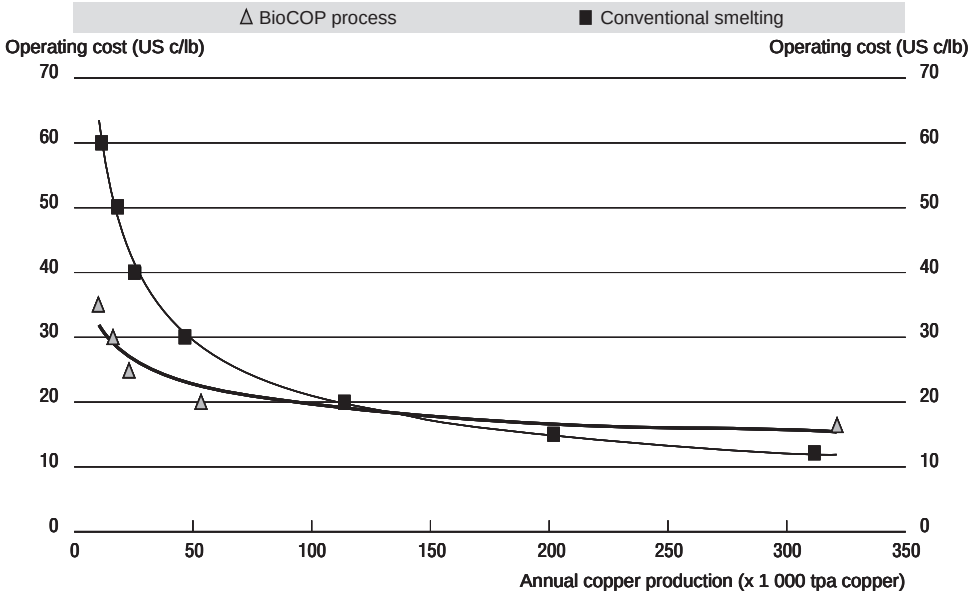
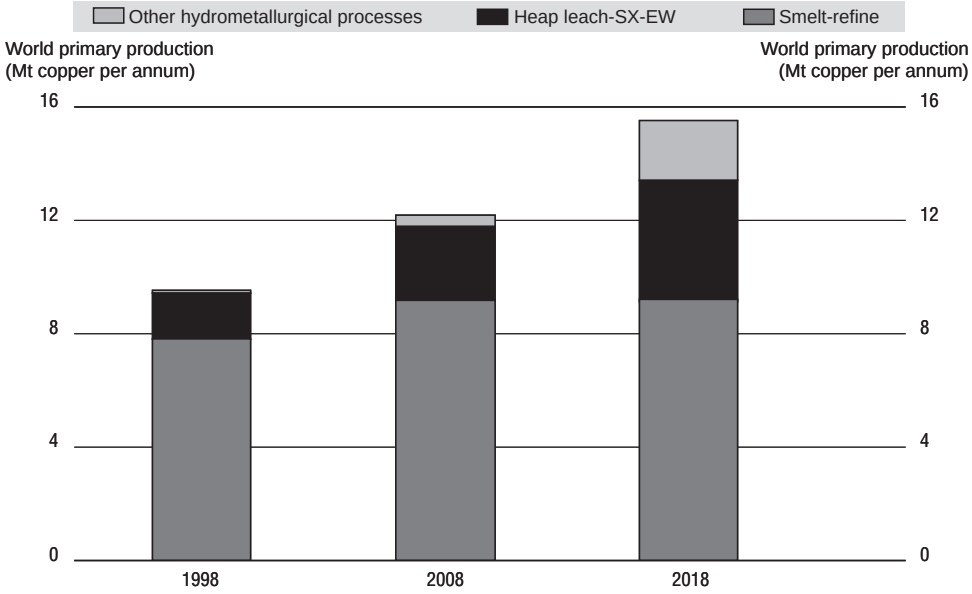
Figure 5. **Comparison of operating costs for smelting and bioleaching**



1. tpa: tons per year.
2. x 1 000 = kilo tons.

Figure 6. **Primary copper production by process route**



It has long been realised that most chalcopyrite concentrates are refractory to mesophilic organisms. Thermophiles, operating between 60 °C and 85 °C will solubilise chalcopyrite, but delicate cell walls make them sensitive to solids abrasion. At the end of 1997, an R&D programme was initiated to investigate the use of thermophiles and by mid-1999 good extraction was being achieved in acceptable residence times.

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A key success factor is the ability to control the rate of microbial growth in the stirred tank reactor. This is influenced by metal concentrations, nutrient addition, CO_2 levels and oxygen supply, pH and temperature. Iron species are required to provide a source of ferric ions but sufficient concentrations are usually available in the ore concentrates.

Description of the process of innovation

Historical overview

Stirred-tank bioleaching technology was developed by the South African company Gencor to replace the badly polluting roasters used to treat an arsenical refractory gold ore at their Fairview Mine. Biotechnology was chosen in favour of ultrafine milling (very power-intensive and lower gold recoveries) and pressure leaching (high capital cost and complex control and maintenance). In 1986, after ten years of in-house R&D, a plant was successfully installed at the Fairview Mine. The process, known as BIOX®, is now widely used and is capable of handling arsenic-containing concentrates and precipitating the arsenic in a stable, environmentally acceptable form. The process is not only cleaner but also gives superior gold recovery.

