

Process Intensification for Substrate-Coupled Whole Cell Ketone Reduction by In Situ Acetone Removal

Kirsten Schroer, Eva Tacha, and Stephan Lütz*

Institute of Biotechnology 2, Research Centre Jülich, 52425 Jülich, Germany

Abstract:

Three different reactor configurations for in situ acetone removal in whole cell biotransformation processes with substrate-coupled cofactor regeneration were applied. The reduction of 2,5-hexanedione to the corresponding (2*R*,5*R*)-hexanediol was catalyzed by recombinant *Escherichia coli* cells expressing an alcohol dehydrogenase from *Lactobacillus brevis*. The reaction was carried out in a substrate-coupled cofactor regeneration approach using 2-propanol as redox equivalent for intracellular cofactor regeneration. In contrast to a process without acetone removal, where 54% yield could be reached, the yield was increased to >90% when a pervaporation system was applied or when acetone was removed by sparging air through the reaction mixture. In a third system, conversion was driven using a biphasic system to extract acetone continuously from the biocatalyst containing aqueous phase and to allow high concentrations of the hydrophobic substrate 1-phenyl-2-propanone. When methyl *tert*-butyl ether was applied as the non-aqueous phase, only 24% yield was achieved. When the ionic liquid 1-butyl-3-methylimidazolium bis((trifluoromethyl)sulfonyl)amide was applied as the non-aqueous phase, >95% yield was reached as a result of the preferential partitioning behaviour of acetone over 2-propanol into the ionic liquid.

Introduction

Chiral compounds are important building blocks in the chemical and pharmaceutical industry for the production of, for example, chemical catalysts, liquid crystals, flavours, agrochemicals, or drugs. In particular, chiral alcohols are of interest as building blocks.¹ There is a wide range of methods for the production of chiral alcohols described in literature. One chemical method is the use of chiral boranes as reagents for the enantioselective reduction of ketones.^{2,3} Furthermore, chemical transition metal catalysts are applied for the asymmetric synthesis of chiral alcohols.⁴

Biological methods apply isolated enzymes or whole resting cells as biocatalysts for the synthesis of chiral alcohols. In particular alcohol dehydrogenases (ADHs) have gained increased interest for the commercial production of chiral alcohols.^{5,6} These biocatalysts, used as isolated enzymes or whole cells, catalyze the stereoselective reduction of prochiral

ketones with remarkable chemo-, regio-, and enantioselectivity. In comparison to isolated enzymes, whole cell applications have several advantages. Whole cell biocatalysts are usually more stable due to the protective cell matrix envelope for the enzyme. Furthermore, there is no need for enzyme purification when whole cells are used as biocatalysts.⁷

ADHs are dependent on cofactors (NADH or NADPH) that act as hydride donors in the reduction of ketones. Since these cofactors are too expensive to be used stoichiometrically,⁸ there is a significant interest in developing efficient cofactor regeneration processes. The regeneration of cofactors can be carried out in an enzyme-coupled or in a substrate-coupled approach. The enzyme-coupled approach is characterized by the use of a second enzyme, which catalyzes the oxidation of a cosubstrate to regenerate the reduced form of the cofactor. Formate dehydrogenase or glucose dehydrogenase can be applied as the second enzyme for cofactor regeneration. In the substrate-coupled approach the alcohol dehydrogenase that catalyzes the reduction of the prochiral ketone to a chiral alcohol also catalyzes the cofactor-regenerating reaction by oxidation of a second cosubstrate. In most cases 2-propanol is used as cosubstrate, which is oxidized to acetone.^{9,10}

The substrate-coupled approach leads to a thermodynamic equilibrium between all four components since all reactions are reversible. Thus the maximum yield is limited by the thermodynamic potentials of the compounds. Furthermore acetone may have a harmful effect on the activity and the stability of the biocatalysts. To overcome such limitations, in situ (co)product removal techniques can be applied.¹¹

Different strategies for in situ acetone removal have already been published and are part of processes carried out in industrial scale. Due to its low vapour pressure, acetone can easily be stripped from a reactor system by gassing the reaction mixture with pressurized air or any other inert gas. It has already been applied in ketone-reducing processes catalyzed by isolated enzymes.¹² The applicability of this strategy for acetone removal has also been shown for processes catalyzed by whole cells.¹³

* To whom correspondence should be addressed. Phone: (+49) (0) 2461-61-4388. E-mail: s.luetz@fz-juelich.de.

(1) Daussmann, T.; Lütz, S. *CHEManager Eur.* **2006**, 3, 8.

(2) Hirao, A. *Chem. Commun.* **1981**, 7, 315–317.

(3) Corey, E. J. *Am. Chem. Soc.* **1987**, 109, 7925–7926.

(4) Tang, W.; Zhang, X. *Chem. Rev.* **2003**, 103, 3029–3070.

(5) Breuer, M.; Ditrach, K.; Habicher, T.; Hauer, B.; Kesseler, M.; Sturmer, R.; Zelinski, T. *Angew. Chem., Int. Ed.* **2004**, 43, 788–824.

(6) Liese, A.; Seelbach, K.; Buchholz, A.; Haberland, J. *Processes. In Industrial Biotransformations*; Liese, A., Seelbach, K., Wandrey, C., Eds.; Wiley-VCH: Weinheim, 2006; pp 147–513.

(7) Wichmann, R.; Vasic-Racki, D. *Adv. Biochem. Eng. Biotechnol.* **2005**, 92, 225–260.

(8) Wandrey, C. *Chem. Rec.* **2004**, 4, 254–265.

(9) Hummel, W.; Kula, M. R. *Eur. J. Biochem.* **1989**, 184, 1–13.

(10) Findrik, Z.; Vasic-Racki, D.; Lütz, S.; Daussmann, T.; Wandrey, C. *Biotechnol. Lett.* **2005**, 27, 1087–1095.

(11) Lye, G. J.; Woodley, J. M. *Trends Biotechnol.* **1999**, 17, 395–402.

(12) Stillger, T.; Bönitz, M.; Vilella, M.; Liese, A. *Chem.-Ing.-Tech.* **2002**, 74, 1035–1039.

(13) Goldberg, K.; Edegger, K.; Kroutil, W.; Liese, A. *Biotechnol. Bioeng.* **2006**, 95, 192–198.

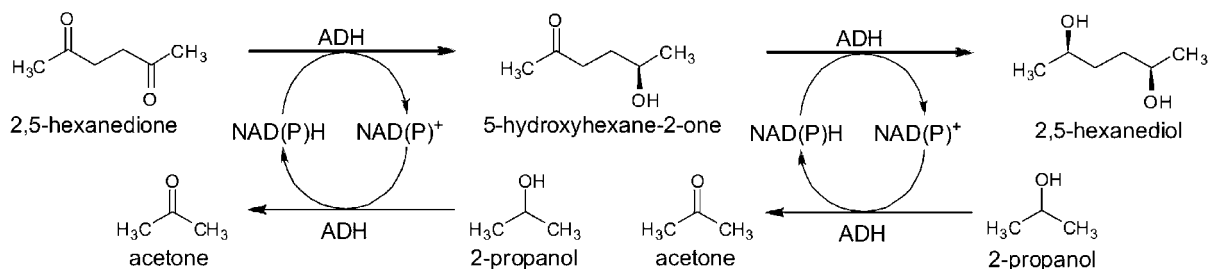


Figure 1. Reduction of 2,5-hexanedione with substrate-coupled cofactor regeneration.

Julich Chiral Solutions and Wacker Chemistry have employed reduced pressure systems to remove the coproduct acetone that is formed during the synthesis of β -keto esters such as (*R*)-methyl hydroxybutyrate and (*R*)-ethyl hydroxybutyrate.^{1,14} Also, acetone removal by pervaporation has been described for biotransformation processes catalyzed by isolated enzymes.¹²

One problem of using biocatalysts for the reduction of prochiral ketones is the low solubility of many ketones in aqueous solutions. For that reason, only low substrate concentrations can be applied to the biocatalysts when the reaction is carried out in an aqueous environment. A straightforward solution to this problem is the application of biphasic systems.^{15,16} In such a system the biocatalysts are located in the aqueous phase while the hydrophic substrate is present in a high concentration in the non-aqueous phase and in small concentrations in the aqueous phase. While there is a yield of substrate in the aqueous phase catalyzed by the cells, new substrate is extracted continuously from the non-aqueous into the aqueous phase. The non-aqueous phase can be used not only as a substrate reservoir but also for the continuous extraction of products or acetone in processes with substrate coupled cofactor regeneration, according to the partition of these compounds between the two phases.

This work describes whole cell biotransformation processes with recombinant *Escherichia coli* overexpressing recombinant alcohol dehydrogenase from *Lactobacillus brevis* (*LbADH*)¹⁷ for the production of several chiral alcohols in a substrate coupled approach with in situ acetone removal. Acetone removal has been achieved by stripping or pervaporation. Furthermore whole cell biotransformation has been carried out in a biphasic system with potassium phosphate buffer and the ionic liquid [BMIM][(CF₃SO₂)₂N] (1-butyl-3-methylimidazolium bis((trifluoromethyl)sulfonyl)amide) to utilize the partition behaviour of acetone in order to remove it from the aqueous phase.¹⁸ In all reactor systems higher yield could be achieved compared to processes without in situ acetone removal. All acetone removal techniques described in this work have already been applied successfully for ketone reducing biotransformation processes catalyzed by isolated enzymes. By contrast only a few examples have been published concerning the combination

