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Biotechnology in the chemical industry

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Abstract - The stereospecificity and regiospecificity of biotechnological synthesis compares favourably with classical organic synthesis and is carried out by a biocatalyst. However, there are limitations in many cases because of the sensitivity of biocatalysts to reaction conditions. The high cost of biocatalysts and problems associated with their regeneration as well as their use in non-aqueous solutions are aspects where research is needed. The successes and failures of several biotechnological conversions are outlined with examples.

Biotechnology is an interdisciplinary science in the focus of many fields of applications (Fig. 1). One of the many definitions around for biotechnology is the production with cells or parts of them (Fig. 2). That means, you should end up with a product. Interrelation between biotechnology and chemistry can be seen from several different points of view.

FROM THE TYPE OF REACTION

There is a classification system for the enzymes, which outlines the various possibilities for enzyme reactions. I have listed six basic types of enzymes for their usefulness for the chemist and given just one example for each reaction.

Hydrolases cleave C-O or other bonds by adding water. I will give you an example for a reaction of this type from our own laboratories in the second part of my talk.

Oxido-reductases are useful, for instance, for a stereospecific reduction of a ketone, or can oxidize aromatic hydrocarbons to phenols (Fig. 3). A classical example for the next group of enzymes is glucose isomerase (Fig. 3).

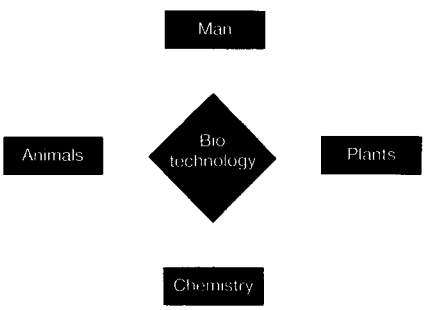

Fig. 1

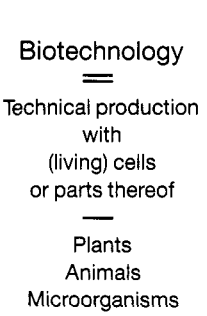

Fig. 2

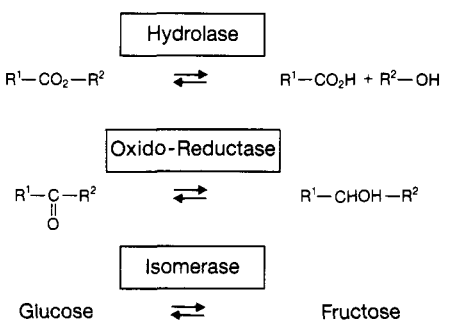

Fig. 3

The lyases add water or ammonia to C = C, C = N or other multiple bonds or vice versa eliminate these small molecules causing a double bond (Fig. 4).

Transferases transfer a whole group, for instance methyl or glycosyl from one compound to another.

Ligases, on the other hand, can link together two molecules. A very interesting feature, but they depend on the breakdown of ATP.

All of you have seen lists of products, which can be made by fermentation or by enzymes, have heard promises of billions of turnover, and seen speculations about the end of all chemistry. It has not happened, and it won't.

