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# Enantioselective enzymatic synthesis of the α-hydroxy ketone (*R*)-acetoin from *meso*-2,3-butanediol

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ABSTRACT

Acetoin (3-hydroxy-2-butanone) is an important flavour compound and is applied in cosmetics, pharmacy and chemical synthesis. In contrast to chemical syntheses or fermentations an enzymatic route facilitates enantioselective acetoin production. The discovery of a (*S*)-selective alcohol dehydrogenase enables a novel production process of (*R*)-acetoin from *meso*-2,3-butanediol. It was shown that the regeneration of oxidised nicotinamide adenine dinucleotide is a key point in preparative application of dehydrogenases for the oxidative route. An electrochemical regeneration system was successful combined with the ADH catalysed reaction. Up to 48 mM (*R*)-acetoin was produced in the reaction system while productivities up to 2 mM $h^{-1}$ were reached. The possibility to apply an electrochemical system in a semi-preparative synthesis will stimulate further research of electroenzymatic processes with oxidoreductases.



## 1. Introduction

Acetoin (3-hydroxy-2-butanone) is an important flavour compound, widely existing in dairy products and some fruits. Because of its unique butter flavour, it is used as flavour enhancer of butter, cheese, coffee and nut containing food. Furthermore, it was classified as one of the 30 platform chemicals which were given the priority to their development and utilisation by the U.S. Department of Energy [1]. It can be widely applied in cosmetics, pharmacy and chemical synthesis. Many efforts have been made to develop acetoin production methods including chemical synthesis and fermentative technologies. Acetoin can be produced chemically by partial reduction of diacetyl, selective oxidation of 2,3-butanediol and oxidation of 2-butanone followed by basic hydrolysis. Furthermore, acetoin is an important physiological metabolite excreted during fermentation by a lot of microorganisms, such as *Serratia marcescens* [2], *Bacillus* species [3,4], *Paenibacillus polymyxa* [5] and *Lactococcus lactis* [6]. Chemical syntheses or fermentations generally lead to the production of racemic mixtures [7]. For the application as flavour component and as intermediate for the synthesis of building blocks the production of racemic acetoin is often sufficient. Enantiopure acetoin is widely used to synthesise novel optically active α-hydroxy ketone derivatives and liquid crystal composites [8]. Furthermore, it was shown that (*R*)-acetoin is a female sex pheromone of the beetle *Amphimallon solstitiale* [9]. (*R*)-Acetoin is highly attractive to swarming males, whereas neither *rac*-acetoin nor the 2,3-butanediols shows activity.

For this wide range of application it is essential to develop an effective process to produce enantiomeric pure acetoin. Due to the fact that enantioselectivity is one key advantage of enzyme-catalysed oxidation we investigated an enzymatic process to produce acetoin. In general, three different classes of enzymes can be used to produce α-hydroxy ketones (Scheme 1). These α-hydroxy ketones can be used as building blocks for compounds of pharmaceutical interest, e.g. antidepressants, HIV-protease inhibitors and antitumorals [10,11]. The oxidative synthesis of enantiopure acetoin starting from 2,3-butanediol would be a highly attractive route due to the availability and costs of the substrate. Different biotechnological routes to produce (*S*)-acetoin from *meso*-2,3-butanediol and (*R*)-acetoin from (2*R*,3*R*)-butanediol are described in literature [12–14]. Until now, no (*S*)-selective ADH for the conversion of

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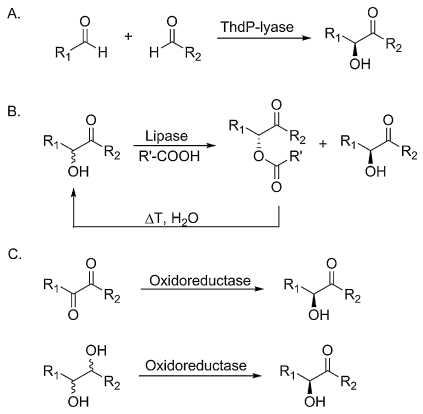


