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Enzymatic Reduction of Ketones in "Micro-aqueous" Media Catalyzed by ADH-A from *Rhodococcus ruber*

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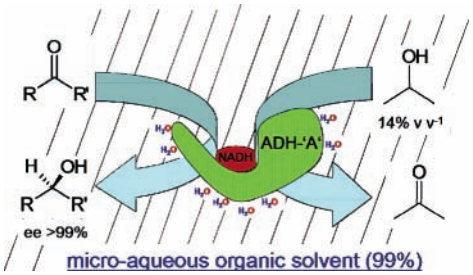
ABSTRACT



Mono- and biphasic aqueous–organic solvent systems (50% v v^{-1}) as well as micro-aqueous organic systems (99% v v^{-1}) were successfully employed for the biocatalytic reduction of ketones catalyzed by alcohol dehydrogenase ADH-A from *Rhodococcus ruber via* hydrogen transfer. A clear correlation between the log *P* of the organic solvent and the enzyme activity—the higher, the better—was found. The use of organic solvents allowed highly stereoselective enzymatic carbonyl reductions at substrate concentrations close to 2.0 M.

The use of alcohol dehydrogenases (ADHs) for the stereoselective reduction of ketones and/or the oxidation of racemic alcohols has gained increasing relevance during the past few years,[1] in particular for the synthesis of important intermediates of pharmaceuticals and bioactive compounds.[2] However, the majority of ketones of interest are highly hydrophobic, and thus possess low solubility in aqueous media, which leads to low substrate concentrations ranging from <5 to 10 mM. One of the traditional methods used to overcome this drawback is the usage of organic solvents, widely employed in the case of hydrolases.[3] However, this technique has been scarcely applied for biocatalytic redox processes. For instance, immobilized and pure horse liver alcohol dehydrogenase (HLADH)[4] and whole cells of baker's yeast[5] or *Geotrichum candidum*[6] were shown to be active in hexane

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(1) (a) Zhang, J.; Witholt, B.; Li, Z. *Adv. Synth. Catal.* **2006**, *348*, 429. (b) Hanson, R. L.; Goldberg, S.; Goswami, A.; Tully, T. P.; Patel, R. N. *Adv. Synth. Catal.* **2005**, *347*, 1071. (c) Zhu, D.; Mukherjee, C.; Hua, L. *Tetrahedron: Asymmetry* **2005**, *16*, 3275. (d) Kalaitzakis, D.; Rozzel, J. D.; Kambourakis, S.; Smonou, I. *Org. Lett.* **2005**, *7*, 4799. (e) Kaluzna, I. A.; Matsuda, T.; Sewell, A. K.; Stewart, J. D. *J. Am. Chem. Soc.* **2004**, *126*, 12827. (f) Kroutil, W.; Mang, H.; Edegger, K.; Faber, K. *Curr. Opin. Chem. Biol.* **2004**, *8*, 120. (g) Nakamura, K.; Matsuda, T. In *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; Wiley-VCH: Weinheim, 2002; Vol. 3, pp 991. (h) Wills, M.; Hannedouche, J. *Curr. Opin. Drug. Discuss. Devel.* **2002**, *5*, 881. (i) Kula, M.-R.; Kragl, U. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Marcel Dekker: New York, 2000; p 839.

(2) Breuer, M.; Ditrich, K.; Habicher, T.; Hauer, B.; Kesseler, M.; Stürmer, R.; Zelinski, T. *Angew. Chem., Int. Ed.* **2004**, *43*, 788.

(3) (a) Faber, K. *Biotransformations in Organic Chemistry*, 5th ed.; Springer-Verlag: New York, 2004. (b) Carrea, G.; Riva, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 2226.

(4) (a) Zaks, A.; Klibanov, A. M. *J. Biol. Chem.* **1988**, *263*, 8017. (b) Grunwald, J.; Wirz, B.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1986**, *108*, 6732. (c) Jones, J. B.; Schwartz, H. M. *Can. J. Chem.* **1982**, *60*, 335.

