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Escherichia coli/ADH-A: An All-Inclusive Catalyst for the Selective Biooxidation and Deracemisation of Secondary Alcohols

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The nicotinamide adenine dinucleotide regeneration system present in *Escherichia coli* cells was exploited for the oxidation and deracemisation of secondary alcohols with the overexpressed alcohol dehydrogenase from *Rhodococcus ruber* DSM 44541 (*E. coli*/ADH-A). Thus, various racemic alcohols were selectively oxidised with lyophilised or resting *E. coli*/ADH-A cells without need for an external cofactor or co-substrate. The addition of these substrates to the *E. coli*/ADH-A cells in buffer afforded the corresponding ketones and the remaining enantioenriched (*R*)-alcohols. This methodology was used for the desymmetrisation of a *meso*-diol and for the synthesis of the highly valuable raspberry ketone. Moreover, a biocatalytic concurrent process was developed with the resting cells of *E. coli*/ADH-A, ADH from *Lactobacillus brevis*, and glucose dehydrogenase for the deracemisation of various secondary alcohols, which afforded the desired enantiopure alcohols in more than 99% *ee* starting from the racemic mixture. The reaction time of deracemisation of 1-phenylethanol was estimated to be less than 30 min. The stereoinversion of (*S*)-1-phenylethanol to its pure (*R*)-enantiomer was also achieved, which provided a biocatalytic alternative to the chemical Mitsunobu inversion reaction.

Introduction

Biooxidation and deracemisation processes performed by whole-cell microorganisms or isolated enzymes are nowadays recognised as valuable tools for the preparation of high value-added compounds such as chiral alcohols used as building blocks for active pharmaceutical ingredients. Oxidoreductases such as alcohol dehydrogenases (ADHs) depend on their (expensive) nicotinamide cofactor nicotinamide adenine dinucleotide (phosphate) [NAD(P)$^+$] for oxidation.[1] These enzymes therefore require an efficient recycling method, as shown with the various coupled systems developed in the past decades,[2] to perform cost-effective enzymatic redox processes at a higher scale.[3]

Although the oxidised nicotinamide cofactors NAD(P)$^+$ are more stable in solution than their corresponding reduced forms NAD(P)H, synthetic schemes for efficient regeneration of NAD(P)$^+$ are far less developed than for NAD(P)H.[2] Thus, several types of enzymes have been used in coupled systems for NAD(P)$^+$ regeneration, such as L-lactate dehydrogenase with pyruvic or glyoxylic acid as a formal oxidant[4] and glutamate dehydrogenase, used to regenerate NAD$^+$ or NADP$^+$ coupled with the reductive amination of α-ketoglutarate.[5] For ADHs, the use of acetone or acetaldehyde in a molar excess has been demonstrated in a "coupled-substrate" approach,[6] although in several cases these conditions are not compatible with the stability of the enzyme. Another category of enzymes, the NAD(P)H oxidases, especially those affording water as a by-product at the expense of molecular oxygen, are an alternative to oxidise NAD(P)H for recycling purposes.[7] This cofactor regeneration system was also described with the simultaneous overexpression of an NAD$^+$-dependent enzyme and an NAD(P)H oxidase (H_2O-producing NADH oxidase from *Lactobacillus brevis*) in a whole-cell biocatalyst.[8]

ADHs are stereocomplementary for oxidation and reduction processes (enzyme-based stereocontrol) and can therefore catalyse the enantioselective oxidative kinetic resolution of *sec*-alcohols to obtain enantioenriched products, though only in a maximum yield of 50% (Scheme 1 a). More attractive and more challenging methods include deracemisation protocols. Thus, a highly valuable optically pure product can be obtained in a theoretical 100% yield and 100% *ee* from a cheap racemic substrate by using a concurrent one-pot process that combines multiple catalysts (Scheme 1 b).[9] In this case, each step must be carefully balanced to ensure that the catalytic processes run at comparable rates and that the various catalytic reactions do not interfere with one another. Efficient systems have been developed for the deracemisation (or stereoinversion) of *sec*-alcohols,[10] which use isolated enzymes solely[7e,11] or in combination with whole-cell biocatalysts.[12]

