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

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 Fe₂(SO₄)₃ in 4.0 M NaOH was added to 5% or 7.5% H₂O₂ 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 β(1→4)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 O₂, ozone, or H₂O₂ (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 H₂SO₄; (3), aqueous FA in combination with stronger oxidisers such as performic acid generated in situ with H₂O₂.

It is well established that the addition of relatively low concentrations of H₂O₂ 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 (Dapia et al., 2000; Ligero et al., 2010; Ligero et al., 2008; Muurinen, 2000). The action of FA/H₂O₂ 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 KMnO₄ 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...
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/H2O2 restriction in peroxide concentration for paper pulp production at atmospheric performic acid pulping of biomass due to excessive concentrations higher than 4% should not be used in the behaviour of Miscanthus under such high pressure, autothermal this is the first detailed laboratory investigation of the degradation cellulose component are investigated. To the best of our knowledge influences of the pretreatment on the enzymatic hydrolysis of the derived from these preliminary investigations are discussed, and the importance for the removal of lignin from biomass for biorefining was made up such that the total mass (liquor and biomass) was.

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 O2 or CO2, respectively, the kinetics of these decomposition reactions are slow, particularly at low H2O2 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, Fe2(SO4)3) could force H2O2/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.ie), 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 H2O2 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 H2O2, 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 Fe2(SO4)3) in a charging vessel fixed to the reactor was injected to the mixture at zero time 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 cellulose 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% H2O2 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 H2O2 (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
both cases with the observation of both lignin and hemicellulose sugars in the liquor. In view of the morphology of the pulps recovered from both pretreatments, this would suggest an almost instantaneous collapse in the secondary structure of the Miscanthus at this point. The time to this disruption, was ascribed to and indicates completion of the decomposition of the peroxo species present. In addition, marks the commencement of both lignin and sugar release to the liquor in each case.

3.2. Lignin and hemicellulose sugar distribution across the liquor and the pulp

The composition of the pulps produced under the conditions investigated is given in Table 1. Comprehending the composition and mass of the starting biomass and the pulp yield at the end of each experiment cellulose recovery, hemicellulose removal and lignin removal were quantified and are given as percentages in Fig. 1. Fig. 1 also shows the temporal evolution of the lignin and sugar concentrations in the liquor expressed as a % of the initial concentrations present in the starting raw material. The treatment with 2.5% H₂O₂ reduced the lignin content of the starting Miscanthus by 79% (Fig. 1) after 25 h, yet only 20% of the initial lignin was detected in the liquor at this time. This can be associated with the oxidation of the solubilised lignin to lignols and lower molecular weight compounds. Because the system did not become auto-catalytic it is hypothesised that peroxide, and by extension oxidising peroxy radical species, were active in the liquor for extended periods. This was evinced by a progressive diminution in the dark.

---

Table 1
Characterisation of untreated Miscanthus and pulps produced after treatment with FA/H₂O₂ at different initial hydrogen peroxide concentration.

<table>
<thead>
<tr>
<th>Component</th>
<th>Untreated Miscanthus</th>
<th>Peroxide concentration w/w %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.50</td>
</tr>
<tr>
<td>Glucose</td>
<td>40.31</td>
<td>52.4</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.64</td>
<td>0.53</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.25</td>
<td>0.17</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>0.21</td>
<td>0.13</td>
</tr>
<tr>
<td>Total C6</td>
<td><strong>41.41</strong></td>
<td><strong>53.23</strong></td>
</tr>
<tr>
<td>Xylose</td>
<td>19.38</td>
<td>22.72</td>
</tr>
<tr>
<td>Arabinose</td>
<td>2.15</td>
<td>2.11</td>
</tr>
<tr>
<td>Total C5</td>
<td><strong>21.53</strong></td>
<td><strong>24.83</strong></td>
</tr>
<tr>
<td>K-Lignin</td>
<td>21.79</td>
<td>5.95</td>
</tr>
<tr>
<td>Extractives</td>
<td>1.81</td>
<td>2.00</td>
</tr>
</tbody>
</table>
colour of the 2.5% liquor aliquots when stored under ambient conditions and by the difficulty of recovering significant amounts of lignin from that liquor.

Only 13% of the starting hemicellulose was solubilised after the 2.5% H₂O₂ treatment, based on analysis of the recovered pulp (Fig. 1). The low concentration of sugars in the liquor would indicate that the hemicellulose was not readily hydrolysed to monomeric sugars, as might be expected in view of the moderate temperature.

