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# Chemoenzymatic Synthesis of a Novel Borneol-Based Polyester

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Terpenes are a class of natural compounds that have recently moved into the focus as a bio-based resource for chemical production, owing to their abundance, their mostly cyclic structures, and the presence of olefin or single hydroxy groups. To apply this raw material in new industrial fields, a second hydroxy group is inserted into borneol by cytochrome P450cam (CYP101) enzymes in a whole-cell catalytic biotransformation with *Pseudomonas putida* KT2440. Next, a semi-continuous batch system was developed to produce 5-*exo*-hydroxyborneol with a final concentration of 0.54 g $L^{-1}$. The bifunctional terpene was then used for the synthesis of a bio-based polyester by a solvent-free polycondensation reaction. The resulting polymer showed a glass transition temperature of around 70 °C and a molecular weight in the range of 2000–4000 g $mol^{-1}$ ($M_w$). These results show that whole-cell catalytic biotransformation of terpenes could lead to bio-based, higher-functionalized monomers, which might be basic raw materials for different fields of application, such as biopolymers.

## Introduction

Terpenes are a wide class of natural hydrocarbons derived from isoprene units, which are abundant in nature.[1] Terpenes have a broad range of possible applications. Some monoterpenes have antibacterial[2] and antifungal[3] properties, whereas higher terpenes such as carotenoids are essential for plants, in which they act as energy transfer molecules to the chlorophylls,[4] and are used as coloring agents in the food industry.[5] Some terpenes are also used in the pharma industry, for example as an ingredient in transdermal drug delivery systems, where terpenes enhance the skin permeation of drugs.[6]

Recently terpenes have been investigated for the production of bio-based polymers. There are several reports of α- and β-pinene[7–10] and limonene[11] being used for polymerization, affording homopolymers or copolymers.[12] These examples make use of the olefinic bonds in many terpenes, which could be used for cationic or radical polymerizations[13] or further converted into epoxides and lactones to enable ring-opening polymerizations. However, monomers to be used in polycondensations require two functional groups (i.e., carboxyl or hydroxy), yet typical monoterpenes do not fulfill this criterion. Dihydromyrcenol, for example, has been used for the production of an amphiphilic biopolymer with emulsifying properties by first introducing a thiol group at the double bond.[14]

In contrast, the modification of non-activated C–H bonds of terpenes is more challenging, but could be achieved by enzymes such as P450 enzymes.[15–19] It has also been shown that recombinant *Pseudomonas putida* is able to transform different terpenes such as limonene or α-pinene into products such as perillyl alcohol or verbenol, which have a higher commercial value for industry than the starting material.[20–22] *P. putida* is a ubiquitous, aerobic, gram-negative bacterium with great metabolic versatility. In the last few decades, it has become a bacterial workhorse for several applications in the field of biotechnology, especially for biotransformation processes.[23,24] Recently, recombinant *P. putida* has been used as a whole-cell catalyst to convert various substrates, such as styrene,[25] octane,[26] or toluene.[27]

Herein we report that parts of the P450cam operon, P450cam (*camC*, monooxygenase), PdX (*camB*, putidaredoxin), and PdR (*camA*, putidaredoxin reductase) can be used to modify borneol in a biotransformation with *Pseudomonas putida*. The terpenoid borneol consists of a bornane scaffold containing one hydroxy group at C2 and exists in two stereo configurations. As a constituent of essential oils of many plants and waste residues, for example, in spruce needles, borneol has been known for many years for its widespread occurrence.[28] Even more importantly, (−)-borneol is also available in high amounts at a cost of approximately 5 USD $kg^{-1}$ from turpentine oil from the cellulose industry by using established chemical transformations, especially as an intermediate in the synthesis of camphor (production route via bornyl chloride) or derived from synthetic camphor (production route via isobornyl acetate).[29–31] Hence, it is an interesting raw material for bio-based polymers. To achieve this, a second functional group has to be introduced. We show the incorporation of a hydroxy group at C5 of (−)-borneol by whole-cell catalysis of recombinant *P. putida* harboring P450cam enzymes to create a novel

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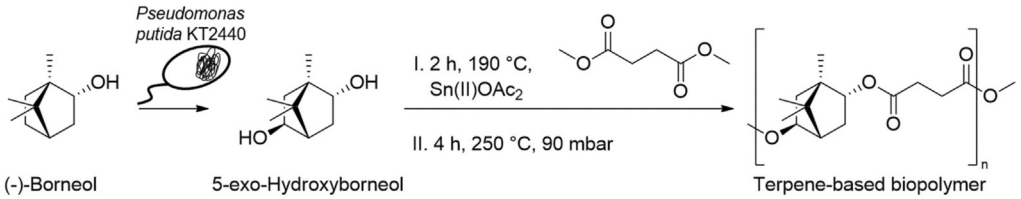


**Scheme 1.** Biotransformation of (−)-borneol to 5-*exo*-hydroxyborneol by engineered *Pseudomonas putida* KT2440. The bio-based monomer was subsequently used for polymerization to produce a terpene-based biopolymer.

monomer that can be used in a polymerization reaction to afford a new bio-based polyester (Scheme 1).

