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A two stage pretreatment process to maximise recovery of sugars from cotton gin trash

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ABSTRACT

We evaluated a two stage pretreatment process for liberation of sugars from cotton gin trash (CGT) for ethanol production. The 1st stage releases C5 sugars for propagation of yeast inoculum intended for C6 sugar fermentations. The effects of a range of dilute acid hydrolysis pretreatment conditions on CGT were assessed. Propagation of recombinant *Saccharomyces cerevisiae* GSF335 in 1st stage liquors yielded up to 66 g yeast biomass kg^{-1} CGT. In the presence of PEG 6000, 2nd stage pretreated fibres were highly acquiescent to enzyme hydrolysis, yielding up to 242 g glucose kg^{-1} CGT. We conclude that the optimum conditions for recovery of sugars from CGT are: 1:6 solids to liquids ratio with 12% H_2SO_4 wt. on solids at 180 °C for 15 min during the 1st stage, with the ensuing pressed unwashed fibre heated to 200 °C for 5 min during the 2nd stage.

1. Introduction

Cotton is a significant broad acre crop in Australia, with more than half a million hectares under cultivation. The process of ginning cotton to produce fibre generates up to 230,000 tons of cotton gin trash (CGT) annually (Anon, 2017). Strategies to manage CGT as a waste material vary but each adds considerable cost to the ginning process (Hassall and Associates, 2005). This combined with increasing disposal fees, stricter regulations on environmental applications and emissions and higher fuel prices, is driving interest in transforming CGT into a value added material by diverting it toward utilisation in bioenergy.

CGT has many compositional attributes indicating strong potential for use as a feedstock in biorefining. These attributes include high polysaccharide content and potentially high-value pharma-nutraceuticals (Egbuta et al., 2017). High yields of fermentable sugars have previously been generated from CGT supporting scalable cellulosic ethanol production systems (McIntosh et al., 2014). Moreover, CGT is an idyllic biorefinery feedstock because it is concentrated at processing sites, consequently minimising operational costs associated with harvesting and transportation.

Like most lignocellulosic material, conversion of CGT to sugars for ethanol fermentation entails a pretreatment step to ensure that structural barriers (lignin and hemicellulose) are removed, thereby permitting enzymes to access and hydrolyse the cellulose to glucose. Among the existing physiochemical pretreatment methods for processing CGT, acid pretreatment with steam explosion is the most frequently reported (Ibrahim et al., 2010). Alkaline pretreatment, either stand alone or in combination with dilute organic acids, are the only notable alternatives (Fockink et al., 2015; Sahu and Pramanik, 2018). Regardless of the approach taken, most pretreatments centre on optimal isolation of fibres with highly digestible cellulose while sacrificing pentose sugar recovery.

As way of example, several authors report that increasing pretreatment severity improves cellulose digestibility of the solid fibre but to the detriment of xylose recovery and often leading to an increase in inhibitory degradation by-products (Jeoh and Agblevor, 2001; McIntosh et al., 2014). The reasons that this occurs are twofold. Firstly, glucose - which is derived from cellulose - is the most abundant sugar and is easily fermented by microorganisms and, secondly, pretreatment conditions that are ideal for cellulose recovery differ to those for hemicellulose hydrolysis. This disparity is a fundamental characteristic of all lignocellulosic material and is known as polydispersity of plant biomass recalcitrance (PPBR) (Zhu et al., 2011). Thus PPBR poses problems in developing a single common set of pretreatment conditions for maximum recovery of both cellulose enriched fibres and xylose laden hydrolysates from plant biomass.

Engineered yeast, such as *Saccharomyces cerevisiae*, preferentially metabolise xylose by respiration rather than fermentation (Kwak and Jin, 2017) because they generally lack the ability to concomitantly ferment C5 and C6 hydrolysates in an economically feasible manner (Jansen et al., 2017). Glucose and molasses based media are currently employed to prepare *S. cerevisiae* inoculum for industrial scale

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fermentations. However, this is a major process and cost component (Qureshi et al., 2015). Thus employing a pretreatment process that maximises xylose recovery has potential to deliver considerable cost savings by providing an easily acquired substrate upon which to cultivate yeast for subsequent fermentation.

Various two stage pretreatment processes have been developed to increase total sugar recovery from lignocellulose. Hydrothermal based treatments such as sequential dilute acid or steam explosion are often used for woody residues. These treatments are contingent on mild conditions during the first pretreatment step to release pentose sugars while the second step is customised to produce cellulosic fibre amenable to enzyme hydrolysis (Inoue et al., 2016). In some instances, the second step consists of an alkaline and solvent treatment or post-alkaline wash which expedites delignification and accordingly improves enzyme hydrolysis of the solid fibre (Brodeur et al., 2016; Martinez-Patino et al., 2017). Although the latter approach generally leads to more digestible fibres, use of additional catalysts and intensive fibre washing between pretreatment steps and enzyme hydrolysis adds costs which counteract any beneficial value derived from alkaline delignification. On the contrary, steam explosion with dilute acid operated in a sequential manner relies on the residual acid in the solid fibre following 1st stage pretreatment for effective defibrillation during the 2nd stage.

Within this context, we conducted a laboratory scale study to investigate and define optimum conditions for a two stage pretreatment process of CGT that produces: (i) pentose rich liquor streams suitable for propagating xylose recombinant yeast for use in subsequent fermentations; (ii) solid fibre holding sufficient residual acid for effective 2nd stage pretreatment; and (iii) sequentially pretreated CGT fibre highly acquiescent to enzyme hydrolysis of glucan to glucose.

