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ORIGINAL ARTICLE

A two-stage C5 selective hydrolysis on soybean hulls for xylose separation and value-added cellulose applications

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Abstract

Soy hulls from dehulling of soybeans are typically disposed of with soymeal or cattle feed. The hulls contain about 38.8% cellulose and 23.8% hemicelluloses and less than 4% lignin. The low lignin content, large volume availability, and being a "captive" feedstock make soy hulls an affordable raw material to produce C5 sugars such as arabinose and xylose from hemicelluloses. In this work, dilute acid hydrolysis of soy hulls using acid concentrations less than 1% (w/w) in solution and at different temperatures (125 °C, 140 °C, and 155 °C) was investigated to generate the kinetics data for sugar and degradation product release and study selectivity towards arabinose and xylose. The primary goal was to produce a hydrolysate rich in C5 sugars with minimal glucose and degradation products. Lower acid concentration (0.4% w/w) at 140 °C and lesser reaction time favoured selectivity towards arabinose release, while xylose release needed higher acid (0.6 to 0.8%) and longer time at the same temperature. From the kinetics data, a two-stage process was devised to achieve two separate hydrolysate streams rich in arabinose (5.00 ± 0.15 g dm^{-3}, 78.1% of total C5 sugars) and xylose (17.20 ± 0.71 g dm^{-3}, 87.7% of total C5 sugars). The xylose-rich stream was used to isolate xylose (powder form) under ambient process conditions using our patented process. The residual soy hulls, post the two-stage hydrolysis, showed high crystallinity with morphology analogous to microcrystalline cellulose, thus making them an effective starting material for high-value cellulose applications such as microcrystalline cellulose and polymer composites.

Keywords Soy hulls · Captive feed stock · C5 sugars · Dilute acid hydrolysis · Selectivity · Microcrystalline cellulose

1 Introduction

In the wake of rapidly depleting non-renewable energy resources, the world is looking towards biomass as a rescue to the ever-growing demand for energy. Biomass is an organic material that has biological origins, and the energy in it is derived from the sun. In general, all products originating from plants or animals are considered biomass. Of all the biomass, ligno-cellulosic biomasses have attracted a constant attention to impart greener sustainability into various processes. The ligno-cellulosic biomasses are typically sourced from sustainable sources such as various agricultural residues to produce biofuels and bioproducts that include biodiesel, biogas, ethanol [1, 2], calorie-free sugars [3], food additives (pectin) [4–6] and engineering materials (cellulose nanocrystals, cellulose nanofibres, membranes, resins, natural fibre reinforced composites, etc.) [7–9]. Soy is one such popular and main agricultural product with a worldwide production of about 350 million metric tons of soybeans that are produced and processed into oil, protein and meal products for a variety of food, feed and industrial applications. The USA alone has a record production of about 123 million tons (4.54 billion bushels) in 2018 [10]. Soybeans are a popular source of proteins and oil and currently serve as a primary alternative for meat and dairy products. Soybean oils are finding continuous applications in industries like tyres, additives for roads, and polymers to impart sustainability. The hull

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portion of the soybeans is separated before processing the beans for oil and protein. At around 4 lbs. of hulls per bushel of soybeans, there are an estimated 8 million tons of soy hulls generated in 2018 from soybeans processing in the USA. The hulls have very low market value, and currently, a small portion of them is used as a dietary fibre feed to the dairy cattle [11], while most of them are left as an agricultural waste [12].

The soy hulls are low lignin (4–10%) containing materials having 35–45% cellulose and 12–25% of hemicelluloses [13–15]. These hemicelluloses when separated and extracted can become a potential source of a variety of C5 sugars such as xylose and arabinose and some specialty chemicals like furfural and 5HMF [12]. Of these, xylose and arabinose are popular in the food industry and are known as low-calorie sugars and have an attractive market value [3]. The cellulosic part of soy hulls when separated from the hull structure can be used as a potential source of micro-sized reinforcement in the production of various polymer composites [16], micro crystalline cellulose [17] and nanocellulose/nanofibrillary cellulose [8, 13]. Until date, soy hulls have been used as a potential raw material for the production of pectin [4, 18, 19], oligopeptides, ethanol [20], and protein-polysaccharide fractions like galactomannan proteins and arabinogalactan proteins [21], carbon adsorbents [22, 23] and anion exchange resins [24].

Acid- or alkali-based hydrolysis is a commonly used technique to separate hemicelluloses from the biomass. Alkali hydrolysis produces different polymeric components of hemicellulose like xylan and arabinan, whereas acid hydrolysis breaks down the hemicellulose into corresponding individual components like xylose and arabinose. Therefore, it is plausible that acid hydrolysis would be a desired path to produce C5 sugars instead of alkali hydrolysis. However, there exist challenges related to selectivity, residual cellulose fibre quality and degradation products like acetic acid, furfural and 5HMF during acid hydrolysis of soy hulls. Hydrolysis as a pre-treatment step to ease the production of ethanol, where the cellulosic part of soy hulls remains as the main source of glucose for the microorganisms to ferment it into ethanol, has been reported in the literature. For example, Corredor et al. [14] reported a combination of dilute acid hydrolysis using H_2SO_4 (2% w/v) and steam explosion (140 °C) as pre-treatment, which increased the effectiveness of enzymatic hydrolysis (> 100%) to produce fermentable sugars for bioethanol. Similarly, the kinetics of acid hydrolysis of soy hulls with varying acid concentrations (H_2SO_4, 1.5–3% w/w) and temperature (110–160 °C) has also been reported [15]. While most of the literature on hydrolysis of soy hulls is targeted towards the extraction of fermentable C5 and C6 sugars, Merci et al. [17] reported an interesting way of preparation of microcrystalline cellulose from soy hulls using reactive extrusion with NaOH and H_2SO_4. This microcrystalline cellulose has potential applications in food, cosmetic and medical industries and can also be used as a reinforcing agent in the polymer composites.

As reported in most of the literature, it has been a conventional practice to use acid concentrations about 2–4% (w/w) for hydrolysis of a variety of biomasses. Cases where the biomass hydrolysis using lower acid concentrations like 1% or lesser with good C5 sugar recovery are very rarely reported. Fonseca et al. [3] reported that using a percolation reactor and a slightly higher reaction temperature reduces the acid loading by 8 times during hydrolysis of dried distillery grains while maintaining the hydrolysate with very low glucose levels and comparable amount of C5 sugars to that reported by earlier researchers using higher acid concentration. Furthermore, they reported the selectivity of C5 sugars by identifying the right conditions to selectively produce xylose- and arabinose-rich hydrolysates for easier recovery of xylose [25].