All of the Gencor non-precious metal assets were transferred to the newly listed company, Billiton Plc, in 1997, including the bioleaching technology. First applied to nickel extraction, the technology was subsequently tried with copper minerals and christened BioCOP™.

A pilot plant has been in operation using mesophilic organisms and more recently thermophilic micro-organisms, at a Codelco mine in Chile since 1997. A commercial prototype plant is planned for construction within the next two years to produce 20 000 tons a year of cathode copper. Finalisation of the process design package is currently in progress. In parallel with the development of this technology, the copper industry was steadily adopting the SX-EW (solvent extraction-electrowinning) hydrometallurgical process that allowed the cost-effective recovery of copper from leach solutions. This approach at the present time accounts for between 20% and 25% of world production.

Process selection

The issue of selection of the most appropriate technology for a particular project is obviously distinct from the issue of the development of a specific technology for commercial exploitation.

Billiton regards the first criterion for technology selection to be that the resulting environmental impact conforms to international best practice. From the resulting shortlist of options, the selection is based on such criteria as product quality, cost effectiveness, appropriate operability requirements, etc. Billiton is primarily a production company and does not compromise its production cost and product efficiency by inappropriate use of its own technology. The company internally ranks competitive technologies addressing any relative shortcomings in its own technology as well as identifying those projects in which it has a competitive advantage.

This type of qualitative ranking is illustrated in Figure 7. In this case the analysis was prepared by a third party/competitor. Billiton would place a higher rating on bioleaching in certain categories but would downgrade this technology in others. In general, this assessment is acceptable, but it is only meaningful when related to a specific project location and set of circumstances.

Leachable copper resources are dwindling and copper SX/EW capacity at typical heap leach operations will gradually become under-utilised with time. Given that primary and secondary sulphide resources frequently underlie copper oxide deposits, sulphide concentrates can be produced by milling and flotation and processed using BioCOP™, the resulting solution enabling full utilisation of the otherwise redundant SX/EW capacity. Since in a typical BioCOP™ plant the SX/EW component forms approximately two-thirds of the total capital cost, the savings in capital expenditure are considerable. Clearly this is a situation where a technology adds significantly to the value of an operation and the supplier of the technology can expect to share in that value.

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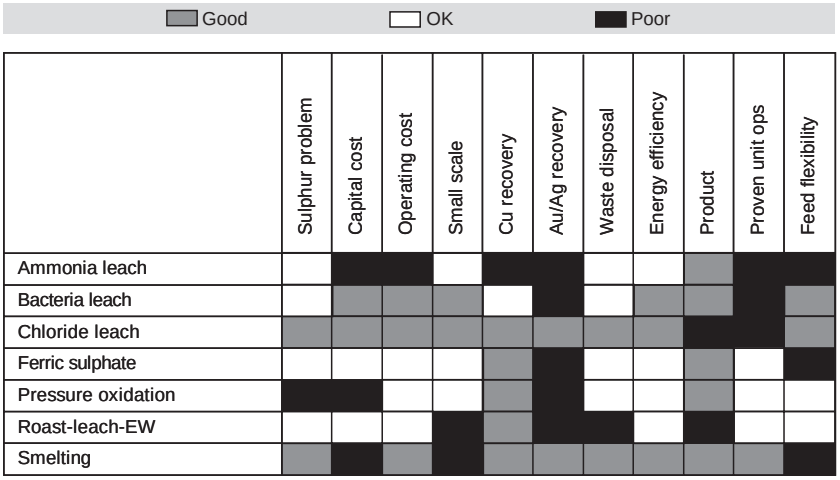
Figure 7. **Qualitative ranking**



Summary and conclusions

The polluting nature of smelting and the resulting high cost to construct and operate clean smelters is favouring hydrometallurgical process options for treatment of base metal sulphide concentrates, particularly for treatment of ores containing problem elements that are difficult to treat by smelting, such as arsenic or bismuth.

Bioleaching is competitive with smelting on a comparative cost basis for low-emission operations. The fact that the bulk of the world's copper resources occur as chalcopyrite prompted the development of bioleaching using thermophilic micro-organisms. The development programme was successful and spin-offs were that thermophiles were found to offer significant improvements for secondary copper, nickel and zinc applications as well. There are many advantages to using bioleaching:

- Naturally occurring components: micro-organisms, water, air.
- Simple modular expandability by adding reactors.
- Simple to operate and maintain.
- Low pressure and temperature process.
- Dust and SO_2 free.
- Ability to handle and dispose of arsenic impurities in a stable form.
- Ability to handle difficult concentrates (containing arsenic).
- In the case of copper, bioleaching is a compatible technology – it produces dilute copper sulphate solutions suitable for SX/EW plants.

Biologically assisted heap and dump leaching, including dump leaching of chalcopyritic mine wastes, has been used with varying degrees of success. However, heap leaching has never been applied to chalcopyrite ores as the main production recovery process. Whilst the technical challenges surrounding such a process are formidable, Billiton believes that its existing expertise in biotechnology can be applied to make large-scale recovery directly from primary copper ores a reality in the medium term. Given that primary, low-grade, copper ores form by far the largest component of global copper resources, the company believes that biologically assisted heap and dump leaching will play a major role in low-cost copper production in the future.

