Impact of water activity on the effectiveness of high solids enzymatic hydrolysis of lignocellulose

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Introduction

Currently, the cost effectiveness of reduced volume to substrate ratios and efficient water usage in the lignocellulosic bioethanol industry has made operation at high dry matter levels (>20%) absolutely essential if said resources are to bring economically viable and competitive products to the modern market. We have for a number of years been working closely with industry on developing technologies for handling high solids lignocellulosic materials during the processing into bioethanol. Although the technologies have now been developed to commercial scale a handful of studies have reported depressed conversions for traditional enzymatic conversion processes as increasing solids concentrations are introduced. Despite the critical nature of the problem only a few have attempted to uncover underlying reasons for this phenomenon. While obvious issues related to effective mixing and end-product inhibition of enzymatic systems likely plays a contributing role, these have been shown to be only minor contributors to a complicated and somewhat delicate systematic problem.

Water, while simple in composition, almost certainly holds a complexity in nature that is nearly equal to its abundance. Water plays a significant role in all living processes and is a critical and often overlooked component of mankind's many industrial endeavors. The modern food industry has long utilized the measurement of water activity (Aw), a thermodynamically relevant property associated with the availability of water in a system. The less water that is freely available in a system the less likely microbial processes will be able to proceed. Today, the most cost effective processes in the biomass to biofuels industry utilize biological conversion processes where water most certainly plays a crucial role. With this study we aim to highlight the criticality of water in this respect and investigate the degree with which water availability can impact the development of optimal processes.

Results and conclusion

During the development of technologies for operating enzymatic hydrolysis and fermentation of pretreated wheat straw at high solids concentrations it was found that the conversion yield decreased almost linearly from 90 to 38% conversion as the solids concentration increased from 2 to 40%. This observation (the "high solids effect") was confirmed using several types of pretreated biomass as well as on pure cellulose substrate and the effect was also observed in SSF experiments.

The "high solids effect" was studied across multiple enzyme activities and plant cell wall substrate types. Using purified enzyme preparations revealed negligible signs of this solids effect. However, separating out the low molecular weight fraction from commercial enzyme preparations and adding them back to the purified enzymes restored the solids effect. Based on this it was concluded that the significant concentration of low molecular weight soluble species that occurs during saccharification and fermentation processes at high dry matter levels may impact water activity and consequently the effectiveness of enzymatic systems. Addition of other low molecular weight species from the process inputs, such as commercial enzyme preparations, to soluble sugars from insoluble lignocelluloses confirmed the observation. By measuring the water activity under various hydrolysis conditions it was possible to show the relationship between depressed conversions under increasing dry solids loadings and the availability of water in the system.

Lastly, we investigated the states of water under various high solids hydrolysis scenarios using low-field NMR spectroscopy. This technique allows for the effective measurement of proton relaxation in systems upon exposure to a defined magnetic field. The information can in turn be utilized to discriminate between the various states in which water exists in the complex hydrolysis environment. In this study the relative constraint and presence of groupings of tightly bound, weakly bound and free water are assessed in systems containing high levels of dry lignocellulose with respect to the increased presence of water soluble species such as glycerol, sugars, surfactants and ethanol. From these measurements it can be seen that as the solids concentration increased in the system, the amount of free and weakly bound water decreased. Addition of water soluble species distorts the distribution of the water pools in this system with already constrained water.

The data presented highlights the critical importance of water in biomass conversion processes and suggests that a greater attention to the complexity of its role may elucidate practical solutions to common cost-impacting bottlenecks in the bioethanol production from lignocellulosic biomass.

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