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Coordination Chemistry Reviews 252 (2008) 767–781
COORDINATION CHEMISTRY REVIEWS
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Review

Selected chiral alcohols: Enzymic resolution and reduction of convenient substrates

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Received 29 June 2007; accepted 27 September 2007
Available online 2 October 2007

Contents

Abstract

Selected synthetic ways of preparation of chiral alcohols are reviewed. In this review, special attention is focused on preparation of enantiomerically pure cycloalkanols by employing enzymic processes, which are considered to be advanced for their sustainable and 'green' nature. Selected processes reviewed are: (a) enzymic resolution through transesterification of racemic alcohols, (b) enzymic resolution through hydrolysis of convenient racemic esters, and (c) enzymic reduction of prochiral ketones. Principles of prediction of the absolute configuration of the products are presented. Due to a number of recent reviews on this topic only a limited number of examples are shown in this review.

Keywords: Lipase; Oxido-reductase; Alcohol; Ester; Ketone; Enantiomer; Diastereoisomer

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doi:10.1016/j.ccr.2007.09.026

1. Introduction

A great number of strategies designed for synthesizing chiral synthons and chiral compounds include different chemoenzymic approaches. Increasing importance of using enzymes and/or microorganisms for relatively easy introduction of chiral centers into the new molecules has been mainly based on the fact that enzymes and microorganisms are able to transform a great variety of organic molecules, both natural and synthetic, into the requested chiral products with high enantioselectivity (natural products, pharmaceuticals, agrochemicals, etc.).

In this review, main attention – but not exclusively – is paid to the synthesis of chiral cycloalkanols. Potential and easy enzyme-mediated ways of their synthesis with high enantioselectivity consist in: (a) enzymic resolution through transesterification of racemic alcohols, (b) enzymic resolution through hydrolysis of convenient racemic esters, and (c) enzymic reduction of prochiral ketones. These procedures employing either lipases (enzyme-mediated resolution processes) or oxido-reductases (biocatalytic reduction of carbonyl compounds) have been frequently reviewed [1–11]. However, most of the reviews pay attention to acyclic aliphatic compounds. Enzyme-mediated synthesis of enantiopure cycloalkanols usually appears in several references within the reviews focused mostly on acyclic compounds. We have studied biocatalytic procedures of preparation of chiral cycloalkanols quite intensively, and, therefore, the examples given in this review are mostly – but not exclusively – focused on enzymic processes resulting in preparation of chiral cyclic alcohols as convenient intermediates in selected chemoenzymic processes using either of the above identified procedure.

2. Processes catalyzed by lipases

2.1. Enzymic resolution through transesterification of racemic alcohols

At present, enzymic catalysis in non-aqueous media has been extensively used where possible (except for enzymic resolution by hydrolysis) [1–3,5,12]. Water is a poor and often inconvenient solvent for nearly all applications in industrial chemistry, because most of the organic compounds of commercial interest are practically insoluble in water. Some organic compounds are sometimes unstable in aqueous media. Even the removal of water may be uneasy and expensive due to its high-boiling point and large heat of vaporization. Side reactions like hydrolysis, racemization, polymerization and decomposition may often decrease the practical value of such enzymic processes. Chemists recognized these limitations of using enzymes in aqueous media and started to develop enzymic procedures in organic solvents already a long time ago [2,12]. Biocatalytic transformations in organic solvents offer the following advantages:

(a) Non-polar substrates are more easily soluble in organic solvents and are converted at a faster rate, due to their increased solubility [12].
(b) Better overall yield and the recovery of the product are facilitated by the use of low-boiling-point organic solvents.
(c) Deactivation and/or substrate or product inhibition are mostly minimized.
(d) Side reactions such as hydrolysis, racemization, polymerization and decomposition are suppressed.
(e) Enzyme immobilization is not required; however, it is advantageous in industrial scale.
(f) The enzyme can be recovered by simple filtration.
(g) Denaturation of enzymes is minimized in most of the organic solvents, especially in those, which are not miscible with water, and where water activity can be controlled.
(h) Thermodynamic equilibrium is shifted in favor of the synthesis.
(i) Microbial contamination is negligible in the case of using living cells in biotransformations.

The use of enzymes in organic solvents, however, has also several disadvantages [12]:

(a) A decrease of enzyme catalytic activity, mainly due to the existence of the heterogeneous catalytic system.
(b) Enzymic reactions may be dependent on substrate or product concentration; if their concentration exceeds a specific level, further progress of the enzymic process can be blocked by inhibition by substrate or product concentration, which finally deactivates the enzymes, and results in a decrease of the reaction rate and enantioselectivity of the process.

2.2. Enzymic resolution through hydrolysis of derivatives of racemic alcohols

Enzymic hydrolysis is usually conducted in aqueous media [1–3,5,12], which presence means a problem to be solved with convenient application of organic substrates, which are mostly insoluble in water. Lipases are the enzymes acting on lipid/water interface, and if convenient shaking or stirring of the reaction mixture is used to extend the size of this interface, then the hydrolytic process takes place easily, and the reaction proceeds towards the chiral product. Conversion of any enzymic resolution process means that just one of the enantiomers present in the substrate racemate is converted into the product, while the remaining unreacted substrate represents a derivative of the opposite enantiomer. The process is kinetically driven, which means in practice that the rates of hydrolysis of both substrate enantiomers present in the original racemate to be resolved are different, however, concentration dependent. Therefore, a careful determination of the conversion rate of the enzyme-mediated resolution process is one of the key factors of efficiency of this enzymic process.

2.3. Prediction of the absolute configuration of the product in lipase-mediated processes

Probably the most detailed approach to predicting the absolute configuration of the products in lipase-mediated resolution was described by Ehrler and Seebach [13] and Hultin and Jones

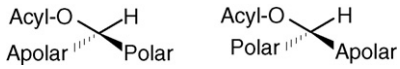


Fig. 1. Models (A – left, B – right) for predicting the absolute configuration of the products in lipase-mediated processes.

[14]. Nevertheless, based on their methods, absolute configuration of the products was not predicted correctly in all examples they presented [13,14]. We have tried to improve their models by introducing another feature of the substituents than simply their molecular volume. Our approach [15] has been based on the modified models A and B (see Fig. 1) in order to compare them with each other, and to compare them with the models built earlier [13,14] in predicting of the absolute configuration of the products expected. The explanation [15] of the nature of the respective substituents located on the chiral center studied is different from that of Hultin and Jones [14], but closer to that mentioned by Ehrler and Seebach [13].

The models A and B (Fig. 1) are based on an assumption that both the hydrophilicity and the hydrophobicity of the "polar" and "apolar" moieties are relative conceptions, which should be extra determined for any structure studied. It seems that the following findings might help in predicting the absolute configuration of the hydrolysis products to be in correspondence with either model A or B (Fig. 1) [15]:

(a) The larger is a distance of the "hydrophilic" moiety from the chiral center studied, the smaller is its effect in the sense of its influence on the "hydrophilicity" of the "polar" site.
(b) An anticipated priority of the "hydrophilic" moiety located in the immediate neighborhood to the chiral center.
(c) An anticipated influence of the space orientation of non-participating substituents on final "hydrophilicity" or "hydrophobicity" of the respective "polar" and "apolar" substituents.

Prediction of the absolute configuration of the product has to follow the above rules [15]. The polar-site prefers moieties more polar in comparison with the apolar-site, but the findings (a)–(c) mentioned in the text should be respected. This means that the polar-site need not be the part of the molecule bearing the most polar moiety present in it, but the part of the molecule which is in agreement with the findings (a)–(c) mentioned in the text in the best way. The apolar-site prefers less polar moieties, the meaning of which can be explained on the same basis as did for the polar-site. The size of both respective sites seems to be inferiorly important. The acyl-site represents the catalytic region for the PPL-mediated hydrolysis. The H-site prefers the smallest substituent possible (a hydrogen atom); small alkyl group are also accepted, however, the larger is the substituent in this site, the worse is the enantiomeric purity of the product obtained.

2.4. Lipases

Lipases (E.C.3.1.1.3) are enzymes [1,16–18] belonging to the family of serine hydrolases and can be found in animals, plants, fungi and bacteria. Because enzymes are usually named after the

Fig. 2. The reversible process of triacylglycerol hydrolysis/formation catalyzed by lipases.

