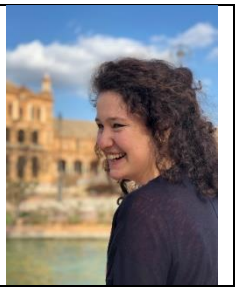
ESR 4

Project title and research strand:	Biocatalytic access to novel functional building blocks and their materials. Strand 3: functional polymers.	
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Abstract

The aim of this PhD was to investigate options to make building blocks for “bio-based” polymers from fatty acids and to invstigate polymer formation. The focus during the first part of the PhD at the University Graz was on the CYP152 family which are P450 peroxygenases that utilise fatty acids as natural substrates and can be used for the biocatalytic □-functionalization of medium chain fatty acids. Enzyme candidates of the CYP152 family were selected, expressed, purified and the conversion of medium-chain fatty acids was investigated. The primary objective was than an upscale of the production of α-hydroxylated fatty acids.

The secondment took place at the company B4Plastics where the main aim was to find and select possible strategies to design and produce bio-based polymers from α-hydroxylated fatty acids in an industrially oriented framework. Initially, a protocol was developed to synthesize alkyl lactides from α-hydroxylated fatty acids. The emphasis was placed on the utilization of a α-hydroxylated fatty acid successfully synthesized in the initial phase of the PhD. For the second step the idea was to synthesise a copolymer using L-lactide. The reaction conditions for ring-opening polymerisation (ROP) were optimized by applying different temperatures, conducting the reaction in an organic solvent or solvent-free and by using different catalyst as $Sn(Oct)_2$, $BiPh_2Br$ and lipases and initiators. Moreover, the effect of the aliphatic side branch was analysed by determining the melting point, molecular weights, glass transition temperature and crystallinity of PLLA and the newly synthesized “bio-based” polymer. Latter is expected to show higher biodegradability than PLLA.

Visual Summary – Poster

Biocatalytic access to novel functional building blocks and their materials

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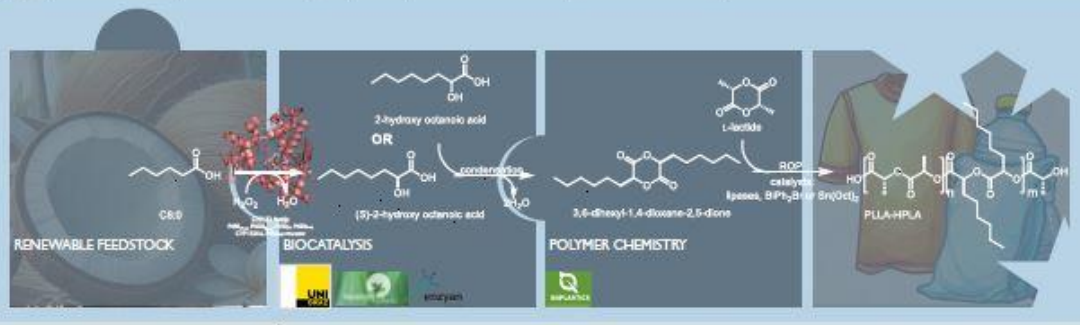
Aim of the project: In this project, where the first part took place at the University of Graz in Austria and the second part at the company B4Plastics in Belgium, novel "bio-based" building blocks and their oligo/polymeric materials were produced based on α-hydroxylated fatty acids obtained via biocatalytic functionalisation of fatty acids.



