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Effect of steam explosion on waste copier paper alone and in a mixed lignocellulosic substrate on saccharification and fermentation

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HIGHLIGHTS

- Steam explosion of copier paper reduces xylose and produces inhibitors.
- Steam explosion at SF 3.6 and 3.9 increased initial rates of saccharification.
- Steam explosion at moderate severities may reduce processing times.
- Co-steam explosion of waste paper and wheat straw reduces inhibitor production.

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ABSTRACT

This study evaluated steam (SE) explosion on the saccharification and simultaneous saccharification and fermentation (SSF) of waste copier paper. SE resulted in a colouration, a reduction in fibre thickness and increased water absorption. Changes in chemical composition were evident at severities greater than 4.24 resulting in a loss of xylose and the production of breakdown products known to inhibit fermentation (particularly formic acid and acetic acid). SE did not improve final yields of glucose or ethanol, and at severities 4.53 and 4.83 reduced yields probably due to the effect of breakdown products and fermentation inhibitors. However, at moderate severities of 3.6 and 3.9 there was an increase in initial rates of hydrolysis which may provide a basis for reducing processing times. Co-steam explosion of waste copier paper and wheat straw attenuated the production of breakdown products, and may also provide a basis for improving SSF of lignocellulose.

1. Introduction

Pre-treatments comprise an important part of the lignocellulosic bioalcohol process, opening up the cellulosic fibre structure through hydrolysis of hemicelluloses and the partial removal of lignin (Trajano et al., 2013). This increases enzyme accessibility and cellulose digestibility. Enzymatic digestion of lignocellulosic substrates is limited in the absence of any pre-treatment (Kumar et al., 2009). There is a large array of pre-treatments including: strong and weak acid and alkali, ammonia fibre expansion (AFEX), hot water and steam explosion, all with their own potential benefits and drawbacks. Acid (Sun and Cheng, 2005) and alkali pre-treatments (Hu and Wen, 2008) require relatively lower processing temperatures but had the drawback of requiring a further step to neutralise the acid/alkali before moving on the further steps as well as having to deal with high salt concentrations, removal and disposal. Similarly AFEX (Holtzapple et al., 1991) required additional steps as it utilises heated ammonia. Hot water treatments (Mosier et al., 2005) require higher temperatures (around 200 °C) but no additional chemicals. Steam explosion (Kokta et al., 1992) is similar to hot water pretreatment in that it uses only water but it uses high pressure steam to break down the biomass, but it can cause the formation of fermentation inhibitors as part of the process.

Waste (shredded) copier paper comprises approximately 50% (w/w) glucan (Elliston et al., 2013) and also forms a significant component of municipal solid waste (23% UK; (Defra, 2008)). It is difficult to recycle because of the reduced fibre quality (Confederation of Paper Industries, 2011) and therefore inevitably goes to landfill. Such waste is, however, a potentially good source of cellulose for second generation biorefining because the paper manufacturing process has effectively removed the lignin

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component and the bulk of the hemicellulose. Chemical pulping during paper manufacture utilises processes similar to those used as pre-treatments in second generation biofuel production (Biermann, 1993; Roberts, 1996), therefore leaving copier paper as a relatively pure cellulose substrate. As a result, waste copier paper can be readily saccharified using cellulase preparations (Elliston et al., 2013, 2014) and converted to bioethanol or other fermentation products (Elliston et al., 2013). Such exploitation could help to both reduce landfill use, the cost of which is rapidly increasing, and help meet demands for renewable energy sources (European Commission, 2010).

Recently, Zhao et al. (2015) demonstrated that the ease of saccharification of cellulose from duckweed (a low lignin dicotyledonous aquatic plant) was significantly improved by steam explosion. Furthermore, Jacquet et al. (2011) demonstrated that pure cellulose could be significantly degraded by steam explosion. The aim of this study was to evaluate whether or not thermophysical pretreatment could improve the biorefining of waste copier paper. This involved assessing the effect of pretreatment on the ease of enzymatic saccharification, and secondly on the nature and extent of the production of fermentation inhibitors particularly from the small but significant levels of non cellulosic sugars present in copier paper. The results of the study demonstrated that the component inorganic filler (calcium carbonate) had a significant attenuating impact on inhibitor generation. This was explored further as a potential means to mitigate formation of inhibitors during pretreatment of other lignocellulosic biomass.

2. Methods

2.1. Materials

M-Real copier paper (The Premier Group, Birmingham, UK), Whatman No. 1 filter paper (Fisher Scientific UK Ltd, Loughborough, UK) and dust extracted wheat straw (Dixon Brothers, Norfolk, UK) were used as the substrates for these experiments, the paper substrates were shredded using a PS-67Cs (Fellowes, Doncaster, UK) cross shredder to 3.9 × 50 mm particle size (Din Security Level 3), the straw was supplied pre-shredded into lengths of approximately 40 mm. Previous work by the authors (Elliston et al., 2013) has shown composition of the paper to be as follows; 4.0% (w/w) moisture, 4.1% (w/w) starch, 46% (w/w) cellulose, 11.9% (w/w) hemicellulose, 1% (w/w) lignin and 33% (w/w) kaolin/calcium carbonate, therefore a total glucan composition of 50.1% (w/w), comparable to other literature analyses (Wang et al., 2012). Also (Merali et al., 2013) details the composition of the wheat straw; 7.9% (w/w) moisture, 37.1% (w/w) cellulose, 23.5 (w/w) hemicellulose, 0.9% (w/w) phenolic acids, 15.8% (w/w) lignin, 8% (w/w) ash.

