Pergamon

Tetrahedron: *Asymmetry* 10 (1999) 923–928

TETRAHEDRON: *ASYMMETRY*

Asymmetric reduction of acetophenone in membrane reactors: comparison of oxazaborolidine and alcohol dehydrogenase catalysed processes

Sebastian Rissom, Juliane Beliczey, Guido Giffels, Udo Kragl † and Christian Wandrey *

Forschungszentrum Jülich GmbH, Institut für Biotechnologie, D-52425 Jülich, Germany

Received 18 January 1999; accepted 10 February 1999

Abstract

The enantioselective reduction of acetophenone was studied in two different ways. Chemical borane reduction using a homogeneously soluble polymer-bound oxazaborolidine catalyst was carried out in a continuously operated membrane reactor and yielded (*R*)-phenylethanol in good enantiomeric excess with high space–time yields. An enzymatic reduction using a dehydrogenase two-enzyme system as the catalyst and formate as the hydrogen source was carried out in an extractive bi-membrane reactor and yielded (*S*)-phenylethanol in excellent enantiomeric excess with a low enzyme consumption. A comparison of the two systems with respect to space–time yield, total turnover number and other parameters is made.

1. Introduction

The enantioselective reduction of prochiral ketones leading to corresponding optically-active secondary alcohols is a topic of great interest.[1] Acetophenone has been used as a model substrate for numerous methods comprising reductions catalysed by hetero- and homogeneously soluble catalysts as well as enzymes as biocatalysts.[2] One chemical route widely used with chiral 1,3,2-oxazaborolidines as catalysts was developed by Itsuno et al.[3–5] and then improved by Corey, Bakshi and Shibata (CBS-reduction).[6–9] Oxidoreductases found in a number of micro- and other organisms[10,11] are the biological analogues to those catalysts. These proteins often show a broad substrate range and generally excellent enantiodifferentiation. We used the carbonyl reductase isolated from *Candida parapsilosis* (*CP*CR)[12–14] as the biocatalyst for the reduction. Use of the same substrate with good activity and selectivity makes it possible to directly compare these approaches. We used homogeneously soluble catalysts in the

* Corresponding author. Tel: +49-2461-615777; fax: +49-2461-613870; e-mail: c.wandrey@fz-juelich.de

† New address: Rostock University, Department of Chemistry, D-18051 Rostock, Germany.

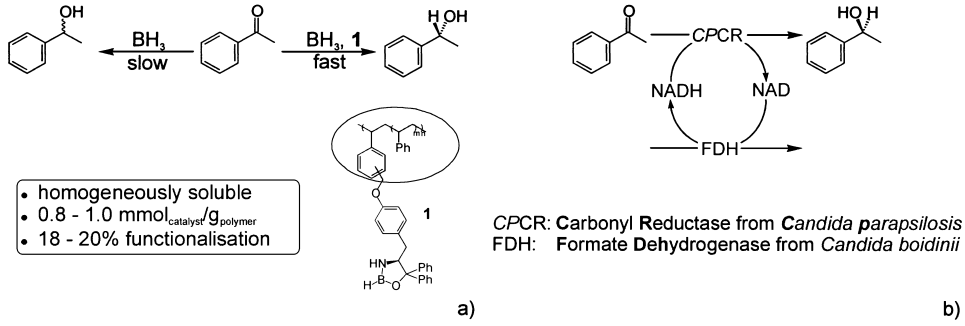
0957-4166/99/$ - see front matter
PII: S0957-4166(99)00050-6

respective reaction medium for both approaches. The organic catalyst was coupled to a polystyrene backbone and thus both catalysts were retained by ultra- or nanofiltration membranes.

2. Results and discussion

2.1. The reaction systems

The synthesis and properties are described for both the polymer-bound oxazaborolidine catalyst[15] as well as the carbonyl reductase.[12] In the case of the chemical catalyst a catalytically-active species is formed and regenerated in situ from the catalyst and borane as the hydrogen donor. Besides the enantioselective catalysed reaction borane reduces the substrate without a catalyst to form a racemic product (Scheme 1a).



Scheme 1. Reaction schemes

The enzyme, on the other hand, receives hydrogen via a nicotinamide adenine dinucleotide (NADH) cofactor. As the cofactor is quite expensive it needs to be recycled. This can either be done by adding a second substate (e.g. isopropanol) that is oxidised by the same enzyme or by adding a second enzyme for regeneration.[16] We chose the second approach and used formate dehydrogenase (FDH) isolated from *Candida boidinii* to recycle the cofactor. This method has several advantages, of which cheap access to the enzyme, the use of formate as a cheap hydrogen source and a favourable equilibrium due to the formation of carbon dioxide as product are the most important.

2.2. Kinetic behaviour

The kinetic behaviour of both systems was determined by measurements of initial reaction rates. The kinetic parameters are shown in Fig. 1. For the oxazaborolidine catalyst, Michaelis–Menten-type kinetics was observed with a K_M of 2.8 mmol L^{-1}.[17,18] For the non-catalysed reaction at constant borane concentration first order kinetics was found. It can clearly be seen that at lower ketone concentrations best ratios between the catalysed and uncatalysed reaction and thus the highest ee values of the product can be reached. These conditions were achieved by running the reaction at high conversions in a continuous stirred tank reactor (CSTR).[19] The enzymatic reaction also showed Michaelis–Menten-type kinetic behaviour with a much lower K_M of 0.04 mmol L^{-1} towards acetophenone and had no competing uncatalysed reaction as neither formate nor cofactor react with the substrate without the action of the enzyme. The ee value of the enzymatic reaction product was >99% under all conditions studied.

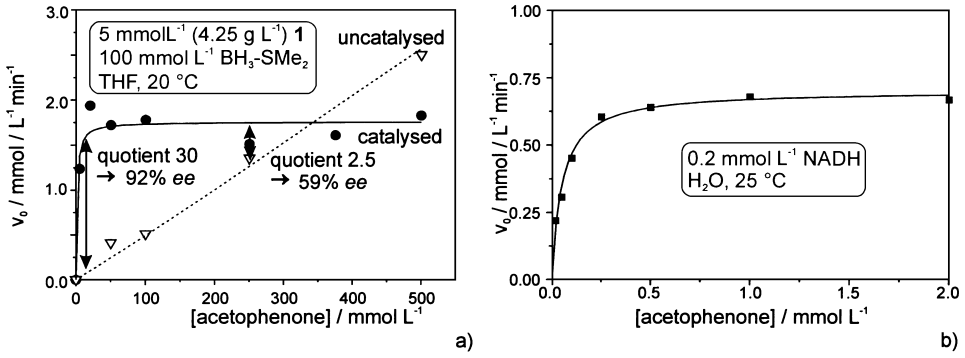
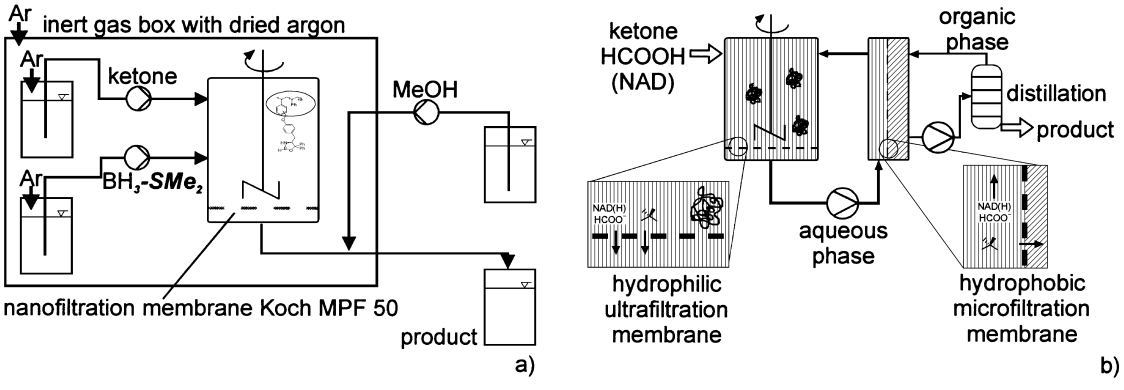


