Enzymes, Specialists in Fibrillation – Prediction of Refining

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- Aims
- Enzymes
- Methods
- Results
  - Total activity
  - Unwanted activity
  - Cellulases on different pulps
  - Cellulases and hornification
  - SEM imaging
  - Grinding
  - Air permeability
- Conclusion
- Outlook

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Aims

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- Potential of enzymes in P&P
  - known > 30 years
  - More efficient enzymes available now
  - Implementation still limiting

  - Develop simple enzyme assays for activity determination
  - Investigate influences of pH, temp and time
  - Enzyme characterisation
  - Affinity of enzymes to real substrates
  - Substrate properties
  - Methods for routine analytics

➢ assist P&P industry in implementation

Enzymes

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- Around 40 enzyme formulations
- 4 fields of application
  - Refining
  - Surface starch conversion
  - Wet end starch
  - Deinking
- Cellulases
  - Increase fibrillation
  - Remove ink particles
- Amylases
  - Surface starch sizing
- Protease
  - Reduce protein impurities in wet end starches
- …

Cellulases

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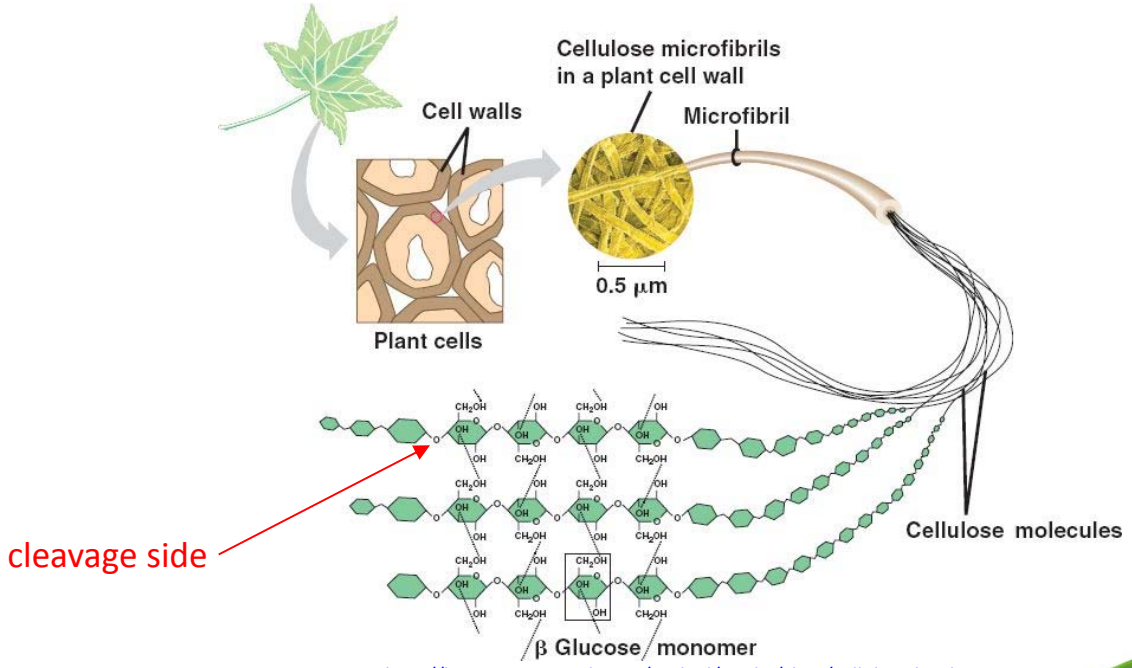
- Cleave β-1, 4 glycosidic bonds in cellulose
- In plants: Rebuilding plant parts
- In fungi: degradation → energy source
- Fungi and bacteria for nutrition (Glucose)
- Complex mechanism of different enzymes



