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Enzymatic Cascade in a Simultaneous, One-Pot Approach with *In Situ* Product Separation for the Asymmetric Production of (4*S*,5*S*)-Octanediol

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ABSTRACT: Stereopure aliphatic diols are an interesting class of compounds because of their potential applications as precursors for chemical catalysts, for high-value polymers, or as precursors for cyclic acetals. We present a simultaneous enzymatic two-step, one-pot cascade for the synthesis of vicinal diols with excellent de and ee values with the exemplary reaction system from butanal to (4*S*,5*S*)-octanediol. This reaction is restricted by an unfavorable reaction equilibrium. For an intensification of the reaction toward higher conversions in equilibrium and increased space time yields (STY), aqueous, microaqueous, and biphasic reaction systems for *in situ* product removal (ISPR) were experimentally investigated and compared. Process concepts for the purification of (4*S*,5*S*)-octanediol from each reaction system were developed and assessed in terms of product-specific energy demand. The two-phase reaction system for *in situ* product removal is favorable for the enzymatic reactions in terms of yield and STY at different time points. In comparison to the aqueous and microaqueous reaction systems, the specific energy demand for (4*S*,5*S*)-octanediol recovery is drastically reduced by approximately a factor of seven when performing ISPR using a biphasic system in comparison to an aqueous reaction system.

KEYWORDS: *one-pot, enzymatic cascade, vicinal diol, MARS, conceptual process design*

■ INTRODUCTION

Aliphatic, vicinal diols are a diverse group of chemicals, used as precursors for polymers or other chemicals[1] and biofuels.[2,3] Additionally, under consumption of CO_2 or polyoxymethylene monomers, they serve as precursors for the production of valuable 1,3-dioxolanes.[4] Some bulk diols, such as 2,3-butanediol, can be produced from glucose by members from the *Klebsiella* family.[2,3] For the production of stereopure vicinal, aliphatic diols, there are routes either via chemical synthesis, such as Sharpless asymmetric dihydroxylation[5] starting from dienes, or enzymatic routes, starting from diketones via a double reduction using alcohol dehydrogenases.[6] Other enzymatic routes to vicinal, diols are shown in the work of Zhang et al.[7] or Qiu et al. for the production of a statin precursor.[8] Although nonaliphatic diols are targeted, a similar approach was used as in this work. There, aliphatic dialdehydes are transformed using a pyruvate decarboxylase to a cyclic hydroxyketone with subsequent reduction to the respective diol. A similar cascade is described by Oeggl et al., starting from aliphatic acetaldehyde and the aromatic 4-methoxy-benzaldehyde.[9] The authors employ a lyase for the cross-aldol condensation reaction toward the hydroxyketone. The intermediate was subsequently reduced by an alcohol reductase from *Ralstonia spec.* (RADH) to the corresponding final diol with high optical purity. In order to reach high substrate concentrations of the hydrophobic 4-methoxy-benzaldehyde without forming a second liquid phase, a microaqueous reaction system (MARS) was employed. Here, the organic solvent cyclopentyl methyl ether (CPME) was used as a reaction solvent enabling substrate concentrations beyond the aqueous solubility limit of the substrate.[9] The enzymes were formulated as lyophilized (death) whole cells (LWC). In comparison to purified enzymes, LWC with a similar enzyme content remain much more active in organic conditions (see SI S4). The cells can be thought of as a shell and thus a very simple form of immobilization that, to a certain extent, shields the enzymes inside from inactivation by harsher reaction conditions.[10] Furthermore, the usage of LWCs can reduce costs by up to 90% due to circumventing purification and already present cofactors. In comparison to the in industry more commonly used crude cell extract, the cost reduction ranges at around 50%.[11,12]

In addition to increased substrate solubility, the MARS offers a new and possibly favorable separation task for enzymatically produced substances.[13,14] Multiple possible solvents were screened in the past, yielding a broad selection to choose from.[14,15] CPME was chosen as solvent in the MARS, since it enabled high activity and is currently declared to be a green solvent.[14,16] The separation of diluted, high boiling diols from their reaction medium is the subject of ongoing research.[3,17,18] Multiple process concepts have been evaluated in conceptual process design studies, such as