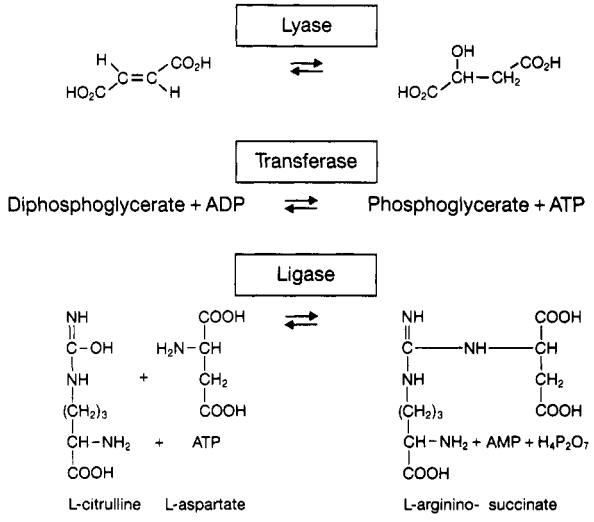

Fig. 4

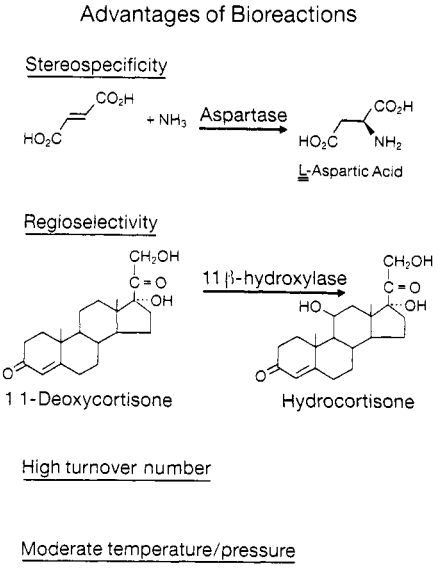

Fig. 5

WHAT IS A BIOPROCESS?

You have a substrate and a catalyst - a biocatalyst in this case - and get a product. It is very similiar to chemistry. Now, what are the advantages of a biotechnological synthesis compared with classical organic chemistry? In many cases, biological reactions have a high stereospecificity (Fig. 5). More and more products come to the market, where for a number of reasons only one stereoisomer is wanted. This has also an impact on chemical intermediates as a starting material for further syntheses. A further advantage of biological reactions is the regiospecificity. You can very often achieve even a 'remote site' reaction, that means there is no directing functional group close to your reaction center. Low temperature, high turnover rate and atmospheric pressure are the typical conditions for a bioconversion.

There are, of course, also some disadvantages in biocatalysts (Fig. 6). The stability of the enzymes or microorganisms: Being usually high molecular proteins, enzymes tend to be sensitive to temperature, pH, oxidation, mechanical shearing and attack by other enzymes like proteases, their lifetime is only limited. In many cases, immobilisation - that means coupling to a usually solid support - is a method of expanding the lifetime of an enzyme catalyst.

Usually bioconversions run in an aqueous medium, and most organic substrates are lipophilic and thus only poorly soluble in water. Though there are many groups of researchers trying to adapt enzymes to organic solvents, and many papers have been published, the major breakthrough in this field for a technical application has not yet been achieved.

Problems of Bioreactions

Stability of the enzyme
Cofactors
Low concentration
Aqueous medium
Product inhibition

Fig. 6

Synthesis with Microorganisms

Side products from energy metabolism
Biomass
Primary metabolites
Secondary metabolites
Bioconversion

Fig. 7

Side Products from Energy Metabolism

Methane
Ethanol
Acetic acid
Polyhydroxy butyric acid

Fig. 8

One other great problem is the requirement of cofactors. These molecules, like NAD or ATP, are very costly and this problem has to be overcome. There are several possibilities. First, you take the whole cells, let them do the job, and throw them away. This, of course, can be done only with very cheap organisms, like baker's yeast, for instance. Another way is to find a cometabolism, e.g. if your product has to be reduced, you add a substrate which is being oxidised by using the same cofactor. This can be a very tiresome and tricky procedure. The best solution seems to be the immobilisation of the cofactor and use of defined enzymes. This has been demonstrated by the leucine process I will mention later on.

I said, there are different standpoints watching bioreactions. This was the chemist's point of view. I will show you now the microorganism's point of view:

Why is it doing, what do we want?

Organisms are producing for their own purpose, of course. Figs. 7 and 8 show you these groups of products, and I will try to give you examples how we make use of them.

PRODUCTS OF THE ENERGY METABOLISM

Methane is obtained by anaerobic waste treatment. Ethanol as a fuel is not a reasonable product from the general economic point of view. There may be circumstances, however which make it locally desirable to save hard currency for crude oil. An interesting example is polyhydroxy butyric acid, which is used by certain bacteria as an energy storage system. It is produced for instance by ICI, but until now it is not an economic success to my knowledge.

To harvest the biomass of an organism can also lead to a product. One good example is Single Cell Protein (SCP), which is also produced by ICI in the largest sterile fermenter existing at the present time. It has about 1500 cubic meters; methanol is the starting material. After extracting the nucleic acids the protein is sold mainly as an animal feed.

Yeasts are a classical product, for baking, brewing, and, as IUPAC Biotechnology Commission has seen last year in the German Democratic Republic, as an animal feed made from diesel oil.

The largest part of the enzymes sold are proteases, for instance for washing powders. Many bulk enzymes are also used in the food industry.

Biomass/Enzymes

SCP
Baker's yeast
Brewing yeast
Proteases
Amylases
Glucose isomerase

Fig. 9

Primary metabolites are so to speak the basic need of an organism. They are readily soluble in water and can be produced in high concentration. Classical examples are the amino acids made by fermentation in quantities up to 200 grams per liter. The largest product of this group is glutamic acid with an annual production of 300.000 tons a year. Another very important product is citric acid, which is made in about the same quantities (see Fig. 10).

Primary Metabolites
Glutamic acid
Citric acid
other
Amino acids

Fig. 10

Secondary Metabolites
Antibiotics
Enzyme inhibitors
Xanthan gum
Scleroglucan

Fig. 11

Bioconversion
Steroids
Leucin
6-APS
Vitamins (Intermediates)
Racemat Resolution
Aspartic Acid
Malic Acid

Fig. 12

Secondary metabolites are substances made in much smaller amounts, they cover 'advanced features' from the organism's point of view. The best know examples are, of course, the antibiotics and enzyme inhibitors. Another group of products one could count in this group are the biopolymers like xanthan gum or scleroglucan (see Fig. 11).

Bioconversion is the strangest thing, we are doing with an organism, from its point of view. The bugs are getting a starting material they have never seen before. In some cases, they just die away, but in other this new product fits somewhere in the metabolism and if we are lucky, they do exactly what we want, otherwise we use our tricks to force them.
There are not so many products which have reached the market by now, just because of the cost. On the other hand, there are many results on the laboratory level waiting for a major breakthrough (see Fig. 12).

I want to go into detail only with two examples:

The leucine process (Fig. 13) developed in W. Germany by a cooperation between our member Professor WANDREY and the industrial manufacturer DEGUSSA. It is a very nice example of solving the different problems I have mentioned before in a special case.

Keto isocaproic acid is converted to leucin by a reductive amination with leucin dehydrogenase. This reaction is a reduction, that means, it needs a cofactor, namely NADH. The price of this cofactor would be prohibitive for any process, if you would use it just once. So, the idea was to recycle it. This was very elegantly solved by coupling an oxidative reaction to keep the electron balance.

Formate is oxidised by formate dehydrogenase to carbon dioxide, which is easily removed from the reaction mixture. The whole process runs in a membrane reactor, that means, a continuous stream of the low molecular compounds is pumped in and removed from the reaction vessel. This causes another problem, explained below.

The cofactor would be washed out. The trick was to immobilise it to polyethylene glycol with an average molecular weight of about 20.000. Thus, it is kept inside the membrane reactor, and Professor WANDREY has reached much more than a 100.000 reaction cycles with one molecule of the cofactor. A very nice example, indeed.

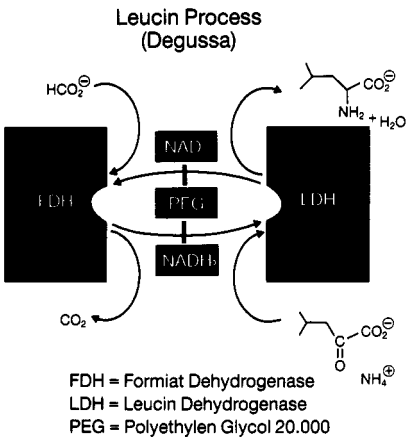

Fig. 13

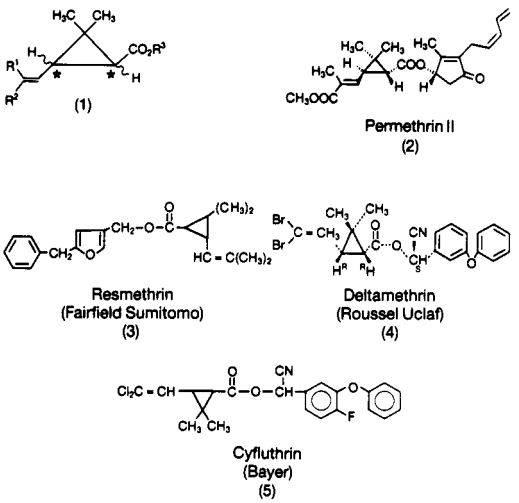

Fig. 14

Now I come to a process developed in our own labs, which is just between lab and pilot scale.

