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A novel oxidative destruction of lignin and enzymatic digestibility of hazelnut shells

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ABSTRACT

This study investigated the effect of sequential oxidative (ozonolysis) pretreatment and enzymatic hydrolysis of hazelnut shells on production of fermentable sugars. Two factors (ozone concentration and pretreatment time), which were determined to be significant by the Box-Behnken response surface methodology, were further maximized for total lignin removal, solid recovery, and total reducing sugar yield via enzymatic hydrolysis of the pretreated biomass using full-factorial design. The ozonolysis was performed by passing 30, 40, and 50 mg/L of ozone gas through a packed bed of ground hazelnut shells for up to 120 min at a flow rate of 0.25 L/min. The highest lignin reduction (20.5%) was obtained in the shells with 30% moisture content subjected to ozone concentration of 50 mg/L for 120 min, which corresponded to a high biomass recovery (94%). The reducing sugar yield also increased from 119.9 mg/g dry untreated biomass to 284.6 mg/g dry untreated biomass for hazelnut shells ozonated at 30 mg/L for 60 min. Thus, these results indicate remarkable benefits of ozonolysis during pretreatment of hazelnut shells.

1. Introduction

Utilization of inexpensive, renewable, and abundant materials in efficient bioconversion processes is one of the state-of-the-art strategies to reduce product costs. Lignocellulosic wastes, especially agricultural and forestry residues, are low cost and widely available for production of fuels, enzymes and chemicals such as ethanol and organic acids. Thus, use of renewable agro-food industrial wastes as feedstock holds great promise. Among these, hazelnut shells are valuable lignocellulosic wastes generated in Turkey's Black Sea region at 250,000 t per year (Dogru et al., 2002), and reported to contain 43.1% lignin, 27.5% hemicellulose, 24.7% cellulose, 3.4% alcohol-benzene extractives and 1.4% ash (Copur et al., 2007). It is well known that lignin content significantly impacts enzymatic hydrolysis of lignocellulosic biomass (Mosier et al., 2005). Lignin is a complex aromatic polymer that serves as a barrier to limit the accessibility of carbohydrates by hydrolytic enzymes. When lignin content in biomass is high, enzymes are blocked by or adsorbed onto lignin. A delignification process (employing chemicals, ozonolysis, peroxide, organosolv and/or biological treatment) to partially remove lignin from cell walls prior to enzymatic hydrolysis of lignocellulosic biomass is therefore required (Mussatto and Teixeira, 2010).

Ozonolysis is a pretreatment technique which reduces lignin content of lignocellulosic materials by attacking aromatic ring structures (Nakamura et al., 2004), thereby increasing yields of fermentable sugars. It is an attractive method due to low energy consumption as the process can take place at room temperature (Contreras Iglesias, 2002) with reported negligible production of toxic residues (Kumar and Wyman, 2009). Ozonolysis has been shown to effectively delignify many lignocellulosic materials such as wheat straw (Ben-Ghedalia and Miron, 1981), bagasse, green hay, peanut, pine (Neely, 1984), cotton straw (Ben-Ghedalia and Shefet, 1983) and poplar sawdust and rye straw (Garcia-Cubero et al., 2010). A net reduction of 66.8% acid insoluble lignin was reported when sugarcane bagasse with 40% moisture content was treated at an ozone concentration of 3.44% (v/v) for 120 min (Travaini et al., 2013). García-Cubero et al. (2009) pretreated wheat and rye straws with ozone and studied the influence of five operating parameters (moisture content, particle size, ozone concentration, type of biomass and air/ozone flow rate) on fermentable sugars from straw in a fixed bed reactor under room conditions. They observed that ozonolysis increased enzymatic hydrolysis yield from 29% and 16–88.6% and 57% in wheat straw and rye straw, respectively, with no furfural and HMF being detected. Kaur et al. (2012) achieved a lignin reduction of over 42% in cotton stalk with ozone pretreatment. Miura et al. (2012) carried out ozonolysis and subsequently wet-disk milling (WDM) on Japanese cedar (Cryptomeria japonica) to improve sugar

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production by enzymatic saccharification. Application of WDM following ozone pretreatment increased glucose and xylose yields by 68.8% and 43.2%, respectively. Panneerselvam et al. (2013) pretreated energy grasses with ozone and observed 51% delignification and enhanced total fermentable sugar production up to 431.9 mg/g.

Research suggests that while many parameters impact ozonolysis efficiency, moisture content and ozone concentration are of significant importance during ozonolysis (Neely, 1984). Neely (1984) suggested that biomass moisture content and ozone concentration ranging from 25% to 35% and 2–6% (w/w) can improve process efficiency. When the moisture content reached more than 40%, ozone consumption decreased, resulting in less delignification (Miura et al., 2012). While application of ozone as a pretreatment has shown promise in various studies, its impact on lignocellulosic feedstocks like hazelnut shells which have significantly higher amounts of lignin is not fully established.

The aim of this study was to determine the effect of ozonolysis on hazelnut shells to improve sugar yields during the enzymatic hydrolysis. Initially, the effect of feedstock moisture content, ozone concentration, and pretreatment time on lignin reduction, solid recovery, and reducing sugar yield was studied by applying a response surface methodology (RSM) design. Subsequently, tests were conducted based on a full factorial design to optimize ozone concentration and pretreatment time, which were found to be the most significant variables.

2. Materials and methods

2.1. Biomass preparation

Hazelnut shells were obtained from a local plant in Ordu, a province of Turkey and dried at 70 °C in a convection oven for 24 h on arrival. The shells were then ground to pass through a 1 mm sieve and stored in sealable plastic bags at room temperature until use.