The data available in the literature on soy hulls clearly lack findings related to the selectivity of C5 sugars possibly due to the severity of hydrolysis conditions, i.e. high acid concentrations. Also, a huge gap exists in the area of hydrolysis of soy hulls with acid concentration less than 1.5% (w/w) in addition to the use of reactors (like percolation reactor) simulating hydrodynamics close to industrial-scale reactors/processors. It is very important to explore these low acid conditions because even a small change in acid concentration will severely impact the economics of the process (operating costs, equipment cost, neutralisation costs, involving in addressing environmental issues like high acid effluent treatment) and further the scalability. Minimising the formation of C5 degradation products and glucose in the hydrolysate significantly improves the economics of xylose isolation and recovery [25]. Therefore, the present work explores dilute acid hydrolysis of soy hulls especially with acid concentrations less than 1% (w/w) with an aim to examine the selectivity of C5 sugars like xylose and arabinose and to minimise the formation of glucose and degradation products. An extensive kinetics data was developed to study the release of sugars and degradation products into the hydrolysate with varying sulfuric acid loading and hydrolysis temperatures. Based on this kinetics data, a suitable C5 sugar selective two-stage process was devised, which gives two separate hydrolysate streams rich in xylose and arabinose while preserving the cellulosic part of the hulls. The xylose-rich stream was further used to produce xylose using our cost-effective and low-energy-consuming xylose isolation process [26]. It is important to note that the economics of our xylose isolation process significantly improves when no other C5 sugar (or C6) is present in the hydrolysate, which further justifies the coherent need for selective separation of C5 sugars during hydrolysis. Finally, the residual hulls from two-stage hydrolysis were analysed and are found to be rich in cellulose with micro-sized morphology and higher crystallinity, thus enabling their use to produce products such as microcrystalline cellulose and lightweight natural fibre–polymer composites.

2 Materials and methods

2.1 Materials

Samples of soy hulls were obtained from Owensboro Grain Company, Owensboro, KY, USA. High-purity standards of D(+)glucose, D(+)xylose, D(+)arabinose, furfural, 5-hydroxyl methyl furfural (5HMF) and acetic acid were purchased from Sigma-Aldrich (St. Louis, MO). Sulfuric acid, 96% is obtained from VWR.

2.2 Dilute acid hydrolysis

All hydrolysis reactions were performed in a 6-L percolation reactor with liquid recirculation (M/K Systems Inc., Peabody, MA) [3]. Soy hulls were screened prior to hydrolysis to remove fines or adhering dust on the hulls using US standard sieve no. 20 (0.85-mm opening). Nearly 90% of the soy hulls retained as coarse fraction were used for hydrolysis experiments. The soy hulls were placed in a perforated basket in the reactor and the liquid percolates through the bed of soy hulls and is recirculated using a pump to the top through a heater. A programmable controller was used to control the heater using the following inputs: set temperature, time to ramp the temperature of the solution form ambient temperature to set temperature and stand time at the temperature. Distributor plates were used on top of the baskets to ensure uniform distribution of the liquor through the soy hull bed. For each hydrolysis experiment, about 300 g of coarse soy hulls were used on dry basis, and liquor of 10 times the soy hulls' weight was used for recirculation. All the hydrolysis reactions were carried out for 2-h stand at three different temperatures, namely 125 °C, 140 °C and 155 °C, each having an initial ramp-up time of 40 min, 50 min and 60 min respectively. Different concentrations of H_2SO_4 loading namely 2%, 4%, 6% and 8% on the basis of dry soy hulls were used at all the stand temperatures tried. Table 1 summarises all the conditions used in the present study for the dilute acid hydrolysis of soy hulls. Each run was labelled based on the percentage acid loading and stand temperature; e.g. 2-125 represents 2% acid loading based on dry soy hulls used at 125 °C temperature. Hydrolysate samples were collected at regular time intervals of 20 min, starting from the onset of stand time using a sampling valve [27]. Once the stand time ended, the liquor in the reactor was cooled for about 30 min and separated from the residual soy hulls. In order to ensure reproducibility, selected experiments were repeated, and it was found that the data is reproducible with an acceptable ± 5% deviation.

Furthermore, to analyse the severity of each experimental condition, combined severity factor (CSF) [27] which combines the effect of both temperature and acid concentration was calculated as:

$$\mathrm{CSF} = \ln\left[t \times \exp\left(\frac{T_{\mathrm{r}} - T_{\mathrm{ref}}}{14.75}\right)\right] - \mathrm{pH}$$

where t is the time of reaction, T_{r} is the reaction temperature in °C, T_{ref} is the reference temperature (100 °C) and pH is the measured pH value of the liquor before the start of the reaction.

2.3 Analysis of soy hulls and determination of percent recovery of monomeric sugars

Soy hulls were analysed for their monomeric sugar content using the procedure reported in NREL/TP-510-42618. This procedure involves hydrolysing known amount of soy hulls using 72% sulfuric acid (10 g/g of soy hulls) for 1 h at 30 °C, followed by dilution with water until the acid concentration is 4%. This diluted mixture is subjected to further hydrolysis at 121 °C for 1 h and cooled before analysing for the quantities of monomeric saccharides like xylose, arabinose and glucose using high-precision liquid chromatography (HPLC).

2.4 Analysis of sugars and degradation products in the hydrolysate

The hydrolysate samples were analysed for monomeric sugars (glucose, xylose, arabinose) and degradation products (acetic acid, 5 HMF and furfural) using HPLC analysis performed in a Waters 600E HPLC system (Waters Corporation, Milford, MA) with an Agilent 1260 Infinity refractive index detector, and an Agilent Hi-Plex H column (300 mm × 7.7 mm, 8 mm). A total of 5 mol m^{-3} sulfuric acid solution at a flow rate of 0.7 mL min^{-1} was used as a mobile phase, while the column temperature was maintained at 60 °C and the refractive index detector temperature at 55 °C. Hydrolysate samples were filtered using 0.45-μm syringe filter and 30 μL of each standard solution, and the samples were injected in duplicate. Sample runtime was monitored for 55 min.

2.5 Characterisation of soy hulls

2.5.1 Morphology

The structure of soy hulls before and after hydrolysis was examined using a scanning electron microscope (SEM, TESCAN USA, Inc.) at a voltage of 10 kV to understand the effects of two-stage hydrolysis on the morphology of hulls. All the soy hull samples were mounted in a required orientation on a conductive carbon tape adhered to a SEM pin stub and are sputter-coated with a conductive 20-nm gold-palladium layer for 20 s prior to microscopy. All the images were scanned using auto tune settings for adjusting focus and working distance present in the instrument software.

Table 1 Conditions used for soy hull hydrolysis

Sample/run name	Acid loading based on dry soy hulls	Hydrolysis temperature, °C	Concentration of H_2SO_4 (w/w) in liquor (mass = 3 kg)
2-125	2%	125	0.2%
4-125	4%	125	0.4%
6-125	6%	125	0.6%
8-125	8%	125	0.8%
2-140	2%	140	0.2%
4-140	4%	140	0.4%
6-140	6%	140	0.6%
8-140	8%	140	0.8%
2-155	2%	155	0.2%
4-155	4%	155	0.4%
6-155	6%	155	0.6%
8-155	8%	155	0.8%

Soy hull loading per run: 300 g, solids to liquor ratio: 1:10

2.5.2 Chemical composition

Fourier transform infrared spectroscopy (FTIR) analysis was also performed on untreated and hydrolysed soy hulls using an attenuated total reflection (ATR)–enabled Perkin Elmer Spectrum 100 FTIR spectrometer to determine the changes in the chemical composition of soy hulls after acid hydrolysis. About 10 mg (< 40 mesh) of finely ground soy hulls was placed on top of the ATR crystal, and the sample was covered using the pressure anvil until the force gauge reads around 80 units. The final resultant scan was usually an average of 4 scans, where all the samples were scanned in the wavenumber range between 650 and 4500 cm^{-1}.

2.5.3 Thermal stability

Differential scanning calorimetry and thermo gravimetric analysis (DSC-TGA) of soy hulls were performed using a SDT TA Q600 system under nitrogen atmosphere to determine the effects of two-stage acid hydrolysis on the thermal stability of soy hulls. A sample of 10 mg was weighed and placed into the alumina sample cups in a furnace under nitrogen carrier gas, and a 20 °C/min heating rate was used to raise the temperature to 600 °C. The resulting TGA weight loss and DSC heat flow curves were monitored and analysed using the software provided along with the instruments.

2.5.4 Crystallinity

XRD analysis of hydrolysed and untreated soy hulls was performed using Bruker D8 powder X-ray diffraction (XRD) with nickel-filtered Cu Kα radiation ($\lambda = 1.5418$ Å) to study the changes in crystallinity caused by two-stage acid hydrolysis. All the samples were finely ground (< 40 mesh) and used for the analysis. All the scans were performed at a scan speed 4° min^{-1} within the fixed range between 10° and 40° (2θ).

3 Results and discussions

3.1 Kinetics of soy hull hydrolysis

Using the NREL method, we found the following polysaccharide composition for the soy hulls used in this study: cellulose 38.73 ± 0.7%, xylan 18.43 ± 0.29% and arabinan 5.38 ± 0.29% on a dry basis. The cellulose and hemicellulose concentrations of soy hulls found in this work were within the range reported in the literature [15]. This composition of soy hulls and its wide availability makes it a potential and cost-effective raw material for an integrated bio industry producing xylose and arabinose [25] and simultaneously as a cellulosic natural fibre reinforced composites [16].

One primary intent of this study is to produce a C5-rich hydrolysate with minimal content of glucose and degradation products. The selectivity towards C5 sugars from soy hulls involves optimising process parameters such as time, temperature and acid concentration. Figure 1 shows the composition of hydrolysates produced using conditions as listed in Table 1. This data would help in optimising the processing conditions to improve the selectivity of xylose over arabinose and glucose and minimising degradation products (acetic acid, 5HMF and furfural) in the hydrolysate. As expected, the data in Fig. 1 shows that with an increase in acid concentration, temperature and time, the concentrations of sugars, i.e. xylose, arabinose and glucose, as well as their degradation products (furfural

