

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



The Effect of Washing Dilute Acid Pretreated Poplar Biomass on Ethanol Yields

Noaa Frederick, Ningning Zhang, Angele Djioleu, Xumeng Ge, Jianfeng Xu and Danielle Julie Carrier

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/56129>

1. Introduction

Ethanol sold in the US or Brazil is produced from feedstocks that contain starch or sucrose: corn starch in the US and sugar cane juice in Brazil. The use of these readily available fermentable sugar sources rouses societal discussions that are anchored on debates involving the use of food commodities for energy production (Wallington et al. 2012). From a sustainability perspective, conversion of cellulosic biomass to ethanol produces less greenhouse gases and particulate matter with a diameter less than 2.5 μm . Furthermore, the cost in dollars per liter in gas equivalent of using corn and corn stover as feedstock are 0.9 and 0.3, respectively (Hills et al. 2009). The production of fuels and biochemicals from cellulosic feedstock is desirable from both societal and environmental perspectives.

Although appealing, the deconstruction of cellulosic biomass into fermentable sugars is problematic. Cellulosic biomass conversion to industrial chemicals and fuels is performed via thermochemical, biochemical or a combination of these platforms. Unfortunately there is no clear technology winner and both conversion platforms have tradeoffs. The thermochemical platform is robust in terms of feedstock processing, but somewhat complicated in terms of the resulting product portfolio (Sharara et al. 2012). On the other hand, the biochemical platform can successfully yield industrial chemicals or fuels, but is delicate in terms of feedstock deconstruction into monomeric sugars (Lynd et al. 2008). This chapter is centered on biomass deconstruction using the biochemical platform.

In the biochemical platform, unfortunately, the deconstruction of plant cell wall into useable and fermentable carbohydrates remains challenging. Feedstock must be reduced in size, pretreated, and hydrolyzed with enzymes to produce a sugar stream that can be fermented

into targeted products (Lynd et al. 2008). The cell wall is designed by nature as an elegant interwoven hemicellulose, cellulose and lignin tapestry that maintains its integrity, resulting in wood products that can sustain daily use for hundreds of years. To release the coveted carbohydrates from plant cell walls, the tapestry must be subjected to some form of pretreatment, ensuring the exposure of sugar polymers, which can subsequently be hydrolyzed.

There are a number of available pretreatment technologies (Tao et al. 2011). However, dilute acid pretreatment, though it contains many drawbacks, is most likely to be adopted at the deployment scale due to its relatively low cost and ease of use (Sannigrahi et al. 2011). Regrettably, dilute acid pretreatment results in the production of inhibitory compounds that inhibit downstream biochemical conversion processing steps. These inhibitory compounds are formed from the degradation of hemicellulose into furfural, acetic acid and formic acid; or lignin-derived phenolic compounds, oligomers and re-polymerized furans named humins (van Dam et al. 1986). Such compounds can inhibit enzymatic hydrolysis by at least 50% (Cantarella et al. 2004). In a sense, the dilute acid-based biochemical platform is caught in a chicken and egg situation: pretreatment is essential to loosen the sugar polymer tapestry, but pretreating biomass causes the formation of inhibitory products that hinder subsequent downstream processing steps. In other words, without pretreatment, the expensive processing enzymes cannot access the complex carbohydrates to release the coveted monomeric sugars, which will be fermented into fuels or bioproducts.

To circumvent the negative effects of dilute acid pretreatment, namely the production of inhibitory products, pretreated biomass is washed prior to enzymatic hydrolysis. Successive washes remove inhibitory products, resulting in biomass amenable to subsequent enzymatic hydrolysis. At the bench scale, inhibitory compounds are removed by washing with up to 30 volumes of water (Djioleu et al. 2012). At the pilot scale, inhibitory compounds are removed from pretreated biomass by washing with at least three volumes of water (Hodge et al. 2008). Washing pretreated biomass will be difficult to replicate at the deployment scale due to the daunting amount of water that will be required. Another approach consists of enhancing our understanding of which compounds critically impede enzymatic hydrolysis, and how to minimize their generation during pretreatment.

The conversion of cellulosic biomass into fuels and biochemicals can be conducted with a range of feedstocks. Cellulosic biomass can be sourced from various streams: forestry products and residues, agricultural byproducts, dedicated energy crops, food processing and municipal solid wastes. In particular, wood energy crops, such as hybrid poplars (*Populus deltoides*), are hardwoods that can find use as biorefinery feedstock. *P. deltoides* is being increasingly planted and managed in the United States as short-rotation plantations for timber, pulp and renewable energy (Studer et al. 2011). The use of *P. deltoides* as a feedstock and its response to various pretreatment technologies combined with enzymatic hydrolysis was reported by the Consortium for Applied Fundamentals and Innovation (CAFI), where the technologies were compared with identical characterized poplar feedstock (Kim et al. 2009). The series of papers were reported in one single 2009 issue of *Biotechnology Progress*. *P. deltoides* is an interesting feedstock that can be deconstructed into fermentable sugars. The production of a fermentable sugar stream was examined by our group (Martin et al. 2011; Djioleu et al. 2012), where high and

low specific gravity poplar was pretreated in 1% (v/v) dilute acid in non-agitated batch reactors and hydrolyzed using Accelerase® 1500 enzymes.

In this work, high specific gravity poplar was pretreated in 0.98% (w/v) dilute acid at 140 °C in a 1 L stirred reactor and the hydrolyzates were fermented with two ethanol producing strains. This work examined the side-by-side effect of washing and not washing the pretreated biomass on sugar yields and its effect on fermentation to ethanol.

2. Materials and methods

2.1. Biomass

High-density poplar was secured from University of Arkansas Pine Tree Branch Station. The material was identical to what was studied by Djioleu et al. (2012) and Martin et al. (2011). The biomass was transformed into chips, which were then ground to 10 mesh using a Wiley Mini Mill (Thomas Scientific, Swedesboro, NJ) as described by Torget et al. (1988). The moisture content was determined with an Ohaus MB45 Moisture Analyzer (Pine Brook, NJ). The poplar used in this study was reported to have a specific gravity of 0.48, as reported by Martin et al. (2011).

2.2. Pretreatment

Twenty-five grams of biomass were weighed and mixed with 250 ml of 0.98% (w/v) sulfuric acid (EMD, Gibstown, NJ), resulting in a solids concentration of 10%. The reaction mixture was placed in a 1 L Parr (Moline, IL) 4525 reaction vessel. The reaction temperature used in these experiments was 140 °C. Reaction time was set as the time when the reactor reached 140 °C. After 40 min, heating was halted and the reactor was cooled under a stream of cold tap water. Temperature decreased from 140 °C down to 100 °C in about four min. When the mixture inside the reactor reached a temperature lower than 60 °C, the contents were retrieved. On average, the cool down period lasted approximately 10 min. The mixture was filtered with a Buchner apparatus fitted with Whatman filter paper. The remainder of the reaction solids were removed from the vessel and likewise filtered through a Buchner apparatus. The volume of the hydrolyzate was recorded and saved for further testing. The mass of filtered solids was recorded and its moisture content determined, using Ohaus MB45 Moisture Analyzer. The filtered solids were either used as is (referred to throughout the work as non-washed) or washed (referred to throughout the work as washed) with three volumes of Millipore water as suggested by Hodge et al. (2008). The wash liquid was saved and kept at 4 °C for further testing.

2.3. Enzyme hydrolysis

The hydrolysis was essentially conducted as in Djioleu et al. (2012), but carried out in a 600 ml Parr reactor described by Martin et al. (2010). Forty grams of washed or non-washed pretreated biomass were placed in the Parr reactor with 20 ml of Accelerase®1500 (Genencor), 200 ml

of pH 4.9 sodium citrate buffer, and 180 ml of Millipore filtered water. The reactor was stirred continuously at a slow speed as reported by Martin et al. (2010) and maintained at 50 °C for 24 hours. The entire sample was collected at the end of the run and stored at 4 °C.

2.4. HPLC analysis

Aliquots from pretreatment hydrolyzates, wash waters and enzyme hydrolyzates were analyzed by high-pressure liquid chromatography (HPLC) for carbohydrates and inhibitory byproducts. Two instruments were used to conduct these analyses. Carbohydrates were analyzed with Waters 2695 Separations module (Milford, MA) equipped with Shodex (Waters, Milford, MA) precolumn (SP-G, 8 µm, 6 x 50 mm) and Shodex column (SP0810, 8 µm x 300 mm). Millipore filtered water (0.2 mL/min) was the mobile phase and the column was heated to 85 °C with an external heater. Carbohydrates were detected with a Waters 2414 Refractive Index Detector (Milford, MA) as described by Djioleu et al. (2012). Inhibitory byproducts were analyzed on a Waters 2695 Separations module equipped with a Bio-Rad (Hercules, CA) Aminex HPX-87H Ion Exclusion 7.8 mm X 30 mm column, heated to 55°C. The mobile phase was 0.005 M H₂SO₄, flowing at 0.6 ml/min. Compounds were detected with a UV index using the Waters 2996 Photodiode Array detector. Furfural and hydroxymethylfurfural (HMF) were detected at 280 nm; whereas, formic acid and acetic acid were detected at 210 nm.

2.5. Fermentation

Fermentation was carried out in 50 ml shake flasks with two strains of yeast, self-flocculating SPSC01 and ATCC4126. The SPSC01 strain was provided by Dalian University of Technology, China (Bai et al. 2004). Preculture of both yeast strains was carried out in medium consisting of 30 g/L glucose, 5 g/L yeast extract and 5 g/L peptone. The overnight grown yeasts were harvested by centrifugation at 4,100 g for 30 min. The pellets of yeast cells were washed twice with de-ionized water, and then re-suspended in 50 mM sodium citrate buffer (pH 4.8) to reach a cell concentration of 2 to 4×10⁹ /ml. The re-suspended yeast cells were inoculated into 10 ml of each hydrolysate to reach a yeast cell concentration of 8×10⁷ /ml. Ethanol fermentations were performed at 30°C on a rotary shaker at 150 rpm for 8 hours. Glucose content of the samples was assayed using a glucose colorimetric assay kit (Cayman Chemical, MI). Produced ethanol was quantified by gas chromatography (GC) on the Shimadzu GC-2010 equipped with a flame ionization detector (FID) and a Stabilwax®-DA column (cross-bond polyethylene glycol, 0.25 mm ×0.25 µm ×30 m), as described early by Ge et al. (2011). Before injection into the GC, 50 µl of fermentation broth was diluted 10 times with de-ionized water and supplemented with 50 µl of 0.1 mg/ml n-butanol as an internal standard.

2.6. Statistical analysis

Experiments were conducted in duplicate (pretreatment and enzymatic saccharification) or triplicate (fermentation). Calculations of carbohydrate and degradation compounds, including HMF, furfural, formic acid, and acetic acid, were calculated using Microsoft Office Excel 2007. Analysis of the variance (ANOVA) was determined using JMP 9.0, LSMeans Differences Student's t, with α= 0.10.

3. Results and discussion

3.1. Pretreatment and enzymatic hydrolysis

Poplar biomass was pretreated at 140 °C for 40 min. This condition corresponded to a combined severity of 1.16 (Abatzoglou et al. 1992). The composition of the hydrolyzate was analyzed by HPLC and calculations were made to express the concentrations in terms of compounds obtained from 100 g of biomass. These pretreatment conditions resulted in the recovery of 12% and 41% of the possible glucose and xylose, respectively; these calculations were based on previously reported high specific gravity compositional analysis (Djioleu et al. 2012). Carbohydrate recoveries are presented in Table 1. Dilute acid pretreatment resulted in the release of xylose from hemicellulose as compared to that of glucose from cellulose, and results presented in Table 1 reflect this trend. Dilute acid hydrolyzates also contained furfural, acetic acid, formic acid and HMF. By determining HPLC concentrations, liquid volumes and initial feedstock masses, amounts of furfural, acetic acid, formic acid and HMF were calculated as 0.71, 1.56, 2.41 and 0.04 g per 100 g, respectively.

After pretreatment, the biomass was either washed with three volumes of water or used as is (non-washed), and the resulting wash waters were analyzed by HPLC. Table 1 presents the compositional analysis of the resulting wash waters; furfural, acetic acid, formic acid and HMF were 0.14, 0.31, 0.41 and 0.01 g per 100 g, respectively. Of the inhibitory compounds monitored, formic acid was generated in the highest concentration. In contrast to dilute acid hydrolyzates, wash waters contained similar proportions of glucose and xylose. Furfural, acetic acid, formic acid and HMF concentrations in the wash waters were at most 18% of those present in dilute acid hydrolyzates, indicating that inhibitory products could remain bound to the pretreated biomass.

The washed and non-washed pretreated pellets were subjected to enzymatic hydrolysis. The results are presented in Figure 1. Washing the pretreated pellet had a significant effect on glucose recovery, where glucose concentrations in the washed condition were 5.3 times higher than those from the non-washed samples. As expected, concentrations of furfural, acetic acid, formic acid and HMF were significantly higher in the enzymatic hydrolyzates of non-washed samples.

g/100 g	glucose	xylose	furfural	acetic acid	formic acid	HMF
Hydrolyzate	0.828 ± 0.030	4.420 ± 0.103	0.710 ± 0.028	1.560 ± 0.323	2.410 ± 0.231	0.037 ± 0.003
Wash water	0.111 ± 0.077	0.103 ± 0.006	0.137 ± 0.023	0.311 ± 0.034	0.412 ± 0.126	0.007 ± 0.002

Table 1. Composition of pretreatment hydrolyzate and wash water of high specific gravity poplar pretreated in dilute acid (0.98 % v/v) at 140 °C for 40 min.

