Journal of Molecular Catalysis B: Enzymatic 114 (2015) 42–49

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

# Medium and reaction engineering for the establishment of a chemo-enzymatic dynamic kinetic resolution of *rac*-benzoin in batch and continuous mode

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ARTICLE INFO

*Article history:*
Available online 27 October 2014

*Keywords:*
Lipases
Dynamic kinetic resolution
Process stability
Water activity
Organic solvents
Deep eutectic solvents
Continuous operation

ABSTRACT

The effect of the reaction parameters water activity and reaction solvent was investigated for the dynamic kinetic resolution (DKR) of *rac*-benzoin with immobilized Lipase TL as biocatalyst for transesterification and the heterogeneous chemo-catalyst Zr-TUD-1 (Si/Zr = 25) for *in situ* racemization. Overall dry reaction conditions led to the best results for both catalysts. The immobilized lipase in a more environmentally benign solvent like cyclopentyl methyl ether (CPME) exhibited a 1.6-fold higher activity and an up to 1.5-fold higher half-life time than in the standard solvents such as toluene and 2-methyltetrahydrofuran (2-MeTHF). Among a variety of deep eutectic solvents (DESs) choline chloride:isosorbide (ChCl:Iso) was found to be suitable for the reaction system. The activity was lower than in the aforementioned solvents, but the very low solubility of the product (*S*)-benzoin butyrate in ChCl:Iso compared to the investigated organic solvents possesses great potential with respect to downstream processing. Optimized reaction parameters (dry CPME) were applied for DKR in batch and continuous mode yielding comparable or slightly better results than in toluene or 2-MeTHF.

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## 1. Introduction

Enzyme catalysis represents an increasingly important research field, where many practical applications for (enantioselective) organic synthesis can be derived, and have been already implemented at industrial scale [1]. The use of non-aqueous media for enzymatic reactions offers several advantages such as high solubility of water-insoluble substrates, less waste water formation and more straightforward downstream processing. In the field of biocatalysis in non-aqueous media lipases have proven to be extremely useful biocatalysts, based on their outstanding resilience to these media, together with the fact that no cofactors are needed for their catalytic performance [2].

To further provide stronger arguments for (industrial) biocatalysis, the use of more environmentally-benign solvents as well as the set-up of continuous processes is presently an area of high interest. Thus, solvents like biomass-derived 2-methyltetrahydrofuran (2-MeTHF) have been reported as a promising alternative to conventional solvents and hence attracted great attention in organic synthesis [3]. Likewise, cyclopentyl methyl ether (CPME) [4] and 1,3-dioxolane [5] have been assessed as environmentally benign alternatives to conventional organic solvents. CPME has been commercially available since 2005 [4b] and has preferable characteristics, *e.g.* a high boiling point, relative stability under acidic and basic conditions, low peroxide formation, high hydrophobicity (ease of drying), and a narrow explosion range [4b]. Similarly, 1,3-dioxolane is considered as a solvent as well as a reagent [5a] owing to its preferable physical, chemical and toxicological properties [5b,c]. Furthermore, deep eutectic solvents (DESs) have been evaluated, which are also environmentally friendly and cost-effective alternatives to many conventional solvents [6]. DESs–eutectic mixtures of an ammonium salt (*e.g.* choline chloride, ethylammonium chloride) and a hydrogen bond donor (HBD) (*e.g.* alcohols, amines,

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http://dx.doi.org/10.1016/j.molcatb.2014.10.011
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amides and carboxylic acids)–have melting points below room temperature, low volatility, high thermal stability, and tailored characteristics similar to ionic liquids, but largely offer diminished toxicity, better biodegradability, large availability at acceptable costs, and simplified preparation [6]. Applicability of DESs in biocatalyzed reactions has been demonstrated for hydrolases [7a–h], and recently for whole-cell redox biocatalysis [7i], for lyases [7j]. Recently, also tandem catalysis of a hydrolase and organocatalysts was demonstrated [7k]. Thus, DESs are currently also receiving great attention as eco-friendly solvents [8].

Following these important emerging fields – biocatalytic continuous processes and the employment of environmentally-benign non-conventional solvents – the present work focuses on the setup of a chemo-enzymatic dynamic kinetic resolution process (DKR) for racemic benzoin (aromatic α-hydroxyketone) in a continuous fashion, while assessing different environmentally-friendly nonaqueous solvents at the same time. Chiral α-hydroxyketones are important building blocks with a broad range of applications in the synthesis of biologically active compounds such as pharmaceuticals, agrochemicals, and pheromones [9]. On this basis, various chemical and biocatalytic approaches for their synthesis have been developed [10]. Among them, enzymatic routes show great potential delivering lower amounts of waste and higher stereoselectivities and overall yields compared to the chemical methods [11]. Biocatalytic syntheses of chiral α-hydroxyketones are possible either by (i) reduction of α-diketones using oxidoreductases (EC 1) [12], (ii) acyloin condensation of aldehydes [13], (iii) kinetic resolution of racemic mixtures *via* C–C bond cleavage both catalyzed by thiamine diphosphate (ThDP) dependent lyases (EC 4) [14], or by (iv) dynamic kinetic resolution (DKR) catalyzed by hydrolases (EC 3) combined with a chemo-catalyst [15]. With regard to lipases, as stated above, their high stability in and good compatibility with organic solvents provide special synthetic advantages.

