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Reviews

Industrial Biocatalysis: Past, Present, and Future

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Abstract:

Industrial biocatalysis is older than one might think. In the past, entire microorganisms have been used, for instance, for the production of acetic acid from ethanol. The racemic resolution of amino acids via the acylase method is regarded as one of the first industrial processes using isolated enzymes (Tanabe, Japan, 1969). Presently, approximately 100 different biotransformations are carried out in industry, mostly for the production of pharmaceutical and agrochemical precursors. In most cases, chiral compounds (fine chemicals) are obtained. Biotransformations are also successful for the production of commodities such as acryl amide. Until now, mainly hydrolases are used industrially with water as solvent. A lot of additional processes are presently under development where oxidreductases are also employed. Another important reaction type is the C−C-bond formation using the reverse reaction of lyases. In the future, we will see a transition from degrading reactions via transforming reactions to synthetic reactions. We will also see more biotransformations in the presence of organic solvents. Probably, there will be a renaissance of whole cell biotransformations where undesired side activities and further metabolising steps (besides the desired reaction) are avoided by means of gene-disruption. Work is going on to establish non-natural cofactor-regenerating systems in whole cells ("designer strains"). Especially the methods of non-natural evolution of enzymes and the possibility to combine such enzymes in one strain can be of great importance for biocatalysis in future. With respect to isolated enzymes it can be predicted that the cost of enzymes will further drop due to an efficient production with genetically engineered microorganisms or higher cells. The gap between enzyme catalysis and homogeneous catalysis will narrow. On one hand we will see enzymes, which have been adapted to industrial biocatalysis by means of evolution under non-natural conditions. On the other hand, homogeneous catalysts will show up, which will mimic principles of enzyme catalysis (synzymes).

This short overview will be restricted to "one-step" biotransformations,[1] even though there are also interesting multi-step examples such as fermentations, which might be regarded as a sequence of biocatalytic steps.

One of the oldest examples for the application of biocatalysis at an industrial scale is the production of acetic acid from ethanol (known since 1815) with an immobilised *Acetobacter* strain. It is remarkable that nearly 200 years ago a process was established using an immobilised biocatalyst (here attached to beech wood shavings).

Another important biotransformation established more than 70 years ago is the production of vitamin C (sorbite−sorbose oxidation). Later, biocatalytic steroid hydroxylations were established industrially. The first hydroxylation was the conversion of progesterone to 11α-hydroxyprogesterone.[2]

The biotransformations mentioned are examples of the use of oxidoreductases from whole cells. A considerable amount of time elapsed before the first isolated oxidoreductases were used in industry! The best known example for the industrial use of an isolated enzyme is the racemic resolution of amino acids via the acylase method.[3] This process was established in 1969 by the Tanabe company, Japan. The industrial breakthrough was achieved by an efficient method of enzyme immobilisation, after lab methods for immobilisation of enzymes had been developed long before.

In the following, the present state and future aspects of industrial biocatalysis are discussed with a few prominent examples following the enzyme nomenclature.

1. Oxidoreductases

Enzymes are often unbeaten, if regio- or stereoselective redox reactions are needed. Until now, the cofactor regeneration problem is mostly solved by using entire cells. A few recently established oxidoreductase processes are mentioned here.

The BASF company (Germany) commercialised a regioselective hydroxylation of (*R*)-2-phenoxypropionic acid to an intermediate used for the production of a herbicide (Scheme 1).[4] Although no chiral centre is formed here, the use of an entire microorganism was the hallmark in this process.

An isolated D-amino acid oxidase is used in a transformation of cephalosporin C to α-keto-adipyl-7-aminocephalosporinic acid, which is an intermediate in the acylase-

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(1) McCoy, M. *Chem. Eng. News* **1999**, *77*, 10.

(2) Peterson, D.; Murray, H.; Epstein, S.; Reineke, L.; Weintraub, A., Meister, P.; Leigh, H. *J. Am. Chem. Soc.* **1952**, *74*, 5933.

(3) Tosa, T. *Enzymologia* **1966,** *31*, 214.

(4) Dingler, C.; Ladner, W.; Krei, G.; Cooper, B.; Hauer, B. *Prestic. Sci.* **1996**, *46*, 33.