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distillation, extraction, adsorption, and membrane processes.[19] However, all these studies tackled the separation of diols from aqueous medium. The advances of a separation from MARS due to lower enthalpy of evaporation have not been investigated or quantified to this date. Another possibility to intensify biocatalytic production processes is *in situ* product removal (ISPR).[20] Multiple well-known unit operations for separation were combined with enzymatic reactions to achieve ISPR and enhance productivity and to overcome limitations caused by an unfavorable reaction equilibria.[21] To be named is the work of Heils et al., who integrated an enzymatic reaction into reactive distillation for increased yields in comparison to the system without ISPR.[22] Hülsewede et al. realized *in situ* crystallization within an enzymatic process for amine synthesis.[23,24] The group of Adlercreutz applied a liquid membrane for the separation of amines.[25] Further, chromatographic ISPR was performed by Wagner et al. for the production of high-value sugars.[26,27] The most widely spread ISPR methodology in biocatalysis, however, is extraction, which was already discussed in the early 1970.[21] Physical and more complex reactive extraction techniques have been applied to enzymatic reaction systems with highly valuable products to increase yield by removing products from the reaction solvent.[28−30] In all these systems, biocompatibility, cross-solubility, and the interfacial tension of the solvent has to be considered in order to increase the overall productivity.[31]

In this work, we present a two-step enzymatic cascade toward (4*S*,5*S*)-octanediol which has not been accessed enzymatically to date, only chemically by osmium-catalyzed asymmetric dihydroxylation.[32] The enzymatic two-step cascade starts with a benzoin-type condensation of two butanal molecules catalyzed by a variant of the pyruvate decarboxylase from *Acetobacter pasteurianus* with a glycine instead of a glutamic acid at position 469 (*Ap*PDCE469G) in order to increase the (*S*)-selectivity.[33] The resulting (*S*)-butyroin is reduced to (4*S*,5*S*)-octanediol by the butanediol dehydrogenase from *Bacillus licheniformis* (*Bl*BDH).[34] The NADH of the reduction step is recycled using the same enzyme with 1,2-propanediol as cosubstrate by oxidizing 1,2-propanediol to hydroxyacetone (coproduct) (Figure 1). The cascade is designed as a two-step, one-pot, simultaneous enzymatic cascade with both enzymes, the substrate, and 1,2-propanediol present at the beginning. This is possible, as *Bl*BDH has a high affinity toward the intermediate product but not to the starting substrate butanal. Conclusively, even in the simultaneous approach, high selectivities toward the target product can be reached. Thereby, the one-pot approach can help to overcome the equilibrium of the first step by removal of intermediate by the reduction reaction[9,35,36] and also reduces necessary separation steps for the reaction.

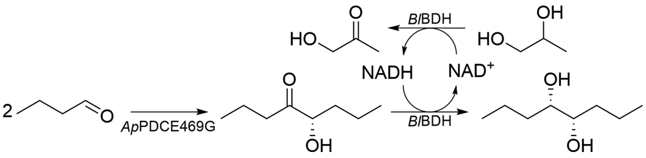

Figure 1. Reaction scheme of the enzymatic transformation of butanal via (*S*)-butyroin to (4*S*,5*S*)-octanediol using a variant of the pyruvate decarboxylase from *Acetobacter pasteurianus* (*Ap*PDCE469G) and the butanediol dehydrogenase from *Bacillus licheniformis* (*Bl*BDH). The cofactor NADH is recycled by oxidation of 1,2-propanediol to hydroxyacetone also by the *Bl*BDH.

As stated above, enzymatic processes can be performed in various reaction systems such as aqueous monophasic organic monophasic (MARS), or biphasic with different benefits and challenges.[13] The process toward (4*S*,5*S*)-octanediol in aqueous reaction solvent is expected to be intensified by applying the MARS and biphasic system. For the latter, CPME is used to form the second phase. The biphasic system will result in ISPR because of the hydrophobicity of the product resulting in a driving force toward the organic phase while the catalyst, cosubstrate, and coproduct are kept to a large extend in the aqueous phase. To identify the most favorable reaction system, we develop and compare process concepts for different reactions systems (aqueous, MARS, and biphasic) with respect to yield and specific energy demand. The reaction and separation units of the process concepts are modeled in continuous mode. The continuous production offers several advantages, such as increased productivity, less manual handling, and consistent product quality.[37] Hence, continuous production is desired, and continuous process concepts are assessed in this study. To enhance the feasibility of the suitable concept, only the well-known unit operations rectification and extraction were applied for the conceptual design of these process concepts.[18,38]

■ MATERIALS AND METHODS

Production of Catalyst. Catalyst was formulated as lyophilized whole cells (LWC). Genes encoding *Ap*PDCE469G[33] and *Bl*BDH[34] (see Supporting Information (SI) for amino acid sequences) were cloned and expressed heterologously in *Escherichia coli* (*E. coli*) (BL21) in autoinduction medium.[39] The cultures grew for 2 h at 37 °C followed by 48 h at 20 °C before the cells were harvested at 4 °C at 8000 rpm for 45 min. The cell pellets were frozen at −20 °C and then lyophilized at −54 °C and 0.01 mbar for 48 h. The dried cell pellets were crushed using a spatula and stored at −20 °C until further use.

Enzymatic Reaction toward (4*S*,5*S*)-Octanediol. *In Aqueous Conditions.* First, 30 mg of *Ap*PDCE469G and 30 mg of *Bl*BDH formulated as lyophilized whole cells were added to 1.5 mL glass vials. Then 1 mL of reaction solution was added, consisting of 50 mM triethanolamine (TEA) buffer pH 9 with 0.01 mM thiamine diphosphate (ThDP) and 2.5 mM $MgCl_2$, 200 mM 1,2-propanediol for cofactor regeneration, and 200 mM butanal. The reaction was started by the addition of the substrate and incubated at 30 °C and 1000 rpm in an Eppendorf shaker for 24 h.

In Organic Conditions (MARS). In the first step, 30 mg of *Ap*PDCE469G and 30 mg of *Bl*BDH formulated as lyophilized whole cells were added to 1.5 mL glass vials. Then 1 mL of reaction solution was added, consisting of cyclopentyl methyl ether (CPME) as a reaction solvent with 200 mM 1,2-propanediol for cofactor regeneration and 200 mM butanal. The reaction was started by the addition of 60 μL of 1 M TEA buffer pH 9 with 0.1 mM ThDP and 2.5 mM $MgCl_2$. The reaction was incubated at 30 °C and 1000 rpm in an Eppendorf shaker for 24 h.

In Biphasic Conditions. First, 225 mg of *Ap*PDCE469G and 225 mg of *Bl*BDH formulated as LWC were added to a 20 mL glass vial. Then 7.5 mL of a reaction solution consisting of CPME saturated 50 mM triethanolamine (TEA) buffer pH 9 with 0.01 mM thiamine diphosphate (ThDP) and 2.5 mM

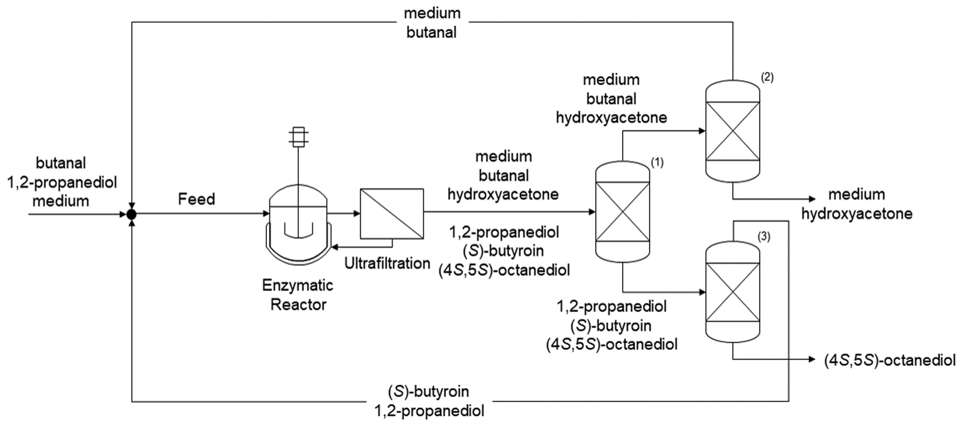

Figure 2. Process concept for the production of (4*S*,5*S*)-octanediol consisting of an enzymatic reactor with an ultrafiltration unit to the retain the enzymes and a separation sequence based on rectification columns. The educts and intermediate (butanal, (*S*)-butyroin, and 1,2-propanediol) as well as the reaction medium are recycled. The coproduct (hydroxyacetone) is purged together with low amounts of reaction medium, while the concentrated product (4*S*,5*S*)-octanediol is gained.

$MgCl_2$ were added to the cells. Additionally, 7.5 mL of buffer saturated CPME were layered on top of the aqueous phase before 1,2-propanediol and butanal were added in concentrations of 200 mM with respect to the entire system to start the reaction. The reaction was incubated at 30 °C for 24 h in an overhead shaker (scientific industries) in a rocking motion with a speed of 20 rpm to minimize the formation of an emulsion.

Analytics. Samples were prepared by taking 20 μL from the reaction mix and adding them to 380 μL of acetonitrile to stop the reaction. For the biphasic system, samples were taken each from the aqueous and organic phase. The samples were centrifuged at 13 000*g* for 3 min to remove cell debris before transferring the supernatant to GC vials for GC analysis. A Thermo Fisher Trace 1310 GC was used with an Agilent CP-Chirasil-Dex CB chiral column (inner diameter 0.25 mm; 25 m × 0.25 μm). The oven was heated in a gradient (40 °C hold 0.5 min, to 55 °C with 17 °C/min, hold 0.5 min, to 73 °C with 10 °C/min, to 82 °C with 15 °C/min hold 2.5 min, to 117 °C with 20 °C/min hold 2.2 min, to 140 °C with 26 °C/min, to 190 °C with 50 °C/min). The elution times of the analytes are in order of appearance as follows: butanal (3.51 min), CPME (6.26 min), hydroxyacetone (5.5 min), 1,2-propanediol (8.12 min), (*R*)-butyroin (9.43 min), (*S*)-butyroin (9.55 min), (4*S*,5*S*)-octanediol (10.42 min), (4*R*,5*R*)-octanediol (10,47), *meso*-4,5-octanediol (10.57 min). The identity of the product and intermediate was determined by commercial racemic standards and based on the known stereoselectivity of the used enzymes for these and similar substrates.[33,34]

