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## PRODUCTION OF BIO-ETHANOL FROM BEECH WOOD PELLETS VIA MILD ACETONE ORGANOSOLV FRACTIONATION

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**ABSTRACT:** Advanced biofuels are produced from renewable resources that comply with stringent sustainability criteria, and lower GHG emissions than fossil fuels. In the BECOOL/BioValue EU-Brazil cooperation project, novel value chains for advanced bioethanol are developed. Second generation (2G) bio-ethanol represents the most important advanced biofuel from sugar streams, but its production costs are higher than those of other fuels, and innovations are needed to improve efficiency and economics of the process. In this work, beech wood pellets were treated using the FABIOLA™ technology, an acetone-based low-temperature organosolv fractionation process. This fractionation results in three main product streams: lignin, a high-purity cellulosic pulp and hemicellulose sugars in solution. The cellulosic pulp was enzymatically hydrolysed producing a glucose-rich (C6) stream and the hemicellulose sugar solution (C5) is conditioned for further biological conversion. The C6 and C5 streams obtained were used for fermentation by the yeasts *Saccharomyces cerevisiae* and *Spathaspora passalidarum*, respectively. While the C6 stream was readily fermentable, the C5 stream was only fermentable after dilution. Therefore the C5 stream was subjected to detoxification by activated carbon. Detoxified C5 streams showed higher fermentability and the production of bio-ethanol reached approximately 0.3 g bio-ethanol/gram xylose consumed. Fermentation experiments at 10-L scale confirmed the results obtained at laboratory scale. Combining FABIOLA™ and detoxification resulted in efficient C6 and C5 utilization for bio-ethanol production.

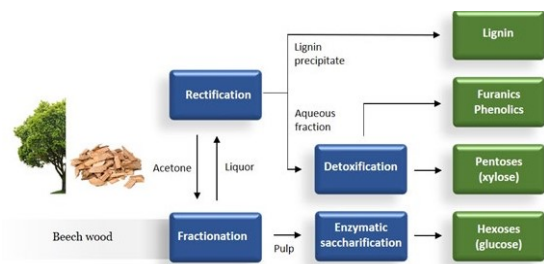
**Keywords:** Bioethanol, Lignocellulosic sources, fermentation, biotechnology

### 1 AIM AND APPROACH

The aim of this study is to develop an efficient pre-treatment and fermentation process for lignocelluloses to bioethanol. To reach this, several approaches have been followed (Figure 1):

- An innovative process for pre-treatment and fractionation (FABIOLA™) was applied to produce fermentable sugar streams and high-purity lignin from beech wood pellets;
- The resulting C6 and C5-rich sugar streams were fermented using different yeasts strains for optimal bioethanol yields;
- A detoxification method for C5-rich stream was developed to enhance fermentability.
- The pre-treatment, detoxification and fermentation processes developed at lab scale were successfully upscaled to pilot scale with confirmed performance.

The FABIOLA™ process uses acetone as solvent at mild process conditions for the effective recovery of the three main components of biomass: Cellulose is rendered hydrolysable via enzymatic saccharification; hemicellulose sugars are recovered in a hydrolysate; and lignin of high quality is recovered for high-value applications such as materials and chemicals (Fig. 1).



**Figure 1.** FABIOLA™ fractionation process for the production of lignin, pentoses (C5) and hexoses (C6) sugars for fermentation.

### 2 MATERIALS AND METHODS

Beech wood pellets fractionation using FABIOLA™ For this study, 70 kg beech wood was fractionated at

pilot scale (460L percolation reactor) at 140 °C with 50 wt% acetone using 1.96% H<sub>2</sub>SO<sub>4</sub> (w/w feedstock) and a liquid to solid ratio of 3.2 w/w. Product pulp was hydrolyzed using Cellic CTec2 (Novozymes). The collected C5 and C6 hydrolysates were concentrated by evaporation before storage at -20 °C.

For the detoxification of C5 streams from fractionation, a granular activated carbon (NORIT® GAC 1240) was used as adsorbent in a glass column (ECOPLUS 15 AB, YMC) with demineralised water as mobile medium.

#### 2.1 Microorganisms

The strains used for ethanol production were: *Saccharomyces cerevisiae* Ethanol Red® from Leaf - Lesaffre, France and *Spathaspora passalidarum* CBS 10155, Westerdijk Fungal Biodiversity Institute, the Netherlands. The yeast strains were stored as 20 % glycerol stocks at -80 °C.

#### 2.2 Sugar fermentation

Small scale batch fermentations were performed with glucose and xylose inoculum solutions (yeast extract-peptone (YP)) or C5 and C6 sugars streams in YP medium, at 25 °C with an agitation speed of 150 rpm. Batch fermentations at 0.2 L scale were carried out in duplicate in YP medium with 180 g/L glucose and 10 g/L xylose (reference) or with 50 % C6 sugar stream (w/v). The conditions were controlled at pH 5.0, temperature of 35 °C, agitation speed of 150 rpm. The medium was inoculated with a suspension of *S. cerevisiae* in saline (20mL) to an OD<sub>600</sub> of 1.0.