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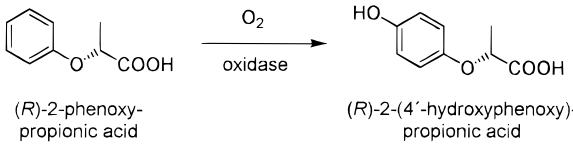
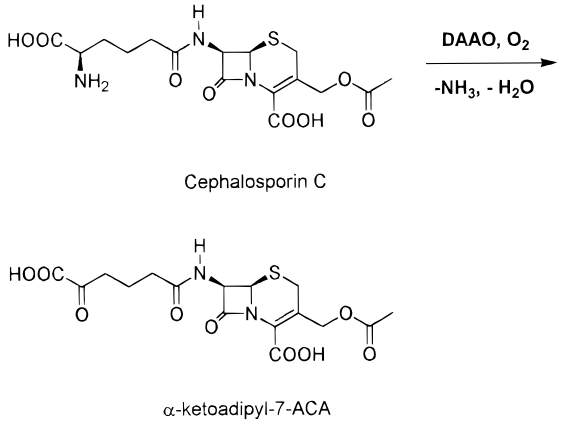
Scheme 1. **Oxidase-catalysed synthesis of (*R*)-2-(4′-hydroxyphenoxy)-propionic acid**



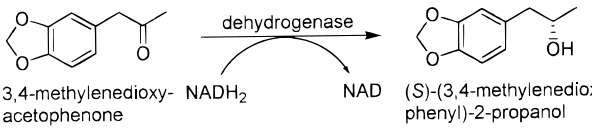
catalysed synthesis of 7-ACA (Scheme 2).[5] This transformation is especially worth mentioning, since this development made an older purely classical chemical process obsolete (e.g., Hoechst, Germany).

Scheme 2. **D-Amino acid oxidase (DAAO)-catalysed synthesis of α-ketoadipyl-7-aminocephalosporinic acid**



The company Lilly (U.S.A.) developed a whole cell biotransformation, where a prochiral ketone is reduced to an optically active secondary alcohol (Scheme 3).[6] This is the key step in a synthesis leading to a pharmaceutical precursor.

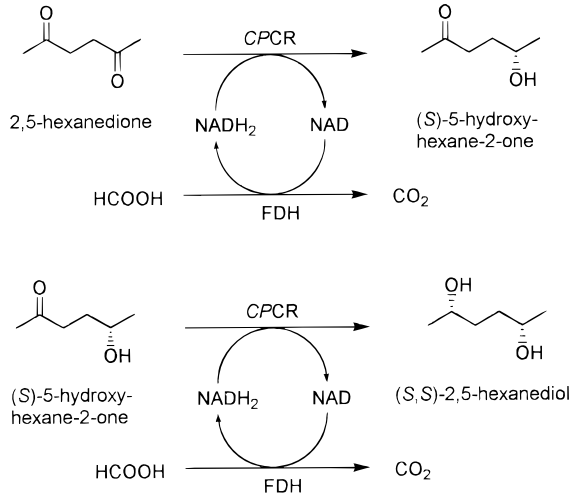
Scheme 3. **Dehydrogenase-catalysed synthesis of (*S*)-(3,4-methylenedioxyphenyl)-2-propanol**



Here an alternative process might be developed in the future. An isolated alcohol dehydrogenase could be used, regenerating the cofactor by means of the formate dehydrogenase/formate system. The problem of low substrate solubility would be overcome by using a membrane contactor where the substrate from the organic phase on one side of the membrane diffuses into the aqueous phase on the other side of the membrane where the catalytic reaction takes place. After biotransformation of the substrate, the product is re-extracted, while the cofactor together with the enzyme involved remains in the aqueous phase.[7]

Multiple reductions of asymmetric ketones lead to optically active polyols. Once again, the formate dehydrogenase/formate system is used for cofactor regeneration. Such diols are building blocks for the production of homogeneous catalysts, where the stereoselectivity is also determined by the optical purity of the building block (Scheme 4).[8] Quite

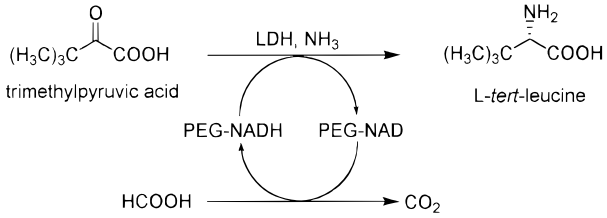
Scheme 4. ***Candida parapsilosis*** **carbonylreductase (*CP*CR)-catalysed synthesis of (*S*,*S*)-2,5-hexanediol and integrated cofactor regeneration with formate dehydrogenase (FDH) from *Candida boidinii***



a number of processes in this field are presently under development.

