Supplementary Material

Nitric-Acid Hydrolysis of *Miscanthus giganteus* to Sugars Fermented to Bioethanol

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1. Material balance for step 1

From data for the recovered solid, it can be determined how much hemicellulose or cellulose is removed into the liquid phase, while from liquid-phase analysis, the amount of sugars in the liquid phase was obtained. Comparing data from the two analyses, the material balance can be checked. However, in the experiment, it cannot recover all of the sugar products from the reaction, because some sugar is adsorbed onto the solid and therefore cannot be transferred into the liquid phase. This adsorption affects the material balance for sugars.

Two definitions are introduced: theoretical recovery and experimental recovery. The theoretical recovery ignores adsorption; it is the maximum amount of sugar recovered after taking into account the amount of degradation. It is calculated based on the assumption that all hydrolysis products are dissolved into the liquid phase.

Theoretical recovery

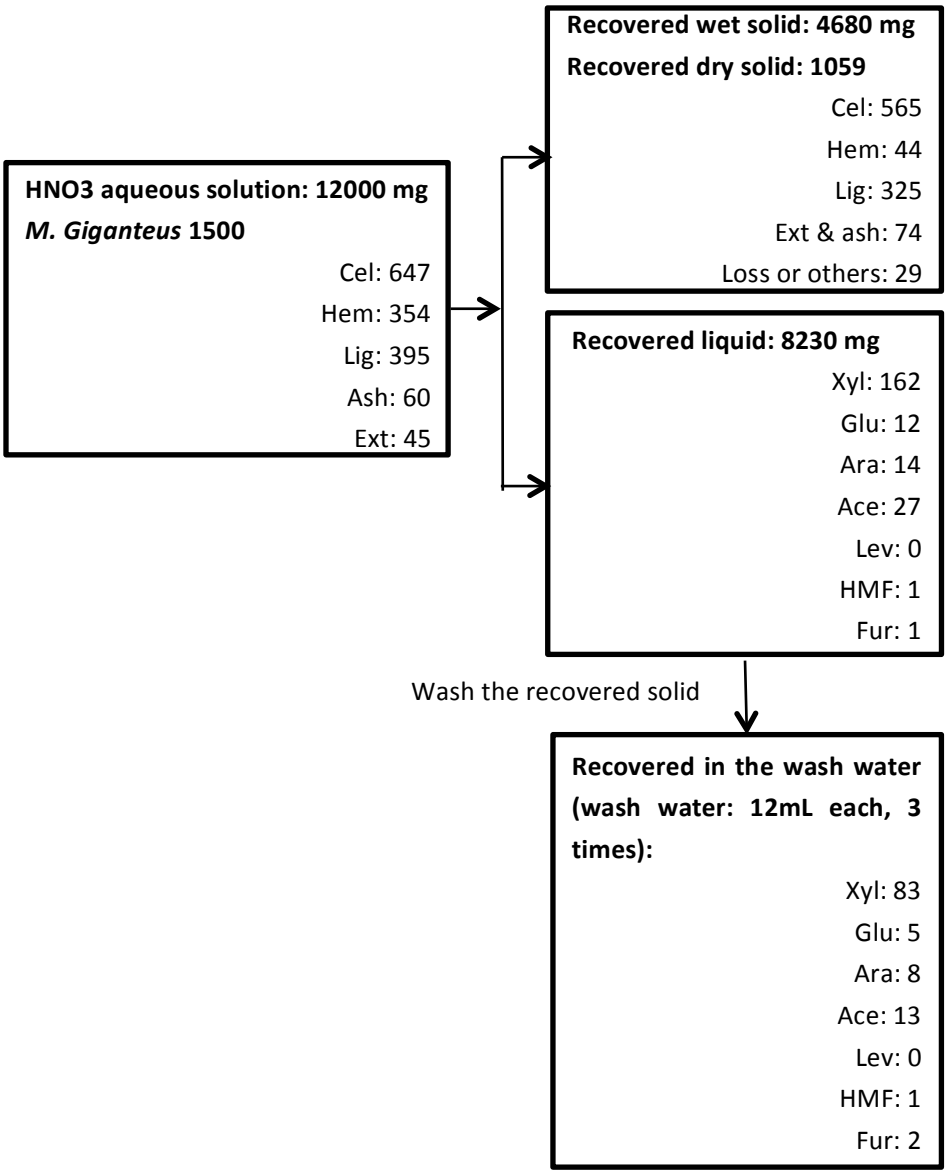
$$= \frac{Equiv.\ sugar\ conc.\ in\ recovered\ liquid}{The\ amount\ of\ cel\ (hemi)\ in\ the\ untreated\ samples \Big/ the\ amount\ of\ aqueous\ solution\ added\ for\ the\ reaction} \quad (4)$$

