Online Monitoring of Enzymatic Degradation of Cellulose

Andreas Prediger1, Britta Laemmerhirt1, Patrick Lindner1, Sascha Beutel1, Andreas Liese6, Thomas Scheper1

1Leibniz University of Hannover, Institute for Technical Chemistry, Callinstr. 5, 30167 Hannover, Germany
2Hamburg University of Technology, Institute of Technical Biocatalysis, Denickestr. 15, 21073 Hamburg, Germany

Introduction

The degradation of cellulose from natural sources to glucose is intensely investigated as it might open up new possibilities for the production of bioethanol. A three-enzyme complex of an endoglucanase, an exoglucanase, and a cellulase is required for the hydrolysis of cellulose to glucose. As the interest in cellulose based degradation processes increases, adequate process monitoring tools are required. Thus an in situ microscope prototype for imaging and online monitoring of the mentioned processes has been developed. A schematic and a picture of the system setup are shown in Fig. 1 and 2. During a hydrolyzation process large cellulose threads are degraded to smaller ones while glucose is released. Therefore the number of detectable objects per image and the proportion of small particles were identified as key variables for the monitoring of the reactions.

All experiments were carried out using ARBOCEL BE 600-10 TG of Rettenmaier & Sohne as substrate while Rohament CL and Ecostone C1 of AB Enzymes were used for the enzymatic degradation.

Automated Image Analysis

For the automatic investigation of the acquired image data the image analysis software CelluloseAnalyzer was developed. A screenshot of the software can be seen in Fig. 3. In a first step the algorithm detects objects using a binarization step. All pixels in the image with a grey value under a threshold of 200 are counted as belonging to an object. Those pixels are subsequently grouped.

In a second step objects which fall short of a size threshold of 75 pixels (17 μm²) are deleted. All remaining objects are counted and analyzed regarding their size and morphological features.

The generated result table is used as a base for further processing with spreadsheet analysis software.

Process Monitoring Results

Data from a model cellulose hydrolyzation are shown in Fig. 4. 2.5 g/l cellulose substrate was degraded at 50°C over 2 days. To catalyze the reaction Rohament CL solution was added until a protein concentration of 0.125 g/l was reached.

As a reference to the in situ microscopy results the glucose formation was monitored offline using an YSI biosensor.

In the beginning of the experiment a fast generation of glucose is observed. As larger threads of cellulose are degraded to smaller ones the proportion of small particles increases. Below a size of 17 μm² the image analysis software does not detect them anymore and thus the number of detected objects decreases rapidly.

This trend can be observed over the course of the experiment. However the velocity of the reaction decreases steadily as the substrate is consumed. After 48 h almost all cellulose has been degraded.

Conclusion and Perspectives

The presented results indicate that monitoring of enzymatic degradation processes of cellulose can be carried out using in situ microscopy. A robust image analysis software for the processing of the acquired image data has been successfully developed. The presented technique allows quick and easy investigation of new enzymes, substrates and inhibitors as long as changes in particle numbers or size distributions can be detected.

Generally in situ microscopy can be used for all kinds of process monitoring ranging from cultivations of yeasts, micro algae and mammalian cell systems over protein crystallization processes to microcarrier based cultivations and new application possibilities are constantly developed. Moreover the image processing algorithms are constantly optimized to allow the detection of objects with complex shapes and the acquisition of additional process information.

Literature: