



# Multiscale Dynamics of Hemicellulose Hydrolysis for Biofuel Production

## Abstract

Hemicelluloses are the earth's second most abundant structural polymers, found in lignocellulosic biomass. Efficient enzymatic depolymerization of hemicelluloses by cleaving their  $\beta$ -(1 $\rightarrow$ 4)-glycosidic bonds to produce soluble sugars is instrumental to the cost-effective production of liquid biofuels. Here we show that the enzymatic hydrolysis of hemicellulose is a multiscale reactive process comprising of molecular scale, pore scale, and reactor scale, and that the complex, coupled interactions between the scales, leading to oscillatory spatial patterns in the reactor, improve sugar yields. We use a tightly coupled experimental and theoretical analysis to quantify the dominant phenomena at each scale during hydrolysis.

Our substrate characterization results show that the average pore size of xylan is 9.8 nm, which is tenfold smaller than its average molecular size (109 nm), which in turn is significantly smaller than the reactor length scale. Thus, the pore scale is the smallest of the three scales involved in hemicellulose hydrolysis, and therefore, the sequence of the length scales is: *Reactor scale* > *Molecular scale* > *Pore scale*, which is a deviation from the classical sequence of scales in most multiscale reactive processes.

Adsorption of the enzyme to the solid particles of xylan is the first step in hemicellulose hydrolysis, followed by the cleaving of  $\beta$ -(1 $\rightarrow$ 4)-glycosidic bonds by an endoenzyme in the solid and liquid phases. Our experiments show that enzyme adsorption occurs primarily on the pore surface, and that the adsorption, which is initially non-equilibrium, attains equilibrium at 5 h. Our particle size analysis shows that the hydrolysis system, which is dominated by the surface reaction/ molecular scale in the first 10 min manifested by 45-54% decrease in particle size, is driven by two to fourfold swelling of the hemicellulose and the reaction at the pore scale, leading its porosity to increase to 84-96% in the first 5 h. Further analyses performed by blocking the pores of the xylan particles show that more than 75% of the total soluble sugars produced are from pore scale hydrolysis. Timescale analysis of various transport and reaction processes show that the pore scale reactive dissolution and particle swelling determine the overall time required for soluble sugar production from solid hemicellulose.

Our experimental results show that the hemicellulose hydrolyses in both liquid and solid phases are found to be non-competitively inhibited at the molecular scale by the products xylose and xylobiose, with the inhibition being more potent in the liquid phase. The optimum solid loading is obtained as 5 mg/ml, above which substrate inhibition sets in. Model-experiment comparisons allow us to quantify the adsorption and desorption rate constants and the xylobiose inhibition constants in the solid and liquid phases.

Our reactor scale analysis shows that reactor mixing does not alter the non-competitive nature of product inhibition, but produces stronger inhibition that results in 8-15% reduction of sugar yield, as the mixing speed increases from 0 to 200 rpm. The kinetic constants ( $K_m, V_{max}, K_x$ ) assume mass-transfer disguised values at no or low mixing speed. An optimal mixing strategy, comprising of 55-70 min of initial rapid convective/ macro-mixing followed by diffusive/ micro-mixing for the rest of the hydrolysis,

increases xylose and reducing sugar yields by 6.3-8% and 13-20%, respectively, over continuous mixing at 200 rpm, for 1-5 mg/ml substrate loading at an optimum enzyme to substrate ratio of 1:20, with an energy saving of 94-96% over 24 h. When using the optimal mixing strategy, the reaction time is the rate-determining timescale in the hydrolysis system till the optimal mixing time, after which the micromixing (or diffusion) time in the reactor becomes the rate-determining timescale for hemicellulose hydrolysis.

The interplay between pore scale processes such as adsorption and reactive dissolution with reactor scale macromixing is further amplified by the interaction between activator-inhibitor type molecular reaction and micromixing. This leads to oscillatory spatial (Turing) patterns in tubular reactors, which increase reducing sugar yields by 9-11%, 16-19%, and 3-6% over no mixing (0 rpm), continuous mixing (150 rpm), and optimal mixing, respectively, while xylose yield increases by 4-5%, 7-9%, and 2.8-3.2%, respectively, for 1-5 mg/ml substrate loading. We simulate a reaction-diffusion model to predict the Turing patterns formed during hemicellulose hydrolysis in tubular reactors, and validate our simulations with the experimentally-obtained spatial patterns. Our model-experiment comparison shows that the activator and inhibitor are out-of-phase, and the amplitudes of the activator's spatial patterns decrease with time, while those of the inhibitor increase significantly. We use this example to illustrate how the complex, coupled interactions between the three scales can be used to engineer an enhancement of soluble sugar and bioethanol yields.

*Keywords:* Multiscale dynamics; Hemicellulose; Pore scale dissolution; Molecular scale non-competitive inhibition; Reactor scale mixing; Turing patterns; Enzymatic hydrolysis; Kinetic and reaction-diffusion modeling