**Scheme 1.** Enzymatic production of α-hydroxy ketones: A – thiamine diphosphate-dependent lyase (ThdP-lyase) can be used to catalyse the carboligation of aldehydes; acetoin is obtained by self-ligation of acetaldehyde, B – lipases can be applied in dynamic kinetic resolutions. Lipase-catalysed kinetic resolutions are combined with racemisation of the substrate, C – oxidoreductases can be used in reductions of diketones and by selective oxidations of vicinal diols (in the synthesis of acetoin $R_1$, $R_2 = CH_3$).

the 2,3-butanediol is described. Recently, it was shown that different alcohol dehydrogenases from the proprietary c-LEcta collection exhibit high activity for the enantioselective oxidation of aldehydes. The ADH-9 which was identified from a biodiversity library was shown to have exceptional high oxidative activity as well as (*S*)-specific activity onto the substrates 2-phenyl propionaldehyde, benzyl aldehyde and benzyl alcohol, hexanal and hexanol, propanal and propanol as well as flurbiprofen aldehyde [15]. In the present study the (*S*)-specific ADH-9 was investigated to produce (*R*)-acetoin by an oxidative route starting from butanediol (Scheme 1).

## 2. Materials and methods

### 2.1. Chemicals

All chemicals were of analytical grade or higher quality and purchased from Fluka (Buchs, Switzerland) or Sigma–Aldrich (Taufkirchen, Germany).

### 2.2. Enzyme production

The ADH-9-gene was expressed in *Escherichia coli* BL21 (DE3) as described [15]. In brief, recombinant *E. coli* cells were cultured in ZYM505 medium [16] at 37 °C to an optical density of 0.7 at 600 nm. Expression was induced by addition of 0.1 mM isopropyl-β-D-thiogalactopyranoside and the cultivation temperature was reduced to 30 °C. Cells were harvested after overnight induction and resuspended with 0.05 M Tris/HCl buffer (pH 7.0) containing 2 mM $MgCl_2$. Cells were lysed by sonication, the supernatant was separated from insoluble matter by centrifugation and freeze dried. The specific activity of the enzyme was 7.14 U per mg enzyme determined by using the substrate *meso*-2,3-butanediol.

### 2.3. Adsorptive immobilisation of ADH-9 onto Amberlite FPA54

100 mg lyophilised ADH-9 was dissolved in 10 mL 0.1 M Tris/HCl buffer pH 9.0. The enzyme solution was added to 7 g Amberlite FPA54 carrier in a crystallisation bowl. The suspension was incubated for 12 h at 23 °C. After incubation, the supernatant was taken and analysed via Bradford assay (Roti-Quant) for calculating the amount of protein. The particles were washed once with 5 mL 0.1 M Tris/HCl pH 9.0 buffer. The immobilisation yield on Amberlite FPA54 was 73%, with 2% protein loss in the wash solution. Consequently an ADH-9 loading of 10 mg ADH-9/g Amberlite FPA54 could be achieved. The specific activity of the immobilised enzyme was 0.01 U per mg carrier determined by using the substrate *meso*-2,3-butanediol.