Pyrethroids are among the most powerful insecticides known at the present time. Figs. 14 and 15 show you a selection of products sold by different companies. Several BAYER products in the market are based on permethrinic acid. There are four stereoisomers possible (Figs. 16 and 17).

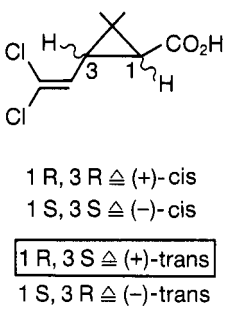

Fig. 15

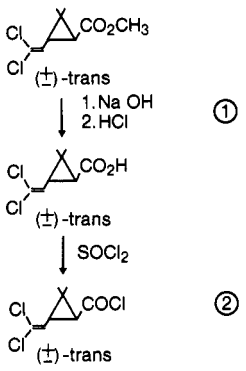

Fig. 16

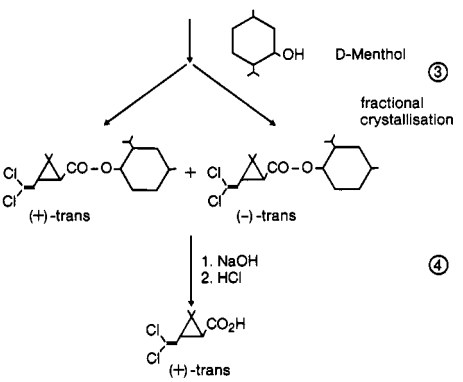

Fig. 17

For several reasons it can be of advantage to have the pure isomers:

1. Toxicology
   Sometimes one stereoisomer has a lower overall or specific toxicity compared with the mixture.
2. Only one or two isomers are carrier of the effect. In most cases there are distinct differences between R and S isomers, sometimes the wanted insecticidial effect is confined with more than 90 % to only one isomer.
3. Cost of the acid and the wasted alcohol part of the molecule. Pyrethroid acids are very costly, and most alcohol components are very expensive, too. So it is worth while to think about possibilities for using only the active isomers.

cis and trans-isomers can be separated by physicochemical methods, e.g. distillation of the corresponding acid chlorides or esters.
The separation of the resulting enantiomers is a more difficult task to achieve.

A multi-step separation process has been developed. However, a resolution by enzymatic hydrolysis is possible by just one step (Fig. 18).

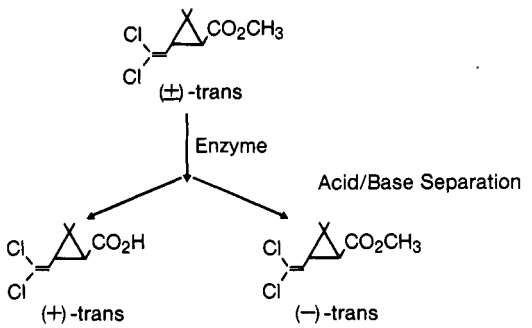

Fig. 18

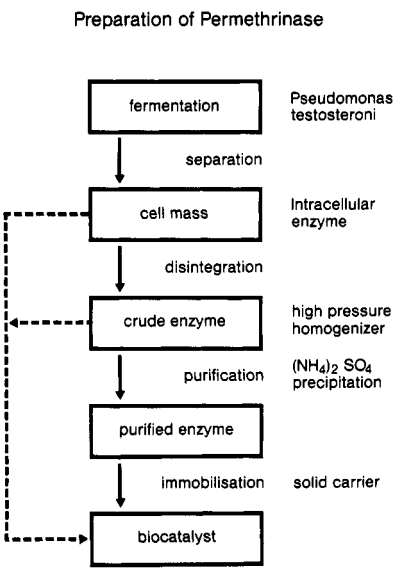

Fig. 19

For practical reasons (the pure trans-form is readily available on an industrial scale) we concentrated on the separation of the 1R-trans and the 1S-transisomers though also a mixture of all four isomers would be possible as the starting material. We started a screening program with commercially available enzymes and ended up with the famous pig liver esterase. It does a good job, however, it is not really a cheap enzyme and the availability in the quantities we needed was not given. Therefore, we continued our search by screening several hundreds of microorganisms, and finally ended up with a pseudomonas strain, the enzyme of this strain has a very good performance with respect to stability, selectivity and esterase activity.

