Uncatalysed steam pretreatment regimes for bagasse and harvest residues in a sugarcane biorefinery

by

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ABSTRACT

Biorefining of sugarcane lignocellulosic wastes, i.e. bagasse and harvest residues, at existing cane sugar mills can diversify product ranges to potentially improve profitability. Bagasse is the fibrous residue after juice extraction from the cane and harvest residues are all agricultural wastes generated during sugarcane harvesting. Pretreatment, the first step in bioprocessing of lignocellulose, must ensure maximum yields of desired sugar intermediates (glucose, xylose and arabinose) according to lignocellulosic feedstock and downstream bioconversion requirements. This study chose steam pretreatment for its proven track record in largescale operations and availability of steam at sugar mills, and was studied uncatalysed to allow operation without chemicals addition.

The originality of this study firstly included the direct comparison of bagasse and harvest residues pretreatment operability in the same equipment. Uncatalysed steam pretreatment of these feedstocks was optimised by response surface methodology in the ranges of 185 – 215 °C and 5 – 15 min for maximum digestibility (indication of cellulose accessibility to enzymes) of the solids, maximum combined sugar yield (CSY) and maximum hemicellulose recovery to identify preferred pretreatment operating regimes. Secondly, deacetylation (acetyl groups removed from lignocellulose with a mild alkaline extraction) upstream of uncatalysed steam pretreatment was proposed as a detoxification step to remove acetic acid in downstream fermentation. Uncatalysed steam pretreatment of raw and deacetylated bagasse and harvest residues was compared on digestibility, fermentability (portion of maximum theoretical ethanol yield that can be obtained) and dewaterability of the pretreated material. Thirdly, the contribution of the explosion step during steam pretreatment to improving digestibility of the pretreated solids was

investigated by comparing material retained and released during the sudden depressurisation at the end of pretreatment.

The most significant findings of this study included that sugarcane harvest residues were better suited than bagasse for biorefining via a sugar platform for the following reasons: (1) harvest residues allowed for robust uncatalysed steam pretreatment at a single condition (between 198 and 200 °C, and between 8 and 12 min) to obtain at least 95% of the maximum digestibility, CSY and hemicellulose recovery values, respectively; (2) maximum digestibility was obtained at lower severity (205.8 °C, 8.7 min as opposed to 215 °C, 15 min for bagasse); (3) pretreated harvest residues did not require detoxification at optimum pretreatment conditions; and (4) pretreated harvest residues displayed better dewaterability, especially when pretreated for maximum hemicellulose recovery, compared to bagasse. Furthermore, it was found that the mechanical impingement experienced by steam pretreated material as it is transported during depressurisation, has a significant contribution to improving digestibility. Also, it was found that deacetylation of feedstocks can be performed prior to uncatalysed steam pretreatment as a detoxification step that could potentially increase fermentability of pretreated pressed slurries (not washed).

Consequently, it is proposed that bagasse continues to be used to generate steam and electricity in a sugar mill, while attention should be given to the recovery, collection and allocation of sugarcane harvest residues as an attractive sugarcane biorefinery feedstock.

OPSOMMING

Bioraffinering van suikerriet lignosellulose-afval, i.e. bagasse en oesreste, by bestaande suikermeulens kan produkreekse diversifiseer en winsgewendheid moontlik verbeter. Bagasse is die veselreste na ekstraksie van suikersap uit suikerriet, terwyl oesreste alle plantaardige afval wat gegenereer word gedurende die oes van suikerriet insluit. Voorbehandeling, die eerste stap in bioprossesering van lignosellulose, moet die maksimale opbrengs van die verlangde intermediêre suikers (glukose, xilose en arabinose) verseker na gelang van die lignosellulosevoer en stroomaf bio-omsettingvereistes. Hierdie studie het stoomvoorbehandeling gekies vir sy bewese prestasies in grootskaalse bedrywe, asook vir die beskikbaarheid van stoom by suikermeulens, en is ongekataliseerd ondersoek om voorsiening te maak vir bedryf sonder die toevoeging van chemikalië.

Die oorspronklikheid van hierdie studie het eerstens die direkte vergelyking in voorbehandelingsbedryfbaarheid tussen bagasse en oesreste in dieselfde toerusting ingesluit. Ongekataliseerde stoomvoorbehandeling van hierdie voere is geoptimeer deur gebruik te maak van responsie-oppervlak metodologie in die bestekke 185 – 215 °C en 5 – 15 min vir maksimum verteerbaarheid (indikasie van sellulose toeganklikheid tot ensieme) van soliede, maksimum gekombineerde suikeropbrengs en maksimum hemisellulose herwinning om voorkeur voorbehandelingsbedryfsregimes te identifiseer. Tweedens, deasetilering (asetielgroep verwydering uit lignosellulose met ‘n matig alkaliese ekstraksie) stroomop van ongekataliseerde stoomvoorbehandeling is as detoksifiseringstap voorgestel vir die verwydering van asynsuur in stroomaf fermentasie. Ongekataliseerde stoomvoorbehandeling van rou en gedeasetileerde bagasse en oesreste is vergelyk op grond van verteerbaarheid, fermenteerbaarheid (gedeelte van maksimum teoretiese

etanolopbrengs wat behaal kan word) en ontwatering van die voorbehandelde materiaal. Derdens, die bydra van die ontploffingstap gedurende stoomvoorbehandeling om verteerbaarheid van voorbehandelde materiaal te verbeter, is ondersoek deur materiaal te vergelyk wat teruggehou is en vrygelaat is gedurende die skielike drukontlasting aan die einde van voorbehandeling.

Die mees beduidende bevindings van hierdie studie het ingesluit dat suikerriet oesreste meer geskik was as bagasse vir bioraffinering via ‘n suikerplatform weens die volgende redes: (1) oesreste het toegelaat vir robuuste ongekataliseerde stoomvoorbehandeling by ‘n enkele kondisie (tussen 198 en 200 °C, en tussen 8 en 12 min) om ten minste 95% van die maksimum verteerbaarheid, gekombineerde suikeropbrengs en hemisellulose herwinning waardes respektiewelik te behaal; (2) maksimum verteerbaarheid is by ‘n minder strawwe kondisie verkry (205.8 °C, 8.7 min teenoor 215 °C, 15 min vir bagasse); (3) voorbehandelde oesreste het nie detoksifisering by die optimale voorbehandelingskondisies benodig nie; en (4) voorbehandelde oesreste het beter ontwatering getoon, veral wanneer daar voorbehandel is vir hemisellulose herwinning, vergeleke met bagasse. Verder is daar gevind dat die meganiese botsing, soos ondervind deur die voorbehandelde materiaal wanneer dit vervoer word gedurende drukontlasting, beduidend bydra tot die verteerbaarheid. Ook is gevind dat deasetilering van die voerstowwe voor ongekataliseerde voorbehandeling uitgevoer kan word as ‘n detoksifiseringstap vir potensieel verbeterde fermenteerbaarheid van gepersde flodders (ongewas).

Gevolglik word dit voorgestel dat bagasse steeds benut word vir die opwekking van stoom en eletrisiteit in ‘n suikermeul, terwyl aandag geskenk moet word aan die

herwinning, versameling en toekenning van suikerrietoesreste as ‘n belowende voerstof tot ‘n suikerrietbioraffinadery.

ZUSAMMENFASSUNG

Die Bioraffinierung von Zuckerrohrlignocelluloseabfällen, i.e. Bagasse und Ernterückständen, in bestehenden Zuckermühlen kann die Produktpalette diversifizieren, um möglicherweise die Rentabilität zu verbessern. Bagasse ist der faserige Rückstand nach der Saftgewinnung aus dem Rohr und Ernterückstände sind alle landwirtschaftlichen Abfälle, die während der Zuckerrohrernte entstehen. Vorbehandlung, der erste Schritt bei der Bioverarbeitung von Lignocellulose, muss maximale Ausbeuten an gewünschten Zuckerzwischenprodukten (Glukose, Xylose und Arabinose) gemäß den Anforderungen an Lignocelluloseausgangsmaterialien und nachgeschalteten Biokonversionsanforderungen sicherstellen. Diese Studie wählte Dampfvorbehandlung aufgrund ihrer nachgewiesenen Erfolgsbilanz im Großbetrieb und der Verfügbarkeit von Dampf in Zuckermühlen, und wurde auch nicht katalysiert untersucht, um einen Betrieb ohne Zugabe von Chemikalien zu ermöglichen.

Die Originalität dieser Studie umfasste erstens den direkten Vergleich der Vorbehandlungbedienbarkeit von Bagasse und Ernterückständen in derselben Ausrüstung. Die nicht katalysierte Dampfvorbehandlung dieser Ausgangsmaterialien wurde optimiert durch eine Erwiderungsoberflächenmethodik in den Bereichen von 185 bis 215 °C und 5 bis 15 min für Verdaulichkeit (Hinweis auf die Zugänglichkeit von Cellulose zu Enzymen) der Feststoffe, maximale kombinierte Zuckerausbeute und maximale Hemicelluloserückgewinnung zur Identifizierung bevorzugter Vorbehandlungs-betriebregime. Zweitens wurde Deacetylierung (Acetylgruppen, die mit einer milden alkalischen Extraktion aus Lignocellulose entfernt wurden) vor der nicht katalysierten Dampfvorbehandlung als Entgiftungsschritt vorgeschlagen, um Essigsäure bei der

nachgeschalteten Fermentation zu entfernen. Die nicht katalysierte Dampfvorbehandlung von rohen und deacetylierten Bagasse und Ernterückständen wurde hinsichtlich Verdaulichkeit, Fermentierbarkeit (Teil der maximalen theoretischen Ethanolausbeute, die erhalten werden kann) und Entwässerungsfähigkeit des vorbehandelten Materials verglichen. Drittens wurde der Beitrag des Explosionsschritts während der Dampfvorbehandlung zur Verbesserung der Verdaulichkeit der vorbehandelten Feststoffe untersucht, indem Material verglichen wurde, das während der plötzlichen Druckentlastung am Ende der Vorbehandlung zurückgehalten und freigesetzt wurde.

Die wichtigsten Ergebnisse dieser Studie waren, dass Zuckerrohrernterückstände aus folgenden Gründen besser als Bagasse für die Bioraffinierung über eine Zuckerplattform geeignet waren: (1) Ernterückstände ermöglichten eine robuste, nicht katalysierte Dampfvorbehandlung unter einer einzigen Bedingung (zwischen 198 und 200 °C und zwischen 8 und 12 min), um mindestens 95% der maximalen Verdaulichkeits-, kombinierte Zuckerausbeute- und Hemicelluloserückgewinnungswerte zu erhalten; (2) maximale Verdaulichkeit wurde bei geringerem Schweregrad erhalten (205,8 °C und 8,7 min im Gegensatz zu 215 °C und 15 min für Bagasse); (3) vorbehandelte Ernterückstände erforderten keine Entgiftung bei optimalen Vorbehandlungsbedingungen; und (4) vorbehandelte Ernterückstände zeigten eine bessere Entwässerungsfähigkeit, insbesondere wenn sie für eine maximale Hemicelluloserückgewinnung vorbehandelt wurden, verglichen mit Bagasse. Darüber hinaus wurde festgestellt, dass das mechanische Auftreffen von dampfvorbehandeltem Material beim Transport während des Druckentlastens einen wesentlichen Beitrag zur Verbesserung der Verdaulichkeit leistet. Es wurde auch gefunden, dass die Deacetylierung von Ausgangsmaterialien vor der nicht katalysierten

Dampfvorbehandlung als Entgiftungsschritt durchgeführt werden kann, der möglicherweise die Fermentierbarkeit von vorbehandelten gepressten Aufschlämmungen (nicht gewaschen) erhöhen könnte.

Infolgedessen wird vorgeschlagen, Bagasse weiterhin zur Erzeugung von Dampf und Strom in einer Zuckermühle zu verwenden, während der Rückgewinnung, Sammlung und Zuteilung von Zuckerrohrernterückständen als attraktives Ausgangsmaterial für die Zuckerrohrbioraffinerie besondere Aufmerksamkeit gewidmet werden sollte.

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All glory to God for opening doors in my life that I could never have dreamt even existed.

This dissertation is dedicated to the loving memory of

Nicholas “Oom Mac” McLaren

(06.04.1945 – 30.05.2018)

(Oom Mac was an influential and beloved figure at Sasol’s petrochemical complex in Secunda, South Africa, who one day, completely unexpectedly, walked into my office and insisted that I should do a PhD study…)

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NOMENCLATURE

AFEX	ammonia fibre explosion
AIL	acid insoluble lignin
ASL	acid soluble lignin
CBP	consolidated bioprocessing
CSF	combined severity factor
CSY	combined sugar yield
C5	molecule with five carbon atoms (pentose sugar in this thesis)
C6	molecule with six carbon atoms (hexose sugar in this thesis)
EH	enzymatic hydrolysis
ELSD	evaporative light scattering detector
FAME	fatty acid methyl ester
FPU	filter paper units
HEFA	hydrotreated esters and fatty acids
HHV	higher heating value
HMF	5-hydroxymethylfurfural
HPLC	high pressure liquid chromatography
HVO	hydrotreated vegetable oil
LCA	life cycle assessment
LHW	liquid hot water
log R_0	severity factor
NREL	National Renewable Energy Laboratory
NIR	near infrared
OD	optical density
p	statistical propability
R_0	reaction ordinate
RI	refractive index
RSM	response surface methodology
SEM	scanning electron microscopy
SHF	separate hydrolysis and fermentation
SPS	simultaneous pretreatment and saccharification
SSCF	simultaneous saccharification and co-fermentation

SSF	simultaneous saccharification and fermentation
t	time (min)
T	temperature (°C)
TSP	thermo separations product
WIS	water insoluble solids
wt	weight
YPD	yeast, peptone and glucose

CHAPTER 1

INTRODUCTION

1.1 Background

The world needs to decouple economic growth from fossil fuel consumption to sustainably support a growing world population with rising standards of living (Obama, 2017). The establishing of a bioeconomy is considered as one of the drivers for achieving a low-carbon economy (Biofuture Platform, 2018). The bioeconomy includes the transitioning away from fossil fuel sources to renewable biomass for the production of biofuels (e.g. ethanol and biodiesel) and non-energy bioproducts (e.g. chemicals and natural fibres) (Menon and Rao, 2012). Already the implementation of transportation biofuel policies, together with large-scale conversion of biomass to liquid transportation biofuels, are now actively pursued worldwide as part of countries' strategies towards a low-carbon economy (Murdock et al., 2019). Even as new transport vehicles are increasingly adopting electricity and hydrogen as energy sources, it is expected that liquid biofuels will remain relevant as a sustainable option for addressing the expanding long-haul transport sector (aviation, marine transport and long-haul trucking) in the future (Fulton et al., 2015). The production of non-energy bioproducts, on the other hand, is currently not as well established, but is expected to grow at 3 – 4% per year (de Jong et al., 2012), and is typically coupled with existing long-standing industries such as paper and pulp, sugar, starch and conventional biofuel production where bioproducts improve business profitability (Biofuture Platform, 2018).

Three conversion pathways are employed for converting biomass into biofuels: chemical, biological and thermochemical, as depicted in Figure 1.1. Ethanol production via biological sugar platforms from plant sugars, starches, cellulose and hemicellulose currently constitutes the largest proportion of annual global biofuel production at approximately 63% in energy terms (Murdock et al., 2019). The latest available information indicates that the annual global production of ethanol biofuel has steadily grown in the last decade, as shown in Figure 1.2, to achieve a record annual production of 112 billion litres in 2018 which represents a 7% growth compared to 2017 (Murdock et al., 2019). The growth is primarily driven by production in the USA (61 billion litres in 2018) and Brazil (33 billion litres in 2018) from corn and sugarcane crops, respectively, and collectively accounted for 83% of the global ethanol biofuel production output in 2018 (Murdock et al., 2019).

Ethanol biofuel has traditionally been produced as first generation biofuel and has reached a maturity where it has become cost competitive with petroleum fuel production (Canilha et al., 2012). First generation biofuels are obtained from the sugars, starches, oils and fats in edible plant biomass. However, the expansion of first generation ethanol biofuel production has become controversial and future growth is deemed unsustainable as first generation biofuel production is linked to increased food competition, an inability to reduce net greenhouse gas emissions, propagation of monoculture, deforestation and increased water intensity (Cerqueira Leite et al., 2009; Fargione et al., 2008; Searchinger et al., 2008).

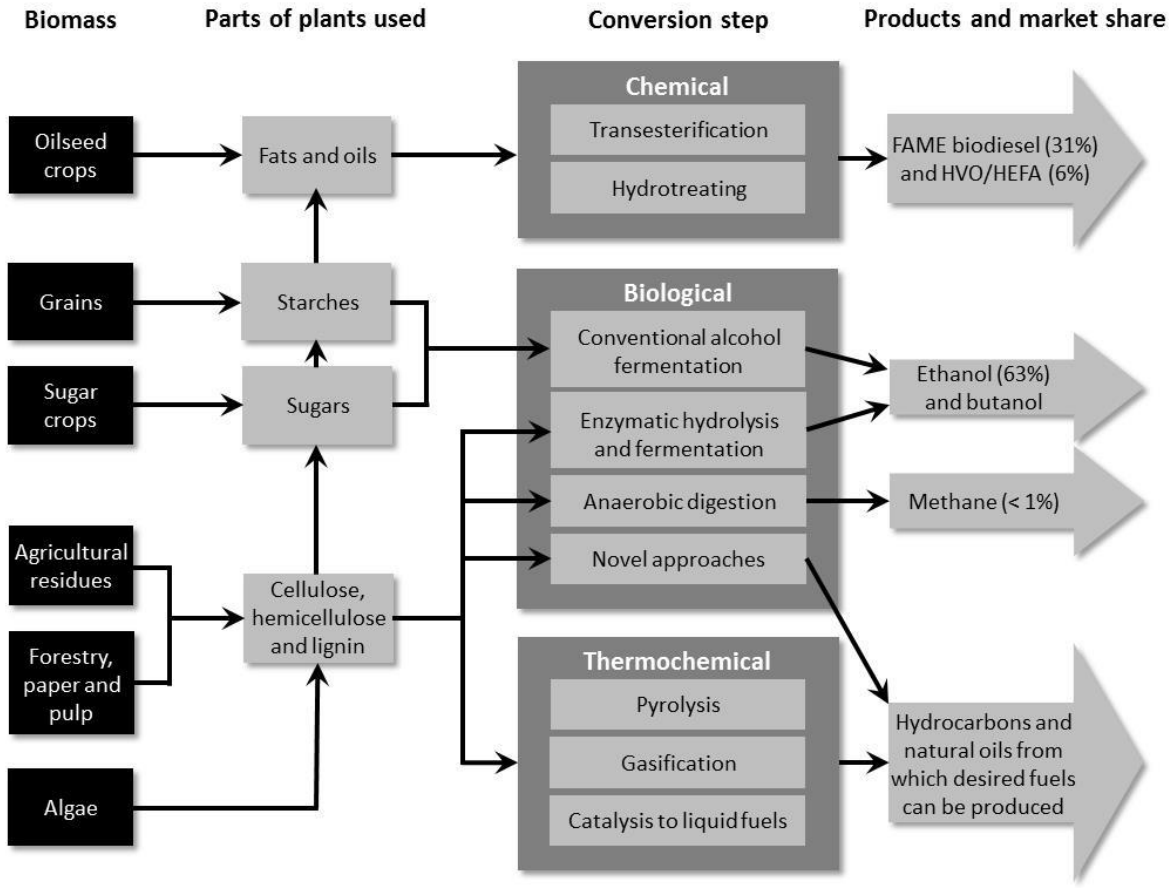

Figure 1.1 Conversion pathways for the production of biofuels from plant biomass (Redrawn and adapted from Pena and Sheehan, 2007). Global transportation biofuel market shares for 2018 are in energy terms and shown in brackets as provided by Murdock et al. (2019). FAME stands for fatty acid methyl ester, HVO stands for hydrotreated vegetable oil and HEFA stands for hydrotreated esters and fatty acids.

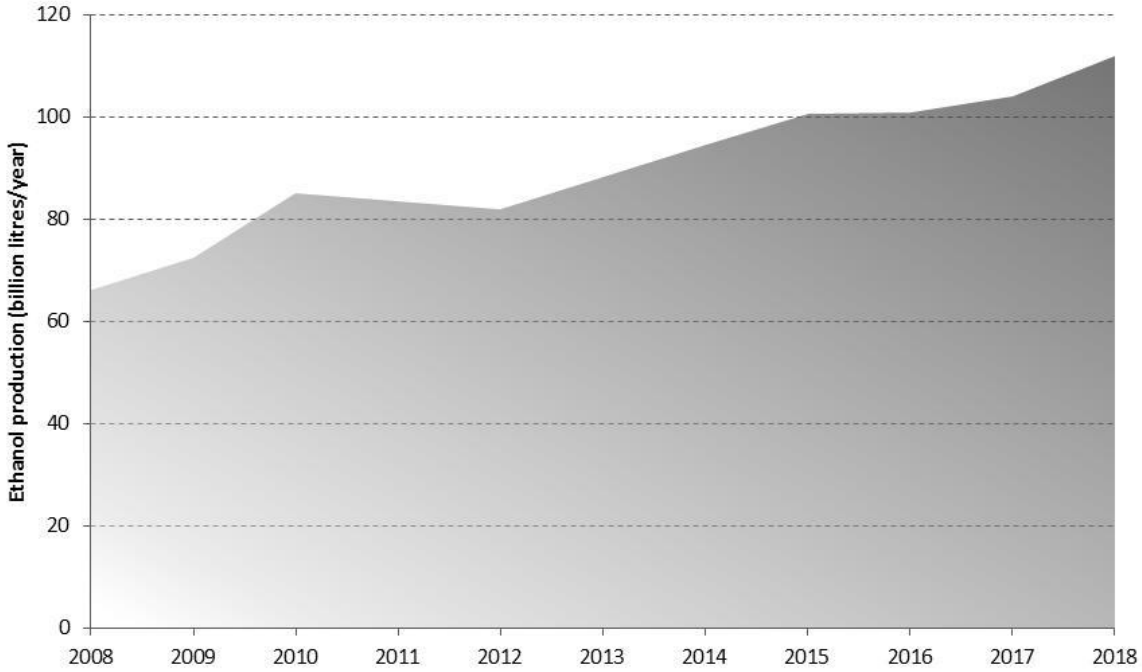

Figure 1.2 The annual global ethanol biofuel production output from 2008 to 2018 (Redrawn and adapted from Murdock et al., 2019).

The growing resistance to the expansion of first generation biofuel production has stimulated research and development of second generation biofuels from lignocellulose (Granda et al., 2007). Lignocellulose constitutes the largest renewable carbon source on earth and is found in the cell walls of plants (Himmel et al., 2007). Consequently, all inedible or waste plant material, including agricultural harvest residues, forestry waste, municipal solid waste and energy crops can potentially serve as feedstocks for the production of second generation ethanol (Ballesteros et al., 2000), known as cellulosic ethanol. Cellulosic ethanol production, therefore, does not directly compete with food and animal feed sources if managed correctly. Furthermore, lignocellulosic feedstocks can be generated without additional land requirements or grown on marginal land with little or no irrigation, fertilisers, pesticides or herbicides (Galbe and Zacchi, 2007). The cost of generating lignocellulosic feedstocks therefore tends to be cheaper than for first generation feedstocks (Claassen et al., 1999; Kim and Dale, 2004). Also, because of much lower farming

requirements, lignocellulosic feedstocks can be more carbon negative over their life cycles than traditional first generation crops (Wang et al., 2007).

Investment in large-scale commercial cellulosic ethanol operations has remained disappointing to date however, despite the advantages over first generation ethanol biofuel production (Brown, 2018; Lynd, 2017; Neto et al., 2018). The high unit cost of cellulosic ethanol is a significant financial risk which has been exacerbated by the sustained low oil prices of recent years. As a result, major cellulosic ethanol role players have recently decommissioned operations or left the business completely, including Abengoa, Beta Renewables, BP and DuPont (Dale, 2018). The main reasons for not attaining profitable operation were driven by the complex and expensive nature of the upstream operational requirements of lignocellulosic feedstock handling and pretreatment (Dale, 2018; Lynd et al., 2017). The few remaining large-scale commercial cellulosic ethanol facilities still in operation with annual production capacities of more than 10 million gallons are listed in Table 1.1.

Table 1.1 Large-scale commercial cellulosic ethanol facilities (> 10 million gallons per year) still in operation at the end of 2018 (Brethauer and Studer, 2015; Lynd et al., 2017; Neto et al., 2018).

Company	Location	Feedstock	Pretreatment	Capacity (10^6 gallon/year)	CapEx (US$ 10^6)
Granbio	São Miguel dos Campos, Brazil	Bagasse, harvest residues	Steam explosion	21.6	265
POET/DSM	Emmetsburg, IA, USA	Corn stover	Two-stage steam explosion	20	275
Raízen	Piracicaba, Brazil	Bagasse, harvest residues	Steam explosion [a]	10.6	102

[a] www.biofuelsdigest.com/bdigest/2014/12/17/raizen-iogen-commence-cellulosic-ethanol-production-in-brazil/

Second generation ethanol biofuel operations require different business models to first generation operations to achieve profitable operation, and therefore have to search for

strategic and synergistic opportunities that can support the production of cellulosic ethanol as a low value commodity (Gurgel et al., 2014; Ragauskas et al., 2006). Even though lignocellulosic feedstocks tend to be inexpensive at the source, the logistical and infrastructure requirements for collecting, transporting, sorting, cleaning, storing, drying, and preparing lignocellulosic feedstocks can significantly add to the cellulosic ethanol unit cost. Furthermore, in contrast to first generation feedstocks, lignocellulose is recalcitrant towards bioconversion to ethanol (Himmel et al., 2007) and requires expensive pretreatment processes and catalysts (Agbor et al., 2011). The unit cost of cellulosic ethanol is further increased by the poor ethanol yields that are typically achieved as a result of glucose recovery that is hindered by the complex structure of lignocellulose on the one hand, but also by glucose loss through degradation reactions on the other hand, when severity of pretreatments are consciously increased for better recovery (Jeoh et al., 2007). Also, higher pretreatment severity, to increase sugar yields, can also increase production of inhibitors, which can negate the benefits of increased sugar yields during the subsequent hydrolysis and fermentation steps (Palmqvist and Hahn-Hägerdal, 2000a, 2000b).

Cellulosic ethanol is to date the largest global effort at industrial-scale bioconversion of lignocelluloses (Wyman and Dale, 2015). However, as cellulosic ethanol biofuel cannot yet cost compete with fossil fuel (Lynd et al., 2017), the operation of commercial cellulosic ethanol facilities, such as the Brazilian endeavours Bioflex 1 of GranBio in São Miguel dos Campos and Costa Pinto of Raízen in Piracicaba (Neto et al., 2018), are often aligned with first generation ethanol and bioproducts production as part of multi-product biorefineries to improve profitability (Neto et al., 2018). To this end, cellulosic ethanol production can be considered a frontrunner in commercialisation of the multi-product lignocellulose

biorefinery concept via the sugar platform and can be considered as a first example of future biorefining technology (Lynd et al., 2017; Wyman and Dale, 2015). The technical knowhow developed around the pretreatment, hydrolysis and fermentation of lignocellulose for the production of cellulosic ethanol can also be adapted to the production of other products of sugars bioconversion. Consequently, isolating sugars, such as glucose, xylose and arabinose, from lignocellulose is a generic approach at the frontend of any sugar platform biorefinery (de Medeiros et al., 2018). The potential downstream production of high-value platform chemicals from these sugar intermediates could financially be more attractive than the production of ethanol (Özüdoğru et al., 2019).

Depending on the type of lignocellulosic feedstock and product requirements, the pretreatment step, the first step in conversion, combined with the subsequent enzymatic hydrolysis step, has to ensure the maximum yield of the required sugar intermediate. For example, if glucose is required as sugar intermediate, then the target of pretreatment will be to produce pretreated solids of maximum digestibility to facilitate cost-effective hydrolysis of cellulose into glucose. As examples, glucose could then be fermented into ethanol, butanol, lactic acid or succinic acid, or be converted via catalysts to 5-hydroxymethylfurfural (HMF), sorbitol or ethylene glycol (Kobayashi and Fukuoka, 2013). Alternatively, if xylose and arabinose are required as sugar intermediates, the target of pretreatment will be to produce prehydrolysate liquor with the maximum recovery of these sugars, which is usually associated with a lower yield of glucose during enzymatic hydrolysis of solid residues (Hendriks and Zeeman, 2009). Xylose and arabinose are both sugar intermediates for the production of furfural, for example (Rasmussen et al., 2014). If both glucose and xylose are required as sugar intermediates, for example for co-fermentation

into ethanol (Chen, 2011), then the target of pretreatment will be to produce the maximum combined sugar yield (CSY) after pretreatment and enzymatic hydrolysis. It is also possible that the maximum CSY are required for the production of multiple products from lignocellulose, such as ethanol from glucose together with furfural from xylose and arabinose (Giuliano et al., 2018).

1.2 Project motivation

The global sugar industry is currently threatened by international sugar prices that are below the production costs of most sugar producers in the world (FAO, 2019), increasing sugar price fluctuations (Maita and Smutka, 2018), a changing consumer behaviour with the introduction of sugar taxations (Marten et al., 2018) and the devastating effects of climate change on current sugar growing regions (Deressa et al., 2005). Incorporating lignocellulosic feedstocks and diversifying product lines in a biorefinery strategy to co-produce biofuels and/or platform chemicals via a sugar platform can help shield the sugar industry against these external shocks (Farzad et al., 2017). Furthermore, recent life cycle assessments of the sugarcane industry have highlighted the financial, socioeconomic and environmental benefits that can be achieved when existing conventional cane sugar mills are upgraded with high pressure boilers and annexed with multi-product lignocellulose biorefineries (Ali Mandegari et al., 2017; Farzad et al., 2017; Melendez et al., 2018; Pachón et al., 2018; Petersen et al., 2018).

The sugarcane industry generates large quantities of lignocellulosic feedstocks, i.e. sugarcane bagasse and harvest residues, which are already partly integrated in the logistical supply chain to sugar mills (Antonio Bizzo et al., 2014). Currently bagasse is typically burned at sugar mills in low-efficiency boilers for on-site electricity and steam generation, but improvements in process efficiencies and investment in high pressure boilers are resulting in surplus bagasse that can be dedicated to bioprocessing (Dias et al., 2013). Harvest residues are either destroyed in pre-harvest burning or left in the fields as soil nourishment after green harvesting (Canilha et al., 2012), but a substantial portion of these can be collected and transported to a sugar mill/biorefinery for conversion, with no negative impacts on

sugarcane agriculture (de Aquino et al., 2018; Lisboa et al., 2018). Therefore, no expansion of sugarcane plantations is required when using the sugarcane bagasse and harvest residues of existing operations for bioprocessing.

The required investment in on-site high pressure steam generation at an upgraded sugar mill to maintain energy self-sufficiency for the sugar mill and additional lignocellulose bioprocessing operations (Carpio and Simone de Souza, 2017; Mandegari et al., 2017), means that high pressure steam would be readily available, and would make steam pretreatment of lignocellulose an attractive choice of pretreatment technology in the sugarcane biorefinery. Steam pretreatment technology has been proven on industrial scale in the production of cellulosic ethanol, as indicated in Table 1.1, but it is critical that its application is optimised for sugarcane bagasse and harvest residues according to the sugar intermediates requirements of multi-product downstream bioconversion processes. In the future, this optimisation could be in near real-time with the introduction of increasingly accurate predictive process control, such as through machine learning (Wu et al., 2019), and through the rapid improvement of online lignocellulose analysing technologies, such as near infrared (NIR) spectroscopy (Uddin et al., 2019).

The motivation for this study therefore recognises the sugarcane industry as a niche opportunity for the potential viable co-production of biofuels, platform chemicals, electricity and heat from bagasse and harvest residues via a sugar platform biorefinery approach using steam pretreatment. This study optimised the uncatalysed steam pretreatment of these feedstocks for the production of sugar intermediates (glucose, xylose, arabinose), but did not optimise the downstream process steps of a sugar platform biorefinery, such as solids/liquid separation, enzymatic hydrolysis and fermentation.

1.3 Novelty of work

Bagasse steam pretreatment has been extensively studied in the literature, but no optimisation study was found for neither catalysed nor uncatalysed bagasse steam pretreatment. Each study was only performed at pre-selected conditions to allow for relative comparison. This study considered the pretreatment severities of all the previous uncatalysed bagasse steam pretreatment studies and ring-fenced them in a pretreatment operating envelope that ranged from 185 – 215 °C and 5 – 15 min. An optimisation study on uncatalysed steam pretreatment between these ranges produced, not only the pretreatment conditions for maximum digestibility, maximum hemicellulose recovery and maximum CSY, but a map that defined distinct ranges of steam pretreatment operation, according to the sugar intermediates required by downstream bioconversions, which can be glucose only, xylose and arabinose or a mixture of glucose, xylose and arabinose. Since very little information is available on steam pretreatment of sugarcane harvest residues, this same methodology was applied in the same pretreatment temperature and time ranges, and in the same pretreatment equipment. This allowed for the first time the direct comparison of uncatalysed steam pretreatment behaviour of bagasse and harvest residues.

Furthermore, this study performed uncatalysed steam pretreatment on bagasse and harvest residues feedstocks that were deacetylated prior to pretreatment to successfully produce detoxified and pretreated substrate for ethanol fermentation. Deacetylation of the raw feedstocks was performed to remove the acetyl groups and therefore served as an upstream detoxification method. Previous studies only considered upstream deacetylation for dilute acid pretreatments, but then an acid would be reintroduced to catalyse the pretreatment. Acetyl groups to ash ratio was investigated as a parameter of lignocellulose

to indicate how sensitive uncatalysed steam pretreatment performance would be to deacetylation. Uncatalysed steam pretreatment of harvest residues, with a low acetyl groups to ash ratio of 0.4 g/g, showed no significant change in digestibility when 82% of the acetyl groups were removed with deacetylation prior to uncatalysed pretreatment. Bagasse, on the other hand, with an acetyl groups to ash ratio of 1.9 g/g, showed a significant drop in digestibility when 90% of the acetyl groups were removed with deacetylation prior to uncatalysed pretreatment.

This study also investigated the contribution of mechanical impingement of the pretreated material during the steam explosion step to the digestibility of the pretreated solids. Pretreated material was either retained with sudden depressurisation or released with sudden depressurisation. In both cases the material was exposed to the same pressure differentials of the steam explosion. This was the first time that such an experiment was attempted to study steam pretreatment.

The process flow diagram in Figure 1.3 illustrates the procedures that were followed to optimise sugar production, and compare digestibility and fermentability of pretreated sugarcane and bagasse. As shown in Figure 1.3, feedstocks were either pretreated in their raw or deacetylated states. Deacetylation was performed with a mild alkaline solution prior to pretreatment to remove most of the acetyl groups from the lignocellulosic structures. After the alkaline solution was removed, the deacetylated material was washed and soaked to have the same neutral pH and moisture as the raw feedstocks before pretreatment. Hemicellulose recovery was determined from measuring the concentrations of monomeric and oligomeric xylose and arabinose in the prehydrolysate liquor that was obtained from the pretreated material (Figure 1.3). Digestibility was determined by measuring the glucose

yield after enzymatic hydrolysis of the pretreated solids (Figure 1.3). Combined sugar yield (CSY) was determined by measuring the total monomeric and oligomeric glucose, xylose and arabinose in the prehydrolysate liquor, as well as all the monomeric glucose, xylose and arabinose in the enzymatic hydrolysis mixture after enzymatic hydrolysis (Figure 1.3). Fermentability was determined by measuring the ethanol yield after simultaneous saccharification and fermentation (SSF) (Figure 1.3).

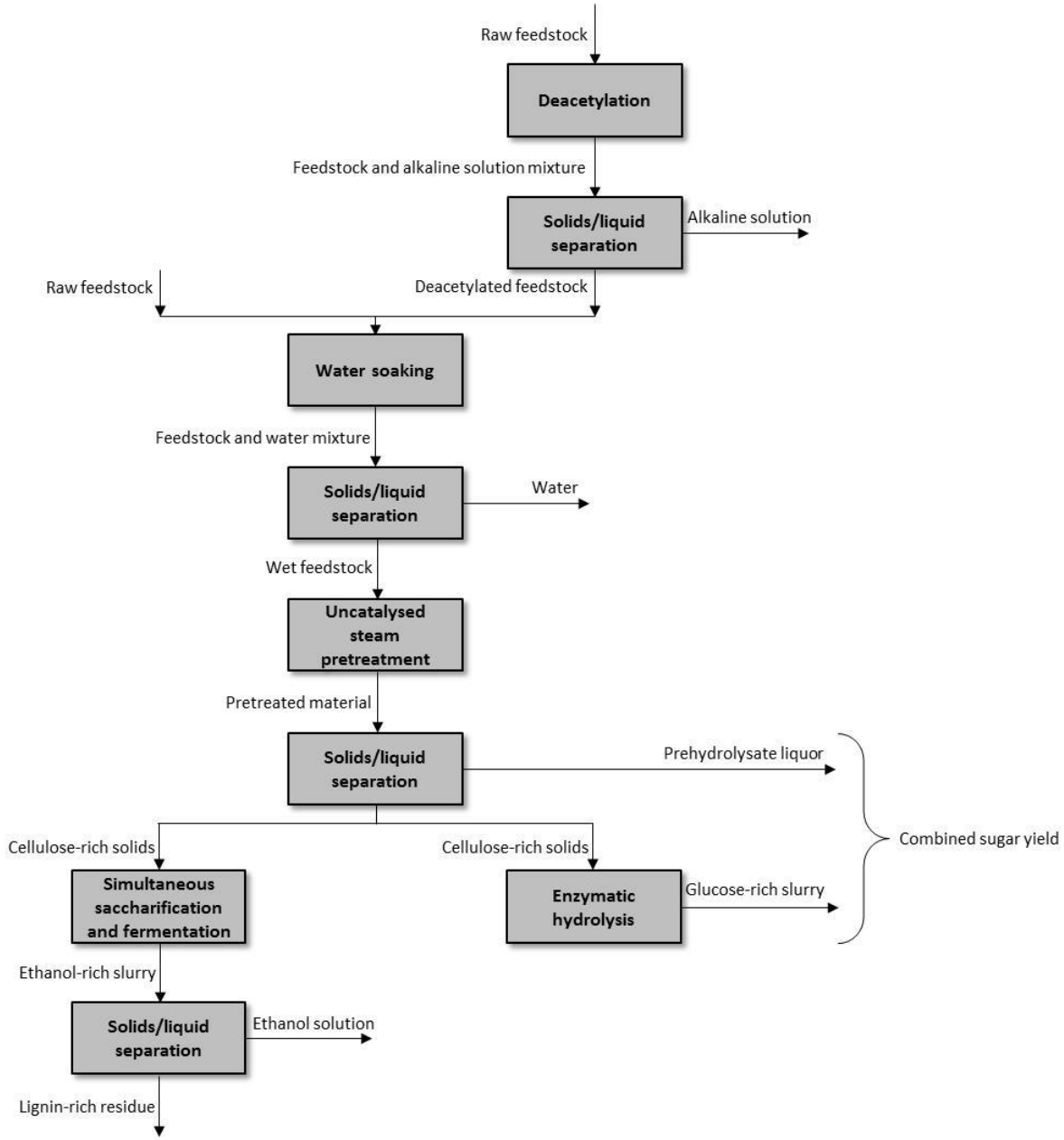

Figure 1.3 Process flow diagram of the procedures that were followed to measure the main parameters of this study. Feedstocks were either pretreated in the raw or deacetylated states. Pretreated material was either fermented in a simultaneous saccharification and fermentation (SSF) setup to determine fermentability or enzymatically hydrolysed to determine digestibility. Fermentability was determined from measuring the ethanol yield after SSF. Digestibility was determined from measuring the glucose yield after enzymatic hydrolysis of the pretreated solids. Hemicellulose recovery was determined from measuring the total monomeric and oligomeric xylose and arabinose in the prehydrolysate liquor. Combined sugar yield (CSY) was determined from measuring the total monomeric and oligomeric glucose, xylose and arabinose in the prehydrolysate liquor, as well as all monomeric glucose, xylose and arabinose in the enzymatic hydrolysis mixture after enzymatic hydrolysis.

