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Optimization of a biocatalytic process to gain (*R*)-1-phenylethanol by applying the software tool *Sabento* for ecological assessment during the early stages of development

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ABSTRACT

Ecological assessment using the software tool *Sabento* was conducted to compare different processes to gain the fine chemical (*R*)-1-phenylethanol from ethylbenzene. The software was applied during the biocatalytic process development using the unspecific peroxygenase (EC 1.11.2.1) of the fungus *Agrocybe aegerita.* The process could be systematically improved with respect to the ecological performance during process development. Compared to a modern chemical process and a further biotechnological process, it now reaches the best environmental key indicator. The software tool *Sabento* proved to be well suited to work out the most important factors determining the ecological burdens in the early stages of process development.

1. Introduction

Biotechnological processes are generally considered ecologically more advantageous than the corresponding chemical processes due to the usage of lower temperatures and pressures as well as mostly renewable raw materials. But not every new biotechnological method is automatically more sustainable and associated with lower risks for the environment compared to an existing chemical or biotechnological production process [1,6]. This has to be proven in each individual case.

Moreover, the essential factors, which affect production costs and environmental burdens of the future production technique, are determined in the very early stages of process development [5]. During this period, information about the process as well as the substances needed or respectively formed is incomplete or not existing. Here it is virtually impossible to perform a complete life cycle assessment (LCA) according to DIN EN ISO 14040 and 14044 [4]. Therefore, we applied here the software tool *Sabento* for an ecological assessment that requires less input data during the development of an innovative, biocatalytic process for the preparation of the chiral fine chemical (*R*)-1-phenylethanol. For comparison, the same ecological assessment was employed on two alternative processes to obtain the required fine chemical.

2. Experimental

The enzyme used to gain (*R*)-1-phenylethanol, was an extracellular, unspecific peroxygenase (EC 1.11.2.1) produced by the agaric basidiomycete *Agrocybe aegerita*, which catalyzes diverse peroxide-dependent oxyfunctionalization reactions [9,7] (Fig. 1).

Starting point of the analysis was the primary procedure for this biotransformation as shown in Fig. 2. In brief, a fed-batch design that varied three process parameters (product concentration in relation to the amount of applied enzyme, prevention of formation of the over-oxidation product acetophenone, and shortening the reaction time) was used to optimize the reaction set-up. As the result, a total turnover number of 4.3×10^4 related to (*R*)-1-phenylethanol and a space-time yield of 60 g L^{-1} D^{-1} were achieved [7]. At the same time, by the ecological assessment during process development, the most important and decisive factors determining the "greenness" of the process were continuously worked out and took into account for process improvement. This was done

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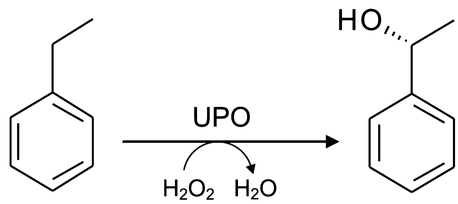

Fig. 1. Stereoselective hydroxylation of ethylbenzene to (*R*)-1-phenylethanol, catalyzed by the uspecific peroxygenase (UPO, EC 1.11.2.1) of the fungus *Agrocybe aegerita* in the presence of hydrogen peroxide (modified according to [7]).

with the help of the ecological assessment method of Heinzle et al. [5,6], established in the software tool *Sabento* for bioprocess development (ifu Hamburg GmbH) (Fig. 3). To be able to evaluate the environmental impact of the new biocatalytic process, the environmental performance was assessed in the same way for two existing production alternatives of (*R*)-1-phenylethanol.

3. Results

In the studied biocatalytic process, the unspecific peroxygenase (UPO) from the fungus *A. aegerita* catalyzes the asymmetric synthesis of (*R*)-1-phenylethanol from the prochiral reactant ethylbenzene with 97–99.5% ee. The procedure was refined by accompanying ecological assessment resulting in a one-step/one-pot reaction that proceeds within 45 s under mild conditions (Fig. 4). After centrifugation and ultrafiltration to separate the enzyme from the reaction mixture, the lipophilic compounds are extracted in a perforator by continuous reflux of the solvent dichloromethane. Column chromatography can be used to separate (*R*)-1-phenylethanol from the by-product acetophenone and the reactant [8].