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Case Study 19

RENEWABLE FUELS – ETHANOL FROM BIOMASS (IOGEN, CANADA)

Introduction

Iogen Corporation of Ottawa, Ontario, is an industrial biotechnology company and is Canada's leading integrated manufacturer and marketer of industrial enzymes. The firm is also a leading developer of technology to make clean fuels from natural fibres. It employs about 100 staff, of which half are engaged in research and development, in its Ottawa facility.

Iogen's primary markets for enzymes are:

- P*ulp and paper*: to reduce chlorine use while achieving the same brightness by attacking and opening up pulp.
- L*ivestock feed*: improves nutrient value of feed for pigs and chickens – in a joint venture with Hoffman LaRoche.
- T*extiles*: pre-softening of denim.

Iogen has two parallel business tracks – enzymes and ethanol – and the company's strategy has been to use the speciality enzyme business to underpin the bioethanol development. Because of Iogen's history of success as a developer and manufacturer of industrial enzymes, making ethanol fuel out of agricultural residues is now becoming a reality and Iogen has become a leading company in the field of development and manufacturing of ethanol fuel from cellulose. Using cellulase enzymes, plant fibre is converted to sugars that are then fermented and distilled to make ethanol.

This fuel makes no contribution to greenhouse gas emissions, and since ethanol contains a high level of oxygen, it reduces smog and local air pollution. Ethanol-blended fuels are safe to use in today's vehicles and all major car manufacturers guarantee the use of 10% ethanol blends. At the same time, Ford, Daimler-Chrysler, and General Motors all sell cars, trucks and mini-vans that can burn fuel with any ethanol content up to 85% (known as flexible fuel vehicles).

History

Iogen's founder became interested in enzymatic treatment of cellulose following the suggestion put forward in the 1970s by the Club of Rome that the world could run short of food. Turning wood into animal feed could alleviate this crisis. Since the early 1970s, Iogen has focused its research on the development of enzymes for fibre modification and has accumulated an impressive record of product and technology firsts that include:

- Developing the steam explosion process to increase the surface area of the feedstock.
- Establishment of Canada's largest industrial enzyme manufacturing business.
- Implementation of the first mill-scale pulp bleaching enzymes in North America.
- First commercialisation of protein-engineered enzymes for pulp bleaching.

In the mid-1970s, with the world on the verge of an energy crisis, Iogen decided to adapt its technology to the production of clean-burning ethanol fuel from waste agricultural fibres. Through an intense research effort in enzyme technology during this time, Iogen proved that enzymes could be used to make ethanol from the sugars found in plant fibres.

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In the mid-1980s, when oil prices fell and interest in alternate fuel sources declined, Iogen pursued research and business opportunities in the speciality enzymes field. In the early 1990s, with proven enzyme technology and a number of successful enzyme products, Iogen became Canada's leading supplier of enzymes to the food, pulp and paper, textile and animal feed industries. Enzyme products are now sold throughout the world, enabling the company to establish a sound financial base and invest in the technology needed to develop an environmentally friendly fuel.

Work on ethanol from cellulose continued over the period and two CAD 7 million pilot plants were built in the 1980s and 1990s. In 1997, Iogen's track record attracted PetroCanada of Calgary, Alberta. Negotiations with PetroCanada led to a strategic partnership in the fall of 1997. PetroCanada's investment helped to build a CAD 30 million, ethanol-from-cellulose commercial validation facility in Ottawa, as the last step before commercial production of ethanol. This facility is in its final stages of commissioning, with production of ethanol expected in 2001.

Process

Iogen's cellulase enzymes are derived from *Trichoderma reesii*. These organisms are stored at –60 °C, grown up in flasks to seed fermentor scale (1 500 ml) and then to full fermentor scale (150 000-180 000 litres), a process which takes 12-14 days. After production, the killed organism is composted while the concentrated enzyme is used in liquid or solid (for textiles) form.

Wheat straw is chopped in a hammer mill. It is then exploded by high temperature cooking and enzyme is added. After enzyme treatment, solids are separated and the liquid stream is fermented with yeast. The solid (lignin) is burned for electricity generation.

Iogen is working with the Universities of Wisconsin and Toronto, which are in turn supported by the US Department of Energy, to develop new and improved yeasts for sugar fermentation. Iogen also has a collaborative R&D programme with the Canadian National Research Council, concentrating on the temperature and pH at which the cellulase enzyme is deactivated. So far they have found that by changing just three amino acids, the deactivation temperature can be increased by 10-12 °C and the pH range widened by 1-2 units. Another line of research is into the physiological control of the organism to give enhanced secretion of the enzyme.

Project

Iogen, together with Petro-Canada, is developing and demonstrating a cost-effective process for the production of ethanol from a wide variety of biomass, including farm residues such as straw and grasses. The Government of Canada announced in January 1999 a CAD 10 million Technology Partnerships Canada (TPC)/Climate Change Action Fund (CCAF) investment as part of the CAD 30 million demonstration project. This repayable federal government investment is supporting the construction and operating of Iogen's demonstration plant. In addition to the federal government's contribution, PetroCanada has invested CAD 15.3 million in this project and Iogen has contributed the remainder.

The demonstration plant has a throughput of 40 tons of raw material/day (the next largest in the world is 1 ton/day) and is situated adjacent to Iogen's current enzyme manufacturing facility in Ottawa. It is in an advanced stage of commissioning, and "shake-down" of the plant should now be complete. The plant is intended to validate manufacturing protocols at significant scale and achieve continuous operation to prove and further develop the technology.

