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Modified simultaneous saccharification and fermentation to enhance bioethanol titers and yields

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ABSTRACT

To maintain our society's sustainability with respect to people, prosperity, and the planet, we must produce liquid transportation fuels such as bioethanol on the renewable basis at a competitive price to petroleum-based fuels. The major challenges to commercialize cellulosic biofuels are low fermentation efficiency, low ethanol titer, and lack of technology to fully utilize the byproduct from bioconversion process such as lignin which has been underutilized. To overcome these technical barriers, we have proposed a novel design to fully utilize each component of lignocellulosic biomass for biofuels and bio-chemicals production, which involves green technologies such as hydrothermal and organosolv pretreatments to produce a cellulose-rich solid with good recovery of clean lignin after solvent recycling for improvement of plant protein-based adhesives as well as xylose remained in the aqueous phase for furfural upgradation. The focus of this study, as a part of the whole biorefinery concept, is to develop modified simultaneous saccharification and fermentation (mSSF) to enhance ethanol titers and yields, which combines the advantages of both separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) via unique decantation process. The mSSF achieved higher ethanol concentration of 58.5 g/L and ethanol yield of 83.5% as compared to the traditional SSF process (49.9 g/L and 71.1%) at the biomass loadings of 20% (w/v). The mSSF also enabled higher ethanol titers of 72.3 g/L at higher loadings of 30% (w/v) with yields of 70.0%. As compared to published high-gravity fermentation, ethanol concentration of 72.3 g/L achieved in this study was the highest one in the lab-scale process, which proved that the proposed mSSF was an effective process to increase ethanol titers without sacrificing ethanol yields. The improved ethanol titers and yields would significantly lower the distillation cost and accelerate the commercialization of cellulosic biofuel production.

1. Introduction

Limited resources of crude oil and environmental concerns for mitigating greenhouse gas emissions have driven global research to explore renewable and sustainable biofuels and bio-chemicals. As the global population expands and the number of vehicles around the world increases, the demand for transportation fuels is expected to increase rapidly [1]. Among potential alternative liquid fuels, bioethanol is considered as the widest utilized transportation fuels [1]. Bioethanol is a renewable alternative fuel derived from various sustainable feedstocks such as sugar-based crops, starch-based crops, and lignocellulosic biomass. Biotechnologies are mature to produce ethanol from sugar and starch-rich crops in large scales and continued to develop advanced cellulosic ethanol production [2]. Currently, commercial production of bioethanol from lignocellulosic biomass is still not economically feasible, mainly facing the technical barriers of low ethanol yield and low ethanol concentration, high enzyme cost, and high water consumption [3,4].

Lignocellulosic biomass is the most abundant and renewable resource with the sustainable worldwide production, including agricultural residues, forestry wastes, dedicated energy crops, and organic municipal solid waste, which represents an indispensable feedstock for the production of commercialized biofuels and renewable chemicals [5,6]. Lignocellulosic biomass is mainly composed of cellulose, hemicellulose, and lignin, in which the fractions of both cellulose and hemicellulose are polysaccharides and thus is a potential source of fermentable sugars [7]. For the biological conversion pathway, a typical process is usually required to sufficiently convert structural sugars of plant cell wall to bioethanol: pretreatment, enzymatic hydrolysis, fermentation, and distillation. Cellulose in native biomass is difficult to digest by enzymes and its sugar yield is usually lower than 20% [8]. Pretreatment of biomass feedstocks was used to breakdown the

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structural barrier and make cellulose more accessible for subsequent saccharification process. Numerous pretreatment methods have been developed to overcome the recalcitrant structure, such as super-size reduction mill, steam explosion, liquid hot water (LHW), dilute acid, lime, ammonia, organic solvent, and ionic liquid pretreatments [9–12]. Challenges in the current leading pretreatment processes include incomplete separation of cellulose and lignin, which could reduce subsequent enzymatic hydrolysis efficiency; formation of inhibitors that affect ethanol fermentation, such as acetic acid from hemicellulose, furans from sugar degradation and phenolic compounds from lignin decomposition; low-concentrated fermentable sugars; low fermentation efficiency at high solids loading; high usage of chemicals and energy-intensive processes and also high cost of waste disposal [8,13].

Hydrothermal pretreatment is an environmentally friendly process as water is used as reaction medium without any addition of chemicals, which is processed at relatively high temperatures (140–220 °C) under mild acidic conditions. Hot water cleaves hemiacetal linkages and liberates acetic acids during pretreatment, which facilitates the breakage of ether linkages in biomass [8]. LHW pretreatment is more effective on agricultural residues and hardwood, but not efficient for softwood species. LHW pretreatment of wheat straw achieved 80% hemicellulose-derived sugar recovery and 91% enzymatic hydrolysis of cellulose [14]. LHW pretreatment has the capability of handling large particle size as the particles are usually broken apart during treatment, therefore, LHW pretreatment reduces the energy cost for particle reduction. The drawback of pretreatments such as hydrothermal pretreatment and acid pretreatment is that significant amount of lignin was retained in the pretreated biomass, which was found to bind enzymes and consequently increase enzymes costs [15].