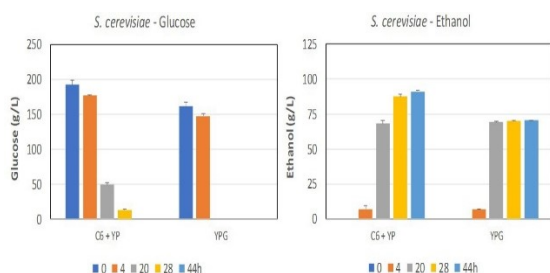
### 2.3 Analytical methods

Fermentation substrates and products were analysed by HPLC. Sugars, organic acids and ethanol were measured in a Waters HPLC system equipped with a refractive index detector (Waters model 2414) and a Shodex KC-811 300 x 8 mm column at 65 °C with 3 mM H<sub>2</sub>SO<sub>4</sub> as mobile phase and a flow rate of 1.0 mL/min. Samples were mixed with an equal volume of 1 M H<sub>2</sub>SO<sub>4</sub> containing 100 mM valeric acid as the internal standard.

## 3 RESULTS

### 3.1 Production of ethanol from C6 sugars streams

After determining best culture conditions, ethanol production from beech C6 sugar stream by *S. cerevisiae* was tested under controlled conditions in a bioreactor. The glucose in C6 medium was completely consumed in 44 h with a maximum rate of 7.9 g/L.h (Figure II). In the reference cultivation on YP medium with a lower glucose content, consumption was completed in 20 h and with a higher rate of at least 9.2 g/L.h. On the other hand, the ethanol yield from C6 medium was higher at 0.47 g per g glucose. Other products were glycerol (7.1 g/L) and acetic acid (0.8 g/L).



**Figure 2.** Rate of glucose consumption (left) and ethanol production (right) by *S. cerevisiae* from YP medium with beech C6 sugar stream (C6 + YP) (50 % w/v) and on YPG medium (10 g/L yeast extract, 20 g/L peptone, 160 g/L glucose). Culture volume, 0.2 L in bioreactor.

### 3.2 Production of ethanol from C5 sugars streams

Beech C5 sugar stream is a mixture of mainly xylose (circa 82 % of all sugars) and low amounts of arabinose (C5) and the C6 sugars glucose, galactose and rhamnose (Table 1). The yeast *Spathaspora passalidarum* was selected because it is able to produce ethanol from C5 sugars [1-2]

**Table I:** Composition of detoxified C5 sugarhydrolysates for fermentation.

| Component      | Units | 1%   | 2%   | 4%   |
|----------------|-------|------|------|------|
|                |       | GAC  | GAC  | GAC  |
| C5 sugars      | g/kg  | 59.5 | 51.4 | 52.6 |
| C6 sugars      | g/kg  | 12.8 | 11.0 | 11.1 |
| Acetic acid    | g/kg  | 1.4  | 1.3  | 1.2  |
| HMF            | mg/kg | 76.2 | 27.4 | 7.2  |
| Furfural       | mg/kg | 1.3  | 0.0  | 0.0  |
| Vanilline      | mg/kg | 3.6  | 0.0  | 0.0  |
| Syringaldehyde | mg/kg | 34.5 | 8.6  | 0.0  |

Growth was performed on YP media supplemented with the C5 streams as indicated (Table 2). Fermentation of the untreated beech C5 sugar stream was inhibited at concentrations of 20 % w/v or higher in nutrient-rich

medium. The detoxified C5 sugar streams were fermentable at sugar concentrations of 50 – 60 g/L by the yeast strain *S. passalidarum*.

In small scale fermentations more than 18 g/L of ethanol was produced from circa 60 g/L of sugars, mainly from xylose with an ethanol yield of 0.32 g per g of consumed sugar (Table II).

**Table II:** Fermentation of *S. passalidarum* cultures in flasks with beech C5 sugars (C5) and supplements, YP (10 g/L yeast extract, 20 g/L peptone). Diluted (dil.) C5 sugar streams were detoxified by adsorption using GAC.

| Medium         | C5 [% w/v] | Treatment of C5 | Time [h] | Ethanol [g/L] | Y <sub>EIOH</sub> [g/g sugar] |
|----------------|------------|-----------------|----------|---------------|-------------------------------|
| C5 + YP        | 10         | -               | 48       | 6.6           | 0.21                          |
| C5 + YP        | 20         | -               | -        | 0             | -                             |
| C5 (dil.) + YP |            | GAC 1 %         | 67       | 15.2          | 0.32                          |
| C5 (dil.) + YP |            | GAC 2 %         | 45       | 18.0          | 0.32                          |
| C5 (dil.) + YP |            | GAC 4 %         | 45       | 18.7          | 0.32                          |

The ethanol yield of 0.32 g per g is circa 63 % of the theoretical yield of 0.51 g per g. In general, in xylose-to-ethanol fermentations by native yeast strains the maximum yield cannot be reached. For these fermentations oxygen is required to neutralize the redox imbalance which originates in a different co-factor preference of the first two enzymes XR and XDH, involved in the conversion from xylose to xylulose

## 4 CONCLUSIONS

- The beech C6 sugar stream was suitable for ethanol production. Only a small effect on the substrate consumption rate was observed as compared to glucose fermentations.

- The combined results suggest that at least phenolic compounds and most likely furans are responsible for the inhibiting effect on fermentation of C5 streams.

- By detoxification and using a specific yeast for C5 fermentation, high yields of bioethanol can be obtained.

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## 6 ACKNOWLEDGEMENT

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