In contrast the behaviour of the system is markedly different under autocatalytic conditions (5.0% and 7.5%) wherein initial peroxide concentration had a significant influence on the extent and rate of lignin and hemicellulose removal. The same overall temporal pattern is observed for the 5% and 7.5% peroxide concentrations. Dissolution of lignin and of carbohydrate into the liquor commences at τₐ, and concentrations of both in the liquor increase rapidly before levelling off at relatively constant values. The time to reach maximum C₅ sugar concentration and maximum lignin concentration in the liquor, ascribed to τₐ, is coincident in both cases. Approximately 65% of the lignin initially contained in the Miscanthus is detected in the liquor after 30 and 140 min in the 7.5% and 5% peroxide liquors, respectively. This increase in the rate of dissolution with increasing peroxide concentration is ascribed to an increase in both the initial oxidising potential and the heat release due to the higher peroxide load. Importantly, and in contrast to the 2.5% peroxide liquor, the aliquots removed following τₐ in both the 5.0% and 7.5% peroxide liquors did not oxidise further on storage in ambient conditions, indicating that a complete decomposition of the peroxide species present had occurred at τₐ.

Analogously significant lignin contents were readily recovered by precipitation from these liquors. An exemplary determination of τₐ and τₐ,max for the 7.5% liquor is shown in Fig. 1d. The present work was undertaken in a batch reactor and the decomposition commenced from a standing cold start, however once the temperature reached 40 °C the decomposition reaction rate became essentially exponential. In a continuous high pressure reactor (plug flow reactor) operating at steady state, it is likely that the required residence time in a high pressure environment could be reduced to as little as 5 min.

Hemicellulose removal from the pulp increased from 13 to 68 and to 89%, respectively, for the 2.5%, 5.0%, and 7.5% initial peroxide concentrations as determined by the concentrations of the component sugars in the pulp. That is attributed mainly to the greater amount of heat released by the higher initial concentrations of peroxide, as well as to the increased oxidising potential of the liquor. The concentrations of higher order oligomers, of furfural, and/or of parallel condensation reaction products were not determined in this study. As expected, the recovery of monomeric sugars from the liquor increased with increasing initial peroxide concentration.

3.3. Characterisation of recovered lignin

FT-IR spectra were obtained for the characterisation of the lignin recovered at the different initial peroxide concentrations (additional Annex C of the Supplementary data). All the spectra contained absorbance bands characteristic of the aromatic molecular building blocks of p-hydroxyphenyl (H), guaiacyl (G), and syringyl groups (S) of lignin including the bands at 1590–1615 cm⁻¹, 1250–1260 cm⁻¹, 1110–1120 cm⁻¹ and 1025–1030 cm⁻¹, as previously assigned by Lin et al. (1992). However, the lignin precipitated from the 2.5% peroxide treatment had significant differences from the lignin precipitated from the pretreatments run under autocatalytic conditions (5.0% and 7.5% H₂O₂). The band at around 1100 cm⁻¹, which is ascribed to C–O groups, has a higher intensity in the autocatalytic lignins compared to the 2.5% lignin. Given that the ether functional group is the principal intra-molecular H, G and S link in the extended lignin molecule, this band may be interpreted as an indication of the degree of lignin oxidation to lower molecular weight lignols. These results are consistent with the observations made during the storage of the samples at ambient conditions and the difficulty of recovering significant amounts of lignin by precipitation due to the continuous degradation in the 2.5% liquor.

Interestingly, the increased oxidation of the lignin at the low peroxide concentration relative to the high peroxide concentrations seems counter intuitive, given the higher oxidation potential of the latter. However, when sufficient peroxide is initially present to force the decomposition reactions to become autocatalytic, complete decomposition of peroxide is achieved in a short time frame, by tₐ, and subsequently the strongly oxidising peroxy species are no longer present in the liquor to degrade the solubilised lignin.

3.4. Cellulose recovery and characterisation

Recovery of cellulose from the raw Miscanthus at 2.5%, 5.0% and 7.5% initial peroxide concentration was 99.7%, 99.6% and 95.63%, respectively, as measured by the glucose content in the starting material and in the pulps, Fig. 1. The slight increase in glucose in the liquor at the highest peroxide treatment is attributed to some degradation of the amorphous cellulose fraction under the more oxidising conditions and elevated temperature. Fig. C-2 (Annex C, Supplementary data) shows SEM and LSCM images of the raw material and pulps recovered following treatment with the different initial peroxide concentrations. The morphologies of the pulps derived under autocatalytic conditions, as in 5.0% and 7.5% was altered relative to the starting material and the 2.5% pulp. The gross morphology of the pulp from the 2.5% peroxide treatment was similar on a macro level to that of the initial biomass, although there was some evidence of swelling of the plant structure at the micro level. In contrast, autocatalytic conditions gave rise to a pulp comprised of largely carbohydrate fibres with an approximate diameter of 10 microns. Likewise the LSCM analysis of the autofluorescence signature of the pulps confirmed increased lignin removal with increasing initial peroxide concentrations (the lignin signal was collected and shown in the blue/red spectral channels under the analytical protocol used; see Fig. C-2 of Annex C). Figure shows clearly a decrease in the red/blue fluorescent signal in going from the raw material to the 7.5% treated biomass.