The preparation of the enzyme is shown in Fig. 19.

The strain ZP 50 is grown in the fermenter, then the cell mass is separated by centrifugation and the cells cleaved by a homogenizer. The obtained crude enzyme is purified by cross filtration and acid and ammonium sulphate precipitation. Then this preparation is immobilised on a solid support by covalent binding. This biocatalyst is used in the bioreaction in the same way as an ordinary catalyst in a chemical process.

Figure 20 shows the production scheme.

In a stirred tank reactor the racemic trans-permethrinic acid methyl ester is reacted as a suspension with the biocatalyst. The left product stream stands for the wanted 1R-trans-isomer, the right stream for the unwanted 1S-trans-isomer. The process runs at a slightly alcaline pH, so that the newly formed acid is dissolved as the sodium salt. The uncleaved ester remains unchanged as an emulsion. The process can be run as a batch-process, but also a semi-batch type is conceivable with consecutive addition of reactants and separation of the product solution. A continuous column process seems not very promising because of problems with the maintenance of the pH. Separation is achieved by toluene extraction, where the unwanted ester is extracted first in the alcaline, and then after acidification the 1R-acid is extracted in a second step. To our surprise very good results were also obtained by cooling the solution and crystallising the products.

Production scheme (proposal)

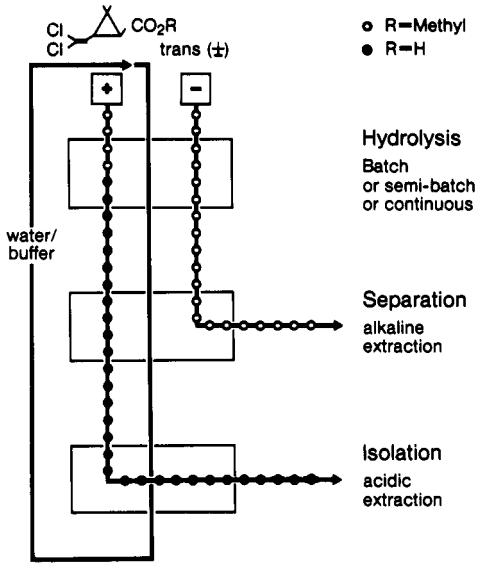

Fig. 20

Activity Yield (fermentation broth)	300-1200 U/L*
Activity Yield (immobilised catalyst)	45%
Specific Activity	15-80 U/g
Half Life Time	>30 days
Ester Concentration	10 g/L ≙ 4.6 g Acid/L
Yield	>95%
Chemical Purity	99%
Optical Purity	99%

*1 U = 1 mg 1 R-trans-acid per liter and hour

Fig. 21

Figure 21 gives an overview on the results we have obtained by now.

The process has been operated in the pilot plant scale in a 100 l reactor, and we have obtained 10 kg amounts of product of the given specificity.

All this leads to several conclusions:

1) Bulk chemicals are close to primary metabolism, and this means only a very limited scope of product of a high solubility in water.

2) Bioconversion means at present a relatively higher production cost - that is specialty chemicals with a higher added value only.

3) The scope of bioreactions in the lab is by far broader and more feasible than the technical application. A main problem to be solved is the low product concentration in bioconversions.

Figure 22 shows you a comparison of estimated world outputs of some bioproducts.

World Output of Biotechnological Products in Tons per Year

Product	t/a
Beer	54.000.000
Wine	28.500.000
Baker's yeast	600.000
Protein yeast	800.000
Citric acid	300.000
Antibiotics	25.000
Glutamic acid	300.000
Vitamin B12	3

Fig. 22

Myself being a Bavarian from Munich, it is a comfort, that beer and wine are still by far ahead!