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Combination of Asymmetric Organo- and Biocatalytic Reactions in Organic Media Using Immobilized Catalysts in Different Compartments

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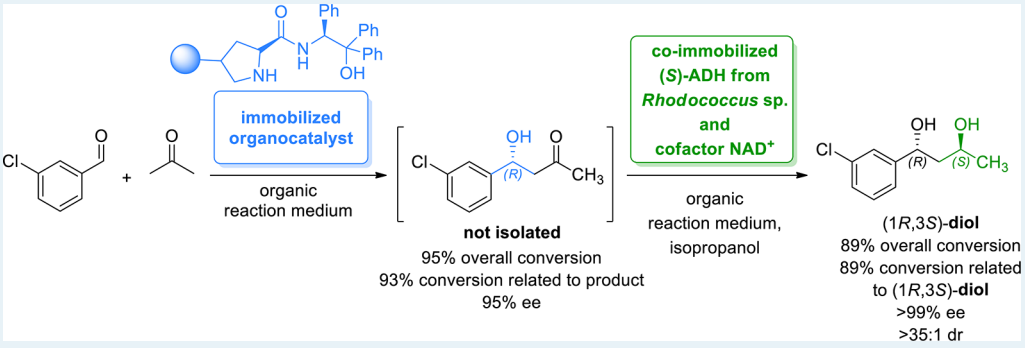
Supporting Information



ABSTRACT: A proof of concept for the combination of an asymmetric organocatalytic reaction with a biotransformation toward a "one-pot like" process for 1,3-diols based on immobilized organo- and biocatalysts, which are utilized in different compartments, is demonstrated. This process which runs completely in organic media consists of an initial proline-derivative-catalyzed aldol reaction and a subsequent reduction of the aldol adduct catalyzed by an alcohol dehydrogenase (ADH) without the need for intermediate isolation. Economically attractive superabsorber-based coimmobilization for the ADH and its cofactor NAD^+ turned out to give a highly efficient biocatalyst with excellent reusability and simple product separation from the immobilizate under avoidance of any tedious extraction steps during the overall process.

KEYWORDS: *aldol reaction, chemoenzymatic synthesis, enzyme catalysis, immobilization, organocatalysis, reduction*

The combination of chemical reactions from different fields of (asymmetric) catalysis toward one-pot type processes represents an attractive opportunity to facilitate the development of both sustainable and economically advantageous production processes without any workup, purification, or isolation of intermediates.[1] One of the particular challenges in this field is the combination of chemo- and biocatalytic reactions since such catalysts are often regarded not to be compatible with each other due to inhibition or deactivation effects and the requirement for different reaction conditions. Accordingly, examples of such combinations toward chemoenzymatic one-pot processes are still rare, in particular when using aqueous phases as the preferred reaction embodiment for enzymes as reaction media.[2−15] Vice versa, the use of "free" biocatalysts in organic solvents as typically preferred reaction media when using synthetic chemocatalysts can be complicated due to stability issues. Besides using the two "worlds" of catalysts (bio- and synthetic chemocatalysts) in "free" form in a batch mode (according to Figure 1, part A), compartmentation of such catalysts according to Figure 1B is a further exciting option for the combination of bio- and chemocatalysis without intermediate isolation due to the following advantages: (i) The catalysts can be easily separated from the reaction mixtures and reused. (ii) Compatibility problems of bio- and chemocatalyst with each other such as inhibitions and deactivations can be avoided. (iii) In spite of avoiding intermediate isolations, both reactions can run at different reaction condtions (e.g., reaction temperature), thus making unit operation steps under optimized conditions for each individual step possible.