Description of the Process Concepts. Three developed process concepts for the purification of (4*S*,5*S*)-octanediol are presented in the following: The product separation via rectification from aqueous reaction medium (AQ-RECT), rectification from MARS (MARS-RECT) and separation via *in situ* extraction from aqueous reaction medium and consecutive rectification (BP-RECT). Based on a butanal feed of 1 kg/h at 30 °C and atmospheric pressure, the feed concentration for the enzymatic reactor is set to 200 mM by addition of fresh reaction medium (aqueous or organic) in combination with the recycle streams. The process can be divided into two areas: the enzymatic cascade as the upstream process and a combination rectification columns for the downstream. The enzymatic cascade is modeled using yield-based reactors and an ultrafiltration unit to retain the enzymes. The downstream consists of three rectification columns in each process concept.

In a first rectification column (1), the cut between the coproduct hydroxyacetone and the cosubstrate 1,2-propanediol is realized. The distillate stream consists of hydroxyacetone, the reaction or extraction medium (water (AQ-RECT), CPME (MARS-RECT, BP-RECT)), and the low boiling substrate butanal. The distillate stream is inserted in a second rectification column (2) to purge hydroxyacetone in the bottom and recycle butanal and reaction medium in the top stream. The bottommix of the first column consists of the high boiling components 1,2-propanediol, the intermediate product (*S*)-butyroin and the target product (4*S*,5*S*)-octanediol. The mixture is inserted to the third rectification column (3), whereby the product is gained in the bottom with the desired purity and the intermediate product and cosubstrate are recycled. In Figure 2, the rectification cascade for the process concepts AQ-RECT and MARS-RECT is presented. In the case of ISPR, an extraction column is added after the ultrafiltration unit. The reaction medium is aqueous in this case, and CPME is used as extraction solvent, to extract the hydrophobic products (butanal, hydroxyacetone, (*S*)-butyroin, (4*S*,5*S*)-octanediol). The extract is inserted into the rectification cascade and is separated analogous to the rectification concepts. The hydrophilic 1,2-propanediol remains in the raffinate and is recycled (see SI Figure 3). CPME was chosen to keep the components for all processes the same. Additionally, CPME is considered as a green solvent with very low cross solubility in water.[40]

Process Modeling in AspenPlus. AspenPlus V11.0[41] was utilized for the assessment of the presented process concepts. To cover nonidealities within the liquid mixtures, the nonrandom two liquid (NRTL) property method was chosen to calculate activity coefficients. The NRTL property method is suitable for polar systems at low pressure far from the critical region.[42] Analogous to the process assessments performed by Kampwerth et al.,[43] all required NRTL parameters were calculated on the basis of the method suggested by Scheffczyk et al. using COSMO-RS.[44,45]

The NRTL model equations and the used parameters are presented in the SI (see SI table S2). The enzymatic cascade in the process concepts is modeled by using two stoichiometric reactors, to account for each enzymatic conversion step

