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Microbiological transformations 49. Asymmetric biocatalysed Baeyer–Villiger oxidation: improvement using a recombinant *Escherichia coli* whole cell biocatalyst in the presence of an adsorbent resin

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Abstract

The biocatalyst used in this work was whole cells of *Escherichia coli* TOP10 [pQR239] into which had been cloned the cyclohexanone mono-oxygenase from *Acinetobacter calcoaceticus* NCIMB 9871. Here we describe how, using whole resting cells of this recombinant strain, the biotransformation of bicyclo[3.2.0]hept-2-en-6-one (**1**) to its corresponding regioisomeric lactones (−)-(1*S*,5*R*)-2-oxabicyclo[3.3.0]oct-6-en-3-one (**2**) and (−)-(1*R*,5*S*)-3-oxabicyclo[3.3.0]oct-6-en-2-one (**3**) could be improved from levels of 1 g l^{-1} (9.3 mM) to 20 g l^{-1} (185 mM). The rate and yield of the biotransformation were improved by (i) increasing the cell concentration, (ii) using a specially designed vessel with high aeration rates, and (iii) adding an adsorbent resin directly to the biotransformation medium. We tested a series of resins and selected Amberlite XAD-4 and Optipore L-493 for further studies. In the presence of 100 g l^{-1} Optipore L-493 resin, 20 g l^{-1} bicycloheptanone (**1**) was successfully converted to a mixture of the two expected lactones at an overall yield of 83% (70% preparative yield after purification). Both these products were obtained in nearly enantiopure form (e.e. > 98%).

Keywords: Asymmetric Baeyer–Villiger oxidation; Biotransformation; Adsorbent resin; *Acinetobacter calcoaceticus* (NCIMB 9871); Cyclohexanone mono-oxygenase (CHMO); Over-expressed Baeyer–Villigerase; *E. coli* TOP10 [pQR239] strain

1. Introduction

The chemical Baeyer–Villiger (BV) oxidation was described more than a 100 years ago. However, attempts to achieve it in an asymmetric manner using conventional chemistry, i.e. using transition metal catalysts, have only relatively recently been met with moderate success [1,2]. Conversely, enzymatic BV oxidation has been proven for over 20 years to be a very efficient way to achieve this reaction in an asymmetric manner thereby enabling enantiopure lactones to be obtained from racemic or prochiral cyclic ketones (for a recent review see for instance [3]). These BV enzymes belong to an NADPH-dependent flavoenzyme family, currently called "Baeyer–Villiger mono-oxygenases" (i.e. BVMOs). Although several such enzymes have been detected and studied [4], their application in asymmetric organic synthesis has

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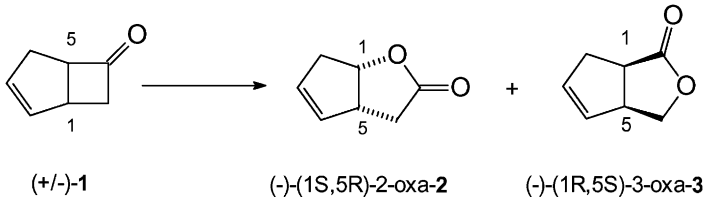

Scheme 1. Oxidation of bicyclo[3.2.0]hept-2-en-6-one (**1**) to its corresponding regioisomeric lactones (−)-(1*S*,5*R*)-2-oxabicyclo[3.3.0]oct-6-en-3-one (**2**) and (−)-(1*R*,5*S*)-3-oxabicyclo[3.3.0]oct-6-en-2-one (**3**).

essentially been limited to the use of the cyclohexanone mono-oxygenase (CHMO) from the bacteria *Acinetobacter calcoaceticus* NCIMB 9871. However, the fact that BVMOs are co-factor dependent enzymes severely limits the use of these enzymes in purified form [5]. Therefore, the use of whole cells, enabling cofactor recycling, has proved to be the method of choice for preparative scale synthesis [6]. For example, we have shown previously that, by using whole cells of this bacteria (or of one of its variants), bicyclo[3.2.0]hept-2-ene-6-one (**1**) was converted regio- and enantioselectivity, thus forming both of the two regioisomeric lactones, (−)-(1*S*,5*R*)-2-oxabicyclo [3.3.0]oct-6-en-3-one (**2**) and (−)-(1*R*,5*S*)-3-oxabicyclo[3.3.0]oct-6-en-2-one (**3**), in nearly enantiomerically pure form [7,8] (Scheme 1).