Accordingly, based on our recent proof of concept for the combination of an asymmetric organocatalytic reaction with a biotransformation toward a one-pot process with both steps running in water,[14] we became interested in the development of

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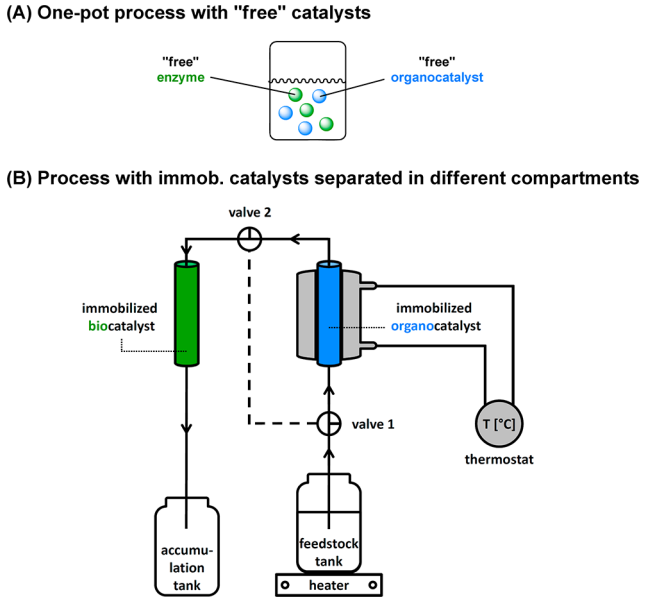
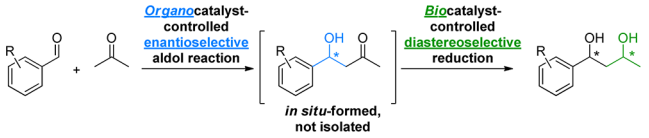


Figure 1. Comparison of different reactor concepts for the combination of organo- and biocatalysis toward a process without workup of the intermediate.

analogous "one-pot like processes" with immobilized catalysts under compartmentation of the catalysts as described above. In detail, the reactions being involved in such a chemoenzymatic one-pot process are an initial proline-derivative-catalyzed aldol reaction and a subsequent reduction of the aldol adducts catalyzed by an alcohol dehydrogenase (ADH) according to the concept shown in Scheme 1.[12,14] A key feature of this process is

Scheme 1. Chemoenzymatic Synthesis of 1,3-Diols



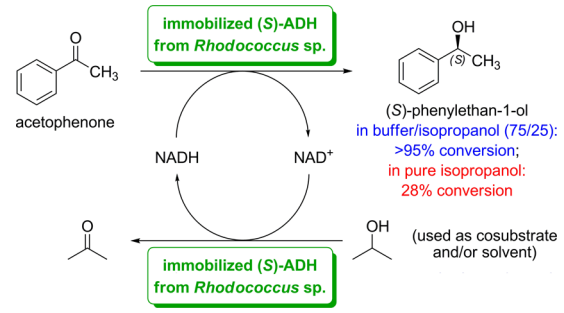
the addition of the ADH after the initial aldol reaction step. The formation of the second stereogenic center of the 1,3-diols is fully controlled by the ADH, thus overriding internal induction of the first stereogenic center and enabling a highly enantio- and diastereoselective access to all stereoisomers.[12] It should be added that for analoguous 1,3-aminoalcohols, the Bäckvall group developed also a chemoenzymatic one-pot process, which is based on a combination of organo-, metal-, and biocatalysis.[13]

