# BIOCATALYSIS: INDUSTRIAL ENZYMES AND THE EXPLOITATION OF MICRO-ORGANISMS

Biocatalysis can be broadly defined as the use of biological molecules (usually enzymes) to catalyse specific chemical reactions. Enzymes are complex protein molecules. They are produced by living organisms to catalyse the biochemical reactions required for life. Although enzymes are formed within living cells, they can continue to function *in vitro* (in the test-tube) and their ability to perform very specific chemical transformations is making them increasingly useful in industrial processes.

Biocatalysis is a major part of biotechnology. Biotechnology is defined by the European Federation of Biotechnology as "the integration of natural sciences and organisms, cells, parts thereof, and molecular analogues for products and services" which can be translated from legalese to "a technology which employs practical applications of living organisms or the components of living organisms".

Biotechnology is not a single field and there is danger viewing it as a unified body of scientific knowledge. It is multi-disciplinary and has its roots in many areas - a smorgasbord of subjects including: engineering, chemistry, plant and animal biology, microbiology, immunology and many more (see **Figure 1**). Industrial Biotechnology encompasses to varying degrees the life sciences, chemistry and engineering and it is now being applied so widely that it has even made skirmishes into such unlikely territories as the manufacture of silicon chips and gold mining.

Biotechnogy is almost as old as history, people having used it to make sourdough, alcoholic beverages, vinegar, yoghurt and cheese as far back as 700 BC. It came of age in the 19th century when Louis Pasteur discovered that it was yeast which converted sugars to ethanol and that bacteria caused spoilage by converting ethanol to acetic acid. War brought further advances - the conversion of sugar into glycerol for making nitroglycerine in Germany in World War 1, and turning it into acetone and butanol in Britain in World War 2.

At present, only about 20 enzymes are produced on a truly industrial scale. These enzymes are made by 35 producers with 4 major companies holding 75% of the market. In 1992, the market value of these enzymes was approximately US$800 million with a predicted yearly increase of around 10-15%. The market is highly competitive, suffers from over-capacity, has small profit margins and is technologically intensive. Nonetheless, there is room for growth as long as new markets can be found. A breakdown of enzyme production and their uses is given in **Table 1.**

## INTRODUCTION

Biocatalysis can be broadly defined as the use of biological molecules (usually enzymes) to catalyse specific chemical reactions. Enzymes are complex protein molecules. They are produced by living organisms to catalyse the biochemical reactions required for life. Although enzymes are formed within living cells, they can continue to function *in vitro* (in the test-tube) and their ability to perform very specific chemical transformations is making them increasingly useful in industrial processes.

## Table 1. The industrial application of enzymes.

| | | |
|---|---|---|
| Laundry Detergents | Proteinase (91%). | Used in pre-soaks to remove protein -based stains. |
| | Lipase (6%). | Now commonly included to digest oils and fats. |
| | Amylase (2%). | Removes resistant starch residues. |
| | Cellulase (1%). | Digests the cotton 'fuzz' which accumulates with excessive washing. |
| Starch Industry | Amylases, amyloglucosidases and glucoamylases | Converts starch to glucose and other sugar syrups. |
| | Glucose Isomerase, | Converts glucose syrups into fructose syrups. |
| Dairy Industry | Rennin from the stomachs of young ruminant animals. | Manufacture of cheese. |
| | Lipases. | Enhances ripening of blue-mold cheeses. |
| | Lactases, | Break down lactose to glucose and galactose. |
| Textiles Industry | Amylase, | Now widely used to remove starch from woven fabrics. Starch is used as an adhesive (or 'size') on the threads of many fabrics to prevent damage during weaving. Traditionally, chemicals were favoured but now bacterial amylases are commonly used. |
| Brewing Industry | Amylases, glucanases, proteinases. | Splits polysaccharides and proteins in the malt. |
| | Proteinases. | Reduces clouding of beers |
| | Amyloglucosidase, | Low Calorie beer production. |
| | ß-glucanase, | Improves filtration characteristics. |
| Baking Industry | α-amylase, | Catalyses the breakdown of starch in flours. Used in the manufacture of bread. |
| | ß-xylanase, | Improves the characteristics and rising of bread. |
| | Proteinases, | Reduces the protein in flour. Used in biscuit manufacture. |
| Leather Industry | Proteinase (trypsin). | The process known as 'bating' treats the leather with proteinases to make it more pliable. Trypsin isolated from both slaughterhouses and micro-organisms replaces the old method of using dog and pigeon faeces. |
| Pulp and Paper Industry | ß-xylanases | Emerging technology for enhancing pulp-bleaching. |
| | Lipases | Reduces 'pitch' which causes paper to stick to rollers and tear. |

