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Optimization of activated carbon detoxification of dilute ammonia pretreated energy cane bagasse enzymatic hydrolysate by response surface methodology

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ABSTRACT

A challenge in syrup production from lignocellulosic biomass is the presence of non-sugar compounds in the hydrolysate, generated during the hydrolytic process, which negatively impact downstream processes. Energy cane bagasse was pretreated with ammonium hydroxide and then hydrolyzed with enzymes Cellic® CTec2 and HTec2. Non-sugar compounds such as formic acid, acetic acid, levulinic acid, furfural, 5-hydroxymethylfurfural (5-HMF), and phenolic compounds were formed during pretreatment and enzymatic hydrolysis. Activated carbon (AC) treatments were carried out to remove these non-sugar compounds while retaining the fermentable sugars, mostly glucose and xylose. Powdered AC and granular AC were compared and parameters including AC dose, hydrolysate pH and contact time were optimized using response surface methodology. Optimum conditions for powdered AC were 9.21% (w/w) dose, at pH 1.96 for 10 min, and for granular AC were 12.64% (w/w) dose, at pH 1.91 for 51.60 min. At these conditions, approximately 40% acetic acid, 75% formic acid and over 90% levulinic acid, HMF, furfurals, and phenolic compounds were removed with minimal fermentable sugar losses. Activated carbon adsorption can significantly reduce the non-sugar compounds present in the hydrolysate with minimal losses of fermentable sugars, which is beneficial in the production of lignocellulosic syrup and value-added products.

1. Introduction

Lignocellulose is a promising renewable resource that can be used in the production of syrups, a feedstock with great potential in the processing of fuels, bio-hydrogen, microbial lipids, and other chemicals (Liang et al., 2012; Pattra et al., 2008; Whitfield et al., 2012). Pretreatment and hydrolysis are required to convert the polymeric sugars cellulose and hemicellulose, present in the lignocellulosic material, into their monomeric sugars, mostly glucose and xylose. These sugars can be concentrated into a syrup which contains reduced water activity to help control microbial growth and preserve the fermentable sugars, thus improving the logistics associated with long-distance transportation, long-time storage, and year-round supply of lignocellulosic materials to processing industries (Eggleston et al., 2013).

In order to provide enzymes better accessibility to the polymeric sugars, and enhance the bio-digestibility of biomass, pretreatment prior to enzymatic hydrolysis is necessary to decrease the crystallinity of cellulose, reduce the lignin content, increase biomass porosity, and soften the hemicellulose-lignin shield that surrounds the cellulose (Behera et al., 2014). Numerous pretreatment methods have been suggested and can be categorized as mechanical (i.e., milling, grinding), physicochemical (e.g. autohydrolysis, liquid hot water, steam, supercritical fluids), chemical (i.e., alkali, acid, organic solvents, oxidizing agents), and biological (i.e., fungi) processes or combinations of these approaches (Aita et al., 2011; Asakawa et al., 2016; Kim et al., 2003; Meighan et al., 2017; Xiao et al., 2017). Alkali-based pretreatments, specially ammonia-based, have demonstrated great success in delignifying lignocellulosic biomass (Aita and Kim, 2011). Ammonia can break down the C—O—C bonds present in the lignin and the ether and ester bonds found between the lignin and hemicellulose, as well as penetrating the crystalline structure in cellulose causing it to swell (Aita et al., 2011). Dilute ammonia pretreatment has been previously reported to be highly effective in improving enzymatic digestibility of numerous lignocellulosic biomass, and was selected in this study due to its advantages such as reduced solubilization of hemicellulose and the formation of non-sugar compounds (Aita et al., 2011; Aita and Kim, 2011; Jönsson and Martín, 2016).

Pretreatment conditions can generate non-sugar compounds such as organic acids (i.e., acetic acid, formic acid, levulinic acid), phenolic compounds (i.e., 4-hydroxybenzoic acid, vanillin, catechol) and

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furaldehydes (i.e., furfural, 5-hydroxymethylfurfural (HMF)) which can alter the final quality and purity of lignocellulosic syrups and interfere with downstream processes such as enzymatic hydrolysis and fermentation (Larsson et al., 1999; Palmqvist and Hahn-Hägerdal, 2000; Ximenes et al., 2011). Organic acids can inhibit cell growth and reduce fermentation yields (Palmqvist and Hahn-Hägerdal, 2000). Furfural and HMF can cause inactivation of cell replication which results in a longer lag-phase (Larsson et al., 1999). Phenolic compounds can damage the integrity of the biological membrane interfering with cell growth and enzyme efficiency (Palmqvist and Hahn-Hägerdal, 2000). Nevertheless, these non-sugar compounds can be recovered and used as platform chemicals in the production of value-added products (Cannella et al., 2014; Carter et al., 2011). For example, acetic acid is widely used in the food industry and in the production of vinyl acetate monomers, acetic anhydride and esters (Le Berre et al., 2000). HMF and furfural can be converted to levulinic acid, dimethylfuran, 2,5-furandicarboxylic acid, and dihydroxymethylfuran, which are building blocks in the manufacture of alternative fuels, polymers, foams, and polyesters (Choi et al., 2015; Rosatella et al., 2011). Therefore, the strategy for producing lignocellulosic syrups should be designed for not only the removal of the non-sugar compounds from the hydrolysates, but their recovery as potential value-added products (Cannella et al., 2014).

Hydrolysate detoxification aims at removing and/or recovering non-sugar compounds with minimal sugars losses (Kamal et al., 2011). Some detoxification methods include evaporation, flocculation, overliming, and adsorption with activated carbon, ion exchange resins and laccases (Deng and Aita, 2018; Lee et al., 2011; Mateo et al., 2013; Moreno et al., 2012; Mussatto and Roberto, 2004; Vallejos et al., 2016; Villarreal et al., 2006). Among these methods, activated carbon is one of the most widely applied methods for removing inhibitors from enzymatic and acid hydrolysates, due to its low cost, high capacity of adsorption, and ease of use (Kamal et al., 2011). Activated carbon type, dose, hydrolysate pH, and contact time are the major factors that affect activated carbon adsorption efficiencies (Mateo et al., 2013; Mussatto and Roberto, 2004). The effectiveness of detoxification also depends on the type of lignocellulose hydrolysate, conditions of pretreatment and enzymatic hydrolysis (Mussatto and Roberto, 2004).

Response surface methodology (RSM) is one of the most popular statistical modeling methods used to obtain optimal process conditions. RSM offers several advantages such as the capability of obtaining large amounts of information from reduced number of experiments, and investigating the interaction effects among the independent variables (Baş and Boyacı, 2007). Central composite design (CCD) is an experimental design that is commonly used in RSM. Compared to multilevel factorial design, CCD requires much fewer experiments without sacrificing critical information (Khataee et al., 2010). CCD consists of a fractional factorial design with center points for replication and a group of axial points.

This study investigated the removal of organic acids, furaldehydes and total phenolic compounds from dilute ammonia pretreated energy cane bagasse enzymatic hydrolysate by activated carbon adsorption for syrup production. Response surface methodology was used to optimize the process parameters (activated carbon type, dose, contact time, and hydrolysate pH) for the maximum removal of non-sugar compounds while minimizing fermentable sugars losses.

2. Material and methods

2.1. Biomass

Energy cane is a cross breed between commercial sugarcane (*Saccharum officinarum*) and wild sugarcanes (*S. spontaneum*) with great potential as an energy crop. Energy cane has a higher fiber content and biomass yield than regular sugarcane (Bischoff et al., 2008; Kim and Day, 2011; Sierra et al., 2008). A non-commercial variety of energy cane (Ho 02-113) was collected from the Sugar Research Station in St. Gabriel, LA. Energy cane juice was extracted by passing the whole stalk, leaves and tops through a roller press (Farrel Corporation, Ansonia, CT) three times. Energy cane bagasse, the solid portion left behind after juice extraction, was stored at −20 °C until further use.