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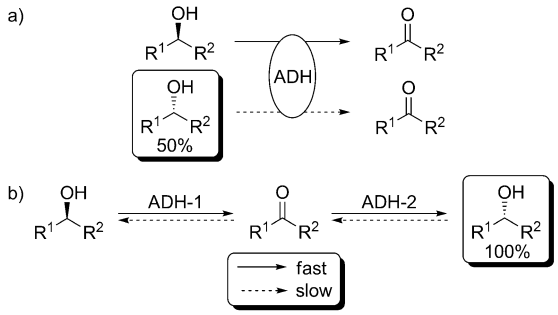
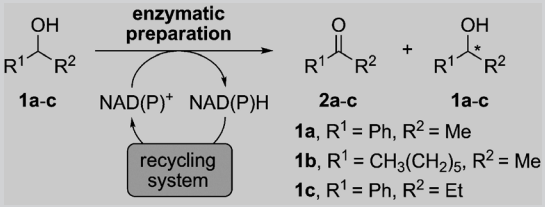


Scheme 1. a) ADH-catalysed oxidative kinetic resolution; and b) deracemisation (or stereoinversion) of a racemic (or enantiopure) *sec*-alcohol catalysed by two stereocomplementary ADHs.

While screening ADHs for oxidation reactions, we observed that *Escherichia coli* cells recycle NAD^+ already on their own without the need to overexpress an additional NADH oxidase. Herein, we describe the use of lyophilised or resting cells of *E. coli* overexpressing the solvent-tolerant ADH from *Rhodococcus ruber* DSM 44541 (ADH-A)[13] as an efficient catalyst to achieve the biooxidative kinetic resolution of several *sec*-alcohols without need for an external cofactor or recycling system. The application of this methodology to deracemise *sec*-alcohols through a biocatalytic concurrent process with NADP-dependent *Lactobacillus brevis* ADH (LBADH)[14] and an NADPH recycling system was also successfully achieved.

Table 1. Study of several enzymatic systems for the biooxidation of substrates **1a–c**.[a]



Entry	Biocatalyst	Substrate	**2a–c** Conv. [%][b]	**1a–c** *ee* [%][c]
1	lyo *E. coli*/ADH-A	**1a**[d,e]	50	>99 (*R*)
2	lyo *E. coli*/ADH-A	**1a**[e]	50	>99 (*R*)
3	lyo *E. coli*/ADH-A	**1a**	50	>99 (*R*)
4	lyo *E. coli*	**1a**	<1	n.d.[f]
5	ADH-A	**1a**	<1	n.d.[f]
6	ADH-A + lyo *E. coli*	**1a**	18	22 (*R*)
7	lyo *E. coli*/TeSADH	**1b**	30	31 (*R*)
8	lyo *E. coli*/ADH-T	**1b**	25	23 (*R*)
9	lyo *E. coli*/RasADH	**1c**	38	38 (*R*)
10	lyo *E. coli*/SyADH	**1c**	6	8 (*R*)
11	lyo *E. coli*/LBADH	**1a**	24	23 (*S*)
12	lyo *E. coli*/LBADH	**1a**[d]	50	>99 (*S*)

[a] Reaction conditions: [substrate] = 40 mM, lyophilised (lyo) cells of *E. coli* or *E. coli*/ADH (20 mg) or commercial ADH-A (3 U), Tris-HCl buffer (50 mM, pH 7.5, total end volume 0.6 mL), shaken at 250 rpm at 30 °C for 24 h; [b] Conversions determined by using GC; [c] Measured by using chiral GC; [d] Acetone (5 vol%) was added as a co-substrate; [e] NAD^+ was added (1 mM); [f] Not determined.

Results and Discussion

Biooxidative kinetic resolution of *sec*-alcohols

As a continuation of our work with recombinant ADHs overexpressed in *E. coli*,[13a,15] the biooxidation of racemic alcohols was explored to perform oxidative kinetic resolutions (Table 1). Thus, *rac*-1-phenylethanol (**1a**, 40 mM) was added to a mixture of *E. coli*/ADH-A, a catalytic amount of NAD^+ (1 mM) and a molar excess of acetone as a co-substrate (Table 1, entry 1). As expected, kinetic resolution was achieved, which afforded 50% of acetophenone (**2a**) and 50% of the remaining enantiopure (*R*)-**1a** after 24 h. As a control experiment, the same reaction was performed without acetone (entry 2), and surprisingly, the same results were observed, which indicated a 50% yield of (*R*)-**1a**. In a subsequent experiment, no additional NAD^+ was added to the reaction medium and again excellent conversion and enantioselectivity were observed (entry 3). These observations led to the hypothesis that a catalytic amount of NADH/NAD^+ present in the lyophilised *E. coli* cells was internally oxidatively recycled and used by the overexpressed ADH-A. The membranes of *E. coli* contain an electron transport chain that oxidises NADH with oxygen as the electron acceptor: various types of enzyme complexes or isolated enzymes oxidising NADH have been described in *E. coli*. Of these complexes, the most studied are NADH dehydrogenases, such as the NADH:ubiquinone oxidoreductase (analogous to the mitochondrial NADH:ubiquinone oxidoreductase called complex I), involved in the aerobic respiratory chain,[16] and flavohaemoglobins (e.g. Hmp),[17] which can act as "NADH oxidase-like" enzymes.[18] Moreover, the NADH:ubiquinone oxidoreductase that accounts for the NADH oxidase activity of *E. coli* is highly dependent on NADH.[19]

