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Enzymatic hydrolysis of beech wood lignocellulose at high solid contents and its utilization as substrate for the production of biobutanol and dicarboxylic acids

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HIGHLIGHTS

- Organosolv pretreated beech wood was hydrolyzed with solid loadings up to 23%.
- Resulting glucose concentrations of up to 152 g L^{-1} have been reached.
- The decreasing influence of the solid content on the glucose yield is examined.
- The hydrolysate was successfully used as substrate for the production of butanol.
- The hydrolysate was also successfully used to produce succinic and itaconic acid.

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ABSTRACT

The development of a cost-effective hydrolysis for crude cellulose is an essential part of biorefinery developments. To establish such high solid hydrolysis, a new solid state reactor with static mixing is used. However, concentrations >10% (w/w) cause a rate and yield reduction of enzymatic hydrolysis. By optimizing the synergetic activity of cellulolytic enzymes at solid concentrations of 9%, 17% and 23% (w/w) of crude Organosolv cellulose, glucose concentrations of 57, 113 and 152 g L^{-1} are reached. However, the glucose yield decreases from 0.81 to 0.72 g g^{-1} at 17% (w/w). Optimal conditions for hydrolysis scale-up under minimal enzyme addition are identified. As result, at 23% (w/w) crude cellulose the glucose yield increases from 0.29 to 0.49 g g^{-1}. As proof of its applicability, biobutanol, succinic and itaconic acid are produced with the crude hydrolysate. The potential of the substrate is proven e.g. by a high butanol yield of 0.33 g g^{-1}.

1. Introduction

The accessibility of alternative substrate sources for the fermentative production of value added chemicals has been one of the primary goals of industrial biotechnology research in the past two decades. Rising oil prices and repeated discussions concerning world food affairs expedite the implementation of biorefinery plants. Several industrial and pilot scale plants have been commissioned by now. These are based on a variety of renewable feedstocks (sugar cane, corn, wheat, barley, rice, wood) and differ in a wide product range from e.g. pyrolysis oils up to fermentation products (Sanchez and Cardona, 2008).

Utilization of wood as feedstock has the advantage of its high and nearly ubiquitous occurrence and an established transport and processing infrastructure throughout the world. Besides the compact, partially crystalline structure of the cellulose in microfibrils, the dense lignin structure of trees provides a highly efficient steric protection against enzymatic attacks (Arantes and Saddler, 2011). Pre-treatment of the wood is necessary to allow a sufficient subsequent enzymatic degradation reaction. For this, several methods have been established – e.g. extraction with hot water, acid or alkaline chemical hydrolysis, steam or ammonia fiber explosion or water–ethanol extraction (Organosolv process) (Pan et al., 2005; Gonzalez et al., 2009).

To establish better economic perspectives towards a lignocelluloses biorefinery, it is reasonable to develop an integrated process for the conversion of the lignocellulose material under full utilization of all co-products. Due to this, the Organosolv process has gained high relevance within the field of biorefinery development,

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as the lignin retains a lot of its polymeric structure and its original composition. This allows the utilization of lignin as e. g. plastic composite substitute (Nägele et al., 2002). On the other hand, the high temperatures applied during Organosolv extraction result in conversion of glucose to furfurals and other side products. While being platform chemicals as well, furfurals act highly inhibitory on several enzymes and microorganisms that have to be considered in substrate upstream processing (Kumar et al., 2009).

Within this value added production chain of lignocellulosic biorefineries, enzymatic hydrolysis of polysaccharides plays a central role, but high solid concentrations are a process challenge to be overcome. The successful application of high solid concentrations during cellulose hydrolysis will increase product concentrations, decrease reactor volumes and lower energy consumption which are important factors for the commercial feasibility of the process. Until now, hydrolysis of wooden cellulose up to concentrations of 10–20% (w/v) (and up to 40% (w/v) in case of corn stover or straw) have been described, whereas loading above 15% (w/w) are typically regarded as 'high-solids systems' (Jorgensen et al., 2007; Roche et al., 2009). In this respect, gravity-based mixing systems are described as an efficient way of performing enzymatic hydrolysis at high solid matter contents (Jorgensen et al., 2007; Roche et al., 2009).

A significant part of the production costs of lignocellulose hydrolysates is caused by the enzyme loading (Klein-Marcuschamer et al., 2012). One method for decreasing the amount of enzyme is to implement a recycle step. Since the cellulases employed in the hydrolysis of lignocellulosic biomass readily bind to the cellulose polymer during catalysis, this undertaking faces various obstacles (Xue et al., 2012; Weiss et al., 2013). Hence, as low enzyme dosages as possible (in the range of 3–6 FPU per g dry matter) currently are a prerequisite for developing cost-effective advanced biorefinery concepts (Jorgensen et al., 2007).