1.4 Layout of dissertation

The layout of this dissertation and the titles of all the chapters are shown in schematic form in Figure 1.4. The deliverables from each chapter become the inputs to subsequent chapters as shown by the arrows in Figure 1.4. Chapters 1 – 3 form the basis of this investigation to arrive at the research questions as discussed in Chapter 3, and to formulate the subsequent objectives of this study. Chapters 4 – 6 use the objectives to answer the research questions as deliverables for these particular chapters and were written as potential articles that will be submitted for publication in peer reviewed journals. Finally, the deliverables from Chapters 4 – 6 are used to draw conclusions on the answers for the research questions and make recommendations in Chapter 7.

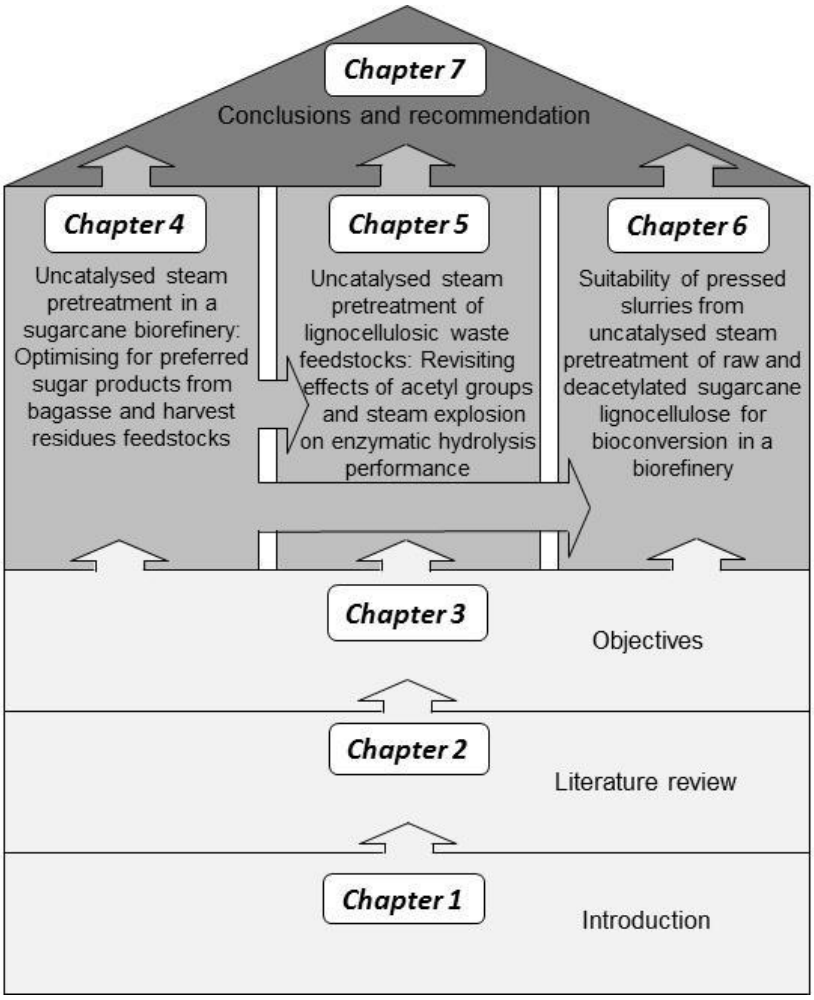

Figure 1.4 Schematic layout of this dissertation.

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CHAPTER 2

LITERATURE REVIEW

2.1 Lignocellulosic feedstocks in the sugarcane industry

2.1.1 Opportunities in the sugarcane industry

The viable production of bioproducts through bioconversion of sugars, especially commodity products such as ethanol biofuel, will foremost require access to inexpensive lignocellulosic feedstocks (Wyman and Dale, 2015). With the logistical supply lines already in place and sugarcane bagasse included in the harvested sugarcane stalks that is transported to the sugar mills (Martín et al., 2006), as shown in Figures 2.1 and 2.2, the integration of sugarcane bagasse and harvest residues bioprocessing with existing sugar mill operations can provide for a competitive edge, because of the low feedstock cost base (Seabra et al., 2010). Globally there is a growing consensus that the sugarcane industry can migrate away from processing cane stalks for the production of sugar and surplus electricity in a conventional sugar mill, to an integrated biorefinery that can bioprocess all of the sugarcane biomass for the co-production of food, biofuel, platform chemicals and electricity (Balakrishnan and Batra, 2011; Canilha et al., 2012; Corrêa do Lago et al., 2012; Solomon, 2011). Platform chemicals are building block chemicals that are typically of higher value than commodities such as ethanol biofuel. If a commodity is part of the product slate, the co-production of value-added chemicals could be of strategic business importance to ensure overall competitiveness (De Bhowmick et al., 2018).

Figure 2.5 Harvested sugarcane stalks delivered by rail on a continuous basis to the Umfolozi Sugar Mill, KwaZulu-Natal, South Africa when the mill is in operation (Own photo.). The fibrous component of these stalks report as bagasse, a waste product, during sugar production.

Figure 2.6 Storage of bagasse on bagasse heaps next to the Umfolozi Sugar Mill, KwaZulu-Natal, South Africa (Own photo.).

2.1.2 Structure of lignocellulose

Lignocellulose is the structural building material of all plant cell walls and comprises mainly of three biopolymers: cellulose, hemicellulose and lignin as depicted in Figures 2.3 – 2.5. These biopolymers are intimately bonded to create a structure that is highly recalcitrant to bioconversion, providing the natural protection for plants against microbial attack. Consequently, complete decomposition of dead plant biomass is a slow process in nature that can take up to several years, even during conditions conducive for microbial activity (Malherbe and Cloete, 2002). The challenge of industrial bioprocessing of lignocellulose is therefore to break the cross-links in lignocellulose to expose and liberate monomeric sugars suitable for bioconversion in a financially viable fashion.

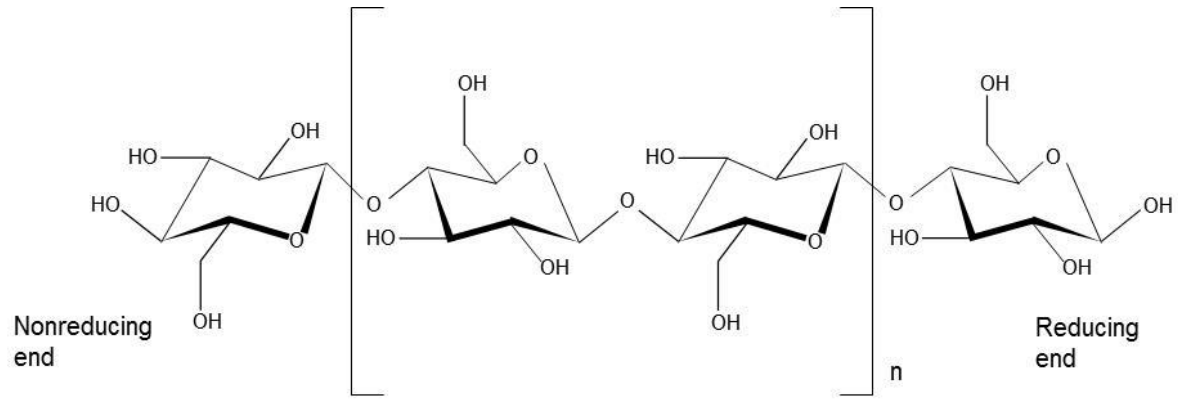

Figure 2.7 The cellulose biopolymer with its comprising β-1,4 linked glucose monomers. Adapted from Brethauer and Studer (2015).

Lignocellulose consists approximately 40 – 50% of cellulose which is the target component for hydrolysis into glucose (Gray et al., 2006). Cellulose biopolymers are linear homopolymers of 7 000 – 15 000 D-glucose monomers linked by β-1,4-glucosidic bonds and are arranged in linear structures with a high degree of polymerisation (Jørgensen et al., 2007). This arrangement allows for intra- and intermolecular hydrogen bonds to create a crystalline structure (Klemm et al., 2005) that is chemically stable as well as being resistant to microbial attack (Ding and Himmel, 2006).

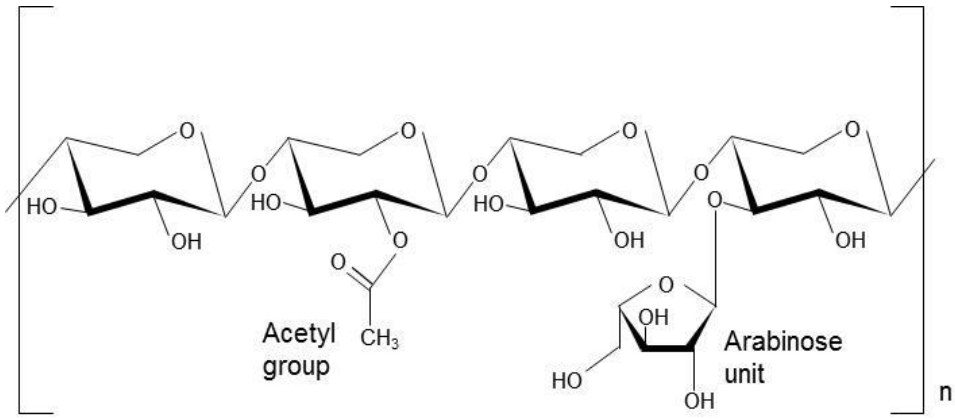

Figure 2.8 An example of a hemicellulose polymer containing acetyl groups and arabinose units on a xylan backbone. Adapted from Rasmussen et al. (2014).

In contrast to cellulose, hemicellulose is a biopolymer made up of various units that are distinctly arranged for different plant biomass such as agricultural crops, hard woods and soft woods (Gírio et al., 2010). Hemicellulose is a branched and complex polymer that surrounds and cross-links the crystalline cellulose structures (Jørgensen et al., 2007). Hydrogen bonding and covalent ester bonding link the hemicellulose with the cellulose microfibrils and lignin, respectively (Pu et al., 2008). This arrangement assists in binding the cell wall matrix in aligned fibrils and imparts flexibility to the cell wall (Carpita, 1996). The hemicellulose biopolymer can contain 40 – 600 units of the pentose sugars D-xylose and L-arabinose, hexose sugars D-glucose, D-mannose and D-galactose, and uronic acids (Gírio et al., 2010). Lignocelluloses from agricultural crops and hardwoods usually have a higher content of pentose sugars than softwoods (Kristensen, 2008). The hydroxyl groups of the sugars can also be substituted with acetyl groups, but this is dependent on the type of plant biomass (Gírio et al., 2010). Hardwoods contain 3.5 – 7 acetyl groups for every 10 xylose sugars, whereas agricultural crops are less acetylated (Alén, 2000).

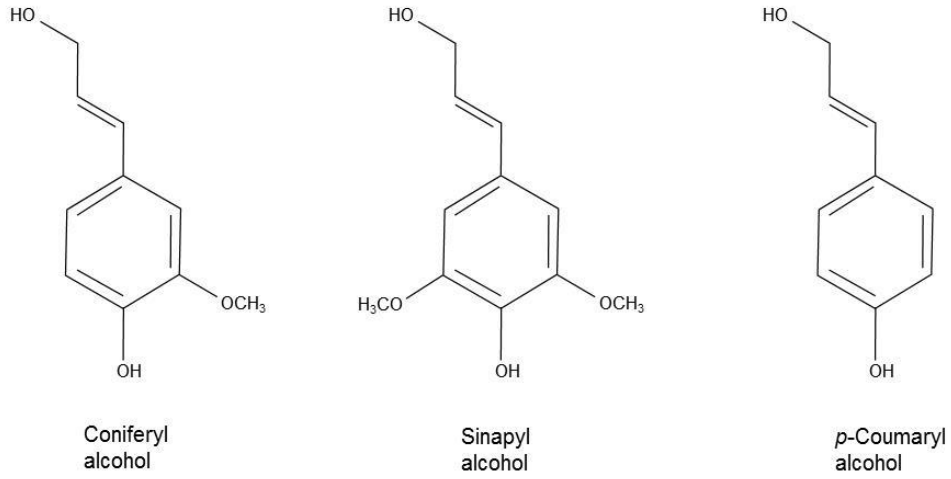

Figure 2.9 The aromatic building blocks of lignin: coniferyl alcohol, sinapyl alcohol and *p*-coumaryl alcohol. Adapted from Brethauer and Studer (2015).

Some plant cells are reinforced with a secondary cell wall that has lignin, an amorphous polymer, as its framework in which the cellulose and hemicellulose are imbedded (Kristensen, 2008). Lignin is a non-polysaccharide that consists of the aromatic phenyl propane units: coniferyl alcohol, sinapyl alcohol and *p*-coumaryl alcohol (McKendry, 2002). These units are polymerised through crosslinked alkyl-aryl, alkyl-alkyl and aryl-aryl ether bonds (Jørgensen et al., 2007). Lignin offers mechanical strength to the cell wall and is highly resistant to chemical and microbial degradation (Kristensen, 2008). The prevalence of lignin in lignocellulose can drastically complicate and hinder the hydrolysis of cellulose and hemicellulose to monomeric fermentable sugars, by bonding covalently to cellulose and hemicellulose to shield it from enzymatic attack (Akin, 2008, 2007), causing inhibition during fermentation (Pan, 2008), limiting the extent to which the cell wall can swell to restrict enzyme accessibility (Mooney et al., 1998), and adsorbing of enzymes during enzymatic hydrolysis (Converse et al., 1990; Eriksson et al., 2002).

2.1.3 Components of the sugarcane plant

The above ground biomass of the sugarcane plant consists of the millable stalk, tops and associated leaves (Antonio Bizzo et al., 2014; Beeharry, 1996) as shown in Figures 2.6 and 2.7. The millable stalk of the mature plant does not include any leaves, and refers only to the older part of the cane where sucrose is stored in the pith. This part of the sugarcane plant has always been of interest for harvesting, whereas the tops and leaves were burned or simply left in the field as ground cover. The tops consist of the green and immature top stalk, representing the growth region of the plant, as well as young green leaves. The leaves associated with the millable stalk include the mature green and brown leaves on the stalk as well as detached leaves that have fallen to the ground.

Figure 2.10 A sugarcane plantation near KwaDukuza, KwaZulu-Natal, South Africa (Own photo.). Here the mature green and brown leaves of the sugarcane plants can clearly be seen.

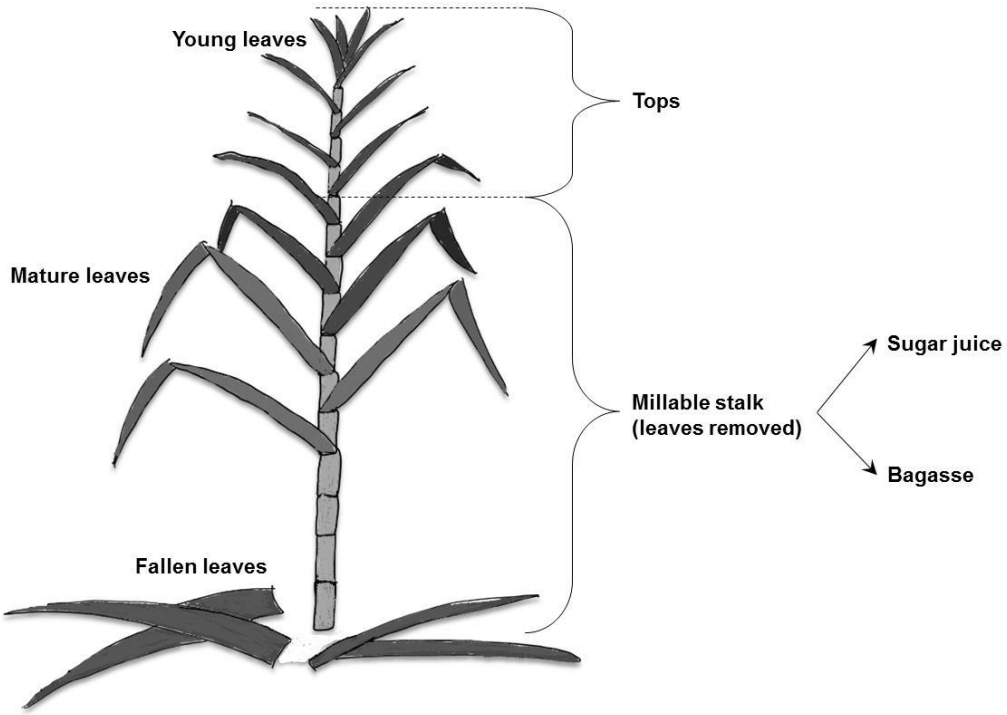

Figure 2.11 The above ground biomass of the sugarcane plant. The millable stalk has all leaves removed before it can be crushed to produce the sugar juice and the remaining bagasse fibrous residues (Own drawing.).

The production of high quality clear juice during sugar milling requires that all tops and leaves are removed from the millable stalks, because of their low sucrose content and high impurity contents, including ash, reducing sugars, colour, organic acids and starch, which adversely impact downstream sugar production (Eggleston et al., 2010). This unwanted plant material is generally referred to as trash or straw in the literature, but the definitions are not always used consistently (Eggleston et al., 2010; Muir et al., 2009). In this dissertation the tops and all associated leaves from the sugarcane plant will be collectively referred to as harvest residues, unless otherwise specified, whereas bagasse will refer to the fibrous residue after extraction of sucrose-containing juice from the sugarcane stalks (free of harvest residues) during milling (Antonio Bizzo et al., 2014).

2.1.4 Bagasse and harvest residues volumes

Sugarcane bagasse is an agricultural waste stream produced at the sugar mill. The burning of bagasse on-site at the sugar mill for the generation of steam and electricity was rather borne from the need for waste material handling (Beeharry, 1996). As a result, these processes were generally designed to be inefficient to leave no surplus bagasse (Antonio Bizzo et al., 2014). However, spurred by rising energy costs and the bioprocessing potential of the total sugarcane biomass, sugar mills are now investing in higher efficiency high pressure boilers for increased electricity generation, thereby introducing greater potential to allocate bagasse for bioprocessing (Coelho et al., 2006; Martín et al., 2006). It was estimated that only 50% of the bagasse production would be required for energy generation in a sugar mill with the installation of high efficiency boilers and improved steam consumption initiatives (Rocha et al., 2012).

Sugarcane harvest residues are another source of lignocellulose available to the sugarcane industry for bioprocessing. In South Africa (Graham and Haynes, 2006), as in many other parts of the world (Balakrishnan and Batra, 2011; Da Silva et al., 2010; Jutakanoke et al., 2012; Muir et al., 2009), the practice of pre-harvest burning of sugarcane fields is still widely employed. In fact, pre-harvest burning still accounts for more than 90% of all harvested sugarcane stalks in South Africa (Madho et al., 2017). This practice removes most of the leaves between the stalks to facilitate passage through the fields, thereby allowing faster and safe manual harvesting of the sugarcane stalks (Müller and Coetsee, 2008). Not only does this practice cause air pollution (Dawson and Boopathy, 2007; França et al., 2012), disrupt soil ecology and deprive cropland of nutrition (Graham et al., 2002; Graham and Haynes, 2006), but it also destroys large quantities of sugarcane harvest

residues, which are potentially valuable lignocellulosic feedstocks that can be processed in a biorefinery.

Increasing manual labour costs (Muir et al., 2009), technical improvements in mechanical harvesters (Leal et al., 2013) and legislature to phase out pre-harvest burning (Leal et al., 2013) are driving the shift towards mechanical green harvesting of sugar cane, and are resulting in growing availabilities of harvest residues. Green harvesting usually requires that the harvest residues are left as soil cover in the fields to reduce soil erosion, improve soil water retention, nourish the soil and limit weed growth (Hassuani et al., 2005). However, too thick a layer of harvest residues left in the fields poses a fire hazard, complicates mechanical cultivation, delays or stops ratooning and promotes the proliferation of pests (Hassuani et al., 2005). The required amount of harvest residues to be left as soil cover for sustainable sugarcane farming is unclear and other factors, such as climate, seem to be more important (Hassuani et al., 2005). A long term study has recently confirmed that 50% of the total harvest residues can be recovered from the fields without affecting soil productivity (de Aquino et al., 2018). Certain conditions, such as the age of the cane and risk of fire, can even allow for the complete recovery of harvest residues (Hassuani et al., 2005; Lisboa et al., 2018).

Harvest residues represent a significant amount of additional biomass available for the sugarcane industry. In a study of Louisiana-grown sugarcane varieties, Eggleston et al. (2009) found that the total amount of harvest residues constituted 16.4% to 19.8% of the above-ground sugarcane plant on a wet mass basis, corresponding to 36.6% on a dry mass basis. Beeharry (1996) reported an even higher percentage of 31% of above ground biomass to be harvest residues for Mauritian sugarcane on a wet basis. In other words,

approximately one third of the total above ground biomass is currently either destroyed in pre-harvest burning or left in the field during green harvesting. The amount of sugarcane harvest residues generated for every millable stalk will however vary according to the cane variety, cane age, soil fertility and climatic conditions (Panray Beeharry, 2001). Table 2.1 gives reported mass ratios of the different harvest residues, together with bagasse, that are generated for every 1 000 kg of millable stalk harvested and sent to the sugar mill. In some cases it was not reported if the mass of the residues was on a dry or wet basis. Table 2.1 shows that approximately equal amounts of bagasse and total harvest residues (wet and dry basis of tops and leaves) are generated as lignocellulosic agricultural wastes during the production of cane sugar. Therefore, approximately 140 kg of bagasse and 140 kg harvest residues are generated on a dry basis for every 1 000 kg of wet millable stalk harvested.

Table 2.2 Mass of sugarcane residues generated for every 1 000 kg of millable stalks harvested (wet basis).

Bagasse (kg)	Tops (kg)	Leaves (kg)	Tops and leaves (kg)	Reference[a]
300 (wet basis)	96 – 102 (dry basis)	63 – 77 (dry basis)	n/a	(1)
140 (dry basis)	n/a	n/a	140 (dry basis)	(2)
300 (wet basis)	300 (wet basis)	150 (wet basis)	n/a	(3)
333 (wet basis)	n/a	n/a	333 (wet basis)	(4)
300 – 340 (wet basis)	160 – 200	40 – 60	n/a	(5)
n/a	n/a	85 – 114 (dry basis)	n/a	(6)
n/a	n/a	n/a	140 (dry basis)	(7)
180 – 280	n/a	n/a	n/a	(8)

[a] 1. Panray Beeharry (2001), 2. Corrêa do Lago et al. (2012), 3. Beeharry (1996), 4. Larson et al. (2001), 5. Solomon (2011), 6. Singh et al. (2008), 7. Dias et al. (2013), 8. Pessoa Jr. et al. (1997).

Using an average harvest productivity of approximately 85 $t.ha^{-1}.year^{-1}$ millable stalks (wet basis) (Antonio Bizzo et al., 2014; Hassuani et al., 2005), therefore translates to an approximate production of 12 $t.ha^{-1}.year^{-1}$ (dry basis) of bagasse and harvest residues, respectively. Even though more harvest residues can be recovered during sugarcane harvesting, a recovery of 50% will be assumed, as suggested by de Aquino et al. (2018) for soil protection and nutrition. Depending on the efficiency of the boilers and the

requirement of the sugar mill to be self-sufficient in energy generation, zero to 50% of the bagasse can be available for bioprocessing (Ali Mandegari et al., 2017; Pachón et al., 2018; Rocha et al., 2012). Consequently, bagasse and harvest residues are estimated to be available as lignocellulosic feedstocks in the ratio of 140 kg (dry) and 70 kg (dry), respectively, for every 1 000 kg of wet millable stalk harvested, but that at least 70 – 140 kg (dry) of bagasse or equivalent amounts of harvest residues will be required for energy generation.

2.1.5 Bagasse and harvest residues compositions

Each part of the sugarcane plant is specialised and adapted for functionality, and each part will therefore differ according to chemical composition and cell structure. As a result, bagasse, tops, green leaves and brown leaves all have different bioprocessing characteristics (Da Silva et al., 2010; Eggleston et al., 2010). However, the physicochemical differences between bagasse and the different harvest residues, and their requirements for bioprocesses are still poorly understood (Eggleston et al., 2010, 2009).

Benjamin et al. (2014) determined the chemical compositions of bagasse from seven different sugarcane cultivars from different origins in South Africa for a representative analysis of typical South African bagasse. The analysis confirmed, as shown in Table 2.2, that sugarcane bagasse is fairly rich in cellulose (indicated by glucan polysaccharide content), with relative low amounts of lignin and ash, which should make it an attractive feedstock for recovering glucose as sugar intermediate as claimed elsewhere for bagasse (Canilha et al., 2012; Ewanick and Bura, 2011).

Table 2.3 Chemical compositions of bagasse from seven sugarcane cultivars in South Africa (% dry weight). Copied and adapted from Benjamin et al. (2014).

	Glucan	Xylan	Arabinan	Total lignin	Acetyl groups	Extractives	Ash
	35.1 ± 0.4	24.6 ± 0.5	2.5 ± 0.2	19.6 ± 0.6	3.2 ± 0.1	9.9 ± 0.3	1.6 ± 0.1
	36.1 ± 0.3	24.3 ± 0.7	2.2 ± 0.1	20.4 ± 0.5	2.8 ± 0.1	7.5 ± 0.1	1.8 ± 0.1
	36.9 ± 0.6	24.0 ± 0.2	1.5 ± 0.1	19.7 ± 0.5	2.9 ± 0.1	7.5 ± 0.3	2.0 ± 0.0
	40.7 ± 1.0	26.3 ± 0.6	2.2 ± 0.1	14.4 ± 0.3	3.2 ± 0.2	7.4 ± 0.9	0.8 ± 0.1
	34.1 ± 1.0	25.5 ± 0.3	2.7 ± 0.1	16.4 ± 0.3	3.2 ± 0.1	7.3 ± 0.2	0.9 ± 0.1
	38.3 ± 1.6	27.2 ± 0.7	2.5 ± 0.1	16.1 ± 0.3	3.3 ± 0.2	6.1 ± 0.2	0.9 ± 0.1
	39.6 ± 0.6	19.5 ± 0.3	1.3 ± 0.1	22.4 ± 0.2	3.2 ± 0.2	5.0 ± 0.5	1.3 ± 0.3
Average	37.3	24.5	2.1	18.4	3.1	7.2	1.3

No literature could be found that described the chemical composition of South African sugarcane harvest residues for comparison, however, and very little information could be found for harvest residues reported internationally, as is shown in Table 2.3. The description of harvest residues in literature, as listed in Table 2.3, refers to different parts of the sugarcane plant and the definitions of trash and straw are unclear (Eggleston et al., 2010). It is therefore difficult to make sensible comparisons between the compositional analyses of bagasse and harvest residues in Tables 2.2 and 2.3, respectively.

Table 2.4 Chemical compositions of various sugarcane harvest residues components (% dry weight).

Description	Cellulose	Hemicellulose	Lignin	Extractives	Ash	Reference[a]
Straw	39.8 ± 0.3	28.6 ± 0.2	22.5 ± 0.1	6.2 ± 0.3	2.4 ± 0.3	(1)
Straw	33.5 ± 0.2	27.1 ± 0.3	25.8 ± 0.5	n/a	2.5 ± 0.2	(2)
Tops	29.85	18.85	25.69	n/a	n/a	(3)
Leaves	38.5	23	15.6	n/a	n/a	(4)
Leaves	33.3	21.2[b]	36.1	n/a	n/a	(5)
Trash	36.68	28.57	20.45	11.50	n/a	(6)
Trash	40	25	18 - 20	n/a	n/a	(7)

[a] 1. Oliveira et al. (2013), 2. Costa et al. (2013), 3. Sindhu et al. (2014), 4. Jutakanoke et al. (2012), 5. Ferreira-Leitão et al. (2010), 6. Antonio Bizzo et al. (2014), 7. Singh et al. (2008)

[b] Calculated as the sum of 18.1% xylan and 3.1% arabinan.

Canilha et al. (2012) and Chandel et al. (2012) have mentioned that harvest residues contain more ash than bagasse, although, when comparing Table 2.2 and the limited information in Table 2.3, this difference does not seem significant. However, in the only steam explosion pretreatment comparison between bagasse and harvest residues in the same pretreatment

unit that could be found in literature, Ferreira-Leitão et al. (2010) found that sugarcane leaves displayed a higher pH buffer capacity than bagasse. Unfortunately Ferreira-Leitão et al. (2010) did not report on the ash compositions of the feedstocks, but it is possible that the higher buffer capacity could be attributed to a higher ash content in the leaves (Szczerbowski et al., 2014).

Furthermore, no information could be found in the literature on the acetyl groups and extractives contents of sugarcane harvest residues. These components have been demonstrated to have important implications for steam explosion pretreatment of lignocellulose. The acetyl groups are released as acetic acid during steam pretreatment which participates as a weak acid in propagating hydrolysis of the hemicellulose (Palmqvist et al., 1999). Extractives include organic acids, inorganic materials, proteins, chlorophyll, waxes and non-structural sugars (Chen et al., 2007), and can condense with lignin as pseudo-lignin during severe pretreatment onto the cellulose to hinder enzymatic hydrolysis (Ballesteros et al., 2011).

2.2 The biorefinery concept

Following conventional petrochemical refining, but using biomaterial as feedstock instead, the aim of a biorefinery is to bioprocess all of the feedstock into a wide range of co-products for improved overall profitability, where high value products subsidise the unit cost of low value commodities (Kamm and Kamm, 2004). The biorefinery processing philosophy is not a new concept and has in fact been implemented for many decades on large scale in the paper and pulp industry to maintain profitable operations with the advent of competition from less expensive fossil-based equivalent products on the world market (Rødsrud et al., 2012). Naturally then, in recent years the biorefinery concept has gained renewed impetus in the green economy as a strategy to co-produce biofuels and platform chemicals from lignocellulose that can better compete with low-cost products obtained from fossil fuels (Banerjee et al., 2010; Chandel et al., 2018; FitzPatrick et al., 2010). Of special interest is the utilisation of agricultural wastes as feedstocks for such lignocellulose biorefineries (Nizami et al., 2017). Biorefining of lignocellulosic agricultural wastes into a value-added product slate has the potential to expand the green economy in rural communities through the establishment of downstream beneficiation industries and circular economies (Clark and Deswarte, 2008).

One example of a lignocellulose biorefinery is where cellulose is converted to glucose and fermented to cellulosic ethanol, whereas hemicellulose is converted to xylose and arabinose for bioconversion into platform chemicals such as furfural and lactic acid (Pachón et al., 2018). Furthermore, if lignin can be recovered, it can be used as a fuel source for generating electricity and heat or can be upgraded to value-added fine chemicals (Doherty et al., 2011). Biorefinery fractionation of sugarcane bagasse and harvest residues into

cellulose, hemicellulose and lignin, and the subsequent conversion into products pose many challenges, since, unlike petroleum feedstocks in a petrochemical refinery, lignocellulosic feedstocks display low thermal stabilities and highly specialised structures (FitzPatrick et al., 2010). Bioprocessing of lignocellulose therefore requires special processes, such as pretreatment and subsequent enzymatic hydrolysis that must be specifically tailored for the lignocellulose type and required downstream products.

Consequently, the integrated structure of biorefinery processing, coupled with the recalcitrant and complex nature of lignocellulose, will, therefore, not have one set of process conditions for the maximum co-production of all products. Changing process conditions to increase the yield of one co-product might adversely affect the yield of another. The selection of process conditions will have to be a trade-off against the relative product yield optima, product preferences, energy requirements, environmental impact and feedstock properties, and is ultimately a decision based on economic and environmental analyses.

2.3 Pretreatment of lignocellulose

2.3.1 Pretreatment principles

Pretreatment represents the first step in the bioconversion of raw lignocellulose to sugar intermediates. A pretreatment step is necessary to alter the recalcitrant lignocellulose structure in such a way that the cellulose within is exposed and becomes accessible to downstream enzymatic hydrolysis, at a yield and rate acceptable for industrial operation (Hendriks and Zeeman, 2009). Acid or enzyme catalysts are utilised to hydrolyse pretreated cellulose into fermentable glucose monomers (Martín et al., 2002). Enzymatic hydrolysis subsequent to pretreatment is considered more attractive than acid hydrolysis, due to higher potential glucose yield and lower environmental impact (Taherzadeh and Karimi, 2007), but its efficiency is limited when access is restricted by the crystalline structure of cellulose and the presence of hemicellulose and lignin (Laureano-Perez et al., 2005). Enzymatic hydrolysis tends to be a very expensive process step and therefore relies on effective pretreatment to limit consumption of expensive enzymes, such as cellulase (Klein-Marcuschamer et al., 2012). For example, the cost contribution of cellulase to the production of cellulosic ethanol from techno-economic analyses can be as high as $1.47/gal (Klein-Marcuschamer et al., 2012; Liu et al., 2016; Luo et al., 2019). However, in light of the recent closures of large-scale cellulosic ethanol plants, cost of enzymes was not cited as a reason for exiting the business, but rather the technical challenges of pretreatment (Dale, 2018).

The aims of pretreatment are to prepare a cellulose substrate with improved accessibility to enzymatic attack that can be readily hydrolysed for maximum glucose yield, fractionate the lignocellulose structure into its cellulose, hemicellulose and lignin

constituents, decrease the crystallinity of cellulose and increase the porosity of the lignocellulose for improved enzyme access (Chandra et al., 2007; Gurgel et al., 2014; Sun and Cheng, 2002). Besides for increased accessibility, and of particular importance for biorefinery operations, pretreatment should also aim to optimise both the pentose and hexose sugar yields by limiting sugar losses through degradation reactions (Chiaramonti et al., 2012). Degradation reactions might include the formation of fermentation inhibition products (Rasmussen et al., 2014). Fermentation inhibitors might necessitate a subsequent detoxification treatment step to minimise their detrimental effect on fermentation (Jönsson et al., 2013) and/or require fermentation with tolerant yeasts (Liu et al., 2005). However, in certain applications the degradation of pentose and hexose sugars during pretreatment could in fact be desired to produce chemicals such as furfural and HMF via chemical conversion (Steinbach et al., 2017), in which case pretreatment conditions will deliberately be set to degrade the sugars. Pretreatment should also recover lignin to simplify downstream processing and to allow for valuable co-production (Yang and Wyman, 2008). Other parameters such as hemicellulose recovery, feed particle size requirement, degree of inhibitor formation and energy demand can also influence the decision on the most appropriate pretreatment method (Alvira et al., 2010; Chiaramonti et al., 2012; Sun and Cheng, 2002).

Pretreatment is considered to be the most expensive unit process, after feedstock cost, in the conversion of lignocellulosic feedstock via enzymatic hydrolysis to sugar intermediates for downstream bioprocessing (Mosier et al., 2005), and is estimated to account for more than 18% of the total production cost (Gnansounou and Dauriat, 2010; Yang and Wyman, 2008). Furthermore, the efficiency of the pretreatment step influences all

downstream costs as it will determine process variables such as material digestibility, enzyme loadings and related hydrolysis rates, stirring power requirements during fermentation, by-product formation and consequent detoxification requirements, fermentation productivity, distillation and waste treatment demands (Chiaramonti et al., 2012; Galbe and Zacchi, 2007; Greenwood et al., 2013; Wyman et al., 2005). A more robust pretreatment step can also reduce the upstream energy and capital cost requirement for feed size reduction (Jørgensen et al., 2007).

2.3.2 Pretreatment technologies

Technologies for the pretreatment of lignocellulose can be categorised into physical, physico-chemical, chemical or biological approaches. Physical pretreatment reduces the particle size of the material for increased mass transfer and reduced crystallinity (Menon and Rao, 2012) and often include milling, irradiation and extrusion (Da Silva et al., 2010; Menon and Rao, 2012). In physico-chemical pretreatment methods, the lignocellulose structure is not only physically disrupted, but is also accompanied by cleavage of chemical bonds to achieve a certain degree of dissolution. Steam explosion (Jacquet et al., 2015), ammonia fibre explosion (AFEX) (Teymouri et al., 2004), wet oxidation (Martín et al., 2006), wet explosion (Biswas et al., 2014), microwave-chemical pretreatment (Zhu et al., 2006) and liquid hot water (LHW) pretreatments (Nitsos et al., 2013) are the most popular physico-chemical methods. Originally developed in the paper industry for pulping with the Kraft process (Doherty et al., 2011), chemical pretreatment represents the most studied lignocellulose pretreatment method (Menon and Rao, 2012). This pretreatment method aims to remove the lignin and/or hemicellulose from the lignocellulose and is achieved through treatments using acid (Benjamin et al., 2013; Castro et al., 2014; de Vasconcelos et

al., 2013; Kabel et al., 2007; Lavarack et al., 2002), alkali (Guo et al., 2013; Lavoie et al., 2010), organic solvent (organosolv) (Zhao et al., 2009) or ionic liquid (Zhu et al., 2012). Little attention has been given to biological pretreatment because of its slow reaction rate, low selectivity and loss of sugar when destructing lignocellulose components, but it does have the advantages of low energy requirement and no chemicals requirement to make it an environmentally friendly option (Sánchez, 2009).

There is no proven best pretreatment method (Gurgel et al., 2014), since the most suitable pretreatment will tend to be application specific. When the cost of pretreatment is the most important criterion in selecting the best pretreatment method, then the use of no or low cost, non-corrosive and recoverable chemicals; low energy and water consumption; minimal inhibitor production and applicability to a wide variety of lignocellulose feedstocks are all factors that need to be considered (Gurgel et al., 2014). This makes it difficult to compare different pretreatment methods on their unit cost contributions, since upstream and downstream operating costs, capital cost, recycling cost and effluent treatment cost are all a function of the pretreatment technology (Jeoh et al., 2007). Kumar and Murthy (2011) published a techno-economic analysis of different pretreatment technologies for a 250 000 tonne/y grass straw cellulosic ethanol plant, which is summarised in Table 2.4. The plant was assumed to employ simultaneous saccharification and co-fermentation (SSCF). Table 2.4 shows that uncatalysed steam explosion pretreatment, although resulting in a lower ethanol yield, has the potential to satisfy the requirements for lower capital and operational cost to a large extent and that this technology can be a candidate pretreatment method in an attempt to lower the unit cost of cellulosic ethanol.

Table 2.5 Summary of techno-economic analysis done by Kumar and Murthy (2011) for a SSCF cellulosic ethanol plant by comparing different pretreatment technologies.

	Dilute acid	Dilute alkali	Hot water	Uncatalysed steam explosion
Projected ethanol yields (L/dry tonne feedstock)	252.62	255.80	255.27	230.23
Capital cost ($/L ethanol)	1.92	1.73	1.72	1.70
Ethanol production cost ($/L ethanol)	0.83	0.88	0.81	0.85
Water usage (kg/L ethanol)	5.96	6.07	5.84	4.36

Steam explosion is the most employed physico-chemical method for pretreatment of lignocellulose (Alvira et al., 2010; Wanderley et al., 2013) and has the ability to treat a wide range of lignocellulose materials including agricultural residues, softwood and hardwood (Ewanick and Bura, 2011). By changing the mechanisms of loading and discharging biomass, steam explosion can be operated batch-wise or, as would rather be preferred in industry, on a continuous basis (Schultz et al., 1984). Steam explosion pretreatment studies reported in literature are mostly performed with batch pretreatment units such as the examples shown in Figure 2.8 that were used in this study. However, continuous steam explosion pretreatment technology has been proven in large-scale operations, being the only pretreatment method employed in the remaining large-scale commercial cellulosic ethanol operations, as indicated in Table 1.1 in Chapter 1. Compared to other pretreatment methods, the steam explosion technology can offer a lower environmental impact; requires lower capital expenditure; is becoming increasingly energy efficient; does not require hazardous chemicals and can accept relatively large feed particle size (Alvira et al., 2010; Avellar and Glasser, 1998; Galbe and Zacchi, 2007; Garrote et al., 1999). Furthermore, the steam explosion pretreatment process can be incorporated in pellet production to improve the logistics of lignocellulosic feedstock supply to a biorefinery (Tang et al., 2018).

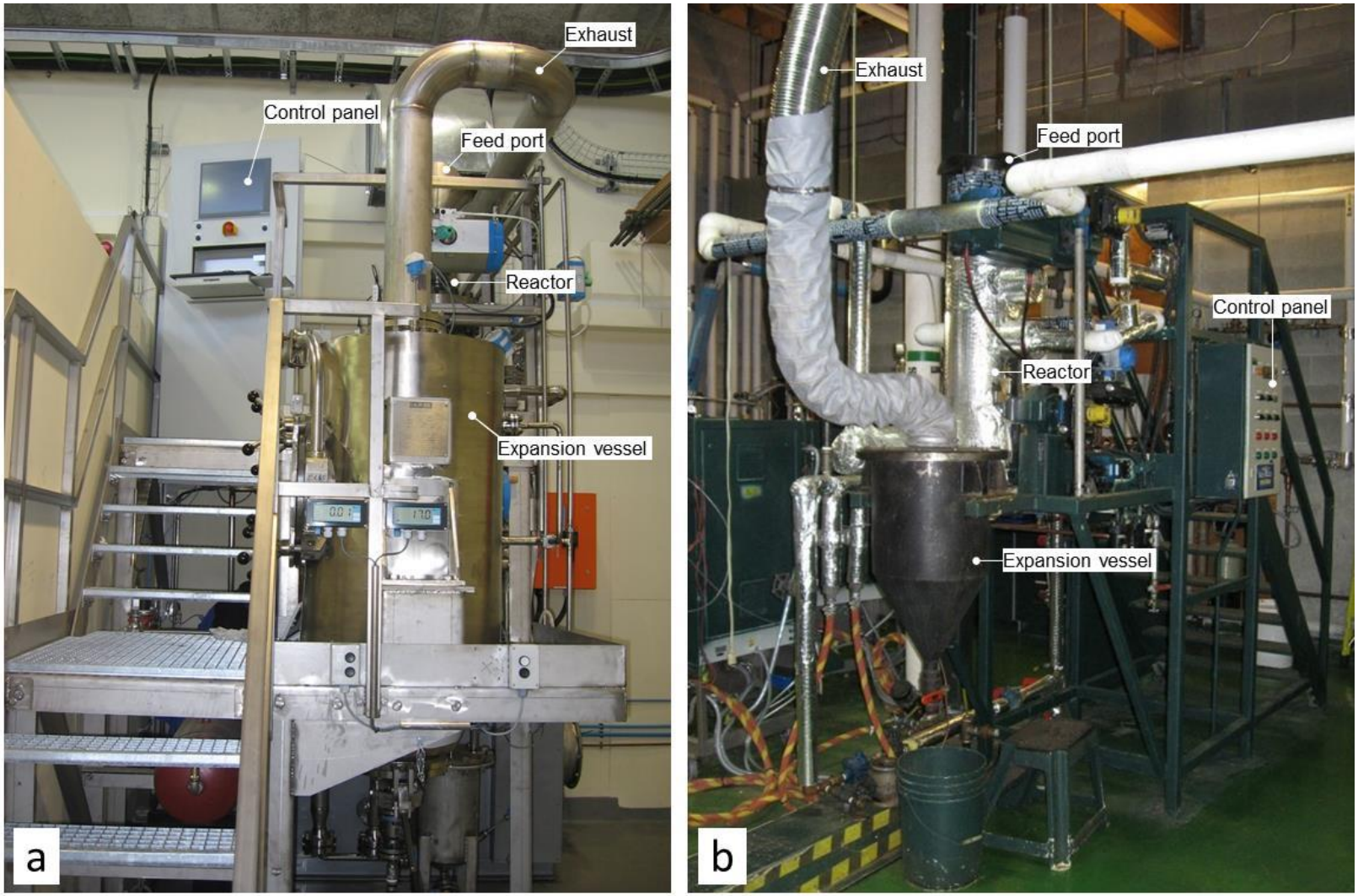

Figure 2.12 Batch lab-scale steam pretreatment units that were used in this study at (a) the Department of Process Engineering, Stellenbosch University, South Africa; and (b) the Department of Wood Science, University of British Columbia, Canada. Feedstock is loaded through the feed port into the reactor where it is steam pretreated and exploded into a receiving expansion vessel that is open to atmosphere via an exhaust line. (Own photos.)

Disadvantages of steam explosion pretreatment include the loss of xylose to volatile compounds, incomplete removal of lignin from the lignocellulose matrix and, depending on the severity of the pretreatment conditions and the type of feedstock, produce phenolic compounds from lignin and sugar degradation products that can inhibit downstream enzymatic hydrolysis and fermentation (Chiaramonti et al., 2012). Horn et al. (2011) have found in their uncatalysed steam explosion pretreatment of wheat straw that a considerable amount of biomass was lost. This was mainly as a result of volatile degraded xylose compounds that escaped the system at the end of the pretreatment process. The loss in biomass increased with an increase in severity of pretreatment conditions. Approximately 20% of the biomass was lost at the harsh treatment conditions of 210˚C and 220˚C, but it was also at these harsh conditions where the most digestible cellulose was obtained. Nevertheless, steam explosion remains an attractive option for large scale operations for its proven track record, ability to handle multiple feedstocks at large particle sizes and potential to operate uncatalysed to reduce operating costs (no chemicals needed) and capital costs (reduced wastewater treatment facilities required as less chemicals are added in the process) (Seidel et al., 2017).

It is doubted whether the explosion step itself contributes significantly to improving the digestibility of the lignocellulose for enzymatic hydrolysis during steam explosion pretreatment (Brownell et al., 1986; Duff and Murray, 1996; Mosier et al., 2005). Consequently, steam explosion pretreatment will from this point on in this dissertation be referred to as steam pretreatment, unless otherwise stated.

2.3.3 Steam pretreatment

2.3.3.1 Mechanism of steam pretreatment

The development of steam pretreatment technology can be traced back to the patents by Mason (1929) and Babcock (1932), and was originally developed for the production of fibreboard from wood chips. Wood chips were steam heated at approximately 285 °C for approximately 2 min, after which the chips were discharged to atmospheric pressure in a steam explosion to create a pulp. Since 1980 this procedure also found application as a method to increase the accessibility of cellulose in various lignocellulosic feedstocks for enzymatic hydrolysis and subsequent fermentation to produce cellulosic ethanol (Schultz et al., 1984).

Generally, steam pretreatment of lignocellulose material is performed with high pressure saturated steam that heats and pressurises the lignocellulose at temperatures of 160 to 240 °C and pressures of 7 to 48 bar, respectively, for periods from 1 to 20 min in a reactor before being suddenly depressurised to atmospheric pressure (Agbor et al., 2011; Galbe and Zacchi, 2012). Furthermore, steam pretreatment is either catalysed with the addition of a chemical, such as an acid or base, to the lignocellulosic feedstock or performed uncatalysed. Uncatalysed steam pretreatment is preferred for its decreased requirements for materials of construction, lower operating and maintenance costs, and reduced impacts on downstream fermentation and wastewater treatment processes (Franden et al., 2009; Humbird et al., 2011). Uncatalysed steam pretreatment can also maintain higher operational availabilities with less downtime for cleaning of carbonaceous deposits, such as insoluble humins, on inside surfaces of equipment as can be experienced with catalysed pretreatment (Shekiro III et al., 2014).

As a physico-chemical pretreatment, uncatalysed steam pretreatment relies on the autohydrolysis achieved during steam heating together with the mechanical forces during depressurisation at the end of the pretreatment. The effect on the lignocellulose structure during the heating period is close to dilute acid hydrolysis (Brownell et al., 1986), but with steam as the heat carrier instead. The high pressure steam penetrates and condenses inside the lignocellulose structure (Oliveira et al., 2013), where the heat releases acetyl groups from the hemicellulose to form acetic acid, causing autohydrolysis of hemicellulose bonds (Galbe and Zacchi, 2012; Garrote and Parajó, 2002). Autohydrolysis acts as mild acid hydrolysis as the pH decreases from almost neutral to about pH 3.5 to 4 with the release of acetic acid (Galbe and Zacchi, 2012). Water also acts as a weak acid at high temperature and contributes to the autohydrolysis mechanism (Alvira et al., 2010). The acetic acid then subsequently catalyses the hydrolysis of the pentosan polysaccharides into short-chain oligomers that is solubilised and diffused from the lignocellulose in the plant cell walls, leaving an increasingly porous structure to accelerate subsequent enzymatic hydrolysis and diffusion (Greenwood et al., 2013). The selective removal of hemicellulose exposes the cellulose structure for better enzyme accessibility (Himmel et al., 2007).

Lignin is only partially removed in steam pretreatment, and rather rearranged in the lignocellulose matrix when it is melted at high temperatures to undergo depolymerisation and repolymerisation reactions (Donaldson et al., 1988; Li et al., 2007; Pan et al., 2005). While lignin is in its depolymerised state, it is soluble in alkaline solutions and certain organic solvents (Schultz et al., 1984). This property allows for delignification when employing steam pretreatment under alkaline conditions or when steam pretreatment is followed with an alkaline or organic solvent treatment (Doherty et al., 2011).

After the heating period, the material and steam at high pressure are suddenly released to an expansion vessel at atmospheric pressure. This sudden decompression ruptures the cell walls and loosens the lignocellulose matrix to increase the accessible surface area (Duff and Murray, 1996). However, it has been shown that the cellulose arrangement itself is not significantly affected in the absence of sudden decompression (Brownell et al., 1986; Donaldson et al., 1988) and the resulting material deconstruction during the steam explosion step is thought to contribute only marginally to improving its digestibility (Mosier et al., 2005). This has again been contradicted by Pielhop et al. (2016) and Seidel et al. (2017) who have shown that increasing the differential pressure of the steam explosion step during steam pretreatment has a significant effect on the cellulose accessibility of the resulting pretreated material.

2.3.3.2 Steam pretreatment severity

The severity of steam pretreatment is determined by the temperature, retention time and pH. These conditions should be selected with great care to avoid loss of sugars through degradation reactions and subsequent formation of inhibitors for downstream fermentation. The fact that different pretreatment severities are required for the optimum recovery of hemicellulose and the optimum digestibility of cellulose (Heitz et al., 1991) complicates the selection of pretreatment conditions. Furthermore, the selection of optimum severity conditions is highly feedstock dependent (Kaar et al., 1998), to the extent where opposite trends for pretreatment efficiency were observed by Rosgaard et al. (2007) when changing severity conditions for wheat and barley straw. Ewanick and Bura (2011) also showed that, while different feedstocks can be similar in chemical composition, they might require different steam pretreatment severities to achieve the same results. In their case, sugarcane bagasse was more recalcitrant and required harsher pretreatment (10 min

at 205 ˚C) compared to switchgrass (7.5 min at 195˚C) to produce solids with comparable cellulose digestibilities.

Compared to lignin and cellulose, hemicellulose is the most thermally labile component (Hendriks and Zeeman, 2009). The hemicellulose will, however, not be removed from lignocellulose when the pretreatment conditions are too mild, but will on the other hand, be degraded when the steam pretreatment conditions are too harsh (Söderström et al., 2002). Hemicellulose is, therefore, hydrolysed and solubilised at lower severities than the pretreatment severities required for cellulose. Hydrolysis of hemicellulose was observed to start at temperatures as low as 150 ˚C in the absence of an acid catalyst (Chiaramonti et al., 2012). Increasing the severity of pretreatment will result in a continued shift from producing hemicellulose oligomers to producing proportionally more monomers, but with a concomitant increase in degradation products, such as furfural (Chiaramonti et al., 2012; Kabel et al., 2007).

Cellulose pretreatment requires more severe conditions, but too harsh pretreatment conditions can cause condensation reactions within the internal surfaces resulting in decreased accessibility to cellulases (Sun et al., 2005). Severe conditions also lead to a loss in cellulose digestibility with the deposition of lignin onto the cellulose to shield it from enzymatic attack (Ballesteros et al., 2000; Pan et al., 2005) as was confirmed by Kristensen et al. (2008) with scanning electron microscopy. Also, thermal degradation at severe conditions converts cellulose via dehydration and depolymerisation into degradation products such as 5-hydroxymethylfurfural (HMF) (Jacquet et al., 2011).

Öhgren et al. (2005) have found for steam pretreatment of SO_2 impregnated corn stover that high temperature and short retention time favoured glucose yield, while lower

temperature and longer retention time favoured xylose yield. The highest combined sugar yield, for the conditions investigated, was obtained at 190 ˚C for 5 min. This means that the pretreatment conditions are usually selected as a compromise to optimise for combined sugar yield (Agbor et al., 2011), i.e. maximal combined xylose and glucose recovery, which implies minimised degradation, but at the cost of less than maximal cellulose digestibility.

The yields of sugar intermediates will be the driving factor in selecting the steam pretreatment conditions in terms of temperature and time, but other considerations might also play a role in deciding pretreatment operation. For example, the production of inhibitors at high temperatures and long pretreatment times could offset the advantage of more accessible cellulose for downstream fermentation processes (Espírito Santo et al., 2019). Fockink et al. (2018) considered the rheological behaviour of the slurries from steam pretreated bagasse as higher complex viscosities translate into higher power consumption. Iroba et al. (2014) found for steam pretreatment of barley straw that the carbon content and the higher heating value of the pretreated solids increase with an increase in pretreatment temperature and time, in the case where pretreated solids are not bioprocessed into glucose, but rather used as fuel. Fermenting the steam pretreated solids and then use the fermentation residues as a fuel source could make more sense in a multi-product biorefinery (Leibbrandt et al., 2011), however, the effect of different steam pretreatment conditions on the heating value of fermentation residues could not be found in literature. Also, in a multi-product biorefinery setup, it will be important to produce pretreated slurries that can easily be dewatered in a solids-liquid separation step to produce cellulose-rich solids and pentose-rich liquid prehydrolysate for separate processing. A high degree of dewaterability will require lower power consumption and lead to improved

inhibitor removal from the solids. No literature could be found on the dewaterability characteristics of pretreated material.

2.3.3.3 Severity factor for steam pretreatment

A severity factor has been introduced for autohydrolysis (uncatalysed) pretreatment methods to provide a basis for developing pretreatment severities and comparing different response variables (Abatzoglou et al., 1992; Overend and Chornet, 1987). The severity factor is a function of the temperature and retention time of the uncatalysed pretreatment. The severity factor concept has subsequently been expanded to the combined severity factor to also account for the enhanced effects of acid and alkaline catalysts during catalysed pretreatment (Chum et al., 1990; Park et al., 2012).

The severity factor, $\log R_0$, is defined as:

$$\log R_0 = \log \int_0^t \exp\left(\frac{T(t) - T_{Ref}}{\omega}\right) \quad dt$$

where ω is normally set equal to 14.75 in literature and T_{Ref} = 100 ˚C. T(t) is the pretreatment temperature in ˚C as a function of retention time t in min. In isothermal pretreatment at temperature T the severity factor is therefore calculated as:

$$\log R_0 = \log\left(t \times \exp\left(\frac{T - 100}{14.75}\right) \right)$$

The severity factor is rather empirical and cannot explain steam pretreatment on a fundamental and cellular level (Kristensen, 2008). Consequently, this measure cannot be regarded as completely accurate (Agbor et al., 2011), but could be used for rough estimates of the degree of pretreatment (Galbe and Zacchi, 2007). Nevertheless, the severity factor has shown to correlate well when comparing results of steam pretreatment experiments in

the laboratory with those obtained in industry (Heitz et al., 1991), and has remained relevant to date as a tool for relative response comparison at different pretreatment severities (Espírito Santo et al., 2019; Fockink et al., 2018; Simangunsong et al., 2020).

The morphological changes of sugarcane bagasse and sugarcane harvest residues after uncatalysed steam pretreatment at different pretreatment severities are shown in Figures 2.9 and 2.10, respectively. Uncatalysed steam pretreatment severity increases with an increase in steam pretreatment temperature and/or time. As is evident from Figures 2.9 and 2.10, an increase in steam pretreatment severity results in smaller particles (Pielhop et al., 2016) and darker material as lignin is redistributed on the surfaces of the fibres (Auxenfans et al., 2017).

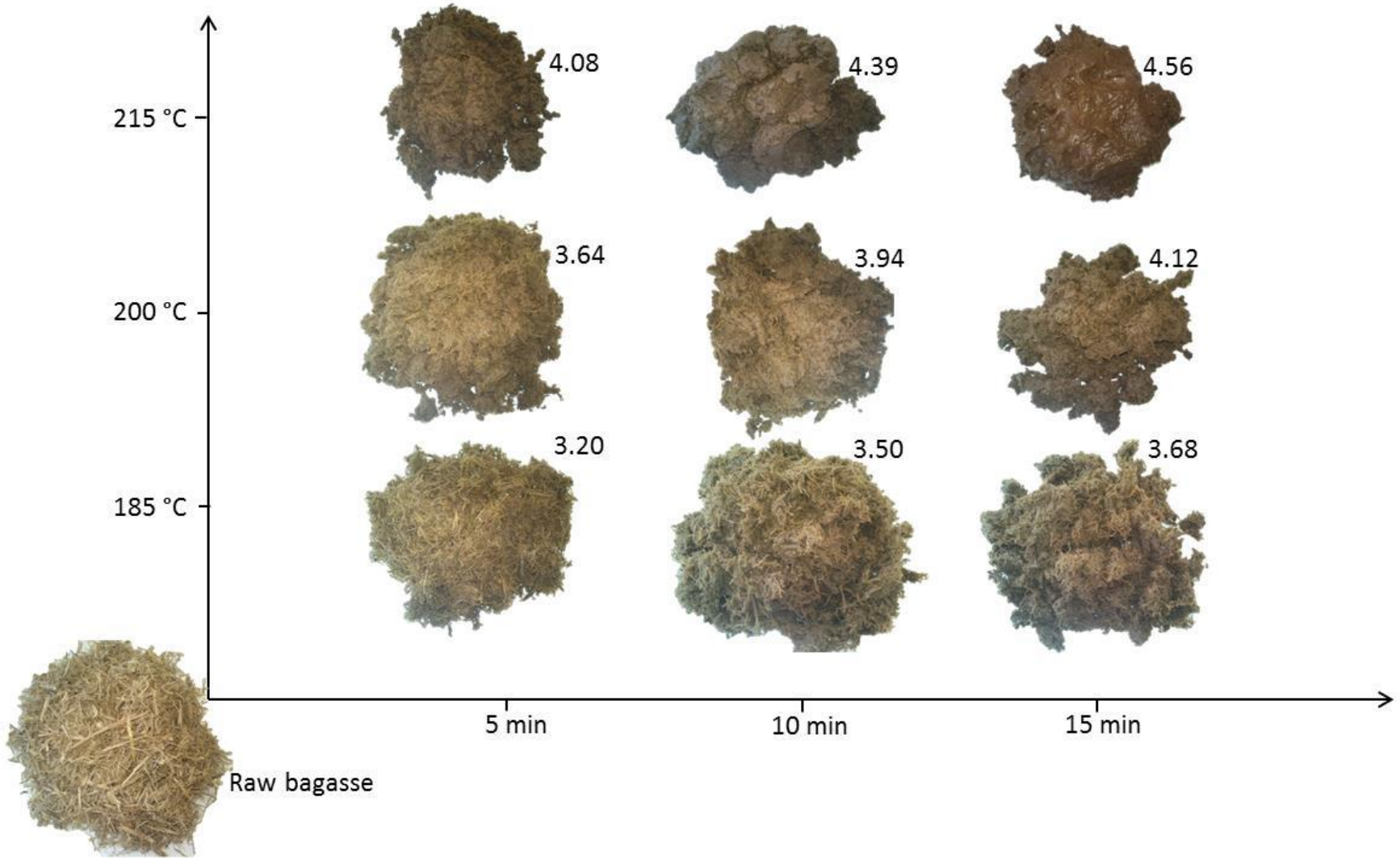

Figure 2.13 Morphological changes of sugarcane bagasse after uncatalysed steam pretreatment at different temperatures (185 °C, 200 °C, 215 °C) and different times (5 min, 10 min, 15 min). A sample of raw bagasse is also shown for reference. The resulting pretreatment severity factors (log R0) are indicated and increase with an increase in temperature and/or time. (Own work.)

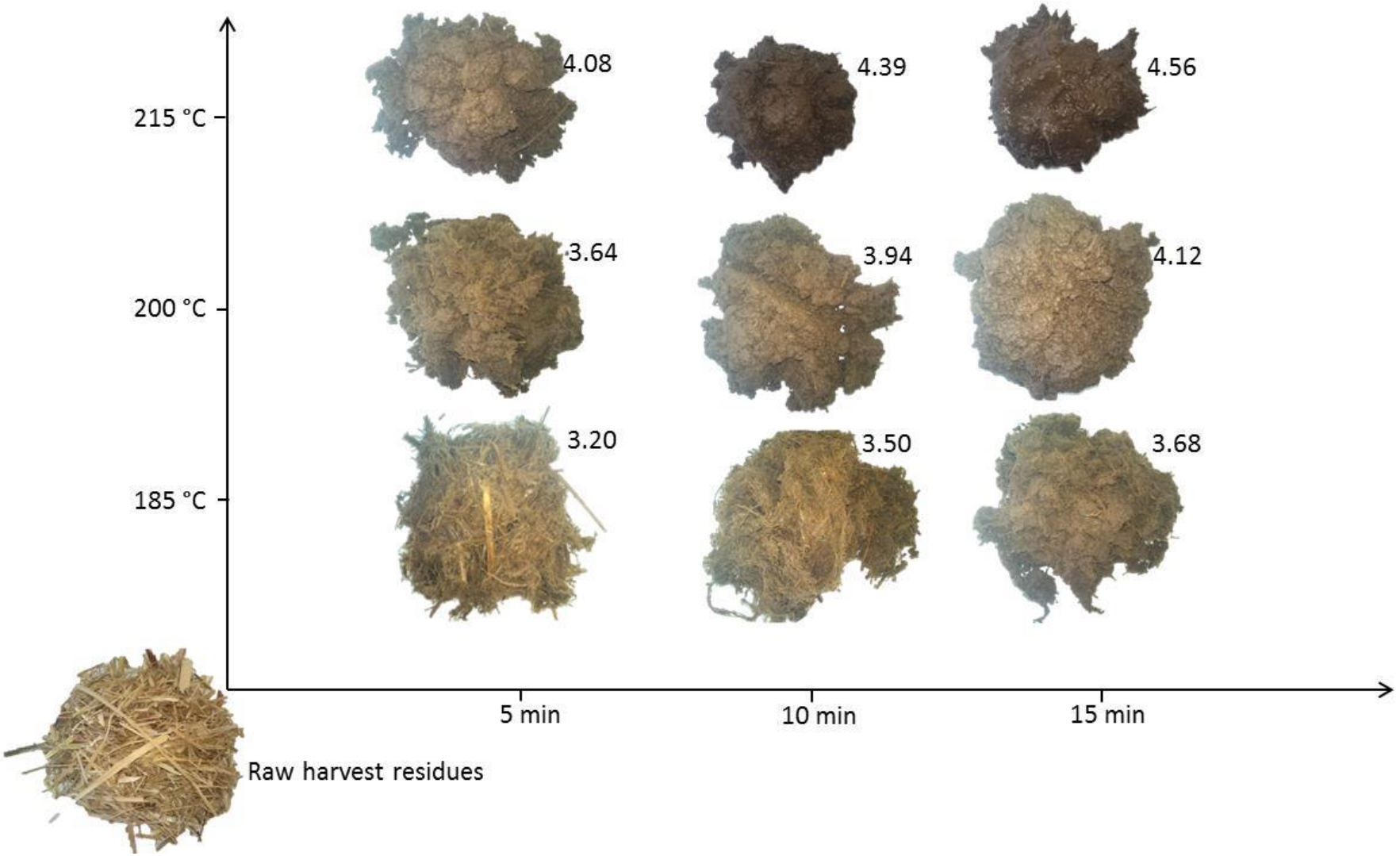

Figure 2.14 Morphological changes of sugarcane harvest residues after uncatalysed steam pretreatment at different temperatures (185 °C, 200 °C, 215 °C) and different times (5 min, 10 min, 15 min). A sample of raw harvest residues is also shown for reference. The resulting pretreatment severity factors (log R_0) are indicated and increase with an increase in temperature and/or time. (Own work.)

The severity factor has also been expanded to the combined severity factor (CSF) to accommodate for catalysed pretreatment. The CSF of an acid catalysed pretreatment is calculated by incorporating the pH of the pretreatment hydrolysate:

$$\mathrm{CSF} = \log \mathrm{R}_0 - \mathrm{pH}$$

whereas the combined severity factor of an alkaline catalysed pretreatment is calculated by incorporating the pOH of the pretreatment hydrolysate:

$$\mathrm{CSF} = \log \mathrm{R}_0 - \mathrm{pOH}$$

Ferreira-Leitão et al. (2010) used the combined severity factor in steam pretreatment by incorporating the measured pH values of the slurry after pretreatment of sugarcane bagasse and harvest residues impregnated with the acid catalysts SO_2 and CO_2. The combined severity factor correlated well with the production of degradation products. Park et al. (2012) also used the combined severity factor for the alkaline impregnation of *Eucalyptus grandis* with NaOH and found that the combined severity factor correlated well with lignin removal and enzymatic hydrolysis. Fockink et al. (2018) confirmed the relevancy of the combined severity factor as a basis for comparing both catalysed and uncatalysed steam pretreatment of sugarcane bagasse.

2.3.4 Steam pretreatment of sugarcane bagasse and harvest residues

The primary aim of pretreatment of lignocellulose is to directly improve the rate of enzymatic hydrolysis of cellulose, or conversely, reduce the enzyme dosage. Pretreatment, therefore, also impacts indirectly on the productivity of downstream bioconversion of the sugar intermediates to bioproducts. The enzymatic hydrolysis and fermentation processes for pretreated lignocellulose can be performed in different process configurations such as simultaneous saccharification and fermentation (SSF), separate hydrolysis and fermentation

(SHF), simultaneous saccharification and combined fermentation (SSCF), consolidated bioprocessing (CBP) and simultaneous pretreatment and saccharification (SPS) (Pandiyan et al., 2019; Rastogi and Shrivastava, 2017).

All the studies on steam pretreatment of sugarcane bagasse and harvest residues (catalysed and uncatalysed) that could be found in literature are listed in Tables 2.5 and 2.6, respectively. These tables highlight the conditions of the pretreatments in terms of catalysts used, pretreatment temperatures and pretreatment times. Only two studies were found for steam pretreatment of sugarcane harvest residues (Table 2.6). Since lignocellulose pretreatment studies have to date mainly focused on producing substrate for cellulosic ethanol production, the conditions and performances of ethanol fermentation are also given, where available.

Pretreatment temperatures in previous studies for uncatalysed steam pretreatment of bagasse (Table 2.5) ranged from 170 to 243 °C. Bernier-Oviedo et al. (2018) used long pretreatment times (15 – 60 min) for uncatalysed steam pretreatment of bagasse at the low temperature of 170 °C. Fockink et al. (2018) only used pretreatment temperatures of 195 – 205 °C for uncatalysed steam pretreatment of bagasse, whereas the low temperatures of 170 – 195 °C were used for bagasse steam pretreatment with phosphoric acid catalyst. The high steam pretreatment temperatures used by (Kaar et al., 1998; Kling et al., 1987; Schultz et al., 1984) for uncatalysed bagasse were combined with short pretreatment times of less than 5 min. Short pretreatment times can, however, be problematic in comparing relative pretreatment severities, as the temperature ramp-up introduces non-isothermal pretreatment.

Table 2.6 Summary of steam pretreatment of sugarcane bagasse experiments found in literature. Where fermentability of the pretreated solids was tested, the best reported production results are given.

Catalyst with steam pretreatment	Temperature range (˚C)	Time range (min)	Pretreatment targets	Fermentability	Reference
Uncatalysed	194, 211, 224	1	Glucose recovery	Not tested	(Schultz et al., 1984)
Uncatalysed	190, 200, 210, 220	1 – 25	Glucose recovery, hemicellulose recovery	Not tested	(Kling et al., 1987)
Uncatalysed	188 – 243	0.5 – 44	Glucose recovery, xylose recovery	Not tested	(Kaar et al., 1998)
Impregnated with 1.1% SO_2 (w/w moisture), 1% H_2SO_4 (w/w) or no catalyst.	205	10	Glucose recovery, xylose recovery, CSY	Fermentation only on prehydrolysates	(Martín et al., 2002)
Uncatalysed or impregnated with 2% SO_2 (w/w moisture).	180, 190, 205	5, 10	Glucose recovery, xylose recovery, CSY	SSF, 5% solids, no detox, whole slurry diluted, 23.5 g/L, 81% theoretical ethanol yield	(Sendelius, 2005)
Uncatalysed	205	10	None	Fermentation only on prehydrolysates	(Martín et al., 2006)
Impregnated with 2% SO_2 (w/w moisture).	190	5	None	SSF, 5 or 7.5% solids respectively, no detox, whole slurry diluted, 0.27 g/L.h-1 ethanol	(Rudolf et al., 2008)
Impregnated with 2% SO_2 (w/w moisture)	180, 190, 205	5, 10	Glucose recovery, xylose recovery, CSY	Fermentation only on prehydrolysates and enzymatic hydrolysates	(Carrasco et al., 2010)

(Table continuing on next page.)

Table 2.5 Summary of steam pretreatment of sugarcane bagasse experiments found in literature. Where fermentability of the pretreated solids was tested, the best reported production results are given. (Continued from previous page.)

Catalyst with steam pretreatment	Temperature range (˚C)	Time range (min)	Pretreatment targets	Fermentability	Reference
Impregnated with 3% CO_2 (w/w moisture), 3% SO_2 (w/w moisture) or no catalyst. (SO_2 impregnation only tested at 190 ˚C for 5 min).	190, 205, 220	5, 10, 15	Glucose recovery, xylose recovery	Not tested	(Ferreira-Leitão et al., 2010)
Dry, water-soaked, SO_2 onto dry and SO_2 onto water-soaked.	205	10	Glucose recovery	SSF, 5% solids, solids were washed	(Ewanick and Bura, 2011)
Soaked for 4 h in 1% H_3PO_4 (w/w) solution. Then pressed to contain 10 g H_3PO_4 / kg dry weight.	160, 170, 180, 190	10	None	SSCF at 10, 12 or 14% solids, whole slurry with no detox, 29.0 g/L ethanol, 0.21 g ethanol/g bagasse	(Geddes et al., 2011)
Immersed in a 0.5% lactic acid (w/w) solution for 3 h. Then pressed and stored for 1 or 2 months. Re-impregnated with 1.5% SO_2 (w/w) or not.	200, 210	5	Glucose recovery, xylose recovery, CSY	Not tested	(Monavari et al., 2011)
Uncatalysed	190	15	None	Not tested	(Rocha et al., 2012)
Uncatalysed	200	7	Glucose recovery	Fermentation only on enzymatic hydrolysate	(Wanderley et al., 2013)
Uncatalysed	220	5	None	SSF, 5% solids, ethyl acetate extraction on slurry, 66.3% theoretical ethanol yield	(Li et al., 2014)

(Table continuing on next page.)

Table 2.5 Summary of steam pretreatment of sugarcane bagasse experiments found in literature. Where fermentability of the pretreated solids was tested, the best reported production results are given. (Continued from previous page.)

Catalyst with steam pretreatment	Temperature range (˚C)	Time range (min)	Pretreatment targets	Fermentability	Reference
Uncatalysed, 0.95% H_3PO_4 (w/w), 0.95% H_2SO_4 (w/w)	195	7.5	Glucose recovery	SSF, 12% solids, no detox, 25.2 g/L ethanol	(Neves et al., 2016)
Soaked for 1 h in 4% H_2SO_4 solution.	190, 210	10, 5	None	SSF, 5% solids, 94.33% theoretical ethanol yield	(You et al., 2016)
Uncatalysed	170	15, 30, 60	Glucose recovery	Fermentation only on enzymatic hydrolysate	(Bernier-Oviedo et al., 2018)
Uncatalysed, H_3PO_4 and H_2SO_4	170, 180, 195, 200, 205	4, 7.5, 10.5, 15	Glucose recovery	Not tested	(Fockink et al., 2018)
Uncatalysed, 0.01 M citric acid, 0.1 M NaOH	180	5	Glucose recovery	Not tested	(Silva et al., 2018)
Uncatalysed and 0.5% H_2SO_4 (w/w)	188, 195, 198, 204	8, 10, 15	Glucose recovery	Not tested	(Espírito Santo et al., 2019)

Table 2.7 Summary of steam pretreatment of sugarcane harvest residues experiments found in literature. Where fermentability of the pretreated solids was tested, the best reported production results are given.

Catalyst with steam pretreatment	Temperature range (˚C)	Time range (min)	Pretreatment targets	Fermentability	Reference
Impregnated with 3% CO_2 (w/w moisture), 3% SO_2 (w/w moisture) or no catalyst. (SO_2 impregnation only tested at 190 ˚C for 5 min).	190, 205, 220	5, 10, 15	Glucose recovery, xylose recovery	Not tested	(Ferreira-Leitão et al., 2010)
Uncatalysed	180, 190, 200	15	Glucose recovery	Not tested	(Oliveira et al., 2013)

In all these studies, as listed in Tables 2.5 and 2.6, no optimisation study was found that predicted and evaluated steam pretreatment conditions (temperatures and times) for pretreatment of sugarcane bagasse and harvest residues for maximum yields of sugar products. In other words, pretreatment was only performed at pre-selected conditions without developing models for predicting the optimum conditions. However, Kling et al. (1987) and Kaar et al. (1998) performed uncatalysed steam pretreatment of bagasse at a large number of different temperature and time combinations that enabled them to develop trends for certain sugar products. Kling et al. (1987) studied uncatalysed steam pretreatment at 190, 200, 210 and 220 °C at 12 treatment times ranging from 1 to 25 min, and found the highest total hemicellulose recovery (approximately 65% (wt) of the total hemicellulose) after 200 °C, 6 min, and the highest glucose yield (approximately 36% (wt) of dry bagasse feed) after 200 °C, ca. 5 -7 min and 210 °C, ca. 3 – 4 min with enzymatic hydrolysis. Kaar et al. (1998) studied uncatalysed steam pretreatment of bagasse at 95 different temperature and time combinations. Temperatures ranged from 188 to 243 °C and times from 0.5 to 44 min, and were combined into pretreatment conditions to produce a severity factor (log R_0) range of 3.7 to 4.3. This means that the higher temperatures were used for shorter times and vice versa. Similarly to the observation by Kling et al. (1987), Kaar et al. (1998) also found that hemicellulose recovery tend to be fairly constant with constant log R_0. Both Kling et al. (1987) and Kaar et al. (1998) found the highest total hemicellulose recovery and highest xylose recovery, respectively, at an uncatalysed steam pretreatment severity log R_0 of approximately 3.8. However, contrary to Kling et al. (1987), Kaar et al. (1998) found, when considering similar pretreatment conditions, the highest glucose recovery after enzymatic hydrolysis when bagasse was pretreated

with a harsher severity log R_0 of 4.1 at 216 °C, 5 min. The higher required severity could probably be attributed to the wet feedstock used by Kaar et al. (1998) (50.4% moisture content) as opposed to the dry feedstock used by Kling et al. (1987). Higher feedstock moisture content has been shown to decrease the severity of steam pretreatment (Brownell et al., 1986).

Optimisation studies, with the help of response surface modelling (RSM), to find the best pretreatment conditions (temperatures and times) were however found in literature for uncatalysed and catalysed steam pretreatment of other agricultural feedstocks. López-Linares et al. (2015) investigated 185 – 215 °C and 2.5 – 7.5 min in uncatalysed pretreatment for optimising digestibility of rapeseed straw and found 215 °C and 7.5 min to be the optimum condition in the studied ranges. Bura et al. (2003) investigated 150 – 230 °C and 1 – 9 min at different SO_2 concentrations to optimise catalysed steam pretreatment of corn fibre and found 190 °C and 5 min at 3% SO_2 as the optimum conditions for producing maximum CSY.

No literature was found that investigated steam pretreatment of blends of lignocellulosic feedstocks, even though Ferreira-Leitão et al. (2010) mentioned the possibility of steam pretreating a blend of bagasse and harvest residues. However, blending of bagasse and harvest residues have been performed for dilute sulfuric acid (1.5%, w/w) pretreatment by Pereira et al. (2015), who blended bagasse, straw (component of harvest residues) and tops (component of harvest residues) in a 1:1:1 ratio. This increased the enzymatic conversion by 55% and the ethanol yield by 25%, compared to bagasse alone as feedstock.

2.3.5 Deacetylation of lignocellulose prior to pretreatment

Deacetylation is an alkaline extraction process where the acetyl groups are removed from the lignocellulose by alkaline de-esterification (Chen et al., 2012a). Chen et al. (2012a, 2012b) and Shekiro et al. (2014) deacetylated corn stover in a mild alkaline extraction process prior to dilute sulphuric acid pretreatment and removed approximately 80% of the acetate from the corn stover. Because the pretreatments were still catalysed by sulphuric acid, the cellulose digestibility was improved, compared to using raw corn stover.

