# Single-Step Microwave-assisted Hot Water Extraction of Hemicelluloses from selected Lignocellulosic Materials – a Biorefinery Approach

Authors: Teklu M. Gezahegn[a,b*], Malin Brodin[b], Annie F. Chimphango[a], Karin Øyaas[b], Bård H. Hoff[c], Johann F. Görgens[a]

a: Stellenbosch University, Process Engineering Department, Stellenbosch 7602, South Africa
b: Paper and Fibre Research Institute (PFI), Høgskoleringen 6B, NO-7491 Trondheim, Norway
c: Norwegian University of Science and Technology (NTNU), Department of Chemistry, Høgskoleringen 5, NO-7491 Trondheim, Norway

## ABSTRACT

The viability of single-step microwave-induced pressurized hot water conditions for co-production of xylan-based biopolymers and bioethanol from aspenwood sawdust and sugarcane trash was investigated. Extraction of hemicelluloses was conducted using microwave-assisted pressurized hot water system. The effects of temperature and time on extraction yield and enzymatic digestibility of resulting solids were determined. Temperatures between 170-200 °C for aspenwood and 165-195 °C for sugarcane trash; retention times between 8-22 minutes for both feedstocks, were selected for optimization purpose. Maximum xylan extraction yields of 66 and 50 %, and highest cellulose digestibilities of 78 and 74 %, were attained for aspenwood and sugarcane trash respectively. Monomeric xylose yields for both feedstocks were below 7 %, showing that the xylan extracts were predominantly in non-monomeric form. Thus, single-step microwave-assisted hot water method is viable biorefinery approach to extract xylan from lignocelluloses while rendering the solid residues sufficiently digestible for ethanol production.

**KEYWORDS**:

Lignocellulosic Biorefinery, Hemicellulose Extraction, Aspenwood, Sugarcane Trash, Microwave-assisted Pressurized Hot Water Method

*** Corresponding Author:** Process Engineering Department, Stellenbosch University, Stellenbosch 7602, South Africa; Tel: +27-641950543
E-mail: 18935001@sun.ac.za (Mihiretu, G.T.)
(Or, gezahegn_teklu@yahoo.com)

## 1. INTRODUCTION

Hemicelluloses, notably xylan, are the second most abundant carbohydrate polymers in lignocellulosic biomasses such as hardwoods and agricultural residues. Biopolymers in the form of xylan-rich hemicelluloses have potential applications as gels, films, adhesives, coatings, stabilizing and viscosity-enhancing agents in the food, biomedical and pharmaceutical industries (Ebringerova, 2006; Canilha et al., 2013). Due to their ability to self-assemble in spatially crosslinked manner, remarkable hydrophilic property and swelling capacity, hemicellulosic biopolymers have become of special interest in the development of biocompatible hydrogels for applications in wound dressing and advanced drug delivery systems (Silva et al., 2011; Ebringerova, 2006). Recent studies on xylan-based hydrogels have also shown the possibility of modifying and further synthesizing them into newly functionalized biomaterials for innovative applications in nanomedicine (Pahimanolis et. al., 2014; Chimphango et. al., 2012; Zhang et al., 2014) and tissue engineering (Venugopal et al., 2014; Tan and Marra, 2010).

Despite their potential applications, the path towards large scale production of hemicelluloses for high-value bio-based products largely remains underexplored (Zhang et al., 2014; Spiridon and Popa, 2008). One viable approach in realizing their ultimate economic value is the development of a lignocellulosic biorefinery system, whereby hemicellulosic biopolymers are co-produced with cellulosic ethanol (Chantal et al., 2012; Ragauskas et al., 2006). However, such a co-production scheme requires consideration of the complex and recalcitrant nature of lignocelluloses (Hayashi and Kaida, 2011; Hill, 2006). The high degree of crystallinity of celluloses and the lignin shield around them is one of the major causes for the recalcitrance of lignocellulosic materials towards enzymatic digestion (Hayashi and Kaida, 2011; Hill, 2006). The

intercalation of hemicelluloses into cellulose microfibrils and their interlinkage with lignin is another important factor for such recalcitrance (Bond et al., 2013; Yang et al., 2011). Thus, aside from loosening up the structural rigidity of celluloses and breaking up the lignin shield, the removal of hemicelluloses from the lignocellulosic matrix forms an essential part of biomass pretreatment for enhanced cellulose digestibility (Yan et al., 2016; Hayashi and Kaida, 2011).

However, hemicelluloses are thermally so labile that their extraction in oligomeric and polymeric forms typically requires a milder set of pretreatment conditions than those required for enhanced cellulose digestibility (Bond et al., 2013; Tutt et al, 2012). With increased severity of pretreatment conditions there is increased decomposition of hemicelluloses, which in turn leads to increased formation of degradation products that have inhibitory effects on the downstream bio-catalytic action of enzymes and fermentation yeasts (Yang et al., 2011; Cardona et al., 2010). Furthermore, an increased solubilisation of lignin is generally expected to take place as the pretreatment conditions get more severe (Yan et al., 2016). Even though lignin removal is desired for enhanced enzymatic digestibility, its presence with the extracted hemicellulose may compromise the quality of the intended biopolymeric product. The downstream separation of lignin components from the hemicellulose fraction as well as detoxification of inhibitory products therein do also have cost and technical implications (Yang et al., 2011; Chen et al., 2012; Karp et al., 2013). Therefore, the development of a biorefinery system meant for co-production of hemicellulosic biopolymers and cellulosic ethanol needs to take into account the potential operational challenges that primarily prevail at the pretreatment stage. Such challenges become even more evident when it comes to defining a single pretreatment step that can both remove

hemicelluloses in oligo- and polymeric form and produce enzymatically digestible solids rich in cellulose (Bond et al., 2013; Trajano and Wyman, 2013).

There are several pretreatment techniques to enhance the enzymatic digestibility of lignocellulosic biomass, but not all of them are suitable for the extraction of hemicelluloses for application as biopolymers/biomaterials (Trajano and Wyman, 2013; Mosier 2013, Ramirez et al., 2013). Among these methods, the extraction of hemicelluloses in their oligo- and polymeric form can be realized under pressurized hot water conditions (Aachary and Prapulla, 2011; Teo et al., 2010). Due to its remarkable dielectric properties and high loss tangent (tan δ), water can be effectively heated up through microwave irradiation (Barba and d'Amore, 2012; Tsubaki et al., 2016). The phenomena of autoionization of water at elevated temperatures and pressures leads to apparent formation of hydronium (H3O+) and hydroxyl (OH-) ions thereby rendering water to behave like polar and apolar solvent (Mosier 2013; Chemat et al., 2012). These properties of subcritical water are used to deconstruct the lignocellulosic matrix and remove extractible components such as hemicelluloses (Mosier, 2013; Chemat et al., 2012). The extraction of hemicelluloses under microwave-induced conditions has been reported (Tsubaki et al., 2016; Gulbrandsen et. al., 2015); as well as the effect of microwave-assisted hot water pretreatment on digestibility of lignocellulosic biomass (Ma et al., 2009; Binod et al., 2012). However, the technological routes for hemicellulose extraction and bioethanol co-production from lignocellulosic biomass have often been considered independently. There is as well a clear gap in defining a set of optimal process conditions for single-step production of hemicelluloses and digestible solids in a biorefinery setup.

