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Detoxification of dilute ammonia pretreated energy cane bagasse enzymatic hydrolysate by soluble polyelectrolyte flocculants

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ABSTRACT

This study investigated the detoxification of dilute ammonia pretreated energy cane bagasse enzymatic hydrolysate using polyelectrolyte flocculants. Non-sugar compounds such as formic acid, acetic acid, levulinic acid, furfural, 5-hydroxymethylfurfural (HMF), and phenolic compounds can be generated during pretreatment, which have negative effects on downstream processes. Flocculation was carried out to remove these non-sugar compounds while retaining the fermentable sugars. The flocculants polyethylenimine (PEI) and poly-diallyldimethylammonium chloride (pDADMAC) were used and parameters including flocculant dose and hydrolysate pH were evaluated. The recyclability of PEI and recovery of non-sugar compounds were also assessed. Optimum conditions for both PEI and pDADMAC were 15 g/L dose at unadjusted pH 4.5. At these conditions, PEI outperformed pDADMAC by having greater adsorption efficiencies towards non-sugar compounds with minimal sugar losses. PEI removed 43% organic acids, 73% total phenolic compounds and 100% furans with less than 10% total fermentable sugar losses. However, after two cycles, only 20% of the adsorbed organic acids and total phenolic compounds, and 80% of the furans were recovered. Sugar losses of less than 10% were observed throughout the recycling process. PEI can significantly remove the non-sugar compounds from dilute ammonia pretreated energy cane bagasse enzymatic hydrolysate with minimum sugar losses. However, recycling of PEI and recovery of adsorbed non-sugar compounds are not recommended for more than two cycles.

1. Introduction

Lignocellulose is a highly sustainable and renewable natural resource that can be used in the processing of syrups, fuels, hydrogen, microbial lipids, biodegradable plastics, and other value-added chemicals (Canilha et al., 2012; Duarte et al., 2010; Liang et al., 2012; Pattra et al., 2008). A year-round supply of lignocellulosic biomass to biorefineries is challenging due to the seasonality of the crops and their high water and sugar content, which make them highly susceptible to microbial degradation. Lignocellulosic syrup production from the concentration of sugars from hydrolysates can significantly reduce water activity, control microbial growth and preserve the sugars thus providing solutions to challenges associated with long-distance transportation, long-time storage, and biomass supply to bio-based fuels and chemicals manufacturing plants (Eggleston et al., 2015).

Pretreatment of lignocellulose prior to enzymatic hydrolysis is necessary in order reduce the lignin content and soften the hemicellulose-lignin shield that surrounds the cellulose to facilitate exposure of the polymeric sugars (Laureano-Perez et al., 2005; Meighan et al., 2017). Among pretreatments, dilute ammonia pretreatment has been effective in removing the lignin and improving enzymatic digestibility (Aita and Kim, 2011). However, regardless of the positive outcomes that result from pretreatment and hydrolysis processes, harsh processing conditions can promote the formation of non-sugar compounds (i.e., organic acids, furans, phenolic compounds) which interfere with downstream processes. Organic acids (i.e., acetic acid, levulinic acid, formic acid) result from the degradation of hemicellulose and can inhibit cell growth and reduce fermentation yields (Palmqvist and Hahn-Hägerdal, 2000). Furans including furfural and 5-hydroxymethylfurfural (HMF) formed from the degradation of pentose and hexose sugars, respectively, interfere with cell replication which results in longer lag-phases (Larsson et al., 1999). Degradation of lignin releases various phenolic compounds such as 4-hydroxybenzoic acid, vanillin and catechol which inhibit cell growth as well as the activity of cellulase and β-glucosidase (Ximenes et al., 2011).

It has been widely agreed that improved fermentation yields were achieved by removing these non-sugar compounds from hydrolysates (Kamal et al., 2011; Lee et al., 2011; Villarreal et al., 2006). On the other hand, these non-sugar compounds can be recovered and serve as platform chemicals to various products in multiple industries. For

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example, acetic acid is used as a condiment in food and animal feed industries and serves as the raw material in the manufacture of polymers (Le Berre et al., 2000). HMF and furfural are widely used in the production 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).

Physical, physicochemical and biological detoxification methods have been studied over the years and some of these methods include evaporation, flocculation, overliming, adsorption with activated carbon, ion exchange resins, and laccases (Lee et al., 2011; Mateo et al., 2013; Moreno et al., 2012; Mussatto and Roberto, 2004; Vallejos et al., 2016; Villarreal et al., 2006). The key to detoxification is separating the non-sugar compounds from the hydrolysate without a significant loss of fermentable sugars. Flocculation is a process known to enhance solid-liquid separations with the potential of flocculant recyclability (Cannella et al., 2014; Carter et al., 2011a). Flocculants can be inorganic salts (i.e., alum, ferric chloride) or organic polyelectrolytes (i.e., polyamines, chitosan) with various mechanisms or mode of action (i.e., ionic strength, hydrophobic interactions) depending on the characteristics of the flocculants (i.e., charge density, chain length, structure) and the properties of the targeted solution (i.e., charge, particle size) (Carter et al., 2011a; Matilainen et al., 2010). Polyethylenimine (PEI) and polydiallyldimethylammonium chloride (pDADMAC) are water soluble cationic polyelectrolytes with relatively low molecular weights and high charge densities. PEI and pDADMAC have been reported to be effective in separating not only solids, but also dissolved components in solutions, such as precipitation of humic and fulvic acids from groundwater, and have also shown promising results in the detoxification of lignocellulosic biomass (i.e., Ponderosa pine slurry, maple wood hydrolysate) (Cannella et al., 2014; Carter et al., 2011b; Matilainen et al., 2010).

This study investigated the effectiveness of flocculation treatment in the removal of organic acids, phenolic compounds, furfural, and HMF from dilute ammonia pretreated energy cane bagasse enzymatic hydrolysate for syrup production. PEI and pDADMAC were evaluated at different doses and pH to determine the optimum flocculation parameters for removing non-sugar compounds while minimizing fermentable sugars losses. The recovery of the non-sugar compounds and the recyclability of flocculants were also evaluated.

2. Materials and methods

2.1. Energy cane biomass

Energy cane is a hybrid of commercial and wild sugarcanes and one of the most attractive lignocellulosic materials due to its higher fiber content, cold resistance, lower water input, and fertilizer requirement as compared to sugarcane (Kim and Day, 2011). Non-commercial energy cane variety Ho 02-113 was harvested (stalks and leaves) at the Sugar Research Station in St. Gabriel, LA. Energy cane samples were passed through a roller press (Farrel Corporation, Ansonia, CT) three times. The solid fraction after juice extraction (referred to as bagasse) was dried in a 45 °C oven for 24 h to a final moisture content of 5%, then finely milled (Wiley Mill, Swedesboro, NJ) and sieved (2 mm mesh). Milled bagasse was stored at −20 °C until further use. The chemical composition of milled bagasse (untreated) consisted of 40.26 ± 0.34% glucan, 19.81 ± 0.57% xylan, 28.74 ± 0.24% lignin, 1.87 ± 0.23% arabinan, 5.45 ± 0.53% extractives, and 3.87 ± 0.77% ash.

2.2. Dilute ammonia pretreatment

Finely milled and dried energy cane bagasse was pre-mixed with ammonium hydroxide (28% v/v solution, Fisher Scientific, Pittsburgh, PA) and water at a ratio of 1: 0.5: 8. The mixture was then transferred to a 4 L reactor (Parker Autoclave Engineers, Erie, PA) where it was heated to 160 °C at100 rpm for 1 h (Aita et al., 2011). The slurry was pressed to remove any excess liquid post pretreatment. The collected solid fraction was dried in a 45 °C oven until a final moisture content of 5% and then stored at 4 °C. The chemical composition of untreated and pretreated energy cane bagasse was analyzed following National Renewable Energy Laboratory (NREL) Laboratory Analytical Procedures (LAP TP-510-42618, 42619, 42620, 42621, 42622).

2.3. Enzymatic hydrolysis

Commercially available enzymes Cellic® CTec2 (cellulase) and HTec2 (xylanase) were supplied by Novozymes (Novozymes, Franklinton, NC). Cellulase activities of CTec2 (132 FPU/mL) and HTec2 (56 FPU/mL) were measured using No. 1 filter paper (Whatman, Maidstone, UK) following NREL LAP-510-42628. β-glucosidase activities of CTec2 (3230 IU/mL) and HTec2 (16.52 IU/mL) were determined using the method documented by Ghose (Ghose, 1987). Xylanase activities of CTec2 (16300 IU/mL) and HTec2 (23300 IU/mL) were tested using the method provided by Bailey et al. (1992). Enzymatic hydrolysis studies were carried out at a 5% (w/w) bagasse loading followed by the addition of 25% (w/w) g/g glucan CTec2, 5% (w/w) g/g glucan HTec2 and 0.05 M citric buffer. The buffer was used to ensure the pH of the hydrolysate remained at 5, which is the optimum pH for CTec2 and HTec2. Samples were incubated at 55 °C for 72 h at 200 rpm. Post hydrolysis, the solid fraction was removed by filtration using a 0.2 μm nylon filter (VWR, Radnor, PA) and the hydrolysate was kept at −20 °C. Hydrolysate samples were analyzed for total phenolic compounds and for sugars and non-sugar compounds (organic acids, HMF and furfural) before detoxification by flocculants as described below.

2.4. Flocculation

PEI (50% w/v, avg. MW 60,000) was purchased from Fisher Scientific (Pittsburgh, PA) and pDADMAC (35% w/w, avg. MW 100,000) was obtained from Sigma-Aldrich (St. Louis, MO). Preliminary experiments and published information have indicated that flocculation with PEI or pDADMAC were not affected by contact time, agitation or temperature. Therefore, flocculation parameters of 30 min contact time, 150 rpm agitation and 22 °C were selected based on published information (Cannella et al., 2014; Carter et al., 2011a). Flocculants were separately added to the hydrolysate samples at various doses (5, 15, 30 g/L) and the mixture agitated at 150 rpm for 30 min at 22 °C. The effect of hydrolysate pH on flocculation was also investigated by adjusting the hydrolysate pH to 2.0, 4.5 (unadjusted) or 8.0 using sulfuric acid or sodium hydroxide as needed. Post flocculation, flocculant polymers were separated by filtration using a 10,000 kDa molecular weight cutoff (MWCO) centrifugal filter (EMD Millipore, Billerica, MA) at 14,000 × g for 30 min. The permeate was analyzed for total phenolic compounds and for sugars and non-sugar compounds (organic acids, furfural and HMF) as described below.

2.5. PEI recycling

PEI was selected for the recycling studies as it was more efficient than pDADMAC in the removal of non-sugar compounds from the hydrolysate. The initial concentration of HMF and furfural in the dilute ammonia pretreated energy cane bagasse hydrolysate prior to detoxification by PEI was low (0.04 g/L furfural and 0.06 g/L HMF). Therefore, in this section, the concentration of furfural and HMF in the hydrolysate was adjusted to 2 g/L (the average concentration for organic acids and total phenolic compounds) by adding pure chemicals to the hydrolysate to accurately evaluate the adsorption efficiency of PEI, and the recovery of furans over the recycling process. HPLC-grade HMF