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Butanol production from hydrothermolysis-pretreated switchgrass: Quantification of inhibitors and detoxification of hydrolyzate

Kan Liu [a], Hasan K. Atiyeh [a,*], Oscar Pardo-Planas [a], Thaddeus C. Ezeji [b], Victor Ujor [b], Jonathan C. Overton [a], Kalli Berning [c], Mark R. Wilkins [a], Ralph S. Tanner [d]

[a] Biosystems and Agricultural Engineering Department, Oklahoma State University, Stillwater, OK, USA
[b] Department of Animal Sciences, The Ohio State University, and Ohio State Agricultural Research and Development Center, Wooster, OH, USA
[c] Agricultural and Biosystems Engineering Department, North Dakota State University, Fargo, ND, USA
[d] Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, USA

HIGHLIGHTS

- pH adjustment and $CaCO_3$ addition to switchgrass hydrolyzate improved ABE production.
- Switchgrass hydrolyzate contained both furanic and phenolic inhibitors.
- Activated carbon detoxification removed detected inhibitors except cinnamaldehyde.
- Detoxification of switchgrass hydrolyzate increased butanol titer from 1 to 11 g/L.
- 17 g/L total ABE was produced with detoxified hydrolyzate.

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ABSTRACT

The present study evaluated butanol production from switchgrass based on hydrothermolysis pretreatment. The inhibitors present in the hydrolyzates were measured. Results showed poor butanol production (1 g/L) with non-detoxified hydrolyzate. However, adjusting the pH of the non-detoxified hydrolyzate to 6 and adding 4 g/L $CaCO_3$ increased butanol formation to about 6 g/L. There was about 1 g/L soluble lignin content (SLC), and various levels of furanic and phenolic compounds found in the non-detoxified hydrolyzate. Detoxification of hydrolyzates with activated carbon increased the butanol titer to 11 g/L with a total acetone, butanol and ethanol (ABE) concentration of 17 g/L. These results show the potential of butanol production from hydrothermolysis pretreated switchgrass.

1. Introduction

Butanol is a traditional bulk chemical that has comparable energy content (27 MJ/mol) to gasoline (32 MJ/mol) (Atsumi and Liao, 2008). The global butanol market in 2008 was 2.8 million tons with a market size estimated to be 5 billion US dollars (Green, 2011). Butanol can be blended with gasoline as a "drop-in" biofuel and upgraded into jet fuel or biodiesel (Simmons, 2011; Yeung and Thomson, 2013).

The production of butanol was typically performed via the acetone–butanol–ethanol (ABE) fermentation process using corn starch and molasses (Jones and Woods, 1986). However, the increase in the price of these food-based feedstocks caused a switch to petroleum as a source of butanol (Qureshi and Ezeji, 2008). Thus, sugar substrates from cheap and sustainable feedstocks for biological fermentation are required to compete with petroleum-derived butanol. Lignocellulosic biomass contains large amounts of fermentable sugars including glucose and xylose (Cheng, 2010), hence, sugars made biomass such as wood, corn fiber, corn stover, switchgrass, barley straw and wheat straw may be used for butanol production (Qureshi and Ezeji, 2008; Qureshi et al., 2010a).

Switchgrass (*Panicum virgatum* L.) is considered an energy feedstock due to its high productivity, suitability for marginal land use, and low water and nutritional requirements (McLaughlin and Adams Kszos, 2005). A previous study on butanol production from switchgrass using dilute acid pretreated switchgrass and

* Corresponding author at: Department of Biosystems and Agricultural Engineering, 214 Ag Hall, Oklahoma State University, Stillwater, OK 74078, USA. Tel.: +1 405 744 8397; fax: +1 405 744 6059.
E-mail address: hasan.atiyeh@okstate.edu (H.K. Atiyeh).

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Clostridium beijerinckii P260 resulted in poor product accumulation (1.0 g/L butanol and 1.5 g/L total ABE), due to the toxic compounds in the hydrolyzate (Qureshi et al., 2010b). In addition, detoxification of switchgrass hydrolyzate by overliming did not improve butanol production (Qureshi et al., 2010b). However, when the hydrolyzate was diluted with water (1:1 ratio) and supplemented with additional pure sugar, 9.6 g/L butanol and 14.6 g/L ABE were achieved (Qureshi et al., 2010b). Dilution of the fermentation medium and addition of pure sugars renders the process less economically feasible. Recently, another study reported the production of butanol from NaOH (1% v/w) pretreated switchgrass hydrolyzate, with titers reaching 13.0 g/L butanol, and 22.7 g/L total ABE using *Clostridium saccharobutylicum* DSM 13864, without detoxification of the hydrolyzate (Gao et al., 2014). However, addition of NaOH during pretreatment may increase process cost due to the need for chemical recycling and treatment of process and waste streams (Pang et al., 2008; Yang and Wyman, 2008). Acid pretreatment also has challenges regarding use of expensive equipment due to acid-mediated corrosion, generation of fermentation inhibitors, loss of sugars during hydrolyzate detoxification, and difficulty in the recovery and recycle of acid (Yang and Wyman, 2008). According to the Department of Energy (DOE) report on biomass conversion to ethanol, pretreatment cost was estimated to be 0.78$/gallon ethanol, which was 35% of total processing price of $2.24/gallon ethanol (DOE, 2011). The high contribution of pretreatment to the overall cost, undoubtedly, is one of the bottlenecks to the bioconversion of lignocellulosic feedstocks to biofuels. Thus, the reduction or elimination in use of chemicals during biomass pretreatment is required to reduce process cost and make it commercially feasible.

Production of ethanol from hydrothermolysis pretreated switchgrass using thermotolerant yeast strains has been previously reported (Pessani et al., 2011), however, this technology has not been employed for butanol production. The advantage of hydrothermolysis over other pretreatment technologies such as dilute acid or alkali pretreatments is the absence of a catalyst (acid or base) and lower cost of reactor due to low corrosion potential (Alvira et al., 2010). While hydrothermolysis has provided similar glucan to glucose yields compared to dilute acid or alkali pretreatments of switchgrass, one of its major drawbacks is the low xylan to xylose conversion (Tao et al., 2011). However, a technoeconomic analysis comparing different switchgrass pretreatment technologies suggested that if both oligomeric and monomeric sugars were fermented, pretreatment by hydrothermolysis would offer a lower selling price for ethanol than dilute acid or alkali pretreatments (Tao et al., 2011). This advantage of lowering cost of production would be applicable to butanol production.

Biomass hydrolyzate typically contains microbial inhibitors such as hydroxymethylfurfural (HMF) and furfural from degradation of hexose and pentose sugars during pretreatment and hydrolysis, and phenolic compounds from the degradation of lignin (Mussatto and Roberto, 2006; Zhang et al., 2012). For example, HMF and furfural concentrations above 3 g/L caused between 5% and 10% decrease in total ABE production by *Clostridium acetobutylicum* ATCC 824 (Zhang et al., 2012). It was also reported that phenolic compounds including p-coumaric acid, ferulic acid, 4-hydroxybenzoic acid, vanillic acid, syringaldehyde, and vanillin at a level of 1 g/L of each compound caused 64–74% growth inhibition of *C. beijerinckii* with no butanol production (Cho et al., 2009). Soluble lignin content (SLC), mainly phenolic compounds in the hydrolyzate, lower than 1.7 g/L is recommended for ABE fermentation (Mussatto and Roberto, 2006; Wang and Chen, 2011). High SLC level in fermentation medium can inhibit ABE fermenting strains by increasing cell membrane fluidity, causing leakage of cellular contents, disrupting the cell redox balance and causing acid crash (Ezeji et al., 2007; Ujor et al., 2014; Wang and Chen, 2011). Therefore, it is critical to quantify inhibitors in the hydrolyzate and to reduce their levels before ABE fermentation. Inhibitors in pretreated Eastern redcedar hydrolyzate resulted in poor ABE fermentation (Liu et al., 2015). However, improved ABE production was reported after the detoxification of redcedar hydrolyzate.

The present study focused on process development for butanol production from switchgrass following hydrothermolysis pretreatment, enzymatic hydrolysis, and fermentation of non-detoxified and detoxified hydrolyzates. The concentrations of inhibitors in the hydrolyzates were measured after pretreatment and during hydrolysis. To the best of our knowledge, this is the first report of butanol production from hydrothermolysis-pretreated switchgrass, inhibitor levels in the hydrolyzate, and ABE fermentation of detoxified and non-detoxified switchgrass hydrolyzate.

2. Methods

2.1. Hydrothermolysis pretreatment

Alamo switchgrass (*P. virgatum L.*) was harvested at the end of July 2012 in Maysville, OK. Switchgrass was ground using a Thomas-Wiley mill equipped with a 2 mm screen. Ground switchgrass was then pretreated by hydrothermolysis in a 1 L Parr reactor (Parr series 4520, Parr Instrument company, Moline, IL, USA) at 200 °C for 10 min and 500 rpm (Pessani et al., 2011). The dry switchgrass to DI water ratio for hydrothermolysis pretreatment was 1:10 (w/w). Finally, the pretreated switchgrass solids were washed four times with deionized (DI) water. Each wash used 500 mL DI water at 60 °C and 600 rpm agitation for 15 min. After each wash, solids and water were separated by vacuum filtration using No. 4 Whatman filter paper. Washed pretreated switchgrass solids were stored in resealable plastic bags at 4 °C until used in enzymatic hydrolysis. The compositions of pretreated and unpretreated switchgrass were analyzed using National Renewable Energy Laboratory (NREL) protocols (Hames et al., 2008; Sluiter et al., 2008).

2.2. Enzymatic hydrolysis

Accellerase 1500, generously provided by DuPont (Rochester, NY, USA), was used for enzymatic hydrolysis of switchgrass. Enzymatic hydrolysis with a total working weight of 100 g in each flask was performed at 50 °C and 250 rpm in 250 mL baffled Erlenmeyer flasks in a shaker incubator (MaxQ 4450, Thermo Scientific, Dubuque, IA, USA). Acetate buffer (50 mM) was used for hydrolysis (pH 5.5). A solid loading of 14% was chosen to achieve an initial concentration of 70 g/L glucose after enzymatic hydrolysis. Samples (2 mL) were withdrawn periodically from each flask and centrifuged at 16,000*g* for 10 min. The enzymatic hydrolysis was performed for 48 h. Enzyme loading of 50 FPU/g glucan was used as in the present study as in the conversion of redcedar to ethanol (Ramachandriya et al., 2013) and butanol (Liu et al., 2015).

2.3. Enzymatic hydrolyzate detoxification

The switchgrass hydrolyzate was collected and centrifuged at 5000*g* for 15 min at 4 °C (IEC MultiRF, Thermo Fisher, Waltham, MA, USA). Then, the hydrolyzate was centrifuged at 48,000*g* for 10 min (Avanti J-E, Beckman Coulter, Inc., Brea, CA, USA) to remove suspended fine particles and obtain a solids-free hydrolyzate. The detoxification of the switchgrass hydrolyzate was performed by loading 10% (w/v) powdered activated carbon (Hydrodarco B, CABOT, Norit American, Inc., Marshall, Texas, USA) or rod shape carbon (AP4-60, Calgon Carbon Corporation, Pittsburgh, PA, USA)