Investigating the Anatomical Features of Ethanol Organosolv Pretreated *Buddleja davidii*

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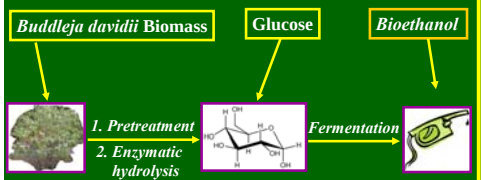
INTRODUCTION

Buddleja davidii is a shrub originated in China, but can be naturalized in different parts of the world, including the U.S.

Basic characteristics of *B. davidii*

- Exhibits a very wide range of growth habit
- Well adapted to poor conditions
- 1.8 – 3.7 m tall
- Spread of 1.2 – 4.6 m
- Perennial
- Very few pests or diseases

Vigorous growth in open and poor soil | Growth in very poor sites | Growth in no soil at all



RESEARCH OBJECTIVES

Understand the effects of ethanol organosolv pretreatment on the cellular structure of *B. davidii*.

- **Pretreatment is the crucial step in the bioconversion process of biomass to bioethanol.**

ANALYSIS

- Compositional analysis of the three fractions.
- Enzymatic conversion of cellulose to glucose using cellulase (20 FPU) and β-glucosidase (40 IU).
- Microscopic investigation of pretreated *B. davidii* to understand the changes in the plant cell wall structure during pretreatment.

PROCEDURE

The ethanol organosolv pretreatment was performed as follows:

Pan et al. *Biotechnology and Bioengineering* 94 (2006) 851 - 861

Ground wood + ethanol + water + H_2SO_4 → Cooking → Unwashed pulp

Sampling liquor for furfural and HMF analysis

Filtration → Spent liquor → Dilution with water

Pulp

Ethanol washes

Precipitation

Warm aqueous ethanol washing

Filtration → Filtrate

Washed pulp

Solid fraction
- **cellulose**
- **residual lignin**
- **residual hemicellulose**

Precipitate

Ethanol organosolv lignin fraction

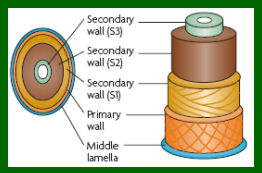
Water-soluble fraction
- **sugars (monomers & oligomers)**
- **depolymerized lignin**
- **furfural and HMF**

Pretreatment Conditions

Condition	Temperature (°C)	Time (min)	Sulfuric Acid (%, w/w oven-dried wood)	Ethanol Concentration (%, v/v)
1	180	60	1.25	50
2	180	40	1.75	50
3	195	60	1.50	65

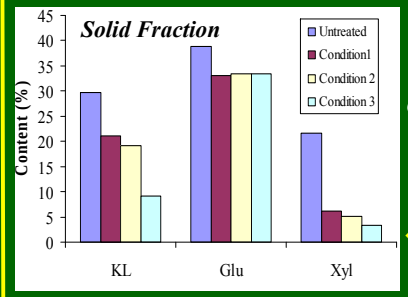
Sample preparation for imaging

- Native and pretreated wood was:
  1- embedded in resin after fixing in FAA and dehydration in graded ethanol series.
  2- sectioned using a microtome set at 2 µm.
  3- stained with 1% (w/v) safranin for 2 min.
  4- imaged using a light microscope.



Sticklen, *Nature Reviews* 9 (2008) 433-443

RESEARCH RESULTS

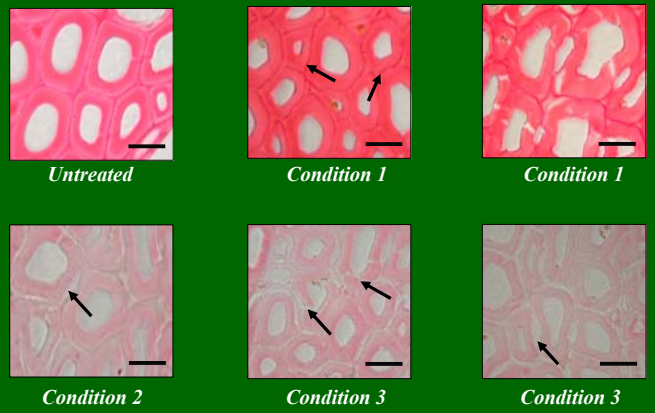


From Condition 1 to Condition 3

- ***More delignification and Carbohydrate hydrolysis/degradation***
- ***Increase in EOL Fraction (4% to 19%)***
- ***Increase in % Lignin in Water-Soluble Fraction (3% to 4%)***
- ***Increase in Furfural and HMF in WS Fraction (0.3% to 1%)***

Enzymatic Conversion of Cellulose to Glucose

- ***Untreated = 4% Condition 1 = 68% Condition 2 = 98% Condition 3 = 98%***



Scale Bar = 10 µm

CONCLUSIONS

✓ **Partial delignification permits cellulases to attack cellulose not only from the lumen side but also from the absent middle lamella region.**

✓ **Lignin content and its physical distribution within the biomass matrix are important with respect to recalcitrance.**

✓ **The chemical and physical changes that occur to the biomass after pretreatment are what dictate efficient enzymatic digestibility. Such changes include: lignin content, hemicellulose content, as well as altering the biomass cell wall structure.**