Most of us use biocatalysis around the home - often without realising. If you use 'biological' detergents or contact lens cleaners, or even cook ham with pineapple you are using biocatalysis. In each one of these actions you are applying a proteinase enzyme to hydrolyse a protein substrate. If you make home-made bread, you are also using biocatalysis. Many commercial preparations of yeast include a purified xylanase, an enzyme that partially breaks down the hemicellulose polymer xylan that can make bread heavy, and the yeast itself, *Saccharomyces cerevisiae*, uses its own α-and ß-amylases to hydrolyse the starch in the flour. Next the yeast converts the sugars to ethanol and $CO_2$ and the $CO_2$ that makes the bread rise. Biocatalysis is also found in industry most commonly in food production and processing. Familiar examples are cheese, yoghurt, beer and wine production, but also many food additives and sugars are produced by enzymes.

In the past, many sectors of the chemical industry were more restrained in embracing this technology, largely because enzymes were perceived as being too delicate to survive the extreme conditions in the reaction vessels. Many enzymes have very specific requirements of pH and temperature before they will function and more often than not, these requirements are quite different from those for which an industrial plant was designed. But attitudes are changing. A new awareness of the diversity of microbial life has pushed microbiology from a rather academic subject to the fore-front of biotechnology. We now know that some micro-organisms can produce enzymes which can survive and function in extreme conditions. What's more, we are not short of new candidates. It is estimated that less than 1% of all known micro-organisms have so far been identified: there are probably in excess of 50 million bacterial species still undiscovered*! We are no longer forced to change industry to accommodate an enzyme; research and development now focuses on finding enzymes which will function in existing industrial processes. Find a natural environment which resembles the conditions in your reaction vessel, and with luck, it will contain many organisms that produce enzymes which fit your needs.

In this article we will focus on biocatalysis and look at a single case study; the application of enzymes in the pulp and paper industry. But first, we will take a broader view of biotechnology, its history and its structure.

## BIOTECHNOLOGY

### What is biotechnology?

Biotechnology is defined by the European Federation of Biotechnology as "the integration of natural sciences and organisms, cells, parts thereof, and molecular analogues for products and services" which can be translated from legalese to "a technology which employs practical applications of living organism or the components of living organisms".

Biotechnology is not is single field and there is danger viewing it as a unified body of scientific knowledge. It is multi-disciplinary and has its roots in many areas - a smorgasbord

*Traditional terms in taxonomic classification such as 'species' or 'genus' are actually rather difficult to apply to bacteria. The clear boundaries which define species in higher organisms are not so distinct with microbial life and in many cases one 'species' merges into another through a spectrum of ever changing intermediates.

of subjects including: engineering, chemistry, plant and animal biology, microbiology, immunology and many more (see figure 1). Industrial Biotechnology encompasses to varying degrees the life sciences, chemistry and engineering and it is now being applied so widely that it has even made skirmishes into such unlikely territories as the manufacture of silicon chips and gold mining.