Raw materials in order of preference are:

- *Agricultural residues*: cereal straws, corn stover and grasses, with a focus on wheat straw, which is widely available not only in Canada but throughout the world.
- *Dedicated energy crops*: In the United States (and Canada) the focus is on a native prairie grass called switch grass which has a high yield. In the United Kingdom, there is interest in Miscanthus (elephant grass, yield about 8 tons/acre) which takes three years to establish but requires no fertiliser thereafter.
- *Forest residues* (hardwoods, such as aspen).

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Straw has the advantage of being easy to collect (it is already baled and stored) and much easier to pre-treat at the plant. Farmers are thought to be unwilling to grow a new crop in the absence of confidence in the market.

Following success with the demonstration plant and a favourable business climate, subsequent plans are to commence construction of the world's first commercial ethanol-from-cellulose plant within two years. By February 2002, the beta version of the demonstration plant should be finished and will provide the design basis for commercial plants.

Economics

Iogen forecasts that ethanol costs will be lower than the product obtained from the technology currently employed in Canada, which utilises wheat or corn. This should lead to the widespread use of 10% ethanol, blended with petrol, as a motor vehicle fuel in Canada. Every litre of ethanol substituted for petrol will reduce CO_2 emissions by more than 90% compared to petrol. Light-duty vehicles could reasonably attain their proportionate share of national reduction if fuels including E10 (10% ethanol and 90% petrol) and E85 (85% ethanol and 15% petrol) were widely employed by 2010. Blending 10% ethanol into all of Canada's petrol by 2010 would result in a decrease of 10.2 megatons of CO_2 emissions per year, assuming the ethanol is derived from cellulose.

In Canada, Iogen expects to pay CAD 35/ton for their wheat straw raw material. This is acceptable to farmers in Western Canada, but in Ontario, for example, straw fetches CAD 80/ton for animal bedding. In the United Kingdom, there is a similarly wide range of prices. Another key element is the cost of the enzymes and the challenge is to drive down this cost.

Ethanol is tax-exempt in Canada. On the basis of alternatives for greenhouse gas exemption, Iogen believes it can put forward a good case for this being so wherever fuel ethanol is used. In Canada, tax is CAD 0.25/litre (equivalent to GBP 0.11); in the United Kingdom, tax is GBP 0.48/litre.

If it is the government's goal to reduce CO_2 emissions, then all alternatives should be treated equally from a taxation point of view. Thus, energy efficiency (high-efficiency lighting, use of aluminium in cars), energy conservation (public transport, walking instead of driving), clean vehicles (fuel cells, electric power) and cleaner fuels (ethanol) all reduce petrol in a similar fashion and should be tax-exempt in similar fashion.

Discussion

In a study undertaken by consultants for the Canadian national climate change process, the emissions reduction of a range of transportation alternatives relative to low-sulphur petrol were estimated (Table 28).

Table 28. **Emissions reduction and cost effectiveness**

	Reduction relative to petrol (%)	Cost effectiveness (CAD/ton of CO_2 reduced)[1]
E10 (corn)	4	151
E10 (cellulose)	6	85
Diesel	23	–34
Low-S gas hybrid	35	6
E85 (corn)	40	155
Low-S diesel hybrid	43	52
H_2 fuel cell (natural gas)	53	236
E85 (cellulose)	64	86
Electric car	70	161

1. Cost effectiveness takes into account capital and running costs.

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When emissions reduction and cost effectiveness are combined, E85 (cellulose) is the outright leader with diesel, petrol and diesel hybrids and electric vehicles close behind.

The success of Iogen's demonstration plant should lead to the widespread use of 10% ethanol, blended with petrol, as a motor vehicle fuel in Canada.

Iogen has not conducted it's own life cycle analyses on cellulose-based ethanol but has relied on work done by the US Department of Energy. These clearly demonstrate the CO_2 advantage of cellulose-derived ethanol over petrol and over grain-based ethanol.

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Case Study 20

THE APPLICATION OF LCA SOFTWARE TO BIOETHANOL FUEL (ICPET, CANADA)

The following is a very abbreviated version of a study prepared by Gloria Z. Fu of the National Research Council of Canada's Institute for Chemical Process and Environmental Technology in March 2000 (Draft Working Document of the National Research Council of Canada).

Introduction

The objective of this case study was to test the capability of the commercial computer software, SimaPro 4.0, and life cycle assessment (LCA) methodology in a study on bioethanol fuel derived from cellulose by enzymatic hydrolysis. To identify the influences of parameters on the LCA results, different scenarios are used, and ethanol-petrol blends are compared with each another and with traditional petrol in terms of different environmental impact categories.

The case aims to demonstrate the performance of SimaPro 4.0, its strengths and weaknesses and, in addition, the environmental performance of ethanol fuel compared with traditional petrol. The study also attempts to identify special demands on LCA methodology and LCA software when applied to biotechnology applications.

Although some studies have shown that the use of ethanol as fuel may be beneficial by reducing emissions, the question of whether or not ethanol should be produced for use as fuel is still quite controversial. Whether or not ethanol use as fuel is environmentally friendly may depend on the production process, the raw material, the distribution and/or use.

Studies carried out at institutions in the United States and at the University of São Paulo show that engines running on ethanol produce 20-30% less carbon monoxide, and insignificant emissions of sulphur dioxide. Ethanol-run cars generate roughly 15% less nitrogen oxide and minimal amounts of polluting carbon particulates.

A study, completed in late 1997 by Argonne National Laboratory, Illinois, stated that, on a per mile of travel basis, the replacement of normal petrol with E10 and E85 fuel meant reduction respectively of 2.8-2.9% and 35-36% of total greenhouse gas emissions (expressed as carbon dioxide equivalents). These are equivalent to 28-29% and 41-42% reductions in greenhouse gas emissions per unit of ethanol production. A study carried out by the US Department of Energy concluded that ethanol-run cars reduce greenhouse gas emissions by more than 90% compared to petrol.

Objective

SimaPro 4.0 is a software tool developed by PRé Consults B.V., the Netherlands, for the purpose of simplifying the work of LCA. SimaPro can create the life cycle of a product in a process tree structure using assemblies and life cycle boxes. Each process is represented by a data sheet, which will contain all received information with respect to inputs (raw material input, energy demand, outputs from other processes), and outputs (emissions and products), etc. With this information about each process and a process tree of the life cycle, SimaPro 4.0 is able to draw up an inventory of all the environmental inputs and outputs associated with the product.

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For impact assessment, SimaPro 4.0 mainly uses two types of technique, the SimaPro method, which includes SimaPro 3.0, and the Ecopoints method, which includes Ecopoints 1997. SimaPro 3.0 is the newest version of the SimaPro method and was developed in the Netherlands, while Ecopoints 1997, the newest version of the Ecopoints method, was developed in Switzerland. The SimaPro and Ecopoints methods are quite similar, both being developed on the theory of distance-to-target. However, the target in SimaPro 3.0 is derived from real environmental data for Europe, while the Ecopoints system uses policy levels instead of sustainability levels. Policy levels are usually a compromise between political and environmental considerations. The normalisation values in SimaPro are based on average European data in 1990, while Ecopoints uses target values rather than average European data.

The LCA is based on the production of ethanol fuel by enzymatic hydrolysis from wood or agricultural fibres. It is assumed that ethanol is used in E10 fuel in new passenger cars at the present situation. The fuel economy is estimated as 28 miles per gallon (mpg) for all new cars. The production, distribution and consumption of ethanol fuel are assumed to be in Ottawa. The required data were collected mainly from individuals, the literature, research reports and recent environmental reports of companies in related fields, which provide the data representing an average over a population of similar processes.

The pre-treatment of the raw material requires explosive decompression with steam. The steam-exploded substrate (wood, hay, straw, etc.) is mixed with an enzyme broth in hydrolysis tanks. The pH of the slurry is controlled at 4.8 with the addition of ammonia, while the temperature is controlled at 48 °C by using low-pressure steam. The hydrolysis process is maintained under anaerobic conditions by sparging with CO_2. The resulting hydrolysate is pumped to centrifuges, and separated into a solid slurry and a clarified hydrolysate. The slurry is then de-watered to about 5% solids, and the resulting lignin-rich filter cake is a by-product. The filtrate and centrifugate are combined, giving a hydrolysate containing about 9% total sugars (hexose and pentose) by weight, which is sent for fermentation.

The process analysed does not involve the fermentation of pentose sugars. Extensive research has been conducted to develop and improve micro-organisms capable of fermenting these and the implementation of this technology could improve ethanol yields by as much as one-third.

There are a number of by-products associated with the enzymatic hydrolysis process, including lignin, pentose sugars and animal feed products. Lignin can be sold as a precursor for lignin chemicals, in the area of phenolic substitution, or can be used to produce steam or electricity for plant use or can be sold directly as fuel. Pentose sugars can be concentrated to 48% syrup, to be sold as animal feed molasses or be used as substrate for yeast production. They can also be used to produce a methane-rich biogas through the anaerobic digestion of the sugars or be converted to furfural, a chemical intermediate.

Steam generation

Two scenarios are considered. One is to assume that steam is generated in the same way as US energy generation, which is 33% from coal, 26% from oil, 29% from gas, and 9% from nuclear power. The complete LCA data for this scenario are taken directly from the SimaPro 4.0 database. Another is to assume that lignin, a by-product of the ethanol production, and other bio-fuels, are used for steam generation.

Petrol manufacturing

Petrol manufacturing is assumed to be made by the average technology used in western Europe. The possible raw materials needed, emissions and energy demand associated with this technology are taken directly from SimaPro 4.0.

To make the inventory data more environmentally relevant and easier to compare, the software groups all the data into different impact categories, such as e nergy demand, greenhouse effect,

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acidification, eutrophication, winter smog, summer smog, ozone depleting substances, heavy metals, carcinogenic substances and solid wastes.

Results and conclusions

Figures 8 and 9 show a comparison of energy demand and the greenhouse gas emissions for various fuels.