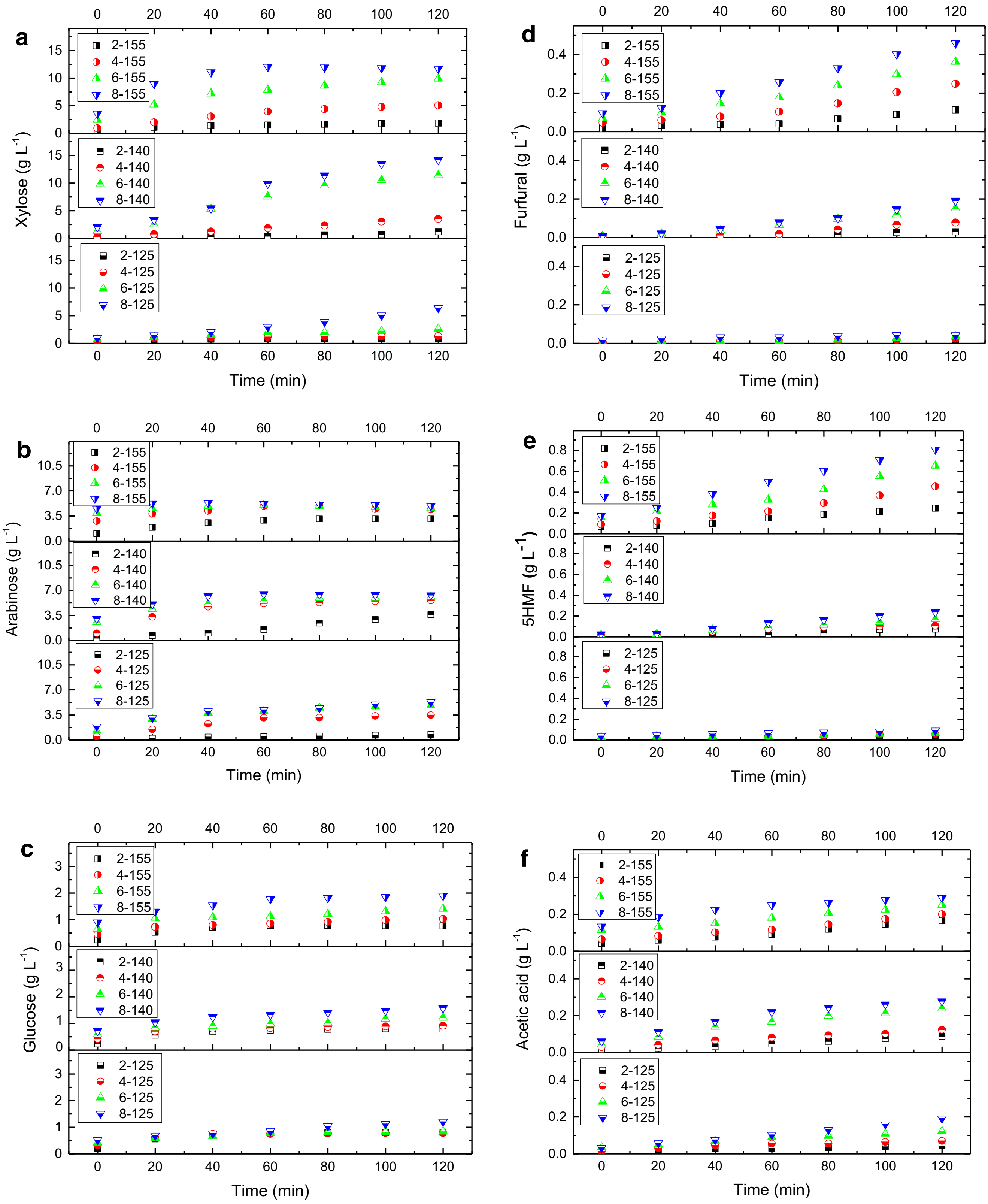


Fig. 1 Impact of temperature and H_2SO_4 loading on the release of xylose, arabinose, glucose, acetic acid and degradation products from soy hull hydrolysis

and 5HMF) and byproducts from side chains of hemicellulose (acetic acid) increased.

The final hydrolysate consists of two significant C5 sugars xylose and arabinose and a minor amount of C6 sugar glucose. Figure 1a and b show that for any given operating temperature, the final concentrations of xylose and arabinose in the hydrolysate have significantly increased with increase in acid loading. However, Fig. 1c shows that glucose has the lowest concentration among all the sugars for the conditions reported. Glucose is usually obtained by hydrolysis of either cellulose into its monomeric form or glucuronic acid side groups attached to the xylan polymer of hemicelluloses [28]. Both xylose and arabinose are obtained from the hydrolysis of xylan, arabinan and arabinoxylan polymers of hemicellulose [29]. Unlike glucose, both xylose and arabinose concentration in the final hydrolysate (after 2-h reaction) was peaking at 140 °C and 0.8% acid (8-140: 14.63 g/L and 6.4 g/L respectively) and not at 155 °C and 0.8% acid (8-155: 11.61 g/L and 4.8 g/L respectively, Fig. 1a and b). Figure 1a shows that there is a noticeable release of xylose (> 1 g/L) even before the onset of the stand temperature for 0.6% and 0.8% acid at 140 °C and 155 °C (6-140, 8-140, 6-155 and 8-155). This implies that the significant release of xylose kicks off at temperatures greater than 125 °C and at higher acid concentrations. Furthermore, an additional 10 and 20 min were provided during the temperature ramp for the liquor to reach the stand temperatures 140 °C and 155 °C respectively compared with that of 125 °C may also be responsible for higher initial xylose (> 1.5 g/L) during the onset ($t = 0$ min) of the reaction at corresponding temperatures. The xylose concentration increased with time for all acid concentrations used at temperatures 125 °C and 140 °C, while at 155 °C and 0.8% acid, the xylose release was highest for the initial 60 min with peak value 12.06 g/L and then took a down trend with the value reduced to (11.6 g/L) at 120 min operating time. A higher release of xylose was observed at 140 °C and 0.8% acid (8-140) compared with that of 155 °C and 0.8% acid (8-155) from 80 min until the end. A similar trend was observed for 0.6% acid at 140 °C (6-140) and 155 °C (6-155) with a crossover point after 60 min. This trend can be attributed to the formation of degradation products like furfural and 5HMF, which amplified with high operation temperature, i.e. 155 °C and higher acid concentrations too (Fig. 1d and e).

On the other hand, final arabinose concentrations in the hydrolysate at the end of operating time showed trends similar to that of xylose, but there is a key difference in the release of arabinose (Fig. 1b) compared with that of xylose. The release of arabinose is very quick when compared with that of xylose, excluding two conditions, 0.2% acid at 125 °C and 140 °C (2-125 and 2-140). This can be possibly explained by the presence of arabinoxylans in soy hull hemicellulose, where arabinose groups are loosely bound to the xylan backbone as side chains [30]. These side chains can be cleaved easily under acidic conditions and at significantly lower temperatures, thus facilitating the faster arabinose release. We can observe that majority of the arabinose released from soy hulls within the first 40 min of operation time and remains more or less constant until the end of the reaction. In most of the cases, 50% of the final arabinose coming out at the end of the reaction was already there during the onset of the hydrolysis stand time (0 min). Maximum value of arabinose 6.51 g/L was observed at 0.8% acid loading and 140 °C (8-140) and at 60 min. Although the extent of arabinose release increased with an increase in acid loading, it looks quite sensitive to temperature. A temperature of 140 °C was found to be more suitable for arabinose release where a higher arabinose release was observed at 0.4%, 0.6% and 0.8% acid when compared with their respective counterparts at 155 °C. Possibly, arabinose being released faster than xylose may act as an initial feed source for the formation of degradation products like furfural [31]. This was especially evident during higher operating temperature 155 °C (Fig. 1d) where a lowering of arabinose concentration in liquor was observed as time proceeds at 0.4%, 0.6% and 0.8% acid (4-155, 6-155 and 8-155).

C-6 sugar glucose concentration increased continuously as the reaction proceeded and increased with an increase in acid loading and temperature. A 2-h long hydrolysis yielded a maximum of 1.9 g/L of glucose for an acid concentration of 0.8% and 155 °C temperature (Fig. 1c). The value is quite low when compared with xylose and arabinose. At lower acid concentrations, i.e. 0.2% and 0.4% acid, the effect of temperature on glucose release with time was minimal, while at higher acid loadings, temperature significantly increased the release of glucose. Cassales et al. [15] reported similar findings in his work stating that glucose release is more impacted by temperature and acid concentration.