http://bio1151.nicerweb.com/Locked/media/ch05/cellulose.html

Synergistic cellulose degradation

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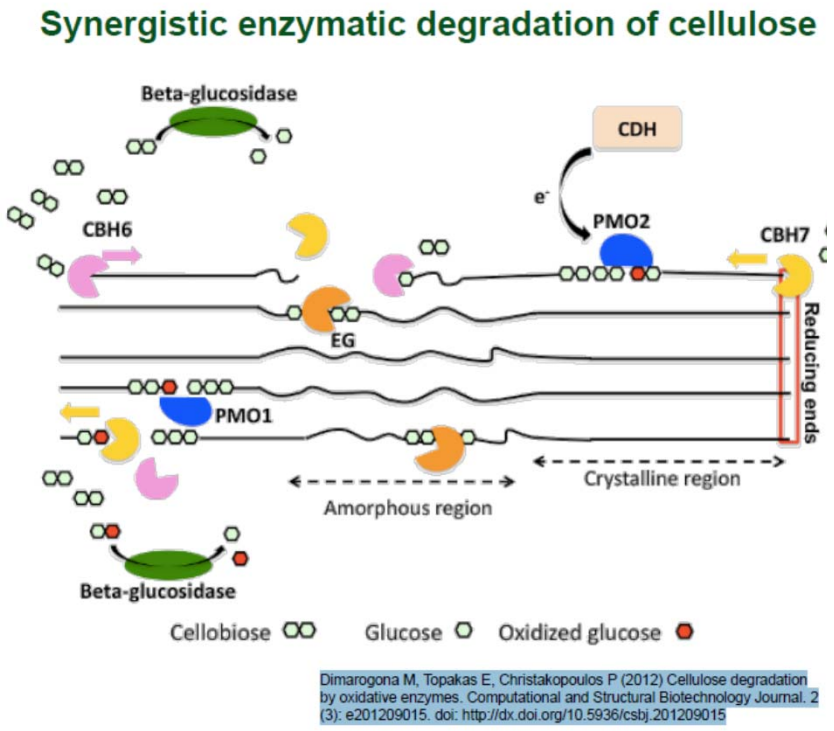
- Acting within the chain (endoglucanases)
  - Introducing new ends for fibrillation
  - Desirable for paper
- Acting on ends (CBH, Glucosidase)
  - Release of small subunits (C1,C2)
  - Unwanted in pulp and paper enzymes
- End product is glucose



Dimarogona M, Topakas E, Christakopoulos P (2012) Cellulose degradation by oxidative enzymes. Computational and Structural Biotechnology Journal. 2 (3): e201209015. doi: http://dx.doi.org/10.5936/csbj.201209015

PMO polysaccharide monooxygenase
EG Endoglucanase
CBH Cellobiohydrolase
CDH: Cellobiose dehydrogenase

- Cellulases are always a cocktail of different enzymes
  - Investigation required

Methods

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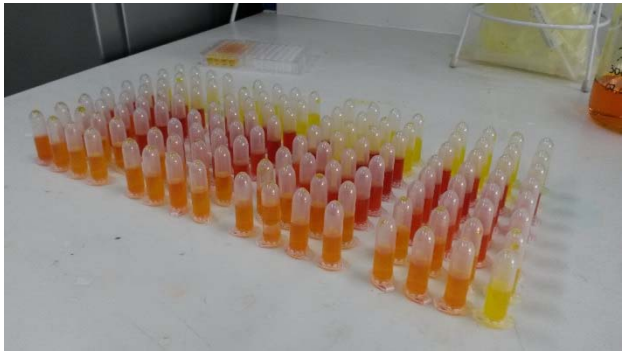
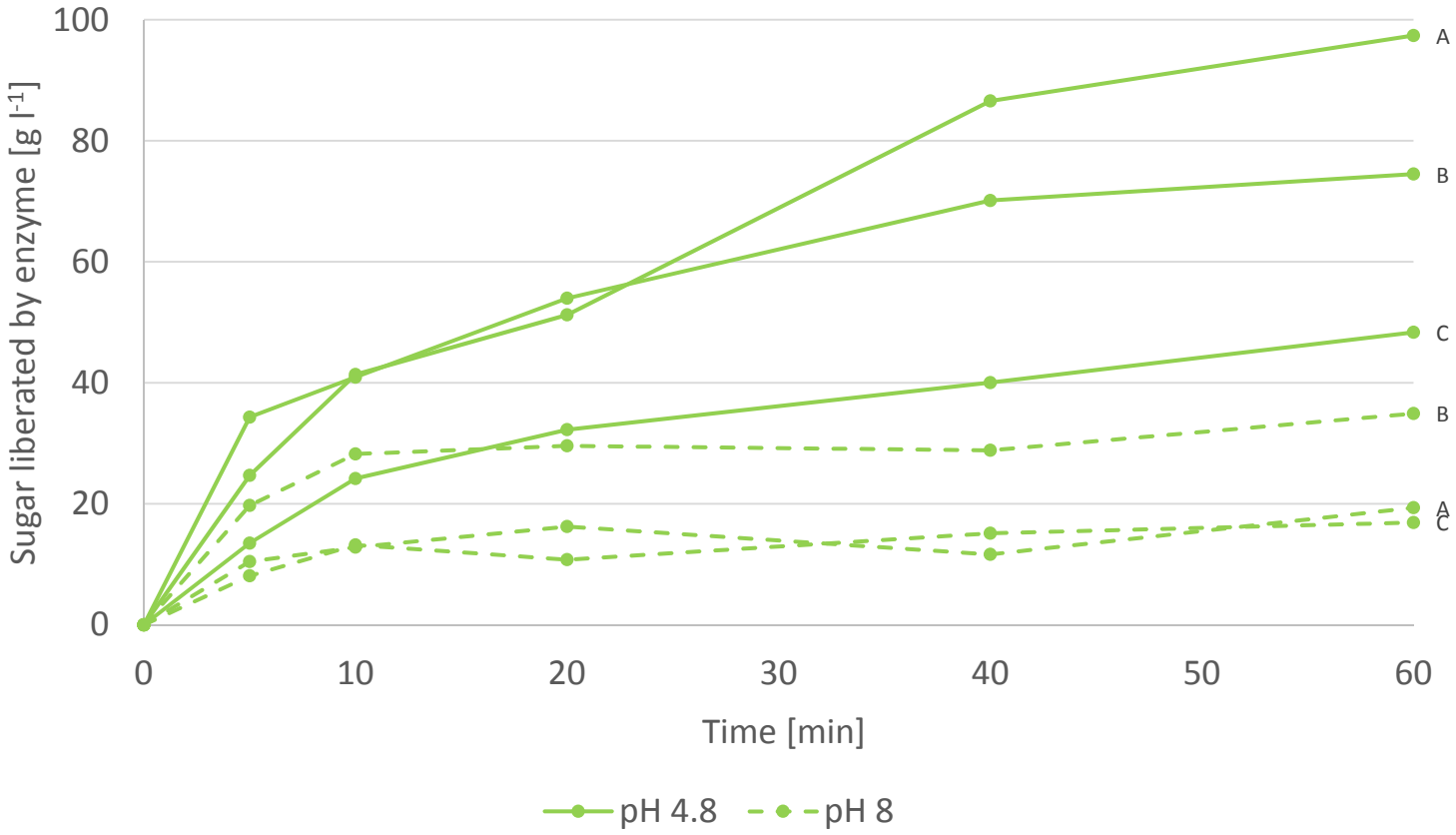
- Protein content / purity
  - Content photometric with Bradford
  - SDS gel electrophoresis
  - How many fractions?
  - Which molecular weight?
- Reducing sugar content
  - Different substrates
  - Selectivity for enzyme fractions
- HPLC
  - Xylose / glucose
- Paper
  - SEM
  - Air permeability (Bendtson, Gurley)
  - Milling degree
  - Work absorption
  - …



Total activity



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- Different pH stability for different formulations

