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# Continuous process for large-scale preparation of chiral alcohols with baker's yeast immobilized on chrysotile fibers

Renato Wendhausen Jr., Paulo J.S. Moran *, Inés Joekes, J. Augusto R. Rodrigues

*Universidade Estadual de Campinas, Instituto de Química, Campinas SP, Brazil*

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**Abstract**

Bioreductions of prochiral ketones in a continuous process were carried out in a Packed-bed-reactor with baker's yeast (*Saccharomyces cerevisiae*) immobilized on chrysotile fibers (IMBY). The performance of the reactor was observed for 30 days, under a constant dilution rate of $1.7 \times 10^{-1}$ $h^{-1}$ using ethylbenzoylformate as substrate. The steady state showed $2.9 \times 10^{-2}$ g $l^{-1}$ $h^{-1}$ of productivity, 50% chemical yield, and 90% ee (enantiometric excess). Using ethylacetoacetate at the same dilution rate for 8 days, the steady state showed 65% chemical yield and 95% ee. No significant change on the IMBY integrity was observed. The results show that chrysotile is an excellent carrier for immobilization of baker's yeast (BY) cells to be used in the continuous process of bioreduction 

*Keywords:* Ketoester reduction; Microbial uses of *Saccharomyces cerevisiae*; Continuous process

## 1. Introduction

Stereoselective reduction of prochiral ketones is a useful method to afford chiral centers. This method can be overcome by isolated dehydrogenases with high ee (enantiometric excess) [1]. These enzymes are NAD(P)H dependent, and these expensive coenzymes have to be regenerated continuously. Alternatively, a whole cell system can be used to avoid coenzyme addition or regeneration system. Bioreductions of prochiral ketones mediated by baker's yeast (BY) are now recognized as a useful method to afford chiral centers [2–4]. However, this method has not been considered to be suitable for large-scale production of chiral alcohols, due to the low concentration of reagents and tedious isolation of products, since these reactions are generally performed in batch process. Some recent works on continuous cell culture system try to overcome these disadvantages [5,6]. Another way is to use immobilized BY for enantioselective reductions of ketones [7–12]. The immobilization of BY on solid support is known to increase cell stability and metabolism [13], and makes a continuous process operation for a long period of time possible. In this communication, we describe a continuous process of bioreduction of carbonyl compounds using BY immobilized by

* Corresponding author. E-mail: moran@iqm.unicamp.br

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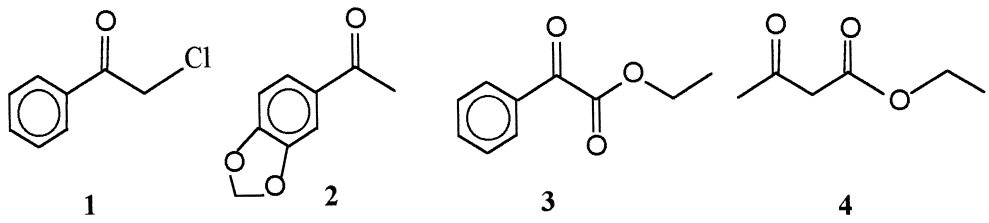



Scheme 1.

adsorption on chrysotile fibers (IMBY). Chrysotile, which is a very inexpensive mineral, has been already used by us as a support for cell immobilization in synthetic applications [14,15].

## 2. Materials and methods

### *2.1. Materials*

The microorganism *Saccharomyces cerevisiae* from commercial BY was purchased from Fleshmann. The medium used was made of substrate ethylbenzoylformate or ethylacetoacetate 0.33 g/l from Aldrich, sucrose 10%, urea (2 g/l), $MgSO_4 \cdot 7H_2O$ (0.5 g/l), KCl (20 g/l) and ethanol (20 ml/l). Ethanol is added in order to facilitate the substrate solubilization. The pH was adjusted to 5.0, with phosphoric acid addition (about 0.2 ml of concentrated acid). The chrysotile used as cell support was obtained from SAMA Mineração de Amianto washed with flowing water and activated by sonication at a controlled pH, as described elsewhere [16].

