Microbiological Transformations 60. Enantioconvergent Baeyer–Villiger Oxidation *via* a Combined Whole Cells and Ionic Exchange Resin-Catalysed Dynamic Kinetic Resolution Process

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Abstract: A dynamic kinetic resolution process was applied to an enantioconvergent microbial Baeyer–Villiger oxidation of benzyloxycyclopentanone, *rac*-**1**. This was achieved by combining a whole cell-based kinetic resolution and an anion exchange resin-catalysed *in situ* racemisation. Several resins were screened according to their capacity to catalyse the racemisation of **1**. As compared to our previous results, the presence of weakly basic Lewatit MP62 in biotransformations of **1** allowed a three-fold improvement of usable substrate concentration and afforded nearly enantiopure lactone (*R*)-**2** (97% ee) in preparative (isolated) yields higher than 84%. Noyori's model was applied to this reaction and allowed kinetic constants determination.

Keywords: asymmetric Baeyer–Villiger oxidation; biotransformations; dynamic kinetic resolution; ion exchange resin; racemisation; whole-cell process

Introduction

The increasing interest and need for enantiomerically pure compounds in pharmaceutical and agricultural industry has led to considerable development of asymmetric catalysis techniques during the last decades.[1] In this context, significant effort has been made to overcome the important productivity limitation linked to the use of classical resolution processes – i.e., the maximum 50% theoretical product yield barrier – and new strategies have been developed to afford the enantiopure product in nearly quantitative yields. One possible way to reach this goal is dynamic kinetic resolution (DKR),[2] an approach which combines an *in situ* racemisation with a kinetic resolution (KR) step (*cf.* Scheme 1). Both substrate enantiomers can thus be converted into a single enantiomer of the product with a 100% theoretical yield. A number of successful examples for DKR methods have been reviewed recently.[3] Some of them involve solely conventional chemical methods,[4] whereas others associate chemical and biocatalytic steps.[5] In this latter case, enzymes or whole cells contribute either to racemisation[6] or to kinetic resolution.[3]

The Baeyer–Villiger oxidation of ketones into lactones is an important reaction in organic chemistry because of its potential applications in synthesis, lactones being valuable building blocks for synthesis of countless natural – or unnatural – biologically active products. Only recently, transition metal-based chemical approaches to asymmetric BV oxidation have been developed with, to date, generally rather moderate success.[7,8] On the other hand, we (and others) have demonstrated that specific enzymes – i.e., Baeyer–Villiger monooxygenases[9] (BVMOs) – were highly efficient to achieve asymmetric BV oxidation with high enantio- or enantiotoposelectivity.[10,11] Moreover, we recently have developed a logistically simple whole cell process involving a resin-based *in situ* SFPR (substrate feeding and product removal) strategy, which allowed us to overcome one of the main bottlenecks[11] met with this type of biotransformation, i.e., the low substrate or product concentration dictated by inhibition phenomena. The successful application of such a process to the biotransformation of *rac*-bicyclo[3.2.0]hept-2-en-6-one has opened the way to highly productive biooxidation.[12]

In the same context, we also have recently reported a first example of DKR.[13] This was applied to 2-benzyloxymethyl-cyclopentanone, *rac*-**1** (Scheme 1), and was

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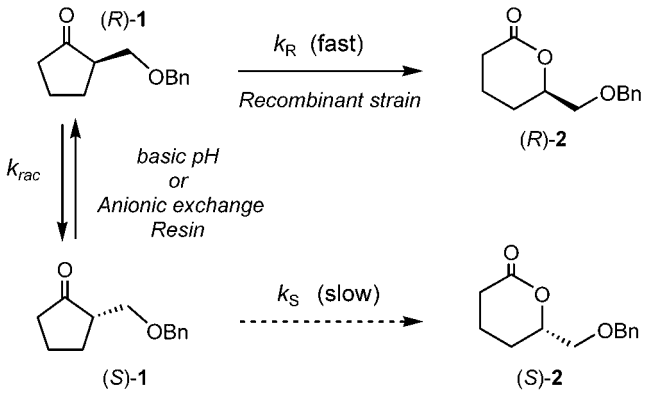

Scheme 1. DKR of *rac*-**1**.

based on the combined use of a recombinant strain overexpressing CHMO (the best known BVMO)[14] and of a base-catalysed substrate racemisation (at pH 8.5). However, the partial loss of enzymatic activity observed at this pH, as well as the rather low optimal usable substrate concentration, seriously hampered the preparative scale applicability of this approach. We therefore have further investigated new alternatives for improving such a DKR process and, in the present work, we describe results based on the combined use of the whole-cells approach and substrate racemisation catalysed, at nearly *neutral* pH, by an ionic exchange resin.