In this study, DKR of racemic benzoin with a lipase from *Pseudomonas stutzeri* (under the trade name Lipase TL) and the chemo-catalyst Zr-TUD-1 (Si/Zr = 25) [16] was investigated (Scheme 1) for solvent needs and continuous operation. Several TUD-1 catalysts with aluminum (Al), zirconium (Zr) and tungsten (W) as incorporated metals had been evaluated for this reaction in a previous study [17]. Among these, Zr-TUD-1 (Si/Zr = 25) excelled by its high racemization rate and hence was chosen for further study. The combination of both catalysts in a heterogeneous formulation leads to a yield and enantioselectivity of >99%, and offers high recyclability in batch reaction systems [17].

For achieving high productivities process parameters such as water activity ($a_w$) [18] and solvent [19] are crucial. Hence, the effect of these parameters on the activity of the catalysts and the stability of the enzyme (*i.e.* half-life time) were in-depth evaluated. The optimized reaction conditions identified for the DKR of *rac*-benzoin were combined and then demonstrated in a continuous stirred tank reactor (CSTR, see SI) under temperature and water activity controlled conditions.

## 2. Experimental

### 2.1. Materials

All chemicals were purchased from Sigma-Aldrich (Schnelldorf, Germany), Carl Roth GmbH (Karlsruhe, Germany) or VWR (Dresden, Germany) and were used as received with a purity ≥98%. Lipase from *P. stutzeri* (trade name Lipase TL) was kindly provided by Meito Sangyo Co., Ltd. (Tokyo, Japan). Accurel MP1001 carrier was obtained from Membrana GmbH (Obernburg, Germany). The humidity sensor HMT337 was purchased from Vaisala GmbH (Bonn, Germany).

**Table 1**
Water activity values of selected saturated salt solutions at 25 °C and 50 °C.

| Salt | $a_w$ [25 °C][a] | $a_w$ [50 °C][a] |
|---|---|---|
| LiCl | 0.11 | 0.11 |
| KAc | 0.23 | 0.19[b] |
| $MgCl_2$ | 0.33 | 0.31 |
| $KCO_3$ | 0.43 | 0.43 |
| $Mg(NO_3)_2$ | 0.53 | 0.45 |
| NaBr | 0.58 | 0.51 |
| NaCl | 0.75 | 0.74 |
| KCl | 0.84 | 0.81 |
| $K_2SO_4$ | 0.97 | 0.96 |

[a] L. Greenspan (1977) [20].
[b] Data measured with a humidity sensor.

### 2.2. Immobilization of lipase TL

Lipase TL solution at 30 g/L concentration was prepared in a potassium phosphate buffer (200 mM, pH 6.2) and stirred (at 1000 rpm) for at least 1 h at room temperature. Insoluble components were removed by centrifugation (2600 × *g*) for 10 min. Accurel MP1001 carrier was mixed with absolute ethanol ($7.6\ mL_{ethanol}/g_{carrier}$) and incubated for 30 min. The lipase solution was added to the pre-treated carrier ($20\ mL_{lipase\ solution}/g_{carrier}$) and the suspension was shaken (at 340 rpm) for 4 h at 40 °C. After incubation, the particles were washed two times with buffer ($20\ mL_{buffer}/g_{carrier}$) and filtered afterwards. The immobilized enzyme preparation was dried over silica gel under reduced pressure for at least five days before further application. For the fragmented immobilized lipase the formulation was then crushed in a mortar.

### 2.3. Analysis of protein loading

Protein loading was determined by measurement of the protein concentration before and after immobilization in the supernatant fraction using Bradford reagent (Roti®-Nanoquant, Carl Roth GmbH & Co. KG) according to the manufacturer's instructions and bovine serum albumin (BSA) as a standard.

### 2.4. Synthesis of deep eutectic solvents (DESs)

DES forming components (*i.e.* ammonium salts and hydrogen bond donors) were weighed in a glass vessel at a defined molar ratio (*e.g.* 1:2 or 1:1.5). The mixture was stirred at 100 °C until a clear liquid was obtained (at least 1 h up to 24 h). Water was removed using a rotary evaporator and the DESs were stored for 72 h over silica gel under reduced pressure.

### 2.5. Adjustment of water activity

Saturated salt solutions were prepared by mixing a large excess of each salt and distilled water and stored at 25 °C in a desiccator for several days before using. Nine reagent grade salts with defined water activities were used: $LiCl_2$, KAc, $MgCl_2$, $K_2CO_3$, $Mg(NO_3)_2$, NaBr, NaCl, KCl, $K_2SO_4$ (Table 1).

For initial experiments, all reaction components (*i.e.* substrates, solvent, and chemo-catalyst) were stored separately in a desiccator over saturated salt solutions for three days at 50 °C. Only in case of the enzyme, the $a_w$ values were adjusted at 25 °C as a decrease in the enzyme specific activity (51% (in toluene) and 54% (in 2-MeTHF) within 72 h) was observed when incubation was performed at 50 °C. Later, all reaction components were incubated with saturated salt solutions at 25 °C as the $a_w$ values at 25 °C and at 50 °C were not significantly different (Table 1). The time for reaching equilibrium $a_w$ (72 h) was previously determined using a Humidity Sensor HMT337.

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