2.2. Steam explosion

Steam explosion was carried out using pilot scale steam explosion apparatus, Supplementary Fig. S1 (Cambi, Asker, Norway), aliquots of 250 g were exploded over a range of severity factors achieved by altering residence time and temperature. Severity factors were calculated from Eq. (1) (Overend et al., 1987). The variables t being residence time (minutes) and T temperature (°C) respectively.

$$\mathrm{SF} = \log_{10}\left[t(\mathrm{min}) \cdot \exp^{\left(\frac{T(^{\circ}\mathrm{C})-100}{14.75}\right)}\right] \quad (1)$$

The steam explosion apparatus was equilibrated to required temperature prior to the addition of material in order to reduce temperature fluctuation during actual explosion. Furthermore the apparatus was pressurised and exploded several times to ensure the removal of all material before severity factors were altered.

2.3. Dry weights

Moisture content was measured using a Mettler LP-16 Infrared Dryer Balance (Mettler–Toledo Ltd, Leicester, UK).

2.4. FT-IR

A Bio-Rad 175 C FTS spectrophotometer (Bio-Rad Laboratories, Hemel Hempstead, UK) was used for experimentation, equipped with an MCT detector and Golden Gate single reflection diamond ATR sampling accessory. Samples were measured in triplicate over a range of 800–4000 cm^{-1} with a resolution of 4 cm^{-1}. Air was used as a background and 64 scans were taken for each spectrum. Triplicate spectra were averaged and the final spectra area normalised but no other manipulation was carried out.

2.5. Enzyme digestion

Steam exploded samples were weighed out to give 0.5 g dry weight in 20 g total, therefore 2.5% (w/v) substrate concentration. 200 μL Accellerase® 1500 (Genencor, Rochester, N.Y., USA) (20 FPU/g of substrate) and 40 μL βeta-Glucosidase (βG); Novozyme 188 (Novozymes Corp, Bagsvaerd, Denmark) (25 U/g of substrate) were added for digestion. Accellerase® 1500 was chosen on the basis of high enzymatic efficacy and industrial suitability. Digestions were carried out at 50 °C while shaking at 120 rpm on an orbital shaker in sodium acetate buffer (pH 5.0) with added thiomersal (0.01% w/v) to prevent microbial contamination. Further experimentation using low enzyme additions evolved the reduction of enzyme addition by a factor of ten (2 FPU/g of substrate Accellerase® 1500 and 2.5 U/g of substrate βG)

2.6. Simultaneous saccharification and fermentation

0.25 g dry weight of each sample was weighed into 20 mL glass bottles and brought to 8.8 mL with Yeast Nitrogen Base (Formedium, Hunstanton, UK) in 0.1 mol/L NaOAc buffer (5.0 pH). NCYC 2826 *Saccharomyces cerevisiae* (National Collection of Yeast Cultures, Norwich, UK) was chosen as the fermenting organism for these experiments due to its high ethanol tolerance (15–20% v/v; (CECT, 2013)). 1 mL NCYC 2826 grown in YM media (Fisher Scientific UK Ltd, Loughborough, UK), with a cell count of 6.45×10^7 cells/mL was added along with 75 μL Accellerase® 1500 and 25 μL βG, 20 FPU/g of substrate and 25 U/g of substrate respectively, giving a total volume of 10 mL liquid. A substrate blank was used to account for any residual fermentable sugars and produced ethanol transferred in the YM inoculum and enzyme addition. Bottles were incubated at 25 °C whilst being shaken at 120 rpm for 24, 48 or 120 h, then 2 mL samples are taken into gas tight screw cap tubes which were boiled to stop further fermentation/saccharification.

2.7. HPLC analyses

2.7.1. Analysis of carbohydrate by HPLC

Sugars present in the residual solid were analysed by HPLC using the Nation Renewable Energy Laboratory (NREL) procedure (NREL, 2011). Samples were filtered through AcroPrep™ 0.2 μm GHP Membrane 96 Well Filter Plates (VWR International Ltd, Lutterworth, UK) in a centrifuge (Eppendorf, UK) at 500 rpm for 10 min into a 96 deep well collection plate (Starlab, Milton Keynes, UK). The plate was sealed and loaded directly onto a Series 200 LC instrument (Perkin Elmer, Seer Green, UK) equipped with a refractive index detector. The analyses were carried out using an Aminex HPX-87P carbohydrate analysis column (Bio-Rad Laboratories Ltd, Hemel Hempstead, UK) with matching guard