**Evaluation of the efficiency of alternative enzyme production technologies**

Enzymes are used in an increasing number of industries. The application of enzymes is extending into the production of lignocellulosic ethanol in processes that economically can compete with fossil fuels. Since lignocellulosic ethanol is based on renewable resources it will have a positive impact on, for example, the emission of green house gasses. Cellulases and hemicellulases are used for enzymatic hydrolysis of pretreated lignocellulosic biomass, and fermentable sugars are released upon the enzymatic process. Even though many years of research has decreased the amount of enzyme needed in the process, the cost of enzymes is still considered a bottleneck in the economic feasibility of lignocellulose utilization. The purpose of this project was to investigate and compare different technologies for production of these enzymes. The filamentous fungus *Trichoderma reesei* is currently used for industrial production of cellulosases and hemicellulases. The aim of the thesis was to use modeling tools to identify alternative technologies that have higher energy or raw material efficiency than the current technology. The enzyme production by *T. reesei* was conducted as an aerobic fed-batch fermentation. The process was carried out in pilot scale stirred tank reactors and based on a range of different process conditions, a process model was constructed which satisfied the course of fermentation. The process was governed by the rate limiting mass transfer of oxygen from the gas to the liquid phase. During fermentation, filamentous growth of the fungus lead to increased viscosity which hindered mass transfer. These mechanisms were described by a viscosity model based on the biomass concentration of the fermentation broth and a mass transfer correlation that incorporated a viscosity term. An analysis of the uncertainty and sensitivity of the model indicated the biological parameters to be responsible for most of the model uncertainty.

A number of alternative fermentation technologies for enzyme production were identified in the open literature. Their mass transfer capabilities and their energy efficiencies were evaluated by use of the process model. For each technology the scale-up enzyme production was simulated at industrial scale based on equal mass transfer. The technical feasibility of each technology was assessed based on prior knowledge of successful implementation at industrial scale and mechanical complexity of the fermentation vessel. The airlift reactor was identified as a potential high energy efficiency technology for enzyme production with excellent chances for success.

Two different pilot plant configurations of the airlift reactor technology were tested in nine fermentations. The headspace pressure was varied between 0.1 and 1.1 barg and the superficial gas velocity in the airlift riser section was varied between 0.02 and 0.06 m/s. The biological model developed in the stirred tank reactor was shown to apply to the airlift reactor with only small modifications: The mass transfer of oxygen in the airlift reactor was studied and a mass transfer correlation containing the superficial gas velocity and the apparent viscosity of the fermentation broth was shown to describe the experimental data well. The mass transfer rate was approximately 20% lower than the literature data for airlift reactors. Mixing in the pilot scale airlift reactor was also studied. As the mixing time was of the same order of magnitude as the characteristic time for oxygen transfer, mixing could also be limiting the process at that scale. The process model for the airlift reactor was also shown to describe the experimental data well for a range of process conditions.

**General information**

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Evaluation of the energy efficiency of enzyme fermentation by mechanistic modeling

Modeling biotechnological processes is key to obtaining increased productivity and efficiency. Particularly crucial to successful modeling of such systems is the coupling of the physical transport phenomena and the biological activity in one model. We have applied a model for the expression of cellulosic enzymes by the filamentous fungus Trichoderma reesei and found excellent agreement with experimental data. The most influential factor was demonstrated to be viscosity and its influence on mass transfer. Not surprisingly, the biological model is also shown to have high influence on the model prediction. At different rates of agitation and aeration as well as headspace pressure, we can predict the energy efficiency of oxygen transfer, a key process parameter for economical production of industrial enzymes. An inverse relationship between the productivity and energy efficiency of the process was found. This modeling approach can be used by manufacturers to evaluate the enzyme fermentation process for a range of different process conditions with regard to energy efficiency.

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Modelling Fungal Fermentations for Enzyme Production

We have developed a process model of fungal fed-batch fermentations for enzyme production. In these processes, oxygen transfer rate is limiting and controls the substrate feeding rate. The model has been shown to describe cultivations of both Aspergillus oryzae and Trichoderma reesei strains in 550L stirred tank pilot plant reactors well. For each strain, 8 biological parameters are needed as well as a correlation of viscosity, as viscosity has a major influence on oxygen transfer. The parameters were measured averages of at least 9 batches for each strain. The model is successfully able to cover a wide range of process conditions (0.3-2 vvm of aeration, 0.2-10.0 kW/m³ of specific agitation power input, and 0.1-1.3 barg head space pressure). Uncertainty and sensitivity analysis have shown that the uncertainty of the model is mainly due to difficulties surrounding the estimation of the biological parameters and to a lesser degree the uncertainty of the viscosity and mass transfer correlations. Until now, the model has been applied to evaluation of energy efficiency at different process conditions and bioreactor designs. Our goal is to expand the model to cover both pilot plant and production scale so that the model may assist downscaling operations as well as production optimization and production planning. Further developments of the model will enable more advanced applications such as model based control and simulated process optimization.

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Towards improved bioprocess operation: monitoring, modeling and control

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Contributors: Gernaey, K., Eliasson Lantz, A., Albæk, M. O., Krühne, U.
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**Example of a process model application: Evaluation of energy efficiency**

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**Investigations of the efficiency of enzyme production technologies using modelling tools**
Growing markets and new innovative applications of industrial enzymes leads to increased interest in efficient production of these products. Most industrial enzymes are currently produced in traditional stirred tank reactors in submerged fed batch culture. The limiting parameter in such processes is often oxygen transfer from the gas to the liquid phase. In many systems there is a trade-off between productivity and efficiency. Often high productivity technologies and high productivity processes are preferred. We have studied the efficiency of oxygen transfer versus productivity in fed batch fermentations of the filamentous fungus Trichoderma reesei in 550 litre pilot scale stirred tank reactors for a range of process conditions. Based on the experimental data a process model has been created, which satisfactorily simulates the effect of the changing process conditions: Aeration rate, agitation speed and head space pressure. Examples of simulation results and outlines of the future uses of the model will be given.

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**Model based bioreactor comparison for cellulase production**

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**Modeling enzyme production with Aspergillus oryzae in pilot scale vessels with different agitation, aeration, and agitator types**
The purpose of this article is to demonstrate how a model can be constructed such that the progress of a submerged fed-batch fermentation of a filamentous fungus can be predicted with acceptable accuracy. The studied process was enzyme production with Aspergillus oryzae in 550 L pilot plant stirred tank reactors. Different conditions of agitation and aeration
were employed as well as two different impeller geometries. The limiting factor for the productivity was oxygen supply to
the fermentation broth, and the carbon substrate feed flow rate was controlled by the dissolved oxygen tension. In order to
predict the available oxygen transfer in the system, the stoichiometry of the reaction equation including maintenance
substrate consumption was first determined. Mainly based on the biomass concentration a viscosity prediction model was
constructed, because rising viscosity of the fermentation broth due to hyphal growth of the fungus leads to significant
lower mass transfer towards the end of the fermentation process. Each compartment of the model was shown to predict
the experimental results well. The overall model can be used to predict key process parameters at varying fermentation
conditions.
Efficiency of enzyme production technologies

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Comparison of Traditional Rushton Disc Turbines with Up-pumping Hydrofoil B2 Impellers in 550 L Pilot Scale Aerobic Submerged Fermentations

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Modelling of an industrial enzyme fermentation by mass transfer estimation

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Gassed and ungassed power draw in a pilot scale 550 litre fermentor retrofitted with up-pumping hydrofoil B2 impellers in media of different viscosity and with very high power draw

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