3.2. Ethanol production from washed and non-washed hydrolyzates

The fermentability of the enzymatic hydrolysates was evaluated using two yeast strains, self-flocculating yeast SPSC01 and conventional *Saccharomyces cerevisiae* ATCC4126. Both yeast strains solely metabolize glucose and not xylose. A total of four hydrolysate samples, two from

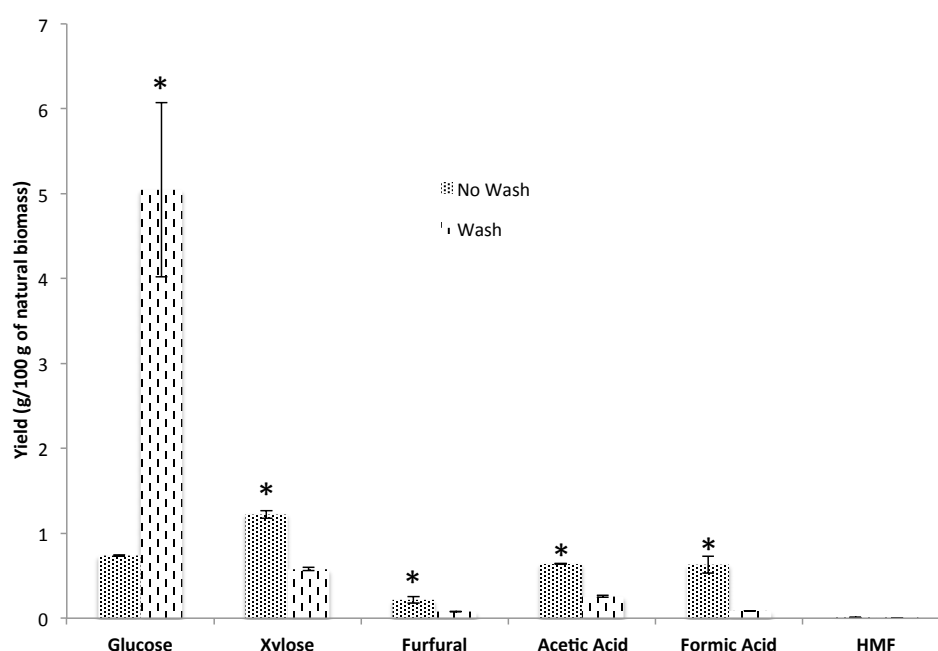


Figure 1. Carbohydrate, furan and aliphatic acid yields of washed and non-washed enzymatically hydrolysed dilute acid pretreated poplar.

washed pretreatments and two from non-washed pretreatments were directly used for the fermentation, and ethanol yields based on glucose ($Y_{E/G}$) were determined. Since the initial glucose concentrations in the hydrolyzates were low (less than 4.0 g/L) due to inefficient enzymatic saccharification (Table 2), all the fermentations were completed within 6 hours, as indicated by pre-experiments (data not shown).

Samples	Glucose content (g/l)	ATCC4126			SPSC01		
		Ethanol (g/l)	$Y_{E/G}^a$ (g/g)	g/100 g	Ethanol (g/l)	$Y_{E/G}^a$ (g/g)	g/100 g
Non-washed-A	0.20±0.00	0	0	0	0.08±0.01 ^b	-- ^c	-- ^c
Non-washed-B	0.19±0.01	0	0	0	0.07±0.01 ^b	-- ^c	-- ^c
Washed-A	2.32±0.06	0.41±0.03	0.18	0.90	0.48±0.05	0.21	1.05
Washed-B	3.76±0.11	1.08±0.09	0.29	5.75	1.46±0.15	0.39	7.74

^a: $Y_{E/G}$ refers to ethanol yields based on the glucose contained in hydrolysates

^c: Not accurately detected because of out of the detection limit of GC

^d: Not calculated due to inaccurately determined ethanol concentration by GC

Table 2. Ethanol yields of the fermentation of four different enzymatic hydrolyzates with two yeast strains ATCC4126 and SPSC01. Of the four hydrolyzate samples, two were prepared from non-washed pretreated biomass and two from washed pretreated biomass. The pretreatments were conducted at 140 °C (A) and 160 °C (B), respectively.