(5) (a) Nakamura, K.; Inoue, K.; Ushio, K.; Oka, S.; Ohno, A. *J. Org. Chem.* **1988**, *53*, 2589. (b) Nakamura, K.; Kondo, S.; Nakajima, N.; Ohno, A. *Tetrahedron* **1995**, *51*, 687.

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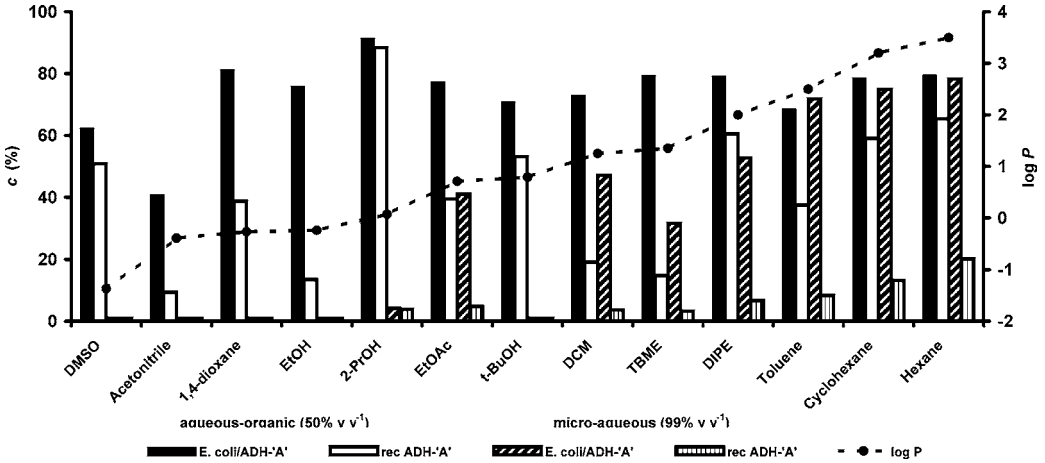
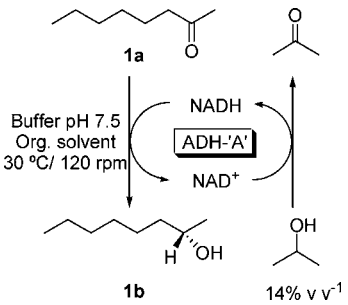


Figure 1. Effect of different organic solvents in aqueous−organic (50% v v^{-1}) and micro-aqueous (99% v v^{-1}) organic solvent systems on the enzymatic reduction of **1a** catalyzed by ADH-A from *Rhodococcus ruber*. Reactions were performed both with *E. coli*/ADH-A cells (30 U) and with recombinant enzyme (1.1 U) (t = 24 h). log P (●) values are plotted to correlate enzyme activities with the hydrophobicity of the organic solvents.

or benzene, respectively. In order to improve activities of whole microbial cells in organic solvents, the cells had to be immobilized.[6,7] Subsequently, when purified enzymes were employed as biocatalysts in biphasic systems, the percentage of employed organic solvent was rather limited,[8] probably due to the unstability of ADHs in this medium.

We have recently demonstrated that alcohol dehydrogenase ADH-A from *Rhodococcus ruber* DSM 44541[9] overexpressed in *E. coli*[10] catalyzes the reduction of the ketones with simultaneous cofactor-recycling in a coupled substrate approach using 2-propanol as hydrogen donor (Scheme 1).[11]

Scheme 1. Enzymatic Reduction of 2-octanone Catalyzed by ADH-A in Aqueous−Organic and Micro-aqueous Media Employing a Coupled Substrate Approach



This enzyme showed impressive operational stability in the presence of high concentrations of 2-propanol (up to 80% v v^{-1}).[12] Encouraged by these results, we decided to study the organic-solvent stability of ADH-A using the recombinant enzymes and lyophilized whole cells of *E. coli* harboring the overexpressed protein (*E. coli*/ADH-A).