### 2.4. Characterisation of the ADH-9

All enzymatic conversions were performed in 0.1 M Tris/HCl (pH 8.0) buffer at 30 °C. NADH-assays were performed measuring absorption of NADH formed during the reaction at 340 nm continuously in a Shimadzu spectrometer or by taking samples after distinct time of incubation and measuring absorption at 340 nm. Experiments measuring the absorption at 340 nm continuously contained 0.01 mg $mL^{-1}$ of soluble ADH-9, 5–100 mM 2,3-butanediol (mix of isomers) and 0.1 mM $NAD^+$. Assays performed by taking samples contained 0.01–0.1 mg $mL^{-1}$ ADH-9 as soluble form or 10 mg $mL^{-1}$ as immobilised form, 25 mM 2,3-butanediol (mix of isomers), 25 mM $NAD^+$. Reaction vessels were shaken at 75 rpm (Certomat-R, B. Braun, Biotech International, Melsungen). Experiments regarding the selectivity were performed using same concentrations as before and each of the enantiomer of the substrate. Samples were incubated for 1 h and extracted with ethyl acetate for GC analysis. Investigations for testing the electrochemical stability of the enzyme and substrate were performed in an electrochemical reactor. The reaction solution containing 0.12 mM 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), and 10 mg $mL^{-1}$ enzyme or 50 mM substrate was polarised at 600 mV vs. Ag/AgCl. The electrode set-up consisted of polished glassy carbon ($A = 2.0\ cm^2$, HTW Hochtemperatur-Werkstoffe GmbH, Thierhaupten, Germany) as working electrode, a platinum plate as counter electrode and a Ag/AgCl (3 M KCl) reference electrode (SE 21, Sensortechnik Meinsberg, Waldheim, Germany). After different incubation times samples were taken and analysed in an NADH-assay (enzyme containing samples) or with GC (substrate containing samples, see Section 2.6).

### 2.5. Electroenzymatic conversion

Electroenzymatic reactions using the immobilised or soluble ADH-9 were performed in the above-mentioned glass reactor with a reaction volume of 20 mL. The reaction solution by using the immobilised enzyme contained 0.1 or 1 mM ABTS, 75 mM *meso*-2,3-butanediol, 0.1 U $mL^{-1}$ immobilisate and 1 mM $NAD^+$. Reaction solutions with soluble ADH-9 contained 1 mM ABTS, 75 mM *meso*-2,3-butanediol, 0.1 or 10 U $mL^{-1}$ soluble ADH-9 and 1 mM $NAD^+$. After different time of incubation samples were taken and extracted with ethyl acetate for GC analysis. The potential of the working electrode was 600 mV vs. Ag/AgCl (3 M KCl). Further reactions have been implemented in a 3-dimensional electrochemical cell working as a loop reactor. The reactor consisted of a packed bed working electrode between two cathodes. The packed bed was built up of 7.2 g splintered glassy carbon particles (1000–2000 μm; HTW Hochtemperatur-Werkstoffe GmbH, Thierhaupten, Germany). The cathodes were iridium-based oxide plated titanium net electrodes (Metakem, Usingen, Germany). The electrodes were separated with a cation exchange membrane (THOMAPOR® CMX, Reichelt Chemietechnik, Heidelberg, Germany). Each cathode had a separate catholyte chamber. The Ag/AgCl (3 M KCl) reference electrode was positioned in one of these chambers (for further information see [17]). The reaction solution containing 1 or 5 U $mL^{-1}$ ADH-9, 75 mM *meso*-2,3-butanediol, 1 mM $NAD^+$ and 1 mM ABTS was pumped vertically upwards through the packed bed anode with a flow rate of 2.5 mL $min^{-1}$. The total reaction volume was 40 mL. After different time of incubation samples were taken and extracted for GC analysis.

### 2.6. Chiral GC analytics

Product formation and substrate consumptions were monitored by GC. A sample of 300 μl was extracted with the same volume of ethyl acetate. After vortexing for 30 s and centrifugation for 15 s the supernatant was dried over $Na_2SO_4$. The GC programme was as follows: 60 °C, hold 3 min, 10 °C $min^{-1}$ up to 80 °C, hold 7 min, 20 °C $min^{-1}$ up to 125 °C, hold 10 min, 45 °C $min^{-1}$ up to 210 °C; 75 kPa, total flow of 223 mL $min^{-1}$, column flow of 0.88 mL $min^{-1}$. The applied GC column was a CP-Chirasil-DEX CB (CP7502). The concentrations of the samples were calculated using a calibration curve. The calibration curve was achieved by measuring samples of acetoin, *meso*-2,3-butanediol, (*S,S*)-2,3-butanediol and (*R,R*)-2,3-butanediol with distinct concentrations.