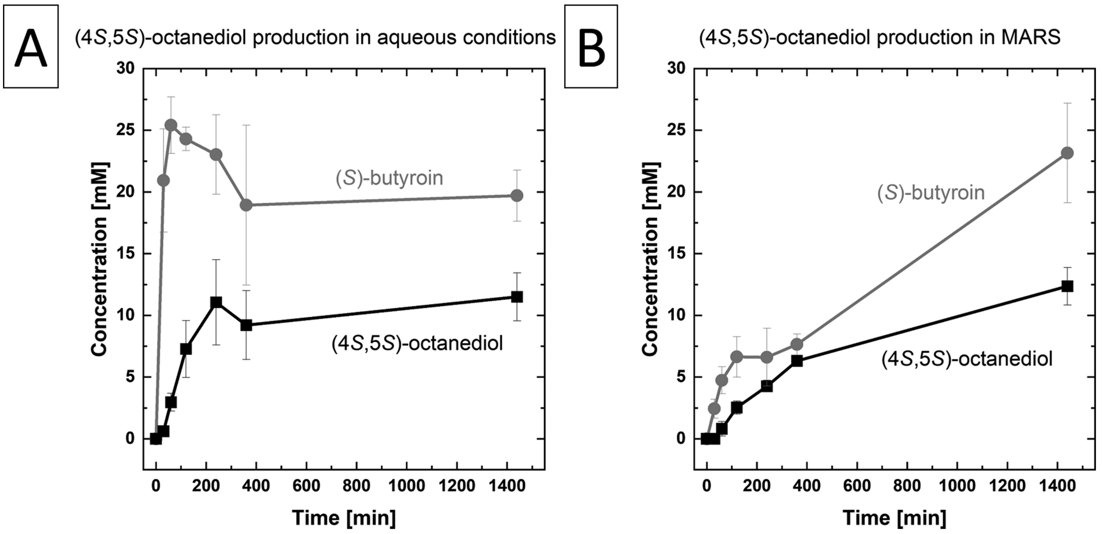

Figure 3. Simultaneous one-pot enzymatic production of (4*S*,5*S*)-octanediol in aqueous and organic reaction conditions. (A) Conversion of 200 mM butanal to (*S*)-butyroin using *Ap*PDCE469G as lyophilized whole cells (LWC) with subsequent reduction using *Bl*BDH formulated as LWC. 200 mM 1,2-propanediol was added for cofactor recycling. The reaction took place in aqueous 50 mM TEA buffer with 0.1 mM ThDP and 2.5 mM MgCl2. (B) Identical reaction as (A) but in a microaqueous reaction system (MARS) with CPME as a reaction solvent and 1 μL/mgLWC 1 M TEA buffer with 0.1 mM ThDP and 2.5 $MgCl_2$. The figure shows the arithmetic mean values of three technical triplicates.

separately and adjust the individual yields of each conversion step. Depending on the choice of reaction system (aqueous, MARS, or biphasic), the yields are adjusted to fit to the experimentally achieved yields. The same conversion yields from batch experiments are assumed to be achieved in continuous mode. In reality, a cascade of multiple continuously stirred tank reactors can be utilized to achieve the same conversion yield as a batch reactor.[46] Since the energy demand of the reactors is neglectable in the overall process, only one reactor was used for each conversion step due to simplification, rather than a cascade. Beside the main substrate and product, also the cosubstrate and coproduct for the enzymatic reaction are considered. For the implementation of the rectification columns, the radfrac model is utilized, since it is applicable for strongly nonideal mixtures.[47] To ensure comparability between the processes, all columns were modeled at atmospheric pressure with 10 theoretical separation plates, a molar reflux ratio of 1 and distillate to feed ratios adjusted via design specifications to gain the desired (4*S*,5*S*)-octanediol in purities of at least 95 wt %. The pressure loss along the columns was not considered. The condenser was modeled as total condenser. The extraction was realized using the multistage extraction column model with 15 theoretical separation plates at 30 °C and atmospheric pressure.

Assessment of the Process Concepts. Process evaluation of the concepts was performed on the basis of product-specific energy demand, which couples the heat demand for the unit operations (mainly rectification and cooling units) with the overall yield. No heat integration was performed, to account for conceptual advantages and the small-scale applicability of the (4*S*,5*S*)-octanediol synthesis.[48] The specific energy demand is defined as

$$\text{specific energy demand} = \frac{\sum \dot{Q}}{\dot{m}_{4,5\text{-}ODO}}$$

where $\dot{Q}$ is the heat duty for the rectification columns and chilling units, while $\dot{m}_{4,5\text{-}ODO}$ is the mass stream of gained (4*S*,5*S*)-octanediol. The solid–liquid separation of whole cells used in the enzymatic reactors can be realized via *in situ* ultrafiltration, which is typically not energy intensive. Thus, ultrafiltration costs are not taken into account in all simulations because they would require a high degree of detail and are in the same order of magnitude for all three process concepts.[19]

RESULTS AND DISCUSSION

In this section, the results of the experimentally conducted (4*S*,5*S*)-octanediol synthesis and the evaluation of the different process concepts is presented.

The cascade from butanal to (4*S*,5*S*)-octanediol tested in batch conditions in monophasic aqueous and organic (MARS) reaction systems is shown in Figure 3. 1,2-Propanediol was chosen as a cosubstrate since the *Bl*BDH only accepts molecules with two vicinal hydroxyl or carbonyl functions in a molecule as a substrate, and 1,2-propanediol was one of the few diols that the *Bl*BDH oxidizes.[34] Because of this substrate specificity, the *Bl*BDH does not reduce butanal to butanol. This is a huge advantage in our context as it cannot reduce the starting material. Therewith it enables a simultaneous cascade mode in which both enzymes are present without any undesired side-activity being detected. However, the *Ap*PDCE469G was inhibited by concentrations of 1,2-propanediol higher than 200 mM in MARS (see SI Figure S1), Conclusively, maximal 200 mM 1,2-propanediol was chosen for the reduction reaction. In the tested substrate range, the lyophilized whole cells already provided the necessary cofactor NADH for the reduction in sufficient amounts; however, ThDP was added in 0.1 mM to increase the carboligation reaction.