The objective of this study is to establish feasible solution space for single-step microwave-assisted pressurized hot water extraction of hemicelluloses from two

selected lignocellulosic materials, while simultaneously enhancing the enzymatic digestibility of the solid residue. More specifically, the effects of microwave-induced temperature and retention time on xylan extraction and enzymatic digestibility of the solid residue were investigated. Sugarcane harvesting residues and aspenwood sawdust were selected for the study. The feedstock selection was made with envisaged application of the study results towards developing an integrated biorefinery system in the sugar and/or paper and pulp mills. To that end, the findings from this study are expected to provide valuable insight on the dynamic behaviour of a lignocellulosic biorefinery system across the interface of hemicellulose extraction conditions and pretreatment conditions for enhanced digestibility of the solid residue.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Sugarcane (*Saccharum officinarum*) trash and aspen (*Populus tremula*) sawdust were used in this study; the former being of South African and the latter of Norwegian origin. Chemicals such as sulphuric acid, potassium hydroxide, sodium hydroxide, citric acid monohydrate, potassium sodium tartrate, 3,5-dinitrosalicyclic acid, phenol, sodium azide and bovine serum albumin as used in this experimental study were of laboratory grade. Ethanol (95 % v/v) was used in the determination of ethanol extractives in raw samples. The enzymes Cellic CTec2 and HTec2, both from Novozymes, were used for enzymatic hydrolysis tests. Glucose standard solutions for cellulase activity evaluation and xylose control solutions as used in acid-hydrolysis tests were prepared from standard-grade glucose and xylose respectively. Unless otherwise mentioned, de-ionized water was used for all test purposes.

### 2.2. Preparation and Characterization of Raw Materials

The sugarcane trash consisted primarily of semi-dried leaves and tops. It was further dried in open air to a moisture level of 8.3 % and shredded to smaller sizes (approx. 5 to 7 cm). To make the sugarcane trash is representative enough as locally-sourced feedstock; the shredded mass was spread on plastic sheet, split into two parts and mixed back manually. This was done twice before they are packed into plastic bags. The shredded sugarcane trash was further reduced in size using Schuttle Buffalo hammer mill fit with 2 mm screen. Likewise, aspenwood sawdust was air-dried to moisture content of 6.3 %, reduced in size using lab-scale knife mill fitted with 2 mm screen.

The resulting milled raw materials were fractioned using lab-scale sieve shaker. Those in the size range of 250 - 1000 µm were uniformly mixed and used for the preparation of the actual test samples. Representative samples from prepared raw materials were characterized for extractives, ash, structural sugars and lignin contents. Determination of extractives was carried out in duplicates based on the NREL two-step method, NREL/TP-510-42619 (Sluiter et al., 2008). Ash content was determined as per the NREL protocol, NREL/TP-510-42622 (Sluiter et al., 2008). Lignin contents (acid-soluble and –insoluble) as well as structural sugars were determined in accordance with the NREL two-stage method, NREL/TP-510-42618 (Sluiter et al., 2012).

**2.3. Hemicellulose Extraction**

The raw biomass meant for extraction test purpose was soaked in water overnight for about 18 h at a soaking loading ratio of 50 mL water per g dry biomass. The soaked material was vacuum filtered to remove the liquid and recover the solid residue. Moisture and dry matter contents of the wet solid residue was measured using Sartorius MA-40 automatic moisture analyser. The extraction feed was prepared by

mixing about 3 g soaked wet residue (dry weight basis) and calculated amount of water in a PTFE-TFM (polytetrafluoroethylene, modified) liner in such a way that the loading ratio is 15 mL water per g dry biomass. The extraction of hemicelluloses was conducted under microwave-assisted pressurized hot water condition at varying combinations of temperature and holding time (=> section 2.8) using Anton Paar Multiwave-3000 microwave system. This system was equipped with a sensor to control an accurate profile of temperature and pressure inside a reference vessel, an infrared (IR) sensor to monitor the temperature at the base of each extraction vessel, and an integrated cooling system. Microwave power of 1000 W, ramp-up time of 10 min and cooling time of 30 min were fixed for all extraction experiments. Following the completion of each extraction test, the slurry was transferred to a 100 mL bottle. The reactor liner was washed with 50 mL of water to recover solid residues and sugar extracts stuck on the inner wall. The wash water was poured into the bottle containing the extraction slurry. The slurry was vacuum filtered using Whatman filter paper to separate the liquid and solid fractions. The solid residue was repeatedly washed with additional 400 (± 25) mL water until the pH of the wash water became neutral. The volume of the liquid fraction and weight of the wet solid residue were recorded. The liquid fraction was stored in schott bottle and kept in refrigerator till required for subsequent hydrolysis tests. About 25 mL samples (2X) were taken from the fresh liquid fraction, syringe-filtered (0.22 µm pore size) into 30 mL plastic bottles and kept in freezer till required for analysis. The wet solid residues were freeze-dried to about 95 % dry matter content using Heto PowerDry PL6000 freeze dryer and kept in plastic bags till required for subsequent hydrolysis tests (acid and enzymatic).

**2.4. Enzymatic Hydrolysis**

Saccharification experiments were conducted on freeze-dried solid samples as per the NREL Protocol NREL/TP-510-42629 (Selig et al., 2008). The tests were run in 20 mL scintillation vials at 2 % (w/w) substrate loading and 10 mL overall hydrolysis volume, i.e. about 0.21 g of solid sample was used per test. Added to each vialled solid sample were 5 mL of sodium citrate buffer (0.1 M, pH 4.9), 0.1 mL of 2 % sodium azide solution, 10 µL of Bovine Serum Albumin (BSA) and the balance with de-ionized water and finally the enzyme preparations. The enzyme combinations used were Cellic CTec2 and HTec2. The former (i.e. CTec2) was loaded at 15 FPU/g of substrate (dry weight basis) and the later (i.e. HTec2) was taken at 25 % (v/v) of CTec2. The enzymatic activity of CTec2 was determined in accordance with the protocol NREL/TP-510-42628 (Adney et al., 2008) and the estimated activity was around 150 (±10) FPU/mL. Enzymatic hydrolysis tests were carried out in an incubator set at 50 °C shaken at 150 rpm. After 72 h, the hydrolysis was terminated by putting the vials in boiling water for about 5 min and subsequently cooled in cold water. Separation of the slurry into liquid and solid fractions was carried out using vacuum filtration. The vials were subsequently washed with 10 mL of de-ionized water so as to wash out solid residue and liquid remaining therein. Samples were taken from the resulting liquid hydrolysate, syringe-filtered at 0.22 µm, bottled and kept in freezer till required for the intended analytical purposes. The enzymatic hydrolysis tests were run in duplicates.

### 2.5. Sugars Analysis

#### 2.5.1. Sugars in Liquid Fraction

Hemicellulosic sugar extracts (xylose in particular) in liquid fractions were analysed based on the NREL two-stage acid hydrolysis method, NREL/TP-510-42623 (Sluiter et al., 2008). At the second stage of acid-hydrolysis, control samples from 66.67

mM xylose solution were simultaneously acid-hydrolysed under the same autoclaved conditions. The xylose standard solution was prepared as suggested in the NREL protocol so as to account for the xylose loss from degradation. The acid-hydrolysis tests on actual samples as well as on samples from on xylose solution were conducted in duplicates. About 8 mL of the acid hydrolysate was taken for sugar analysis purpose. The pH of the analytical sample was adjusted in the range of pH 4 to pH 6 using 6 M potassium hydroxide and 1 M sulphuric acid solutions. The sample was subsequently filtered using syringe-filter with 0.22 µm pore-size and analysed by High Pressure Liquid Chromatography (HPLC) method using Biorad Aminex HPX-87H column (7.8x300 mm) with 5 mM sulphuric acid as a mobile phase. Column temperature was set at 65 °C. Samples were injected at a volume of 30 µL, eluted at a flowrate of 0.6 mL/min and detected with an RI-detector.

