Bioresource Technology 109 (2012) 173–177

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Autothermal, single-stage, performic acid pretreatment of Miscanthus *x giganteus* for the rapid fractionation of its biomass components into a lignin/hemicellulose-rich liquor and a cellulase-digestible pulp

Donncha Haverty [a], Karla Dussan [a], Anna V. Piterina [a,b], J.J. Leahy [a,b], M.H.B. Hayes [a,*]

[a] *Carbolea, Chemical and Environmental Science Department, University of Limerick, Ireland*
[b] *Materials and Surface Science Institute (MSSI), University of Limerick, Ireland*

ARTICLE INFO

Article history:
Received 11 November 2011
Received in revised form 2 January 2012
Accepted 3 January 2012
Available online 11 January 2012

Keywords:
Performic acid pretreatment
Delignification
Biorefining
Cellulose hydrolysis
Lignocellulose

ABSTRACT

A novel approach to the performic acid pulping of biomass enables effective delignification and fractionation in a time frame not achieved heretofore. An autothermal decomposition reaction was triggered when 100 mg/L $Fe_2(SO_4)_3$ in 4.0 M NaOH was added to 5% or 7.5% H_2O_2 in aqueous formic acid containing chipped Miscanthus *x giganteus*. Peroxy-decomposition resulted in pressures of 19 and 35 bar in the 5% and 7.5% peroxide liquors and reduced the lignin content in the resulting pulps to <6% within 140 and 30 min, respectively. Solubilised lignin was available for recovery from the liquor by subsequent dilution with water. Hemicellulose removal to the liquor was 68% and 89% for the 5% and 7.5% peroxide solutions. Crystalline cellulose yields were >99% and >95% and the rate of glucose release from cellulase digestion of the pulps in 24 h was more than 20-fold that for the raw Miscanthus.

1. Introduction

Although holocellulose in lignocellulosic biomass is plentiful in the biosphere, and is often available as a waste stream, it is significantly more difficult to convert than the carbohydrates of food crops to useful products by hydrolysis processes. This arises from the chemical stability conferred by the $\beta(1\rightarrow4)$glycosidic linkages and to its association with lignin within the biomass structure. Thus much of the energy and cost inputs to 2nd and 3rd generation biorefining involve disassociating the lignin from the holocellulose and presenting, particularly the cellulose, in a form that is amenable to hydrolysis.

Pretreatments have been described that include mechanical comminution, aqueous thermal swelling through steam explosion and other analogous high pressure processes, dilute acid or alkaline hydrolysis, fractionation through the use of solvents other than water (Organosolv), oxidative treatments utilising O_2, ozone, or H_2O_2 (Hayes, 2009; Hendriks and Zeeman, 2009; Kumar et al., 2009).

Formic acid (FA) has attracted significant research and commercial interest as an Organosolv medium to fractionate and potentially hydrolyse lignocellulosic material (Muurinen, 2000). Having physical properties similar to water, and being relatively stable it is amenable to recovery and recycling from upstream or downstream operations. Three general types of formosolv media have been investigated: (1), aqueous FA, optionally in combination with other low molecular weight carboxylic acids such as acetic acid; (2), aqueous FA in combination with mineral acid catalysts, principally HCl and H_2SO_4; (3), aqueous FA in combination with stronger oxidisers such as performic acid generated *in situ* with H_2O_2.

It is well established that the addition of relatively low concentrations of H_2O_2 to FA at elevated temperature generates peroxyformic acid *in situ*. In combination with the solubility of lignin in FA, the presence of performic acid, peroxide and the peroxy radicals that derive from their decomposition partially oxidises lignin enhancing its dissolution and fractionation from the carbohydrate in lignocellulose (Dapía et al., 2000; Ligero et al., 2010; Ligero et al., 2008; Muurinen, 2000). The action of FA/H_2O_2 mixtures is utilised to produce pulps for the paper industry in the Milox process (Laamanen et al., 1990), wherein the objective is optimal reduction of Kappa number (the volume of 0.1 M $KMnO_4$ consumed by 1 g of dry pulp, indicating the degree of delignification or the bleachability of the pulp) while maximising pulp yield.

Ligero et al. (2010) investigated the optimisation of two stage performic acid pulping of Miscanthus and determined that an optimum Kappa number (17.2) and pulp yield (54.5%) was achieved with a first treatment of 90% FA and 1.5% peroxide at 67 °C for 60 min. A second stage treatment for 30 min at the same temperature and peroxide concentration was predicted to further reduce the Kappa number to 3–5 with little degradation in the quality of the pulp. Higher peroxide concentrations oxidised lignin to

---

* Corresponding author. Tel.: +353 61 20 26 31.
E-mail address: michael.h.hayes@ul.ie (M.H.B. Hayes).

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2012.01.007

low-molecular weight lignols and phenols, and degraded the quality of the pulp. Villaverde et al. (2012) suggest that peroxide concentrations higher than 4% should not be used in the atmospheric performic acid pulping of biomass due to excessive oxidation of both the lignin and carbohydrate. However this restriction in peroxide concentration for paper pulp production may not apply in biorefining where, the objectives of a FA/H_2O_2 treatment are on the one hand, maximum lignin removal, and on the other, physical alteration of the lignocellulosic structure to a form that renders the carbohydrate more amenable to hydrolysis.

While this is not achievable at atmospheric pressure in a single stage process using low concentrations of peroxide, even with the addition of external heat, it was postulated that it may be achieved at higher peroxide concentrations without the addition of external heat, should a number of issues be resolved. Even though hydrogen peroxide and performic acid decompose exothermically to yield water and O_2 or CO_2, respectively, the kinetics of these decomposition reactions are slow, particularly at low H_2O_2 concentration. Furthermore at atmospheric conditions much of the heat released is readily consumed in vapourising the solution preventing the reactions from becoming autocatalytic.

The present study has sought to determine if the introduction of a sudden change in pH (by the addition of an alkaline solution) coupled with a decomposition catalyst (Ferric sulphate, $Fe_2(SO_4)_3$) could force H_2O_2/FA in contact with Miscanthus *x giganteus* in a closed system to become autocatalytic and thereby achieve:

1. The release of the thermal energy required to heat the system;
2. An initial aggressive oxidising environment (peroxy radicals) to break the lignin-holocellulose association and facilitate fractionation; and
3. A sufficiently short-lived aggressive oxidising environment, thus avoiding over oxidation of both the solubilised components and the remaining pulp.

The information provided by this approach will be especially important for the removal of lignin from biomass for biorefining by acid and enzymatic hydrolysis. The liquor and solid phases derived from these preliminary investigations are discussed, and the influences of the pretreatment on the enzymatic hydrolysis of the cellulose component are investigated. To the best of our knowledge this is the first detailed laboratory investigation of the degradation behaviour of Miscanthus under such high pressure, autothermal conditions.

2. Methods

Roughly chopped Miscanthus *x giganteus* (85% >2 cm), a fast growing perennial woody type grass, was sourced from JHM Crops Ltd. (www.jhmcrops.i.e.), in Adare, Co. Limerick, Ireland (52°33'50" N 8°47'24"W) and used without further size reduction. Formic acid 98% w/w, sodium hydroxide, ferric sulphate, and 30% w/w H_2O_2 were of analytical grade and supplied by Lennox Laboratory Supplies, Dublin, Ireland.

2.1. Pretreatment procedure

Miscanthus (300 g) was mixed with liquor (2700 g) and sealed in an 8 L Parr reactor, with a maximum operating pressure of 130 bar (13 MPa) and modified with additional ports. The liquor was made up such that the total mass (liquor and biomass) was 2.5%, 5% and 7.5% w/w with respect to H_2O_2, using the requisite amount of 30% aqueous peroxide solution in each case, and adjusting the formic acid weight fraction accordingly. The reactor was equipped with a stirrer which operated at 1500 rpm. A solution of NaOH (125 mL, 4 M, containing 100 mg/L $Fe_2(SO_4)_3$) in a charging vessel fixed to the reactor was injected to the mixture at time zero by means of nitrogen back pressure. The temperature and pressure of the contents were monitored and logged to a PC. The reactor was fitted with a liquid sampling port through which aliquots (20 mL) were removed at regular intervals to determine the temporal composition of the liquor. The solids content at the end of each run was filtered, washed with FA and water, and dried at room temperature to a constant weight.

2.2. Analytical procedures

As it was expected that the pretreatment would remove lignin and hemicellulose fractions from the Miscanthus, the extent of delignification and hydrolysis was monitored by measuring the lignin and monosaccharide content of the liquor during the course of the experiments. The recovered solids (pulps) after treatment were analysed to quantify their C6 sugar, C5 sugar, lignin, ash and extractives content enabling determination of the complete mass balance for each set of experimental conditions investigated. Laser Scanning Confocal Microscopy (LSCM) of the biomass and pretreated pulps provided confirmation of the extent of lignin removal and provided additional insight to the alterations effected in the biomass by the various pretreatments. The morphology of the cellulosic pulps on a micro- and macro-level, (fibre length, width, homogeneity and surface degradation) was further elucidated using Scanning Electron Microscopy (SEM). To determine the influence of the pretreatments on the crystallinity of the pulps X-ray Diffraction (XRD) was utilised to measure the crystallinity index (CI) of the pulps using standard protocols. The accessibility of the pretreated pulps towards enzymatic hydrolysis was studied *in vitro* using a typical mixture of cellulose enzymes and the raw material as a comparative control. Lignin recovered from the liquor after the pretreatment was characterised by FTIR to elucidate any chemical changes effected in the lignin under the varying pretreatment conditions used. Individual analytical procedures used are described fully in Appendix A of the Supplementary data.

3. Results and discussion

3.1. Thermal potential of the peroxide/FA mixtures

The temperature profiles of the FA and 2.5%, 5.0% and 7.5% peroxide mixtures are shown in Fig. 1 *a–c*, respectively. Insufficient peroxide was available for the decomposition reaction to become autocatalytic at the 2.5% loading. Consequently the maximum temperature reached was only 45 °C after 4 h of reaction. However, at higher peroxide concentrations the decomposition reactions became autocatalytic and significant heat was released. Additional information regarding the theoretical amount of energy released due to hydrogen peroxide decomposition at the concentrations investigated is given in Appendix B of the Supplementary data. In both the 5.0% and 7.5% peroxide concentrations, the temperature curve is characterised by a rapid increase in temperature followed by a plateau at or about the boiling point of the FA/water azeotrope (107 °C). The reactor system was not insulated, but the duration of the plateau at 7.5% H_2O_2 as compared with 5.0% is consistent with more heat being released for the 7.5% concentration. Furthermore, the pressure profiles for both concentrations are consistent with the calculated pressures arising from the expected amount of oxygen released from the decomposition of H_2O_2 (max. pressure 19 and 35 bar, respectively), indicating that peroxide decomposition is the dominant reaction.

A temperature fluctuation is observed following the rapid heat-up phase of the 5.0% and 7.5% peroxide treatments, coincident in