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Production of fermentable sugars and polyhydroxybutyrate from hybrid poplar: Response surface model optimization of a hot-water pretreatment and subsequent enzymatic hydrolysis

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ABSTRACT

In this study, the target was the generation of fermentable monosaccharides from hardwoods that can be converted into value-added chemicals, such as polyhydroxybutyrates (PHB). Poplar flour was subjected to a hot-water pretreatment. The pretreatment conditions were optimized using a response surface methodology (RSM) on a 2^3 full central composites design by varying temperature, reaction time and solid loading. The optimal pretreatment condition for producing sugars was 200 °C, 22 min and 20% solid loading. After pretreatment the solid residue was treated with a commercial cellulase/xylanase preparation and released sugars quantified. The total sugars yield was applied as response variable to the RSM. A maximum yield of 96% sugars was obtained. Potential fermentation inhibitors were also detected at low concentrations (acetic acid, furfural, hydroxymethylfurfural). A preliminary trial using wood hydrolysates in a fed-batch bioreactor using a mixed microbial consortia afforded PHB (14% on biomass basis) and maximum PHB productivity of 0.18 g/L/h.

1. Introduction

In the United States Pacific Northwest (PNW), hybrid poplar has been cultivated as an energy crop and gone from the research and developmental stage into a commercial enterprise occupying about 25,000 ha with a biomass growth rate up to 20×10^3 kg/ha/y (500,000 tonnes/y) on 6–8 year rotations [1]. A significant amount of hybrid poplar saw mill residues is available (e.g. the GreenWood Resources mill can process 190,000 m^3/y [2]) and can be converted into biofuels and/or biomaterials and this would benefit the nation's energy security and economy by displacing imported petroleum [3]. The technique for conversion of lignocellulosic biomass to fuel ethanol has been well developed. Cellulosic ethanol, however, has economic barriers to overcome to be economically competitive [4,5]. Therefore, upgrading cellulosic biomass, after bioconversion, to higher value products such as PHB (polyhydroxybutyrate, a biodegradable polymer produced intracellularly by a wide variety of microbes) would gain better

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commercial value compared to cellulosic ethanol. The cost of the carbon substrate reportedly contributes more than 40% of the production cost of PHB [6,7]. So using inexpensive renewable energy crop feedstocks (e.g. hybrid poplar) could be a tremendous advantage to the economics of PHB production [3].

Hybrid poplar is a fast growing hardwood with low lignin (~20%) and high carbohydrate (cellulose plus hemicellulose) contents and has been highlighted as a good biomass resource for chemical production [8]. Cellulose is present in poplar (~50%) and is a semi-crystalline polymer comprised of D-glucose units linked by β–O–4 linkages [9]. The major proportion of cellulose exists in the crystalline form. However, cellulose is more susceptible to degradation in its amorphous form [10]. Thus, breaking down cellulose crystalline structure to make it more accessible to cellulase enzymes usually requires pretreatment with heat, long reaction time and addition of catalysts [8]. Xylose is the main constituent of hardwood hemicellulose, as acetyl-4-O-methylglucuronoxylan [9]. Recently, studies showed that xylose can be obtained via a pretreatment process using dilute sulfuric acid [11].

Extractives and solubilized lignin from poplar may act as fermentation inhibitors, so that a detoxification step such as over-liming, ion exchange and extraction is necessary [10]. A pretreatment process can not only depolymerize lignin, but also remove some lignin [12]. Enzymatic hydrolysis is the most common method for converting woody biomass to sugars. Compared with acid hydrolysis, enzymatic hydrolysis yields limited amount or even no fermentation inhibitors such as furfural and it does not need neutralization and detoxification [4]. The only disadvantage of enzymatic hydrolysis is longer reaction time required for releasing sugars. However, enzymatic hydrolysis is a better choice if further fermentation or bioconversions are required to produce value-added chemicals [4].