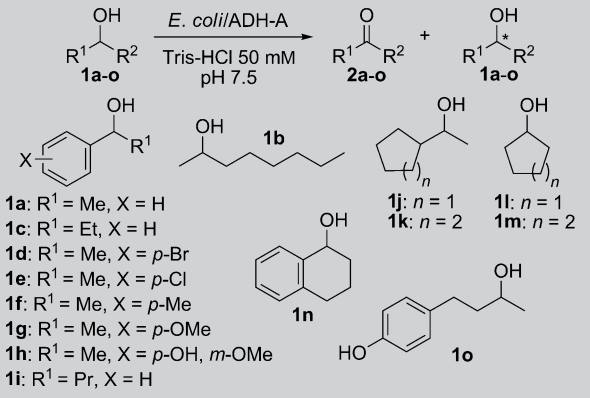
To confirm the presence of an integrated NAD^+ regeneration system in *E. coli*, lyophilised *E. coli* cells (TOP10) as well as purified commercially available ADH-A were tested separately under the same reaction conditions, which leads to no detectable conversion (entries 4 and 5). On combining lyophilised *E. coli* cells and purified ADH-A, 18% of **2a** was obtained (entry 6). Other lyophilised *E. coli* preparations overexpressing NADP-dependent ADHs were also explored, such as ADH from *Lactobacillus brevis* (LBADH),[14] *Ralstonia* sp. (RasADH),[20] *Sphingobium yanoikuyae* ADH (SyADH),[21] *Thermoanaerobacter ethanolicus* (TeSADH)[22] or *Thermoanaerobium* sp. ADH (ADH-T),[23] however, conversions obtained with their respective model substrates were modest (entries 7–11) because of the higher cytosolic concentration of NAD^+/NADH with respect to $NADP^+$/NADPH in *E. coli*.[24] In addition, the aerobic respiratory chain is more efficient for NADH than for NADPH.[25] Therefore, because these ADHs are more selective towards NADPH, the overall system is less efficient. Nonetheless, on adding acetone (5 vol%) as a co-substrate with *E. coli*/LBADH, 50% of **2a** and 50% of the remaining enantiopure alcohol (*S*)-**1a** were obtained (entry 12).

The reverse reaction, that is, the reduction of **2a** to **1a** with either *E. coli*/ADH-A or *E. coli*/LBADH, was also achieved by adding 2-propanol (5 vol%) without external addition of NADH, which afforded **1a** with 85 and 86% conversion, re-

spectively (from a 40 mM substrate concentration), and more than 99% *ee* of *S* enantiomer with ADH-A and more than 99% *ee* of (*R*)-enantiomer with LBADH. The presence of the nicotinamide cofactor in *E. coli* cells thus proves to be useful to reduce the cost for these redox reactions.

Once the lyophilised cells of *E. coli*/ADH-A were established as a suitable preparation to achieve the biooxidative kinetic resolution of a *sec*-alcohol under simple conditions without additional co-substrate or NAD^+, different alcohols were tested to determine the substrate scope of this straightforward process (Table 2).

Table 2. Substrate screening with lyophilised cells of *E. coli*/ADH-A.[a]



Entry	Substrate	2a–o Conv. [%][b]	1a–o *ee* [%][c]
1	(*S*)-**1a**	92	>99 (*S*)
2	**1b**	48	4 (*R*)
3	**1c**	34	77 (*R*)
4	**1d**	15	21 (*R*)
5	**1e**	16	24 (*R*)
6	**1f**	42	91 (*R*)
7	**1g**	47	94 (*R*)
8	**1h**	42	92 (*R*)
9	(*S*)-**1i**	11	>99 (*S*)
10	**1j**	47	94 (*R*)
11	**1k**	49	97 (*R*)
12	**1l**	52	n.a.[d]
13	**1m**	80	n.a.[d]
14	**1n**	17	21 (*R*)
15	(*S*)-**1o**	91	>99 (*S*)

[a] Reaction conditions: [substrate] = 40 mM, lyophilised cells of *E. coli*/ADH-A (20 mg), Tris-HCl buffer (50 mM, pH 7.5, total end volume 0.6 mL), shaken at 250 rpm at 30 °C for 24 h; [b] Conversions determined by using GC; [c] Measured by using chiral GC; [d] Not applicable.