The simultaneous, one-pot cascade results in similar product concentrations of about 12 mM in both MARS and aqueous conditions. Since two butanal molecules are ligated to form one molecule of (*S*)-butyroin, the theoretical maximal product concentration is 100 mM. Conclusively, by far, no full conversion is reached. In aqueous conditions, the maximal product concentration is reached after 4 h, whereas the reaction proceeds slower in MARS, presumably due to a lower activity of the *Ap*PDCE469G. The stereoselectivity of the cascade is excellent with an ee and de over 99% for the (4*S*,5*S*)-octanediol, regardless of the solvent system chosen (Table 1). In addition to (4*S*,5*S*)-octanediol, (2*S*,3*S*)-butanediol was also produced using the same enzymatic cascade. Here, acetaldehyde was used as the substrate, showing the adaptiveness of the cascade toward other aldehydes and products. However, this approach was only successful for the aqueous system, likely because of inhibition effects of high concentrations of acetaldehyde at the aqueous cells in MARS (see SI Figure S2). Also, the stereoselectivity in the (2*S*,3*S*)-butanediol synthesis has excellent ee of over 99% but a maximal de of 77% (see SI Table S1). Additionally to acetaldehyde and butanal, other aldehydes such as propanal yielding (3*S*,4*S*)-hexanediol can be used as preliminary experiments have shown (unpublished data).

Table 1. Enantiomeric (ee) and Diastereomeric (de) Excess of the Enzymatic Production of (4*S*,5*S*)-Octanediol under Either Aqueous, MARS, or Biphasic Reaction Conditions

product	system	de	ee
(4*S*,5*S*)-octanediol	aqueous	>99%	>99%
	MARS	>99%	>99%
	biphasic	>99%	>99%

MARS conditions provide a more straightforward downstream processing; however, the aqueous conditions showed a higher initial activity. Due to that, a biphasic system was tested which combines the higher activity of the enzyme in the aqueous phase with product extraction to the organic CPME phase. In Figure 4, the intermediate and product concentration in the organic phase is presented, showing the activity of the enzymes in the biphasic system including ISPR.

Similar to the aqueous system, the highest product concentrations in the ISPR system were reached already after 4−6 h with 14 mM (4*S*,5*S*)-octanediol. The intermediate and product concentration in the aqueous phase was much lower. They are in the range of 1−3 mM throughout the reaction, showing an efficient extraction into the organic phase. Given the higher product concentration in the organic phase, the STY in the biphasic system is also higher with 8.6 g L^{-1} d^{-1} in comparison to aqueous (5.3 g L^{-1} d^{-1}) and especially to MARS (3.7 g L^{-1} d^{-1}) because of the slower reaction rate.

The presented process concepts for separation from aqueous (AQ-RECT), MARS (MARS-RECT), and biphasic (BP-RECT) reaction systems were evaluated with regard to product-specific energy demand. The evaluation was performed assuming the yields from batch experiments are the same as the yields in continuous mode. As stated in the description of the process modeling, a cascade of multiple continuously stirred tank reactors can be utilized to achieve the same conversion yield as a batch reactor. In the presented process concepts, one reactor for each conversion step is used for simplification. In addition to the yields achieved after 24 h, the yields after 6 h were considered to account for the space time yield (see Figure 5).

The AQ-RECT process concept serves as the reference process, since its aqueous reaction medium is considered to be conventional. The specific energy demand for the AQ-RECT is 1519 kJ/g assuming a yield after 24 h in batch mode and 1775 kJ/g when applying the yield after 6 h. The increase of the specific energy demand is caused by the lower yield after 6 h, which leads to higher dilution and thus higher heat demand for evaporation. The overall yield, including recycle and purges, is reduced from 82.8% to 74.5%, accounting for a further increase of the specific energy demand.

The MARS-RECT process concept utilizes the nonconventional reaction environment for the two enzyme catalyzed steps. CPME is utilized as the solvent. The specific energy demand of this process concept drastically reduced compared with the AQ-RECT. When assuming the yield after 24 h, the

