

Morphological Alteration of Wood Fibres in the Process of Enzymatic Hydrolysis of Cellulose

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Introduction

- Pretreatment is a key step in bioconversion
 - Costs; accessibility; enzyme effectiveness
- Biomass structure and chemical composition
 - physical and chemical barrier to cellulose accessibility
- Fiber morphology changes can reveal the pathways of enzymatic hydrolysis of cellulose

Objectives

- Develop techniques for observing biomass physical structure on both *micro-* and *nano-* scale
- Develop techniques for studying fiber morphology change *in situ* during enzymatic hydrolysis
- Ultimately develop economically viable biomass pretreatment technologies

Experimental

– Substrates

- Poplar steam explosion pulp
- Aspen bleached kraft pulp (Aspen BKP)
- Softwood thermomechanical pulp (R14)
- Softwood bleached kraft pulp (Softwood BKP)
- Wheat straw unbleached soda-AQ pulp

Experimental

- Enzymatic hydrolysis
 - Enzymes
 - 15 FPU/g cellulose Celluclast 1.5 L (cellulase)
 - 22.5 CBU/g cellulose Novozymes 188 (β -glucosidase)
 - Hydrolysis, pH=4.8, 50°C, 2% of consistency, on shaking incubator at 160 rpm
 - Stopping Hydrolysis, boiling for 5min

Experimental

– Analytical techniques

FE-SEM and SEM

- Hitachi SU-70 Field Emission Gun (FEG) SEM
- JEOL 6400 SEM
- Samples dried with CPD

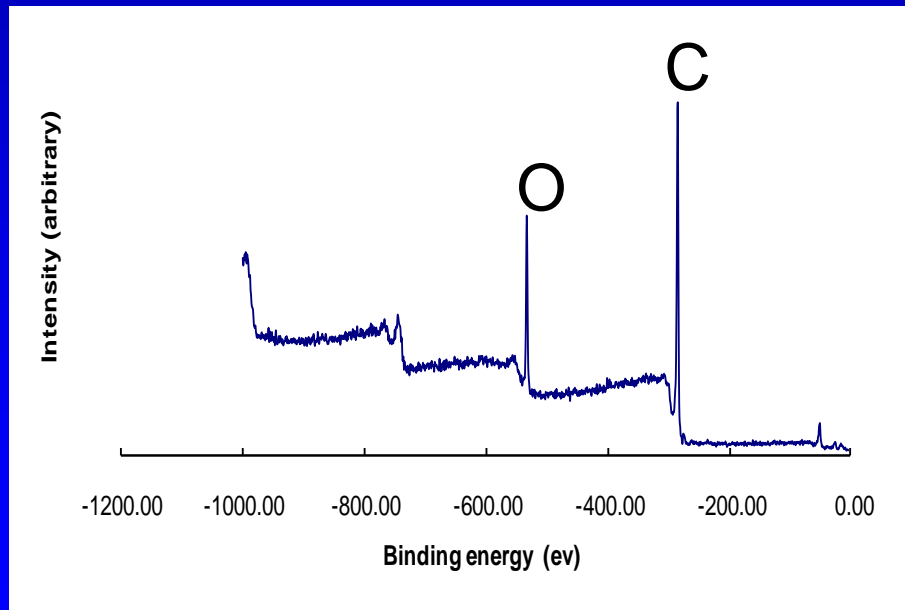
TEM

- JEOL 2011 Scanning Transmission Electron Microscopy
- Samples embedded in resin block and sliced to ultra-thin sections

Experimental

– Analytical techniques

X-ray photoelectroscopy (XPS)

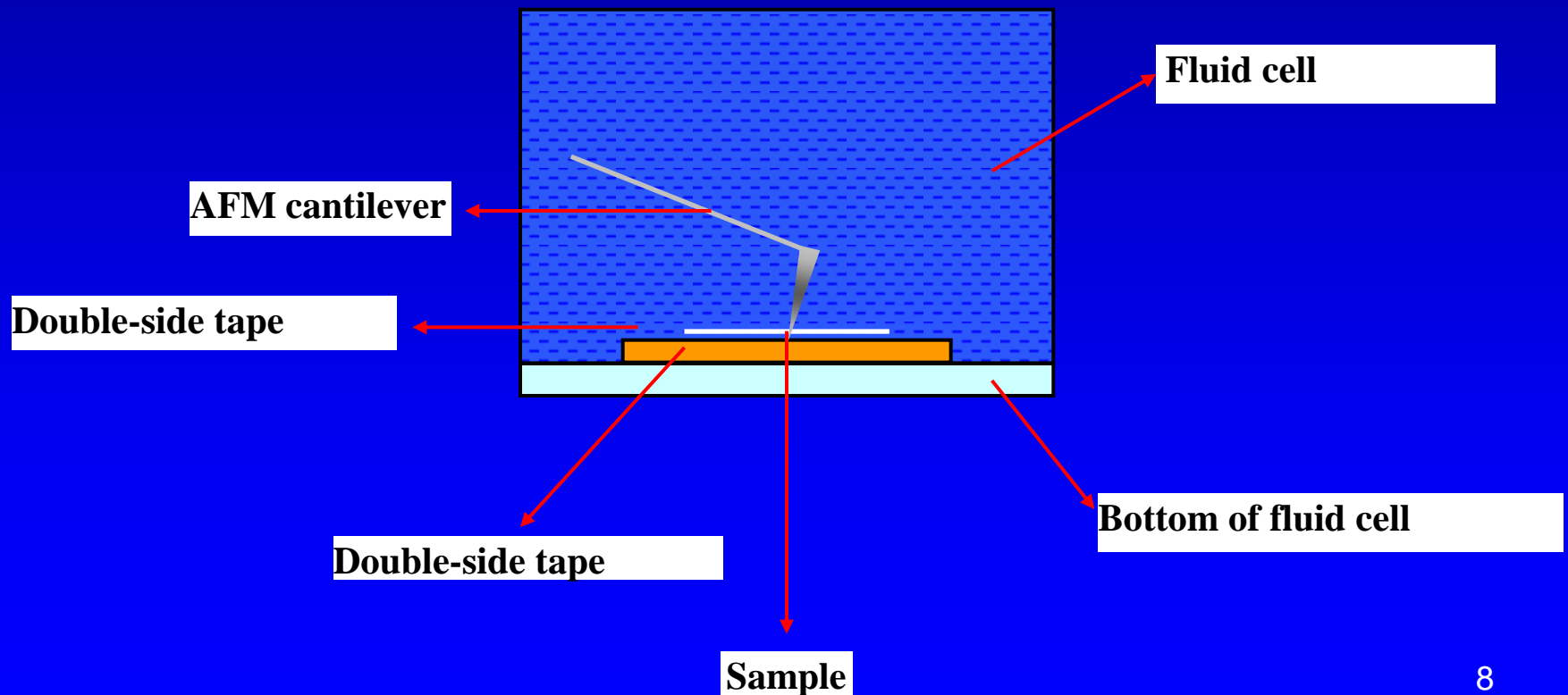


*O/C ratio of
Lignin (0.33),
Carbohydrate (0.83),
extractives (0.12)*

Experimental

- Analytical Techniques

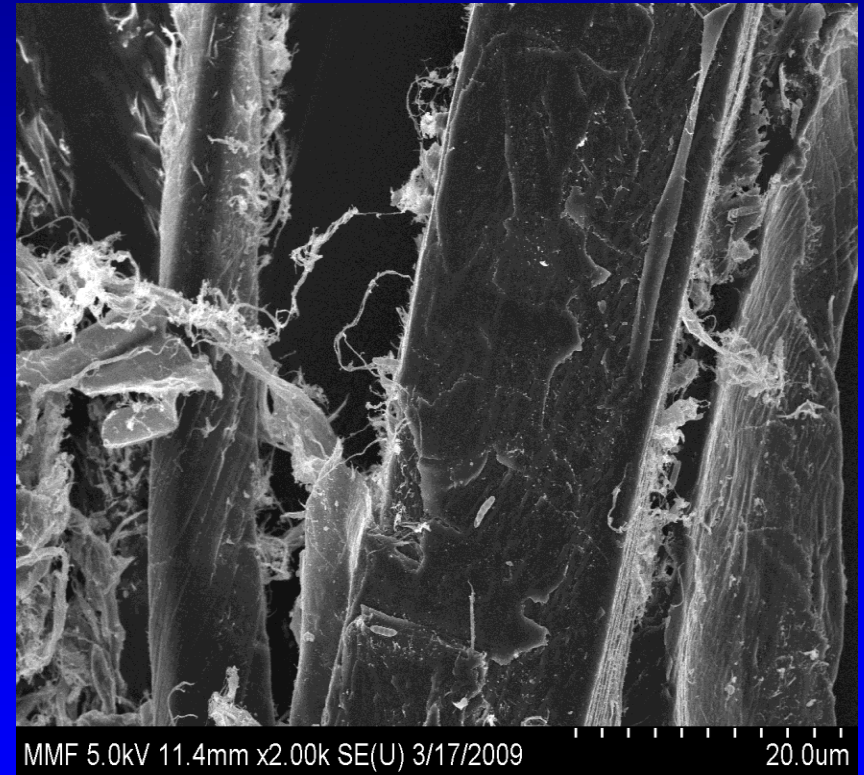
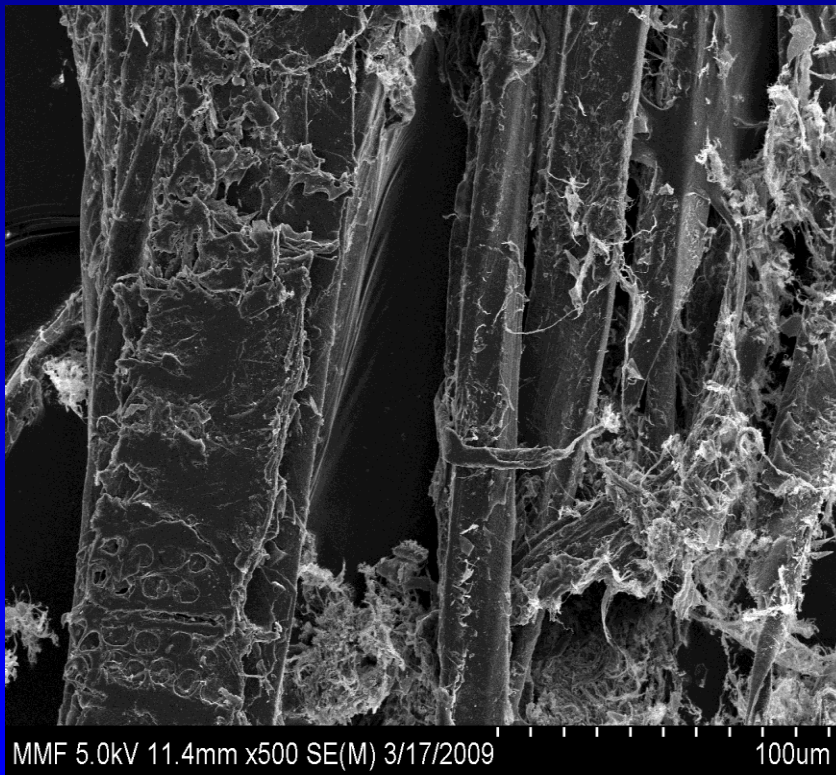
AFM, enzyme hydrolysis conditions in a fluid cell



Preliminary results

Poplar steam explosion pulp

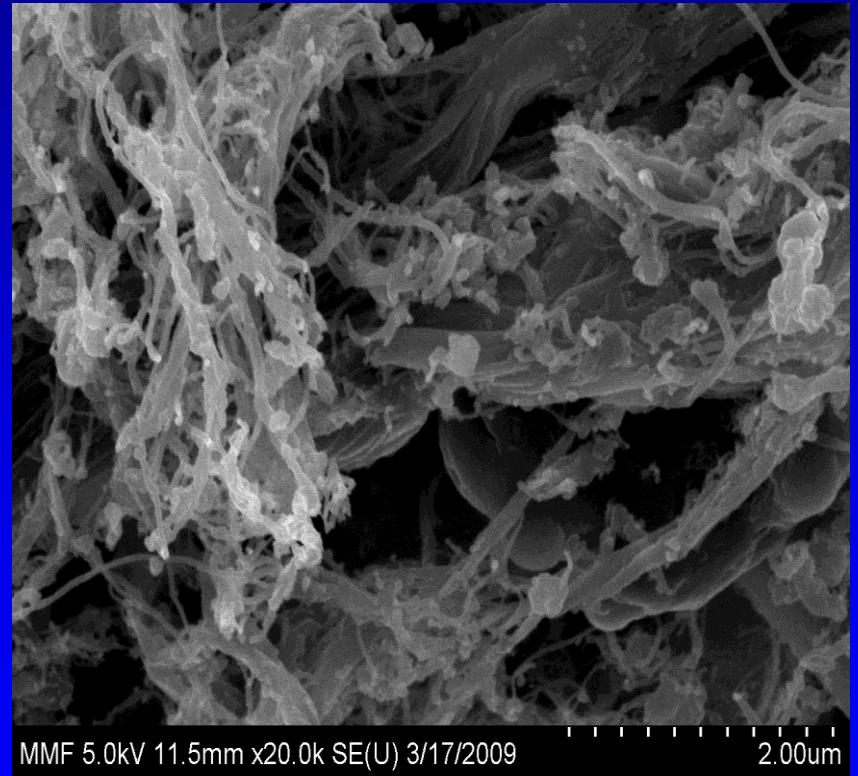
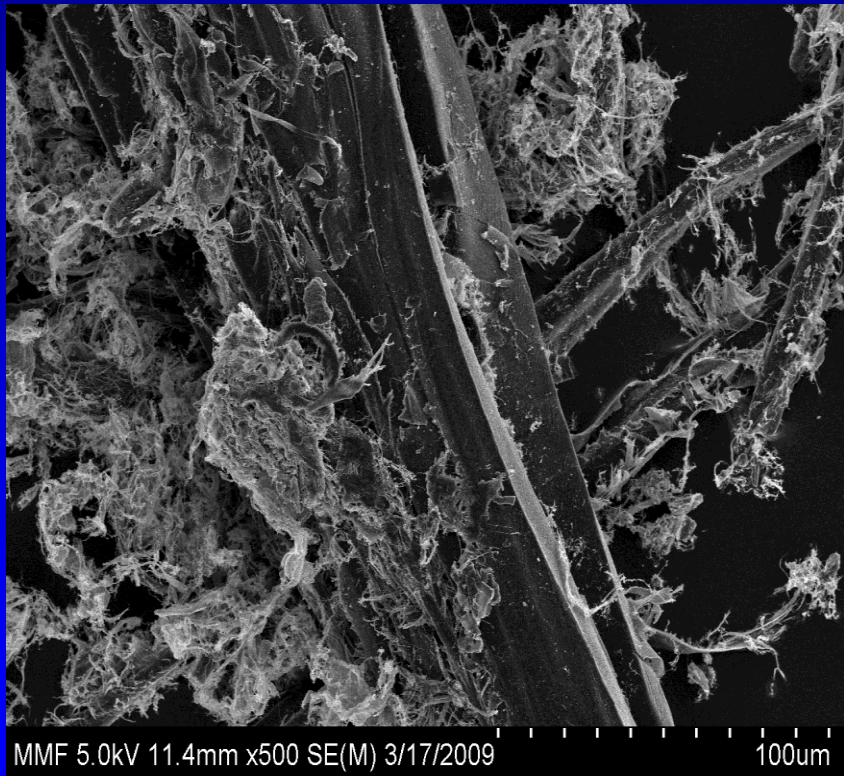
- FE-SEM (original fibers)



Fibres with fibrillation

Poplar steam explosion pulp

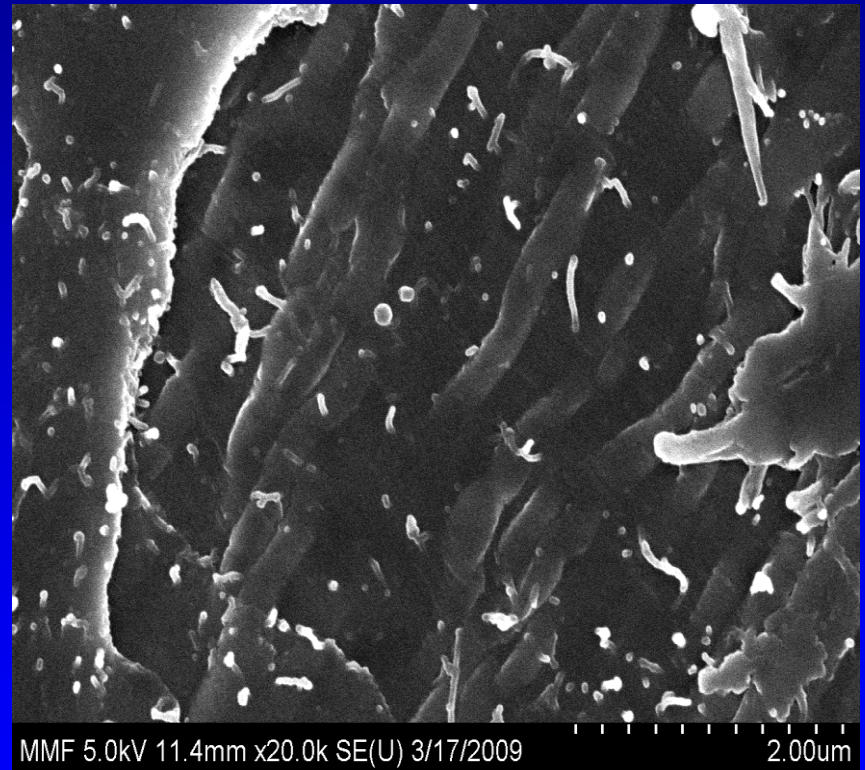
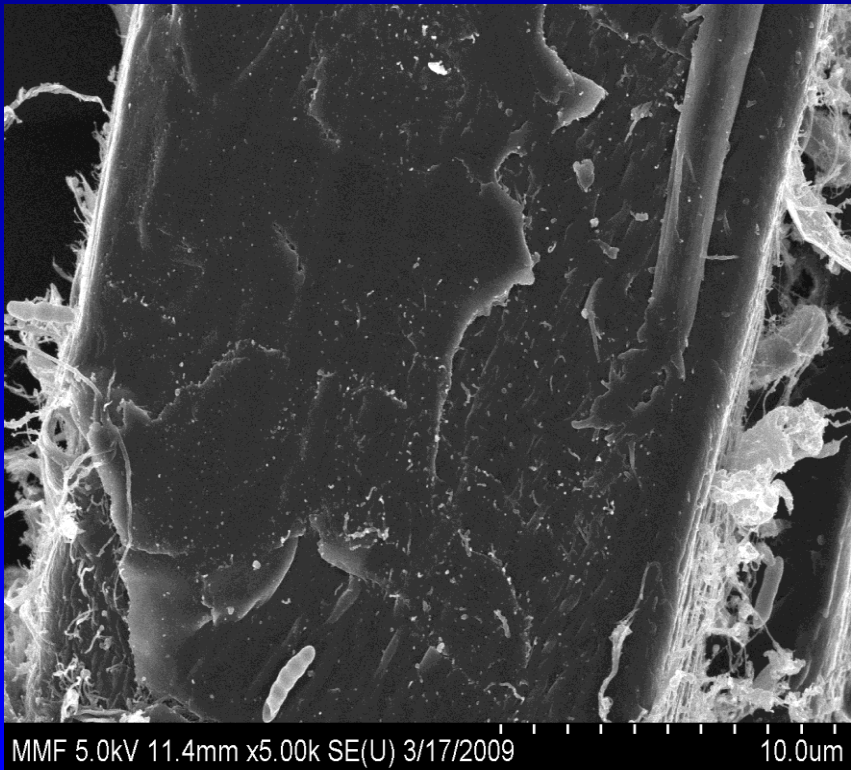
- FE-SEM (original fibers)



