Distinct Roles of Residual Xylan and Lignin in Limiting Enzymatic Hydrolysis of Organosolv Pretreated Woody Biomass

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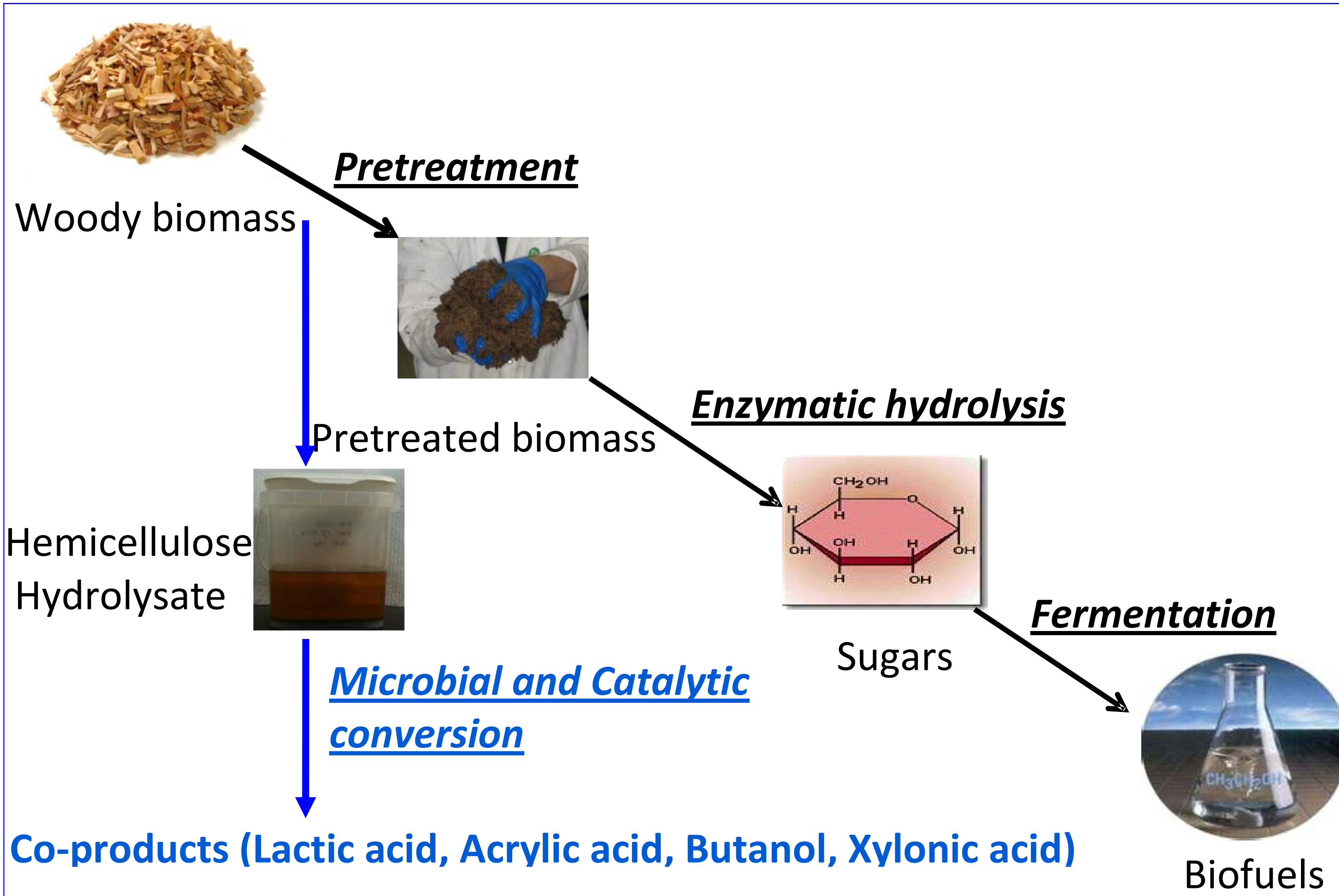
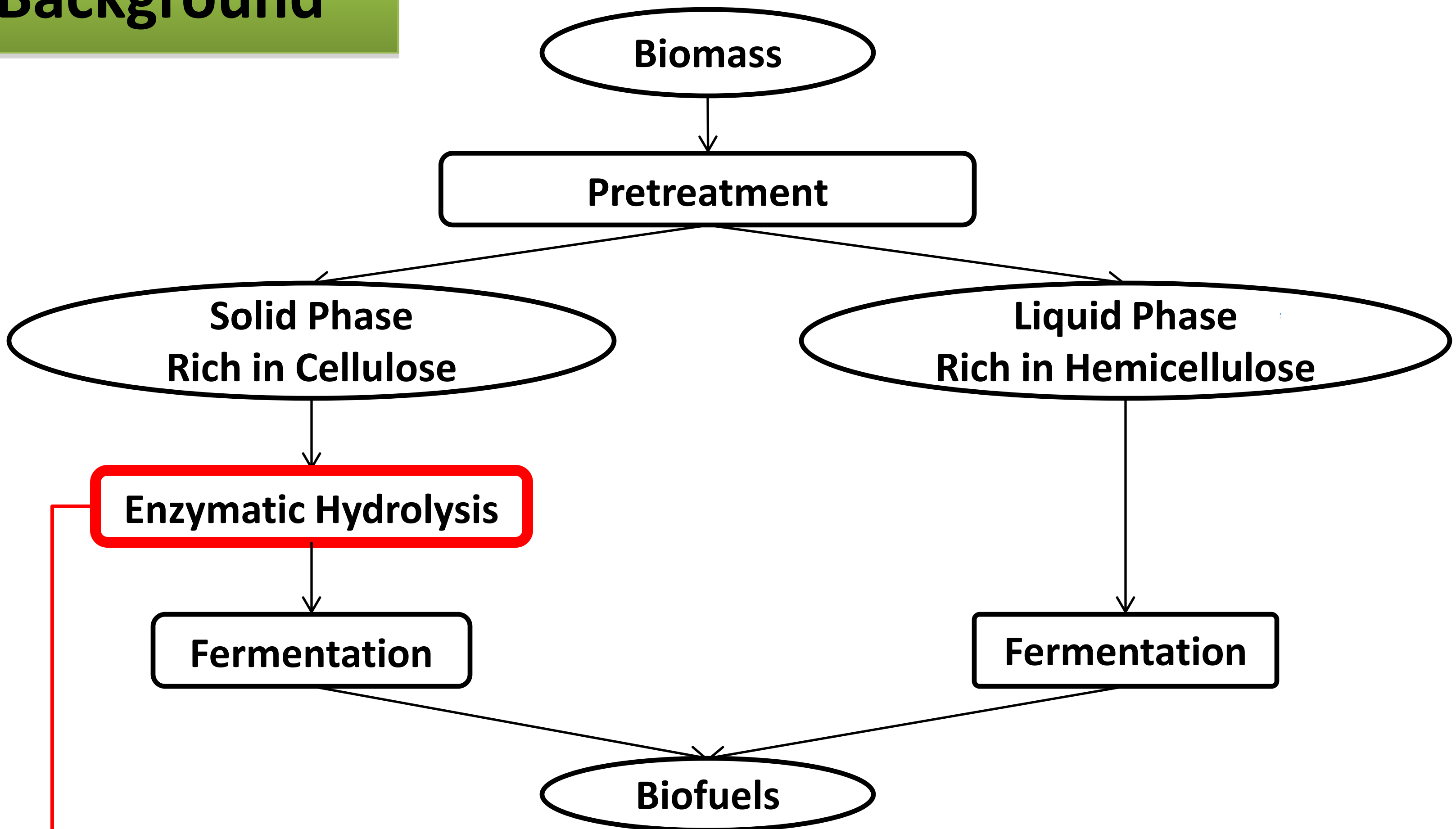


Outline

- **Background and Goals**
- **Effects of residual xylan/lignin on enzymatic hydrolysis**
- **Effects of pectinase/xylanase supplementation on enzymatic hydrolysis**
- **Ethyl xyloside production in SSF process**
- **Conclusion**

Background



Enzymatic hydrolysis limiting factors

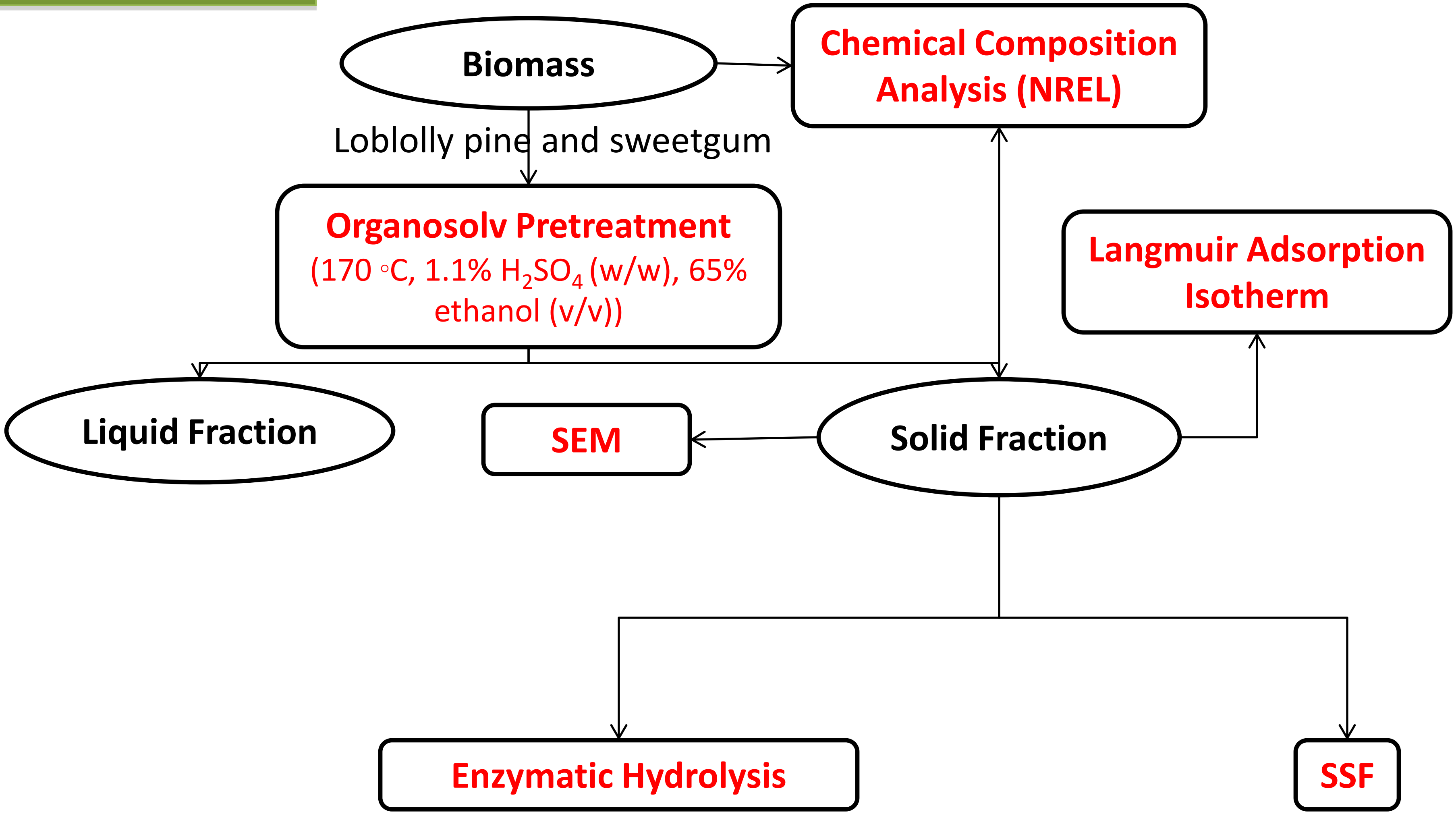
- **Residual xylan and lignin**
- Other factors (DP, crystallinity, pore size and accessible surface area)
- End product inhibition

Goals

- **Goals**
  - **Distinguish the different roles of residual xylan and lignin in enzymatic hydrolysis of pretreated woody biomass**
  - **Improve enzymatic hydrolysis of woody biomass**

- **Hypotheses**
  - **Residual xylan affects Initial hydrolysis rate**
  - **Residual lignin affects Final hydrolysis yield**

Methods


Biomass
Chemical Composition Analysis (NREL)
Loblolly pine and sweetgum
Organosolv Pretreatment
(170 ◦C, 1.1% H_2SO_4 (w/w), 65% ethanol (v/v))
Langmuir Adsorption Isotherm
Liquid Fraction
SEM
Solid Fraction
Enzymatic Hydrolysis
SSF

Methods

Langmuir Adsorption Isotherm

$$\Gamma = \frac{\Gamma_{\max} KC}{1 + KC} \qquad R = \Gamma_{\max} \times K$$

Ʈ: The surface concentration of adsorbed cellulase enzymes;
Ʈ$_{max}$: The surface concentration of protein at full coverage;
K: Langmuir constant;
C: The free protein concentration in the bulk solution;
R: The distribution coefficient

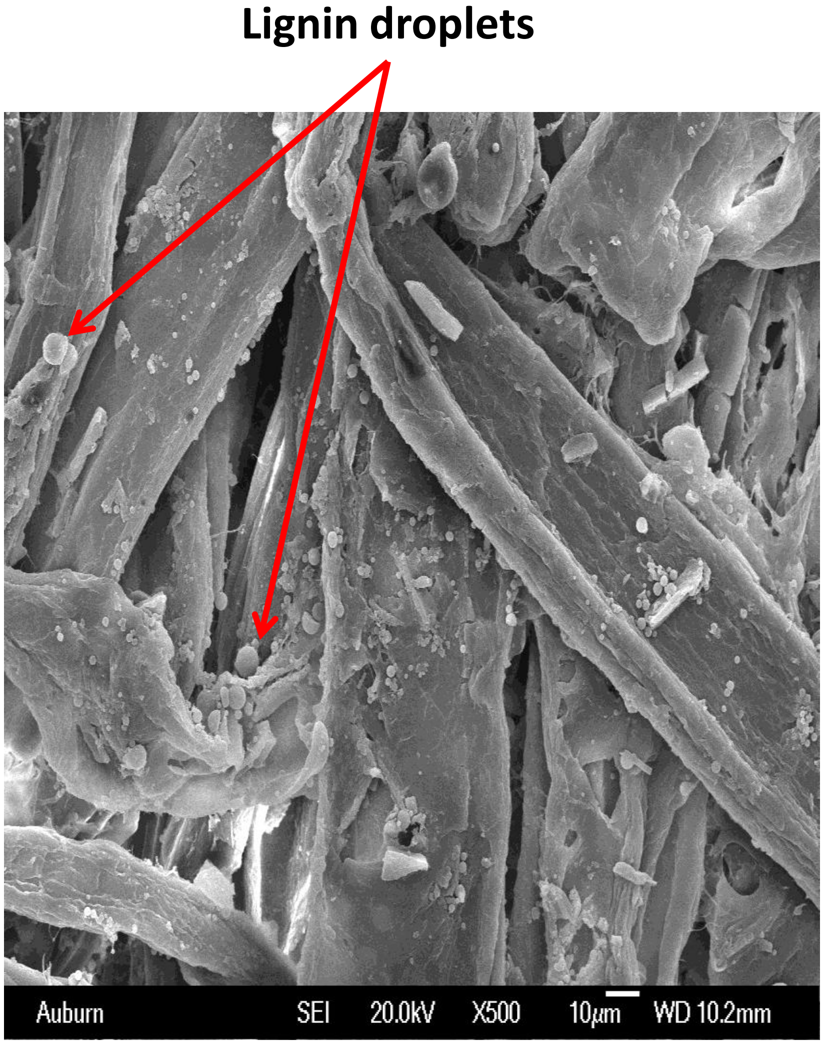
Results and discussion

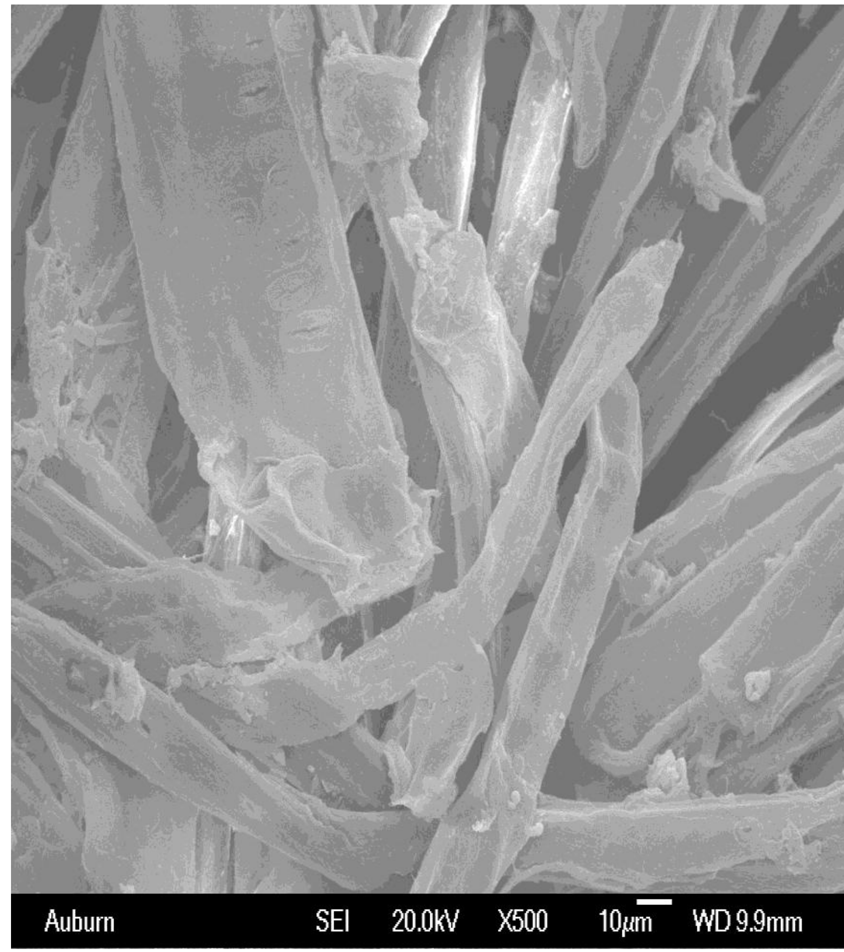
Table 1. Chemical composition of untreated biomass and pretreated biomass