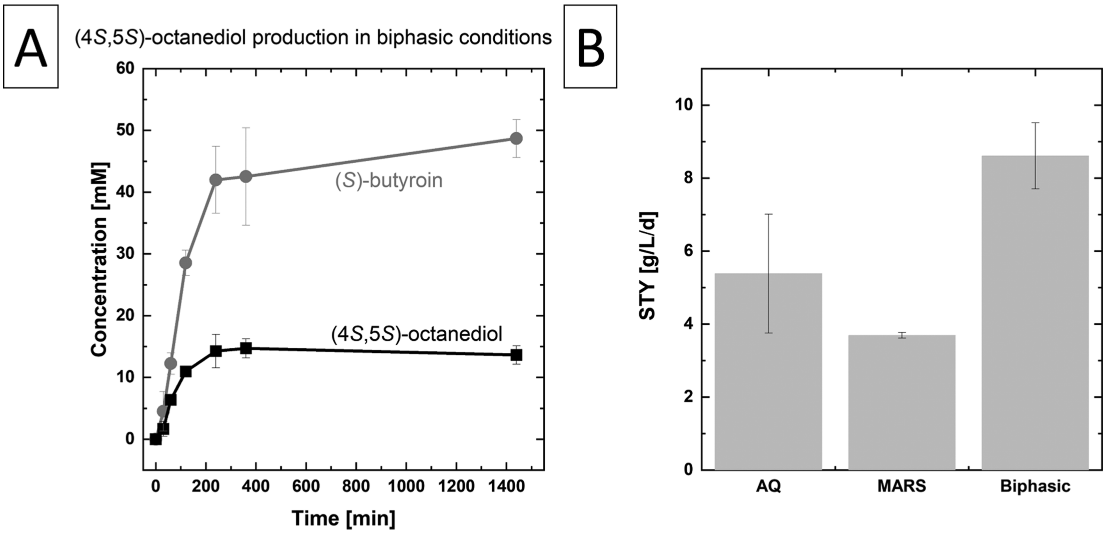

Figure 4. Simultaneous one-pot enzymatic production of (4*S*,5*S*)-octanediol in biphasic conditions (A) and space-time yield (STY) of biphasic, aqueous, and MARS reaction conditions (B). Conversion of 200 mM butanal to (*S*)-butyroin using *Ap*PDCE469G as lyophilized whole cells (LWC) with subsequent reduction using *Bl*BDH formulated as LWC. 200 mM 1,2-propanediol was added for cofactor recycling. The reaction took place in aqueous 50 mM TEA buffer with 0.1 mM ThDP and 2.5 mM $MgCl_2$. The second reaction phase consists of CPME, and both phases have a volume of 7.5 mL. STY of the enzymatic conversion of butanal to (4*S*,5*S*)-octanediol under biphasic, MARS, and aqueous conditions. STY was calculated on the basis of the product concentration after 6 h and the volume of the reaction phase. In the biphasic system, the concentration in the organic phase was used for calculation. The figure shows the arithmetic mean values of three technical triplicates.

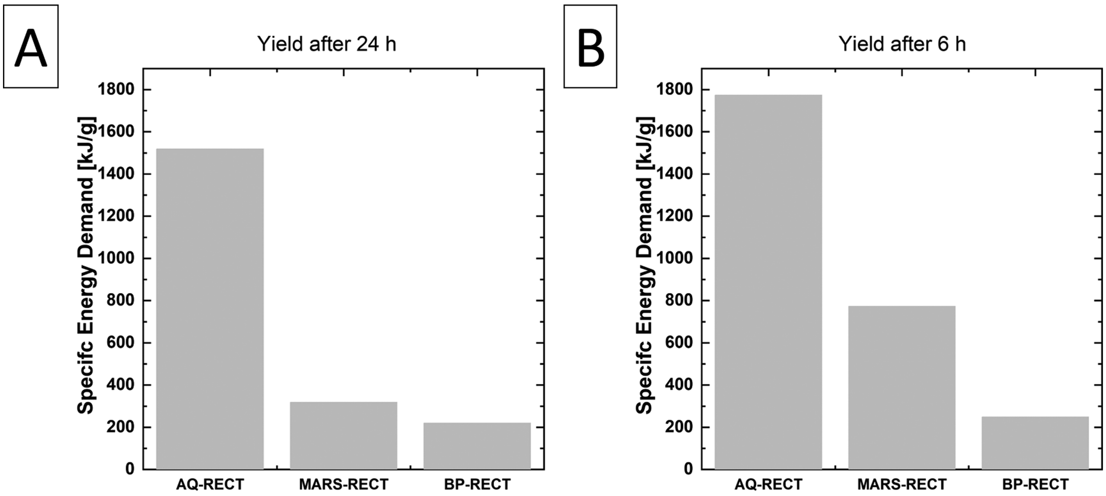

Figure 5. Product-specific energy demand of different process concepts (aqueous rectification (AQ-RECT), MARS-rectification (MARS-RECT), and biphasic rectification (BP-RECT)) concerning yield after 24 h (A) and yield after 6 h (B).