The amount of xylose extract in the liquid fraction was quantified based on the respective xylose concentration from HPLC analysis. The HPLC-read xylose concentration for the actual liquid samples was first adjusted for dilution and further corrected for xylose loses from degradation. The corrected xylose concentration was used to determine the overall amount of xylose extract in the liquid fraction, which in turn was used to determine the overall xylose yield. Furthermore, samples from un-hydrolysed liquid fractions were also directly HPLC-analysed so as to quantify monomeric xylose present therein. The amount of xylose that was in non-monomeric form was determined by subtracting the amount of monomeric xylose from the overall xylose. The yield for xylose extracts was calculated against the original xylose in the initial raw sample.

### 2.5.2. Sugars in Solid Samples

The content of sugars such as glucose, xylose and arabinose in freeze-dried pretreated samples was determined as per the NREL two-stage acid hydrolysis method NREL/TP-510-42618 (Sluiter et al., 2012). The preparation of analytical samples as well as the HPLC setup for sugar analysis was the same as described under section 2.5.1. The concentration of sugars from the HPLC results was used to determine the composition of the raw and pretreated solids for such major structural sugars as glucose and xylose.

### 2.5.3. Sugars in Enzymatic Hydrolysates

The hydrolysate samples from enzymatic hydrolysis tests (section 2.4) were analysed for sugars (glucose and xylose) under the same HPLC setup as described in section 2.5.1. The HPLC results on the sugar concentrations were used to quantify the amount of enzymatically released sugars. The enzymatic sugar yields for glucose and xylose were calculated as the percentage of the respective sugar in the initial raw sample that was enzymatically released. Enzymatic sugar yield for glucose and xylose were also determined for raw (un-pretreated) samples of SCT and AW.

## 2.6. Degradation Products in Liquid Fractions

The analytical samples prepared from extraction liquid fractions (section 2.3) were analysed for acetic acid, formic acid, furfural and HMF following the NREL method, NREL/TP-510-42623 (Sluiter et al., 2008). Samples were analysed by similar HPLC setup as in 2.5.1 and as per the same NREL protocol.

## 2.7. Lignin Content

The content of lignin in the raw lignocellulosic materials as well as in pretreated solids was determined in accordance with the NREL protocol, NREL/TP-510-42618

(Sluiter et al., 2012). Acid-soluble lignin contents were determined based on absorbance readings taken at 205 nm on liquid samples against a background with de-ionized water. The analysis was conducted using UV-Vis Spectrophotometer (UV-1800, SCHMADZU).

### 2.8. Experimental Design

The design of experiments on hemicellulose extraction and enzymatic saccharification tests as well as statistical analysis of results thereof was carried out using Design-Expert 8.0.2. Experiments were statistically designed based on central composite design (CCD) as a response surface methodology. Microwave-induced liquid hot water temperature and retention time were varied as per the design to investigate their effects on sugar yields. Parametric values (minimum, central and maximum) for temperature and retention time were chosen based on preliminary test results (section 3.1). The minimum, central and maximum temperature values for aspenwood were respectively set at 185, 175 and 195 °C; similarly, for sugarcane trash, the temperature values we set at 170, 180 and 190 °C respectively. For both feedstocks, the minimum, central and maximum values for retention time were set at 10, 20 and 20 min respectively.

The response-factor relationship (response surface equation) – both for the extraction yield and enzymatic sugar yield – was represented by a quadratic model taking the following form:

$$Y = B_0 + B_1*X_1 + B_2*X_2 + B_{12}*X_1*X_2 + B_{11}*X_1\text{^}2 + B_{22}*X_2\text{^}2 \text{ ------ (Eq. I)}$$

Where, Y [% w/w] is the yield figures (extraction sugar yield, enzymatic sugar yield) which are the output (dependent) parameters; X1 and X2 are the input (independent) parameters representing temperature [°C] and retention time [min] respectively.

## 3. RESULTS AND DISCUSSIONS

### 3.1. Selection of Experimental Set Points

Two batches of preliminary tests were carried out on the extraction of hemicelluloses from selected lignocellulosic feedstock under microwave-induced pressurized hot water conditions. The aim was to identify reasonable ranges of values for temperature and retention time, for optimization using a central composite design as response surface methodology. The underlying reason for the design of main experiments (see section 2.8) was to establish the microwave-induced conditions where the extraction yield for hemicelluloses, xylan in particular, would be enhanced, while formation of monomeric sugars as well as degradation products thereof is minimized. The first batch was conducted at a fixed time (10 min) and three temperatures (165, 175 and 185 °C for sugarcane trash and 175, 185 and 200 °C for aspenwood) selected based on previous works on liquid hot water methods (Teo et al., 2010; Aachary and Prapulla, 2011; Sun et al., 2014; Sukhbaatar et al., 2014). The second batch tests were conducted based on the steepest ascent methodology to follow the direction of increments in the yields. Preliminary test results (data not shown here) showed that extraction yields for both feedstocks were predominantly influenced by temperature and, to a lesser extent, by retention time. For an extraction time of 10 min, the extraction yields for sugarcane trash and aspenwood were observed to increase steeply with an increase in temperature starting from 180 °C and 185 °C respectively. It was also observed that monomeric sugars and degradation products thereof could noticeably form at these same temperatures. These temperatures were thus chosen as centre-point values in designing main extraction tests. For the purpose of this experimental study, an extraction time of 15 min was selected as central value.

### 3.2. Effects of Temperature and Retention Time on the Extraction of Hemicellulose from selected Lignocellulosic Materials under Microwave-induced Liquid Hot Water Conditions

The extraction of hemicelluloses, xylan in particular, under controlled microwave conditions was carried out as per the experimental design under section 2.8. Discussed hereinbelow are the effects of microwave-induced pressurized hot water temperature and retention time on the extraction yield of xylan (expressed as “xylose yield”) from aspenwood and sugarcane harvesting residues.

#### 3.2.1. Effects on Xylan Extraction from Aspenwood

Results obtained on the extraction of xylan from aspenwood under microwave-induced conditions are shown in Table-1. The xylose yield figures were directly influenced by variations made in both controlled extraction parameters. An increase in retention time from 10 to 20 min increased the xylose yield by less than 10 % for temperatures below 175 °C; by 12 to 40 % for temperatures from 175 to 185 °C; and by up to 66 % for temperatures higher than 185 °C. For extractions at high temperatures (i.e. 185 °C and above) and under the span of retention times (i.e. 8 to 22 min), the overall xylose yield was in the range of 40 to 70 % (w/w). Under the same conditions, the fraction of the xylan extracts in monomeric form was in the range of 2.2 to 5.3 %, showing that the extracted xylan was to high extent (more than 90 %) in non-monomeric form.

[Insert **Table-1** Here]

The quantitative relationship between the response (xylose yield) and input parameters (temperature and time) were statistically analysed using central composite design (CCD) method as a response surface methodology. The statistical model results (ANOVA for reduced quadratic model with significant model terms, coefficients for the

quadratic equation, as well as the R-Square values) both for the overall xylose yield (O-XY) and non-monomeric xylose yield (NM-XY) are shown in Table-2. With very low p-value ($p<0.0001$) and high R-Squared value (~0.97), the fitted quadratic models had a high significance (with 95% confidence interval, i.e. CI=95 %) to reflect the response-factor relationship. ANOVA results indicated that the xylose yield was significantly influenced by the positive linear effects from both temperature and retention time as well as the negative quadratic effect from temperature. Pareto chart analysis was also carried out to compare the size of standardized effects of the extraction temperature and retention time on the xylose yield. The resulting chart (not shown here) showed that both extraction temperature and retention time had significant positive effect on the xylose yield, with temperature (t-value of 20.67) having a more significant impact than retention time (t-value of 7.35), when compared with the Bonferroni and standardized t-value limits, which were 3.96 and 2.78 respectively.