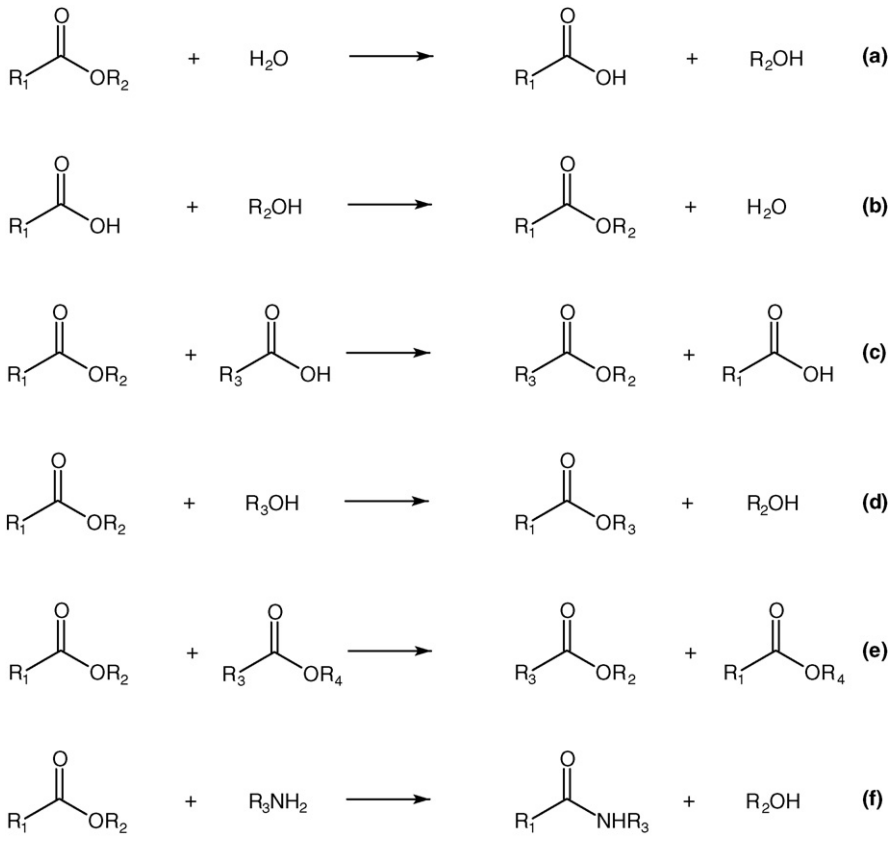
type of reaction they catalyze, lipases are sometimes redefined as carboxylesterases acting on long-chain acylglycerols, and are often termed triacylglycerol hydrolases. Because the reaction they catalyze is reversible, they can also catalyze the formation of acylglycerols from free fatty acids and glycerol (Fig. 2). In the industrially developed countries, the lipids present in the human diet consist mainly of triacylglycerols (TAGs), from 100 to about 150 g/day, i.e., 30% of each individual's daily caloric intake and these TAG molecules cannot cross the intestinal barrier.

A series of hydrolytic and absorption stages are therefore necessary to produce the chemical energy resources present in the hydrocarbon chains of biologically usable TAGs. Lipases in the digestive tract play an important role in nutrition processes, both in humans and in higher animals [19].

Some lipases show ability for controlling access to their active site. However, most of the lipases, which are used in laboratory investigations and/or in industrial production, are substrate tolerant enzymes, which accept a large variety of natural and synthetic substrates for biotransformation. Lipases do not require cofactors. They are often used in both, free or immobilized forms. They are commercially available, relatively inexpensive, and display relatively high stability. They act at the lipid–water interface and, therefore, they do not require water-soluble substrates. This function distinguishes lipases from other hydrolytic enzymes, and their efficiency in conducting transformations in organic solvents under mild conditions increases their importance as useful tools in organic synthesis [1–3,5,12,16–18]. In principle, lipases can be used in synthetic organic chemistry to catalyze several resolution processes (Scheme 1). These processes are: (a) enzymic hydrolysis (usually performed in aqueous media), (b) enzymic esterification, (c) enzymic transesterification by acidolysis, (d) enzymic transesterification by alcoholysis, (e) enzymic interesterification and (f) enzymic aminolysis.

2.4.1. Lipases in organic solvents

Transesterification of racemic alcohols (acyl transfer reactions) can be similarly conducted in a water-free medium (Scheme 1) and can be divided into alcoholysis, acidolysis and interesterification (ester–ester interchange) [1–3,20,21]. Direct esterification and alcoholysis of racemic alcohols have been frequently used in lipase-mediated synthesis of enantiomerically pure alcohols. Conditions favoring rapid and irreversible reactions are necessary for achieving of high enantioselectivity in a kinetic resolution process. The enantiomeric purity of the product and of the remaining unreacted substrate decreases if the reverse reaction (hydrolysis) appears significantly, mainly due to the reversible nature of the esterification and the interesterification in biocatalytic kinetic resolutions. Employing enol esters (vinyl or isopropenyl esters) results in a formation of



Scheme 1. Resolution processes catalyzed by lipases.

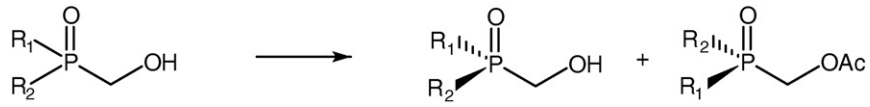
vinyl or isopropenyl alcohol which undergo to a keto–enol tautomerization, and the enzymic reaction becomes practically irreversible.

The direction of the reaction is dependent on the adequate solvent medium used, e.g., aqueous or organic solvents. Davison et al. [22] discussed several types of vapor-phase enzyme reaction schemes that are theoretically possible and formulated nomenclature and methodologies for non-aqueous biocatalysis. Catalysis of vapor-phase reactions employing whole cell systems (e.g., biofiltration of organic vapors) has been well studied [23]. The majority of these systems are not intended for the synthesis of fine chemicals, but for removal and/or destruction of organic gases and odors. Comparatively, progress for synthetic reactions is lacking. Lamare and Legoy [24] and Parvaresh et al. [25] have investigated lipase-catalyzed transesterification reactions in the gas phase and discussed various characteristics associated with solid–gas biocatalysis. Barton et al. [26] reported vapor-phase enzymatic synthesis of ethyl esters in bench-scale reactors and were successful in achieving considerable yields. However, limitations due to the types of organic vapors used were noticed. In practice, enzyme activity in such systems increased substantially as water activity increased with concomitant decrease in thermostability. More recently, using lipases in non-aqueous media has been extended to include supercritical fluids [27–29], and ionic liquids [9,30,31]. Application of these media may reduce the quantities of waste volatile compounds, which is an important step in the direction of sustainable and green chemistry.

2.4.2. Lipases in supercritical carbon dioxide

The advantage of application of biocatalysts in organic solvents was the basis for searching also for other, possibly less toxic reaction media, which could show more sustainable and green features. Among the considered media, supercritical fluids proved to have unique properties, which can be applied to a wide range of advanced chemical processes [32–36]. Supercritical carbon dioxide (SC-CO_2), defined as carbon dioxide existing only under the conditions above its critical point, has the added benefits of sustainable nature, inflammability, low toxicity and availability. It exhibits similar properties to organic solvents, and is able to dissolve a large variety of natural or synthetic organic compounds. When required or helpful for improving its properties, it can be mixed with different modifiers, co-solvents usually miscible with water. In turn, it differs from ordinary organic solvents by having a combination of gas-like properties (e.g., low viscosity and high diffusivity), and liquid-like properties (e.g., solubilizing power) [37]. These properties can be further modified by changing pressure and/or temperature, and by addition of a modifier (Fig. 3), and show SC-CO_2 as convenient green and sustainable medium for designing enzymic processes [37].

The reports on enzyme-catalyzed reactions in SC-CO_2 have started to appear since 1980s (Randolph et al. [38], Hammond et al. [39], Nakamura et al. [40], Mori et al. [41,42] and Kamat et al. [43,44]. The beneficial effects of SC-CO_2 for lipase-mediated reactions using either flow-type or batch-type reactors have been demonstrated by Matsuda et al. [37] (Fig. 3). Among many lipases tested, *Candida antarctica* lipase (CAL) was the best



Scheme 2. Transesterification of racemic substrates by vinyl acetate catalyzed by *Candida antarctica* lipase (CAL).

