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Lipase-catalyzed transesterification of ethyl formate to octyl formate

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ABSTRACT

The preparation of octyl formate via lipase-catalyzed transesterification of ethyl formate with 1-octanol is demonstrated. To shift the equilibrium of the reaction, ethyl formate was added in surplus but could be partially recovered for subsequent reactions. The same was true for the biocatalyst (Novo435), which could be reused at least 27 times.

This method gives simple access to a hydrophobic formic acid ester, which can be used as a reactive organic phase in biocatalytic redox reactions. The enzymatically prepared octyl formate can be utilized by formate dehydrogenase to regenerate NADH from NAD^+.

1. Introduction

The use of hydrolases (E.C. 3.1) for the interconversion of carboxylic acids, ester, and amides is nowadays a well-established procedure also utilized on industrial scale [1–3]. Interestingly, the formation of formic acid esters, despite several promising early contributions [4], has so far failed to be the focus of research. This is rather astonishing as some formyl esters are valuable compounds, e.g. ingredients in flavors and fragrances [5].

We became interested in the enzymatic synthesis of formic acid esters in the course of an ongoing project evaluating formic acid esters, such as octyl formate, as hydrophobic formate equivalents to serve as an organic phase in two-liquid phase reaction setups for oxidoreductases [6]. This project was inspired by the work of Bertau and co-workers who first demonstrated that formate dehydrogenase also accepts formic acid esters [7]. Therefore, we envisioned a simple and scalable method to produce formic acid esters. Aiming at an environmentally acceptable process, lipases are amongst the first catalysts to be considered. Overall, we designed an enzymatic production system of formic acid esters from commercially available ethyl formate (Scheme 1). As biocatalyst we chose the immobilized lipase B from *Candida antarctica* commercialized as Novo435.

To attain a commercially and ecologically feasible synthesis of formyl esters, a range of challenges has to be addressed. First, the cost contribution of the biocatalyst to the overall process has to be reduced, e.g. by recycling [8]. Second, the enormous surplus of substrate, needed to shift the equilibrium of the transesterification reaction, should be reduced [9].

2. Materials and methods

2.1. Chemicals and enzymes

All chemicals were purchased from Sigma–Aldrich (Zwijndrecht, The Netherlands) in the highest purity available and used without further purification.

The immobilized lipase B from *Candida antarctica* (trade name Novo435) was kindly donated by Novozymes (Bagsværd, Denmark) and used as received.

Formate dehydrogenase (FDH) was obtained by expressing *fdh* in *E. coli* JM101 carrying the plasmid pBTac2 (C23S) following a published procedure [10]. For the experiments reported here, crude cell extracts of the recombinant *E. coli* cells were used.

2.2. Methods

Throughout the manuscript enzyme activities are expressed as international units (U) with 1 U being the enzyme activity producing 1 μmol of product (octyl formate in case of the lipase-catalyzed transesterification and NADH in case of the formate dehydrogenase-catalyzed reduction of NAD^+) per minute.

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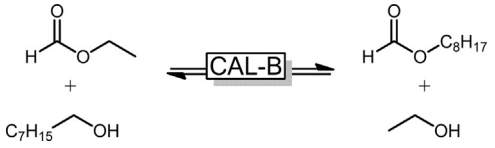

Scheme 1. Lipase-catalyzed transesterification of ethyl formate with various alcohols.

All transesterification reactions were carried out using a three-necked flask equipped with magnetic top-stirring, temperature control and reflux cooling. The reaction mixture was exposed to the ambient atmosphere and no extra drying or degassing measures were taken. Unless indicated otherwise, the reagents were added to the flask (amounts are specified in the captions of the figures and tables) and incubated for at least 15 min at the envisaged reaction temperature. Prior to starting the reaction by addition of the biocatalysts, a zero-sample was taken. Samples were taken at different time intervals to follow the reaction progress. The stirring was interrupted during the sampling to allow the biocatalyst to settle; then a sample (0.1 ml) was taken from the reaction mixture, diluted with 0.9 ml heptane (containing 100 mM dodecane as internal standard) mixture and analyzed by GC.

2.3. Recycling experiments

After every cycle, the biocatalyst was filtrated and washed two times with 20 ml portions of 1-octanol. When stored over night or over the weekend, the biocatalyst was kept under 1-octanol at room temperature (this was done in order to prevent possibly remaining ethyl formate to hydrolyze; the resulting formic acid has been previously demonstrated to inactivate the biocatalyst). Ethyl formate was (partially recovered) from the reaction mixture by distillation under reduced pressure. Unless otherwise indicated, the distillate was weighted, supplemented with fresh ethyl formate (to a total of 48 g, typically 15–20 g of fresh ethyl formate were added), 5 g of 1-octanol and used for the next reaction cycle. The remaining crude octyl formate product was collected and used for the FDH-activity assay.