## 3. Results and discussion

### 3.1. Enzyme characterisation

Initially, the substrate spectrum of ADH-9 in oxidative direction was investigated with 0.6 mg $mL^{-1}$ ADH-9, 0.5 mM $NAD^+$ and 0.5 mM 2,3-butanediol, 2,5-hexanediol, 1,4-butanediol, 2-methyl-1-butanol, 2-methyl-2-butanol and isopropanol in buffer using an NADH-assay (Table 1). The substrates 2,3-butanediol, 2-methyl-2-butanol and isopropanol were oxidised by ADH-9. The NADH formation rate with the vicinal 2,3-butanediol was more than 200-fold higher compared to the other substrates. The isomer 1,4-butanediol was not converted, which indicates that the vicinal position of the hydroxyl groups is important for the enzyme activity.

For further characterisation, the influence of the concentrations of *meso*-2,3-butanediol, (*S,S*)-2,3-butanediol and the mix of the isomers on the velocity of enzymatic oxidation with ADH-9 were investigated in NADH-assays. An increase of substrate conversion was observed with increasing the substrate concentration up to 100 mM. From these data the $k_m$ und $V_{max}$ values were calculated by using the nonlinear curve fit. The $k_m$-value of the ADH-9 with *meso*-2,3-butanediol is 6.5 mM and the $V_{max}$-value 1.47 μM $s^{-1}$ (see SI). By using the mix of isomers similar values can be calculated, the $k_m$-value with this substrate is 5.1 mM and the $V_{max}$-value 1.27 μM $s^{-1}$. The kinetic parameters of the mixture were determined by *meso*-2,3-butandiol. The affinity of the ADH-9 towards (*S,S*)-2,3-butanediol was 27-times lower compared to the *meso*-form of the substrate. It was also shown that a substrate concentration up to 100 mM had no inhibition effect onto the reaction. The specific activity of the ADH-9 was 7.14 U per mg protein.

For a further characterisation of the reaction, control reactions with different enzyme concentrations of soluble ADH-9 were performed and analysed by using NADH-assay. The concentration of NADH, indicating substrate conversion, was measured by determining the absorption at 340 nm. Fig. 1 shows substrate conversion measured by increasing concentration of the reduced cofactor. In all reactions a maximum substrate conversion of just 10% (corresponding to a product concentration of 2 mM) was achieved, although the added $NAD^+$ concentration was high enough to enable 75% conversion (Fig. 1). To explain this effect, the influence of the addition of different NADH concentrations prior starting control reaction was tested. It was shown that even low concentrations of added NADH (2 mM; corresponding to an $NAD^+$/NADH ratio of 7.3 at the end of the reaction) led to a reduced conversion of 6.7%. This reveals that an enhanced $NAD^+$/NADH ratio is required to improve the conversion rate.

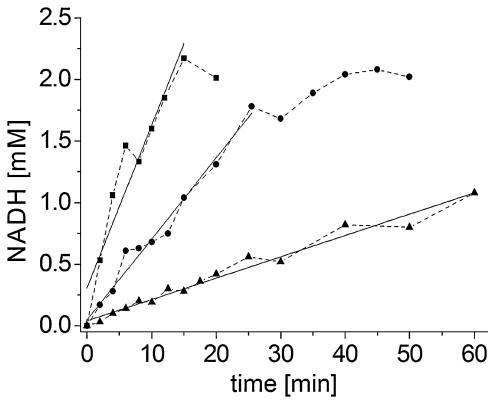


**Fig. 1.** Photometric determination of NADH production over time with different ADH-9 concentrations (▲: 0.01 mg $mL^{-1}$, ●: 0.05 mg $mL^{-1}$, ■: 0.1 mg $mL^{-1}$). Conditions: 0.01–0.1 mg $mL^{-1}$ ADH-9, 25 mM 2,3-butanediol (mix of isomers), 25 mM $NAD^+$.