Potential disadvantages of using wild-type strains include (a) low CHMO activity, (b) the presence of a constitutive lactone hydrolase which, depending on the substrate used, may lead to product degradation, and (c) low optimum substrate concentrations, due to both substrate and/or product inhibition (i.e. the biotransformation of only 1 g l^{-1} of ketone **1** was possible with the wild-type strain *A. calcoaceticus*). In order to circumvent these drawbacks, CHMO has been recently cloned and over-expressed in different hosts [9–11], including *Escherichia coli* TOP10 [pQR239] [12]. The CHMO gene from *A. calcoaceticus* had initially been cloned into *E. coli* JM105 but in this system the CHMO expression levels were no better than those observed with the wild-type host [13]. The recombinant *E. coli* TOP10 [pQR239] strain is particularly attractive for the following reasons: (a) the CHMO content is much higher (by a factor of 25) than in the wild type strain; (b) it does not contain any lactone hydrolase activity which would lead to degradation of the product (nor should contain any alcohol dehydrogenase that competitively consumes the ketone substrate); (c) this strain is non-pathogenic and grows faster than the *A. calcoaceticus* wild-type; and (d) the enzyme production is induced by L(+)-arabinose, which has the advantage of being a relatively cheap inducer. We have previously described the use of this strain to perform the oxidation of **1** and have observed that, primarily due to substrate and product inhibition, the best compromise for performing this biotransformation was a moderate substrate concentration of about 1 g l^{-1} (the optimum concentration of **1** was 0.2–0.4 g l^{-1} and the maximum was 3 g l^{-1}) [14]. Here we describe the optimisation of this biotransformation up to a substrate concentration of 20 g l^{-1}, thus making this procedure particularly attractive for fine organic synthesis.

2. Materials and methods

2.1. Growth of recombinant E. coli TOP10

E. coli TOP10 [pQR239] contains a pBAD plasmid into which has been cloned the CHMO gene from *A. calcoaceticus* NCIMB 9871. The expression of the CHMO gene is induced by L(+)-arabinose. *E. coli* TOP10 [pQR239] was cultivated essentially as described previously [12]. The medium (pH 6.8) contained 10 g l^{-1} of each of glycerol, NaCl, yeast extract, peptone, and 100 mg l^{-1} of ampicillin. Routinely, a 330 ml pre-culture was used to inoculate an 1 l fermentor (New Brunswick Scientific BIOFLO 3000, New Brunswick, NJ, USA) at 37°C. The fermentor was stirred at 500 rpm and aerated with an air flow of 0.5 vvm via a submerged sparger. When the culture reached an A_{600} of 2–3, L(+)-arabinose (0.05% w/v) was added to induce the CHMO activity. After a further 4 h growth (A_{600} of approximately 12), the

cells were harvested by ultrafiltration at 4°C. The cell yield from an 11.5 l working volume fermentor was typically 21.7 g WW l^{-1} (5.4 g DW l^{-1}).

2.2. Cell immobilisation

A cell solution (0.95 g DW per 10 ml) was mixed gently with an equal volume of 4% aqueous alginate paste. The resulting mixture was passed through a syringe needle dropwise into a cold 2% $CaCl_2$ solution. After 16 h at 4°C, the beads were recovered by filtration and washed with 50 mM Tris–HCl, pH 7.5 containing 0.2% $CaCl_2$. Biotransformations using immobilised cells were performed in 50 mM Tris buffer pH 7.5 to prevent solubilisation of the beads by phosphate ions.

2.3. Whole cell biotransformations using resting cells

The biotransformations were routinely performed on a 25 ml scale in 250 ml Erlenmeyer flasks in an agitated water bath maintained at 30°C. Typically, 0.44 g DW cells were added to 25 ml 50 mM phosphate buffer, pH 7.5. Bicyclo[3.2.0]hept-2-en-6-one (**1**) (Merck) was added at the concentrations specified. Samples (1 ml) were removed periodically and then analysed by GC.

The aerated vessel is essentially a 450 ml bottle, which at the base consists of a sintered glass. Air was sparged at a flow rate of 0.9 vvm and further agitation was not necessary to ensure good mixing. A water jacket maintained the temperature at 30°C. Routinely, the biotransformations were performed at a scale of 100 ml containing 1.8 g DW cells.