Deacetylation of lignocellulose prior to steam pretreatment could be an attractive consideration, as it will remove inhibitors for downstream fermentation. Unlike with dilute acid pretreatment, the digestibility of pretreated material from uncatalysed steam pretreatment will likely decrease with deacetylation. There would potentially be a trade-off in digestibility of pretreated material versus the amount of inhibitors during fermentation with the introduction of deacetylation prior to uncatalysed steam pretreatment. No literature on deacetylation of lignocellulose prior to steam pretreatment could be found.

2.4 Conclusions

The following three main areas, as listed below, were identified from the literature review where a lack of reported information remains regarding the development of an integrated sugarcane biorefinery process with uncatalysed steam pretreatment of sugarcane bagasse and harvest residues for the co-production of a sugar platform:

1) Little research is available on the bioprocessing of sugarcane harvest residues as a source of lignocellulose

While sugarcane bagasse is widely recognised as a potential lignocellulosic feedstock for bioprocessing with extensive research on its preparation and pretreatment, sugarcane harvest residues have largely been ignored. The gaps in the literature were categorised as:

1.a) Little sugarcane harvest residues feedstock data available

Very few investigations commenting on composition have been conducted for sugarcane harvest residues, as indicated by the limited studies in Table 2.3. Also, as shown in Table 2.3, many studies did not report extractives and ash contents, and no study reported on acetyl groups content. The little information available on sugarcane harvest residues, including leaves and tops, is further compounded by the variability in the reported characterisation of the feedstock, especially due to the absence of standardisation of the plant components investigated. Studies dealing with harvest residues often refer to different parts of the sugarcane plant, generically referred to as trash, straw, tops, leaves or residues, resulting in large variations in the reported compositions and an inability to draw direct correlations between published data.

1.b) Bioprocessing properties of sugarcane harvest residues are largely unknown

As discussed in Section 2.3.3.2, different lignocellulosic feedstocks, such as bagasse and harvest residues, can behave very differently during steam pretreatment and could require different pretreatment conditions for maximum yields of the subsequent sugar intermediates. Only one study could be found that compared steam pretreatment of bagasse and harvest residues in the same pretreatment unit, but was compared at only a few selected conditions (Ferreira-Leitão et al., 2010).

No study was found that investigated steam pretreatment of blends of bagasse and harvest residues, even though Ferreira-Leitão et al. (2010) mentioned the possibility. Blending of bagasse and harvest residues improved the enzymatic conversion and ethanol yield, compared to bagasse only for a dilute acid pretreatment by Pereira et al. (2015).

2) Steam pretreatment of bagasse and harvest residues not considered for overall biorefinery operation

The gaps in the literature were categorised as:

2.a) No optimisation studies to identify operational regimes of steam pretreatment of bagasse and harvest residues in a biorefinery process configuration

No studies could be found that identified different steam pretreatment regimes of preferred operating conditions of any lignocellulosic feedstock for the production of pretreated sugar products at maximum digestibility of cellulose, maximum hemicellulose recovery and maximum combined sugar yield, as will be required in a biorefinery depending on feedstock and market conditions. In

literature steam pretreatment of bagasse and harvest residues were all investigated at discrete and pre-selected pretreatment conditions with no further optimisation studies. Furthermore, while steam pretreatment of bagasse has been extensively investigated, steam pretreatment of harvest residues are poorly defined with only two publications found in literature that described steam pretreatment of harvest residues at different conditions (Table 2.6).

2.b) Little consideration for downstream implications of steam pretreatment conditions

In literature steam pretreatment performance is usually discussed in terms of sugar yields after enzymatic hydrolysis. However, no information is available in the literature on the impacts of bagasse steam pretreatment on dewaterability of pretreated solids. In the few fermentability studies available on pretreated bagasse material, the material is usually detoxified, diluted or washed (Table 2.5). Sometimes no mention is made on the detoxification steps. No dewaterability and fermentability tests were found for steam pretreated harvest residues.

No studies on deacetylation of feedstocks prior to steam pretreatment were found. Literature was only found for deacetylation prior to dilute acid pretreatment, which therefore meant that the pretreatment remained catalysed. The impacts on fermentability of deacetylated and uncatalysed steam pretreated material are therefore not available.

Heating values of the resulting residues after fermentation of steam pretreated bagasse and harvest residues could not be found in literature.

3) The understanding of the mechanism of autohydrolysis during uncatalysed steam pretreatment is still unclear

The gaps in the literature were categorised as:

3.a) The mechanism and effects of steam explosion during steam pretreatment are still unclear

It is still doubted if the explosion step contributes to improving the digestibility of steam pretreated material, as mentioned in Section 2.3.3.1 (Mosier et al., 2005). Three studies have tried to systematically elucidate the effect of steam explosion on steam pretreatment performance, but the results are contradictory (Brownell et al., 1986; Pielhop et al., 2016; Seidel et al., 2017).

3.b) The acetyl groups to ash ratio requirement for effective uncatalysed steam pretreatment is not reported

No studies are available on the impact of uncatalysed steam pretreatment performance for different lignocellulosic feedstocks with different acetyl groups to ash ratio contents when deacetylated prior to steam pretreatment.

2.5 References

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CHAPTER 3

OBJECTIVES

The aim of this study was to experimentally identify preferred uncatalysed steam pretreatment operating regimes for sugarcane lignocellulosic wastes, i.e. bagasse and harvest residues, for the subsequent production of sugar intermediates: glucose, xylose and arabinose. These sugar intermediates would create the sugar platform in a biorefinery setup that would be utilised for the downstream production of biofuels and platform chemicals. The pretreatment was chosen to be uncatalysed for its lower operating and capital cost implications in a biorefinery approach, even though pretreatment yields of sugars are generally lower, as opposed to catalysed pretreatment. The pretreatment operating regimes of each feedstock had to respectively produce pretreated solids of maximum digestibility, maximum hemicellulose recovery and maximum CSY. Pretreated solids of maximum digestibility were preferred for the readily production of glucose via hydrolysis, maximum hemicellulose recovery in the prehydrolysate allowed for the maximum yield of xylose and arabinose, and maximum CSY in the pretreated material provided the maximum overall yield of glucose, xylose and arabinose after downstream hydrolysis.

Knowledge of the pretreatment operating regimes of each feedstock allows for the maximum yields of the target sugar intermediates after pretreatment and hydrolysis as required by the downstream products. However, maximum yields of the required sugar intermediates are not necessarily the only metrics in sugar platform

biorefinery operation. Other bioprocessing aspects such as dewaterability of the pretreated slurries, fermentability of the pretreated products and the gross energy yield could also be important considerations in choosing the preferred pretreatment operating regime and preferred feedstock. The dewaterability of pretreated slurry is an indication of the ease with which the prehydrolysate can be separated from the solids in the pretreated slurry and will determine the energy requirements of the solids-liquid separation step, the efficiency in recovering the prehydrolysate and the efficiency in removing fermentation inhibitors from the solids. Fermentability is an indication of the amount of inhibitors the pretreated material contain. Even at high yields of the sugar intermediates, low fermentability could be undesirable, as the pretreated material would require additional detoxification and washing steps prior to bioconversion. The gross energy yield could be another factor in determining the preferred pretreatment operating regime in the case where biofuels are produced and where lignin-rich fermentation residues are produced as a heating fuel by-product.

Given the gaps in literature as identified in Section 2.4, the following research questions were developed from the aim of this study:

- What are the preferred uncatalysed steam pretreatment operating regimes of bagasse and harvest residues in terms of pretreatment temperatures and times to achieve maximum digestible solids, maximum hemicellulose recovery and maximum CSY for each feedstock?
- Do these three pretreatment operating regimes differ substantially for each feedstock and how do they compare between the feedstocks? Why are these differences observed in the pretreatment behaviour of the two feedstocks?

- Are the yields of the sugars glucose, xylose and arabinose significantly different between these three pretreatment operating regimes for each feedstock?
- How would deacetylation of bagasse and harvest residues prior to uncatalysed steam pretreatment impact on pretreatment performance and downstream fermentation?
- What contribution does the explosion step in uncatalysed steam pretreatment make to the physical changes in the material and the digestibility thereof?
- What would be the gross energy yield from the various pretreatment conditions in terms of ethanol production and heating value of the fermentation residues?
- How does a blended feedstock of bagasse and harvest residues impact the pretreatment operating regimes compared to the pure feedstocks? Why are these differences observed in the pretreatment behaviour of the blended feedstock?
- What would be the preferred feedstock between bagasse and harvest residues for bioprocessing in a sugarcane biorefinery?
- How can variations in different harvest residues characteristics such as chemical composition, leaves to tops ratio, age, cultivar and origin be used to predict steam pretreatment behaviour?

It was consequently decided to reach the aim of this study and answer the research questions with the following objectives:

Objective 1

Optimise uncatalysed steam pretreatment of bagasse and harvest residues to develop steam pretreatment-based biorefinery processes

This objective was addressed with the work in Chapter 4. Little information is available in literature on sugarcane harvest residues composition and its steam pretreatment behaviour relative to sugarcane bagasse. This objective entailed the determining of compositional information of the two feedstocks and comparing their uncatalysed steam pretreatment behaviour in the same pretreatment reactor. Based on typical uncatalysed steam pretreatment conditions in literature for bagasse (Section 2.3.4), both feedstocks were separately steam pretreated in the ranges 185 – 215 °C and 5 – 15 min without catalyst and the pretreated products characterised in terms of digestibility of the pretreated solids, the recovery of hemicellulose from the prehydrolysate and the CSY in the pretreated products. These responses were used to build response surface models (RSM) to indicate preferred operating regimes of uncatalysed steam pretreatment in terms of temperature and time as required by the sugar platform for downstream processing.

Objective 2

Investigation of effects of uncatalysed steam pretreatment strategies on enzymatic hydrolysis and fermentation

This objective was addressed with the work in Chapters 4, 5 and 6, and expanded onto the optimal uncatalysed steam pretreatment conditions of Objective 1. Objective 2 included strategies such as blending of bagasse and harvest residues into a single feedstock, deacetylation of feedstocks prior to uncatalysed steam pretreatment and investigating the mechanism of particle refinement in steam explosion.

A feedstock blend of bagasse and harvest residues could potentially be synergistic to improve autohydrolysis at low pretreatment severities and buffer pH at high pretreatment severities. Bagasse and harvest residues were blended in a 1:1 mass ratio and steam pretreated at the three optimum pretreatment conditions for bagasse for maximum digestibility, maximum hemicellulose recovery from the prehydrolysate and maximum CSY. The blend was also pretreated at one pretreatment condition that was identified as an optimum condition for pretreating harvest residues. Blended feedstock that was steam pretreated as raw and deacetylated was also assessed for dewaterability, fermentability and gross energy yield.

Deacetylation of the lignocellulosic feedstocks prior to pretreatment could provide for a way to remove most of the acetyl groups upstream in a biorefinery setup as a potential detoxification step for downstream fermentation. However, this would be predicated on the performance of uncatalysed steam pretreatment with significantly less available acetyl groups in the lignocellulose. In this work, deacetylation briefly entailed the removal of most of the acetyl groups from the hemicellulose in the feedstocks prior to pretreatment with a dilute alkaline extraction process. The feedstocks were pretreated in the raw, as well as in the deacetylated state. Digestibilities of the resulting pretreated solids were compared with each other based on glucose yields from enzymatic hydrolysis. Pretreated material was also pressed and fermented to produce ethanol in an SSF setup in fed-batch to a maximum solids concentration of 15%. Afterwards, the gross energy yields from the various pretreatment conditions were calculated in terms of ethanol production and heating value of the fermentation residues. The dewaterability and fermentability of the

pretreated slurries, as well as the gross energy yield were used as indicators for feedstock suitability for bioprocessing in a sugarcane biorefinery.

An improvement in the steam explosion step could potentially decrease the required severity of steam pretreatment to preserve the sugar platform in a biorefinery. Objective 2 therefore also attempted to elucidate the mechanism by which the steam explosion step refines the pretreated material to improve digestibility. The effect of rapidly transporting pretreated material out of the pretreatment reactor during steam explosion was investigated by either retaining pretreated material in the reactor during explosion or releasing it from the reactor during explosion. Digestibilities of the resulting pretreated solids were compared with each other based on glucose yields from enzymatic hydrolysis.

CHAPTER 4

UNCATALYSED STEAM PRETREATMENT IN A SUGARCANE BIOREFINERY: OPTIMISING FOR PREFERRED SUGAR PRODUCTS FROM BAGASSE AND HARVEST RESIDUES FEEDSTOCKS

This chapter appears as a draft manuscript.

Title: "Uncatalysed steam pretreatment in a sugarcane biorefinery: Optimising for preferred sugar products from bagasse and harvest residues"

Authors: Martin Louis Hamann, Eugéne van Rensburg, Johann Ferdinand Görgens

Objectives of this chapter in the dissertation

The objective of this chapter was to establish the preferred uncatalysed steam pretreatment operating regimes for bagasse and harvest residues to produce pretreated products that could feed the sugar platform in a sugarcane biorefinery with the sugar intermediates: glucose, xylose and arabinose. The preferred pretreatment operating regimes included pretreatment for producing maximum digestibility of the pretreated solids, maximum combined sugar yield (CSY) in all the pretreatment products and maximum hemicellulose recovery in the prehydrolysate. Pretreated solids of maximum digestibility were preferred for the readily production of glucose via hydrolysis, maximum hemicellulose recovery in the prehydrolysate allowed for the maximum yield of xylose and arabinose, and maximum CSY in the pretreated material

provided the maximum overall yield of glucose, xylose and arabinose after downstream hydrolysis. Knowledge of the different pretreatment operating regimes for the different feedstocks would aid in selecting the most appropriate feedstock for bioprocessing and provide the optimal pretreatment conditions to produce the maximum sugar intermediates as required by the downstream processing of a sugarcane biorefinery.

The objective of this chapter was purely to optimise for pretreatment products with maximum sugar intermediate yields and not to perform further conversions, such as fermentation of sugars into ethanol. Fermentability was therefore not considered as a metric for optimisation and inhibitors in the pretreatment products were not reported.

The pretreatment conditions for producing maximum CSY from bagasse and harvest residues respectively were used in the following study in Chapter 5 as reference pretreatment conditions of lignocellulosic feedstocks. In Chapter 6 the optimum pretreatment conditions found in Chapter 4 were used to pretreat bagasse, harvest residues and a blend of these feedstocks in the raw state, as well as in the deacetylated state to compare fermentability and dewaterability of respective pretreated slurries, as well as gross energy yields.

Declaration by the candidate:

With regards to Chapter 4, page numbers 100 to 145 of this dissertation, the nature and scope of my contributions were as follows:

Nature of contribution	Extent of contribution (%)
Experimental planning	95
Executing experiments	100
Interpretation of results	90
Writing the chapter	100

The following co-authors have contributed to Chapter 4 pages 100 to 145 in the dissertation in the following manner:

Name	E-mail address	Nature of contribution	Extent of contribution (%)
Eugéne van Rensburg	eugenevrb@sun.ac.za	• Experimental planning • Interpretation of results • Reviewing chapter	5 5 30
Johann Ferdinand Görgens	jgorgens@sun.ac.za	• Interpretation of results • Reviewing chapter	5 70

Candidate signature:……

Date:……

Declaration with signature in possession of candidate and supervisor.

Declaration by co-authors:

The undersigned hereby confirm that

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 4 pages 100 – 145 in the dissertation,
2. no other authors contributed to Chapter 4 pages 100 – 145 in the dissertation besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 4 pages 100 – 145 of this dissertation.

Signature	Institutional affiliation	Date
	Stellenbosch University	
	Stellenbosch University	

Declaration with signatures in possession of candidate and supervisor.

Uncatalysed steam pretreatment in a sugarcane biorefinery: Optimising for preferred sugar products from bagasse and harvest residues feedstocks

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ABSTRACT

The sugarcane wastes of bagasse and harvest residues present lignocellulosic feedstocks to produce sugar intermediates for a sugar platform in an integrated sugarcane biorefinery. This work compared the uncatalysed steam pretreatment of bagasse and harvest residues in the same pretreatment setup. Pretreatment was optimised to produce preferred pretreatment operating regimes to produce maximum digestibility of the solids, maximum hemicellulose recovery in the prehydrolysate and maximum combined sugar yield (CSY) after enzymatic hydrolysis for the respective feedstocks. Pretreatment conditions were evaluated between 185 and 215 °C for times of 5 to 15 min. Optimum pretreatment conditions were relatively far apart for bagasse at 215 °C, 15 min for maximum digestibility, 202.2 °C, 5 min for maximum hemicellulose recovery and 215 °C, 5 min for maximum CSY. In contrast, the optimum conditions were closer for harvest residues to allow for potential stable pretreatment operation at a single condition. Harvest residues pretreatment within temperatures of 198 and 200 °C, and times of 8 and 12 min was predicted to achieve digestibility, hemicellulose recovery and CSY values to more than 95% of the predicted maxima.

4.1 Introduction

Biorefining is making inroads as a processing philosophy for converting organic wastes, such as lignocellulosic wastes, into a wide range of raw materials for the bio-based economy (Nizami et al., 2017). Lignocellulosic wastes contain cellulose and hemicellulose that can be recovered through bioprocessing as sugar intermediates, including glucose, xylose and arabinose, for the downstream production of value-adding alcohols, organic acids and other chemicals (Kobayashi and Fukuoka, 2013). However, depending on these products, bioprocessing options have different sugar intermediate preferences. Furthermore, cellulose is thermally and chemically more resistant than hemicellulose to degradation reactions, so that maximum cellulose and hemicellulose recoveries are often mutually exclusive in bioprocessing (Hendriks and Zeeman, 2009). Biorefinery operation therefore requires a tailored approach to bioprocessing to maximise recovery of preferred sugar intermediates.

Biorefining of the lignocellulosic wastes generated in the sugarcane industry, i.e. bagasse and harvest residues (Bizzo et al., 2014), for additional revenue is of considerable interest as current global sugar prices remain subdued (FAO, 2019). Conventional cane sugar mills can then be upgraded to integrated biorefineries for processing the whole sugarcane plant to co-produce additional products to cane sugar (Chandel et al., 2012; Corrêa do Lago et al., 2012; Seabra et al., 2010). Biorefinery flexibility in accepting both bagasse and harvest residues will decrease unit costs of products from better utilisation of logistical supply lines and installed processing capacities, as well as increased economies of scale (Macrelli et al., 2012; Valdivia et al., 2016; Zhu and Yao, 2011). Besides for multiple feedstocks, profitable operation of large-scale sugarcane biorefining will also depend on the capacity to maintain several

product lines (Rødsrud et al., 2012). Recent techno-economic analyses have confirmed the integration synergies when incorporating multi-product sugarcane biorefining from combined bagasse and harvest residues feedstocks in a sugar mill (Farzad et al., 2017; Mandegari et al., 2017; Nieder-Heitmann et al., 2018).

Feedstock pretreatment is the first step in bioconversion of lignocellulose to sugar intermediates (Hendriks and Zeeman, 2009). Consequently, process conditions during lignocellulose pretreatment in a multi-product biorefining with multiple feedstocks must adapt according to feedstocks and product requirements. Lignocellulosic feedstock management therefore becomes integral in ensuring that the most suitable feedstock or blend of feedstocks is sent to pretreatment for bioprocessing while still ensuring that enough lignocellulosic feedstock is bypassed as boiler fuel for the self-sufficient energy generation of the sugarcane biorefinery (Ali Mandegari et al., 2017).

Steam pretreatment was the preferred technology choice for this study for its ability to handle various feedstock types (Ewanick and Bura, 2011), as well as for the availability of high pressure steam on-site at a sugar mill. Furthermore, steam pretreatment allows for operation without added catalysts, as was performed in this study, with consequent savings in the materials of construction for less corrosion allowance (Biezma and San Cristóbal, 2005), limited downtime as no cleaning of scaling is required (Shekiro III et al., 2014), as well as limited downstream impacts on fermentation and wastewater treatment (Franden et al., 2009).

Steam pretreatment of bagasse has been extensively studied, including investigations to find maximum combined sugar yield (CSY) for acid catalysed (Carrasco

et al., 2010; Viridiana Ferreira-Leitão et al., 2010; Sendelius, 2005) and uncatalysed pretreatment (Kaar et al., 1998) by varying both the pretreatment temperatures and durations. The steam pretreatment behaviour of harvest residues is not well documented and no study was found that investigated the combined effects of temperature and duration for harvest residues.

This study followed a multi-product approach to steam pretreatment of bagasse and harvest residues as alternative feedstocks with the aim to identify preferred pretreatment regimes that would maximise the release of the desired sugars at maximum cellulose digestibility, maximum hemicellulose recovery or maximum CSY. This approach is in contrast to pretreatment optimisation studies of lignocellulose in literature that aim for a single sugar target, usually maximum CSY, at a single pretreatment condition.

4.2 Materials and methods

4.2.1 Raw material

Approximately 1 000 kg sugarcane bagasse and 1 000 kg harvest residues were collected from two prominent sugarcane growing regions in the 2014 harvest season in South Africa: Malalane, Mpumalanga and Durban, KwaZulu-Natal. The sugarcane harvest residues consisted of leaves and tops in an approximate 1:1 mass ratio. The received material was air-dried inside a greenhouse for 12 days and the moisture content of the plant material decreased to between 6 and 9%. A laboratory tooth mill and a hammer mill with a sieve size of approximately 20 mm were used for the comminution of the bagasse and harvest residues, respectively, to produce particle lengths of approximately 20 to 200 mm. Both the bagasse and the harvest residues were sieved again with a 600 µm x 600 µm sieve to remove sand and pith. The respective bagasse and harvest residues from the two regions were blended to produce representative samples of the bagasse and harvest residues available in South Africa.

4.2.2 Steam pretreatment

4.2.2.1 Design of experiment

The bagasse and harvest residues were separately steam pretreated in range finding experiments with each feedstock evaluated at nine pretreatment conditions that included temperatures of 185, 200 and 215 °C in combination with retention times of 5, 10 and 15 min. The minimum and maximum values of these ranges were ring-fenced around the most conditions used in other studies that steam pretreated bagasse uncatalysed (Espírito Santo et al., 2019; V. Ferreira-Leitão et al., 2010; D.H. Fockink et al., 2018; Li et al., 2014; Martín et al., 2002, 2006; Neves et al., 2016; Rocha et al.,

2012; Sendelius, 2005; Wanderley et al., 2013). A minimum time of 5 min was chosen to minimise the impact of reactor temperature ramp-up time (less than 90 sec) on the pretreatment temperature and a maximum time of 15 min was chosen as a realistic limit for productivity in industry. The equipment could only support a constant maximum temperature of 215 °C and initial test runs confirmed that temperatures below 185 °C was inadequate in this time range. It was decided to use these same ranges for harvest residues as very little steam pretreatment studies on harvest residues are available in literature.

The results from the range finding experiments were statistically analysed in 3^2 full factorial designs using Statistica 13.0 (Dell Inc., Tulsa, OK, USA) software to obtain regression models to predict the target responses of digestibility of the water insoluble solids (WIS), hemicellulose recovery in the prehydrolysate and CSY within the studied range of pretreatment conditions for bagasse and harvest residues. The predicted pretreatment conditions that produce maximum target responses were then validated with the relevant feedstocks as well as a blend of bagasse and harvest residues in a 1:1 mass ratio. All measurements were performed in triplicate and results are reported as averages with variances indicated as standard errors.

4.2.2.2 Equipment and steam quality

The feedstocks were pretreated in a batch steam pretreatment unit (IAP GmbH, Graz, Austria) with a 200 L electrical boiler that provided saturated steam to a 19 L pretreatment reactor. The boiler unit contained a steam accumulator to ensure that adequate steam was readily available as demanded during the steam pretreatment process. Steam was automatically bled from the accumulator at frequent intervals to avoid build-up of incondensable gases. Before every run the reactor with its loaded

material was first purged with steam to displace all air from the reactor. The whole steam pretreatment unit was insulated with all equipment downstream of the boiler heated with electrical heat tracing to limit condensation. Any condensate in the supply line to the reactor was removed in a steam trap before the reactor. Pure saturated steam of a very high steam quality could therefore be supplied to the reactor to ensure consistent heating and reduced condensation in the reactor as would be expected for industry during continuous operation.

4.2.2.3 Operating philosophy

The reactor was preheated with steam prior to pretreatment to limit condensation inside the reactor during pretreatment. Two thermocouples measured the temperatures on the inside of the reactor and on the outside of the reactor shell, respectively. The reactor was preheated until the outside of the shell attained a temperature that was less than 30 °C colder than the target temperature of the intended pretreatment on the inside of the reactor. This relatively narrow temperature differential was maintained throughout the pretreatment runs and ensured rapid heating ramp-ups, consistent temperature control and limited condensation inside the reactor.

Batches of 500 g of dried material were steam pretreated at a time. The material were impregnated with water to reach a moisture content of approximately 65% prior to pretreatment by soaking the material in tap water overnight followed by centrifugation. Even though wetter material will result in milder pretreatment conditions (Brownell et al., 1986), moist feedstocks are industrially relevant as it better resembles material at the sugar mill leaving the extraction process (Oliverio et al.,

2014). Increasing the moisture content also ensured a constant moisture content of all the material before pretreatment throughout the experiment.

The pretreatment severities could be estimated with the severity factor, log R_0, as described by Overend et al. (1987) for relative comparison of uncatalysed pretreatment severities. The severity factor considers the combined effect of temperature and retention time, and for constant temperature pretreatment can be simplified to:

$$\log R_0 = \log\left(t \times \exp\left(\frac{T-100}{14.75}\right)\right) \quad (1)$$

where t is the retention time (min) and T is the pretreatment temperature (°C). Batch pretreatment, however, contains a temperature ramp-up period before the pretreatment temperature is reached and therefore requires the actual severity factor to be integrated over the total time, including the ramp-up period. Temperature logging functionality of the steam pretreatment unit enabled integration of the complete temperature profiles of the steam pretreatment runs. It was confirmed that the actual severity of the pretreatment conditions that were studied deviated less than 5% from the calculated severity factor when using the constant pretreatment temperature. The small deviation was achieved by setting the retention time from the moment a trigger temperature of T – 4 °C was reached while setting the target temperature to a value of T + 1 °C. Once the retention time was reached, a discharge valve at the bottom of the pretreatment reactor instantaneously opened, while the supply of steam from the boiler was automatically stopped. The pretreated material was transported in a steam explosion from the pretreatment reactor to an expansion vessel as the high pressure steam expanded to atmospheric pressure. All the

pretreated material in the expansion vessel was collected and weighed. The pretreated material was obtained as a relative dry slurry as a result of limited condensation during steam pretreatment, and depending on the pretreatment severity, the moisture content ranged from 72% - 88%. The samples were separated into pressed solid and liquor (prehydrolysate) fractions by filter pressing with a hydraulic jack. The prehydrolysates were subsequently filtered through Whatman No.1 filter paper and the pH measured to expand on the severity factor to calculate the combined severity factor (CSF) (Chum et al., 1990):

$$\mathrm{CSF} = \log \mathrm{R}_0 - \mathrm{pH} \qquad (2)$$

The CSF has been developed to compare severities of catalysed pretreatments, but has also been used successfully in other studies (Viridiana Ferreira-Leitão et al., 2010; Douglas Henrique Fockink et al., 2018) to explain differences in autohydrolysis achieved at different uncatalysed steam pretreatment severities.

Pretreated material was sampled in triplicate and stored at 4 °C to be processed within days.

4.2.3 Enzymatic hydrolysis

The digestibility of the pretreated solids was determined by subjecting the WIS component to enzymatic hydrolysis. WIS was prepared by removing the residual soluble solids from the pressed solids samples obtained after steam pretreatment, as explained in 4.2.2.3, by washing with excess distilled water at 50 °C at a mass ratio of 1:10. Wet WIS were recovered at a moisture content of approximately 80% - 85% after vacuum filtration through Whatman No.1 filter paper and used as substrate for enzymatic hydrolysis.

Enzymatic hydrolysis was carried out in 250 ml Erlenmeyer flasks with a working volume of 100 ml at a WIS solids loading of 2% dry weight. Cellic CTec2 (Novozymes) cellulases were dosed at a loading of 15 FPU per gram of dried WIS. A pH of 5 was maintained with a 0.05 M citrate buffer. Sodium azide at 0.02% (w/v) was added as disinfectant. Blank flasks without WIS were also prepared in triplicate to measure the sugar concentrations introduced by the cellulases formulation and to confirm that the WIS in the other flasks contained negligible amounts of prehydrolysate. A 1 ml sample was taken from each flask at the onset and end of the enzymatic hydrolysis experiment to measure the initial and final sugar concentrations, respectively, and denatured at 100 °C. Enzymatic hydrolysis of the substrates was allowed to continue for 72 h in a rotating incubator at 50 °C with an agitation of 150 rpm.

4.2.4 Chemical composition analyses

All chemical compositions of the bagasse and harvest residues raw material and pretreated products were determined as set out in the standard laboratory analytical procedures prepared by NREL (Sluiter et al., 2011, 2006, 2005b, 2005a). Conversion factors for back calculating sugar and acetyl group fractions in the lignocellulose were employed as recommended by NREL (Sluiter et al., 2011).

The concentrations of monomeric sugars and acetic acid in all other liquid streams emanating from steam pretreatment, compositional analyses and enzymatic hydrolysis were analysed with a TSP HPLC System using an Aminex HPX-87H column equipped with a Cation-H Micro-Guard Cartridge (Bio-Rad, Johannesburg, South Africa). The column temperature was set at 65 °C and analytes eluted isocratically at a flow rate of 0.6 mL/min with 5 mM sulfuric acid as the mobile phase. An RI detector

(Shodex, RI-101, Munich, Germany) operated at 45 °C measured the concentrations of the monomeric sugars and acetic acid.

4.2.5 Definitions of responses

Digestibility was defined as the net mass of glucose produced per 100 g of dry WIS subjected to the enzymatic hydrolysis protocol described, and therefore did not represent an overall glucose yield, but indicated the relative accessibility of the cellulose in the pretreated solids. Hemicellulose recovery was expressed as the total mass of monomeric and oligomeric xylose and arabinose in the prehydrolysate of the pretreated material per 100 g of dry feedstock fed to steam pretreatment. The CSY was calculated as the total mass of all monomeric glucose, xylose and arabinose released during enzymatic hydrolysis, as well as the total mass of all monomeric and oligomeric glucose, xylose and arabinose recovered in the prehydrolysate during steam pretreatment per 100 g of dry feedstock fed to steam pretreatment.

4.3 Results and discussion

4.3.1 Chemical composition of feedstocks

The chemical composition of the raw sugarcane bagasse and harvest residues were analysed for the main components, as reported in Table 4.1. The composition of the bagasse was comparable to the compositions found by a study of a range of South African sugarcane bagasse (Benjamin et al., 2014). The definition of what constitutes sugarcane harvest residues with the respective ratios of dry leaves, green leaves and tops are not used consistently in the literature, resulting in considerable variance between reported compositions (Leal et al., 2013; Pereira et al., 2015). However, the higher ash content of 7.03 g/100 g dry harvest residues found in this study, compared to 2.19 g/100 g dry bagasse, agreed with comparisons made in other studies (Antonio Bizzo et al., 2014). The analysis also revealed an acetyl groups content of 4.13 g/100 g dry bagasse that was significantly higher than the 2.78 g/100 g for dry harvest residues ($p < 0.05$), whereas the harvest residues contained significantly higher total extractives of 14.79 g/100 g dry feedstock, compared to 6.77 g/100 g dry bagasse ($p < 0.05$).

Table 4.8 Chemical compositions of the raw sugarcane feedstocks.

Compound	Bagasse (g/100 g dry)	Harvest residues (g/100 g dry)
Glucan	39.67 ± 1.34	32.44 ± 0.84
Xylan	20.31 ± 0.33	18.18 ± 0.25
Arabinan	1.61 ± 0.00	3.03 ± 0.04
Water extractives	5.57 ± 0.21	12.94 ± 0.29
Ethanol extractives	1.21 ± 0.03	1.85 ± 0.05
Total extractives	6.77 ± 0.23	14.79 ± 0.27
Acid insoluble lignin	18.85 ± 0.38	15.44 ± 0.15
Acid soluble lignin	1.79 ± 0.03	1.99 ± 0.03
Total lignin	20.64 ± 0.36	17.43 ± 0.17
Acetyl groups	4.13 ± 0.09	2.78 ± 0.03
Ash	2.19 ± 0.09	7.03 ± 0.03

Compositions are on a dry basis. Variations are expressed as standard errors of triplicate samples.

4.3.2 Range finding pretreatment experiments

Uncatalysed steam pretreatment in the range finding experiments (185 – 215 °C and 5 – 15 min) revealed that bagasse experienced pretreatment at a higher CSF than harvest residues for the same pretreatment temperature and time (Table 4.2). The resulting pH of the prehydrolysate from bagasse pretreatment was 0.17 to 0.45 lower than for harvest residues at the same pretreatment condition, as shown in Table 4.2. This difference in pH was the result of the higher acetyl group content of bagasse (Table 4.1), leading to acetic acid formation that increased autohydrolysis for bagasse pretreatment (Öhgren et al., 2007). It is also possible that the harvest residues displayed a higher pH buffer capacity than bagasse, as was found by Ferreira-Leitão et al. (2010), possibly as a result of the higher ash content of harvest residues (Table 4.1), which could reduce the effect of autohydrolysis (Szczerbowski et al., 2014).

Table 4.9 Pretreatment severities of the range finding experiments.

Pretreatment condition	Severity factor (log R_0)	Bagasse prehydrolysate		Harvest residues prehydrolysate	
		Average pH	Combined severity factor (CSF)	Average pH	Combined severity factor (CSF)
185 °C, 5 min	3.20	4.06	-0.86	4.41	-1.21
185 °C, 10 min	3.50	3.83	-0.32	4.28	-0.78
185 °C, 15 min	3.68	3.68	0.00	3.97	-0.29
200 °C, 5 min	3.64	3.68	-0.03	3.88	-0.24
200 °C, 10 min	3.94	3.38	0.56	3.67	0.27
200 °C, 15 min	4.12	3.24	0.88	3.38	0.74
215 °C, 5 min	4.08	3.17	0.92	3.34	0.74
215 °C, 10 min	4.39	3.04	1.35	3.23	1.15
215 °C, 15 min	4.56	3.04	1.52	3.21	1.36

Similar to the uncatalysed steam pretreatment comparison by Fockink et al. (2018), it was decided to report the response values from uncatalysed range finding experiments rather against CSF, as shown in Figures 4.1.a – c. Harvest residues were found to be more amenable to steam pretreatment, compared to bagasse, as it managed to produce more digestible WIS at all pretreatment severities lower than CSF of 0.27 (Figure 4.1.a), as has indeed been found by Ferreira-Leitão et al. (2010) for uncatalysed and catalysed steam pretreatment of bagasse and harvest residues. Pereira et al. (2015) have also found in their comparison of dilute acid pretreated material (1.5% w/w H_2SO_4, 1:10 solids loading, 121 °C, 30 min), that tops and straw (components of harvest residues) were more digestible than bagasse. In the range finding experiment a maximum of 53 g glucose/100 g dry WIS was produced from harvest residues pretreated at 200 °C and 10 min (CSF = 0.27), whereas the pretreated bagasse pretreated at the same temperature and time (CSF = 0.56) produced an average of 34 g glucose/100 g dry WIS (Figure 4.1.a).

The digestibility of pretreated bagasse WIS, however, continued to increase with an increase in severity above CSF of 0.27, whereas the digestibility of WIS from harvest residues concomitantly decreased with such increases in CSF (Figure 4.1.a). The decrease in digestibility of harvest residues WIS at these higher severities could possibly be ascribed to the much higher total extractives content in the feedstock (Table 4.1), compared to bagasse, which can condense with lignin as pseudo-lignin during severe pretreatment onto the cellulose to hinder enzymatic hydrolysis (Ballesteros et al., 2011). On the other hand, no apparent decrease in digestibility of bagasse WIS was observed with increase in pretreatment severity, as digestibility at the four most severe pretreatment conditions studied (CSF > 0.56) did not differ significantly ($p > 0.05$). This can be attributed to the

transition, with increasing pretreatment severity, of high overall yield of glucan in the WIS to lower overall yield of glucan in the WIS (data not shown), because of glucan degradation, but with improved accessibility to enzymes.

Both feedstocks displayed considerable variation in repeatability of the measured glucose at the end of enzymatic hydrolysis that seemed independent of pretreatment severity (Figure 4.1.a). The high variance in certain cases could possibly be attributed to the fact that the feedstocks were respective blends of cultivars from different regions that were not thoroughly blended in the large-scale blending of this study. Nevertheless, the results from the chemical compositions in Table 4.1 displayed a narrow variance. This apparent discrepancy in variances was also observed by Brienzo et al. (2015) who found the digestibility of dilute acid pretreated bagasse from different sugarcane cultivars to exhibit significant differences, even though the chemical compositions of the different raw bagasse were similar. Total hemicellulose recoveries from the pretreatment prehydrolysates displayed smaller variations in repeatability (Figure 4.1.b). Consequently, the large variation of the CSY values (Figure 4.1.c) was a result of the variation found in the digestibility results.

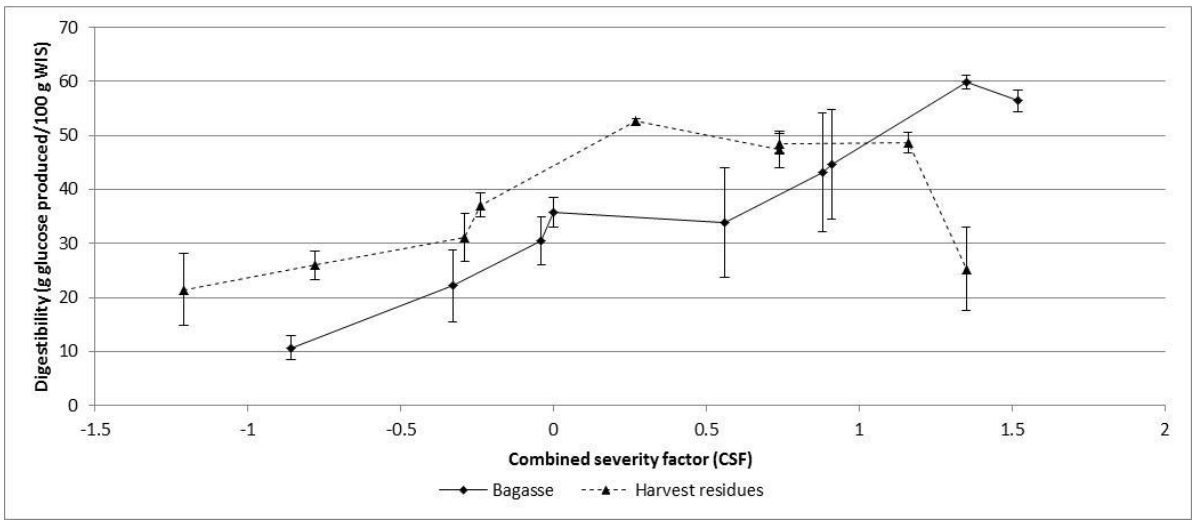


Figure 4.15.a Digestibility of steam pretreated bagasse and harvest residues WIS as a function of CSF of steam pretreatment. Error bars represent standard errors of triplicate pretreated slurry samples.