More than 10 years ago a process with continuous cofactor regeneration using isolated enzymes was developed (Scheme 5).[9] Here the reductive amination of trimethylpyruvate to

Scheme 5. **Reductive amination of trimethylpyruvic acid with leucine dehydrogenase (LDH) and integrated cofactor regeneration with formate dehydrogenase (FDH)**



L-*tert*-leucine was achieved using a combination of leucine dehydrogenase and formate dehydrogenase. This process has now reached ton-scale.[10] Nevertheless, there is still a prejudice that cofactor regeneration with isolated enzymes is an obstacle hard to overcome in industrial biotransformations.[11]

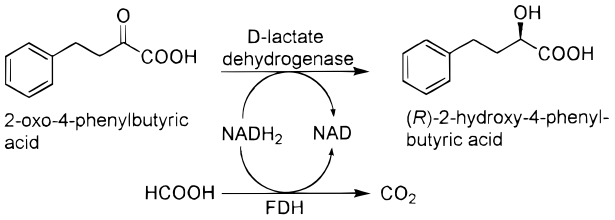
The same production technique might become industrially relevant when considering the reduction of α-keto acids to the corresponding α-hydroxy acids. In this way, (*R*)-2-hydroxy-4-phenylbutyric acid (an important precursor for ACE inhibitors) can be obtained (Scheme 6).[12]

2. Transferases

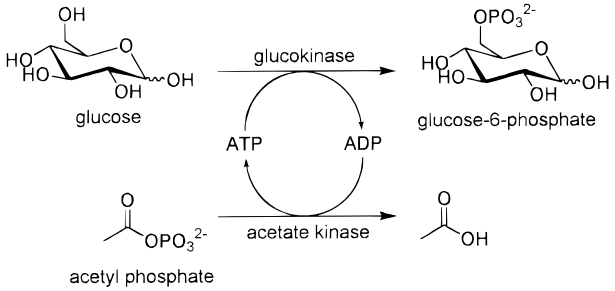
In the field of transferases glucose-6-phosphate is synthesised by means of a glucokinase starting from glucose in combination with an acetate kinase (Scheme 7).[13]

(5) Tanaka, A.; Tosa, T.; Kobayashi, T. *Industrial application of immobilized biocatalysts*, Marcel Dekker: New York, 1993.
(6) Anderson, B.; Hansen, M.; Harkness, A.; Henry, C.; Vinzenzi, J.; Zmijewski, M. *J. Am. Chem. Soc.* **1995**, *117*, 12358.
(7) Kruse, W.; Hummel, W.; Kragl, U. *Recl. Trav. Chim. Pays-Bas.* **1996**, *115*, 239.
(8) Rissom, S. *Membranverfahren für Redoxenzyme*, Ph.D. Thesis, University of Bonn, 1999.
(9) Kula, M.-R.; Wandrey, C. *Methods Enzymol.* **1987**, *136*, 9.
(10) Kragl, U.; Vasic-Racki, M.; Wandrey, C. *Bioprocess Eng.* **1996**, *14*, 291.

Scheme 6. **D-lactate dehydrogenase-catalysed reduction of 2-oxo-4-phenylbutyric acid**

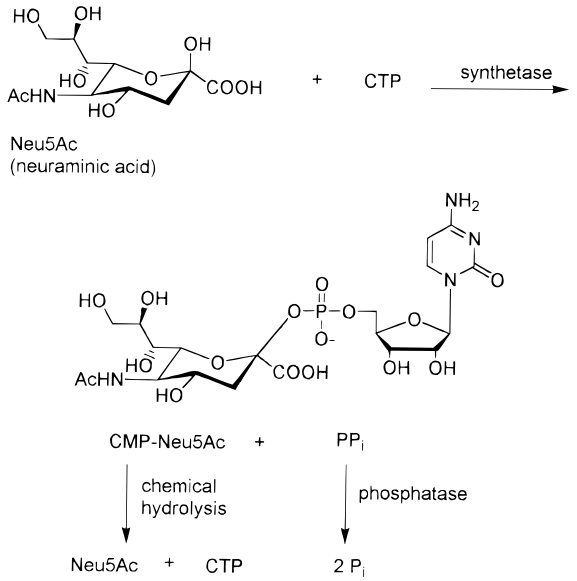


Scheme 7. **Synthesis of glucose-6-phosphate by using a glucokinase in combination with an acetate kinase**