At the end of the biotransformations, the products were continuously extracted into dichloromethane for approximately 20 h before GC analysis to calculate the total lactone yields.

2.4. Adsorption of ketone and lactone on adsorbent resins

We tested a series of resins including: Amberlite XAD-7, XAD-4, XAD-2000 and XAD-2, Duolite S-761, Sepabeads SP-207, Dowex Optipore L-323 and L-493 (Supelco). To estimate the adsorption capacity of each of these resins, a 10 g l^{-1} aqueous bicycloheptenone (**1**) (or racemic lactone (−)-(1*S*,5*R*)-2-oxabicyclo[3.3.0]oct-6-en-3-one (**2**) and (−)-(1*R*, 5*S*)-3-oxabicyclo[3.3.0]oct-6-en-2-one-3) solution was added to the resin (which had been washed and dried under vacuum prior to use) giving a final resin concentration of 40 g l^{-1} (20 g l^{-1} for the experiments with the lactone). After 1 h gentle longitudinal agitation, 1 ml of the aqueous solution was removed (without resin) and analysed by GC. The amount of adsorbed compound was determined by subtracting the concentration of free compound in solution.

Before being used in the biotransformation, the resin was agitated gently for 1 h in the presence of the specified amount of bicycloheptanone (**1**) and 50 mM phosphate buffer (pH 7.5). At the end of the biotransformation, the resin phase was separated from the aqueous phase by filtration. The lactone (and any remaining ketone) was extracted from (i) the resin phase using acetone, and (ii) the aqueous phase by dichloromethane. The residual ketone and lactone yields were determined by GC analysis. The lactone yield in the Optipore L-493 experiment was also determined by weighing the pure lactone obtained after purification by flash chromatography (silica gel, pentane/ether).

2.5. Gas chromatography (GC) analysis: yield and e.e. determination

During the course of the biotransformation, 1 ml samples were removed and added to 1 ml of 1 g l^{-1} undecane in ethyl acetate. Undecane served as an internal standard for quantification of the ketone and the corresponding lactones. Samples were injected onto an Optima 5 fused silica capillary column (diameter 0.5 μm, length 30 m) (Macherey–Nagel GmbH & Co., Duren, Germany) maintained at 110°C and with helium as the carrier gas. The e.e. was determined using a Macherey–Nagel fused silica capillary column of Lipodex E maintained at 130°C.

3. Results and discussion

The bioconversion of **1** at a concentration of 1 g l^{-1} typically produced a mixture of the two regioisomeric lactones (−)-(1*S*,5*R*)-2-oxabicyclo[3.3.0]oct-6-en-3-one (**2**) and (−)-(1*R*,5*S*)-3-oxabicyclo[3.3.0]oct-6-en-2-one (**3**) in 120 min, which corresponds to an activity

of 45 μmol min^{-1} g^{-1} DW. The e.e. of **2** and **3** were 98 and 99.5%, respectively. These results correspond to those reported previously [12]. In order to optimise this biotransformation, we explored the effect of (a) co-solvent, (b) reaction temperature, (c) cell concentration, (d) substrate concentration, (e) aeration of the medium, (f) cell immobilisation, (g) using water miscible or non-miscible solvents, and (h) using adsorbent resins.

3.1. Optimisation of the biotransformation in shake flasks

3.1.1. Effect of glycerol and ethanol

Co-factor recycling within whole cells can be achieved by the addition of an alcohol to the biotransformation reaction. An added advantage of adding a co-solvent is the increased solubility of bicycloheptenone (**1**). The use of glycerol or ethanol as possible co-substrates was investigated. The improvement of the biotransformation rate was more pronounced in the presence of glycerol (5%) than in the presence of ethanol (1%) (Fig. 1). Addition of 0.5, 5 or 10% glycerol significantly improved the biotransformation rate, whereas at levels of 20% glycerol, an inhibitory effect was observed. The improvement in the biotransformation was presumably due to co-factor recycling with the glycerol acting as a reductant. Cells from the 11 l fermentor were routinely stored at −20°C in 50 mM phosphate buffer, pH 7.5, containing 50% glycerol. Before being used in the biotransformation, the cells were washed with buffer to avoid any inhibition. When the biotransformation was performed in a biphasic medium (i.e. 60% octane), the addition of 0.5% glycerol improved the rate of the biotransformation, whereas 5% glycerol totally inhibited the reaction, presumably because the glycerol was concentrated in the aqueous phase.

