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Pulp properties resulting from different pretreatments of wheat straw and their influence on enzymatic hydrolysis rate

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HIGHLIGHTS

- Process parameters for grinding and pulp storage were optimized.
- Comparison of pulp digestibility after three very different pretreatments.
- Pulp hydrolysis using the novel *Penicillium verruculosum* cellulase complex.
- Lignin removal is not crucial for enzymatic hydrolysis.
- Autohydrolysis pretreatment enables complete conversion of cellulose to glucose.

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ABSTRACT

Wheat straw was subjected to three different processes prior to saccharification, namely alkaline pulping, natural pulping and autohydrolysis, in order to study their effect on the rate of enzymatic hydrolysis. Parameters like medium concentration, temperature and time have been varied in order to optimize each method. Milling the raw material to a length of 4 mm beforehand showed the best cost–value-ratio compared to other grinding methods studied. Before saccharification the pulp can be stored in dried form, leading to a high yield of glucose. Furthermore the relation of pulp properties (i.e. intrinsic viscosity, Klason-lignin and hemicelluloses content, crystallinity, morphology) to cellulose hydrolysis is discussed.

1. Introduction

Wheat straw is a fast-growing and abundant agricultural by-product. For instance, 8–13 million tons per year are readily available in Germany without risking humus reduction (Zeller et al., 2011). As it has no application in the food industry, it could serve as an excellent starting material for the production of cellulose, basic chemicals, lignin and bioethanol in the biorefinery framework. Especially the latter attains increasing importance, since the increasing level of greenhouse gases, the depletion of fossil fuels and the unstable oil market lead to a general interest in alternative transportation fuels (Solomon et al., 2007; Talebnia et al., 2010).

For bioethanol production using lignocellulosic material at least four operations including pretreatment, hydrolysis, fermentation and distillation are necessary (Talebnia et al., 2010). Different pretreatment procedures have been developed in order to increase the accessibility of cellulose by removing hemicelluloses, by breaking down the lignin structure and disrupting the crystalline structure of cellulose (Wang et al., 2012). Furthermore, the removal of lignin can be of advantage, as it has been reported that high lignin contents in the substrates may exert inhibitory effect on the enzymes used for hydrolysis (Rahikainen et al., 2011; Sewalt et al., 1997). Thus different methods have been investigated in order to prepare the carbohydrate fraction for enzymatic hydrolysis, which can be classified into physical (e.g. milling), physico-chemical

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(e.g. autohydrolysis, steam explosion), biological (e.g. white rot-fungi) and chemical processes (e.g. organosolv, alkaline pulping) (Kumar et al., 2009; Talebnia et al., 2010). This study focuses on chemical and physico-chemical treatments.

One of the advantages of using annual plants is that traditional pulping methods like Kraft-pulping are not necessary. Thus sulfur-free and hence more environment-friendly procedures can be applied. That is why several studies have been engaged with less severe pulping methods like alkaline pulping using sodium hydroxide or calcium hydroxide (Epelde et al., 1998; García et al., 2009; Khristova et al., 1998). The alkaline condition leads to the dissociation of phenolic hydroxyl groups in the lignin and increases its solubility. The latter is furthermore enhanced by lignin fragmentation and release of methanol, which, though released in small quantities, can work as an additional solvent (Epelde et al., 1998; Gierer, 1985). Due to this method a very good delignification of the pulp can be achieved and the accessibility of cellulose is increased due to swelling and removal of hemicelluloses. On the other hand fermentable sugars are lost and, because of the high silica content of annual plants, irrecoverable salts are formed and incorporated into the biomass (Kumar et al., 2009). However, Lei et al. (2010) showed that the silica content can be effectively lowered by a hot-water pretreatment. Thus, with further studies the process and the recovery of chemicals could still be improved.

In contrast to alkaline pulping the use of organic solvents for pulping has the advantage of easily recovering the initial chemicals. Furthermore, very pure lignin can be obtained, so that a complete usage of the lignocellulosic material is possible. Among the organic solvents two main groups have been in the focus of study: organic acids (e.g. formic acid, acetic acid) and alcohols (e.g. ethanol). The so called NATURAL PULPING process, which is similar to the MILOX process, uses formic acid and hydrogen peroxide to generate peroxyformic acid *in situ* (Siegle, 2001). This leads to an oxidation and depolymerization of lignin and thus enhances its solubility (González et al., 2010; Ligero et al., 2010). However, the corrosive character of peroxyformic acid makes it necessary to work with glass or enameled steel.

Compared to the pretreatments already described, autohydrolysis is the most environmental friendly procedure, as the biomass is treated only with hot water or steam at temperatures usually in the range of 150–230 °C for only short residence times (Carvalheiro et al., 2008; Garrote et al., 1999; Wang and Sun, 2010). Autohydrolysis leads to specific hemicellulose removal resulting in a cellulose and lignin rich solid fraction. Due to cell penetration and breakdown of the lignocellulosic structure the cellulose accessibility is increased. This process also has the advantage to produce a hemicellulosic liquid stream that can also be used for fermentation purposes or for recovering value-added compounds. However, a careful optimization of the operational conditions is important in order to minimize the formation of degradation compounds like furfural and hydroxymethylfurfural, which might act inhibitory on microorganisms used later on in the process (Gírio et al., 2010).

After the pretreatment procedure the lignocellulosic material is prepared for the hydrolysis step. Two main procedures are used to hydrolyze cellulose: acid and enzymatic treatments. Unlike acid hydrolysis, enzymatic hydrolysis is highly specific and occurs under mild reaction conditions. Thus, it is considered to be more promising for the inclusion into a biorefinery concept (Wang and Sun, 2010). For this application a fungal cellulase complex is used. It includes endo-glucanase, exo-glucanase and β-glucosidase activities. They are known to be influenced by the pretreatment of cellulosic material as well as the hydrolyzing conditions. Most cellulase enzyme complexes show an optimum activity at θ = 45–55 °C and pH = 4–5 (Talebnia et al., 2010). For this investigation an alternative enzyme complex obtained from *Penicillium verruculosum* strain was used. In comparison to the worldwide employed complex from *Trichoderma reesei*, *P. verruculosum* cellulases are characterized by higher content of β-glucosidase and higher resistance towards ethanol (Morozova et al., 2010).

The objective of this work was to investigate the influence of pretreatment processes, i.e. alkaline pulping, natural pulping and autohydrolysis on the enzymatic hydrolysis of wheat straw pulp. Pulping parameters have been varied to optimize each method and to examine their impact on hydrolysis rate using an enzymatic complex of *P. verruculosum*. Furthermore the effect of a mechanical treatment of the wheat straw prior to pulping has also been investigated.

2. Experimental

2.1. Raw material

The wheat straw used in this study was obtained from Agrargenossenschaft Rossau eG, Germany. It was prepared in three different ways: chopped to a length of 4 to 10 cm, milled to pass a 4 mm mesh and as thermo mechanical pulp (TMP) using a 12-zoll-refiner and a disk gap of 0.15 mm. The composition of the wheat straw is as follows: cellulose 44.2%, hemicellulose 27.3%, lignin 19.3%, extract 3.9% and ash 3.0% on a dry weight basis. With the parameters used, TMP refining was not found to have a significant effect on the chemical composition of wheat straw.

2.2. Methods

2.2.1. Alkaline Pulping

Alkaline pulping was carried out in a 2 L digester. The cooking conditions were: liquid–solid ratio 6.2 mL/g, concentration of sodium-hydroxide (1, 3, 6, 9) wt%, maximum temperature (120, 140, 160) °C, time at maximum temperature (30, 90, 180) min.

The pulp was separated from the black liquor over a Büchner funnel, washed with sodium hydroxide solution (0.1 M) and afterwards with deionized water. Enzymatic hydrolysis was conducted directly or after storing the pulp at −18 °C or after drying it at 105 °C to analyze the influence of different storage conditions.

2.2.2. Natural Pulping

Natural pulping was carried out according to Siegle (2001) using a 3 L round bottom flask with reflux condenser. The following parameters were used: liquid–solid ratio 27 mL/g, concentration of formic acid (30, 50, 60, 70, 80) wt%, maximum temperature 101 °C, time at maximum temperature (20, 40, 60) min. After cooling down, the pulp was separated, washed and dried.

2.2.3. Autohydrolysis

The hydrothermal treatments (autohydrolysis) were performed in a stainless steel reactor with a total volume of 2 L. The reactor was fitted with two four-blade turbine impellers, heated by an external mantle, and cooled by cold water circulating through an internal stainless steel loop. The wheat straw was mixed with water in the reactor in order to obtain a liquid-to-solid ratio of 10 mL/g. A maximum temperature of (180, 190, 200, 210, 220) °C at an average rate of temperature increase (from 100 °C) of approximately 4.7 °C/min was applied. When the desired temperature was reached, the reactor was cooled down and the liquid and solid phases were recovered by pressing in a hydraulic press up to 200 bar. The solid phase was washed with water, pressed again and dried.