Compositions	Untreated loblolly pine (%)	Untreated sweetgum (%)	OPLP substrate (%)	OPSG substrate (%)
Acetone extractives	1.64±0.08	0.99±0.09	8.53±0.19	8.47±0.21
Acid-insoluble lignin	28.48±0.04	23.56±0.28	18.38±0.17	8.16±0.03
Acid-soluble lignin	0.26±0.03	2.24±0.02	0.23±0.06	0.87±0.03
Glucan	41.33±0.49	41.19±0.73	63.32±1.24	69.80±1.78
Xylan	6.34±0.13	16.18±0.50	2.93±0.79	9.74±0.53
Galactan	2.16±0.09	1.87±0.32	NA	NA
Arabinan	1.30±0.11	0.83±0.11	0.90±0.21	NA
Mannan	12.17±0.39	3.33±0.49	4.50±0.59	3.01±0.28
Total	93.69	90.20	98.79	100.05

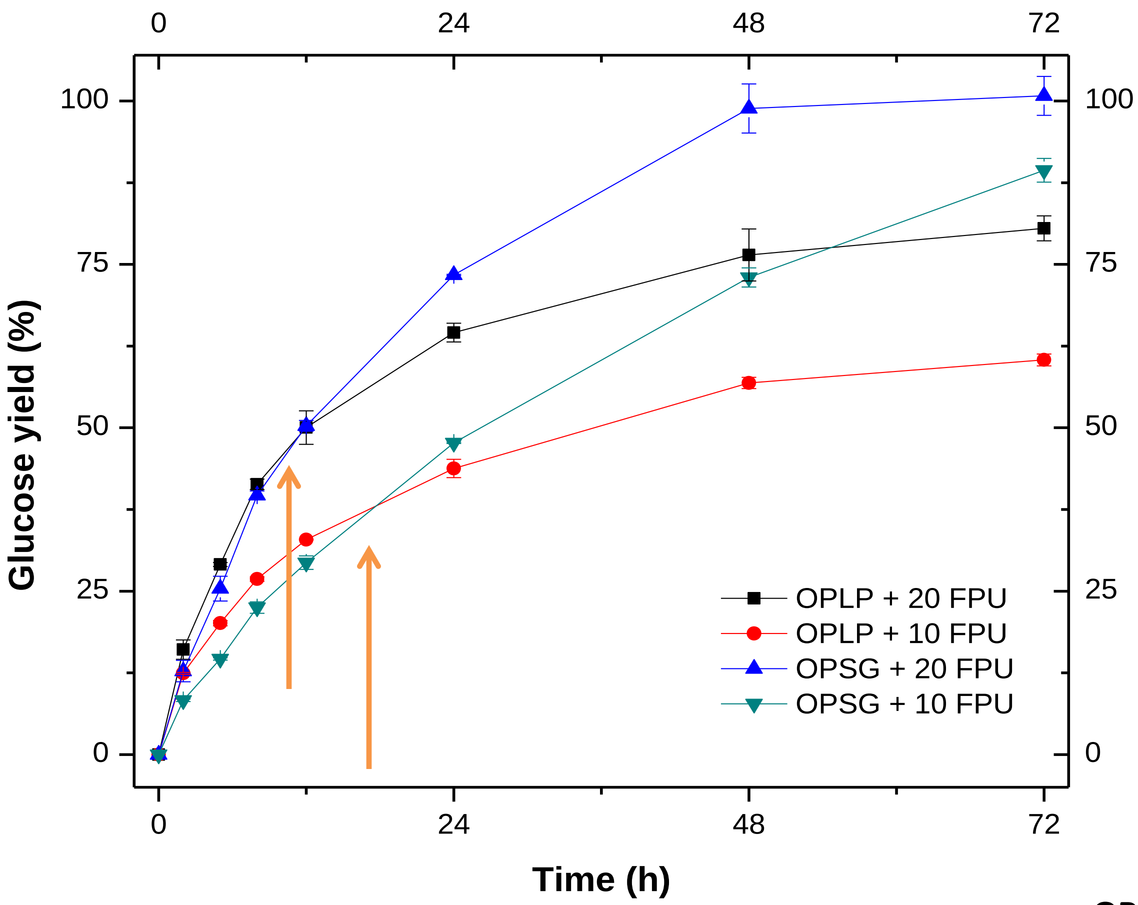
OPLP: organosolv pretreated loblolly pine;
OPSG: organosolv pretreated sweetgum.

SEM images of OPLP (left) and OPSG (right)





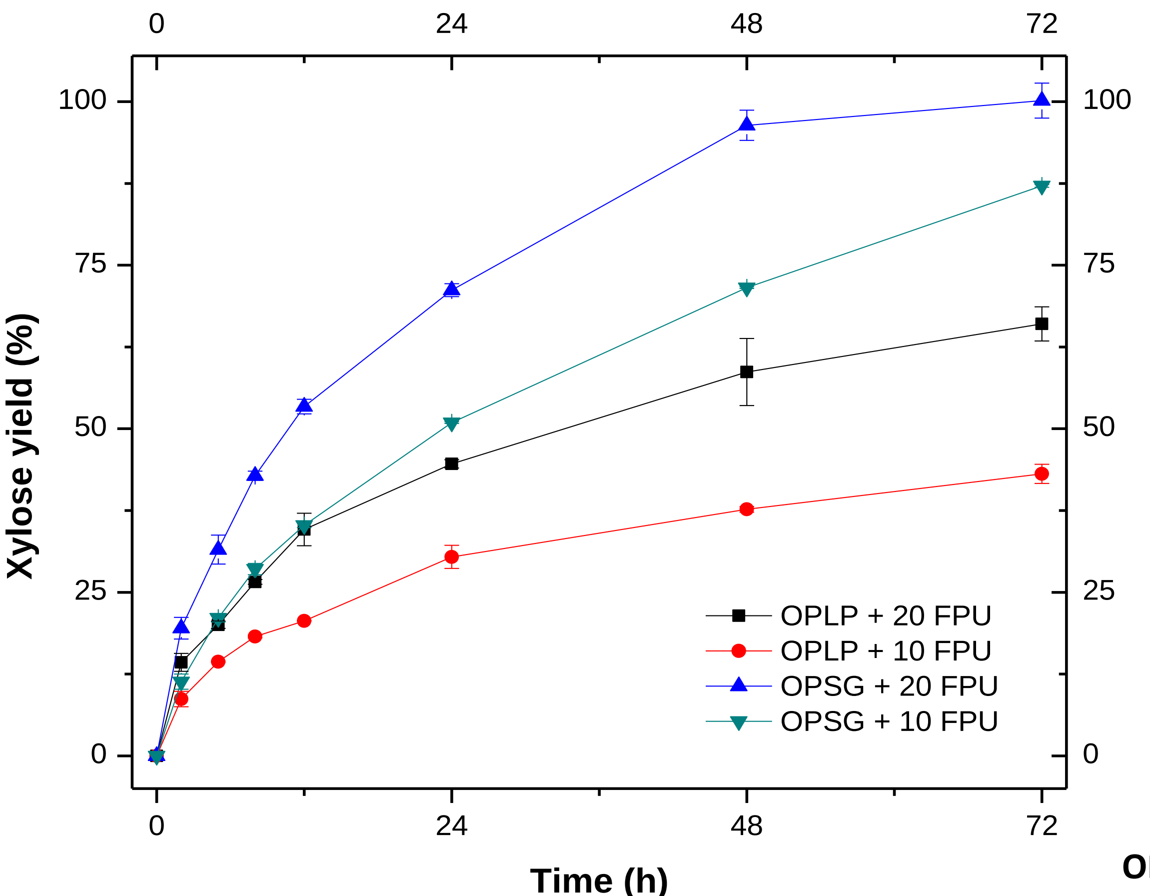
Effect of enzyme loading on the hydrolysis of glucan in OPLP and OPSG



1. **Initial glucan hydrolysis rate (g L^{-1} h^{-1}):**
   - OPLP >OPSG
   - 1.0 vs 0.7 (10 FPU)
   - 1.5 vs 1.2 (20 FPU)
2. **Final glucan hydrolysis yield:**
   - OPSG>OPLP
   - 89% vs 60% (10 FPU)
   - 100% vs 81% (20 FPU)
3. **Crossing point at 17 h**

OPLP: organosolv pretreated loblolly pine;
OPSG: organosolv pretreated sweetgum.

