Available at www.sciencedirect.com

ScienceDirect

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

Ethanol fermentation from hydrolysed hot-water wood extracts by pentose fermenting yeasts

*Alan M. Shupe, Shijie Liu**

Department of Paper and Bioprocess Engineering, SUNY ESF, 1 Forestry Drive, Syracuse, NY 13210, USA

ARTICLE INFO

Article history:
Received 19 March 2010
Received in revised form
11 April 2011
Accepted 17 August 2011
Available online 9 September 2011

Keywords:
Bio-refinery
Ethanol fermentation
Hemicellulosic hydrolysate
Pichia stipitis
Yeast

ABSTRACT

Two strains of each yeast *Candida shehatae* and *Pichia stipitis* were used to ferment sugar maple wood extracts to ethanol. Of these four strains the most promising was the *P. stipitis* NRRL Y-11543 strain. This strain produced a maximum of 13.51 g L^{-1} ethanol from wood extracts containing 5- and 6- carbon sugars. The main carbon source for fermentation in these extracts was xylose monosaccharide at 36.7 g L^{-1} with other sugars concentrations ranging from 1.04 to 2.08 g L^{-1}. Through dilute acid hydrolysis the oligomers in the extracts were converted to xylose and other monosaccharides. The initial sugar maple dilute acid hydrolysate used in this study has a xylose concentration of 86.8 g L^{-1}. Nano-membrane filtration removed the majority of the acetates, formates, furfurals and methanol, which were liberated during hydrolysis; however the sulfates that were introduced remained. High levels of sulphate and aromatic compounds in the hydrolysate were inhibitory to the fermentation. *P. stipitis* did not produce much ethanol from this hydrolysate without dilution with distilled water. The highest ethanol concentration reached was 18.4 g L^{-1} after a 1:1 dilution with distilled water. Further improvements in the hydrolysis of hot water extracts also resulted in higher sugars concentrations. Reducing the amount of sulfates as well as better control of aeration rate by carrying out the fermentation in a benchtop fermentor resulted in higher ethanol concentrations of 35.4 g L^{-1} in only 76 h as opposed to 7 days in shake flasks.

1. Introduction

With depleting fossil energy sources and the environmental impacts of rising atmospheric carbon levels, renewable energy sources are attracting significant attention. The bio-refinery is a conceptual process to convert plant biomass to chemicals and energy [1]. While there are many pathways, incremental deconstruction of woody biomass and biochemical conversion seem to be very promising. The lignocellulosic bio-refinery is an effective process for the utilization of biomass and will play an important role in the future of both the chemical and energy industries [2].

Large quantities of hemicelluloses in woody biomass need to be converted to biofuels in addition to the cellulose in order for a wood based bio-refinery to be economically feasible. Hemicellulose can be depolymerized (or hydrolysed) to produce 5- and 6- carbon sugars, as well as acetic acid, all of which are platform chemicals. Six carbon sugars are the substrate of choice for most microorganisms. Therefore, a major bottleneck in a wood based bio-refinery is the biological conversion of five carbon sugars. Microorganisms which utilize five carbon sugars to produce ethanol or other biofuels are vital for a successful bio-refinery.

* *Corresponding author*. Tel.: +1 315 470 6885; fax: +1 315 470 6945.
E-mail addresses: amshupe@syr.edu (A.M. Shupe), sliu@esf.edu (S. Liu).
0961-9534/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biombioe.2011.08.010

The conventional process for producing ethanol and other liquid fuels from lignocellulosic biomass is composed of four main steps as shown in Fig. 1 [3]. The first is pretreatment where the lignocellulosic matrix is broken down. The second step is hydrolysis, which can be performed either by enzymes or acid. The third step is the fermentation where the sugars are converted to fuels by microorganisms, usually by yeast strains. The final step is the distillation step which consists of separation and purification of the fermentation broth. In this schematic, hemicellulose becomes largely a waste material. At SUNY ESF, the pretreatment step is replaced by a hot-water extraction process where hemicellulose is extracted then hydrolyzed. A more detailed description can be found in Amidon et al. [1] and Amidon & Liu [4].

For lignocellulosic biomass to become the main feedstock for the chemical and energy industries and replace fossil fuels effective utilization of all components is required [2]. Efficient use of pentoses, in addition to the hexoses, which are the focus of a cellulosic ethanol plant, will greatly increase the economic feasibility of the process. It was demonstrated that for the process of wood to ethanol, complete use of xylose can reduce the price of ethanol [5]. During hot-water extraction process, pentose and hexose sugars can degrade to furfural and hydroxymethylfurfural (5-HMF). Both are toxic compounds that can inhibit cell activity and affect the specific growth rate. Delgenes et al. [6] showed that at low concentrations of furfural and 5-HMF (under 1 g L^{-1}) *Pichia stipitis* can still achieve relatively high ethanol production. Nanomembrane filtration can effectively improve the fermentability of the hydrolysate by reducing the concentrations of furfural, 5-HMF and acetic acid to under 1 g L^{-1}.

