Exploring Biocatalyzed Lactone Building Blocks Toward Biobased Polyesters

Summary

Both polymers and enzymes are omnipresent in our lives. While the first ones are fully integrated in our environment, the second ones are essential to most biological functions taking place in our bodies. Enzymes are not only needed for biological processes, they are also of paramount importance for the chemical industry, where they act as catalysts for the synthesis of molecules.

The goal of this thesis is to expand the use of oxidative enzymes for the synthesis of building blocks, which are used as precursors for polymeric materials. More specifically, the application of Baeyer-Villiger monooxygenases is explored, targeting the preparation of alkyl substituted lactones by oxidation. Ring opening polymerization of these lactones affords aliphatic polyesters. Depending on the structure of the lactones, the properties of the polyesters can be tuned, thus allowing their use in different types of applications.

In **Chapter 2**, an overview of functional lactones for the preparation of aliphatic polyesters by ring opening polymerization is given. Although these lactones are currently predominantly obtained by chemical processes, it is also possible to use enzymes, namely Baeyer-Villiger monooxygenases, to synthesize them. This chapter highlights the role that these oxidative enzymes can play for the synthesis of lactones for polymers and gives the state-of-the-art regarding their application in large scale bioreactions.

Baeyer-Villiger monooxygenases are however not yet mainstream for the synthesis of lactones. One reason is that they typically suffer from poor stability, which is a major drawback for process intensification. In addition, enzymes applied to the synthesis of chemicals at large scale must be pushed to their limits in order to achieve high process metrics, for example high product concentration and high productivity. This is due to their initial function, which is to catalyze biochemical reactions in biological systems.

In order to expand the industrial applicability of Baeyer-Villiger monooxygenases, several of these enzymes were screened for the oxidation of alkyl substituted cyclic ketones in **Chapter 3**. A particularly thermostable enzyme was identified for the oxidation of 3,3,5-trimethylcyclohexanone: cyclohexanone monooxygenase from *Thermocrispum municipale*. This reaction is further investigated by reaction engineering in **Chapter 4**, where improved process metrics are targeted. Substrate feeding strategies are employed to overcome substrate inhibition while ensuring that high product concentrations are achieved. Coimmobilization of this Baeyer-Villiger monooxygenase and the

selected coenzyme is shown to be particularly relevant for the reutilization of these enzymes over several reaction cycles. Lastly, up-scaling of this reaction with soluble enzymes is achieved in the first gram-scale biocatalyzed synthesis of the alkyl substituted lactones, which are obtained as a mixture of regio-isomers.

The ring opening polymerization of these alkyl substituted regio-isomeric lactones is explored in **Chapter 5**. In addition to metal-based catalysts, organocatalysts such as bases and bifunctional catalysts are shown to be efficient for the polymerization of these lactones. A difference of reactivity upon polymerization is observed between the two regio-isomeric lactones. The most substituted lactone is shown to be more reactive for all catalysts evaluated in this chapter. Computational studies confirm that this difference of polymerization behavior is due to their thermodynamic properties, and can be predicted.

The effect of the structure of lactones upon copolymerization is assessed in **Chapter 6**. Copolyesters from alkyl substituted lactones with a more polar lactone are evaluated. The determination of the reactivity ratio indicates a tendency for random copolymers. Different copolymerization strategies were employed to avoid the formation of homopolymers and to tune the microstructure of the copolyesters. In **Chapter 7**, a potential application for copolyesters based on a biobased macrolactone is evaluated as dispersing agents in inks and coatings. The aim is to increase the solubility range by copolymerization with alkyl substituted lactones. An important decrease in crystallinity is achieved. Control over the microstructure of the copolymers is directly correlated to their crystalline properties, and thus solubility range.

The environmental impact of (bio)chemical processes is a growing concern. While most enzymatic reactions are typically perceived as being greener than their chemical counterparts, this is however usually not supported by quantified studies. In **Chapter 8**, this perception is analyzed by comparing the environmental impact of the synthesis of lactones *via* two routes, using either a Baeyer-Villiger monooxygenase or an organic oxidant. This early-stage life cycle assessment highlights the importance of laboratory scale studies for the identification of key process metrics.

Lastly, the main conclusions, outlook and recommendations of this thesis are given in **Chapter 9**. A valorization of the work presented in this thesis is given in **Chapter 10**.