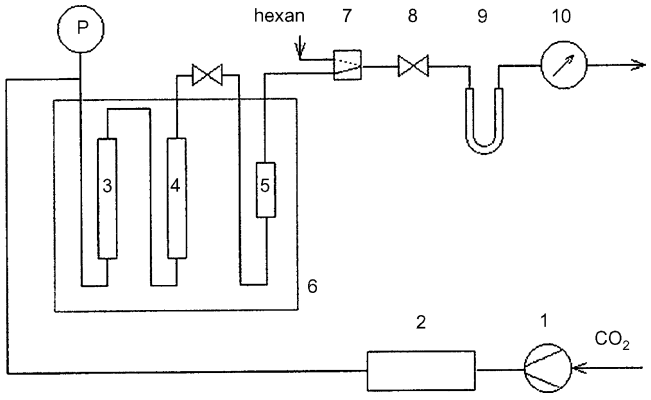


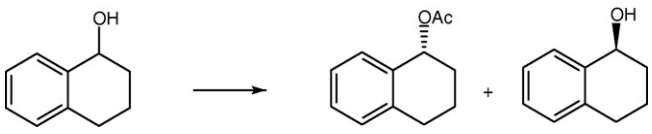
Fig. 3. System for performing enzymic reactions in SC-CO_2: (1) compressor, (2) pressure control unit, (3) and (4) optional mixing or saturation units, (5) reactor, (6) oven, (7) rinsing valve, (8) micrometer valve, (9) trap, (10) gas meter.

enzyme in conducting the enantioselective transesterification using vinyl acetate as an acyl donor in SC-CO_2 (Scheme 2). Both the reactivity and stereoselectivity of the reaction could be largely improved by changing the pressure. Thus, at pressures up to 8 MPa, i.e., below the critical density, the reaction did not proceed at all. When changing the pressure, the reaction conversion could be improved. The author concluded that it was important to increase the pressure so that the density of carbon dioxide exceeds its critical density. The reaction was the fastest when the pressure was close to the critical pressure: at 11 MPa, the reaction rate reached its maximum, and then gradually decreased if the pressure was increased up to 15 MPa. The optimal conditions were obtained at 13 MPa, at which point the reaction stopped at approx. 50% conversion and the enantioselectivity was high. Controlling the reaction course by changing the solvent properties is an advantage of supercritical fluids.

Other examples, mentioned above, of the lipase-mediated reaction in SC-CO_2 have been reviewed [27–29]. Scheme 3 shows the resolution of 1,2,3,4-tetrahydronaphthalen-1-ol by transesterification [29].

2.4.3. Lipases in ionic liquids

A considerable amount of work has been performed to understand solvent effects on the structure and function of enzymes in order to select more stable and efficient biocatalysts [45–47], recently reviewed by Yang and Pan [9]. Most of the organic



Scheme 3. Resolution of 1,2,3,4-tetrahydronaphthalen-1-ol by transesterification in SC-CO_2.

media have a number of disadvantages, including toxicity to the environment, volatility and flammability [48].

Ionic liquids are solutions composed entirely of ions [30,49,50]. They are relatively polar solvents and promote the dissolution of a vast array of pharmaceutical intermediates and final drug substance (target) molecules [9,51]. The replacement of conventional solvents in biocatalytic processes by ionic liquids could therefore overcome many of the disadvantages associated with organic solvents. The ability to modify the physico-chemical properties of these solvents by simple structural modifications to the cations or changes in the anions increases the importance of ionic liquids in organic chemistry [9,30,31,52,53].

Lipases are active in anhydrous ionic liquids [54]. Sheldon coworkers [54] examined the activity of *C. antarctica* lipase B (CAL-B) in ionic liquids for alcoholysis, aminolysis and perhydrolysis, discovering similar reaction rates, compared to the reactions performed in organic solvents such as propan-2-ol and butan-1-ol. Subsequently, other groups have investigated lipases in ionic liquids, due to the widespread use of these enzymes in industry [55]. These workers have generally reported good enzyme activity and stability [56]. Park and Kazlauskas [57] investigated the regioselective acylation of glucose in ionic liquids and obtained much higher yields and selectivities than in commonly used organic solvents, due to the high solvation properties of ionic liquids for both hydrophobic and hydrophilic reactants. They also demonstrated the influence of an additional purification procedures following ionic liquid synthesis to remove impurities and enhance enzyme activity. This is of particular significance when silver salts (e.g., $AgBF_4$ and $AgPF_6$) are used to prepare ionic liquids, silver being a well-known enzyme inhibitor [57]. Berberich et al. [58] have demonstrated the importance of controlling the water content in ionic liquids, in order to achieve higher conversion. Roberts et al. [52] reported the CAL-B-catalyzed resolution of 2,3,4,5-tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic acid methyl ester, a key lotrafiban intermediate, in six ionic liquids to afford (2*S*)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic acid leaving the unreacted (2*R*)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic acid methyl ester and (*R*)-2,3,4,5-tetrahydro-4-methyl-3-oxo-1*H*-1,4-benzodiazepine-2-acetic acid methyl ester in optically pure form. The results have been compared with the industrial process operated in *tert*-butanol [59]. The authors [58] concluded that replacing the organic solvent by an ionic liquid under otherwise identical conditions reduced the rate of conversion.

Sheldon coworkers [53] studied the effect of ionic liquids on the activity of Novozym 435 (an immobilized version of CAL-B) on a model example consisting of the CAL-B-catalyzed alcoholysis (transesterification) of ethyl butanoate with butan-1-ol to

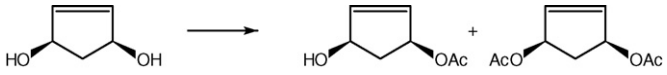
give 1-butyl butanoate and compared the results with that carried out in *tert*-butanol. The results revealed that, when the reaction was performed in ionic liquids containing a nitrate, ethyl sulfate or lactate anion, in which the CAL-B is soluble, the reaction rate was at least 10-fold slower. The authors [53] concluded that the ionic liquids interact with the protein sufficiently and strongly affect its dissolution, but also induce a structural change that leads to a loss of activity. Lozano et al. [60] have combined ionic liquids with supercritical carbon dioxide in lipase catalysis. Thus, free and immobilized *C. antarctica* lipase B (CAL-B) dispersed in ionic liquids (1-ethyl-3-methylimidazolium bistriflimide and 1-butyl-3-methylimidazolium bistriflimide) were used as catalysts for the continuous kinetic resolution of *rac*-1-phenylethanol in supercritical carbon dioxide at 120 or 150 °C and 10 MPa to afford the (*R*) ester product, and leaving the (*S*) unreacted alcohol. Excellent activity, stability and enantioselectivity levels were observed in the continuous operation (Fig. 3).

2.4.4. Lipases immobilized by sol–gel doped material

Catalysis by sol–gel doped materials, porous metal oxides confining an active species, obtained by a hydrolysis/condensation reaction of suitable precursors, has become in the last 20 years a prominent tool to synthesize a vast number of useful molecules both in the laboratory and in industrial plants [61–64]. Biological species such as enzymes, whole cells, antibodies and even bacteria [65] can all be entrapped in silica sol–gel matrix with only minor loss of activity. Generally, however, conventional sol–gel entrapment may largely reduce the original enzyme activity. A major point, in fact, concerns the secondary and tertiary structures of these giant molecules, which is often altered (denaturated) by the constraints imposed by confinement in the sol–gel cages and by the alcohol released in the condensation process.

Showing its unmatched potential in materials synthesis, the sol–gel method profound versatility was recently exploited to solve the problem by simply replacing the silicon alkoxides precursors with glycerol-derived polyol silicates [66,67]. In this manner, enzymes and *cells* are easily immobilized in biosilica gels, which display activities approaching those of the free biologicals, together with the high stabilities and robustness that characterize sol–gel bioceramics. The method can be extended to metallosilicate, alkylsiloxane, functionalized siloxane and composite sol–gels, allowing the fabrication of a physico-chemically diverse range of bio-doped polymers, which will find a multitude of applications in the near future.

Catalytic sol–gel lipase immobilizates rapidly reached the market after their invention in 1995 because of their remarkable activity and stability. The original procedure for the encapsulation produced by the fluoride-catalyzed hydrolysis of mixtures of $RSi(OCH_3)_3$ and $Si(OCH_3)_4$ has been improved considerably with higher enzyme loading, variation of the alkylsilane precursor, and the use of additives [6]. Fluka commercialized a vast set of these sol–gels which are also excellent catalysts in the kinetic resolution of chiral alcohols and amines, and are recyclable without any substantial loss in enantioselectivity (residual activity of 70% even after 20 reaction cycles). Another fundamental sol–gel biocatalytic process is commercially carried out by the Italian company Indena, which produces taxol (paclitaxel) over silica-entrapped *Taxus* cells, using the process developed by Carturan in 1989 in which an aqueous TEOS sol is sprayed over a suspension of living cells [68] resulting, in this case, in a bioreactor system which affords the synthesis of a precious anticancer drug without destroying the *Pacific yew* trees from whose bark the compound was extracted in very low yields. The process prevents the cells death and affords a whole set of bioactive materials whose enormous potential awaits full exploitation in catalysis and in many other fields.



Scheme 4. Resolution of a cyclic vicinal diol by transesterification with vinyl acetate in organic solvents catalyzed by Novozyme 435.

Avecia licensed the Johnson Matthey's sol–gel technology for preparing organic–inorganic silica hybrid gels doped with chiral ligands [69,70]. These catalysts allow easy conversion of aldehydes and ketones into chiral cyanohydrins, high value building blocks and useful precursors for hydroxyamino acids and amino alcohols.

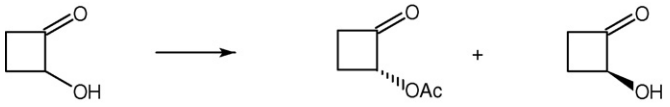
The new sol–gel technology, named Chiral Technologies Interface System, dramatically improved process economics for large scale pharmaceutical manufacturing as it raises the turnover number of the catalyst compared with its homogeneous version, while maintaining enantiomeric excess above 90%. Clearly, the competitive advantage offered here by these sol–gel catalysts will push many other companies to adopt commercial sol–gel catalysts for their fine chemicals productions, while Johnson Matthey itself is continuing to develop a line of chiral sol–gel catalytic materials in joint development with Avecia [64].

Companies such as BASF, for instance, operate large units of research working on sol–gel catalysts, and are easily envisaged to reach the market with proprietary technology to deliver more economic and sustainable manufacture of chiral compounds and fine chemicals by molecular sol–gel catalysts [64].

2.5. Lipase-mediated resolution of racemic substrates: synthesis of enantiomerically pure secondary alcohols

Secondary alcohols and their esters are frequently used as excellent targets in lipase-catalyzed kinetic resolutions [71,72]. These compounds are often used as chiral synthons in organic synthesis. Higher enantioselectivity in resolution can be achieved with the secondary alcohols, compared to primary and tertiary ones, which are difficult to achieve [73,74]. Transesterification of a substrate (Scheme 4) catalyzed by Novozyme 435 using vinyl acetate in organic solvents afforded the (*R*)-ester in >99% enantiomeric purity, and the unreacted (*S*)-substrate was also recovered with high enantiomeric purity (up to 99%) [75,76].

The reduction of acetophenones with sodium borohydride in the presence of neutral alumina in *n*-hexane followed by enantioselective acylation catalyzed by lipases was performed in one pot. Starting from the readily available



Scheme 5. Resolution of 2-hydroxybutanone by transesterification achieved by catalysis with several lipases in organic solvents.

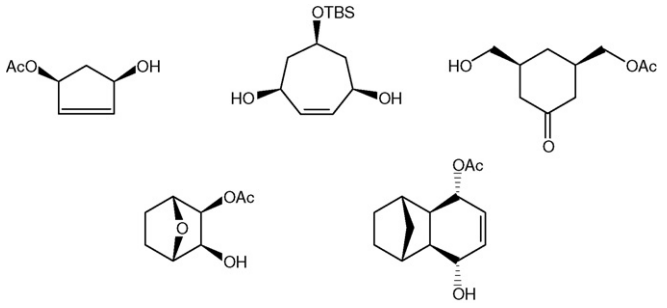


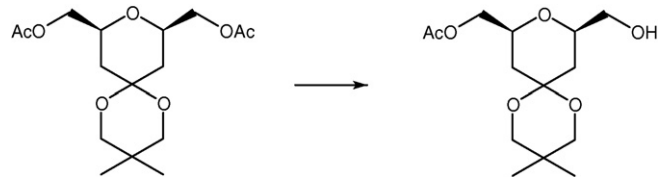
Fig. 4. Examples of enantiomerically pure products of enzymic resolution of the corresponding racemic substrates in organic solvents; The racemic substrates bear more than one functionality.

2-hydroxycyclobutanone (Scheme 5) or the corresponding acetals, Hazelard et al. [77] reported the preparation of optically active 2-hydroxycyclobutanone and derivatives, by enantioselective transesterification with various lipases in organic solvents. Due to the racemization drawback, the alcohol was recovered in almost racemic form and in a low yield, while the (*R*)-ester was obtained in up to 89% enantiomeric purity, depending on the lipase, the acyl donor and the solvent used.

Other secondary alcohols containing benzofuran, azide, alkylthio, carboxylic acid ethyl ester and α-methylene-β-hydroxy ester moieties have been successfully resolved (cf. Fig. 4 and Scheme 6) [2,18].

A rapid screening of different hydrolases for the enantioselective hydrolysis of esters of difficult to resolve substrates such as 1-methoxy-2-propanol, 3-butyn-2-ol, 3-hydroxy-tetrahydrofuran and other compounds was performed by Baumann et al. [78].

Biaryls and arylpyridines form the basic structure of many biologically active compounds [79], and are found in new materials such as electroluminescent conjugated polymers [80] and semiconductors [81]. Furthermore, their optically active forms have found widespread application as ligands in catalytic asymmetric synthesis [82]. These applications became the basis for investigations of the lipase-catalyzed resolution of 1-biaryl- and 1-(pyridylphenyl)alkan-1-ols [83]. Lipase B from *C. antarctica* (CAL-B) was used in the enantioselective transesterification of some 1-biaryl-2-yl-, -3-yl- and -4-yl-ethanols and -propan-1-ols, as well as 1-(2-, 3- and 4-pyridylphenyl)ethanols with vinyl



Scheme 6. Resolution by lipase-mediated hydrolysis.

acetate as the acyl donor to afford enantiomerically pure products. In the case of the resolution of 1,3- and 1,4-disubstituted substrates these were transformed within several hours (conversion degrees ranging from 23 to 50%), but the reaction rates for propan-1-ol derivatives were slower than those for ethanol derivatives. The transesterifications of 2-substituted alcohols took several days. These reactions were, however, accompanied by a chemoenzymic side reaction, which could be suppressed by the presence of isopropenyl acetate as the acyl donor. Using isopropenyl acetate as the acyl donor also resulted in increase of the enantioselectivity of the process [83].