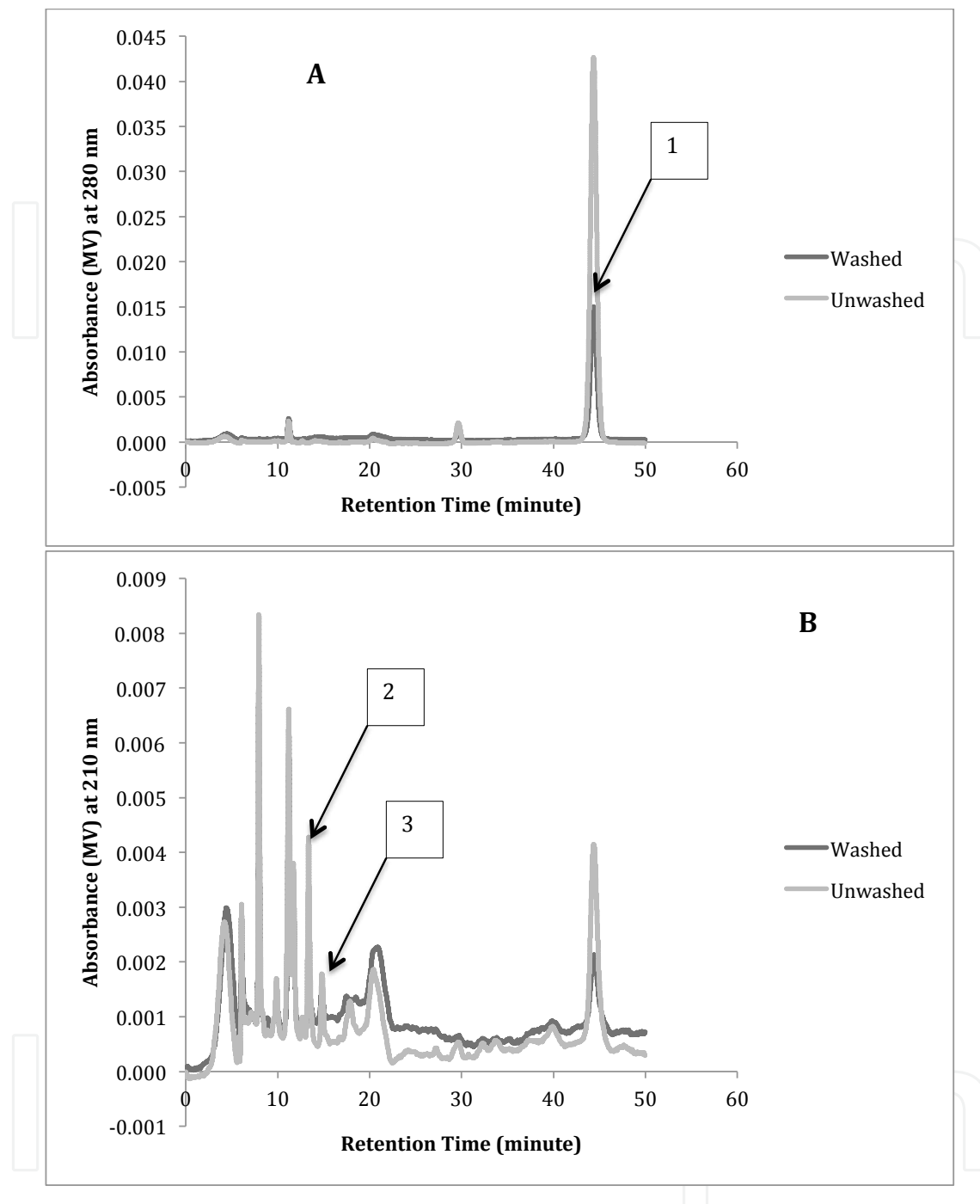


Figure 2. Chromatogram of enzymatic hydrolyzate of washed and unwashed biomass after dilute acid pretreatment. Compounds detected at A) 280 nm and B) 210nm. Compounds are 1) Furfural, retention time = 44.35 min; 2) Formic acid, retention time = 13.6 min; 3) Acetic acid, retention time = 14.7min

It is shown in Table 2 that trace amounts of ethanol were detected in the fermentation broth of the non-washed enzymatic hydrolysates. In contrast, significant amounts of ethanol were generated from the washed enzymatic hydrolysates, in particular from the hydrolyzate sample that contained a glucose concentration of 3.76 g/L, producing 1.08 g/L and 1.46 g/L of ethanol by the ATCC4126 and SPSC01 strains, respectively. However, the ethanol yields $Y_{E/G}$ deter-

mined in this study, 0.18~0.39 g/g were generally lower than those obtained earlier with the fermentation of the enzymatic hydrolyzates from several other energy crops, such as switchgrass, miscanthus and gamagrass, using identical yeast strains (Ge et al. 2011, 2012). This should be attributed to the presence of substantial amount of fermentation inhibitors in the poplar hydrolyzates, such as furfural, acetic acid and formic acid HMF (see discussion below), though the pretreated biomass has been extensively washed with water. It should be noted that enzymatic hydrolyzates prepared from other energy crops largely lacked these inhibitors because these biomass were pretreated with concentrated (84%, w/v) phosphoric acid under moderate reaction conditions (50°C for 45 min) (Ge et al. 2011, 2012). However, this phosphoric acid-based pretreatment approach is regarded as too expensive to be economically feasible.

Of particular interest is the observation that the self-flocculating SPSC01 yeast always produced higher ethanol yields than the ATCC4126 strain from the same enzymatic hydrolyzates (Table 2). While no ethanol was detected from the fermentation of the unwashed hydrolyzates by the ATCC strain, marginal levels of ethanol could be produced by the SPSC01 strain. When the washed hydrolyzates were tested for fermentation, the SPSC01 yeast could produce up to 35% more ethanol than the ATCC strain. The SPSC01 yeast is an industrial strain that has been reported to have high ethanol productivity, high ethanol tolerance and lower capital investment required for yeast cell recovery (Bai et al. 2004; Zhao and Bai, 2009; Zhao et al. 2009). It has been successfully used for continuous ethanol fermentation at commercial scales in China (Bai et al. 2008). The results from this study indicated that this self-flocculating strain could also have a higher tolerance to fermentation inhibitors than the non-flocculating yeast, thus being able to produce higher ethanol yields. Fermentation with the self-flocculating yeast may represent a promising strategy to increase the production of cellulosic ethanol.

