**Atomic Force Microscopy Study of Cellulose Thin Films**

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**System**

- **Cellulose**
  - Bacterial cellulose grown in Clarke laboratory from Acetobacter xylinum
  - Read beat in methanol with 2.5 mm beads for approximately 15 mins
  - Small samples centrifuged to remove small glass particles

**Enzymes**

- **β-glucosidase**
- **Cellulohydrolyase**
- **Endoglucanase**
- **Hole**: sequential degradation
- **Common Design**:
  - Carbohydrate binding domains (CBM)
  - Catalytic domains
- **Susceptibility of reaction**:
  - Degree of cellulose polymerization
  - Cellulose crystallinity
  - Surface area

**Carbohydrate Binding Domain**

- **Responsible for binding the enzyme to cellulose**
- **More than 30 different families of CBMs have been identified**
- **Example**: Wedge shaped structure with two flat surfaces

**Film Preparation**

1. **Modify Gold - Self Assembly of Thiols**
   - Gold on glass slides used as cellulose substrate for LB transfers
   - Thin films of cellulose will not stick to surface in solution
   - Cellulose in solution needed for enzyme studies and electrochemistry
   - Need to make surface hydrophilic
   - Use short thiols:
     - Thioglycerol
     - Thioglycolic acid

2. **Cellulose Film - Langmuir-Blodgett Transfer**
   - Spread cellulose dispersion and let volatile solvent evaporate.
   - Compress monolayer and transfer to a solid substrate (gold slide)

**Motivation**

**Cellulose Biodegradation**

- Availability of bioenergy and biofuels depends on our ability to overcome issues with the conversion of biomass
- A key step is the efficient release of glucose from feedstock

**Imaging the enzymatic activity of the cellulose enzymes on a cellulose substrate will help to better understand the synergistic and mechanistic effects of the biodegradation of cellulose.**

**Studying the biodegradation of cellulose is of significant interest, as cellulose currently constitutes a large source of waste biomass. The complete hydrolysis of cellulose leads to an easily fermentable sugar glucose.**

**When glucose is biologically converted into other products such as ethanol, it can provide environmental, economic, and strategic benefits on a large scale. With the depletion of fossil fuels the hydrolysis of cellulose is becoming an increasingly important biotechnology.**

**It is therefore of great interest to completely understand the process of enzymatic degradation and propose industrial applications.**

**Principles**

**Atomic Force Microscopy**

- A magnetic field is used to drive a magnetically coated cantilever at or near its resonance frequency
- The oscillating tip was moved close to the surface so that it just tapped the surface.
- There is a reduction in the oscillation amplitude contributes to the identification of surface features.
- MAC mode is ideal for soft and fragile samples like the proteins used in this study.

**Results**

**Representative Cellulose Fibers**

- Imaging conditions:
  - Thioglycerol: Dried Sample; Contact mode; Water

**High Resolution of Fibers**

- Imaging conditions:
  - Contact mode; Phosphate buffer pH 7.4

**Degradation Studies**

- Target Fiber: 3 mins 30 mins 60 mins
- Sigma Cellulase on cellulose in phosphate buffer (pH 7.4) at Low Temperature (~7 °C)
- Imaging conditions:
  - Au-Thioglycerol and Thioglycolic acid
  - MAC mode AFM
  - Add 0.1 ml 1 mg/ml
  - CenA in phosphate buffer
  - Image over 20 hrs

**Enzymes on Fibers**

- Before enzyme: 3 hrs
- After enzyme: 19 hrs

**Never Dried**

- Conditions:
  - Thioglycerol, no drying
  - MAC mode, phosphate buffer

**Future Work**

- Binding Studies:
  - Improve resolution of enzymes on fibers
- Determine specific binding sites
- Degradation:
  - Use active enzymes on amorphous cellulose samples
- Perform temperature and pH studies

**References:**

1. www.molec.com
2. www.nsrc.org

**Funding:**

[NSERC CRG]

**Collaboration:**

[NSERC CRG]

**CenA on Gold**

- Endoglucanase
  - 25.5 nanometer maximum diameter
  - Heat: catalytic domain
  - Tail Binding domain (10 nm)
  - 47 Å, 8 nm

**Future Work:**

- Bind enzymes to cellulose fibers
- Determine specific binding sites
- Use active enzymes on amorphous cellulose samples
- Perform temperature and pH studies