Gembloux Agro-Bio Tech
Université de Liège

Les Journées Condorcet
5 & 6 juillet 2016

Structure Fédérative de Recherche Condorcet

# Novel butanol pretreatment significantly improves delignification and saccharification of different lignocellulosic biomasses

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## Context

This study investigated a novel organosolv pretreatment using n-butanol and diluted sulfuric acid to separate cellulose, hemicelluloses and lignin into three distinct phases[1]. To demonstrate the effect of the organic solvent, the pretreatment was also performed without addition of n-butanol for comparison. The effects on biomasses of interest in Belgium (tall fescue, beech wood and sugar beet pulp) and in Japan (eucalyptus, Japanese cedar and sugarcane bagasse) were assessed.

## Experimental

- **Conditions**: 6g biomass in 20:60 mL n-butanol/$H_2SO_4$ 1%, 45min cooking time at 200 rpm at 180°C.
- **Saccharification**: 100 mg $mL^{-1}$ by the cellulase Cellic CTec2 at a load of 6.6 FPU $g^{-1}$ at 50°C for 72h[2].
- **Surface morphology**: micrographs were acquired on a JEOL JSM-7500F Field Emission (FE) Scanning Electron Microscope (SEM).

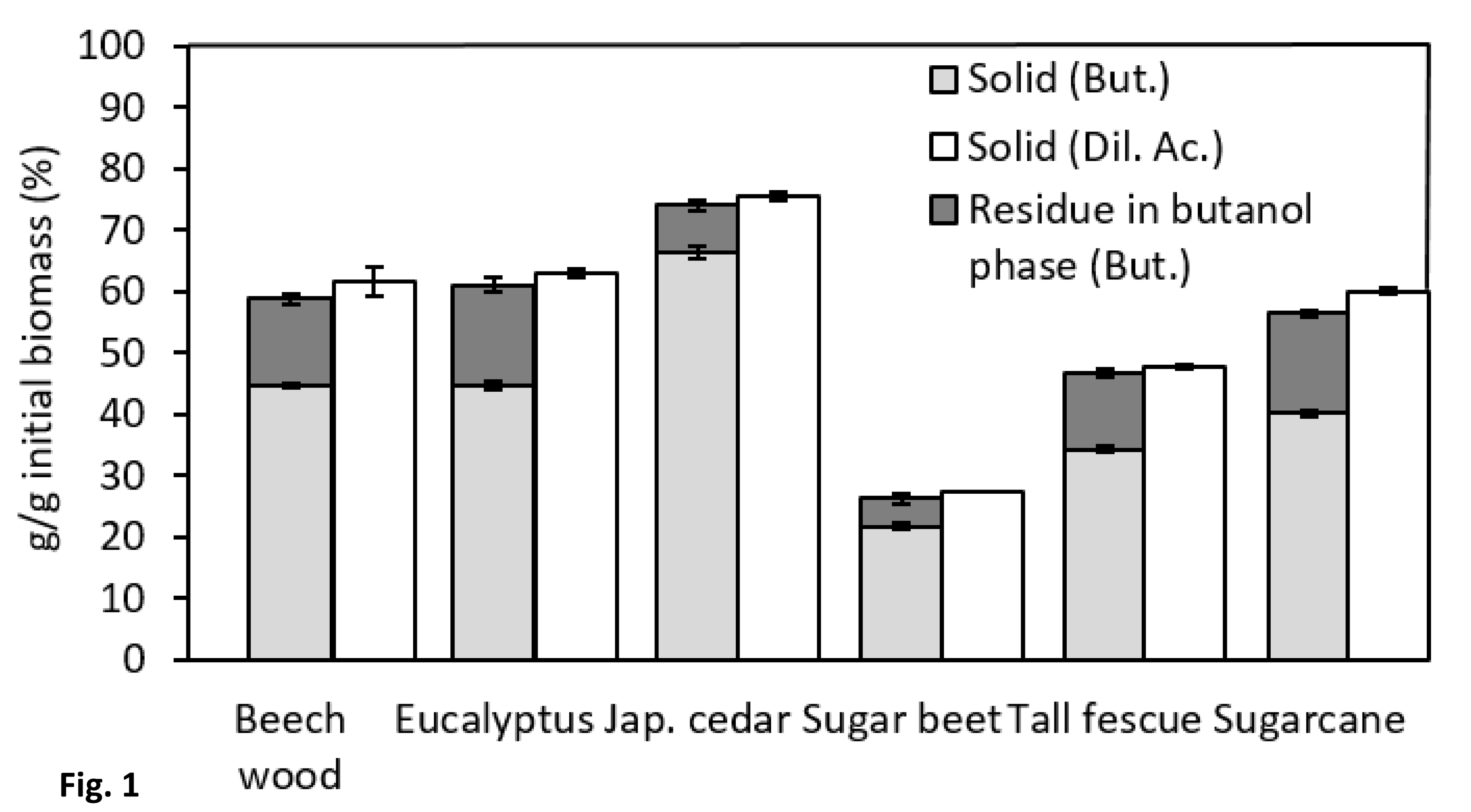


Fig. 1

The amount of recovered solid after butanol pretreatment (But.) is diminished relative to what remained after the dilute acid pretreatment (Dil. Ac.) (**Fig.1**). The decrease is due to a solubilization of lignin in the butanol phase **Fig.2.1.** Hemicelluloses were hydrolyzed and found in the aqueous phase **Fig.2.2.** The major part of the cellulose was recovered in the solid residue **Fig.2.3.**

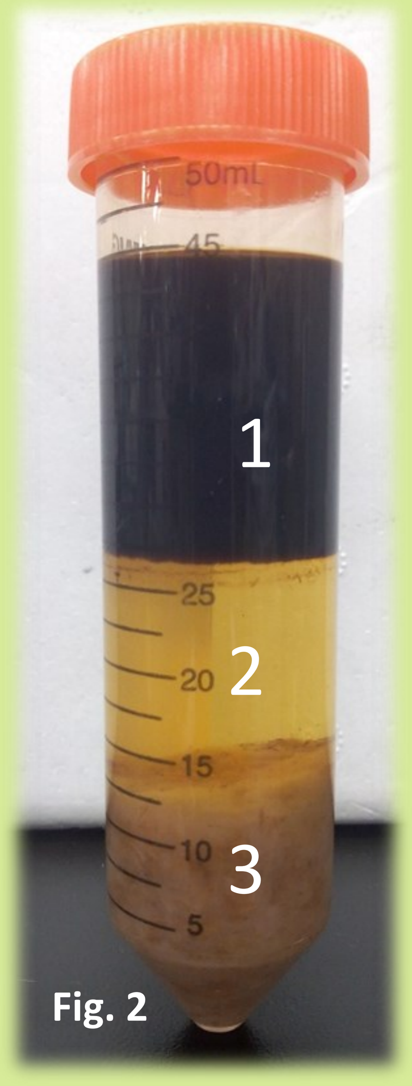


Fig. 2

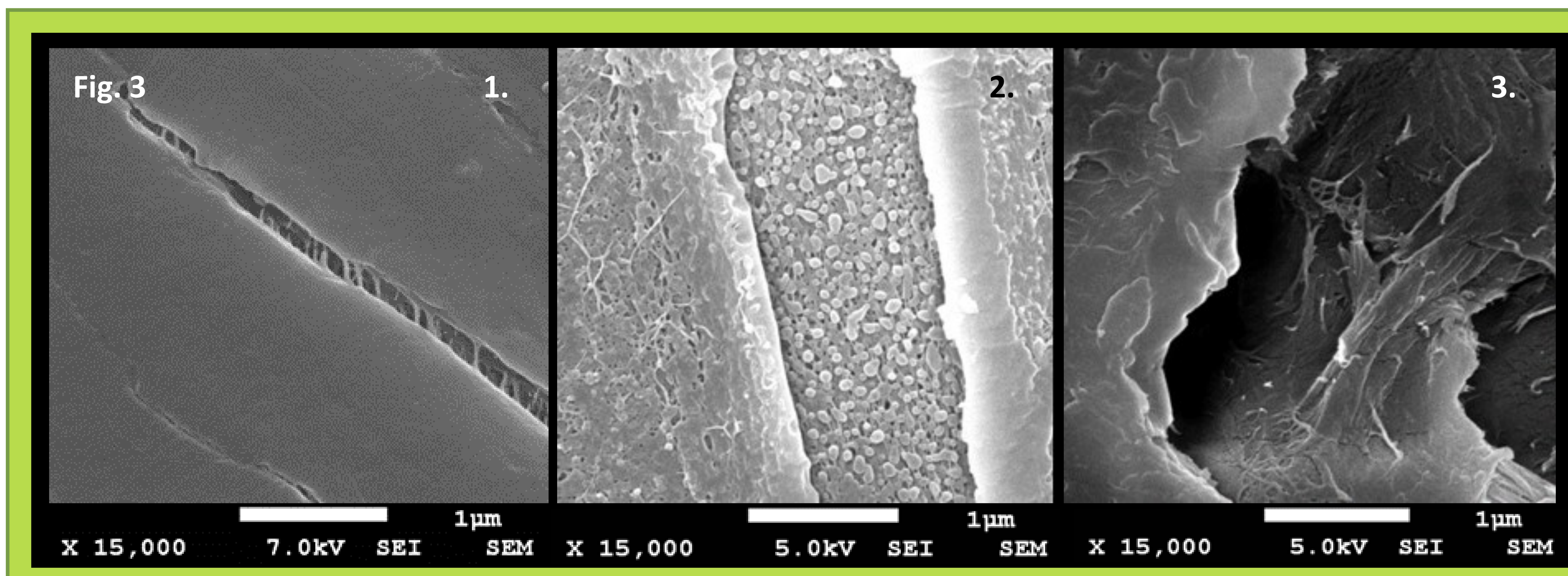


Fig. 3

Micrographs were acquired by SEM from sugarcane bagasse as an example. The surface of raw bagasse (**Fig. 3.1.**) appears smooth apart from some cracks. The bulk of the biomass is inaccessible. The effect of dilute acid pretreatment is clearly revealed in **Fig. 3.2.** in which spherical droplets are observed on the surface. It suggests that the heat applied during pretreatment is sufficient to melt lignin which can then migrate through the matrix, ultimately coalescing as spherical droplets on the surface[3]. By contrast, **Fig 3.3**. which represents pretreated bagasse after butanol pretreatment is free of droplets. These images suggest that butanol efficiently prevents the formation of lignin deposits on the surface of biomass samples. In addition, the structure is particularly more open and the fiber are more apparent.

**Table 1.** Yield of saccharification performed on cellulosic pulp.

| | Pretreatment | Cellulose[a] (%) | Saccharification[b] (%) | Yield[c] (%) | $[C]_{glucose}$[d] (mg $mL^{-1}$) |
|---|---|---|---|---|---|
| **Beech Wood** | Dilute Acid | 52.3 ± 0.8 | 3.5 ± 0.6 | 6.7 | 1.5 ± 0.1 |
| | Butanol | 71.0 ± 1.5 | 46.0 ± 2.0 | 64.8 | 9.7 ± 0.4 |
| **Eucalyptus** | Dilute Acid | 53.8 ± 0.3 | 7.5 ± 0.3 | 13.9 | 2.0 ± 0.0 |
| | Butanol | 74.0 ± 0.4 | 48.5 ± 2.4 | 65.5 | 9.5 ± 0.1 |
| **Jap. Cedar** | Dilute Acid | 40.1 ± 1.5 | 0.0 ± 0.0 | 0.0 | 0.4 ± 0.0 |
| | Butanol | 43.9 ± 0.5 | 0.5 ± 0.0 | 1.1 | 0.6 ± 0.0 |
| **Sugar Beet Pulp** | Dilute Acid | 60.5 ± 2.9 | 46.7 ± 3.3 | 77.2 | 8.9 ± 0.0 |
| | Butanol | 68.1 ± 0.4 | 69.1 ± 2.5 | 100 | 11.6 ± 0.6 |
| **Tall Fescue** | Dilute Acid | 51.6 ± 1.2 | 20.2 ± 2.9 | 39.1 | 3.7 ± 0.1 |