Two different solvent systems were employed for the reduction of the model substrate 2-octanone (**1a**) to (*S*)-2-octanol (**1b**): (a) organic cosolvent at 50% v v^{-1} and (b) micro-aqueous organic solvent at 99% v v^{-1}.[13]

(a) Mono- or biphasic aqueous−organic solvent systems: As shown in Figure 1, lyophilized whole cells of *E. coli*/ADH-A were fully active in all mono- or biphasic aqueous−organic solvent systems at 50% v v^{-1}. A moderate decrease of conversion after 24 h was observed for recombinant ADH-A, indicating probably a slight deactivation of the enzyme. Detailed analysis of the solvent-effects showed a continuous decrease of reaction rate upon gradual increase of solvent concentration (detailed data are given in the Supporting Information). In all cases, the stereoselectivity of the enzyme remained completely intact leading to enantiopure (*S*)-**1b**, emphasizing the robustness of the enzyme.

(6) Nakamura, K.; Inoue, Y.; Matsuda, T.; Misawa, T. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2397.

(7) (a) De Temiño, D. M.-R.; Hartmeier, W.; Ansorge-Schumacher, M. B. *Enz. Microb. Technol.* **2005**, *36*, 3. (b) Gervais, T. R.; Carta, G.; Gainer, J. L. *Biotechnol. Prog.* **2003**, *19*, 389. (c) Luo, D.-H.; Zong, M.-H.; Xu, J.-H. *J. Mol. Catal. B: Enzym.* **2003**, *24*−*25*, 83.

(8) (a) Hu, J.; Xu, Y. *Biotechnol. Lett.* **2006**, *28*, 1115. (b) Lu, J.; Lazzaroni, M. J.; Hallett, J. P.; Bommarius, A. S.; Liotta, C. L.; Eckert, C. A. *Ind. Eng. Chem. Res.* **2004**, *43*, 1586. (c) Gröger, H.; Hummel, W.; Buchholz, S.; Drauz, K.; Van Nguyen, T.; Rollmann, C.; Hüsken, H.; Abokitse, K. *Org. Lett.* **2003**, *5*, 173.

(9) (a) Poessl, T. M.; Kosjek, B.; Ellmer, U.; Gruber, C. C.; Edegger, K.; Faber, K.; Hildebrant, P.; Bornscheuer, U. T.; Kroutil, W. *Adv. Synth. Catal.* **2005**, *347*, 1827. (b) Stampfer, W.; Edegger, K.; Kosjek, B.; Faber, K.; Kroutil, W. *Adv. Synth. Catal.* **2004**, *346*, 57. (c) Stampfer, W.; Kosjek, B.; Faber, K.; Kroutil, W. *J. Org. Chem.* **2003**, *68*, 402. (d) Stampfer, W.; Kosjek, B.; Moitzi, C.; Kroutil, W.; Faber, K. *Angew. Chem., Int. Ed.* **2002**, *41*, 1014.

(10) Edegger, K.; Gruber, C. C.; Poessl, T. M.; Wallner, S. R.; Lavandera, I.; Faber, K.; Niehaus, F.; Eck, J.; Oehrlein, R.; Hafner, A.; Kroutil, W. *Chem. Commun.* **2006**, 2402.

(11) Kosjek, B.; Stampfer, W.; Pogorevc, W.; Faber, K.; Kroutil, W. *Biotechnol. Bioeng.* **2004**, *86*, 55.

(12) Stampfer, W.; Kosjek, B.; Kroutil, W.; Faber, K. *Biotechnol. Bioeng.* **2003**, *81*, 865.

(13) Definition of "micro-aqueous": Micro-aqueous solvent systems are "monophasic" and are generally composed of ≤1 % water and ≥99% organic solvent, see: Yamane, T. *Biocatalysis* **1988**, *2*, 1.