2.2. Ozone treatment

Ozonolysis was performed in a glass column reactor tube (Aceglassware, NJ, USA) of 5 cm diameter and 30 cm length. One end of the reactor was plugged with glass wool to support the biomass. Ozone, produced on-site by an ozone generator (Model: OL80 A Ozone lab instrument, Canada) supplied with industrial grade oxygen (Airgas National Welders, Raleigh, NC) at a flow rate of 0.25 L/min maintained using a mass flow controller (Model no: FMA5516, Omega, CT, USA), was introduced into the reactor from the top (set up adapted from Panneerselvam et al., 2013). The bottom of the reactor tube was connected to an ozone destructor through an exit line.

Five gram (dry basis) of ground hazelnut shell was prepared for ozonolysis by adding different amounts of moisture, equivalent to 25, 30 or 35% (dry basis) according to the experimental plan shown in Table 1, and allowed to equilibrate for an hour. Three ozone concentrations, 30, 40, and 50 mg/L, were tested and after each experiment ozonated hazelnut shells were washed with 200 mL of distilled water. Pretreated samples were stored in ziplock bags at room temperature until further use for compositional analysis including total solids, AIL and ASL, ash, and reducing sugar content and enzymatic hydrolysis typically within a week.

Table 1
Coded and uncoded process variables used in BBD.

Variable	Low (−1)	Center (0)	High (+1)
Ozone concentration (mg/L)	30	40	50
Moisture content (%)	25	30	35
Pretreatment time (min)	60	90	120

2.3. Enzymatic hydrolysis

Ozone pretreated hazelnut shells were enzymatically hydrolyzed at 5% solids loading (dry basis) in 10 mL volume made up by 50 mM sodium citrate buffer (pH 4.8), 40 μg mL tetracycline hydrochloride (ICN Biomedicals, Inc. CA, USA) (an antibiotic added to avoid microbial contamination) and Cellic® Ctec2 cellulase enzyme complex (with a cellulase activity of 110 FPU/mL) (density 1.1457 g/mL) (Novozymes North America Inc., Franklinton, North Carolina) at a loading of 0.5 g enzyme/g biomass supplemented with 0.2 g enzyme/g biomass HTec2 xylanase enzyme complex (density 1.1548 g/mL) (Novozymes North America Inc., Franklinton, North Carolina). Samples were hydrolyzed in 50 mL centrifuge tubes in a shaking water bath (Model:89032-226, VWR International, PA, USA) at 50 °C and 150 rpm for 72 h. After hydrolysis, the samples were centrifuged at 10,000 rpm for 10 min and the supernatant used for fermentable sugar analysis. Untreated samples with equivalent enzyme loading were also hydrolyzed as control. Enzymatic conversion (η) (%) was calculated as (Eq. (1))

$$\text{Enzymatic conversion } (\eta)\ (\%) = \left(\frac{\text{Concentration of reducing sugar in hydrolysate (mg/g)}}{\text{Concentration of reducing sugar in pretreated solids (mg/g)}}\right) \text{x } 100 \quad (1)$$

Ground hazelnut shells were pretreated with ozone to identify optimal conditions for pretreatment on the basis of minimum acid insoluble lignin (AIL) content and maximum solid recovery in the pretreated solids as well as maximum 'reducing sugar yield' in the enzymatic hydrolysate (Table 2). Carbohydrate content (referred to as 'reducing sugar recovery') of the pretreated solids was also measured as total reducing sugars and used to eventually estimate the enzymatic conversion (η). Pretreatment conditions were optimized further to maximize reducing sugar yield in the enzyme hydrolysate (Table 3). Alkali pretreatment with sodium hydroxide was performed as a control measure to provide a baseline comparison of ozonolysis with a conventional pretreatment method.

2.4. Analytical methods

The composition of ground untreated/raw and ozonated hazelnut shells including total solids, acid insoluble lignin (AIL), acid soluble lignin (ASL) and ash (untreated only) was determined using the procedures described by National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008a, 2011, 2008b). Three hundred mg of dry hazelnut

Table 2
Box-Behnken design matrix for identifying key process variables, solid recovery (%) and lignin (%) content of ozone pretreated samples.

Run No.	Moisture content (%)	Ozone conc. (mg/L)	Time (min)	Solid recovery (%)	Acid insoluble lignin (%)	Acid soluble lignin (%)
1	25	40	120	91.0 ± 0.8	40.2 ± 1.8	1.5 ± 0.1
2	30	30	120	92.5 ± 0.6	40.9 ± 0.6	1.5 ± 0.1
3	30	50	120	94.0 ± 1.4	37.5 ± 0.9	1.9 ± 0.1
4	35	40	120	89.1 ± 2.3	41.6 ± 1.4	1.4 ± 0.1
5	25	30	90	95.5 ± 2.1	42.2 ± 1.1	1.3 ± 0.1
6	25	50	90	93.2 ± 1.5	41.4 ± 0.9	1.3 ± 0.0
7	30	40	90	92.9 ± 1.6	40.2 ± 0.2	1.5 ± 0.1
8	30	40	90	91.6 ± 0.9	40.3 ± 0.1	1.6 ± 0.0
9	30	40	90	93.3 ± 1.0	41.5 ± 2.4	1.5 ± 0.0
10	35	30	90	92.8 ± 0.5	43.1 ± 1.0	1.2 ± 0.1
11	35	50	90	92.4 ± 1.4	39.3 ± 1.5	1.5 ± 0.0
12	25	40	60	94.8 ± 1.4	40.5 ± 0.3	1.8 ± 0.1
13	30	30	60	93.6 ± 0.5	42.9 ± 1.2	1.3 ± 0.1
14	30	50	60	92.8 ± 1.8	40.9 ± 1.3	1.4 ± 0.1
15	35	40	60	93.0 ± 0.5	41.2 ± 0.3	1.7 ± 0.0