specific energy demand is 319 kJ/g, while the overall yield is only slightly lower (75.6%). This reduction of the specific energy demand can be attributed to the smaller enthalpy of evaporation of CPME (289.26 kJ/kg) compared with H_2O (2260 kJ/kg).[49,50] However, as presented in Figure 3, the STY in MARS is lower compared with the aqueous system, leading to a reduction of the overall yield in MARS from 75.6% to 59%. To account for this weakness, the specific energy demand for the yield after 6 h is assessed, which is 773 kJ/g for the MARS-RECT process concept. Given the low conversion of the MARS after 6 h (Figure 2), the specific energy demand is approximately doubled compared with the yield after 24 h. Still, the MARS system has lower specific energy demand than the AQ-RECT when comparing the yield after 6 h (Figure 5). Hence, the MARS system is advantageous over conventional reaction medium when assessing the overall process performance.

The BP-RECT process concept has the lowest specific energy demand for both scenarios, the yield after 6 and 24 h. This is a result from multiple factors. First, the general processing costs are reduced due to lower recycling streams, since the hydrophilic cosubstrate 1,2-propanediol remains in the raffinate (bottom stream of the extraction column) and is not inserted in the rectification cascade. Second, the yields of the enzymatic conversion after 24 and 6 h are the highest in biphasic reaction conditions leading to the highest concentrated product stream from the enzymatic cascade reactor and thus the lowest energy demand for evaporation. Additionally, because of the extraction into CPME, the heat of evaporation reduced drastically compared with the AQ-RECT as with the MARS-RECT. Lastly, the amount of used solvent for the extraction is low compared with the aqueous reaction solvent allowing for a product accumulation in the organic solvent. In this study, a phase ratio of solvent to aqueous phase of 0.75 is utilized, which could be further reduced. These reasons lead to the lowest specific energy demand for a process realizing yield after 24 h (220 kJ/g) and assuming the yield after 6 h (249 kJ/g). The overall yield (including conversion and separation), however, is suboptimal with 69.2% after 24 h and 62% after 6 h, respectively. This results from the recycle and purge of the raffinate combined with no full recovery of (4*S*,5*S*)-octanediol as well as of the substrates during extraction. Nevertheless, the BP-RECT process concept is the most promising of the presented concepts because of its lowest specific energy demand, easy cosubstrate handling, and remaining potential for optimization in terms of minimum amount of solvent for the extraction.

A summary of the merits and demerits of each approach including the STYs and specific energy demands is given in Table 2.

Table 2. Space Time Yields and Specific Energy Demands for the Aqueous, MARS, or Biphasic Reaction System Calculated Based on the Conversions after 6 or 24 h

	STY [g·l^{-1}·d^{-1}]		specific energy demand [kJ·g^{-1}]	
reaction system	24 h	6 h	24 h	6 h
aqueous	1.7	5.4	1519	1775
MARS	1.8	3.7	319	773
biphasic	2	8.6	220	249

■ CONCLUSION

In this work, we present a simultaneous, one-pot, two-step cascade from butanal to (4*S*,5*S*)-octanediol consisting of a carboligation step using *Ap*PDCE469G and a subsequent reduction including cofactor recycling by *Bl*BDH. While the final product was produced with excellent ee and de values of >99%, the yields were still comparably low. It was observed that the intermediate is not fully converted to the product hinting toward the reduction being the bottleneck. For further optimization, a different cosubstrate with a higher affinity to the enzyme might increase the productivity. Here racemic 2,3-butanediol or a glucose dehydrogenase with glucose for NADH recycling could be helpful; however, in this cascade, 1,2-propanediol was chosen because of the costs and simplicity as the same enzyme could be used for the main reaction and cofactor recycling. Another possibility is the increase of the cosubstrate concentration.[50] Experiments have shown higher conversions to up to 85% in the aqueous system with very high 1,2-propanediol concentrations (see SI Figure S3). Out of the three solvent systems tested, in a biphasic system with CPME as a second phase performed best. The highest volumetric productivities for enzymatic transformation and the lowest specific energy demand for product purification was reached. Concerning the monophasic systems, the MARS showed also much lower specific energy demands because of the lower enthalpy of evaporation of the organic solvent CPME compared with a buffered system. The organic solvent can be recycled to a large extent. Thus, the preparation costs would not affect the overall energetic assessment. The much higher initial costs for the organic solvents will equalize and meet a break-even point eventually in comparison to the aqueous system. This underlines the benefits of nonaqueous reaction systems for enzymatic reactions when not only the transformation itself but also the performance of the overall process including DSP is taken into account.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.1c00433.

> Figures on the enzyme inhibition, effect of 1,2-propanediol and synthesis of (2*S*,3*S*)-butanediol is given; a comparison of enzyme formulations in MARS, the process concept for the (4*S*,5*S*)-octanediol production, and the related information on the NRTL model in AspenPlus (PDF)

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Notes

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