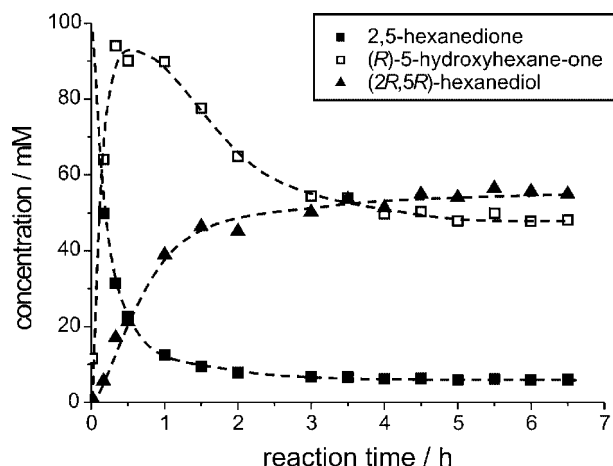


Figure 2. Concentration time course for the reduction of 2,5-hexanedione without acetone removal. Conditions: $V = 0.01$ L, 40°C , pH 6.0, 0.05 mol L^{-1} potassium phosphate buffer, $50\text{ g}_{\text{cww}}\text{ L}^{-1}$ rec *E. coli*, 0.1 mol L^{-1} 2,5-hexanedione, 1 mol L^{-1} 2-propanol. Dashed lines (---) are drawn as visual aides.

of whole cell biotransformation processes, in particular catalyzed by recombinant microorganisms, and acetone removal techniques.

In Situ Acetone Removal by Stripping

2,5-Hexanedione has been reduced to (*2R,5R*)-hexanediol by recombinant *E. coli* cells expressing recombinant *LbADH*. Because the conversion of 2,5-hexanedione to 2,5-hexanediol is a two-step reaction, an excess of the cosubstrate 2-propanol is necessary to achieve sufficient regeneration of cofactors (Figure 1).

A batch reaction experiment without acetone removal showed that there is a thermodynamic limitation that results in low yield. In this batch experiment a high 2-propanol concentration of 1 mol L^{-1} was applied. Despite almost complete consumption of the substrate, only 55 mmol L^{-1} of the product (*2R,5R*)-hexanediol could be produced. The intermediate (*R*)-5-hydroxyhexane-2-one was not converted completely; a concentration of 45 mmol L^{-1} could be determined after equilibrium was reached (Figure 2).

The coproduct acetone is the most volatile compound in this reaction system. Thus it is possible to remove acetone from the reaction system by passing a continuous air stream through the reaction mixture. The pressurized air was first passed through a rotameter and then through a water/2-propanol mixture in order to saturate the air stream with water and 2-propanol. Finally the air stream was passed into the reaction solution thermostated at 40°C .

(14) Daussmann, T.; Rosen, T. C.; Dünkelfmann, P. *Eng. Life Sci.* **2006**, *6*, 125–129.

(15) Liese, A.; Zelinski, T.; Kula, M. R.; Kierkels, H.; Karutz, M.; Kragl, U.; Wandrey, C. *J. Mol. Catal. B: Enzymatic* **1998**, *4*, 91–99.

(16) Vilella, M.; Stillger, T.; Müller, M.; Liese, A.; Wandrey, C. *Ang. Chem., Int. Ed.* **2003**, *42*, 2993–2996.

(17) Hummel, W. *Adv. Biochem. Eng. Biotechnol.* **1997**, *58*, 145–184.

(18) Eckstein, M.; Vilella, M.; Liese, A.; Kragl, U. *Chem. Commun.* **2004**, *9*, 1084–1085.

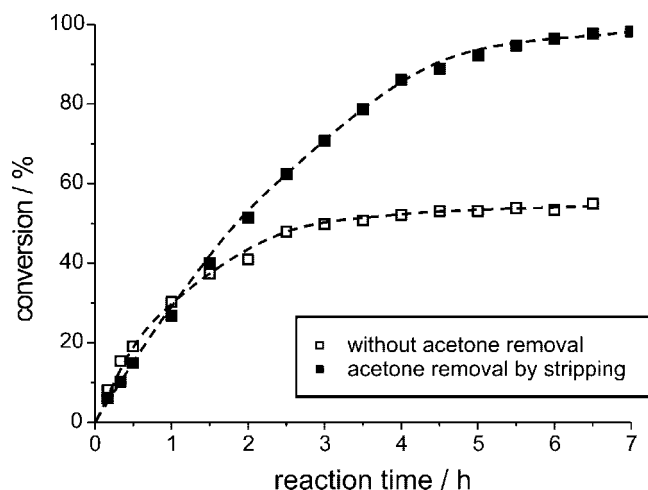


Figure 3. Yield during production of (2*R*,5*R*)-hexanediol with acetone removal by stripping. Conditions: $V = 0.05$ L, 40 °C, pH 6.0, 0.05 mol L⁻¹ potassium phosphate buffer, 50 g_{cell} L⁻¹ rec *E. coli*, 0.1 mol L⁻¹ 2,5-hexanedione, 1 mol L⁻¹ 2-propanol, air stream 1 L min⁻¹. Dashed lines (---) are drawn as visual aides.