Yeast and bacteria are essential for the fermentation of xylose. The thicker cell walls, better growth at lower pH, less stringent nutritional requirements and greater resistance to contamination give yeasts the advantage in the commercial production of ethanol [7]. Furfural and 5-HMF, though strong inhibitors to fermentation and growth are able to be detoxified by yeasts by reducing them to furan methanol and furan-2,5-diethanol [8]. There are several natural xylose fermenting yeasts, such as *P. stipitis*, *Candida shehatae*, and *Pachysolen tannophilus* which may be of interest in the fermentation of hemicellulosic materials. Of these natural xylose fermenting yeasts *P. stipitis* and *C. shehatae* have been described as the best D-xylose fermenting yeasts [9]. There are some yeasts that produce xylan-degrading enzymes such as *P. stipitis*, making it of great interest as only a few of the xylan hydrolysing yeasts are capable of fermenting xylan to ethanol [10].

In xylose fermenting microorganisms, bacteria use an isomerase pathway to channel pentoses to the central metabolism, whereas in yeast and fungi a reductase/dehydrogenase pathway is used [3]. Xylose metabolism employs pathways distinctly different from those for the utilization of glucose, in most xylose fermenting yeasts xylose metabolism requires aerobic conditions at which cellular respiration is promoted. Unlike *Saccharomyces cerevisiae*, in which fermentation is induced by sensing the presence of glucose, *P. stipitis* initiates fermentation in response to oxygen limitation. Jeffries et al. [11,12] showed that the genes responsible for cycling fructose-6-phosphate (F6P) as well as those which take F6P through glycolysis were elevated by oxygen limited conditions when growing on xylose. This confirms previously reported data that the maximum specific ethanol productivity of *P. stipitis* occurred under microaerobic conditions [13].

The monosaccharides in sugar maple hemicellulosic hot water wood extracts used in this study consists primarily of xylose with minor amounts of glucose, mannose, arabinose, galactose, and rhamnose. *C. shehatae* can degrade D-xylose and D-glucose, and produce ethanol from glucose and xylose simultaneously [14]. *P. stipitis* also has the ability to ferment xylose and glucose as well as mannose and galactose, and can produce cell mass from L-arabinose [15]. The ability to utilize xylan is also present in *P. stipitis* by secreting xylanase enzymes into the medium, however all naturally occurring xylan-degrading *P. stipitis* strains produce only low levels of xylanolytic enzymes [9,16]. The objective of this study is to examine the fermentation of concentrated hot water wood extracts and hydrolysates from sugar maple by the yeasts *C. shehatae* and *P. stipitis*.

2. Materials and methods

2.1. Microorganisms and maintenance

The two strains of *P. stipitis* used were NRRL Y-11543 and NRRL Y-7124. *C. shehatae* NRRL Y-12858 and 12854 were also tested. All yeast strains were maintained at 4 °C on YM agar slants. The media used for inoculum contained (g L^{-1}): xylose, 50; yeast extract, 3; peptone 3; KH_2PO_4, 10; and $(NH_4)_2SO_4$, 4. The pH of the media was adjusted to 5.5 +/− 0.01 and autoclaved for 20 min at 121 °C. The sugar and salts were autoclaved separately then mixed together to prevent caramelization of the xylose. To prepare the inoculum four 125 mL Erlenmeyer flasks containing 50 mL of medium each, were inoculated from fresh agar slants. All flasks were then

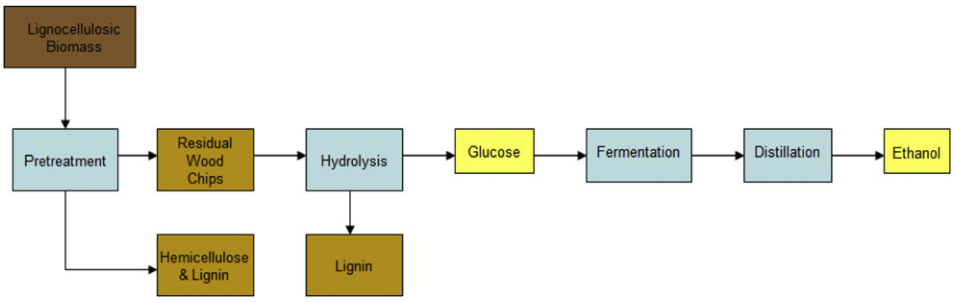

Fig. 1 – Conventional process for producing ethanol from lignocellulosic biomass.