Effect of enzyme loading on the hydrolysis of xylan in OPLP and OPSG



1. **Xylan hydrolysis (rate): OPSG>OPLP**
   - ➢ **0.14 vs 0.03**
   - ➢ **0.21 vs 0.05**
2. **Xylan hydrolysis (yield): OPSG>OPLP**
   - ➢ **87% vs 43%**
   - ➢ **100% vs 66%**

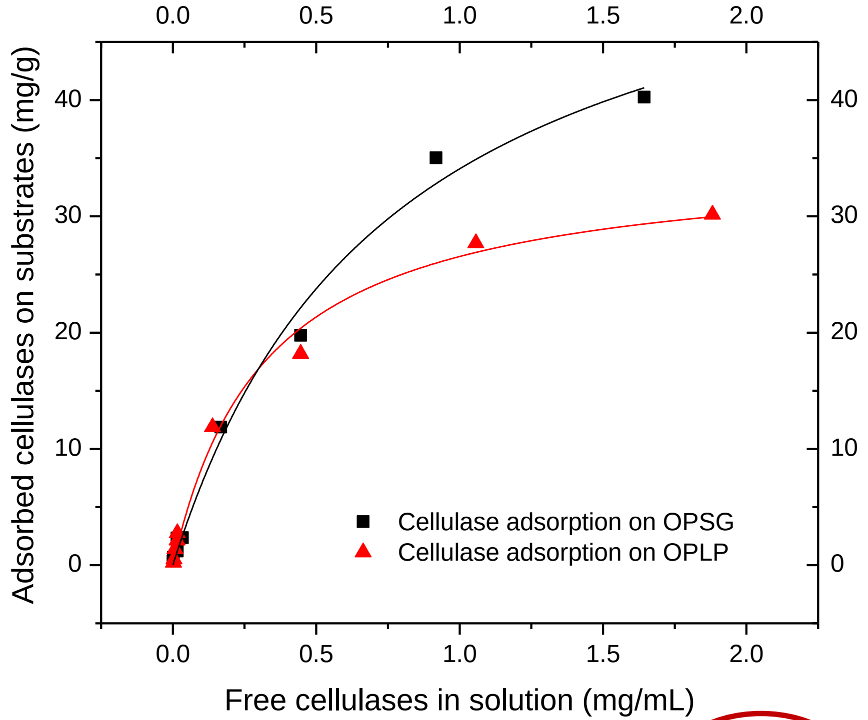
OPLP: organosolv pretreated loblolly pine;
OPSG: organosolv pretreated sweetgum.

Results and discussion

- **Initial glucan hydrolysis rate:**
  - OPLP (1.0 $g \cdot L^{-1} \cdot H^{-1}$)>OPSG (0.7 $g \cdot L^{-1} \cdot H^{-1}$) 10 FPU
  - OPLP (1.5 $g \cdot L^{-1} \cdot H^{-1}$)>OPSG (1.2 $g \cdot L^{-1} \cdot H^{-1}$) 20 FPU
- **Final glucan hydrolysis yield**:
  - OPSG (89%) > OPLP (60%) 10 FPU
  - OPSG (100%)>OPLP (81%) 20 FPU
- **Xylan hydrolysis** (initial rate and final yield):
  - OPSG>OPLP

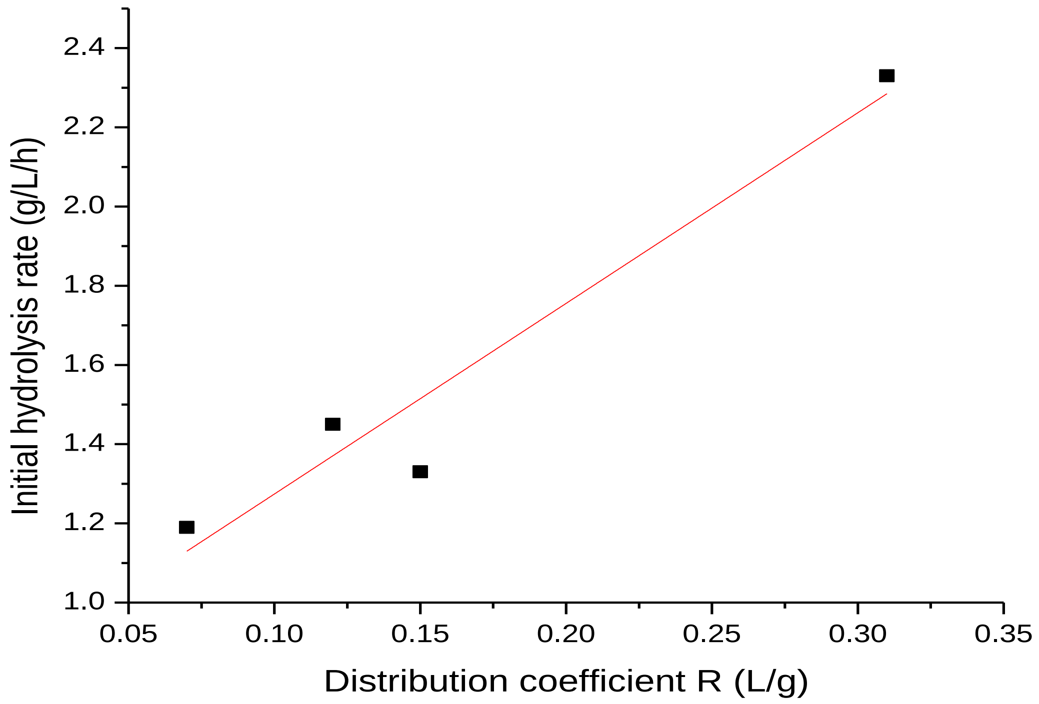
- **Question:**
  - Why the initial hydrolysis rate (glucan) was higher in OPLP than that in OPSG?

Cellulase adsorption isotherms on OPLP and OPSG



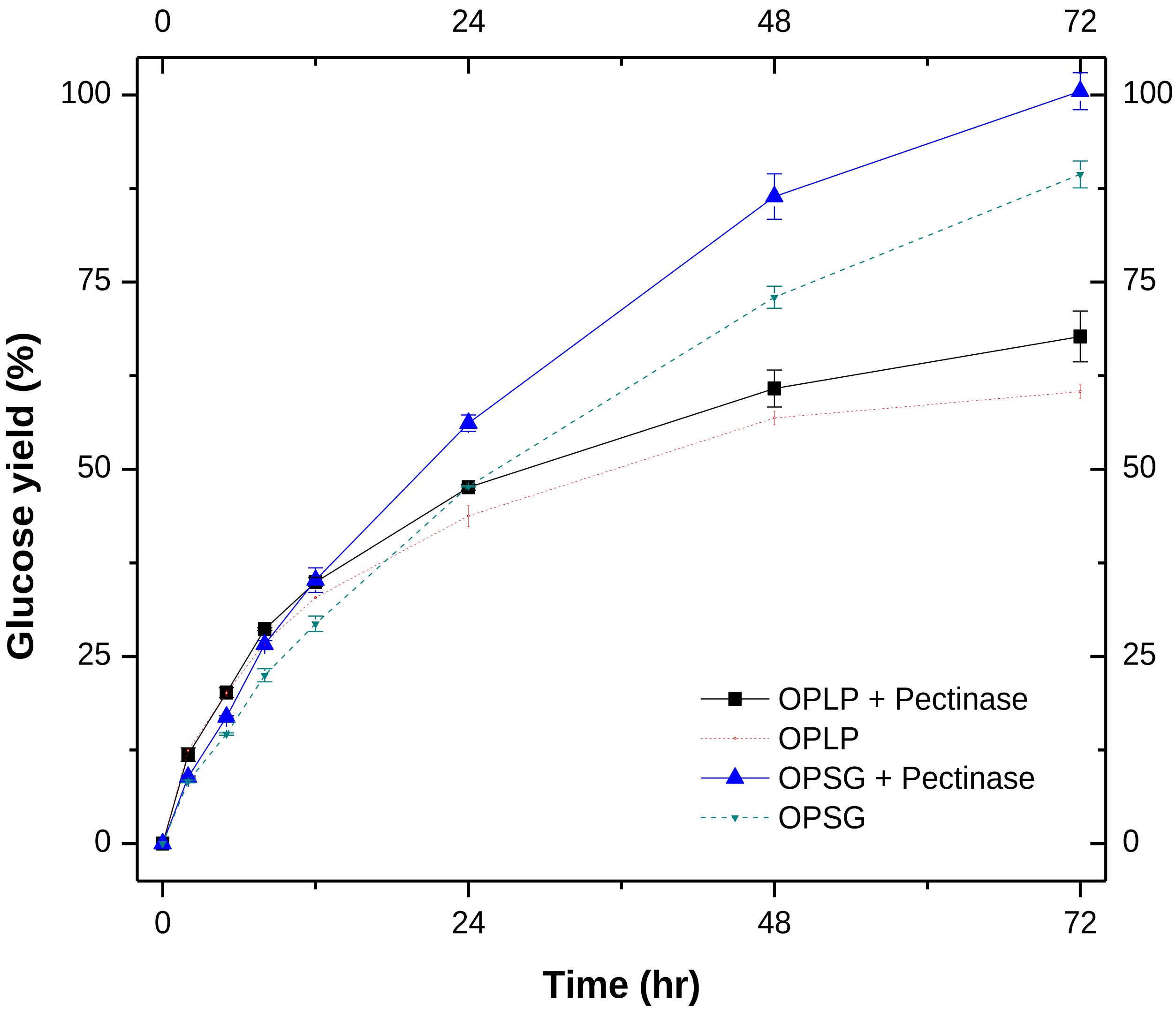
Cellulases	τ_{max} (mg/g)	*K(mL/mg)*	*R(L/g)*
Cellulases on OPLP	35.09	3.11	0.11
Cellulases on OPSG	60.19	1.31	0.08
Celluclast on EPLP*	87.69	3.48	0.31
Celluclast on SELP*	101.05	1.45	0.15

Correlation between distribution coefficient and initial hydrolysis rate



R^2=0.9, initial hydrolysis rate is well correlated to the Distribution coefficient (R)

Effect of pectinase on enzymatic hydrolysis of OPLP and OPSG



Pectinase addition (3.6 mg/g glucan)

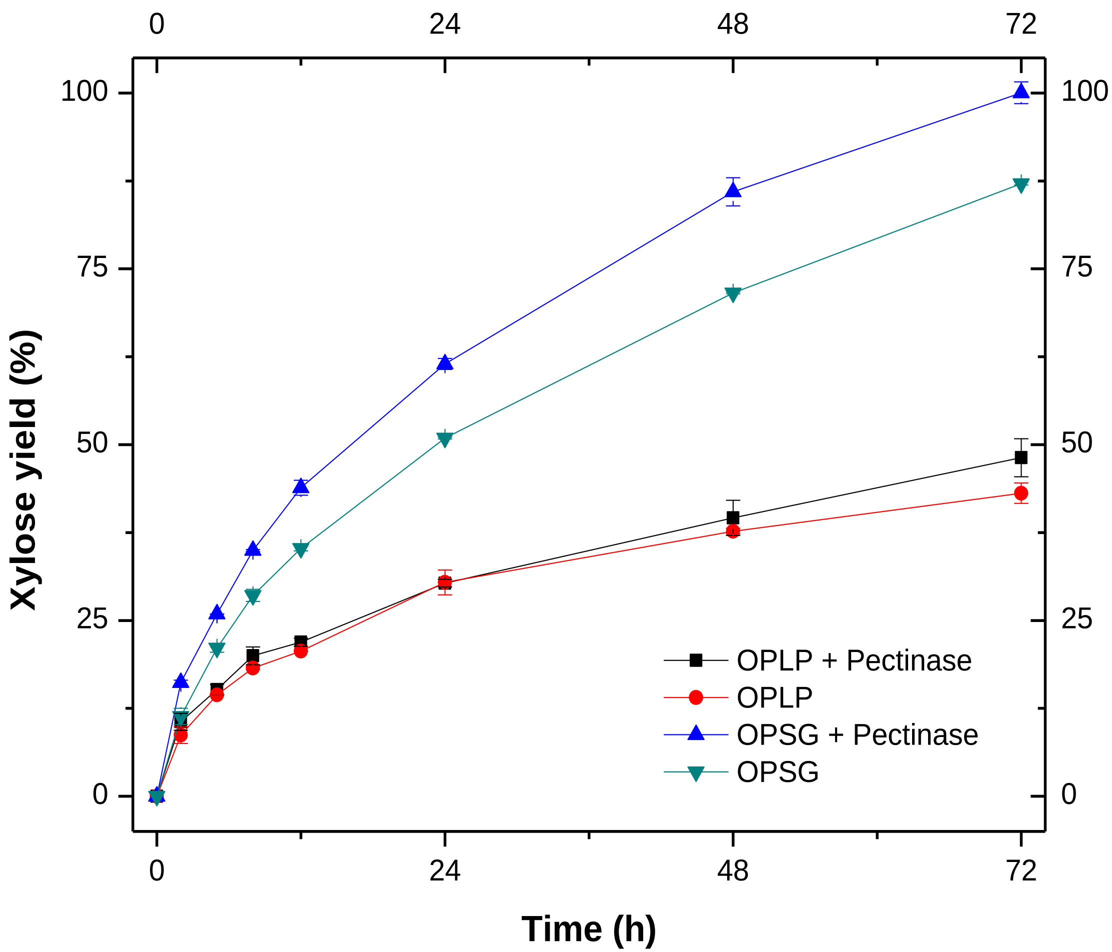
1.Initial rate (g L^{-1} h^{-1})

- OPLP >OPSG
- 1.0 vs 0.7 (10 FPU)
- 1.0 vs 0.8 (+Pectinase)

2. Final yield

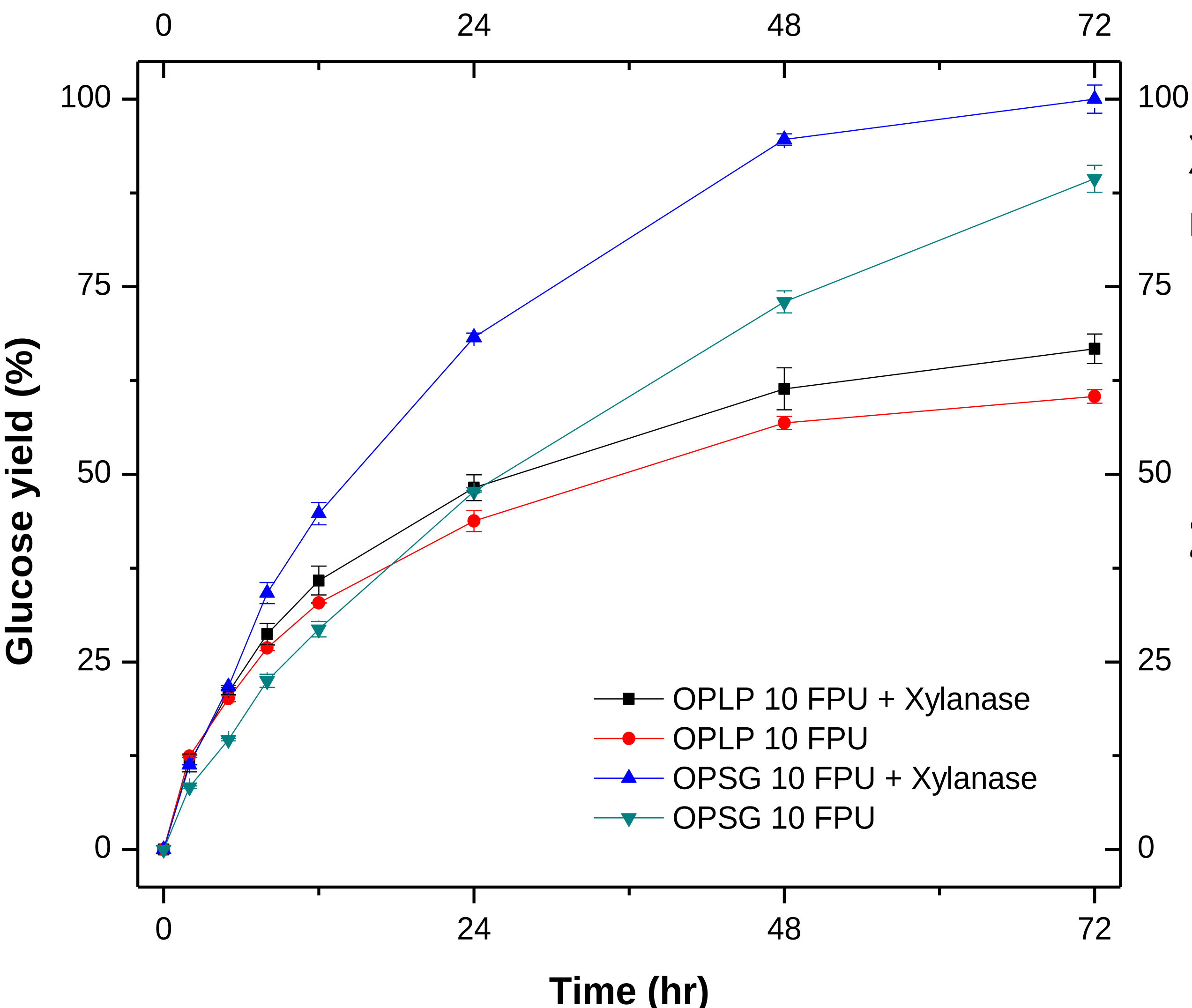
- 60% to 67% in OPLP
- 89% to 99.9% in OPSG

Effect of pectinase on enzymatic hydrolysis of OPLP and OPSG



1. Xylose yield increased 43% to **48%** in OPLP, 87% to 99.9% in OPSG

Effect of xylanase on enzymatic hydrolysis of OPLP and OPSG



Xylanase addition (2.7 mg/g glucan)

1.Initial rate (g L^{-1} h^{-1}):