A first series of 1-phenylethanol derivatives (**1a**, **c**–**h**) were used as substrates for this system, which generally gave good results. (*S*)-**1a** was fully oxidised under these conditions (92% conversion). 1-Phenyl-1-propanol (**1c**; Table 2, entry 3) was also oxidised to the corresponding propiophenone (**2c**; 34% conversion), which gave enantioenriched (*R*)-**1c**. Among a series of *para*-substituted derivatives, those bearing electron-withdrawing groups (entries 4 and 5) afforded lower conversions than did those bearing electron-donating groups (entries 6–8). Previous experiments performed with LBADH and ADH-A demonstrated that the oxidation of the alcohol slowed down if the substrate had an electron-withdrawing group.[26] A bulky substrate such as (*S*)-1-phenyl-1-butanol (**1i**) was oxidised with this system to some extent (entry 9). 2-Octanol (**1b**; entry 2) was used as a substrate; however, although conversion was close to 50%, *rac*-**1b** remained. Aliphatic substrates were previously shown to oxidise with ADH-A in a less selective manner than in their corresponding bioreductions.[27] 1-Cycloalkylethanol compounds **1j** and **k** were also oxidised, which afforded the remaining enantioenriched alcohols in high *ee* (entries 10 and 11). Cyclic alcohols **1l** and **m** were also used, which afforded good conversions into the corresponding ketones (entries 12 and 13), whereas for 1-tetralol (**1n**; entry 14), a lower conversion was observed.

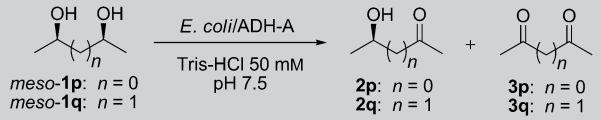
The preparation of an important ketone, the raspberry ketone **2o**, was also envisaged by using this mild biooxidation methodology.[28] Raspberry ketone is currently extensively used in industry, especially for flavouring and as a food additive. Thus, (*S*)-4-(4-hydroxyphenyl)-2-butanol (**1o**) was converted into **2o** with lyophilised cells of *E. coli*/ADH-A in buffer, which led to 91% conversion (entry 15). An upscale of the reaction (60 mg) also achieved good conversion (88%) and isolated yield (73%).

Desymmetrisation of *meso*-diols

The regio- and stereoselective reduction of diketones and oxidation of diols via biocatalytic hydrogen transfer have been performed previously with nicotinamide cofactor recycling systems to obtain chiral hydroxy ketones or diols.[29] These derivatives are important building blocks of many natural compounds such as pheromones or antitumour agents, and they can also be used as precursors for fine chemicals in the flavour and fragrance, agrochemical and pharmaceutical industries. Particularly interesting is the mono-oxidation of *meso*-diols because, in the best case, they can afford an enantiopure hydroxy ketone in a theoretical yield of 100%. Therefore, *meso*-diols 2,3-butanediol (**1p**) and 2,4-pentanediol (**1q**) were investigated by using lyophilised cells of *E. coli*/ADH-A; the *S*-configured *sec*-alcohol should be oxidised preferentially, affording the enantiopure (*R*)-hydroxy ketone. The results of the reactions with lyophilised cells of *E. coli*/ADH-A after 24 h at 30 °C are summarised in Table 3.

For **1p**, ADH-A demonstrated no stereospecificity, and thus, 3-hydroxy-2-butanone (**2p**) was obtained in the racemic form, along with 3% of 2,3-butanedione (**3p**; Table 3, entry 1). For **1q**, excellent conversion (>99%) and *ee* (99%) were achieved, which afforded (*R*)-4-hydroxy-2-pentanone (**2q**) in the absence of 2,4-pentanedione (**3q**, entry 2). Therefore, the use of lyophilised cells of *E. coli*/ADH-A without adding any external cofactor and co-substrate proved to be an economical route for the desymmetrisation of a *meso*-diol, which generated an enantiopure hydroxy ketone that is tedious to synthesise by using other chemical pathways.

