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Influence of enzyme loading on enzymatic hydrolysis of cardboard waste and size distribution of the resulting fiber residue

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HIGHLIGHTS

- Fiber size of biomass during enzymatic hydrolysis has been investigated.
- Different cellulase and hemicellulase loadings were used.
- Cellulase was mainly responsible for the extent of saccharification.
- The size reduction of fibers occurred rapidly after the enzyme addition.
- The mean fiber length was reduced, at most, by 20%.

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ABSTRACT

Enzymatic hydrolysis of lignocellulosic biomass to sugars alters the properties of the cellulosic fibers. Several process variables, including enzyme loading, play an important role in these changes. Many physical properties of fibers are affected: their length and width, porosity, specific surface area, and degree of fibrillation, for instance, may undergo dramatic changes when subjected to enzymatic degradation. In this study, the influence of enzyme loading on the fiber size was investigated using milled cardboard waste as the raw material. The effect of cellulases and hemicellulases on the monosaccharide production and the resulting fiber size was studied using commercial enzyme products. It was shown that the cellulase loading largely determined the amount of sugars produced. The fiber length was reduced during the course of hydrolysis, although the size reduction was not especially dramatic. Based on the SEM images, no significant damage to the fiber surfaces occurred during the process.

1. Introduction

Various lignocellulosic wastes, residues and crops are under consideration for industrial bioethanol production, in part due to their relatively high cellulose contents and excellent availability. Driven by rising oil prices and the increasing demand for sustainably-produced transportation fuels, the first commercial bioethanol plants for the demonstration of industrial production have recently been constructed (Larsen et al., 2012; Huang et al., 2009). Enzymatic hydrolysis, performed in order to cleave cellulosic polymers to monosaccharides, has been recognized as the key process stage to enable feasible bioethanol production. On the other hand, the main difficulties in the process are related to this challenging process stage which, together with required pretreatment and enzyme production, may contribute to over 40% of the total cost of bioethanol production (Banerjee et al., 2010). Several process configurations for bioethanol production have been proposed, but a number of technical difficulties remain (Cardona and Sanchez, 2007; Hamelinck et al., 2005; Huang et al., 2008).

Cellulases and hemicellulases are the enzymes most typically used to cleave cellulose and hemicellulose to monosaccharides. There are many characteristics that affect the enzyme choice and requirement, such as the substrate type, composition and lignin content (Van Dyk and Pletsche, 2012). The practicality of enzyme loading, in turn, is determined by cost factors, i.e. the cost of enzyme and the price of the end product, and can be optimized (Newman et al., 2013). In the case of industrial processes, in which a high sugar concentration must be obtained, the initial suspended solid concentration in the hydrolysis should be high, preferably over 200 g solids/kg suspension. However, high solid loadings are known to reduce the obtainable yield (Kristensen et al., 2009), which adversely affects the process economy.

Development of effective bioethanol production from lignocellulosic raw materials can be facilitated by an in-depth understanding

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of fiber properties during the enzymatic saccharification. During the degradation of solid biomass (cellulose and hemicelluloses) to their structural sugars, the suspended solids content in the solid–liquid system decreases while the concentration of dissolved solids correspondingly increases. The changes in the physical and chemical composition of the biomass, as well as rheological characteristics of the suspension (Nguyen et al., 2013), may be dramatic.

Previous studies have shown that the both the initial composition and upstream pretreatment of the feedstock have a large influence on the success of enzymatic hydrolysis. The selection of the pretreatment method is influenced, for instance, by the type of raw material and the costs of enzymes (Jorgensen et al., 2007). Pretreatment with steam and/or acids has been widely applied and recognized as effective, in spite of some drawbacks, such as formation of inhibitory compounds (Galbe and Zacchi, 2012). Reduction of the particle size and fiber dimensions of the biomass may improve enzymatic saccharification greatly in many cases (Hoeger et al., 2013; Yeh et al., 2010) but not without exceptions (Del Rio et al., 2012). The enzyme loading also has an important role in the process (Soares et al., 2011). Although high enzyme loading typically results in an enhanced degree of conversion, the relative improvement may be rather poor (Kinnarinen et al., 2012). The main focus of the intensive research on enzymatic hydrolysis has been on the pretreatment stage. However, there are some interesting industrial waste fractions, such as cardboard waste, that can be hydrolyzed even without any other pretreatment than particle size reduction. Consequently, when there is no pretreatment stage, the hemicellulosic sugars can be potentially recovered simultaneously with the main hydrolysis product, glucose. In this process, not only the saccharification itself is interesting, but the properties of non-degraded fiber residue largely determine its potential for utilization. The fiber residue can be pumped into the fermentation stage, recycled back to hydrolysis, perhaps after enzyme recovery by desorption (Moniruzzaman et al., 1997), deliquored (Kinnarinen et al., 2012) and dried for combustion, or utilized in some other way. Selection of the utilization method depends on the fiber properties, from which the size of the fibers is particularly important. Changes in fiber size during the enzymatic hydrolysis of lignocellulosic biomass have, up to date, only been evaluated in a few studies. Most of the previous attempts to study the subject have been made using strongly diluted fiber suspensions, which may have led to excessively high sugar yields and overestimation of the fiber length reduction, as compared to hydrolysis at more realistic solid loadings. The mechanism of fiber length reduction, caused by enzymatic attack, has been recently investigated by Clarke et al. (2011), who observed substantial fiber cutting already during the initial period of hydrolysis.