Fines attached to fibres

Poplar steam explosion pulp

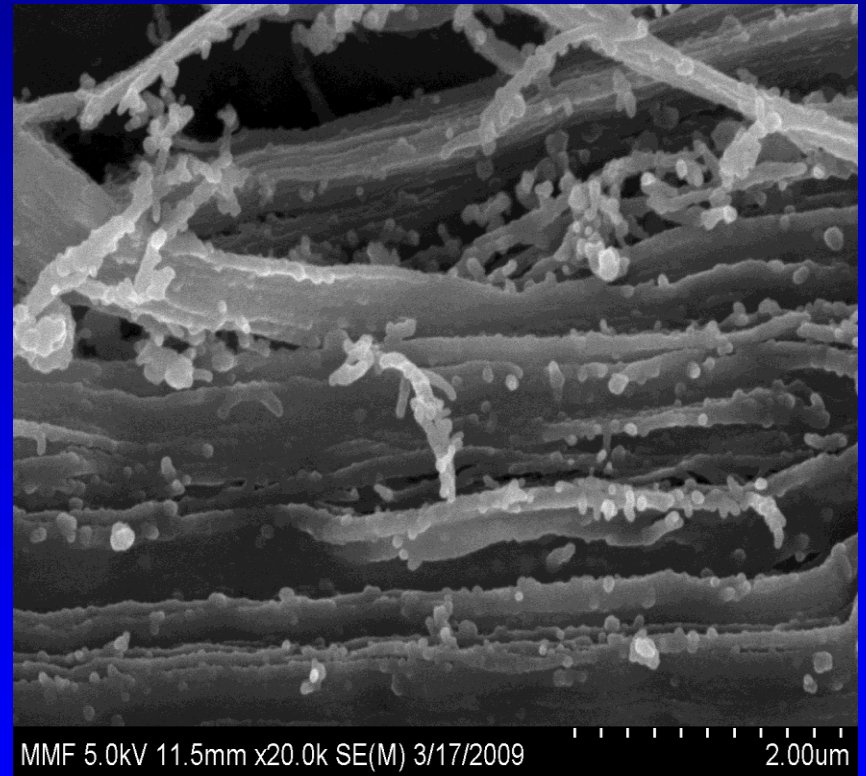
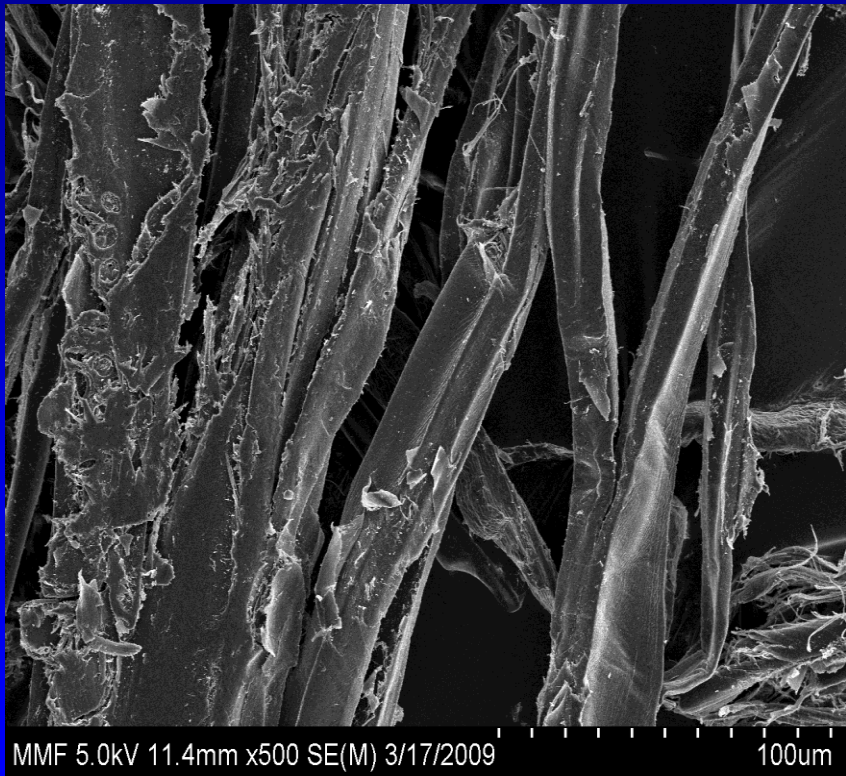
- FE-SEM (original fibers)



*Lignin covering on the fibre surface,
XPS: surface lignin concentration 27% vs. 18% in bulk¹²*

Poplar steam explosion pulp

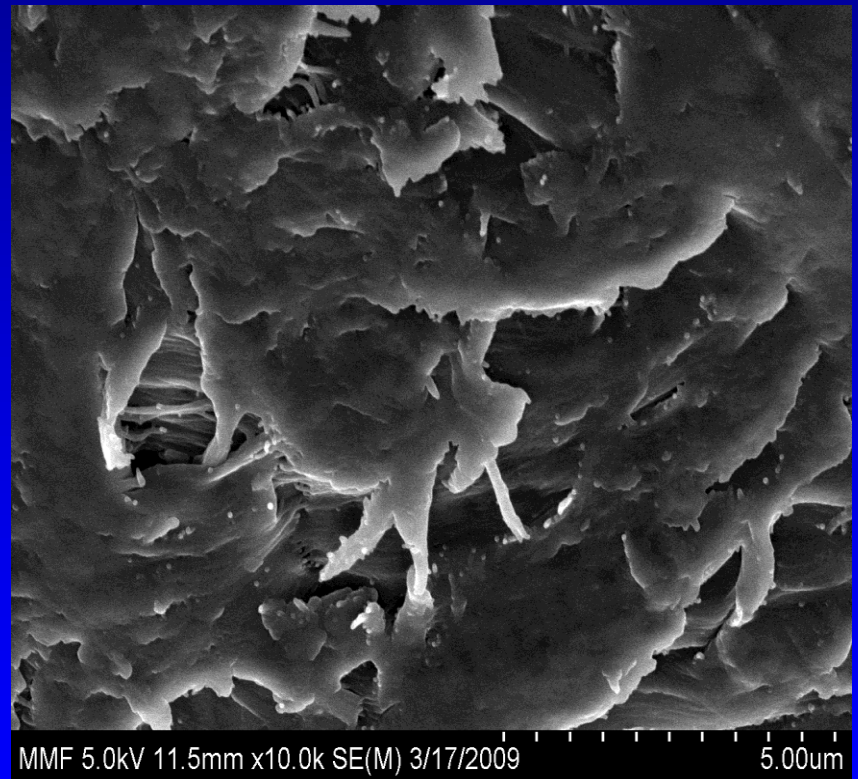
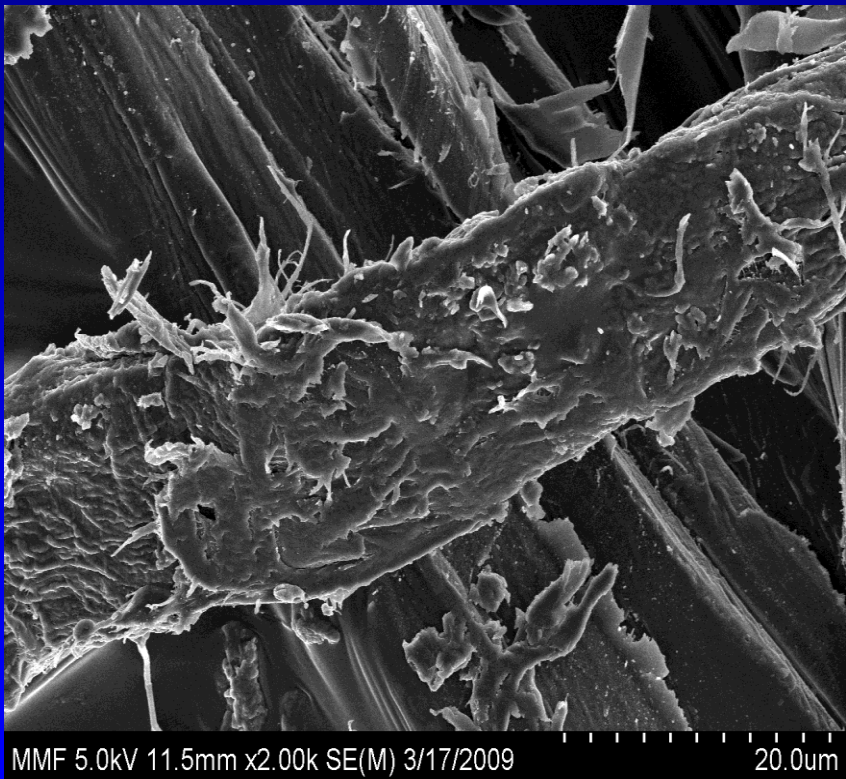
- FE-SEM (hydrolysis 144hrs)



Changes at fibre and fibril levels

Poplar steam explosion pulp

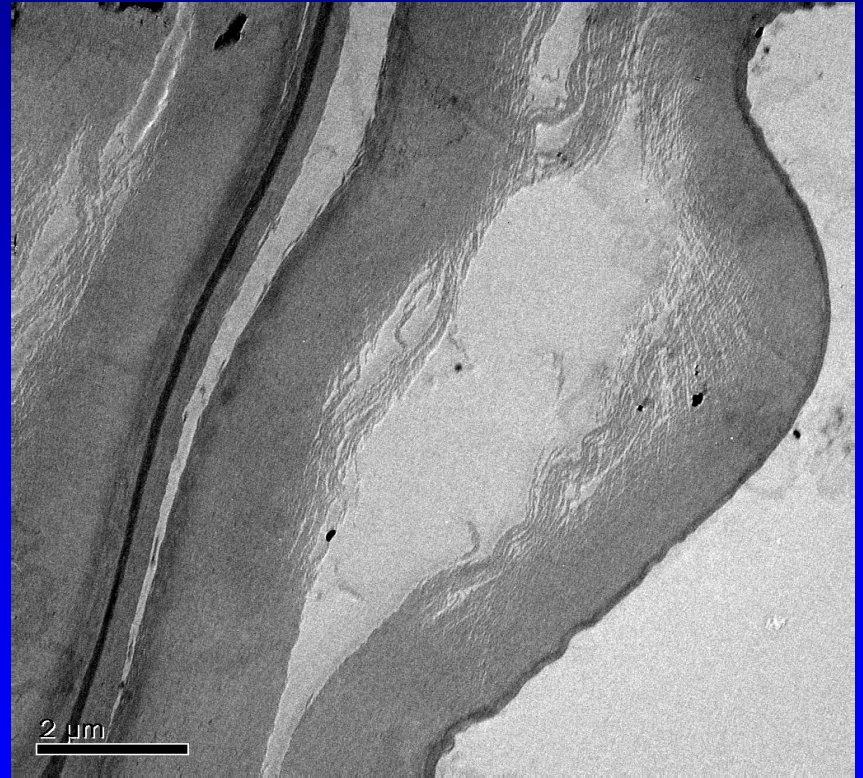
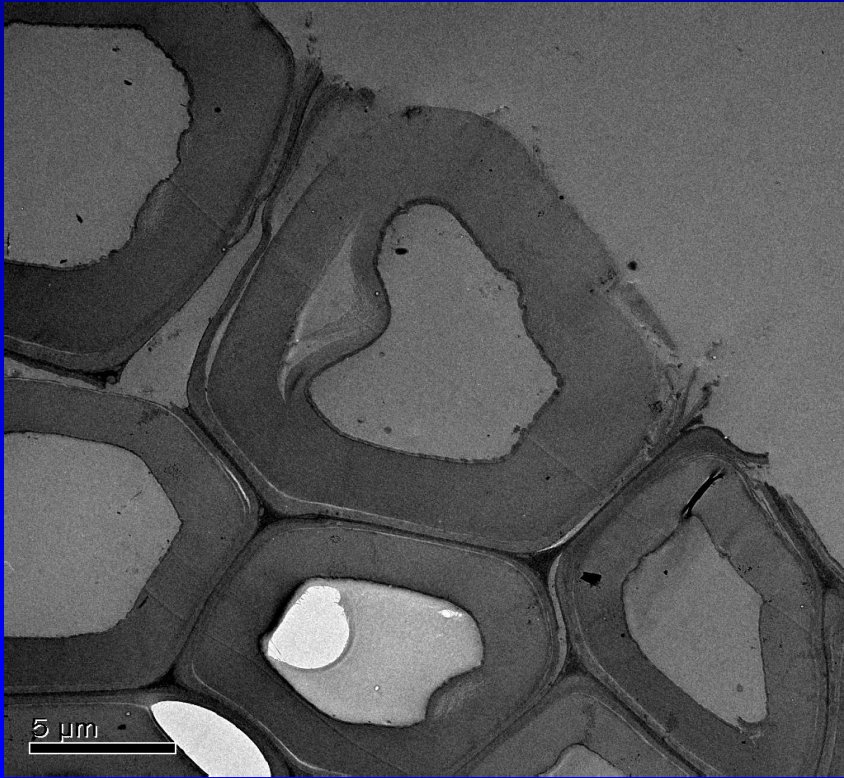
- FE-SEM (hydrolysis 144hrs)



Lignin or hemicellulose residues

Poplar steam explosion pulp

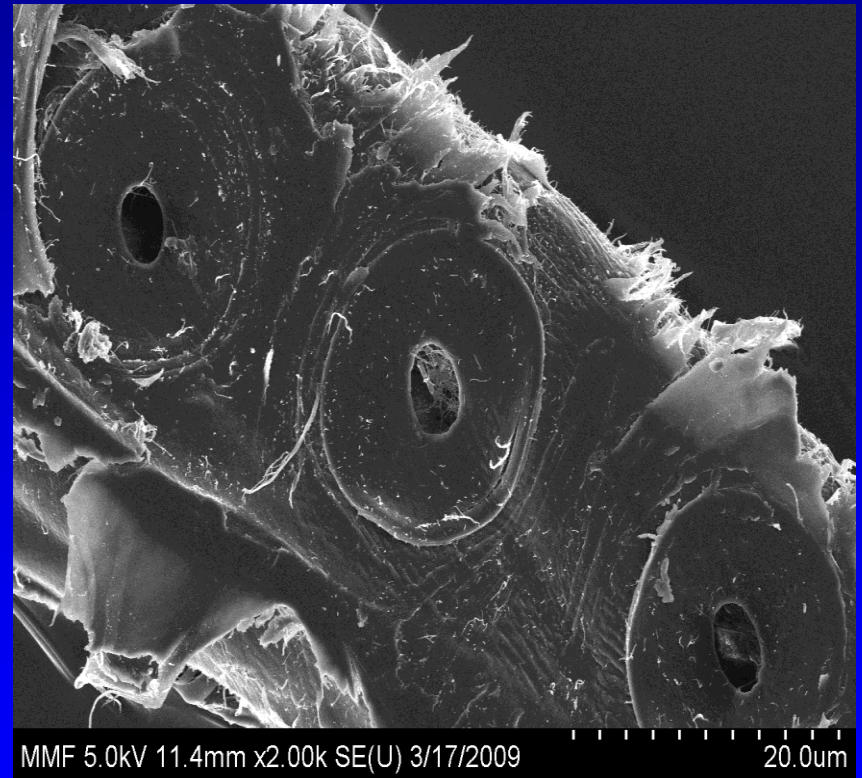
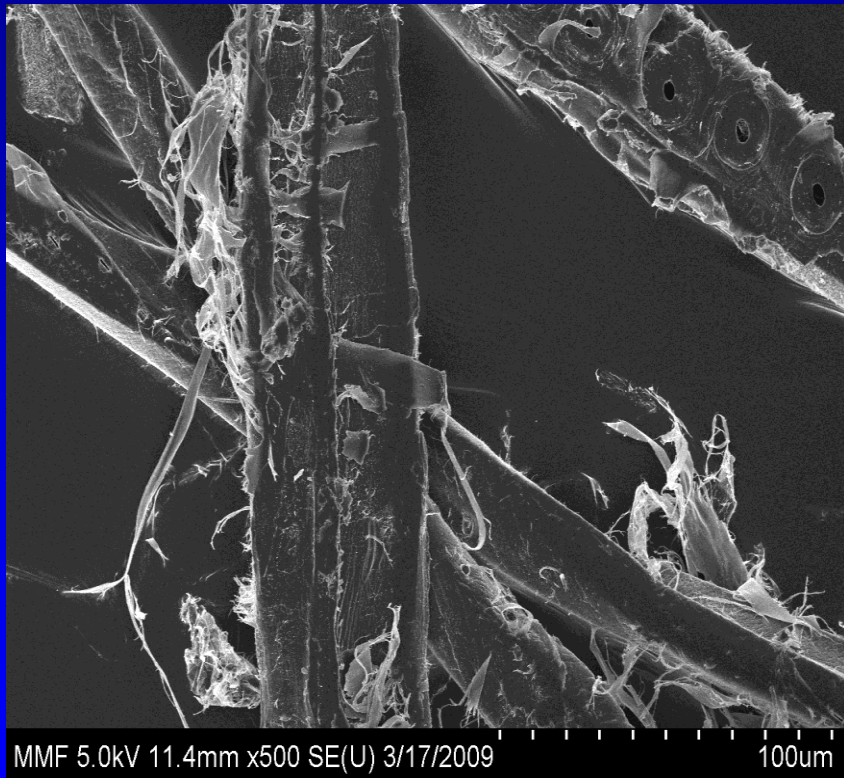
- TEM (hydrolysis 48hrs)