Organosolv pretreatment is a process of using aqueous organic solvents as reaction medium to fractionate the major biomass components, of which cellulose and lignin are usually recovered as precipitated solid streams while hemicellulose and sugar degradation products are dissolved into a water-soluble fraction. Organosolv pretreatment is able to obtain a clean lignin component from biomass, while the lignin is usually burned as an energy source in other pretreatment processes [16]. Ethanol is a commonly used organic solvent and using acid or alkaline catalysts to assist in the delignification process. A two-step process involving dilute-acid presoaking and aqueous-ethanol organosolv pretreatment of Miscanthus x giganteus yielded a solid residue with 95% initial glucan recovery, of which 98% was converted to glucose after 48 h of enzymatic saccharification, and 73% initial xylan recovery as well as 71% lignin recovery as ethanol organosolv lignin [17].

Achieving high ethanol concentration is as critical as obtaining high ethanol conversion efficiency because the minimum ethanol concentration of 40 g/L is required for economical ethanol distillation [4]. Traditional method to reach high ethanol concentration is to increase the amount of biomass loadings (> 16 wt%), so-called high-gravity processing. However, ethanol yields usually decrease as biomass loadings increase due to poor mass transfer and accumulated inhibitors [18,19]. Advanced bioreactor capable of handling high solids loading is needed to ensure sufficient mixing of substrate and enzymes, low energy input and low stress to enzymes and yeast cells [20].

Enzymatic saccharification of treated biomass conducted separately from the fermentation step is referred as separate hydrolysis and fermentation (SHF), while enzymatic saccharification of cellulose performed in the presence of the fermentative microorganism is referred as simultaneous saccharification and fermentation (SSF) [8]. SSF is superior to SHF in terms of reduced amount of reactors and low risk of contamination, however, yeast recycling is very difficult when using SSF, in addition, the optimal temperatures for enzymes (50 °C) and yeasts (30 °C) are different, which indicates the conditions used in SSF cannot be optimal for both enzymes and yeast [21]. We proposed a new design of modified SSF (mSSF) with focus on enhancing ethanol concentration as a part of the whole biorefinery process are shown in Fig. 1. Details of mSSF scheme are also shown in Fig. 1. Treated biomass is first subjected to SHF in one large-volume fermenter at the optimal conditions for enzymes and then the saccharified liquor (low-concentrated sugars) is decanted to small-volume fermenters with the addition of another set of treated biomass to perform SSF. SSF as a traditional method to produce ethanol will be conducted and compared to our proposed design [22].

In the proposed biorefinery concept, hemicellulose component is hydrolyzed by hot water and used to generate furfural, which has been successfully produced in a commercial scale [23,24], thus, conversion of hemicellulose to furfural is not the focus of this study. Lignin extracted through organosolv treatment exhibits high purity and more active functional groups [16]. Soy protein adhesives (SPA) have shown great potentials to replace petroleum-derived phenol-formaldehyde or urea-formaldehyde resins commonly used for wood adhesives, while water resistance of SPA can’t compete with phenol formaldehyde and isocyanate-based adhesives for exterior applications [25,26]. In contrast, lignin has an aromatic and cross-linked structure and can react with soy protein to form protein-lignin polymer which improves the wet adhesive strength due to the hydrophobic property of lignin [27–29]. As a part of the whole biorefinery process, solvent-extracted lignin will be reacted with soy protein to form protein-lignin polymers as bio-adhesive and the adhesive performance of protein-lignin adhesives will be evaluated, particularly the wet strength as compared to protein only and commercial Kraft lignin in separate subsequent studies.

To the best of our knowledge, we are the first to propose this novel integrated process (mSSF) to enhance ethanol concentration without sacrificing ethanol yield at the time of writing. Thus, for this study, we aim to optimize hydrothermal and organosolv pretreatment conditions for achieving the highest ethanol yield and test the integrated process for enhancing ethanol concentration.

2. Materials and methods

2.1. Materials

Switchgrass was harvested at the Kansas State University Research farm (Manhattan, KS). After grinding into < 1 mm particle size with a cutting mill (SM 2000, Retsch Inc., Newton, PA, USA), the switchgrass samples was sealed in a plastic bag and stored at room temperature. The chemical composition of switchgrass was determined according to the National Renewable Energy Laboratory (NREL) procedure as shown in Table 1 [30]. In the NREL procedure, switchgrass was first subjected to warm water and ethanol extraction, and then samples were treated with sulfuric acid (72%) at 30 °C for 60 min and hydrolyzed by dilute acid (4%) at 121 °C for another 60 min. After acid hydrolysis, carbohydrates including cellulose and hemicellulose were converted to monosaccharide, which was measured by high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) equipped with an RCM monosaccharide column (300 × 7.8 mm) (Phenomenex, Torrance, CA) and a refractive index detector (RID10A, Shimadzu, Kyoto, Japan). The mobile phase was 0.6 mL $\min^{-1}$ of double-distilled water, and the oven temperature was 80 °C. Lignin consists of acid insoluble and acid soluble lignin. Acid insoluble lignin was weighed from the solid after oven heating overnight at 105 °C (the weight of acid insoluble lignin and ash) and then at 575 °C for at least 6 h to measure the ash content. All chemicals used for this research were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Hydrothermal pretreatment

The primary objective of pretreatment is to open the recalcitrant structure and increase enzymatic accessibility to plant cell wall surfaces. Hydrothermal pretreatment was carried out in a Parr reactor (Parr Instrument Co., Moline, IL). The stainless steel reactor with the