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Mimicking the Fenton reaction-induced wood decay by fungi for pretreatment of lignocellulose

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HIGHLIGHTS

- The Fenton reaction is naturally used by fungi for wood decay.
- The Fenton reaction using $FeCl_3$ and H_2O_2 was employed to pretreat rice straw.
- After the Fenton pretreatment, the enzymatic digestibility increased to 93.2%.

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ABSTRACT

In this study, the Fenton reaction, which is naturally used by fungi for wood decay, was employed to pretreat rice straw and increase the enzymatic digestibility for the saccharification of lignocellulosic biomass. Using an optimized Fenton's reagent ($FeCl_3$ and H_2O_2) for pretreatment, an enzymatic digestibility that was 93.2% of the theoretical glucose yield was obtained. This is the first report of the application of the Fenton reaction to lignocellulose pretreatment at a moderate temperature (i.e., 25 °C) and with a relatively high loading of biomass (i.e., 10% (w/v)). Substantial improvement in the process economics of cellulosic fuel and chemical production can be achieved by replacing the conventional pretreatment with this Fenton-mimicking process.

1. Introduction

Lignocellulose is one of the most abundant renewable resources available on the earth (Lynd et al., 2008). There have been many attempts to commercialize the production of biofuels such as ethanol with lignocellulose as the biomass feedstock (Banerjee et al., 2010; Jung et al., 2014). However, owing to the physical and chemical rigidity and recalcitrance of lignocellulose, it is difficult and highly expensive to produce sugars from carbohydrates in lignocellulose (Mosier et al., 2005). Lignocellulose is mainly composed of cellulose, hemicellulose, and lignin. Cellulose is a key carbohydrate used for producing glucose, which is a major raw material in the synthesis of biofuels and bio-based chemicals by microbial fermentation. Since cellulose is physically and chemically protected by the combination of hemicellulose and lignin, physical and/or chemical pretreatment is required prior to the enzymatic hydrolysis of cellulose to produce glucose (Jung et al., 2011; Kim et al., 2005). Although these physicochemical pretreatment methods have been extensively studied, they still have major problems such as high operating temperatures leading to high energy consumption and generation of byproducts that are toxic to enzymes and microorganisms (Jung et al., 2013; Jung and Kim, 2014). Therefore, process improvements for the pretreatment of lignocellulose are necessary to enable it to be adopted as a raw material in the industrial production of fuels and chemicals.

Although lignocellulose is highly rigid and recalcitrant, there are natural solutions for effectively destroying it by fungal action. The decay of lignocellulose by fungi is induced not only by enzymes such as cellulase, hemicellulase, and ligninase, but also by chemical reagents such as the Fenton's reagent (Arantes et al., 2012). For example, in the degradation of wood by fungi, the Fenton reaction that occurs in the presence of the Fenton's reagent (i.e., iron and

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H_2O_2) facilitates the chemical degradation of lignocellulose (Arantes et al., 2011; Rättö et al., 1997). The Fenton reaction involves the oxidation of Fe^{2+} to Fe^{3+} by H_2O_2 and then the reduction of Fe^{3+} to Fe^{2+}, resulting in the formation of H_2O, O_2, and two kinds of oxygen radicals, namely $HO^{\cdot}$ and $HOO^{\cdot}$ (Arantes et al., 2011; Fenton, 1894; Haber and Weiss, 1934; Hammel et al., 2002) (Supplementary Fig. S1). The oxygen radicals initiate chain reactions for the oxidation of the lignocellulosic components and the further formation of other reactive oxygen species (Neyens and Baeyens, 2003).

The Fenton reaction does not require extreme conditions such as high temperature, high pressure, or high concentrations of chemicals. Therefore, it is considered to be an environmentally benign process (Banerjee et al., 2011). The Fenton reaction has been employed to degrade organic compounds and toxic chemicals in wastewater (Zazo et al., 2005). Furthermore, since the Fenton reaction is found to be effective in destroying wood in the nature, it can be applied to the pretreatment of lignocellulose for increasing the enzymatic digestibility. Recently, although the Fenton reaction was applied to the pretreatment of lignocellulose (Jain and Vigneshwaran, 2012; Kato et al., 2014), it is premature to conclude the Fenton reaction is applicable to lignocellulose pretreatment. It is because their solids loadings of biomass feedstocks were too low (<5%, w/w) and their enzymatic digestibility test results are not possible to be compared with those from other pretreatment studies. To simulate actual pretreatment conditions, a higher initial solids loading (~10%, w/w) is necessary.

In this study, by mimicking the degradation of plant cell walls by fungal action, the Fenton reaction was applied to the pretreatment of rice straw at a 10% biomass loading and at room temperature. To our knowledge, this is the first study of mimicking natural phenomena for developing a novel lignocellulose pretreatment process at a relatively high solids loading of biomass.