➢ Individual adaption to industrial requirements

Unwanted activity – Xylanase

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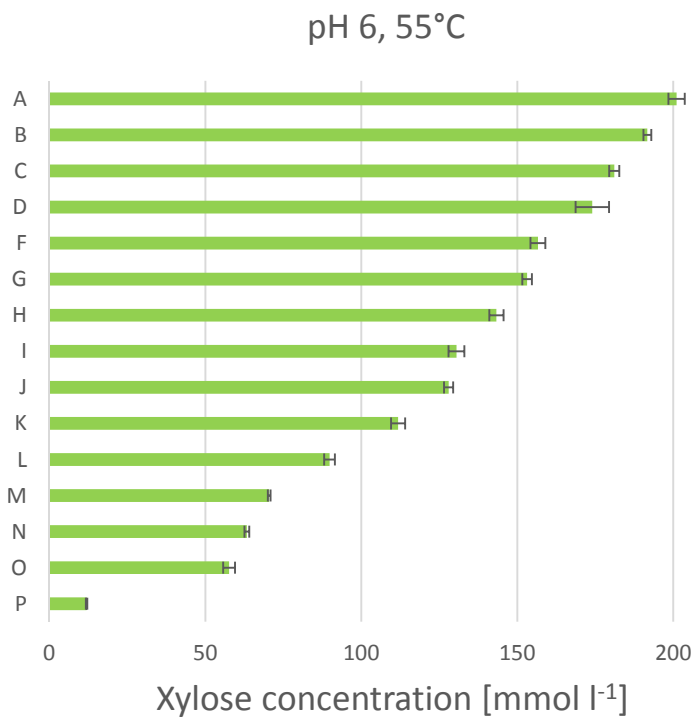

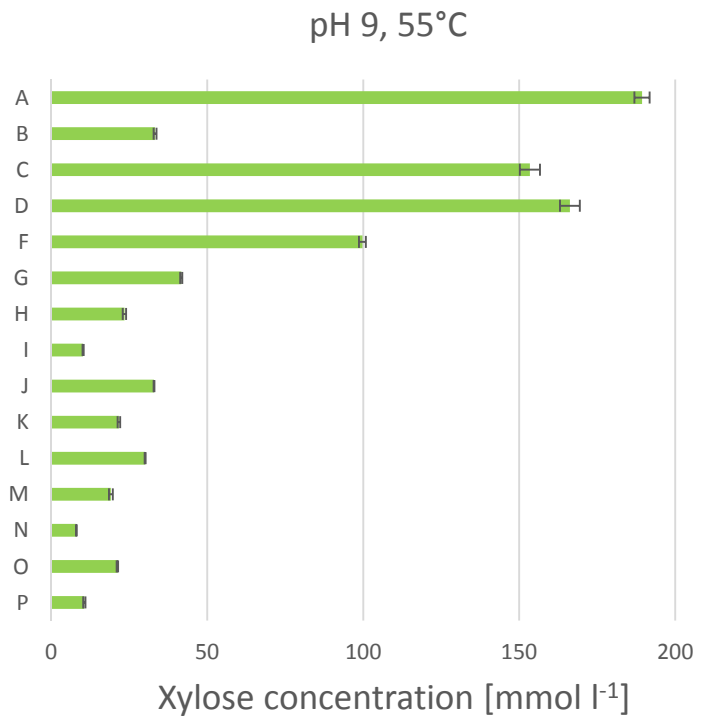

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- Degradation of Hemicellulose
- Cellulases (i.e. Endoglucanases)
- Unwanted xylanase activity was found in all refining enzymes
- Activity is pH and temperature dependent

➢ Screening of single enzyme components

Unwanted activity – β-Glucosidase

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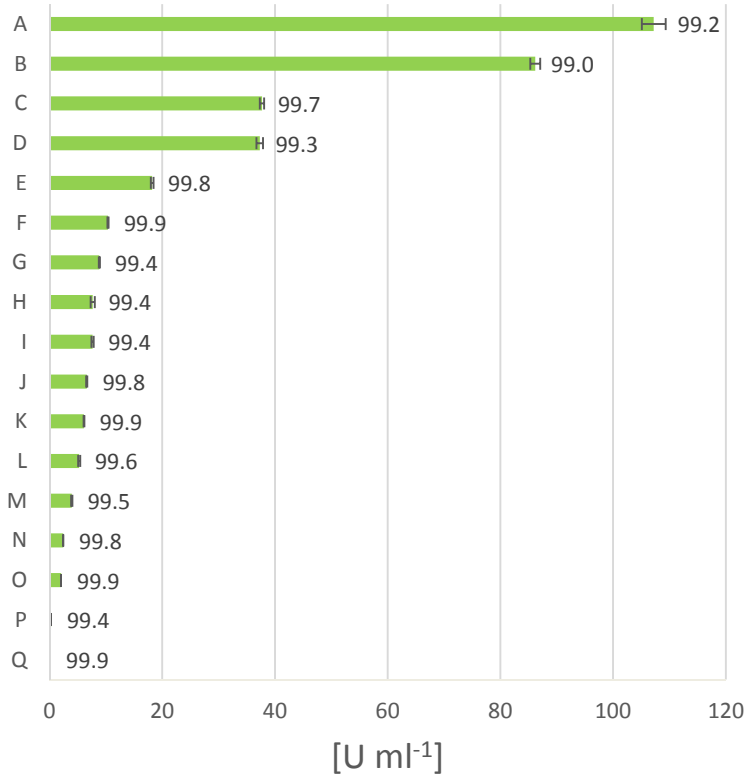
- No fibrillation events
- Only endoglucanase activity for fibrillation
- β-glucosidase seems to correlate with total activity
- [U ml^{-1}] amount of glucose [µmol] liberated in one minute by 1ml enzyme
- All investigated enzymes show some activity



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Cellulases on different pulps

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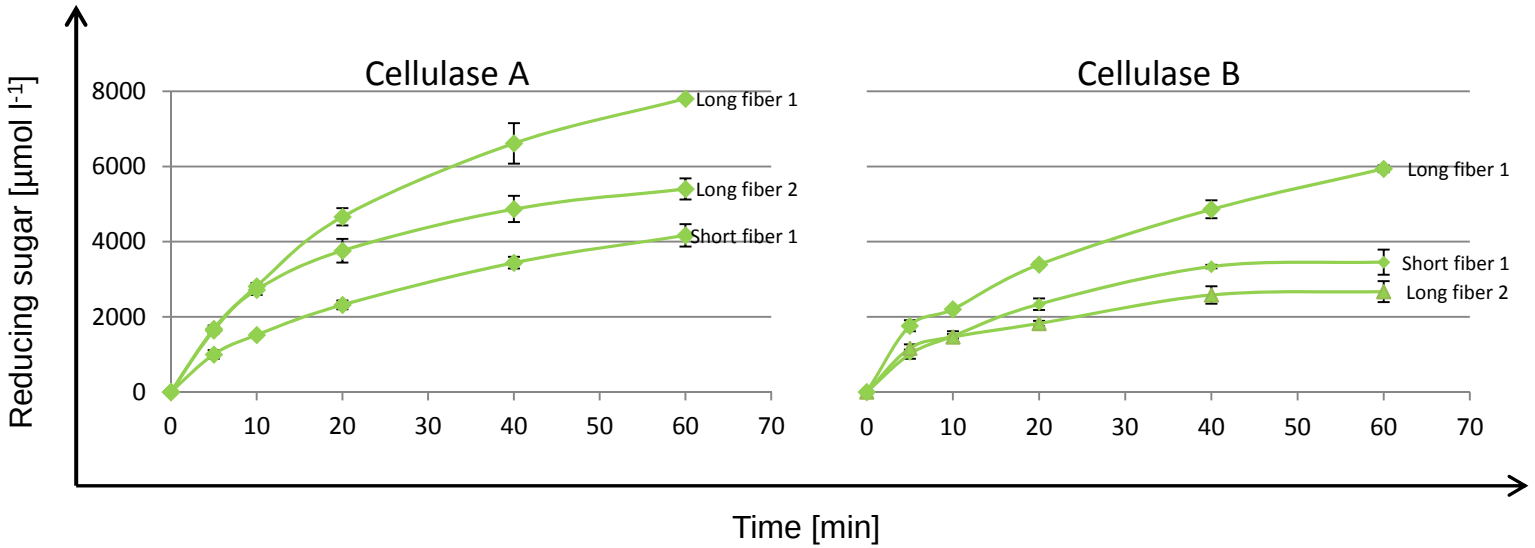

- Higher affinity to long fiber pulps
- Chain length is influencing activity
  - Longer chains → less end positions for exocellulases
- Crystallinity?
- Hemicellulose?

Influences of Hornification

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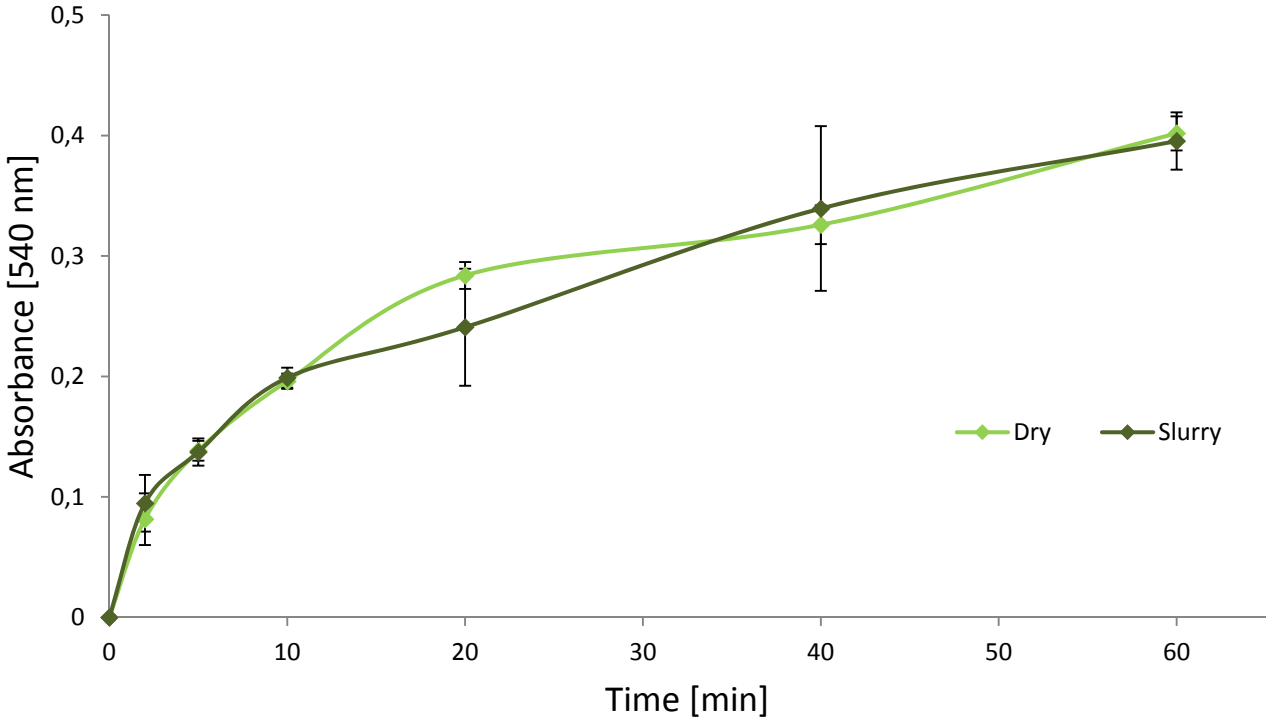
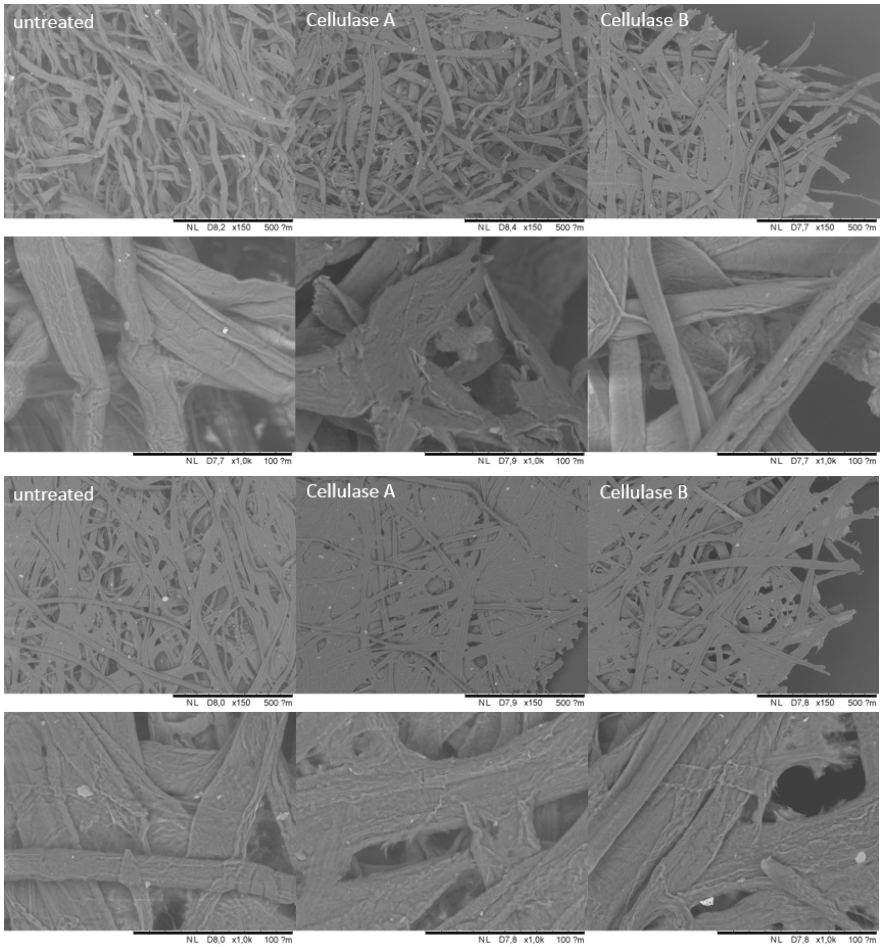