Lignin barrier

Softwood thermomechanical pulp

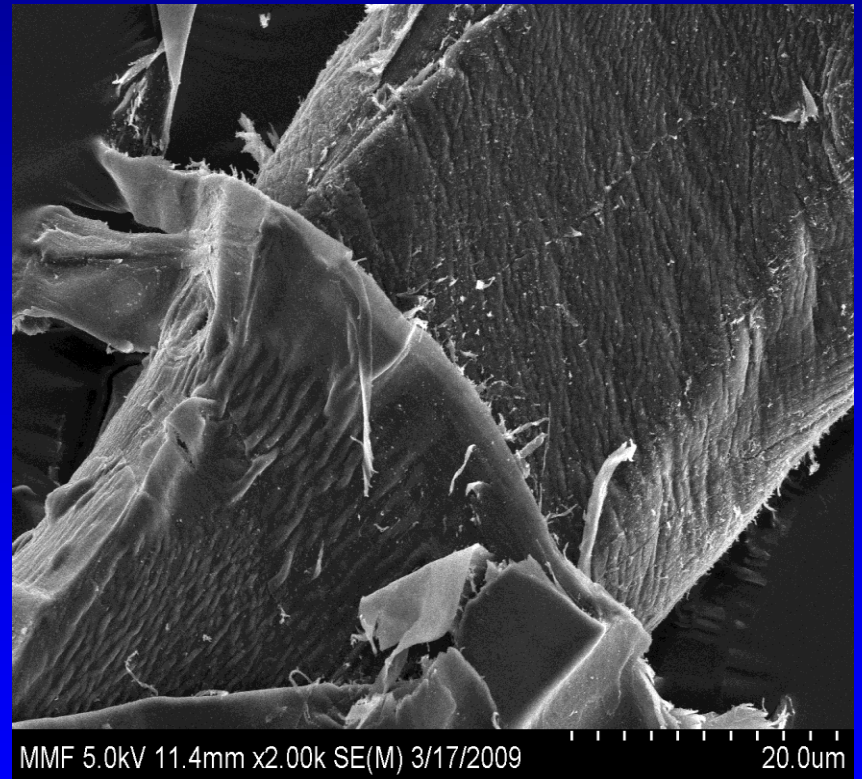
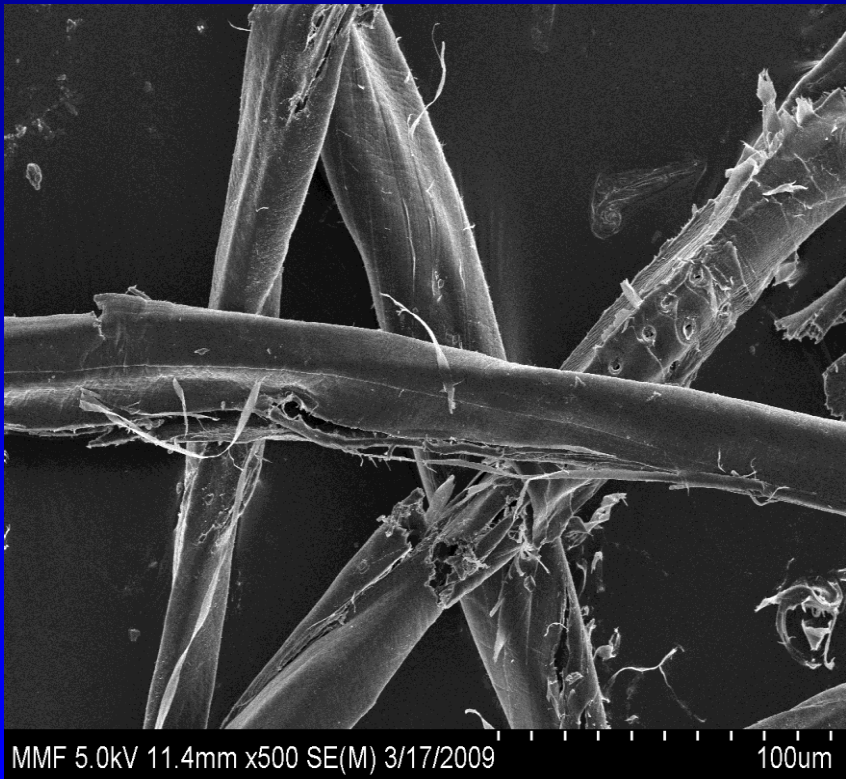
– FE-SEM (original fibers, R14)



Fibres with fibrillation

Softwood thermomechanical pulp

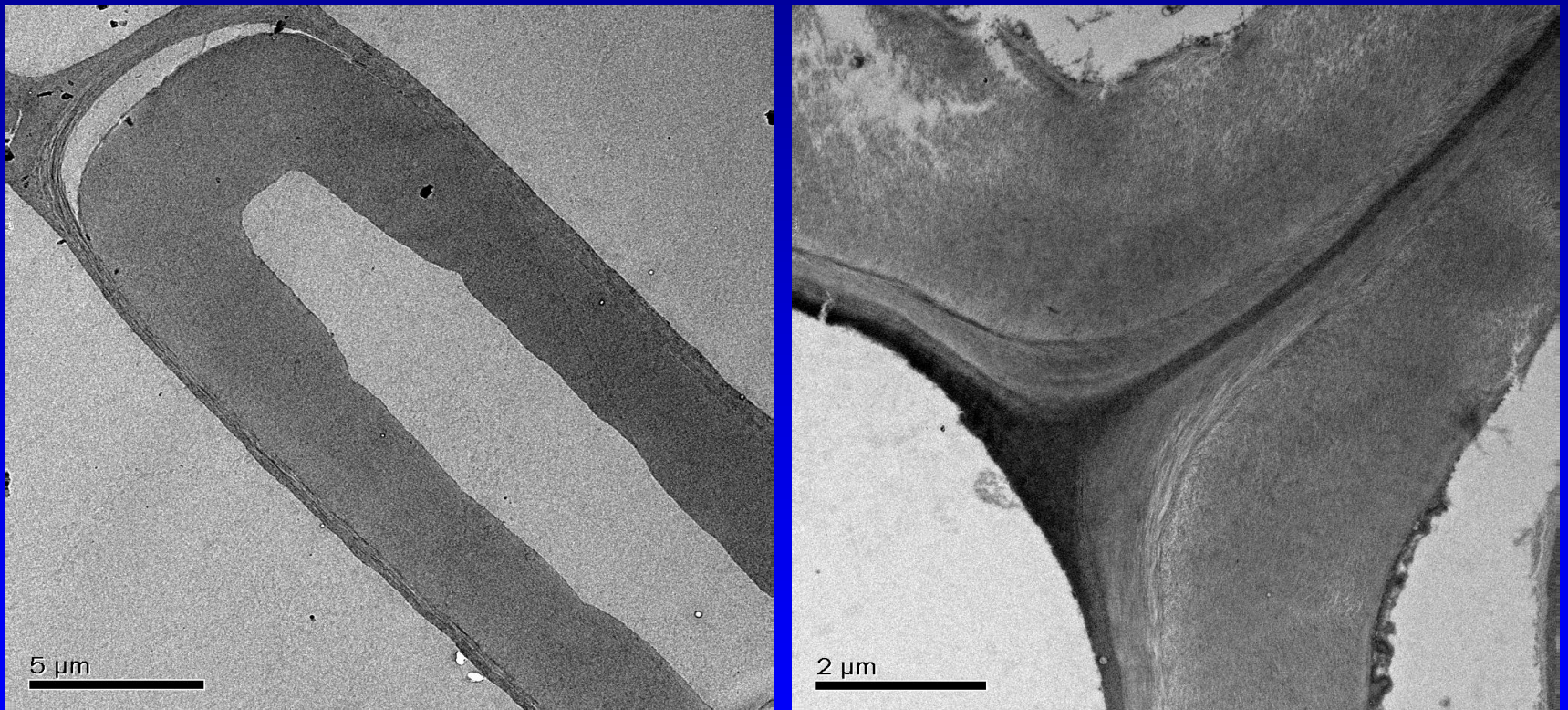
- FE-SEM (hydrolysis 10hrs.)



Cleaner surface

Softwood thermomechanical pulp

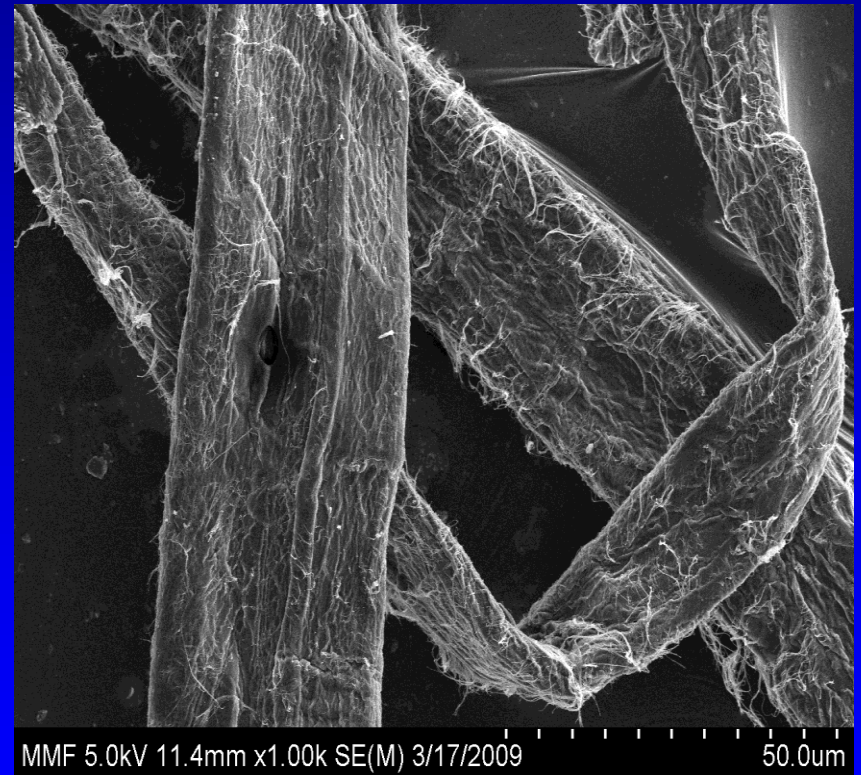
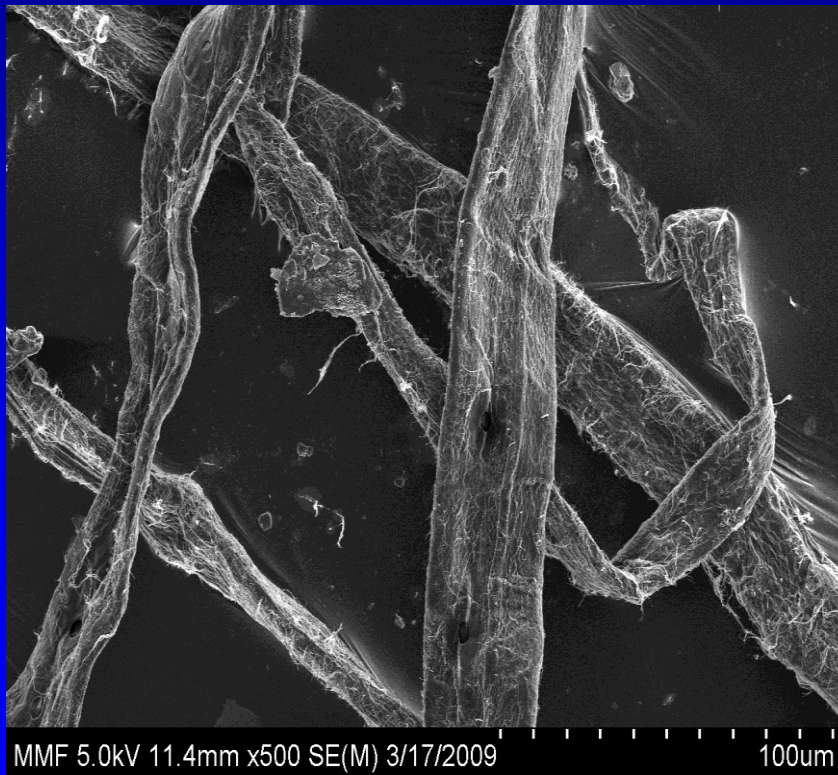
- TEM (hydrolysis 10hrs.)



Lignin barrier

Softwood bleached kraft pulp

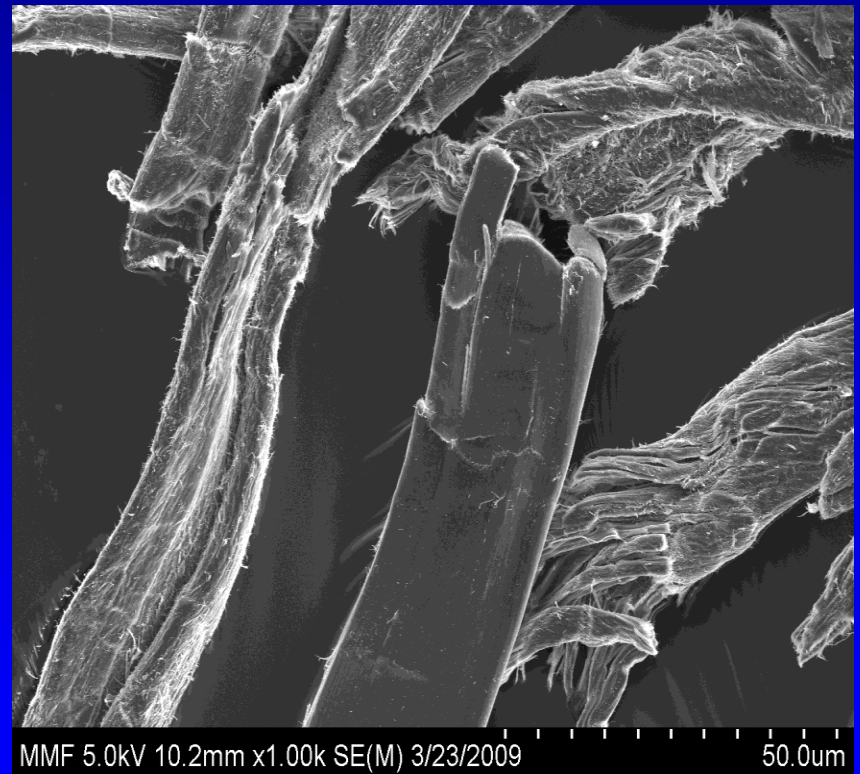
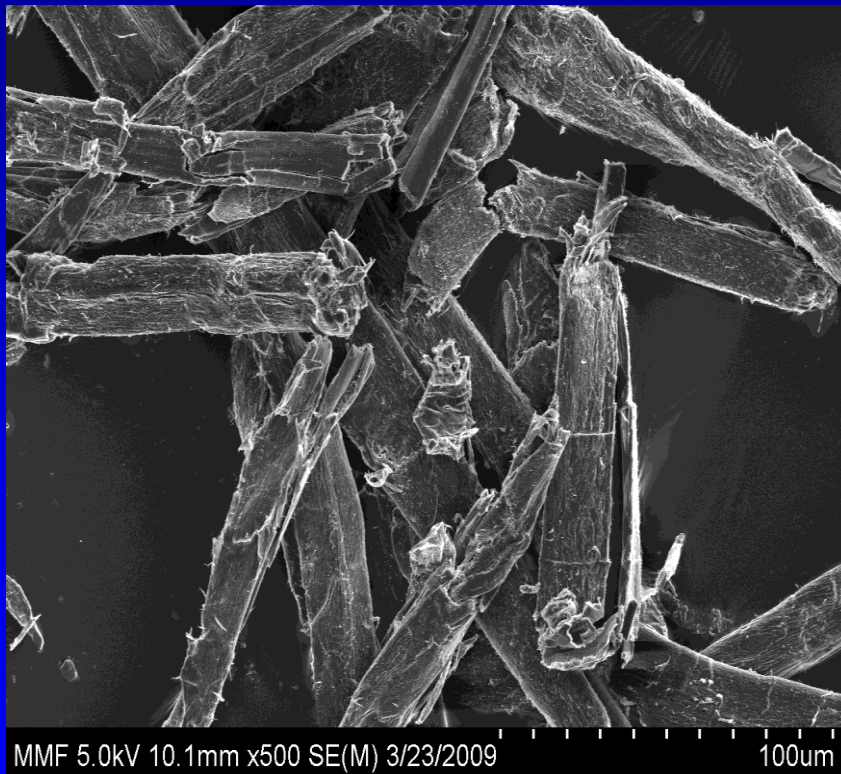
- FE-SEM (original fibers, R14)



Original long fibres

Softwood bleached kraft pulp

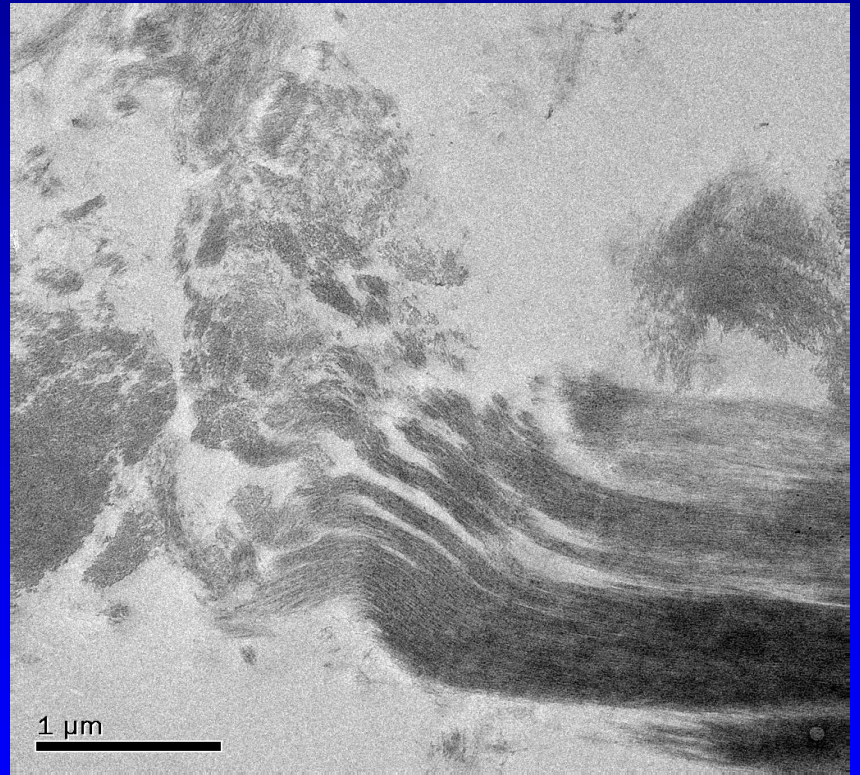
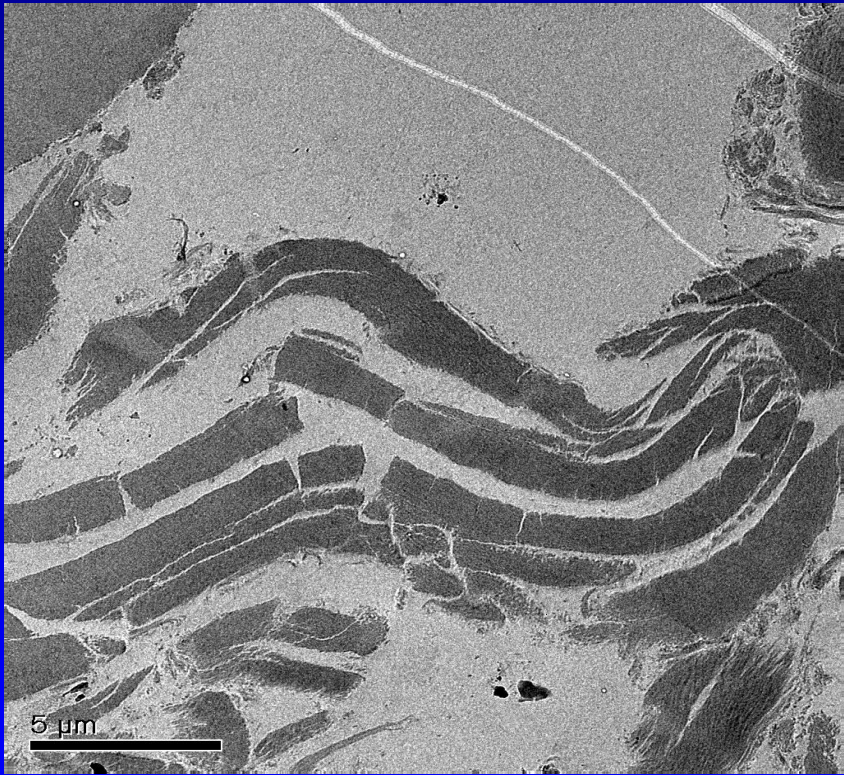
– FE-SEM (hydrolysis 8hrs.)