## Results and Discussion

### Engineering of a *P. putida* whole-cell catalyst for biotransformation of borneols

The cam operon[32] is responsible for camphor degradation in *P. putida* ATC17453, where in the first step camphor is hydroxylated at C5 by the enzyme P450cam and then further metabolized.[33] For this hydroxylation two more proteins, PdR and PdX, are necessary as they act as an electron transporter system towards the P450cam.[33] To obtain a *P. putida* strain capable of borneol hydroxylation without further degradation, parts of the cam operon were cloned into the broad host vector pBBR122. It is known that the camD gene codes for a dehydrogenase transforming the 5-hydroxy group into a ketone[34,35] and the camR repressor negatively regulates the operon,[34,36] both of which would result in a reduced yield of the hydroxylated borneols. Hence, a synthetic operon that does not contain these two regions was designed. After successful cloning and sequencing, the plasmid was transferred to *P. putida* KT2440 to yield a whole-cell catalytic system. Afterwards, this strain was used to convert (−)-borneol into the corresponding hydroxyborneol in a biotransformation. To determine the stereochemistry of the product and to clarify if isomers are produced, NMR spectroscopy and GC-MS analysis of the purified product and substrates were performed (see Supporting Information). As there is no 5-*endo*-hydroxyborneol available, (−)-borneol and its isomer (−)-isoborneol were analyzed by GC-MS and could be differentiated, giving rise to two separate signals (Figure S8 in the Supporting Information). NMR spectroscopy indicated that (−)-borneol is hydroxylated in only one orientation as well (Figures S1 and S7). Furthermore, it was reported that hydroxylation of camphor by P450cam enzymes is highly site-specific[37] and solely leads to 5-*exo*-hydroxycamphor.[37,38] Therefore the resulting product of the biotransformation should be enantiomerically pure 5-*exo*-hydroxyborneol.

### Optimization of the biotransformation of (−)-borneol

For optimal production of hydroxylated (−)-borneol, the substrate conversion was analyzed for its dependence on different temperatures, as well as different buffers and buffer systems, as the activity of P450cam enzymes is influence by both. Michizoe et al. tested different buffers at different pH values for the hydroxylation of camphor by the P450cam monooxygenase and obtained an optimum at pH 7.4.[39] For the conversion with purified enzymes, it could be shown that the buffer substance itself had an influence on the stability of PdX, which was more stable in Tris-HCl buffer than in phosphate-buffered systems.[39] Hence, the buffered systems itself, using Tris-HCl, MOPS and sodium-potassium-phosphate (S/P) buffers, as well as the pH values were varied (pH 6.0–8.0) to identify the optimal parameters for the conversion of (−)-borneol. Conversion rates of (−)-borneol and yields of 5-*exo*-hydroxyborneol decreased in all buffers at alkaline pH values (Table 1). Contrary to the pH optimum at pH 7.4, as mentioned above, the optimum for the conversion of (−)-borneol was observed at pH 6. This difference may occur because whole cells are used as catalysts. Nevertheless, Spolitak et al. reported similar activities even with purified P450cam at pH 6.2, 7.4 and 8.0 for the reaction with peracids,[40] so pH optimum for each conversion is also dependent on the substrate. Interestingly, in S/P and Tris-HCl buffers the selectivity increased at alkaline pH values, whereas it remains nearly constant in MOPS. The selectivity is also slightly influenced by the buffer substance as high activity was obtained in Tris-HCl and phosphate buffer by contrast to the MOPS buffered system. As mentioned above, the purified enzymes showed a lower activity in phosphate buffers.[39] Using whole cells for the conversion protects the enzymes, as the yields are similar in Tris-HCl- and phosphate-buffered systems at pH 7 and 8 (Table 1). Based on these results, the preferred buffer system for the conversion of (−)-borneol is S/P buffer at pH 6.