(b) For micro-aqueous organic solvent systems at 99% v v^{-1}, the picture drastically changed (Figure 1): water-miscible solvents (DMSO, acetonitrile, 1,4-dioxane, EtOH) led to complete deactivation of *E. coli*/ADH-A and recombinant ADH-A. Marginal activity was retained in 2-propanol, which also acts as cosubstrate. In contrast, water-immiscible solvents proved to be more biocompatible: reaction rates fully recovered with *E. coli*/ADH-A and even with cell-free ADH-A, deactivation was modest.[14] Overall, a clear correlation between the hydrophobicity of the solvents (expressed as log *P*)[15] and enzyme activity can be seen: whereas hydrophilic (water-miscible) organic solvents (log $P < 0$) lead to complete deactivation, high biocompatibility is found for hydrophobic solvents (log $P > 2$), such as toluene, cyclohexane and hexane.

The influence of the substrate concentration (**1a**) on the activity and stereoselectivity of *E. coli*/ADH-A cells was studied in 99% v v^{-1} hexane (Figure 2). Although apparent

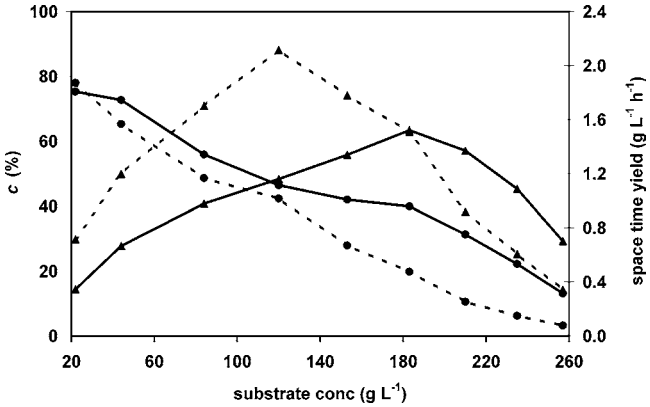


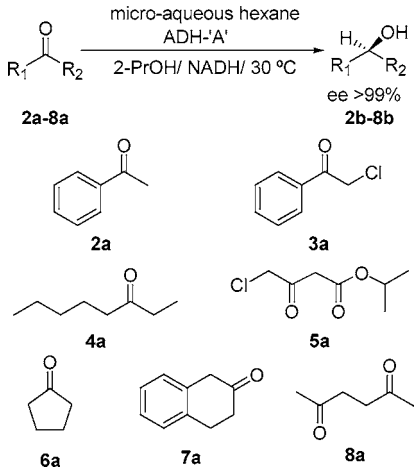
Figure 2. Effect of substrate concentration (**1a**) on conversion (●) and space time yield (▲), expressed as g of ketone consumed per L of solution h^{-1} of *E. coli*/ADH-A (dashed line, 24 h) and purified ADH-A (solid line, 48 h) in micro-aqueous hexane (99% containing 1% buffer−NADH solution).

conversions were lower at elevated substrate concentrations, the space time yield (expressed as g of 2-octanone **1a** consumed per L of solution h^{-1}) increased, reaching a maximum at 0.94 M (120 g L^{-1}). A similar substrate concentration-conversion profile−albeit with a slightly reduced maximum−was obtained for recombinant ADH-A, which exihibited a maximum space time yield at 1.42 M substrate concentration **1a** (180 g L^{-1}). Surprisingly, even at concentrations close to 2.0 M, recombinant ADH-A exhibited reasonable activity, which is an important step for up-scaling. The stereoselectivity of the enzyme remained completely unchanged at elevated substrate concentrations and (*S*)-**1b** was obtained in enantiopure form.

One of the major advantages of using micro-aqueous organic solvents is the facilitated recovery of enzymes by filtration.[3] Thus, the feasibility of recycling *E. coli*/ADH-A was investigated for the reduction of **1a** in 99% hexane and toluene. Reusing *E. coli*/ADH-A afforded the same excellent stereoselectivity during all the cycles, thus enantiopure (*S*)-**1b** was obtained. It was also observed that the *E. coli* cells showed activity until the fifth cycle, in which no reaction was observed for both solvents any more, allowing to recycle the biocatalyst during four times until its total deactivation.

The flexibility of ADH-A (*E. coli*/ADH-A or in partially purified form) concerning its substrate spectrum was demonstrated in micro-aqueous hexane. As shown in Table 1, a

Table 1. ADH-A-Catalyzed Bioreduction of Ketones in Micro-aqueous Hexane (99% v v^{-1})[a]



		E. coli/ADH-A		rec ADH-A	
substrate	product[b]	time (h)	*c* (%)	time (h)	*c* (%)
2a	(*S*)-**2b**	42	67.3	42	63.1
3a	(*R*)-**3b**[c]	40	96.3	42	48.1
4a	(*S*)-**4b**	30	14.4	24	20.1
5a	(*R*)-**5b**[c]	30	>99	30	68.8
6a	**6b**	24	56.1	30	76.6
7a	(*S*)-**7b**	32	35.4	24	41.8
8a	(2*S*,5*S*)-**8b**[d]	24	76.4	42	74.1

[a] For experimental details see the Supporting Information. [b] ee >99%. [c] Switch in CIP priority. [d] Sole product (2*S*,5*S*)-hexanediol (**8b**).

set of aromatic, linear and cyclic ketones (**2a**−**7a**) was reduced to the corresponding alcohols **2b**−**7b**. Depending on the steric requirements of the substrate, variable reaction times were required to reach moderate or good conversions. For instance, enantiopure (*R*)-**3b**, an important pharma-intermediate,[16] was obtained with 96% conversion in 99% hexane after 40 h using *E. coli*/ADH-A. Finally, a diketone (**8a**) was employed as substrate. Similar to the results obtained in buffer,[17] enantiopure (2*S*,5*S*)-2,5-hexanediol (**8b**) was achieved as the sole product. We were pleased, that no trace of the intermediate keto-alcohol was observed after 24

(14) Detailed experiments showed that at least 0.5% v v^{-1} of water is required for activity.

(15) Logarithm of the partition coefficient of a given compound in a standard *n*-octanol/water two-phase system.

(16) Hamada, H.; Miura, T.; Kumobayashi, H.; Matsuda, T.; Harada, T.; Nakamura, K. *Biotechnol. Lett.* **2001**, *23*, 1603.

h (*c* 76%, *E. coli*/ADH-A) or 42 h (*c* 74%, recombinant ADH-A). In each case, the newly generated *sec*-alcohol moieties were formed with absolute specificity (*ee* >99%).

It has been shown in this study that the recently cloned and overexpressed ADH-A can successfully be employed for the highly enantioselective reduction of a broad set of ketones employing nonconventional aqueous−organic solvent systems. The exceptionally robust enzyme efficiently worked in micro-aqueous media composed of 99% of a very hydrophobic organic solvent and buffer. These solvent systems allowed to employ high substrate concentrations of ~2.0 M, which represents a 10-fold improvement in comparison to previous examples.[8c] Furthermore, an interesting clear correlation between log *P* and the biocompatibililty of the organic solvent—the higher, the better—was observed, which is in contrast to recent observations using ADHs.[18]

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Supporting Information Available: Experimental and kinetic data as well as the achiral and chiral GC data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Edegger, K.; Stampfer, W.; Seisser, B.; Faber, K.; Mayer, S. F.; Oehrlein, R.; Hafner, A.; Kroutil, W. *Eur. J. Org. Chem.* **2006**, 1904.

(18) Filho, M. V.; Stillger, T.; Müller, M.; Liese, A.; Wandrey, C. *Angew. Chem., Int. Ed*. **2003**, *42*, 2993.