2.4. GC analytics

Product analysis was performed on a Shimadzu 2014 gas chromatograph (direct injection, 320 °C) using a CP SIL 5 CB column (50 m, 0.53 mm, 1.0 μm) and the following temperature program (50 °C hold for 4 min, raise to 110 °C (20 °C per min) hold for 3 min, raise to 350 °C (20 °C per min) hold for 1 min).

Sample preparation entailed dilution of the sample (0.1 ml) with 0.9 ml heptane (containing 100 mM dodecane as internal standard).

Octyl formate and 1-octanol formations were based on calibration curves using authentic standards.

2.5. Formate dehydrogenase assay

To test the enzymatic activity of FDH with the crude octyl formate product, a spectroscopic assay following the characteristic absorbance of reduced nicotinamide cofactors at 340 nm ($\varepsilon = 6220\ M^{-1}\ cm^{-1}$) was used. For this assay, 10 ml TRIS buffer (50 mM, pH 7.5) containing 1 mM NAD^+ were supplemented with 25 μl FDH crude extract (approximately 5 U ml^{-1} FDH activity in the stock). To this solution, 1 ml of the crude octyl formate product (pooled products from recycling experiments 1–20) was added. The resulting emulsion was gently shaken at 30 °C. Samples were taken at different time intervals, then centrifuged and analyzed on a Shimadzu UV-2401 PC spectrophotometer.

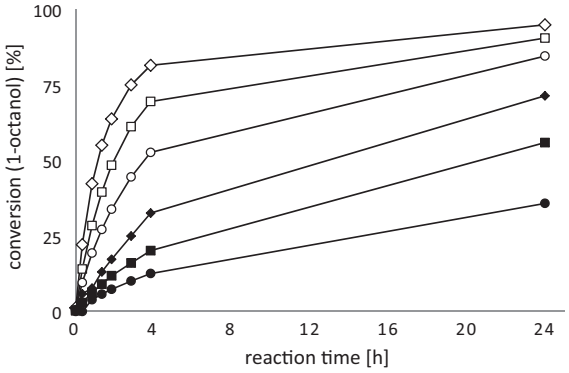

Fig. 1. Influence of the ratio of 1-octanol to ethyl formate on the time-course of the transesterification reaction. General conditions: *m*(Novo435) = 0.1 g; *m*(ethyl formate) = 48 g (0.65 mol); *m*(1-Octanol) = 2 g (◊), 5 g (□), 10 g (○), 20 g (♦), 40 g (■), 80 g (●) (0.025, 0.062, 0.12, 0.25, 0.5 and 1 mol mol^{-1}); *T* = 40 °C.

3. Results and discussion

We began our study by investigating the effect of varying ratios of the starting materials (ethyl formate and 1-octanol) over the time course of the transesterification reaction (Fig. 1). It is worth mentioning that all experiments reported here were performed under neat conditions.

When using approximately equimolar concentrations of the starting material, only 35% conversion was observed after 24 h. Upon prolonged reaction time (3 days), this value increased to 48% indicating an equilibrium constant of the transesterification reaction of around 1. In this set of experiments, because both initial concentrations of 1-octanol and ethyl formate were varied simultaneously (neat reaction conditions) kinetic parameters could not be determined at this stage. It is, however, interesting to note that the biocatalyst's specific activity (as determined in the first hour) gradually increased from 1.77 U $mg^{-1}_{Novo435}$ to 4.99 U $mg^{-1}_{Novo435}$.

For subsequent experiments, we used a molar ratio of 94:6 between ethyl formate and 1-octanol. The resulting product composition of approximately 9:1 of octyl formate to 1-octanol appeared acceptable in view of the envisioned application as reactive organic phase for NADH-dependent reactions (vide infra).

When using inactivated catalyst under otherwise identical reaction conditions, less than 2% conversion was observed within 24 h. Also, formic acid was not efficiently converted by Novo435, which we attribute to the inactivity of CALB in the presence of strong acids [11]. The temperature-dependence of the transesterification rate is shown in Fig. 2. For further experiments we chose a reaction temperature of 40 °C as it appeared to be a good compromise between biocatalyst activity (increasing with temperature) and undesired evaporation of ethyl formate.

Catalyst regeneration is of the utmost importance to attain economic feasibility of an enzymatic process, especially if low-value-added compounds are envisaged. Therefore, we evaluated the recycling of the catalyst Novo435 (Fig. 3). It should be mentioned here that we also aimed at recycling the non-reacted starting material (ethyl formate). Thus, after each reaction, not only the catalysts were recovered (by filtration) but also the non-reacted ethyl formate was distilled off the crude reaction mixture. For the next cycle, the distillate was supplemented with fresh ethyl formate and 1-octanol. In these experiments, a molar ratio of 94:6 between ethyl formate and 1-octanol was used, which would allow for a maximal conversion of 1-octanol of approximately 90% (Fig. 1). We assumed that this purity would be sufficient for the envisaged application as 'hydrophobized formic acid' since 1-octanol will represent the major side-product of this concept (Scheme 2).