The batch reduction of 2,5-hexanedione was also carried out in a batch experiment with acetone removal by stripping and compared to a process without acetone removal (Figure 3). In this two-step reduction yield was defined as amount of product (2*R*,5*R*)-hexanediol divided through the initial amount of substrate (2,5-hexanedione):

$$\text{yield} = \frac{c_i(\text{product})}{c_0(\text{substrate})} \quad (1)$$

During the first 90 min, the course of reaction was almost equal for both experiments. In the experiment without acetone removal a yield of 55% could be achieved when equilibrium was reached. In the experiment with acetone removal by stripping the reaction went on without deceleration until completion. In comparison to the process without acetone removal, thermodynamic limitation could be overcome and almost quantitative yield of >95% was achieved.

In Situ Acetone Removal by Pervaporation

The reduction of 2,5-hexanedione (Figure 1) has also been carried out in a reaction system with acetone removal by pervaporation. The pervaporation process is characterized by a selective transfer of compounds through a membrane.¹⁹ Thereby the physical conditions of these compounds change from liquid to gaseous. In pre-investigations several organophilic membranes have been characterized with respect to their ability to remove acetone selectively from the reaction mixture. A membrane composed of polydimethoxysiloxane was chosen for further investigations due to the high acetone mass flow in comparison to other tested membrane types. The mass transfer of 2-propanol is 50% of that of acetone. Thus 2-propanol must be added during the reaction in order to ensure sufficient concentration of 2-propanol. The mass transfer of a compound

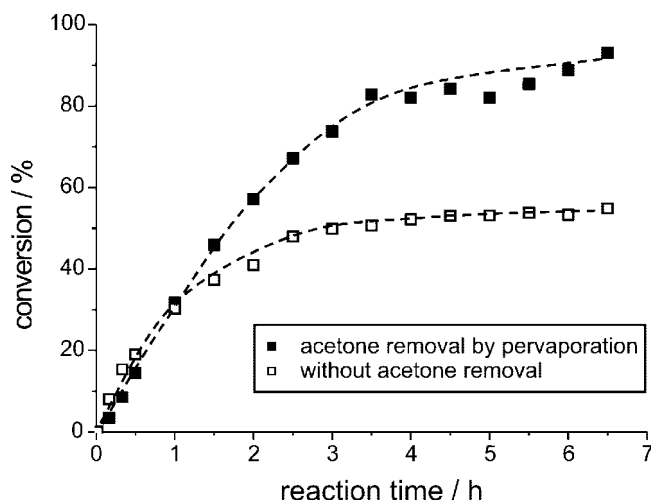


Figure 4. Yield during production of (2*R*,5*R*)-hexanediol with acetone removal by pervaporation. Conditions: $V = 0.15$ L, 40 °C, pH 6.0, 0.05 mol L⁻¹ potassium phosphate buffer, 50 g_{cell} L⁻¹ rec *E. coli*, 0.1 mol L⁻¹ 2,5-hexanedione, 1 mol L⁻¹ 2-propanol, vacuum pressure 15 mbar. Dashed lines (---) are drawn as visual aides.

in a pervaporation process strongly depends on the temperature. For that reason 40 °C was chosen as the reaction temperature, which is an acceptable compromise between sufficient acetone mass transfer and thermostability of the biocatalyst.

Figure 4 shows the increasing yield in biotransformation processes without acetone removal and with in situ acetone removal by pervaporation. During the first 90 min, the course of reaction was almost equal for both experiments. In the experiment without acetone removal the yield just slightly increased after 90 min. In the reactor system with the pervaporation unit the reaction continued. In this reaction where equilibrium is shifted to a much higher degree of conversion as a result of the acetone removal by pervaporation, the yield could be increased to >90%.

Since the synthesis of (2*R*,5*R*)-hexanediol from the product 2,5-hexanedione is a two-step reaction there are 2 equiv of acetone produced for each equivalent of ketone fully reduced. In the experiment without acetone removal the acetone concentration increased up to 180 mmol L⁻¹ (Figure 5). In the biotransformation processes with in situ acetone removal, the acetone concentration did not reach more than 75 mmol L⁻¹ over the course of the reaction. In general, the rate of acetone removal did not significantly differ between the two methods. From the concentration–time curve it becomes obvious that there is a drastic increase in acetone concentration at the beginning of the reaction due to the high catalytic activity of the whole cell catalysts, even when in situ acetone removal methods are applied. After 30 min, a decreasing acetone concentration can be observed in the experiments with acetone removal.

In Situ Acetone Removal by Extraction

The experiments with in situ acetone removal by extraction were carried out using the substrate 1-phenyl-2-propanone, which was reduced to 1-phenyl-2-propanol (Figure 6). Since both compounds, substrate and product, are extremely hydrophobic compounds, they preferably remain in the non-aqueous

(19) Lüttz, S.; Rao, N.; Wandrey, C. *Chem. Eng. Technol.* **2005**, 77, 1669–1682.