Table 3. Desymmetrisation of *meso*-diols **1p** and **q** with lyophilised cells of *E. coli*/ADH-A.[a]



Entry	Substrate	**1p,q** [%][b]	**2p,q** [%][b]	*ee* [%][c]	**3p,q** [%][b]
1	*meso*-**1p**	<1	97	<1	3
2	*meso*-**1q**	<1	>99	99 (*R*)	<1

[a] Reaction conditions: [substrate] = 30 mM, lyophilised cells of *E. coli*/ADH-A (20 mg), Tris-HCl buffer (50 mM, pH 7.5, total end volume 0.6 mL), shaken at 250 rpm at 30 °C for 24 h; [b] Percentage of compounds measured by using GC; [c] Measured by using chiral GC.

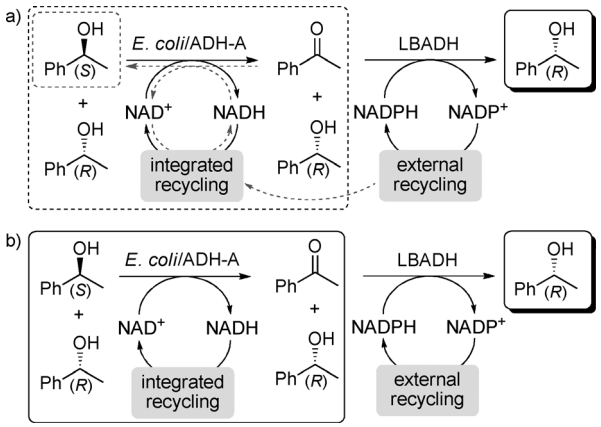
Table 4. Deracemisation process of **1a** with lyophilised cells of *E. coli*/ADH-A, LBADH and GDH or G6PDH.[a]

Entry	[**1a**] [mM]	Cofactor recycling system	[G6P] or [Glucose] [mM]	**2a** [%][b]	**1a** [%][b]	**1a** *ee* [%][c]
1	40	G6PDH	20	17	83	<1
2	40	G6PDH	100	4	96	40 (*R*)
3	40	GDH	100	<1	>99	35 (*R*)
4	40	GDH[d]	100	<1	>99	8 (*R*)
5	40	GDH	250	<1	>99	65 (*R*)
6	30	GDH	250	<1	>99	86 (*R*)
7	30	GDH[e]	250	<1	>99	65 (*R*)
8	30	GDH	500	<1	>99	86 (*R*)
9	30	GDH	750	<1	>99	92 (*R*)
10	30	GDH	1000	<1	>99	94 (*R*)

[a] Reaction conditions: substrate, lyophilised cells of *E. coli*/ADH-A (20 mg), LBADH (3 U), GDH or G6PDH (3 U), glucose or G6P, Tris-HCl buffer (50 mM, pH 7.5, 1 mM NADPH, total end volume 0.6 mL), shaken at 250 rpm at 30 °C for 24 h; [b] Percentage of compounds determined by using GC; [c] Measured by using chiral GC; [d] Additional NAD^+ (0.5 mM) was added; [e] A total of 6 U was added.

Deracemisation of *sec*-alcohols

Considering the NAD^+ preference of the above system, a biocatalytic concurrent process was envisaged for the deracemisation of *sec*-alcohols (Scheme 2a). This process was accomplished by coupling *E. coli*/ADH-A with the stereocomplementary NADP-dependent LBADH[14] by using an external recycling system that independently regenerated NADPH and was the driving force of the process leading to the enantiopure alcohol product starting from the racemic mixture.[7e] First, either glucose dehydrogenase (GDH) or D-glucose-6-phosphate dehydrogenase (G6PDH) were tested with varying concentrations of glucose and glucose-6-phosphate (G6P), respectively, to determine the best external recycling system for NADPH (Table 4).



Scheme 2. Concurrent biooxidation and reduction processes for the deracemisation of *rac*-**1a** with a) lyophilised *E. coli*/ADH-A cells and b) resting *E. coli*/ADH-A cells, along with purified LBADH, external NADPH and an NADP cofactor recycling system. The undesired NADH recycling catalysed by using the external cofactor regeneration system in panel a is highlighted in red.

