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# Development of a continuous process for the lipase-mediated synthesis of peracids

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ABSTRACT

Biocatalytic processes can be used for the in situ production of harmful reagents, e.g., percarboxylic acids which are intermediates in epoxidations. Chemo-enzymatic epoxidations of unsaturated fatty acids using these enzymatically synthesized peracids are currently receiving increased interest by the academic and industrial community as safe and environmentally friendly alternatives to chemical synthesis. The applicability of these reaction cascades is still limited due to enzyme inhibition and deactivation which impair the first – enzymatic – step and thus hinder the following chemical step. To overcome these limitations, a new strategy for the synthesis of peracids was investigated. This is based on a continuous stirred tank reactor and the avoidance of liquid/liquid interfaces by performing the reaction in a single-phase organic solution. With this strategy, full enzymatic activity could be maintained for 50 h, doubling the literature benchmark. The peroxyacetic acid yield with respect to hydrogen peroxide was up to 70%. The designed process enables high peracid space-time yields with a reduced demand of enzymes.


## 1. Introduction

Biocatalytic applications usually provide high selectivity and are carried out at mild reaction conditions. They can also be used for the in situ production of dangerous reagents, which are hard to store and/or handle. One example is the lipase-mediated synthesis of percarboxylic acids which can serve as intermediates in epoxidations. With production capacities of several Mt per year, epoxides are industrially important commodity chemicals [1]. Rather than utilizing hazardous peroxy acids or strong mineral acids which are commonly used as oxidizing agents in the Prileshajev reaction, in a lipase-catalyzed system, the highly reactive percarboxylic acid can be synthesized in situ from carboxylic acids and hydrogen peroxide. This makes the chemo-enzymatic reaction a safer and more environmentally friendly alternative to the chemical synthesis. Furthermore, the undesired ring opening of the epoxide occurring in the traditional process can be avoided [2,3]. These systems have a high ecological potential: in situ regeneration of precursors/intermediates and reduction of downstream processing steps decreases production costs and generation of waste [4,5], which is directly linked to a higher "green index" [6] and E-factor (kg of waste produced per kg of product) [7]. The combination of the strengths of the chemical and biological approaches makes these hybrid systems a powerful tool for organic synthesis and a "hot topic in applied biocatalysis" [8].

*Candida antarctica* lipase B (CalB) is a highly efficient lipase for catalyzing the peroxidation of carboxylic acids [9,10] and has been used in the combined chemo-enzymatic epoxidation of various substrates [11,12]. Most studies on the chemo-enzymatic epoxidation with CalB have been carried out in two-phase systems using a combination of the substrates with an additional solvent (mostly toluene) to improve enzyme stability [11,13,14]. With a focus on the development of "greener" processes, so-called solvent-free processes have also been investigated [15,16]. However, a loss of enzyme activity occurs when the hydrogen peroxide concentration in the reaction medium is too high [13,14,17] which on the other hand is required for high initial reaction rates and yields [16]. A reduced enzyme deactivation was observed using a stepwise addition of aqueous hydrogen peroxide [9,10,13]. In a continuous process, high epoxidation yields of 99.1% were reported for

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**Nomenclature**

*Symbols*

| | |
|---|---|
| c | Concentration [M, g $L^{-1}$] |
| d | Stirrer diameter [mm] |
| D | Reactor diameter [mm] |
| h | Filling height [mm] |
| H | Total height [mm] |
| K | Partition coefficient (–) |
| $K_M$ | Half-saturation constant (mol $L^{-1}$) |
| m | Mass (g) |
| N | Amount (mol) |
| n | Stirrer speed ($min^{-1}$) |
| $\dot{R}$ | Reaction rate (mM $h^{-1}$) |
| $\dot{r}$ | Specific reaction rate (mmol $h^{-1}$ $g^{-1}$) |
| t | Time (h) |
| τ | Residence time (h) |
| V | Volume (mL) |
| $\dot{V}$ | Flowrate (mL $min^{-1}$) |
| Y | Yield (–) |

*Subscripts and superscripts*

| | |
|---|---|
| EA | Ethyl acetate |
| in | Influent |
| PAA | Peroxyacetic acid |

1-methylcyclohexene by Wiles et al. using a capillary filled with Novozym® 435 at a residence time of 2.6 min and a temperature of 70 °C, with a 2:1 molar ratio of hydrogen peroxide to the alkene in the influent [18].

In a fed-batch process using an enzyme recycle reactor with gradual hydrogen peroxide feeding for the epoxidation of linseed oil, a deactivation of the enzyme occurred, e.g., after 300 min at 60 °C with 20% aqueous hydrogen peroxide [19]. In contrast, Wiles et al. [18] observed no deactivation within 24 h of continuous operation at 70 °C. Nevertheless, an optimal economic and ecologic combination of process operation (mode) and operating parameters has not been described yet.