In this study, the influences of cellulase and hemicellulase loading on the degree of saccharification and fiber size were investigated. Commercially available cellulase and hemicellulase preparations (Cellic CTec2 and HTec, Novozymes, Denmark) were used. The fiber size distributions during the 24 h of hydrolysis were determined using a standard fiber testing instrument, in which the fibers were measured with an image analysis technique. In order to determine the respective sugar concentrations, the liquid phases of the same samples were analyzed using high-performance liquid chromatography (HPLC). Additionally, visual characterization of the fibers was performed using scanning electron microscopy (SEM).

2. Methods

2.1. Composition of cardboard waste

In the experiments, air-dry cellulosic waste was used as the raw material. The raw material consisted mainly of shredded corrugated cardboard, collected from Finland. In addition to cellulose, hemicelluloses and lignin, several impurities were present: pieces of plastic, metals and inorganic minerals were observed in the raw material. Prior to the analyses and experiments, the sample was milled, using a hammer mill, in order to reduce its particle size. The initial particle size is presented in the Results and Discussion Section. An approximate chemical composition of the raw material is presented in Table 1.

The composition was comparable to that of old corrugated cardboard, reported by Yáñez et al. (2004). The cellulose content of the raw material was determined according to the method of Black (1951), utilizing the anthrone reagent in strong sulfuric acid. The proportion of lignin was measured using a liquid chromatographic method (Phenomenex Luna 3u C18(2) column, 20 mM ammonium hydroxide/methanol, 50/50 vol-%, as eluent), described in more detail by Kinnarinen et al. (2012). The hemicellulose content was calculated, not measured, assuming that no extractives were present. Due to the presence of inorganic matter, such as calcium carbonate, used as a filler in paper and cardboard products, the ash content was relatively high. The ash content was determined according to the ISO 1762:2001 standard.

2.2. Preparation of fiber suspensions

The fiber suspensions of 10 wt.% were prepared in sealed plastic bottles ($V = 50\ cm^3$). In each bottle, the same weight of the milled raw material was added and extremely pure RO water (Millipore) was added to form the suspension. After thorough mixing, in a shaker, for one hour, sulfuric acid ($2.0\ mol\ dm^{-3}$) was added to the bottles to gradually adjust the pH to 5.0. In order to obtain an optimum temperature for the hydrolysis experiments, a water bath with bottle holders was prepared and set to a constant temperature of 46.0 °C, which was at an optimum level for the process, according to the enzyme manufacturer.

The enzymes used were commercial preparations (Novozymes, Denmark), consisting of different types of enzymes designed to degrade cellulose and hemicellulose. The cellulase was of the CTec2 type, while the product type of the hemicellulase was HTec. The activity of the cellulase product was not measured, but it was approximately $150\ FPU\ cm^{-3}$, according to Zhou et al. (2013). Based on literature (Eckard et al., 2012), the xylanase activity of Cellic HTec was $1090\ FXU\ cm^{-3}$. Both cellulase and hemicellulase were added simultaneously into the bottles, after which the bottles were closed, shaken manually for 30 s and placed in the temperature-controlled water bath. The experimental plan with the applied enzyme loadings is shown in Table 2.

The enzyme loadings were selected based on prior experience to obtain effective saccharification and enable easy sampling already during the initial period of hydrolysis.

2.3. Experimental procedure

In typical saccharification and fermentation processes for lignocellulosic materials, either separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF) is applied for 48–72 h. This study aimed at investigating specifically

Table 1
Approximate composition of the cardboard waste used as the raw material for the experiments.

Component	Concentration (wt.%)	Method or reference
Cellulose	63 ± 1.6	Black (1951)
Hemicellulose	14	Calculated
Lignin	12 ± 0.4	Kinnarinen et al. (2012)
Ash	11 ± 0.2	ISO 1762:2001