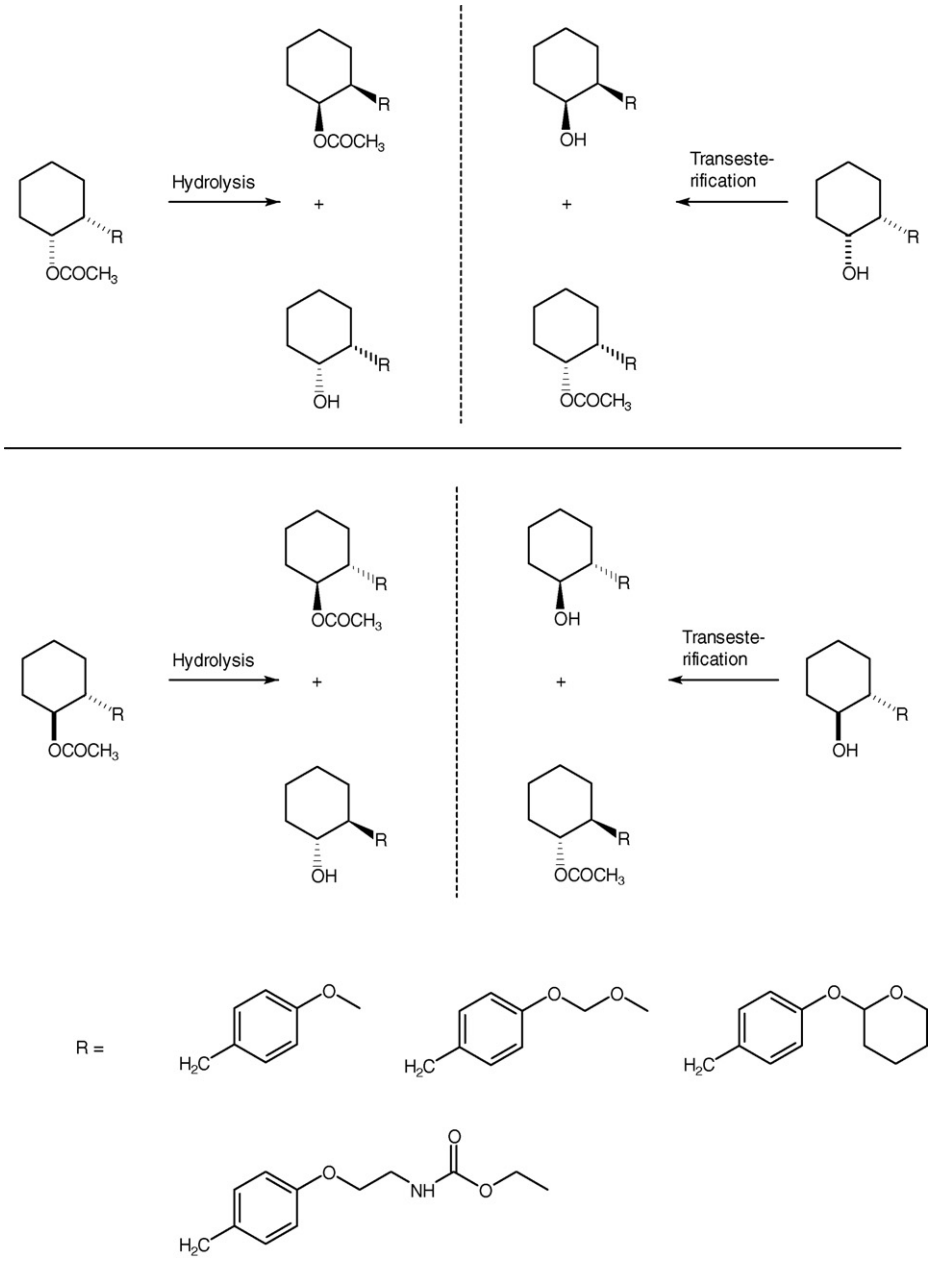
One of the most important advantages of lipase catalysis over traditional chemical catalysis that is evident in the above-mentioned examples of polymer synthesis is that lipases are not oxygen sensitive and benefit from the presence of water in reactions. In contrast to lipase catalysts, chemical catalysts are usually water sensitive and require moisture safeguards [84,85].

In our team, we have intensively studied lipase-mediated resolution processes using 2-(4-alkoxybenzyl)cycloalkan-1-ols and their ester derivatives (mostly acetates and butanoates) as substrates. Those compounds are general precursors of a series of insect juvenile hormone bioanalogs, we have been investigating for many years [15,86–101]. Lipase-catalyzed resolution processes (Scheme 7) enable in principle preparation of all possible stereoisomers of 2-(4-alkoxybenzyl)cycloalkan-1-ols. In practice, enantiomeric purity >99% of the product of the lipase-catalyzed resolution procedure has been achieved with a number of lipases employed. However, lower enantiomeric purity of the unreacted substrate, and the yields rarely exceeding 40% (i.e., 80% conversion) still creates important limitation against selecting this enzymic procedure as general approach in the synthesis of all possible stereoisomers of 2-(4-alkoxybenzyl)cycloalkan-1-ols. More details on our results can be found the respective original papers [15,86–101]. Lipase-catalyzed hydrolysis was performed in aqueous media, because this environment is close to the natural environment in which hydrolytic processes are realized by nature itself. In addition, we have also employed several ionic liquids as process auxiliaries to study their effect on enantioselectivity of this enzymic reaction, as well as their effect of the absolute configuration of the products. Lipase-catalyzed transesterification is the reaction dependent on water activity. Relatively high tolerance of lipases towards organic solvents was the basis for using different organic solvents as media for performing this type of lipase-catalyzed reactions.

3. Processes catalyzed by oxido-reductases

3.1. Enzyme-mediated reduction of carbonyl compounds

Enzymic reduction of carbonyl group represents one of the most important reaction employed in the synthesis of chiral alcohols [10]. Enzymes that can be used for catalysis of reduction of carbonyl group are dehydrogenases and oxido-reductases, which require the presence of a coenzyme, such as NADH or NADPH. The hydride anion is transferred from the cofactor to the carbonyl compound (the substrate), and NAD^+ or $NADP^+$ are formed. This is a limiting factor for application of the isolated

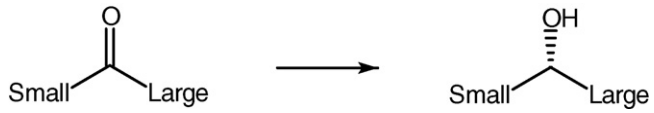


Scheme 7. Synthesis of all possible stereoisomers of 2-substituted cyclohexanols through lipase-mediated resolution processes.

dehydrogenase or oxido-reductase enzymes, because expensive and unstable coenzyme has to be added to the reaction mixture. In designing the bioreduction process to get chiral alcohols, microorganism whole cells can be advantageously used. Each cell represents a small factory fully equipped for the reduction of the substrate bearing the carbonyl moiety. The process designer has only to plan, in which way the ketonic substrate should be added, and which medium should be used for the designed reaction to make the conditions for the action of cell factories as good as possible. Nowadays, a wide variety of media and auxiliaries are available for enzyme-mediated reduction of carbonyl compounds [10].

In principle, employing microorganism whole cells is favored over using isolated enzymes, especially under industrial scale. In turn, methodologies are already developed, which enable use of isolated dehydrogenases, and which use auxiliary reagents for recycling the coenzymes:

(a) Alcohols, such as ethanol, 2-propanol, etc., which oxidize into easily volatile aldehydes or ketones.
(b) Sugars, such as glucose and glucose-6-phosphate.
(c) Formate, from which gaseous carbon dioxide is formed.
(d) Molecular hydrogen.
(e) Light energy, i.e., application of photochemical methods under the presence of a *Cyanobacterium*, a photosynthetic biocatalyst.
(f) Electric power, i.e. electrochemical regeneration of NADH and NADPH, which does not require additional enzyme.



Scheme 8. A model for predicting absolute configuration of the products in oxido-reductase catalyzed reductions.

3.2. A model for predicting diastereoselectivity in oxido-reductase catalyzed reductions

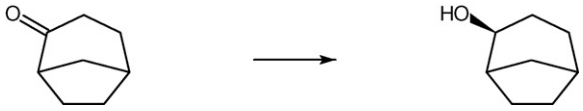
A model for predicting diastereoselectivity was created for using redesigned substrates for baker's yeast reductions, as well as on a proposed working model that rationalizes the diastereoselectivity obtained by reduction of α-substituted β-dicarbonyl compounds. Nowadays, this model is used for predicting diastereoselectivity with any biocatalytic reduction, in which microorganisms, mostly yeasts, are employed. A more general approach was developed by extrapolation of Prelog's rule [102]. This rule was formulated to account for the observed enantioselectivity in the reduction of decalones by *Curvularia lunata*, and has proved useful in predicting baker's yeast reductions as well [103]. In light of that knowledge [104], this generalization simply stated that predominant oxido-reductases deliver hydride anion to the *Re*-face of a prochiral ketone, producing an (*S*) chiral alcohol as illustrated for the small (S) and large (L) groups of the ketone (Scheme 8).

Following similar reasoning as in the formulation of the Prelog's rule, the size and hydrophobicity of the α-substituent is compared to that of the ester ligand. During the course of the reduction from the *Re*-face of the ketone, the larger ligand would reside in the same plane as the carbonyl, and the smaller, less hydrophobic ligand would fit in a smaller site opposite to the incoming hydride equivalent. The major product would exhibit a 3*S* hydroxyl as well as the adjacent configuration. When there is no clear difference in size between the α-substituent and the ester, mixtures of the two diastereoisomers would be produced.

The enantioselectivity of baker's yeast reductions of a given substrate is the result of the cumulative action of competing oxido-reductases of opposite chirality operating at varying rates, with the enzyme(s) that has the higher V_{max}/K_M ratio determining the stereochemical outcome [104]. Although the active sites of the dominant oxido-reductases of baker's yeast have not yet been defined, the proposed working model rationalizes the diastereoselectivity observed for all of the reported examples of baker's yeast reductions of α-substituted β-keto esters, and should be useful in predicting diastereoselectivity and in redesigning substrates for baker's yeast reductions.

Baker's yeast has proved to be an organism that can be advantageously used by the preparative organic chemist [10] as a chiral reducing agent. Although the substrate concentration is important [10,104] due to the presence of several reductase enzymes in the yeast organism, the yield and selectivity of reduction is quite reproducible.