Figure 8. **Comparison of the total energy demand for the production of traditional petrol and E10 fuel in different scenarios**

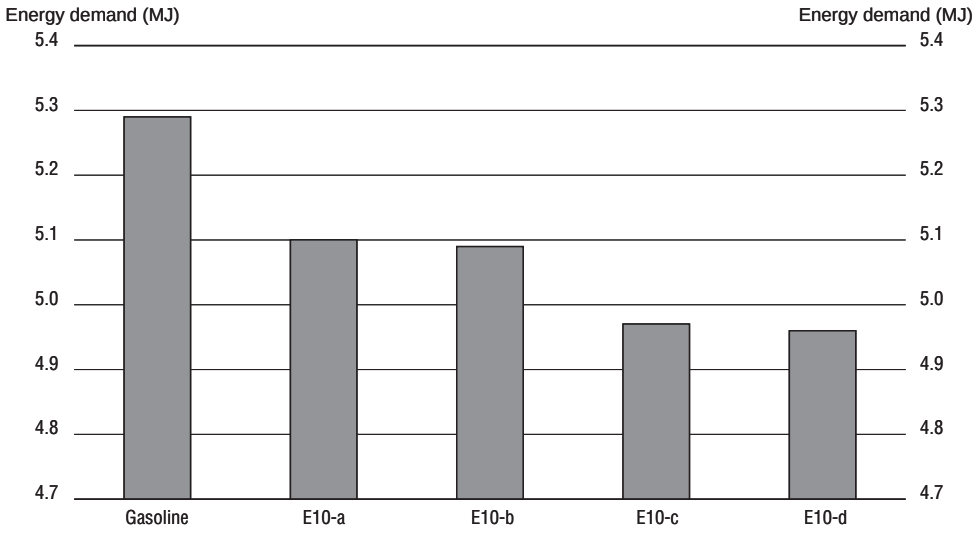
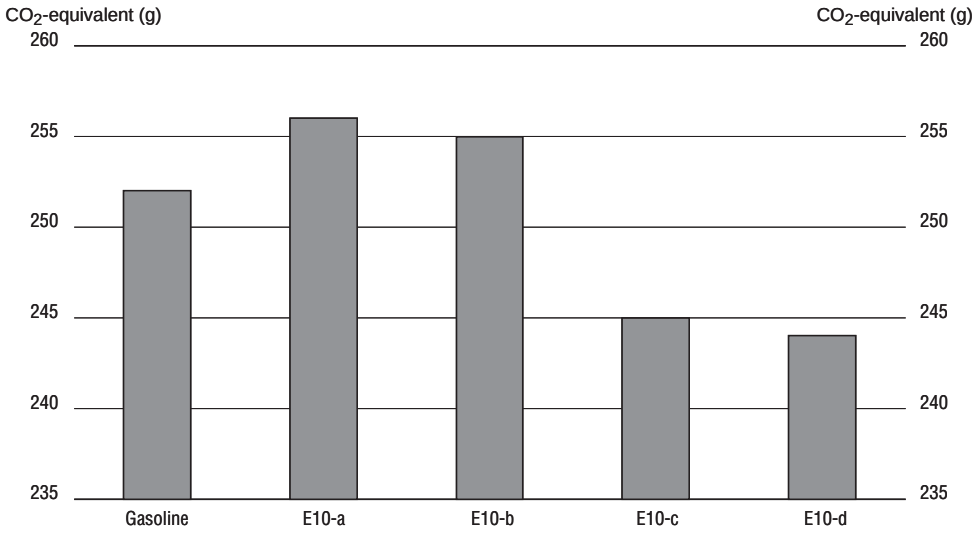


Figure 9. **Comparison of greenhouse gas emissions from the whole life cycle of traditional petrol and E10 fuel in different scenarios**



Note: CO_2 emissions of biomass origin are treated as zero.

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The four E10 alternatives are:

– E10-a: feedstock cultivation is included. Steam is generated: 33% by coal, 26% oil, 29% gas and 9% nuclear power.

– E10-b: feedstock cultivation is excluded. Steam is generated as in E10-a.

– E10-c: feedstock cultivation is included. Steam is generated by lignin and other bio-fuels.

– E10-d: feedstock cultivation is excluded. Steam is generated as in E10-c.

The analysis demonstrates that:

- Ethanol fuel may help to reduce greenhouse gas emissions if steam is generated by burning lignin or bio-fuel rather than fossil fuel for pre-treatment of the feedstock.
- The most effective way of improving the environmental performance of E10 may be to improve the environmental performance of petrol manufacturing and the combustion efficiency of car engines, rather than to improve ethanol manufacturing.
- Replacing traditional petrol by E10 fuel may save energy, emit less summer smog and ozone-depleting substances and discharge less heavy metals. It can, however, cause more eutrophication, acidification and winter smog, and generate more solid wastes.
- For bioethanol production from waste fibres, the steps requiring improvement are in enzyme manufacturing, energy use in breaking down feedstock and transportation.
- Feedstock cultivation impacts almost all environmental categories, but mainly acidification, eutrophication, heavy metals and carcinogenic substances. It can also be expected to give rise to biodiversity, landscape and land-use problems.

Interpretation of results

For products made by biotechnology, a complete LCA perhaps should include production inputs to agriculture (seeds, fertilisers, pesticides, etc.), cultivation, manufacturing, storage and distribution, packaging, and waste management. For performing an LCA on such a complicated system, there are many variables which can influence the results, such as the type of production technology, raw materials used in the manufacturing process and the geographic location.

All LCA data for petrol manufacturing were taken directly from SimaPro 4.0. They are the average production data in the Netherlands. Although most of the data come from major companies and can be considered to be representative of the general technology, no data, however, are directly from ethanol production sites. The results should not be considered as referring to any specific company, but rather can serve as a general reference for decision makers.

It also must be kept in mind that the calculations for the whole life cycle of E10 are done on the basis of the assumptions and the different data sources described. The comparisons between traditional gasoline and E10 are based on the assumption that gasoline manufacturing and E10 manufacturing are investigated and compared at the same level of detail.

