Applied Energy 193 (2017) 210–219

Contents lists available at ScienceDirect

Applied Energy

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

Biofuel production from birch wood by combining high solid loading simultaneous saccharification and fermentation and anaerobic digestion

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HIGHLIGHTS

- A combined process was developed in order to gain high-energy yields.
- Detoxification with reducing agents improved fermentation performance.
- Fed batch SSF was effective for enhancing ethanol titer.

ARTICLE INFO

Article history:
Received 23 November 2016
Received in revised form 28 January 2017
Accepted 14 February 2017

Keywords:
Detoxification
Reducing agent
Fermentation
Biomethane
Microbial community

ABSTRACT

Inhibitors generated during pretreatment of lignocellulosic biomass may affect the subsequent biochemical conversion to biofuels. In the present study, we tested 6 different reducing agents for their ability to detoxify steam-exploded birch used for ethanol production in a simultaneous saccharification and fermentation process. Cysteine, which was the most efficient detoxifying agent, increased both ethanol productivity and ethanol yield from 0.10 (non-detoxified) to 0.91 g/L/h and from 0.17 (non-detoxified) to 0.46 g/g, respectively. Gradual fed-batch feeding mode with a final total solid loading of 35% (w/w) resulted in an ethanol titer of 53.2 g/L within 72 h and a final ethanol concentration of 83.2 g/L after prolonged incubation. Moreover, residual waste (stillage) remaining after bioethanol production was subsequently used for biogas production to make the process more economically feasible. The methane yield from the stillage was 188.1 mL/g volatile solids (VS). The microbial community at the end of the biomethane process was characterized by 16S rRNA analysis. The phyla *Firmicutes* and *Bacteroidetes* were dominant members of the bacterial community, whereas the archaeal communities were dominated by methanogenic Euryarchaeota belonging to the families *Methanobacteriaceae* and *Methanosaetaceae*.

1. Introduction

Biofuels have emerged as a renewable alternative to fossil fuels and have the potential to generate about one quarter of the world's energy need by 2035 [1]. Today the annual production of biofuels comprises about 24 billion liters of biodiesel and 89 billion liters of bioethanol globally [2]. Current biofuels are mainly produced from either oil plants or sugar and starch containing crops, representing so-called 1st generation biofuels. The main production of 1st generation biofuels using corn and sugar cane takes place in the US and Brazil, respectively. The development of 2nd generation biofuels based on lignocellulosic biomass such as residues from forestry and agriculture is of high interest since such feedstocks are abundant, cheap and do not compete with food applications [3]. Thus, the production of bioethanol and biomethane from lignocellulosic biomass is a sustainable alternative to gasoline or natural gas. In this context, the production of multiple types of biofuels and energy products from a commercial biorefinery represents a compelling alternative to petroleum to maximize the energy value of available biomass resources.

Lignocellulose consists mainly of three polymeric components namely the polysaccharides cellulose and hemicellulose and the aromatic non-polysaccharide lignin. Due to its rigid and recalcitrant structure, the biochemical conversion of lignocellulose to fermentable sugars is challenging. Prior to fermentation of the sugars, pretreatment and subsequent hydrolysis of the lignocellulosic biomass are key processes. Pretreatment is necessary to make the biomass more accessible to enzymes in order to ensure efficient saccharification. Different pretreatment methods have been developed including acid treatment, alkali treatment, ammonia fiber expansion, ball mill, liquid hot water treatment, microwave irradiation, steam explosion and organosolv treatment [4,5].

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http://dx.doi.org/10.1016/j.apenergy.2017.02.042
0306-2619/

Thermal based pretreatments such as steam explosion have turned out to be one of the simplest, most efficient and eco-friendly methods [6,7]. However, a major drawback is the thermal associated formation of compounds such as phenols, furan aldehydes, aliphatic acids and organic acids, which may inhibit the saccharification and fermentation processes [8,9]. Thus, several methods such as alkaline, biological, sulfonation, ion exchange, overliming and treatments with reducing agents have been investigated to reduce the amount of inhibitors in hydrolysates through detoxification. Washing of the pretreated biomass has been applied to reduce the amount of these inhibitory compounds, resulting in less inhibition. However, washing will dilute the pretreated biomass, and thus require subsequent water removal. Additionally, washing will lead to loss of soluble sugars. These factors will make the process more costly and could make the biofuel process economically unfeasible [10,11]. It has been shown that chemical modification of inhibitor compounds by reaction with reducing agents generate compounds being less harmful to the fermenting microbes and thereby improving the fermentability of lignocellulosic hydrolysates [8,12]. A drastic improvement in fermentability can be achieved by using relatively low concentrations of reducing agents [13,14]. In contrast to washing, the use of reducing agents do not require additional processing steps since the chemicals can be added directly to the fermentation vessel. Additionally, treatment with reducing agents may also decreases problems with inhibition of enzymatic hydrolysis [8,12]. Reducing agents are used in the pulp and paper industry and the textile industry for reductive bleaching and dyeing, respectively. However, the use of reducing agents also adds cost to the process, and what kind of detoxification that is most economical has to be evaluated for each specific case.