**Table 1.** (−)-Borneol substrate consumption and 5-*exo*-hydroxyborneol product formation in different buffer systems after 6 h at 30 °C. Selectivity is calculated based on conversion and yield.

| | Conversion [%] | Yield [%] | Selectivity [%] |
|---|---|---|---|
| S/P buffer pH 6 | 100 ± 0.0 | 78.6 ± 11.1 | 78.6 |
| S/P buffer pH 7 | 76.3 ± 2.0 | 69.8 ± 3.8 | 92.6 |
| S/P buffer pH 8 | 63.0 ± 1.5 | 63.6 ± 1.6 | 100 |
| Tris-HCl buffer pH 7 | 100 ± 0.0 | 70.2 ± 2.8 | 70.2 |
| Tris-HCl buffer pH 8 | 74.9 ± 5.4 | 70.7 ± 3.7 | 94.4 |
| MOPS buffer pH 6 | 100 ± 0.0 | 64.8 ± 1.7 | 64.8 |
| MOPS buffer pH 7 | 100 ± 0.0 | 67.1 ± 0.9 | 67.1 |
| MOPS buffer pH 8 | 76.6 ± 1.9 | 51.9 ± 4.0 | 67.8 |

Substrate conversion by P450cam enzymes is also influenced by the temperature as shown for whole-cell biotransformation of camphor with recombinant *E. coli*.[41] Furthermore, *P. putida* tolerates a wide range of temperatures,[42] so the influence of different temperatures on the biotransformation was investigated (Figure 1). Conversion rates were similar in the temperature range of 30–40 °C and decreased at lower temperatures. In contrast, product yields decreased at higher temperatures, reaching their maximum at 30 °C. Mouri et al. reported that lower temperatures for the hydroxylation of camphor by P450cam enzymes in recombinant *E. coli* resulted in faster conversion.[41] The authors used inducible cells for the protein expression, which were harvested, concentrated, and then used for the hydroxylation of 2 mM camphor.[41] Following this procedure an optimal temperature of 20 °C was identified.[41] In contrast, the herein-reported hydroxylation of (−)-borneol was performed by using a constitutive expression of the enzymes with growing cells. Therefore 30 °C seemed to be the optimal temperature for the conversion, as it also is the optimal growth temperature of *P. putida*.[42]

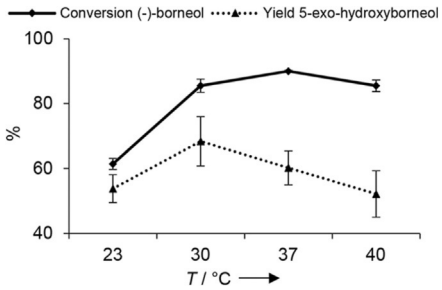


**Figure 1.** Conversion of (−)-borneol and obtained yield of 5-*exo*-hydroxyborneol at different temperatures after 5 h reaction time using the P450cam enzymes in recombinant *P. putida* KT2440 as a whole-cell catalytic system.

Both experiments showed the formation of 5-oxocamphor as a side-product at lower pH values and higher temperatures, respectively. Side product formation is attributed to different effects: (i) Products are further converted by P450cam enzymes due to unspecific oxidation as shown for camphor, which is oxidized to 5-oxo-camphor,[43] (ii) a high oxygen level in the media is also responsible for further oxidation of products by P450cam enzymes.[44] To prevent further product oxidation the biotransformation was performed in a two phase system, where the product should be protected by dissolving in the organic phase. Moreover, high volatility as well as insolubility of (−)-borneol in aqueous media as two major challenges could be circumvented by the two phase system as well. As *Pseudomonas* species are known to have a high tolerance to different organic solvents,[45,46] they are excellent candidates for biotransformations in presence of organic solvents. *n*-Hexane and *n*-dodecane, both of which are reported to minimize toxic effects of substrate or product,[47] containing (−)-borneol were used and space-time yields (STY) were calculated accordingly. STYs for both solvents (*n*-hexane and *n*-dodecane) were similar ($STY \approx 32\ mg\,L^{-1}\,h^{-1}$) and also comparable to the conversion without an organic solvent ($STY_{P\text{-}media} \approx 34\ mg\,L^{-1}\,h^{-1}$). Similar yields were reported for α- and β-pinene ($STY = 22$ and $41\ mg\,L^{-1}\,h^{-1}$), which were converted by resting cells of *Pseudomonas* strain PIN.[48] The major product of the metabolism of this strain was *p*-cymene, which was further metabolized to give a broad spectrum of terpenes (limonene, α-terpinolene, α-terpineol).[48] Schewe et al. investigated the biotransformation of α-pinene by using recombinant *E. coli* as a whole-cell catalytic system.[49] They made use of the $P450_{BM\text{-}3}$ monooxygenase from *Bacillus megaterium* for the conversion of α-pinene into α-pinene oxide, *trans*-verbenol, and myrtenol.[49] As a major product, $20\ mg\,g_{cdw}^{-1}$ α-pinene oxide was obtained in 1.5 h reaction time.[49] The reaction's conversion rates remained constant for the first 30 min and then decreased. The authors suggested that the main problem was the toxicity of the substrate α-pinene.[49] To determine any toxicity of (−)-borneol or 5-*exo*-hydroxyborneol to *P. putida*, which would affect the conversion rates, a toxicity test was performed by using 2 mM substrate or product. Bacterial growth was analyzed over 24 h, but neither (−)-borneol nor 5-*exo*-hydroxyborneol showed any toxic effect on the cells.