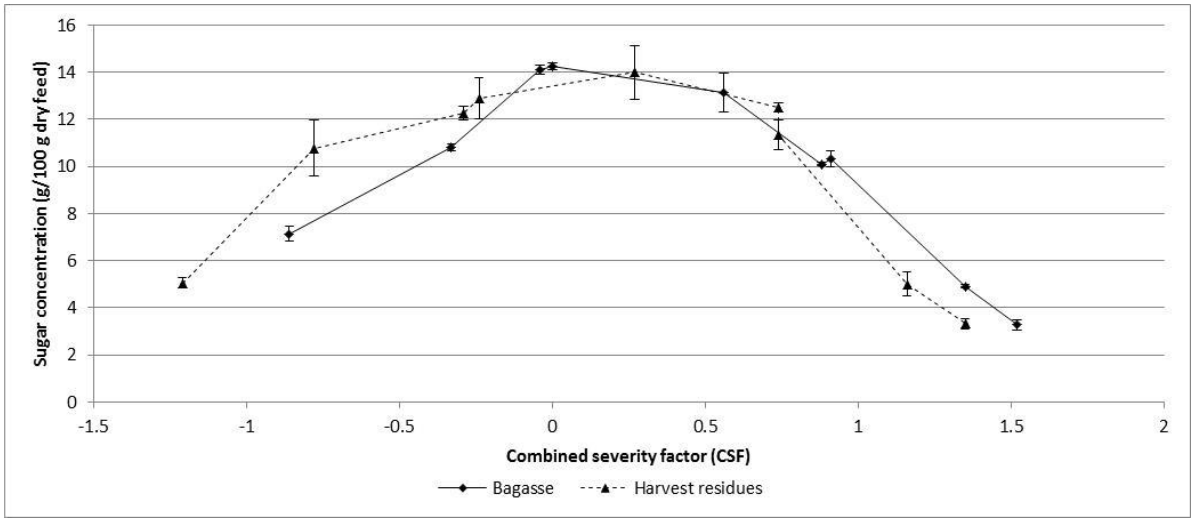


Figure 4.16.b Total xylose and arabinose sugar recovered from bagasse and harvest residues in the pretreatment prehydrolysate as a function of CSF of steam pretreatment. All sugar yields are reported in the monomeric form. Error bars represent standard errors of triplicate pretreated slurry samples.

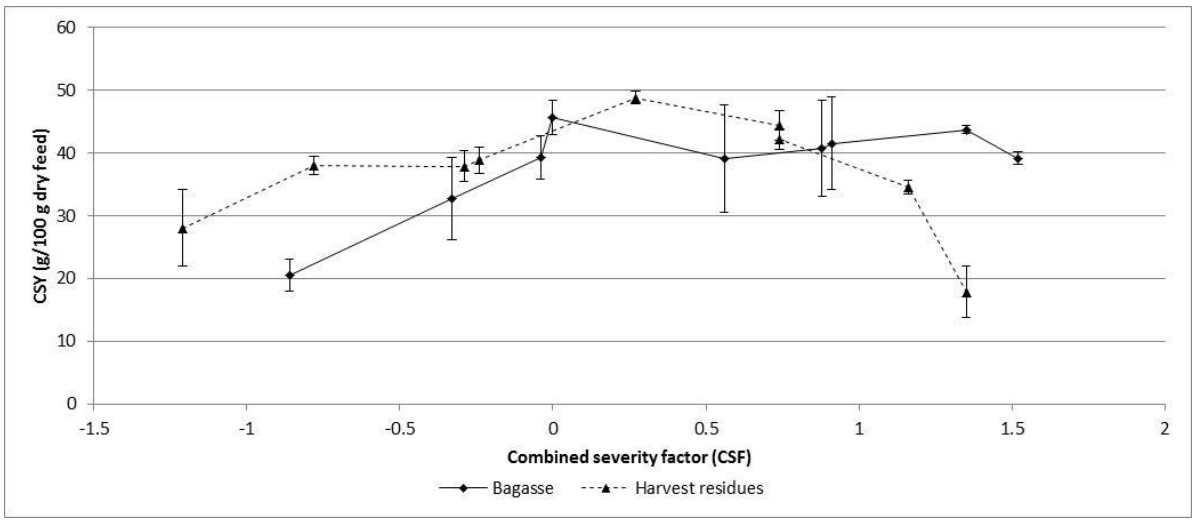


Figure 4.17.c CSY obtained from bagasse and harvest residues as a function of CSF of steam pretreatment. All sugar yields are reported in the monomeric form. Error bars represent standard errors of triplicate pretreated slurry samples.

4.3.3 Modelled steam pretreatment regimes

Uncatalysed steam pretreatment target responses from the range finding experiments, as displayed in Figures 4.1.a – c, were incorporated in response surface methodologies to obtain the maximum predicted values in the temperature and time ranges studied for digestibility of WIS, recovery of hemicellulose in the prehydrolysate and CSY for bagasse and harvest residues, respectively. The generated regression equations (available in the

Addendum in Section 4.7.2) were used to model preferred steam pretreatment regimes by ring-fencing all the pretreatment conditions that were predicted to produce responses to within 95% of the maximum values of the respective target responses. The modelled steam pretreatment regimes for bagasse and harvest residues are depicted in Figures 4.2.a and 4.2.b, respectively.

Distinct regimes of pretreatment conditions that did not overlap were found for producing target responses at more than 95% of the predicted maxima for bagasse, since the predicted pretreatment conditions for the maximum target responses were far removed at 215 °C and 15 min, 202.2 °C and 5 min, and 215 °C and 5 min for digestibility of the WIS, recovery of hemicellulose in the prehydrolysate and CSY, respectively (Figure 4.2.a). These predicted conditions of the maximum target responses all bordered the ranges in which experimental data was collected, indicating that higher response values could be obtained outside the studied pretreatment conditions. In the ranges studied, 215 °C, 5 min is a pretreatment operating condition that provides a compromise between hemicellulose recovery and digestibility to provide the maximum predicted CSY of approximately 45 g/100 g dry feed. However, by using the developed regression equations (Section 4.7.2), it can be predicted that changing pretreatment conditions in the direction of temperatures higher than 215 °C and times less than 5 min will improve digestibility, hemicellulose recovery and CSY the most.

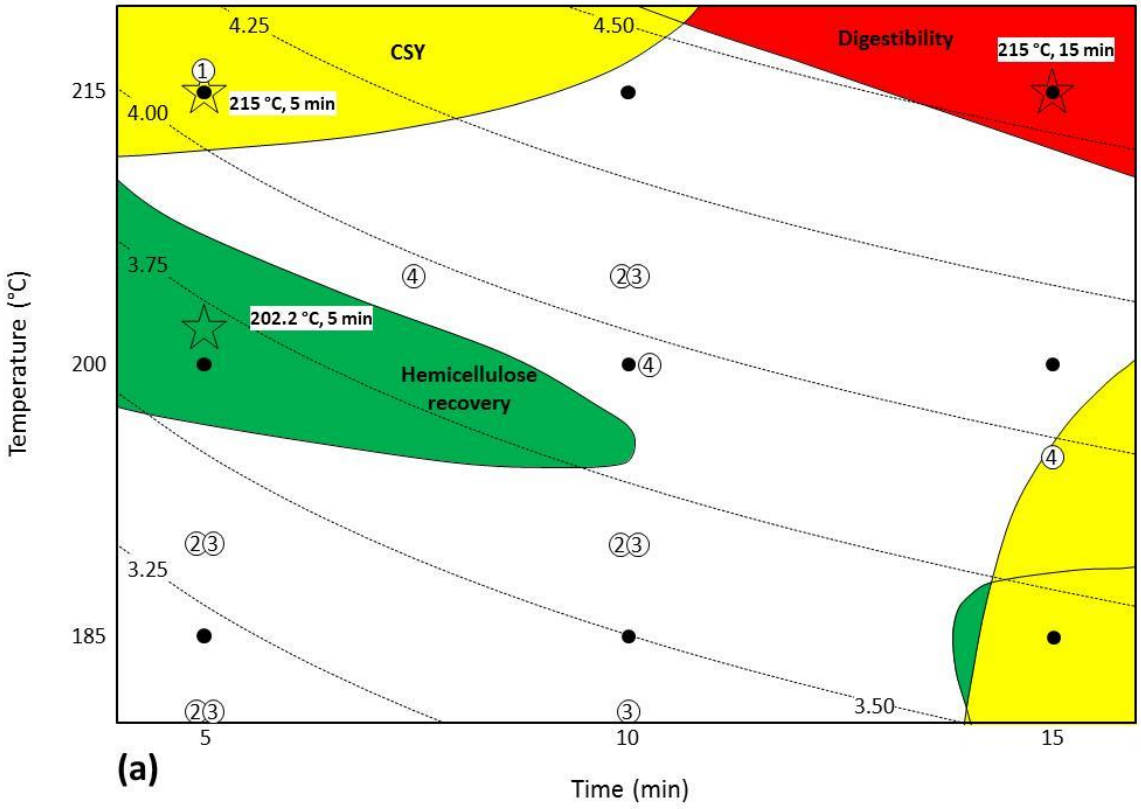
In contrast to bagasse, the predicted maxima of the target responses for harvesting residues were within the pretreatment ranges of the study (Figure 4.2.b). Also, for producing at 95% of the predicted maxima, pretreatment regimes overlapped (Figure 4.2.b). In other words, a common, single pretreatment regime existed for harvest residues, where

pretreatment could simultaneously almost satisfy maximum digestibility of the WIS, maximum hemicellulose recovery from the prehydrolysate and maximum CSY. This common area of overlap was contained between temperatures of 198 and 200 °C, and times of 8 and 12 min. It is therefore difficult to selectively optimise for a particular sugar product (glucose, xylose or CSY) from uncatalysed steam pretreatment of harvest residues. However, selection of pretreatment conditions anywhere within the operating window will provide 95% or more of the maximum yields for the any of the sugar products of interest, giving a very robust industrial process.

The closer optimum conditions of harvest residues could possibly be explained by the better accessibility to cellulose in pretreated harvest residues, compared to bagasse. Only a small increase in pretreatment severity is required to move from maximum hemicellulose recovery in the prehydrolysate to maximum digestibility. Similarly, it has been demonstrated that the optimum conditions can be moved closer by acid impregnating material before steam pretreatment and improving its accessibility to cellulose (Linde et al., 2008; Sassner et al., 2005).

The modelled pretreatment regimes in Figures 4.2.a and 4.2.b were corroborated by experimental results reported previously that made comparisons of uncatalysed steam pretreatment of bagasse and harvest residues at different pretreatment conditions. Four previous reports made comparisons for bagasse (numbers 1 to 4 in Figure 4.2.a) and two reports made comparisons for harvest residues (numbers 5 and 6 in Figure 4.2.b). Kaar et al. (1998) studied 95 different pretreatment conditions for bagasse and found 216 °C, 5 min the optimum for glucose yield, whereas maximum xylose yield was found at a constant log R_0 of approximately 3.8, in agreement with the pretreatment regimes for maximum CSY and

hemicellulose recovery in Figure 4.2.a. The pretreatment conditions investigated by Sendelius (2005) and Carrasco et al. (2010) were outside any of the identified pretreatment regimes for bagasse in Figure 4.2.a, but nevertheless corresponded, as CSY increased closer to the identified pretreatment range for maximum CSY and highest digestibility was found at 205 °C, 10 min. The CSY from bagasse at the pretreatment conditions (195 °C, 15 min; 200 °C, 10.5 min; 205 °C, 7.5 min) studied by Fockink et al. (2018) with constant log R_0 of 3.97 did not differ significantly from each other with overall yields of more than 80%, and did not reflect the higher predicted CSY at 195 °C, 15 min in Figure 4.2.a. Ferreira-Leitão et al. (2010) studied three pretreatment conditions for sugarcane harvest residues, all at 5 min, and found the highest CSY and overall xylose yields at 210 °C in agreement with the predicted pretreatment regimes for CSY and hemicellulose recovery shown in Figure 4.2.b. Oliveira et al. (2013) also studied pretreatment conditions for harvest residues at a constant time of 15 min, and found an increase in hemicellulose solubilisation from 67.1% at 180 °C to 85% at 190 °C and 92.7% at 200 °C, with the latter two conditions in or close to the predicted pretreatment regime for maximum hemicellulose recovery in Figure 4.2.b.



1 - Kaar et al. (1998)

2 - Sendelius (2005)

3 - Carrasco et al. (2010)

4 - Fockink et al. (2018)

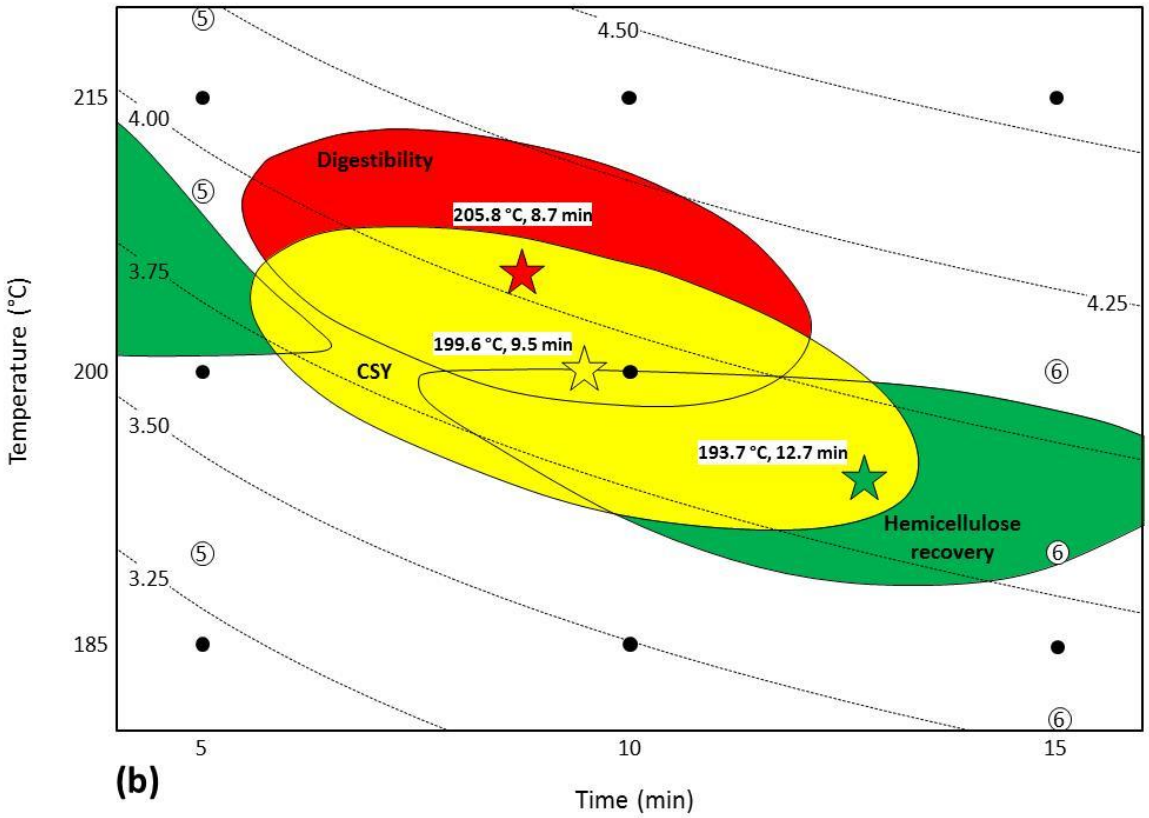


Figure 4.18 Modelled uncatalysed steam pretreatment regimes for (a) bagasse and (b) harvest residues that contain predicted response values within 95% of the respective predicted maximum values for digestibility, hemicellulose recovery and CSY. Predicted maxima in the studied ranges are indicated with stars in corresponding colour of their pretreatment operating regimes: red (digestibility), green (hemicellulose recovery) and yellow (CSY). Contours of constant severity factor (log R_0) are included for comparison of relative severity between bagasse and harvest residues pretreatment.

5 - Ferreira-Leitão et al. (2010)

6 - Oliveira et al. (2013)

4.3.4 Validation of pretreatment conditions

The modelled steam pretreatment regimes of bagasse were validated at the three optimised pretreatment conditions for digestibility, hemicellulose recovery and CSY (Figure 4.2.a), whereas a candidate steam pretreatment condition of 200 °C and 10 min was chosen for validation of the common area of overlapping pretreatment regimes modelled for harvest residues (Figure 4.2.b). It was conceived that these four pretreatment conditions could define the entire range of pretreatment conditions of operation in a sugarcane biorefinery. Subsequently, blends of bagasse and harvest residues in a mass ratio of 1:1 were also steam pretreated at these pretreatment conditions to assess the applicability of the proposed pretreatment range. The target responses of the validation runs are summarised in Table 4.3 and the overall mass balances of the resulting sugar product spreads from the validation runs at the four pretreatment conditions, followed by enzymatic hydrolysis of the WIS, are shown in Figures 4.3 – 4.5 with all values reported in average equivalents of the respective lignocellulosic polymer. The remaining relevant oligomeric and polymeric sugars in the residues after enzymatic hydrolysis were not measured, but calculated by subtracting the sum of all the other related sugar fractions from the original sugar fraction in the raw feedstock. The amount of relevant sugar lost through degradation during steam pretreatment was determined from the difference in sugar compositions in the raw feedstock and the pretreated material, as well as measuring the loss in total solids. The total monomeric and oligomeric sugar concentrations in the prehydrolysates are shown in Figures 4.6.a and b in the Addendum in 4.7.1.

The validation runs found the actual target response values to deviate up to 15.5% and up to 8.6% from the predicted target response values at the four identified

pretreatment conditions for bagasse and harvest residues, respectively (Table 4.3). These deviations were to be expected, given the variation in the digestibility data of the range finding experiments that were used to obtain the regression equations of the models. Nevertheless, the validation runs proved that substantially different sugar product spreads can be obtained by changing the steam pretreatment conditions of bagasse and that the single pretreatment condition for harvest residues achieved all target responses close to the predicted maxima (Figures 4.3.a, 4.4.a and 4.5.a).

The pretreatment condition predicted to produce maximum digestibility for bagasse (215 °C, 15 min) only yielded 69.8% of overall glucose after enzymatic hydrolysis, even though the WIS was practically completely digestible, since almost no glucan remained in the residues (Figure 4.3.a). This relatively low overall glucose yield is the result of extensive glucan lost (30.3% of available glucan in bagasse) in degradation reactions at this severity. However, when pretreating bagasse at the pretreatment condition predicted for maximum CSY (215 °C, 5 min), an overall glucose yield of 93.5% was obtained after enzymatic hydrolysis with only 3.3% of the glucan in the bagasse lost in degradations reactions (Figure 4.3.a). The arbitrary chosen pretreatment condition (200 °C, 10 min) for harvest residues confirmed a sugar product spread that achieved both high digestibility and high overall glucose yield as the glucan that reported in the enzymatic hydrolysis residues and lost to degradation was negligible, while yielding 99.8% of the overall glucose after enzymatic hydrolysis (Figure 4.3.a). This was achieved with a CSF of 0.27 that is lower than the CSF values of the bagasse pretreatments in Figure 4.3.a, indicating, as was seen earlier, that harvest residues are more amenable to steam pretreatment than bagasse at lower pretreatment severities, while also better preserving glucan from degradation reactions.

The pretreatment condition for maximum hemicellulose recovery in the prehydrolysate for bagasse managed to recover 61.9% of the total xylan and arabinan (monomeric and oligomeric) in the prehydrolysate (Figures 4.4.a and 4.5.a) with an overall hemicellulose yield, when including monomeric sugars released during enzymatic hydrolysis, of 78.4%. Harvest residues pretreated at the candidate condition recovered 53.9% of the total hemicellulose in the prehydrolysate with an overall hemicellulose yield of 69.1%, which are lower than for the optimum bagasse condition, but could be acceptable when also considering the high overall glucose yield and high digestibility of this pretreatment condition.

The contributing acetyl group and ash compositions from bagasse and harvest residues in the blended feedstock changed the resulting CSF of the pretreatment conditions, compared to the pretreatment of the respective feedstocks on their own as shown in Figures 4.3.b, 4.4.b and 4.5.b. The sugar product spreads were furthermore influenced by the different proportion of sugars in the raw blended feedstock which was calculated as the average values of bagasse and harvest residues compositions (Table 4.1), and included 36.06 g glucan, 19.25 g xylan and 2.32 g arabinan per 100 g dry feed.

Interestingly, the most severe pretreatments of the blended feedstock at 215 °C produced WIS and overall glucose yields that were not significantly different ($p > 0.05$) from the pretreatments of bagasse at these pretreatment conditions (Table 4.3 and Figure 4.3), even though the blended feedstock contained less glucan and harvest residues were shown to be less digestible at high pretreatment severities, respectively. It is possible that the lower CSF of the blended feedstock runs, compared to bagasse alone, helped to preserve more of the glucan in the bagasse or that the higher CSF of the blended feedstock runs,

compared to harvest residues alone, improved the removal of lignin from the cellulose. However, the WIS digestibility decreased significantly ($p < 0.05$) from 54.9 to 42.8 g glucose/100 g dry WIS (Table 4.3), supported by an increase from 0.17 to 9.96 g glucose/100 g dry feed (Figure 4.3.b) of unhydrolysed glucan in the residues after enzymatic hydrolysis, when harvest residues were replaced with the blended feedstock at the pretreatment condition of 200 °C and 10 min. This could be ascribed to the low digestibility of bagasse at this relatively low CSF of 0.53. Consequently, the CSY of the blended feedstock at this pretreatment condition was also significantly lower ($p < 0.05$), compared to pretreating harvest residues (Table 4.3).

Table 4.10 Target responses of the validation runs.

Pretreatment condition	Digestibility (g glucose/100 g dry WIS)		Hemicellulose recovery (g/100 g dry feed)		CSY (g/100 g dry feed)	
	Bagasse	**Blend**	**Bagasse**	**Blend**	**Bagasse**	**Blend**
215 °C, 15 min	60.3 ± 2.8 (55.3%)	54.5 ± 1.4 (51.2%)	2.5 ± 0.1	2.8 ± 0.0	31.2 ± 1.3	28.2 ± 0.7
215 °C, 5 min	61.7 ± 2.2 (58.3%)	56.5 ± 1.0 (50.9%)	* 7.4 ± 0.1	* 6.7 ± 0.1	* 46.5 ± 1.3	* 39.3 ± 0.6
202.2 °C, 5 min	36.7 ± 0.9 (52.7%)	34.7 ± 2.4 (50.9%)	13.6 ± 0.3	14.0 ± 0.1	41.5 ± 0.6	39.2 ± 1.5
	Harvest residues	**Blend**	**Harvest residues**	**Blend**	**Harvest residues**	**Blend**
200 °C, 10 min	* 54.9 ± 0.3 (51.5%)	* 42.8 ± 0.4 (54.2%)	11.4 ± 0.4	12.5 ± 0.2	* 47.0 ± 0.6	* 40.0 ± 0.4

Hemicellulose and CSY values are expressed as sums of sugars in their polymeric forms. Variations are expressed as standard errors of triplicate samples. Responses between pure and blended feedstocks that differ significantly ($p < 0.05$) at the same pretreatment condition are indicated with *. The average glucan content of the WIS is indicated in parentheses in the Digestibility columns.

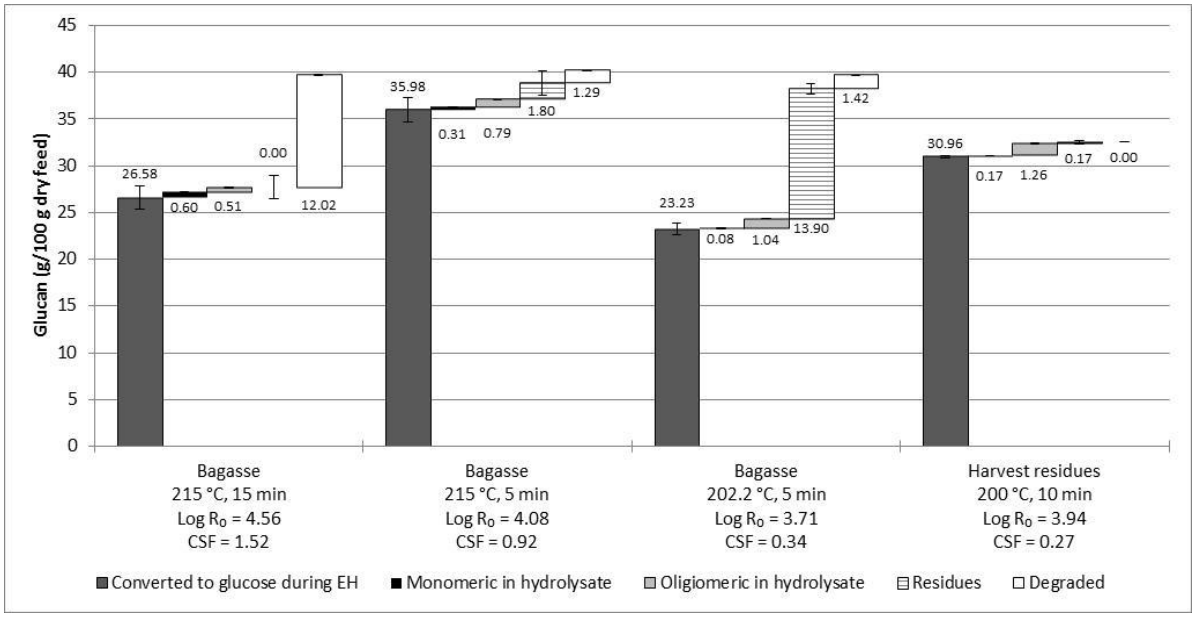


Figure 4.19.a Mass balances of glucan products from respective bagasse and harvest residues feedstocks after steam pretreatment at optimum conditions and enzymatic hydrolysis. All values are reported in average glucan equivalents of the respective raw feedstocks. Error bars represent standard errors of triplicate sampling.

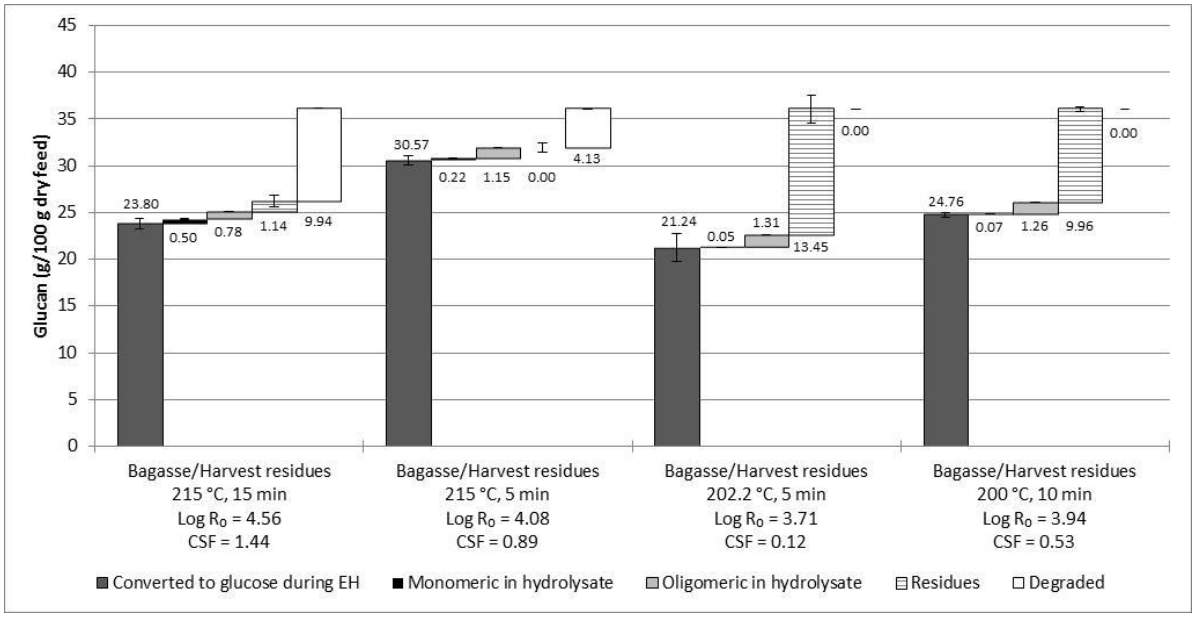


Figure 4.20.b Mass balances of glucan products from bagasse and harvest residues feedstocks blended in a 1:1 mass ratio after steam pretreatment and enzymatic hydrolysis. All values are reported in average glucan equivalents of the blended raw feedstocks. Error bars represent standard errors in triplicate sampling.

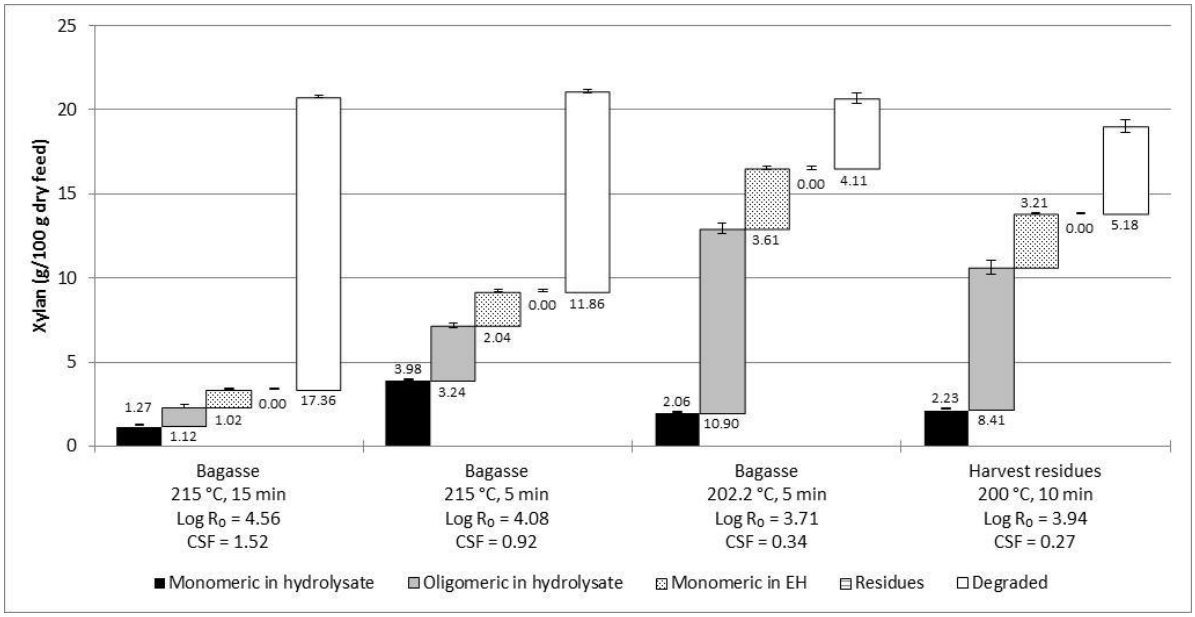


Figure 4.21.a Mass balances of xylan products from respective bagasse and harvest residues feedstocks after steam pretreatment at optimum conditions and enzymatic hydrolysis. All values are reported in average xylan equivalents of the respective raw feedstocks. Error bars represent standard errors of triplicate sampling.

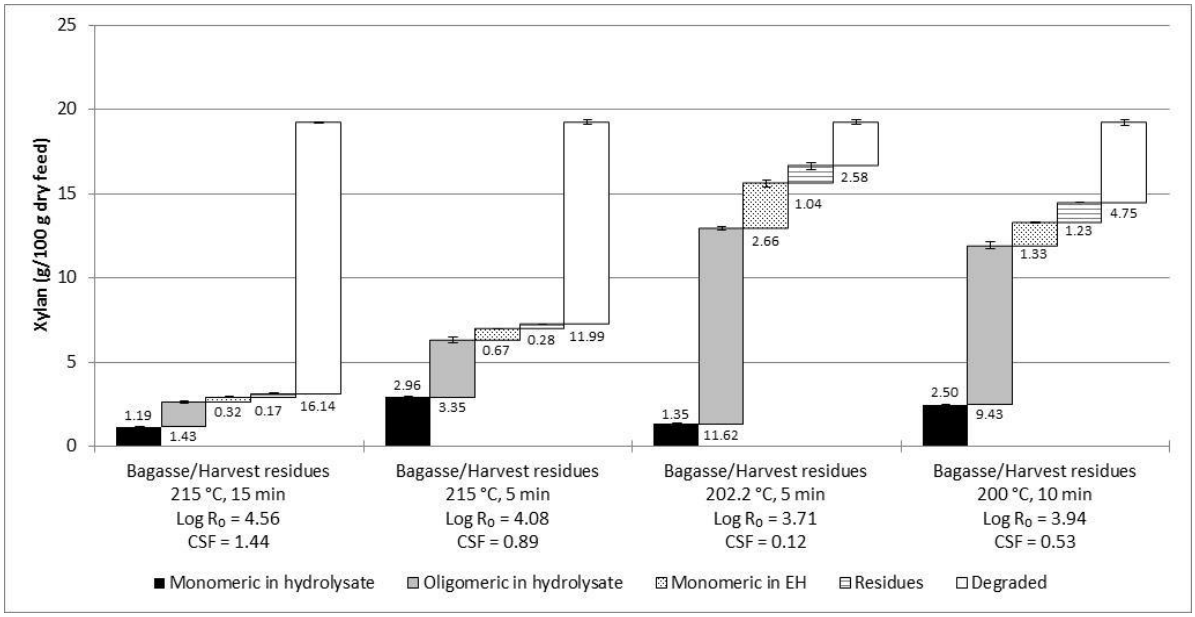


Figure 4.22.b Mass balances of xylan products from bagasse and harvest residues feedstocks blended in a 1:1 mass ratio after steam pretreatment and enzymatic hydrolysis. All values are reported in average xylan equivalents of the blended raw feedstocks. Error bars represent standard errors in triplicate sampling.

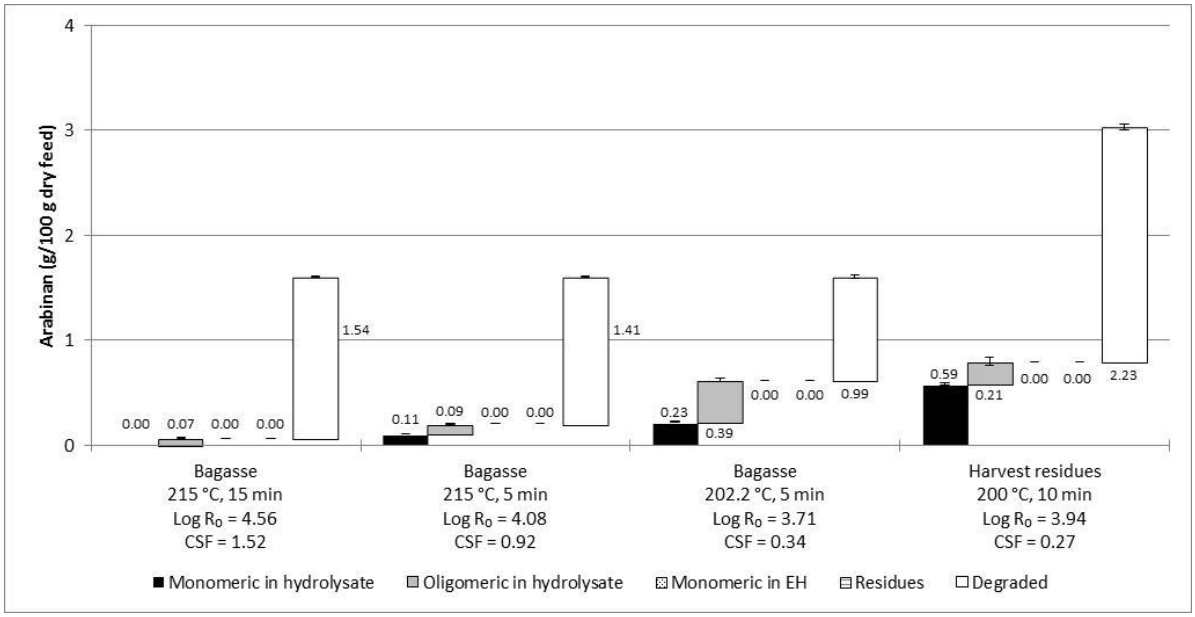


Figure 4.23.a Mass balances of arabinan products from respective bagasse and harvest residues feedstocks after steam pretreatment at optimum conditions and enzymatic hydrolysis. All values are reported in average arabinan equivalents of the respective raw feedstocks. Error bars represent standard errors of triplicate sampling.

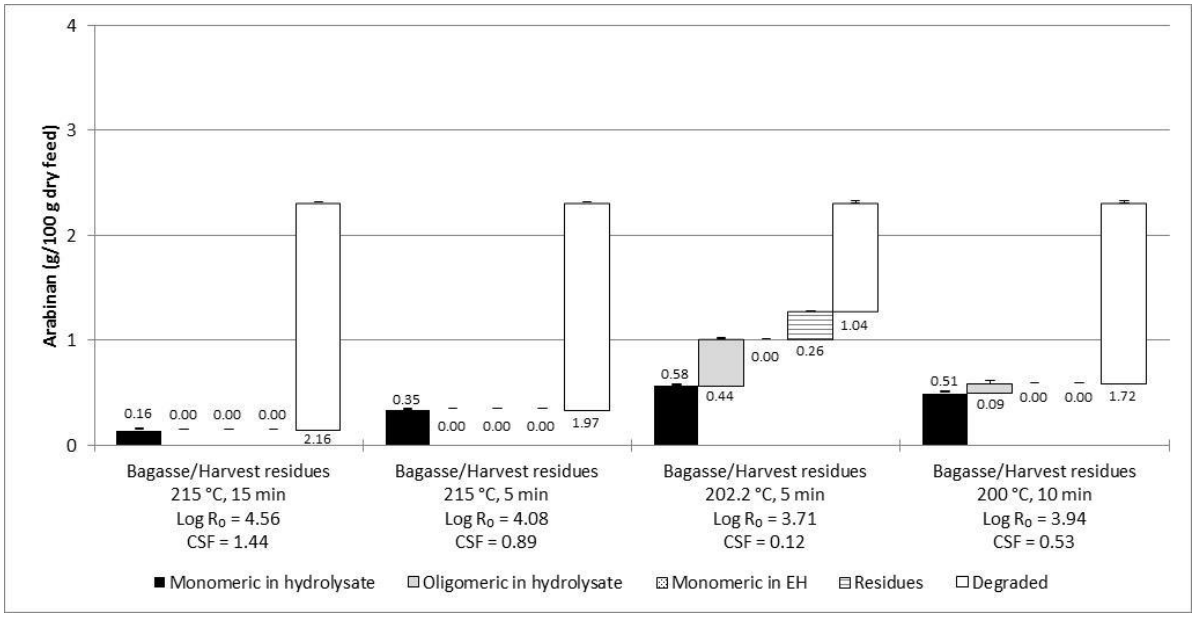


Figure 4.24.b Mass balances of arabinan products from bagasse and harvest residues feedstocks blended in a 1:1 mass ratio after steam pretreatment and enzymatic hydrolysis. All values are reported in average arabinan equivalents of the blended raw feedstocks. Error bars represent standard errors in triplicate sampling.