Figure 1. Kinetic behaviour

2.3. Reactors used

Both reactions were performed in a continuously operated membrane reactor. These reactors made use of the high molecular weight of the polymeric catalysts and used membranes to retain the catalysts in the reaction volume while continuously re-supplying the substrates. In the case of chemical reduction the system had to be kept under strictly anhydrous conditions due to the deactivation of the catalyst in the presence of water. THF was used as the solvent and methanol added for quenching excess borane at the reactor outlet. On the other hand, the enzymatic reaction was carried out in an aqueous buffer system. The aqueous phase was recirculated and continuously resupplemented in the substrate. Catalytic amounts of cofactor were added to compensate for its thermal deactivation. pH was adjusted by titration with formic acid.[20] After reaction the product was extracted with iso-octane by means of a membrane-based extraction device.[‡,21] The schemes of both reactors used are shown in Scheme 2.



Scheme 2. Reactors used

2.4. Discussion and comparison of significant parameters

Both reactions were run for a number of residence times in the continuously operated reactor and are thus directly comparable. Fig. 2 shows the concentration time-curves for two typical experiments. Some of the important parameters are shown in Table 1 where only the best results obtained are compiled. In

‡ The membrane extractor is available from Hoechst Celanese under the brand name of Liqui-Cel®.

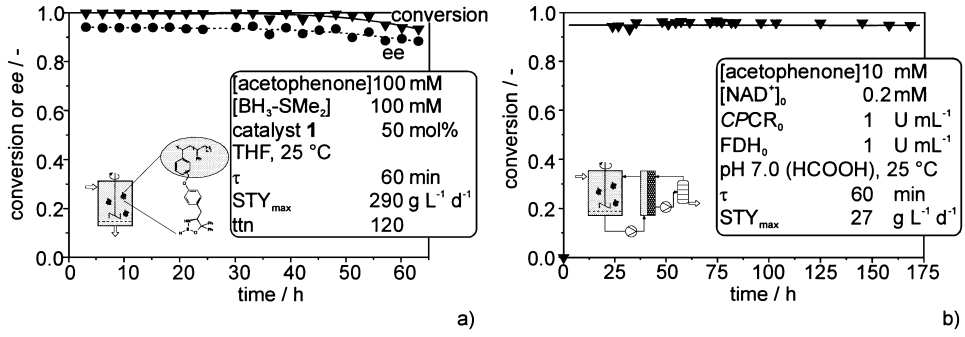


Figure 2. Conversion plots of the continuous syntheses

Table 1
Significant parameters of both reactions (best results shown)

parameter	unit	oxazaborolidine reduction	enzymatic reduction
[substrate]	mmol L^{-1}	**250**	40
[cofactor]	mmol L^{-1}	**no cofactor needed**	0.2
[catalyst]	mmol L^{-1}	35	**$6.8 \cdot 10^{-6}$**
total turnover number	-	560	**$2.4 \cdot 10^{8}$**
turnover frequency	min^{-1}	0.3	**$2.3 \cdot 10^{4}$**
molecular weight of cat.	g mol^{-1}	14000	**78000**
active centres per catalyst	-	**~ 16**	1
mass per active centre	g	**850**	78000
half-life of catalyst	days	1.2	**31.1**
space-time yield	g L^{-1} d^{-1}	**1400**	88
ee value	%	94	**> 99**

the case of the enzymatic reaction the data for *CP*CR as the producing enzyme have been taken into account.

