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Optimisation of dilute alkaline pretreatment for enzymatic saccharification of wheat straw

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ABSTRACT

Physico-chemical pretreatment of lignocellulosic biomass is critical in removing substrate-specific barriers to cellulolytic enzyme attack. Alkaline pretreatment successfully delignifies biomass by disrupting the ester bonds cross-linking lignin and xylan, resulting in cellulose and hemicellulose enriched fractions. Here we report the use of dilute alkaline (NaOH) pretreatment followed by enzyme saccharifications of wheat straw to produce fermentable sugars. Specifically, we have assessed the impacts of varying pretreatment parameters (temperature, time and alkalinity) on enzymatic digestion of residual solid materials. Following pretreatment, recoverable solids and lignin contents were found to be inversely proportional to the severity of the pretreatment process. Elevating temperature and alkaline strengths maximised hemicellulose and lignin solubilisation and enhanced enzymatic saccharifications. Pretreating wheat straw with 2% NaOH for 30 min at 121 °C improved enzyme saccharification 6.3-fold when compared to control samples. Similarly, a 4.9-fold increase in total sugar yields from samples treated with 2% NaOH at 60 °C for 90min, confirmed the importance of alkali inclusion. A combination of three commercial enzyme preparations (cellulase, β -glucosidase and xylanase) was found to maximise monomeric sugar release, particularly for substrates with higher xylan contents. In essence, the combined enzyme activities increased total sugar release 1.65-fold and effectively reduced cellulase enzyme loadings 3-fold. Prehydrolysate liquors contained 4-fold more total phenolics compared to enzyme saccharification mixtures. Harsher pretreatment conditions provide saccharified hydrolysates with reduced phenolic content and greater fermentation potential.

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1. Introduction

The search for sustainable transportation fuels is instigated by diminishing crude oil supplies, impact on climate change and green house gas (GHG) emissions. Global petroleum demands have steadily increased from 57×10^6 barrels/day in 1973 to 82×10^6 barrels/day in 2004 and is anticipated to rise another

50% by 2025 [1]. Allowing for current rates of production and existing reserves, we will soon approach Hubbert's predicted 'peak oil' levels [2]. In selecting an alternative, consideration must be given to fuels which serve to combat climate change and produce cleaner air. Since the industrial revolution atmospheric CO₂ levels have increased from ~275–~380 ppm owing to the burning of fossil fuels. Consequently,

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atmospheric temperatures have risen by 0.6 ± 0.2 °C during the twentieth century. If left unchecked, CO₂ levels could easily surpass 550 ppm by the middle of this century [3].

Biofuels, fuels derived from plant biomass are currently the only sustainable class of liquid fuels [4]. First-generation biofuels such as ethanol are currently produced from plants rich in storage carbohydrates (i.e. sugar and starch). However, as the demands for the feedstock intensify so will the debate between ‘food’ versus ‘fuel’. Moreover, 1st generation bioethanol does not significantly diminish green house gas (GHG) emissions [5]. These shortcomings can be addressed by producing ethanol from lignocellulosic material (2nd generation biofuels), such as agricultural and forest waste residues. Second-generation biofuels are derived from the inedible and/or unexploited part of the plant (lignocellulose) and can be sourced from plant residues or organic waste such as crop straw, forestry thinnings or municipal waste.

Potential agricultural biomass residues from major grain crops in Australia can be exploited for bioethanol production and is presently estimated at 65 Mt [6,7]. Making allowances for current uses like ground cover, soil carbon and fodder, leaves approximately 45% available for production of 2nd generation biofuels [6]. The largest individual contributor to this biomass derives from wheat cultivation. Presently, the total planted area in Australia stands at approximately 13 million hectares with an estimated grain yield of 22 Mt [8]. Assuming the harvest index statement that for every 4 ton of grain at least 6 ton of residue is produced holds true, the total straw yield from wheat cultivation in Australia would approach 33 Mt.