Once the monosaccharides (primarily glucose and xylose) have been gained from the wood feedstock, these can be further processed by value adding fermentation steps. Currently, bioethanol is a widely-produced biotechnological product fermented from sugar beet, starch or lignocellulose. Nevertheless, bioethanol has some distinct disadvantages as gasoline substitute, as hygroscopic characteristics and a high difference of the specific combustion energy density in comparison to gasoline. Especially the mixing ratio dependency of the combustion energy density of ethanol-gasoline fuels is a challenge for motor operations. In comparison, butanol can be readily added to gasoline in any ratio and shows no hygroscopicity (Pfromm et al., 2010). Furthermore, the variety of chemical building blocks derivable from the C4 molecule is higher, components as acrylate, methacrylate esters, glycol ethers, butyl acetate can be derived from the butanol (Tashiro and Sonomoto, 2010). Besides the interesting product characteristics, the application of clostridia strains, typically used for butanol fermentation, offers the advantage of higher substrate utilization because in contrast to most yeast strains used for ethanol production clostridia are naturally capable of diauxic glucose and xylose metabolization (Ounine et al., 1985).

However, lignocellulose biorefineries should not be restricted to solvent production. Recent research interest has had a focus on the twelve top value added chemicals from biomass derived sugars identified by the U.S. Department of Energy's biomass program (U.S. Department of Energy, 2004). Two promising compounds from this list are succinic and itaconic acid. Succinic acid has a variety of applications in the fields of specialty chemicals, Life Science & Pharma and as plastic composite. The molecules structure is very close to maleic anhydride and the composite can thus readily be integrated in established chemical processes (Zeikus et al., 1999). Less notice has been taken of the unsaturated itaconic acid. It is an auspicious platform chemical and can be used as co-monomer in several polymer applications. With high probability the compound will be able to supplement a considerable amount of the chemistry currently based on acrylic-/methacrylic acid (Willke and Vorlop, 2001). Thus, cheap substrates for the competitive production of these two acids are of high interest.

This article gives an insight into an enzymatic hydrolysis process including a solid–liquid separation step. As substrate, crude beech wood cellulose fractions derived by an Organosolv process are used up to solid concentrations of 23% (w/w) at a very low enzyme dosage. The gained crude monosaccharide hydrolysate is used for different platform chemical fermentations. As medium value product, butanol has been produced from wood in high yields. Furthermore, the high value building blocks succinic and itaconic acid can readily be produced from the beech wood derived monosaccharides as substrate.

2. Methods

2.1. Reagents

Glucose and xylose used as standard reagents were purchased from Sigma–Aldrich (Steinheim, Germany), while citric acid and trisodium phosphate were used to prepare phosphate buffer (pH 5). All named chemicals were purchased in the highest purity available.

The cellulose for hydrolysis was a crude Organosolv pretreated beech wood fraction kindly provided by the Johann Heinrich von Thünen Institute (Hamburg, Germany). These lignocellulose substrates are, in average, composed of 70% (w/w) cellulose, 10% (w/w) hemicellulose, 10% (w/w) lignin. The rest of the fiber mass is made up of water and ash.

Commercial enzyme solutions, a cellulase complex (NS22086, 1000 biomass hydrolysis units per g), a ß-glucosidase (NS22118, 250 cellobiase units per g) and a xylanase (NS22083, 2500 fungal xylanase units per g) were obtained from Novozymes Cellic® CTec2 and HTec2-Kit (Novozymes A/S, Bagsvaerd, Denmark). All enzymes were technical liquid preparations.

2.2. Enzymatic hydrolysis

Different batches of Organosolv pretreated beech wood cellulose were used as substrate. The enzyme loadings were 6 mg g^{-1} NS22086, 0.25 mg g^{-1} NS22083 and 0.6 mg g^{-1} NS22118 (mg solubilized enzyme preparation per g substrate). Enzyme activity for cellulose substrate was tested according to the IUPAC standard method and was 75 FPU mL^{-1}. The resulting loading was therefore 5 FPU per g substrate.

Hydrolysis experiments were performed in 300 mL Erlenmeyer flasks with 10 g substrate at 200 rpm. The shake flasks were placed in an Ecotron incubation shaker (Infors HT, Bottmingen/Basel, Switzerland) at 50 °C. The reaction slurry was buffered to pH 5 using phosphate buffer. These hydrolysis experiments were run in duplicate. Saccharification reactions with solids concentration $\geqslant$17% (w/w) were carried out in a solid-state bioreactor with 0.52 kg substrate for 17% (w/w) and 1.1 kg substrate for 23% (w/w) at 10 rpm, Terrafors (maximal working volumes of 3–4 kg of solids, Infors HT, Bottmingen/Basel, Switzerland). To maintain a pH of 5 during hydrolysis 0.5 M NaOH-solution was added.

The hydrolysate utilized for the fermentation was manufactured from 18% (w/w) of crude cellulose fiber. To adjust to a comparable standard glucose concentration of 40 g L^{-1} for subsequent fermentations, the hydrolysate was diluted with deionized water when necessary.

2.3. Glucose and xylose analysis

Glucose and xylose concentrations in the supernatant were measured via HPLC analysis, using a refractometric detector. The