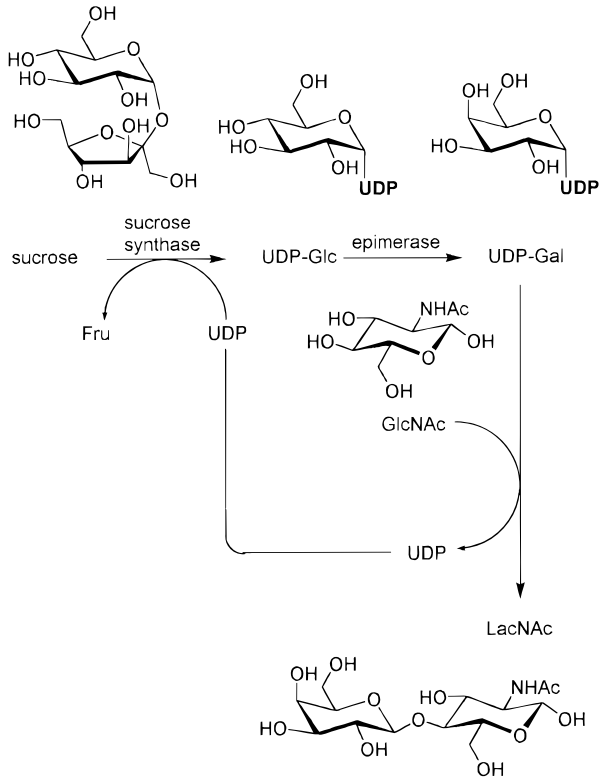
This process is carried out in multi-kilogram scale by the Japanese company Unitika. In a somewhat smaller scale, cytidinemonophosphate *N*-acetylneuraminic acid is obtained from neuraminic acid and CTP using a transferase (called CMP-Neu5Ac synthetase, Scheme 8).[14]

Scheme 8. **Synthetase-catalysed synthesis of CMP-Neu5Ac**



In the field of activated sugars "SuSy" (sucrose synthase, a transferase) might become important. In principle, this enzyme is able to use the energy of the glycolytic bond in sucrose to transfer UDP to other sugars (Scheme 9).[15] In

Scheme 9. **Synthesis of UDP-galctose and *N*-acetyl-lactosamine (LacNAc)**



this manner, quite a number of activated sugars can be obtained (e.g., UDP-galactose).

3. Hydrolases

In the field of hydrolases a large number of industrial biotransformations have been commercialised. Over the years, the acylase process using an immobilised enzyme has been replaced by a process with a soluble acylase using an enzyme membrane reactor (Scheme 10).[16]

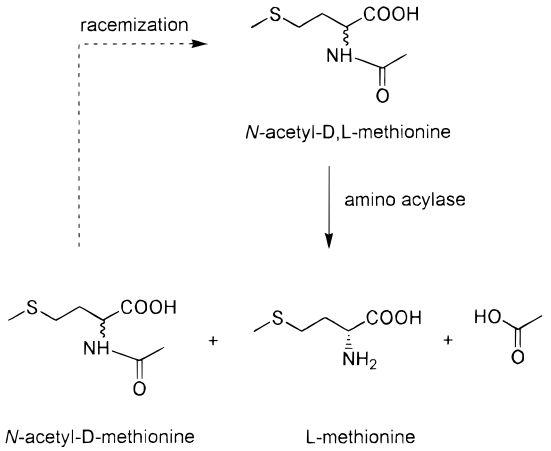
Another new development is the production of D-pantolactone out of the corresponding racemate (Scheme 11). A lactonase opens the lactone ring stereospecifically.[17]

A classical lipase-catalysed resolution is used to obtain an intermediate for diltiazem. Here methyl-*p*-methoxyphenyl glycidate is stereospecifically hydrolysed by means of a lipase (Scheme 12). The interesting aspect of this process is the application of an immobilised lipase in a hollow fibre module.[18] The enzyme is located at the interfacial boundary layer between an organic and an aqueous phase (membrane-supported liquid/liquid interphase).