3.3. Differences between washed and non-washed hydrolyzates

Figure 2 presents HPLC chromatograms from washed and non-washed enzymatic hydrolyzates; analysis was conducted at 280 (A) and 210 (B) nm. Retention times of furfural, acetic acid and formic acid HMF were 44.4, 14.7, 13.6 minutes, respectively. Peaks at 9 and 12 minutes remain unidentified. Examination of the UV traces showed that, for the most part, washing did not remove any compounds, but decreased peak intensity. Results from Figure 1 demonstrate that washing biomass is critical to maximize sugar recovery; however UV traces at 280 and 210 nm are qualitatively similar. Mass spectrometry analysis of the hydrolyzates would have most likely revealed more peaks, aiding in identifying which peaks need to be removed and/or minimized prior to enzymatic hydrolysis and fermentation.

In related work, aliphatic acid and furans from wet distillers grain (Ximenes et al. 2010), corn stover (Hodge et al. 2008), wheat straw (Panagiotou and Olsson 2007) and poplar wood (Cantarella et al. 2004) wash waters were analyzed. Having detected and quantified compounds in wash waters, solutions were reconstituted and tested for their effect on saccharification cocktails. Cantarella et al. (2004) pretreated poplar in steam at a severity of 4.13 and tested the effect of washing the pretreated biomass. Cantarella et al. (2004) washed poplar-pretreated material with either 12.5 or 66.7 volumes of water to biomass ratio prior to enzymatic hydrolysis and fermentation steps. They reported that using washed biomass resulted

in the production of at least 20 g/L of ethanol, while the use of the non-washed control produced no ethanol.

Cantarella et al. (2004) showed that increasing formic acid concentrations of washed steam pretreated poplar from 3.7 to 11.5 mg/ml decreased sugar recovery from enzymatic hydrolysis by 60%. Formic acid was also shown to inhibit enzymatic hydrolysis by Arora et al. (2012). They showed that adding 5 or 10 mg/ml formic acid to washed dilute acid pretreated poplar biomass resulted in recovery of 47% and 14%, respectively, of potential sugars. Using steam explosion-pretreated wheat straw as their system, Panagitou and Olsson (2007) reported the effects of adding 4 and 15 mg/ml of formic acid to their hydrolyzate; the higher concentration annihilated sugar recovery.

In work reported by Moreno et al. (2012), wheat straw was pretreated by steam explosion; the pretreated slurry was incubated with *Pycnoporus cinnabarinus* or *Trametes villosa* laccases prior to fermentation with *Kluyveromyces marxianus*. Biomass loadings of 5, 6 and 7% were tested. No differences in ethanol yields at 5% and 6% were observed; however, loadings at 7% resulted in an 86% reduction in ethanol yields compared to the control, which was not prior incubated with laccases. These results indicate that inhibitory byproducts are present in the pretreatment hydrolyzates. Incubation with *T. villosa* laccases removed almost 100 % of vanillin, syringaldehyde, p-coumaric acid and ferulic acid from pretreated hydrolyzates, enabling ethanol to glucose yields greater than 0.33 g/g.

Although this report is centered on the effects of aliphatic acids and furans on enzymatic hydrolysis and fermentation, it is important to note that other generated products may play key roles in inhibiting enzymatic hydrolysis and fermentation (Palmqvist and Hahn-Hägerdal 1999; Moreno et al 2012). Lignin derivatives can result in nonproductive binding of the saccharification cocktail with lignin derivatives (Berlin et al. 2006); and released sugars and their degradation compounds can deactivate or obstruct enzyme active sites (Kumar and Wyman 2008). It is critical to establish a better understanding of pretreatment chemistry in terms of generated degradation products. By understanding which compound plays a critical role in inhibiting enzymatic hydrolysis and fermentation, attempts can be made to minimize their generation, thereby improving processing yields. Pretreatments at 0.98% (w/v) dilute acid, 140 °C for 40 min resulted in the recovery of 12% and 41% of possible glucose and xylose, respectively. The authors recognize that these were low carbohydrate yields. Pretreatment were re-conducted at 0.98% (w/v) dilute acid, 160 °C for 40 min. Glucose recovery from non-washed and washed biomass was 0.92 and 19.85 g/100g, respectively, indicating that a 20 °C increase in temperature significantly augmented sugar recovery. Conversely, formic acid contents were 0.65 and 0.04 g/100 g non-washed and washed biomass, respectively; higher content was determined in non-washed biomass as for the 140 °C pretreatment conditions.