| | Butanol | 70.4 ± 2.4 | 73.7± 1.5 | 100 | 11.0 ± 0.2 |
| **Sugarcane Bagasse** | Dilute Acid | 53.0 ± 1.0 | 9.9 ± 0.4 | 18.7 | 2.3 ± 0.3 |
| | Butanol | 80.7 ± 1.9 | 77.5 ± 0.7 | 96.0 | 11.6 ± 0.3 |

[a] Amount of cellulose in the solid residue after pretreatment
[b] Yield of dried residue hydrolyzed by cellulase Cellic CTec2
[c] Percentage of hydrolyzed cellulose [d] Concentration of aq. glucose

Delignification achieved by butanol pretreatment improves significantly the enzymatic saccharification yield although almost no improvement was observed on Japanese cedar (low delignification rate: 12%). Saccharification of sugarcane was improved to 77.5% from 9.9%, consisting in the best improvement achieved by butanol and corresponding to a cellulose hydrolysis of 96% **Table 1**.

## Conclusion

Delignification and saccharification were successfully achieved through the addition of a small amount of n-butanol to a dilute sulfuric acid pretreatment. Overall, the greatest improvement were achieved on sugarcane bagasse. However, Japanese cedar was an exception to the general conclusions.

### References

1. Sasaki K, et al.: Mechanical milling and membrane separation for increased ethanol production during simultaneous saccharification and co-fermentation of rice straw by xylose-fermenting Saccharomyces cerevisiae. *Bioresource Technol*. 2015 **185** : 263-268.
2. Teramura H, et al.: Organosolv pretreatment of sorghum bagasse using a low concentration of hydrophobic solvents such as 1-butanol or 1-pentanol *Biotechnol. Biofuels* 2016 **9**(27).
3. Selig MJ, et al.: Deposition of lignin droplets produced during dilute acid pretreatment of maize stems retards enzymatic hydrolysis of cellulose. *Biotechnol Prog*. 2007 **23** : 1333-1339.