These results all indicate that the best parameters for the conversion of (−)-borneol are a low oxygen level in the media (low amount of side-products), S/P buffer pH 6 as the medium (high product yields) and 30 °C as the conversion temperature (fastest conversion with the highest yield).

## Development of a semi-continuous batch system for the production of 5-*exo*-hydroxyborneol

For the production of larger quantities of 5-*exo*-hydroxyborneol, a semi-continuous batch system was developed, which allowed the reuse of the cells as a catalyst (Figure 2).

After complete substrate conversion was observed, the product was separated from the cells by cross flow filtration and extracted with ethyl acetate. Consequently fresh substrate was added to the cells, which could be reused up to three times (Figure 3).

Substrate conversion was high in every batch process (>98%; Table 2), which confirmed that the cells are reusable

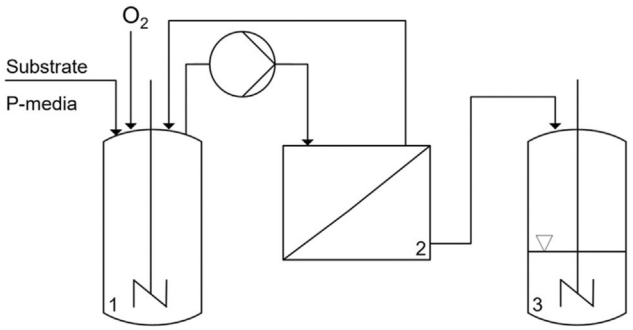


**Figure 2.** Flow chart of the biotransformation process of (−)-borneol. Biotransformation is performed in a 1.5 L air-flushed stirred tank reactor (1). After complete conversion, the product is separated from the cells by cross-flow filtration (2) and subsequently extracted with an organic solvent (3), whereas the cells are returned to the reactor and used for further biotransformations after addition of fresh media and substrate.

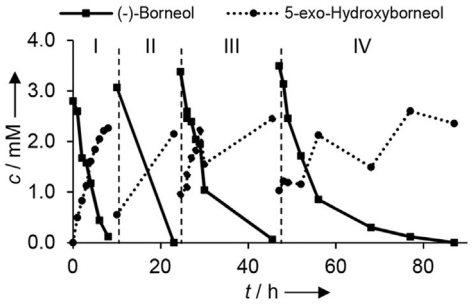


**Figure 3.** Substrate consumption and product formation during the biotransformation of (−)-borneol with cell recycling. Each dotted vertical line indicates the addition of new substrate. Overall, cells could be used four times for the conversion of (−)-borneol (Phases I–IV).

**Table 2.** Parameters obtained during the biotransformation (see Figure 3). STYs refer to wet biomass. For calculation, see Experimental Section.

| | Phase I | Phase II | Phase III | Phase IV |
|---|---|---|---|---|
| *t* [h] | 8 | 13 | 21 | 40 |
| Conversion [%] | 99.8 | 99.6 | 98.0 | 98.8 |
| Yield [%] | 80.9 | 75.3 | 81.7 | 83.3 |
| STY [mg $L^{-1}$ $h^{-1}$] | 48 | 28 | 20 | 11 |
| STY [mg $mg^{-1}$ $h^{-1}$] | 12 | 6.0 | 4.0 | 2.0 |

as a catalyst for the conversion of (−)-borneol. The time to reach full conversion increased from 8 to 40 h, but was shorter than other hydroxylation reactions by P450cam enzymes (e.g., α- and β-ionone whole-cell biotransformation with recombinant *E. coli*).[50] Product yields varied slightly with the addition of fresh substrate, but were similar to those in the preliminary tests and also those reported for other hydroxylation reactions (hydroxylation of limonene by *P. putida* MTCC 1072 cells).[21]

By implementing the semi-continuous process, several advantages could be achieved compared to a batch process. The batch process is limited to a substrate concentration of 5 mM, because the substrate solubility is low and, after the conversion, the product has to be isolated from the cells. With the semi-continuous process, it is possible to convert more substrate over time, thereby increasing the total product yield. Additionally, the amount of product per cell could be increased, as the cells can be reused. Furthermore, when cells are present during the extraction with ethyl acetate, an interphase is formed and so extraction after cell removal by cross-flow filtration was more efficient.

