

Insight into the Effect of Mechanical Milling on Cellulose Accessible Surface Area and Enzymatic Digestibility

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Background

Cellulose accessible surface area has been identified as a key factor influencing enzymatic deconstruction, as an intimate contact between cellulose and cellulase is a primary step for initiation of enzymatic hydrolysis. The cellulose accessibility is believed to be influenced by its structural characteristics and spatial interactions with other cell wall components.

Quantification of cellulose surface area in wet state is believed to reveal the accessible characteristics better for understanding its digestibility, such as probe molecules adsorption etc.. However, most of the current research only use one technique for evaluating accessibility, which is inadequate for reflecting physical structural changes. Mechanical milling pretreatment can produce highly digestible substrates without significant chemical composition removal.

Therefore, a comprehensive measurement of cellulose accessible properties in combination with knowledge of cellulose structure change should yield important information about mechanical pulverization mechanism and enzymatic digestibility.

Objectives

- To delineate the effect of mechanical pulverization on cellulose accessibility
- To provide insight into the role of cellulose accessibility in biomass recalcitrant fundamentals

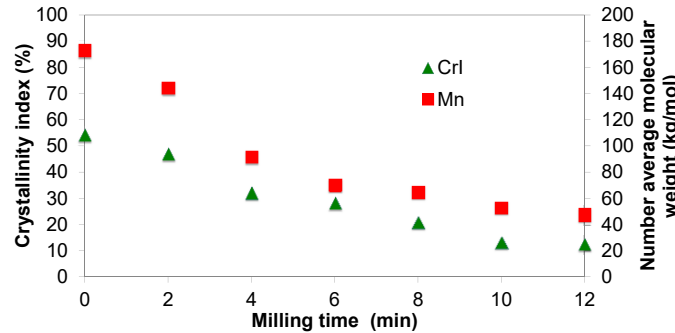
Research approaches

- Mechanical pulverization with Ring & Puck mill
- Crystallinity by X-ray diffraction
- Molecular weight by size exclusion chromatography
- Langmuir enzyme adsorption
- Surface area by dye adsorption
- Water activity by Vapor adsorption
- Morphology by electronic microscopy
- Digestibility by enzyme assay

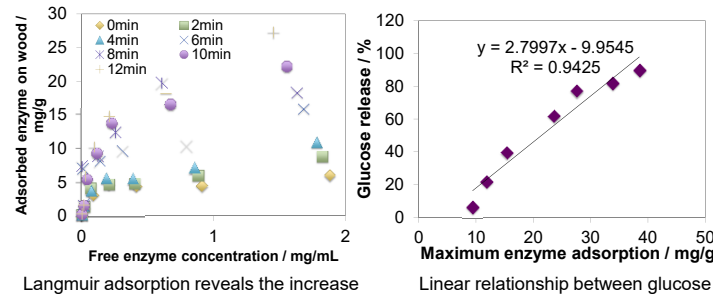
Main results

Correlation of digestibility with accessible properties

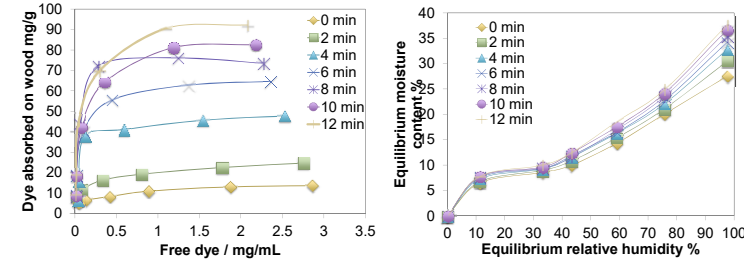
Milling time (min)	Glucose yield %	CrI %	M _n (g/mol)	Γ _m mg/g	A _{DR20} mg/g	A _{H2O} mg/g	A _{DO} mg/g	O/B
0	6.2	54.2	173050	9.4	14.5	50.8	10.5	0.21
2	21.7	46.8	144307	11.8	25.3	62.8	17.2	0.27
4	39.3	32.1	91604	15.4	50.0	66.4	25.9	0.36
6	62.1	28.2	69462	23.6	65.8	67.3	29.4	0.38
8	77.3	20.7	64450	27.5	76.3	68.3	39.4	0.44
10	81.8	13.0	52498	33.8	86.2	71.5	45.3	0.49
12	89.8	12.5	47459	38.5	95.2	75.1	59.5	0.54
Correlation	--	0.97	0.94	0.94	0.98	0.83	0.9	0.96



XRD and SEC reveal physicochemical structure destruction of milled wood cellulose.

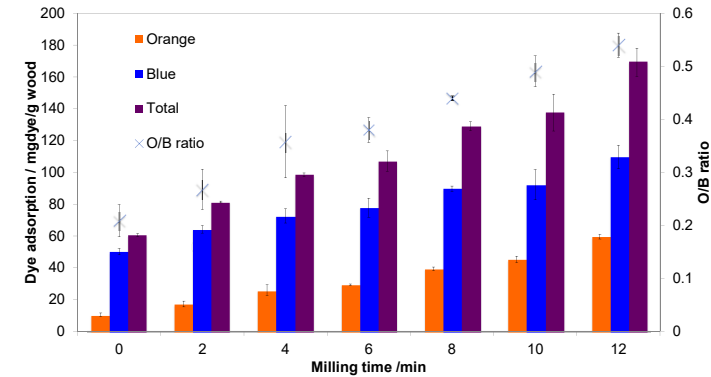


Langmuir adsorption reveals the increase of maximum adsorbed enzyme of milled wood. Release and maximum enzyme adsorption.

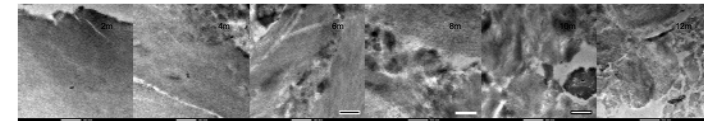


Congo red adsorption reveals increase of total cellulose surface area of milled wood

Vapor adsorption reveals increase of hydrophilicity of milled wood.



Simons' stain reveals increase of cellulose surface area accessible to cellulase.



Ultrastructure alteration of milled wood cell wall contributes to increase of accessibility.

Conclusion

- Accessible surface area of cellulose is an important factor governing the extent of milled wood enzymatic hydrolysis.
- Macro/micro/nano morphological and physicochemical alteration of wood cell wall contribute to increased accessibility and susceptibility of cellulose.