During the first part of the PhD an atom economical process for the regio- and stereoselective biocatalytic α-hydroxylation of medium chain fatty acids was developed, requiring hydrogen peroxide as the only stoichiometric reagent. The enzyme $P450_{Bsβ}$[1] showed high conversions, regio- and stereoselectivity for the substrates caproic acid [C6, conv. 95%; 70% α-2-OH C6 e.e. 81% (S)] and was active towards longer chain dicarboxylic acids like azelaic acid (nonanedioic acid, conv. 68%) and sebacic acid (decanedioic acid, conv. 99%) with a remarkable chemoselectivity for mono-hydroxylation. $P450_{Bsβ}$[2] allowed to achieve excellent conversion for the fatty acids caprylic (C8) and capric acid (C10, >99%) with up to >99% (S) e.e.. These results allowed an efficient and scalable process to produce α-hydroxylated fatty acids. In summary, TONs of up to 42000 were achieved for the conversion of C8 on preparative scale using $P450_{Bsβ}$ as catalyst at substrate concentrations up to 150 mM giving the desired product in gram quantities(**Table 1**).

Table 1: Preparative scale transformations of C8, C10 and sebacic acid with $P450_{Bsβ}$ and $P450_{Bsα}$; adapted from Bangert *et al.*[2]

Entry	Substrate	Substrate [mM]	Enzyme	Total H_2O_2 [mM]	GC-MS conv. [%]*	Isolated yield [%]/ Purity [%]^b	TON^c
1	C8	10	$P450_{Bsβ}$	20	>99	99 (80 mg)/ >99	3333
2	C8	50	$P450_{Bsβ}$	100	>99	89 (401 mg)/ 94 (6% β-OH)	16667
3	C8	100	$P450_{Bsβ}$	150	>99	87 (1260 mg)/>99^d	33333
4	C8	150	$P450_{Bsβ}$	100	80	n.d.	42000
5	C8	150	$P450_{Bsβ}$	150	90	n.d.	42000
6	C10	10	$P450_{Bsβ}$	20	97^e	n.d.	3233
7	sebacic acid	10	$P450_{Bsβ}$	20	>99	29 (126 mg)/ >99	3333
8	sebacic acid	50	$P450_{Bsα}$	100	25	n.d.	833

Reaction conditions: Reactions were performed in a 250 mL reaction flask containing reaction buffer (100 mM KPi buffer, pH 7.4), DMSO (5% v/v), fatty acid (10, 50, 100 or 150 mM) and purified enzyme (2 μM), in a final volume of 50 mL (100 mL for entry 2 and 25 mL for entry 6). H_2O_2 was added continuously via a syringe pump (entry 1-4 and 7: 1.4 mM/h over 12 h to a final concentration of 20 mM (stock: 200 mM); entry 5, 6 and 8: 8.3 mM/h over 12 h to a final concentration of 100 mM (stock: 200 mM), entry 2 and 5: 12.5 mM/h over 12 h to a final concentration of 150 mM (stock: 400 mM)). N.d. not determined.

*Conversion was determined by GC-MS using lauric acid (5 mM) as ISD by comparison with a sample at t = 0.

[b]Isolated yields (%) were calculated based on the measured mass (mg) of isolated and dried product and the maximum theoretical yields (mg). Purity (%) was calculated based on GC-MS data.

[c]TON = turnover number which is defined as mmol substrate converted per mmol catalyst.

[d]Yield after second purification step.

[e]α-OH 61% GC-Area; β-OH and γ-OH product 26% GC-area and 9% GC-area, respectively.

The synthesis of the 3,6-dihexyl-1,4-dioxane-2,5-dione as monomer for polymer synthesis was successfully established. The condensation reaction was carried out in toluene using *p*TsOH as catalyst and resulted in a crystalline product [7 g, (79%)]. Initial experiments were carried out to replace toluene and *p*TsOH with D-limonene as solvent and Amberlite™ IRC120 H as catalyst. Furthermore the synthesis of PLLA and PLLA-HPLA using three different catalyst, lipases, $Sn(Oct)_2$ and $BiPh_2Br$, was investigated. Further optimizations are required for enzymatic ring-opening polymerisation, as it currently yields only short oligomers. The polymers that were obtained using $Sn(Oct)_2$ or $BiPh_2Br$ as catalyst were of low molecular weight (**Table 2**). Interestingly, GC analysis indicated faster consumption of 3,6-dihexyl-1,4-dioxane-2,5-dione (85.2%, **Table 2 entry 2**) than L-lactide (37.1%, **Table 2 entry 1**). Thus, introduction of hexyl chains into PLLA resulted in a less crystalline PLLA-HPLA using $Sn(Oct)_2$ as catalyst. When a higher mol% of 3,6-dihexyl-1,4-dioxane-2,5-dione was used a low molecular weight polymer (M_n = 295 g/mol) was obtained that did not precipitate in DCM/MeOH and could therefore not be further analyzed (**Table 2 entry 4**).

