



# Comparison of liquid hot water, very dilute acid and alkali treatments for enhancing enzymatic digestibility of hazelnut tree pruning residues

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## ABSTRACT

The effect of pretreatments on the composition of the hazelnut tree pruning residue (HTPR) and on the digestibility of the cellulose was investigated. The liquid hot water (LHW) and the very dilute acid (VDA) treatments were effective in solubilizing hemicellulose. The cellulose conversion increased up to around 60% (corresponding to 32–36 g/L glucose) with decreasing hemicellulose concentration in the pretreated HTPR. The alkali treatment provided partial delignification, however, the glucose production was comparably lower. Combining the hemicellulose removal and the delignification effect of different pretreatments in two-stage processes (LHW-alkali and VDA-alkali treatments) enhanced the cellulose concentration in the solids, but not the amount of glucose released in the enzymatic digestion. These results suggested that the hemicellulose was the main barrier against the conversion of cellulose in the LHW and VDA treated HTPR and the glucose in the hydrolysis medium inhibited the cellulase activity, which prevented the complete conversion of cellulose.

## 1. Introduction

Lignocellulosic materials are composed mainly of cellulose, hemicellulose, and lignin and they are recognized as a sustainable feedstock for the production of bio-based chemicals, such as pharmaceuticals, fine chemicals, bulk chemicals and fuels (Fiorentino et al., 2017). However, lignocellulosic materials are recalcitrant and they resist to enzymatic saccharification in their native form. Therefore, pretreatments are required to render the cellulose amenable to enzymatic hydrolysis. Hemicellulose and lignin parts of the lignocellulosic biomass have been generally considered as physical barriers that restrict the accessibility of cellulose by cellulolytic enzymes.

Several physical, chemical, physicochemical and biological pretreatment methods are available for disrupting the rigid lignocellulosic network. Liquid hot water (LHW) treatment (also known as autohydrolysis, hot compressed water, and subcritical water treatment), which is carried out in pressure reactors using subcritical water, draws attention since it does not require any catalyst and corrosion resistant reactors and it releases relatively lower amount of sugar degradation products (Alvira et al., 2010). In LHW treatment, water auto-ionizes and the acetyl groups on the hemicellulose are released as acetic acid; both provide hydronium ions for the hydrolysis reactions between hemicellulose and lignin and within the carbohydrates (Heitz et al., 1986). LHW has been demonstrated to be a successful method to remove part of the hemicellulose in several lignocellulosic materials,

including sugar cane bagasse (Laser et al., 2002), corn stover (Mosier et al., 2005), wheat straw (Pérez et al., 2008), and sunflower stalks (Monlau et al., 2012). Concentrated acids can hydrolyze cellulose directly, however, they are corrosive and hazardous. Dilute acid (DA) treatment at elevated temperatures, on the other hand, targets hemicellulosic carbohydrates allowing removal and hydrolysis of those. This releases hemicellulosic monomers, such as xylose, mannose, and arabinose, and facilitate enzymatic hydrolysis of cellulose (Sun and Cheng, 2002). DA treatment generally carried out using 0.5–5% sulphuric acid (or other acids) at temperatures of 121–220 °C (Lee et al., 2015a; Sun and Cheng, 2002). This treatment suffers from carbohydrate degradation and consequent inhibitor formation (Zabed et al., 2016). The other well-established method is the alkali pretreatment. Under alkaline conditions, the ester linkages in hemicellulose and lignin are easily broken. This significantly promotes the solubilization of hemicelluloses and lignin, and increases porosity and surface area, resulting in the exposure of cellulose to enzymes (Kim et al., 2016; Zheng et al., 2009). Unlike acid-based treatments, sugar degradation does not occur in alkali treatment (Zabed et al., 2016).

Following the pretreatment processes, the lignocellulosic materials are subjected to enzymatic hydrolysis for saccharification. The conditions of the pretreatment process, such as temperature, time and catalyst concentration as well as of the enzymatic hydrolysis should be optimized to ensure maximum cellulose conversion. An effective pretreatment is necessary to achieve an efficient enzymatic

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saccharification.

Hazelnut (*Corylus avellana* L.) is one of the main agricultural products in Turkey with an annual production of 420,000 tons, accounting for 56% of the total production worldwide (FAOSTAT, 2016). During hazelnut agriculture and processing, high amount of lignocellulosic biomass, in the form of shell, skin, husk, leaves, and woody biomass is discarded. The residues of hazelnut production have no economic value; i.e., they are usually burned in fields or in heaters and not utilized for production of value-added chemicals and materials (Çöpür et al., 2013). The potential of hazelnut residues has been shown in some research reports. Hazelnut shell, skin, husk, and leave were reported to contain compounds with antioxidant activity (Esposito et al., 2017; Shahidi et al., 2007; Surek and Buyukkileci, 2018). Çöpür et al. (2013) treated the steam-exploded husks with NaOH, H<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub> or NaBH<sub>4</sub>, and obtained 52.6 g ethanol per kg husks. Surek and Buyukkileci (2017) recovered 62% of the xylan in the shell in the form of xylooligosaccharides using autohydrolysis treatment. Colantoni et al. (2015) showed that hazelnut prunings could be used to produce biochar.