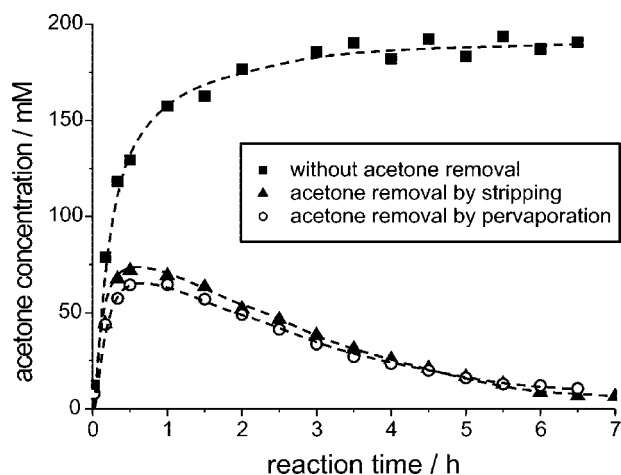


Figure 5. Acetone concentration during reduction of 2,5-hexanedione with and without in situ acetone removal. Dashed lines (---) are drawn as visual aides.

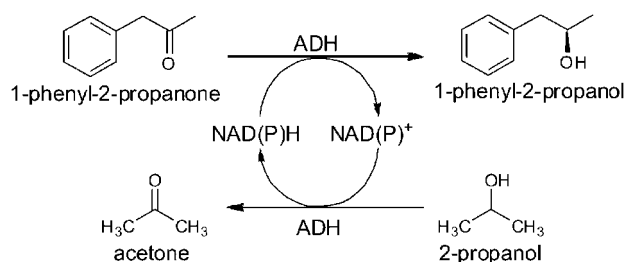


Figure 6. Reduction of 1-phenyl-2-propanone with substrate-coupled cofactor regeneration.

Table 1. Partition coefficients for 2-propanol and acetone¹⁸

system	2-propanol	acetone
MTBE/buffer	1.0	1.1
[BMIM][(CF ₃ SO ₂) ₂ N]/buffer	0.4	2.0

phase. Thus their partitioning behaviour will not lead to any shift in the thermodynamic equilibrium of the reaction, when different non-aqueous phases are applied. However, calculating equilibrium conversions in biphasic systems is a complex task. Recently, new approaches have been reported to estimate suitable reaction conditions for enzyme-catalyzed reactions in biphasic systems and to obtain reliable data for calculation of equilibrium constants.^{20,21}

It is known that the partition behaviour of 2-propanol and acetone in a biphasic system containing buffer and the ionic liquid [BMIM][(CF₃SO₂)₂N] significantly differ from their partitioning behaviour in buffer/methyl *tert*-butyl ether (MTBE) (Table 1). Thus a positive effect on the biotransformation can be expected due to the acetone extraction.

The reduction of 1-phenyl-2-propanone with substrate-coupled cofactor regeneration was carried out in biphasic systems containing MTBE or the ionic liquid [BMIM][(CF₃SO₂)₂N] as non-aqueous phase. In the biphasic system including MTBE as non-aqueous phase, the produced

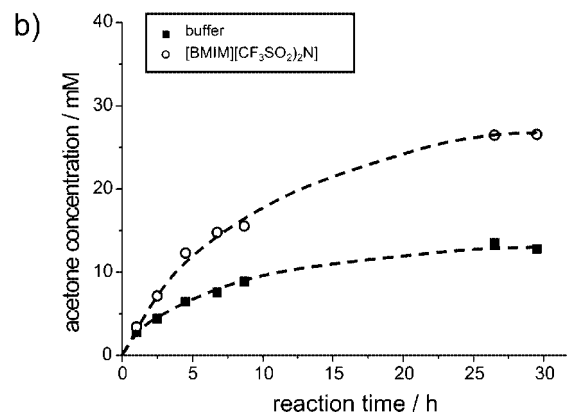
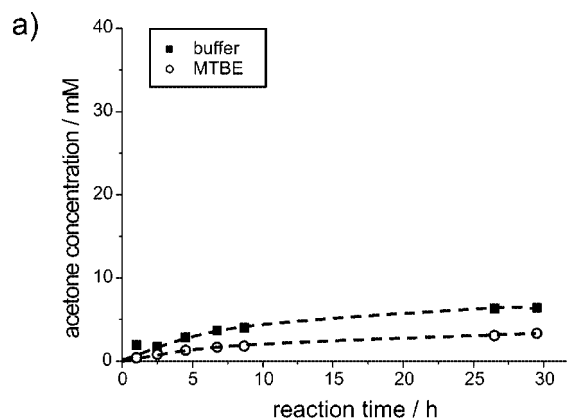


Figure 7. Acetone concentration in both phases during reduction of 1-phenyl-2-propanone in (a) buffer/MTBE biphasic system and (b) buffer/[BMIM][(CF₃SO₂)₂N] biphasic system. Conditions: $V = 0.005$ L KP_i buffer pH 6.0, 0.005 L non-aqueous phase, room temperature, 20 g_{cell} L⁻¹ *rec. E. coli*, 0.02 mol L⁻¹ 1-phenyl-2-propanone, 0.4 mol L⁻¹ 2-propanol. Dashed lines (---) are drawn as visual aides.

acetone prefers to remain in the aqueous phase according to its partition coefficient (Figure 7a). In the biphasic system with the ionic liquid [BMIM][(CF₃SO₂)₂N] as non-aqueous phase, the acetone concentration in the aqueous phase is only 50% of that in the non-aqueous phase (Figure 7b). As a result of the more effective extraction in the biphasic system with buffer and [BMIM][(CF₃SO₂)₂N], an increased yield of >95% could be achieved. In the system with buffer and MTBE, a yield of only 24% was observed (Figure 8).