Figure 1d and e show the kinetics of the release of the sugar degradation products furfural and 5 HMF into the hydrolysate with different acid concentrations and temperatures used. It is quite evident that higher temperature and acid concentration accelerate their rate of formation of furfural and significantly when compared with that of milder conditions. Hydrolysis conditions having acid concentrations 0.4%, 0.6% and 0.8% at 155 °C (4-155, 6-155 and 8-155) yielded higher degradation products compared with 0.8% acid at 140 °C (8-140) which showed the highest yield of xylose and arabinose in the liquor. The onset of formation of degradation products at higher temperature 155 °C (4-155, 6-155 and 8-155) and acid concentration, possibly contributed to the loss of xylose. Figure 1f shows the kinetics of the release of acetic acid, which is a byproduct of the hydrolysis and is usually obtained from the side chains of xylan polymer. Although in most of the cases, its concentration increased with temperature, acid concentration and time, condition sets 6-140 and 6-155 and 8-140 and 8-155 yielded almost similar concentrations into the hydrolysate with time. Furthermore, we can observe that a rise in the concentration of acetic acid at these conditions attains a plateau after a runtime of 60 min.

Figure 2 shows the dependency of percentage yields of xylose and arabinose in the final hydrolysate with CSF. Following the observations from Fig. 1, the data in Fig. 2 show that high severity factors (combination of high temperature and high acid concentration) contributed to the loss of percentage yields of xylose and arabinose and simultaneously increased the concentration of degradation products. While the percentage yields consistently increased with increase in CSF for all conditions at temperatures 125 °C and 140 °C, the yields at all acid concentrations at 155 °C with higher CSF values are lower than that of corresponding 140 °C. So it makes sense that CSF can be effective in predicting the yields of C5 sugars from soy hull hydrolysis for reaction temperatures 140 °C and lesser and acid concentrations less than 1% where the sugar degradation reactions are minimal. As discussed earlier, the xylose recovery was maximum at 67.5% for 0.8% acid and 140 °C (8-140), whereas 99% of the arabinose seems to be extracted in the same condition. Even 0.4% acid at 140 °C (4-140) gives almost 87% of arabinose recovery while only extracting 16% xylose, which gives arabinose-rich hydrolysate. Cassales et al. [15] reported similar values of xylose and arabinose recovery for a similar operating temperature but at higher acid loading (2.1% acid and 153 °C) and only 60 min operation time. Although higher acid loading reported by Cassales et al. has reduced the operation time significantly but the hydrolysate simultaneously had a higher amount of glucose (3–4 g/L), arabinose (6 g/L) and degradation products (almost 3 to 4 times of values reported in our work), which significantly impacts the selective separation.

3.2 Optimal hydrolysis conditions for pentose selectivity

Data from Figs. 1 and 2 show a reasonable difference between the rates of release of arabinose and xylose as well as the time needed to begin the release of the C5 sugars. Arabinose release began even at lower temperatures and acid concentrations, while xylose release relied more on the amount of acid loading and time. Based on this observation, a two-stage time-specific selective operation was evaluated to exclusively extract arabinose first from the soy hulls in stage 1 (operation time = 40 min), and then extracting the xylose using the remaining biomass from the first stage in stage 2 (operation time = 60 min). Fonseca et al. [3] followed the same strategy by identifying the right conditions for selectively extracting arabinose and xylose separately from dried distillery grains. These conditions and the final concentrations of arabinose and xylose in the final hydrolysate after each stage are reported in Table 2. The hydrolysate resulting from stage 1 hydrolysis (Table 2) had a higher arabinose (78.1% of C5 sugars) concentration of 5.0 to 1.4 g/L of xylose, while the stage 2 hydrolysate had a 17.2 g/L xylose (88% of C5 sugars) to that of a mere 2.3 g/L arabinose—thus, stage 1 operation shows a greater selectivity towards arabinose and the stage 2 operation shows a greater selectivity towards xylose. It is noteworthy to say that the selected total combined reaction time of the two-stage operation is just 100 min and relatively less compared with that of the experiments used to determine the kinetics data (120 min), further causing lower degradation of sugars and cellulose. The xylose-rich hydrolysate from stage 2 with low concentrations of other sugars and degradation products was found to be a suitable starting material for patented xylose

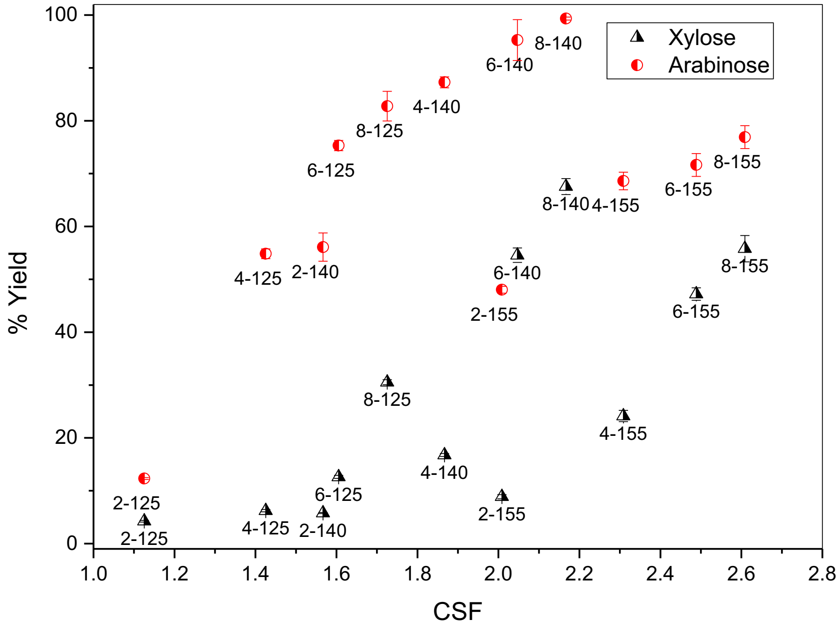


Fig. 2 Plot showing the dependency of the percentage yields of xylose and arabinose at the end of 2-h reaction on combined severity factors (CSF) of all the experiments used

Table 2 Conditions of selected two-stage hydrolysis and concentrations of sugars and degradation and byproducts of respective hydrolysates

	Stage 1	Stage 2
Hydrolysis conditions		
% Acid loading based on dry soy hulls	4%	6%
Soy hulls to liquor ratio	1:10	1:7
Acid concentration in the liquor	0.4%	0.85%
Temperature, °C	140	140
Stand time, min	40	60
Hydrolysate composition		
Xylose, g dm^{-3}	1.40 ± 0.06	17.20 ± 0.71
Arabinose, g dm^{-3}	5.00 ± 0.15	2.34 ± 0.10
Glucose, g dm^{-3}	0.82 ± 0.01	1.96 ± 0.05
Acetic acid, g dm^{-3}	0.07 ± 0.00	0.27 ± 0.01
5HMF, g dm^{-3}	0.05 ± 0.00	0.34 ± 0.02
Furfural, g dm^{-3}	0.01 ± 0.00	0.13 ± 0.00

isolation and separation process (US Patent # 10,407,453) developed by biomass and biofuels group at University of Louisville (U*of*L) [26]. This hydrolysate stream from the second stage was evaluated for xylose isolation using this process, and pure xylose as shown in Fig. 3 was produced.

