## **Electronic supplementary information**

# Consolidated bioprocessing of lignocellulose by a microbial consortium

### Simone Brethauer and Michael Hanspeter Studer

## 5 Fermentation of Avicel with S. cerevisiae employing only βglucosidase

In order to investigate the cellulolytic activity of  $\beta$ -glucosidase, which was added to all MBM fermentations, we performed a shake flask fermentation of *S. cerevisiae* on 17.5 gL<sup>-1</sup> Avicel and

- 10 added only β-glucosidase. This blank reaction was performed in 125 mL Erlenmeyer flasks that contained a total reaction mass of 25 g and were placed on a shaking incubator featuring a throw of 25 mm (Minitron, Infors-HT, Bottmingen, Switzerland) that rotated at 150 rpm at 28°C. The flasks were closed using screw
- 15 caps with apertures together with PTFE-lined silicone septa and vented trough 20-gauge needles (VWR, Dietikon, Switzerland). An enzyme loading of 30 CBU g<sub>glucan</sub><sup>-1</sup> was applied using Novozymes 188. Otherwise the conditions were similar to the MBM experiments described in the main manuscript.
- 20 No ethanol was produced in this experiment (Fig. S1) thereby showing that  $\beta$ -glucosidase has no significant cellulolytic activity.



25 Fig. S1 β-glucosidase blank. In the fermentation of *S. cerevisiae* on 17.5 gL<sup>-1</sup> Avicel in the presence of only β-glucosidase (30 CBU g<sub>cellulose</sub><sup>-1</sup>) no ethanol is produced

#### **Co-fermentation of xylose and Avicel**

- 30 As most lignocellulosic biomass contains not only hexose sugars but also pentoses such as xylose that cannot naturally be fermented by *S. cerevisiae* strains, we enhanced the consortium with *S. stipitis* cells and used xylose and Avicel as carbon sources for first proof of concept experiments. Here, also the performance
- 35 of the MBM system was compared to simultaneous saccharification and co-fermentation (SScF) using commercially available cellulase and the same yeast strains. In the MBM reactor, 8.4 gL<sup>-1</sup> (78% yield) ethanol were produced in 150 h and xylose was completely consumed (Fig. S2a). During SScF,
- 40 7.4 gL<sup>-1</sup> (77%) ethanol were produced in 164 h and 0.5 gL<sup>-1</sup> xylose remained unmetabolized (Fig. S2b).



Fig. S2 Co-fermentation of Avicel / xylose mixtures. Shown are the xylose (□) and total ethanol (○) concentrations. (a) Consolidated bioprocessing of Avicel and xylose to ethanol in the MBM system employing *T. reesei*, *S. cerevisiae* and *S. stipitis*. *T. reesei* was grown for 4 d on 7.5 gL<sup>-1</sup> Avicel. Then, *S. cerevisiae* and *S. stipities* were added together with each 10 gL<sup>-1</sup> Avicel and xylose. t=0 designates the incoculation with yeast, the fungi only phase is not 5 shown. (b) Commercial enzyme based SScF. 10 gL<sup>-1</sup> Avicel and 7.8 gL<sup>-1</sup> xylose were converted to ethanol employing a cellulase loading of 15 FPU g<sub>cellulose</sub><sup>-1</sup> and a co-culture of *S. cerevisiae* and *S. stipitis*.

## 10 Batch consolidated bioprocesssing of Avicel to ethanol with Dekkera bruxxelensis

In order to circumvent the addition of  $\beta$ -glucosidase to the MBM fermentations, we exchanged *S. cervesiae* against the cellobiose fermenting yeast *Dekkera bruxellensis* (DSMZ 70739). In this

- 15 experiment, we used the two step carbon source addition procedure: The 32 mL reactor was filled with Mandels base medium containing 7.5 gL<sup>-1</sup> Avicel and autoclaved for 20 min at 121°C. The remaining medium ingredients were added and the reactor was inoculated with 5% v/v *T. reesei* preculture. After
- 20 4 d, 10% of the liquid reaction phase was removed and corn steep liquor, Avicel and yeast inoculum were added. The final concentration of corn steep liquor was 3 gL<sup>-1</sup>, the final Avicel concentration was 10 gL<sup>-1</sup> and the final optical density at 600 nm (OD<sub>600</sub>) of the yeast cells was 0.5.
- 25 The control experiment using *S. cerevisiae* was performed similarly with the only difference that  $\beta$ -glucosidase was added together with the yeast to final activity of 30 CBU g<sub>glucan</sub><sup>-1</sup>.



Fig. S3 Consolidated bioprocessing of Avicel to ethanol in the MBM 30 reactor employing *T. reesei* in conjunction with *S. cerevisiae* ( $\circ$ ) or with the cellobiose fermenting yeast *D. bruxxelensis* ( $\Box$ ).  $\beta$ -glucosidase was added only to the *S. cerevisiae* culture.