In a first set of experiments, the effect of the nicotinamide cofactor-regenerating enzyme (GDH or G6PDH) was studied (entries 1–3) using 40 mM of *rac*-**1a** and 20 or 100 mM of glucose or G6P. With G6PDH, the ketone **2a** was detected, whereas with GDH, no traces were observed and (*R*)-**1a** was afforded in 35% *ee*.[30] Although an excess of G6P or glucose was used, the deracemisation process was still incomplete, perhaps owing to the partial disruption of the lyophilised cells; this disruption allows G6PDH or GDH, which can accept both NADPH and NADH, to interfere and recycle NADH taken up by ADH-A and thus reduces the ketone **2a** back to the alcohol (*S*)-**1a** (Scheme 2a). The addition of NAD^+ to the reaction mixture did not improve the system (entry 4). To shift the equilibrium towards the alcohol (*R*)-**1a** and achieve a complete process, higher glucose concentrations (250 mM; entry 5) or lower substrate concentrations (30 mM; entry 6) were attempted, improving the *ee* to 86%. Doubling the amount of GDH led to lower *ee* (65%; entry 7), which confirmed the interference between both oxidation and reduction reactions, as mentioned above. Finally, increasing the molar excess of glucose up to 1 M (entries 8–10) led to the alcohol (*R*)-**1a** in 94% *ee* after 24 h, without any trace of acetophenone (entry 10).

Nevertheless, the deracemisation system required a high concentration of glucose (more than 30 equiv. with regard to the substrate) at a 30 mM substrate concentration to achieve more than 90% *ee*. To further improve this methodology, we envisaged compartmentalising the oxidation reaction from the reduction process by using resting cells of *E. coli*/ADH-A (Table 5) with their cell wall intact. In this manner, the interference of the external recycling enzyme was minimised while still allowing the transport of the alcohol and the ketone (Scheme 2b). An amount of 35 mg of wet resting cells was subsequently used for all reactions on the basis of the best results obtained. Under these conditions, only 100 mM of glucose (a 10-fold decrease) was required to obtain 96% *ee* of (*R*)-**1a** with no trace of **2a** after 24 h (entry 1). A concentration of 200 mM of glucose was found to be the minimum needed to obtain enantiopure (*R*)-**1a** (entries 2 and 3), whereas an increase up to 500 mM did not have any detrimental effect (entries 4 and 5). Using these optimal conditions, the deracemisation of *rac*-**1a** was upscaled (50 mg), which afforded the enan-

Table 5. Deracemisation process of **1a** with resting cells of *E. coli*/ADH-A, LBADH and GDH.[a]

Entry	[Glucose] [mM]	**2a** [%][b]	**1a** [%][b]	**1a** *ee* [%][c]
1	100	<1	>99	96 (*R*)
2	150	<1	>99	98 (*R*)
3	200	<1	>99	>99 (*R*)
4	250	<1	>99	>99 (*R*)
5	500	<1	>99	>99 (*R*)

[a] Reaction conditions: [substrate] = 40 mM, wet resting cells of *E. coli*/ADH-A (35 mg), LBADH (3 U), GDH (3 U), glucose, Tris-HCl buffer (50 mM, pH 7.5, 1 mM NADPH, total end volume 0.6 mL), shaken at 250 rpm at 30 °C for 24 h; [b] Percentage of compounds determined by using GC; [c] Measured by using chiral GC.

tiopure (*R*)-**1a** with an isolated yield of 82% after purification. The concentration of **1a** could also be increased to 60 mM to obtain more than 99% *ee* of *R* enantiomer, whereas at 80 mM, a lower *ee* of 90% of *R* enantiomer was observed.

To demonstrate the wide applicability of this method, substrates that performed well in the biooxidative process (Table 2) were also deracemised (Figure 1). Thus, 1-(4-methoxyphenyl)ethanol (**1g**) and 1-cyclopentylethanol (**1j**) were obtained in their (*R*)-enantiopure form whereas the other substrates (**1f**,**h**,**k**) afforded the enantioenriched *R* enantiomer in modest to high *ee* (81–91%). The lower enantioenrichment observed with **1b** (59%) was due to the fact that ADH-A is not selective for its oxidation (Table 2, entry 2); therefore, in this case, both enantiomers were oxidised and then the ketone was subsequently reduced.

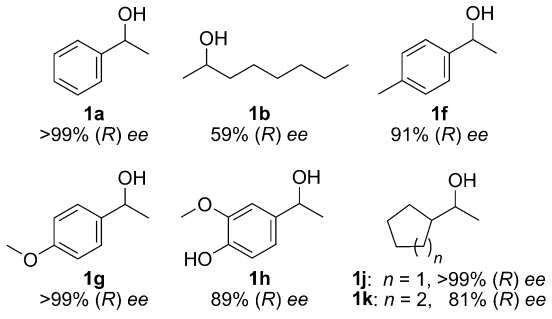


Figure 1. Deracemisation of *sec*-alcohols **1a**,**b**,**f**–**h**,**j**–**k** at a concentration of 40 mM.