Experimental recovery is based on the amount of sugar recovered after reaction. Comparison between the theoretical and experimental recoveries indicates how much sugar is not recovered under the experimental conditions due to adsorption onto the solid.

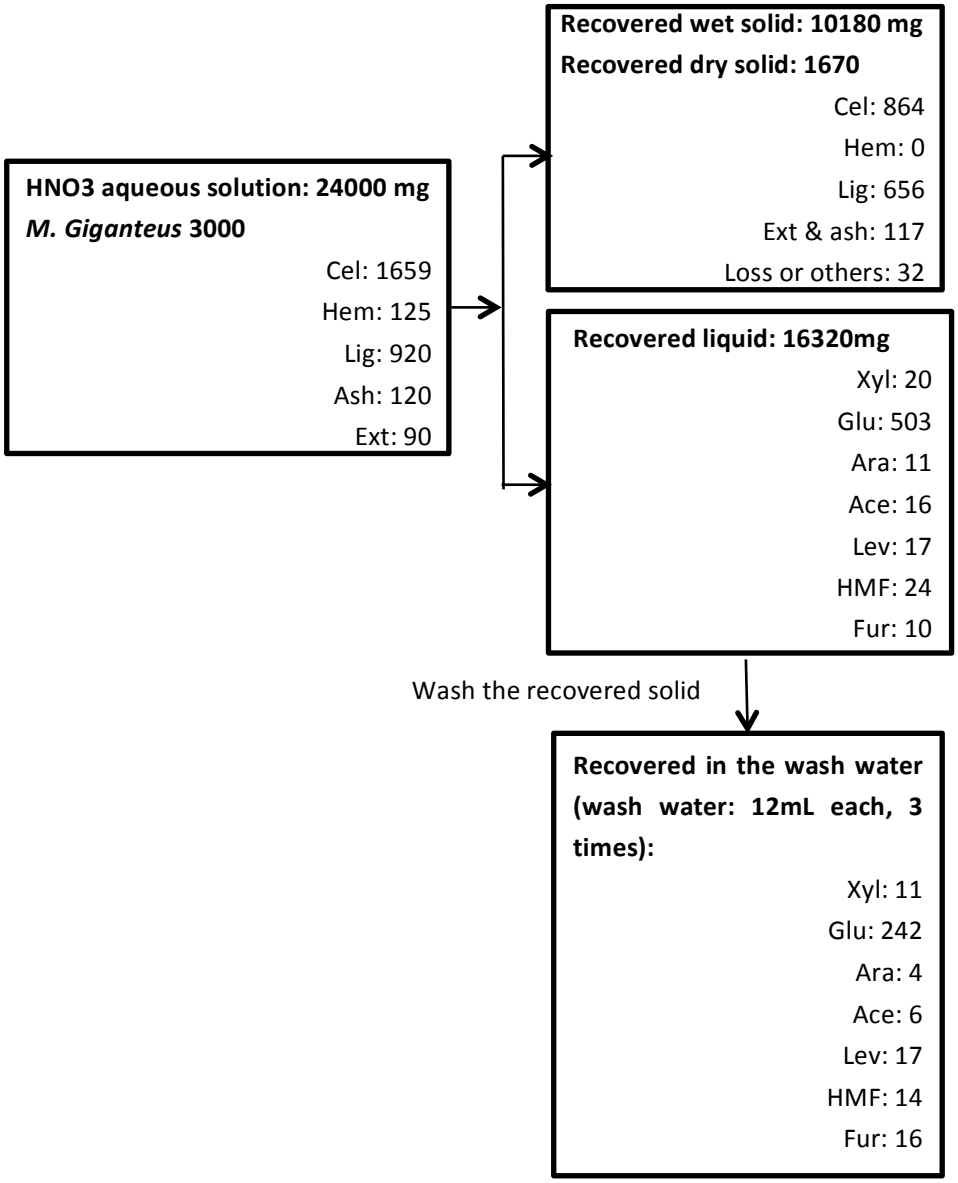
After 20 minutes at 140°C, from the solid analysis, 88% of hemicellulose is in the liquid phase. However, only 40% of xylose was obtained from the recovered liquid. To recover the adsorbed sugar, the solid was washed after the reaction three times and analyzed the collected wash-water. An additional 21% of xylose was recovered.