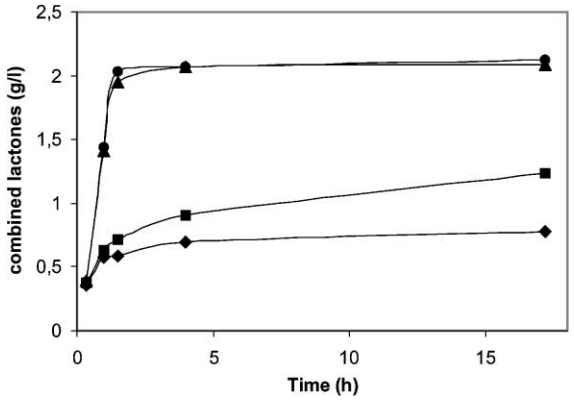

Fig. 1. The effect of glycerol and ethanol as co-substrate on biotransformations of 2 g l^{-1} **1** in shake flasks by *E. coli* TOP10 [pQR239] whole cells: (◆) no co-substrate; (▲) 5% glycerol; (■) 1% ethanol; (●) 5% glycerol and 1% ethanol.

3.1.2. Effect of temperature

The biotransformation of 3 g l^{-1} **1** was performed in shake flasks at 23, 31 and 38°C and followed for 6 h. Under these conditions the optimum temperature was found to be 31°C and only very low levels of lactone were formed at 38°C. Control experiments in which the biotransformation was performed in the absence of cells suggested that the evaporation of bicycloheptenone (**1**) (loss of approximately 11% h^{-1} at 38°C) was greatly reduced at the lower temperatures (loss of approximately 8% h^{-1} at 30°C). Previously, it was reported that the optimum temperature was 37°C [14]. However, this was when the activity was only measured for the initial 15 min and so substrate evaporation was not observed and the enzyme stability was not taken into consideration.

3.1.3. Effect of cell concentration

The effect of increasing the cell concentration was explored over the range of 1.7–35 g l^{-1}. At cell concentrations of 1.7 g DW l^{-1}, the bioconversion of 1 g l^{-1} bicycloheptenone (**1**) to lactones **2** and **3** was complete in approximately 120 min (corresponding to an activity of 45 μmol min^{-1} g^{-1} DW), whereas at cell concentrations of 17 g DW l^{-1} the reaction was complete in 50 min (10.9 μmol min^{-1} g^{-1} DW). In flask cultures containing 4 g l^{-1} **1**, only a slight increase in the biotransformation rate was observed when a cell concentration of 35 g DW l^{-1} was used instead of 17 g DW l^{-1}. After 180 min, the amount of combined lactone formed was 2.24 g l^{-1} (56% yield) when the cell concentration was 17 g DW l^{-1}, whereas 2.63 g l^{-1} total lactone (66% yield) was formed when the cell concentration was doubled. The addition of fresh cells during the course of the biotransformation had no effect on the rate of the biotransformation or on the final yield of lactone. This result confirms that at a cell concentration of 17 g DW l^{-1}, the cells were in excess even at high bicycloheptanone concentrations.

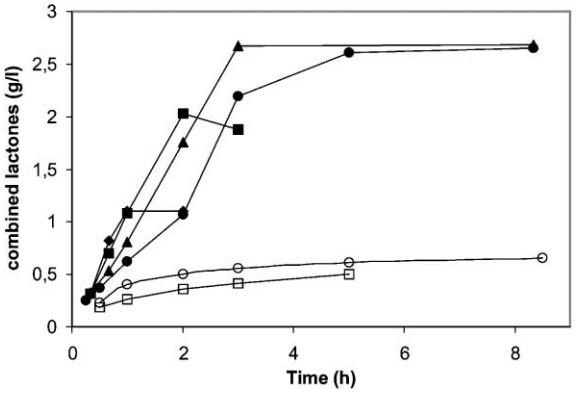

Fig. 2. Biotransformations at various concentrations of bicycloheptenone in shake flasks by *E. coli* TOP10 [pQR239] whole cells ($17\,g\,DW\,l^{-1}$): (◆) $1\,g\,l^{-1}$; (■) $2\,g\,l^{-1}$; (▲) $3\,g\,l^{-1}$; (●) $4\,g\,l^{-1}$; (□) $5\,g\,l^{-1}$; (○) $6\,g\,l^{-1}$.

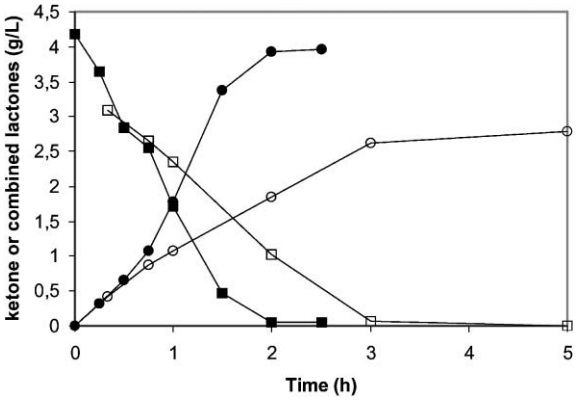

Fig. 3. Biotransformation of $4\,g\,l^{-1}$ bicycloheptenone (**1**) in shake flasks or in the well aerated vessel. The black lines represent the lactone concentration and the grey lines represent the ketone concentration: (□) ketone disappearance in shake flasks; (○) lactone formation in shake flasks; (■) ketone disappearance in the aerated vessel; (●) lactone formation in the aerated vessel.

3.1.4. Effect of bicyclo[3.2.0]hept-2-en-6-one concentration

The biotransformation of **1** in shake flasks was investigated at concentrations varying from 1 to $6\,g\,l^{-1}$ (Fig. 2). The flasks were maintained at 30°C and contained 0.44 g DW of cells (i.e. $17\,g\,DW\,l^{-1}$) in 25 ml phosphate buffer, pH 7.5, containing 0.5% glycerol. As can be seen from the graph, the rate of the biotransformation was the same when 1, 2 or $3\,g\,l^{-1}$ **1** was used and the yields approached 100% in all three cases. However, at $4\,g\,l^{-1}$ bicycloheptenone a slower rate was observed and the reaction reached a plateau after the production of approximately $3\,g\,l^{-1}$ total lactone, i.e. at a yield of 75%. On further increasing the concentration of **1** to 5 and $6\,g\,l^{-1}$, very little lactone was formed showing a clear inhibitory effect. Feeding bicycloheptenone (**1**) in batches of $1\,g\,l^{-1}$ over 6 h had no beneficial effect on the biotransformation.

3.2. Better aeration leads to improved yields

3.2.1. Biotransformations in buffer

Since the reaction requires molecular oxygen, it was to be expected that the rate of biotransformation could be improved by increasing the aeration. A reaction vessel was used in which compressed air was sparged from beneath the reaction mixture via a sintered glass. The biotransformations in the aerated vessel were routinely carried out using the optimum conditions as determined above with shake flasks, i.e. $17\,g\,DW\,l^{-1}$ cell concentration, 0.5% glycerol, pH 7.5, 30°C. The results obtained are shown in Fig. 3. It clearly appeared that the rate of biotransformation of **1** (at a concentration of $4\,g\,l^{-1}$ in buffer) was better in the well aerated vessel than in shake flasks. The yields of combined lactone obtained (after extraction into dichloromethane) were 83 and 75%, respectively. When a concentration of $6\,g\,l^{-1}$ bicycloheptenone was used in the aerated vessel, the amount of combined lactone measured (after extraction into dichloromethane) was equivalent to a concentration of $4\,g\,l^{-1}$. This result confirms that, above this level, the lactone inhibits the reaction, as we have already observed using lower cell concentrations [14]. Consequently, we investigated various methods to prevent or minimise enzyme inhibition and thereby improve the lactone yield.

3.2.2. Effect of cell immobilisation

Cell immobilisation was investigated in an attempt to improve cell stability in the biotransformation. Previously, the immobilisation of cells in alginate beads was shown to protect the bacteria against the toxicity of phenol [15,16]. However, a potential disadvantage of using cells entrapped in calcium alginate gel beads is mass transfer limitation [17].

Cells of *E. coli* TOP10 [pQR239] were immobilised onto alginate beads and used in the aerated vessel. The

biotransformation of 6 g l^{-1} **1** was very slow and less than 2 g l^{-1} total lactone was formed, which was lower than with free cells in suspension. This may be due to mass transfer limitation of the substrate or oxygen diffusion limitations even when improved aeration was obtained by using the specially designed vessel.

3.3. Toxic substrate and product removal using two-phase systems

3.3.1. Effect of a biphasic system using water non-miscible organic solvents

One possible approach for avoiding substrate and/or product inhibition is performing the reaction in a biphasic system using water non-miscible organic solvents [18,19]. This may result in partitioning the substrate and/or product in the aqueous or organic phases and is potentially useful for product recovery.

Various organic solvents were tested including *n*-octane, cyclohexane, isopropyl ether, hexane, dichloromethane, *n-tert*-butyl methyl ether, ethyl acetate, toluene. The only solvent which improved the biotransformation rate and yield was *n*-octane. Other solvents tested inhibited the reaction, presumably since they were harmful to the cells, even in a biphasic system.

In shake flasks (30°C, pH 7.5, 0.5% glycerol, 17 g DW l^{-1}) in the presence of buffer alone, 3 g l^{-1} **1** was totally converted to **2** and **3** in 180 min, whereas in the presence of octane the reaction was complete in 100 min. Moreover, in the aerated vessel, the biotransformation at 4 g l^{-1} substrate concentration was also improved in the presence of an octane phase. Yields of combined lactone obtained (after extraction) were 83% for buffer and 92% for octane (90 ml octane:10 ml buffer).

At concentrations of 6 g l^{-1} **1**, the effect of *n*-octane was even more pronounced (Fig. 4). It is noteworthy that in the buffer a long lag (>3 h) was observed at 6 g l^{-1} **1**. During this lag time, the concentration of **1** dropped to below 4 g l^{-1} due to evaporation, i.e. dropped to below inhibitory levels. The yields of total lactone obtained after extraction were 60% for the reaction conducted in buffer and 75% in the presence of octane. During the biotransformation in the presence of octane, an emulsion formed and a centrifugation step was necessary at the end of the biotransformation to separate the two phases. Furthermore, almost 40% of the lactone recovered was in the aqueous phase and 60% in the octane phase.

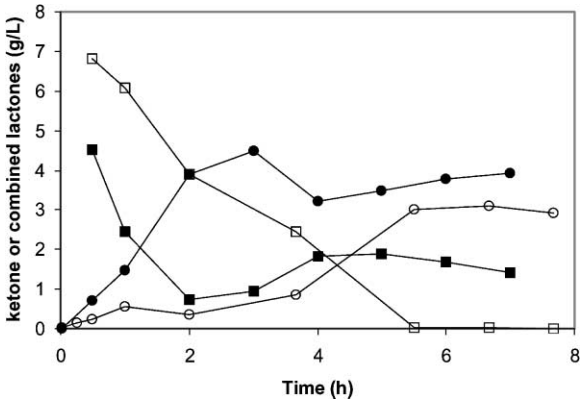

Fig. 4. Biotransformation of 6 g l^{-1} bicycloheptenone (**1**) in phosphate buffer or in a biphasic system of *n*-octane (60 ml octane:40 ml buffer) in the well aerated vessel: (□) ketone disappearance in buffer medium; (○) lactone formation in buffer medium; (■) ketone disappearance in biphasic medium; (●) lactone formation in biphasic medium.

3.3.2. Effect of adsorbent resin addition

The addition of a resin can be considered as a two-phase system in which the product (and reactant) are removed from the reaction mixture via adsorption onto the solid resin phase. Addition of an adsorbent resin into the reaction medium itself, or a combination of substrate feeding and toxic product removal by

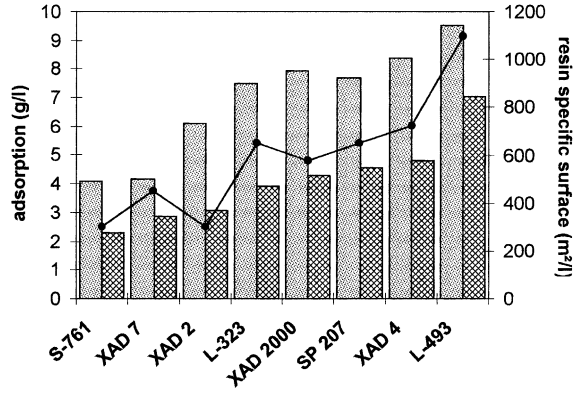

Fig. 5. Adsorption capacity of various resins towards ketone **1** and racemic lactone **2**: (▨) adsorbed ketone concentration (for 40 g l^{-1} of resin); (▩) adsorbed lactone concentration (for 20 g l^{-1} of resin); (●) specific surface area of resin.