Results and Discussion

As already underlined by Faber,[2] the success of a DKR process requires that the rate constant for racemisation (k_{rac}) is equal to (or greater than) the rate constant for transforming the substrate into product (k_{fast}). Obviously, the compatibility of both reactions (racemisation *and* enantioselective transformation) is the critical facet of such a methodology, since substrate racemising conditions must not adversely affect the resolution reaction, or interfere with the chiral integrity of the product. Thus, the main challenge in our study was to find substrate-racemising conditions while not affecting the efficiency of the enzymatic resolution.

As a model substrate for α-substituted cyclopentanones, 2-benzyloxymethylcyclopentanone, *rac*-**1**, was synthesised from commercial ethyl 2-oxocyclopentanecarboxylate, following described experimental procedures (four steps, 50% overall yield).[15] We had previously reported that microbial BV oxidation of *rac*-**1**, carried out at nearly neutral pH, was very efficient using either a culture of the wild-type bacteria *Acinetobacter calcoaceticus* NCIMB 9871[13] or a culture of the recombinant *E. coli* TOP10[pQR239] strain overexpressing CHMO.[16,17] Thus, the (*R*)-**1** enantiomer gave preferentially nearly enantiomerically pure (*R*)-6-benzyloxymethyltetrahydro-2-pyrone **2**, whereas (*S*)-**1** was insignificantly oxidised. Using *E. coli* TOP10[pQR239], both these compounds were isolated in, respectively, 54 and 33% yield and 84 and 82% ee, which corresponds to an apparent E value of *ca.* 30. On the other hand, the DKR of *rac*-**1** (300 mg, 1.5 mmol) carried out at pH 8.5 (using a 1-L cell broth) afforded (*R*)-**2** (275 mg, 87% yield) in 96% ee.[13]

However, drawbacks in this interesting DKR process were (a) limited ketone concentration (0.3 g L^{-1}), owing to potential toxicity or inhibition effects of *rac*-**1** towards the microbial cells and/or the enzyme[18] and (b) significant (35–40%) loss of enzymatic activity at the rather high pH (8.5) yet essential for a fast enough racemisation of **1** (*cf.* Figure 1). As a consequence, a stringent condition for improving this process would be to conduct the biotransformation at nearly neutral pH (which we have shown to be optimum as far as cell activity is concerned – *cf.* Figure 1), while still performing fast enough *in situ* racemisation. Moreover, since the rate ratio between the biooxidation on one side and the racemisation on the other determines the efficiency of a DKR procedure, the enzymatic activity enhancement allowed by the use of an optimum (neutral) pH necessitated an – at least similar – improvement in the racemisation rate.

Racemisation Studies

To the best of our knowledge, only few studies have described accurate racemisation kinetics.[19,20] Zwanenburg and co-workers have recently summarised and classified, in an excellent review, different racemisation techniques.[21] The most common method is a base-catalysed process generally performed at rather high pH, which necessitates that the substrate bears an acidic hydrogen at the stereogenic centre. However, a few examples using anionic exchange resins have also been describ-

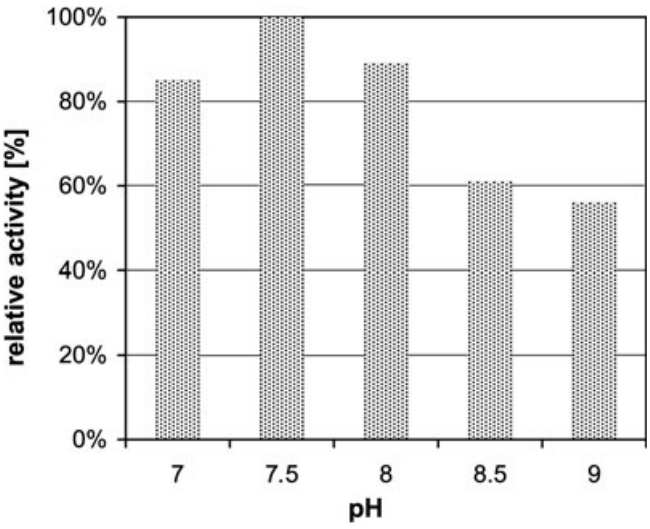

Figure 1. Relative specific activity of *E. coli* [pQR239] cells at various pH values (measured against racemic bicyclo[3.2.0]-hept-2-en-6-one).

Table 1. Characteristics of anion exchange resins and effect on the racemisation of **1**.