4. Conclusions

Dilute acid pretreatment processes resulted in the production of inhibitory byproducts, such as furfural, acetic acid, formic acid, and HMF that hindered both the enzymatic saccharification

and fermentation steps. Washing the pretreated biomass with water did not entirely remove the inhibitory compounds, but significantly decreased their concentrations, which resulted in recovery of 5.3 times more glucose and substantially increased ethanol yields. The self-flocculating yeast strain SPSC01 showed higher tolerance to fermentation inhibitors than the non-flocculating ATCC4126 yeast, resulting in up to 35% increase in ethanol yield.

Acknowledgements

The authors would like to thank the University of Arkansas, Division of Agriculture, and the Department of Biological and Agricultural Engineering, for financial assistance. The authors would also like to acknowledge Department of Energy award 08GO88035 for pretreatment equipment and support; CRREES National Research Initiative award no. 2008-01499 for the HPLC instrument, and the Plant Powered Production (P3) Center through an NSF RII Arkansas ASSET Initiative (AR EPSCoR) for the support of undergraduate and graduate student stipend and equipment.

Author details

Noaa Frederick¹, Ningning Zhang², Angele Djioleu¹, Xumeng Ge², Jianfeng Xu² and Danielle Julie Carrier¹

1 Department of Biological and Agricultural Engineering, University of Arkansas, Fayetteville, AR, USA

2 Arkansas Biosciences Institute and College of Agriculture and Technology, Arkansas State University, Jonesboro, AR, USA

References

- [1] Abatzoglou, N, Chornet, E, & Belkacemi, K. (1992). Phenomenological kinetics of complex systems: The development of a generalized severity parameter and its application to lignocellulosics fractionation. *Chemical Engineering Science*, 153, 375-380.
- [2] Arora A, Martin E, Pelkki M and Carrier DJ. (2013). "The effect of formic acid and furfural on the enzymatic hydrolysis of cellulose powder and dilute acid-pretreated poplar hydrolysates." *Sustainable Chemistry and Engineering* 1: 23-28.
- [3] Bai, F. W, Chen, L. J, Anderson, W. A, & Moo-young, M. (2004). Parameter oscillations in a very high gravity medium continuous ethanol fermentation and their at-

tenuation on a multistage packed column bioreactor system. *Biotechnology and Bioengineering* 88, 558-566.

- [4] Bai, F. W, Anderson, W. A, & Moo-young, M. (2008). Ethanol fermentation technologies from sugar and starch feedstocks. *Biotechnology Advances* , 26, 89-105.
- [5] Berlin, A, Balakshin, M, Gilkes, N, Kadla, J, Maximenko, V, Kubo, S, & Saddler, J. (2006). Inhibition of cellulase, xylanase and β -glucosidase activities by softwood lignin preparations. *Journal of Biotechnology* , 125, 198-209.
- [6] Cantarella, M, Cantarella, L, Gallifuoco, A, Spera, A, & Alfani, F. (2004). Subsequent enzymatic hydrolysis and SSF. *Biotechnology Progress* , 20, 200-206.
- [7] Van Dam, H, Kieboom, A, & Van Bekkum, H. (1986). The conversion of fructose and glucose in acidic media: Formation of hydroxymethyl furfural. *Starch/Stärke* 3: S, 95-101.
- [8] Djiroleu, A, Arora, A, Martin, E, Smith, J. A, Pelkki, M, & Carrier, D. J. (2012). Sweet-gum sugar recovery from high and low specific gravity poplar clones post dilute acid pretreatment/enzymatic hydrolysis. *Agricultural and Analytical Bacterial Chemistry* (in press).
- [9] Ge, X. M, Burner, D. M, Xu, J, Phillips, G. C, & Sivakumar, G. (2011). Bioethanol production from dedicated energy crops and residues in Arkansas, USA. *Biotechnology Journal* , 6, 66-73.
- [10] Ge, X. M, Green, S, Zhang, N, Sivakumar, G, & Xu, J. (2012). Eastern gamagrass as a promising cellulosic feedstock for bioethanol production. *Process Biochemistry* , 47, 335-339.
- [11] Ge, X. M, Zhao, X. Q, & Bai, F. W. (2005). Online monitoring and characterization of flocculating yeast cell flocs during continuous ethanol fermentation. *Biotechnology and Bioengineering* , 90, 523-531.
- [12] Hills, J, Polasky, S, Nelson, E, Tilman, D, Huo, H, Ludwig, L, Neumann, J, Zheng, H, & Bonta, D. (2009). Climate change and health costs of air emissions from biofuels and gasoline. *Proceeding of National Academy of Sciences* , 106, 2077-2082.
- [13] Hodge, D, Karim, M, Schell, D, & Macmillan, J. (2008). Soluble and insoluble solids contributions to high-solids enzymatic hydrolysis of lignocellulose. *Bioresource Technology* , 99, 8940-8948.
- [14] Kim, Y, Mosier, N, & Ladisch, M. (2009). Enzymatic digestion of liquid hot water pretreated hybrid poplar. *Biotechnology Progress* , 25, 340-348.
- [15] Kumar, R, & Wyman, C. (2008). Effect of enzyme supplementation at moderate cellulose loadings on initial glucose and xylose release from corn stover solids pretreated by leading technologies. *Biotechnology and Bioengineering* , 102, 457-467.