- Cellulases showed no significant differences in activity for dried pulp and pulp slurry

SEM imaging



150 x

1000 x

Long fiber pulp

150 x

1000 x

Short fiber pulp

- Effects for cellulase A on long fiber pulps
- No effects on short fiber pulps
- Relation enzyme activity / fibrillation ?

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Grinding Degree

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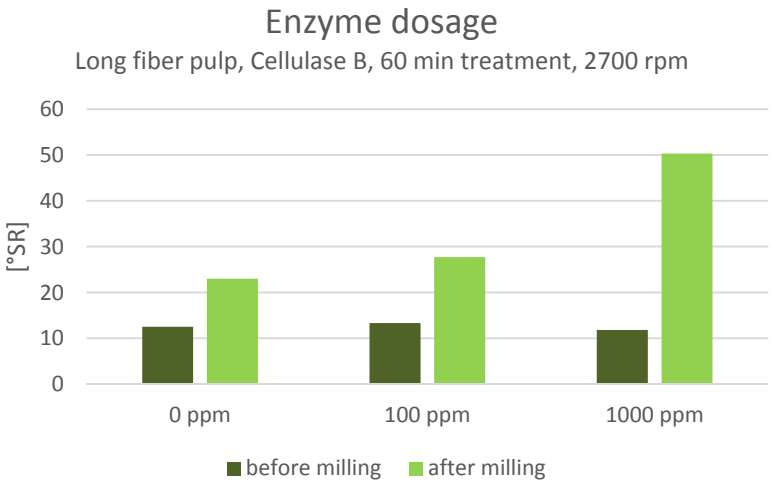

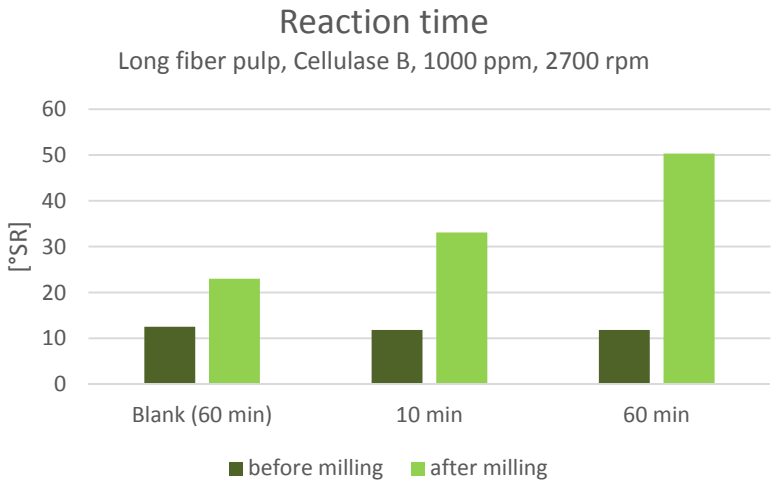

- No effects before milling → challenges in assay development
- High enzyme dosage
- Long reaction time
- Glucose liberation before milling
- High reproducibility