The amount of hazelnut tree pruning residue (HTPR) in Turkey was reported to be over 2 million tons per year (Bascetincelik et al., 2006). It contains a substantial amount of polysaccharides, thus it can be considered as a potential source of fermentable carbohydrates (Surek and Buyukkileci, 2017). In this study, the potential of HTPR was tested through pretreatments followed by enzymatic hydrolysis of cellulose into glucose. It was treated for the first time with LHW, very dilute acid (VDA), and alkali solutions under various conditions. All of the LHW and VDA treated HTPR samples and one of the alkali treated HTPR samples were then subjected to hydrolysis using a commercial cellulase. Although the pretreatments applied are well-known and have been tested on various lignocellulosic biomass, their effect on the particular biomass could be different and the optimization of the operational

reaction time (15 min or 45 min), the reactor was cooled to 60 °C within 20–25 min with tap water circulating in the cooling coil. The liquid and solid phases were separated by filtration through Whatman No.1 filter under vacuum. The solid phase was washed repeatedly with distilled water until the pH of the water after the rinsing was almost neutral. After drying at 60 °C until constant weight, the solid recovery was measured gravimetrically.

In the DA treatment, HTPR was treated with 0.1% H<sub>2</sub>SO<sub>4</sub> (w/v) at 130 °C, 150 °C, 170 °C, and 190 °C for 15 min in the pressure reactor. The solid-liquid ratio, reactor operating conditions, and processing of the treated solids were the same as described for the LHW treatment. Due to the relatively low concentration of the H<sub>2</sub>SO<sub>4</sub>, the process was called “very dilute acid (VDA) treatment” in this study.

The alkali treatment of HTPR was performed at 121 °C for 60 min in an autoclave, at a solid-liquid ratio of 1:10, using 0%, 0.5% and 2% NaOH solution. The treated biomass was processed as described for LHW treated solids. In two-stage treatments, the LHW and the VDA treated HTPR samples were subjected to alkali treatment as described above.

For each treatment, solid recovery (Eq. (1)) and the fraction of cellulose recovered in the pretreated HTPR (Eq. (2)) were calculated. Similarly, the hemicellulose and lignin removed from the HTPR in the treatments were calculated (Eq. (3)). All calculations were done on a dry weight basis.

$$\text{Solid recovery (\%)} = \frac{\text{Amount of insoluble solid after pretreatment(g)}}{\text{Initial amount of biomass before pretreatment(g)}} \times 100 \quad (1)$$

$$\text{Cellulose recovery (\%)} = \frac{\text{Amount of cellulose in the pretreated biomass(g)}}{\text{Amount of cellulose in the raw material(g)}} \times 100 \quad (2)$$

$$\text{Hemicellulose/Lignin removal (\%)} = \frac{\text{Hemicellulose/Lignin in the raw material(g)} - \text{Hemicellulose/Lignin in the treated solid}}{\text{Hemicellulose/Lignin in the raw material(g)}} \times 100 \quad (3)$$

conditions is needed. The removal of hemicellulose and lignin from the lignocellulosic network can potentially increase the enzymatic digestibility of cellulose. Therefore, the extent of hemicellulose and lignin removal in the pretreatments were measured and their effects on cellulose digestibility were quantified. Alternatively, pretreatment was applied in two stages (LHW-alkali or VDA-alkali) in order to combine the effects of different treatments.

## 2. Materials and methods

### 2.1. Materials

The HTPR, which was composed of thick branches with a diameter of 1.5–3.0 cm, was obtained from hazelnut producers in Ordu, Turkey. The HTPR was dried in an oven at 60 °C for two days and milled to a particle size less than 2 mm. The dry samples stored at room temperature until use.

All chemicals were of analytical grade and purchased from Sigma-Aldrich (Steinheim, Germany) and Merck Millipore (Darmstadt, Germany). The cellulase (Accellerase 1500) and the  $\beta$ -glucosidase (Accellerase BG) enzymes were kind gifts from DuPont, Finland.

### 2.2. Pretreatments

The LHW treatment was carried out in duplicates in the pressure reactor (BR-300, Berghof, Eningen, Germany). The biomass (25 g) was mixed with deionized water (250 mL) in the stainless-steel tank with a 600 mL total volume and the mixture was heated to 170 °C, 190 °C, and 210 °C by the surrounding heating block. The content was continuously stirred with the paddle agitator rotating at 300 rpm. At the end of the

### 2.3. Enzyme assays

The cellulase activity in Accellerase 1500 was determined by measuring the released glucose after incubation of the enzyme solution and the substrate for a certain time (Adney and Baker, 2008). Filter-paper strip (1.0 × 6.0 cm) in 1.0 mL 50 mM sodium citrate buffer (pH 4.8) was incubated with 0.5 mL enzyme solution at 50 °C for 60 min in a water bath. The reaction was stopped by the addition of 3.0 mL of DNS reagent and the absorbance of the solution was read at 540 nm. One filter paper unit (FPU) of enzyme activity was defined as the amount of enzyme that released 1  $\mu$ mol of reducing sugar in 1 min at 50 °C and pH 4.8.

The  $\beta$ -glucosidase activity in Accellerase BG and Accellerase 1500 was determined according to Grover et al. (1977) using p-nitrophenyl- $\beta$ -D-glucopyranoside (pNPG) as the substrate. The enzyme solution (500  $\mu$ L) was mixed with 1000  $\mu$ L 6.67 mM pNPG in acetate buffer (133 mM, pH 4.8) and the mixture was incubated at 50 °C for 30 min in a water bath. The reaction was stopped by 2 mL of 200 mM Na<sub>2</sub>CO<sub>3</sub> and the absorbance of the solution was measured at 400 nm. One unit of  $\beta$ -glucosidase activity was defined as the amount of enzyme which produced 1  $\mu$ mol of p-nitrophenol in 1 min at 50 °C and pH 4.8. The activity was calculated by taking the p-NPG extinction coefficient as 18.1 cm<sup>2</sup>/ $\mu$ mol.

### 2.4. Enzymatic saccharification

Saccharification of the pretreated HTPR was carried out in duplicates as described by Selig et al. (2009) except that higher solid loading

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