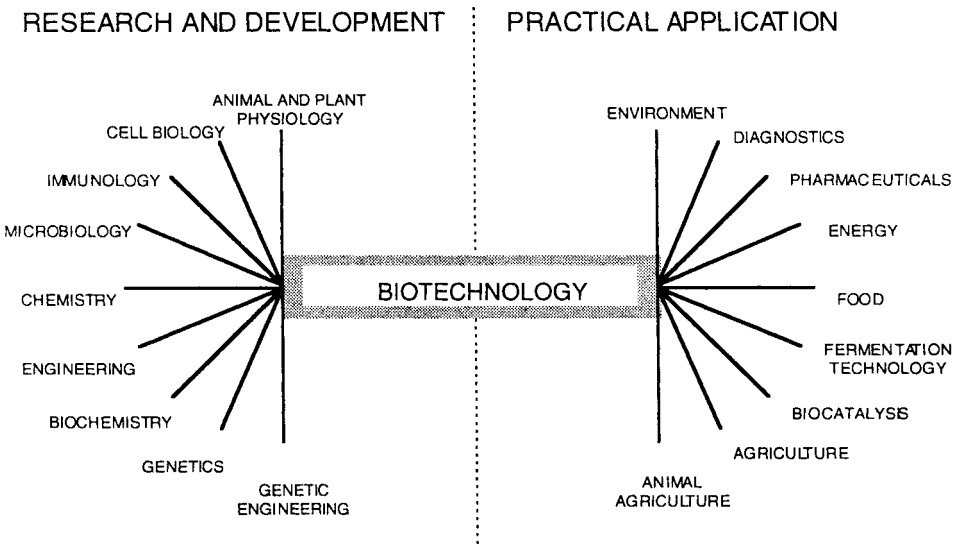


**Figure 1. Biotechnology is not a single subject but encompasses a wide range of scientific disciplines.**

## A Brief History

The word 'biotechnology' may be a relatively new addition to the English language, but the application has been around for many years. Micro-organisms have been used for millennia in the production of beer, wine, vinegar, yoghurt and cheese. The Egyptians, Sumerians and Babylonians produced alcoholic beverages from barley, and the Greek epic poems *The Iliad* and *The Odyssey*, written around 700 BC, both refer the uses of calf's and kid's stomachs (sources of rennet) for the production of cheese. Sour dough bread appeared in Europe around 800 BC and early Christian and Sanskrit writings describe fermented dairy products. The first purified chemical to be produced by biotechnology was ethanol, which by the 14th Century, was being produced to fortify wines and beers.

Throughout the centuries, nobody understood the underlying chemistry or even that living organisms were involved. Biotechnology came of age in the 19th Century. French wine merchants were searching for a way to prevent spoilage when their beers and wines were shipped over long distances. They enlisted the help of Louis Pasteur who discovered that it was yeast converting the sugars to ethanol and that spoilage was caused by bacteria which converted the ethanol to acetic acid. Pasteur developed the method of 'Pasteurisation' which kills the bacteria without spoiling the flavour. Not everybody was in agreement with Pasteur. He had a vocal adversary in the chemist Justus Von Liebig who maintained it was nothing to do with living organisms but fermentation was a simple chemical reaction. Both Von Liebig and Pasteur died before the dispute was finally settled and in a way, they were both partly correct. In 1897, the Buchner brothers proved that yeast extract could convert glucose to ethanol and $CO_2$ without the need for any living organisms. Such reactions were called 'unorganised ferments' and William Kühne named the agents responsible for catalysing the reactions 'enzymes'. This wasn't a great leap in understanding, the word 'enzyme' is simply

Greek for ‘in yeast’.
Meanwhile, a Japanese scientist named Takamine was developing a fermentation process for industrial production of enzymes from the fungus *Aspergillus oryzae*. The product, called ‘Takadiastase’ was a mixture of amylases and other glycolytic enzymes and although rather crude, Takadiastase production can claim to be the true beginnings of enzyme technology. (Takadiastase can still be purchased in many Eastern countries where it is considered an aid to digestion). Shortly afterwards in Europe, the textile industry moved from chemicals to enzymes for ‘desizing’. Size is starch which is added to strengthen the warp during weaving, but once the fabric has been woven, the size must be removed. The first attempts used malt extract or pancreas extract, but by 1917, the industry was converted to bacterial amylases from *Bacillus subtilis.*

A rather less attractive traditional biotechnological process was ‘Bating’ carried out by the leather tanning industry. Bating is the method by which leather is rendered softer and more pliable by the action of proteinases. The traditional source of these enzymes was dog and pigeon faeces. To compound the unpleasantness, the breakdown of sulphur-containing compounds in hair produced $H_2S$ gas. Your neighbourhood took a sharp ‘nose’-dive if a tannery moved in next door! The image of the tanning industry remained at rock-bottom until the German industrial magnate Otto Röhm became convinced that the excrement contained secretions from the digestive tract. He produced an extract of pancreas which seemed to work well and he took this as confirmation of his theory. In fact, later experiments showed that it wasn’t the animal enzymes at all but enzymes produced by bacteria living in the gut. Röhm also brought us the first ‘biological’ detergent which despite his 1913 patent, didn’t score a hit for another 50 years. The early preparations, marketed under the brand name of *Burnus* had limited success. The enzyme ‘pancreatin’ (Trypsin) was not particularly active in the alkaline conditions used in detergent washes. Modern detergents use bacterial alkaline serine proteinases which are far more suited to high pHs.

As is often the case, it was war which provided the biggest impetus for the development of biotechnology. During World War 1, a British naval blockade prevented Germany from importing vegetable oils - the raw material for glycerol and hence nitroglycerine. In response German biochemists developed a method using yeast grown in the presence of sodium bisulphite. Bisulphite blocks key enzymes in the biochemical pathway for the conversion of sugars to alcohol and so the yeast is forced to use an alternative pathway which has glycerol as its end product (see **Figure 2**).

Meanwhile on the other side, Britain was having difficulties in obtaining butanol and acetone which were required for the production of explosives and rubber. Scientists turned to a bacterium called *Clostridium acetobutylicum* which produces these two chemicals as end-products of its rather unusual biochemical utilisation of sugar. Unfortunately, *C. acetobutylicum* also produces $H_2$ gas and the combination of the two volatile solvents and hydrogen resulted in a number of plants being destroyed by explosions. But despite its hazards, the process proved so successful that it was used until the 1950s in Europe and the last plants were only recently closed in South Africa which had limited access to raw materials during the apartheid trade embargo.

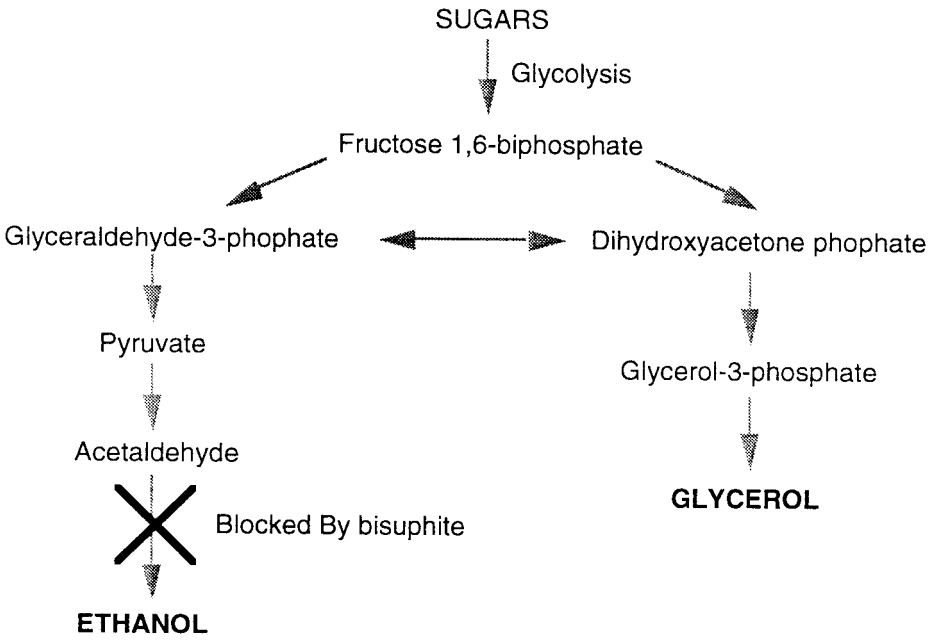


**Figure 2. The production of glycerol from yeast - an early example of industrial scale biotechnology.**

From the 1940s to the 1960s, the most dramatic developments came from the pharmaceutical industry. New antibiotics were discovered and organisms were genetically altered to enhance production. The first (and still the most successful) of these antibiotics was penicillin but soon afterwards, pharmaceutical companies realised the utility (and profitability) of antibiotics and so an intense research program was implemented. Streptomycin was isolated from *Streptomyces griseus* and many hundreds of new antibiotics from filamentous bacteria of the genus *Actinomyces.* The micro-organisms needed to be grown in large quantities without contamination. New processes were developed such as the use of steam for sterilisation, stirred tank bioreactors, aeration with sterile air and pressurisation of bioreactors which prevents the in-flow of contaminated air. In parallel, microbiologists were improving the production organisms. The first strain of *Penicillium* produced only 2 mg of penicillin per litre of culture. Today's *Penicillium* strains produce in excess of 20g per litre - a 20,000-fold improvement! By the 1960s the technology had developed sufficiently that plant and animal cells could be cultured and now many viral vaccines are produced from animal tissue cultures.

**Liquid fuels**

An expanding biotechnology is the production of ethanol as a liquid fuel. Ethanol has been known as a fuel for many years. In fact the Model T Ford was originally designed for ethanol and at around that time, ethanol was considered to be the obvious fuel for the future. It is plentiful and cheap and whereas most fuels (petrol, diesel, natural gas etc.) are produced from fossil hydrocarbons, ethanol is produced from renewable biological feedstocks, such as agricultural crops and forestry by-products. Furthermore, ethanol is safer, less polluting, requires lower engine compression and less cooling. So why did petrol take over the market? There are two key reasons: first, per kilometre petrol originally cost less, and second, the large investments made by the oil and auto industries in capital, human skills and technology made the entry of a new industry difficult. However, in countries with a surplus agricultural capacity, liquid fuels from biomass can be a viable alternative to petrochemicals.

The biggest fuel ethanol user is Brazil where approximately 3.2 billion gallons are made from sugar cane for automotive use every year. Brazil began its ethanol program (called 'Proalcool') in 1979, in an effort to use its sugar cane crops and to reduce dependence on oil imports. Since then, many people cite the noticeable improvement in air quality as justification for continuing the effort. Most of Brazil's vehicles are fuelled by 22% ethanol blends, and more than 4 million operate on 95% ethanol. Ten years ago, 96% of the cars sold in Brazil were made to run solely on alcohol. Today the trend has reversed somewhat, mainly because popular imported cars are not designed for ethanol. The down-turn is more indicative of Brazil's lonely pioneering rather than a failure of the technology.

In other countries, ethanol is used mainly as a fuel additive. In America, ethanol-blended petrol accounts for about 9% of the sales. In a country as large as the USA (and a country so dependent on the car) this is a significant amount, equating to 1.2 billion gallons - roughly the entire Canadian consumption of petrol. Blending began in the USA in the late 1970's and it is estimated that over two trillion kilometres have been travelled using fuel ethanol blends. Environmentally, ethanol blends have been shown to reducing carbon monoxide and nitrogen dioxide emissions and in countries where the farming economy has slumped, the fuel market has help to stabilise farmers' incomes. Fuel ethanol may be relatively old biotechnology, but it is destined to have a large impact in the future.

**The Starch industry**

Enzymes have proved to be of great value in the starch industry for around 20 years. In the 1950s, fungal amylase was used to manufacture syrups that contained sugars which could not be produced by conventional acid hydrolysis, but it was amyloglucosidase which revolutionised the industry in the 1960s. Amyloglucosidase can completely break down starch into glucose and so within a few years, almost all glucose production converted from chemical to enzyme hydrolysis. Among the benefits were: greater yield, higher degree of purity and easier crystallisation. The modern enzymes are more thermostable than their predecessors and these are favoured because starch is heated (liquefaction) to facilitate enzyme hydrolysis and pumping. Since then, interest has widened to other enzymes; in particular glucose isomerases. Using these enzyme, glucose containing an aldehyde group can be converted into the corresponding ketone sugar, fructose. This has the same calorific value as glucose, but is twice as sweet and hence less is needed - which is popular with dieters and food manufacturers. Considering that starch is universally available, and frequently cheaper than cane sugar and beet sugar, it makes economic sense to use 'High Fructose Corn Syrup' (HFCS) in many products where sugar has hitherto predominated.

## BIOTECHNOLOGY TODAY

Biotechnology today extends from public and private companies and includes multi-national corporations and governments. In 1996, the journal *Nature Biotechnology* estimated total biotechnology revenue at around US$8.5 billion (give or take a million or two) with 230 public companies sharing in this income. However, if you include funding for Research and Development, this figure is closer to US$30 billion. Despite the large figures for income, many companies are still making an annual loss in this area because of their investment in R & D, but clearly, the high level of investment reflects confidence in the technology. The processes involved in the production of a commodity by modern industrial biotechnology are summarised as a flow diagram in **Figure 3**.

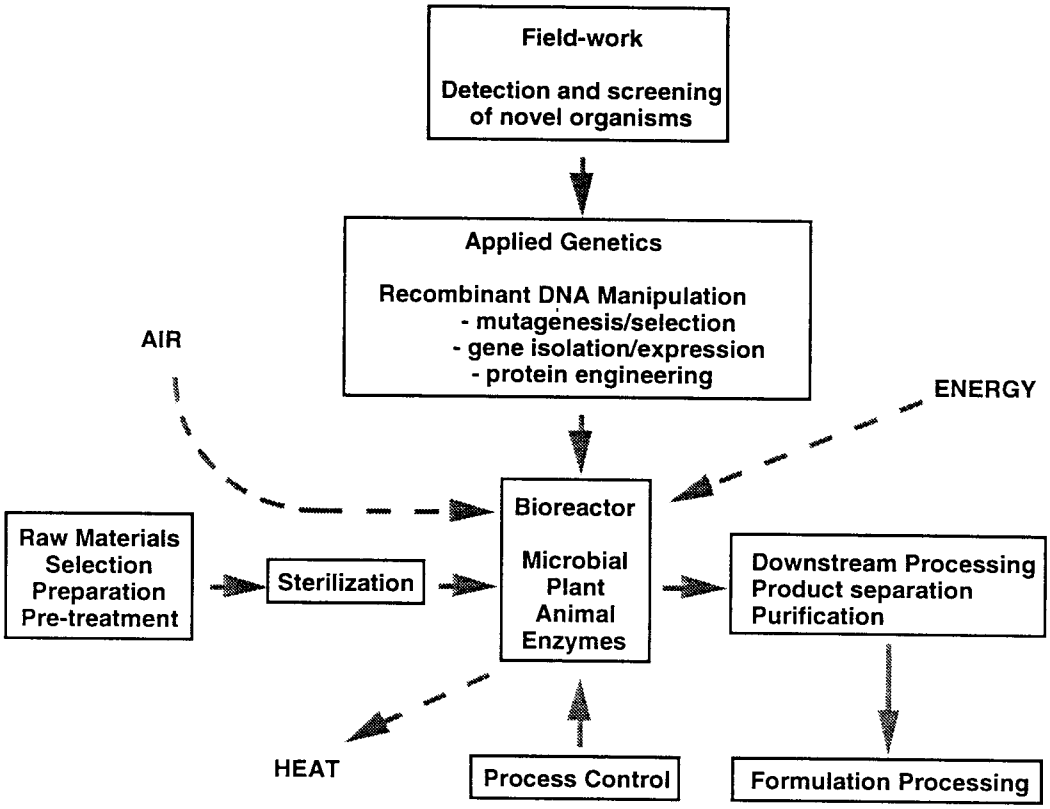


**Figure 3. The stages of a biotechnological process. When balancing the books, it must not be forgotten that a crucial part of the input is research and development. Often R & D can take many years before a process becomes feasible or cost effective.**

Details vary considerably from process to process but in all cases, the technology should be able to compete with existing industries. Raw materials should be cheap and accessible and preferably a waste product from another industry. The bioreactor itself may use whole organisms (bacteria, fungi, plant, or animal cells) or enzymes. Energy input is required for mechanical functions such as stirring, pumping and aeration, and as most biocatalysis reactions are exothermic, refrigeration is also required to prevent the organisms from being cooked or the enzymes denatured. Sterility is critical - particularly with whole organisms. Very often, organisms are genetically modified to enhance production levels and as the modifications are not for benefit of the organism, they almost invariably produce strains which are weaker and which are rapidly outgrown by their wild-type counterparts. With traditional enzymes and the more familiar organisms, contamination is a considerable problem but new, high-temperature enzymes and thermophilic organisms (which are discussed later) can alleviate this by allowing processes to be run at temperatures where common contaminating organisms cannot survive.

**Enzyme Technology**

Enzyme-catalysed processes are gradually replacing chemical processes in many areas of industry. Enzymes have all the properties of true catalysts. In the presence of an appropriate enzyme, a chemical reaction occurs at a much higher rate but the enzyme is not consumed by the reaction. Their ability to perform very specific chemical transformations (biotransformations) has made them increasingly popular in industries where less specific chemical processes produce unwanted by-products. Purity and predicability are of particular

importance in food manufacture where by-products may be harmful or affect flavour, and because of their specificity, pharmaceutical companies favour biotransformations in the development of novel therapeutic agents. In addition, enzymes are chiral catalysts which means that they can be used to produce optically active, homochiral compounds of a kind that are often difficult to make using traditional organic chemistry. Recently, a greater awareness of conservation issues have forced industries with a history of polluting to consider alternative, cleaner methods so there is now significant growth of biotechnology outside of the pharmaceutical and food industries.

At present, only about 20 enzymes are produced on a truly industrial scale. These enzymes are made by 35 producers with 4 major companies holding 75% of the market. In 1992, the market value of these enzymes was approximately US$800 million with a predicted yearly increase of around 10-15%. The market is highly competitive, suffers from over-capacity, has small profit margins and is technologically intensive. Nonetheless, there is room growth as long as new markets can be found. A breakdown of enzyme production and their uses is given in **Table 1.**

Matching an enzyme with a process is the greatest challenge to a research and development program. Often an industrial plant can be modified to accommodate the limitations of an enzyme but this is costly and a better approach is to find an enzyme more suited to the existing process. Increasingly, new organisms are being found living in unusual environments and these are proving an excellent source of novel enzymes. Living organisms are now generally divided into three groups (or domains): the Eukarya (still often called the eukaryarotes), the Bacteria and the Archaea (or archaebacteria). The Eukarya, which include all animals and plants are limited in their ability to withstand hostile conditions such as extreme ranges of temperature or pH. Some worms that can live above 60□C have been found living around deep ocean volcanic vents, but these are exceptional. Bacteria and Archaea are not so constrained and can thrive in quite unbelievable conditions; from freezing to boiling water and from an acidic pH 2 to alkaline pH 12. The record holder for temperature at present is the wonderfully named Archaea *Pyrococcus furiosa* which grows optimally at around 113°C and finds it too chilly if temperatures fall to 100°C. These are the organisms of the future for biotechnology. Many industrial processes are designed to run at elevated temperatures where viscosity is reduced and chemical reactions are faster. By using enzymes with optimal activities at these temperatures, changes to existing industrial plants can be minimised. Furthermore problems with contamination are reduced, and less cooling is required where the reactions are exothermic.

It was once thought that the ‘thermophiles’ (Greek for ‘heat-lovers’) survived by having a rapid turnover of cellular components. Nobody really believed that proteins or other bio-molecules could withstand such extremes of temperature. In the early 1980’s this theory was proven to be largely incorrect. The macromolecules used by thermophilic organisms are intrinsically heat-stable and optimally active at the same temperature as the environment in which their owners grow. In fact, a close look at the reaction kinetics of biochemical reactions above 100°C suggest that it is us that are living in a hostile, frigid environment and that the natural place for life is in boiling water. Where we have a large energy expenditure to create macromolecules, in the chemical and physical environment of a geothermal hot pool many of the same reactions are energetically favoured (have a negative $\Delta G$) - it seems that thermophiles actually gain energy when building the same molecules which cost us so much to make. The properties of an ‘extremophile’s’ protein (such as thermal stability) are a direct result of the protein’s sequence of amino acids. This means that enzymes can be isolated and

purified and they will still function at high temperatures. The application of thermostable biocatalysis is still relatively limited with most thermostable enzymes being used in the detergent or starch industries. However, they are rapidly expanding into other areas and usurping their mesophilic (mid-temperature loving) counterparts. Outside of industry, thermostable enzymes have specialised uses in medical diagnostics and molecular genetics research. Perhaps the best example is a DNA polymerase isolated from *Thermus aquaticus* which is used in the Polymerase Chain Reaction (PCR). The half-life of this enzyme is 40 minutes at 95°C and so *Taq* DNA polymerase can be repeatedly heated during the DNA denaturation steps of the reaction. PCR has so revolutionised molecular genetics that its inventor, Kary Mullis, shared the Nobel Prize for Chemistry in 1993.