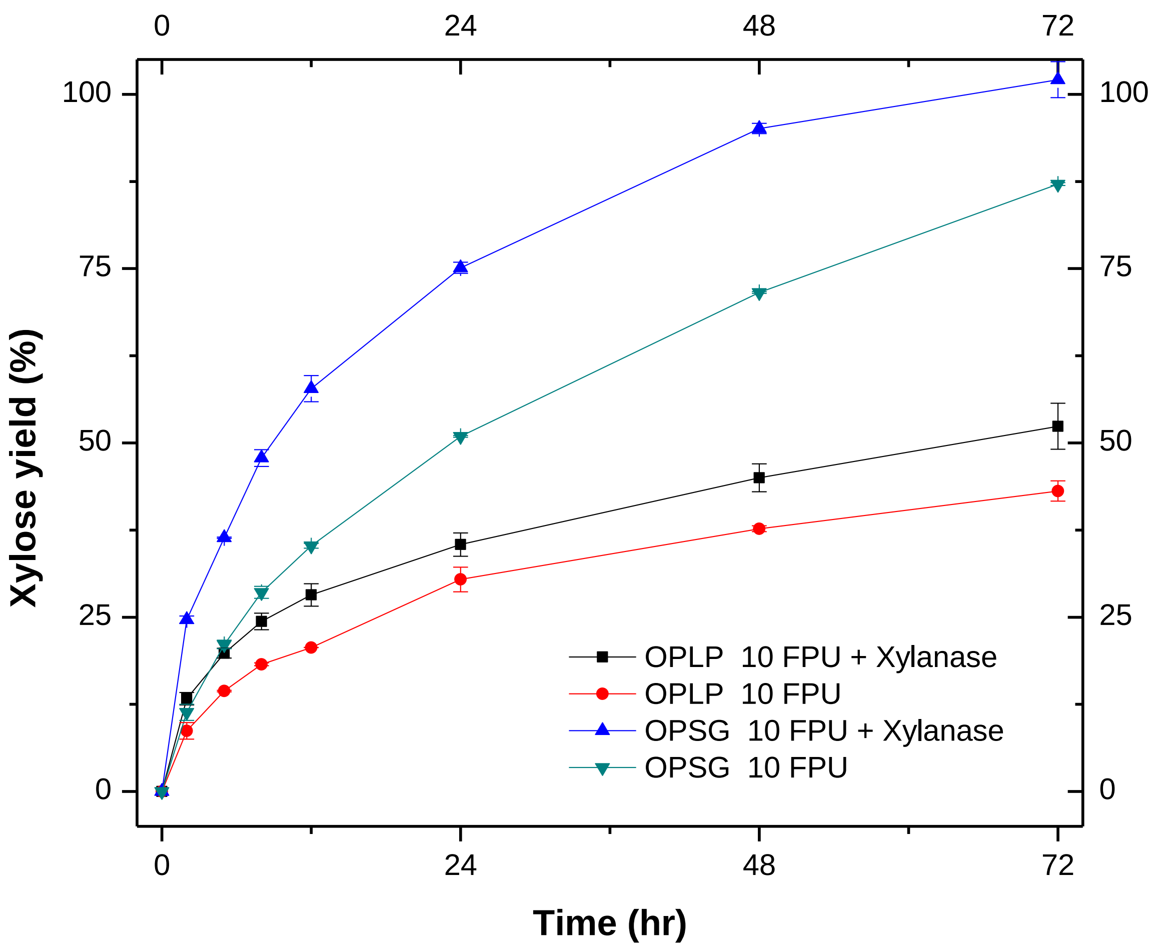
- OPLP >OPSG
- 1.0 vs 0.7 (10 FPU)
- 1.0 vs 1.0 (+Xylanase)

2. Final yield

60% to 67% in OPLP

89% to 99.9% in OPSG

Effect of xylanase on enzymatic hydrolysis of OPLP and OPSG



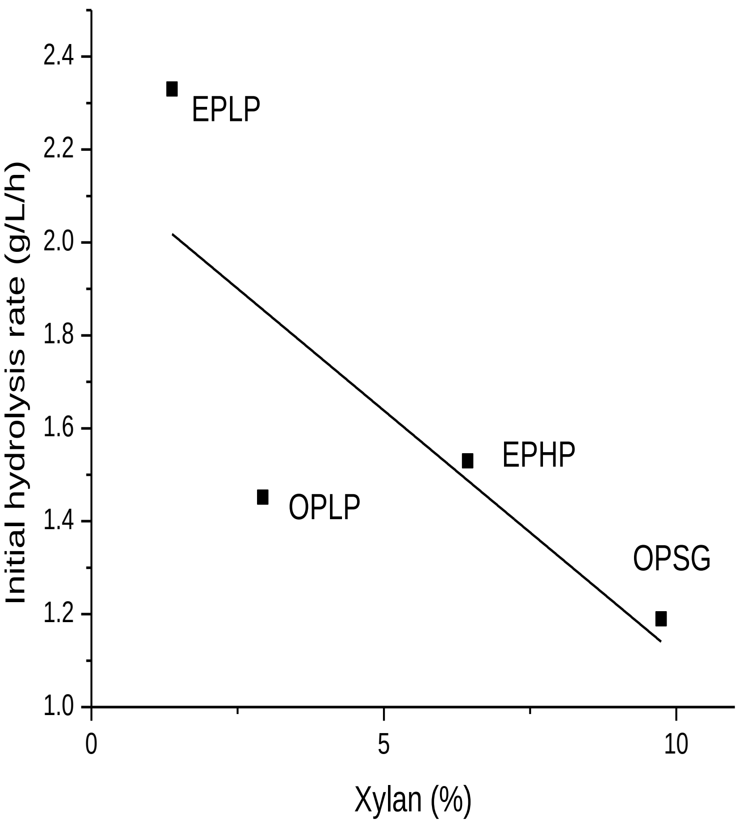
1. Xylose yield increased
   43% to **52%** in OPLP
   87% to 100% in OPSG

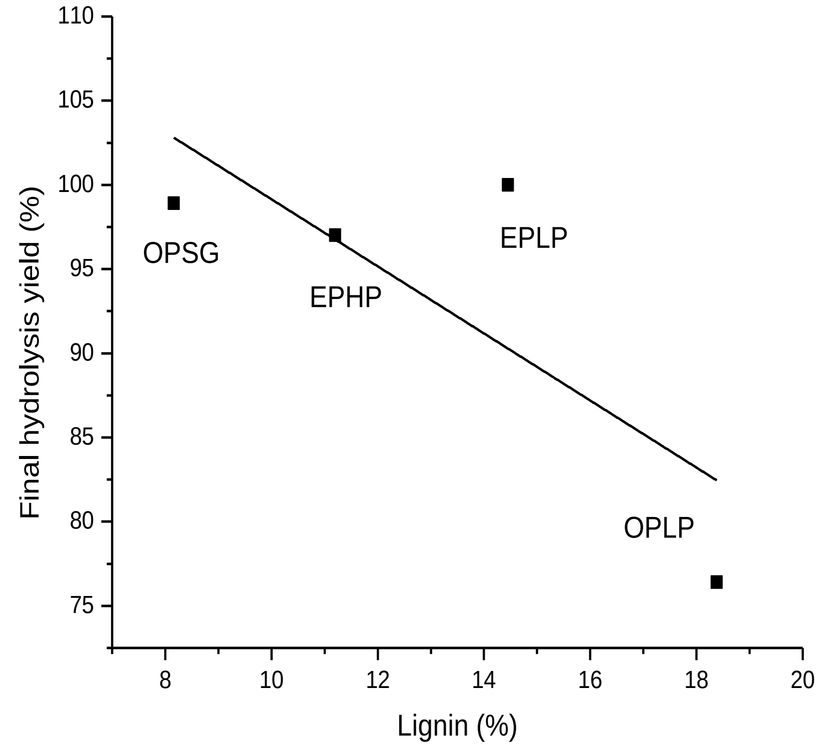
Results and discussion

- **Pectinase supplementation** (3.6 mg/g glucan)
  - Initial hydrolysis rate (OPSG) did not change much (0.7 vs 0.8)
  - Initial hydrolysis rate (OPLP) kept the same (1.0)
  - Final hydrolysis yield increased in both substrates
    - From 60% to 67% in OPLP; from 89% to 99.9% in OPSG

- **Xylanase supplementation** (2.7 mg/g glucan)
  - Initial hydrolysis rate (OPSG) increased by 50% (0.7 vs 1.0)
  - Initial hydrolysis rate (OPLP) kept the same (1.0)
  - Final hydrolysis yield increased in both substrates
    - From 60% to 67% in OPLP; from 89% to 99.9% in OPSG