After selectively separating out xylose and arabinose in a two-stage process, the residual soy hulls are expected to be rich in cellulose. In general, these residual hulls are resultant from physical treatments following hydrolysis to separate the hydrolysate adhering to the hulls. Following stage 1 hydrolysis, the soy hulls were mildly pressed to squeeze out the hydrolysate and then fed for the second stage. At the end of the stage 2 operation, the hydrolysate was separated from soy hulls by squeezing them, and furthermore, the soy hulls are washed to remove any residual acid and dried in ambient conditions. These dried hulls were further examined using SEM, XRD, FTIR and TGA to analyse the changes in morphology, crystallinity and thermal stability.

Fig. 3 Xylose produced from second-stage hydrolysate using U*of*L process

3.3 Morphology of the residual soy hulls after two-stage hydrolysis

Figure 4 shows a microscopic morphology of as-received, stage 1 and stage 2 soy hulls. Figure 4a shows the outside and inside surfaces and Fig. 4b shows the cross-section of the as-received soy hulls. A higher magnification of SEM was used in Fig. 4b to show the entire cross-sectional view and the three-layered structure consisting of a thin inside layer, an intermediate pillar-like cellulosic fibres and a thick outside layer (thick layer) all cemented together by a waxy substance. These pillar-like fibres when separated from the hull can be used as good dispersible reinforcements in polymer composites. It can be noted from Fig. 4c that after stage 1 hydrolysis, a significant loosening of three layers was achieved exposing the intermediate fibres. Figure 4d shows that stage 2 hydrolysis successfully detached the fibres from the hulls. These fibres are measured to have a length of approximately 100 μm and a diameter of 5 μm.

3.4 Effect of two-stage hydrolysis on chemical composition of soy hulls

Fourier transformed infrared spectroscopy (FTIR) was performed on as-received, stage 1 and stage 2 soy hulls, and the results are presented in Fig. 5. The analysis provides a qualitative comparison of the changes occurring in functional groups among different hydrolysis treatments. In all three FTIR curves (Fig. 5), the baselines were normalised against the peak at 1020 cm^{-1} that corresponds to C–O–H stretching intensity. All samples showed OH (3339 cm^{-1}), C–O (990–1275 cm^{-1}) and C=O (1741 cm^{-1}) and amide (1550 cm^{-1}) groups indicating the hydrophilicity of soy hulls along with C=C (1681 cm^{-1}) and C–H bending (1300–1470 cm^{-1}) and C–H stretching (2930 cm^{-1}–2868 cm^{-1}) [32–36]. The amide peak of as-received soy hulls can be attributed to the protein content in them, and it decreased with stage 1 and stage 2 processes suggesting removal of acid-soluble protein during hydrolysis. Further reduction in the intensity of C=C peak with each stage of hydrolysis demonstrates possible removal of unsaturated fatty acids, which are associated with the presence of oils on the hulls. The intensity of O–H peak for soy hulls from stage 2 hydrolysis appears to be smaller than as-received and stage 1 soy hulls. This can be attributed to the removal of the majority of hemicelluloses from the soy hulls particularly in stage 2 hydrolysis resulting in a marginal increase in hydrophobicity. The increase in hydrophobicity is desirable for natural fibres used in lightweight natural fibre–

