Impact of mixed feedstock of wheat straw, willow and Miscanthus on enzymatic hydrolysis and inhibitor production after microwave hydrothermal pre-treatment in Europe

Kamaljit Moirangthem†, Darren Greetham‡, Jim Craigon and Gregory Tucker

School of Biosciences, University of Nottingham, Loughborough, UK

ABSTRACT
Lignocellulosic feedstocks for biorefinery are likely to be seasonal and the supply of feedstock to cellulosic biorefineries remains a challenge. One way to overcome this is by utilizing a mixed feedstock which facilitates the maintenance of a year-round feedstock supply. This study investigated the impact of mixing of three industrially relevant lignocellulosic feedstocks—wheat straw, willow and Miscanthus using two major performance indicators—sugar yield and fermentation inhibitor production. A microwave hydrothermal pre-treatment regime of 200°C for 5 min was applied to each feedstock individually and to 1:1 (w/w) mixes and the predicted sugar yield in the mixes was compared to the observed values. All the mixes resulted in improved sugar yields with willow + Miscanthus (15.4%, p = 0.015) and wheat + willow (13.6%, p = 0.010) showing a statistically significant improvement. Saccharification kinetics, inhibitor production, impact on yeast metabolic activity and growth were compared and no adverse impacts of mixing were observed. The use of mixed feedstocks in a hot water based commercial production of biofuels is unlikely to have any adverse effects on productivity and may indeed prove beneficial.

Introduction
Lignocellulosic biomass, such as energy crops and agricultural residues represent a potential biorefinery feedstock for the production of fuel or other chemicals. Some of the challenges to making this economical are: (i) to secure a year round sustainable supply of feedstock as they are likely to be seasonal with annual variability in yields [1] and may thus have limited availability and supply [2], (ii) overcoming recalcitrance of the feedstock in terms of saccharification to fermentable sugars that requires pre-treatments to disrupt the lignocellulose matrix [1], and (iii) reducing inhibitory compound formation during pre-treatment which could interfere with any subsequent enzymatic hydrolysis and fermentation steps [3].

Microwave technology as a pre-treatment for lignocellulosic biorefineries is becoming popular [4]. Water is the only solvent used and the process has a faster heat transfer when compared to a conventional heating process [5]. Microwave hydrothermal pre-treatment, as utilized in this research, is generally performed by pre-treating biomass at temperatures between 140 and 220°C under high pressure to maintain water in the liquid state [6]. This treatment is mild compared to dilute acid pre-treatment, but has similar effect in terms of the chemical and physical changes that occur in the structure of lignocellulose [7]. The reaction medium becomes acidic due to the release of acetic acid and other weak acids derived from hemicellulose and to the auto-dissociation of water at elevated temperatures. The release of acetic acid from biomass facilitates the solubilisation of hemicellulose, and the formation of monomeric sugars and sugar degradation products or inhibitors such as 5-hydroxyl methyl furfural (HMF), furfural, formic acid or levulinic acid [8]. The breakdown of lignin also releases phenolic compounds which at certain concentrations can be inhibitory to a subsequent microbial fermentation [9].

Amongst the inhibitory compounds furfural and HMF are often used as representative for the general content of inhibitory compounds [10]. They have been subjects of extensive investigation due to their negative effects on microbial physiology [11], and are considered major inhibitors in microbial conversion processes [12]. Presence of furfural or HMF inhibits the growth of yeast cells and subsequent fermentation in a dose-dependent manner [13]. Although furfural and HMF can act synergistically, yeast cells are more sensitive to growth inhibition by furfural than by HMF at the same concentration [14–16]. However, their effects on yeast are dose dependent [17]. Hence, their concentration after pre-treatment is an essential parameter to be considered.