Stability Investigations/Comparison

To evaluate the stability of the biocatalysts applying different methods of in situ acetone removal, the cells were incubated under certain conditions and their remaining catalytic activity was determined periodically. In a first experimental set-up the stability of the cells was determined in processes applying the acetone removal techniques. In a second set-up there was no acetone removal but all other conditions (e.g., temperature, stirring speed) were kept constant, so that the differences in stability were influenced only by the acetone removal procedure. In the case of the biphasic system the stability of the cells in the [BMIM][(CF₃SO₂)₂N]/buffer biphasic system was compared to that stability of the cells in an aqueous one-phase system with the same parameters such as pH value and temperature. The results can be seen from Table 2.

(20) Eckstein, M. F.; Peters, M.; Lembrecht, J.; Spiess, A. C.; Greiner, L. *Adv. Synth. Catal.* **2006**, 348, 1591–1596.

(21) Eckstein, M. F.; Lembrecht, J.; Schumacher, J.; Eberhard, W.; Spiess, A. C.; Peters, M.; Roosen, C.; Greiner, L.; Leitner, W.; Kragl, U. *Adv. Synth. Catal.* **2006**, 1597–1604.

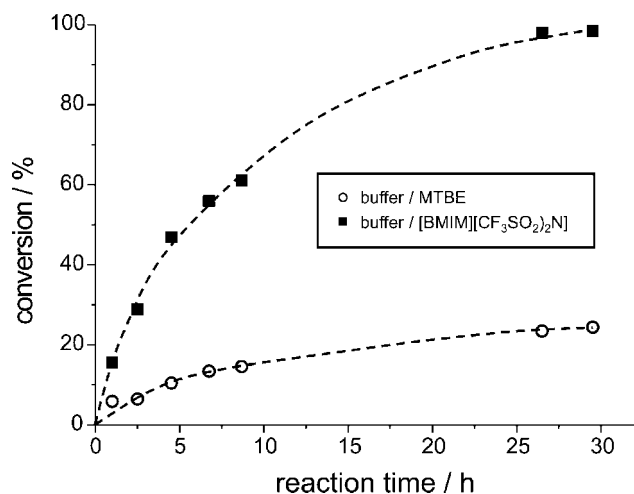


Figure 8. Yield during reduction of 1-phenyl-2-propanone in biphasic systems. Conditions: $V = 0.005$ L KP_i buffer pH 6.0, 0.005 L non-aqueous phase, room temperature, 20 g_{cww} L⁻¹ rec *E. coli*, 0.02 mol L⁻¹ 1-phenyl-2-propanone, 0.4 mol L⁻¹ 2-propanol. Dashed lines (---) are drawn as visual aides.

It became obvious that the stripping procedure has the most drastic effect towards the stability of the whole cell biocatalysts. The relative activity was only 25% in comparison to the cells incubated under the same conditions but without passing an air stream through the reaction mixture. The pervaporation process is a more gentle way for acetone removal since there is a relative stability of 82% compared to cells incubated in a process without applying the pervaporation procedure. The relative stability of cells incubated in a biphasic system consisting of [BMIM][(CF₃SO₂)₂N] and buffer was determined to be 63% in comparison to cells incubated in 50 mmol L⁻¹ potassium phosphate buffer, pH 6.0.

Conclusions and Summary

By applying different in situ acetone removal techniques, whole cell ketone reduction processes with substrate-coupled cofactor regeneration can be intensified significantly. In particular for two-step reductions such as the reduction of 2,5-hexanedione such a technique is an important tool since only low yield could be obtained when the coproduct acetone is not removed from the reaction mixture.

The reduction of 2,5-hexanedione by recombinant *E. coli* cells resulted in almost similar yields when acetone was removed by either applying the stripping procedure or a pervaporation step. The pervaporation process is a very gentle way of acetone removal which results in excellent stability of the biocatalyst compared to processes applying acetone removal by the stripping method. Nevertheless, the stripping process is characterized by a simple reactor set-up that can be run cost-effectively. In industry, there are also some ketone reducing processes established using methods for acetone removal employing evaporation by reduced pressure.^{1,14} To our knowledge there are no processes established in industry using the pervaporation method for in situ acetone removal in biotransformation processes catalyzed by whole resting cells. The majority of examples for in situ product removal in whole cell biotechnology are dealing with product removal from fermentation processes, for example, removal of ethanol from fermenta-

tion processes with *Saccharomyces cerevisiae* or removal of butanol and acetone from fermentation processes applying *Clostridium species*.²² On the laboratory scale, this method had been restricted to ketone-reducing processes using isolated enzymes as biocatalysts. This is the first work that deals with the combination of whole cell biotransformation and in situ acetone removal by pervaporation.