A lot of cutting

Softwood bleached kraft pulp

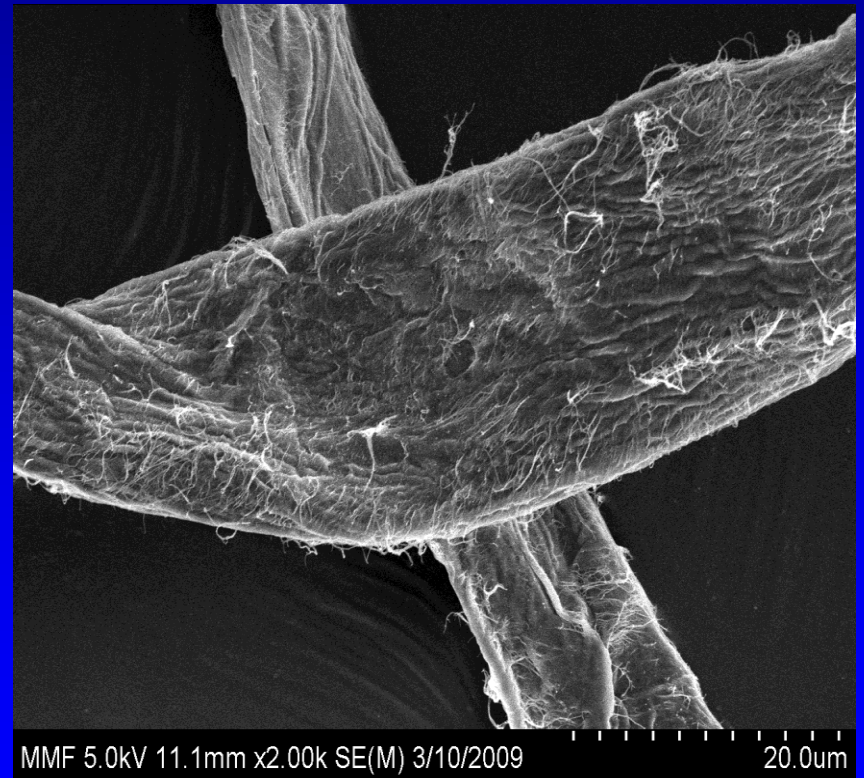
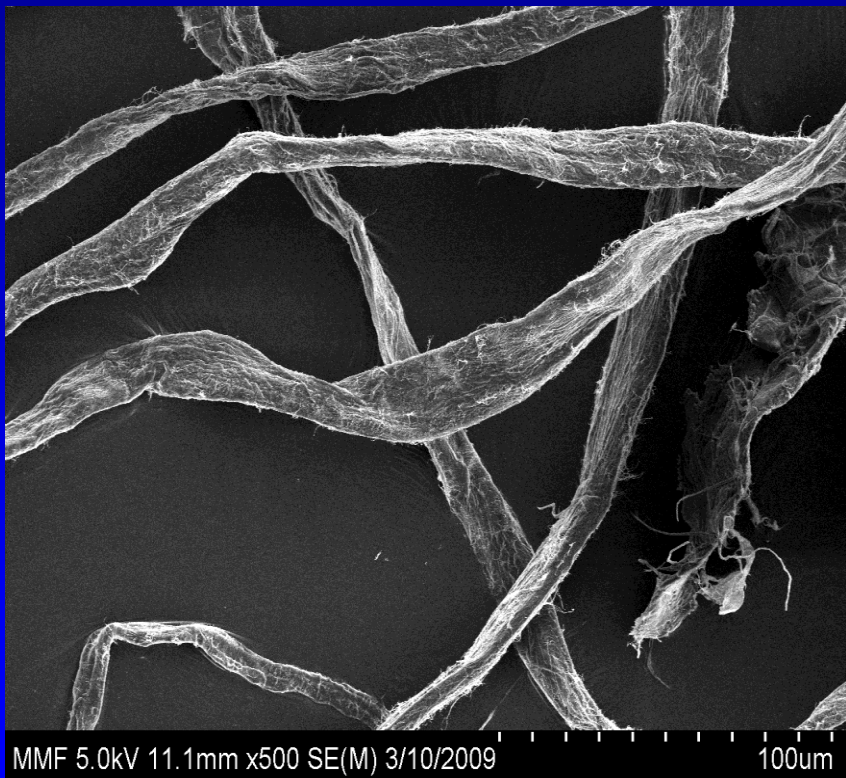
– TEM (hydrolysis 8hrs.)



Almost complete cell wall disintegration

Aspen bleached kraft pulp

- FE-SEM (original fibers)

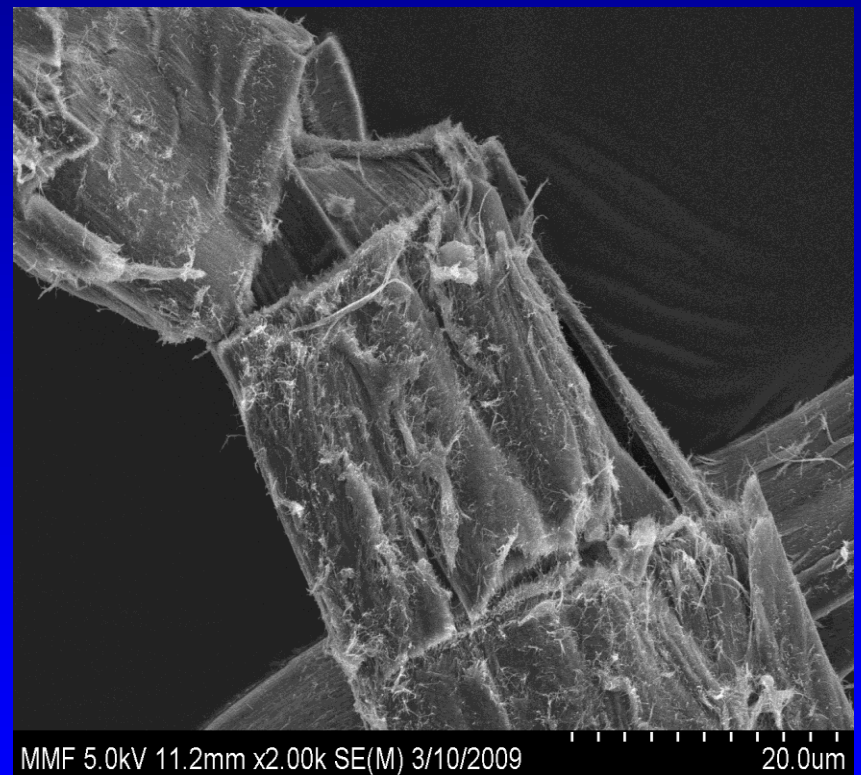
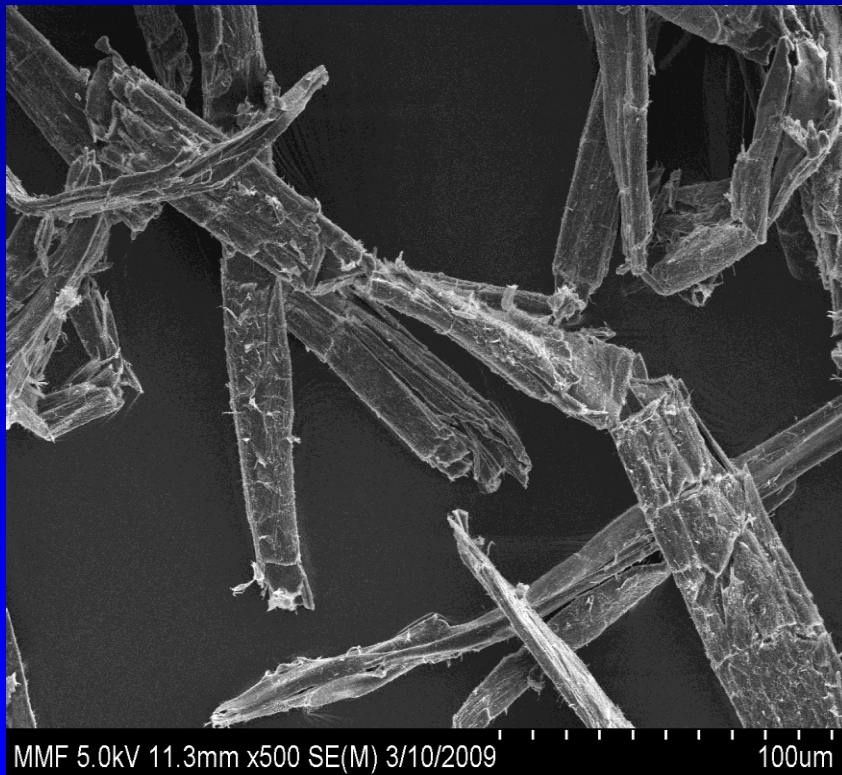


Similar results found with

- Aspen BKP, and
- Wheat straw unbleached soda-AQ

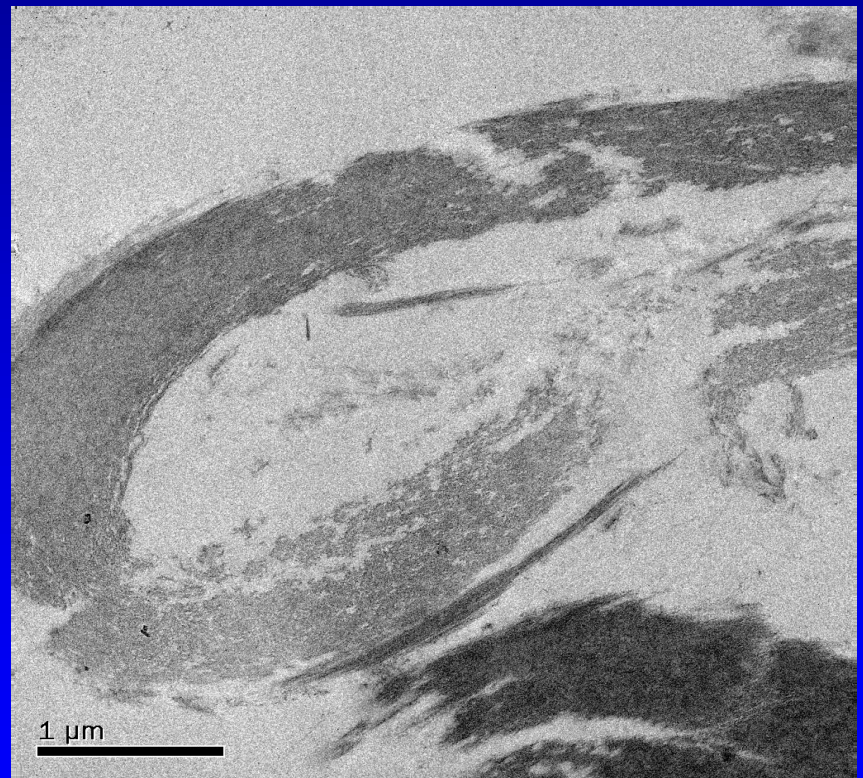
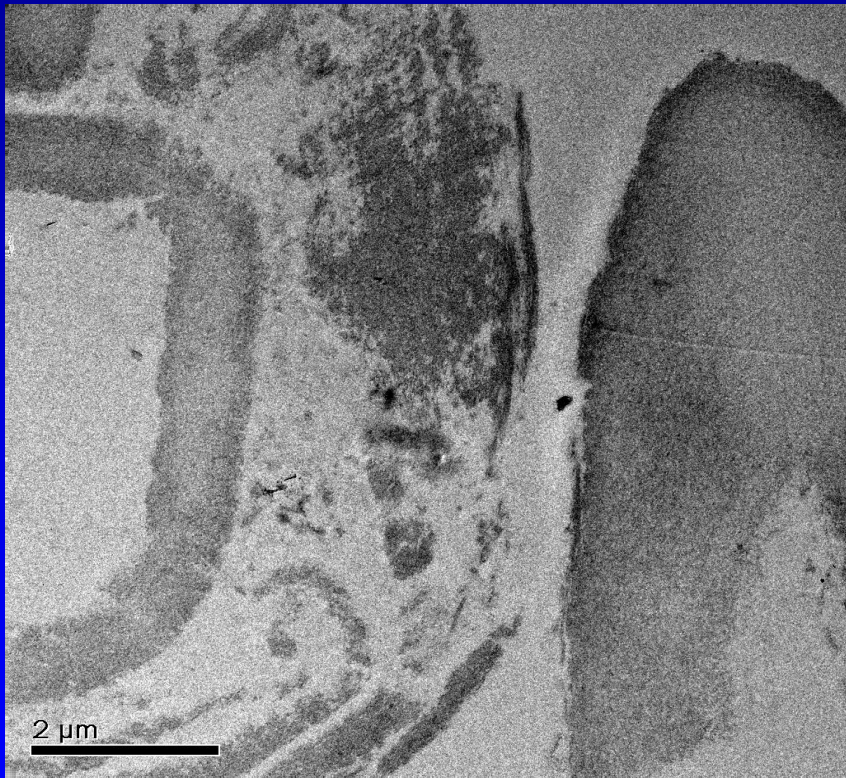
Aspen bleached kraft pulp

- FE-SEM (hydrolysis 6hrs.)



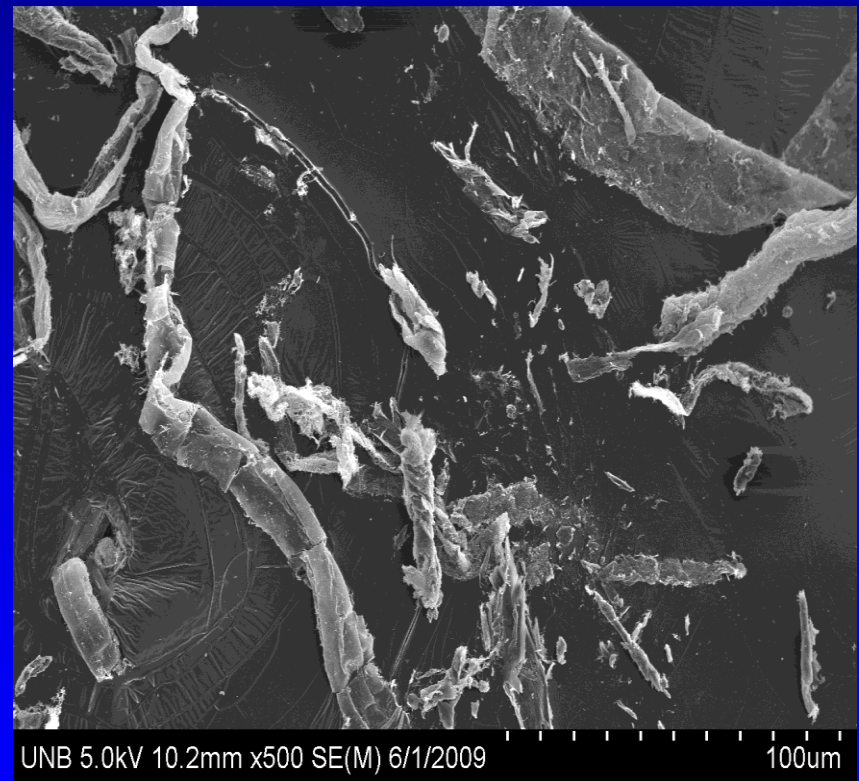
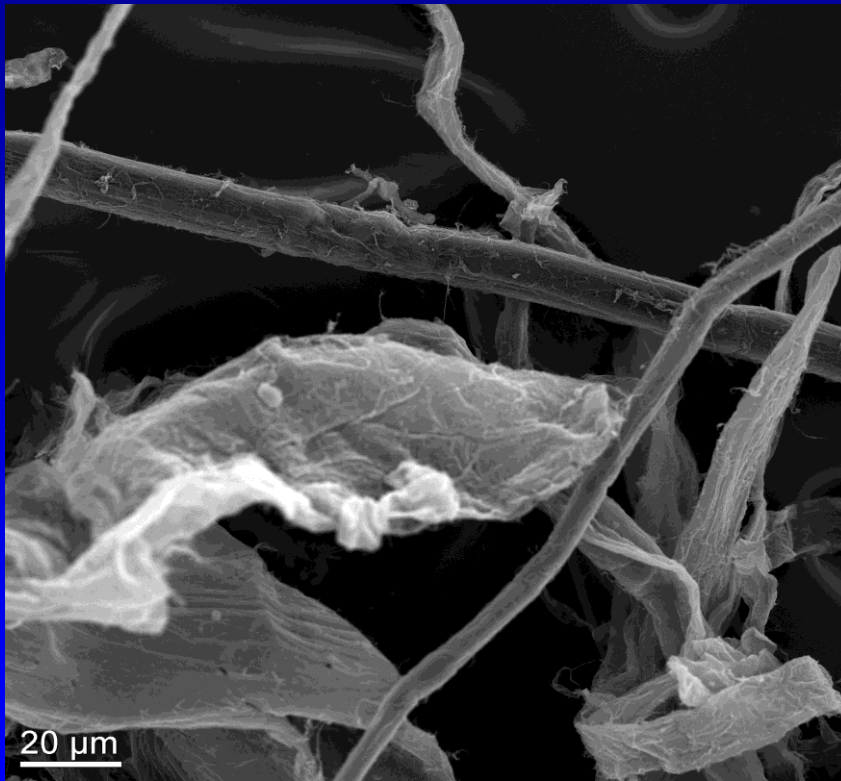
Aspen bleached kraft pulp

- TEM (hydrolysis 6hrs.)