Literature review
There have been limited studies on the effect of mixing feedstocks on process efficiency and inhibitor...
concentrations [18]. However, mixed feedstocks are increasingly being considered for lignocellulosic biofuel production [19] as they could potentially buffer the variation seen with a single feedstock and have been shown to provide more economic advantage [20, 21]. One study looked at the effect of mixing municipal solid waste paper and corn stover in a 1:1 ratio using an ionic liquid pre-treatment [22]. This study monitored the effect of mixing on saccharification and reported a slight improvement in glucose yield. Another study mixed eucalyptus residues, wheat straw and olive tree pruning in a 50:25:25 ratio using a hydrothermal pre-treatment and monitored sugar recovery in both the pre-treated biomass, liquor and inhibitor composition [23]. This study showed that mixtures of lignocellulosic materials can be efficiently processed by autohydrolysis and generate a consistent product composition that was independent of the different proportions of each feedstock. Concentration of inhibitors was also reported to be low for the three different mixes (furfural 0.33–0.49 g/L and HMF 0.17–0.29 g/L). A further study examined the effect of mixing aspen, balsam and switch grass in 1:1 ratios using an acid pre-treatment and monitored saccharification and inhibitor composition and found glucose yields for the mixed feedstocks were very similar to the predicted values [24]. Oke et al. provided a more in depth study on the prospects and challenges of mixed feed stock biorefineries [18]. While Shi et al. obtained a 90% sugar yield after 24 h of saccharification of ionic liquid pre-treated mixed feedstocks of equal portion of switchgrass, lodgepole pine, corn stover and eucalyptus—in flour and pellet form [25]. Furthermore, Baral et al. through supply and value chain analysis determined that a mixing ratio of corn stover, Miscanthus, and switchgrass of 36%, 50%, and 14%, respectively, minimized the selling price of sugar [26].

Within the context of a UK or EU biorefinery, the major feedstocks are likely to include agricultural waste (wheat straw) and dedicated energy crops such as willow and Miscanthus. Wheat straw is a promising feedstock for bioethanol production in Europe due to its large production and high carbohydrate content [27] and majority of agricultural straw is derived from wheat in nearly all regions of the UK, except Scotland where barley prevails [28]. For bioenergy crops in Europe, research has been focused on Miscanthus [29] with the sterile hybrid Miscanthus × giganteus currently being the main commercially exploited species of this genus for biomass purposes due to its high yield potential. Willow has many desirable characteristics for feedstock and biomass production. With its coppicing ability and vigorous juvenile growth, it can produce high yields. With its coppicing ability and vigorous juvenile growth, it can produce high yields. Willow—tor a and Miscanthus—goliath were kindly provided by Prof Iain Donnison (Aberystwyth University, UK). Willow straw was air-dried, while willow and Miscanthus were oven dried at 60 °C until dry weight was constant, prior to milling. The three feedstocks were milled separately to 2 mm mesh size using a FRITSCH Pulverisette 19 knife mill and stored at 4 °C in bags with airtight seals.

**Composition analysis**

**Sugar analysis**

Sugar composition was measured following Saeman hydrolysis [31]. 1 mL of 12 M H2SO4 was added to 30 mg of sample at 37 °C for 1 h. This was followed by addition of 11 mL of MilliQ water (reducing the molarity to 1 M) and incubation at 100 °C for 2 h. The hydrolysate was analyzed using High Performance Anion Exchange Chromatography (HPAEC).

**HPAEC—high performance anion exchange chromatography**

Analysis of sugars was performed on a Dionex ICS-3000 comprised of a high-pressure GD 50 gradient pump, a guard column (Carbopac PA1, 4 mm × 50 mm), an analytical column (Carbopac PA20, 4 mm × 250 mm) and a pulsed amperometric detector (PAD). 10 mM NaOH was used as the mobile phase and the column was flushed with 200 mM NaOH between runs. All chromatographic analyses were carried out at 30 °C with a flow rate of 1.0 mL min⁻¹. Samples were diluted 1:100 in 10 mM NaOH and centrifuged at 4472 x g for 10 min before loading to Dionex vials. Sugar standards ranged from 0.250 to 2 g L⁻¹ of arabinose, galactose, glucose and xylose, respectively.

**Microwave hydrothermal pre-treatment and enzymatic saccharification**

**Microwave hydrothermal pre-treatment**

Pre-treatment was conducted in a Monowave 300 microwave generator (Anton Paar, Germany). 1 g of sample was mixed with 10 mL of MilliQ water, left at room temperature for 2 min and then heated at different combinations of temperature and holding times ranging from 180–220 °C in steps of 20 °C and 5–20 min in steps of 5 min, respectively. The resultant pre-treated mixture was centrifuged for 10 min at 4472 x G to decant the hydrolysate. The hydrolysate composition was analyzed using HPAEC and after measuring its pH, it was stored at -20°C. The residue was air dried for 72 h, weighed and stored at 4 °C for further analysis.

Pre-treatment severity (log R0) was calculated using Eq. (1) [32]:

\[
R_0 = t \times e^{-\frac{(T-100)}{14.75}},
\]

where \(t\) is the time (minute) and \(T\) the temperature (°C).

**Materials and methods**

**Feedstock**

Wheat straw—Revelation—was obtained from the Sutton Bonnington farm (University of Nottingham, UK). Willow—tora and Miscanthus—goliath were kindly provided by Prof Iain Donnison (Aberystwyth University, UK). Willow straw was air-dried, while willow and Miscanthus were oven dried at 60 °C until dry weight was constant, prior to milling. The three feedstocks were milled separately to 2 mm mesh size using a FRITSCH Pulverisette 19 knife mill and stored at 4 °C in bags with airtight seals.

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**Enzyme saccharification**

Pre-treated biomass residue, produced as described above, was hydrolyzed using Cellic® CTEc3 (kindly provided by Novozyme A/S, Denmark). This was carried out using a slight modification to the method described by the National Research Energy Laboratory (NREL) [33]. Enzyme solution was made up using 5 mL of Cellic® CTEc3 in 1 L of 50 mM Sodium citrate buffer at pH 3.76. 40 mL of this solution was added to 200 mg of sample and incubated at 50 °C for 72 h at 150 rpm (MaxQ 4358 shaking incubator, Thermo Scientific, UK). This level of enzyme loading was used to avoid enzyme being a limiting factor in the digestion. Aliquots were taken at 6 different time points—15 min, 3 h, 6 h, 24 h, 33 h and 72 h and monomeric sugar composition assessed using HPAEC. The sugar concentration present in the enzyme solution was also analyzed and subtracted to allow accurate calculation of % glucose released.

Kinetics analysis of the enzyme hydrolysis was carried out assuming the reaction followed first order behaviour. This was found to be valid for the data sets of this study, thus avoiding the need for more complex kinetic algorithms, and allowing simple comparison of kinetic parameters [34]. A non-linear optimization method was used to fit a simulated curve to the experimental points using the Solver function in Microsoft Excel 2016. A function was used describing a single first-order exponential growth process, according to Eq. (2), where S is the glucose yield at time t, x is the rate constant and A is the final equilibrium glucose yield.

\[ S = A(1 - e^{-xt}) \]  

(2)

**Inhibitor composition**

Inhibitors furfural and 5-hydroxyl methyl furfural in the hydrolysate were measured by HPLC using UV detection at 280 nm (2695 HPLC system and 996 Photodiode Array Detector, Waters, USA) with UV spectra for secondary confirmation [36]. The chromatographic conditions were as follows: Techsphere ODS C18 column (5 μm, 4.6 mm × 250 mm; HPLC Technologies, UK) at ambient temperature. The mobile phase was a binary mixture of 1% acetic acid (solvent A) and methanol (solvent B) with an overall flow rate of 1.0 mL min⁻¹ and compounds were detected at 280 nm. The system was operated in gradient mode, ramping from 20% to 50% methanol over 30 min with a 100% methanol column cleaning phase and a 9-min re-equilibration period. The sample injection volume was 10 μL. Data were recorded using Millennium Chromatography software (Waters, USA). Quantification was performed by comparison of peak areas of authentic standards (0.025–0.4 g/L concentration range) of HMF and Furfural.

**Experimental design and statistical analysis**

For the laboratory analysis, samples of each milled feedstock were placed in three separate test tubes. Similarly each 50:50 mixture of feedstocks was also made up in three separate test tubes. Thereafter, the material from each single feedstock or mixture tube was processed, measured and recorded separate and independent from the material in the other tubes. There were thus 18 tubes of material representing a completely randomised design with 6 treatments [the 3 single and 3 mixed feedstocks] each with 3 replicates.

The data were analysed by one-way Analysis of Variance (ANOVA) using Genstat 18.1 (VSN International Ltd). For the comparison of observed and expected values, three predefined contrasts were included in the ANOVA to directly compare each mixed feedstock to the average of its two components when they were used alone. A significant effect would indicate that the components were responding differently when mixed with the same feedstock.

**Results and discussion**

In the first instance, a screen was carried out to assess the impact of various hydrothermal pre-treatment conditions on the saccharification of wheat straw, willow and Miscanthus individually (Additional file 1: Figure S1–S3). The impact of the various pre-treatments on the sugar composition of the residue, and release of sugars into the...
hydrolysate liquor was similar in all three feedstocks. As expected, there was an increased loss of xylose into the liquor as severity of treatment increased and, in all cases, there was little impact on glucose retention in the residue. Severity factors for this scoping work are shown in Additional file 1: Table S1 and ranged from 3.05–4.84. Although pre-treatment at 180 °C for 20 min had a higher severity factor (3.66) than at 200 °C for 5 min (3.64), the latter resulted in higher saccharification. The same was true for 200 °C for 20 min (4.25) compared to 220 °C for 5 min (4.23). This indicates that the effect of temperature was more important than residence time for reduced recalcitrance, which was also suggested previously by Alvira et al. using wheat straw [27].

From this scoping experiment a pre-treatment regime of 200 °C for 5 min was selected and this was applied to each feedstock individually and to 1:1 (w/w) mixtures. This was a severity that resulted in around 50–60% glucose digestibility in all three feed stocks and as such would allow any synergistic enhancement of digestibility by mixing to be detected.

**Effect of mixed feedstocks on sugar composition of pre-treated residue**

Sugar composition of the residues from individual pre-treated feedstocks was measured (Figure 1). These values were then used to calculate expected compositions from 1:1 mixes and these compared to those obtained experimentally (Figure 1).

The sugar composition of the residue obtained after pre-treatment did not alter significantly when compared to their expected values, and glucose compositions of all the 3 mixes were at a similar level. Silva-Fernandes et al., reported similar findings: mixtures of lignocellulosic materials processed by auto hydrolysis generated consistent product composition independently of the different proportions of each feedstock [23].

**Enzyme saccharification**

For each case, the impact on saccharification of the resultant residue was determined by monitoring glucose and xylose release (Figure 2). Enzyme saccharification focussed on the yield of major sugars (glucose and xylose) as the impact on yields would be more apparent for the major sugars. Experimental glucose and xylose yields of the mixes were then compared against theoretical expected values (Table 1). For the individual feedstocks, it can be seen that wheat straw was the least recalcitrant followed by willow and then Miscanthus. ANOVA test between the 3 individual feedstocks showed that their responses to the pre-treatment were statistically different for wheat versus willow (p = 0.0013) and wheat versus Miscanthus (p = 0.0005) but, not for willow versus Miscanthus (p = 0.0763), respectively.

When the observed glucose and xylose yields after 72 h of the 3 different mixes were compared with their respective theoretical yields (Table 1), the observed yields were found to be higher than expected yields in all the cases. Since the results in Figure 1 showed very little differences in the sugar composition of the pre-treated residues, it is unlikely that this is the reason for the observed improvements in saccharification. The cause may therefore be due to a reduction in recalcitrance possibly due to improved response to pre-treatment brought about by the mixed nature of the feedstock. Microwave radiation has been known to result in a decrease of cellulose crystals size enhancing lignocellulose hydrolysis [4].

The Miscanthus + willow with an expected 0.127 g glucose/g original biomass and an observed 0.147 g glucose/g the observed glucose and xylose yields after 72 h of the 3 different mixes were compared with their respective theoretical yields (Table 1), the observed yields were found to be higher than expected yields in all the cases. Since the results in Figure 1 showed very little differences in the sugar composition of the pre-treated residues, it is unlikely that this is the reason for the observed improvements in saccharification. The cause may therefore be due to a reduction in recalcitrance possibly due to improved response to pre-treatment brought about by the mixed nature of the feedstock. Microwave radiation has been known to result in a decrease of cellulose crystals size enhancing lignocellulose hydrolysis [4].

The Miscanthus + willow with an expected 0.127 g glucose/g original biomass and an observed 0.147 g glucose/g
original biomass; and wheat + willow mixes with an expected 0.155 g glucose/g original biomass and an observed 0.176 g glucose/g original biomass, demonstrated the largest enhancement between expected and observed values these being 15.4% and 13.6%, respectively—both being statistically significant at the 5% probability level (p = 0.015** and 0.010**, respectively). For the wheat + Miscanthus mix while the actual glucose yield of 0.165 g glucose/g original biomass was still higher than the expected 0.151 g glucose/g original biomass, there was no evidence of an effect (p = 0.065).

The observed xylose yields from all three mixtures were again higher than expected however, in the case of the two mixtures—Wheat + wheat and wheat + willow + wheat, there was no evidence of an effect (p = 0.499, 0.255, respectively). Only the mixed Miscanthus and willow feedstock showed any significant enhancement in xylose yield (11.8%, p = 0.019**).

Previous studies reported glucose yields of 84% from municipal solid waste paper and corn stover mix after ionic liquid pre-treatment compared to a 82.2% expected, with a glucose yield of 52.51% compared to 2.2% expected [24]. However, Aspen and switch grass in the same study gave a glucose yield of 52.51% compared to an expected value of 53%, with an observed xylose yield of 3.81% compared to the expected 4.8%.

### Saccharification kinetics

Data shown in Figure 2 was used to calculate rate constants describing the kinetics of glucose release for either individual feedstocks or their 1:1 mix (Table 2).

For the individual feedstocks, wheat straw and Miscanthus showed the fastest saccharification rates (p = 0.0549). In contrast, willow showed a significantly slower rate of glucose release than either of the other two feedstocks [wheat vs. willow (p = 0.001) and willow vs. Miscanthus (p = 0.0006)]. This could be attributed to the differences in their cell wall composition, where willow has a higher hemicellulose and lignin content compared with wheat straw and Miscanthus [28] or higher bulk density of willow (210 kg/m³) compared to Miscanthus (180 kg/m³) and wheat straw (20 kg/m³) [37]. Mixing wheat and Miscanthus showed no evidence of an effect on the rate constant for glucose release. The rate of release for the wheat and willow mix was intermediate between that for the two feedstocks individually while, the rate for the willow and Miscanthus was similar to that for the willow alone. Thus, the enhancement in glucose yields seen above (Table 1) when willow was mixed with either of the two ‘grasses’, is not associated with an increased rate of initial release.

### Effect of mixed feedstocks on inhibitor production

The impact of the pre-treatment liquors (hydrolysates) on the metabolic activity and growth of yeast was assessed. Concentrations of two major inhibitors furfural and HMF in the pre-treated liquor were also measured (Table 2). Studying both yeast metabolism and growth is crucial as inhibitory compounds could result in either (i) a reduction in the conversion of sugars into biomass (growth) or (ii) reduced metabolic output, or both simultaneously. It has also been shown that yeast metabolism does not always correlate with growth [35].

For the individual feedstocks, the inhibitory impact on metabolism ranged from about 30% inhibition for Miscanthus to an actual apparent stimulation in the case of willow (Table 2). This observed stimulation may be related to an increased metabolic response to stress. In terms of the mixed feedstocks the observed inhibitory responses were in the same range as for the individual feedstocks.

The impact on yeast growth is probably a better indicator on the potential impact of the liquor on yeast performance than metabolism (Table 2). In this case, wheat demonstrated the greatest inhibition and willow again had the least effect. In contrast to the effect on metabolic rate, the impact on growth was quite substantial ranging from 70 to 60% inhibition of growth as compared to the control. The range of the inhibitory effect of the liquors from the mixed feedstocks was very similar to that for the individual feedstocks.

The concentrations of two major inhibitory compounds—furfural and HMF in the liquors were analyzed (Table 2). Miscanthus had the highest level of furfural and wheat the lowest while willow had the highest level of HMF and wheat again the lowest. Amongst the individual feedstocks, willow showed the highest overall inhibitor content. This could be attributed to the higher hemicellulose content of willow cell wall [28] which resulted in a larger amount of xylose/galactose sugars for the production of these inhibitors during pre-treatment at 200 °C for 5 min. The only major difference exhibited between the observed and expected levels of these inhibitors in the mixed feedstocks was for the level of furfural in the wheat + willow

### Table 1. Comparison of observed and predicted glucose and xylose yields at 72 h.

| Categories of performance indicators | Individual feedstock | Mixed feedstock |
|--------------------------------------|----------------------|----------------|
|                                     | Wheat                | Willow         | Miscanthus   | Wheat + Willow* | Wheat + Miscanthus* | Willow + Miscanthus* |
| Glucose yield (g/g original biomass) | 0.178 ± 0.008        | 0.132 ± 0.006  | 0.123 ± 0.004 | 0.176 ± 0.012** | 0.165 ± 0.018     | 0.147 ± 0.004**    |
| Theoretical                          | n.a                  | n.a            | n.a          | 0.155           | 0.151             | 0.127             |
| % yield gain                         | n.a                  | n.a            | n.a          | +13.6%          | -9.4%             | +15.5%            |
| Xylose yield (g/g original biomass)  | 0.144 ± 0.005        | 0.082 ± 0.002  | 0.116 ± 0.001 | 0.118 ± 0.008   | 0.133 ± 0.010     | 0.111 ± 0.006**   |
| Theoretical                          | n.a                  | n.a            | n.a          | 0.113           | 0.150             | 0.099             |
| % yield gain                         | n.a                  | n.a            | n.a          | +4.6%           | -2%               | +11.8%            |

*The performance of the individual feedstocks was used to calculate an expected yield of glucose and xylose in the 1:1 mixed experiments. These were then compared to the observed yields. Results are expressed as g/g of original biomass and are the mean ± SD (n = 3), p > 0.05 are not shown as they were not deemed significant; *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.
mix which was higher than expected. The highest furfural concentration recorded was 0.135 g/L. Given that the reported toxicity threshold for yeast is 2.4 g/L of furfural [38], it is unlikely that the inhibitory effects on growth seen in the present experiment are directly due to furfural but are more likely to have arisen from additional or synergistic impacts of a wider range of inhibitory compounds.

Synergistic effect of inhibitors such as HMF and vanillin or HMF and furfural have been reported on yeast performance where it stopped or slowed yeast activity [39]. Additionally, presence of low concentrations of acetic acid has been shown to have beneficial effect on fermentations and improved yeast tolerance to other inhibitory compounds. However, at higher concentrations of acetic acid (>25 mM) a synergistic effect on yeast metabolism is observed with other inhibitory compounds (furfural, vanillin, HMF, etc.) [39]. This could contribute to the higher yeast metabolism seen with the willow hydrolysate. In a study with aspen, switch grass and balsam, furfural concentrations at the optimal pre-treatment conditions were
0.36 g/L for the aspen: balsam blend (0.33 g/L expected), and 0.67 for the aspen: switch-grass blend (1.125 g/L expected) [24]. While the study with wheat straw, olive pruning and Eucalyptus residues, furfural levels were 0.33–0.44 g/L and HMF levels were 0.17 to 0.29 g/L depending on the mixes [23]. The inhibitors levels produced in this study are much lower when compared to previous studies. This may reflect the different solid loadings and pre-treatment regimes used in each study, or, directly as a result of the different feedstocks used. Hydrothermal pre-treatment used in this study is known to solubilize hemicellulose mainly as oligomers and not monomers, which reduces the risk of degradation of the soluble hemicellulose fraction [40] and the resultant inhibitors.

Conclusion

Mixing feedstocks of wheat straw, willow and Miscanthus does not appear to have any adverse effects on the effectiveness of hot water pre-treatment. In fact, all the mixes gave higher than expected sugar yields with wheat + willow and willow + Miscanthus mixes in particular showing statistically significant improvement. Hence, it could be possible to reduced reliance on single biomass and overcome potential feedstock bottle neck in a biorefinery by utilizing mixed lignocellulosic biomass while achieving significant improvement in sugar yield. The biochemical or biological basis for this enhanced sugar release or synergistic effect is unclear. The impact of mixing on inhibitor productions seems to be minimal and does not adversely further impact on either metabolic activity or growth of yeast. Mixing feedstocks may prove technically difficult on a commercial scale and that wheat as a sole biomass inputted into the system appears to be the best option. However, if willow and Miscanthus are the only available feedstocks, then mixing either these two together or willow with some wheat could provide significant improvements in yields. This warrants a further investigation to ascertain whether or not these benefits are seen on a larger scale and with feedstocks grown under a range of environmental conditions.

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Disclosure statement

The authors indicate no potential conflicts of interest.

ORCID

Kamaljit Moirangthem http://orcid.org/0000-0002-5146-8995

References

[1] Yue D, You F, Snyder SW. Biomass-to-bioenergy and biofuel supply chain optimization: overview, key issues and challenges. Comput Chem Eng. 2014;66:36–56.
[2] Balat M. Production of bioethanol from lignocellulosic materials via the biochemical pathway: a review. Energy Convers Manage. 2011;52(2):858–873.
[3] Ko JK, Um Y, Park Y, et al. Compounds inhibiting the bioconversion of hydrothermally pretreated lignocellulose. Appl Microbiol Biotechnol. 2015;99(10):4201–4212.
[4] Kucharska K, Rybarczyk P, Holowacz I, et al. Pretreatment of lignocellulosic materials as substrates for fermentation processes. Molecules. 2018;23(11):2937.
[5] Chiaramonti D, Prussi M, Ferrero S, et al. Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. Biomass Bioenergy. 2012;46:25–35.
[6] Kim Y, Kreke T, Ko JK, et al. Hydrolysis-determining substrate characteristics in liquid hot water pretreated hardwood. Biotechnol Bioeng. 2015;112(4):677–687.
[7] Pu Y, Hu F, Huang F, et al. Assessing the molecular structure basis for biomass recalcitrance during dilute acid and hydrothermal pretreatments. Biotechnol Biofuels. 2013;6(1):15.
[8] Gurrum RN, Datta S, Lin YJ, et al. Removal of enzymatic and fermentation inhibitory compounds from biomass slurries for enhanced biorefinery process efficiencies. Bioreourc Technol. 2011;102(17):7850–7859.
[9] Palmqvist E, Hahn-Hägerdal B. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Bioreourc Technol. 2000;74(1):25–33.
[10] Galbe M, Wallberg O. Pretreatment for biofermenters: a review of common methods for efficient utilisation of lignocellulosic materials. Biotechnol Biofuels. 2019;12(1):2–26.
[11] Almeida JRM, Bertilsson M, Gorwa-Grauslund MF, et al. Metabolic effects of furaldehydes and impacts on biotechnological processes. Appl Microbiol Biotechnol. 2009;82(4):625–638.
[12] Guarnieri MT, Franden MA, Johnson CW, et al. Conversion and assimilation of furfural and 5-(hydroxy)methylfurfural by Pseudomonas putida KT2440. Metab Eng Commun. 2017;4:22–28.
[13] Almeida JR, Modig T, Petersson A, et al. Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by Saccharomycyes cerevisiae. J Chem Technol Biotechnol. 2007;82(4):340–349.
[14] Sanchez B, Bautista J. Effects of furfural and 5-hydroxymethylfurfural on Saccharomyces cerevisiae. J Biotechnol. 2001;82(4):352.
[15] Sanchez B, Bautista J. Effects of furfural and 5-hydroxymethylfurfural on the fermentation of Saccharomycyes cerevisiae and biomass production from Candida guilliermondii. Enzyme Microb Technol. 1988;10(5):315–318.
[16] Taherzadeh MJ, Gustafsson L, Niklasson C, et al. Physiological effects of 5-hydroxymethylfurfural on Saccharomycyes cerevisiae. Appl Microbiol Biotechnol. 2000;53(6):701–708.
[17] Liu ZL, Slininger PJ, Dien BS, et al. Adaptive response of yeasts to furfural and 5-hydroxymethylfurfural and new chemical evidence for HMF conversion to 2,3,5-trihydroxymethylfuran. J Ind Microbiol Biotechnol. 2004;31(8):345–352.
[18] Liu ZL, Slininger PJ, Gorsich SW. Enhanced biotransformation of furfural and hydroxymethylfurfural by newly developed ethanologenic yeast strains. In: Davison BH, Evans BR, Finkenstein M, McMillan JD, editors. Twenty-sixth symposium on biotechnol- ogy for fuels and chemicals. Totowa, NJ: Humana Press; 2005. p. 451–460.
[19] Oke MA, Annuar MSM, Simarani K. Mixed feedstock approach to lignocellulosic ethanol production—prospects and limitations. Bioenerg Res. 2016;9(4):1189–1203.
[20] Althuri A, Gujala LKS, Banerjee R. Partially consolidated bioprocessing of mixed lignocellulosic feedstocks for ethanol production. Bioreourc Technol. 2017;245(Pt A):530–539.
[21] Michelin M, Teixeira JA. Liquid hot water pretreatment of multi feedstocks and enzymatic hydrolysis of solids obtained thereof. Bioreourc Technol. 2016;216:862–869.
[22] Ashraf MT, Schmidt JE. Process simulation and economic assessment of hydrothermal pretreatment and enzymatic
hydrolysis of multi-feedstock lignocellulose - Separate vs combined processing. Bioresour Technol. 2018;249:835–843.

[22] Sun N, Xu F, Sathitsuksanoh N, et al. Blending municipal solid waste with corn stover for sugar production using ionic liquid process. Bioresour Technol. 2015;186(C):200–206.

[23] Silva-Fernandes T, Duarte LC, Carvalheiro F, et al. Hydrothermal pretreatment of several lignocellulosic mixtures containing wheat straw and two hardwood residues available in Southern Europe. Bioresour Technol. 2015;183(C):213–220.

[24] Brodeur-Campbell M, Klinger J, Shonnard D. Feedstock mixture effects on sugar monomer recovery following dilute acid pretreatment and enzymatic hydrolysis. Bioresour Technol. 2012;116(C):320–326.

[25] Shi J, Thompson VS, Yancey NA, et al. Impact of mixed feedstocks and feedstock densification on ionic liquid pretreatment efficiency. Biofuels. 2013;4(1):63–72.

[26] Baral NR, Davis R, Bradley TH. Supply and value chain analysis of mixed biomass feedstock supply system for lignocellulosic sugar production. Biofuels, Bioprod Bioref. 2019;13(3):635–659.

[27] Alvira P, Negro MJ, Ballesteros I, et al. Steam explosion for wheat straw pretreatment for sugars production. Bioethanol. 2016;2(1):1–10.

[28] Di Maio D, Turley D. 2014. Lignocellulosic feedstock in the UK. York, UK: NNFC The Bioeconomy Consultants; p. 1–45. https://www.nnfcc.co.uk/files/mydocs/LBNet%20Lignocellulosic%20feedstock%20the%20UK_Nov%202014.pdf

[29] Lewandowski I, Clifton-Brown JC, Scurlock JMO, et al. Miscanthus: European experience with a novel energy crop. Biomass Bioenergy. 2000;19(4):209–227.

[30] Volk TA, Abrahamson LP, Cameron KD, et al. Yields of willow biomass crops across a range of sites in North America. Asp Appl Biol. 2011;112:67–74.

[31] Saeman JF, Bubl JL, Harris EE. Quantitative saccharification of wood and cellulose. Ind Eng Chem Anal Ed. 1945;17(1):35–37.

[32] Overend RP, Chornet E. Fractionation of lignocellulosics by steam-aqueous pretreatments [and discussion]. Philos Trans R Soc. 1987;321(1561):523–563.

[33] Selig M, Weiss N, & Ji Y. Enzymatic saccharification of lignocellulosic biomass: laboratory analytical procedure (LAP). Colorado, USA: NREL - National Renewable Energy Laboratory; 2008. p. 1–8.

[34] Ibbott R, Gaddipati S, Hill S, et al. Structural reorganisation of cellulose fibrils in hydrothermally deconstructed lignocellulosic biomass and relationships with enzyme digestibility. Biotechnol Biofuels. 2013;6(1):33.

[35] Greetham D, Wimalasena T, Kerruish DWM, et al. Development of a phenotypic assay for characterisation of ethanologenic yeast strain sensitivity to inhibitors released from lignocellulosic feedstocks. J Ind Microbiol Biotechnol. 2014;41(6):931–945.

[36] Wilkinson S, Smart KA, Cook DJ. Optimisation of alkaline reagent based chemical pre-treatment of Brewers spent grains for bioethanol production. Ind Crops Prod. 2014;62:219–227.

[37] Carroll JP, Finnan J. The relationship between bulk density and energy input, in biomass pellet production. Adv Anim Biosci. 2010;1(1):331–331.

[38] Allen SA, Clark W, McCaffery JM, et al. Furfural induces reactive oxygen species accumulation and cellular damage in Saccharomyces cerevisiae. Biotechnol Biofuels. 2010;3(1):2–10.

[39] Greetham D, Hart AJ, Tucker GA. Presence of low concentrations of acetic acid improves yeast tolerance to hydroxymethylfurfural (HMF) and furfural. Biomass Bioenergy. 2016;85(C):53–60.

[40] Mosier N, Hendrickson R, Ho N, et al. Optimization of pH controlled liquid hot water pretreatment of corn stover. Bioreasor Technol. 2005;96(18):1986–1993.