Evaluation

It should be noted that no generally accepted evaluation method exists. While highly aggregated information is clear, it is also less precise. Less aggregated information, on the other hand, is less uncertain and probably more credible.

This LCA shows that SimaPro 4.0 is a good tool to perform such a study. It simplifies the work of analysis, it follows correct LCA procedures and correctly interprets the cause and effect chain of the pollutant. It is a useful tool for mapping out the overall environmental impacts of the ethanol production from the cradle to the grave. It can help to reveal the steps in the production process which effect environmental improvements and create a complete picture of what replacing one product by another really means to the environment. SimaPro 4.0 was, however, developed in Europe. The

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database includes mainly European data and reflects European energy production, technology and environmental situation. Other data may be more appropriate to other locations.

There are a number of programmes for LCA analysis available, each with its own strengths and weaknesses. Other software may have more appropriate databases.

To investigate how the LCA method can be applied to biotechnology, more case studies need to be done to investigate which methodological issues are important. When evaluating environmental impacts, the dilemma of how to assess the effects on biodiversity, landscape and land use are often encountered. These are, in practice, very difficult to quantify.

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Case Study 21

USE OF ENZYMES IN OIL-WELL COMPLETION (M-I, BP EXPLORATION, UNITED KINGDOM)

Introduction

Drilling fluids ("muds") are essential components in oil-well drilling both to lubricate the drill string and to hold open the wellbore. Traditional drilling fluids were muds – dispersions of clay minerals in water or oil where the clays provide the required viscosity as well as controlling fluid loss to the formation. Oil-based muds have better lubricating properties and operate better over longer drilling distances, but on the other hand cost more and have environmental disadvantages (especially with regard to the disposal of cuttings).

Drilling fluids are designed to deposit a low permeability filter cake on the borehole wall by depositing a layer of solids on the surface of the formation. The primary purpose of this "cake" is to limit the leakage of drilling fluids into the formation and prevent solids invasion into production zones. However, after the well is drilled to the desired depth and before production can start this barrier must be partly, or preferably completely, removed in order to maximise production rates. Completions which do not use a cemented and perforated liner can depend critically on effective removal of this filter cake.

Drill-in fluid systems have developed as one of the key components for successful horizontal well drilling and completion. These fluids are formulated to provide the same functionality as drilling muds (*i.e.* rheology, hole cleaning, etc.) while minimising the clean up problems associated with conventional drilling muds. Today, clay-free water-based drilling fluids are preferred. These fluids obtain the necessary drilling properties (viscosity/fluid loss control) from organic polymers. One polymer (usually xanthan gum) provides the viscosity and another (starch or cellulose) acts as a binder. The other fundamental components are a solid of controlled particle size to bridge the rock pores (bridging agent) and a brine to give the required density without adding high volumes of solids. One advantage of water-based over oil-based muds is that they are safer to dispose of into the environment.

There is little benefit in engineering a non-damaging reservoir-drilling fluid if the chemicals used in the pre-production cleanup will damage the lifetime productivity of the well. Although drill-in fluids are designed to be inherently less damaging than the conventional drilling muds, it is recognised that the mechanisms of formation damage associated with conventional muds (solids invasion, chemical incompatibilities between filtrate and reservoir fluid, incomplete filter cake removal, etc.) can also occur to some extent using drill-in fluids. In particular, partial removal of filter cake can significantly impede flow capacity and cause considerable reduction in well productivity, or lead to increased flow though smaller areas and subsequent early failure of sand control screens.

Conventional process

To realise the full potential of open-hole horizontal completions, formation damage from residual filter cake must be eliminated. A common approach to minimising such damage is the application of so-called "breaker" systems which dissolve filter cake solids and chemically destroy ("break") the polymers in the fluid. Conventional oilfield breakers are acids or strong oxidising agents, such as hydrochloric acid and sodium perborate solutions. Field experience show that these work relatively

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well over short distances but are less easy to apply effectively over a greater length of wellbore, possibly as a consequence of their reactive nature.

Acids and oxidising agents are non-specific, *i.e.* they will react with anything that is acid-soluble or can be oxidised, including metal components, such as tubulars, screens and formation components. As a result, downhole tools may be corroded heavily and the breaker will be at least partially used up before reaching the filter cake. Their high reactivity may also result in "wormholes" through the filter cake as breakthrough occurs, opening a path of least resistance into the formation.

An added effect of acid on tubulars is that the corrosion reaction increases the level of iron ions in solution, which can promote crude oil sludging. Breaker solutions lost to the formation can affect the mineralogy and serve as a continuous bleed of corrosive compounds back into the production equipment.

In low-temperature reservoirs, acids and oxidising agents often fail to remove the filter cake completely, leaving residues that can plug screens and dramatically reduce production. Uneven breakdown of the filter cake may lead to only a partial opening of the reservoir leaving production (and profits) behind. Additionally, there is a risk of downhole corrosion and formation damage.

Biotechnological process

Novel alternatives to these breakers are enzymatic breakers which hydrolyse the polymers, breaking down the filter cake into easily dispersed fragments. Furthermore, these enzymes do not react with substances other than their specific polymer and, being catalysts, are not used up in the process. The slow, specific reaction of an enzymatic breaker thus provides a material that can be pumped evenly across the length of the production zone.