The stereochemistry of the ADH-9 catalysed acetoin formation was investigated with the three stereoisomers of 2,3-butanediol. The product and substrate concentrations were determined by chiral GC. *Meso*-2,3-butanediol was converted in (*R*)-acetoin as sole product. By using (*S,S*)-2,3-butanediol as substrate (*S*)-acetoin was formed. The product concentration was 13.5 times lower compared to the conversion with the *meso*-form. The (*R,R*)-2,3-butandiol was not converted. Therefore, the ADH-9 can be defined as (*S*)-selective in this conversion of 2,3-butanediol. Solely the (*S,S*)-2,3-butanediol and the *meso*-2,3-butanediol are converted into the (*S*)- or (*R*)-enantiomer of acetoin. This is the first described (*S*)-selective ADH for the conversion of the 2,3-butanediol (Table 3). Therefore, the production of (*R*)-acetoin from *meso*-2,3-butanediol was further investigated.

### 3.2. Electroenzymatic conversions

To increase the performance of the reaction system the enzymatic conversion was coupled to an electrochemical $NAD^+$ regeneration approach, which should hold the $NAD^+$/NADH ratio on an adequate level. $NAD^+$ is regenerated in a chemical oxidation reaction between NADH and the mediator ABTS [17,18]. This mediator shuttles electrons derived from NADH towards the electrode and is thereby re-oxidised closing the regeneration cycle. The applied electrochemical system results in a cosubstrate- and coproduct-free reaction setup. Initially, the stability of substrate and enzyme under electrochemical conditions by applying the oxidation potential of the mediator were tested. Solutions containing ABTS in TRIS buffer and 10 mg $mL^{-1}$ ADH-9 or 50 mM substrate were incubated applying a potential of 600 mV vs. Ag/AgCl, which was used in the latter experiments for cofactor regeneration. Samples were taken from the reactor after different incubation times and the enzyme activity or substrate concentration were determined. The enzyme activity as well as the substrate concentration was constant during the tested time period. This means that there was no conversion, adsorption or polymerisation of the substrate due to applying the electrochemical conditions. These experiments showed that both, enzyme and substrate were stable under electrochemical conditions.

**Table 1**
Determination of specific activity of ADH-9 using different substrates.

| Substrate | Substrate oxidation rate [μM $min^{-1}$] |
|---|---|
| 2,3-Butanediol | 66 |
| Isopropanol | 0.1 |
| 2-Methyl-1-butanol | 0.3 |
| 2-Methyl-2-butanol, 2,5-Hexanediol, 1,4-Butanediol | 0 |

**Table 2**
Comparison of different reaction settings and their productivities. Substrate, cofactor and mediator concentration were constant (75 mM, 1 mM and 1 mM, respectively).

| Entry | Enzyme<br>#Immobilised<br>*Soluble [U mL$^{-1}$] | Reaction system | Reaction time [h] | Product (*R*)-acetoin [mM] | Productivity initial (average) [mM h$^{-1}$] | Conversion to (*R*)-acetoin [%] |
|---|---|---|---|---|---|---|
| 1 | #0.1 | Electrochemical cell | 28 | 3.5 | 0.57 (0.24) | 4.7 |
| 2 | *0.1 | Electrochemical cell | 42 | 11.0 | 3.3 (0.32) | 14.7 |
| 3 | *10 | Electrochemical cell | 28 | 8.3 | 3.3 (0.45) | 11.1 |
| 4 | *1 | 3-Dimensional reactor | 11 | 13.4 | 4.8 (1.52) | 17.9 |
| 5 | *5 | 3-Dimensional reactor | 50 | 48.44 | 5.7 (2) | 64.6 |