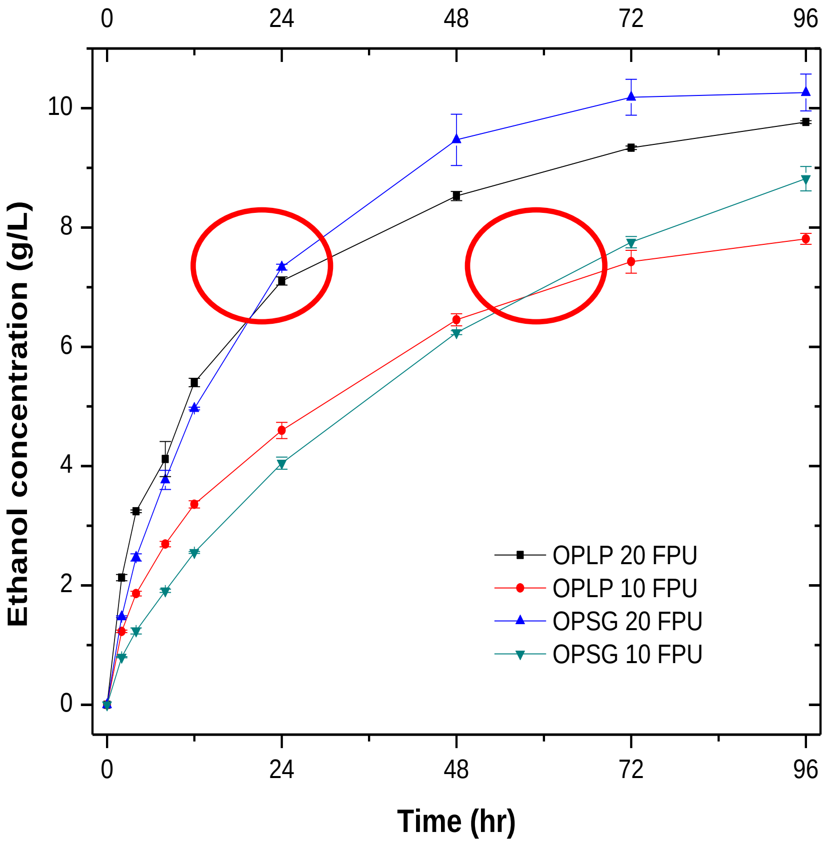
Correlation between residual xylan/lignin and enzymatic hydrolysis

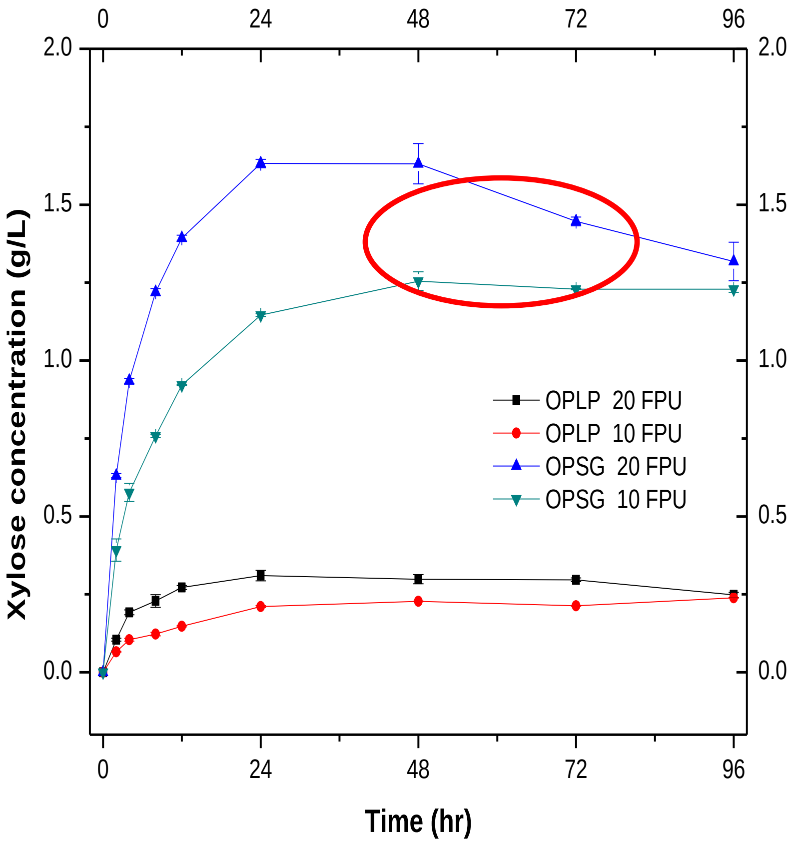




Organosolv pretreated lodgepole pine (EPLP), loblolly pine (OPLP), sweetgum (OPSG) and hybrid polar (EPHP).

Effects of enzyme loading on SSF





1. Initial ethanol production rate (OPLP>OPSG):
   - 0.5 vs 0.3 (10 FPU)
   - 0.8 vs 0.6 (20 FPU)
2. Final ethanol yield: OPSG>OPLP
   - 8.8 vs 7.8 (10 FPU), 10.3 vs 9.8 (20 FPU)

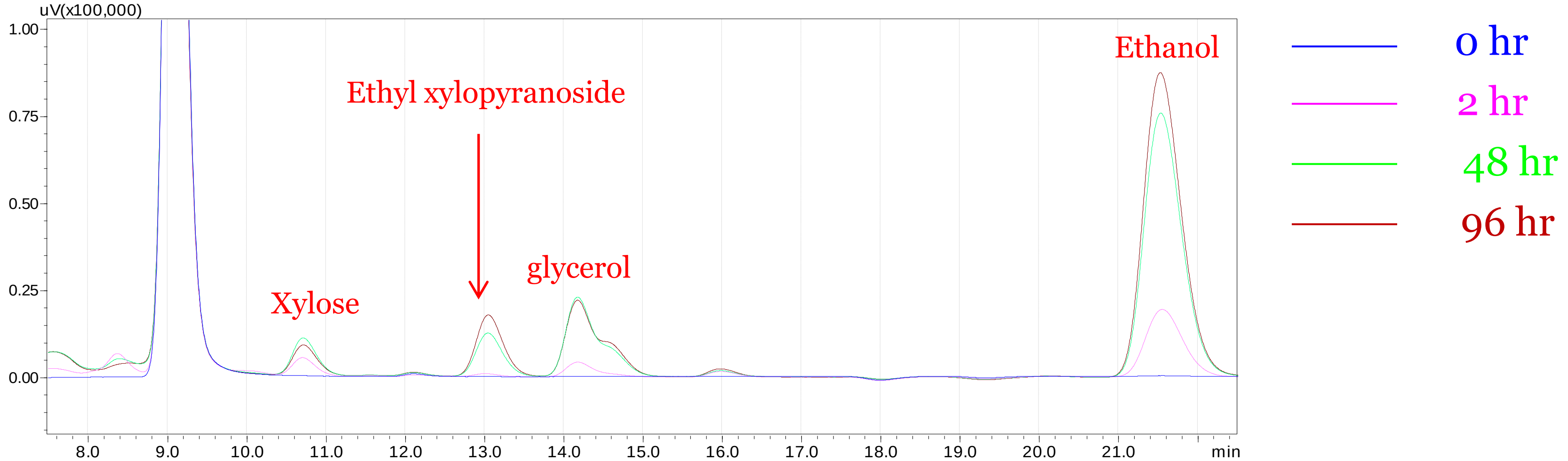
1. Decreased in OPSG after 48hr with 20 FPU loading ?

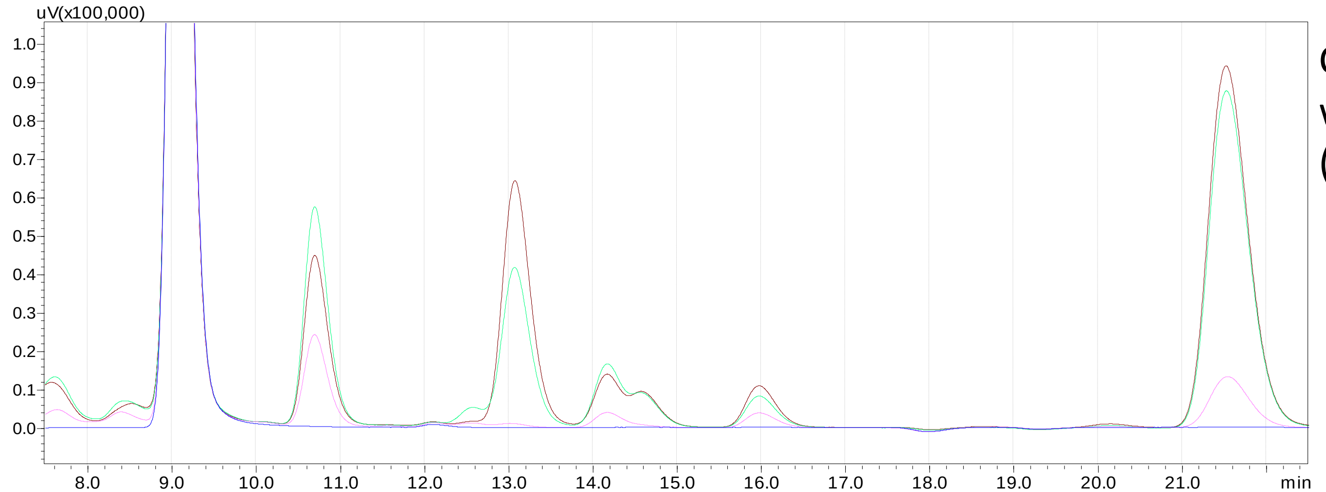
Results and discussion

- **Ethanol production**
  - Initial ethanol production rate: OPLP>OPSG
  - Final ethanol yield: OPSG>OPLP
  - Same trend as glucan hydrolysis but the crossing point changed (58h)