Crude blends of enzymes, typically hemicellulase, cellulase, amylase and pectinase in non-specific ratios were initially used to degrade polymer. However, once bound to the filter cake, non-specific enzymes cannot release or react and thus can block linkage sites from contact with the correct enzyme.

BP led a multi-company study, including Shell, Amoco, Chevron, Statoil and Hydro, of new horizontal well completion technology in 1994 and this was possibly the first time the use of enzymes in mudcake clean-up was seriously considered. At this time environmental aspects were not a principal factor. Although work was undertaken by the service companies, little emerged until 1997 by which time the environmentally friendly nature of this technology was important.

Advances in biotechnology led to the isolation and production of polymer-specific enzymes (PSEs) in 1993, optimised to improve hydrolysis of appropriate polymeric linkages in the targeted polymer (starch, cellulose or xanthan). One such PSE is "Wellzyme A", a starch-specific enzyme designed specifically for open-hole-completed reservoirs by M-I, a Smith/Schlumberger company. A number of "service" companies, including M-I, BJ Services and Cleansorb, now offer enzyme-based technologies. Some muds contain calcium carbonate, which may also have to be removed at clean-up. One supplier has developed a two-enzyme process in which one enzyme removes the organic polymer and the other, an esterase, when fed a suitable substrate, generates mildly acidic conditions which dissolves the carbonate.

BP, along with several other operators, is funding research within the service companies and their enzyme suppliers. The operator challenges them to find a solution to a defined problem. The service companies are researching not only the use of enzymes but also novel compositions for drilling fluids such as the incorporation of ceramic beads.

Suppliers are asked to define the operating envelopes of their products and the operator tests of the enzyme, looking at its compatibility, in the laboratory. BP has reasonable confidence that its laboratory rig models conditions in the field.

It is usually difficult to have a control experiment in the field, although similar wells, with and without specific treatments, have been compared. Controls are therefore all tested in the laboratory. It sometimes occurs, as when clean-up fluid leaks away into an unexpected fault, that the consequences of not using clean-up are tested in the field.

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A "joint venture" research programme, the EU-funded "Well Productivity 2002" project, involving both operators and service companies, is currently looking at the minimisation of formation damage.

Advantages and disadvantages

Compared with conventional mineral or organic acids or peroxide breakers, enzyme breakers have a number of advantages:

- They are polymer-specific with no undesirable side reactions.
- They are catalysts and are not consumed, while the reaction of acids and oxidising agents is stoichiometric.
- The large molecular size means that enzymes do not bypass partially broken-down polymer and thus promote more even degradation of the filter cake and hence even inflow.
- Less risk of formation damage as a result of sudden fines ingress, often associated with very rapid filter cake breakthrough.
- More environmentally friendly.
- Lower HSE (health, safety and environment) risk for the rig crew/completions team (in solution, the enzymes are harmless to handle).

More recent tests have indicated that enzymatic hydrolysis of the starch or cellulose alone, *i.e.* degrading the binding polymer without breakdown of the xanthan, will result in removal of the filter cake.

Use of enzymes is, however, limited with respect to temperature and pH. Although thermophilic enzymes do exist, few can withstand temperatures above 100 °C for long before becoming "denatured". In practical applications, perceived limitations on the use of enzymes include high density, high salinity conditions and also temperatures above 100 °C. In certain wells the temperature reaches 150-175 °C. The drive is to find novel enzymes that will function in these harsh conditions.

The primary disadvantages of the non-enzymatic treatments can be summarised thus:

- Acids are corrosive towards completion tools and can cause tool failures.
- Acids are highly reactive towards drilling mud filter cake and the calcium carbonate "cement" in the reservoir. As such, once the filter cake is broken through, acid tends to leak off into the formation, rather than attacking the filter cake evenly. This causes uneven breakdown of filter cake.
- Acids are consumed by the reaction with calcium carbonate/starch/steel and so if insufficient acid is exposed to the filter cake, incomplete breakdown will occur. As enzymes are catalysts, and therefore not used up in the reaction, they will continue to react with the specific chemical substrate until no more exists, or the enzyme becomes denatured.
- Once fully reacted, spent acids may precipitate solids deep in the formation, impairing production rates.

The main reasons why enzymes are not more widely used, in situations where the physical conditions would permit, are:

- Extra materials cost as compared to acids.
- The well stimulation effect that acids can bring to the operation.
- The "comfort factor" from using tried and tested procedures.

If an enzyme fulfils its potential of rapid, complete polymer destruction downhole, the acid flush used in many field treatments should not be necessary. To many operators, saving acid use means higher efficiency combined with large cost saving. However, the use of enzymes is a relatively new technology to this industry, and as such it is taking time for people to appreciate the advantages.

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Practical performance

Laboratory tests performed by a number of oil companies have confirmed the advantages of the enzyme breaker and very recent reports have been made of the use of enzymes in commercial drilling in the Gulf of Mexico, the North Sea, the Caspian and Alaska. In the Gulf of Mexico example, pre-testing showed that over 95% of filter cake could be degraded consistently. In this application, the enzyme breaker resulted in a three to fourfold increase in fluid production over normal vertical completions. The use of a customised drill-in fluid and the enzyme breaker system yielded savings in one case of USD 75 000 and in a second case of USD 83 000. In BP's Harding field, for example, enzymes were not effective because of the need for high salinity drilling fluids. However, in Baku (offshore Caspian), the use of enzymes appears to give improved performance.

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Annex

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