Table 2: Polymerization of L-lactide and 3,6-dihexyl-1,4-dioxane-2,5-dione (dihexyl-lactide) using $Sn(Oct)_2$ as catalyst:

Entry	Monomer	ROP	Time [h]	M_n [g/mol]	m [g]	M_w [g/mol]	PDI	T_g [°C]	T_m [°C]	Conv./ Yield [%]	χ_c [%]
1	L-lactide	Sn-ROP/ toluene Init. BnOH	1.5	5828 Lit: 4020[1] NMR: 3015	1.6	7200	1.235 Lit.1.1[1]	48.5	149.7	98 (NMR)/ 80	37
2	L-lactide dihexyl-lactide (5 mol%)		3	3826	0.5	5359	1.401	42.5	141.8 - 146.4	97 (NMR) L-lactide: 37.1 dihexyl-lactide: 85.2 (GC)/ 25	4
3	L-lactide		2.5	3853 Lit: 25000[2]	4.8 3	4156 Lit: 27500[2]	1.079 Lit: 1.1[2]	47.2	147.4 - 155.2	76 (GC)/ 81	7
4	L-lactide dihexyl-lactide (10 mol%)		2.5	NMR: 295	-	-	-	-	-	L-lactide: 87.0 dihexyl-lactide: 79.7 (GC)	-

M_n: number-average molecular weight; M_w: weight-average molecular weight; PDI: Polydispersity Index were determined via GPC; T_g = glass transition temperature, T_m = melting temperature and χ_c = degree of crystallinity were analysed via DSC.

Substrate Consumption via GC [%] = $(1 - \frac{\text{Initial Relative Substrate Concentration}}{\text{Relative Substrate Concentration}}) \times 100\%$; trans-cinnamic acid served as internal standard

Conversion via NMR [%] = $\frac{\text{Integral (polymer)}}{\text{Integral (monomer)} + \text{Integral (Polymer)}}$; Yield $\eta = \frac{\text{isolated polymer yield}}{\text{starting monomer amount}} \times 100\%$

Lit.: [1]Trimaille, T *et al. J. Polym. Sci. Part A: Polym. Chem.* 2004, 42, 4279–4291; [2]Kricheldorf *et al. Macromol. Chem. Phys.* 2013, 2100464.

Impact: The aim of this PhD project was to produce a "bio-based" polymer from α-hydroxylated fatty acids (PLA-HPLA). To avoid persistency, we envisioned a material that can be faster degraded than PLA. We achieved a cooperation between different stakeholders that were researchers in the field of biocatalysis and industrial partners in the field of polymer chemistry and biocatalysis. The first stage of the process has an impact on sustainability as we used renewable resources and achieved high atom economy by maximizing the incorporation of all materials used in the process. Apart from that we utilized selective biological catalysts and the amount of waste and required chemicals produced was kept at a minimum. This project brings value to the environment as less hazardous chemicals are used and as the consumption of fossil-based resources is reduced. Furthermore, we kept in close contact with all the stakeholders to consider risks and costs. For the second stage we tried to use environmental solvents (e.g. D-limonene and 2M2B) and catalysts (e.g. lipases) to achieve an efficient and sustainable upscaling by reducing waste and using less toxic chemicals and less energy. Refining the reaction conditions for increased yields and conducting biodegradation tests are essential steps to draw conclusions regarding biodegradability. Furthermore to see how the monomer is incorporated thermal fractionation techniques are necessary.

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Biobased Value Circle