- **Xylose release**
  - Decreased after 48hr with 20 FPU loading
  - Potential enzyme-catalyzed reaction between xylose and ethanol

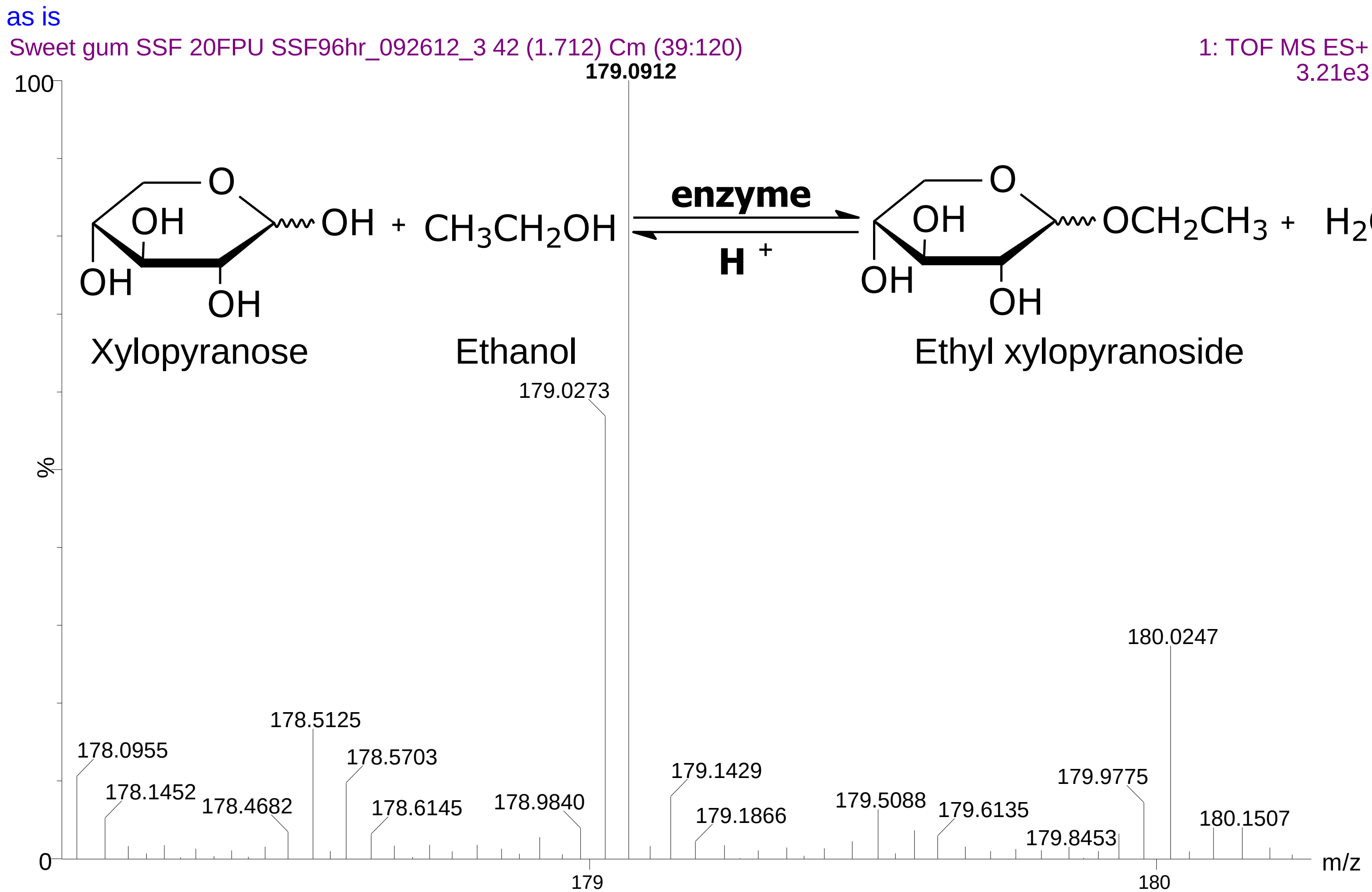
Formation of ethyl xylopyranoside in the process of SSF





Chromatograms in SSF with 20FPU/g glucan (a)softwood; (b) hardwood

LC/MS analysis of potential reaction product between xylose and ethanol



Conclusion

- **Interactions between xylan/lignin and cellulase affect the enzymatic hydrolysis of pretreated biomass**
  - Residual xylan controls the initial hydrolysis rate
  - Residual lignin controls the final hydrolysis yield
  - Two-phase hydrolysis

- **Xylanase supplementation increases initial hydrolysis rate by removing xylan**

- **Linear correlation between initial hydrolysis rate and distribution coefficient**

- **Potential enzyme-catalyzed production of ethyl xyloside**

Acknowledgement

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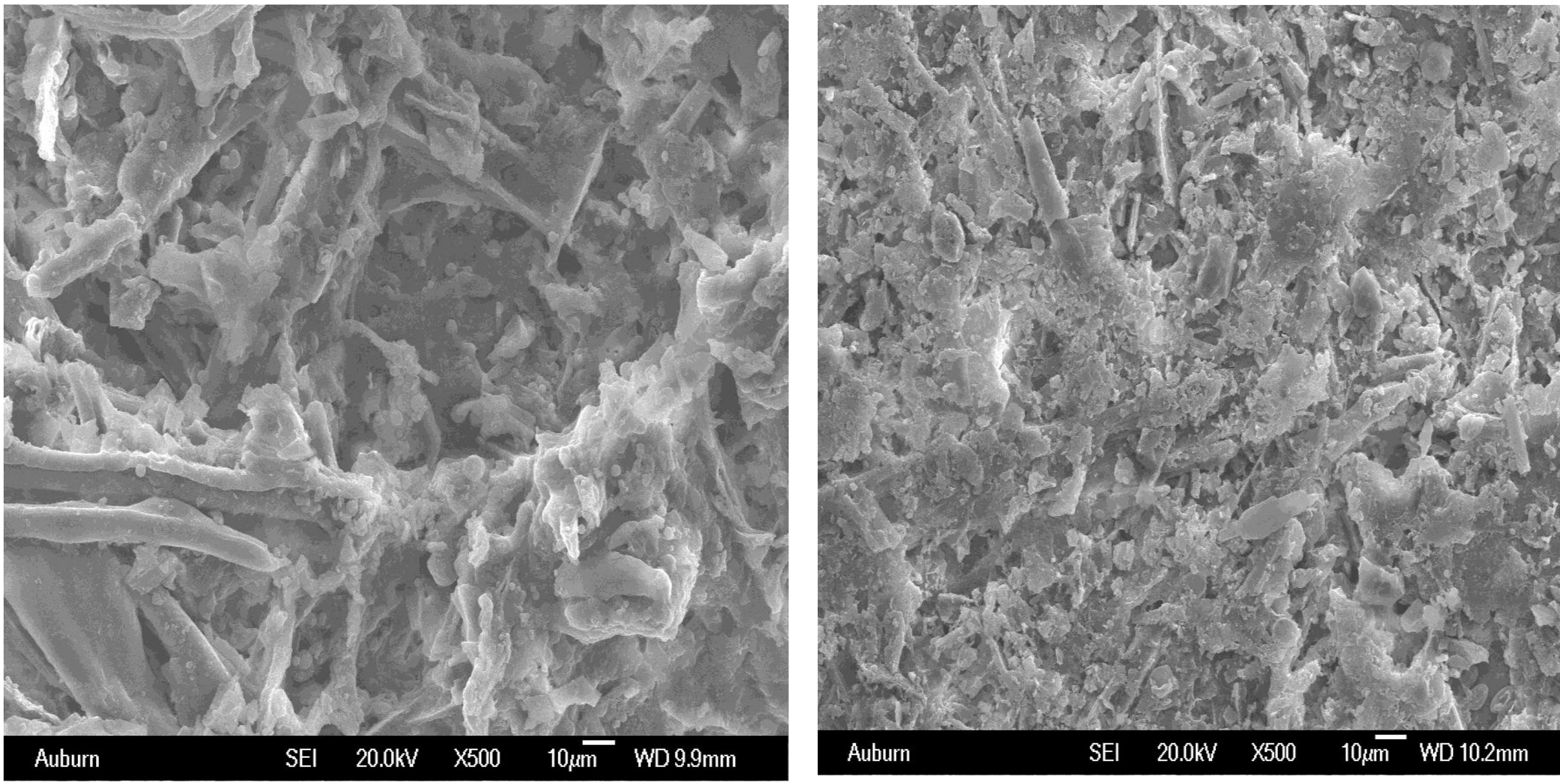
- **Dr. Sushil Adhikari** (Biosystems Engineering)
- **Graduate students (**Mi Li, Rui Xie, Lu Lu and Jing Li**)**

SEM images of enzymatic hydrolyzed OPLP and OPSG



Enzymatic hydrolyzed biomass OPLP(left) and OPSG (right)) after 72 hr with 10 FPU