[Insert **Table-2** Here]

The contour plot generated based on the quadratic model, derived from CCD experimental results (see Table-2) is shown in Fig-1. Considering the region defined by the design points (i.e. Temp: 170 – 199 °C; Time: 8 – 22 min), where the yields are well supported by experimental data, the constant-yield lines (i.e. the overall xylose yield figures) are increasing in the up-right-direction showing that there was net positive effect on overall xylose yield with increases from both input parameters. However, from the extended version of the contour lines, it could be observed that there are inflection points around a temperature of 200 °C, beyond which the constant–yield lines continue to increase in the up-left direction, due to the positive effect from retention time, while the net effect of temperature has become negative (reflected in the growing size of the negative quadratic effect from increased temperature). These inflection points, in the

study context here, can be viewed as good indicators of the maximum temperature and the shortest retention time suitable for xylose yields higher than 45 % (e.g. at 200 °C, a xylose yield of 45 % could be achieved in less than five minutes extraction time). For a given extraction time, the xylose yield can be increased when the temperature is increased up to 200 °C; for higher temperatures though, the yield can be expected to decrease, as discussed below.

[Insert **Fig-1** Here]

The increase in xylose yield at temperatures of 185 °C and above could primarily be the result of increased acidity level of the extraction medium, mainly from acetic acid. Under these conditions noticeable formation of acetic acid in the extraction hydrolysate was observed (0.36 to 0.72 g/100 g $_{\text{initial dry raw sample, dry weight basis}}$, see Table-1). The observed increase of acetate concentration in the extraction medium could lead to increased thermal effect of microwave irradiation through ionic dissipative mechanisms (Barba and d'Amore, 2012; Tsubaki et. al., 2016). Such increased acidity level in the extraction medium might have hastened the progressive depolymerisation of xylan and its ultimate conversion into monosaccharides (Trajano and Wyman, 2013; Mosier, 2013). In fact, it is these acetic-acid-derived hydronium ions to which much of the observed auto-catalytic effect under such severity of conditions might be attributed (Carvalheiro et al., 2016; Tsubaki et. al., 2016). To a lesser extent, increased self-ionization and so increased auto-catalytic action of water at such high temperatures can be part of the reason for enhanced dissolution of hemicelluloses (Trajano & Wyman, 2013; 2012; Mosier, 2013). Furthermore, with increased temperature subcritical water can exhibit high rate of diffusion, low viscosity and low surface tension – properties that can enhance the solubility and extraction of hemicellulosic components from a lignocellulosic biomass (Teo et al., 2010).

A closer look at the results on xylan extraction from aspenwood (Table-1) showed that temperatures of 185 °C and above were not only high enough for enhanced yields, but also severe enough to lead to monomeric xylose formation. It is important here to note that, at such high temperatures, extended extraction time could have significant effect on the formation of monomeric xylose as well as on degradation products thereof. For instance, at 185 °C, increasing the time from 8 to 22 minutes and, at 195 °C, increasing the time from 10 to 20 min led to over 100 % increase in the monomeric xylose yield (from results in Table-1). At the same conditions, the amount of acetic acid in the extraction hydrolysate was observed to increase substantially with increased retention time. Furfural, a degradation product from xylose, was also observed to form at such severe extraction conditions and results thereof (see Table-1) show that its formation was highly influenced by increases in retention time. For instance, at 185 °C, increasing the retention time from 8 to 22 minutes led to a three-fold increase (from 20.8 mg to 65.0 mg of furfural per 100 g $_{\text{dry initial sample}}$). Whereas at 195 °C, as the retention time was increased from 10 to 20 minutes, an eight-fold increase in furfural formation was observed (from 29.7 mg to 244.4 mg per 100 g $_{\text{initial dry sample}}$).

As discussed earlier, for aspenwood, the linear positive effect of time on xylose extraction yield would mean that any reduction in this extraction parameter (at such elevated temperatures) would lead to corresponding reduction in xylose yield – both overall and monomeric. The minimization of monomeric xylose formation was thus only possible by compromising the overall extraction yields, which increased with increased time. Therefore, at such high temperatures (i.e. 185–200 °C), a trade-off needs to be made between high non-monomeric xylose yield and low monomeric xylose yield. In this context, the choice of microwave-assisted pressurized hot water method for the purpose of extracting hemicelluloses from aspenwood is well justified, as high

temperatures can be achieved in a relatively short time as used in the present experimental study. In other words, the rapid microwave irradiation effect in reducing the severity of extraction conditions through reduced retention time (Tsubaki et al., 2016; Barba and d'Amore, 2012) can potentially be exploited towards enhanced xylan extraction from aspenwood, while minimizing the formation of monomeric sugars and degradation products thereof.

### 3.2.2. Effects on Xylan Extraction from Sugarcane Trash

For sugarcane trash, results on xylan extraction (presented in Table-3) show that the xylose yield figures were predominantly influenced by the extraction temperature. Under the extraction times investigated here (i.e. 10 to 20 min) and for temperatures below 170 °C, the xylan extraction yield increased only slightly to values of less than 10 %. There was no observed formation of monomeric xylose, nor were organic acids and degradation products observed, under these low-severe conditions. For temperatures higher than 170 °C, however, the xylan extraction yield increased significantly (from 20 to 50 % w/w), with noticeable formation of monomeric xylose, degradation products (furfural and HMF) as well as organic acids such as acetic acid and formic acid. The low monomeric xylose yield of 3 to 6 % w/w shows that the xylan extracted from sugarcane trash was to a large extent in non-monomeric form.

[Insert **Table-3** Here]

Statistical analysis of the response-factor relationship was carried out using CCD and results obtained both for overall and non-monomeric xylose yields (O-XY and NM-XY) are presented in Table-4. As per the quadratic model generated, which itself was of low significance (p-value=0.0333 and R-Squared=0.77), temperature was identified as the sole significant model term (with p-value = 0.0023) with positive linear

effect on the xylose yield. The effect from retention time was rather insignificant within 95 % confidence interval. Pareto chart analysis was carried out to see the independent effect each extraction factor had on the xylose yield, and results thereof (not shown here) did confirm the relative (in-) significance of the input parameters as was deduced from the ANOVA results.

[Insert **Table-4** Here]

A contour plot based on the statistically generated model (shown in Fig-2) also portrays the yield profile over a wide range of temperatures and times. The effect of retention time seems to level out with increased temperature showing that the observed increases in overall xylan extraction yield were mainly in response to changes in the extraction temperature. One plausible explanation hereto could be made on the basis of the high level of ash content in sugarcane trash, which was estimated at 7.89 % (w/w). In the course of the extraction process, those inorganic minerals constituting the ash, once they find their way into the extraction medium, would dissociate into the respective cations and anions. Such inorganic ions may compete for those hydronium ($H_3O^+$) and hydroxide (OH-) ions from self-ionization of sub-critical water as well as the dissolution of organic acids such as acetic acid and formic acid. As the effectiveness of microwave-assisted hot water extraction process is directly dependent on the apparent concentration of $H_3O^+$ and $OH^-$, the reaction they undergo with the inorganic ions and the resulting neutralization effect might have undermined the extent of hemicellulose extraction under such low-acid auto-hydrolytic conditions (Tanjore et al., 2011; Trajano and Wyman, 2013).