4.4 Conclusions

Sugarcane bagasse and sugarcane harvest residues behave differently during uncatalysed steam pretreatment and require different regimes of pretreatment conditions for the optimal production of a certain sugar product. The pretreatment regimes of harvest residues that could attain more than 95% of the predicted target response values of maximum digestibility, maximum CSY and maximum hemicellulose recovery, were shown to overlap between temperatures of 198 and 200 °C, and times of 8 and 12 min. The predicted pretreatment conditions for the maximum target responses were also relatively close to each other. Harvest residues can therefore provide for the stable production of different sugar products at the same pretreatment conditions in a sugarcane biorefinery. It is recommended to investigate pretreatment temperatures higher than 215 °C and times less than 5 min as potential single point pretreatment conditions for the uncatalysed steam pretreatment of bagasse that will favour digestibility, hemicellulose recovery and CSY. Further work is also required in finding optimum pretreatment conditions for blended feedstocks within the identified pretreatment operating envelope, as the blended feedstocks failed to improve the target responses obtained with pure feedstocks.

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4.7 Addendum

4.7.1 Sugar in prehydrolysates

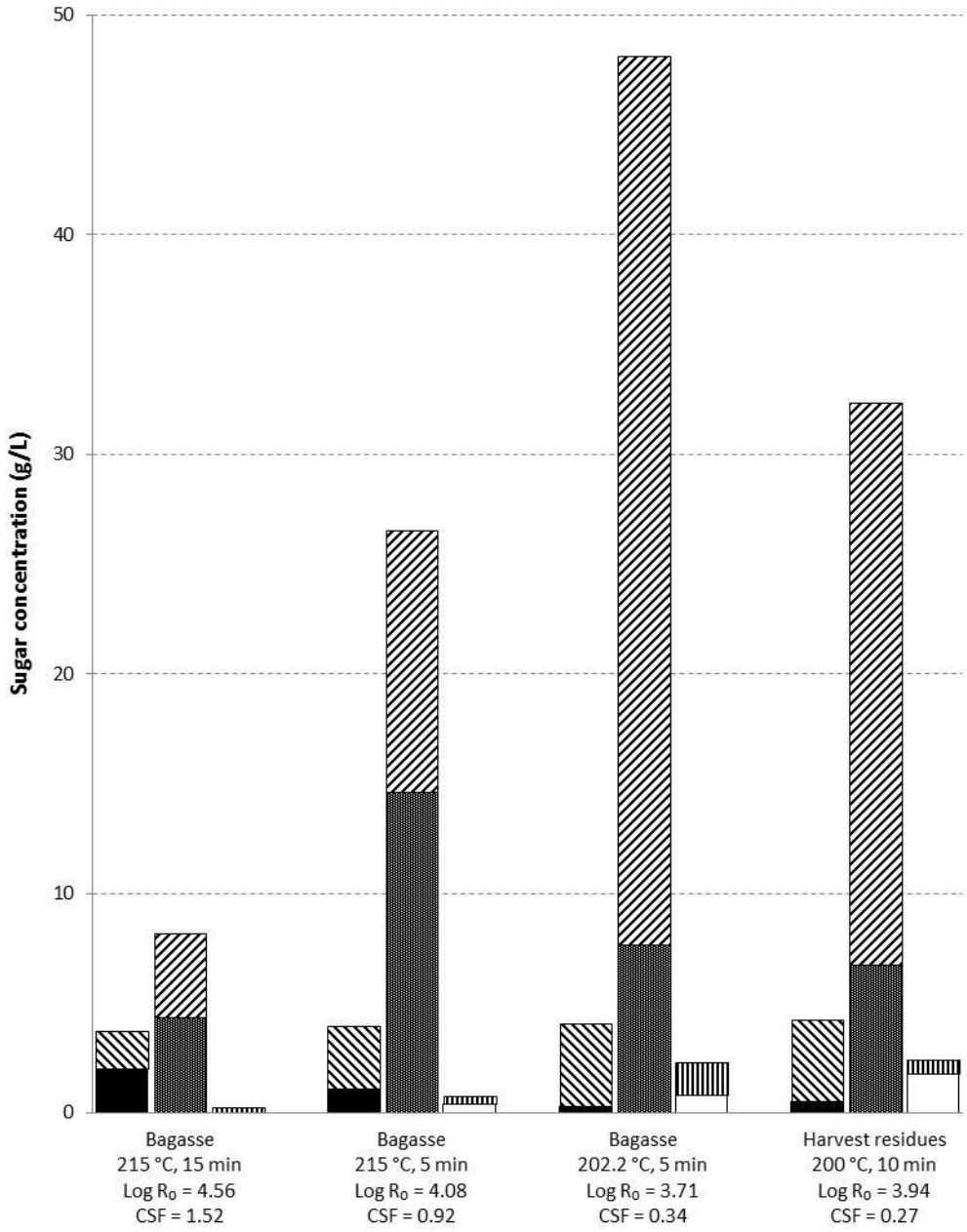

Figure 4.6.a Total sugar concentrations in the prehydrolysates after pretreating bagasse and harvest residues separately at the respective optimal conditions. The first column of every condition is glucose, second column of every condition is xylose and third column is arabinose. The top striped part of every column represents the oligomeric form of the sugar and the bottom solid part of every column is the monomeric form of the sugar.

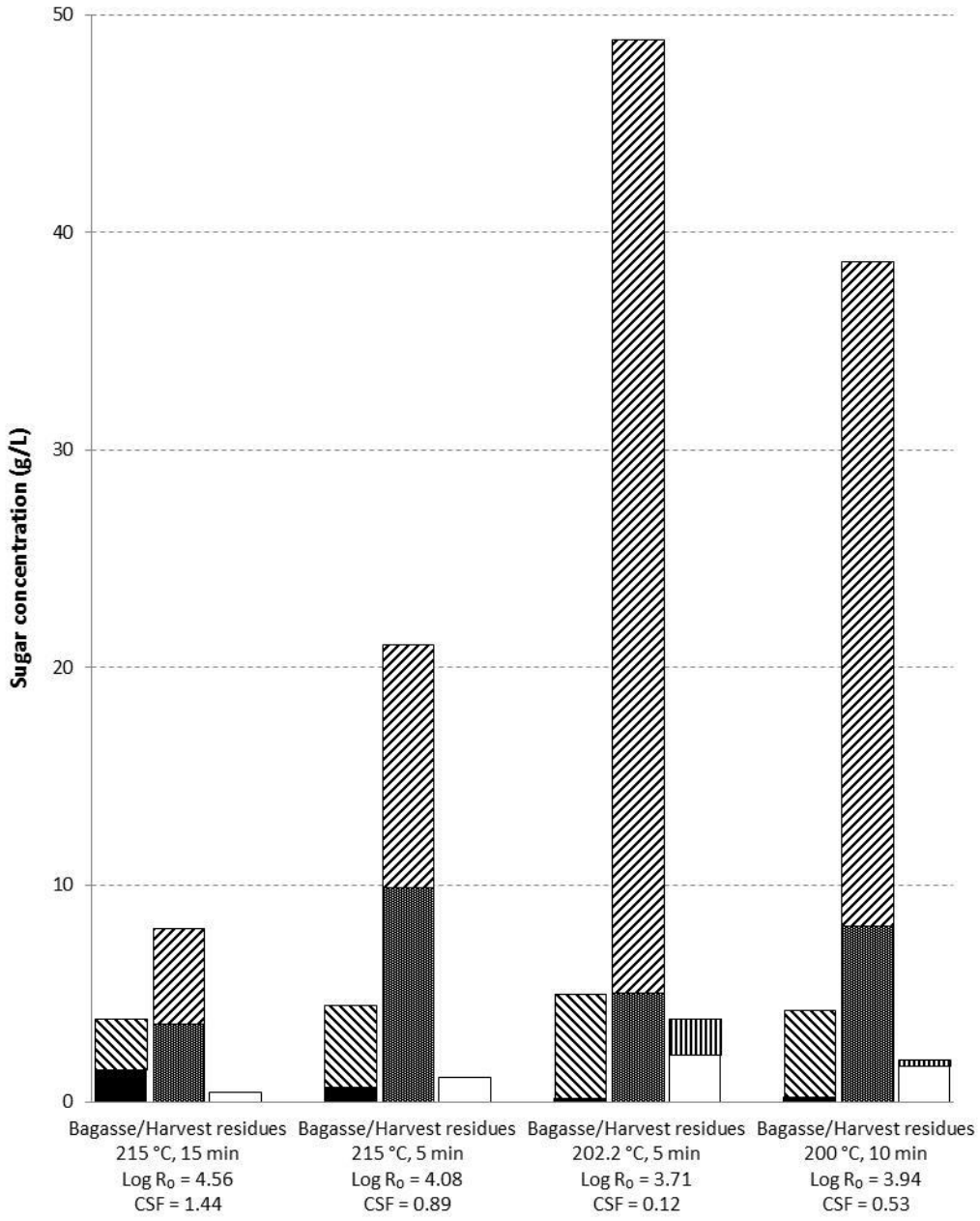

Figure 4.6.b Total sugar concentrations in the prehydrolysates after pretreating bagasse and harvest residues blends at the respective optimal conditions of the respective feedstocks. The first column of every condition is glucose, second column of every condition is xylose and third column is arabinose. The top striped part of every column represents the oligomeric form of the sugar and the bottom solid part of every column is the monomeric form of the sugar.

4.7.2 Regression equations

Regression equations for bagasse:

Digestibility: = -184.56 + 1.6493 x T + 1.0275 x t

CSY: = -210.39 + 19.151 x T + 1.2015 x t - 0.091715 x T x t

Hemicellulose:= -375.51 - 183.41 x T + 13.21 x T^2 + 3.3446 x t - 0.0070103 x t^2 + 1.9385 x T x t - 0.0050964 x T x t^2 - 0.13516 x T^2 x t + 0.0003445 x T^2 x t^2

Regression equations for harvest residues:

Digestibility: = -2467.4 + 27.7 x T -0.29097 x T^2 + 23.312 x t - 0.054309 x t^2 - 0.10996 x T x t

CSY: = -2125.1 + 26.999 x T - 0.22479 x T^2 + 20.498 x t - 0.048655 x t^2 - 0.114 x T x t

Hemicellulose:= -1086.3 + 34.097 x T - 1.1986 x T^2 + 10.079 x t - 0.022927 x t^2 - 0.16923 x T x t + 0.0059209 x T^2 x t

CHAPTER 5

UNCATALYSED STEAM PRETREATMENT OF LIGNOCELLULOSIC WASTE FEEDSTOCKS: REVISITING EFFECTS OF ACETYL CONTENT AND EXPLOSION STEP ON DIGESTIBILITY

This chapter appears as a draft manuscript.

Title: "Uncatalysed steam pretreatment of lignocellulosic waste feedstocks: Revisiting effects of acetyl groups and steam explosion on enzymatic hydrolysis performance"

Authors: Martin Louis Hamann, Richard Chandra, Mandy Lin, Jack Saddler, Johann Ferdinand Görgens

Objective of this chapter in the dissertation

This chapter investigated the impacts of alkaline deacetylation of the feedstock, which affected acetyl groups to ash ratio, as well as mechanical impingement of pretreated material during explosion, on the performance of uncatalysed steam pretreatment of lignocellulose to produce digestible material. Both feedstock acetyl groups to ash ratio and steam explosion intensity (pressure differential) are important in determining digestibility for uncatalysed steam pretreatment, but acetyl groups can have inhibitory effects in downstream fermentation and high pressure steam explosion requires increased operating

costs, as well as potentially degrading of sugars, especially xylose and arabinose, at the associated high temperatures.

In addition to bagasse and harvest residues, poplar, a hardwood feedstock, was also studied in this chapter to enable a broader comparison of lignocellulosic feedstocks with different compositional ratios of acetyl groups to ash. The range of lignocellulose ratios of acetyl groups to ash were further expanded by also studying these feedstocks in a deacetylated state in uncatalysed steam pretreatment. Deacetylation briefly entailed the removal of most of the acetyl groups from the hemicellulose in the raw feedstocks with a dilute alkaline extraction process. Deacetylated feedstocks were then washed to neutral pH prior to pretreatment. The resulting digestibilities of the pretreated solids were determined from enzymatic hydrolysis to understand the impact of deacetylation on autohydrolysis in uncatalysed steam pretreatment.

Concomitantly, the impact of the steam explosion step during steam pretreatment on the digestibility of the pretreated solids were investigated by either retaining or releasing pretreated material during the sudden depressurisation of the steam explosion step. The pressure differential was kept constant in both material handling methods for the same feedstock. By understanding the mechanism of mechanical deconstruction of pretreated material during depressurisation, it could be possible to increase the digestibility of the pretreated solids at lower pretreatment severities, while also better preserving xylose and arabinose. This suggestion to increase the CSY and save on operating costs is discussed in Chapter 7 as a recommendation for future studies and design for improved cost-effective steam pretreatment.

The potential application of deacetylation of feedstocks during uncatalysed steam pretreatment found in this chapter encouraged its use as a method to detoxify pretreated material in a biorefinery process in Chapter 6.

Declaration by the candidate:

With regards to Chapter 5, page numbers 146 to 178 of this dissertation, the nature and scope of my contributions were as follows:

Nature of contribution	Extent of contribution (%)
Experimental planning	95
Executing experiments	95
Interpretation of results	90
Writing the chapter	100

The following co-authors have contributed to Chapter 5 pages 146 to 178 in the dissertation in the following manner:

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Candidate signature:……

Date:……

Declaration with signature in possession of candidate and supervisor.

Declaration by co-authors:

The undersigned hereby confirm that

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 5 pages 146 – 178 in the dissertation,
2. no other authors contributed to Chapter 5 pages 146 – 178 in the dissertation besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 5 pages 146 – 178 of this dissertation.

Signature	Institutional affiliation	Date
	University of British Columbia	
	University of British Columbia	
	University of British Columbia	
	Stellenbosch University	

Declaration with signatures in possession of candidate and supervisor.

Uncatalysed steam pretreatment of lignocellulosic waste feedstocks: Revisiting effects of acetyl groups and steam explosion on enzymatic hydrolysis performance

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ABSTRACT

High acetyl groups contents and increased pressure differentials are important for improved digestibility of uncatalysed steam pretreated lignocellulose, but deacetylation prior to pretreatment and better mechanical deconstruction during the explosion step could benefit downstream processing and operating costs, respectively. Raw poplar, sugarcane bagasse and sugarcane harvest residues with acetyl groups to ash ratios of 14.6, 1.9 and 0.4 g/g, respectively, were compared with their deacetylated forms with ratios of 1.7, 0.3 and 0.1 g/g, respectively, in uncatalysed steam pretreatment. Concomitantly, pretreated material was either released with steam explosion or retained in the reactor with explosion. High acetyl groups to ash ratios were found to be beneficial to improving digestibility, but that at low ratios in the feedstock, deacetylation prior to uncatalysed steam pretreatment had no significant effect on digestibility. Also, the explosion step does not directly contribute to reducing particle size of pretreated material, but rather the mechanical impingements of pretreated material transported through an obstruction.

5.1 Introduction

Lignocellulosic wastes from forestry and agricultural operations present sustainable carbon sources for the production of a wide range of bioproducts from sugar intermediates without impacting on current food production and land usage (Thorenz et al., 2018). Lignocellulose is, however, inherently recalcitrant to liberating its constituent sugars, and requires energy intensive pretreatment at the front-end of the hydrolysis-fermentation bioconversion process (Hendriks and Zeeman, 2009). As a result, the pretreatment operation is highly integrated with the downstream processes (Greenwood et al., 2013), and the method of pretreatment should therefore be selected to benefit the bioprocess as a whole.

Steam pretreatment is a candidate pretreatment technology for large-scale industrial bioprocessing of lignocellulose (Brethauer and Studer, 2015). The technology becomes especially attractive for applications without acid catalyst requirements, since it decreases the requirements for materials of construction, operating and maintenance costs, and impacts on downstream fermentation and wastewater treatment processes (Franden et al., 2009; Humbird et al., 2011). Uncatalysed pretreatment is therefore typically limited to hardwood and agricultural feedstocks for their relative high acetyl group contents (Galbe and Zacchi, 2012). Also, these advantages of uncatalysed pretreatment are traded off against the higher pretreatment severities required to produce pretreated material of comparable digestibility (Carrasco et al., 2010). It is hypothesised that at these consequent elevated temperatures and steam pressures, uncatalysed steam pretreatment improves the digestibility of lignocellulose through the combined effects of autohydrolysis and a physical disrupting steam explosion step at the conclusion of treatment (Chornet and Overend, 1988).

Autohydrolysis during uncatalysed steam pretreatment benefits from the acetyl group content in the hemicellulose, but the associated acetic acid formation could be detrimental to downstream fermentation (Palmqvist et al., 1999). Deacetylation prior to pretreatment allows for the bulk removal of acetyl groups from the hemicellulose polymers without significantly affecting glucan and xylan contents (Chen et al., 2012a), and has been proven to be more cost effective than removing acetic acid after pretreatment from the prehydrolysate (Aden et al., 2002). Deacetylation holds several advantages for the bioconversion of lignocellulose, including the potential recovery of acetic acid as a value-adding chemical, improved enzymatic hydrolysis of xylose oligomers and improved fermentability with the removal of acetic acid as a prominent inhibitor (Chen et al., 2012a, 2012b). Feedstocks were typically deacetylated with a mild alkaline extraction process and then washed with water to attain pH 7 – 8 in other studies (Chen et al., 2012a, 2012b; Shekiro et al., 2014). Water consumption for washing the deacetylated material can be excessive and time consuming as one study used wash water at a rate of 48 L/kg dry solids for 1 h (Chen et al., 2012b). In practice, therefore, deacetylated material would not be washed to near neutral and would rather be pretreated alkaline, or would be dosed with acid for acid catalysed pretreatment. Pan et al. (2016) assessed a two-stage sequence of deacetylation followed by dilute acid pretreatment and calculated a wash water consumption of approximately 0.3 L/kg dry mass.

The mechanism of the steam explosion step is explained as high pressure steam diffusing into the lignocellulosic structures where it condenses to fill the structures with water that becomes superheated with the sudden depressurisation at the end of steam pretreatment (Allen et al., 2001; Hu and Ragauskas, 2012). Flashing of the water from inside

the material is believed to occur in an explosion that disrupts the lignocellulosic matrix to increase the accessible surface area. During the explosion the high pressure steam is expanded to atmospheric pressure with its potential for heat and work dissipated. Steam consumption is consequently the largest component of steam explosion operating cost (Yu et al., 2012). However, there is doubt whether the explosion step contributes significantly to improving enzymatic hydrolysis (Duff and Murray, 1996; Mosier et al., 2005). Brownell et al. (1986) demonstrated that the enzymatic hydrolysis of aspen wood chips was not significantly affected when the steam pressure differential was decreased by 80% by slowly bleeding off steam just before the explosion. On the contrary, other recent studies (Pielhop et al., 2016; Seidel et al., 2017) found, by similarly comparing full pressure differential steam explosions with manipulated mild steam explosions, that for relatively high pretreatment severities the explosion step did indeed make a significant improvement to the enzymatic hydrolysis of different lignocellulosic feedstocks. The improvement was attributed to an effective reduction in particle size when pretreated with full explosion (Pielhop et al., 2016; Seidel et al., 2017). Possible explanations for the contradicting enzymatic hydrolysis results included that the discernible benefit of steam explosion disappears when pretreating amenable feedstocks, such as the hardwood aspen (Pielhop et al., 2016), and when low enzyme dosages are used during enzymatic hydrolysis (Seidel et al., 2017).

The impacts of autohydrolysis via the accumulation of acetic acid and the steam explosion step on the lignocellulosic structure during steam pretreatment are still not well understood and the relative contributions of these two mechanisms to the digestibility of the pretreated material unknown (Rodríguez et al., 2017). Furthermore, the influence of deacetylated material on the digestibility of the pretreated material has to date only been

investigated for dilute acid pretreatments (Chen et al., 2012b, 2012a; Shekiro et al., 2014). In addition, the only systematic studies on the effect of steam explosion on enzymatic hydrolysis were done by comparing full explosion with mild release (Brownell et al., 1986; Pielhop et al., 2016; Seidel et al., 2017), with contradictory results. The objective of this article was to elucidate the effects of feedstock acetyl content and full pressure differential steam explosion during uncatalysed steam pretreatment of different lignocellulosic feedstocks on the enzymatic digestibility of pretreated solids.

5.2 Materials and methods

5.2.1 Lignocellulosic feedstocks

Three different lignocellulosic feedstocks were compared in this study, namely poplar wood chips, sugarcane bagasse and sugarcane harvest residues. All of these feedstocks were sourced from already prepared stocks that had been used and described in recent steam pretreatment studies (Chapter 4, Tang et al., 2018). While no further size reduction was done on the bagasse and harvest residues, the poplar chips were fractioned in vibrating sieves and the fraction reporting between 1 mm and 8 mm used for this study. This selection of feedstocks represented a range of feedstocks with different combinations of acetyl group and ash contents. The hardwood poplar had a relatively high acetyl group content, but with an insignificant ash content, whereas the harvest residues were, in contrast, characterised by relatively low acetyl groups and relatively high ash contents. The bagasse contained intermediate amounts of acetyl groups and ash compared to the poplar and harvest residues. The moisture content of these air-dried raw feedstocks ranged from 6 to 8%.

5.2.2 Deacetylation

Deacetylation of the feedstocks prior to steam pretreatment was achieved with a dilute alkaline extraction step, as described by (Chen et al., 2012a), in which the feedstocks were immersed in a 0.1 M solution of NaOH at a solids to liquid ratio of 1:12 for 3 h at 70 °C. The extraction liquid was subsequently analysed for acetic acid and sugars, while the deacetylated material was repeatedly washed with water until neutral pH. The washed material was then left to soak in water overnight to ensure uniform water penetration

between deacetylated material and material that was only soaked in water, prior to pretreatment.

5.2.3 Steam pretreatment

The feedstocks were steam pretreated in a batch operated 2 L Stake Tech II reactor (Stake Technologies (now SunOpta), Norval, ON, Canada) with saturated steam. The blow valve at the bottom of the reactor opened fully from closed in less than 0.5 second at the end of a steam pretreatment run to create an instantaneous depressurisation in the form of a steam explosion. The feedstocks were all prepared for steam pretreatment in batches of 50 g raw material by either soaking in water overnight at room temperature or by deacetylation followed by soaking in water overnight at room temperature. For both cases the soaked material was subsequently vacuum filtered to a moisture content of approximately 70% before fed to steam pretreatment. In addition, poplar was also fed dry (8% moisture) to steam pretreatment.

Two different loading methods were employed for the batch steam pretreatment of the feedstocks. The first was the conventional loading method where the feedstock was piled into the reactor volume (Figure 5.1.a). The subsequent steam pretreatment pressurised the reactor volume with saturated high pressure steam that was, after a certain pretreatment time, exploded and discharged the pretreated material out of the reactor into a downstream collection vessel at atmospheric pressure with the sudden opening of a blow valve. In the second method the feedstock was packed inside a cylindrical steel basket (dimensions: diameter of 50 mm and height of 300 mm) with perforated sides and lowered by a handle through the feed valve into the reactor (Figure 5.1b). The perforation created an effective outside surface coverage of the basket of approximately 50% and therefore still

allowed steam to freely enter between the interior biomass particles during steam pretreatment. The material was nevertheless retained within the steel basket and remained stationary inside the reactor during the sudden depressurization to atmospheric pressure at the end of a pretreatment run. After depressurisation, the basket with the pretreated material was immediately lifted out of the reactor by a handle to remove the material from the hot reactor. The condensate with dissolved material was collected downstream in the collection vessel as the prehydrolysate.

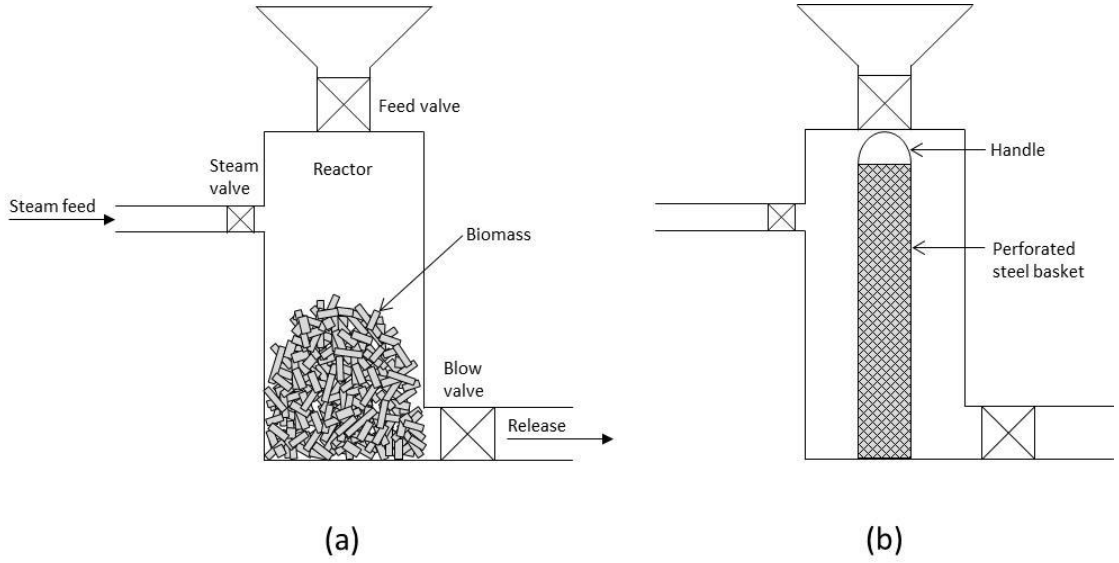


Figure 5.25 Different methods of loading the feedstocks in the reactor for batch steam pretreatment: (a) conventional loading that resulted in the explosive discharge of the pretreated material out of the reactor at the end of pretreatment and (b) packed loading inside a perforated steel basket that remained stationary with the sudden depressurisation at the end of pretreatment and retained the pretreated material.

Based on previous studies of these exact same feedstock lots (Chapter 4, Tang et al., 2018), each feedstock was pretreated at process conditions that produced maximum combined sugar yield (CSY) from uncatalysed steam pretreatment with conventional loading and subsequent explosive discharge. The steam pretreatment conditions for the poplar, sugarcane bagasse and sugarcane harvest residues were 210 °C for 5 min, 215 C for 5 min and 200 C for 10 min, respectively, and represented pressure differentials of approximately

19, 21 and 15 bar, respectively between the reactor and atmosphere. Each pretreatment condition was repeated in triplicate with the pretreated material from each condition thoroughly mixed to produce composite slurry samples in the case of the conventional loaded runs, and composite samples of retained material and condensate, respectively in the case of basket packed runs.

5.2.4 Enzymatic hydrolysis

The solids and prehydrolysate liquor from the conventional loading runs were recovered from the collected slurries through vacuum filtration with Whatman No.1 filter paper. These recovered pretreated solids, as well as the retained material from the packed loading runs, were respectively washed with excess reverse osmosis water at 50 °C at a mass ratio of 1:10. The water insoluble solids (WIS) were again recovered through vacuum filtration with Whatman No.1 filter paper. WIS recovered from the retained pretreated material was also disintegrated in a blender as an additional WIS substrate for enzymatic hydrolysis comparison.

Cellic CTec2 (Novozymes) enzymes at a loading of 10 mg/g glucan were used for the enzymatic hydrolysis of all the WIS at a solids loading of 2% dry weight. A 0.05 M citrate buffer maintained a pH of 5. Enzymatic hydrolysis of the WIS was performed in triplicate and continued for 72 h at 50 °C, after which samples were taken, denatured at 100 °C and submitted for HPLC analyses.

5.2.5 Analytical methods

The chemical composition of the pretreated material was determined according to the TAPPI Standard Method T-222. The concentrations of oligomeric and monomeric sugars were measured with HPLC as described by Bura et al. (2009). The concentration of acetic

acid was measured with HPLC as described by Chen et al. (2012b). All of the above analytical methods were performed in duplicate. The sugar and acetyl group compositions of the feedstocks and WIS were determined from converting HPLC results with the conversion factors as provided by Sluiter et al. (2011).

5.3 Results and discussion

5.3.1 Lignocellulosic feedstock compositions

The lignocellulosic feedstocks of this study were selected for their different acetyl groups to ash ratios (Table 5.1). Raw poplar, with its relatively high acetyl groups to ash ratio of 14.6 g/g, was expected to be the most amenable to uncatalysed steam pretreatment from the feedstocks investigated, whereas the raw harvest residues with its significantly lower acetyl groups to ash ratio of 0.4 g/g expected to perform the poorest. This is because the autohydrolysis effect of hemicellulose by steam treatment at high temperatures is further propagated with the release of acetic acid from the hemicellulose (Holopainen-Mantila et al., 2013). The ash content of lignocellulose has however been showed to buffer acid catalysed reactions (Szczerbowski et al., 2014), and could therefore decrease the effect of acetic acid on autohydrolysis.

The dilute alkaline extraction process achieved different deacetylation efficiencies for the different feedstocks, namely 93%, 90% and 82% for poplar, bagasse and harvest residues, respectively (Table 5.1). These are significantly higher removal efficiencies of acetyl groups than the approximate two-third removal found by Chen et al. (2012a) with the same deacetylation protocol for corn stover. As a result, the different deacetylated feedstocks had similar acetyl group contents of approximately 0.5 g/100 g dry. Since ash removal during deacetylation was found to be more consistent with approximately one-third of the ash component removed from the different feedstocks (Table 5.1), the acetyl groups to ash ratio remained the highest for deacetylated poplar and the lowest for deacetylated harvest residues. Furthermore, in accordance with (Chen et al., 2012a), the

dilute alkaline extraction did not significantly affect the glucan and xylan compositions of the raw feedstocks, and removed less than 2.5% of the respective sugars.

Table 5.11 Acetyl groups and ash contents of raw and deacetylated feedstocks

	Poplar	Bagasse	Harvest residues
Raw feedstocks			
Acetyl groups (g/100 g dry)	7.3 ± 0.2	4.1 ± 0.2[a]	2.8 ± 0.1[a]
Ash (g/100 g dry)	0.5 ± 0.0	2.2 ± 0.2[a]	7.0 ± 0.1[a]
Acetyl groups : ash ratio (g/g)	14.6	1.9	0.4
Deacetylated feedstocks			
Acetyl groups (g/100 g dry)	0.5 ± 0.2	0.4 ± 0.0	0.5 ± 0.1
Ash (g/100 g dry)	0.3 ± 0.0	1.5 ± 0.1	4.8 ± 0.1
Acetyl groups : ash ratio (g/g)	1.7	0.3	0.1

All variances are expressed as standard deviations from duplicate samples.
[a] From Chapter 4 with variances recalculated to express standard deviations.

5.3.2 Effect of mechanical disruption by impingement on particle sizes of pretreated solids

The steam explosion step at the end of each steam pretreatment run subjected the feedstock to the full pressure differential between the reactor and atmosphere, regardless of the feedstock loading method employed. The pressure differentials of the steam explosions employed were inside the range where Pielhop et al. (2016) observed discrepancies in particle sizes between material that was steam exploded with the full and partial pressure differential. Steam pretreatment with conventional loading and discharge of the feedstock produced a slurry in the downstream collection vessel that contained finely disrupted solid particles. However, when feedstocks were packed in the basket and retained in the reactor with steam explosion, the structure of the pretreated material seemed to be visually intact, although it was darker and brittle to the touch. It was deduced that steam penetration was uniform throughout the packed material in the basket during steam pretreatment, as no differentiation in colour or structure between outer and inner layers of

the pretreated material was observed. The only exception was the material near the surface of the steel basket in the section that faced the steam inlet that was much darker. This was probably because of the exposure to the latent heat transfer of the supplied steam. However, there was no evidence of material forced outwards though the holes of the perforated steel basket, as would be expected if material was exploded from the inside outwards.

Scanning electron microscope (SEM) analyses of steam pretreated poplar revealed the unravelled fibres of the pretreated material when discharged with explosion, as opposed to an intact structure when retained with explosion (Figure 5.2). These observations suggested that no material destruction occurred from within the material when subjected to steam explosion during steam pretreatment, as is generally described in literature.

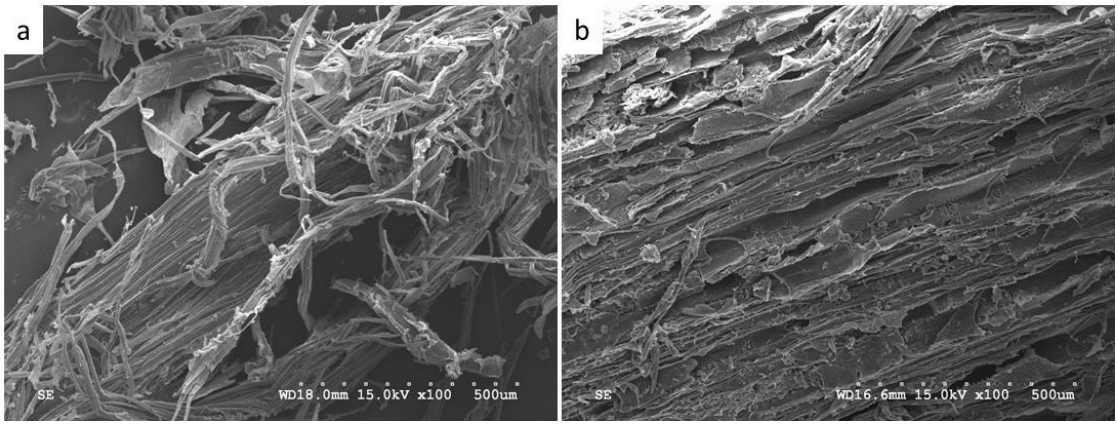


Figure 5.26 SEM images (x100 enlargement) of steam pretreated poplar chips that were (a) loaded in the conventional way and discharged at full explosion; and (b) loaded in the basket and retained during full explosion. In these cases the raw poplar was soaked in water with subsequent steam pretreatment at 210 °C for 5 min followed by full steam explosion at a pressure differential of approximately 19 bar.

Rapid depressurisation without the mechanical forces by which material moves out of the reactor therefore did not directly disrupt the lignocellulosic structure. Disruption was rather caused by the resulting impinging movement of pretreated material as it was transported out of the reactor through downstream piping as has been observed before for different

feedstock loading patterns in the reactor (Sui and Chen, 2015). On the other hand, material did not experience mechanical disruption when it was retained with explosion, even when it was exposed to the full and rapid depressurisation. This finding was also supported by the previous studies that bled 80% of the pressure before steam explosion to effectively create a milder removal of material from the reactor with little effect on pretreated particle size (Brownell et al., 1986; Pielhop et al., 2016). It therefore followed that the application of a higher pressure differential during the steam explosion step would consequently provide more energy for the impinging movement to cause greater disruption and ultimately produce pretreated solids with smaller particle sizes (Yu et al., 2012). The extent of this disruption would be dependent on the pretreatment severity to soften the material.

5.3.3 Steam pretreatment sugar recoveries

Steam pretreatment of each feedstock was based on temperatures and times that have shown to produce maximum CSY when uncatalysed and exploded (Chapter 4; Tang et al., 2018). These pretreatment conditions were employed for each respective feedstock in the various feedstock scenarios, i.e. state of acetylation of the feedstock and loading method of the feedstock.

The recoveries of glucan and xylan from the raw feedstocks after steam pretreatment are shown in Figure 5.3. The glucan recoveries for all feedstocks were relatively unaffected by the different feedstock scenarios during steam pretreatment, as almost all of the glucan in the feedstocks remained in the WIS after steam pretreatment (Figure 5.3.a). This is indicative for steam pretreatment of feedstocks at conditions for maximum CSY, and would require that hemicellulose is solubilised and partially lost through degradation reactions as is shown by the xylan recoveries in Figure 5.3.b (Kabel et al., 2007). Significant differences

($t < 0.05$) were however observed in the recoveries of xylan for each feedstock between raw and deacetylated feedstocks, as well as between discharged and retained pretreated material (Figure 5.3.b). Steam pretreatment of deacetylated feedstocks resulted in lower solubilisation rates of xylan when compared to the same treatment of the corresponding raw feedstocks (Figure 5.3.b). Solubilised oligomeric xylose therefore also experienced less hydrolysis to monomeric xylose, as negligible amounts of monomeric xylose were produced when feedstocks were deacetylated. These were to be expected for uncatalysed pretreatment with lower concentrations of released acetic acid. Interestingly, the total xylan recoveries were lower for every feedstock that was packed in the steel basket and retained during the steam explosion at the end of pretreatment, compared to the same feedstocks pretreated in the conventional manner with explosive discharge. It was therefore hypothesised that the retained material therefore had to experience higher effective pretreatment severities that resulted in increased xylan losses through degradation reactions, even though pretreatment runs were performed at the same temperatures and times for poplar, bagasse and harvest residues, respectively. The higher severities could possibly be ascribed to the direct contact of the material in the basket to the supplied steam in the reactor, as opposed to the piled material that was partly immersed in the formed condensate at the bottom of the reactor (Brownell et al., 1986). The observation mentioned in 5.3.2 that the material that faced the steam inlet was slightly darker, supported the hypothesis that material directly exposed to steam experienced harsher pretreatment.