Anion exchange resin	Strength and type of resin	Functional group	Exchange capacity [meq g^{-1}]	Measured pH[a]	Racemisation acceleration
Amberlite IRA 900	Strong (type I), macroreticular	quaternary amine	3.5	6.9	–
Dianion HPA 25	Strong (type I), highly porous	quaternary alkyl amine	2.2	6.9	–
Dowex Marathon MSA	Strong (type I), macroporous	trimethylbenzylammonium	4.1	7.0	–
Lewatit OC1950	Strong (type I), gel	quaternary amine	1.1	7.4	–
Amberlite IRA 910	Strong (type II), macroreticular	dimethylethanolamine	3.5	7.3	–
Lewatit MP64	Medium, macroporous	Tertiary and quaternary amine	1.4	7.8	++
Lewatit MP62	Weak, macroporous	Tertiary amine	1.7	7.9	+++
Amberlite IRA 67	Weak, gel	polyamine	4.9	7.7	+

[a] pH measured after resin addition (12.5 meq L^{-1}) into a culture medium at initial pH 6.7.

ed.[22,23] We therefore hypothesised that racemisation of the structurally simple α-substituted ketone *rac*-**1** could, similarly, be performed at nearly neutral pH *via* a ketone/enol equilibrium using such a resin. In order to check this possibility, we first prepared enantiomerically enriched (*S*)-**1** (65% ee) by performing the *E. coli* TOP10[pQR239]-catalysed biotransformation of 0.5 g of *rac*-**1** at neutral pH. Using this enantiomerically enriched substrate, several strongly, medium and weakly basic anion exchanger resins (gel and macroporous types listed on Table 1) were screened for their racemisation performances.

Racemisation assays were carried out in the culture medium at two different temperatures (30 and 37 °C) in the presence of 5 equivalents of resin with respect to the substrate. Racemisation is supposed to be a pseudo-first-order reaction and rate constants (k_{rac}) were determined by a least square fit of the calculated and measured ee of **1** according to Equation (1):

$$ee(t) = ee(0)e^{-2k_{rac}t} \quad (1)$$

The results are reported in Figure 2. Because addition of resin slightly modified the pH of the medium (*cf.* Table 1) and in order to avoid a subsequent bias of outcomes, the rate constant was compared with those of the pH-dependent racemisation (black lines) at the considered pH. Thus, we observed that with type I and type II strong anionic resins, which are the most frequently used in experiments involving a heterogeneous base-catalysed racemisation,[22,23] the racemisation rates are similar to those observed in the culture medium at the corresponding pHs. A weak anion exchanger (IRA 67) only led to modest improvement whereas the best results were obtained with the medium anionic resin Lewatit MP64 as well as with the weakly anionic resin Lewatit MP62. Interestingly, both these resins are macroporous and are the only ones to bear tertiary amines.[24] Lewatit MP62 was selected for further studies because, in the presence of this resin, racemisation of **1** at 37 °C (pH 7.7) was faster than at pH 9 at 30 °C (*cf.* Figure 2).

Preliminary Controls

As a consequence of the above observations and proposal for optimal experimental conditions, it remained to verify the compatibility of these conditions with the preservation of CHMO enzymatic activity and determine the optimum usable substrate concentration. The CHMO activity of the recombinant *E. coli* strain was checked by using racemic bicyclo[3.2.0]hepten-2-one as a test substrate.[12] We observed that: (a) the anion exchanger Lewatit MP62 (12.5 meq L^{-1}) only scarcely affected the CHMO activity and (b) at pH 7, the temperature rise from 30 to 37 °C approximately led to a two-fold enzymatic activity increase (results not shown). We therefore proceeded to the evaluation of the DKR of *rac*-**1** using these experimental conditions.

In order to define the optimal ketone concentration that could be used, substrate and product inhibitory levels had also to be determined. The solubility of *rac*-**1** in

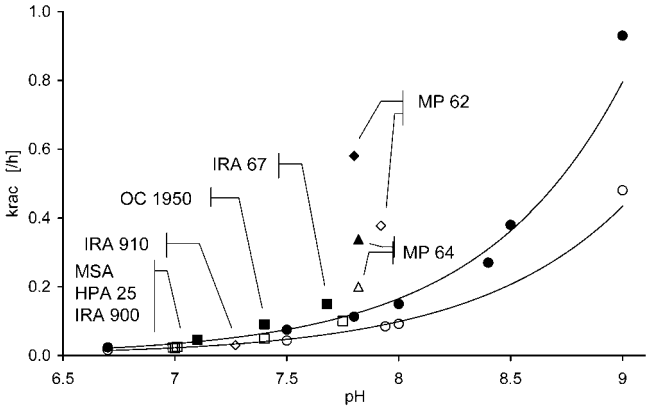

Figure 2. Racemisation constants (k_{rac}) with respect to pH. *Empty symbols:* racemisation at 30 °C, *filled symbols:* racemisation at 37 °C; *full lines*, black circle and empty circle: pH-dependent racemisation (addition of KOH to the culture medium); *other single symbols:* racemisation with various anionic resins (12.5 meq L^{-1}), ◆ and ◇ Lewatit MP62, ▲ and △ Lewatit MP64, ■ and □ other resins.

the culture medium was determined to be about 1.3 g L^{-1}. Thus, several experiments achieved at different concentrations (pH 7, 30 °C, 250 mL shake flasks – results not reported) showed that *rac*-**1** was not inhibitory until a concentration close to its solubility (no decrease of the reaction rates). Nevertheless, biooxidation did not go to completion at concentrations higher than 1 g L^{-1}.