Regrettably, due to dilution, the concentrations of sugars in the washes are low. As a result of washing, the total experimental recovery of xylose is 61%. For theoretical recoveries, about 62% of hemicellulose converted to xylose was obtained, 6% to arabinose, 11% to other degradation products and 3% to oligomeric saccharides. Because of washing, the experimental recovery almost equals the theoretical recovery, as shown in SM-1. Consistent with the literature [16], more sugars can be recovered when washing is used between the first and second steps. However, comparing the solid and liquid analyses, a 6% difference remains. This difference probably follows from errors in the solid and liquid analyses, and from losses during experimental operations such as removal of sample from the reactor to the filter, and washing.

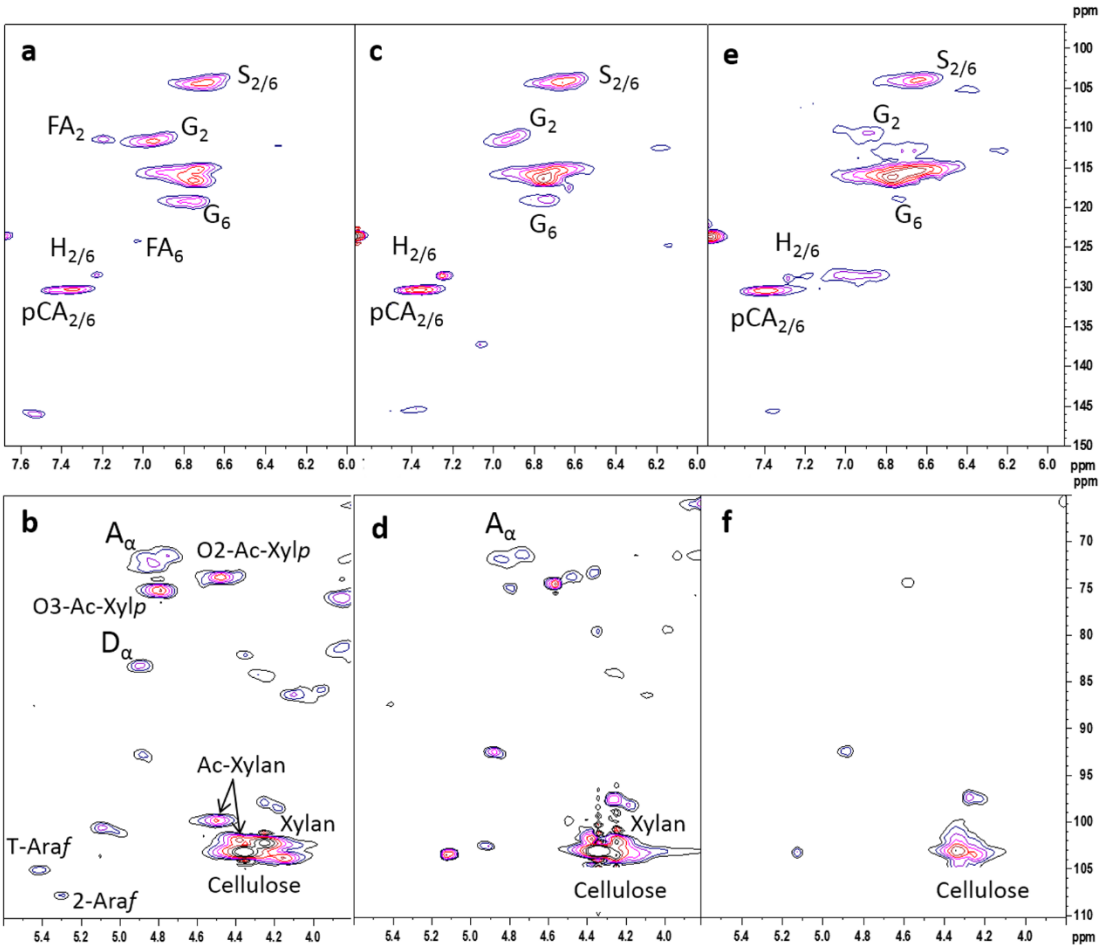
Nearly all of the sugars can be recovered if the wash step is included. For comparison, Nguyen et al. reported a 56-63% hemicellulosic sugar yield from wood chips impregnated with 0.35% H_2SO_4 at 212°C for 105 seconds [17].