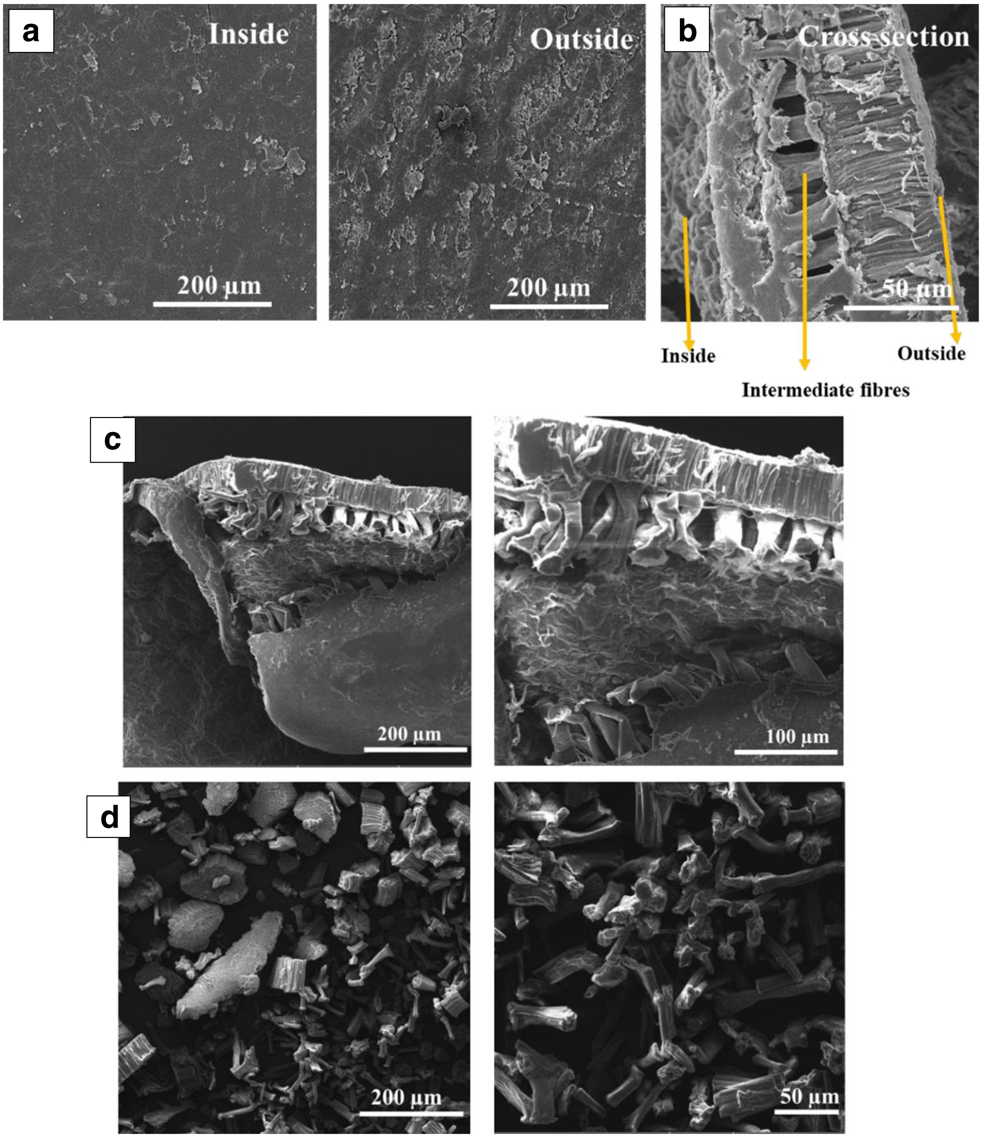


Fig. 4 The SEM micrographs of **a** as-received soy hulls showing inside and outside views, **b** cross-sectional view of as-received soy hulls at a higher magnification, **c** residual hulls after stage 1 hydrolysis at two different magnifications showing hull surface and fibres and **d** residual hulls after stage 2 hydrolysis at two different magnifications showing separated soy hull fibres

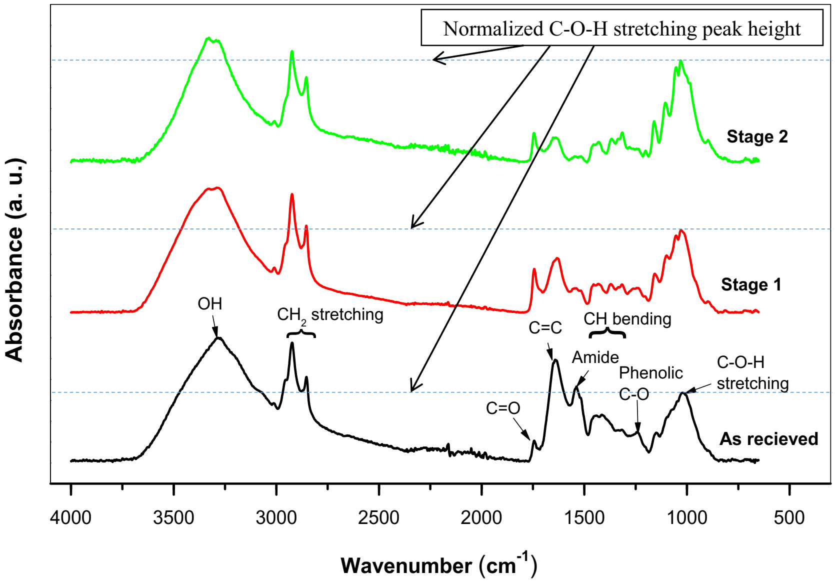


Fig. 5 Comparison of FTIR spectra of hydrolysed (stages 1 and 2) and untreated soy hulls

polymer composites. Compared with other biomasses, absence of prominent aromatic peaks (1505 cm^{-1} and 1600 cm^{-1}) can be noted in treated and untreated soy hulls due to the low percentage of lignin in it. However, the phenolic C–O–C peak that could be assigned to lignin is further reduced in the treated samples indicating removal of acid-soluble lignin during acid hydrolysis.

3.5 Thermal stability behaviour of residual soy hulls post two-stage hydrolysis

DSC, TGA and derivative TGA analysis of as-received, stage 1 and stage 2 soy hull was performed. Figure 6 shows the effect of each stage of hydrolysis on thermal stability. All the samples exhibited weight loss under 100 °C as a result of moisture removal, which can be observed in TGA and derivative TGA data (peaks around 70–80 °C in derivative TGA curve). From TGA data, it can be deduced that the as-received and stage 2 soy hulls had a moisture ~ 5.8%, while the stage 1 soy hulls had slightly high moisture of 8.2%, while the leftover residual mass post the analysis varied as 26.2%, 18.5% and 23.18% respectively for as-received, stage 1 and stage 2 soy hulls. The derivative TGA data of as-received soy hulls shows that the onset of thermal degradation starts at 200 °C, with two peaks at 260 °C and 345 °C and finally end at 390 °C, which represents a typical pattern of any cellulosic biomass. Soy hulls from stage 1 hydrolysis also showed a similar onset and end temperatures but with a single peak at 347 °C. The disappearance of degradation peak at 260 °C in stage 1 soy hulls can be attributed to the removal of any of the adherent protein and fatty acid portion of the soy hulls during stage 1 hydrolysis, while preserving most of the cellulose and hemicellulose content. Derivative TGA curve of stage 2 soy hulls showed a noticeable change in the peak degradation temperature which dropped from 347 °C in stage 1 to 290 °C. This peak degradation temperature of stage 2 soy hulls complies with the value of native microcrystalline cellulose reported in the literature [37]. This drop in thermal stability of soy hulls post stage 2 can be attributed to the removal of the majority of the amorphous hemicellulose which acts as a binder thus imparting structural stability of the cellulosic fibres [38]. Furthermore, the exposure of micro-sized cellulosic fibres after stage 2 hydrolysis induced an increased surface area that is exposed to a heat source, thus reducing the thermal stability significantly [37]. Typically, cellulosic materials do not exhibit any phase change properties like melting, rather they disintegrate thermally, which can be clearly observed from the DSC curves. DSC analysis indicates that there is a drop in heat flow (endothermic) at around 350 °C for all the samples indicating the peak degradation temperature. Furthermore, on close observation, one can note a small dip in the heat flow between 270 and 320 °C especially for stage 2 soy hulls showing a good allegiance with its respective derivative TGA data.

