Biomass and Bioenergy 105 (2017) 402–410

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: http://www.elsevier.com/locate/biombioe

Research paper

Development and evaluation of poplar hemicellulose prehydrolysate upstream processes for the enhanced fermentative production of xylitol

Sai Swaroop Dalli [a], Misha Patel [b], Sudip K. Rakshit [a, b, *]

[a] Department of Chemistry and Material Sciences, Lakehead University, Thunder Bay, Ontario P7B 5E1, Canada
[b] Department of Chemical Engineering, Lakehead University, Thunder Bay, Ontario P7B 5E1, Canada

ARTICLE INFO

Article history:
Received 1 April 2016
Received in revised form
25 July 2017
Accepted 3 August 2017

Keywords:
Hemicellulose prehydrolysate
Acid hydrolysis
Detoxification
Fermentation
Xylitol
Candida guilliermondii

ABSTRACT

A major bottleneck to the utilization of hemicellulose streams obtained on pretreatment of lignocellulosic residues is the toxins formed during the hydrolysis of the pentose polymers. Low acid (1.75% (w/w) and 120 °C) hydrolysis yielded a 3-fold increase in xylose concentration with low byproduct formation. Efficient detoxification method using vacuum evaporation and solvent extraction techniques removed the major inhibitors, acetic acid (80%) and furfural (98%) with little loss of sugars. The effectiveness of hydrolysis and subsequent detoxification was ascertained by the fermentation of detoxified hydrolysate with *Candida guilliermondii.* The xylitol concentration and yield obtained was 28.78 g L^{-1} and 0.59 g g^{-1} respectively, in 37 h. The productivity of 0.81 g L^{-1} h^{-1}, the highest reported from wood prehydrolysates, demonstrates that the upstream processing methods are effective. Wood prehydrolysates treated in this way could be used to produce fermentative products like xylitol and other value added products economically and beneficially.

1. Introduction

The use of wood based resources for the production of useful chemicals instead of fossil based resources will help mitigate climate change. Lignocellulosic biomass has the potential to serve as a renewable feedstock to produce value-added chemicals and precursor platform chemicals. The utilization of cellulose in various industries like pulp and paper, textiles (rayon, cellophane), pharmaceuticals (fillers in drugs), fuel (ethanol, butanol), and many others have been studied extensively [1]. However, in order to make biorefining of lignocellulosics economical, the other components of wood such as hemicelluloses and lignin need to be integrated and utilized to produce value added products. We have recently presented the importance of such integration in biorefining processes, in order to compete successfully with the fossil fuel industry [2].

There is considerable potential for use of the hemicellulose stream which contains a variety of pentose sugars to produce various useful products like platform chemicals, fuels and biologically active compounds [3]. Hemicelluloses from corn cob, sugarcane bagasse, wheat straw, switch grass, spent grains and barley have been used as carbon sources to produce some products like xylitol, a non-fermentable sugar alcohol, through microbial fermentation [4]. Since xylose is the major monomeric sugar in hemicellulose, it is relatively easy to produce xylitol, which has good commercial value with an estimated annual market of US $537 million, from this sugar [5].

Current industrial production of xylitol involves catalytic hydrogenation of xylose at high temperatures (80–140 °C) and pressure (50 atm). Some examples of catalysts used in this process are Nickel (Ni) and Ruthenium (Ru) [6,7]. The major drawback of this process is in upstream processing, as the substrate (xylose) needs to be in pure form requiring detoxification and removal of impurities along with other sugars. This purification process of xylose increases the cost of production of xylitol [4]. Microbial fermentation of xylose to produce xylitol does not need such high substrate purity and thus gained attention for its economical and ecofriendly nature. Therefore, the hemicellulose prehydrolysates of different types of biomass can be used for the production of xylitol using various microbes in the fermentation process. Though many

* Corresponding author. Department of Chemical Engineering, Lakehead University, Thunder Bay, Ontario P7B 5E1, Canada.
E-mail address: srakshit@lakeheadu.ca (S.K. Rakshit).

http://dx.doi.org/10.1016/j.biombioe.2017.08.001
0961-9534/

researchers report the use of various microbes that produce xylitol [8,9], the microbial route has not been commercialized yet. This is because these microbial methods are not able to compete with the current industrial production of xylitol through chemical processes in the production and commercial aspects. As the use of such renewable resources have many advantages, considerable efforts on producing xylitol from hemicelluloses by biological routes is being carried out, in order to develop an efficient method that can be commercialized.