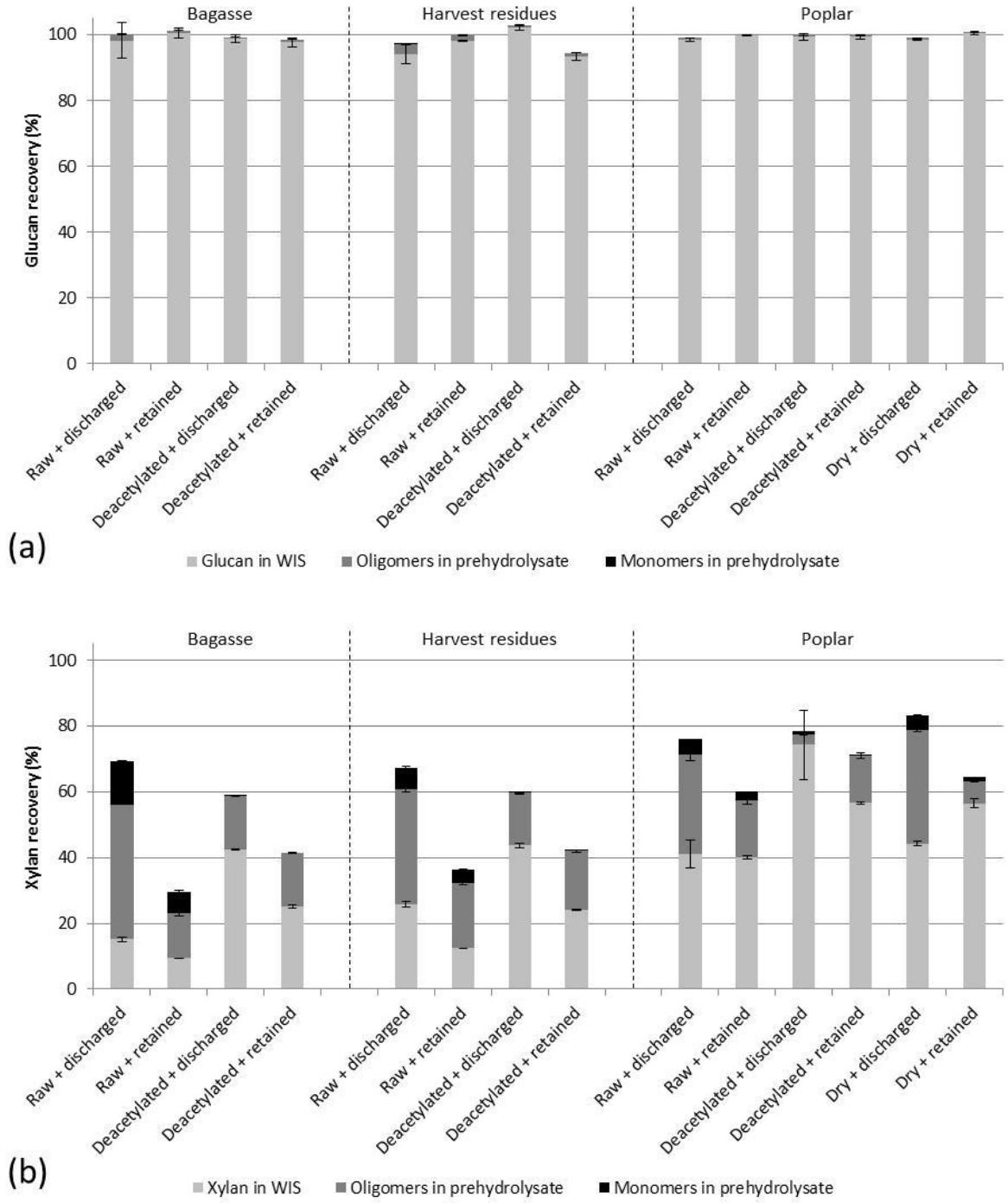


Figure 5.27 Recoveries of (a) glucan and (b) xylan from the raw feedstocks after steam pretreatment in the WIS and prehydrolysate liquor. All variances are expressed as standard deviation from duplicate samples.

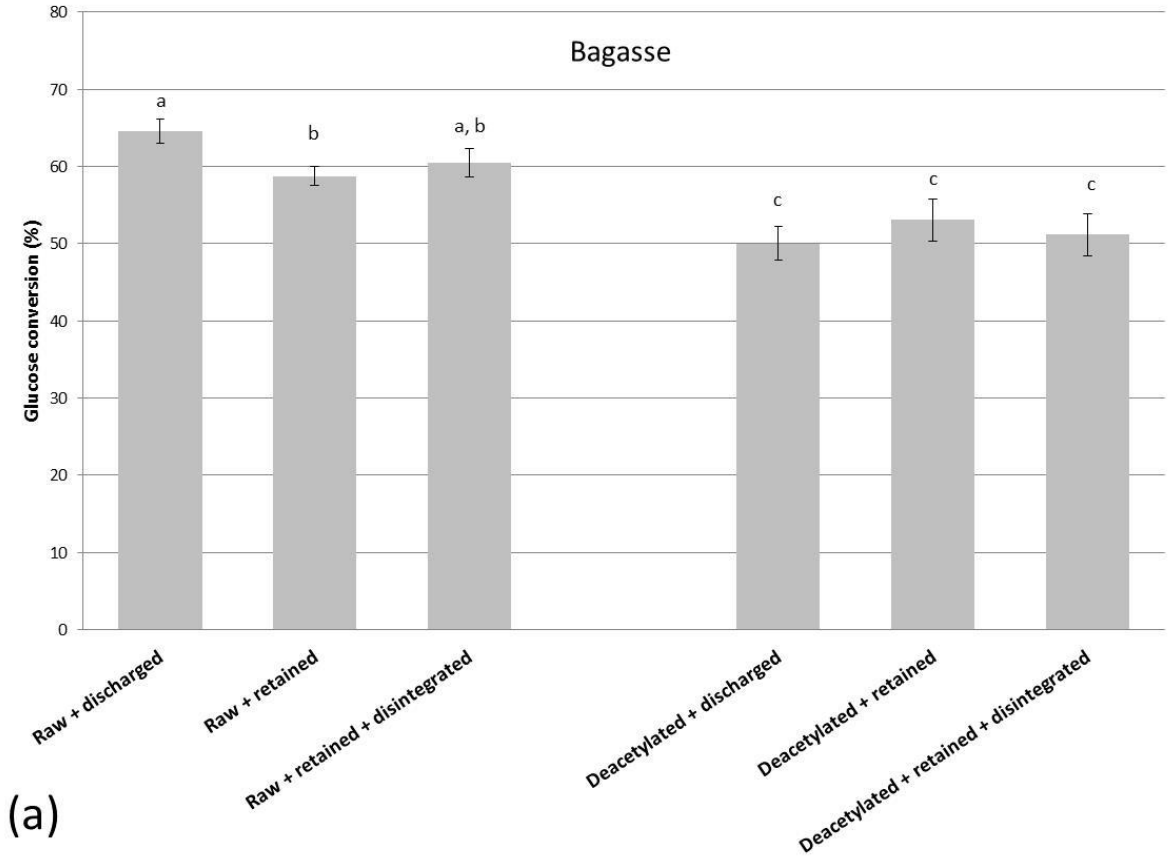
5.3.4 Glucose conversion yields from enzymatic hydrolysis of pretreated solids

It was expected, since acetyl groups are required for autohydrolysis during uncatalysed pretreatment, a reduction of available acetyl groups with deacetylation would adversely affect the digestibility of the subsequent pretreated WIS. However, for harvest residues WIS

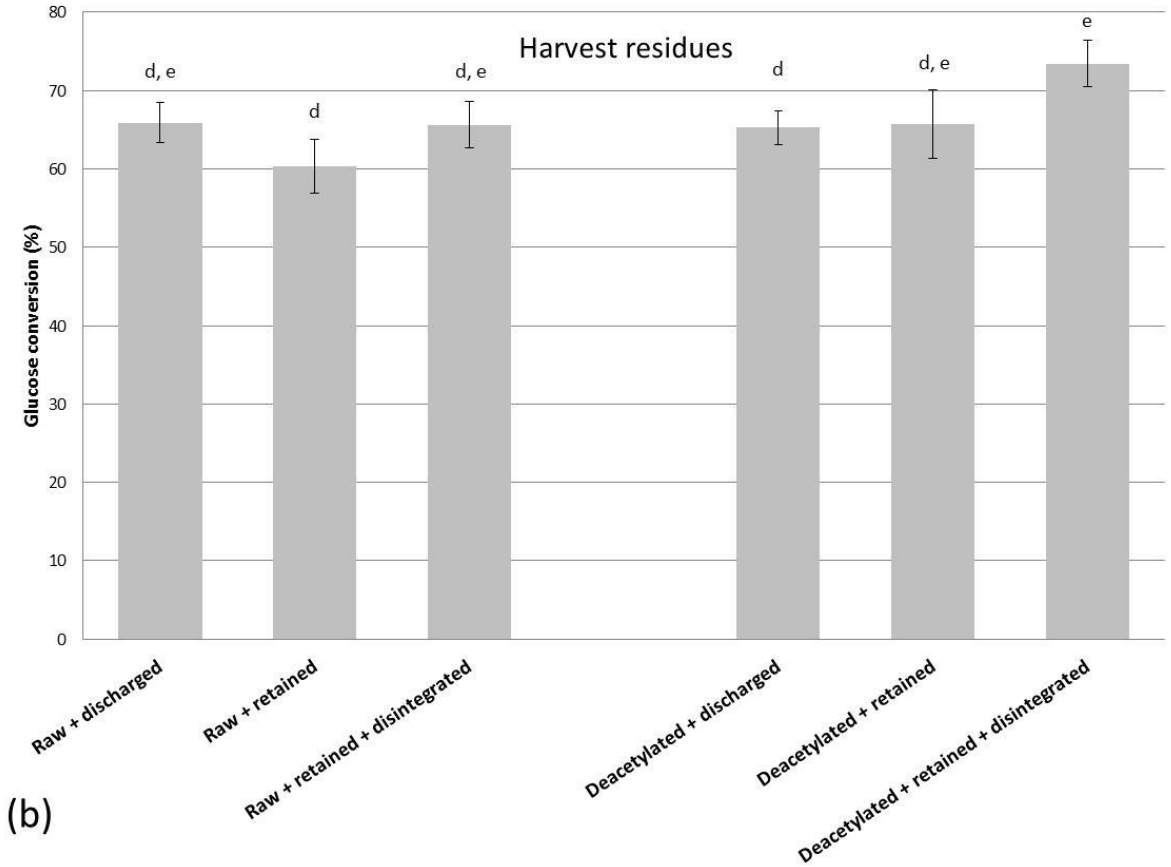
did not exhibit a significant difference ($p > 0.05$) in glucose conversion yields between raw and deacetylated feedstocks, regardless if pretreated material was discharged or retained during steam explosion (Figure 5.4.b). This indifference could be attributed to the relative low acetyl groups to ash ratio of 0.4 g/g for raw harvest residues. In comparison, all pretreated WIS from bagasse and poplar showed significant decreases ($p < 0.05$) in glucose conversion yields when the feedstocks were deacetylated, except for the case of discharged poplar where the decrease was, nevertheless, moderately significant at $p = 0.08$. Deacetylation of raw bagasse and raw poplar resulted in decreases of glucose conversion yields from the different WIS hydrolysed that ranged from 6% - 15% and 14% - 17%, respectively. The acetyl groups to ash ratios of raw bagasse and poplar were therefore possibly high enough to significantly influence the impacts of autohydrolysis on digestibility of pretreated WIS (Table 5.1).

The enzymatic hydrolysis results in Figure 5.4 also support the conclusion from the visual observations of the pretreated material that steam explosion did not significantly contribute to disrupting the lignocellulosic structure for increased enzymatic accessibility (Chandra et al., 2007). In the cases of raw bagasse and dry poplar (Figures 5.4.a and c), pretreated material that was retained during the steam explosion yielded significant lower ($p < 0.05$) glucose conversion yields than pretreated material discharged during steam explosion. However, disintegration of the retained pretreated material improved digestibility of the pretreated material to result in glucose conversion yields equivalent ($p > 0.05$) to the respective discharged raw materials (Figures 5.4.a and c). In other words, disintegration of the retained pretreated material presented a replacement for the disruption achieved with explosive discharge (Jin and Chen, 2006). In all the other cases

(Figure 5.4), material retained during steam explosion yielded glucose conversions that were not significantly different ($p > 0.05$) to pretreated material discharged during steam explosion. It is possible that these pretreated feedstocks were already amenable to enzymatic hydrolysis at the end of steam pretreatment and that no significant improvement in digestibility could be achieved with the size reduction provided by explosive discharge (Pielhop et al., 2016). However, material packed in the basket was more exposed to the latent heat transfer of condensing steam and effectively experienced higher pretreatment severities than the conventionally loaded material during steam pretreatment. It is therefore possible that these higher effective pretreatment severities improved the digestibility of the WIS from retained material. This will also explain the significant ($p < 0.05$) and moderate ($p = 0.07$) glucose conversion improvements of 8% and 13% achieved with deacetylated harvest residues (Figure 5.4.b) and raw poplar (Figure 5.4.c), respectively over discharged material when retained material was disintegrated after pretreatment. Consequently, disintegrated raw poplar (Figure 5.4.c) had a digestibility that did not differ significantly ($p > 0.05$) from steam pretreated dry poplar (discharged or retained with disintegration), which represented a much harsher pretreatment severity (Brownell et al., 1986).



(a)



(b)

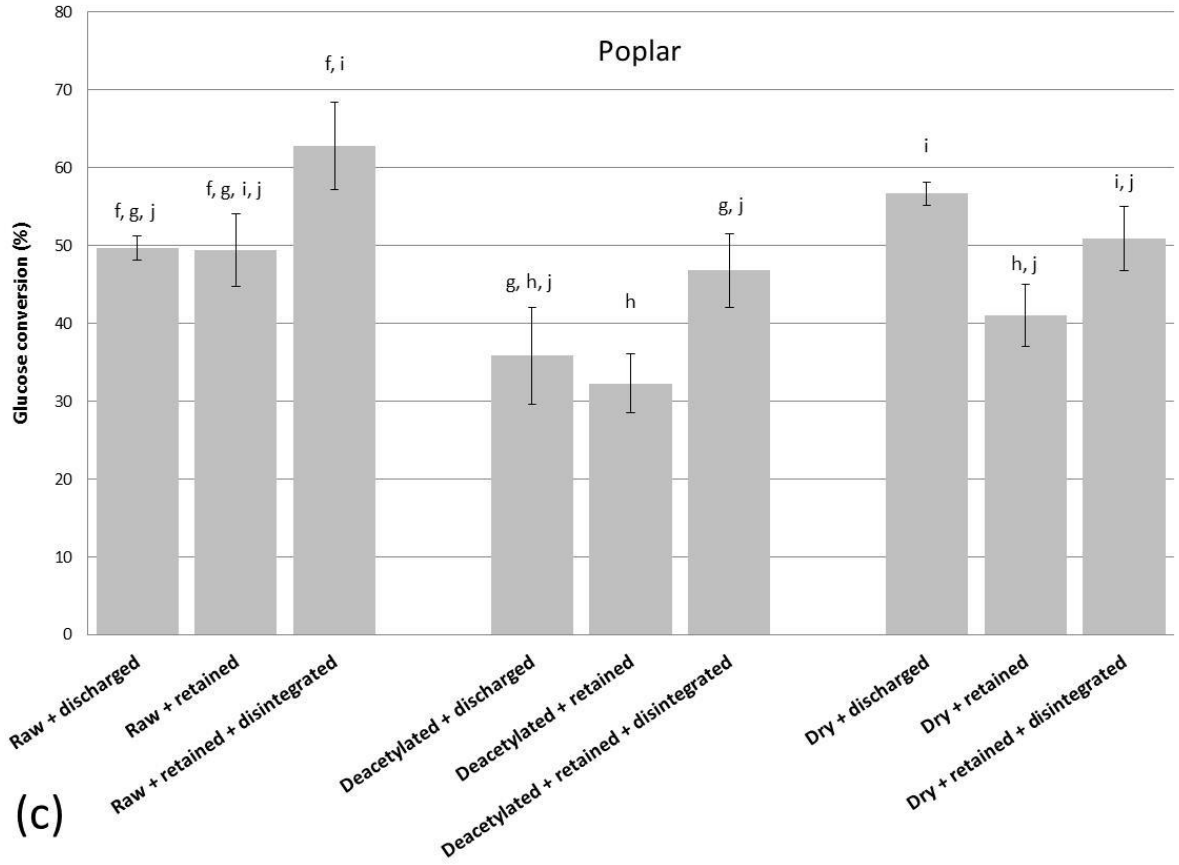


Figure 5.28 Overall glucose conversions from raw feedstock glucan after 72 h of enzymatic hydrolysis of WIS from (a) bagasse, (b) harvest residues and (c) poplar. Similar letters indicate glucose conversions that were not significantly different ($p > 0.05$). All deviations are expressed as standard deviation from triplicate samples.

5.4 Conclusions

High acetyl group content to ash ratios in lignocellulose are beneficial in steam pretreatment to improve enzymatic hydrolysis. Depending on the acetyl group content to ash content ratio, deacetylation of the lignocellulosic feedstock prior to pretreatment will not significantly affect the digestibility of uncatalysed steam pretreated solids. The rapid depressurisation during steam explosion step does not directly contribute to reducing particle size of pretreated material, but rather the mechanical impingements of pretreated material transported through an obstruction. Improving the mechanical design for increased mechanical damage to the pretreated material during the material discharge with steam explosion could therefore increase digestibility, and potentially allow for pretreatment at lower temperatures and pressures.

5.5 Acknowledgements

The authors gratefully acknowledge the Canada-Africa Research Exchange Grants (CAREG), the National Research Foundation (NRF) of South Africa and the Centre for Renewable and Sustainable Energy Studies (CRSES), Stellenbosch, South Africa for financial support. The authors also thank Novozymes (Bagsværd, Denmark) for donating the enzymes used in this study.

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CHAPTER 6

SUITABILITY OF PRESSED SLURRIES FROM UNCATALYSED STEAM PRETREATMENT OF RAW AND DEACETYLATED SUGARCANE LIGNOCELLULOSE FOR BIOCONVERSION IN A BIOREFINERY

This chapter appears as a draft manuscript.

Title: "Suitability of pressed slurries from uncatalysed steam pretreatment of raw and deacetylated sugarcane lignocellulose for bioconversion in a biorefinery"

Authors: Martin Louis Hamann, Eugéne van Rensburg, Johann Ferdinand Görgens

Objective of this chapter in the dissertation

The objectives in this chapter were to assess the dewaterability and fermentability of the pretreated slurries of uncatalysed steam pretreatment of bagasse and harvest residues when pretreated at optimum pretreatment conditions as identified in Chapters 4 and 5. Pretreated slurries from the optimum pretreatment conditions were all pressed in the same manner and fermented in a fed-batch fashion until a maximum added solids concentration of 15%. This chapter also used the deacetylation process tested in Chapter 5 to assess the impact of deacetylation of feedstocks prior to uncatalysed steam pretreatment on the downstream fermentability of the pretreated slurries. Despite the negative impact of

deacetylation on the digestibility of pretreated solids from uncatalysed steam pretreatment of lignocellulose with high acetyl groups to ash ratios (Chapter 5), deacetylation was pursued in this chapter as a means to detoxify the pretreated lignocellulose. Fermentability was therefore improved by limiting the formation of acetic acid.

Declaration by the candidate:

With regards to Chapter 6, page numbers 179 to 213 of this dissertation, the nature and scope of my contributions were as follows:

Nature of contribution	Extent of contribution (%)
Experimental planning	95
Executing experiments	100
Interpretation of results	95
Writing the chapter	100

The following co-authors have contributed to Chapter 6 pages 179 to 213 in the dissertation in the following manner:

Name	E-mail address	Nature of contribution	Extent of contribution (%)
Eugéne van Rensburg	eugenevrb@sun.ac.za	• Experimental planning • Interpretation of results	5 5
Johann Ferdinand Görgens	jgorgens@sun.ac.za	• Reviewing chapter	100

Candidate signature:……

Date:…….

Declaration with signature in possession of candidate and supervisor.

Declaration by co-authors:

The undersigned hereby confirm that

1. the declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 6 pages 179 – 213 in the dissertation,
2. no other authors contributed to Chapter 6 pages 179 – 213 in the dissertation besides those specified above, and
3. potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 6 pages 179 – 213 of this dissertation.

Signature	Institutional affiliation	Date
	Stellenbosch University	
	Stellenbosch University	

Declaration with signatures in possession of candidate and supervisor.

Suitability of pressed slurries from uncatalysed steam pretreatment of raw and deacetylated sugarcane lignocellulose for bioconversion in a biorefinery

Martin Hamann, Eugéne van Rensburg and Johann Görgens

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ABSTRACT

In a sugar platform biorefinery, pretreated lignocellulose slurry should ideally be easily dewaterable and fermentable to allow for energy efficient separation of cellulose-rich solids from the pentose-rich prehydrolysate and high volumetric productivity of sugar fermentation, respectively. This work compared uncatalysed steam pretreatment of raw and deacetylated sugarcane bagasse and sugarcane harvest residues at optimum conditions for maximum digestibility, maximum hemicellulose recovery and maximum combined sugar yield (CSY). Pretreated slurries were pressed at 250 kPa (g) for 1 min to a cake and subsequently fermented in a fed-batch setup to a maximum of 15% solids. Pressing failed to remove sufficient inhibitors from the raw bagasse slurries when pretreated at 215 °C, 15 min and 215 °C, 5 min for maximum digestibility and maximum CSY, respectively, and were not fermentable. However, when deacetylated prior to pretreatment, fermentability of the pressed slurries improved to ethanol volumetric productivities of 0.42 and 0.37 g/L.h^{-1}, respectively. In contrast, pressed slurry of raw harvest residues was fermentable when pretreated at conditions for maximum digestibility, maximum hemicellulose recovery and maximum CSY with ethanol volumetric productivities of 0.42, 0.35 and 0.33 g/L.h^{-1}. Harvest residues could therefore be more suited than bagasse for bioprocessing in a biorefinery.

6.1 Introduction

The global sugar industry is currently threatened by international sugar prices that are below the production costs of most sugar producers in the world (FAO, 2019), increasing sugar price fluctuations (Maita and Smutka, 2018), a changing consumer behaviour with the introduction of sugar taxations (Marten et al., 2018) and the devastating effects of climate change on sugar growing regions (Deressa et al., 2005). Incorporating lignocellulosic feedstocks and diversifying product lines in a biorefinery strategy can help shield the sugar industry against these external shocks (Farzad et al., 2017). Recent life cycle assessments (LCAs) of the sugarcane industry have highlighted the financial, socioeconomic and environmental benefits that can be achieved when existing conventional cane sugar mills are upgraded with high pressure boilers and expanded into multi-product lignocellulose biorefineries (Ali Mandegari et al., 2017; Farzad et al., 2017; Melendez et al., 2018; Pachón et al., 2018; Petersen et al., 2018). These integrated sugarcane biorefineries can co-produce biofuels and platform chemicals via a sugar platform by pretreatment and bioconversion of sugarcane lignocelluloses, i.e. bagasse and harvest residues (Kobayashi and Fukuoka, 2013).

Surplus bagasse can be produced by sugar mills through upgrading of boilers to increase efficiency (Ali Mandegari et al., 2017; Dias et al., 2011) and, together with the increase in available harvest residues generated from green harvesting of sugarcane (Leal et al., 2013), will be potential lignocellulosic feedstocks for biorefining. In order to maintain energy self-sufficiency and limit net increases in greenhouse gas emissions in, additional biorefining energy demands must be made-up with more lignocellulosic feedstocks as boiler fuel (Carpio and Simone de Souza, 2017; Mandegari et al., 2017). The sugarcane lignocellulose LCAs and techno-economic studies found in literature usually make no

distinction between the allocation of bagasse and harvest residues for boiler fuel or for bioprocessing feedstock, while treating them as a single lignocellulosic feedstock with properties approximated to that of bagasse.

Furthermore, investigations of multi-product sugarcane biorefinery scenarios with lignocellulose pretreatment have identified the need to recover all of the hemicellulose rich prehydrolysate to maximise production volumes (Farzad et al., 2017; Nieder-Heitmann et al., 2019; Özüdoğru et al., 2019). The complete recovery of the prehydrolysate with successive washing steps (Li and Chen, 2008) will not be desirable in large-scale operation, because of increased water consumption and increased energy consumption (García-Aparicio et al., 2006). Filtration or centrifuging of the slurry from pretreatment could be industrially viable, but a fraction of the prehydrolysate, containing valuable hemicellulose, as well as inhibitors to enzymatic hydrolysis and fermentation (Palmqvist and Hahn-Hägerdal, 2000; Rasmussen et al., 2014), will remain as the moisture content in the filtered/pressed slurry. Easily dewatered pretreated slurries are therefore preferred for lower energy consumption, higher hemicellulose recovery and less inhibitors for downstream fermentation.

Overliming of the prehydrolysate is a common practice for removing inhibitors to improve fermentability (Jennings and Schell, 2011), but Mohagheghi et al. (2006) found that 7 – 34% and 7 to 21% of the xylose and arabinose in the prehydrolysate were degraded with overliming at 50 °C for 30 min at pH 9 to 11 that would significantly affect yields in a multi-product biorefinery. Furthermore, overliming requires additional downstream pH adjustment and solids/liquid separation steps to condition prehydrolysate (Mohagheghi et al., 2006). These steps are water intensive and were estimated by Pan et al. (2016) to

require approximately 2.5 L/kg dry biomass for a dilute acid pretreatment process. The remaining dissolved salts can cause severe scaling in downstream equipment to increase process cleaning requirements (Mohagheghi et al., 2006). As opposed to overliming after pretreatment, deacetylation prior to pretreatment has been proposed as a detoxification step in a biorefinery context (X. Chen et al., 2012) for process simplification (Humbird et al., 2011), comparatively lower water consumption at approximately 0.3 L/kg dry biomass (Pan et al., 2016) and the potential to recover acetic acid as a by-product (Pan et al., 2016).

In this study sugarcane bagasse and harvest residues were steam pretreated without catalyst as a cost effective pretreatment method with limited downstream impacts for large-scale operation (Pielhop et al., 2016). This study assessed for the first time the impacts of deacetylation of raw feedstocks prior to uncatalysed steam pretreatment on downstream fermentability as a technique to increase the fermentability of pretreated material. The pretreated slurries were all subjected to the same pressing pressure to compare dewaterability and to produce pressed slurries that were directly fermented without any detoxification.

6.2 Materials and methods

6.2.1 Feedstocks

Sugarcane bagasse and sugarcane harvest residues were sourced from two sugar mills in South Africa during the 2014 harvest season. These raw feedstocks were prepared, as described in Chapter 4, and finally blended into respective raw feedstocks of bagasse and harvest residues.

These raw feedstocks were prepared prior to steam pretreatment by either water impregnation or by deacetylation followed by water impregnation, according to preferred conditions identified in Chapters 4 and 5. Water impregnation was achieved by soaking the raw feedstocks in water overnight and then centrifuging the soaked feedstocks to a moisture content of approximately 65%. Deacetylation was performed as described by Chen et al. (2012) and briefly entailed that the raw feedstocks were immersed in a 0.1 M solution of NaOH for 3 h at 70 °C with a solids to liquid ratio of 1:12. During this dilute alkaline extraction process the bulk of the acetyl groups was removed from the hemicellulose polymers. The deacetylated feedstocks were subsequently washed to a neutral pH and then also subjected to the same water impregnation protocol, as described above, prior to pretreatment.

6.2.2 Steam pretreatment

Prepared feedstocks of bagasse, harvest residues and a blend of these feedstocks in a 1:1 mass ratio were steam pretreated in batches of 500 g (dry). Uncatalysed steam pretreatment was performed with saturated high pressure steam in a batch steam pretreatment reactor (IAP GmbH, Graz, Austria) with a capacity of 19 L by following the same operating procedures as described in Chapter 4. Bagasse and harvest residues were

steam pretreated at the conditions that were predicted in Chapter 4 to produce maximum digestibility of the pretreated solids, maximum combined sugar yield (CSY) and maximum hemicellulose recovery in the pretreatment liquor within the pretreatment ranges of 185 to 215 °C and 5 to 15 min (Table 6.1). Steam pretreatment of the feedstock blend was however not at optimised conditions, but at 215 °C and 5 min, to produce pretreated material with both high digestibility and high CSY (Chapter 4 and Table 6.1). Steam pretreated material was discharged out of the reactor with sudden depressurisation at the end of steam pretreatment and collected as a slurry in a collection vessel.

Table 6.12 Previously determined steam pretreatment conditions used in the ranges 185 – 215 °C and 5 – 15 min to achieve different targets for the pretreated bagasse and harvest residues slurries (Chapter 4).

	Temperature (°C)	Time (min)
Bagasse		
Maximum digestibility (BD)	215.0	15.0
Maximum CSY (BC)	215.0	5.0
Maximum hemicellulose recovery (BH)	202.2	5.0
Harvest residues		
Maximum digestibility (HD)	205.8	8.7
Maximum CSY (HC)	199.6	9.5
Maximum hemicellulose recovery (HH)	193.7	12.7
Bagasse / harvest residues (1:1 mass blend)		
High digestibility and high CSY (B/H)	215.0	5.0

The abbreviations between parentheses indicate the naming of the pretreated slurries used in this study.

6.2.3 Fed-batch simultaneous saccharification and fermentation (SSF)

The steam pretreated material in this study could not be frozen or sterilised as these procedures could affect the lignocellulosic structures to confound the actual impact of the steam pretreatment conditions on the feedstocks. Preparing the pretreated material substrate was therefore executed in parallel to growing of the yeast culture to ensure that substrates with limited exposure to outside microbial contact was available at the onset of pre-saccharification, as shown in Figure 6.1. The time pre-saccharification was started was

denoted as t - 0 and the execution of all other SSF activities were in reference to this time point.

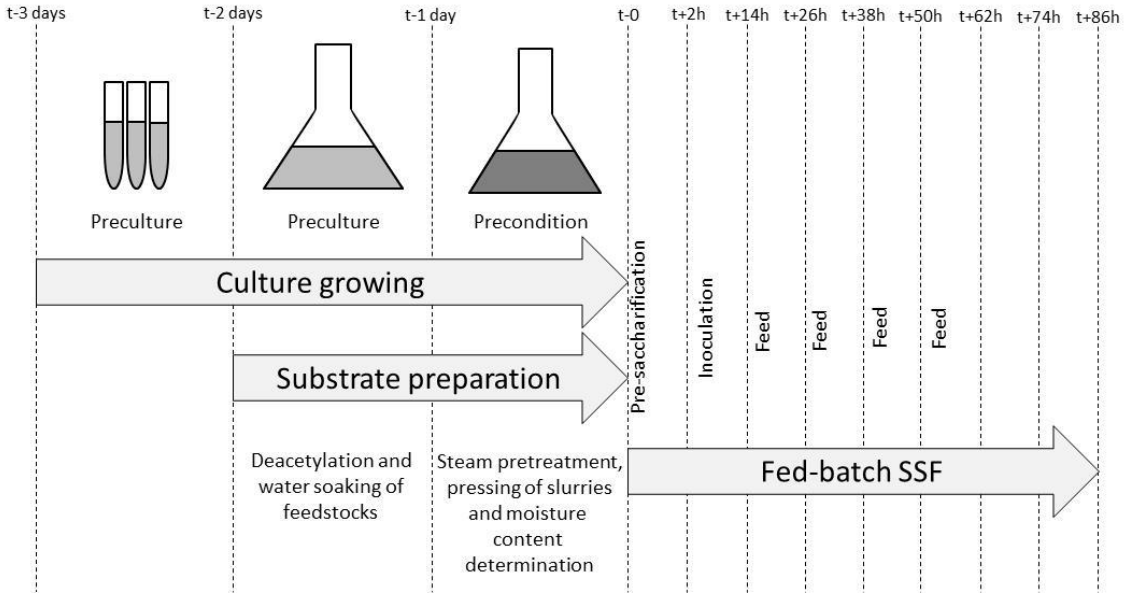

Figure 6.29 SSF protocol with parallel culture growing and substrate preparation.

6.2.3.1 Yeast culture growing

Saccharomyces cerevisiae M2N (previously known as MH1000) (Van Zyl et al., 2011) is an industrial strain yeast that was grown throughout the experiment from the same stock culture stored at -87 °C. The culture was aseptically seeded three days before the onset of pre-saccharification (t – 3 days; Figure 6.1) in three test tubes with each containing 5 mL of sterile growth medium that was prepared from glucose (20 g/L), peptone (20 g/L) and yeast extract (10 g/L). The preculture was incubated in an orbital shaker for 24 h at 30 °C with an agitation of 150 rpm. On t – 2 days the precultures in the test tubes were aseptically transferred to a 1 L Erlenmeyer flask containing 250 mL of the growth medium and further incubated for 24 h at 30 °C and 150 rpm (Figure 6.1). Preconditioning of the preculture was started on t – 1 day with the introduction of both the pretreatment liquors from steam pretreated bagasse and harvest residues to ensure that no bias tolerance was developed towards a feedstock. The liquors were obtained from the slurries of steam pretreated

bagasse and harvest residues that were pretreated at the respective conditions for maximum digestibility (Table 6.1). Preconditioning was performed in sterile conditions in a 1 L Erlenmeyer flask that contained 12.5 mL preculture (5%), 175 mL growth medium (70%), 31.25 mL sterile filtered bagasse pretreatment liquor (12.5%) and 31.25 mL sterile filtered harvest residues pretreatment liquor. Preconditioning was allowed for 24 h at 30 °C and 150 rpm.

6.2.3.2 Pressing of pretreated slurries

Steam pretreatment and collection of pretreated slurries were performed one day before pre-saccharification (t – 1 day), as shown in Figure 6.1. Batches of 50 g steam pretreated slurry were pressed in a hydraulic press at a pressure of 250 kPa (gauge) for 1 min to remove a fraction of the prehydrolysate. The pressed material was then stored overnight at 4 °C, while samples of the pretreated slurries and pressed material were oven dried overnight at 105 °C for moisture determination and to calculate the relative dewatering achieved with pressing.

6.2.3.3 Simultaneous saccharification and fermentation (SSF)

Pressed material was loaded into 250 mL fermentation flasks at an initial solids loading of 5% (dry w/w), together with 5% (w/w) of 1 M phosphate buffer and 10% (w/w) of a peptone (10 g/L) and yeast extract (5 g/L) mixture. Ampicillin was added at 0.005% (w/w) to avoid bacterial growth during SSF as the pressed material was not sterilised. Cellic CTec2 (Novozymes) with a cellulase activity of 150 FPU/mL was dosed at 10 FPU/g of total pretreated solids (15% dry w/w). Pre-saccharification was found to improve ethanol yields for high solids fermentations (Mesa et al., 2011; Neves et al., 2016) and was employed for 2 h in this work from time point t – 0 at 50 °C and 150 rpm. Two 0.5 mL samples were taken

from every flask after the addition of enzymes (t – 0) and again after pre-saccharification (t + 2 h), but before inoculation, for HPLC analysis and pH measurement. The pH was adjusted to 5 with 3 M KOH.

Yeast was harvested from the preconditioned culture through repeated centrifugation and washing with 0.9% (w/v) sterile saline solution. Inoculation was performed at t + 2 h with the addition of 10% (w/w) yeast suspension at an optical density of 1 (OD_{600nm}). SSF was continued at 35 °C and 150 rpm for 84 h until t + 86 h.

The fermentation flasks were batch fed with 2.5% (dry w/w) of the required pressed material every 12 h after inoculation until the total added solids reached 15% (dry w/w) (t + 14 h, t + 26 h, t + 38 h, t + 50 h). Fed-batch feeding allowed for effective mixing during fermentation at high solids loadings. Two 0.5 mL samples were taken from each flask just after feeding for HPLC analysis and pH measurement, respectively. The sampling was continued every 12 h after t + 50 h (t + 62 h, t + 74 h, t + 86 h). SSF was stopped at t + 86 h.

6.2.3.4 Definitions

The theoretical maximum ethanol concentration (g/L) was defined as:

= [Solids fed (g/L) x Solids glucose fraction (g/g) +
Pre-hydrolysate fed (g/L) x Pre-hydrolysate glucose fraction (g/g)]
x 0.511 (1)

The ethanol yield as a fraction of the theoretical maximum (%) was defined:

= Experimental ethanol concentration (g/L) /
Theoretical ethanol concentration (g/L) x 100 (2)

Ethanol volumetric productivity (g/L.h^{-1}) was defined at the completion of an SSF run at t + 86 h after 84 h of fermentation as:

= Final theoretical ethanol concentration (g/L) / 84 (h) (3)

6.2.4 Higher heating value (HHV) determination

The HHVs of the raw feedstocks and the fermentation residues were measured in duplicate with a bomb calorimeter (Cal2K Eco) according to ASTM standard D5865-11a. A sample size of 0.25 g was used in an oxygen atmosphere at 2 MPa.

6.3 Results and discussion

6.3.1 Pretreated slurry dewaterability

Dewaterability of the pretreated slurries is an important factor that will influence the recovery of hemicellulose rich prehydrolysate for the co-production of value-adding chemicals, as well as the removal of inhibitors to improve subsequent fermentation of the slurries. Similarly to other rheological properties of pretreated slurries such as complex viscosities (Fockink et al., 2018; Lou et al., 2014), a low degree in dewaterability will require higher capital and operating costs, while also limiting the minimum moisture content that can be achieved with mechanical processing. The relative dewatering achieved for the different pretreated slurries under the same set of pressing conditions is shown in Figure 6.2. All the pretreated slurries had a similar moisture content of approximately 80% before pressing.

Dewaterability of pretreated bagasse slurries seemed to increase with increase in steam pretreatment severity (Figure 6.2). Prehydrolysate removal increased significantly ($p < 0.05$) from 24% for bagasse pretreated for maximum hemicellulose recovery (202.2 °C, 5 min) to 36% and 52% for bagasse pretreated for maximum CSY (215 °C, 5 min) and maximum digestibility (215 °C, 15 min), respectively. In accordance, the resulting decrease in pretreatment severity of deacetylated bagasse also resulted in significantly lower dewaterability ($p < 0.05$). This behaviour is however contrary to sludge dewaterability studies that predict decreasing dewaterability with decreasing particle sizes (Karr and Keinath, 1978), when considering that slurry particle sizes decrease with increase in steam pretreatment severity (Pielhop et al., 2016). It is possible that other factors such as compressibility (Karr and Keinath, 1978) which increases with pretreatment severity as

more lignin flowing occurs (Stelte et al., 2011), dominates in the pressure range of the studied pressing test. Interestingly for bagasse, optimum steam pretreatment for hemicellulose production results in pretreated slurry that is the most difficult to dewater from all the conditions studied here.

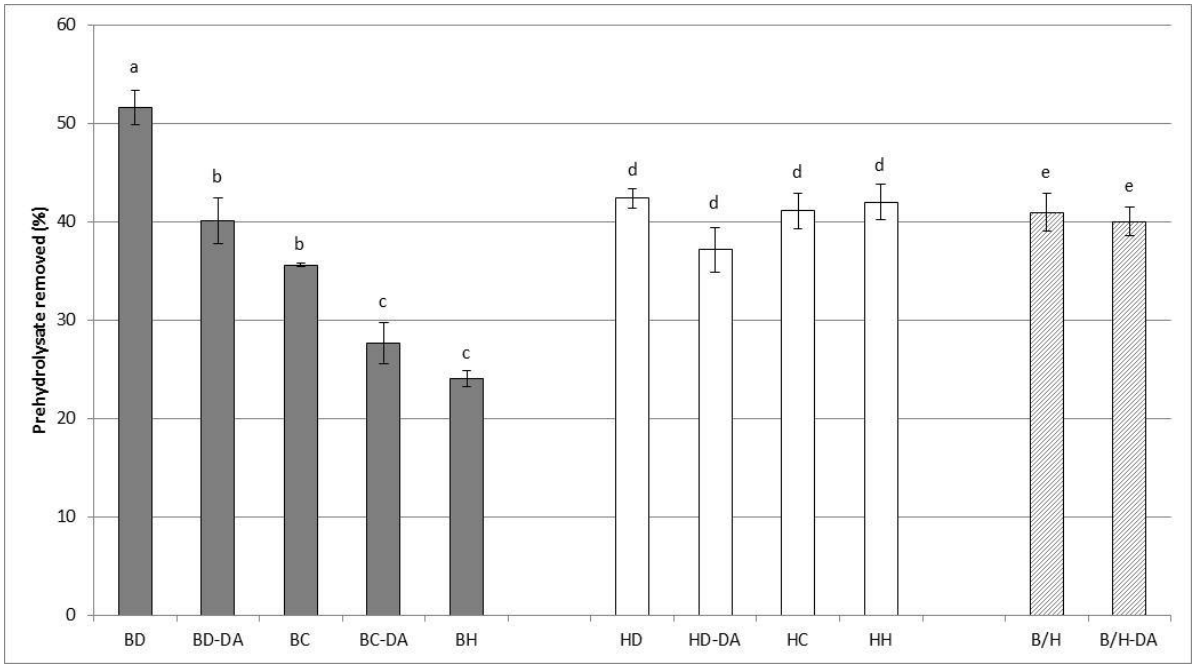

Figure 6.30 Prehydrolysate removals achieved in pressing 50 g of pretreated slurries at a pressure of 250 kPa (gauge) for 1 min. BD, BC, BH are the pretreated bagasse slurries from steam pretreatment conditions for maximum digestibility, maximum CSY and maximum hemicellulose recovery in the hydrolysate, respectively. HD, HC, HH are the pretreated harvest residues slurries from steam pretreatment conditions for maximum digestibility, maximum CSY and maximum hemicellulose recovery in the hydrolysate, respectively. B/H is the pretreated blend slurry. DA means that the feedstock was deacetylated prior to steam pretreatment. Similar letters above the bars indicate results that were not significantly different ($p > 0.05$).

All the slurries from pretreated harvest residues displayed similar dewatering ($p > 0.05$) of approximately 40% as the steam pretreatment temperatures and times, and therefore pretreatment severities, were relatively close to each other (Table 6.1). Blended feedstock (B/H and B/H-DA in Figure 6.2) pretreated at 215 °C and 5 min dewatered significantly better ($p < 0.05$) than bagasse at the same pretreatment conditions for both raw and deacetylated feedstocks (BC and BC-DA in Figure 6.2). This was probably because the pretreatment temperature of 215 °C is relatively harsh for the harvest residues component.

Deacetylation did not affect the dewaterability of harvest residues and the blend significantly ($p > 0.05$), because of the relatively low acetyl groups content of harvest residues, as discussed in Chapter 5.

6.3.2 Fermentability of pressed slurries

A summary of the final fermentability results of the fed-batch SSF experiment is given in Table 6.2. Fermentability of pretreated harvest residues increased as the pressed slurry became more digestible, with increases in ethanol concentration (28.0 to 35.7 g/L), ethanol yield (66.7 to 80.8%) and ethanol volumetric productivity (0.33 to 0.42 $g/L.h^{-1}$), at pretreatment conditions for maximum digestibility compared to pretreatment at maximum hemicellulose recovery (Table 6.2). However, steam pretreatment of bagasse at the higher pretreatment severities for maximum digestibility and maximum CSY, which included pretreatment temperature of 215 °C, produced pressed slurries that were completely inhibitory to the yeast with no ethanol production (Table 6.2). However, when bagasse was deacetylated prior to steam pretreatment at these same pretreatment conditions (BD-DA at 215 °C, 15 min and BC-DA at 215 °C, 5 min), the resulting pressed slurries were increasingly fermentable as pretreatment temperature and time (pretreatment severity) increased (Table 6.2). Similarly, the pressed slurry from steam pretreated blended feedstock (B/H) at 215 °C and 5 min was completely inhibitory, but produced a fermentable pressed slurry (B/H-DA) when the blended feedstock was deacetylated prior to steam pretreatment (Table 6.2).

Table 6.13 Summary of final fermentability results of the fed-batch SSF experiment.

Pressed slurry (pretreatment conditions)		Final ethanol concentration (g/L)	Final theoretical ethanol yield (%)	Final ethanol volumetric productivity ($g/L.h^{-1}$)
BD	(215 °C, 15 min)	ND	0	0
BD-DA	(215 °C, 15 min)	35.0 ± 0.5	75.3 ± 1.1	0.42 ± 0.01
BC	(215 °C, 5 min)	ND	0	0
BC-DA	(215 °C, 5 min)	31.0 ± 0.8	63.0 ± 1.6	0.37 ± 0.01
BH	(202.2 °C, 5 min)	24.5 ± 2.4	54.4 ± 5.3	0.29 ± 0.03
HD	(205.8 °C, 8.7 min)	35.7 ± 1.6	80.8 ± 3.5	0.42 ± 0.02
HD-DA	(205.8 °C, 8.7 min)	33.7 ± 0.5	78.4 ± 1.3	0.40 ± 0.01
HC	(199.6 °C, 9.5 min)	29.6 ± 2.0	67.6 ± 4.6	0.35 ± 0.02
HH	(193.7 °C, 12.7 min)	28.0 ± 2.1	66.7 ± 5.0	0.33 ± 0.03
B/H	(215 °C, 5 min)	ND	0	0
B/H-DA	(215 °C, 5 min)	34.9 ± 1.4	80.0 ± 3.2	0.42 ± 0.02

BD, BC and BH represent bagasse pretreated at optimum conditions for digestibility, CSY and hemicellulose recovery, respectively.
HD, HC and HH represent harvest residues pretreated at optimum conditions for digestibility, CSY and hemicellulose recovery, respectively.
B/H represents a blend of bagasse and harvest residues in a 1:1 mass ratio pretreated at 215 °C and 5 min.
DA indicates deacetylated feedstocks.
ND stands for not detected.

The fermentability results of the fed-batch SSF runs with time are shown in Figures 6.3, 6.4 and 6.5 for the pressed slurries of steam pretreated bagasse, harvest residues and blended feedstock, respectively. In Figures 6.3 and 6.5 it is shown that the pressed slurries of steam pretreated bagasse BD (215 °C, 15 min) and BC (215 °C, 5 min), as well as of the blended feedstock B/H (215 °C, 5 min) were completely inhibited from the start when only 5% solids were fed as no ethanol was produced after inoculation (Figures 6.3.b and 6.5.b). The enzymes were however not completely inhibited, and enzymatic hydrolysis continued throughout the runs with glucose concentrations increasing even 86 h after enzymatic hydrolysis was started (Figures 6.3.a and 6.5.a).

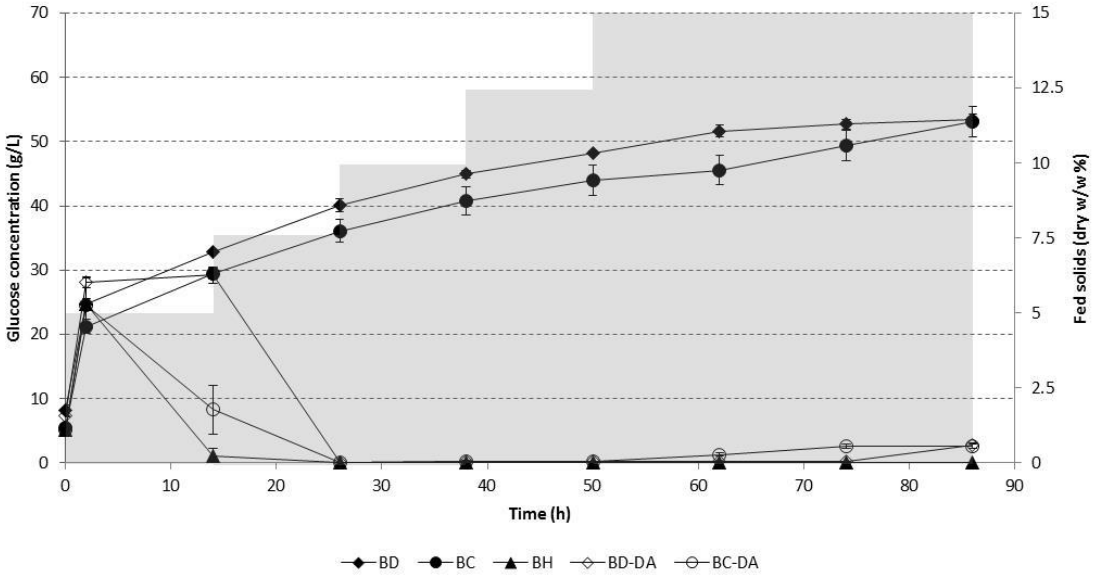

Figure 6.31.a Glucose concentrations during fed-batch SSF of pressed bagasse slurries. The grey area indicates the total added solids concentration. BD, BC and BH represent bagasse pretreated at optimum conditions for digestibility, CSY and hemicellulose recovery, respectively. DA indicates deacetylated feedstocks.

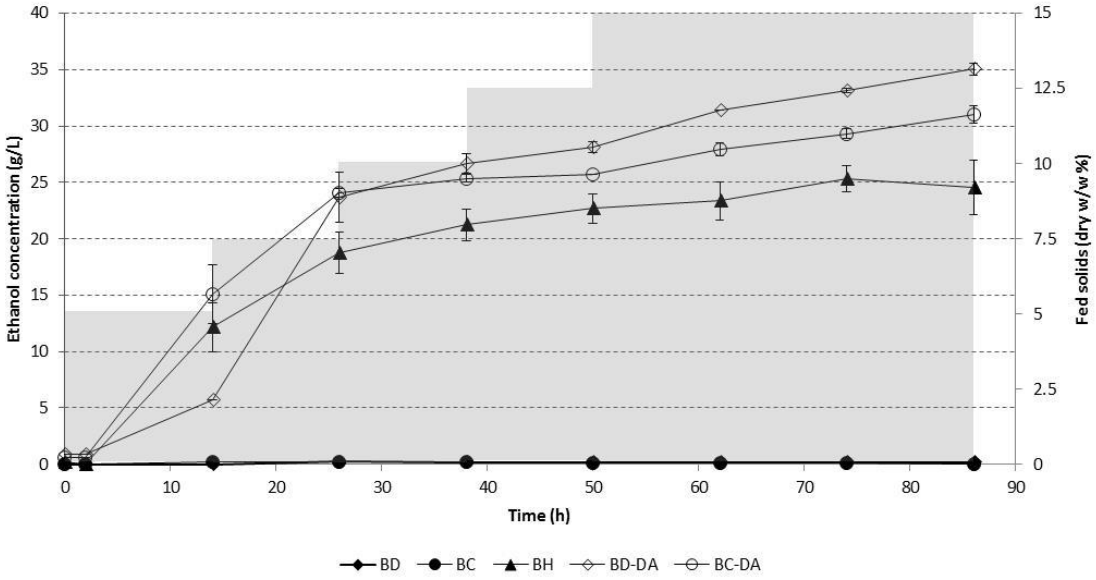

Figure 6.32.b Ethanol concentrations during fed-batch SSF of pressed bagasse slurries. The grey area indicates the total added solids concentration. BD, BC and BH represent bagasse pretreated at optimum conditions for digestibility, CSY and hemicellulose recovery, respectively. DA indicates deacetylated feedstocks.

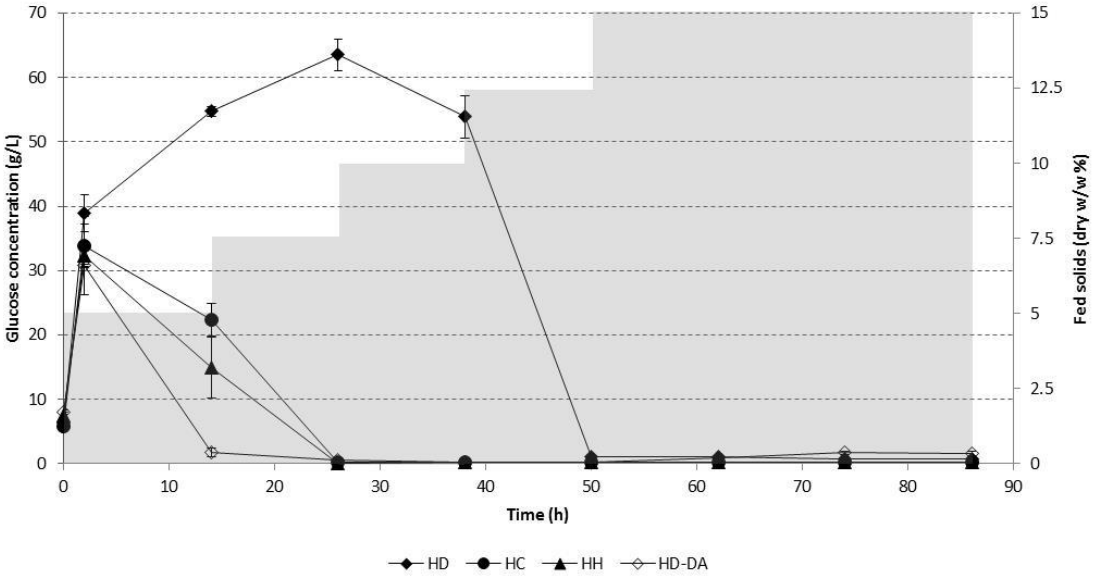

Figure 6.33.a Glucose concentrations during fed-batch SSF of pressed harvest residues slurries. The grey area indicates the total added solids concentration. HD, HC and HH represent harvest residues pretreated at optimum conditions for digestibility, CSY and hemicellulose recovery, respectively. DA indicates a deacetylated feedstock.

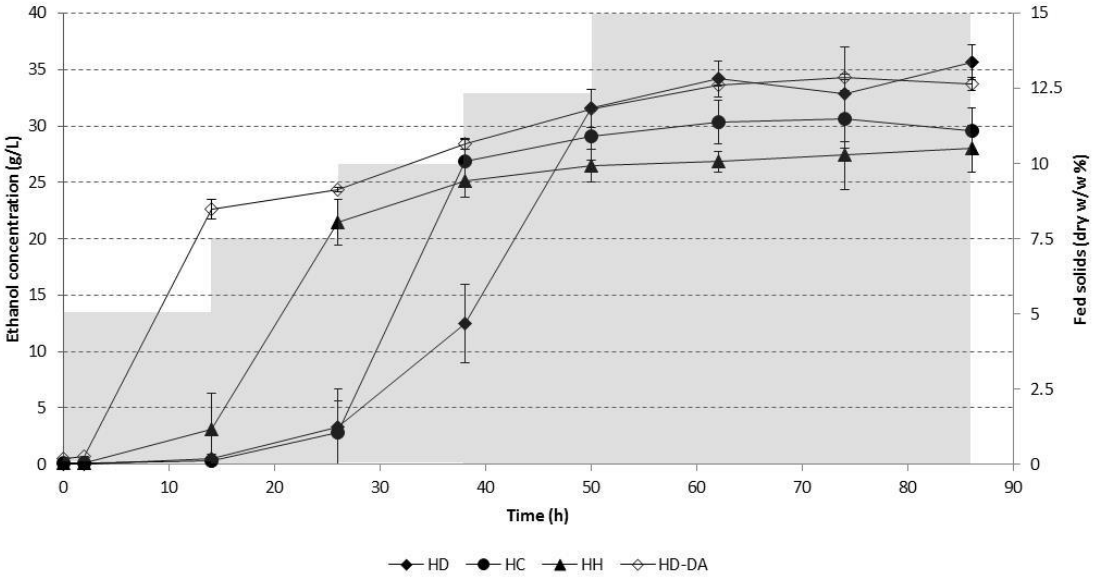

Figure 6.34.b Ethanol concentrations during fed-batch SSF of pressed harvest residues slurries. The grey area indicates the total added solids concentration. HD, HC and HH represent harvest residues pretreated at optimum conditions for digestibility, CSY and hemicellulose recovery, respectively. DA indicates a deacetylated feedstock.

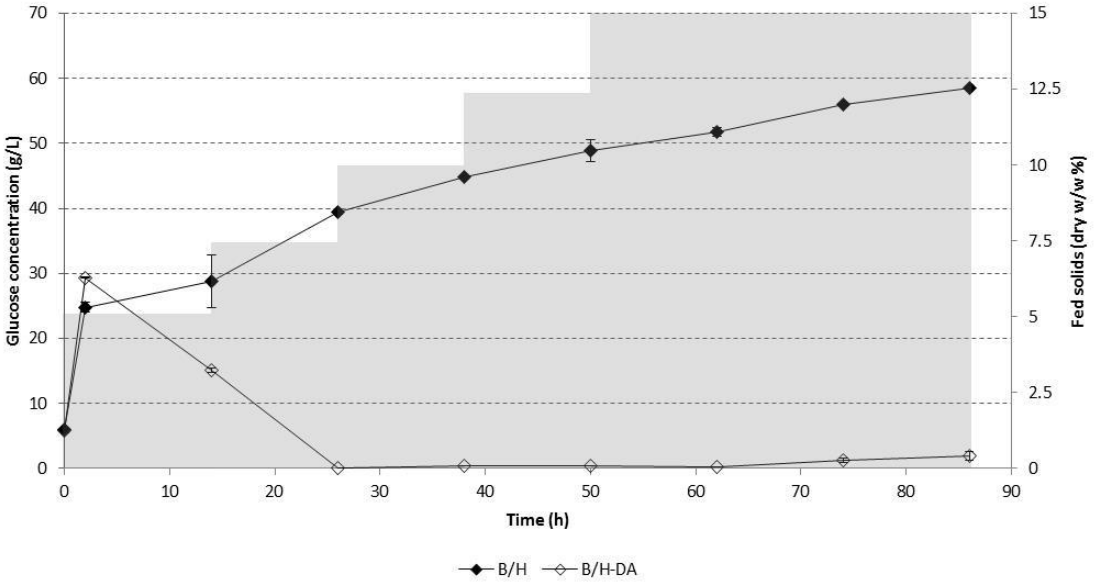

Figure 6.35.a Glucose concentrations during fed-batch SSF of pressed bagasse and harvest residues blend slurries. The grey area indicates the total added solids concentration. B/H represents a blend of bagasse and harvest residues in a 1:1 mass ratio pretreated at 215 °C and 5 min. DA indicates a deacetylated feedstock.

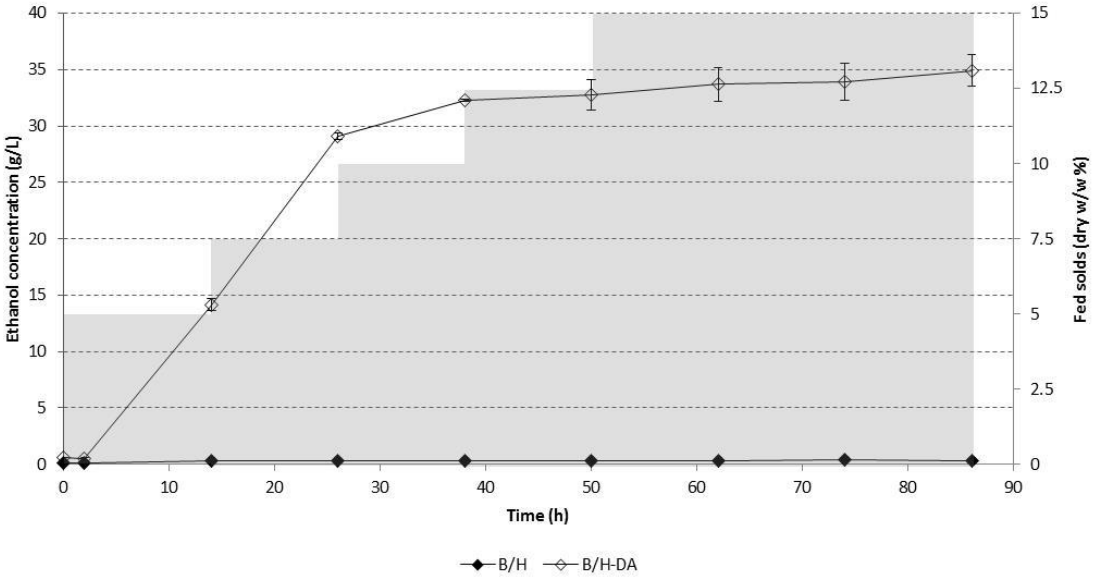

Figure 6.36.b Ethanol concentrations during fed-batch SSF of pressed bagasse and harvest residues blend slurries. The grey area indicates the total added solids concentration. B/H represents a blend of bagasse and harvest residues in a 1:1 mass ratio pretreated at 215 °C and 5 min. DA indicates a deacetylated feedstock.

The yeast could ferment all of the pressed slurries from steam pretreated harvest residues HD (205.8 °C, 8.7 min), HC (199.6 °C, 9.5 min) and HH (193.7 °C, 12.7 min), even though the initial ethanol volumetric productivities were different as the yeast adapted to the different inhibitory environments after inoculation (Figure 6.4). A lag period of at least 12 h after inoculation was observed for the pressed slurry from steam pretreated harvest residues HD (205.8 °C, 8.7 min) in which no ethanol was produced (Figure 6.4.b).

The initial concentrations of selected inhibitors in this study, as measured after pre-saccharification, but before inoculation of the different fermentation runs, are shown in Table 6.3. It has been shown that acetic acid, HMF and furfural are the dominant inhibitors produced during the pretreatment of agricultural feedstocks with high acetyl groups contents (Jönsson et al., 2013). However, it has been observed that synergistic effects exist between these inhibitors, as well as with different phenolic components (Klinke et al., 2003; Palmqvist et al., 1999; Zaldivar and Ingram, 1999). Palmqvist et al. (1999) have shown that the ethanol yields from bakers' yeast could be stimulated with acetic acid concentrations below 10 g/L in the absence of furfural and slightly increased with furfural concentrations below 2 g/L in the absence of acetic acid. However, acetic acid together with furfural negatively affected ethanol yield. In Table 3 the highest initial concentrations of acetic acid and furfural in the presence of each other that still allowed for fermentation was 1.9 g/L acetic acid for pressed bagasse slurry BH (202.2 °C, 5 min) and 0.3 g/L furfural for pressed harvest residues slurry HD (205.8 °C, 8.7 min). The initial combination of 1.5 g/L acetic acid and 0.3 g/L furfural for pressed harvest residues slurry HD proved almost too toxic for the yeast and resulted in a net increase in glucose production for at least 24 h after inoculation. Any higher initial concentrations of both these inhibitors resulted in complete inhibition

(Table 6.3). No grouping of the measured phenolic compounds in Table 6.3 could be identified as main effects for inhibition of the yeast.

Table 6.14 Concentrations of selected inhibitors at t + 2 h after pre-saccharification and before inoculation. The shaded rows indicate the pressed slurries that were completely inhibited with no ethanol fermentation.

Pressed slurry	Acetic acid (g/L)	HMF (g/L)	Furfural (g/L)	3,4-Dihydroxy-benzoic acid (mg/L)	Vanilic acid (mg/L)	Syringic acid (mg/L)	Vanilin (mg/L)	*p*-Coumaric acid (mg/L)	Syring-aldehyde (mg/L)	Ferulic acid (mg/L)	Conifer-aldehyde (mg/L)
BD	4.2 ± 0.4	0.3 ± 0.0	0.6 ± 0.0	5.7 ± 0.1	15.8 ± 0.3	42.1 ± 1.2	64.6 ± 0.6	79.4 ± 3.0	41.8 ± 0.5	20.1 ± 0.4	14.3 ± 0.3
BD-DA	0.5 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	1.5 ± 0.0	4.3 ± 0.1	22.9 ± 0.2	15.8 ± 0.8	8.3 ± 0.3	12.3 ± 0.5	1.6 ± 0.1	3.7 ± 0.2
BC	3.0 ± 0.9	0.0 ± 0.0	0.3 ± 0.0	1.4 ± 0.1	10.3 ± 2.3	123.4 ± 13.1	31.4 ± 4.0	99.1 ± 10.0	14.5 ± 2.6	27.9 ± 3.1	7.5 ± 0.7
BC-DA	0.4 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	1.1 ± 0.0	5.8 ± 0.9	49.4 ± 0.9	13.0 ± 0.2	20.0 ± 1.4	8.3 ± 0.2	6.0 ± 0.3	3.4 ± 0.1
BH	1.9 ± 0.7	0.0 ± 0.0	0.1 ± 0.0	1.3 ± 0.1	7.3 ± 0.0	172.2 ± 14.2	41.7 ± 3.4	203.1 ± 22.1	16.9 ± 0.9	55.9 ± 4.2	15.2 ± 1.6
HD	1.5 ± 0.1	0.0 ± 0.0	0.3 ± 0.0	4.9 ± 0.3	14.6 ± 0.6	15.1 ± 0.5	75.2 ± 3.6	78.6 ± 6.3	10.2 ± 1.5	38.6 ± 2.9	14.8 ± 0.6
HD-DA	0.5 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 0.3	4.7 ± 0.2	6.0 ± 0.4	19.1 ± 0.7	24.4 ± 0.8	5.3 ± 0.1	10.3 ± 0.4	5.2 ± 0.2
HC	1.4 ± 0.5	0.0 ± 0.0	0.2 ± 0.0	2.9 ± 0.0	7.9 ± 0.8	8.8 ± 0.5	42.0 ± 1.3	50.2 ± 1.7	5.6 ± 1.5	24.6 ± 0.0	8.5 ± 0.2
HH	1.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.0	2.9 ± 0.0	10.2 ± 0.0	9.9 ± 0.0	45.9 ± 0.2	59.9 ± 0.3	8.1 ± 0.0	28.3 ± 0.4	9.6 ± 0.2
B/H	1.8 ± 0.1	0.0 ± 0.0	0.4 ± 0.0	4.8 ± 0.1	16.7 ± 0.5	20.8 ± 0.6	71.4 ± 0.6	147.6 ± 2.8	21.7 ± 0.0	53.6 ± 0.7	17.9 ± 0.2
B/H-DA	0.5 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	1.2 ± 0.0	5.0 ± 0.0	6.9 ± 0.2	18.6 ± 0.3	24.3 ± 0.8	9.5 ± 0.9	8.9 ± 0.3	4.9 ± 0.1

BD, BC and BH represent bagasse pretreated at optimum conditions for digestibility, CSY and hemicellulose recovery, respectively.
HD, HC and HH represent harvest residues pretreated at optimum conditions for digestibility, CSY and hemicellulose recovery, respectively.
B/H represents a blend of bagasse and harvest residues in a 1:1 mass ratio pretreated at 215 °C and 5 min.
DA indicates deacetylated feedstocks.

6.3.3 Fuel products

Lignin rich residue remaining after fermentation can be used to supplement fuel for cogeneration of electricity and steam. The HHV of raw bagasse and harvest residues were measured as 17.6 ± 0.2 and 16.4 ± 0.1 MJ/kg, respectively, whereas the HHV of the resulting fermentation residues only marginally increased to 18.8 ± 1.0, 19.1 ± 0.5 and 19.3 ± 1.5 MJ/kg at the end of the fermentation runs for pressed slurries from pretreated bagasse, pretreated harvest residues and pretreated blended feedstock respectively. The HHVs of the residues were comparable to measurements done elsewhere for residues from simultaneous saccharification and co-fermentation (SSCF) of bagasse and other agricultural lignocellulose, even though the HHV of pure lignin was measured as 26.7 MJ/kg (Liu and Bao, 2017). The slight increase in HHV of fermentation residues could be ascribed to low HHV impurities in the residues such as ash, yeast and sugars (Liu and Bao, 2017).

The potential HHV of the residue and ethanol resulting from the studied steam pretreatment conditions per 1 000 kg of dry raw feedstock are shown in Figure 6.6. Deacetylation, deterioration during steam pretreatment and conversion during fermentation contributed to losses of water insoluble solids with overall recoveries of fermentation residues ranging from 273 to 425 kg dry solids per 1 000 kg dry feedstock. Consequently, the total HHV of the residue varied considerably from 5 400 to 8 400 MJ per 1 000 kg dry feedstock (Figure 6.6). The potential ethanol production increased with an increase in pretreatment temperature for harvest residues and ranged from 156 – 204 kg ethanol/1 000 kg dry harvest residues (Figure 6.6), with the highest production for pressed slurry pretreated at the condition for maximum digestibility (205.8 °C and 8.7 min).

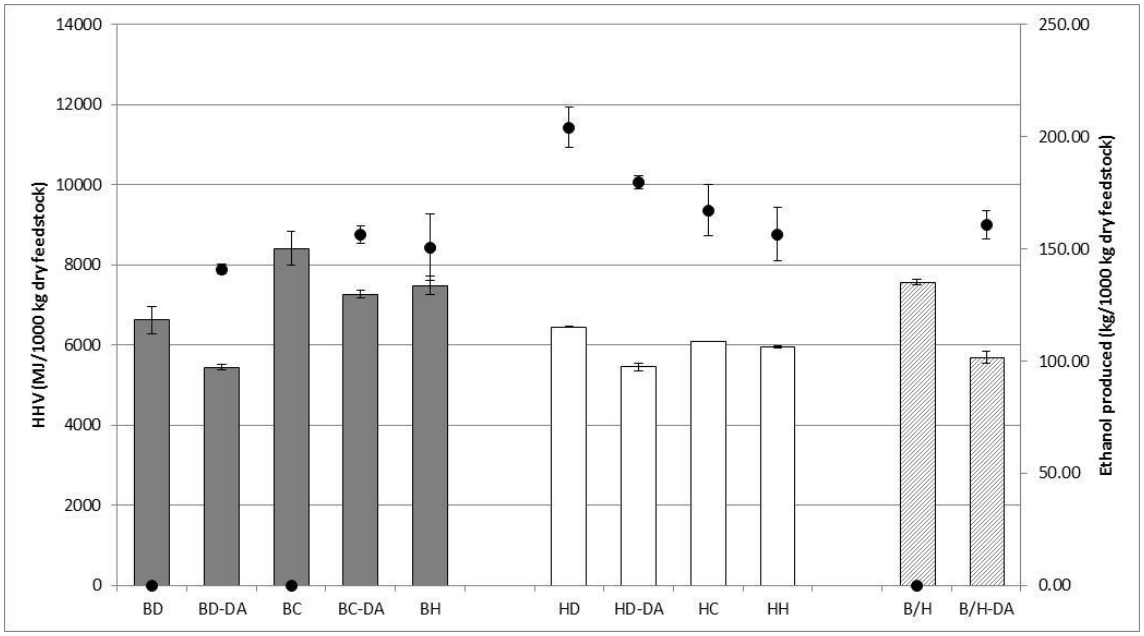

Figure 6.37 Overall fuel products produced per 1 000 kg dry feedstock feed steam pretreated at different conditions in this study. The bars indicate the HHV available in the fermentation residues produced per 1 000 kg dry feedstock pretreated and (●) indicate the ethanol produced per 1 000 kg dry feedstock pretreated. BD, BC, BH represent steam pretreatment of bagasse for maximum digestibility, maximum CSY and maximum hemicellulose recovery in the hydrolysate, respectively. HD, HC, HH represent steam pretreatment of harvest residues for maximum digestibility, maximum CSY and maximum hemicellulose recovery in the hydrolysate, respectively. B/H represent steam pretreatment of a 1:1 mass ratio blend of bagasse and harvest residues at 215 °C and 5 min. DA means that the feedstock was deacetylated prior to steam pretreatment.

6.4 Conclusions

Pressed slurries from steam pretreated bagasse and harvest residues, when pretreated at their respective optimal pretreatment conditions, exhibit different dewaterability and fermentability properties. The pressed slurries from bagasse steam pretreatment, when pretreated for maximum digestibility and maximum CSY, were not fermentable with the fast feeding method applied in this study. Harvest residues were found to be better suited for robust bioprocessing (bioconversion) into ethanol and chemicals than bagasse. Pretreated slurries from all the optimum pretreatment conditions for harvest residues displayed similar dewatering properties and the pressed slurries could be fermented at 5-15% solids loadings without detoxification with ethanol volumetric productivities ranging from 0.33 – 0.42 g/L.h^{-1} and theoretical ethanol yields ranging from 66.7 – 80.8%. Bagasse should preferably be allocated to boiler fuel. Blends of excess bagasse with harvest residues might negatively affect the fermentability properties of the feedstock, but more research is required in finding the optimum pretreatment conditions for blends of bagasse and harvest residues. Deacetylation was shown to improve the fermentability of completely inhibited pressed slurries of bagasse and a 1:1 mass ratio blend of bagasse and harvest residues to ethanol volumetric productivities ranging from 0.37 – 0.42 g/L.h^{-1} and theoretical ethanol yields ranging from 63.0 – 75.3%.

6.5 Acknowledgements

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CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

Harvest residues are better suited for bioprocessing in a sugarcane biorefinery than bagasse. Optimum uncatalysed pretreatment conditions were found for harvest residues in the temperature and time ranges studies that were relatively close to each other, namely 205.8 °C and 8.7 min, 199.6 °C and 9.5 min, and 193.7 °C and 12.7 min for producing pretreated material with target responses at maximum digestible solids, maximum CSY and maximum hemicellulose recovery in the prehydrolysate, respectively. Pretreatment conditions could be grouped around these conditions to create pretreatment regimes with responses predicted to be at least 95% of the predicted maximum target responses. The optimum pretreatment conditions of harvest residues were close enough, that when the regimes were set to contain all predicted target responses of at least 95% of the predicted maxima, the regimes overlapped. This overlap contained the approximate pretreatment conditions of 198 – 200 °C and 8 – 12 min.

In other words, harvest residues can be stably pretreated in a single pretreatment regime of overlap to produce pretreatment products of acceptable digestibility, CSY and hemicellulose recovery in the prehydrolysate. Bagasse, on the other hand, did not display an overlap of these pretreatment regimes at 95% of predicted maxima, as the predicted optimum pretreatment conditions were relatively far apart at the limits of the ranges

investigated at 215 °C and 15 min, 215 °C and 5 min, and 202.2 °C and 5 min for maximum digestibility of the solids, maximum CSY and maximum hemicellulose recovery in the prehydrolysate, respectively.

Furthermore, bagasse steam pretreated for maximum digestibility, as well as maximum CSY, ironically resulted in pretreated slurries that, when pressed, could not be fermented, because of too high concentrations of inhibitors. In contrast, all the pressed slurries from the harvest residues pretreated at the optimum pretreatment conditions for harvest residues were fermentable. The fermentability and ethanol production potential of harvest residues increased with pretreatment temperature and were the highest when pretreated for maximum digestibility (205.8 °C and 8.7 min). Final ethanol volumetric productivity and ethanol production potential ranged from 0.33 – 0.42 g/L.h-1 and 156 – 204 kg ethanol/1 000 kg dry harvest residues, respectively.

Deacetylation of feedstocks prior to uncatalysed steam pretreatment could successfully remove inhibitors, especially acetic acid, to improve fermentability of pressed slurries from pretreatment, even pressed slurries that otherwise would be completely inhibited. Deacetylation was however shown to significantly decrease the enzymatic hydrolysis performance of bagasse.

The steam explosion step at the end of steam pretreatment is not directly responsible for increasing accessibility of the cellulose by reducing the particle size. Mechanical deconstruction of the pretreated material occurs while the material is transported out of the pressure reactor with sudden depressurisation as the material is impinged against obstructions in the flow conduit.

7.2 Recommendations

7.2.1 Consider feedstock management in models

Bagasse and harvest residues are usually treated as a single lignocellulosic feedstock in sugarcane biorefinery models and, because of a lack of information on harvest residues, approximated as bagasse. However, future modelling should take cognisance of the different bioprocessing properties of bagasse and harvest residues. It is recommended that bagasse is allocated for cogeneration of electricity and steam, and harvest residues allocated to bioprocessing as far as possible.

7.2.2 Study the effects of different ratios of bagasse and harvest residues blends

In the case where bioprocessing is more attractive than electricity generation, surplus bagasse can be blended with harvest residues as feedstock for bioprocessing into chemicals. Further research is however still required to find the optimum pretreatment conditions for different ratios of bagasse and harvest residues blends. In this study blends of 1:1 mass ratios of bagasse and harvest residues were studied at a few steam pretreatment conditions and it was found that the resulting properties are not necessarily linear interpolations. The resulting contents of acetyl contents and ash of the blended feedstock have different effects on the CSF of the pretreatment. Further research is still required on the dewaterability and fermentability of blended feedstocks.

7.2.3 Increasing the recovery of harvest residues

Even though the maximum sustainable long-term recovery of harvest residues from the fields is still not known, life cycle and techno-economic assessments usually assume a 50% recovery of harvest residues from the fields. Since harvest residues are preferred for bioprocessing, further studies should be conducted to assess the impacts of higher harvest

residues recoveries from the fields and possible mitigating steps that can be taken to offset potential negative impacts of high recoveries of harvest residues.

7.2.4 Investigate different sugarcane harvest residues

Very little information is available in literature on the pretreatment behaviour of sugarcane harvest residues. The information that is available is typically from different types of harvest residues such as tops and/or leaves to further complicate comparison. Sugarcane harvest residues (tops and leaves in an approximate 1:1 mass ratio) from two locations in South Africa were blended into one feedstock (Malalane and Durban in an approximate 1:1 mass ratio) for this study. Further studies should be conducted to understand the variability in pretreatment behaviour of different harvest residues (tops, leaves and blends of tops and leaves), as well as harvest residues from different cultivars, locations and seasons.

7.2.5 Design steam pretreatment with increased mechanical impingement

The pressure differential of the steam explosion step does not directly contribute to the material deconstruction of pretreated material to improve digestibility, but it is rather the degree of mechanical impingement that is experienced by the pretreated material as it is transported from out of the reactor. It could therefore be possible to increase the deconstruction of pretreated material by improving the outlet design for increased mechanical impingement. Potentially this could offset the pretreatment severity required to allow for lower temperatures and pressures during steam pretreatment to decrease operating costs and sugar degradation. Further future study is recommended by comparing retained and released pretreated material under a range of pressure differentials during the explosion step. The range of pressure differentials can be created by gradually releasing steam from the pretreatment reactor just before the explosion. Material characteristics on a

cellular level should also be taken into account, as even though the explosion step has been shown not to contribute significantly to increasing digestibility on a particle level, it is possible that the explosion step could increase porosity or deconstruct material on a cellular level. Methods such as the Simon's stain method could help determine differences in surface area.

7.2.6 Valorisation of acetic acid

Deacetylation of bagasse and harvest residues prior to steam pretreatment has shown in this study to improve fermentability of downstream pretreated slurries. In a biorefinery approach the removal of acetic acid from the feedstocks could represent an additional value-adding by-product. It is therefore recommended to investigate processes for recovering acetic acid and reusing the alkaline stream.

7.2.7 Studying of acetyl groups to ash ratios

Deacetylation in this study removed a small fraction of the ash as well so that acetyl groups so that the ash constant could not be kept constant when comparing raw and deacetylated pretreated material. Future studies would benefit from either keeping acetyl groups content or ash content constant when comparing changes in the acetyl groups to ash ratio of a feedstock.

7.2.8 Optimisation of process steps downstream of uncatalysed steam pretreatment

The process steps downstream of uncatalysed steam pretreatment of bagasse and harvest residues in a sugar platform biorefinery, such as enzymatic hydrolysis and fermentation, should be optimised by utilising the optimum pretreatment conditions found in this study.

7.2.9 Optimum pretreatment conditions for bagasse

Uncatalysed steam pretreatment of bagasse seemed to have benefitted digestibility, hemicellulose recovery and CSY when pretreated above 215 °C for times less than 5 min. Future work should explore this pretreatment envelope to determine a single point of pretreatment operation for bagasse that still delivers acceptable digestibility, hemicellulose recovery and CSY.

7.2.10 Investigate alkaline steam pretreatment following deactylation

Washing of the feedstocks after deacetylation proofed to consume excessive amounts of water. In order to improve water intensity of a biorefinery operation, steam pretreatment of unwashed or slightly washed deacetylated material should be investigated.