3.6 XRD analysis of residual soy hulls post two-stage hydrolysis

The effect of hydrolysis on the cellulose crystallinity of different soybean hulls is presented in Fig. 7. The XRD peaks were found to match very well with the cellulosic material of soybean hulls exhibiting amorphous and crystalline peaks at $2\theta = 15$ and 23 respectively [14]. Distinguishable difference was observed in the peak intensity and width among different samples. The as-received hulls showed broad peaks between $2\theta = 10$ and 25. These peaks gradually transformed to narrow peaks with high intensity, which clearly indicates an increase in the crystalline constituents in the hydrolysed soybean hulls. Among the

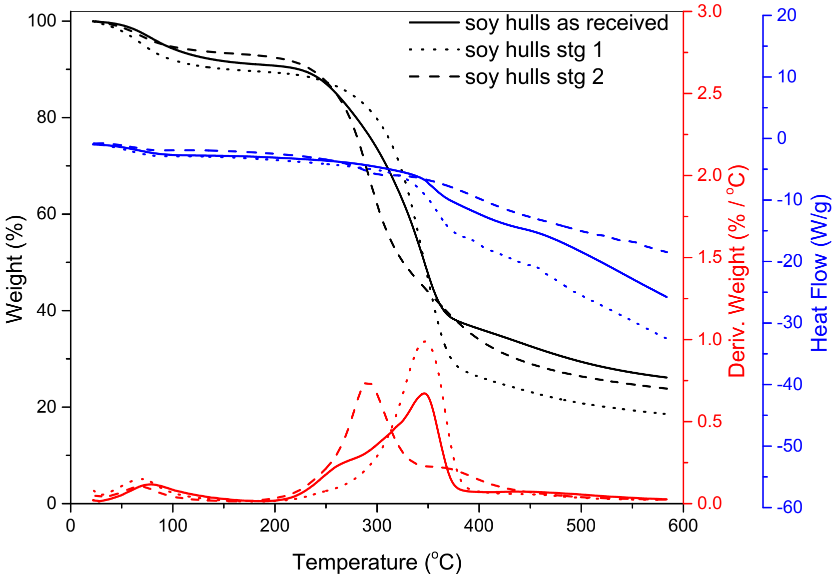
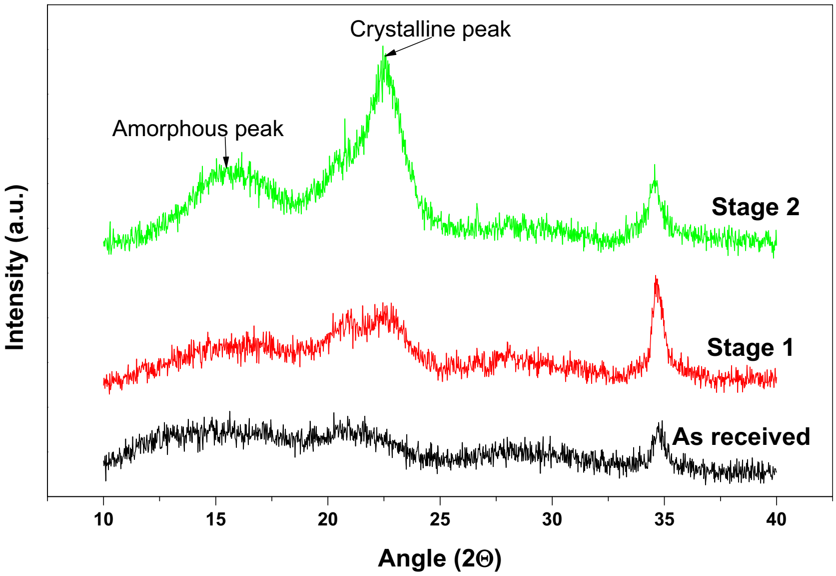


Fig. 6 TGA (black colour), derivative TGA (red colour) and DSC (blue colour) analysis of as-received, stage 1 and stage 2 soy hulls showing the variation of thermal properties with two-stage hydrolysis

Fig. 7 Comparison of XRD spectra of soy hulls as-received and after stage 1 and stage 2 hydrolysis showing clear increase in the intensity of crystalline peaks after stage 2 hydrolysis



treatments, stage 2 hydrolysis was found to provide more crystalline hulls compared with stage 1 treatment. Corredor et al. [14] reported similar changes, and the amorphous peaks correspond to hemicellulose; waxes and fatty acids in untreated soy hulls showed a predomination over the crystalline peaks. Crystallinity index is a very common way of measuring the crystallinity which is a measure of the ratio of crystalline to amorphous masses present in polymers. Crystallinity index values of present soybean hulls were calculated using peak subtraction method [39] and found to be 61%, 76% and 84%, respectively for as-received, stage 1 and stage 2 soy hulls. This significant increase in the crystallinity index with each stage of operation can be attributed to the removal of amorphous groups from soy hulls especially waxes, fatty acids and some hemicelluloses in stage 1 and majority of hemicelluloses in stage 2. The crystallinity index of stage 2 soy hulls is in line with that of microcrystalline cellulose reported in literature [17]. From this analysis, it appears that the highly individualised micro-sized soy fibres (Fig. 4d) with high crystallinity after stage 2 hydrolysis (Fig. 7) can be effective in the production of microcrystalline cellulose and reinforcement fibres for lightweight natural fibre–polymer composites. These treated hulls were also employed as reinforcing agents in thermo plastic copolyester (TPC)–soyhull composites, which showed promising compatibility [40, 41].

4 Conclusions

In this study, we showed that dilute sulfuric acid hydrolysis of the soy hulls with concentrations less than 1% can be very effective to selectively separate out C5 sugars and improve the crystallinity of cellulose in the remaining soy hull biomass. The kinetics study in the given range of acid concentration and temperature range helped to devise a two-stage process leading to arabinose-rich and xylose-rich hydrolysates respectively after stage 1 and stage 2. The arabinose and xylose yields increased with an increase in hydrolysis temperature in the range 125 and 140 °C and dropped at 155 °C as the degradation products started to form. A temperature of 140 °C was found to be the suitable reaction temperature that provided higher yields of both arabinose and xylose as well along with negligible levels of degradation products and glucose, while acid concentration and reaction time decided the selectivity towards arabinose and xylose. The combined severity factor appeared to be effective in predicting the xylose and arabinose yields within the reaction temperature range of 125–140 °C. The two-stage hydrolysis was also effective in deconstructing the soy hull and releasing the cellulosic fibres. There was a consistent increase in the crystallinity of the residual soy hulls with each stage of hydrolysis, but the thermal stability increased slightly after stage 1 and decreased after stage 2 hydrolysis. Dry xylose product was successfully isolated from the second-stage hydrolysate using a patented xylose isolation process (US Patent # 10,407,453) developed by the University of Louisville. The residual soy hulls coming from the two-stage process showed morphology, thermal stability and crystallinity behaviour similar to micro crystalline cellulose. Thus, the two-stage hydrolysis of soy hulls is effective in producing arabinose- and xylose-rich hydrolysates as well as hydrolysed soy hull biomass suitable for the production of microcrystalline cellulose and reinforcement fibres in lightweight natural fibre–polymer composite applications.

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