Industrial Crops and Products 70 (2015) 410–416

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Ambient-temperature sulfuric acid pretreatment to alter structure and improve enzymatic digestibility of alfalfa stems

Shengfei Zhou, Qiang Yang, Troy M. Runge*

Department of Biological System Engineering, University of Wisconsin, Agricultural Engineering Building, Madison, WI 53706 USA

ARTICLE INFO

Article history:
Received 14 October 2014
Received in revised form 18 March 2015
Accepted 20 March 2015
Available online 6 April 2015

Keywords:
Ambient-temperature sulfuric acid pretreatment
Pore size
Pore volume
Surface area
Crystallinity
Enzymatic digestibility

ABSTRACT

A novel pretreatment with sulfuric acid at ambient temperature had demonstrated positive effects on ethanol production from alfalfa stems. To better understand these effects and help create a process control system, physical parameters (pore dimensions, surface area, and crystallinity index) and the enzymatic digestibility of alfalfa stems were studied in present work. The SEM images showed that more fine particles were present on the surface of alfalfa stems after acid pretreatment. The BET pore size, pore volume, surface area, and crystallinity were increased with increasing acid during pretreatment, might be due to the acid pretreatment hydrolyzed some hemicellulose and amorphous cellulose. Ensiling and wet storage decreased crystallinity, probably due to the swelling increased the pore size of substrates. The enzymatic hydrolysis of alfalfa stems was improved by acid pretreatment because surface damage, improved pore size, pore volume and surface area improved cellulose exposure. Ensiling also improved the enzymatic hydrolysis, likely due to the swelling resulted in increased pore size and broken hydrogen bonding of substrates.

1. Introduction

With an increasing global population and expanding resource demand, it may be not realistic to produce large amount of biofuels from food related agricultural commodities such as corn starch and cane sugar. Ethanol produced from cellulosic biomass is considered as a feasible replacement of fossil fuels for transportation (Kuhad et al., 2011), allowing both food and fuel production on farm if agricultural residues, mainly cellulosic biomass, can be used for fuel production. Alfalfa, which is good protein and carbohydrate source (Balliette and Torell, 2014), can be a feedstock candidate for cellulosic ethanol production. Alfalfa has been widely used as animal feed, and it is also beneficial to land and farm including soil, water and nutrient retention, reduced nitrogen fertilizer input, and increased subsequent crop yield (Zhou et al., 2014), which are all important issues on farms. As more environmental concerns are raised for farming to be sustainable, we expect more and more alfalfa will be included in crop rotations. To encourage alfalfa planting, fewer harvests would be required to save labor and fuels, as current practices require harvesting there or four times per season for forage use (Sheaffer et al., 2000). If alfalfa leaves and stems can be harvested separately, we could reduce seasonal harvests as the alfalfa leaves with high protein and palatability would be separated from the low digestibility stems and used as animal feed. The mature alfalfa stems, with high carbohydrates content (Dien et al., 2011) would need to be utilized to encourage this practice, and have been considered as feedstock candidate for liquid fuel (ethanol) production (Digman et al., 2010a,b,c).

A typical process for cellulosic ethanol production includes substrate preparation, pretreatment, hydrolysis, fermentation, and ethanol recovery. The pretreatment step is essential for overcome the recalcitrance (resistance of plant cell walls to deconstruction) of lignocellulosic biomass. The recalcitrance is mainly caused by the highly crystalline structure of cellulose and the complicated matrix of cellulose, as well as the complications from hemicelluloses and lignin (Brodeur et al., 2011). The goal of pretreatment is to open the cell wall structure to improve cellulose accessibility to cellulase. There are physical, chemical, and biological pretreatments as well as combinations thereof, which can change the biomass chemically and physically including removing hemicelluloses, reducing the degree of polymerization of lignin polymer, increasing substrate surface area, pore volume and size, and altering cellulose crystalline structure (Chiaramonti et al., 2012; Singh et al., 2014).

Dilute acid hydrolysis at elevated temperature (DA) is a widely studied pretreatment, and considered one of the most promising pretreatment techniques for industrial application (Alvira et al.,

---

* Corresponding author. Tel.: +1 608 890 3143; fax: +1 608 262 1228.
E-mail addresses: szhou34@wisc.edu (S. Zhou), qyang4@wisc.edu (Q. Yang), trunge@wisc.edu (T.M. Runge).

http://dx.doi.org/10.1016/j.indcrop.2015.03.068
0926-6690/

2010). It is usually carried out with a very low sulfuric acid concentration of 0.2–2.5% w/w, at elevated temperature between 130 °C and 210 °C (Brodeur et al., 2011). Sulfuric acid is commonly used might due to its low cost, though other acids have also been studied (Kumar et al., 2009). It has been proposed that the main effects of DA pretreatment include hemicellulose removal and structure changes of the substrates (Knappert et al., 1980). The disadvantages of acid pretreatment at elevated temperature include relatively high capital investment, inhibitor production and increased hydrophobic components against effective enzyme adsorption (Shuai et al., 2010). Well-known inhibitors are weak acid such as acetic acid, furan derivatives including furfural and 5-(hydroxy methyl) furfural (HMF), and phenolic compounds (Palmqvist and Hahn-Hägerdal, 2000). Weak acids can inhibit cell growth because of uncoupling or intracellular anion accumulation, and furan derivatives have inhibition effects because they can be metabolized in cell and the metabolites inhibit cell growth, while the phenolic compounds can affect the functions of cell membranes (Palmqvist and Hahn-Hägerdal, 2000).