[Insert **Fig-2** Here]

In the experimental case here, the presence of organic acids such as acetic acid and formic acid was noticeable for temperatures of 180 °C and above (see Table-4),

where the xylan extraction yields as well as their depolymerisation were enhanced. Increased acidity of the extraction medium (subcritical water) might have led to the formation of degradation products such as furfural and HMF, which coincided with conditions where monomeric xylose was formed. This is a strong indication that, for sugarcane trash, temperatures starting from 180 °C could be severe enough to cause not only the decomposition of extracted xylo-oligomers but also their subsequent degradation (dehydration) into furfural. While temperatures starting from 180 °C and retention time in the range of 10 to 20 min may lead to enhanced extraction of hemicelluloses, it is important to take into consideration the possible depolymerisation of xylan extracts as a result of increased acidity level (i.e. severity of conditions) in the extraction medium.

### 3.3. Effect of Microwave-assisted Pressurized Hot Water Temperature and Retention Time on Cellulose digestibility (Enzymatic Hydrolysis Glucose Yield)

#### 3.3.1. Effects on Enzymatic Glucose Yield – Aspenwood

Results from enzymatic hydrolysis test (shown in Table-1) revealed that the cellulose content as well as the enzymatic digestibility of aspenwood solids were substantially enhanced following their pretreatment under microwave-assisted conditions. For raw aspenwood, the enzymatic hydrolysis glucose yield was 18.85 % (w/w); this yield figure could be enhanced close to 80 % (w/w) after pretreatment, indicating that the cellulose in aspenwood was rendered highly digestible under microwave-induced conditions. The effects of temperature and retention time on the enzymatic hydrolysis sugar yield were statistically analysed (results shown in Table-5) and the response-factor relationship was well represented by a quadratic model with high level of significance ($p$-value<0.0001, R-Squared value=0.99, CI=95%). Accordingly, temperature appears to have positive linear effect and negative quadratic

effect on the glucose yield, whereas only a linear positive effect of time was identified as significant. Pareto chart analysis results (data not shown) also showed that temperature (with t-value of 14.27) had more significant effect than retention time (t-value of 6.25).

[Insert **Table-5** Here]

The observed increase in cellulose digestibility of the pretreated solids may primarily be attributed to the removal of hemicelluloses (xylose in particular), both during pretreatment and enzymatic hydrolysis stages. As is shown in Figs-3a and b, the cellulose digestibility of pretreated solids was directly correlated with variations in the values of overall xylose yields. With increased removal of xylose, the pretreated solid would not only get more cellulose-enriched (see Fig-3c), but the solid ligno-carbohydrate matrix would also become more open-structured – effects which make the solid residue become more accessible to enzymatic attack (Ioelovich & Morag, 2012; Yang B. et al., 2011). Furthermore, the removal of lignin from the solid residue could also be another factor for enhanced digestibility as delignification does likewise lead to a more porous structure. Under the conditions investigated here (see Table-1), the degree of lignin removal was observed to increase 5 to 20 % w/w with increased severity of conditions.

[Insert **Fig-3** Here]

### 3.3.2. Effects on Enzymatic Hydrolysis Glucose Yield – Sugarcane Trash

For sugarcane trash, hydrolysis test results (shown in Table-3) revealed that the extent of enzymatically released glucose as well as apparent cellulose content of the pretreated solids were both enhanced significantly following its pretreatment under

microwave-induced conditions. About 19 % (w/w) of the cellulose present in raw sugarcane trash could be enzymatically digested without pretreatment; whereas, under the pretreatment conditions here, up to 75 % (w/w) of the cellulose could be digested, i.e. released in glucose form. The response-factor relationship were statistically analysed and the resulting quadratic model was statistically significant (with p-value=0.0021; R-Squared value=0.91; CI=95%). Results thereof (shown in Table-6) could show that the enzymatic hydrolysis glucose yield for sugarcane trash was predominantly influenced by temperature. Pareto chart analysis results thereon also showed temperature (with t-value of 3.77) to be the significant factor; whereas retention time to be rather insignificant (t-value=0.75, which is below the reference t-value=2.78).

[Insert **Table-6** Here]

Similar to aspenwood, the enzymatic digestibility of the cellulose in sugarcane trash was generally observed to increase with increased removal of hemicelluloses – both during pretreatment (Fig-4a) and enzymatic hydrolysis (Fig-4b). With increased removal of hemicelluloses, both the content and structural porosity of the cellulosic component in the lignocellulosic solid apparently increases (Fig-4c) thereby rendering the pretreated biomass more amenable for enzymatic attack (Ioelovich & Morag, 2012; Yang B. et al., 2011). The cellulose digestibility was evidently increasing with increased removal of both hemicelluloses and lignin (Fig-4d).

[Insert **Fig-4** Here]

## 4. CONCLUSION

Single-step microwave-induced pressurized hot water pretreatment was demonstrated as a viable technique for extracting xylan from aspenwood and sugarcane trash, while enhancing their enzymatic digestibility for cellulosic ethanol production. Viable pretreatment conditions for enhanced xylan extraction and cellulose digestibility were established for each feedstock. About two-third of the original xylan in aspenwood and over half of that in sugarcane trash were extracted, with more than 90 % being in non-monomeric form. The cellulose digestibility for both lignocellulosic materials was improved by four-fold. Thus, microwave-induced hot water method can be regarded as viable route for advancing second-generation biorefineries.

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# TABLES AND FIGURES

## A) TABLES

### Table-1

### Extraction Results (Sugar Yield, Lignin Removal, Degradation Products), Sugars in Pretreated Solids and Enzymatic Hydrolysis Sugar Yields for Aspenwood

| Pretreatment Conditions | | Sugar Extract (Xylose), Lignin and Degradation Products in Liquid Fraction | | | | | | | | Sugars in Pretreated Solids [%w/w] | | Enzymatic Hydrolysis Sugar Yield [%] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Xylose Extraction Yield [%w/w] | | | Lignin Removal [% w/w] | Degradation products [mg] per g raw sample | | | | | | | |
| Temp [°C] | Time [min] | Overall | Non-Monomeric | Monomeric | | Acetic Acid | Formic Acid | Furfural | HMF | Glucose | Xylose | Glucose | Xylose |
| 171 | 15 | 6.2 | 6.2 | [n.d] | 8.1 | [n.d] | [n.d] | [n.d] | [n.d] | 59.6 | 19.9 | 32.7 | 25.5 |
| 175 | 10 | 12.5 | 12.5 | [n.d] | 10.5 | [n.d] | [n.d] | [n.d] | [n.d] | 61.1 | 19.9 | 40.8 | 33.4 |
| 175 | 20 | 21.9 | 21.9 | [n.d] | 13.5 | [n.d] | [n.d] | 0.09 | [n.d] | 62.8 | 18.7 | 50.9 | 43.3 |
| 185 | 8 | 40.4 | 39.5 | 2.2 | 16.2 | [n.d] | [n.d] | 0.21 | [n.d] | 68.8 | 15.8 | 58.5 | 52.4 |
| 185 | 15 | 43.0 | 41.9 | 2.4 | 16.4 | 3.68 | [n.d] | 0.30 | [n.d] | 67.2 | 15.1 | 63.1 | 56.1 |
| 185 | 22 | 55.9 | 53.3 | 4.6 | 17.7 | 4.05 | [n.d] | 0.65 | 0.04 | 70.3 | 13.1 | 69.2 | 57.4 |
| 195 | 10 | 46.8 | 45.6 | 2.5 | 18.9 | 3.34 | [n.d] | 0.30 | [n.d] | 67.3 | 14.4 | 65.3 | 60.2 |
| 195 | 20 | 66.1 | 62.6 | 5.3 | 19.4 | 7.16 | 2.10 | 2.44 | 0.06 | 67.6 | 11.6 | 77.8 | 64.6 |
| 199 | 15 | 57.8 | 55.1 | 4.6 | 19.5 | 4.63 | [n.d] | 0.62 | 0.04 | 71.2 | 12.2 | 73.8 | 64.3 |
| Raw Sample Composition [%w/w] | | | 18.7 | | 22.0 | [--] | [--] | [--] | [--] | 50.3 | 18.7 | 18.8 | 15.6 |

## Table-2

## Statistical Analysis Results for Xylose Extraction Yield from Aspenwood

Response: Xylose Extraction Yield, XY [% w/w]

ANOVA for Response Surface Reduced Quadratic Model

Analysis of Variance Table [Partial sum of squares - Type III]

| Source | Sum of Squares | | df | Mean Square | | F-Value | | p-value | |
|---|---|---|---|---|---|---|---|---|---|
| | O-XY* | NM-XY** | | O-XY | NM-XY | O-XY | NM-XY | O-XY | NM-XY |
| Model | 3486 | 3117 | 3 | 1162 | 1039 | 110 | 134 | < 0.0001 | < 0.0001 |
| A-Temp [oC] | 2873 | 2562 | 1 | 2873 | 2562 | 272 | 331 | < 0.0001 | < 0.0001 |
| B-Time [min] | 322 | 265 | 1 | 322 | 265 | 30 | 34 | 0.0004 | 0.0002 |
| A^2 | 292 | 290 | 1 | 292 | 290 | 28 | 38 | 0.0005 | 0.0002 |
| Residual | 95 | 70 | 9 | 11 | 8 | | | | |
| Lack of Fit | 81 | 60 | 5 | 16 | 12 | 4 | 5 | 0.0860 | 0.0756 |
| Pure Error | 14 | 10 | 4 | 4 | 2 | | | | |
| Cor Total | 3581 | 3186 | 12 | | | | | | |

Model Terms and Significance

| Level of Significance | Factor | Coefficient | |
|---|---|---|---|
| | | O-XY | NM-XY |
| significant | Intercept | 44.0 | 42.8 |
| | Temp | 19.0 | 17.9 |
| | Time | 6.3 | 5.8 |
| | (Temp)^2 | -6.4 | -6.4 |

| Level of Significance | R-Squared (R-Sq) Values | | |
|---|---|---|---|
| | | O-XY | NM-XY |
| not significant | R-Sq | 0.97 | 0.98 |
| | Adj R-Sq | 0.96 | 0.97 |

Note: *O-XY= Overall Xylose Yield; ** NM-XY= Non-monomeric Xylose Yield

**Table-3**

**Extraction Results (Sugar Yield, Lignin Removal, Degradation Products), Sugars in Pretreated Solids and Enzymatic Hydrolysis Sugar Yields for Sugarcane Trash**

| Pretreatment Conditions | | Sugar Extract (Xylose), Lignin and Degradation Products in Liquid Fraction | | | | | | | | Sugars in Pretreated Solids (SF) [%w/w] | | Enzymatic Hydrolysis Sugar Yield [%] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Xylose | | | Lignin Removal [%w/w] | Degradation Products [mg], per g raw sample | | | | | | | |
| Temp [°C] | Time [min] | Overall | Non-Monomeric | Monomeric | | Acetic Acid | Formic Acid | Furfural | HMF | Glucose | Xylose | Glucose | Xylose |
| 167 | 15 | 9.5 | 9.5 | [n.d] | 9.1 | [n.d] | [n.d] | [n.d] | [n.d] | 47.7 | 26.6 | 41.4 | 23.8 |
| 170 | 10 | 18.6 | 18.6 | [n.d] | 10.6 | [n.d] | [n.d] | [n.d] | [n.d] | 47.7 | 23.1 | 49.5 | 34.5 |
| 170 | 20 | 23.2 | 23.2 | [n.d] | 8.6 | [n.d] | [n.d] | [n.d] | [n.d] | 48.4 | 25.2 | 53.2 | 32.4 |
| 180 | 8 | 20.2 | 19.5 | 3.5 | 18.0 | 5.24 | 1.87 | 2.05 | 0.04 | 49.9 | 23.1 | 52.6 | 35.9 |
| 180 | 15 | 21.7 | 20.9 | 3.8 | 16.1 | 5.69 | 1.89 | 2.36 | 0.05 | 50.3 | 22.9 | 54.4 | 40.1 |
| 180 | 22 | 24.2 | 23.3 | 3.7 | 14.3 | 5.10 | 1.85 | 2.68 | 0.05 | 49.2 | 20.7 | 56.8 | 42.8 |
| 190 | 10 | 29.2 | 28.3 | 3.2 | 17.8 | 9.58 | 2.56 | 3.21 | 0.05 | 53.2 | 21.2 | 62.9 | 47.6 |
| 190 | 20 | 29.2 | 28.2 | 3.6 | 15.5 | 6.76 | 1.88 | 4.14 | 0.06 | 53.2 | 20.5 | 65.4 | 49.8 |
| 194 | 15 | 50.9 | 47.7 | 6.2 | 21.4 | 11.10 | 2.28 | 3.29 | 0.18 | 61.3 | 15.3 | 74.3 | 53.3 |
| Raw Sample Composition [%w/w] | | | 23.2 | | 18.8 | [--] | [--] | [--] | [--] | 38.7 | 23.2 | 19.2 | 6.5 |

## Table-4

## Statistical Analysis Results for Xylose Extraction Yield from Sugarcane Trash

Response: Xylose Extraction Yield, XY [% w/w]

ANOVA for Response Surface Quadratic Model

Analysis of Variance Table [Partial sum of squares - Type III]

| Source | Sum of Squares | | df | Mean Square | | F-Value | | p-value | |
|---|---|---|---|---|---|---|---|---|---|
| | O-XY* | NM-XY** | | O-XY | NM-XY | O-XY | NM-XY | O-XY | NM-XY |
| Model | 833 | 707 | 5 | 167 | 141 | 5 | 5 | 0.0333 | 0.0369 |
| A-Temp | 706 | 590 | 1 | 706 | 590 | 20 | 19 | 0.0029 | 0.0034 |
| B-Time | 18 | 16 | 1 | 18 | 16 | 1 | 1 | 0.5037 | 0.5001 |
| AB | 5 | 6 | 1 | 5 | 6 | 0 | 0 | 0.7104 | 0.6864 |
| A^2 | 104 | 95 | 1 | 104 | 95 | 3 | 3 | 0.13 | 0.1249 |
| B^2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0.9429 | 0.91 |
| Residual | 248 | 219 | 7 | 35 | 31 | | | | |
| Lack of Fit | 224 | 195 | 3 | 75 | 65 | 13 | 11 | 0.0165 | 0.0209 |
| Pure Error | 24 | 24 | 4 | 6 | 6 | | | | |
| Cor Total | 1081 | 926 | 12 | | | | | | |

Model Terms and Significance

| Significance | Factor | Coefficient | |
|---|---|---|---|
| | | O-XY | NM-XY |
| significant | Intercept | 21.7 | 20.9 |
| | A-Temp | 9.4 | 8.6 |
| | B-Time | 1.5 | 1.4 |
| | AB | -1.2 | -1.2 |
| | A^2 | 3.9 | 3.7 |
| | B^2 | 0.2 | 0.3 |

R-Squared (R-Sq) Values

| Significance | | O-XY | NM-XY |
|---|---|---|---|
| | R-Sq | 0.77 | 0.76 |
| | Adj R-Sq | 0.61 | 0.59 |

## Table-5

## Statistical Analysis Results for Enzymatic Hydrolysis Glucose Yield for Aspenwood (AW)

Response: Enzymatic Hydrolysis Glucose Yield [%w/w], AW

ANOVA for Response Surface Reduced Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

| Source | Sum of Squares | df | Mean Square | F-Value | p-value Prob > F | Significance |
|---|---|---|---|---|---|---|
| Model | 1872 | 3 | 624 | 203 | < 0.0001 | significant |
| A-Temp [oC] | 1501 | 1 | 1501 | 488 | < 0.0001 | |
| B-Time [min] | 179 | 1 | 179 | 58 | < 0.0001 | |
| A^2 | 192 | 1 | 192 | 62 | < 0.0001 | |
| Residual | 28 | 9 | 3 | | | |
| Lack of Fit | 14 | 5 | 3 | 1 | 0.5745 | not significant |
| Pure Error | 13 | 4 | 3 | | | |
| Cor Total | 1900 | 12 | | | | |

Model Terms

| Factor | Coefficient |
|---|---|
| Intercept | 63.8 |
| Temp | 13.7 |
| Time | 4.73 |
| (Temp)^2 | -5.21 |

R-Squared Values

| | |
|---|---|
| R-Squared | 0.99 |
| Adj R-Squared | 0.98 |

## Table-6

## Statistical Analysis Results for Enzymatic Hydrolysis Glucose Yield for Sugarcane Trash (SCT)

Response: Enzymatic Hydrolysis Glucose Yield, SCT [% w/w]

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

| Source | Sum of Squares | df | Mean Square | F-Value | p-value Prob > F | Significance |
|---|---|---|---|---|---|---|
| Model | 716 | 5 | 143 | 13 | 0.0021 | significant |
| A-Temp | 649 | 1 | 649 | 58 | 0.0001 | |
| B-Time | 21 | 1 | 21 | 2 | 0.2126 | |
| AB | 0 | 1 | 0 | 0 | 0.8659 | |
| A^2 | 44 | 1 | 44 | 4 | 0.0879 | |
| B^2 | 3 | 1 | 3 | 0 | 0.6238 | |
| Residual | 78 | 7 | 11 | | | |
| Lack of Fit | 60 | 3 | 20 | 4 | 0.091 | not significant |
| Pure Error | 18 | 4 | 5 | | | |
| Cor Total | 794 | 12 | | | | |

Model Terms (Equation)

| Factor | Coefficient |
|---|---|
| Intercept | 53.7 |
| A | 9.0 |
| B | 1.6 |
| AB | -0.3 |
| A^2 | 2.5 |
| B^2 | 0.7 |

R-Squared Values

| | |
|---|---|
| R-Squared | 0.90 |
| Adj R-Squared | 0.83 |

**B) FIGURES**

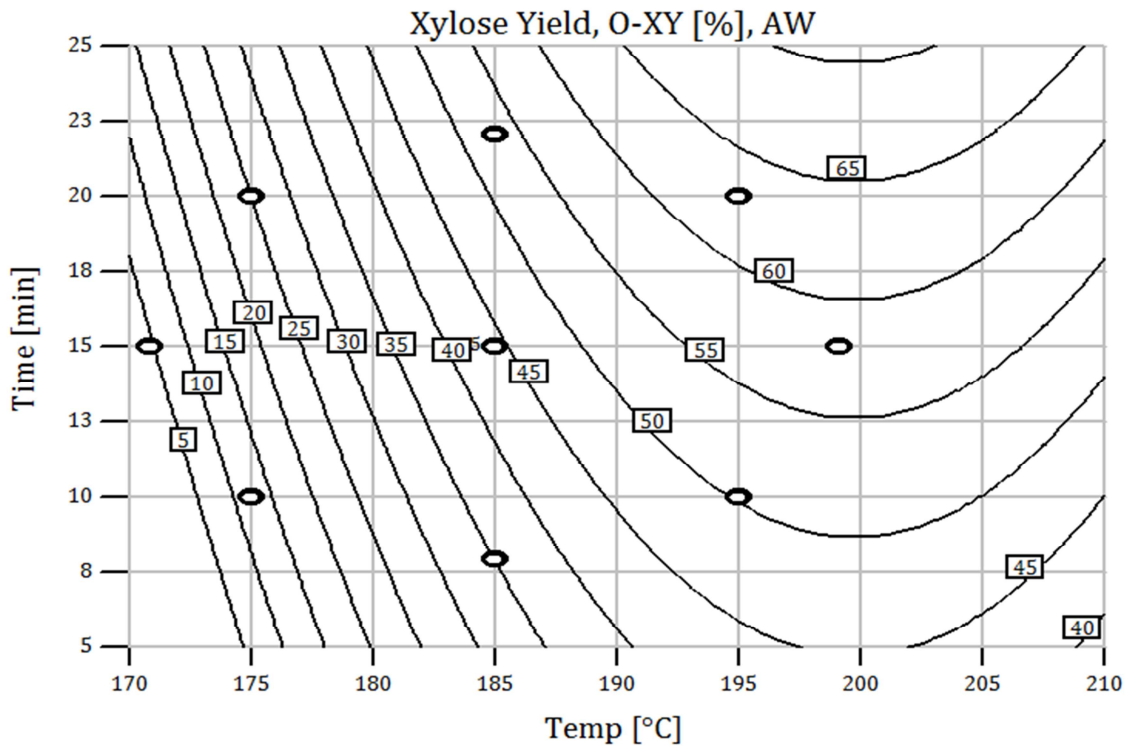


**Fig-1** Contour Plot for overall Xylose Yield (O-XY) for Aspenwood (AW) against Temperature and Retention Time

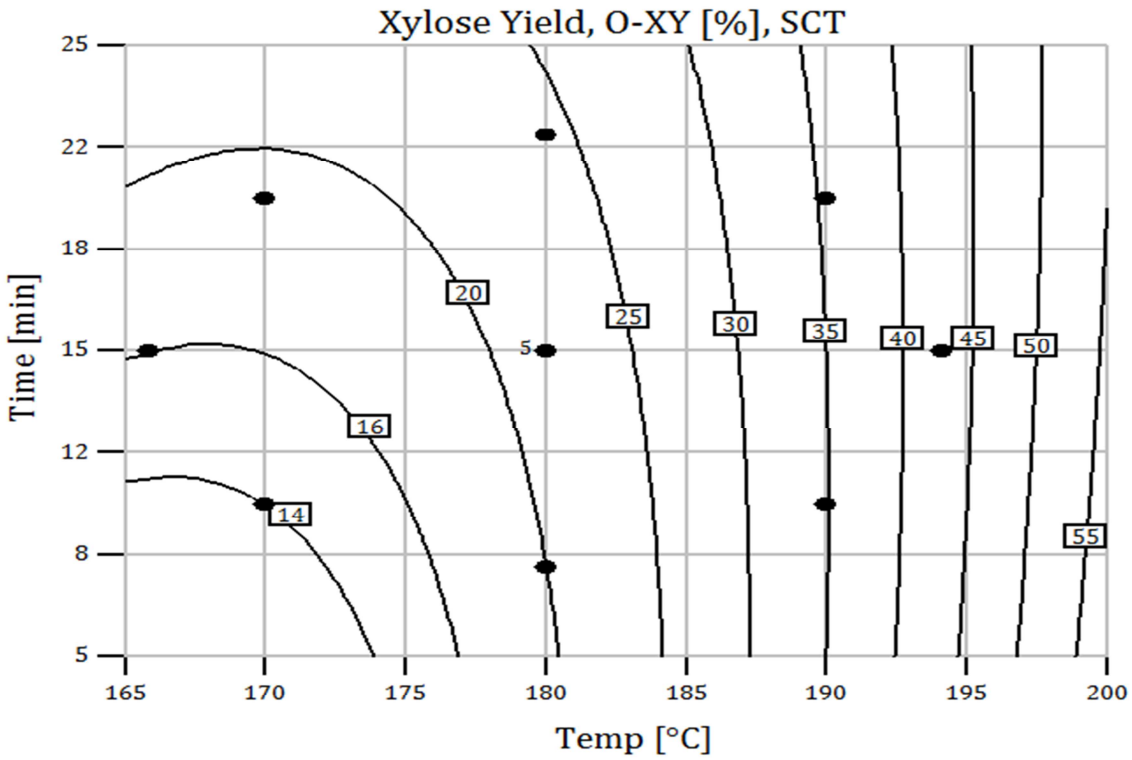


**Fig-2** Contour Plot for overall Xylose Yield (O-XY) for Sugarcane Trash (SCT) against Temperature and Retention Time

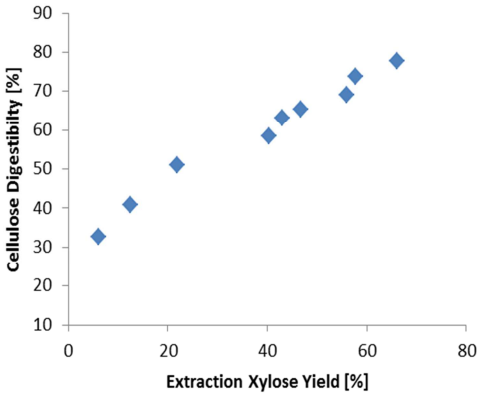


Fig-3a) Cellulose Digestibility vs Extraction Xylose Yield (Aspenwood)

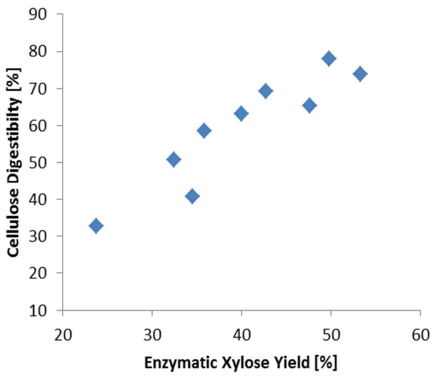


Fig-3b) Cellulose Digestibility vs Enzymatic Hydrolysis Xylose Yield (Aspenwood)

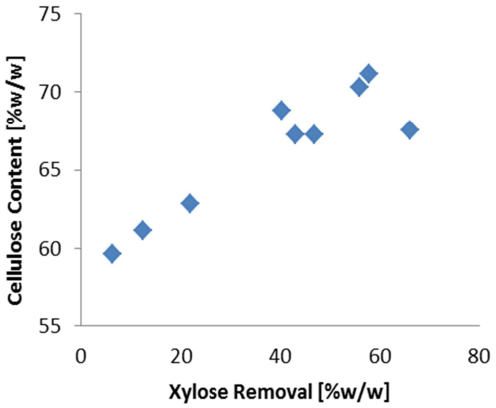


Fig-3c) Cellulose content in pretreated solids against xylose (hemicellulose) removal, (Aspenwood)

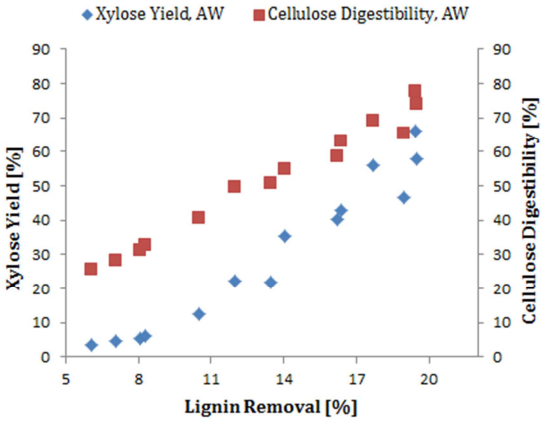


Fig-3d) Cellulose Digestibility and Extraction Xylose Yield against lignin removal (Aspenwood)

**Fig-3** Cellulose content and digestibility of pretreated solids against degree of removal of Xylose and/or Lignin (Aspenwood)

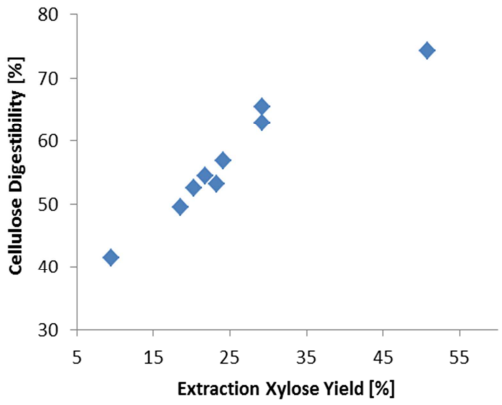


Fig-4a) Cellulose Digestibility vs Overall Extraction Xylose Yield (Sugarcane Trash, SCT)

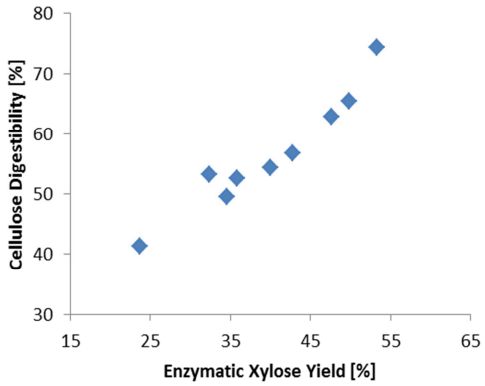


Fig-4b) Cellulose Digestibility vs Enzymatic Hydrolysis Xylose Yield (SCT)


Fig-4c) Cellulose content in pretreated solids against xylose (hemicellulose) removal (SCT)

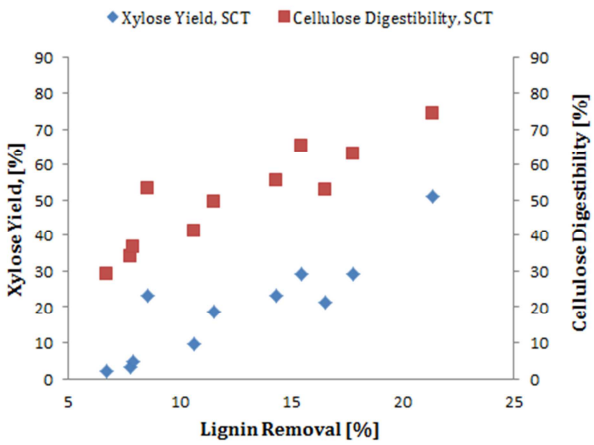


Fig-4d) Cellulose Digestibility and Extraction Xylose Yield against lignin removal (SCT)

**Fig-4** Cellulose content and digestibility of pretreated solids against removal of xylose (Hemicellulose) and/or lignin (Sugarcane Trash)