In contrast to a two-phase system, a single phase avoids effects caused by interfaces, e.g. mass transfer limitations at the liquid/liquid interface or high local concentrations of hydrogen peroxide. Furthermore, a liquid/liquid interface can be harmful to enzymes [20]. Although the use of CalB in an organic single-phase epoxidation system would be of advantage, these processes have been less investigated until now. Ankudey et al. used ethyl acetate and water free urea hydrogen peroxide in combination with immobilized lipase (NZ435) to epoxidize different alkenes with high yields of up to 100% in batch experiments [21]. In contrast to the overall reaction in a single-phase reaction system, the first enzymatic reaction step was less investigated on its own [22]. So currently, the individual contributions of the enzymatic and the chemical step cannot be distinguished. In a multistep reaction like the chemo-enzymatic epoxidation, the knowledge of each individual step is essential for overall process optimization which otherwise would remain a black box. The chemical epoxidation has frequently been described as the rate determining step [12,22,23]. In the kinetics of these reactions an increase in peracid concentration was found to increase the epoxidation rate [22,24]. Therefore, the parameters which influence the peracid concentration, e.g. hydrogen peroxide, also influence the epoxides formation (see Fig. S1).

To the best of our knowledge, there is a no literature dealing with the enzymatic peracid production under continuous process conditions in a single phase system. Consequently, this work addresses

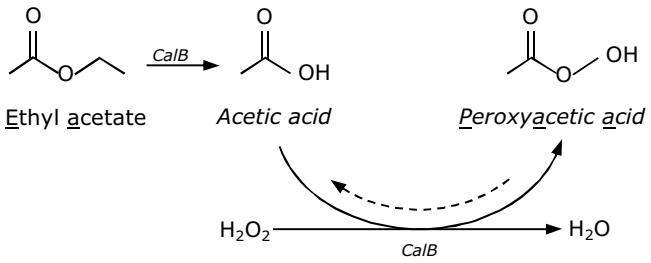


**Fig. 1.** Simplified lipase-mediated peroxyacetic acid production as a first step of the chemo-enzymatic epoxidation in ethyl acetate.

two main topics: the development of a continuous single-phase process where the deactivation of the enzyme can be minimized, and the quantitative evaluation of operating parameters that influence the enzymatic step. Therefore, the enzymatic reaction was investigated in detail under solvent-free conditions in a continuous stirred tank reactor CSTR. Besides the co-substrate hydrogen peroxide, ethyl acetate was chosen as the model substrate (Fig. 1) and excess medium because it is environmentally friendly, non-toxic and high conversions were reported using ethyl acetate [18,21].

## 2. Experimental

### 2.1. Enzymes and chemicals

CalB immo (immobilized lipase B from *Candida antarctica* on methacrylate carrier) with a specific activity of 8100 LU/g was obtained from c-LEcta GmbH (Germany).

Hydrogen peroxide (50 wt% in water) and all chemicals used for HPLC analyses and derivatization: methyl *p*-tolyl sulfide (MTS 99%), triphenylphosphine (TPP 99%), methyl *p*-tolyl sulfoxide (MTSO 97%), triphenylphosphine oxide (TPPO 98%) were purchased from Sigma-Aldrich, Germany. Acetonitrile (ACN ≥99.9% gradient grade) was obtained from VWR (Germany). Ethyl acetate (≥99.8% for HPLC) was purchased from Th. Geyer (Germany).

### 2.2. Determination of the peroxyacetic acid and hydrogen peroxide concentration

To determine the concentration of peroxyacetic acid and hydrogen peroxide in ethyl acetate simultaneously, precolumn derivatization according to Pinkernell et al. was applied [25]. The stepwise quantitative reaction of the peroxyacetic acid with MTS and hydrogen peroxide with TPP produces the corresponding MTSO and TPPO. The substances were analyzed by HPLC (module 1, Waters, USA) equipped with a LiChrosorb 100-5 RP 18, 250 × 4.6 mm column (Knauer GmbH, Germany) and the Millenium 2.10 chromatography software was used. The injection volume was 20 µL and the UV detection wavelength 230 nm. A constant flow rate of 1 mL $min^{-1}$ and a gradient of acetonitrile and water (0–0.1 min 100% ACN, 0.1–7.5 min 75% ACN ramped to 100% ACN; 7.5–20 min 100%) were applied. The linear range ($R^2 > 0.99$) of the calibrations for MTSO and TPPO were 0.25–3.75 mM and 0.08–1.2 mM, respectively. Samples with higher concentrations were diluted accordingly with ethyl acetate. Retention times were 7.5 min for MTSO and 8.5 min for TPPO, respectively. Every sample was analyzed twice.

### 2.3. Determination of the partition coefficient of hydrogen peroxide

To determine the partition coefficient K of hydrogen peroxide between the aqueous and organic phase (Eq. (1)), a set of experi-