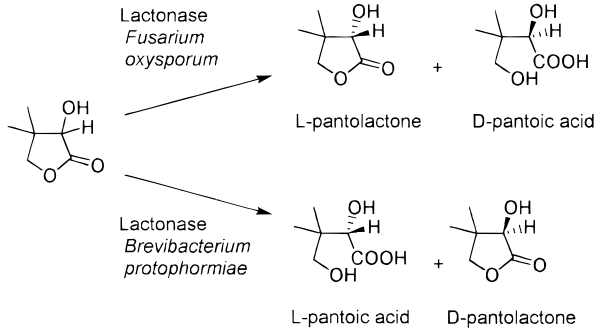
Recently, a process to obtain (*S*)-1-phenylethylamine from the corresponding racemate was commercialised by BASF,

(11) Thomas, K.; Woodley, J. *Rational Selection of Cofactor Regeneration Processes*; 2nd European Symposium on Biochemical Engineering Science; Porto, 1998; Vol. 201.
(12) Schmidt, E.; Ghisalba, O.; Gygax, D.; Sedelmeier, G. *J. Biotechnol*. **1992**, *24*, 315.
(13) Nakajima, H.; Kondo, H.; Tsurutani, R.; Dombou, M.; Tomioka, I.; Tomita, K. *ACS Symp. Ser*. **1991**, *466*, 111.
(14) See ref 10.
(15) Zervosen, A.; Elling, L. *J. Am. Chem. Soc.* **1996**, *118*, 1836.
(16) Bommarius, A.; Drauz, K.; Klenk, H.; Wandrey, C. *Ann. N.Y. Acad. Sci.* **1992**, *672*, 126.
(17) Shimizu, S.; Ogawa, J.; Kataoka, M.; Kobayashi, M. In *New Enzymes for Organic Synthesis*; Scheper, T., Ed.; Springer: New York, 1997; p 45.
(18) Lopez, J.; Matson, S.; Quinn, J. In *Extractive Bioconversions*; Mattiasson, B., Ed.; Marcel-Dekker: New York, 1991; p 27.

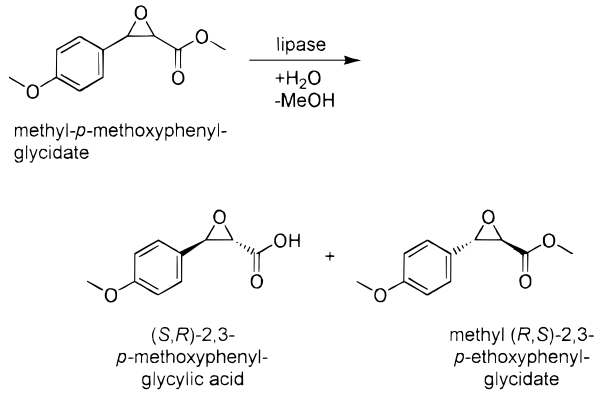
Scheme 10. **Amino acylase-catalysed enantioselective hydrolysis of *N*-acetyl-D,L-methionine**



Scheme 11. **Kinetic resolution of pantolactone catalysed by a lactonase**



Scheme 12. **Lipase-catalysed synthesis of (*S*,*R*)-2,3-*p*-methoxyphenylglycylic acid**

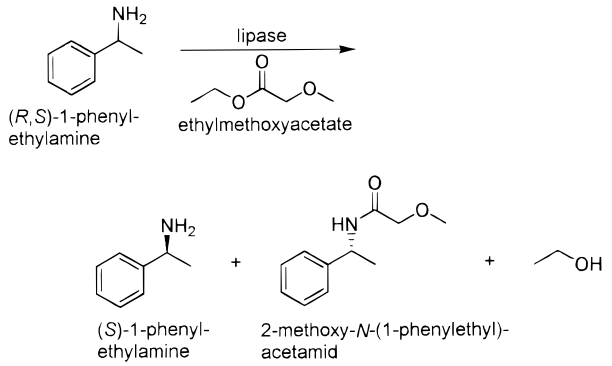


Germany (Scheme 13).[19] A lipase is used in a transesterification where an amine is used as a nucleophile instead of an alcohol. This process has meanwhile reached ton-scale.