The CI of the pulps was determined (Appendix B, Fig. B-1), and are compared with the CI of the starting raw material in Table 2. Values of CI calculated by the method of Segal et al. (1959) were significantly higher than those calculated by the method of Park et al. (2010). Observed variations in CI were small regardless of the pretreatment, and ranged from 69.6% for the raw material to 62.9% for the 5.0% peroxide-treated pulp. Overall, the trend of decreasing CI in going from the raw material to the 2.5% and 5.0% treated materials is consistent with the swelling of the biomass and some disruption in the hydrogen bonding within the cellulose structure. However, this trend was reversed when the peroxide concentration was increased from 5.0% to 7.5% (in the case of the latter, the CI was 66.7%). This fact is attributed to a depolymerisation of some of the amorphous cellulose in the increasingly aggressive environment, leaving a greater fraction of crystalline cellulose in this pulp. The increased amount of glucose (3.5% w/w of that in the starting material, Fig. 1c) observed in the 7.5% peroxide liquor is evidence of this depolymerisation, as is the LSCM analysis of the 7.5% pulp which confirms the commencement of the degradation of the surface of the cellulose fibrils (Fig. 2C l).

3.5. Enhanced enzymatic digestibility of the cellulosic pulps produced under auto-catalytic pretreatment conditions

Fig. 2 compares the enzymatic digestibility of the pulps produced under autocatalytic conditions, i.e. 5.0% and 7.5% with those
of the raw material and Avicel at 45 °C. While the digestibility of the raw material was low, the cumulative glucose release measured after enzymatic hydrolysis for 24, 48, and 72 h, reveals that the pulps were highly amenable to enzymatic hydrolysis. Despite the fact that all the pulps and the raw material had a similar CI (Table 2), a 20-fold increase in the rate of glucose release was observed for the pulps compared with the raw material under the same conditions of biomass and enzyme loadings. The overall enhancement of the pulp digestibility is attributable to the delignification, homogenous size distribution, and high surface area of the fibrous material. Several factors affecting the absolute enzymatic hydrolysis rate were outside the scope of the present study and were not optimised. These include: (1) solids loading; (2) enzyme loading; (3) the effect of various additives; (4) the enzyme cocktail used; and (5) the temperature and duration of digestion.

In summary, the novel FA/peroxide process described here effectively transforms lignocellulosic biomass into cellulosic fibres simultaneously removing lignin and hemicellulose to the liquid phase. The autothermal process can greatly reduce the energy consumption associated with typical hydrolysis processes and physical comminution operations required for biomass conversion. Potentially, these advantages offer a more feasible route to second and third generation biorefinery process that are economic, sustainable, and efficient.

4. Conclusions

Miscanthus can be fractionated in 30 min by autocatalytic decomposition of hydrogen peroxide (7.5% w/w) and formic acid, and with no external energy input, into a lignin/hemicellulose-rich liquor and a fibrous pulp. Lignin and hemicellulose removal (89% and 88% respectively) yielded a pulp with higher enzymatic digestibility than untreated Miscanthus (up to 20-fold). Lignin was recovered from the liquor in a further aqueous dilution step. FA/peroxide under autocatalytic conditions can reduce the energy consumption required in physical comminution operations. The fibrous nature of the material obtained mitigates mass transfer effects in subsequent hydrolysis of the cellulosic fraction, whether enzymatic or chemical.

Acknowledgements

The authors acknowledge financial support via a research grant from EU-FP7-DIBANET project and via the a Postdoctoral Fellowship (A.V.P.) from the Enterprise Partnership Scheme co-funded by the Irish Research Council for Science, Engineering and Technology (IRCSET) (www.ircset.ie).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2012.01.007.

References

Muurinen, E., 2000. Organosolv pulping, a review and distillation study related to peroxyacid pulping, Department of Process Engineering, University of Oulu, Oulu, Finland.