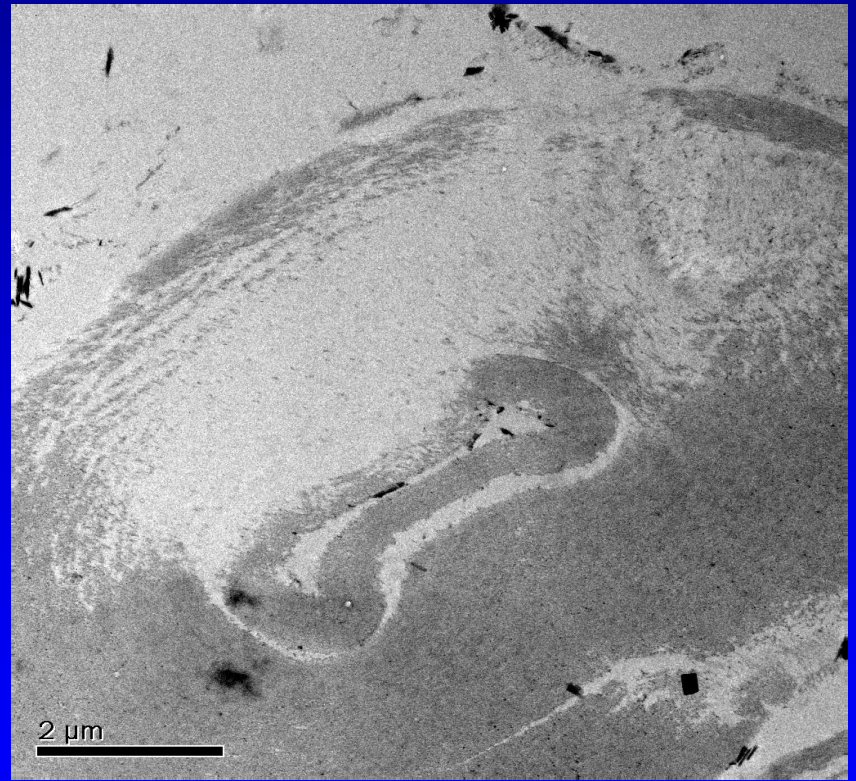
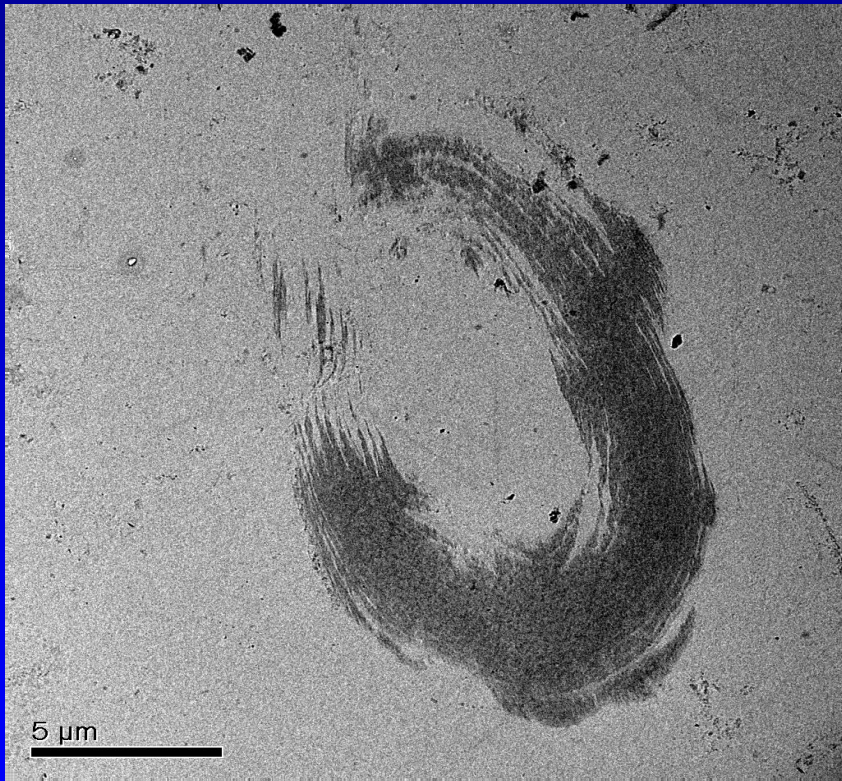
Wheat straw unbleached soda-AQ pulp

- SEM (original fibers)
- FE-SEM (hydrolysis 6hrs)



Wheat straw unbleached soda-AQ pulp

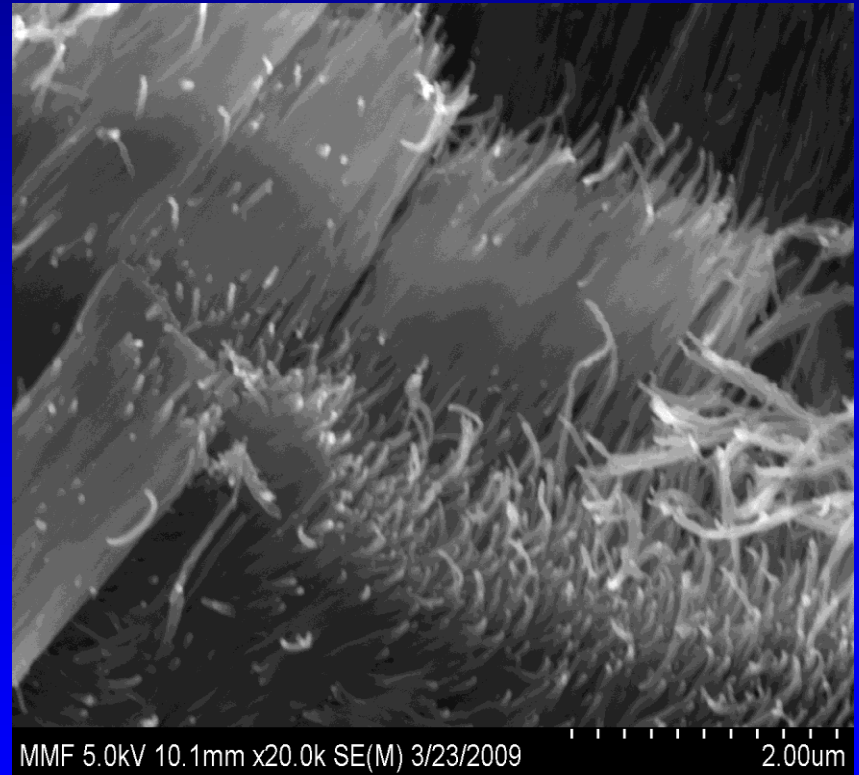
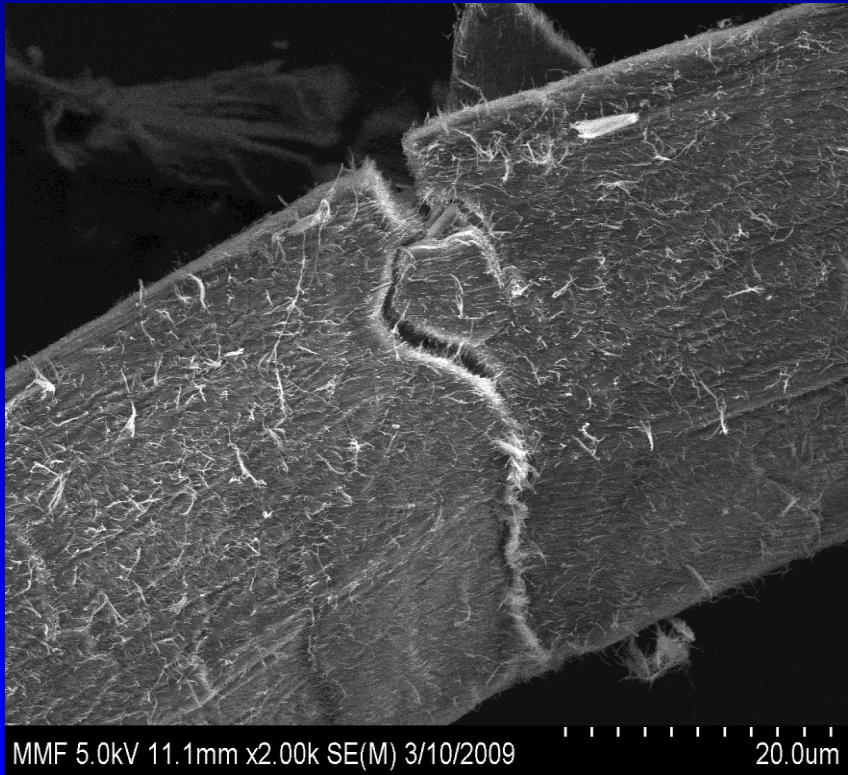
- TEM (hydrolysis 6hrs.)



Some Morphological Features in Hydrolysis by FE-SEM

Some Morphological Features in Hydrolysis by FE-SEM

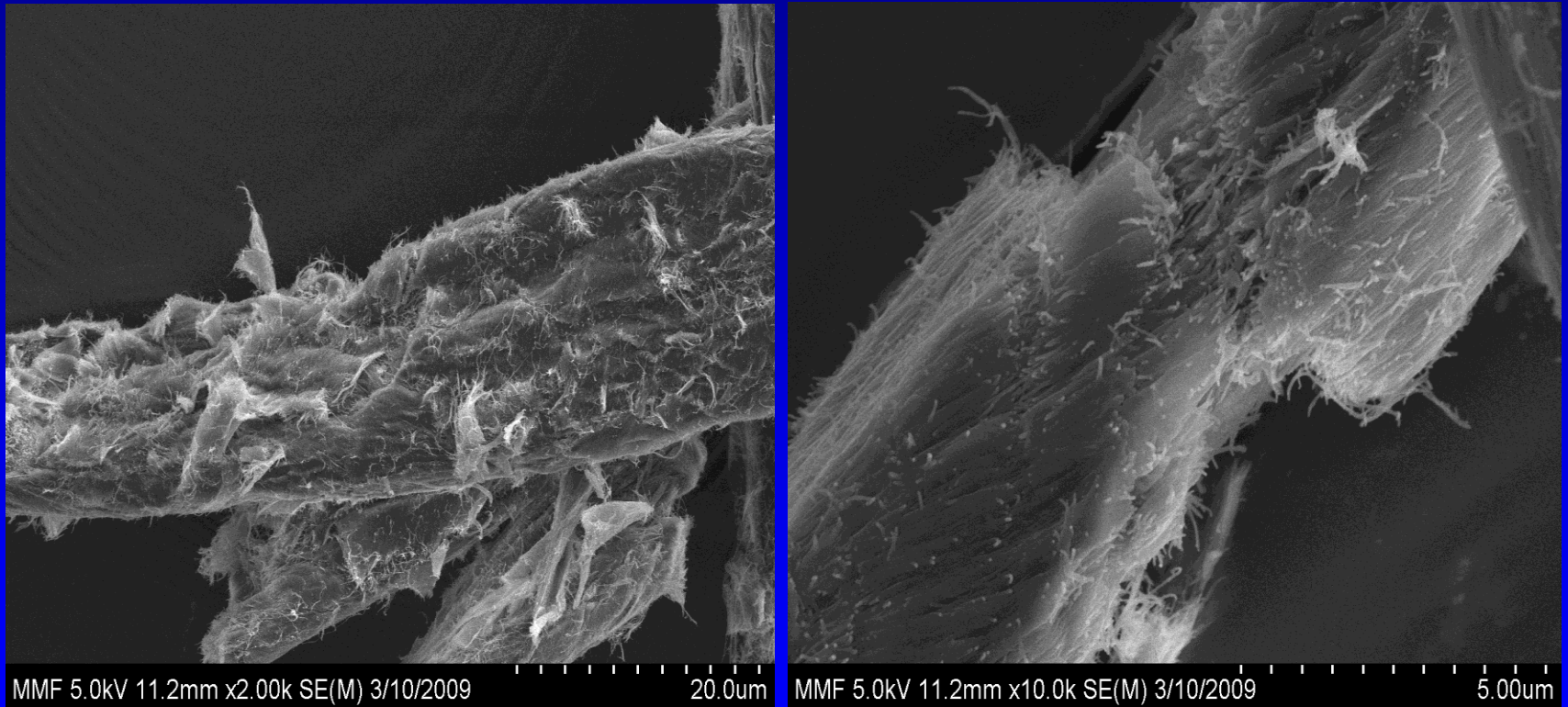
- *Aspen BKP, after 2hrs*



Cutting

Some Morphological Features in Hydrolysis by FE-SEM

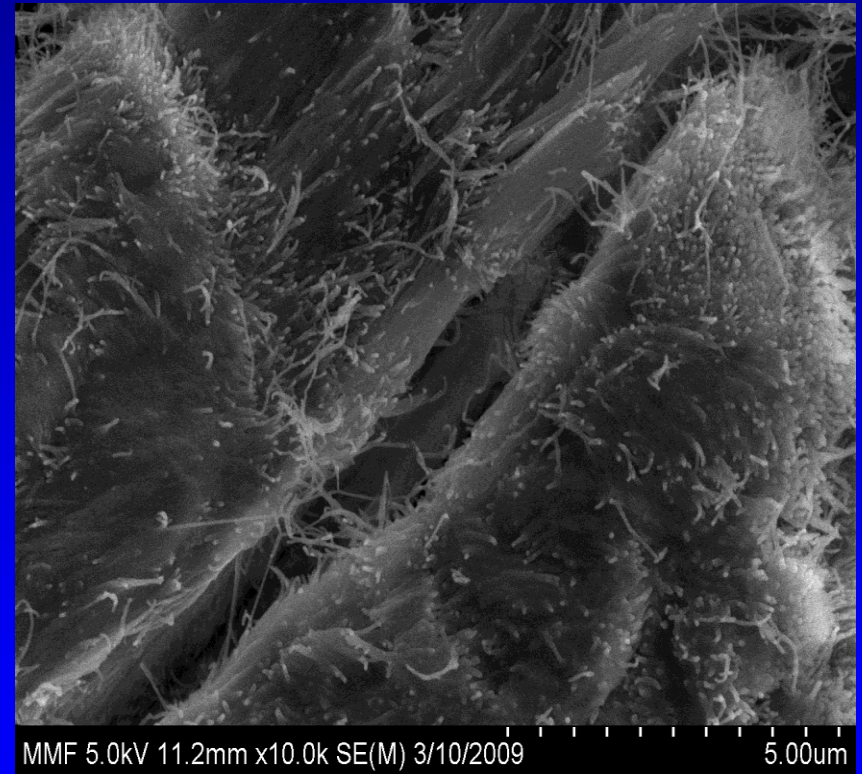
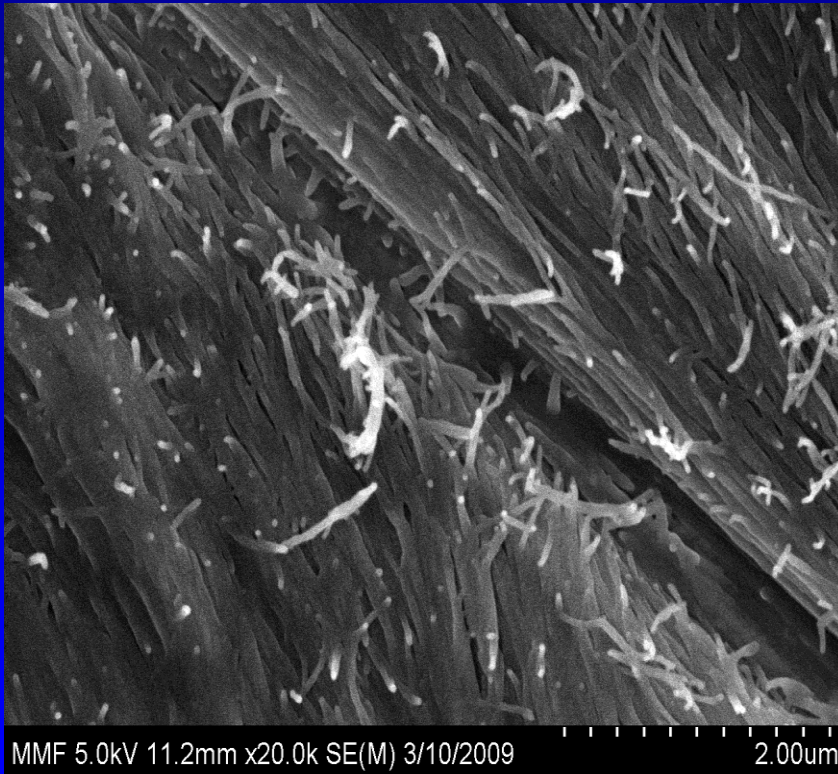
- *Aspen BKP*, after 2hrs