- [16] Lynd, L, Laser, M, Bransby, D, Dale, B, Davison, B, Hamilton, R, Himmel, M, Mcmillan, J, Sheehan, J, & Wyman, C. (2008). How biotech can transform biofuels. *Nature Biotechnology* , 26, 169-172.
- [17] Martin, E, Bunnell, K, Lau, C, Pelkki, M, Patterson, D, Clausen, E, Smith, J, & Carrier, D. J. (2011). Hot water and dilute acid pretreatment of high and low specific gravity *Populus deltoids* clones. *Journal of Industrial Microbiology* 38, 355-361.
- [18] Martin, E, Duke, J, Pelkki, M, Clausen, E, & Carrier, D. J. (2010). Sweetgum (*Liquidambar styraciflua* L.): Extraction of shikimic acid coupled to dilute acid pretreatment. *Applied Biochemistry and Biotechnology* , 162, 1660-1668.
- [19] Moreno, A, Ibarra, D, Fernández, J, & Ballesteros, M. (2012). Different laccase detoxification strategies for ethanol production from lignocellulosic biomass by the thermotolerant yeast *Kluyveromyces marxianus* CECT 10874. *Bioresource Technology* , 106, 101-109.
- [20] Palmqvist, E, Almeida, J, & Hahn-hägerdal, B. (1999). Influence of furfural on anaerobic glycolytic kinetics of *Saccharomyces cerevisiae* in batch culture. *Biotechnology and Bioengineering* , 62, 447-454.
- [21] Panagiotou, G, & Olsson, L. (2007). Effect of compounds released during pretreatment of wheat straw on microbial growth and enzymatic hydrolysis rates. *Biotechnology and Bioengineering* , 96, 250-258.
- [22] Sannigrahi, P, Kim, D, Jung, H, & Ragauskas, A. (2011). Pseudo-lignin and pretreatment chemistry. *Energy Environmental Sciences* , 4, 1306-1310.
- [23] Sharara, M, Clausen, C, & Carrier, D. J. (2012). An overview of biorefinery technology" in *Biorefinery Co-Products: Phytochemicals, Primary Metabolites and Value-Added Biomass Processing* (Wiley Series in Renewable Resource). Bergeron C, Carrier DJ and Ramaswamy S. (eds). John Wiley & Sons. , 1-18.
- [24] Studer, M, Demartini, J, Davis, M, Sykes, R, & Davison, B. Tuskan, Keller M, Tuskan G and Wyman C. (2011). Lignin content in natural *Populus* variants affects sugar release. *Proceeding of National Academy of Sciences* , 108, 6300-6305.
- [25] Tao, L, Aden, A, Elander, R, Pallapolu, V, Lee, Y, Garlock, R, Balan, V, Dale, B, Kim, Y, Mosier, N, Ladisch, M, Falls, M, Holtzapple, M, Sierra, R, Shi, J, Ebrik, M, Redmond, T, Yang, B, Wyman, C, Hames, B, Thomas, S, & Warner, R. (2011). Process and technoeconomic analysis of leading pretreatment technologies for lignocellulosic ethanol production using switchgrass. *Bioresource Technology* , 102, 11105-11114.
- [26] Torget, R, Himmel, M, Grohmann, K, & Wright, J. D. (1988). Initial design of a dilute sulfuric acid pretreatment process for aspen wood chips. *Applied Biochemistry and Biotechnology* , 17, 89-104.

- [27] Wallington, T, Anderson, J, Mueller, S, Kolinski, E, Winkler, S, Ginder, J, & Nielsen, O. (2012). Corn ethanol production, food exports, and indirect land use change. *Environmental Science & Technology* 46:6379–6384.
- [28] Ximenes, E, Kim, Y, Mosier, N, Dien, B, & Ladisch, M. (2010). Inhibition of cellulases by phenols. *Enzyme and Microbial Technology* , 46, 170-176.
- [29] Zhao, X. Q, & Bai, F. W. (2009). Yeast flocculation: New story in fuel ethanol production. *Biotechnology Advances* , 27, 849-856.
- [30] Zhao, X. Q, Xue, C, Ge, X. M, Yuan, W. J, Wang, J. Y, & Bai, F. W. (2009). Impact of zinc supplementation on the improvement of ethanol tolerance and yield of self-flocculating yeast in continuous ethanol fermentation. *Journal of Biotechnology* , 39, 55-60.

