

Energy Biosciences Institute

Surface Kinetic Mechanisms of Enzymatic Cellulose Deconstruction

E. Rodriguez Porcel, S. A. Maurer, and C. J. Radke

Chemical Engineering Department, University of California

Berkeley, CA 94702-1462

radke@berkeley.edu

Project EBIO7-J122

Abstract

The rate-determining step in the biological decomposition of lignocellulosic feedstocks is the enzymatic cleavage of lignocellulose found in plant cell walls to simple sugars. Currently, this process is accomplished through the reaction of solid lignocellulose crystals suspended in an aqueous solution containing various enzymes and cellulase-producing bacteria. Product saccharides are subsequently fermented into alcohols or transformed through other non-biological processes into sources of liquid fuel.

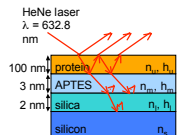
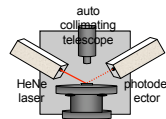
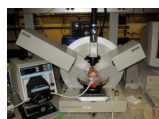
The rate of lignocellulose deconstruction is governed by surface interactions between crystalline lignocellulose and aqueous cellulase. Rate data currently available on cellulose deconstruction kinetics has been obtained through batch studies; little is known about the molecular events that trigger the release of cellobiose from the surface of crystalline lignocellulose. To explore these molecular events, specifically the sorption and catalytic behavior of cellulase(s) at an immobilized cellulose surface, this project focuses on measuring and modeling enzyme kinetics at the aqueous/solid cellulose interface. The ultimate goal, in collaboration with the Energy Biosciences Institute, is to provide a mechanistic understanding of cellulose decomposition and to build a theoretical framework upon which improved cellulases can be designed and synthesized for use in biofuel production.

Goals

- Explore surface enzyme kinetics of **rate-determining step** in decomposition of lignocellulosic feedstocks
- Utilize flow ellipsometry to establish kinetic parameter for **sorption/catalytic behavior** of aqueous cellulase(s) on an immobilized cellulose surface
- Utilize AFM and XRD to characterize **surface of reacted cellulose**
- Describe **molecular events** that trigger release of cellobiose from lignocellulose surface
- Provide structural characteristics and parameters that can be used for **improved cellulase design**

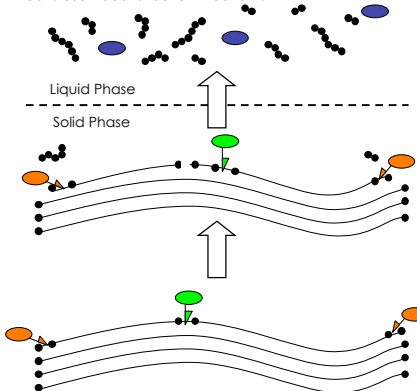
Instrumentation

Flow ellipsometer, as previously applied to enzymatic cleavage of protein stains.



Background

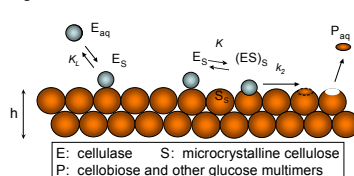
Cellulose Deconstruction Mechanism



Cellulase Classification

- **Endoglucanase** cleaves in middle of cellulose chains, producing smaller units
- **Exoglucanase** cleaves ends of cellulose chains, producing soluble multimeric sugars consisting of 2-6 monomers
- **β -glucosidase** cleaves β -bond in soluble sugars, producing glucose monomers

Langmuir-Michaelis-Menten Kinetic Model



Modification of standard Michaelis-Menten enzyme kinetics to account for adsorption of enzyme to immobilized substrate surface

$$\rho_s \frac{dh}{dt} = -\frac{k_2 K_T \Gamma_{\max} K_L [E]_{\text{bulk}}}{1 + K_L [E]_{\text{bulk}} (1 + K_T S)}$$

Criteria for a "Good Enzyme"

1. Enzyme binding is reversible
2. Enzyme binds strongly at low substrate concentrations (large K_L , Γ_{\max})
3. Enzyme catalyzes reaction well (large K , k_2)
4. Free from allosteric inhibition

Methodology

Materials

Enzyme: Cellulase from *Trichoderma reesei* ATCC 26921 (Novozyme Corp., Sigma #C2730)
Substrate: Avicel® Cellulose PH-101 (Sigma # 11363)
Surfactant: Sodium dodecyl sulfate (SDS) (Sigma # 436143)
N-methylmorpholine-N-oxide (NMMO) (Sigma # 67873)

Sample Preparation

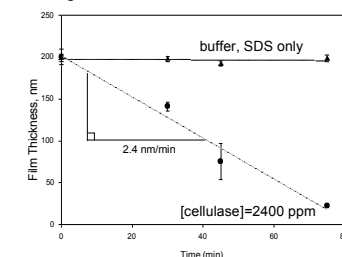
- Plasma clean 1 cm x 1 cm silicon wafer
- Soak wafers in 0.1% poly(diallyldimethylammonium chloride) solution
- Spin coat NMMO (59%), H₂O (15.85%), Avicel® cellulose (2%) and DMSO (24.15%) solution at 3000 rpm

Thickness Measurements

- Soak cellulose-coated wafers in an aqueous solution of enzyme for specified times
- Use ellipsometer to determine cellulose film thickness.

Preliminary Results

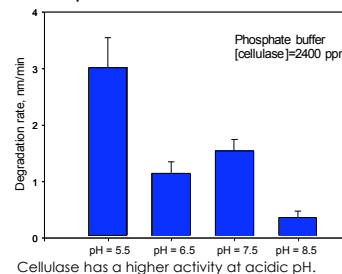
Film Degradation



- No degradation observed when wafer is washed with water, buffer, and SDS.

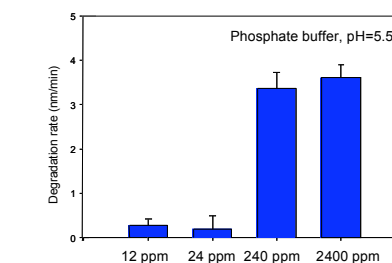
- Linear degradation confirms film homogeneity.

Effect of pH



Cellulase has a higher activity at acidic pH.

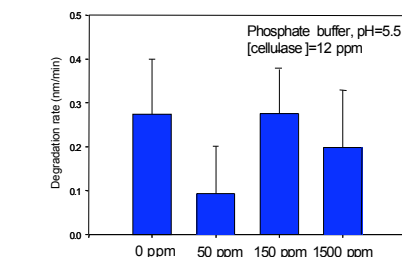
Effect of Enzyme Concentration



- An increase in the enzyme concentration from 240 to 2400 ppm does not affect the degradation rate, because all the binding sites on the cellulose surface are occupied at these high concentrations.

- Consistent with Langmuir-Michaelis-Menten kinetic model.

Effect of SDS Concentration



Studied concentrations of SDS do not affect the degradation rate.

Conclusion

Measuring surface deconstruction kinetics of microcrystalline cellulose by cellulases is feasible.

Future Work

- Explore roughness and crystallinity of cellulose substrate surface
- Characterize molecular structure and function of cellulases
- Obtain surface kinetic parameters for cellulose decomposition
- Investigate adsorption behavior of enzyme via inactivation
- Examine product inhibition, allosteric or otherwise
- Modify substrates to reflect the reality of biofuel production