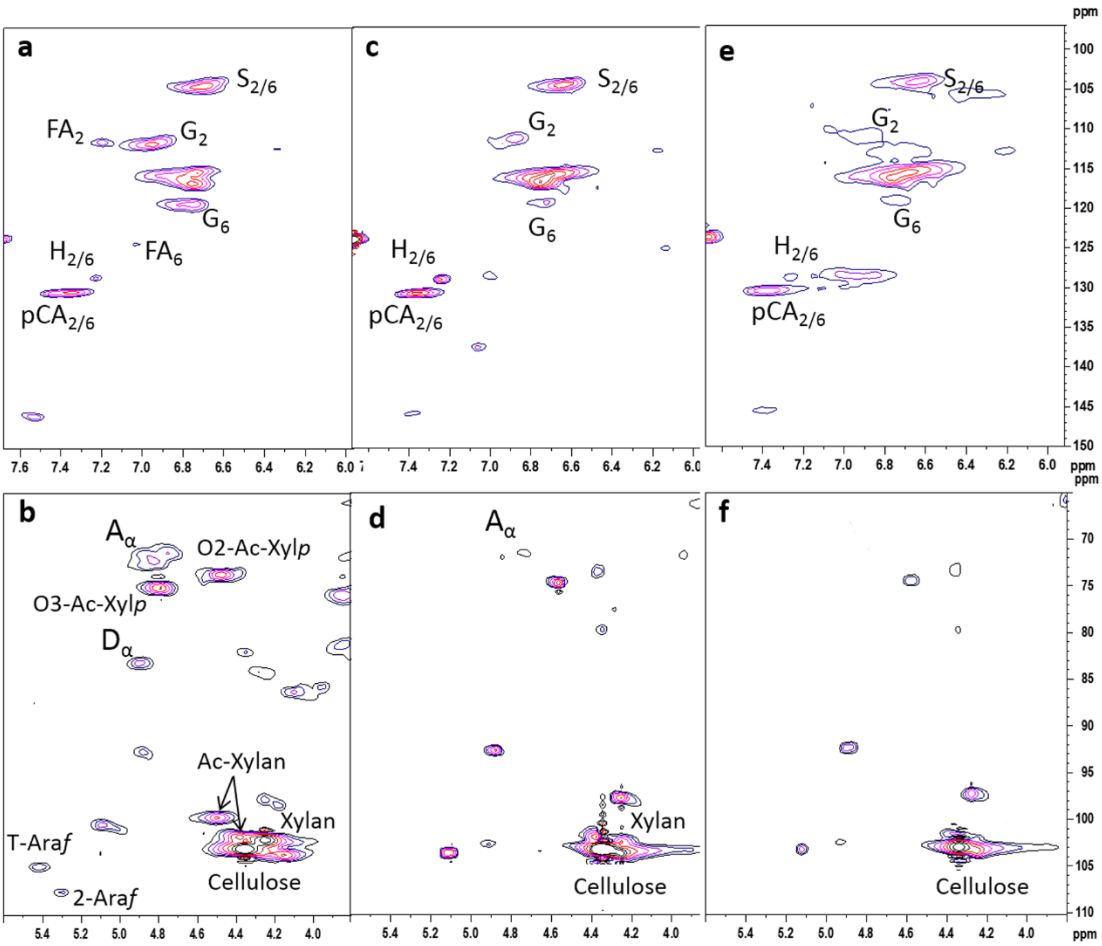
SM-1 Experimental mass balance for step 1 (140°C, 20 minutes, 0.5 wt. %HNO3) (unit: mg). Cel: cellulose; hem: hemicellulose; lig: lignin; ext: extractables; xyl: xylose; glu: glucose; ara: arabinose; ace: acetic acid; lev: levulinic acid; fur: furfural.