The way forward sounds fairly straightforward; find an organism growing at a suitable temperature and extract its enzymes. Unfortunately it is not so simple. Many enzymes are produced in very small quantities or the organisms that produce them are extremely difficult grow in the laboratory. In fact with most micro-organisms, we still don't know how to grow them at all. Even if suitable growth conditions can be found, many grow in such difficult conditions (such as 110°C, 2000 atmospheres pressure and in the total absence of oxygen) or at such pitiful cell densities, that to extract as little as milligram of enzyme would be all but impossible and yet kilograms or tons of enzyme are often needed by an large industrial end-user. To the rescue came recombinant DNA technology or 'Genetic Engineering'.

**Genetic Engineering**

Genes are the fundamental basis of all life. The genetic code is similar to a computer program but instead of using a binary code of '1's and '0's, the basis for the genetic code is the chemical sequence within DNA (deoxyribonucleic acid). DNA is 'heteropolymer' - a polymer of more than one monomeric unit. With DNA there are four units or 'bases': A = adenine, C = cytosine, G= guanine and T = thymine. DNA is often described as a 'blueprint', but this is misleading. A better description is a 'recipe'. When you see a blueprint of a building you can immediately tell whether it is the plan of a house, a block of flats or a bridge. If you were given a recipe for a building (a set of instructions without pictures) you would have to perform a lot of the instructions before it became apparent what you were building. It is the same with DNA. DNA is a set of instructions which cannot be interpreted at a glance and DNA from one organism looks pretty much like that from another. However, as our knowledge increases, our ability to identify and discriminate between genes and organisms increases; but we still have a long way to go.

The sequence of the four bases along a DNA molecule, is translated into the amino acid sequence of proteins and the rules are the same for all organisms (except for a few minor differences here and there). As a consequence of the common language, a gene can be transferred from one organism to another and it will be translated correctly. The characteristics of an enzyme are defined by the sequence of its amino acids, and in turn, the amino acid sequence is defined by the gene from which it is translated. Therefore, if we can locate and isolate a gene from the chromosome of an organism which we have difficulty growing, we can insert it into a new host which is much easier to handle and use this to produce our enzyme. Isolating genes is becoming increasingly simplified as new methods are devised. In fact, methods have advanced to such an extent that we don't even need the original organisms. PCR allows us to isolate genes directly from environmental samples. You

simply treat the mixture of organisms as a gene pool. The latest strategy for obtaining suitable enzymes for industry is:

1) Find a pool which is similar in temperature and pH to the conditions within an existing process.
2) Extract genes directly from the pool water or sediment by using the polymerase chain reaction.
3) Transfer the genes to a production host.
4) Engineer the new host to produce the gene product in large quantities.

If you are having trouble finding a pool which is a natural counterpart to your process, you may find suitable organisms actually living within your existing industrial plant. If reactions are carried out in conditions which aren't too harsh, then micro-organisms may be growing on vessel walls or in the outlet and waste pipes.

Once you have cloned your gene, enhancing production levels of the 'recombinant' enzyme can be relatively simple if the production organism is well understood. A gene comprises the translatable code and DNA sequences upstream and downstream which specify when to activate the gene and how much of the product to make. It is possible to swap these control sequences and trick the host into believing it is making something else - something useful for its own survival. It is also possible to modify the gene so that the organism secretes the protein through the cell wall into the surrounding medium and this action greatly simplifies purification. Genetic systems have now been developed where it is possible to produce more than 20 grams of protein for every litre of culture. With this sort of production level, costs are reduced dramatically and the amounts of enzyme needed by industry become achievable goals.

Written by Dr David J. Saul (School of Biological Sciences, University of Auckland, Private Bag 90219, Auckland, New Zealand), Dr Moreland D. Gibbs and Professor Peter L. Bergquist (both of the School of Biological Sciences, University of Macquarie, Sydney, New South Wales 2109, Australia)..