passing the reaction mixture through an external loop with a fluidised bed of resin, have been reported by different authors to be a satisfactory way to overcome substrate and/or product inhibition [20–25]. Therefore, we investigated the possibility of applying this technique to the Baeyer–Villiger oxidation of **1**. Preliminary experiments were performed to determine the adsorption capacity of different commercially available resins towards ketone **1**, as well as lactones **2** and **3**. The results obtained are illustrated in Fig. 5. Not surprisingly, the adsorption capacity of the resins was proportional to the specific surface area per weight of material. Amberlite XAD-4 and Optipore L-493 gave the best results, i.e. were the most adsorbent resins, and were selected for further studies. Bioconversions of **1** were performed in the presence of each of these resins.

Our results showed that the BV oxidation could be achieved at concentrations of up to $20\,g\,l^{-1}$ ketone **1**. Preparative scale biotransformations (100 ml) of $20\,g\,l^{-1}$ **1** were carried out in the aerated vessel at a cell concentration of $17\,g\,DW\,l^{-1}$ (0.5% glycerol, pH 7.5, 30°C) and in the presence of $100\,g\,l^{-1}$ of XAD-4 resin or L-493 resin (i.e. 5 g of resin g^{-1} **1**). At the start of the biotransformation, the concentrations of ketone **1** free in the aqueous phase were 1.9 and $0.8\,g\,l^{-1}$ for the resins XAD-4 and L-493, respectively. The biotransformations were stopped after 45 h and the results obtained after extraction and GC analysis are shown in Table 1. The best result was obtained with Optipore L-493, which was the most adsorbent resin, giving a yield of 70% of pure lactone products **2** and **3** after flash chromatography (83% overall yield before purification). The presence of the resin did not change the enantio- and regioselectivity of the reaction. In both cases, the e.e. of the obtained (−)-(1*S*,5*R*)-2-oxabicyclo[3.3.0]oct-6-en-3-one (**2**) and (−)-(1*R*,5*S*)-3-oxabicyclo[3.3.0]oct-6-en-2-one (**3**) were 98 and 99.9%, respectively.

Doubling the cell concentration to $35\,g\,DW\,l^{-1}$ did not improve the biotransformation yield. However, these results were not extensively optimised and it is possible that further work to investigate the optimal resin concentration could result in the successful biotransformation of even higher concentrations of **1**.

4. Conclusion

The present work was aimed at trying to improve the experimental conditions for performing the biocatalysed Baeyer–Villiger oxidation of bicyclo[3.2.0]hept-2-en-6-one (**1**) using whole cells of the recently constructed recombinant organism *E. coli* TOP10 [pQR239]. This *E. coli* strain, which is induced using L(+)-arabinose, over-expresses the cyclohexanone mono-oxygenase cloned from *A. calcoaceticus* NCIMB 9871. This enzyme is known to catalyse the Baeyer–Villiger oxidation of **1** in a combined regio- and enantioselective manner. Previously, this biotransformation was limited to a substrate concentration of about $1\,g\,l^{-1}$, due to substrate and product inhibition. Here we describe how this inhibition could be overcome by modifying the experimental protocol. The use of a biphasic system with organic solvents was of limited success and only *n*-octane gave better results than with buffer alone. However, a combination of increased cell concentration, improved aeration and, essentially, addition of the adsorbent resin Optipore L-493, allowed the substrate concentration to be increased from $1\,g\,l^{-1}$ (9.3 mM) to $20\,g\,l^{-1}$ (185 mM). As previously described, this allows the preparation of lactones **2** and **3** in nearly enantiopure form, an asymmetric oxidation which is still hardly accessible using conventional (i.e. metal-catalysed) chemistry. This improvement obviously makes the biocatalysed Baeyer–Villiger oxidation approach even

Table 1
Biotransformation of $20\,g\,l^{-1}$ bicycloheptenone (**1**) in the presence of $100\,g\,l^{-1}$ XAD-4 resin or L-493 resin and a cell concentration of $17\,g\,DW\,l^{-1}$

	XAD-4			L-493		
	Resin	Buffer	Total	Resin	Buffer	Total
Residual ketone **1** (%)	7	0	7	3	0	3
Combined lactones (%) (**2**:**3**)	56 (1.2:1)	8 (1.3:1)	64 (1.2:1)	76 (1.1:1)	7 (1.1:1)	83 (1.1:1)

more attractive for preparative scale fine organic synthesis.

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