Erosion

Some Morphological Features in Hydrolysis by FE-SEM

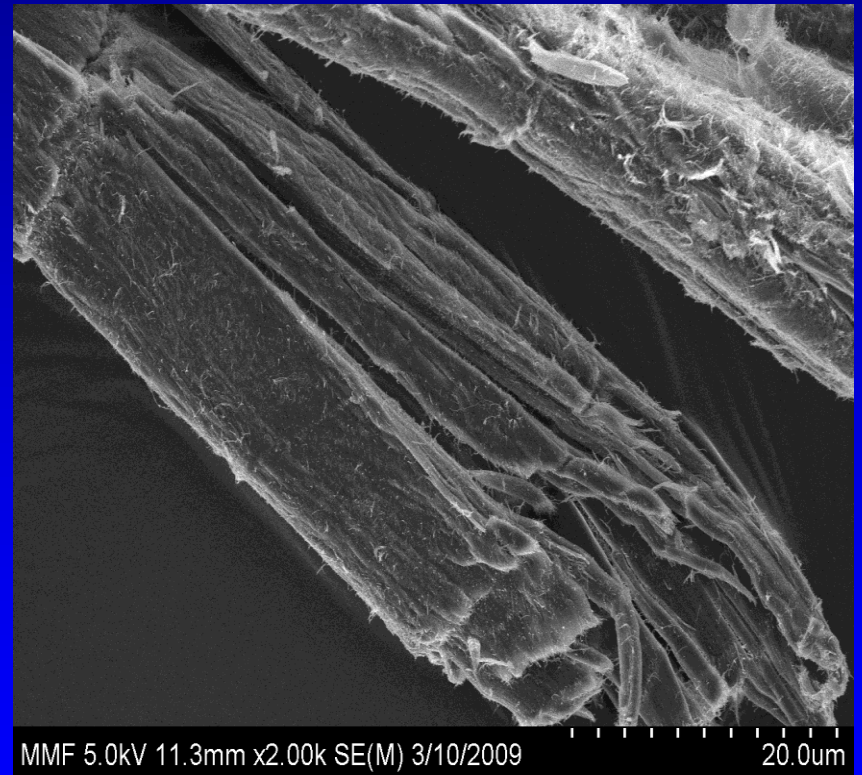
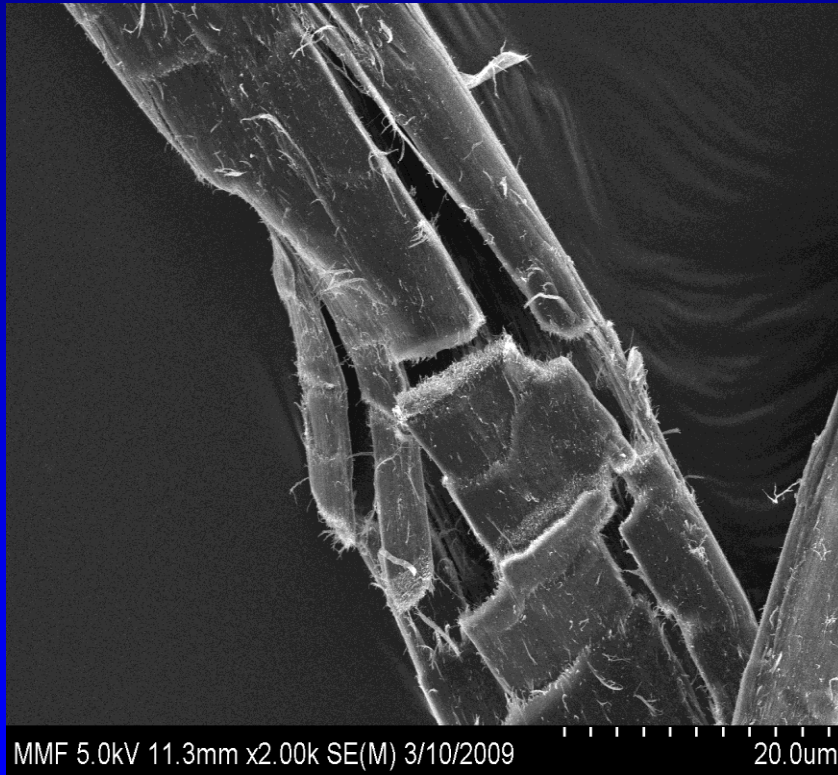
- *Aspen BKP, after 2hrs*



Splitting and fracturing

Some Morphological Features in Hydrolysis by FE-SEM

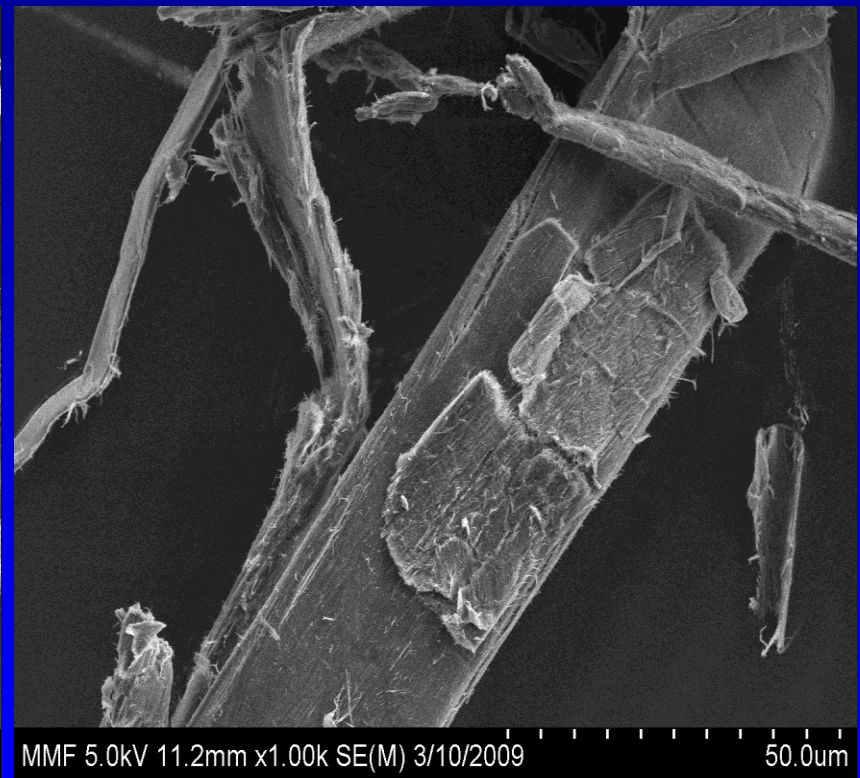
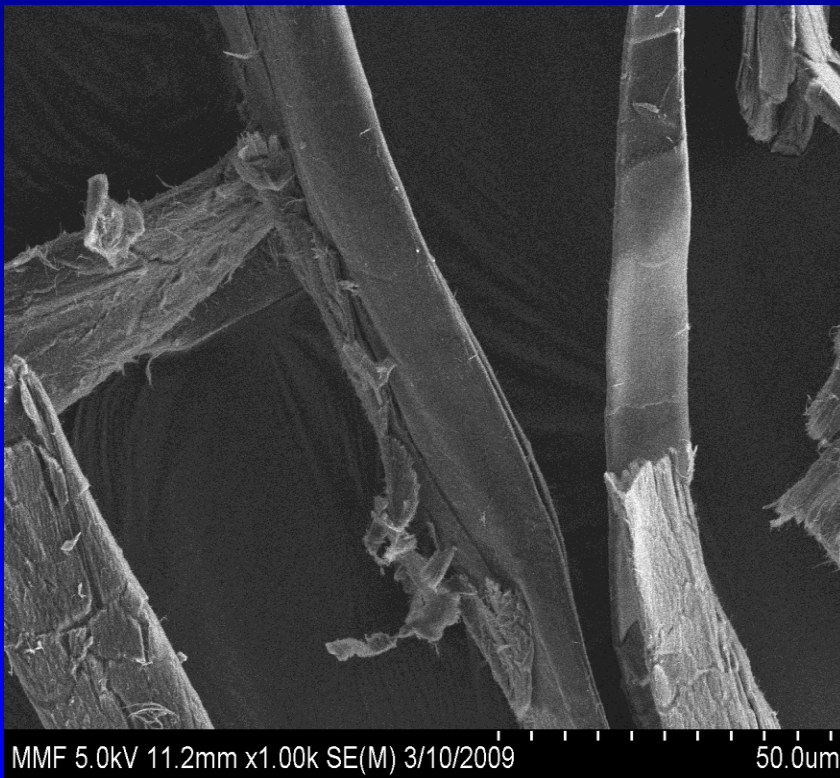
- *Aspen BKP*, after 6hrs



More splitting and fracturing

Some Morphological Features in Hydrolysis by FE-SEM

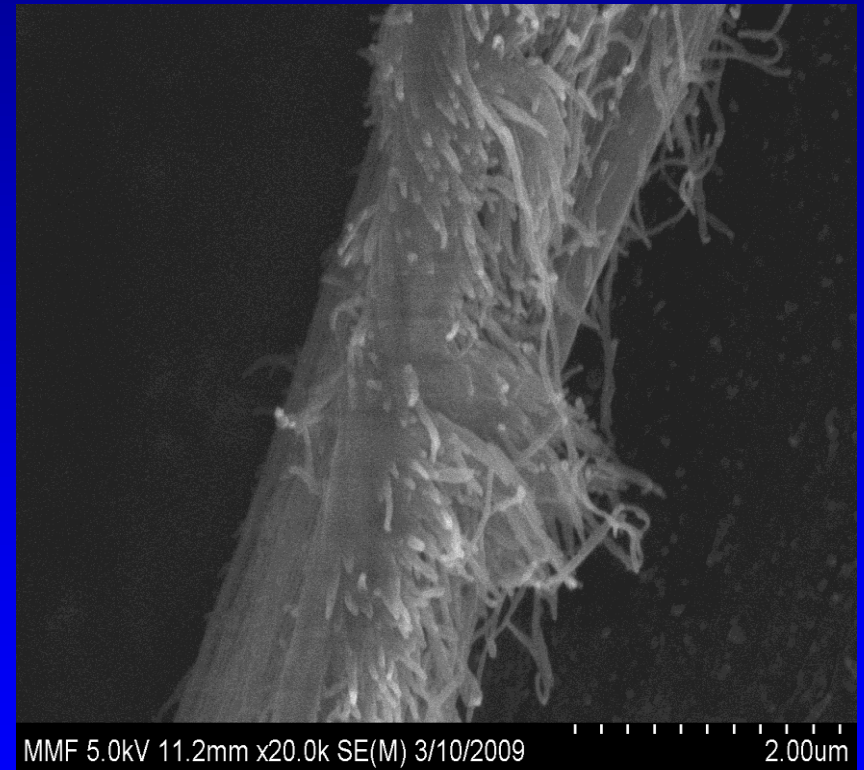
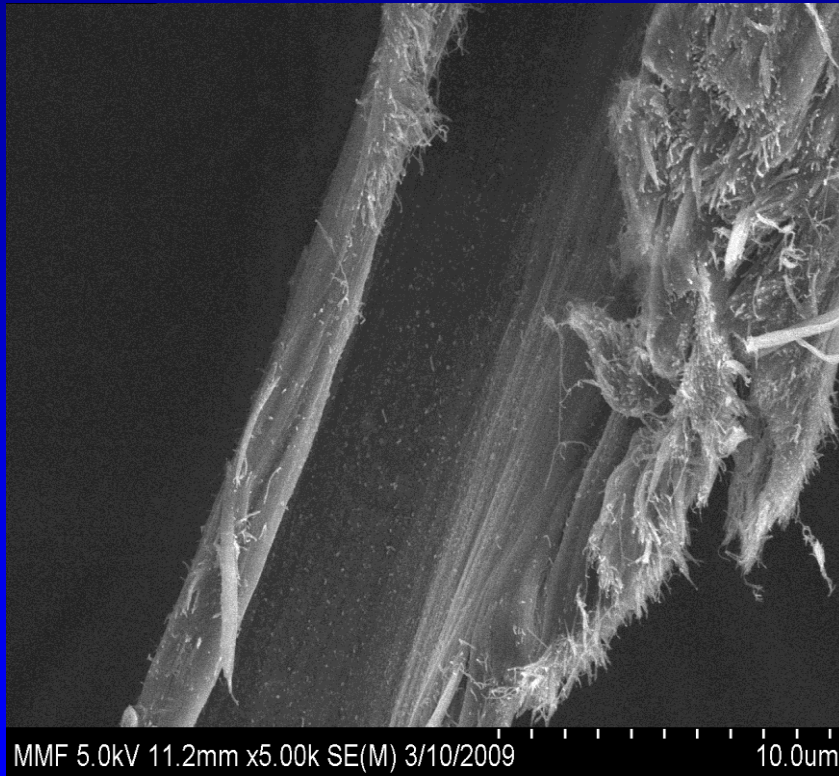
- *Aspen BKP*, after 6hrs



Peeling

Some Morphological Features in Hydrolysis by FE-SEM

- *Aspen BKP*, after 6hrs



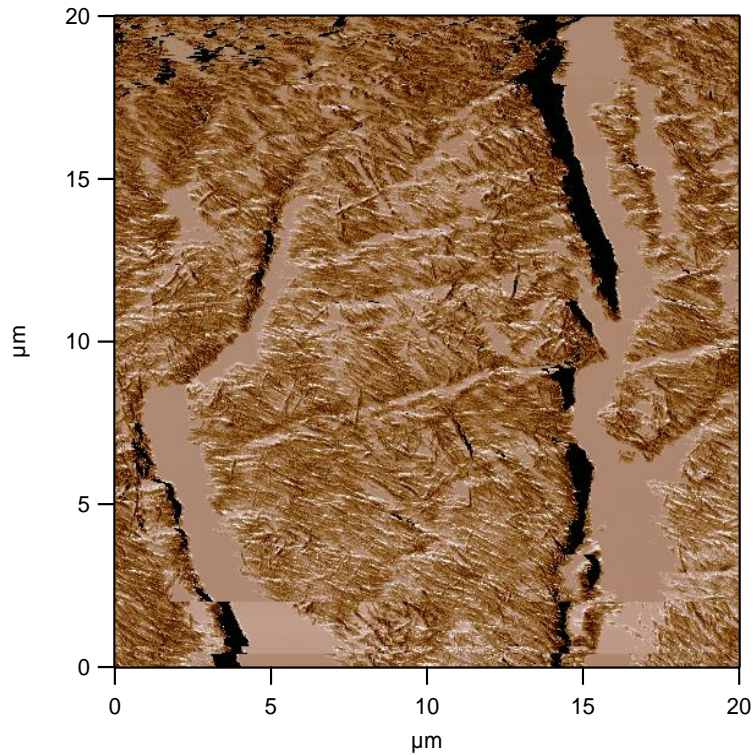
Cell wall thinning

Some Morphological Features in Hydrolysis by AFM

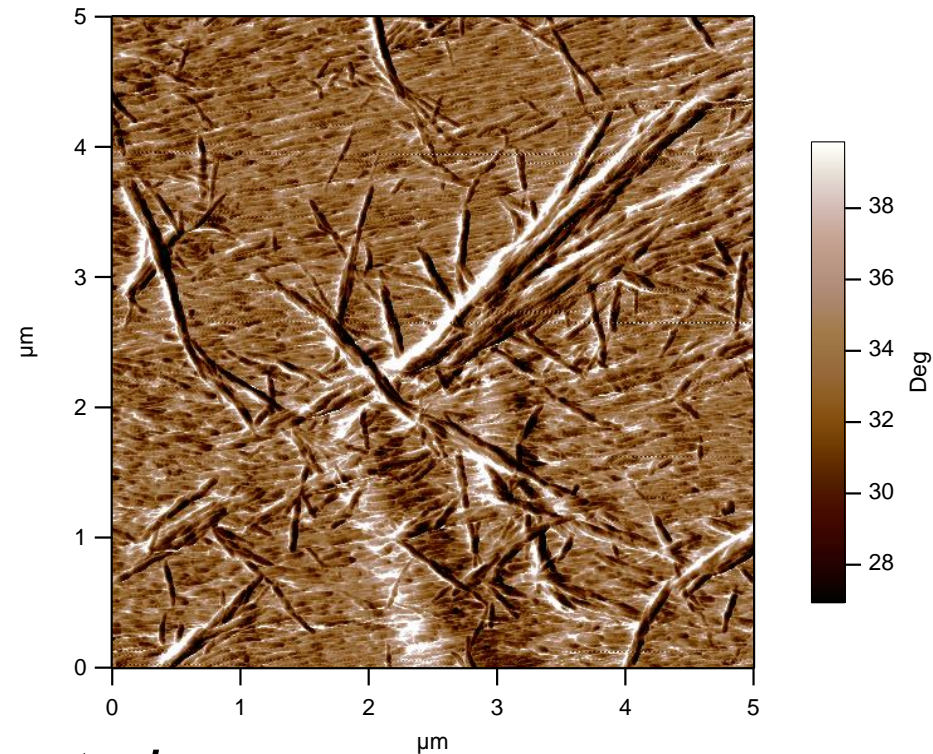
Softwood bleached kraft pulp

– AFM (phase images of fiber surface)