Utilization of lignocellulosics requires effective pretreatment to free cellulose from its lignin seal and open up the crystalline structure so that enzymatic hydrolysis can take place [9]. Although a range of chemical, physical and biological processes have been configured to release constituent sugars from lignocellulose, they suffer several shortcomings such as cost, infrastructure needs and technological impasses [10]. Alkali treatment processes are generally very effective in the pretreatment of agricultural residues such as wheat straw and herbaceous crops [11,12].

Alkali-based pretreatment efficiently delignifies lignocellulose by disrupting the ester bonds cross-linking lignin and xylan, leading to fractions enriched in both cellulose and hemicellulose [9]. Its mechanism of action closely resembles soda or kraft pulping. Alkali pretreatment processes generally utilize lower temperatures, pressures and residence times compared to other pretreatment technologies, the degree dependant directly on the nature of the biomass feedstock, mainly its lignin content [13]. Sodium hydroxide, ammonia, peroxide and lime have received a great deal of attention as pretreatment agents, owing in part to cost-effective practises such as chemical and water recycling (processes well developed in the pulping industry) and partly because lower enzyme loads are generally required to convert cellulose to glucose [14].

Several studies have assessed with varying degrees of success, the utility of pretreating lignocellulosic materials with dilute alkali followed by enzyme hydrolysis with cellulases [15–18]. For the most part, these studies examined and reported conditions for hydrolysing the lignocellulosic biomass without detailing the specific affects of individual treatment parameters on saccharification yields and changes

in monosaccharide compositions. Moreover, these studies failed to highlight the fact that they employed extremely high enzyme dosages, extended reaction and fermentation times above what would be considered the cost-benefit threshold. Overcoming the toxic effects of inhibitors generated from sugar and lignin decomposition (furans, phenols, carboxylic acids) and salts was the major reason for these economically unsustainable practises.

In this study, we report sugar yields and profiles from post-grain harvested wheat straw following dilute alkaline processing and low enzyme dose saccharification. Specifically, we examine and describe three characteristic phases: 1) ascertaining the function of key pretreatment parameters (alkaline strength, temperature and residence time) and their impact on sugar solubilisation, lignin reduction and solid losses; 2) enzymatic hydrolysis efficacy of pretreated solid residues and variations in sugar composition with respect to pretreatment parameters; and 3) the role of individual and combined enzyme activities and their impact on the rates and yields of sugar release. Due to the impact of phenolic compounds on downstream processes we also discuss their release during pretreatment and saccharification. Understanding these key elements will enable further process optimisation of wheat residues and assist in determining the efficacy of the conversion strategy.

2. Materials and methods

2.1. Materials

Post-grain harvested wheat straw (*Triticum aestivum*) was sourced from the Liverpool plains Northern NSW, Australia. The wheat straw was dried at 55–60 °C for 48 h, ground in a rotary mill (Thomas Wiley Laboratory Mill) and passed through a 1.5 mm screen. All chemicals used were of reagent or analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Pretreatment

The effect of pretreatment parameters including: alkalinity, residence time and temperature were evaluated using a $4 \times 3 \times 2$ factorial design. The procedure described by McIntosh and Vancov [19] was employed in this study. Briefly, dilute sodium hydroxide (NaOH) at 0.75% 1.0% and 2.0% (w/v) was used to pretreat milled wheat straw samples at a solid loading of 10% (w/v). Treatments were performed at 60 °C in a water bath and in an autoclave at 121 °C (15 psi) with residence times of 30, 60 and 90 min. Insoluble solids were separated from liquor fractions following pretreatment using a Buchner funnel fitted with glass fibre filters. Solid material was washed with water until the pH was 7, sealed in plastic bags and stored at –20 °C.

2.3. Enzyme assays

Cellulase (NS50013), β-glucosidase (NS50010) and xylanase (NS50030) preparations were kindly supplied by Novozymes (Bagsværd Denmark). Enzyme activities, as described by supplier,

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