Finally, an estimation of the reaction time for the deracemisation of **1a** was obtained using resting cells of *E. coli*/ADH-A (Figure 2). After only 5 min of reaction, 66% *ee* of (*R*)-**1a** was observed. Within 10 min, 98% *ee* of (*R*)-**1a** was obtained. To obtain more than 99% *ee* of (*R*)-**1a** with no trace of **2a**, only 30 min were necessary, which demonstrates the usefulness of this quick and cost-effective process for the deracemisation of *sec*-alcohols.

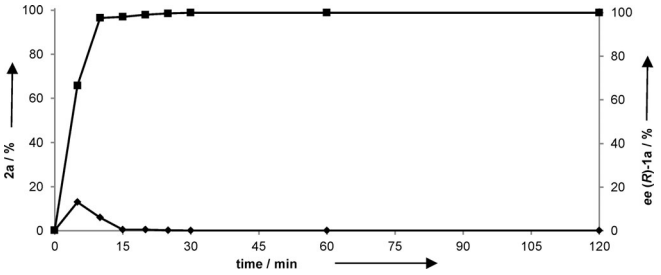
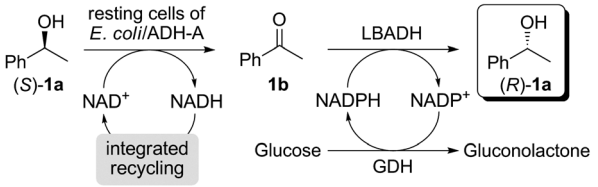


Figure 2. Time frame of the deracemisation of *rac*-**1a**. ◆: **2a**, ■: *ee* (*R*)-**1a**.

Stereoinversion of a *sec*-alcohol

The deracemisation process developed above was also used to perform the stereoinversion of a *sec*-alcohol starting from (*S*)-**1a** to obtain the enantiopure (*R*)-**1a** (Scheme 3). Thus, with 30 mM of (*S*)-**1a** in buffer and with the combined *E. coli*/ADH-A and LBADH system described previously, the (*R*)-enantiomer was obtained exclusively in more than 99% *ee* after 24 h.



Scheme 3. Concurrent biooxidation and reduction processes for the stereoinversion of (*S*)-**1a**.

Conclusions

More efficient, cheaper and simpler systems are increasingly required to meet the growing demand of chiral intermediates needed for pharmaceuticals, fine chemicals or other applications. Although previous methodologies described the use of whole cells for bioreductions by simply adding glucose in the absence of additional cofactors,[31] examples for biooxidative protocols were still missing. Herein, we have demonstrated the use of a simple methodology to perform selective oxidative transformations with only lyophilised or resting cells of *E. coli*/ADH-A in an aqueous medium, without need for an external cofactor regeneration system. Hence, various racemic *sec*-alcohols were oxidised, which afforded the corresponding enantioenriched (*R*)-alcohols, a *meso*-diol was desymmetrised to afford an enantiopure hydroxy ketone that can be used as a chiral building block, and a valuable derivative such as the raspberry ketone was obtained in high isolated yield. This system also enabled the deracemisation of racemic 1-phenylethanol (*rac*-**1a**) through a biocatalytic concurrent process to form enantiopure (*R*)-**1a** in more than 99% *ee* under 30 min when coupled with the stereocomplementary ADH from *Lactobacillus brevis* and an external recycling system for NADPH (glucose dehydrogenase with glucose). Several aromatic and aliphatic *sec*-alcohols were also obtained enantioenriched. This deracemisation process avoids the time-consuming and yield-

lowering protection–deprotection chemistry and can be used for stereoinversion processes as an equivalent to the Mitsunobu reaction. At this stage, more studies are needed to determine the exact nature of this internal NAD^+ recycling system, which could lead to the application of this methodology to other NAD^+-dependent enzymes overexpressed in *E. coli* at higher substrate concentrations. This method offers the potential to reduce costs and increase efficiency owing to shorter time and fewer steps for the production of high value-added (enantioenriched) compounds.