Simultaneous saccharification and fermentation (SSF) which are carried out in the same vessel, reduces the capital and operational costs. SSF processes minimize the inhibition effect of cellobiose and glucose on enzymatic hydrolysis through continuous utilization of hydrolyzed sugars by the fermenting microbes. By means of a fed-batch SSF, high final ethanol titers can be achieved. Fed-batch SSF has been shown to reduce the viscosity of the slurry at high solid loadings and minimize the negative effects of inhibitors present in lignocellulosic hydrolysates [10,15]. Yeast strains such as *Saccharomyces cerevisiae* is well studied and a suited microorganism for industrial ethanol production from lignocellulosic biomass. However, pentose sugars would not be efficiently fermented by *S. cerevisiae* and will remain in the biomass residue known as stillage. In order to improve the economy of such processes, additional conversion of residual sugars to ethanol or biochemicals is necessary. Utilization of anaerobic digestion for combined bioethanol and biogas production is also a promising and feasible strategy.

In the present study, we tested different reducing agents for their ability to detoxify steam exploded birch used for the production of ethanol with the yeast *S. cerevisiae* through a SSF process. Potential inhibitor compounds were quantified in non-detoxified as well as detoxified lignocellulosic biomass. A fed-batch SSF process with high solids loading was developed for efficient biomass conversion into high concentrations of ethanol. Moreover, the remaining stillage was subsequently subjected to biogas production to make the process more economically feasible. In addition, we characterized the microbial community at the end of the biomethane process.

2. Materials and methods

2.1. Chemicals and enzymes

The protein content of the enzyme cocktail (Cellic Ctec2, Novozymes) was 64.1 mg mL^{-1}, as determined by the BioRad protein assay (Bio-Rad, Hercules, CA, USA) using bovine serum albumin (BSA) as standard. Reducing agents were purchased from Alfa Aesar and Sigma-Aldrich (St. Louis, MO). Glucose, mannose, galactose, xylose and arabinose were obtained from Fluka (Milwaukee, WI) and Alfa Aesar (Ward Hill, MA). Acetic acid, furfural, hydroxymethylfurfural (HMF) and ethanol were purchased from Alfa Aesar (Fair Lawn, NJ). All the reducing agents (gallic acid, dithiothreitol (DTT), cysteine, glutathione, sodium sulfide and sodium sulfite) were purchased from Sigma-Aldrich (St. Louis, MO). All chemical reagents were of chromatographic grade.

2.2. Experimental setup

The study was carried out according to flow diagram shown in Fig. 1.

2.3. Raw material and pretreatment

Stem wood of birch without bark were shredded (20–30 mm chips) and dried using a drum dryer, then milled to pass a sieve of 6 mm (SM2000, Retsch, Haan, Germany), and stored at room temperature. The dry matter content (DM) of the milled materials were 90%. The milled birch wood was pretreated by steam explosion (Cambi AS Asker, Norway) at 210 °C with a residence time of 10 min as described previously [16].

2.4. Characterization of raw and pretreated birch

The total solids, structural carbohydrate and lignin contents of the raw and pretreated biomasses were analyzed using standard laboratory analytical procedures (LAP) developed by the National Renewable Energy Laboratory (NREL) [17], as shown in Table 1. The ash content of the solid fraction was determined by incineration of dried samples at 550 °C for 3 h.

In order to determine structural modifications caused by steam explosion on the birch, structural and functional group modifications were investigated. FT-IR spectra were obtained on a Nicolet iS50 FT-IR spectrophotometer (Thermo Scientific, USA). The sample was deposited on the surface of the glass disc and measured against pre-established background and the spectrum was recorded in the range of 4000–500 cm^{-1}. Scanning electron microscopy (SEM) imaging was carried out using a Zeiss EVO 50VP (Cambridge, UK) scanning electron microscope. All samples were dried at 105 °C and coated with gold using a 550X Sputter Coating device. The Zeiss EVO 50VP was operated at an acceleration voltage of 20 kV. X-ray diffractometer (PANalyticalX'pert Pro MPD) was used to measure the crystallinity of the samples with Cu Ka radiation at 40 kV and 300 mA. The crystallinity index (CrI) was calculated by the following equation (Eq. (1)).

$$\mathrm{CrI} = (I_{002} - I_{am}) \times 100/I_{002} \quad (1)$$

where I_{002} was the intensity of the 002 peak at $2\theta = 22.5°$ and I_{am} was the intensity of $2\theta = 18.7°$.

2.5. Media and pre-cultivation of yeast

The *S. cerevisiae* strain Thermosacc® dry (Lallemand Inc., Canada) was used in all fermentation experiments. It was precultured in CBS medium with 20 g/l of glucose as carbon source for 24 h at 35 °C. The cells were then harvested and the pellet was re-suspended in a sterile solution of 0.9% NaCl to yield a yeast suspension of 15 g/L.