### *2.2. Immobilization*

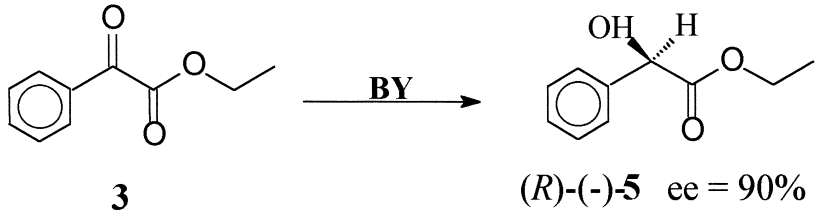
The BY (100 g) was dispersed in water (8 l) and activated chrysotile (100 g) was added under gentle stirring. After 30 min, the IMBY was filtered.

### *2.3. Bioreactor and bed preparation*

The continuous bioreductions were carried out in a jacketed glass column as a Packed-bed type reactor with a total volume of 1 l, equipped with an external system of thermostated water to keep an optimum fermentation temperature. The bed was prepared by gently mixing the cell/support complex with commercial sand ($\phi = 2$ mm), in order to obtain a structure inside the reactor suitable for the flow of solution and $CO_2$ gas, as described elsewhere [17]. Caution: wet chrysotile is used to avoid unhealthy conditions.

### *2.4. Purification and enantiomeric excess determination*

Ethyl (*R*)-(−)-mandelate (**5**) was extracted with ethyl acetate from the reaction mixture, purified by column chromatography and the yield was evaluated, taking into account the initial amount of reagent. The ee was determined by GC analysis using a chiral column [stationary phase: heptakis-(2,6-methyl-pentyl)-$\beta$-cyclodextrine].




Scheme 2.

Table 1
Chemical yield and enantiomeric excess of **5** as a function of concentration of **3** at dilution rate of $1.7\times10^{-1}$ $h^{-1}$

| Substrate conc. (g/l) | Chemical yield (%) | ee (%) |
|---|---|---|
| 0.33 | 50.0 | 90.0 |
| 0.50 | 49.1 | 90.0 |
| 0.66 | 46.6 | 90.3 |
| 1.00 | 44.8 | 90.0 |

Table 2
Chemical yield and enantiomeric excess of **5** as a function of dilution rate of **3** at a concentration of 0.33 g/l

| Dilution rate ($10^{-3}$ $h^{-1}$) | Chemical yield (%) | ee (%) |
|---|---|---|
| 2.8 | 50.0 | 90.0 |
| 4.5 | 45.4 | 89.6 |
| 7.5 | 38.5 | 89.9 |
| 9.4 | 36.9 | 90.1 |

Ethyl (*S*)-(+)-3-hydroxybutyrate (**6**) was extracted with ethyl acetate from the reaction mixture and was purified by distillation in Kugelrohr system and the ee evaluated by optical rotation was compared with the literature data [18].

## 3. Results and discussion

The reactor was fed continuously with an ascendant flow of a sterilized solution of ketone and cell nutrients, with the help of a peristaltic pump. A mixture of water/ethanol (0.3 to 0.5% of ethanol) was used as solvent. The temperature was kept at 30°C by circulating thermostated water.

The compounds **1, 2, 3,** and **4** (Scheme 1) were submitted to bioreduction in the continuous process.

The compound **1** (α-chloroacetophenone) has an electrophilic activated center and is already documented to show a fast reduction (less than 4 h) by BY [15]. However, after two days of operation, the reactor shows complete deactivation. The chlorine derivatives are in general toxic to cells and the enzymes responsible for the reduction (dehydrogenases NADH dependent) [19] are not able to regenerate the cofactor through cell glycolysis metabolism and the reduction process is stopped. The compound **2** (3,4-methylenedioxyacetophenone) was not reduced, probably due to the deactivated electrophilic center. Finally, the compounds **3** and **4**