Experiments were similarly carried out after *prior* addition of lactone *rac*-**2** (synthesised by chemical BV oxidation of **1**) into the biotransformation medium (pH 7), at various concentrations (*cf.* Figure 3). The initial presence of lactone **2** at a concentration of about 1 g L^{-1} noticeably slowed down the reaction rate. At 1.5 g L^{-1} initial lactone concentration, biotransformation was totally inhibited. Consequently, it appeared that the optimal concentration of *rac*-**1** usable for this reaction was close to 1 g L^{-1}. It is to be stressed that the observed inhibition might be due either to the cyclic (lactone) or to the open (hydroxy acid) form of **2**, since both exist in equilibrium in these experimental conditions.[25] In contrast, at pH 8.5, only the hydroxy-carboxylate would exist that might explain the lower usable concentration of **1** (0.3 g L^{-1}) at this pH,[13] suggesting that the open anionic form would be more inhibitory than the protonated one.

Dynamic Kinetic Resolution of *rac*-1

Studies on the BV biooxidation of *rac*-**1** were carried out at analytical and preparative scales, using a cell broth regulated at pH 7.5. However, due to the above cited equilibrium between the open and closed form of **2**, and, to some extent, to a partial adsorption of **2** onto the resin, accurate measurement of the lactone concentration was not possible during the biotransformation. For preparative scale experiments, acidification of cell broth before extraction and use of a Dean–Stark apparatus was necessary to ensure the cyclisation of hydroxy acid into lactone.

Analytical Scale Experiments

In order to check the influence of the resin on the biotransformation of *rac*-**1**, we carried out experiments either in absence or in presence of Lewatit MP62 and, in the latter case, the resin was added either at the beginning of the biotransformation or after 3.5 h. These experiments were conducted on an analytical scale in an aerated bottle at 1 g L^{-1} of *rac*-**1**. The concentration and ee of the unreacted ketone **1** are reported on Figure 4. In the absence of resin, the ketone ee raised up to 70% before stabilising but, as soon as the resin was added, it decreased rapidly down to about 15%, a value similar to the one reached when the resin was added at the start of the bioconversion. We observed also a faster disappearance of the ketone in presence of the resin. After extraction in recyclisation conditions, (*R*)-**2** was obtained with a yield as high as 83% and excellent ee (> 98%). These experiments unambiguously illustrate the interest of this resin which makes possible performing this DKR of *rac*-**1** at nearly neutral pH.

Further Improvements

In order to improve this process, we further explored the possible influence of reaction temperature and the proportion of resin used. As far as temperature is concerned, we had previously observed that the BVase activity of our *E. coli* recombinant strain (against bicycloheptenone) was nearly doubled at 37 °C as compared to

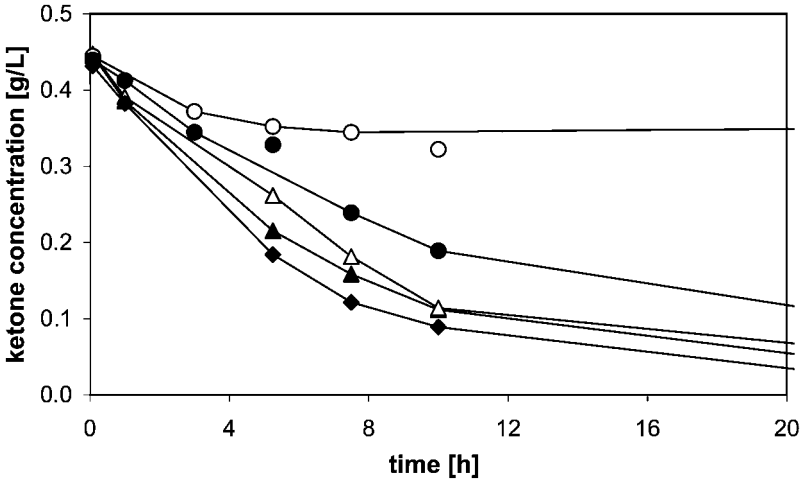

Figure 3. Determination of the inhibitory level of lactone **2**. Time dependent concentration of *rac*-**1** in the presence of various initial concentrations of *rac*-**2**: ◆ 0, ▲ 0.5, △ 0.7, ● 1.0 and ○ 1.5 g L^{-1} (*other conditions:* 30 °C, pH 7, 0.5 g L^{-1} of rac-**1**).

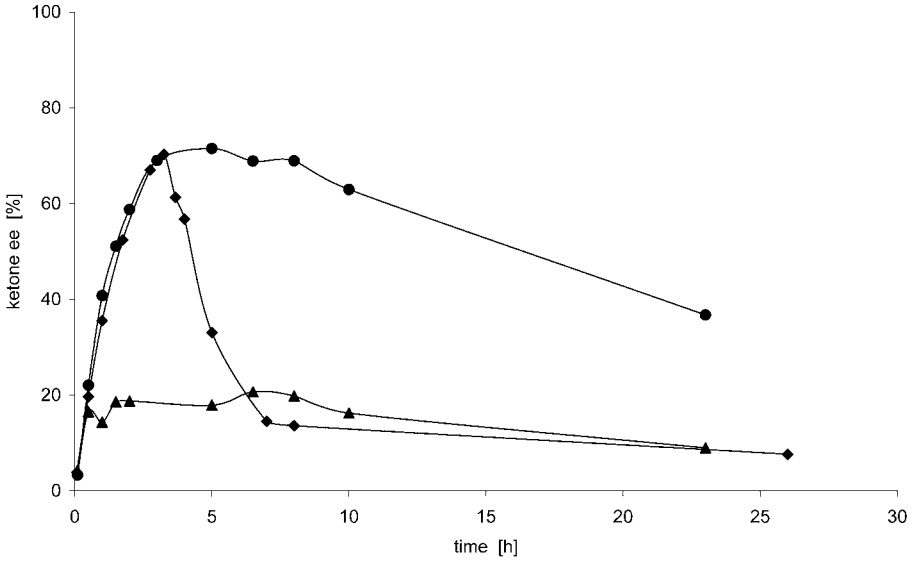

Figure 4. Analytical scale DKR of *rac*-**1**. Effect of Lewatit MP62 on unreacted ketone ees. No resin: ●; addition of resin (24.5 meq L^{-1}) at time 0: ▲; after 3.5 h: ◆ (*other conditions:* 1 g L^{-1} of *rac*-**1**, pH 7.5, 30 °C).

30 °C. As the temperature enhancement also increased the racemisation rate, that could be an interesting improvement factor, provided that the increase of the racemisation rate would be at least equivalent to that of the enzymatic activity. Therefore, we carried out a series of analytical experiments at 37 °C (a) in the absence of resin and (b) in the presence of 2.5 and 5 equivalents of Lewatit MP62 (corresponding, respectively, to 4 and 8 meq L^{-1}). Results are reported in Figure 5 and Table 2. They indicate that the "stabilised" ketone ee reached a value higher than in the previous experiments at 30 °C. Moreover, the ee of the formed lactone decreases until a value of 68% after 6 h of bioconversion when the reaction was conducted in the absence of resin, whereas values of 83 and 86% were observed in the presence of, respectively, 2.5 and 5 equivalents of Lewatit MP62 (*cf.* Table 2).

Kinetic Calculations

Interestingly, these observed drops of ee could be explained using a theoretical kinetic model previously developed by Noyori and co-workers.[26] It was based on two main hypotheses: (a) the reaction rates (k_{rac}, k_R and k_S) of such a DKR process are pseudo-first order in substrate concentration and (b) the kinetic resolution is an irreversible reaction. Assuming these hypotheses are fulfilled in our experiments, we calculated the kinetic constants of these reactions using a least squares fit based on our experimentally determined ketone and lactone ees. The results obtained are indicated in Table 2.

The good correlation we observed between the calculated k_{rac} values and those determined independently in the course of base-catalysed racemisation experiments (*cf.* Table 2) as well as between calculated and experimental values (black lines and symbols – *cf.* Figure 5), seems to validate, in a first approximation, the applicability of Noyori's kinetic model to our case. Calculations

Table 2. Kinetic constants determined from experimental ketone and lactone ees following Noyori's equations.

Resin concentration [meq mL^{-1}]			
	0	4	8
Observed maximum ketone ee [%]	88	70	60
Observed lactone ee after 6 h reaction [%]	68	83	86
Calculated k_{rac} [h^{-1}]	0.08 (0.08[a])	0.2	0.4 (0.5[a])
Calculated k_R [h^{-1}]	1.4	1.1	1.3
Calculated k_S [h^{-1}]	0.05	0.03	0.04

[a] k_{rac} values determined separately during racemisation experiments.

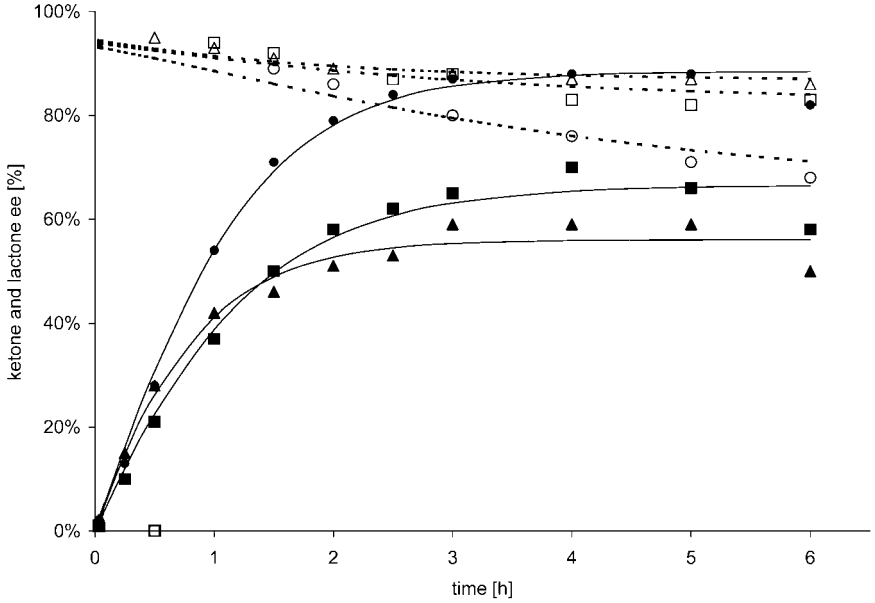

Figure 5. Analytical scale DKR of *rac*-**1**: Experimental and simulated effect of Lewatit MP62 on ketone and lactone ees. Experimental (symbol) and simulated (line) ketone (empty symbol) and lactone (black symbol) ees. *No resin*: ○ and ●; *Lewatit MP62* 4 meq L^{-1}: □ and ■; 8 meq L^{-1}: △ and ▲ (others conditions: 0.3 g L^{-1} of **1**, pH 7.5, 37 °C).

led to nearly unchanged k_R and k_S values in the presence or absence of resin, whereas a noticeable increase of k_{rac} with resin concentration was observed – which translated into an increase of the ee of the formed lactone. These outcomes illustrate the necessity of a fast enough racemisation rate for achieving a successful DKR process. Thus, with an observed k_R/k_S ratio of *ca.* 30, k_{rac} has to be equal to, at least, one third of the k_R value in order to provide a lactone ee higher than 90%. Also, it is noteworthy that a two-fold increase of the resin proportion only had a very negligible – if any – effect on the increase of the lactone ee (86 *versus* 83%).

Preparative-Scale Experiments

In order to validate the above determined optimal conditions, and also to check that the formed lactone was not irreversibly adsorbed onto the resin, three gram-scale experiments were performed, using different experimental conditions.

A first run was carried out using 1 g of *rac*-**1** (4.9 mmoles), at 30 °C and in the presence of 5 equivalents of Lewatit MP62. The two others were conducted on, respectively, 1 g and 1.3 g of *rac*-**1**, at 37 °C and using 2.5 equivalents only of resin. In all cases, the pH was automatically set at 7.5 until completion of the reaction. The results are reported in Table 3. First of all, in all three cases and whatever resin proportion, a very satisfactory yield (higher than 84%) of purified lactone (*R*)-**2** was obtained, indicating that the biooxidation smoothly occurred indeed and that an efficient extraction of the lactone could be achieved. Further information can also be drawn from these experiments: (a) a noticeable acceleration of the biooxidation rate was observed at 37 °C. Thus, in these experiments (entry 2 and 3), 0.6 g L^{-1} of **2** were formed after 5 h biotransformation, as compared to about 0.35 g L^{-1} in the 30 °C run

Table 3. Preparative DKRs of *rac*-**1**.

Entry	Temperature [°C]	Initial ketone concentration [g L^{-1}]	Lewatit MP62		Residual ketone **1**		Lactone **2**	
			Resin equivalents	concentration [meq L^{-1}] (mass [g])	yield[a] [%]	ee max. [%]	yield[a] [%] (mass [g])	ee [%]
1	30	1	5	24 (14.6)	7	17	84 (0.90)	97
2	37	1	2.5	12 (7.3)	5	29	85 (0.93)	91
3	37	1.3	2.5	16 (9.5)	17	17	89[b] (1.04)	96

[a] Isolated yield.
[b] Yield based on the recovered ketone.