In contrast to the preliminary tests, no side-product formation was observed, which is most likely due to the controlled lower oxygen level in the media. The preliminary tests were performed in shake flasks, where the oxygen level is supposed to be high. However, in the semi-continuous process, the oxygen level is controlled by an external oxygen supply. By varying the level of oxygen, specific product yields could be increased and best results were obtained at an airflow of 2.75 $L_{air}$ $L_{culture}^{-1}$ $min^{-1}$. In each cross-flow filtration step, a certain amount of the medium remained in the reactor; otherwise cell density would increase and cells would be damaged by increased shear stress, so product yields remained at approximately 80%. There could also be some loss of substrate during the biotransformation because of the volatile nature of the substrate.

### Synthesis of a bio-based borneol polyester

The melting point of 5-*exo*-hydroxyborneol obtained from the biotransformation was determined (m.p. = 130–140 °C) and purity could be increased by recrystallization (> 98%). To see whether the purified product could be used for polymerization, a proof of concept was done by the polycondensation of 5-*exo*-hydroxyborneol and succinic acid dimethyl ester. Succinic acid could be obtained from renewable resources such as carbohydrates by fermentation.[51] There are several reports on the biotechnological production of succinic acid by metabolic engineered bacteria[52] and yeasts,[53] which can produce succinic acid at high titers (45 g $L^{-1}$).[53] Using this monomer for the polycondensation reaction, the resulting biopolymer is a novel all bio-based polyester. Furthermore, this monomer was chosen to produce a polymer that is somewhat similar to polybutylene terephthalate (PBT), where a $C_4$ alkyl chain and a ring structure are also present. In the bio-based polyester, the aromatic ring is exchanged for the bicyclic alkyl ring of the terpene monomer and the ester bond is reversed (Figure 4).

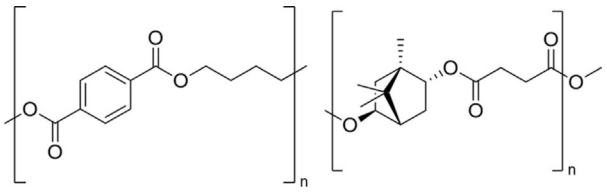

**Figure 4.** Structure of the commercial polybutylene terephthalate (PBT, left) compared to the terpene-based biopolymer (right).

During the first step of the solvent-free polymerization, methanol was released and used as a marker to follow the reaction progress. First, polymerizations were carried out at lower reaction temperatures and times, which resulted in viscous, glutinous reaction mixtures. On increasing reaction temperature and time, an amber-colored, transparent solid was obtained. To further investigate the new material, the thermal properties and molecular mass distribution of the biopolymer and an industrial PBT (as reference) were analyzed by using differential scanning calorimetry (DSC; Figure 5) and gel-permeation chromatography (GPC), respectively. DSC analysis of the crude biopolymer showed a glass transition temperature ($T_g$) in the range of 35–55 °C. After the removal of residual monomers, $T_g$ increased to about 70 °C, which is higher than that of PBT (45–60 °C). The terpene-based copolymer had a molecular weight in the range of $M_w$ = 2000–4000 g $mol^{-1}$ (PDI = 1.9), which is much lower compared to the industrial PBT ($M_w$ = 55 000 g $mol^{-1}$). The difference in the molecular weights might be attributed to the non-optimized polymerization procedure. Polycondensation of modified terpenoids is a rather underexplored area in the wide field of terpene-based polymers. There

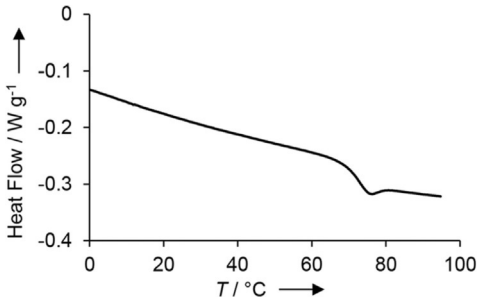


**Figure 5.** DSC analysis of the terpene-based biopolymer after removal of residual monomers. The second scan of the sample is shown and the glass transition temperature was calculated by integration.

are a lot of reports, which use other polymerization techniques for terpene-based polymers. Often double bonds are used for the polymerization of terpenes, as a straightforward approach shown for cationic polymerizations reported for the homopolymerization of β-pinene.[54] By using this method, Satoh et al. obtained poly(β-pinene) with high molecular weights ($M_n$ up to 50 000) and a high transparency.[55] Terpenes without double bonds often have to be modified before they are suitable for polymerization. (−)-Menthone for example, carrying a single carbonyl group, can be converted into lactams[56] or lactones,[57] which could be used in ring-opening polymerization (ROP) reactions to give polyamides ($M_w$ = 1700–3400 g mol$^{-1}$) and polyesters ($M_n$ up to 90 000 g mol$^{-1}$), respectively. One of the most common techniques for the synthesis of terpene-based polyesters is the combination of ROP with lactones as monomers. For example carvone-derived lactones lead to homopolymers with molecular weights in the range of $M_n$ = 700–10 500 g mol$^{-1}$ by ROP.[58] Compared to these terpene-based polymers, the novel borneol-based biopolymer is an interesting starting point for further investigations, especially by using polycondensation as a polymerization technique. Thus, novel bio-based materials could be obtained and, by optimizing the polymerization procedure, the production of high molecular weight borneol-based biopolymers should also be possible.

## Conclusion

In this study, we have shown that it is possible to hydroxylate (−)-borneol to yield a bifunctional terpenediol by whole-cell catalysis. A process was developed that allowed the semi-continuous production of 5-*exo*-hydroxyborneol with direct product extraction, in which cells could be reused as the catalyst for repeated biotransformations. In a proof of concept the purified product was used for the polymerization with succinic acid dimethylester. The biopolymer had a $T_g$ of around 70 °C and a $M_w$ in a range of 2000–4000 g mol$^{-1}$. To increase molecular weights, the polymerization procedure has still to be optimized. Therefore, a screening of different parameters affecting the polymerization, such as (i) the amount and kind of catalyst, (ii) polymerization temperatures and pressures, and (iii) polymerization time, will be performed next. Further investigations on the bio-based borneol polyester, for example, determination of its impact resistance or ultimate strength, will be performed, even with the low molecular weight polymers, to identify suitable applications for the material.

## Experimental Section

### GC-MS

Cell samples were mixed with an equimolar volume of ethyl acetate and centrifuged after vigorous mixing. A sample of the upper organic phase (150 μL) was mixed with ethyl acetate (350 μL) and GC-MS was performed using auto-injector AOC-5000, the Shimadzu GC-2010 Plus and a SGE BPX-column (30 m × 0.25 mm inner diameter). Separation of the substances on the GC-MS was achieved at an oven-temperature of 50 °C followed by a temperature programming from 50 °C to 120 °C (15 °C min$^{-1}$), then to 170 °C (5 °C min$^{-1}$), finally to 200 °C (20 °C min$^{-1}$) and holding this temperature for 10 min under the constant flow of 1.69 mL helium min$^{-1}$. The resultant chromatograms were analyzed with the Shimadzu GCMSsolution software.

### DSC

Thermal properties of polymer materials (e.g., glass transition temperature ($T_g$)) were recorded with differential scanning calorimetry using the METTLER Toledo DSC 1 Star system. Samples of solid polymer (10–15 mg) were heated from 50 °C to 300 °C (25 °C min$^{-1}$), hold for 2 min and then cooled down to −20 °C (−25 °C min$^{-1}$) and hold for 5 min. Next, the samples were heated to 100 °C (10 °C min$^{-1}$), then to 400 °C (30 °C min$^{-1}$) and finally cooled down to 50 °C (−30 °C min$^{-1}$). The heating program was performed under constant flow of 50 mL nitrogen min$^{-1}$. The DSC curves obtained were analyzed with the METTLER STAR$^e$ SW 13.00 software and all $T_g$ values were obtained from the second scan, after removing the thermal history.