The choice of biphasic systems is beneficial when hydrophobic substrates should be applied in biocatalytic reactions. It could be shown that by using the ionic liquid [BMIM][(CF₃SO₂)₂N] as the non-aqueous phase hydrophobic ketones such as 1-phenyl-2-propanone can be applied in high concentrations. Furthermore this ionic liquid shows a favourable partitioning behaviour for acetone and 2-propanol so that it can be used not only as substrate reservoir but also for the in situ extraction of acetone. The influence of biphasic systems applying the ionic liquid [BMIM][(CF₃SO₂)₂N] as the non-aqueous phase on the stability and activity of the whole cell biocatalyst has not been investigated satisfactorily until now. In literature there are some other examples for biphasic systems with ionic liquids in whole cell biotransformation processes. Pfründer et al. described the reduction of 4-chloro acetophenone using *Lactobacillus kefir* cells in biphasic systems consisting of buffer and different ionic liquids.²³ Other applications of ionic liquids in biocatalysis, which mainly deal with isolated lipases, have recently been reviewed several times.^{24,25}

Experimental Section

Bacterial Strains and Plasmids. Plasmid pBtacLB-ADH (X-Zyme, Düsseldorf, Germany) carrying the *adh* gene and ampicillin resistance was employed as expression vector. *E. coli* BL21 Star (DE3) (Invitrogen, Karlsruhe, Germany) was used for gene expression and whole cell biotransformation. The *E. coli* strain was transformed by the method described by Hanahan.²⁶

Biomass Production. Recombinant *E. coli* were cultivated on modified Luria Bertani (LB) medium: 10 g L⁻¹ casein peptone, 5 g L⁻¹ yeast extract, 10 g L⁻¹ NaCl, 4 g L⁻¹ glucose. Then 50 mL of medium containing 50 mg L⁻¹ carbenicillin was inoculated with 500 μL of a glycerine stock culture. After incubation overnight at 30 °C and 150 rpm, 200 mL of the same medium was inoculated with 500 μL of the preculture. Main cultures were incubated for 6 h at 37 °C and 150 rpm. Then gene expression was induced by adding 0.2 mmol L⁻¹ IPTG. Afterwards the cultures were incubated for 18 h at 27 °C and 150 rpm. The cells were harvested by centrifugation (Beckman Coulter Avanti J-20 XP, 8000 rpm, 20 min, 4 °C) and washed once with 50 mmol L⁻¹ potassium phosphate buffer, pH 6.0. The cells were stored as a 100 g L⁻¹ cell suspension in 50 mmol L⁻¹ potassium phosphate buffer, pH 6 at 4 °C.

(22) Stark, D.; von Stockar, U. *Adv. Biochem. Eng./Biotechnol.* **2003**, 80, 149–175.

(23) Pfründer, H.; Amidjojo, M.; Kragl, U.; Weuster-Botz, D. *Ang. Chem., Int. Ed.* **2004**, 43, 4529–4531.

(24) Kragl, U.; Eckstein, M.; Kaftzik, N. *Curr. Opin. Biotechnol.* **2002**, 13, 565–571.

(25) Van Rantwijk, F.; Lau, R. M.; Sheldon, R. A. *Trends Biotechnol.* **2003**, 21, 131–138.

(26) Hanahan, D. *J. Mol. Biol.* **1983**, 166, 557–580.

Table 2. Relative stability of the biocatalyst and biocatalyst specific productivity during biocatalysis with in situ acetone removal^a

method of acetone removal	relative stability [-]	biocatalyst specific productivity [$\text{g}_{\text{product}} \text{g}_{\text{cww}}^{-1} \text{d}^{-1}$]
without acetone removal	1	25.5
stripping	0.25	46.3
pervaporation	0.82	44.0
[BMIM][$(\text{CF}_3\text{SO}_2)_2\text{N}$] / buffer	0.63	2.59

^a Stripping and pervaporation experiments were carried out using 2,5-hexanedione as substrate whereas the biphasic system was applied for the reduction of the hydrophobic substrate 1-phenyl-2-propanone.