In our study of immobilizates for the purpose of synthesizing 1,3-diols in a one-pot fashion, we identified a suitable immobilized organocatalyst for the aldol reaction of acetone with 3-chlorobenzaldehyde,[16,17] whereas for the diastereoselective reduction of the β-hydroxy ketone obtained in this initial organocatalytic aldol reaction, superabsorber-based ADH-immobilizates showed dramatic leaching of enzyme and cofactor, respectively, when using an aqueous reaction medium (buffer/isopropanol 75:25).[18] For example, in contrast to >95% conversion for the first cycle, only 40% conversion and no conversion were observed for cycles 2 and 3 with recycled superabsorber-immobilized ADH.[18] As an acrylate-based superabsorbent polymer, Favor SXM 9155 (Evonik Industries AG) was used. Similar leaching effects were observed earlier for similar noncovalently bound enzymes and immobilizates based on a physical bonding of biocatalysts to a support, respectively, due to the weak and incomplete restraint.[19] Although a switch to other types of immobilization methods (e.g., covalent binding) is a further option, our focus was on finding a solution for superabsorber support-based ADHs (a method developed earlier by Jeromin),[20] because this support offers significant advantages like (i) its readily availability at a very low price, thus being an economically highly attractive solid support, (ii) no need for long and/or complex support syntheses, (iii) an easy and fast preparation of the immobilized enzyme (<30 min), and (iv) the nontoxicity of the superabsorber material.

In the following, we report our results on the extension of the superabsorber-based coimmobilization method for an ADH and its cofactor toward 1,3-diol synthesis leading to a surprising highly efficient biotransformation process in organic media and to a fully compatible subsequent chemoenzymatic one-pot process with virtually complete reusability of the biocatalyst while the intermediate does not have to be isolated and the reaction mixture can be easily separated from the catalyst. In addition, combination with an immobilized organocatalyst toward the envisaged combined organo- and biocatalytic "one-pot like process" with the catalysts in different compartments will be shown as well.

The initial step consisted in the choice of an appropriate organic solvent system for the isolated biotransformation step in order to avoid the described enzyme and cofactor leaching in water-rich media.[18] When using isopropanol for a substrate-coupled in situ cofactor regeneration of NADH, the most favorable reaction system consists of only the starting material, ADH, cofactor, and isopropanol (as both cosubstrate and solvent), which is transformed into acetone during the biotransformation (Scheme 2).

Scheme 2. Substrate-Coupled Cofactor Regeneration



Such systems are described for the enzymatic reduction of prochiral ketones using lyophilized whole cells overexpressing an alcohol dehydrogenase from *Rhodococcus ruber*[21] and a carbonyl reductase from *Candida parapsilosis*.[22] In our case, when applying acetophenone as a model substrate in this initial study for the (*S*)-selective reduction with superabsorber-based immobilized ADH from *Rhodococcus* sp. (Rsp-ADH) in pure isopropanol, however, a conversion of only 28% in comparison to >95% was found when operating in an aqueous medium (as shown earlier,[18]), thus indicating a significant destabilization or deactivation of the enzyme when using pure isopropanol as solvent. This result can be explained by the effect of isopropanol extracting water from the superabsorber matrix and therefore substantially lowering the water activity (a_w)[23−25] in the hydrogel microcompartment and drying out this aqueous

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compartment. The resulting high concentration of isopropanol in the superabsorber matrix, thus causing enzyme deactivation, might be a further reason.

Consequently, we applied water-immiscible organic solvents of different hydrophobicity to increase water restraint in the superabsorber compartment and therefore preserve high a_w values. All used solvents were pre-equilibrated with water prior to use in order to avoid alterations in water activity and drying-out effects. When conducting experiments as illustrated in Scheme 3 with solvents ranging from log(P) = 0.7 to log(P) = 4.5,[26] a significant correlation between conversion rates and hydrophobicity was observed (Scheme 3). The experiments showed a low conversion of 24% for the polar solvent ethyl acetate (being able to be solubilized to a significant extent in the aqueous phase of the immobilizate), whereas a high conversion of 93% was obtained for isooctane (as a nonpolar, hydrophobic solvent with negligible solubility in water). The resulting lower amount of organic solvent in the water phase in the case of isooctane could be beneficial due to expected less enzyme deactivation. In addition, when using highly hydrophobic isooctane as a solvent, the polar compounds (*R*)-**1** and (1*R*,3*S*)-**2** should be dissolved to a higher degree in the aqueous phase (compared to the use of the polar ethyl acetate with an increased solubility for (*R*)-**1** and (1*R*,3*S*)-**2**), which also might contribute to the high conversion of 93%. These findings on the solvent impact on enzyme activity (and thus, conversion) are in good correlation to published results for the use of lyophilized whole cells of *E. coli* harboring the overexpressed Rsp-ADH in microaqueous (99% organic solvent) reaction media[21] as well as the application of high-log(P) solvents with *Lactobacillus kefir* ADH immobilized in PVA hydrogel beads.[27] Side- or byproducts were not formed during the biotransformations.