The conversion of *meso*-2,3-butanediol coupled with an electrochemical $NAD^+$ regeneration was tested using soluble and immobilised ADH-9. As reaction system an electrochemical cell with an electrode surface-to-volume ratio of 0.1 cm$^2$ mL$^{-1}$ was used. A detailed study about the used electrochemical regeneration system was described previously [17]. The use of an immobilised enzyme may offer advantages regarding stability and reusability over using the soluble form [19,20]. Therefore, ADH-9 was immobilised onto the carrier Amberlite FPA54 [21] yielding a specific activity 0.01 U per mg carrier and both enzyme preparations were compared regarding the process performance. Fig. 2 shows the time course of the product formation for both ADH-9 preparations. It was shown that although the applied enzyme units per mL were constant (0.1 U mL$^{-1}$) the process with the soluble enzyme showed an even higher productivity (Table 2, entries 1 and 2). Furthermore, the product concentration by using the soluble ADH-9 was 2.3-fold higher compared to the immobilised enzyme.

We assumed an adsorptive effect of the carrier. Thus, it was tested whether the substrate or product or both adsorbs onto the immobilisation carrier. Therefore, 5 mM substrate and product were incubated in separate assays with the immobilisate. The final concentrations were compared to the initial concentrations. Indeed, an adsorption of 52% of the substrate and 12% of the product were found after 1 h. Although the use of immobilised enzymes would be a smart reaction system in regard to the expected advantages over soluble enzymes, we decided to use the soluble ADH-9 in further experiments. To identify the limiting step of the reaction and to increase the productivity the influence of 100-

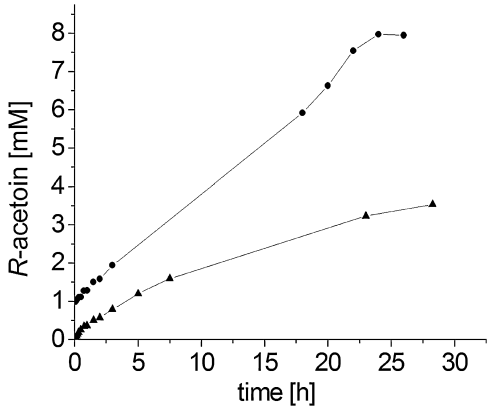


**Fig. 2.** Conversion of meso-2,3-butandiol to (*R*)-acetoin with 0.1 U mL$^{-1}$ soluble ADH-9 (•) or immobilisate of ADH-9 (▲).

**Table 3**
Enantioselective oxidation of 2,3-butanediol (2,3-BD) to acetoin.

| Biocatalyst | Substrate | Product | Reference |
|---|---|---|---|
| | glycerol dehydrogenase, 2,3-butanediol dehydrogenase<br>*meso*-2,3-butanediol → (*S*)-acetoin | | |
| | glycerol dehydrogenase, 2,3-butanediol dehydrogenase<br>(*R*,*R*)-2,3-butanediol → (*R*)-acetoin | | |
| Glycerol dehydrogenase from *H. polymorpha* expressed in *E. coli* | (*R*,*R*)-2,3-BD<br>*Meso*-2,3-BD | (*R*)-Acetoin<br>(*S*)-Acetoin | [14] |
| (2R,3R)-2,3-Butanediol dehydrogenase from *S. cerevisiae* | (*R*,*R*)-2,3-BD<br>*Meso*-2,3-BD | (*R*)-Acetoin<br>(*S*)-Acetoin | [29] |
| (2R,3R)-2,3-Butanediol dehydrogenase from *Paenibacillus polymyxa* | (*R*,*R*)-2,3-BD<br>*Meso*-2,3-BD | (*R*)-Acetoin<br>(*S*)-Acetoin | [12] |
| (2R,3R)-2,3-Butanediol dehydrogenase expressed in *E. coli* | (*R*,*R*)-2,3-BD<br>*Meso*-2,3-BD | (*R*)-Acetoin<br>(*S*)-Acetoin | [13] |
| | ADH-9<br>*meso*-2,3-butanediol → (*R*)-acetoin | | |
| Isolated ADH-9 | *Meso*-2,3-BD | (*R*)-Acetoin | This study |