### GPC

Sample preparation was performed by drying (4 h at 80 °C) the solid polymer samples (5–15 mg) under vacuum. Dried samples were dissolved in appropriate volume of HFIP containing NaTFA (0.05 mol L$^{-1}$). For the calibration curve polymer standards of the ReadyCal-Kit Poly(methyl methacrylate) low were dissolved in HFIP (1.5 mL) containing NaTFA (0.05 mol L$^{-1}$). Samples and polymer standards were stored for two days at RT protected from light. Samples were then filtered (0.2 μm) and GPC analysis was performed using the SECcurity GPC System (Inline degaser and oven TCC600 (Polymer Standards Service), 1260 Infinity pump, 1260 Infinity auto-sampler, 1260 Infinity RI-detector (Agilent Technologies)), a PSS PFG pre-column (50 mm × 8 mm, particle size 7 μm) and two PSS PFG columns (300 mm × 8 mm, particle size 7 μm, porosity 100 Å and 1000 Å). The GPC curves obtained were analyzed using PSS WinGPC UniChrom software (Polymer Standards Service).

### NMR

NMR-spectra were measured on a *JNM-ECA* 400 MHz spectrometer from *JOEL* at 25 °C with the application of standard pulse programs. The chemicals shifts (δ) are reported in ppm with reference to tetramethylsilane. Coupling constants *J* are reported in Hertz (Hz). For determining $CH_2$-signals, the DEPT135°-technique was used. For correct assignment of the signals, 2 D NMR methods, like

COSY, HSQC and HMBC. For more information see the Supporting Information.

### Cloning

The P450cam operon was amplified by PCR with genomic DNA from *Pseudomonas putida* ATCC 17453. For cloning the camCAB part of the operon primer P1 and P2 (P1: 5′-CAATAAGAACGAGG-TAATGCATGA CGACTGAAACCATACAAAG-3′; P2: 5′-TAATGCGGCCGCGTCCCGG GATTGGCCATTG-3′) were used for amplifying the *camC*, *camA* and *camB* genes and adding an overlap sequence at the 3′ end of the PCR product. For amplification of the promotor region primer P3 and P4 (P3: 5′-GTACT-GAATTCGTTGCCCGGCTCGATCCGAG-3′; P2: 5′- GTATGG TTTCAGTCGTCATGCATTACCTCGTTCTTATTG-3′) were used which added an overlap sequence at the 5′ end. Both PCR products were then used in an overlap extension PCR yielding camCAB. This PCR product was then digested with EcoRI and NotI and ligated into the vector pBBR122 digested with the same enzymes yielding pBBR122_camCAB. The plasmid was transformed in *E. coli* DH10B and plated on LB plates containing kanamycine (30 mg $L^{-1}$). Single clones were used for plasmid DNA preparation and sequencing. After the sequencing confirmed the success of cloning the plasmids were transformed in *P. putida* KT2440 by electroporation.

### Biotransformation of (−)-borneol

Single *P. putida* clones were used for inoculation of P-media [25 mL; 2 g $L^{-1}$ $KH_2PO_4$, 4 g $L^{-1}$ $Na_2HPO_4$, 1 g $L^{-1}$ $(NH_4)_2SO_4$, 2.5 g $L^{-1}$ glucose, 30 mg $L^{-1}$ kanamycine, and 0.4% trace metal solution (TMS)]. The TMS contained 10 g $L^{-1}$ MgO, 2 g $L^{-1}$ $CaCO_3$, 5.6 g $L^{-1}$ $FeSO_4{\cdot}7H_2O$, 1.44 g $L^{-1}$ $ZnSO_4{\cdot}7H_2O$, 0.85 g $L^{-1}$ $MnSO_4{\cdot}H_2O$, 0.25 g $L^{-1}$ $CuSO_4{\cdot}5H_2O$, 0.28 g $L^{-1}$ $CoSO_4{\cdot}7H_2O$, 0.062 g $L^{-1}$ $H_3BO_3$, and 50 mL $L^{-1}$ HCl. The cells were incubated in Erlenmeyer flasks overnight (30 °C, 150 rpm). This preculture was then used to inoculate 1 L of fresh media with an optical density measured at 600 nm ($OD_{600}$) of 0.2. The cells were then further incubated (30 °C, 150 rpm) for 24 h. Afterwards (−)-borneol (3–5 mM final concentration after addition to cell broth) was dissolved in dodecane or ethyl acetate and added to the cell broth. Cells were further incubated (30 °C, 150 rpm) and samples were taken at different time intervals and analyzed by GC-MS.