Empirical studies appeared in the past years that make use of specific activation of enzymes via the use of mutants (reviewed by Nakamura and Matsuda [10]).



Scheme 9. Reduction of a bicyclic ketone by *Diplogelasinospora grovesii* IMI171018 with >98% enantiomeric purity.

3.3. Oxido-reductases

In searching for new biocatalysts, screening has been one of the most powerful methods for the identification of the suitable biocatalysis. Various novel methods for screening as well as classical methods have been reported (cf. Nakamura and Matsuda [10]). A screening of 416 strains (71 bacterial strains, 45 actinomycetes, 59 yeast, 60 basidiomycetes, 33 marine fungi and 148 filamentous fungi) has been performed to look for microorganisms that display reductase activity in the absence of oxidase activity [105]. A new microorganism, *Diplogelasinospora grovesii* IMI171018 was isolated and showed very high activity and stereoselectivity in the reduction of cyclic ketones. For example, the bicycloketone were reduced in >98% enantiomeric purity (Scheme 9).

Biocatalysts, discovered by screening, can been prepared in a large quantity by over-expression of the enzymes in transformed *Escherichia coli* [106–109]. For example, the gene encoding (6*R*)-2,2,6-trimethyl-1,4-cyclohexanedione (levodione) reductase was cloned from the genomic DNA of the soil-isolated bacterium *Corynebacterium aquaticum* M-13 (Scheme 10) [106]. The enzyme was sufficiently produced in recombinant *E. coli* cells and the enzyme purified from *E. coli*-catalyzed stereo- and regioselective reduction of levodione. The enzyme was strongly activated by monovalent cations, such as K^+, Na^+, and NH_4^+.

The chiral intermediate (*S*)-1-(2′-bromo-4′-fluorophenyl) ethanol was prepared by the enantioselective microbial reduction of 2-bromo-4-fluoroacetophenone [110]. Organisms from genus *Candida*, *Hansenula*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Sphingomonas* and Baker's yeast reduced the ketone to the corresponding alcohol in >90% yield and 99% enantiomeric purity. In an alternative approach, the enantioselective microbial reductions of methyl, ethyl, and *tert*-butyl 4-(2′-acetyl-5′-fluorophenyl)butanoates were demonstrated using different strains of *Candida* and *Pichia* species. Reaction yields of 40–53% and enantiomeric purity of 90–99% were obtained for the corresponding (*S*)-hydroxyester. The reductase, which catalyzed the enantioselective reduction of keto esters, was purified to homogeneity from cell extracts of *Pichia methanolica* SC13825. It was cloned and expressed in *E. coli* with recombinant cultures used for the enantioselective reduction of keto methyl ester to the corresponding (*S*)-hydroxymethylester. On a

Scheme 10. Reduction of (6*R*)-2,2,6-trimethyl-1,4-cyclohexanedione (levodione) by recombinant *E. coli* cells; The gene encoding levodione reductase was cloned from *Corynebacterium aquaticum* M-13 into *E. coli* cells.

preparative scale, a reaction yield of 98% and an enantiomeric purity of 99% were obtained [10,110].

The synthesis of ethyl (*R*)-4-chloro-3-hydroxy-butanoate ((*R*)-ECHB) from ethyl 4-chloroacetoacetate (ECAA) was studied using whole recombinant cells of *E. coli* expressing a secondary alcohol dehydrogenase of *Candida parapsilosis* [107]. Using 2-propanol as an energy source to regenerate NADH, the yield of (*R*)-ECHB reached 36.6 g/l (more than 99% enantiomeric purity, 95.2% conversion yield) without addition of NADH to the reaction mixture. On the other hand, a novel carbonyl reductase (KLCR1) that reduced ECAA to ECHB was purified from *Kluyveromyces lactis* [108]. KLC catalyzed the NADPH-dependent reduction of ECA enantioselectivity but not the oxidation of (*S*)-ECHB [108].

New bioreduction system for the production of chiral alcohol using an *E. coli* transformant co-expressing genes of carbonyl reductase and cofactor-regenerating enzymes has also been investigated [109]. An NADPH-dependent carbonyl reductase (S1) isolated from *Candida magnoliae*, which catalyzed the reduction of ethyl 4-chloro-3-oxobutanoate (COBE) to ethyl (*S*)-4-chloro-3-hydroxybutanoate (CHBE), with a 100% enantiomeric purity was used to construct the system. The S1 gene comprises 849 bp and encodes a polypeptide of 30 kDa, and the deduced amino acid sequence showed a high degree of similarity to those of the other members of the short-chain alcohol dehydrogenase superfamily [111]. The *E. coli* cells expressing both the carbonyl reductase gene and the glucose dehydrogenase (GDH) gene from *Bacillus megaterium* were used as the catalyst for reduction of ethyl 4-chloro-3-oxobutanoate to ethyl (*S*)-4-chloro-3-hydroxybutanoate [112]. In an organic–aqueous two-phase system, 450 g/l of the product was obtained in 89% yield and 100% enantiomeric purity.

3.4. Alternative medium engineering

Biocatalytic reduction has also been performed in non-aqueous solvents to improve the efficiency of the reaction. This section explains the use of organic solvent, supercritical fluids and ionic liquid.

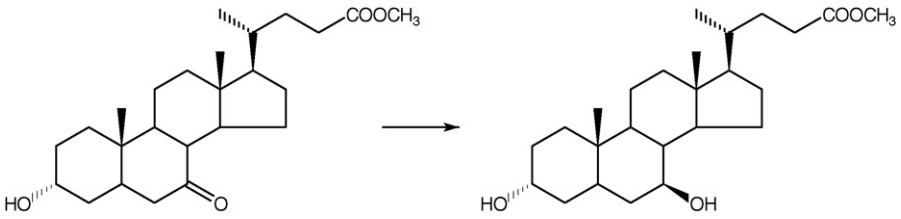
3.4.1. Oxido-reductases in organic solvents

Aqueous–organic two-phase reaction has been widely performed [113–115]. One of the purposes to use two-phase reaction system is to control the substrate concentration in aqueous phase where the biocatalysts exist. Hydrophobic substrate and products dissolve easily in the organic phase, so that the concentration in the aqueous phase decreases. The merits to control and decrease the substrate concentration in the aqueous phase are that:

(a) Substrate and product inhibition can be prevented.
(b) When whole cell containing plural enzymes with opposite selectivity and different Michaelis–Menten constant (K_M) values are used, problems of low selectivity occur. If the substrate concentration decreases, one of the enzymes with low K_M value catalyzes the reaction so that the selectivity can be improved.
(c) The decomposition of unstable substrate/product by aqueous buffer can be prevented by dissolving the substrate and product in the organic phase.

Therefore, organic solvents have been widely used for the biocatalytic reductions. One of the interesting examples for the stereochemical control by using organic solvent for the reduction is as follows. *Geotrichum candidum* IFO 4597 catalyzes the reduction of ketones although the reduction of acetophenone by the resting cell in water afforded only (*R*)-alcohol with 28% enantiomeric purity in a 52% yield [113]. The cells were immobilized on a water-absorbing polymer to spread it on the large surface of the polymer to use it in hexane [113]. The reduction in hexane by the immobilized cell barely proceeded because of the unfavorable partition of ketone and alcohol between the organic and aqueous layers for the reduction. However, when an alcohol such as hexan-2-ol was added to the reaction system the reduction proceeded smoothly to afford (*S*)-alcohol in excellent enantiomeric purity. Hexan-2-ol as well as other 2-alkanols from 2-propanol to 2-octanol worked when they were used in large excess and exerted saturation on the yield at 10–15 mol equiv. The mechanism of the enantioselectivity improvement by this method can be explained as follows: (a) Enantiomeric purity is poor for the reduction in water because both the *S*-enzyme(s) and *R*-enzyme(s) catalyze the reduction; (b) when hexane is used, both enzymes are inhibited; and (c) when alkan-2-ol was added, only an *S*-enzyme was re-activated and catalyzed the reduction (alkan-2-ols are selective to the *S*-enzyme). Various aryl methyl ketones were also reduced smoothly by the same system using 12 equiv. of hexan-2-ol to produce the corresponding (*S*)-alcohols in excellent enantiomeric purity. The reduction of 2-substituted acetophenones gives a relatively better yield than of 4-substituted acetophenones, probably because the reverse reaction, oxidation, does not proceed for the 1,2-disubstituted substrates. The better a substrate is for oxidation the worse it is for reduction of the substituted acetophenone derivatives.

In another example, methyl 7-ketolithocholate (Me-7KLCA) was reduced with *Eubacterium aerofaciens* JCM 7790 in anaerobic interface bioreactor and dihexyl ether was used as the solvent [114,115]. The microbe system reduced 12 g/l of Me-7KLCA to methylursodeoxycholate (Me-UDCA) in more than 50% yield. The product is the precursor of ursodeoxycholic acid which is used as a cholesterol gallstone-dissolving agent (Scheme 11). Derivatives of substituted 1,2,3,4-tetrahydronaphthalen-2-one can be reduced affording the corresponding (*S*) alcohol, as well as other cyclic ketones and β-keto esters (Scheme 12). Cyclic diketones were also reduced selectively by biocatalysts. Reduction of 1,2-indanone by the yeast *Trichosporon cutaneum* MY 1506 afforded (1*S*,2*R*)-1,2-indandiol in 75% yield and with >99% enantiomeric purity (Scheme 13) [116]. A screening of the bioreduction of bicyclo[2.2.2]octane-2,6-dione was performed using 262 strains of different yeasts, of which a major number afforded the (1*R*,4*S*,6*S*)-*endo*-product, while a minor number of strains afforded the (1*S*,4*R*,6*S*)-*endo*-product (Scheme 13) [117]. The (*S*)-monoalcohol was obtained in high enantiomeric purity by the reduction of 1,3-cyclohexanediones



Scheme 11. Reduction of methyl 7-ketolithocholate (concentration 12 g/l) to methyl ursodeoxycholate by *Eubacterium aerofaciens* JCM 7790 in anaerobic bioreactor using dihexyl ether as the solvent.

bearing two identical substituents *R* at C(2) of the cycle by the yeast (Scheme 13) [118].

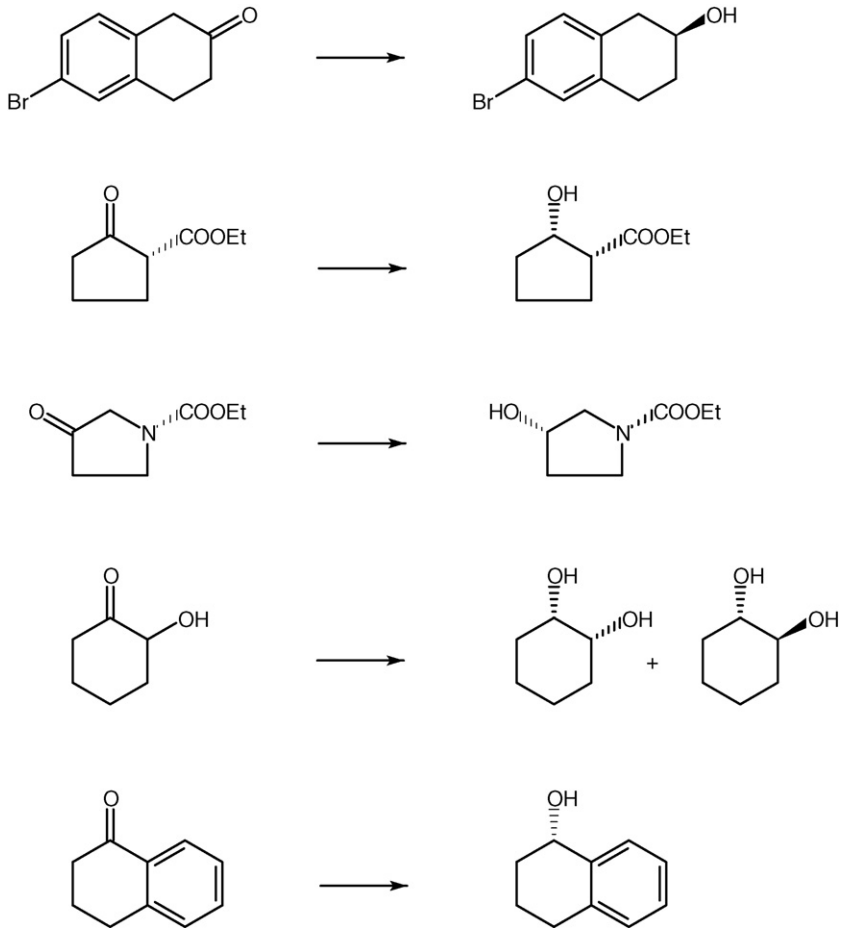
3.4.2. Oxido-reductases under the presence of hydrophobic resin (XAD)

Instead of using organic solvents, hydrophobic resin, Amberlite TM XAD, has been used to control the substrate concentration [119–121]. When XAD is added to the reaction mixture, substrate and products are adsorbed to the hydrophobic resin, XAD, since the substrate and product are usually hydrophobic. Therefore, the affective concentration of the substrate around the enzyme is decreased.

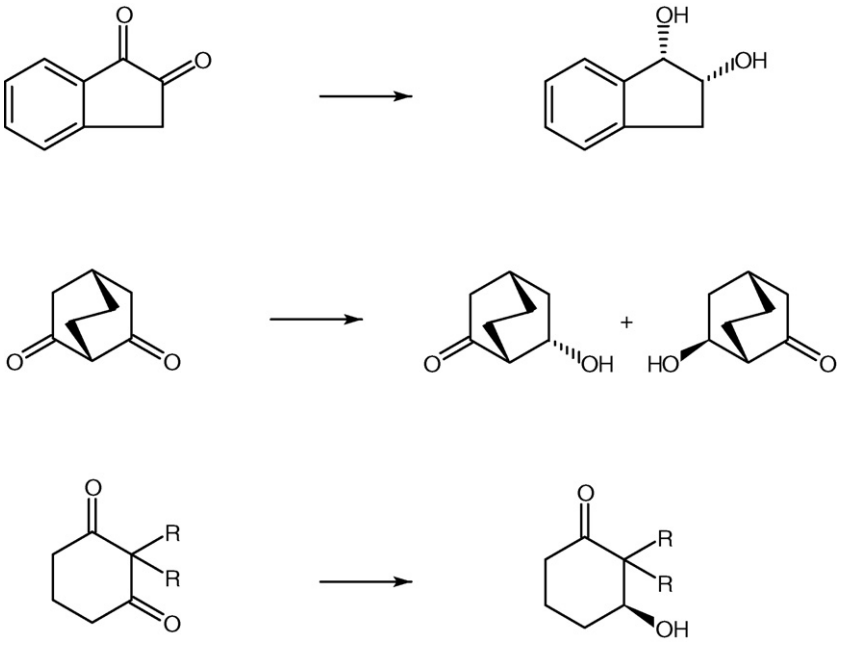
Recently, XAD was used as material to control the stereochemical course of microbial reductions [119]. In the presence of XAD, simple aliphatic and aromatic ketones were reduced to the corresponding (*S*)-alcohols in excellent enantioselectivity while low enantioselectivity was observed in the absence of the polymer.

In the reduction of benzoyloxypropanone, the hydrophobic polymer, XAD-7 was used to prevent product inhibition and to increase substrate concentration [120]. Thus, the reduction proceeded in 70 g/l substrate concentration and afforded 87% (12.4 g) of (*S*)-1-benzoyloxy-2-propanol in >99% enantiomeric purity. The butanone derivative could be reduced with the same method and afforded (*S*)-alcohol in 72% yield and >99% enantiomeric purity, however, the pentanone derivative could not reduced.

An acyclic enone, 2-ethyl-1-phenylprop-2-en-1-one, was reduced with the yeast *Pichia stipitis* CCT 2617 [121]. The reduction proceeded chemo- and enantioselectively to afford (*S*)-



Scheme 12. Examples of reduction of different cycloalkanones to enantiomerically pure products.



Scheme 13. Examples of reduction of α- and β-diketones by yeasts.

2-ethyl-1-phenylprop-2-en-1-ol (65% yield, >99% enantiomeric purity). XAD-7 was used to decrease and control the concentration of both substrate and product.

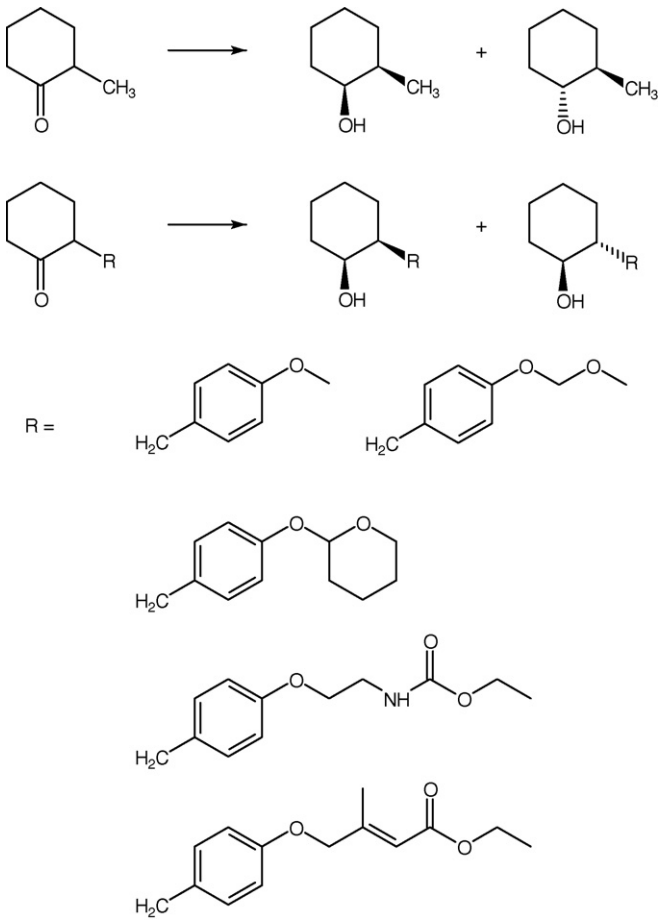
3.4.3. Oxido-reductases in supercritical carbon dioxide

Reduction using alcohol dehydrogenases is usually conducted in aqueous media. The difficulties encountered in such reactions are the extraction of products which dissolve in aqueous media at low concentration, and organic solvent is usually used. However, by using SC-CO_2 this becomes unnecessary because CO_2 transforms into a gas as the pressure decreases. Therefore, reduction of carbonyl group using alcohol dehydrogenases was conducted in SC-CO_2. For example, immobilized cell of *Geotrichum candidum* were used for the reduction in SC-CO_2 [27,28]. Since the whole resting cells was used, the addition of expensive coenzymes was avoided. Moreover, the solubility of the coenzymes in SC-CO_2 did not need to be considered. At first, the reduction of *o*-fluoroacetophenone in SC-CO_2 at 10 MPa was conducted using 2-propanol as a reductant (hydrogen donor) which afforded (*S*)-1-(2-fluorophenyl)ethanol in 81% yield (determined by GC) after 12 h [27]. The time course of the reaction shows that the yield increased with the reaction time, which proved that the alcohol dehydrogenase catalyzed the reduction in the supercritical condition.

The substrate specificity was investigated, and it was found that the enzymatic reduction in SC-CO_2 proceeded for various ketones. Acetophenone, acetophenone derivatives, benzyl acetone and cyclohexanone were used as substrates, and it was found that all of them were reduced by the alcohol dehydrogenase in SC-CO_2 with 2-propanol. The effects of fluorine substitution at the *ortho*, *meta* and *para*-positions of acetophenone were obvious. Compared with the unsubstituted analogue, substitution at the *ortho* or *meta* position increased the yield, whereas substitution at the *para* position decreased the yield. Concerning enantioselectivity, very high values (>99% enantiomeric purity) were obtained for the reduction with the majority of the substrates tested, while slightly lower enantioselectivity was observed for a few of them. Enantioselectivity values obtained in this system are superior or at least equal to those for most other biocatalytic and chemical systems.

The immobilized resting-cell of *G. candidum* was also used as a catalyst for the reduction of 2-fluoroacetophenone and cyclohexanone in a semi-continuous flow process using SC-CO_2 [28]. With flow reactors, the addition of a substrate to the column with a catalyst yielded the product and CO_2, which is a gas at ambient pressure, whereas, with the batch reactor, separation of the product from the biocatalyst was necessary after depressurization. Therefore, the flow type was superior to the bath type for achieving virtually no solvent reaction. Moreover, the size of the reactors using the flow process to generate an amount of product comparable with the corresponding bath reactors is smaller, which is particularly attractive for a supercritical fluid system. This reaction using a semi-continuous flow process also resulted in a higher space–time yield than that of the corresponding batch process.

An isolated enzyme is also used for the reduction in SC-CO_2 [122]. Horse liver alcohol dehydrogenase (HLADH) was used for reduction of butanal. In this case, addition of coenzyme is necessary, and the sample with a soluble coenzyme, fluorinated coenzyme, demonstrated the highest activity. A sample with enzyme alone and a sample with added NAD^+ showed similar rate of butanol production. NAD^+ added separately from the enzyme did not change native HLADH activity because the enzyme and NAD^+ remained separate during the reaction. The HLADH lyophilized with NAD^+ produced very little butanol due to mass transfer limitations.



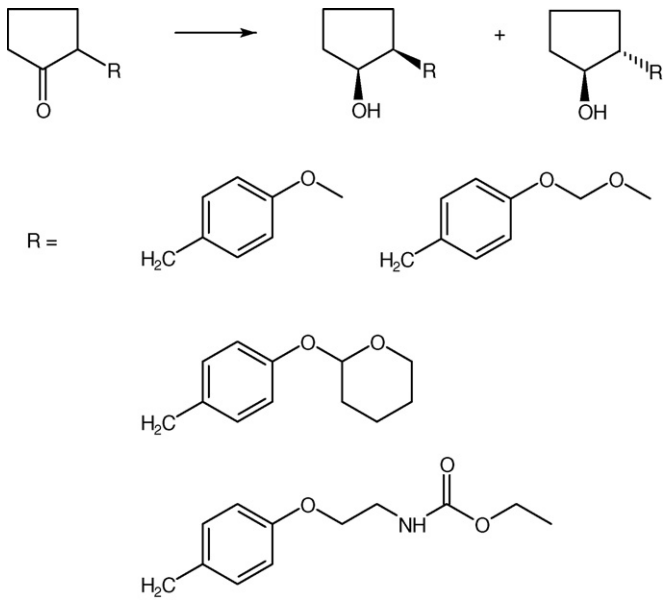
Scheme 14. Reduction of 2-substituted cyclohexanones by *Saccharomyces cerevisiae* and *Geotrichum* sp. whole cells under different conditions.

3.4.4. Oxido-reductases in ionic liquids

Ionic liquids can be used as a solvent in yeast reduction [123]. The reduction of ketones with baker's yeast immobilized on alginate in a 100:10:2 [BMIM]PF_6 ionic liquid/water/methanol mixture proceeded, and afforded chiral alcohols [123].

3.4.5. Enantioselective reduction of 2-(4-alkoxybenzyl)cycloalkan-1-ones

In our team, different strains of *Saccharomyces cerevisiae* and *Geotrichum* species were employed for stereospecific reduction of 2-(4-alkoxybenzyl)cycloalkan-1-ones [124–132] (Schemes 14 and 15). Aqueous media proved to be convenient media for performing the reactions mediated by non-immobilized microorganisms. We have also applied organic solvents as media in these processes, however, due to substantially lower yields of the products, and lower enantioselectivity of the process, we have preferably studied these processes in aqueous media. We made a number of modifications of the processes mediated by the yeasts, testing a number of different strains of both yeast species, adding substrates in different stages of the process, performing the bioreduction process both in cultivation media and in a fresh buffer solution, and also adding the hydrophobic polymer XAD-7 to the reaction mixture. In all cases, with exception of the processes performed under the



Scheme 15. Reduction of 2-substituted cyclopentanones by *Saccharomyces cerevisiae* whole cells under different conditions.

presence of the hydrophobic polymer XAD-7, the absolute configuration of the products followed the Prelog's rule, and the products were usually obtained at least with >95% enantiomeric purity. Even with XAD-7, the absolute configuration of the products was (*S*) on the carbon center bearing the newly formed hydroxyl, however, the enantiomeric purity of the products was substantially lower. A conclusion can be made on the basis of this finding that the presence of the hydrophobic polymer XAD-7 probably affects the enantioselectivity of the process, and may partly enable the access of the hydride anion from the *Si*-face (*anti*-Prelog rule). However, we did not had the strain of *G. candidum* described by Nakamura et al. [133] to our disposal, and, probably XAD-7 was unable to suppress the (*S*)-isoenzyme against the (*R*)-isoenzyme in the microorganism, as the Japanese authors describe in their papers [133]. Further elaboration of this process will be needed in a near future.

Acknowledgment

A financial support from the National Research Program II through the project 2B06024 (SUPRAFYT) is gratefully acknowledged.

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