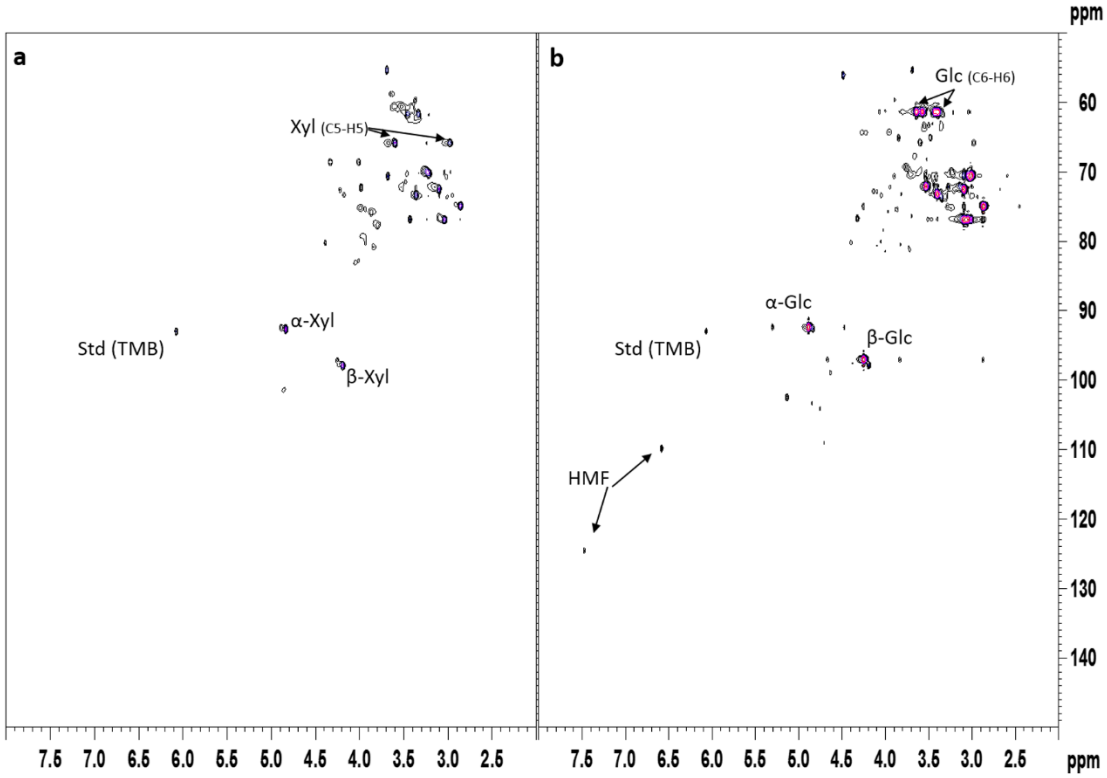
SM-2 Experimental mass balance for step 2 (195°C, 3 minutes, 0.5 wt. %HNO3) (unit: mg). Cel: cellulose; hem: hemicellulose; lig: lignin; ext: extractables; xyl: xylose; glu: glucose; ara: arabinose; ace: acetic acid; lev: levulinic acid; fur: furfural.