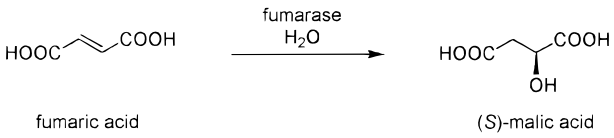
4. Lyases

In the field of lyases (synthases) the two most prominent examples use fumarate as substrate. In one case (*S*)-malic acid is obtained by means of a fumarase (Scheme 14),[20] in the other case (*S*)-aspartic acid is obtained by means of an

Scheme 13. **Lipase-catalysed synthesis of (*S*)-1-phenylethylamine**

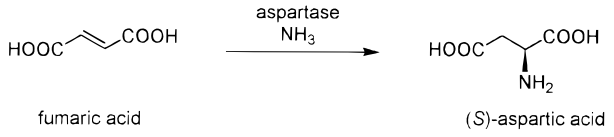


Scheme 14. **Synthesis of (*S*)-malic acid by means of a fumarase**



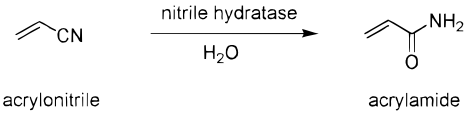
aspartase (Scheme 15).[21]

Scheme 15. **Synthesis of (*S*)-aspartic acid by means of an aspartase**



The process leading to acrylamide from acrylonitrile is also of importance in the field of lyases (Scheme 16).[22] This

Scheme 16. **Synthesis of acrylamide**



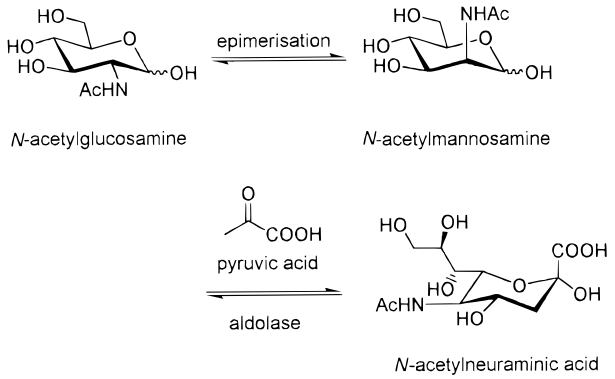
process is meanwhile being carried out in Japan at a scale of more than 10,000 tons per year. It might still be the most prominent example where a non-chiral commodity from classical organic chemistry is now made by means of an enzyme.

Aldolases also belong to the group of lyases. Here the production of *N*-acetylneuraminic acid from *N*-acetylmannosamine/*N*-acetylglucosamine and pyruvate has reached multi-kilogram scale and will probably reach ton-scale (Scheme 17).[23] The company Glaxo Wellcome GB, uses *N*-acetylneuraminic acid as a drug precursor.

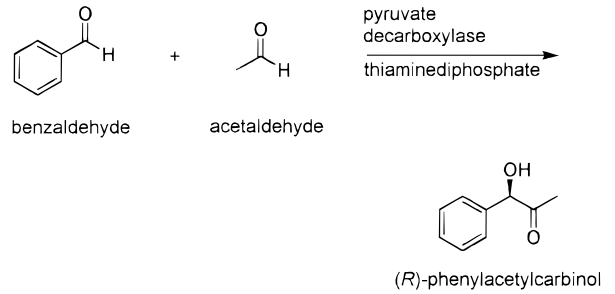
In future, the microbial process to produce L-ephedrine via (*R*)-phenylacetylcarbinol might be replaced by a process using a lyase (pyruvate decarboxylase, Scheme 18).[24] By

(19) Balkenhohl, F.; Ditrich, K.; Hauer, B.; Ladner, W. *J. Prakt. Chem.* **1997**, *339*, 381.
(20) Daneel, H.-J.; Faurie, R. *(*AMINO GmbH). Process for the production of (*S*)-malic acid from fumaric acid. German Patent DE 4424664, 1994.
(21) See ref 5.
(22) See ref 17.
(23) Mahmoudian, M.; Noble, D.; Drake, C.; Middleton, R.; Montgomery, D.; Piercey, J. E.; Ramlakhan, D.; Todd, M.; Dawson, M. *J. Enzy. Microb. Tech.* **1997**, *45*, 393.
(24) Pohl, M. In *New Enzymes for Organic Synthesis*; Scheper, T., Ed.; Springer: New York, 1997; p 15.