### Optimization of the biotransformation

*P. putida* cells containing the pBBR122 camCAB plasmid were incubated (2 d, 30 °C, 120 rpm) in shake flasks. To check whether different buffers at different pH affect conversion, cells were harvested by centrifugation (4000 *g*, 30 min, 4 °C), washed twice with the corresponding buffer (pH 6.0–8.0), and set to an $OD_{600}$ of 1.0. All buffers (20 mM) contained equal amounts of glucose (2.5 g $L^{-1}$) and TMS (0.4%) as the P-media. Cells were treated with (−)-borneol (1 mM), further incubated (30 °C, 120 rpm) and samples for GC-MS were taken after 0 h and 6 h. For the conversion at different temperatures ($T$ = 23–40 °C), cells were produced as above and also incubated (30 °C, 120 rpm) with (−)-borneol (1 mM) at different temperatures (23, 30, 37, and 40 °C). Samples for GC-MS were taken at regular time intervals. Conversion was performed in a two-phase system using *n*-hexane and *n*-dodecane as solvents (each 20% *v*/*v*) containing (−)-borneol (50 mM). Cells were incubated (30 °C, 120 rpm), samples for GC-MS were taken at different time intervals and space-time yields (STY) were calculated [Eq. (1) and (2)].

$$\mathrm{STY}\ [\mathrm{g\,L^{-1}\,h^{-1}}] = \frac{m_{\mathrm{product}}\ [\mathrm{g}]}{V\ [\mathrm{L}] \times t\ [\mathrm{h}]} \quad (1)$$

$$\mathrm{STY}\ [\mathrm{g\,g^{-1}\,h^{-1}}] = \frac{c_{\mathrm{product}}\ [\mathrm{g\,L^{-1}}]}{m_{\mathrm{wet\ biomass}}\ [\mathrm{g\,L^{-1}}] \times t\ [\mathrm{h}]} \quad (2)$$

For the toxicity tests on the substrate, product, or corresponding solvent, the cells were incubated (30 °C and 120 rpm) with (−)-borneol, 5-*exo*-hydroxyborneol (each 2 mM) or ethyl acetate for 48 h and $OD_{600}$ values were measured with the Ultraspec 10 cell density meter (Amersham Biosciences) at different time intervals and compared to a control.

### Production of the diol compound

For the biotransformation of borneol to the corresponding diol, cells were produced as described above and (−)-borneol was used as the substrate. The $OD_{600}$ of the main culture was set to 1.0 and 1 L of the culture broth was then transferred to a stirred tank reactor with a total volume 1.5 L. The broth was incubated (30 °C, 215 rpm, air flow of 2.75 L $L^{-1}$ $min^{-1}$) and (−)-borneol (3–5 mM) was added as substrate. Samples withdrawn at regular time intervals were analyzed by GC-MS. After full conversion of (−)-borneol, the wet biomass weight was determined and the product was separated from the cells by cross flow filtration with a Vivaflow 200 membrane (MWCO = 30 kDa, Sartorius). In addition, the reactor was filled up with fresh media and substrate. This step was repeated three more times. The aqueous product was extracted with the same amount of ethyl acetate and the organic solvent was removed by evaporation.

### Purification of the diol compound by recrystallization

To each gram of product, 6.2 g of ethyl acetate were added and heated to 77 °C. After 2–3 min, the clear reaction mixture was allowed to cool to RT, whereby white crystals precipitated. The suspension was incubated for 24 h at RT, followed by an incubation of another 24 h at 4 °C. After an additional incubation for 30 h at −20 °C, the suspension was filtered under vacuum and white crystals were obtained. The resulting filtrate was purified once more by the same procedure.

### Polymerization

For the transesterification of succinic acid dimethyl ester (1.29 g, 8.8 mmol) with 5-*exo*-hydroxyborneol (1.05 g, 6.2 mmol) in the presence of 0.5 mol% tin(II) acetate (7.1 mg, 0.029 mmol, on 5-*exo*-hydroxyborneol), a two-necked round-bottom flask equipped with a magnetic glass stirrer and a reflux condenser with a distillation bridge were used. The mixture was heated at 105 °C for 16 h. The educts were added under an atmosphere of nitrogen and transesterification was conducted in the absence of solvent for 2 h at 190 °C under a nitrogen atmosphere. In the second step, the reaction mixture was heated to 245 °C and the excess of succinic acid dimethyl ester was removed under reduced pressure ($p$ = 90 mbar). After 4 h, the reaction mixture was cooled to RT.

For the removal of residual monomers, polymer samples (200–250 mg) were pulverized and then taken up in methanol (10 mL) and stirred for 16 h at RT. After filtration, followed by washing with methanol (5 mL), the solvent was removed by evaporation and the filtered solid was dried under reduced pressure.

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## *Conflict of interest*

*The authors declare no conflict of interest.*

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