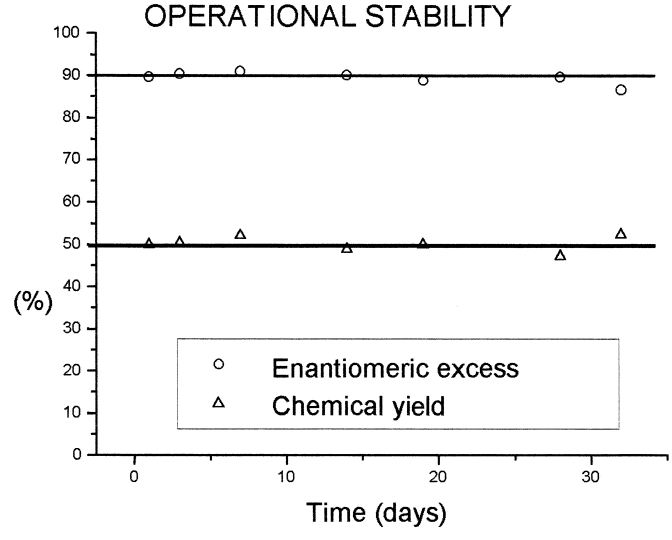
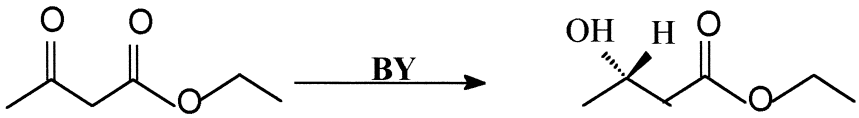



Fig. 1. Operational stability related to chemical yield (Δ) and enantiomeric excess (○), observed during a period of 30 days for the continuous bioreduction of **3**.




**4** (*S*)-(+)-**6** ee = 98%

Scheme 3.

succeeded on bioreduction. The bioreduction of **3** gave **5** ($[\alpha]_D^{20} = -124°$, and ee = 90%), as shown in Scheme 2.

First of all, some variations on the concentration of substrate **3** and dilution rate were tested to find the optimum reactor conditions. The results in Tables 1 and 2 show a maximum of 50% chemical yield and 90% ee. The performance of the reactor was observed for 30 days under a constant dilution rate of $1.7 \times 10^{-1}$ $h^{-1}$ and 0.33 g/l of substrate concentration. The steady state showed $2.9 \times 10^{-2}$ g $L^{-1}$ $h^{-1}$ of productivity, 50% chemical yield, $[\alpha]_D^{20} = -124°$ and ee of 90%. Fig. 1 shows the operational stability related to chemical yield and ee for this period of time.

The same reactor and biocatalyst used for bioreduction of **3** was pumped with a solution of nutrients for one day and after that it was used for bioreduction of **4** to afford compound **6** (Scheme 3).

The reactor was fed with a solution of **4** (0.66 g/l) and cell nutrients, at a dilution rate of $1.7 \times 10^{-1}$ $h^{-1}$ and temperature of 30°C for a period of 8 days. The results can be seen in Table 3.

In the steady state, 65% chemical yield and 95% ee were reached. The reactor did not show any appreciable change in the bed appearance as well as in the IMBY (cell/support/inert structural material) integrity after this period of operation.

## 4. Conclusion

The reactor packed with baker's yeast immobilized on chrysotile was shown to be suitable for use in continuous processes of bioreduction of ethylbenzoylformate (30 days) and ethylacetoacetate (8 days). After these two successive transformations, the biocatalyst was still active for further reactions.

## 5. Nomenclature

| | |
|---|---|
| BY | baker's yeast |
| IMBY | immobilized baker's yeast |
| ee | enantiomeric excess |
| $[\alpha]_D^{20}$ | optical rotation |
| NAD(P)H | reduced nicotinamide adenine dinucleotide and reduced nicotinamide adenine dinucleotide phosphate |

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Table 3
Data of chemical yield and enantiomeric excess of **6** produced by bioreduction of **4**, at dilution rate of $1.7 \times 10^{-1}$ $h^{-1}$ and 30°C

| Time (days) | Chemical yield (%) | $[\alpha]_D^{20}$ (deg) | ee (%) |
|---|---|---|---|
| 1 | 64.5 | + 38.3 | 87.4 |
| 4 | 65.7 | +41.7 | 95.2 |
| 8 | 64.8 | +41.7 | 95.2 |

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