2.2. Dilute ammonia pretreatment

Energy cane bagasse was dried in a 45 °C oven for 24 h to a moisture content of 5%, finely milled in a Wiley mill (Swedesboro, NJ) and sieved with a 2 mm mesh sieve prior to pretreatment. Milled bagasse was then stored at 4 °C until further use. Pretreatment was conducted in a 4 L reactor (Parker Autoclave Engineers, Erie, PA) by mixing energy cane bagasse, ammonium hydroxide (28% v/v solution, Fisher Scientific, Pittsburgh, PA) and water at a ratio of 1: 0.5: 8. Dilute ammonia pretreatment has been reported to significantly remove the lignin and improve cellulose and hemicellulose enzymatic digestibility (Aita et al., 2011). The reactor was heated to 160 °C for 1 h, and then cooled down to 50 °C (Aita et al., 2011). Post pretreatment, all the bagasse was carefully collected from the reactor and pressed to remove excess liquid. Pretreated bagasse was dried in a 45 °C oven for 24 h to a moisture content of approximately 5% and stored at 4 °C. Compositional analysis was conducted for both untreated and pretreated bagasse following standard Laboratory Analytical Procedures (LAP TP-510-42618, 42619, 42620, 42621, 42622) documented by the National Renewable Energy Laboratory (NREL).

2.3. Enzymatic hydrolysis

Cellic® CTec2 and HTec2 are commercially available enzymes and were supplied by Novozymes (Franklinton, NC). Cellic® CTec2 contains cellulase, β-glucosidase and hemicellulase. Cellic® HTec2 is an endoxylanase complex with cellulase that converts hemicellulose to fermentable sugars. Cellulase activity was measured using No. 1 filter paper (Whatman, Maidstone, UK) as described by NREL's LAP-510-42628. β-glucosidase activity was determined by the Ghose method using cellobiose as the substrate (Ghose, 1987). Xylanase activity was analyzed using the method described by Bailey et al. (1992). CTec2 enzyme activities were 132 FPU/mL cellulase, 3230 IU/mL β-glucosidase and 16290 IU/mL xylanase. HTec2 enzyme activities were 56 FPU/mL cellulase, 16.52 IU/mL β-glucosidase and 23300 IU/mL xylanase. CTec2 was added at 25% (w/w) g/g glucan and HTec2 was added at 5% (w/w) g/g glucan to a bagasse loading of 5% (w/w). Citric acid buffer (0.05 M) was used to ensure the pH of the hydrolysate was kept at optimum 5.0. The mixture was incubated at 50 °C for 72 h at 200 rpm. Post hydrolysis, solids were separated by passing the hydrolysate through a 0.2 μm filter (VWR, Radnor, PA). The hydrolysate was stored in a −20 °C freezer until further analysis.

2.4. Activated carbon detoxification and experimental design

Powdered AC (Norit® CN1 (Cabot Corporation, Alpharetta, GA)) with a surface area of 1400 m^2/g and granular AC (DARCO® 12 × 40 (Cabot Corporation, Alpharetta, GA)) with a surface area of 650 m^2/g were evaluated. Enzymatic hydrolysates from dilute ammonia pretreated energy cane bagasse were mixed with powdered AC (Table 1) or granular AC (Table 2) at different conditions (AC dose, hydrolysate pH and contact time). Range of treatment conditions were selected based on published literature and preliminary results (Lee et al., 2011; Mateo et al., 2013). All AC treatments were agitated at 200 rpm and incubated at 22 °C. Previous studies suggested that contact time was not a significant factor in the adsorption efficiency of powdered AC (Lee et al., 2011). Thus, the contact time for powdered AC treatment was fixed at 10 min, which was the adequate time to achieve maximum removal of non-sugar compounds. AC was removed from hydrolysates by filtration using 0.2 μm syringe filters (VWR, Radnor, PA). Sugars (glucose and xylose) and non-sugar compounds (formic acid, acetic acid, levulinic