Air Permeability

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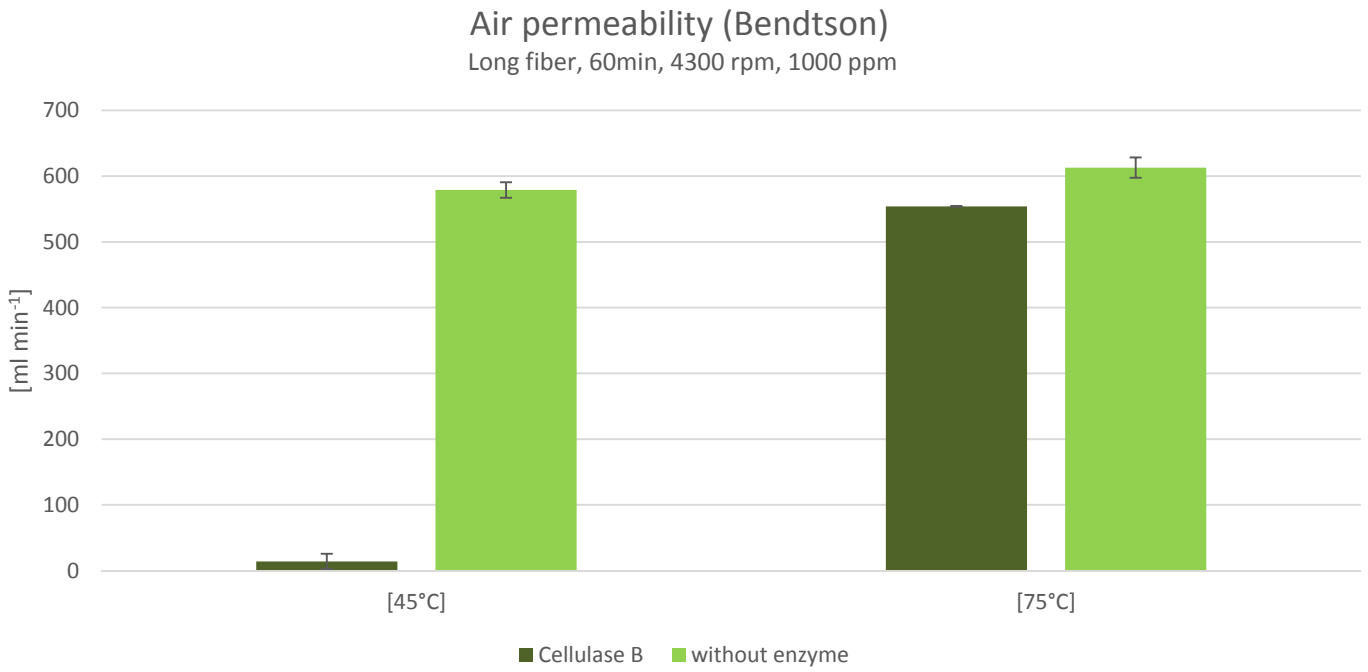

- Strong influences of enzymes on air permeability
- Match of activity and air permeability for temperature
- Effects also visible for other paper related parameters

Conclusion

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- Extensive cellulases characterization
- Correlation of lab tests with reality:
  - No activity under lab conditions →means no activity in reality
- Standard enzyme activity (red. sugars release) does not always correlate to fibrillation effect
- Different modes of actions for reducing sugar release and fibrillation Effects on paper visible only after milling
- Considerable:
  - pH
  - Temp
  - Pulp
  - Enzyme composition
  - Enzyme dosage
  - Side activities

Outlook

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- Testing single enzyme components on pulps
- Simple but specific assays for endoglucanase activity
  - Viscometry
- Monitor fibrillation
- Implementation of a standard procedure for new enzymes

Thank you for your attention

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