Analytics. Quantification of all substrates and products of biotransformation processes was carried out on an Agilent HP-6890A gas chromatograph with a Permabond Carbowax 20M column (50 m \times 0.32 mm i.d., Macherey-Nagel, Dueren, Germany) with a flame ionization detector and helium as carrier gas. To minimize the injection error, *n*-butanol was used as an internal standard. All compounds from experiments with in situ acetone removal were detected by a method using the following temperature program: 5 min 50 °C, 40 °C min⁻¹, 9 min 160 °C. Typical retention times were acetone 2.5 min, 2-propanol 4.0 min, *n*-butanol 7.1 min, 1-phenyl-2-propanone 10.2 min, 1-phenylpropanol 10.9 min, 2,5-hexanedione 10.3 min, 5-hydroxyhexane-2-one 11.7 min, and 2,5-hexanediol 15.6 min. All compounds from batch experiments for investigation on the catalyst stability were detected by a method using the following temperature program: 6 min 70 °C, 25 °C min⁻¹, 3 min 160 °C. Typical retention times were acetone 1.7 min, 2-propanol 2.2 min, *n*-butanol 5.6 min, methylacetoacetate 9.9 min, and methylhydroxybutyrate 10.6 min.

Reactor Performance. *Stripping Experiments.* Biotransformation processes with acetone removal by stripping were carried out on a 50 mL scale in a jacketed glass reactor (glass workshop of the Research Centre Juelich, Germany) with a porous glass frit as bottom. The reactor solution was gassed from the bottom with pressurized air at a flow rate of 1 L min⁻¹. The flow rate was measured by a rotameter (Sho-rate, Brooks Instrument, Veenendaal, Netherlands). To avoid outgassing of 2-propanol, the pressurized air was humidified with a 50% (v/v) mixture of water and 2-propanol. One drop of antifoam (Biospumex 153K, Cognis GmbH, Monheim am Rhein, Germany) was added to the reaction solution in order to avoid foam formation. The biotransformation process was carried out in 50 mmol L⁻¹ potassium phosphate buffer, pH 6.0, at 40 °C. The biomass concentration was 50 g_{cww} L⁻¹.

Pervaporation. Biotransformation processes with acetone removal by pervaporation were carried out in a reverse osmosis system (P-28, cmcelfa, wen-Schwyz, Switzerland). The stainless steel cell provided 44.2 cm² of active membrane area. The polydimethoxysiloxane membrane PA-HP-02, (PolyAN, Berlin, Germany) was used as pervaporation membrane. The reaction solution was pumped through the reactor by a flow rate of 0.3 L min⁻¹ (flexible-tube pump 505U, Watson-Marlow, Rommerskirchen, Germany). The feed volume was 0.15 L. A vacuum of 15 mbar was maintained throughout the whole reaction time (diaphragm vacuum pump MZ 2C, vacuubrand, Wertheim, Germany). The biotransformation process was carried out in 50 mmol L⁻¹ potassium phosphate buffer, pH 6.0, at 40 °C. The biomass concentration was 50 g_{cww} L⁻¹.

Biphasic Systems. For the biotransformation process in biphasic systems, 5 mL of potassium phosphate buffer containing 40 g_{cww} L⁻¹ biomass was introduced into a centrifuge tube together with 5 mL of the non-aqueous medium. The tubes were mixed on a shaker (VXR basic IKA Vibrax, IKA Labortechnik, Staufen, Germany) with 300 rpm.

Sampling. A 200 μL sample of reaction solution was removed from the reaction system and centrifuged (Centrifuge 5415 D, Eppendorf, Hamburg, Germany) at 16000 rpm for 30 s to remove the cells. To avoid further reaction catalyzed by leaked enzyme, the supernatant was transferred into a second tube and incubated for 60 s at 99 °C (ThermoStat plus, Eppendorf, Hamburg, Germany). After cooling at 4 °C the sample was prepared for gas chromatography analysis.

Tests for Stability. For the investigations on the catalyst stability recombinant *E. coli* cells were incubated under the conditions applied in biotransformation processes with in situ acetone removal. Periodically the cells were tested for their remaining catalytic activity. Therefore the cells were used as biocatalysts for the reduction of methyl acetoacetate under the following conditions: $V = 1 \text{ mL}$ (Eppendorf tube), 30 °C, 300 rpm (Thermomixer compact 5350, Eppendorf, Hamburg, Germany) pH 6.0, 0.05 mmol L⁻¹ potassium phosphate buffer, 5 g_{cww} L⁻¹ rec *E. coli*, 0.1 mol L⁻¹ methyl acetoacetate, 0.2 mol L⁻¹ 2-propanol. For calculation the catalytic activity the increasing concentration of the product (*R*)-methyl hydroxybutyrate was measured by gas chromatography as described above.

Acknowledgment

This work was financially supported by BMBF (Project no. 0313440C). The authors thank Prof. Dr. C. Wandrey for ongoing support and Prof. Dr. A. Liese (TU Hamburg-Harburg) for fruitful discussions. Help in plasmid amplification and strain construction by Dr. Bringer-Meyer (IBT-1, Research Centre Juelich) is gratefully acknowledged. Furthermore the authors thank Dr. Matuschewski (PolyAN, Berlin, Germany) for providing the pervaporation membrane.

Supporting Information Available

Additional data on the impact of acetone on the biocatalysts, reactor schemes, and stability measurements in the pervaporation setup. This information is available free of charge via the Internet at <http://pubs.acs.org>.

Received for review March 6, 2007.

OP700055E