Using ecological evaluation during this biocatalytic process development, the following aspects of the procedure for the preparation of (*R*)-1-phenylethanol were considerably improved:

- The acetonitrile content of the reaction mixture was reduced from primarily 25% (Fig. 2) to 3% (Fig. 4). In the primary procedure, this compound turned out to have the most adverse effect in the ecological assessment, expressed as a high potential environmental impact (PEI) value. Nevertheless, a low amount of acetonitrile is still needed to enhance the solubility of ethylbenzene in aqueous solution.
- By the continuous extraction of the products using a perforator, the amount of solvent could be reduced to 50% compared to single solvent extraction.
- The ecological performance was further improved by adopting column chromatography to separate the products from the reactant. In the primary procedure, the by-product acetophenone was removed by derivatisation with semi-carbazide hydrochloride, and product and reactant were separated by energy consuming distillation.

The refinement of the peroxygenase process reduced the PEI from primarily 14,431 to 832, thus by a factor of 17 (Table 1). Due to the shortening of the reaction time from previously one hour to now 45 s, the product recovery was slightly decreased. But simultaneously, the amount of the undesired by-product acetophenone was reduced to less than one-third.

Analysis of the waste flows revealed that acetonitrile – despite being used at a much reduced rate and being recycled – still is the single substance with the highest effect on the environmental key indicator PEI. All other substances used do only marginally contribute to PEI of the revised peroxygenase process.

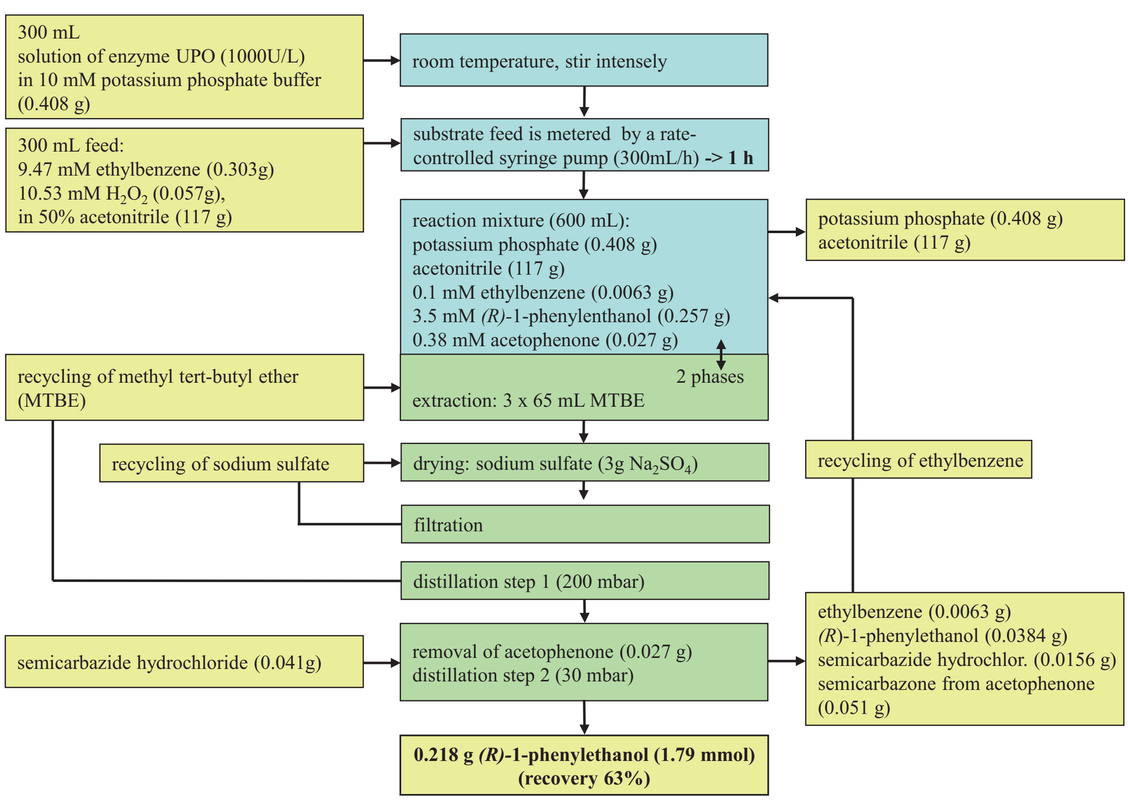

Fig. 2. Primary procedure of the biocatalytic conversion of ethylbenzene to (*R*)-1-phenylethanol, utilizing the unspecific peroxygenase (UPO) from *A. aegerita* (data are based on [7]).