A number of these parameters will be discussed here and conclusions drawn.

2.4.1. Substrate concentration

The substrate concentration in the chemical process was set to a maximum of 250 mmol L^{-1}, but higher concentrations could be possible as the substrate is completely miscible with THF. The substrate concentration in the enzymatic process is restricted by the limited solubility of the substrate in the aqueous reaction solution and was set at a maximum of 40 mmol L^{-1} near the maximum solubility. The cofactor concentration was set to 0.25 mmol L^{-1} with a continuous re-feeding of 0.025 mmol L^{-1} per residence time, which corresponded to a total turnover number of the cofactor of 1600 at complete conversion.

2.4.2. Amount of catalyst used, total turnover number

The oxazaborolidine catalyst was added to a concentration of 35 mmol L^{-1}, which resulted in a total turnover number (ttn) of the catalyst (defined as the amount of product produced per amount of catalyst)[§]

§ TON often used in chemical catalysis.

of 560 over the reaction time. In contrast, the specific activity of the enzyme used was much higher. It can convert 1.9 mmol of substrate per minute and mg of active protein. Thus, at a molecular weight of 78000 g mol^{-1} the concentration of active biocatalyst in the reactor is only about 6.8 nmol L^{-1} which is equivalent to a ttn of 2.4×10^8. A similar ratio is found when the turnover frequency (tof) of the catalyst (defined as the amount of product produced per amount of catalyst and time) is considered.

2.4.3. Stability of the catalyst

A deactivation of the chemical catalyst of 1.8% per hour, which is equivalent to a half-life of 1.2 days, was measured under reaction conditions. The stability of the enzyme system was determined with a deactivation of 1.7% per day (=half-life of 31.1 days).[22] This high stability permits the reactor to be run under stable conditions for several weeks.

2.4.4. Space–time yield

The space–time yield (STY) of a reaction is defined as the amount of product produced per litre of reactor volume and day. In the case of the chemical reaction a quite high STY of up to 1.4 kg L^{-1} day^{-1} was reached, whereas the maximum of the enzymatic reaction is only 88 g L^{-1} day^{-1}. This is due to the lower solubility of substrate and product in water compared to the high solubility in THF.

2.4.5. Enantiomeric excess value of the product

The enzymatic reaction showed an ee of >99% throughout the reaction time as the enzymes used acted almost completely stereoselectively and no uncatalysed reaction took place. For the chemical reduction the ee decreased during the reaction time due to catalyst deactivation and thus enhanced uncatalysed by-reaction. Thus the ee of the synthesis was limited to 90–94% with this particular catalyst. To obtain enantiopure compounds without additional purification steps only the enzymatic alternative was applicable.

The parameters discussed above clearly show that both approaches have their advantages and disadvantages. The enzyme is obviously superior in terms of ttn of the catalyst and amount of catalyst needed. Specially when extremely high ee values are needed for the product this will be the approach of choice. On the other hand, the chemical system allows a much higher STY to be achieved due mainly to the higher substrate concentrations possible.

In terms of a catalyst approach the organic chemist might prefer the chemical approach as the catalysts are easily accessible with normal laboratory equipment and can be fine-tuned quite easily. The opposite enantioselectivity of the reaction can be achieved by synthesising the opposite enantiomer of the catalyst using D-tyrosine as a starting material. Enzymes, on the other hand, are becoming commercially available to a greater extent and the optimisation of these biocatalysts by directed evolution is quite a promising topic.[23] Reversal of the enantioselectivity using the same enzyme remains difficult but enzymes from other organisms such as the alcohol dehydrogenase from *Lactobacillus kefir*[24,25] allow the synthesis of the (*R*)-enantiomer.

In conclusion, both approaches will be complementary as each offers different advantages to the chemist. While the chemical process is superior in terms of catalyst access and space–time yield the enzymatic process has a low catalyst consumption, high total turnover number for the catalyst and, most important, extremely high ee values.

Acknowledgements

The authors wish to thank Professor Dr. M.-R. Kula for supplying enzymes used. Part of this work was financially supported by the BMBF programme ‘Katalyse’ and the ‘Katalyseverbund NRW’.

References

1. Singh, V. K. *Synthesis* **1992**, 605.
2. Noyori, R. *Asymmetric Catalysis in Organic Synthesis*; John Wiley Sons: New York, 1994.
3. Hirao, A.; Itsuno, S.; Nakahama, S.; Yamazaki, N. *J. Chem. Soc., Chem. Commun*. **1981**, 315.
4. Itsuno, S.; Iro, K.; Hirao, A.; Nakahama, S.; Yamazaki, N. *J. Chem. Soc., Chem. Commun*. **1983**, 469.
5. Itsuno, S.; Ito, K. *J. Org. Chem*. **1984**, *49*, 555.
6. Corey, E. J.; Bakshi, K. R.; Shibata, S. *J. Am. Chem. Soc*. **1987**, *109*, 5551.
7. Corey, E. J.; Reichard, G. A. *Tetrahedron Lett*. **1989**, *30*, 5207.
8. Corey, E. J.; Link, J. O. *Tetrahedron Lett*. **1992**, *33*, 331.
9. Corey, E. J.; Helal, C. J. *Tetrahedron Lett*. **1995**, *36*, 9165.
10. Drauz, K.; Waldmann, H., Eds. *Enzyme Catalysis in Organic Synthesis*; VCH: Weinheim, 1995.
11. Peters, J. In *Dehydrogenases — Characteristics, Design of Reaction Conditions, and Applications*; Kelly, D. R., Ed. Biotechnology. Wiley-VCH: Weinheim, 1998; Vol. 8a (Biotransformations I); pp. 391.
12. Peters, J.; Minuth, T.; Kula, M.-R. *Enzyme Microb. Technol*. **1993**, *15*, 950.
13. Peters, J.; Minuth, T.; Kula, M.-R. *Biocatalysis* **1993**, *8*, 31.
14. Peters, J.; Zelinski, T.; Minuth, T.; Kula, M.-R. *Tetrahedron: Asymmetry* **1993**, *4*, 1683.
15. Giffels, G.; Beliczey, J.; Felder, M.; Kragl, U. *Tetrahedron: Asymmetry* **1998**, *9*, 691.
16. Hummel, W.; Kula, M.-R. *Eur. J. Biochem*. **1989**, *184*, 1.
17. Segel, I. H. *Enzyme Kinetics*; John Wiley: New York, 1975.
18. Cornish-Bowden, A., Ed. *Fundamentals of Enzyme Kinetics*, revised edn. Portland Press: London, 1995.
19. Biselli, M.; Kragl, U.; Wandrey, C. In *Reaction Engineering for Enzyme-Catalyzed Biotransformations*; Drauz, K.; Waldmann, H., Eds. Enzyme Catalysis in Organic Synthesis. VCH: Weinheim, 1995; pp. 89.
20. Kruse, W.; Hummel, W.; Kragl, U. *Recl. Trav. Chim. Pays-Bas* **1996**, *115*, 239.
21. Ho, W. S. W.; Sirkar, K. K., Eds. *Membrane Handbook*, 1 edn; Chapman & Hall: New York, 1992.
22. Liese, A.; Zelinski, T.; Kula, M.-R.; Kierkels, H.; Karutz, M.; Kragl, U.; Wandrey, C. *J. Mol. Cat. B Enzymatic* **1998**, *4*, 91.
23. Arnold, F. H.; Moore, J. C. *Biochem. Eng*. **1997**, *58*, 1.
24. Hummel, W. *Appl. Microbiol. Biotechnol*. **1990**, *34*, 15.
25. Hummel, W. *Journal of Biotechnology Letters* **1990**, *12*, 403.