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The work carried out during the Ph. D. project was part of the European research project called the Babilafuente Bioethanol Project and was focussed on meeting challenges arising from this project in relation to the enzymatic saccharification of pretreated substrates relevant for the project. The work involved evaluation of 1) possible ways to increase the glucose release from the commercial cellulase product Celluclast by boosting with other enzyme activities to increase the enzymatic hydrolysis, 2) comparing differently pretreated feedstock substrates and 3) evaluating a fed-batch substrate feeding strategy to increase the substrate loading in the hydrolysis reaction. The substrate for the enzymatic hydrolysis was primarily steam pretreated wheat and barley straw since these substrates were the primary feedstocks for the Babilafuente Bioethanol process. The initial work showed that there was indeed potential to boost the enzyme activities in Celluclast (arising from Trichoderma reesei) by addition of small amounts of fermentation broth from fungal sources other than T. reesei at optimal reaction conditions for Celluclast, pH 5, 50 °C. The activity(ies) related to the boosting effect were indicated to arise from more efficient or different endoglucanase activities than those found in Celluclast. Evaluating the extent of hydrolysis using the 4 major enzyme activities in Celluclast, which constituted a complete set of enzymes for hydrolysis of cellulose, showed that the most efficient mixture resulted in a glucose release corresponding to ~84 % of the glucose release from Celluclast. It was therefore suggested that other enzyme activities than the 4 four main cellulase activities in Celluclast are necessary for optimal hydrolysis of lignocellulose. Even though Celluclast is a multicomponent cellulase mixture, there are still possibilities for further improvement in terms of providing the most efficient cellulase mixture for lignocellulose hydrolysis. It was shown that substrates evaluated all had some residual hemicellulose in the solid cellulose fraction after pretreatment. This residual hemicellulose was speculated to be interlocking the cellulose moiety wherefore hemicellulolytic activities might benefit the glucose release from cellulase hydrolysis. It is therefore suggested that the boosting effect of enzymes in the fungal fermentation broth might to some extent account for the boosting effect and that the hemicellulolytic activities (and remaining cellulolytic activities not evaluated) might account for the lower glucose release obtained with monocomponent activities from T. reesei compared to Celluclast. Evaluation of barley and wheat straw substrates subjected to different pretreatment conditions; hot water extraction and acid- or water impregnation followed by steam explosion showed there were slight differences between the effect of pretreatment conditions in relation to the overall yield from enzymatic hydrolysis. The highest glucose concentration was found for barley straw subjected to acid impregnation followed by steam explosion; however when the glucose concentration was related to the glucose potential in the substrates, the highest yield was obtained with hot water extracted. Analysis of the supernatants from the pretreatments by mass spectrometry showed that the water impregnated straw contained primarily pentose oligomers arising from hemicellulose solubilisation in contrast to the supernatants from acid impregnation. A substrate fed-batch strategy, that is, sequential addition of substrate or substrate + enzymes during the enzymatic hydrolysis was evaluated in terms of viscosity of the reaction mixture, the glucose release, and overall yield. The fed-batch reactions consistently provided lower concentrations of glucose and yield compared to reaction where all substrate was added at the beginning of the hydrolysis. In terms of glucose release and cellulose conversion it a compromise was necessary to achieve high glucose release and high cellulose conversion. In terms of keeping the viscosity of the substrate slurry at a low level throughout the enzymatic hydrolysis reaction the strategy proved effective; the reactions which were added substrate during the hydrolysis had consistently lower viscosity. The low level of viscosity was thought suggest that mixing of substrate and enzyme would be more efficient. The work showed that the commercial cellulase product Celluclast can be improved with enzyme activities from other fungal sources and suggested that supplementation of the current multicomponent cellulase product is feasible as a first step to identify promising enzyme activities for lignocellulose hydrolysis. The importance of other enzyme activities other than the main cellulase components was indicated suggesting that increasing the hydrolytic performance could involve addition of hemicellulase activities to complement the cellulase activities found in Celluclast. Further improving the hydrolysis process in relation to the Babilafuente Bioethanol process might be achieved applying a substrate fed-batch strategy, if optimised in relation to timing of the substrate addition, to achieve high substrate loading since this would ensure a low level of viscosity to ensure efficient mixing of substrate and enzymes.

General information
State: Published
Organisations: Technical University of Denmark
Contributors: Rosgaard, L.
Publication date: Aug 2007

Publication information
Original language: English
Electronic versions:
PhD dissertation Rosgaard.pdf
Source: orbit
Source-ID: 228514
Research output: Research › Ph.D. thesis – Annual report year: 2007