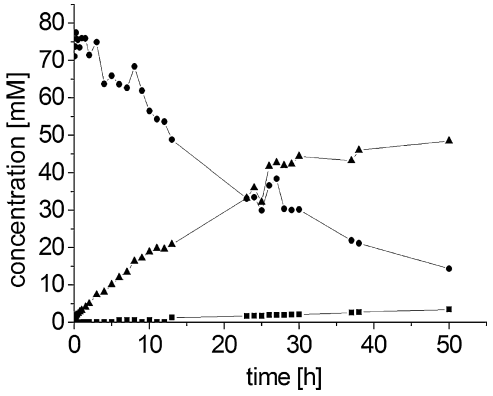


**Fig. 3.** Conversion of *meso*-2,3-butandiol with 5 U mL$^{-1}$ soluble ADH-9 in electrochemical reactor. Addition of enzyme (5 U mL$^{-1}$) after 12.5, 25 and 37.5 h. Value of the pH was controlled and adjusted over the whole experiment. ●: *Meso*-2,3-butandiol, ■: (*S*)-Acetoin, ▲: (*R*)-Acetoin.

fold increased enzyme concentration was investigated (Table 2, entry 3). The initial as well as the overall productivity was more or less constant in the reactions. Therefore, it can be assumed that the regeneration system was the limiting step of the reaction system. One of the main features of electroenzymatic production systems are limitations caused by the heterogeneous character of the electrochemical step. An enhanced surface of electrode should improve the cofactor regeneration. Therefore, a 3-dimensional packed bed electrode was used as an electrochemical reactor. Initially, the conversion of *meso*-2,3-butanediol was driven by using 1 U mL$^{-1}$ soluble ADH-9. The initial and average productivity were 4.8 mM h$^{-1}$ and 1.52 mM h$^{-1}$ (Table 2, entry 4). Compared to the reaction using 10 U mL$^{-1}$ ADH-9 in the standard electrochemical reaction cell the productivity was even higher, although the enzyme concentration was 10 times lower by using the electrochemical flow cell (Table 2, entries 3 and 4). This demonstrates that the overall reaction in the standard cell was limited by the electrode surface. The application of an electrochemical flow cell with enhanced electrode area and an improved ratio between the reaction volume and the electrode surface overcomes this limitation. In the next step a slight improvement of the productivity was achieved with 5-fold increased enzyme concentration (Table 2, entry 5). The electroenzymatic conversion in the electrochemical reactor with 5 U mL$^{-1}$ soluble ADH-9 is shown in Fig. 3. Within 50 h 65% of the substrate was converted to (*R*)-acetoin, resulting in a product concentration of about 50 mM. The conversion demonstrates again the need for an efficient regeneration system resulting in a driving force for the production of (*R*)-acetoin. The initial productivity was 5.7 mM h$^{-1}$ and the average productivity was 2 mM h$^{-1}$. The decreasing substrate concentration fits well with the time course of the produced (*R*)-acetoin. After 12.5 h the product formation stopped. It was assumed that this might be due to enzyme degradation, so additional enzyme was dosed into the reaction system. Immediately product formation proceeded. In contrast to the results regarding the stability of the enzyme found in the standard cell the enzyme was shown to be merely stable up to a particular time. As the enzyme degraded after 12.5 h fresh enzyme was added each 12.5 h. A slight chemical racemisation of the product into the (*S*)-enantiomer was observed. The optically active acetoin undergoes racemisation at a basic pH [22]. As the product formation decreased after 35 h and an addition of fresh enzyme had nearly no effect as well as protein denaturation was not observed a product inhibition was assumed. To investigate an inhibition an $NAD^+$-assay with 50 mM acetoin was performed. In this assay no cofactor conversion takes place. This product inhibition cannot be overcome through a further increase of the cofactor regeneration system or by higher concentrations of substrate although the overall reaction will be thermodynamically favourable [23]. The only effective method for minimising the effects of product inhibition is to remove the product from the reaction system. To overcome the product inhibition continuous reactor concepts, e.g. based on *in situ* product removal [24] or, after further optimisation, the above mentioned immobilisation technique onto the carrier Amberlite FPA 54 would be promising reaction systems.