Experimental Section

General

Alcohols **1a**–**n**, *meso*-diol **1p**, ketones **2a**–**o**, hydroxy ketones **2p** and **q** and diketones **3p** and **q** were commercially available. (*S*)-**1o** was obtained as described previously.[28] *rac*-**1q** was purchased from commercial sources, and the racemic and *meso* isomers were separated by using column chromatography (Et_2O) as described previously.[29] All other commercial reagents and solvents were purchased with the highest purity available and used as received. The NMR spectra were recorded on a Bruker DPX-300 or Bruker AV-300 spectrometer at 300 (^{1}H NMR) and 75 (^{13}C NMR) MHz. GC analyses were performed on a Varian 3900 gas chromatograph equipped with a flame ionisation detector. HPLC analyses were performed by using a Hewlett–Packard 1100 chromatograph equipped with a Chiralpak AS chiral column and UV detector. Commercial enzymes D-GDH 002 (30 U mg^{-1}), G6PDH (640 U mg^{-1}), ADH-A (20 U mg^{-1}) and LBADH (300 U mL^{-1}) were purchased from Codexis, along with the sodium salts of the nicotinamide coenzymes $NAD(P)^+$ and NAD(P)H (purity > 99%). The following ADHs overexpressed in *E. coli* were obtained as described previously:[13a,20,21] *R. ruber* (ADH-A), LBADH, RasADH, SyADH, TeSADH or ADH-T. A Tris-HCl buffer (50 mM, pH 7.5) was used for all ADH-catalysed experiments.

Method for the biooxidative kinetic resolution of a *sec*-alcohol

For the biooxidation of alcohols **1a**–**o** in an Eppendorf tube, the lyophilised cells of *E. coli*/ADH-A (20 mg) and Tris-HCl buffer (600 μL, 50 mM, pH 7.5) were added and the mixture was shaken for 30 min at 30 °C and 250 rpm. Then, the substrate (40 mM) was added. The reaction mixture was shaken for 24 h at 30 °C and 250 rpm. The reaction mixture was then extracted with EtOAc (2× 0.5 mL) and centrifuged after each step (13 000 rpm, 90 s). The combined organic layers were dried with Na_2SO_4, and the resulting crude was analysed by using GC (see the Supporting Information for details).

Synthesis of the raspberry ketone 2o

For the biooxidation of (*S*)-**1o** to the raspberry ketone **2o** in an Eppendorf tube, the lyophilised cells of *E. coli*/ADH-A (20 mg) and Tris-HCl buffer (600 μL, 50 mM, pH 7.5) were added and the mixture was shaken for 30 min at 30 °C and 250 rpm. Then, (*S*)-**1o** (40 mM) was added. The reaction mixture was shaken for 24 h at 30 °C and 250 rpm. The reaction mixture was filtered through Celite and then extracted with EtOAc (2×0.5 mL). The combined organic layers were dried with Na_2SO_4, and the resulting crude was analysed by using HPLC (see the Supporting Information for details). The upscale (60 mg of the substrate) involved the same reaction conditions, with the same equivalences in a 15 mL Falcon tube. The resulting crude reaction product was dried and analysed by using HPLC and NMR spectroscopy, which led to good conversion (88%). Flash column chromatography (CH_2Cl_2/MeOH 9:1) afforded the pure isolated raspberry ketone **2o** in 73% yield.

Method for the deracemisation of a *sec*-alcohol with resting *E. coli*/ADH-A cells

For the deracemisation of the racemic *sec*-alcohols in an Eppendorf tube, fresh resting cells of *E. coli*/ADH-A (35 mg) were suspended in Tris-HCl buffer (420 μL, 50 mM, pH 7.5) and LBADH (3 U, 10 μL), GDH (3 U, 10 μL), glucose (100 μL, 250 mM), NADPH (60 μL, 1 mM) and the racemic *sec*-alcohol (40 mM) were added. The reaction mixture was shaken at 30 °C and 250 rpm for 24 h. The reaction mixture was then extracted with EtOAc (2×0.5 mL) and centrifuged after each step (13 000 rpm, 90 s). The combined organic layers were dried with Na_2SO_4, and the resulting crude was analysed by using GC (see the Supporting Information for details). The stereoinversion of (*S*)-**1a** was performed in the same way, but with a substrate concentration of 30 mM.

For the upscale deracemisation of **1a** in a Falcon tube, fresh resting cells of *E. coli*/ADH-A (700 mg) were suspended in Tris-HCl buffer (8.4 mL, 50 mM, pH 7.5) and then LBADH (60 U, 200 μL), GDH (100 U, 200 μL), glucose (2 mL, 250 mM), NADPH (1.2 mL, 1 mM) and *rac*-**1a** (50 μL, 35 mM) were added. The reaction mixture was stirred at 30 °C in an orbital shaker for 24 h at 250 rpm, and after filtration through Celite, it was extracted with EtOAc (3× 5 mL). The solvent was evaporated, and (*R*)-**1a** was isolated as a clear colourless oil (40.3 mg, 81% isolated yield) in more than 99% purity and more than 99% conversion by using GC (see the Supporting Information for details).

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