Unraveling Substrate Dynamics and Identifying Inhibitors in Hydrolysates of Lignocellulosic Biomass by Exometabolomics

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OUTLINE

Lignocellulosic biomass is the 2nd generation feedstock for biofuel production through fermentation processes. The material has a rigid structure, which needs to be broken down by a pretreatment procedure to expose cellulose for hydrolysis. The hydrolysis products, so called biomass hydrolysates, contain next to the sugar monomers, toxic compounds released and formed during the pretreatment process. These compounds inhibit the growth of the fermenting host(s). To improve the fermentability of biomass hydrolysates, identification of these inhibitory compounds is of great importance. **Chapter 1** of this thesis reviews the approaches and techniques that have been used to study the inhibitors in various biomass hydrolysates, and introduces a non-targeted methodology to systematically identify biomass hydrolysate inhibitors: the exometabolomics approach. To identify hydrolysate inhibitors through an exometabolomics approach, a wide range of biomass hydrolysates needs to be prepared. **Chapter 2** describes the detailed procedures of four pretreatment methods and the overall fermentability of the generated hydrolysates. The hydrolysis efficiency of the carbohydrate polymers in pretreated biomass was analyzed by using high-performance anion-exchange chromatography coupled with mass spectrometry (HPAEC-MS), and the results of this analysis are presented in **Chapter 3**.

The last three chapters of the thesis focus on identifying inhibitory compounds in lignocellulosic biomass hydrolysates and studying their effects on fermenting yeasts during fermentation processes. **Chapter 4** examines the fermentability of a series of biomass hydrolysates, in relation to the presence and dynamics of a target group of inhibitors in these hydrolysates. **Chapter 5** reports the detailed experimental procedure and results of the actual exometabolomics approach introduced in Chapter 1. The research question, identification of inhibitors in biomass hydrolysates, was answered by statistically correlating the fermentability of different biomass hydrolysates with their biochemical compositions. Finally, in search for potential ethanologenic host organisms resistant to biomass hydrolysate inhibitors, a *Pichia anomala* strain was isolated. In **Chapter 6**, the properties and fermentation performance of this strain in biomass hydrolysates were tested. Through further research and possibly genetic modifications, the strain has the potential to become a suitable yeast for fermenting lignocellulosic biomass hydrolysates.