Table 2 summarises results achieved by using different settings and set-ups for the conversion of *meso*-2,3-butanediol into (*R*)-acetoin with coupled electrochemical cofactor regeneration. Using soluble ADH-9 was shown as even higher productivity compared to using immobilised form (entries 1 and 2). In a simple glass reactor with an electrode surface-to-volume ratio of 0.1 cm$^2$ mL$^{-1}$ an increase of enzyme concentration had nearly no effect onto productivities (entries 2 and 3). Using the 3-dimensional electrochemical with an adequate surface-to-volume ratio up to 6000 cm$^2$ mL$^{-1}$ improved productivities can be achieved.

## 4. Conclusion

Oxidations are central transformations in organic chemistry. In the past decades, tremendous advances in transition metal- and organocatalytic oxidation and oxyfunctionalisations have been achieved. Next to these, biocatalysis is emerging as additional pillar for environmentally benign oxidation catalysis [25]. ADHs are enjoying increasing interest as versatile and selective biocatalysts in the context of both, academic research and industrial implementation [15]. On the way to the practical application of ADH in preparative synthesis different topics must be addressed. The identification of a suitable enantioselective enzyme is always the starting point for the development of a novel bioprocess. In the present study, an alcohol dehydrogenase was identified to be (*S*)-selective in the oxidation of *meso*-2,3-butanediol to (*R*)-acetoin. This provides an unique feature compared to published conversions of 2,3-butanediol with dehydrogenases (Table 3). The substrate of the bioconversion 2,3-butanediol is produced as a natural fermentation product by several bacteria and several species of yeast. Product stereo specificity is found to vary from organism to organism [26]. *K. pneumonia* is known to produce a mixture of *meso*- and (*S,S*)-stereoisomers whereas *P. polymyxa* produces (*R,R*)-2,3-butanediol at optical purities as high as 98%. Nielsen et al. engineered an *E. coli* strain which was able to produce up to 1.1 g L$^{-1}$ *meso*-2,3-butanediol [26]. Production of *meso*-2,3-butanediol from glucose was also reported in recombinant *E. coli* harbouring a fragment of the *K. pneuominae* IAM 1063 genome encoding the *meso*-2,3-butanediol biosynthetic pathway [27]. The application of an engineered *E. coli* resulted in product concentration up to 15.7 g L$^{-1}$ *meso*-2,3-butanediol [28]. The discovery of the (*S*)-selective enzyme enables a novel production process of (*R*)-acetoin from *meso*-2,3-butanediol. Admittedly, the produced acetoin concentration of 50 mM is much lower as the concentrations which can be reached in fermentative processes [3–5]. However, the enzymatic route facilitates the production of a valuable enantiopure product. Certainly, a production process of (*R*)-acetoin with whole-cell system over expressing a *meso*-2,3-butanediol dehydrogenase would be possible. Due to the cofactor challenge, possible formation of side-products as well as back reactions a process with isolated enzymes seems to be more convenient.

The oxidation of reduced cofactors NADH is a key point in preparative application of dehydrogenase for alcohol oxidation [30]. Besides the economical and ecological impact of a cofactor regeneration system, most enzymatic oxidations are thermodynamically unfavourable. Only efficient cofactor regeneration can shift the ratio of the oxidised and reduced cofactor in the direction of $NAD^+$ and therefore enable a preparative oxidation process. The applied electrochemical reaction system was shown to be a suitable

concept for the application in combination with an ADH catalysed oxidation. The possibility to apply an electrochemical system in a semi-preparative synthesis of 2 mmol product will stimulate further research of electroenzymatic processes with oxidoreductases [31–33]. Further process optimisation, e.g. of the immobilisation process, development of a continuous process to avoid acetoin racemisation as well as directed evolution of the enzyme, will lead to an ongoing improvement of the production process. Furthermore, the described process will be investigated to produce different α-hydroxy ketones.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcatb.2013.08.016.

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