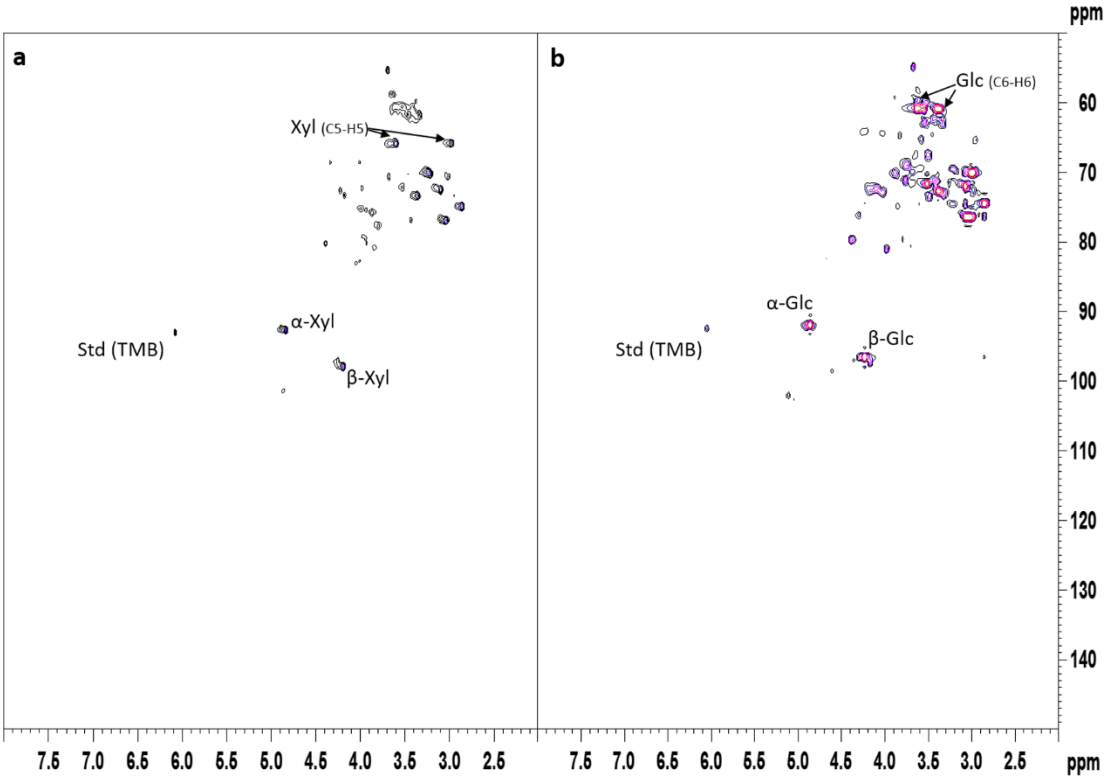
SM-3 2D HSQC NMR spectra of (a and b) non-treated *M. giganteus*; (c and d) recovered solid residue after first step at 140°C for 20 minutes; (e and f) recovered solid residue from second step at 195°C for 3 minutes. Upper spectra are for the aromatic region and lower spectra are for anomeric and aliphatic lignin side-chain regions.