Hot-water pretreatment with controlled pH has been shown to improve enzymatic digestibility of lignocellulosic biomass. Acetic acid and other organic acids are released from the hemicelluloses, which help autocatalyze hemicellulose hydrolysis and disrupt cellulose and lignin structure. The pH of the pretreatment liquor needs to be kept between 4 and 7 to minimize decomposition of sugars [4]. Furfural and 5-hydroxymethylfurfural (5-HMF) are the two major sugar degradation products from hot-water pretreatment. When the concentration of furfural is greater than 2 g/L, it is significantly toxic for fermentation microbes [4]. The present combination of acetate and sugar degradation products would also give strong inhibition effects in normal fermentation processes [13]. However, PHB can be biosynthesized from acetate by polyhydroxyalkanoate (PHA) producing bacteria [14].

Research on PHA has been increasing rapidly in recent decades due to the rise of petroleum prices as well as environmental concerns related to plastic pollution. PHB is the most studied homopolymer in the PHA family which can be used as an alternative for polypropylene [15]. PHB can be produced from waste or renewable resources with the advantage of its biodegradability and biocompatibility. Lee [15] reviewed that using hemicelluloses hydrolysates for the production of PHB would cost $0.34/kg PHB to yield 0.20 g PHB/g substrate, which was much cheaper than using pure acetate ($1.56/kg PHB) as a substrate. Several reports are available for the production of PHB using plant hydrolysates and pure microbial strains, including bagasse hydrolysates by *Ralstonia eutropha* [16], water hyacinth hydrolysates by *Cupriavidus necator* [17] and hydrolyzed rice straw by *Bacillus firmus* [18]. To the best of our knowledge, there is no report on using wood hydrolysates and mixed cultures for the production of PHB.

For the purpose of scale-up or industrial production, determining the optimal pretreatment conditions by using statistical approach is important. The experimental design works for variety of species, chemical reagents, temperature and reactor features. The aim of this study was to find optimal conditions to obtain total sugars (mainly glucose and xylose) via a hot-water pretreatment followed by enzymatic hydrolysis. A response surface methodology (RSM) was chosen to determine the optimal pretreatment conditions for total sugars yield in the hydrolysates. Temperature, reaction time and solid loading were the three variables tested in this design. Proof of concept PHB synthesis from wood hydrolysates was also investigated.

2. Materials and methods

2.1. Raw materials

Hybrid poplar (Potlatch Corp., ID, USA) was milled to <1 mm using a Wiley mill (Thomas Scientific, NJ, USA), dried and stored in sealed plastic bags (moisture content of 5.4%). Chemical composition analysis (ash, extractives, Klason lignin and neutral carbohydrate composition) of raw and residual materials was determined using procedures described by Jain et al. [19]. Commercial PHB was obtained from Sigma–Aldrich.

2.2. Hot-water pretreatment

Pretreatment was conducted in a 75 mL pressure reactor, (Model 4740, Parr Instrument Co., IL, USA) connected to a temperature controlled block heater built in-house. Wood meal (5.0 g, oven dry basis) was introduced to the reactor to which water was added giving a solid loading range from 13.2 to 46.8% and sealed. The reaction temperature ranged from 160 to 210 °C. A 2^3 full factorial design for temperature, time and solid loading was conducted (Table 1). An additional temperature probe was used for monitoring the temperature of the reactor vessel. The reaction vessel took 5, 10 and 15 min to reach 170, 185 and 200 °C, respectively. Timing was started when the vessel reached the desired temperature. After pretreatment, the vessel was placed in an ice-water bath to quench the reaction. The pretreated samples then were washed with hot-water (Milli-Q, 150 mL, 90 °C) [4,5] to extract out the sugars and acids generated, centrifuged (10,000 rpm) to separate the solid and liquid fractions. The liquid fraction was named pre-liquor (PL) and pH was measured. After sampling 1 mL from the PL for inhibitor compounds and 5 mL for sugars determination, the PL together with the solid residue collected were used for enzymatic hydrolysis trials.