After 2hrs



After 2hrs

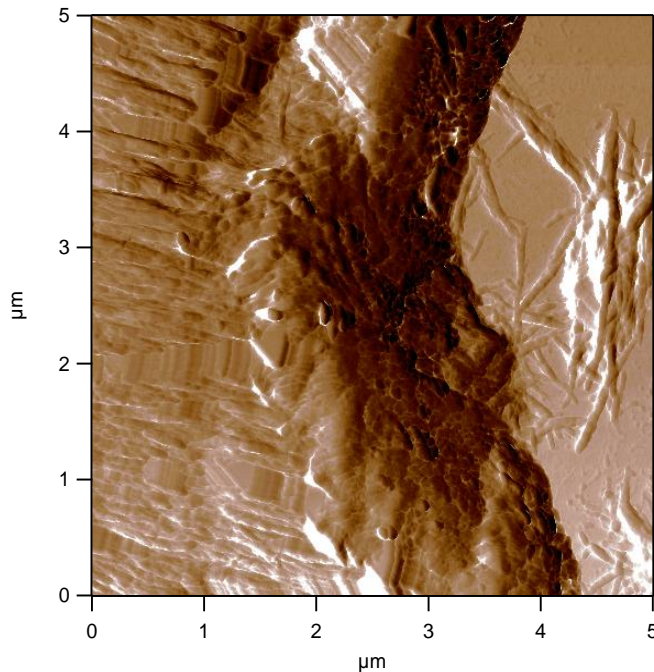


Cell wall fracturing

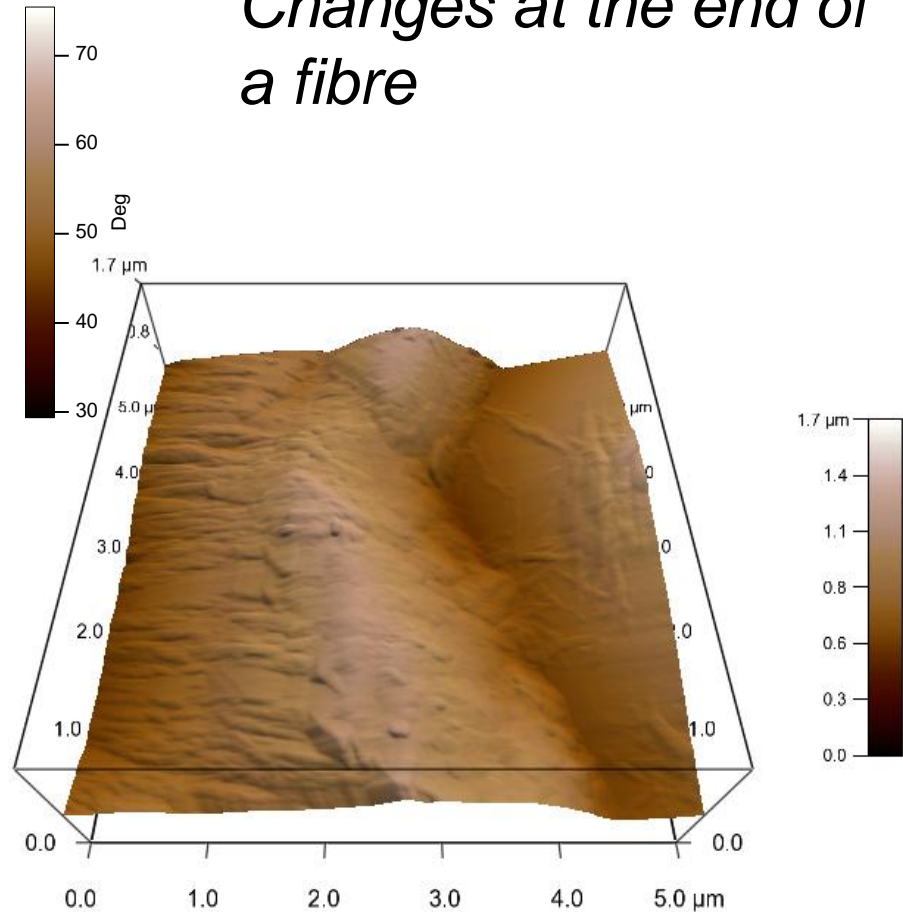
Softwood bleached kraft pulp

- AFM (phase and height 3D images of fiber surface)

after 6hrs



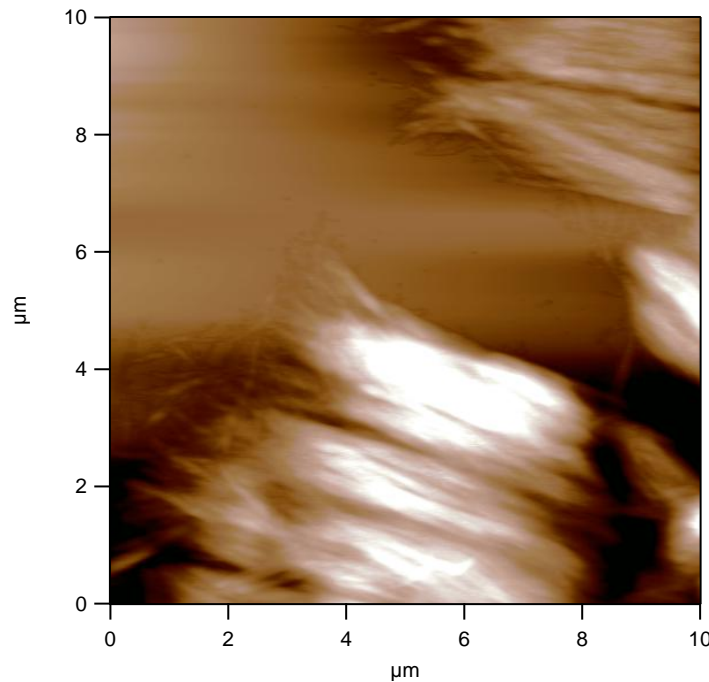
Changes at the end of a fibre



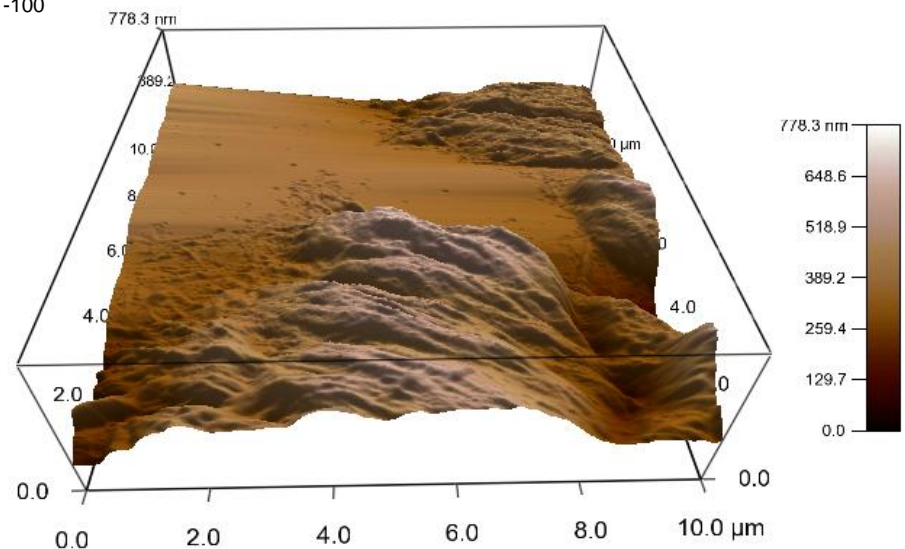
Softwood bleached kraft pulp

- AFM (phase and 3D images of fiber surface)

After 2hrs



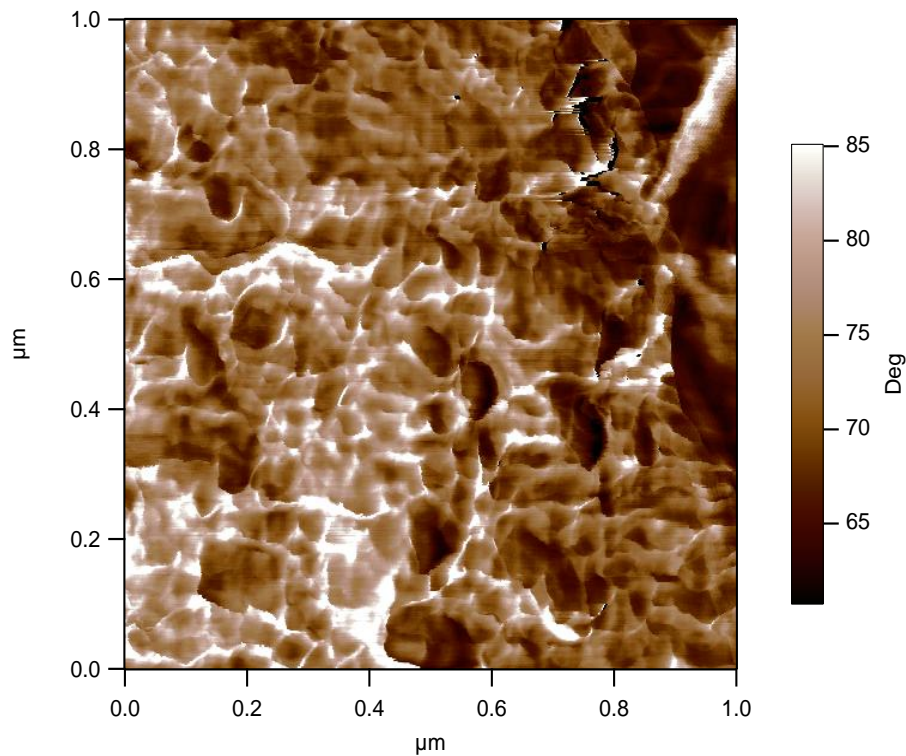
Changes at the end of microfibrils, and lignin/hemicellulose residues



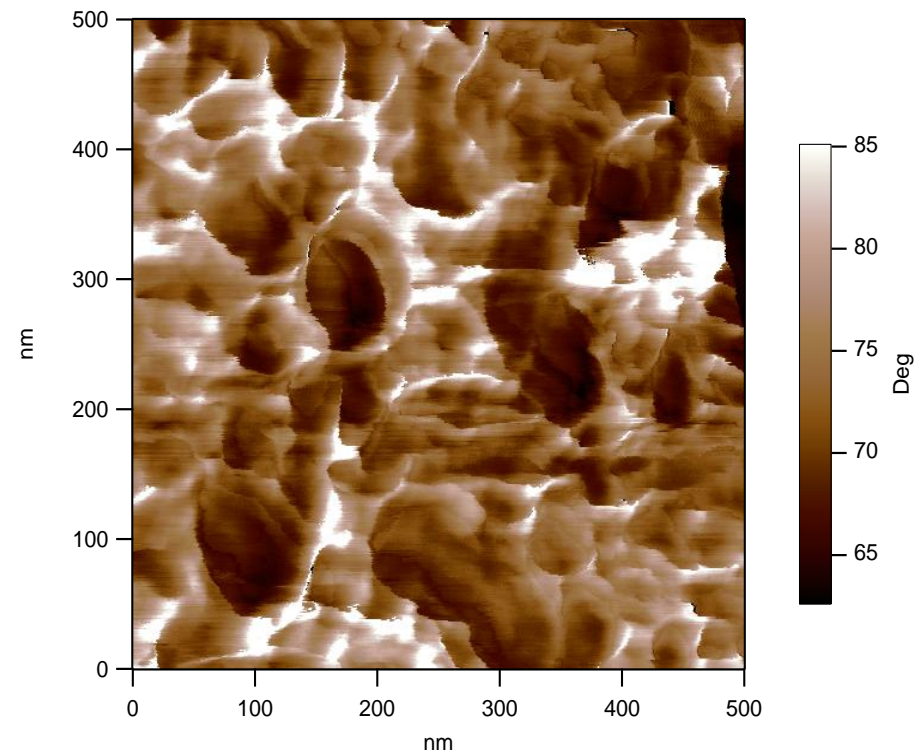
Softwood bleached kraft pulp

– AFM (phase images of fiber surface)

Hydrolysis 2hrs



Hydrolysis 2hrs

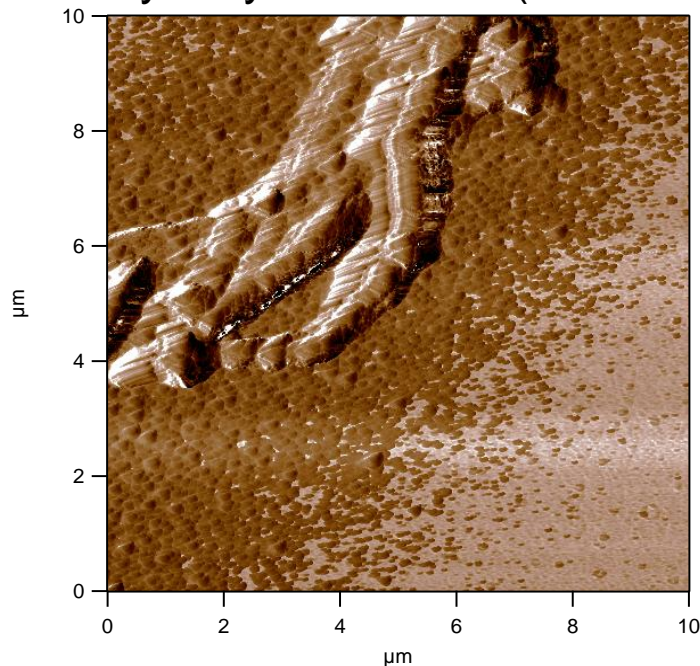


Discrete microfibrils

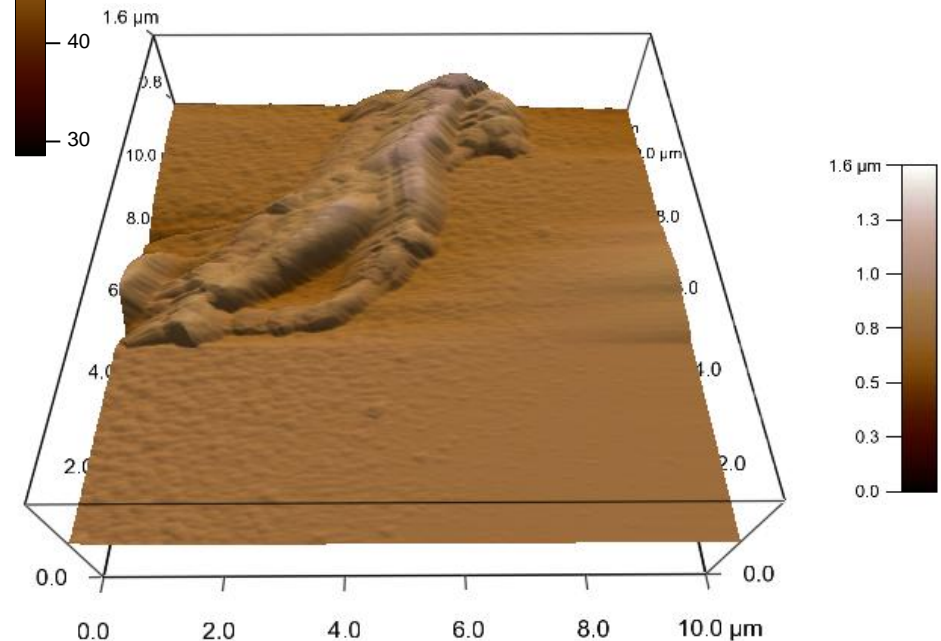
Softwood thermomechanical pulp

- AFM (phase images)

Hydrolysis 144 hrs (unwashed, fines)

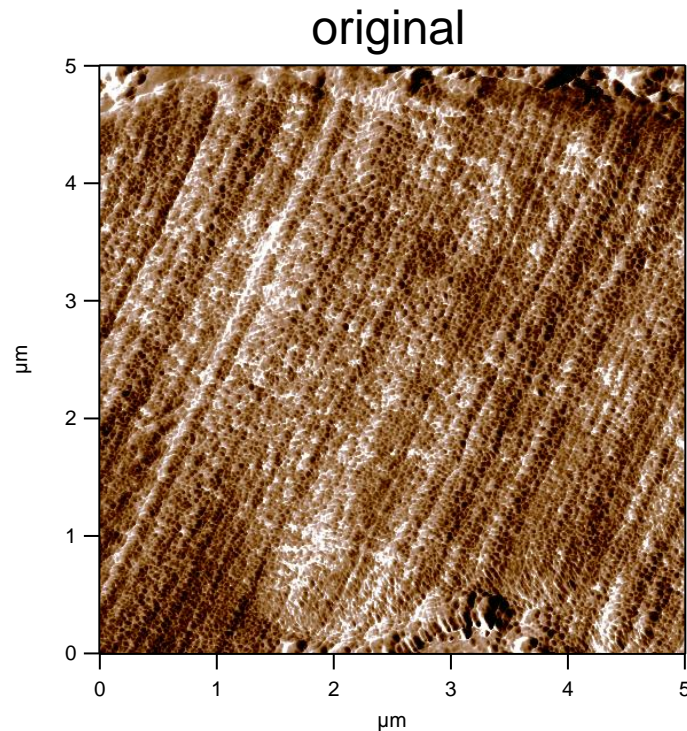


*Microfibrils, and
lignin/hemicellulose
residues*

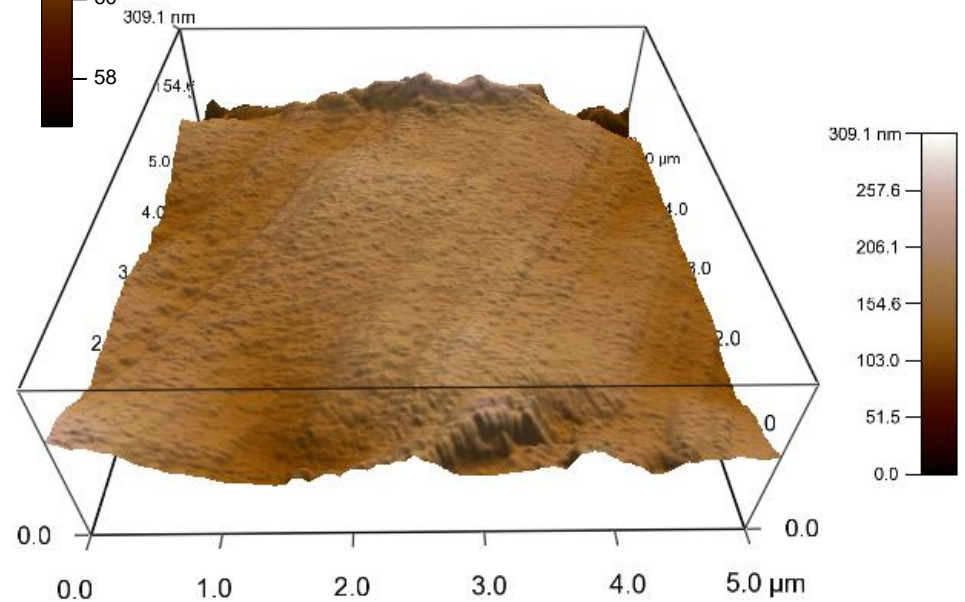


Poplar steam explosion pulp

- AFM (phase and height 3D images of cross section)

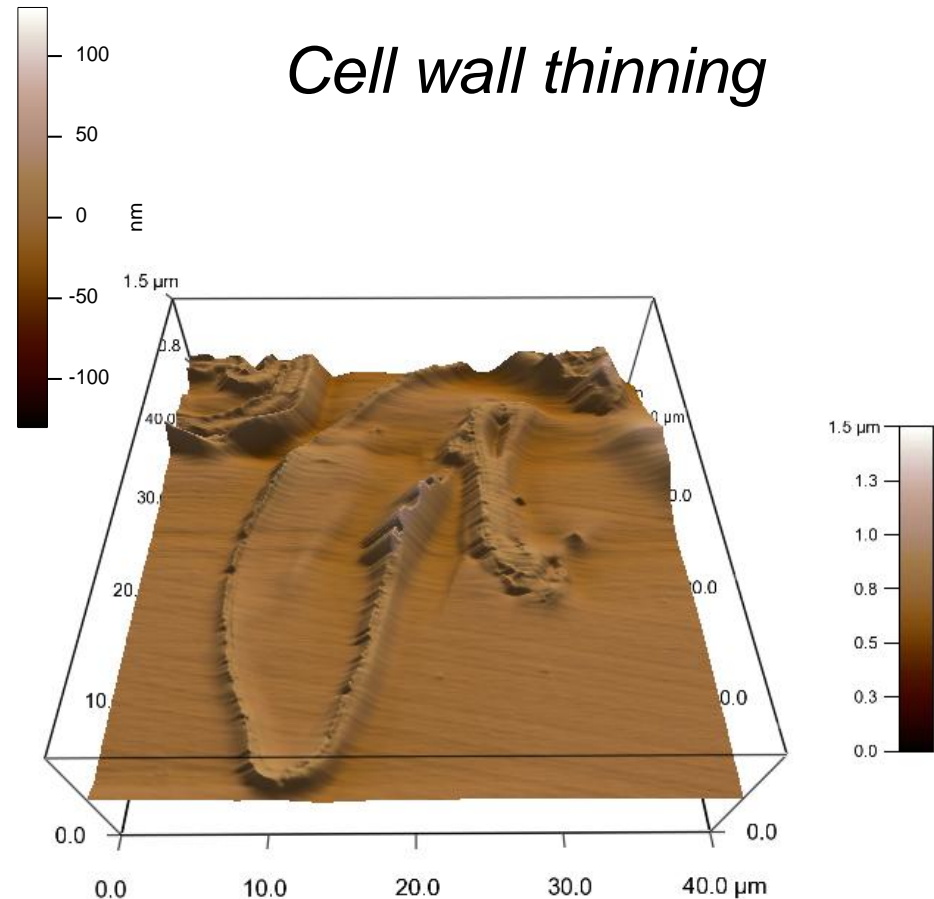
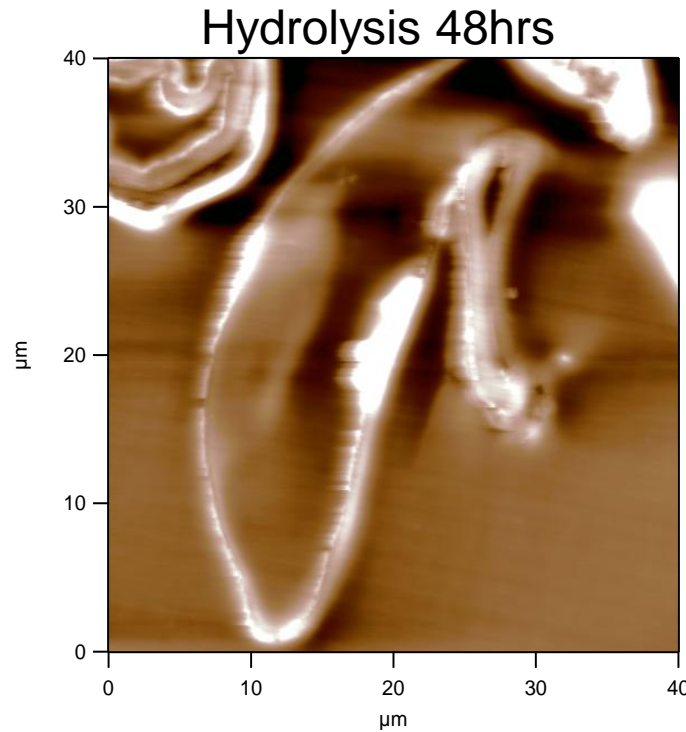


*Microfibrils embedded in
lignin/hemicellulose
matrix*



Poplar steam explosion pulp

- AFM (height and 3D images of cross section)

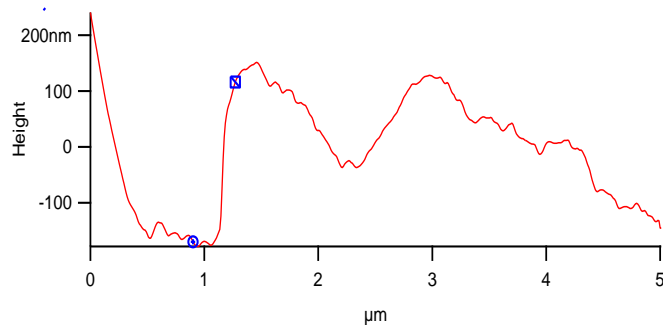
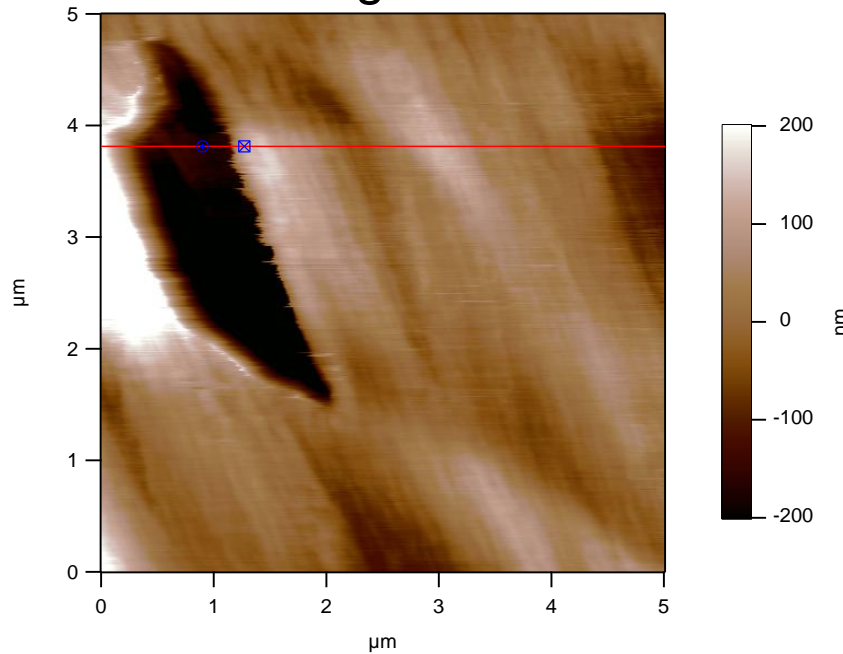


Cell Wall Changes *in situ* during Hydrolysis by AFM

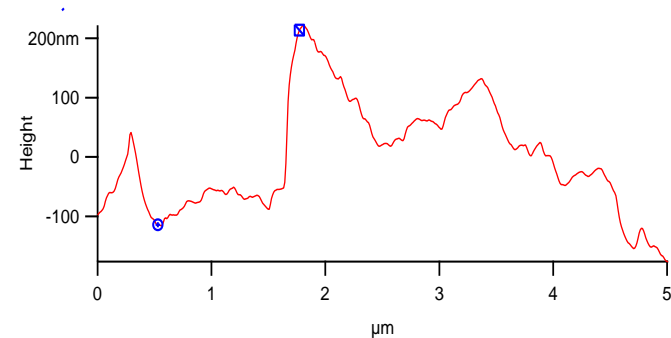
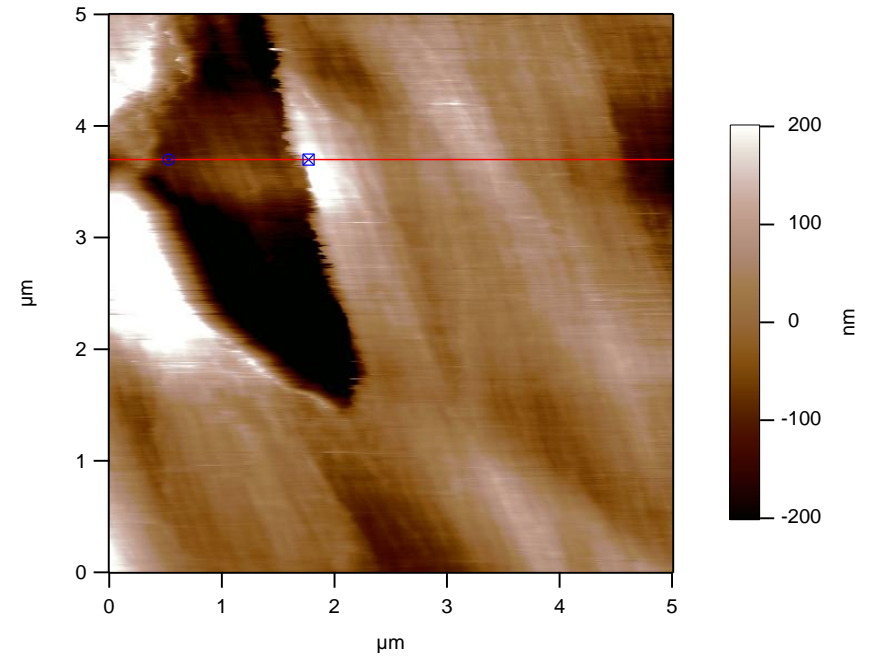
Softwood bleached kraft pulp

- AFM (height images)

Original

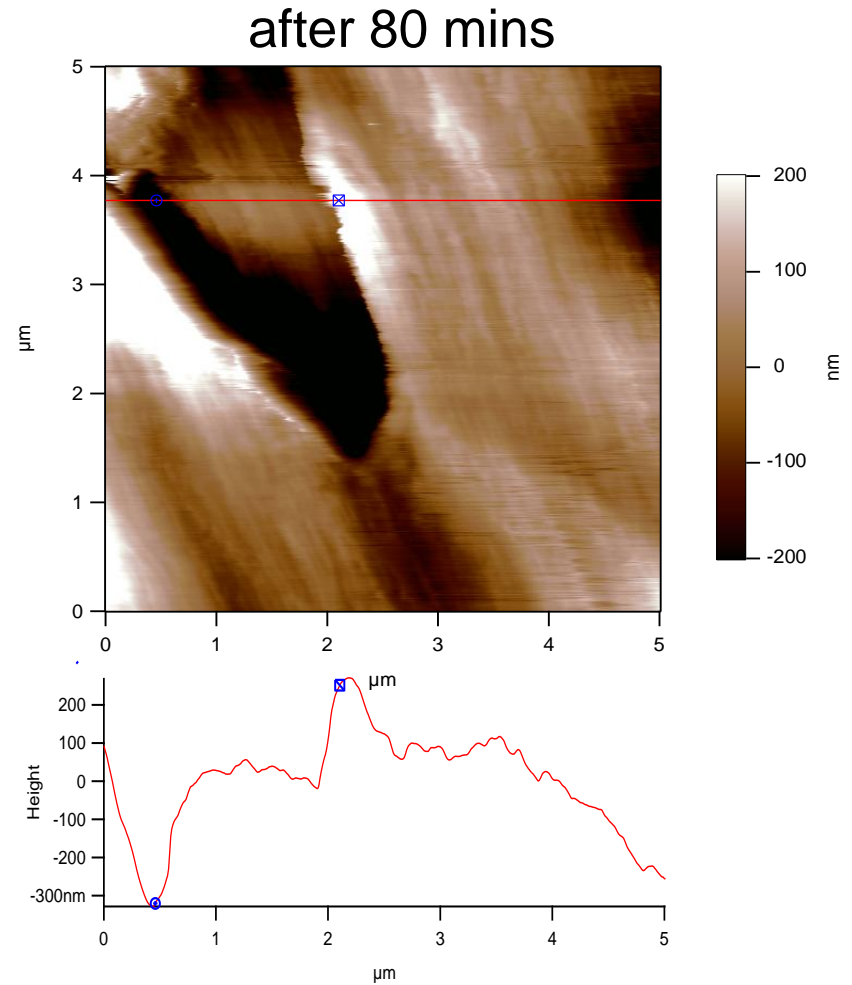
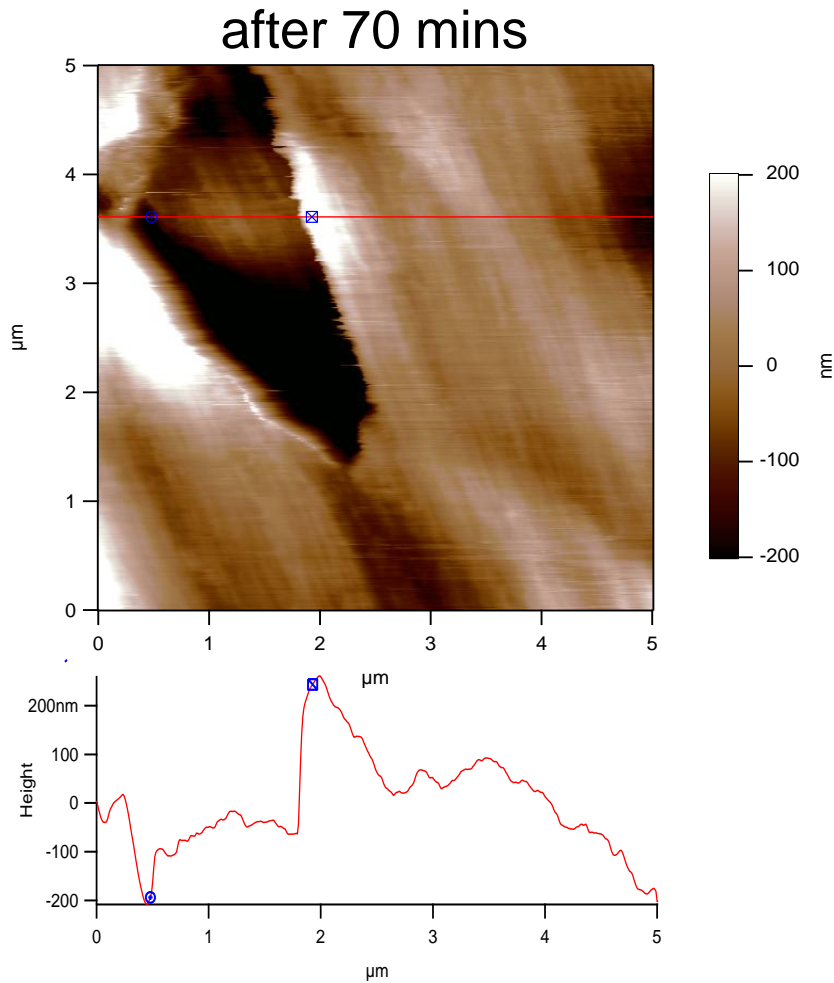


After 60 mins



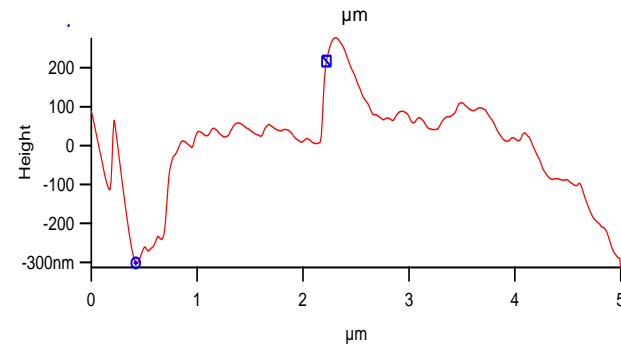
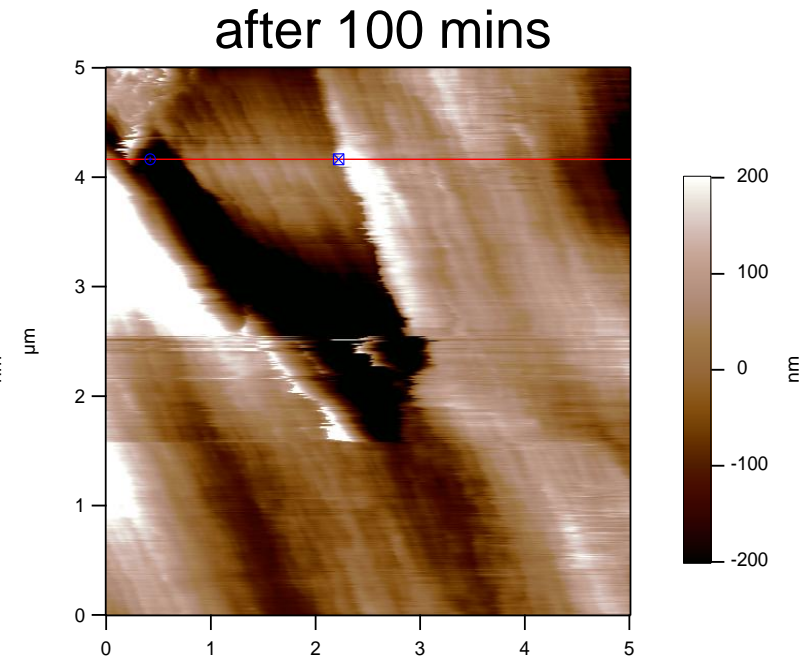
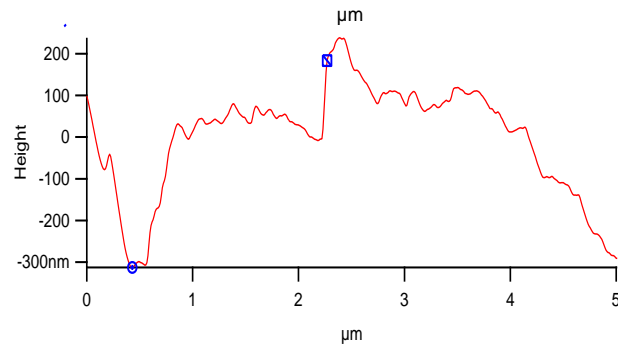
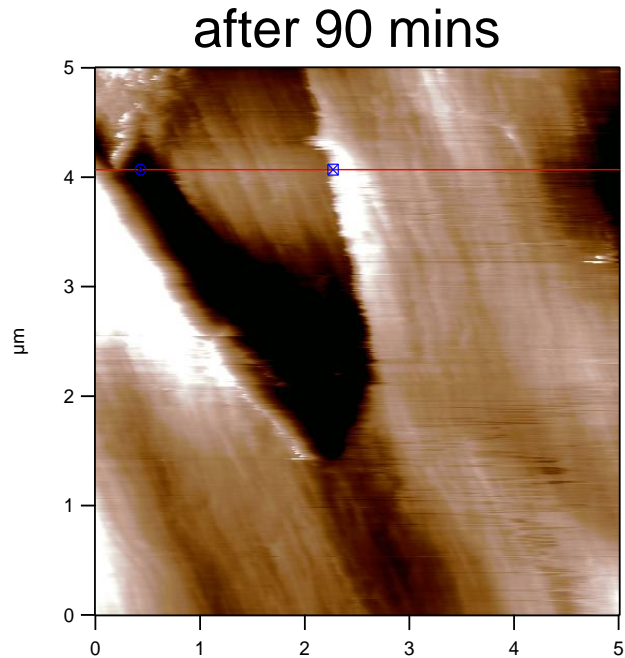
Softwood bleached kraft pulp

- AFM (*In-situ* height images)



Softwood bleached kraft pulp

- AFM (In-situ height images)



Summary

- Changes at both fibre level and fibril level (nano-scale) can be observed in several ways
- It is possible to observe changes *in situ* with AFM
- Changes of substrates with lignin and without lignin are apparent
- Next, to combine chemical analysis and morphological observation
- Ultimately to elucidate the pathways of enzyme actions during hydrolysis

Acknowledgements

- NSERC
- CFI
- AIF
- NBIF
- Irving, NewPage, Novozymes, Andritz, and Tembec