(entry 1), (b) however, for the 37 °C run (entry 2), the final ee of the formed lactone **2** was lower whereas the ee of the remaining ketone stayed higher throughout the biotransformation, indicating that the racemisation rate was not fast enough in these experimental conditions, (c) interestingly, increasing the resin concentration (entry 3) had, as a result, a better ee (96%) of the lactone, (d) on the other hand, a higher substrate concentration (1.3 g L^{-1}) severely slowed down the biooxidation rate after 10 h (17% of ketone still remained in the medium), confirming the inhibitory effect of the lactone at a concentration of *ca.* 1 g L^{-1}.

Conclusion

We have shown in this study that a very satisfactory dynamic kinetic resolution of ketone *rac*-**1** could be set up by combining whole-cell catalysed Baeyer–Villiger oxidation (using a culture of a recombinant *E. coli* strain overexpressing cyclohexanone monooxygenase from *Acinetobacter calcoaceticus*) and *in situ* racemisation catalysed by the weakly basic anion exchanger Lewatit MP62. The choice of the adequate resin was the key point of this work. Also, optimisation of the experimental conditions allowed us to define the parameters leading to both a high enzymatic activity and a fast enough racemisation rate of the unreacted substrate. Noyori's model was applied to this DKR in an attempt of rationalisation and allowed kinetic constants determination. As compared to our previous results conducted at pH 8.5, a three-fold substrate concentration could be used, which translated into an equivalent increase of productivity of this process. Starting from *rac*-**1**, nearly enantiopure lactone (*R*)-**2** (97% ee) was obtained in a preparative (isolated) yield higher than 84%. Further studies are in progress in our laboratory to improve this type of DKR methodology.

Experimental Section

General Remarks

Gas chromatography analyses were performed using a Shimadzu GC-14A chromatograph with helium as the carrier gas.

E. coli TOP10[pQR239] was constructed by Prof. J. Ward (University College, London). Stock cultures were grown on nutrient agar at 37 °C and stored at −20 °C in a 40% glycerol solution. Growth of microorganisms was carried out in an 11-L (New Brunswick) fermentor equipped with a DOT probe, pH control and antifoam automatic addition. Analytical scale biotransformation experiments were carried out in a specially built 500-mL glass bubble column equipped with a sintered glass at the bottom[12a, c] and preparative experiments in a 2-L (Setric) fermentor.

Anion exchanger resins were purchase from Supelco or Fluka. Before use, they were washed with NaOH solution (3 N), then with water until neutrality and filtrated under vacuum. The number of resin equivalents (n_{eq}) used in this paper and the resin concentration (c_{resin}) were defined as follows, Equation (2) and Equation (3):

$$n_{eq} = \frac{m \times C}{n} \quad (2)$$

and

$$c_{resin} = \frac{m \times C}{V} \quad (3)$$

m being the mass of resin (g), *n* the ketone amount *(*mmoles), *V* the volume (L) and *C* the resin exchange capacity expressed in milli-equivalents per gram of resin (meq g^{-1}).

Preparation of the Biocatalyst

The strain *E. coli* TOP10[pQR 239] contains a pBAD plasmid into which the CHMO gene from *A. calcoaceticus* NCIMB 9871 has been cloned. The expression of the CHMO gene was induced by L(+)-arabinose. The culture medium contained 10 g L^{-1} each of glycerol, yeast extract (Fischer Scientific), soybean peptone (Fluka), NaCl, as well as 100 mg L^{-1} of ampicillin. An 11-L fermentor was filled with 5 L of culture medium and was inoculated with 450 mL of a 12 h preculture at 37 °C. The fermentor was stirred at 500 rpm and set at 37 °C, and the vessel was aerated with an air flow of 1.3 vvm. The pH was adjusted at 7.0 and controlled by automatic addition of 1 N H_3PO_4 and 1 N KOH solutions. L(+)-arabinose (0.05% w/v) was added to induce the enzymatic activity during the exponential growth phase (typically, $OD_{590\,nm} = 8$). After a further 1–2 h growth period, the cells were harvested and kept at 4 °C overnight. They were directly utilised for biotransformation.

Analytics

Broth aliquots (3–5 mL) were extracted with ethyl acetate containing octadecane (0.5 g L^{-1}) as internal standard. The organic phase was then analysed by GC on a Optima-5 column (Macherey-Nagel) at 230 °C to determine the ketone **1** concentration. The ee values of **1** (respectively, lactone **2**) were determined using a Chirasil-Dex CB column (Chrompack) at 150 °C [respectively, a Lipodex®E column (Macherey-Nagel) at 170 °C].