Renewable resources such as poplar wood prehydrolysate, byproduct stream rich in hemicellulosic sugars mainly obtained from the pretreatment process of pulp and paper industries, can also be used to produce xylitol. There are many pretreatment techniques reported in literature about the production of hemicellulose prehydrolysates from various renewable lignocellulosic resources [10]. Xylose sugar present in all these prehydrolysate streams exist as poly and oligo-saccharides, and need to be hydrolysed to release the xylose monomers. Sulfuric acid is commonly used catalyst in acid hydrolysis of prehydrolysate. However, the usage of high acid concentrations is not environmentally acceptable and also produce large amounts of inhibitory by-products. In the first part of this study, an efficient acid hydrolysis method was optimized to produce high concentration of fermentable monomers using low acid concentration. During acid hydrolysis, the formation of some inhibitors like acetic acid, furfural and other phenolics is unavoidable. These substances limit microbial growth and production of valuable metabolites [10]. Attempts have been made to remove these inhibitors through detoxification methods such as ion exchange chromatography [11], solvent extraction, evaporation [12], membrane distillation [13] and adsorption techniques [14]. In the second part of this study, a detoxification method using vacuum evaporation and solvent extraction was attempted to reduce the concentration of inhibitors to the levels that did not result in inhibition of the fermentation by microorganisms. The effectiveness of the hydrolysis and detoxification process was demonstrated by the enhanced production of xylitol by fermentation using *Candida guilliermondii* and the detoxified hydrolysate as substrate. This paper presents an efficient process to utilize the poplar hemicellulose prehydrolysate stream to produce xylitol in high yields and productivity. The effectiveness of the low acid hydrolysis and subsequent efficient detoxification on xylitol production indicates that these monomeric sugar streams could be used for other fermentation products as well.

2. Materials and methods

2.1. Prehydrolysate substrate

2.1.1. Composition analysis

Hemicellulose prehydrolysate liquor (PHL) of poplar wood was provided by Green Field Ethanol Inc. (Chatham, ON, Canada). The crude liquid PHL was obtained using a two stage pretreatment process including a steam percolation system [15]. The poplar wood chips were passed through twin extruders in presence of steam and the three major components (cellulose, hemicellulose, lignin) of lignocellulose were separated in two different streams viz. solid (cellulose and lignin) and aqueous streams (hemicelluloses). The latter crude PHL stream was used as starting material in our experiments.

2.1.2. Determination of total solids

The total dissolved and undissolved solids were determined by following the standard NREL procedure recommended by the Department of Energy (US) using a conventional oven method [16]. 1 mL of PHL was weighed in a pre-heated aluminum dish and the sample was oven dried for 6 h at 105 °C. The weight of the sample was then recorded and the total solid content was determined by using the following formula:

$$\%\,Total\ Solids = \frac{weight\ of\ dry\ pan\ plus\ dry\ sample - weight\ of\ dry\ pan}{weight\ of\ liquid\ sample} \times 100$$

For the determination of total dissolved solids, another sample of PHL was centrifuged at 4400 rpm for 30 min and then filtered through a 0.2 μm filter (Millex® filter unit) to remove the minute solid particles present in the PHL and then the supernatant was dried overnight in oven at 105 °C. The filtered PHL was stored at 4 °C for further experiments.

2.2. Acid hydrolysis

Crude PHL was hydrolysed with various concentrations of sulfuric acid ranging from 1.0 to 2.5% (w/w) at 120 °C for 2 h to determine the lowest possible concentration for higher polysaccharide conversion and simultaneous low inhibitor formation. The hydrolysate was then centrifuged and vacuum filtered to remove the traces of black solid particles called ‘humins’ formed due to the reaction between furfural and xylose at high temperatures [17]. The resulted hydrolysate was then neutralized with 3N NaOH, before being used for further experiments.

2.3. Detoxification of hydrolysate

Acid hydrolysis of PHL results in rise of monomeric xylose amounts along with organic acids (e.g. acetic acid), furfural and phenolic compounds. It is necessary to remove these byproducts which act as inhibitors in the microbial fermentation of the hydrolysate. In this study, the detoxification methods of ion exchange resin technique and a method involving vacuum evaporation and solvent extraction which was developed in our laboratory were compared.

2.3.1. Ion exchange resin based detoxification

In this study, Amberlite IRA 400 (Cl^-) resin was initially used to remove inhibitors. In order to regenerate the chloride ions on the resin, it was treated with 1 M HCl and left soaked in it overnight [18]. A column (50 × 3 cm) was packed with the HCl treated resin and washed with water to remove free chloride ions unassociated with the resin. The hydrolysate was then added to the column and allowed to react for 30 min. The collected samples were subsequently analyzed for sugars, acetic acid and furfural using an HPLC. The column was washed with water and the resin was regenerated with 1 M HCl and stored for further use.

2.3.2. A unified detoxification technique with vacuum evaporation and solvent extraction

Vacuum evaporation using a Buchi rotovap has been done to