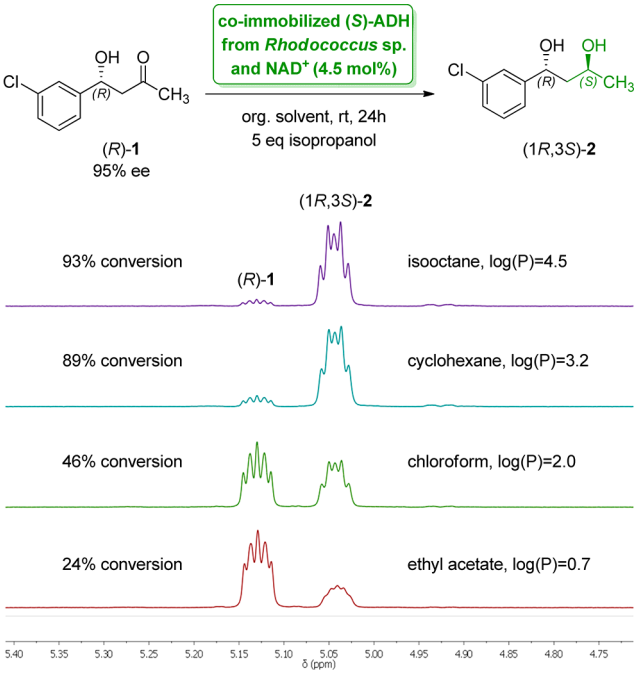
In contrast, a reversed tendency in terms of impact of the hydrophobicity of the organic solvent on the extraction efficiency in the workup was found. For the workup, decantation and subsequent rinsing of the immobilizate is all that is necessary to isolate the product, thus avoiding tedious extraction steps. Notably, the highly hydrophobic solvent isooctane gave only a low recovery rate with a maximum of 80% for (*R*)-**1** and (1*R*,3*S*)-**2** (owing to the polar nature of this substrate and product, and thus a less preferred substrate transfer from the aqueous hydrogel matrix to the organic phase), whereas use of all other, less hydrophobic solvents (cyclohexane, chloroform, ethyl acetate) enabled a recovery rate for (*R*)-**1** and (1*R*,3*S*)-**2** after workup of >95%.

The best compromise as a solvent in terms of achieving both high conversion and product recovery appeared to be cyclohexane with a log(P) of 3.2, which still led to a high conversion (89%) but also enabled high product recovery (>95%). Accordingly, cyclohexane was used in the subsequent experiments as the "solvent of choice".

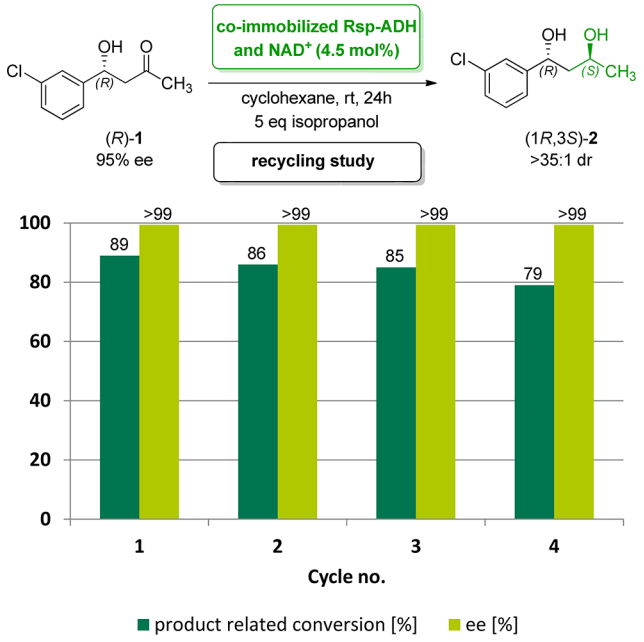
Because we now had in hand a technique for an efficient reduction process with coimmobilized Rsp-ADH and its cofactor running in organic solvents, we were further interested to evaluate the recyclability of this immobilized biocatalyst. As cyclohexane turned out to be the preferred solvent due to both excellent recovery and conversion, we conducted four subsequent biotransformation cycles with a single immobilizate, containing the alcohol dehydrogenase as well as the cofactor (Scheme 4). For the immobilization, an activity of ADH (32 U/mmol of (*R*)-**1**) and coenzyme amount (4.5 mol %) was used, which corresponds to the range of the enzyme activities applied when operating earlier with "free"[14] and superabsorber-based immobilized[18] ADH in aqueous media.

After each reaction cycle, the reaction mixture is decantated, followed by rinsing of the immobilizate and evaporation of volatile materials in vacuo to isolate (*R*,*S*)-**2** with an excellent selectivity of >99% ee and >35:1 dr. The superabsorbed ADH is subsequently used in following cycles without any further treatments or purifications. During four subsequent biotransformations of (*R*)-**1**, only a minor loss in conversion of 3% on average was detected. Again, no side- or byproducts were

Scheme 3. Correlation of Conversion with log(P)



Scheme 4. Recycling of Superabsorbed ADH



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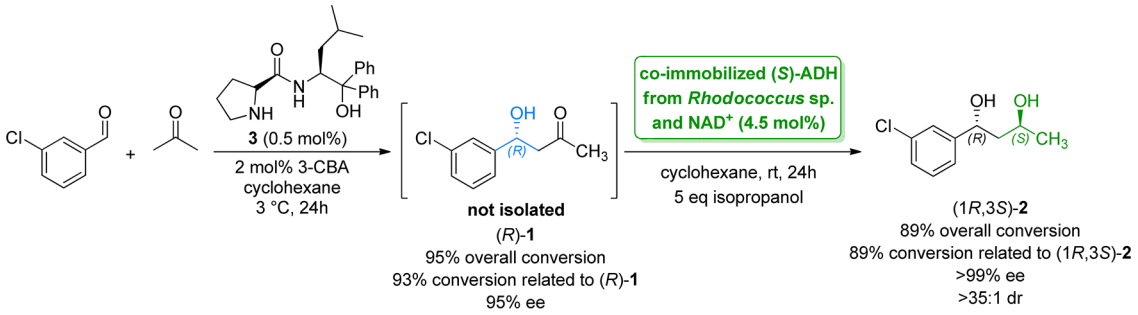
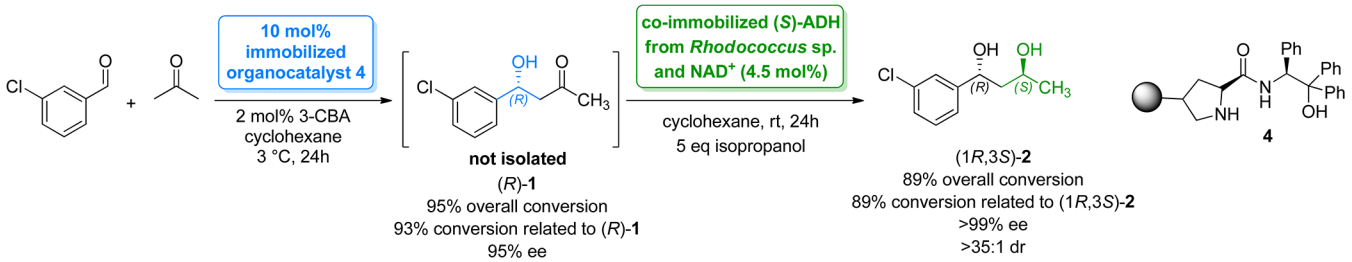
Scheme 5. Combination of Organo- and Biocatalysis Towards the Synthesis of (*R*,*S*)-2 with "Free" Organocatalyst