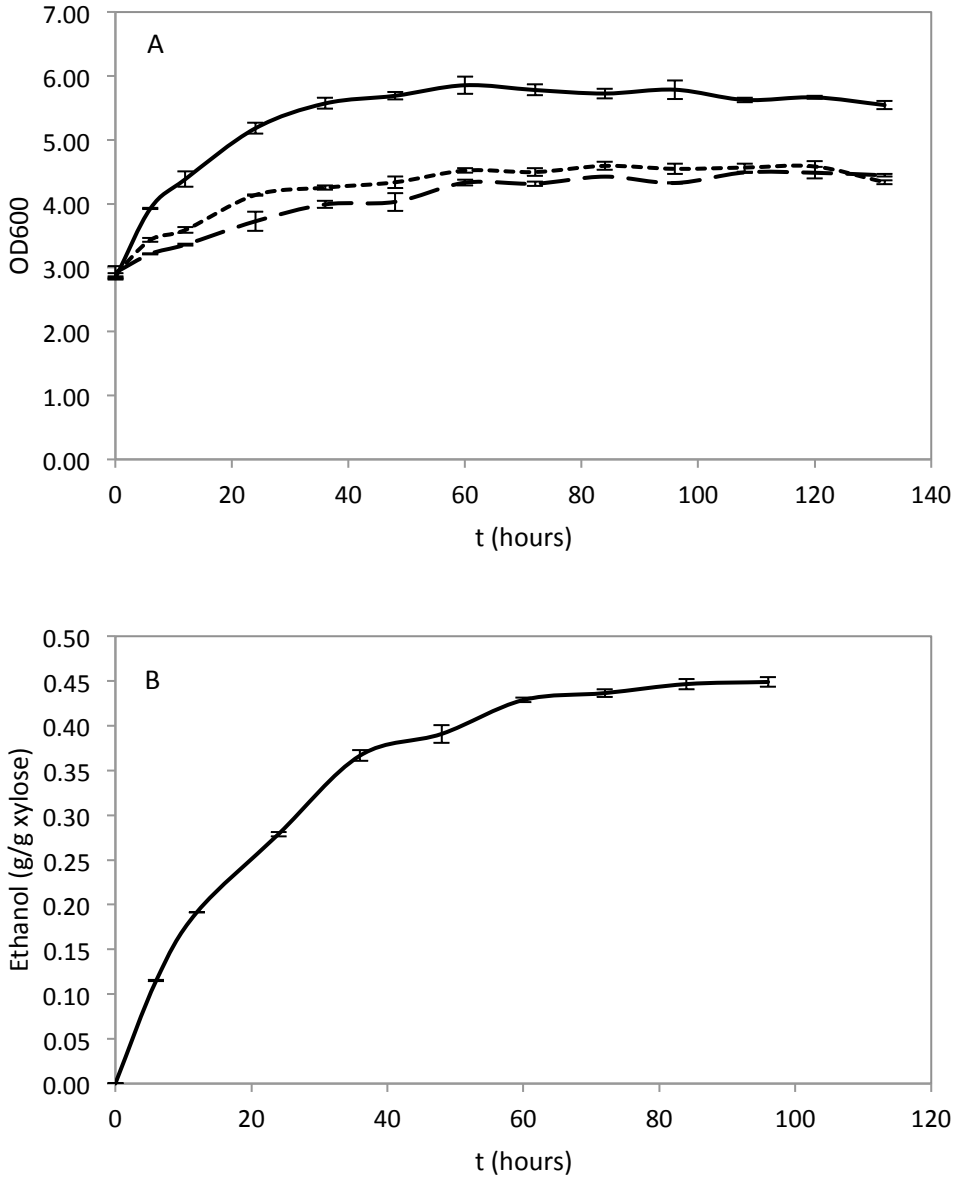
SM-4 2D HSQC NMR spectra of: (a and b) non-treated *M. giganteus*; (c and d) recovered solid from first step at 160°C for 5 minutes; (e and f) recovered solid from second step at 195°C for 3 minutes. Upper spectra are for the aromatic region and lower spectra are for anomeric and aliphatic lignin side-chain regions.



