



Reducing sugar loss in enzymatic hydrolysis of ethylenediamine pretreated corn stover



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HIGHLIGHTS

- The effect of ethylene diamine (EDA) on enzymatic hydrolysis was investigated.
- The Maillard reaction between EDA and carbohydrates led to sugar loss.
- The browning between EDA and sugar was weakened by Na₂SO₃ and H₂O₂.
- Lower temperature during enzymatic hydrolysis reduced sugar loss.

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ABSTRACT

In this study, the effect of ethylenediamine (EDA) on enzymatic hydrolysis with different cellulosic substrates and the approaches to reduce sugar loss in enzymatic hydrolysis were investigated. During enzymatic hydrolysis, xylose yield reduced 21.2%, 18.1% and 13.0% with 7.5 mL/L EDA for AFEX pretreated corn stover (CS), washed EDA pretreated CS and CS cellulose. FTIR and GPC analysis demonstrated EDA reacted with sugar and produced high molecular weight (MW) compounds. EDA was prone to react with xylose other than glucose. H₂O₂ and Na₂SO₃ cannot prevent sugar loss in glucose/xylose-EDA mixture, although they inhibited the browning and high MW compounds formation. By decreasing temperature to 30 °C, the loss of xylose yield reduced to only 3.8%, 3.6% and 4.2% with 7.5 mL/L EDA in the enzymatic hydrolysis of AFEX pretreated CS, washed EDA pretreated CS and CS cellulose.

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1. Introduction

Lignocellulosic biomass, the most abundant and renewable carbohydrate resource over the world, has great potential for the production of biofuels and biochemicals (Eom et al., 2015; Zhao et al., 2012). The utilization of lignocellulose would ensure power security and food safety to a large extent (Daystar et al., 2015; Hu and Ragauskas, 2012). Among the potential lignocellulosic biomass, the agricultural residues will most probably get success because they were waste and the use of them could increase rural income (Sharma et al., 2016; Thurlow et al., 2015). There are more than 0.73 billion ton of agricultural residues produced annually in China (Ma et al., 2012). However, the natural resistance of plant

cell walls to microbial and enzymatic deconstruction, which was known as “biomass recalcitrance” extremely increased the difficulty and cost of lignocellulose utilization (Cavka et al., 2015; Hu and Ragauskas, 2012; Singh et al., 2015).

Pretreatment is essential to reduce the biomass recalcitrance by increasing enzyme accessibility of cellulose and destroying the lignocellulose structure (Feng et al., 2013; Singh et al., 2015). Ethylenediamine (EDA) is an active amide and is able to react with many components in biomass. EDA pretreatment transformed the allomorph of cellulose and induced lignin degradation and relocation, which was proved an effective method to increase glucose and xylose yields in enzymatic hydrolysis (Qin et al., 2015; Wada et al., 2008). EDA pretreatment is a “dry-to-dry” process at atmospheric pressure, which preserved the sugars and microbial nutrients in the solids, saved water and reduced capital costs. Thus, EDA pretreatment is an available method for lignocellulosic bioconversion.

However, solid recovery after EDA pretreatment was more than 100%, indicating the EDA residue in pretreated solid (Qin et al.,

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2015). The remained EDA might have influence on enzymatic hydrolysis and fermentation (Qin et al., 2016). In addition, the Maillard reaction between EDA and reducing sugar have been reported (Kevin et al., 2015). Generally, the development of Maillard reaction products was associated with the loss of reducing sugars and free amino groups (Ahmad et al., 2013; Matmaroh et al., 2006).

In this paper, we explored the effect of EDA on enzymatic hydrolysis of different biomasses. Glucose/xylose-EDA model systems were built to investigate the reaction between EDA and sugars. We characterized the changes of carbohydrates during the Maillard reaction. We further investigated the approaches to reduce carbohydrates loss in enzymatic hydrolysis with the addition of EDA.

2. Materials and methods

2.1. Materials

Corn stover (CS) used for this study was harvested in suburb of Tianjin, China. CS was milled coarsely using a beater pulverizer and the fractions between 20 and 80 meshes were screened. Air-dried CS had an average moisture content of 9% (dry weight basis, dwb). The main composition of untreated CS was 36.3% glucan, 19.4% xylan, 18.2% acid insoluble lignin (AIL), and 14.0% ash (dwb).

Avicel PH-101 (Sigma-Aldrich, MO, US), washed EDA pretreated CS, AFEX pretreated CS, and CS cellulose (CSC) were used as cellulose substrates in the experiment of EDA effect on enzymatic hydrolysis. AFEX pretreated CS was gifted from Ningbo University, Ningbo, China. AFEX pretreatment conditions were as follows: moisture content, 60% (w/w); 1 g ammonia per g CS; 140 °C; 15 min. At the completion of the residence time, the pressure was explosively released by rapidly turning the exhaust valve. The treated biomass was removed and placed in a fume hood overnight to remove any residual ammonia. CSC were prepared according to previous researches (Mittal et al., 2011; Qin et al., 2015). In brief, CS was hydrolysis with 0.6% sulfuric acid for 56 min to remove majority of hemicellulose and solubilized lignin. After that, mixture solid residues with 0.67 g sodium chlorite and 1 mL glacial acetic acid per gram dry matter, then placed in water bath at 60 °C for 2 h to delignification to obtain CSC.

Commercial cellulase Accellerase 1500 (89 mg protein/mL, 77 FPU/mL, where FPU stands for filter paper units) and hemicellulase Multifect xylanase (42 mg protein/mL) were purchase from Genencor (California, USA).

EDA (99%, chemical pure) was purchased from Tianjin Yuanli Co., China. Other chemicals used were of analytical grade.

2.2. EDA pretreatment

EDA pretreatment was carried out following a previously published procedure (Qin et al., 2015) with some modifications. 10 g CS was mixed with 6 mL EDA and transferred into a lab-scale Schott-Duran bottle. The sealed bottles were then heated to 140 °C in an oven. After 20 min, mixture was poured into a plate and dried in the oven at 120 °C for 3 h to remove EDA from CS. Dried solid was washed with a ratio of per g dry solid to total 120 mL water (for three times), and dried in 105 °C overnight to obtain washed EDA pretreated CS.

2.3. Composition analysis

The contents of samples were determined according the Laboratory Analytical Procedure (Rahikainen et al., 2013) of the National Renewable Energy Laboratory (NREL, 2008). The acid-hydrolysate

was centrifuged at 12,000 rpm for 5 min, filtered with a 0.22 µm filter to remove impurities and then for sugar analysis. Glucose and xylose concentrations in hydrolysate were quantified by HPLC equipped with a Waters (Milford, MA) 1515 pump, a reflective index detector (Waters 2414) and an Aminex HPX-87H column (Bio-Rad, Hercules, CA). Running temperature was 65 °C and 5 mM H₂SO₄ was used as mobile phase at a flow rate of 0.6 mL/min. The weight of solid residue dried overnight at 105 °C was recorded and used to calculate the AIL content.

Acid titration was used to determine the EDA concentrations in enzymatic hydrolysis with modifications (Zhou et al., 2010). Different amounts of EDA pretreated CS samples were mixed with deionized water, then shaken at 200 rpm and 50 °C for 3 h. Slurry was centrifuged at 4000 rpm for 5 min. 1 mL supernatant was diluted 10× and titrated with 0.1 M H₂SO₄ to a pH of 2.4. The amount of acid needed to reach the equivalence point was used to calculate the total amine concentration in solution.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis experiments were performed in accordance with the NREL standard protocol (LAP-009). Experiments at 1% glucan loading were conducted with 20 mL reaction volume containing 50 mM citrate buffer (pH 4.8) and 40 mg/L tetracycline in 100 mL Erlenmeyer flasks. Commercial cellulase Accellerase 1500 (15 FPU/g glucan) and xylanases Multifect xylanase (4.6 mg protein/g glucan) was used for the enzymatic hydrolysis. The flasks were incubated at 50 °C or 30 °C and 200 rpm for 72 h in an orbital incubator. After enzymatic hydrolysis, the mixture was centrifuged at 12,000 rpm for 5 min to separate hydrolysate from solid residue. The hydrolysates were frozen at −20 °C for subsequent sugar analysis. All experiments were conducted in duplicate.

2.5. Glucose/xylose-EDA model system Preparation

Glucose/xylose-EDA model system was built. 0.2 g glucose and 0.1 g xylose was respectively added into 20 mL 50 mM citrate buffer (pH 4.8) with different EDA concentrations (0–7.5 mL/L). The flasks were incubated at 30 or 50 °C and 200 rpm in the incubator and 0.5 mL aliquot was withdrawn for sugar analysis.

After 72 h reaction, 2 mL of solution were freeze-dried and stored in a desiccator until used for analysis.

10 g/L Na₂SO₃, 10 mL/L H₂O₂ or 10 g/L NaClO were added respectively before the start of reaction to test their inhibition effect on Maillard reaction in glucose/xylose-EDA model system. EDA concentration was 7.5 mL/L. The pH of solutions was adjusted to 4.8 if necessary after thorough mixing.

2.6. FTIR measurement

All infrared spectra of glucose/xylose-EDA reaction products were obtained with an FTIR620 spectrometer (BRUKER) by using the potassium bromide (KBr) pellet method and were recorded by an average of 64 scans at a resolution of 4 cm^{−1}. Background noise was corrected with pure KBr data.

2.7. Estimation of molecular weight distribution

The method used was according to previous report (Huang et al., 2011) with some modifications. The molecular weight (MW) of products derived from glucose/xylose-EDA model system with and without chemical inhibitor addition were determined using a Waters 600 liquid chromatograph (Waters Co., Milford, MA, USA) equipped with a Waters 2487 UV detector and Empower workstation. Data analysis was performed using gel permeation chromatography (GPC) software (Waters corporation, Milford,

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