Scheme 17. **Aldolase-catalysed synthesis of *N*-acetylneuraminic acid**



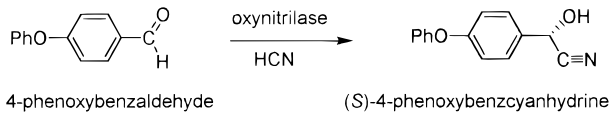
Scheme 18. **Synthesis of (*R*)-phenylacetylcarbinol by means of a pyruvate decarboxylase**



means of genetic engineering it has been possible to make benzaldehyde and acetaldehyde react directly to (*R*)-phenylacetylcarbinol.

Another industrial process using a lyase might be the use of oxynitrilase in order to obtain (*S*)-4-phenoxybenzcyanhydrine (a pyrethroide) (Scheme 19).

Scheme 19. **Oxynitrilase-catalysed synthesis of (*S*)-4-phenoxybenzcyanhydrine**

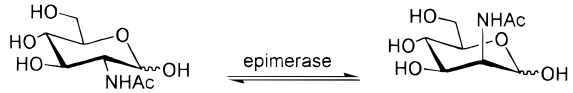


5. Isomerases

In the class of isomerases the "classical" industrial example is the use of glucose isomerase for the production of high-fructose corn syrup.[25] The epimerase for the transformation of *N*-acetylglucoseamine to *N*-acetylmannosamine (as precursor for *N*-acetylneuraminic acid) might become a new example for the industrial use of an isomerase[26] (Scheme 20).

Racemases which can be used simultaneously with an enzyme-catalysed racemic resolution are of general interest. An acetyl amino acid racemase used with acylase for the racemic resolution of amino acids has already been reported in the literature. Until now, an industrial breakthrough is missing due to nonsatisfactory activity and stability of the

Scheme 20. **Epimerase-catalysed isomerisation of *N*-acetylglucosamine to *N*-acetylmannosamine**



racemase. In the future, technical evolution of racemases might become a "hot" field.

6. Ligases

In the field of ligases, examples of industrial biocatalysis are more or less missing. Two examples of some practical interest can be mentioned: In non-ribosomal peptide synthesis (e.g., synthesis of gramicidin-S) ligases are involved, and in genetic engineering DNA-ligases play an important role.

The production of enantiopure compounds is of increasing importance to the chemical and biotechnological industries. Bioorganic transformations are predestined to meet this demand due to their inherent regio- and stereoselective nature. Indeed, a growing amount of enantiopure chemicals for pharmaceutical purposes are being produced biocatalytically today, in contrast to the amount of racemic bulk commodities produced in the past.

New advances in industrial biocatalysis might be seen in multi-step catalysis. This can be achieved with isolated enzymes and more promisingly with genetically modified organisms. The possibility to discriminate between undesired parallel and consecutive reactions by means of genetic engineering will bring a renaissance to whole cell biotransformations. By means of elaborate reactor engineering (multiphase bioreactors) or by development of solvent-resistant organisms/enzymes, the use of organic solvents for enzymatic reactions will probably be increasingly possible.

With the help of new evolutionary techniques such as directed evolution of enzymes in combination with improved screening methods, the time and money spent for research and development of new biocatalysts can be shortened considerably. These advances will make bioprocessing as competitive as any other process under development, when it comes to launching a product in good time. In certain cases, biocatalysis will enable production costs to be cut since cheaper starting materials can be used instead of precious enantiopure intermediates commonly encountered in traditional synthetic processes. The possible integration of a biocatalytic step in a sequence of reactions is considered more often today than has been the case in the past. This is probably due to the increasing variety of available biocatalysts as well as the growing awareness that biocatalysts are not difficult to work with.

In light of the increasing demand for environmentally friendly production processes, biocatalysis are gaining more ground in industry. The integration of enzyme-catalysed reactions with traditional synthetic methods will probably become more pronounced.

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(26) Kragl, U.; Gygax, D.; Ghisalba, O.; Wandrey, C. *Angew. Chem.* **1991**, *103*, 854.