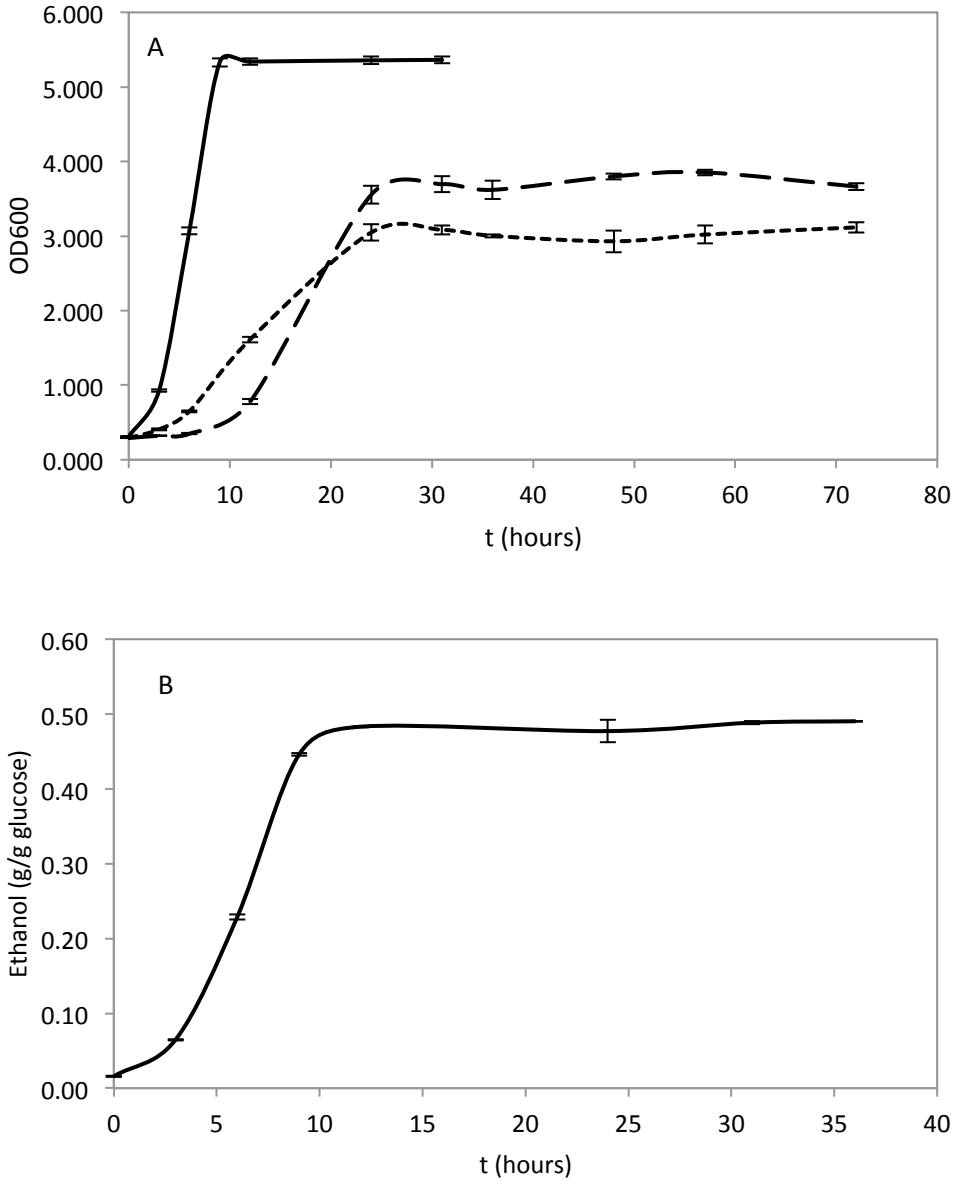
SM-5 2D HSQC NMR spectra of the liquor (a) after first step at 140°C for 20 minutes; (b) after second step at 195°C for 3 minutes.



SM-6 2D HSQC NMR spectra of the liquor (a) after first step at 160°C for 5 minutes; (b) after second step at 195°C for 3 minutes.



SM-7 Anaerobic fermentation of first-step hydrolysate using *S. cerevisiae* SR8. (A) OD600 of the cells, — control without inhibitors, ··· control with inhibitors, – – hydrolysate; (B) control without inhibitors.



SM-8 Anaerobic fermentation of second-step hydrolysate using *S. cerevisiae* SA-1. (a) OD600 of the cells, — control without inhibitors, ··· control with inhibitors, – – hydrolysate; (b) control without inhibitors.