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Production of selected chiral diols via synthetic enzymes cascade in unconventional media

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PURPOSE OF THE ABSTRACT
Stereopure vicinal diols are important building blocks used for fine chemicals and pharmaceutical compounds.1 2,3-Butanediol is a well-known chiral diol, which is used in practical applications such as plasticizers and antifreeze agents.2 These diols can also serve as precursors for bio-fuels starting from bio-resources. For this application, product concentration is more important than stereoselectivity.3 Due to the high selectivity of enzymes and their high reactivity under mild conditions, enzymes are potent catalysts for green chiral diol synthesis.4 In our enzymatic cascade we start with a ThDP dependent lyase forming a C C bond between two aldehydes molecules resulting in a 2-hydroxy ketone. By using an oxidoreductase in the second step, the carbonyl group is reduced and a diol with two chiral centers is formed.
In a recent study, enzymatic and chemical steps are combined to form diols and cyclic acetals in an environment-friendly manner.3 Biotransformation in a green organic solvent was done to provide a reaction environment suitable for the enzymatic and the chemical step. The integration of H2 and CO2 into the reaction renders the process even more ecological. Here, we show a method of synthesizing diols with mainly excellent stereoselectivity by using enzymes. In addition, intensification of the process including the use of unconventional media led to an increase in concentrations and conversion from 84 mM and 84% to 900 mM and 90%, respectively.

Results
Stereoselective formation of all diols as precursors for fine chemicals
The need of stereoselective synthesis has increased in the last decades as more than 50% of used drugs are chiral.5 Due to the high stereoselectivity of modularly combined enzymes, we were able to successfully gain all 12 out of 12 possible stereoisomers of 4 diols with different chain length with good to high concentrations and good to excellent isomeric contents (ic) (Table 1). For example, full conversion was achieved by producing almost 100 mM of (5S,6S)-decanediol with an ic value > 99 %.
Pure stereoisomers of all diols as precursors for bio-fuels

Due to the highly negative impact of CO2 produced from transportations on the climate, alternatives to fossil fuels help cutting CO2 emissions. Diols are seen as suitable precursors for bio-hybrid fuels, as starting material for the diols can be aldehydes produced via microbial cell factories from second generation feedstocks.6 Besides their chiral building block potential, the diols shown in table 1 can be further transformed to dioxolane via chemocatalysis integrating CO2. These dioxolanes show high potential due to optimal properties as blends for bio-fuels.3

When used as blend, yields are more important than selectivities. For this aim, only the ligation step was done enzymatically, the further reduction and cyclisation will be performed using (unselective) ruthenium catalysis. By intensifying the enzymatic carboligation step, an increase of the product concentration from 84 mM up to 900 mM was achieved. This was mainly achieved using enzyme catalysis in a micro-aqueous reaction system.

micro-aqueous reaction system (MARS)

Aqueous buffers are the first choice when it comes to reaction media with enzymes, as most of them are evolved in an aqueous environment. However, many interesting substrates are hydrophobic and this results in low substrate loads and therewith product concentration, when a second phase should be avoided. Applying biocatalysis in an organic solvent, like cyclopentyl methyl ether (CPME), allows to overcome this problem. Therefore, enzymes are formulated as lyophilized whole cells. Protecting the entrapped enzymes in the remaining cell envelope to a certain extent enables high yields under these reaction conditions. Moreover, the MARS is a convenient environment to combine enzymatic and chemical steps for dioxolane synthesis in one reaction environment and additionally facilitates product purification.7

FIGURES

stereoisomer	concentraction [mM]	*ic* [%]	stereoisomer	concentraction [mM]	*ic* [%]
(2*S*,3*S*)-butanediol	21.3	98	(4*S*,5*S*)-octanediol	34.7	>99
meso-2,3-butanediol	17.45	>99	*meso*-4,5-octanediol	38.1	98
(2*R*,3*R*)-butanediol	65.66	98	(4*R*,5*R*)-octanediol	64.9	98
(3*S*,4*S*)-hexanediol	5.17	>99	(5*S*,6*S*)-decanediol	98.66	>99
meso-3,4-hexanediol	4.10	>99	*meso*-5,6-decanediol	76.32	70
(3*R*,4*R*)-hexanediol	7.44	75	(5*R*,6*R*)-decanediol	62.2	80

parameter	initial	optimized
buffer/solvent	50 mM TEA	CPME
substrate addition	no	feeding
reaction time	1 day	4 days
new LWC	no	after 2 days
substrate con.	200 mM	400 mM
product con.	86 mM	900 mM

FIGURE 1

Table 1:

Concentrations and ic values of gained stereoisomers. ic= isomeric content (target isomer/sum of all isomers [%]). Starting concentration of substrate: 200 mM

FIGURE 2

Table 2:

Optimized parameters of the carboligation step. LWC: lyophilized whole cells. CPME: cyclopentyl methyl ether. TEA: triethanolamine

KEYWORDS

enzymatic cascade | vicinal chiral diols | carboligation | oxidoreduction

BIBLIOGRAPHY