Absolute configurations of ketone **1** and lactone **2** were deduced by comparison with GC retention times of authentic samples as described in ref.[13]

Racemisation Studies

Racemisation experiments with optically active cyclopentanone **1** (isolated from the preparative kinetic resolution performed at pH 7 using same the *E. coli* strain) were performed into 2-mL Eppendorf vials, shaken on a reciprocal shaker at the appropriate temperature (30 or 37 °C). Ketone **1** (predis-

solved in an EtOH solution) was added to obtain a final concentration of 0.5 g L^{-1}. Racemisation studies *versus* pH were performed using the culture medium adjusted to the selected pH value by addition of a KOH solution. Racemisation studies with ionic exchange resins were carried out in the culture medium after addition of 5 equivalents of resin per moles of **1** – i.e., at a concentration of 12.5 meq L^{-1}. Ketone ees were monitored by chiral GC analysis of samplings (80 μL) kept at +4 °C after AcOEt extraction. Results are summarised in Table 1 and Figure 2.

Activity Measurement

Whole cell CHMO activity was measured against commercial (±)-bicyclo[3.2.0]hept-2-en-6-one (0.5 g L^{-1}) using 100 mL culture broth in a 500-mL bubble column reactor. Air was sparged through a sintered glass bottom at a flow rate of 1.3 vvm and further agitation was not necessary to ensure good mixing. A water jacket maintained the temperature at the chosen value. pH was controlled by automatic addition of 1 N H_3PO_4 and 1 N KOH solutions. The experiments were carried out at different pH values, i. e., 6.8, 7.5, 8 and 9, and at two different temperatures: 30 and 37 °C. One assay was performed by addition of 1.45 g (2.5 resin equivs.) of Lewatit MP62 at pH 7.5 and 30 °C. Aliquots were taken at regular time intervals, extracted with ethyl acetate containing undecane as internal standard and analysed by GC (Optima 5 column, 110 °C). Specific whole-cell activity was calculated in U/g cells (dry weight) from the initial velocity of combined lactone formation.

Analytical Whole-Cell Biotransformations

Inhibition Studies: Biotransformations were performed at a 25 mL analytical scale in 250 mL Erlenmeyer flasks, using a reciprocal agitated water bath maintained at 30 °C. They were followed by GC analysis of samplings.

Inhibition levels of ketone **1** were determined by addition of **1** (dissolved in the minimum amount of ethanol) at a final concentration of 0.3, 0.5, 1.0 and 1.3 g L^{-1}, respectively, to a *E. coli* TOP10[pQR239] culture at pH 7. Nearly total conversion of **1** was observed at the initial ketone concentrations lower than 1.3 g L^{-1}. Some 25% of the compound **1** remained after 24 h of biotransformation at higher concentration.

Inhibition levels against lactone **2** were determined by prior addition of **2** to the cell broth at a final concentration of 0.5, 0.7, 1.0 and 1.5 g L^{-1}, respectively. Then ketone **1** was added at a final concentration of 0.5 g L^{-1}. Nearly total consumption of substrate **1** was detected in the presence of 0.5 g L^{-1} and 0.7 g L^{-1} additional lactone **2**. At a lactone concentration of 1 g L^{-1}, 20% of substrate **1** remained after more than 24 h. As much as 70% of starting ketone **1** remained after 24 h at a lactone 2 concentration of 1.5 g L^{-1}.

Dynamic Kinetic Resolutions: Typically, racemic 2-benzyloxymethylcyclopentanone **1** was transformed using 100 mL of *E. coli* TOP10[pQR 239] culture, using the above described 500-mL bubble column reactor, at pH 7.5 (controlled by automatic addition of 1 N H_3PO_4 and 1 N KOH) and with an aeration flow rate of 1.3 vvm. Experiments were monitored by withdrawing aliquots (3–5 mL) from the medium that were analysed for yield and ee as described in the Analytics paragraph.

Calculations: Kinetic constants (k_{rac}, k_R, k_S) were determined by a least squares fit from Noyori's equations[26] using ketone and lactone ees measured as described above during the DKR (Scientis software).

Preparative Scale Biotransformations

Typically, preparative scale biotransformations of cyclopentanone **1** (dissolved in 10 mL EtOH) were carried out in a 2-L fermentor filled with 1 L of a *E. coli* TOP10[pQR239] cells. Agitation was fixed at 500 rpm and the air flow at 16 L h^{-1}. The appropriate pH value (pH 7.5) was adjusted and controlled by automatic addition of KOH (3 N) or H_3PO_4 solution (l N). Biooxidation was monitored by GC analysis of aliquots and stopped at completion by addition of 1 N H_2SO_4 until pH 6. The medium was then continuously extracted with dichloromethane 24 h before being acidified down to pH 2 and extracted again for 24 h. Crude extracts were pooled and refluxed in anhydrous toluene (using a Dean–Stark apparatus) containing Amberlite IR 120 resin before immediate purification by flash chromatography (pentane/AcOEt).

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