2. Materials and methods

2.1. Materials

CGT was sourced from the Yarraman Gin, NSW and was dried at 50 °C for 48 h and then ground in a rotary mill (Gelder & Co., NSW, Australia) fitted with a No. 5 sieve (ASTM). To improve uniformity and further reduce particle size, samples were pulverised for 60 s (Labtechnics Pulveriser, WA, Australia). Using standard NREL methods (Sluiter et al., 2008), the composition of the milled CGT was determined as: 27.0% glucan, 8.25% xylan, 0.45% arabinan, 23.0% lignin, 11.3% ash, 14.0% water and 11.0% ethanol extractives. Milled material was stored at room temperature in air-tight containers until use.

2.2. Initial screening of pretreatment conditions

The effects of various dilute acid (DA) pretreatment conditions on CGT were determined using a 2 L Parr stirred reactor (Parr Instruments, USA). In each instance 100 g milled CGT was subjected to a range of combined severity factors (CSF; Pedersen and Meyer, 2010) as outlined in Table 1.

Combined severity factor was used to assess the pretreatment trials. A combined severity factor (CSF) was used to integrate the effects of hydrolysis time, temperature and pH into a single variable. The CSF is defined as:

$$CSF = \log R_0 - pH \quad (1)$$

R_0 represents the severity factor which and is determined by Eq. (2) while pH relates to that of the aqueous acid solution.

$$R_0 = \frac{t \times \exp[(T - T_R)]}{14.75} \quad (2)$$

where t is pretreatment time in min, T is the pretreatment temperature in °C, T_R is the reference temperature of 100 °C.

For each reaction the biomass to liquid ratio was 1:6 (wt./wt.) and the pretreatment temperature was held for 15 min at a constant mixing speed of 60 rpm. An external circulation jacket was used to heat and cool the reaction vessel. When the pretreated material had cooled to 90 °C, the slurry was immediately decanted and the liquors and insoluble fractions (solids) were separated by vacuum filtration using Whatman® glass microfibre GF/A filters mounted in a Büchner funnel. All pretreatments were performed in two independent runs with duplicates included within each run and all chemicals used were of reagent grade (Sigma-Aldrich, USA).

Table 1
Pretreatment conditions and resulting CSF values.

Temp (°C)	Actual CSF at H_2SO_4 concentration (wt% on solids)							
	1.2	2.4	3.6	4.8	6	9	12	15
180	−1.93				−0.13	1.06	1.94	2.27
190	−1.35				−0.01	1.05	2.06	2.56
200	−1.03	−0.69	−0.41	−0.13	0.14	1.34	2.33	2.73
210	−0.61	−0.24	−0.03	0.17	0.48	1.54	2.55	2.98
215	−0.21							
220	−0.11				0.83	1.74	2.73	3.18
225	0.07							
230	0.33	0.47						
240	0.48	0.69	0.78					

2.3. Second stage pretreatment

Three 1st stage pretreated CGT fibres - subjected to DA pretreatment during initial screening of pretreatment conditions - were selected for 2nd stage pretreatment experiments based on specific attributes as discussed in Section 3. These treatments are defined as U, AF and AO (Table 2). The 2nd stage pretreatments were carried out in 30 mL stainless steel tubing modified with threaded cap fittings to pressure seal each end (Swagelok, Australia). Each vessel was loaded with 12 g pressed 1st stage pretreated fibre (dry weight equivalent of 3.8 g, 4.0 g and 4.1 g for U, AF AO 1st stage pretreated fibres, respectively) and 9 g type 1 water for a total reaction mass of 21 g. The contents were thoroughly mixed and end caps tightened to seal prior to placing vessels onto a bed of sand inside a muffle furnace (Carbolite-Gero, United Kingdom). Reaction vessels were heated to either 200 °C, 215 °C or 230 °C for 5 min, removed from the muffle furnace and then rapidly cooled by submerging in cold water. After cooling, reaction vessels were opened and 30 mL type 1 water was added. The contents were then mixed and filtered through a 1.2 µm glass microfibre filter (Filtech, USA) to separate solids and liquids. All pretreatments were performed in two independent runs with duplicates included in each run and samples were stored at −20 °C.

Table 2
Composition of prehydrolysate liquors used in yeast biomass propagation trials.

		Prehydrolysate code					
		U	AH	AF	AI	AO	AP
Pretreatment conditions	°C	180	190	180	190	180	190
	H_2SO_4[a]	9	9	12	12	15	15
	CSF	1.06	1.05	1.94	2.06	2.27	2.56
% release relative to ODM	Glucose	3.8	4.5	7.4	9.1	11.5	12.6
	Xylose	30.5	36.6	56.1	60.6	75.1	70
	Total gluc.	11.9	10.4	12.4	12.5	12	12.3
	Total xyl.	80.9	61.7	87.2	77.6	81.7	71.3
$g\,L^{-1}$	Acetic acid	0.39	1.13	1.34	1.51	0.9	1.08
	HMF	0.18	0.25	0.26	0.38	0.24	0.36
	Furfural	0.38	0.49	0.51	0.8	0.71	1.04

Total glucose and xylose levels were determined following dilute acid hydrolysis. ODM – original dry matter.
[a] Percent on weight.