Scheme 6. Combination of Organo- and Biocatalysis Towards the Synthesis of (*R*,*S*)-2 with Immobilized Catalysts



detected, and recovery rate of (*R*)-**1** and (1*R*,3*S*)-**2** was almost quantitative (>95%) in all cases.

The next step was to set up a chemoenzymatic process based on the initial organocatalytic aldol reaction[12,14,17] with a subsequent reduction of the nonisolated nor purified aldol adduct catalyzed by the superabsorber-based immobilized ADH in an organic solvent (e.g., cyclohexane). Although the used organocatalyst, developed by Singh,[28] was stated to give high yields in water[29] but a significantly lower activity under neat conditions,[28] we were pleased to find that formation of the aldol product (*R*)-**1** proceeded smoothly in cyclohexane at 3 °C when adding 3-chlorobenzoic acid as a cocatalyst (Scheme 5). After 24 h, volatile materials were evaporated to remove excess of acetone, leaving nonconverted 3-chlorobenzaldehyde (<5%), the acid cocatalyst 3-CBA, and proline-derivative **3** along with the aldol product (*R*)-**1** (93% product-related conversion) in the reaction mixture, which was then directly used in the biotransformation step without further purification. The enzymatic reduction with immobilized ADH and cofactor also proceeds efficiently, leading to the desired product (1*R*,3*S*)-**2** with high conversion of 89% (related to the formation of this diol) and with excellent diastereo- and enantioselectivity (d.r. >35:1, >99% ee, Scheme 5). Thus, neither residual catalyst **3** nor 3-chlorobenzaldehyde or the acid cocatalyst showed inhibition of the coimmobilized ADH when the aldol reaction mixture was used in the enzymatic reduction step without any purification.

In addition, we were pleased to find that also the use of an immobilized form of the organocatalyst[17] is possible (although a higher catalyst loading is required, Scheme 6), thus enabling a chemoenzymatic synthesis of (1*R*,3*S*)-**2** under compartmentation of both organo- and biocatalyst in fixed-bed reactors (according to the concept shown in Figure 1B). Also, this heterogeneously conducted organocatalytic aldol reaction turned out to be fully compatible with the subsequent biotransformation, leading to the formation of (1*R*,3*S*)-**2** in cyclohexane with a product related conversion of 89% and excellent diastereo- and enantioselectivity (d.r. >35:1, >99% ee, Scheme 6).

In summary, we have developed a proof of concept for the combination of an asymmetric organocatalytic reaction with a biotransformation toward a "one-pot like" process for 1,3-diols based on immobilized organo- and biocatalysts, which are utilized in different compartments. This process which runs completely in organic media consists of an initial proline-derivative-catalyzed aldol reaction and a subsequent reduction of the aldol adduct catalyzed by an alcohol dehydrogenase (ADH) without the need for intermediate isolation. Economically attractive superabsorber-based coimmobilization for the ADH and its cofactor NAD^+ (prepared in less than 30 min) turned out to give a highly efficient biocatalyst with excellent reusability and simple product separation from the immobilizate under avoidance of any tedious extraction steps during the overall process. The desired 1,3-diol (1*R*,3*S*)-**2** was obtained with high conversion and excellent diastereo- and enantioselectivity (d.r. >35:1, >99% ee).

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and analytical data. This information is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

ADH, alcohol dehydrogenase; Rsp-ADH, alcohol dehydrogenase from *Rhodococcus* sp; NAD^+, nicotinamide adenine dinucleotide (oxidized form); 3-CBA, 3-chlorobenzoic acid; PVA, polyvinyl alcohol

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