2. Methods

2.1. Lignocellulose and its compositional analysis

Rice straw used in all the experiments was harvested from Younggwang, Korea, in 2011. The rice straw was washed and ground using a high-speed rotary cutting mill (MF 10, IKA, Staufen, Germany) to yield a powder with particle sizes less than 1 mm. The compositional analysis of the rice straw was conducted following the Laboratory Analytical Procedure (LAP) published by the National Renewable Energy Laboratory (NREL; Golden, CO) (Sluiter et al., 2012). In brief, 0.3 g of pretreated or untreated rice straw was hydrolyzed in a two-step process using 3 mL of 72% (w/w) H_2SO_4 at 30 °C for 1 h, followed by 4% H_2SO_4 at 121 °C for 1 h. The hydrolysate was separated through a crucible by means of vacuum filtration, neutralized with $CaCO_3$, and analyzed using high performance liquid chromatography (HPLC; Agilent 1100, Agilent Technologies, Waldbronn, Germany). The HPLC equipment was equipped with a Shodex SP0810 column (Showa Denko, Kawasaki, Japan) and a refractive index detector (G1362A, Agilent Technologies, Waldbronn, Germany) for analyzing sugars. The solid residue in the crucible was washed with distilled water, dried at 105 °C for 4 h, and transferred into a furnace to measure the acid-insoluble lignin using a gravimetric methodology by measuring the weights before and after burning in the furnace (Daihan Labtech, Namyangju, Korea). The total solids and ash contents were measured following the LAP of NREL (Sluiter et al., 2005, 2008). All the analyses were conducted in triplicate.

2.2. Pretreatment of rice straw by using the Fenton's reagent

$FeCl_3$ (Sigma Aldrich, St. Louis, MO) and H_2O_2 (50% (w/w), Sigma Aldrich) were diluted to the desired concentrations, and were mixed at different ratios ranging from 1:10 to 1:100. Milled rice straw (5 g) was suspended in a $FeCl_3$ solution at a solids loading of 10% (w/v) in a 500 mL Erlenmeyer flask placed in a shaking incubator (Labcamp, Seoul, Korea) at 25 °C and 200 rpm. The pretreatment was initiated by adding H_2O_2 to the mixture of rice straw and $FeCl_3$ solution. After incubating for desired pretreatment durations, the pretreated rice straw slurry was filtered through a filtration cloth (22–25 μm pore size, Calbiochem, La Jolla, CA) to separate the solid and liquid fractions. The filtered insoluble solids were washed with distilled water until the pH of the filtrate reached 6–7, and were subsequently dried in a vacuum-drying oven at 45 °C for 3 days. To quantify the amount of pretreatment byproducts such as 5-hydroxymethylfurfural (HMF), furfural, acetic acid, glycerol, levulinic acid, and formic acid in the liquid fraction of the pretreated rice straw slurry, an Aminex HPX-87H column (Bio-Rad, Hercules, CA) was used for HPLC.

2.3. Enzymatic digestibility measurements of pretreated rice straw

The pretreatment effectiveness was evaluated by performing the enzymatic saccharification test. Pretreated and washed or untreated rice straw was enzymatically hydrolyzed with a 1% (w/v) glucan loading and various loadings of Accellerase 1000 (Genencor, Rochester, NY), namely 15, 30, and 60 filter paper units (FPU)/g of glucan. The enzymatic reaction mixtures of rice straw in 10 mL of citrate buffer (0.05 M, pH 4.8) were incubated at 50 °C and 180 rpm for 72 h in a shaking incubator. The enzymatic saccharification yield was expressed as a percentage of the theoretical maximum yield of glucose. HPLC equipped with an SP0810 column was used to measure the amount of glucose obtained from the enzymatic hydrolysis.

2.4. Analyses of Fe^{2+} and H_2O_2

Ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine; Sigma Aldrich] forms a stable colored complex by reacting selectively with ferrous ions in a solution mixture containing ferrous and ferric ions (Stookey, 1970). To measure the concentration of Fe^{2+} during the Fenton reaction, a sample of the Fenton reaction mixture (0.1 mL) was diluted appropriately and reacted with 0.1 mL of 10 mM ferrozine solution prepared in a 1 M tris buffer (pH 7.5). The concentration of Fe^{2+} was measured using a xMark microplate spectrophotometer (Bio-Rad, Hercules, CA) at a wavelength of 562 nm. $FeCl_2$ (Sigma Aldrich) was used as the standard for Fe^{2+}.

H_2O_2 in the Fenton reaction mixtures was quantified by using the titanium oxalate method (Eisenberg, 1943). Briefly, titanium(IV) oxysulfate ($TiSO_5$; Sigma Aldrich) was diluted to 0.128 M using 0.2% (w/v) sulfuric acid solution. A sample of the Fenton reaction mixture (0.1 mL) was diluted appropriately and added to 1 mL of the $TiSO_5$ solution. The above mixture was made up to a total volume of 20 mL by adding distilled water and the final solution was incubated at room temperature for 5 min to allow the development of an orange color. The concentration of H_2O_2 in the liquid mixture was subsequently quantified using a spectrophotometer at a wavelength of 390 nm (Sellers, 1980).

3. Results and discussion

3.1. Effect of $FeCl_3$ and H_2O_2 concentrations on the enzymatic digestibility of rice straw

The concentration and the ratio of the constituents of Fenton's reagent (i.e., $FeCl_2$, $FeCl_3$, and H_2O_2) would be key parameters in the Fenton reaction (Barb et al., 1951). In this study, $FeCl_3$ was used