Recently, the ambient-temperature acid pretreatment, which was carried out at environmental temperature with relatively long time, has been reported as a novel pretreatment for biomass to improve ethanol production (Digman et al., 2010b; Zhou et al., 2014). On-farm storage can be up to one to two years and even more to provide animal feed for whole year around because of seasonal harvesting (Aragón, 2012). Thus, the ambient-temperature acid pretreatment was designed to be used on farm combining storage, taking advantage of the long time of the treatment instead of traditional higher temperature. Thus, energy input and danger are potentially reduced, and a reduction of inhibitors compared to DA pretreatment. Our previous work showed that the ambient-temperature acid pretreatment had greatly improved ethanol yields (Zhou et al., 2014). This process was demonstrated to be effective on different farm crops including switchgrass, reed canarygrass and alfalfa at different conditions including different acid loadings (up to 100 g/kg dry matter (DM)) and pretreatment duration (as long as ten months). Studies were initiated in lab scale, while pilot- and full-scale studies had also been done to demonstrate that this process had the potential to be applied on farm (Digman et al., 2010a,b; Zhou et al., 2014).

A pretreatment cause not only chemical component changes, but also physical structure changes in substrate. Many studies have been carried out to demonstrate the correlations between substrate physical parameters and enzymatic digestibility. The surface area of substrates has been proposed to be very important because the direct physical contact between the enzymes and substrates might be a prerequisite to enzymatic hydrolysis (Fan et al., 1981). The pore size is believed to affect the enzymatic hydrolysis as it limits the access of enzymes into the substrates (Mansfield et al., 1999). Accessible pore volume is related to the surface area and has a similar effect (Mansfield et al., 1999). The effect of cellulose crystallinity on enzymatic hydrolysis is somewhat controversial, with some researchers finding the crystallinity affected enzymatic hydrolysis (Gharpuray et al., 1983), while others concluded that the crystallinity was not an important factor (Mansfield et al., 1999). These physical parameters not only helped researchers to understand the mechanism beneath experimental observations, but also have the potential to be applied in process control during production. Grethlein (1985) created very simple linear correlations between the initial glucose yield and the substrate pore volume or specific surface area. Fan et al. (1981) created model to predict the extent of substrate hydrolysis based on specific surface area and crystallinity index. Gharpuray et al. (1983) also created similar model to predict the relative extent of substrate hydrolysis based on specific surface area crystallinity index and lignin content.

In order to explain the effect of ambient-temperature acid pretreatment on ethanol production from alfalfa stems, our previous study (Zhou et al., 2014) investigated ethanol production and chemical components of the substrates. However, the physical structure changes of substrates and their enzymatic digestibility may also be important, because different changes can happen to substrates on multiple aspects during a pretreatment. These aspects of other pretreatments including DA pretreatment have been studied a lot. However, the structure changes caused by our ambient-temperature acid pretreatment have rarely reported before, remaining the question as how this pretreatment altered substrate structures and what correlations were between these physical parameters and enzymatic hydrolysis or ethanol production. To address this, we conducted a study on the structure changes of our substrates and their enzymatic hydrolysis. Though this study was carried out on substrates pretreated with long time (ten months), similar mechanism and correlations would hold for substrates pretreated with short time, which also improved ethanol production from farm biomass (Digman et al., 2010b) and may be more applicable for on-farm production and storage. Thus, the present study was focusing on the physical structure changes of the substrates, their enzymatic digestibility, and some correlations among them. Firstly we examined the surface changes of pretreated alfalfa stems through SEM images. Then, the pore size, pore volume and surface area, as well as the crystallinity of alfalfa stems were measured. These parameters were mainly compared against the glucose yields of enzymatic hydrolysis, while they were also compared against ethanol production, allowing mechanism to be understood and correlations to be created.

2. Materials and methods

2.1. Materials and chemicals

Alfalfa stem powder was prepared according to the methods employed by Zhou et al. (2014). In brief, alfalfa stems were pretreated with different sulfuric acid loading (0, 35, or 75 g/kg dry matter (DM) of biomass) and different moisture content (30, 50, or 65% (w.b.)), and inoculated with lactic acid bacteria. To 1 L Tulpenform borosilicate glass canning jars (Weck-Rundrandglas 100, J. Weck GmbH and Co., Öflingen, Germany) was filled with alfalfa stems (110–170 g DM) and ensiled for 10 months. After ensiling, alfalfa stems were liquefied with a Büchi Mixer B-400 in a 1 L glass beaker, neutralized with 4 M NaOH to pH ∼7, freeze-dried and milled on a Wiley mill fitted with a 1 mm screen.

Avicel PH-101 cellulose powder (∼50 μm particle size), and Sigmacell 50 microcrystalline cellulose (type 50, 50 μm) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sulfuric acid and sodium hydroxide were purchased from Fisher Scientific (Fair Lawn, NJ, US). D(−)-Arabinose, D(+)-galactose, D(+)-glucose (anhydrous), D(+)-mannose, D(+)-xylose (all 99+%) and citric acid monohydrate (99.5%) were purchased from Acros Organics (Geel, Belgium). Tetracycline hydrochloride (96%) was purchased from Alfa Aesar (Heysham, LA3 2XY, England).

Cellic® CTec2 enzyme, which was generously provided by Novozymes (Franklinton, NC), had an activity of 105 filter paper unit (FPU)/mL which was determined in lab according to an NREL procedure (TP-510-42628) (Adney and Baker, 2008).

2.2. Scanning electron microscope (SEM)

SEM images were taken for alfalfa stem powder both before and after simultaneous saccharification and fermentation (SSF). The SSF was carried out according to Zhou et al. (2014). The residues of alfalfa stems after SSF were washed three times as following: