SELECTION OF PREFERRED SWEET SORGHUM CULTIVARS AND THEIR PRETREATMENT OPTIMISATION FOR BIO-ETHANOL PRODUCTION

by

Paul Andrew McIntosh

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Supervisor
Prof. J.F. Görgens

Co-Supervisor
Dr. H.Cheng

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DECLARATION

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Summary

Currently the world is facing a global energy crisis due to rising energy demands, dwindling fossil fuels and increased greenhouse gas emissions. Countries are therefore looking at reducing fossil fuel reliability, mitigating emissions and meeting sustainable development goals. One such initiative has been the replacement of fossil fuel with bio-ethanol, which is both renewable and sustainable. Locally in the South African context, one of the potential bio-ethanol feed stocks under investigation is sweet sorghum. This feedstock is high in both soluble and non-structural sugars, it is drought resistant, has low input requirements, has high yields and can be grown over a wide range of climatic conditions.

Thirty-six sweet sorghum cultivars were therefore collected from the University of Kwa-Zulu Natal, where they were grown, before being transported to the University of Stellenbosch where they were they underwent a selection process coupled with a pretreatment optimization process aimed at maximizing potential bio-ethanol yields. The initial thirty-six cultivars were screened at a small scale with dilute sulfuric acid pretreatment at 170°C, 15 minutes and 0.7% H_2SO_4 combined with enzymatic hydrolysis which was carried out at an enzyme loading of 15FPU/g water insoluble solids (WIS), a pH of 4.8, a temperature of 50°C and a residence time of 72 hours Results showed statistically that increasing lignin and ash content negatively affected pretreatment response resulting in a range of combined sugar yields for the initial thirty-six cultivars of between 32.64g/100g raw material and 44.04g/100g raw material. From these results, the thirty six cultivars were reduced to ten by inclusion of pretreatment response yields and agronomic factors into total estimated ethanol yields from the whole plant. The top ten ranking ethanol producing sweet sorghum cultivars selected were SS27, AS254, AS246, AS103, AP6, AS106, MSJH13, AS245, AS248 and AS79.

Utilising two low severity pretreatments namely, 190°C, 5 minutes, 0.25% (w/w) H_2SO_4 and 200°C, 5 minutes, 0.07% (w/w) H_2SO_4 combined with two enzyme loadings of 3.75FPU/g WIS and 15FPU/g WIS, the previously selected ten cultivars were further evaluated and reduced to five through ranking of the average total potential ethanol yields for the two

pretreatment conditions and their corresponding enzyme loadings. The five cultivars which performed well under these conditions were AP6, SS27, AS103, MSJH13 and AS246 which subsequently underwent further optimization. Conditions investigated were 180 - 190°C, 5 – 15 minutes and 0.25% H_2SO_4 which resulted in an increase in combined sugar yields to between 48.83 and 54.5g/100g raw material on an oven dry basis. Selection of three preferred cultivars was based on the best average total potential ethanol yields calculated for the two seasons at each cultivars optimum pretreatment condition. Further one of the selected cultivars had to have poor pretreatment response at a small scale to allow for the effect of chemical composition to be evaluated in the steam explosion pilot plant. The three selected cultivars were AP6, SS27 and AS246.

Of the five previously selected sweet sorghum cultivars, AP6, SS27 and AS246 underwent optimization in a steam explosion reactor under air dried, water soaked and SO_2 catalysed conditions. Cultivars performed similarly under air dried and water soaked conditions but varied under SO_2 catalysed conditions. A 3% SO_2 catalysed steam explosion was most promising with yields of 87.2% to 91.48% of theoretical sugar content in the native biomass for the 3 preferred Sweet Sorghum cultivars. Combined with agronomic data this translated into potential bio-ethanol yields from the whole plant of between 7131 and 8678 L/ha grown under South African conditions. While these results are promising, further development of the three preferred sweet sorghum cultivars, AP6, SS27 and AS246, is necessary to implement these as dedicated bio-energy feed stocks.

Opsomming

Tans die wêreld in die gesig staar 'n wêreldwye energiekrisis as gevolg van stygende energie vereistes, kwynende fossielbrandstowwe en verhoogde kweekhuisgasvrystellings. Lande is dus op soek op die vermindering van fossielbrandstof betroubaarheid, die vermindering van die uitstoot en die bereiking van volhoubare ontwikkeling doelwitte. Een so 'n inisiatief is die vervanging van fossielbrandstof met bio-etanol, wat beide hernubare en volhoubare. Plaaslik in die Suid-Afrikaanse konteks, een van die potensiële bio-etanol feed lêers wat ondersoek is Sweet Sorghum. Hierdie grondstof is hoog in beide oplosbare en nie-strukturele suikers, dit is droogtebestand, het 'n lae inset, het 'n hoë opbrengste en kan oor 'n wye verskeidenheid van klimaatstoestande gekweek word.

Ses-en-dertig soet sorghum-kultivars is dus versamel van die Universiteit van Kwa-Zulu Natal, waar hulle groot geword het, voordat dit vervoer word na die Universiteit van Stellenbosch waar hulle was dat hulle 'n seleksie proses, tesame met 'n behandeling optimalisering proses wat daarop gemik is die maksimalisering van potensiële bio ondergaan -etanol opbrengste. Die aanvanklike 36 kultivars is gekeur op 'n klein skaal met verdunde swawelsuur suur behandeling by 170 ^{o} C, 15 minute en 0.7% H_2SO_4 gekombineer met ensiematiese hidrolise wat is uitgevoer uit op 'n ensiem laai van 15FPU / g water onoplosbare vastestowwe (WIS), 'n pH van 4.8, 'n temperatuur van 50 ^{o} C en 'n verblyf tyd van 72 uur Resultate het getoon statisties dat die verhoging van lignien en ash inhoud negatief beïnvloed behandeling reaksie wat lei tot 'n reeks van gekombineerde suiker opbrengste vir die aanvanklike 36 kultivars van tussen 32.64g/100g rou materiaal en 44.04g/100g rou materiaal. Uit hierdie resultate is, is die 36 kultivars tot tien verminder deur die insluiting van behandeling reaksie opbrengste en agronomiese faktore in totale geraamde etanol opbrengste van die hele plant. Die top tien posisie etanol vervaardiging van soet sorghum cultivars gekies was SS27, AS254, AS246, AS103, AP6, AS106, MSJH13, AS245, AS248 en AS79.

Benutting van 2 lae erns vir wysigings naamlik, 190 ^{o} C, 5 minute, 0.25% (w / w) H_2SO_4 en 200 ^{o} C, 5 minute, 0.07% (w / w) H_2SO_4 gekombineer met 2 ensiem beladings van 3.75FPU /

g WIS en 15FPU / g WIS , is verder die voorheen gekies tien cultivars geëvalueer en na vyf verminder deur die posisie van die gemiddelde totale potensiële etanol opbrengste vir die twee behandeling voorwaardes en hul ooreenstemmende ensiem beladings. Die vyf kultivars wat goed presteer onder hierdie toestande was AP6, SS27, AS103, MSJH13 en AS246 wat daarna verdere optimalisering ondergaan. Voorwaardes ondersoek was 180 - 190 ^{o} C, 5 - 15 minute en 0,25% H2SO4 wat gelei het tot 'n toename in die gekombineerde suiker opbrengste tot tussen 48.83 en 54.5g/100g rou materiaal op 'n oond droog basis. Keuse van drie voorkeur kultivars is gebaseer op die beste gemiddelde totale potensiële etanol opbrengste bereken vir die twee seisoene by elke kultivars optimale behandeling toestand. Verdere een van die geselekteerde kultivars het swak behandeling reaksie te hê op 'n klein skaal toe te laat vir die effek van chemiese samestelling om geëvalueer te word in die stoom ontploffing pilot plant. Die drie geselekteerde kultivars was AP6, SS27 en AS246.

Van die vyf voorheen gekies soet sorghum cultivars, AP6, SS27 en AS246 optimalisering in 'n stoom-ontploffing reaktor onder lug droog, water geweek en SO2 gekataliseerde voorwaardes ondergaan. Kultivars uitgevoer insgelyks onder lug gedroogde en water geweekte toestande, maar varyied onder SO$_2$ gekataliseerde voorwaardes. 'N 3% SO2 gekataliseerde stoom ontploffing was die mees belowende met opbrengste van 87,2% tot 91.48% van die teoretiese suiker inhoud in die inheemse biomassa vir die dire voorkeur sweet sorghum cultivars. Gekombineer met agronomiese data vertaal in potensiële bio-etanol opbrengste van die hele plant van tussen 7131 en 8678 L / ha gekweek onder Suid-Afrikaanse toestande. Terwyl hierdie resultate is belowend, verdere ontwikkeling van die drie voorkeur soet sorghum kultivars, AP6, SS27 en AS246, is nodig om hierdie as toegewyde bio-energie voer lêers uit te voer.

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1. Literature review

1. 1. Introduction:

For the last few years intensive research has been carried out in the field of renewable energies with the hope of finding one or a number of alternative sources of energy to fossil fuel. This interest has been driven largely by volatile oil prices, green house gas emissions and current world views on climate change, renewable energy and global warming.

Fossil fuels although previously cheap sources of energy are not considered to be sustainable, and as both the supply diminishes and the demand increases, the price of production will climb to levels which will affect individual economies. Furthermore, the burning of these fossil fuels results in a net release of CO_2 into the atmosphere which is known to be a direct contributor to increased trends in global warming observed over the past few decades [1].

Unfortunately during the 20th century major emphasis was placed on the development of fossil fuels as the principal energy source due to a number of reasons which include energy content, abundance and production price to name but a few. This has resulted in fossil fuels accounting for approximately 80% of the current primary world energy consumption, of which roughly 57% is consumed in the transportation sector alone [2].

An alternative to fossil fuel, which has been described as renewable, is solar energy which is directly incorporated into biomass through the photosynthetic pathway [3]. There is therefore a large global supply of solar energy in the form of plant biomass which includes energy crops and lignocellulosic residues both of which are important sources in the up and coming renewable energy revolution. Although the burning of these biomass type fuels, also commonly referred to as biofuels, release CO_2 into the atmosphere, the net release of green house gas emissions is minimal due to the fact that CO_2 will be consumed during photosynthetic growth of the applicable biomass[3]. Biomass energy is therefore regarded as an important and valuable alternative to fossil fuel and it is expected that this form of energy will become more prominent in the 21st century.

1.1.1. Current status and perspectives on world biofuel production

Trends in biofuel production have shown a dramatic increase over the last few years as countries have begun to realize their significance and importance. For example, between 1980 and 2005 an increase in world biofuel production from 4.4 to 50.1 billion liters occurred as countries realized that sustainable biofuel production is a significant contributor in reducing reliance on oil, meeting development goals and combating rising green house gas emissions [4].

Biofuel policies and implementation generally vary from country to country depending on a number of factors including the specific economics of biofuel production, the available feedstocks, political agendas and environmental concerns. Implementation issues such as incorporating biofuels into current fuel infrastructures and engine technologies also play a role in the rate at which fossil fuels can be supplemented with biofuels within each individual country. A number of limitations on the technical and commercialization aspects of biofuels have further slowed the rate of implementation resulting in vast amounts of resources being spent in overcoming these limitations through research into new feedstocks and production processes [4].

A number of alternatives to fossil fuel have been suggested including alcohol fuels which can be used as a replacement to petrol in spark ignition engines, while di-methyl ester, green diesel and biodiesel can be used as a substitute for diesel in compression engines. A number of hydrocarbon fuels can also be produced in the Fischer-Tropsch process of which the principal product is a fuel with similar characteristics to diesel [4].

Up until now, the majority of research in the development of biofuels has been centered on the production of a variety of liquid biofuels which could be used to replace both gasoline and diesel. Consider Table 1-1 below in which the main producers of biodiesel and bio-ethanol can be seen. The main region for biodiesel production is situated in Europe, in which France, Germany and Italy are the main producers. Europe currently also produces a little bio-ethanol but the majority of biofuel production in the form of bio-ethanol is produced in the USA and Brazil [2].

Table 1-1: Main bio-ethanol and biodiesel producers.

Bio-ethanol			Biodiesel		
Country	Million liters	Raw material	Countries	Million liters	Raw material
Brazil	16 489	Sugarcane	Germany	1919	Rapeseed
USA	16217	Corn	France	511	Soybean
China	1998	Corn & wheat	USA	291	Rapeseed
European Union	950	Sugar beet, wheat and sorghum	Italy	227	Rapeseed
India	299	Sugarcane	Austria	83	Rapeseed

Source: Escobar et. al [2].

1.1.2. South African perspective on biofuels

In 2006 South Africa imported roughly 21.5% of its total principal energy supply in the form of crude oil at an import value of R65 456 Million [5, 6]. The amount of crude oil imported per annum has since risen to R 98 613 million in the year October 2010 until the end of September 2011. Furthermore South Africa also imported R13 367 Million of distillate fuel and R10 645 million of petrol from October 2010 until September 2011. These three products, crude oil, distillate fuel and petrol accounted for 18.1% of South Africa's imports respectively and were the 1, 3rd and 4th most valuable commodities imported in this time period [7]. Therefore the development of a local biofuels industry would improve South Africa's energy security and reduce reliance on imported energy sources.

The feed stocks from which South Africa's liquid fuels have been produced are broken up as follows: 50% imported crude oil, 10% domestic crude oil, 30% coal and 8% natural gas [8]. In 2009 alone South Africa utilized 11.3 billion liters of petrol of which 10% could be substituted with ethanol, without modification to existing engines [9].

To date not much has been done in terms of creating a sustainable biofuel economy in South Africa even with the five year industrial biofuels strategy that was announced by the South African Department of Minerals and Energy on 5 December 2007. In this the Department set a 2% penetration target of biofuels into the South African market by 2012 utilising sugar cane and sugar beet as potential crops for bio-ethanol and sunflower, canola and soya beans

as crops for bio-diesel [10]. It is approximated that 100 000 to 150 000 direct jobs could be formed over the next decade as the blending target is lifted to 10% [11]. So far this target is a long way from being reached with the main reason attributed to the fact that mandatory blending of biofuel with current fuels has not been passed. Since the 2007 paper, the Department of Energy released a draft set of regulations in a recent Government Gazette with regards to mandatory blending of biofuels with fossil fuels, which once passed will provide stimulus to the industry [12]. While the draft set would have provided much needed stimulus to South Africa's biofuel economy, the actual regulations released in 2012 fell short and only stipulated how biofuels can be blended with current fuels but failed to enforce the actual blending of biofuels with petroleum fuels [13]. Further regulations are required to enforce and stimulate blending of biofuels with petroleum based products. In the meantime further research into biofuel production is needed in South Africa based on climatic conditions, utilization of South African crops, implementational issues, process technologies, economies of scale and so on to show that this technology is a viable alternative.

1.2. Lignocellulosic biomass as a renewable fuel

Lignocellulosic biomass is anticipated to be a major feedstock source for future energy demands based on both its abundance relative to food crops and its chemical composition [14]. Lignocellulosic biomass sources can be divided into three distinct groups as listed by plant taxonomists; namely softwoods (gymnosperms), hardwoods (woody angiosperms) and herbaceous crops (herbaceous angiosperms) [15]. The main structural and chemical component's of lignocellulose biomass includes holocellulose, lignin and extractive and non-extractive materials. Of these components holocellulose, which consists of the polysaccharides hemicelluloses and cellulose, makes up 60 – 70% of lignocellulose [15]

The composition of lignocellulosic biomass therefore varies according to the percentages of the three main components, cellulose, hemicelluloses and lignin, which can be useful in helping to identify and classify different biomass types [15]. The proportion of these three components for the three main groups of lignocellulosic biomass as classified by plant taxonomists can be seen in Table 1-2.

Table 1-2: Proportions of main components in different compounds

	Cellulose (%)	Hemicelluloses (%)	Lignin (%)	Lignin H/G/S-ratio (%)[a]
Softwood	41 – 50	11 – 33	19 – 30	2 – 18/82 – 98/ trace
Hardwood	39 – 53	19 – 36	17 – 24	0/22 -66/44 – 86
Herbaceous material	24 - 50	12 – 38	6 – 29	5 -26/27 – 54/23 – 67

[a]H/G/S ratio refers to relative lignin composition of 4-hydroxybenzyl (H), guaiacyl (G) and syringyl (S) units.
[b]Source: Klinke et al. [15]

Within each main group of lignocellulosic biomass, the composition will vary for each plant variety, cultivar as well as the climatic and agricultural conditions that the plant was grown under. A number of herbaceous plant materials can be seen in the Table 1-3 with regards to their chemical composition.

Table 1-3: Chemical composition of different herbaceous materials

	Sweet Sorghum bagasse[a]	Paja brava[b]	Corn stover[c]	Wheat straw[d]	Sugarcane bagasse[e]	Switch grass[f]	Bermuda grass[g]	Rye straw[h]
Cellulose	34 ~ 41.3	34.4	36.8	32.6	40.19 ~ 43.4	35.2	32.36	33.2
Hemicelluloses								
- Xylan	17.4 ~ 27.1	25.2	22.2	20.1	22.54 ~ 24.3	21.7	19.37	19.46
-Arabinan	2 ~ 4.8	3	5.5	3.3	2	2.8	4.33	2.47
Lignin	15.2 ~ 25.4	24.7	23.1	26.5	22.8 ~ 25.15	27.4	20.33	19.8
Extractives	ND	2.4	ND	3.3	1.6	ND	ND	ND
Ash	0.4 - 7.02	6.1	6.5	4.6	1.9	3.7	4.17	6.15
Acetyl	ND	1.2	1.7	2	2	ND	ND	ND

a – Mehmood et. al [16], Herrera [17], Zhang et al. [18], Gyalai-Korpos et al. [19], Kim et al. [20], Vasquez et al. [21]

b,c,d – Carrasco et al. [22]

e– Carrasco et al. [23], Neureiter et al. [24]

f – Ewanick and Bura [25]

g, h – Sun and Cheng [26]

1.2.1 Components of Lignocellulose

The following is a description of the different components present in lignocellulose.

1.2.1.1 Cellulose

Cellulose is a high molecular weight polysaccharide consisting of glucose polymers [15] . It consists of a continuous chain of D-glucose molecules which are linked in the β-1-4 configuration, as can be seen in the Figure 1-1. The cellulose chains/micelles are bunched closely together to form thread like micro fibrils that are extremely resistant to enzymatic attack. The bonds between the micro fibrils and linkages are strong intra and intermolecular bonds which give rise to crystalline regions which are hydrophobic in nature and further increase resistance to degradation [15]. An example of the continuous cellulose chain can be seen in Figure 1-1 below.
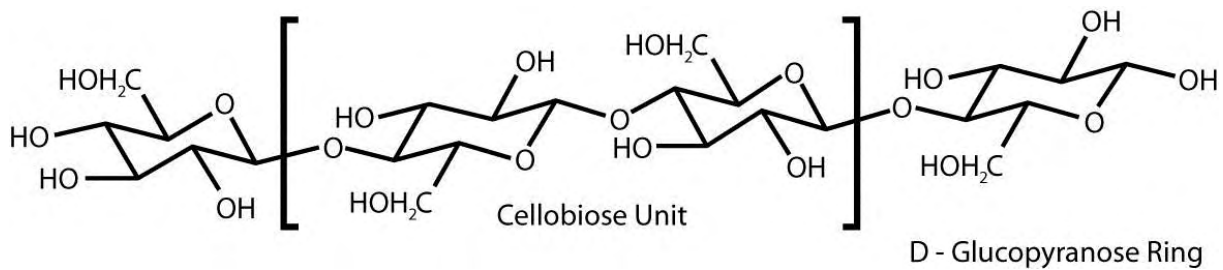
Figure 1-1: Cellulose, redrawn from Lachke et al. [27]

1.2.1.2 Hemicelluloses

Generally the hemicellulosic fraction of a specific plant material will consist of between 15 – 35% of the dry weight of the material, and will comprise of a number of components which could include the pentoses (β-D-xylose and α-L-arabinose), the hexoses (β-D-mannose, β-D-glucose, and α-D-galactose) and the uronic acids (α-D-glucuronic, α-D-4-O-methylgalacturonic and α-D-galacturonic acids). In both hardwoods and herbaceous material, the main hemicellulosic component of the secondary cell wall is xylan, while for softwood it is the mannan type hemicelluloses such as glucomannan and galactoglucomannan that make up the secondary cell wall [28]. For the non-woody material that we are investigating in this project and for other agricultural crops, the main component of the hemicellulosic fraction is arabinoglucuronxylan (arabino-4-O-metylglucuronoxylan). This type of hemicellulose consists of a linear β-(1,4)-D-xylopyranose backbone which contains 4-O-methyl-α-D-glucopyranosyl uronic acid and α-L-arabinofuranosyl which are

linked by α-(1,2) and α-(1,3) glycosidic bonds [28]. The linkages of xylan can be seen in the Figure 1-2.
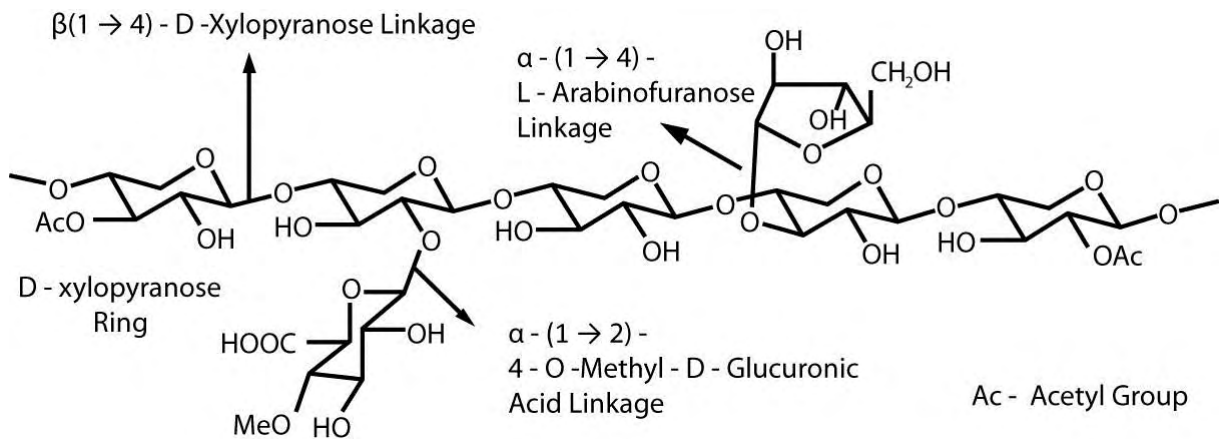
Figure 1-2: Xylan structure, redrawn from Lachke et al. [27]

1.2.1.3 Lignin

The third major chemical component of lignocellulose is lignin, a polycondensate of dehydrogenated products that is made up from the three lignin precursors, p-coumaryl alcohol, coniferyl alcohol, sinapyl alcohol. The three predominant types of aromatic rings usually present in the lignin monomers are p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S), which are found in varying ratios depending on the respective taxonomy of the plant in question [15]. The three aromatic rings can be seen in the following diagram.
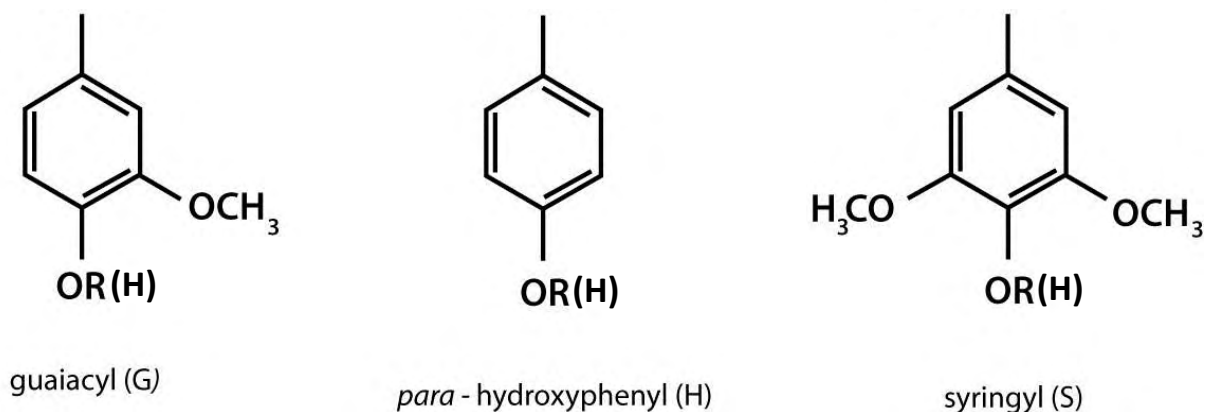
Figure 1-3: Lignin rings, redrawn from Klinke et al. [15]

Lignin is one of the major components that has been found to contribute to the recalcitrant nature of lignocellulose. It is known to interfere with the ability of hydrolytic enzymes to

effectively access the carbohydrate portion by adsorption of the enzymes onto the lignin while also obstructing enzymes due to the carbohydrates being embedded in the complex lignocellulose matrix which includes lignin [29]. Furthermore, lignin has been known to restrict cellulose from swelling which in turn reduces and limits the accessible surface area available to hydrolytic enzymes [30]. One other mechanism of inhibition by lignin is that during pretreatment the molecular weight of lignin is reduced and coupled with its hydrophobic tendency and its surface tension with water result in the deposition of small spherical deposits of lignin on fiber surfaces which is thought to further limit the accessibility of enzymes to cellulose and hemicelluloses [30].

1.2.1.4 Extractives in lignocellulose

Extractives is the group name given to low weight molecular substances present in biomass and may consist of waxes, tannins, fats, resins, alcohol, organic acids, nitrogenous material, chlorophyl, inorganic ions and non-structural sugars depending on the biomass [31, 32, 33].

Under strong acidic pretreatment it has been reported that some of these components can condense and form insoluble substances that act similarly to lignin and lower the enzymatic hydrolysis yield of pretreated material [33, 34]

1.2.1. Sweet sorghum

1.2.1.1. Overview

Plant materials that are high in soluble or non-structural sugars are preferred for bio-ethanol production as they offer a highly convertible carbohydrate which could be utilized as a high worth product. The major benefit of soluble sugars is that they are readily available, require low energy inputs for extraction and processing, require lower amounts of chemicals and the technology used to extract the sugars is efficient and well established. One such plant is Sweet Sorghum which is both highly adaptable and sugar rich [35].

In terms of production, this crop is the world's 5th most important grain crop after wheat, maize, rice and barley. Sorghum has now been bred into four main distinct groups; namely grain (flour, beer), fiber (fiber board, paper and cardboard), multi-purpose (grain, sugars, fiber and fodder) and sweet (primarily sugars) [36]. Both the grain and sweet sorghum varieties are of agricultural interest due to their drought resistance, high yields, relatively low input requirements and their ability to be grown over a broad range of climatic and

environmental conditions [35, 37]. These specific qualities make sorghum a prospective feedstock that could be utilized in climatic and environmental regions that are not favourable to farm starch rich crops such as maize or sugar cane [38].

Of the agricultural residues sweet sorghum has been of particular interest due to its large potential for expression of fermentable sugars [35]. Sweet sorghum is also a dual purpose crop as it can produce both high yields of grain (primarily present in the head of the plant) and sweet juice (primarily present in the stem of the plant) and has shown to have the potential to produce 3000 – 8000 L bio-ethanol per hectare [39]. Furthermore, Sweet sorghum is not favoured for refined sugar production due to its high starch content which is known to interfere with sucrose crystallization while accelerating the inversion of sucrose to the sugar fructose and the sugar glucose [40]. This plant originates or belongs to the *Sorghum bicolor* L. Moench species and is a part of the *Andropogoneae* tribe of the family *Poaceae* which also includes grain and fiber sorghum as well [41, 42]. There are virtually no observed taxonomic or biological differences between the three cultivated forms (sweet, grain and forage) and their difference originates mainly from both human and natural selection based on desired end use [37]. Sweet Sorghum uses the C4 photosynthetic pathway which allows it to more efficiently convert available sunlight into stored energy mostly by eliminating photorespiration through concentrating carbon dioxide around Rubisco [43]. It is speculated that this C_4 crop was initially farmed in east Africa and was then spread firstly to other African countries followed by Europe, Australia, Asia and the United States [41].

Sweet sorghum accumulates high yields of sugars in its stalk and up until recently has mainly been used for syrup and forage production, where the syrup can be converted to sugar and/or ethanol and the forage can be used for animal fodder. In forage application the leaves, stalks and grain can be used by ruminant livestock [41, 44]. As an added advantage, it can be incorporated into the sugarcane processing industry due to its similarities with sugar cane, and can be grown to be harvested during the idle period in the sugar cane industry [36, 40]. Furthermore it has been said that the cultivation cost of sweet sorghum is roughly a third that of sugar cane, which can partly be attributed to its lower water and fertilizer requirements [37]. Sweet sorghum will also outperform sugarcane when it comes to total biomass production over short periods [36]. Recently Gnansounou et al. [41] studied four processing options for Sweet Sorghum in which the sweet juice could either be converted to

white sugar or ethanol and the bagasse could be converted to ethanol or burnt for energy. Interestingly they found that the best economic option was to convert the bagasse to ethanol no matter whether the sweet juice was converted to ethanol or white sugar. It is therefore desirable that Sweet Sorghum be evaluated in the South African context to determine whether it is suitable for bio-ethanol production, both from the juice and the fibrous components.

In terms of the biomass production characteristics of *Sorghum Bi.color* L.Moench, two different sorghum cultivars were recently cultivated at three different locations in Texas, USA. It was found that total biomass yields for these cultivars varied according to location from around 10 – 100 tons/ha showing the importance of selecting a good location for planting and harvesting of sorghum [45]. Further, the composition of sorghum may vary greatly between the different types of Sorghum, whether it is a grain, sweet, and forage sorghum [44].

1.2.2. Sweet sorghum in the South African context

Within the South African context *Sorghum Bicolor* L.Moench is the third most valuable grain crop after maize and wheat and the second most important in Africa [46]. Between 2002 and 2007, the average production of grain sorghum in South Africa was around 225 000 tons per annum of which 90% was used for food production in the form of malt, meal, rice and grits (for brewing) and 10% for animal feed [47]. Of this, the Free State produced on average around 54% of the total domestic sorghum crop, followed by Mpumalanga (28%), Limpopo (7%), North West province (5.8%) and Gauteng (5%) [47]. To date no research has been done into second generation ethanol production in the South African context from sorghum. In South Africa, sorghum cultivars are generally classified according to three general categories. They are [47]:

- Class GM – sorghum containing low tannin content, known as sweet sorghum and which is suitable for malting and milling.
- Class GL – sorghum containing low tannin content, known as sweet sorghum and which is suitable for milling and animal feed purposes. Sweet sorghum also has a high soluble sugar content which makes it suitable for production of ethanol.
- Class GH – sorghum that has a high tannin content, also known as bitter sorghum and which is used mainly for industrial malting

1.3. Bio-ethanol:

Bio-ethanol is an attractive feedstock as it is a renewable bio-based resource. When used as an oxygenate, bio-ethanol offers many advantages over petroleum based fuels due to its oxygen content of 35% which results in a reduction in the particulate and NO_x emissions which are released during combustion [3]. Furthermore compared to petroleum based fuels, bio-ethanol has also been found to have a wider range of flammability limits, an increased octane number, faster flame speeds and increased heats of vaporization than petrol, which results in a much shorter burn time, a leaner burning engine, and a higher compression ratio. These benefits theoretically lead to efficiency advantages of using bio-ethanol as a substitute for petrol in an internal combustion engine. The main disadvantages of using bio-ethanol as a substitute to petrol includes its decreased energy density, which is 66% of that found in petrol, its corrosiveness (due to its hydroscopic nature), lower flame luminosity, its miscibility with water and its decreased vapour pressure making engine starts from cold difficult [48].

A major benefit of bio-ethanol is its potential for use as a mixed fuel with petrol, without much modification of existing internal combustion engines [3]. Mixing ethanol with petrol has been found to improve both the quality and performance of petrol as a result of ethanol's specific characteristics [4]. In a fuel mixture, bio-ethanol can be mixed with petrol from a 10% ethanol to fuel ratio known as E10 up until E85 which consists of 85% fuel ethanol. For the lower mixtures, i.e. E10, normal internal combustion engines can be used while for the higher ethanol mixtures, i.e. from E10 up to E85 or E100, engines that are known as flexi fuel engines which can handle the higher ethanol content should be used [48].

Production of bio-ethanol is usually classified based on the type of feedstock utilized for production. The three main feed stock options are i) sucrose containing feed stocks (such as sugar cane, sugar beet and sweet sorghum) ii) starchy materials (such as corn, barley and wheat) and iii) lignocellulosic biomass (such as wood, agricultural residues and grasses). The first two options (sucrose and starch containing options) are classified as first generation feed stocks, while the third option (lignocellulosic biomass) is classified as a second

generation feedstock [48]. The potential ethanol that can be produced using these three feedstock sources can be found in Table 1-4.

Table 1-4: Bio-ethanol potential of different feed stocks

Feedstock	Feedstock type	Bio-ethanol potential (l/ton dry mass)
Sugar cane	Sucrose containing	70
Sugar beet	Sucrose containing	110
Sweet sorghum	Sucrose containing	60
Potato	Starchy	110
Cassava	Starchy	180
Maize	Starchy	260
Rice	Starchy	430
Barley	Starchy	250
Wheat	Starchy	340
Bagasse and other cellulosic biomass	Lignocellulose	280

Source: Balat et al. [48]

In 2009, the production of fuel ethanol rose to 76 billion liters, increasing 10% from 2008 [49] showing a growing interest in bio-ethanol. The majority of this was produced through first generation processes, while a small fraction was produced through second generation processes. In terms of second generation production of bio-ethanol, the majority of this production is from demonstration plants [50]. Once this second generation technology has been demonstrated to be viable it is bound to take off.

1.3.1. 1st generation bio-ethanol

The most well established 1st generation biofuel is currently bio-ethanol. This is produced by converting the sugars extracted from food and starch containing crops into ethanol through fermentation [4]. Generally the *Melle-Boinot* process is the most common fermentation process which is used to ferment monomeric sugars such as sucrose to ethanol in a batch process. In this process the feedstock is first weighed, then sterilized and the Brix value is

adjusted with H_2SO_4 to a value of 14 - 22° before being fermented. Following fermentation, the product is first separated, and the yeasts are then recycled and the ethanol distilled out [3].

In Brazil the process that is mainly employed for ethanol production is a fed batch fermentation in which the substrate (i.e. fermentable sugar syrup) is kept at a low concentration while allowing ethanol to accumulate in the medium. This process is advantageous as one can avoid both substrate and end-product inhibition by diluting both of these during the majority of the fermentation. This allows one to achieve higher volumetric productivities than one can in batch fermentation alone [3]. There are a number of other process methods by which one can produce ethanol such as repeated batch, continuous and continuous removal of ethanol but as this project is concerned mainly with second generation production of ethanol from lignocellulose these processes will not be discussed here.

1.3.2. 2nd generation bio-ethanol

Second generation bio-ethanol production refers predominantly to processes in which bio-ethanol is produced from lignocellulosic biomass through the conversion of both the cellulosic and hemicellulosic portions of the respective feedstock to monomeric sugars which are subsequently converted to ethanol through fermentation. There are a number of ways to produce bio-ethanol from biomass, of which the most important is the thermo chemical or biochemical route [51].

Using the thermo chemical route, there are two options available. The first route utilizes a combination of thermo chemical and biochemical processes in which the lignocellulosic biomass is first gasified to release synthesis gas consisting of predominantly hydrogen and carbon monoxide, which is then bubbled through specialized fermenters containing genetically engineered organisms capable of fermenting this gas to ethanol [51]. The second thermo chemical route does not utilize microorganisms at all and rather the syngas is passed through a reactor with specific catalysts that are able convert the syngas to ethanol [51],[52].

In terms of the biochemical route, most process configurations generally have similar designs including feedstock handling, hydrolysis, fermentation, distillation and steam production. The major difference is observed in the way that the hydrolysis step is performed. This gives rise to three main process routes, based on the hydrolysis, which can be utilized for the conversion of biomass to ethanol but it must be noted that lignocellulosic ethanol produced via these routes must be assessed against sugar and starch based bio-ethanol production in terms of (i) efficient depolymerisation of the available carbohydrates to soluble sugars (ii) efficient simultaneous fermentation of C5 and C6 sugars, and (iii) advanced process integration to minimise production cost [51, 53].

The first process route to be discussed is based on enzymatic hydrolysis which is preferred over acid hydrolysis as the formation of sugar degradation products is avoided and the utility and capital costs associated with enzymatic hydrolysis are lower than with acid hydrolysis [54, 55]. Enzymatic hydrolysis requires a pretreatment step to overcome the recalcitrant nature of lignocellulose which renders lignocellulose inaccessible to enzymes [51, 56]. There are a number of different pretreatments that can be employed to overcome the recalcitrant nature of lignocellulose such as steam explosion, dilute acid pretreatment, ozonolysis or physical pretreatment; but these are all responsible for the same thing, breaking up the lignocellulosic structure to make the substrate more susceptible for enzymatic hydrolysis [18, 24, 57 – 59]. Currently there is no single micro-organism capable of effectively and efficiently converting both C5 and C6 sugars into ethanol while producing an enzyme to digest the C6 sugar present in the solids fraction into simple monomeric sugars [51, 60]. For this reason, the product resulting from pretreatment is split into a solid and liquid fraction to produce a solid fraction which is rich in cellulose (C6 sugar) and lignin, and a liquid fraction which is rich in hemicelluloses (C5 sugars). The solid fraction is then subjected to enzymatic hydrolysis to release the C6 sugars which in the following step are fermented to ethanol. The enzymatic hydrolysis step can also be combined with fermentation in a single step in what is known as simultaneous saccharification and fermentation [61 – 63]. The liquid stream which is rich in C5 sugars can be fermented separately in a C5 fermentation or co-fermented with the C6 sugars in a simultaneous saccharification and co-fermentation [64]. The final product from the fermentation is distilled to produce ethanol with the remaining lignin used for power or steam generation [53].
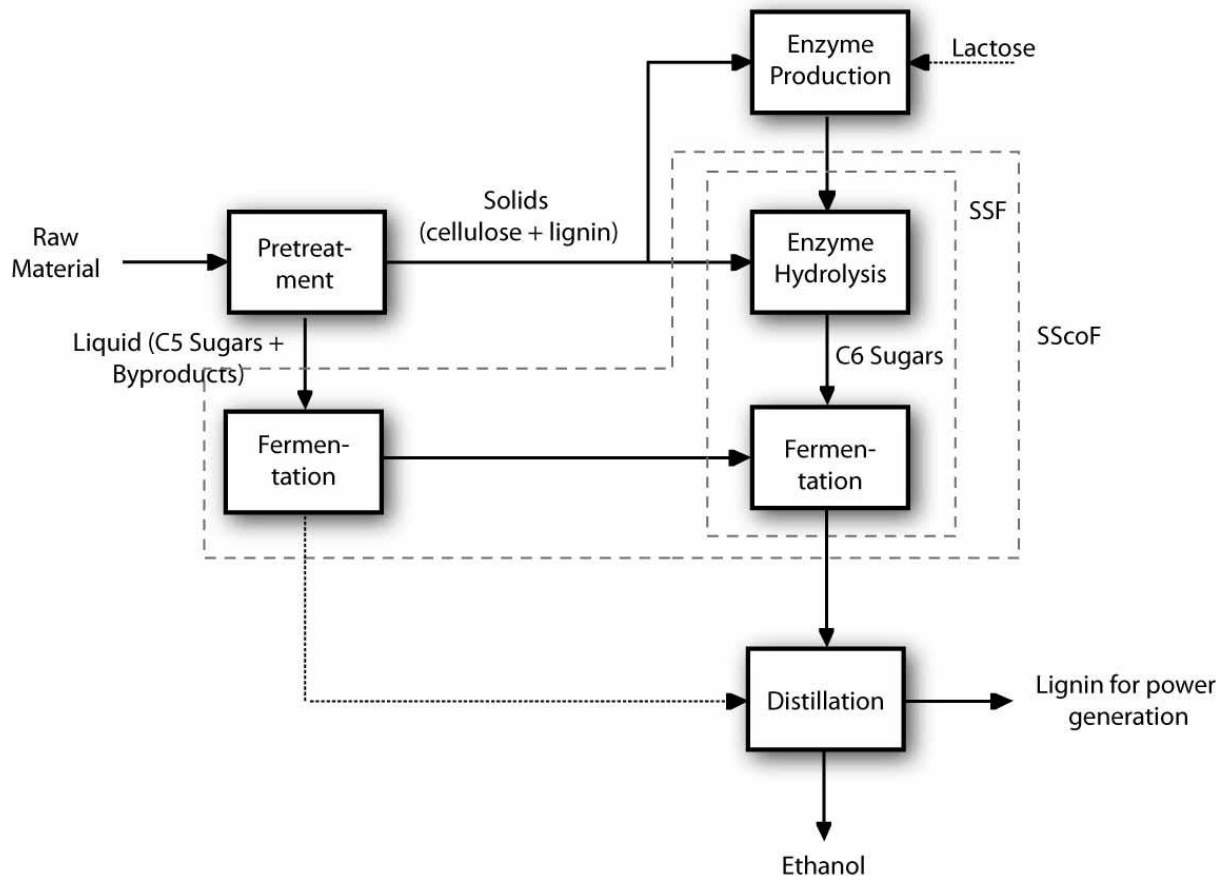
Figure 1-4: Bio-chemical production of ethanol, via pretreatment and enzymatic hydrolysis. Redrawn from Balat et al. and Von Sivers et al. [51, 53].

Currently the major barriers to commercial implantation of this technology include (i) the recalcitrant nature of lignocellulosic biomass to enzymatic attack, (ii) fermentative organisms that are inefficient in utilizing all of the available sugars simultaneously in a single fermentation, namely C5 and C6 type sugars [51, 56]. To overcome some of these barriers ongoing research is being carried out in the following process steps; feedstock preparation, pretreatment, hydrolysis, fermentation and separation/distillation of the final product [65].

Due to the recalcitrant nature of native lignocellulosic biomass, it is important to modify both the physical and chemical properties of the biomass to ensure that sufficient release of sugars can occur during hydrolysis that will theoretically result in high yields of ethanol [66]. An inter dependence can therefore be observed between the substrate (whether it be a grass, a hardwood, a softwood or an agricultural residue), the pretreatment and the way that the product will be eventually be processed. Furthermore, the choice of pretreatment conditions as well as type of pretreatment will substantially influence the physiochemical

characteristics of the pretreated biomass which will have a consequential effect in the following process steps including enzyme loading, choice of enzymes and microbes, whether preconditioning of microbial organisms is necessary, how by-products will be utilized, how waste will be processed, and hydrolysis and fermentation strategies to name but a few [65,67]. It should therefore be recognized that pretreatment will have a major cost implication on each of the downstream processing steps [67]

In terms of economic considerations including capital investment, labour, and other plant running costs; feedstock, pretreatment (including feedstock handling and milling, and associated equipment), biological processing (cellulase production, SSF, pentose conversion and associated equipment), distillation, power cycle and other costs have been described to represent 47.3%, 17.2%, 20.1%, 6.6%, 1.4% and 6.5% per unit cost of bio-ethanol. Leaving out feedstock costs, the cost breakdown to produce bio-ethanol is 32.7% for pretreatment, 39.6% for the biological component, 12.6% for distillation, 2.7% for the power cycle, 12.4% for other costs [67].

One of the areas in which costs need to be brought down to make the enzymatic route feasible is the cost associated with production of cellulase enzymes [67, 68]. Apart from re-use and recycling, reducing the enzyme loading, increasing the enzyme activity, reducing the cost of protein and increasing the overall hydrolysis yield are areas in which cost related improvements could be made [68]. In terms of reducing the enzyme loading, an effective pretreatment is required to remove lignin and to open up the lignocellulose matrix. Removal of lignin will prevent unproductive binding of enzymes to lignin and opening up the lignocellulose matrix will increase the surface area available for enzyme adsorption as well to increase accessibility of enzymes to the cellulosic fraction of the biomass. Bio-prospecting for enzymes is another way of looking for new enzymes that have high activities that would offer decreased enzyme production costs. Coupling this with new combinations of enzymes would offer further scope for cost reduction [68].

The second process route for biochemical production of ethanol from lignocellulose does not employ enzymes in the hydrolysis of the lignocellulose biomass but rather makes use of dilute acid in a two stage hydrolysis process [51, 53]. In the first stage the dilute acid is employed at low temperatures (130°C - 190°C) and longer residence times 10 – 30 minutes

in hydrolyzing of the hemicellulosic portion of the lignocellulose while the second stage utilizes dilute acid at higher temperatures (190°C - 265°C) and much shorter residence times (a few seconds to 5 minutes) with the aim of hydrolyzing the remaining cellulose fraction. The sugars from the 1st stage hydrolysis and 2nd hydrolysis can be either fermented separately or together in co-fermentation of C5 and C6 sugars. The product from the fermentation is then distilled to produce ethanol and a waste stream of lignin [53].
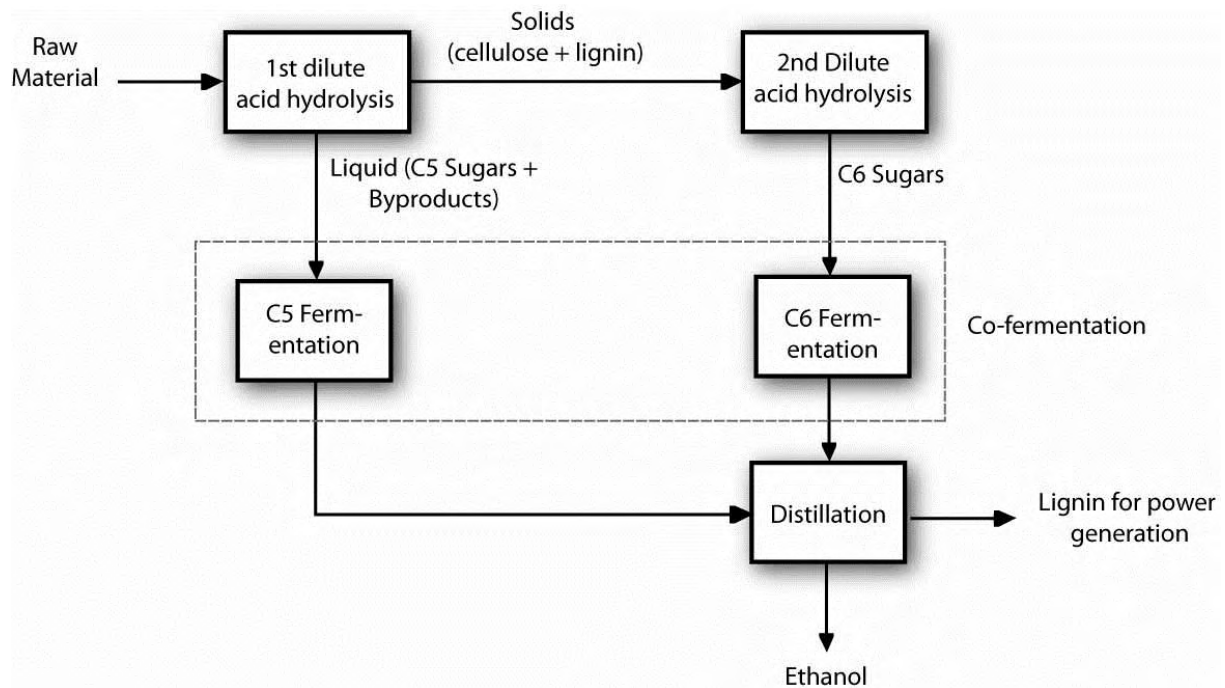
Figure 1-5: Bio-chemical production of ethanol, via pretreatment and fermentation. Redrawm from Balat et al., Von Sivers et al. and Balat et al. [51, 53, 56].

As a third process alternative, see Figure 1-6 below, concentrated acid is used to hydrolyse both the hemicelluloses and cellulose fractions simultaneously in a single step. Acids and acid concentrations that have been used efficiently but not limited to, include 41% HCL, 72% H_2SO_4, or 100% TFA [28]. Temperature is kept low (35 - 100°C) and reaction time varied between 5 and 60 minutes. This alternative has the added advantage that it is a single step hydrolysis process which is able to realize efficient and high sugar recoveries, up to 90% of the theoretical [53, 69]. The disadvantages are that the process requires an acid recovery system to recover and reuse the acid which is most often the most expensive part of the concentrated acid process and that there is high capital cost required for equipment that is resistant to acid corrosion. An additional requirement for this process is that the biomass must first be dried to prevent dilution of the concentrated acid [51, 53].
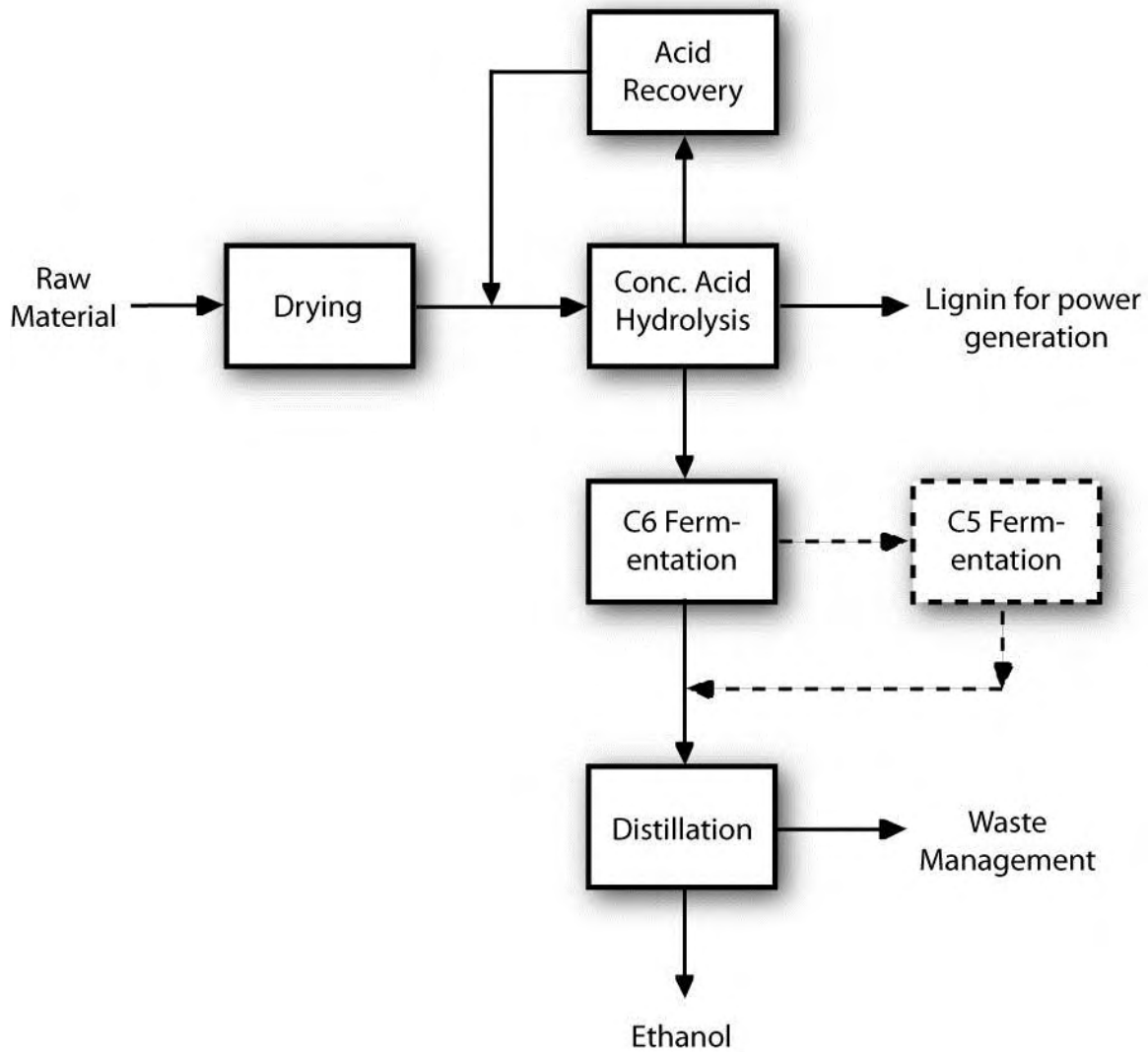
Figure 1-6: Bio-chemical production of ethanol via concentrated acid pretreatment. Redrawn from Von Sivers et al. [53].

Each of these main process steps are important in realizing high yields of ethanol with each step having a major effect on the subsequent ones. Pretreatment is concerned with facilitating the dissimilation of the lignin matrix so that lignin, hemicelluloses and cellulose can be separated [51, 56, 57]. Following this the exposed carbohydrates are hydrolysed into monomers through enzymatic hydrolysis which are then fermented in ethanol during fermentation. The final product from fermentation is then distilled to separate the ethanol out [4]. In terms of second generation production some of the main producers and the quantities they produce can be seen in the Table 1-5.

Table 1-5: Production of second generation ethano

Country	Company	Process	Quantity
Canada	Iogen – Demonstration	Modified steam explosion, enzyme hydrolysis, pretreatment	580 000L/year
Denmark	Inbicon	Hydrothermal pretreatment, enzyme hydrolysis, Fermentation	5.4 million L/year
Norway	Weylands	Concentrated acid hydrolysis and fermentation	200 000L/year

Source: Bacovsky et al. [50]

1.4. Pre-treatment

Pre-treatment methods generally refer to the solubilisation and separation of one or a number of the lignocellulosic biomass components, namely lignin, hemicelluloses, cellulose or extractives from the original lignocellulose structure making the remaining biomass more accessible to further biological or chemical processing [52]. While not all pretreatments solubilise and separate all of the above mentioned components, this process step is necessary in the efficient conversion of lignocellulosic biomass to bio-ethanol [51, 56].

1.4.1. Introduction: Goals of pre-treatment

The specific purpose of pretreatment is to disrupt and disorder the structure of lignocellulosic biomass and in some cases remove compositional and structural impediments that would inhibit and prevent efficient cellulose and or hemicelluloses hydrolysis (the process by which cellulose and hemicelluloses are broken down into their monomeric sugars)[51]. The main aim of pretreatment is therefore to increase the rate and extent of hydrolysis as well as the final yield of fermentable sugars, all of which would increase the cost effectiveness of lignocellulose biomass to ethanol [70]. Pretreatment is considered to

be the main determinant as to whether the lignocellulose to ethanol process is both a viable and cost effective technology as without this step, yields are far too low and the process too inefficient [51].

An effective pretreatment must therefore satisfy the following pretreatment goals. It must (i) increase the rate and extent of hydrolysis resulting in high yields of fermentable sugars, (ii) avoid the loss of both hemicelluloses and cellulose by degradation, (iii) prevent the formation of byproducts that would inhibit hydrolytic enzymes (if enzymatic hydrolysis is employed over chemical hydrolysis) and fermentative organisms in the succeeding hydrolysis and fermentation steps, (iv) be cost effective, (v) reduce the energy requirements, (vi) consumption of little or no chemicals, and (vii) reduce the need for size reduction of feed stocks [14, 51], 56]. The following diagram shows the disruption of the lignocellulose matrix to yield a hydrolysable material.
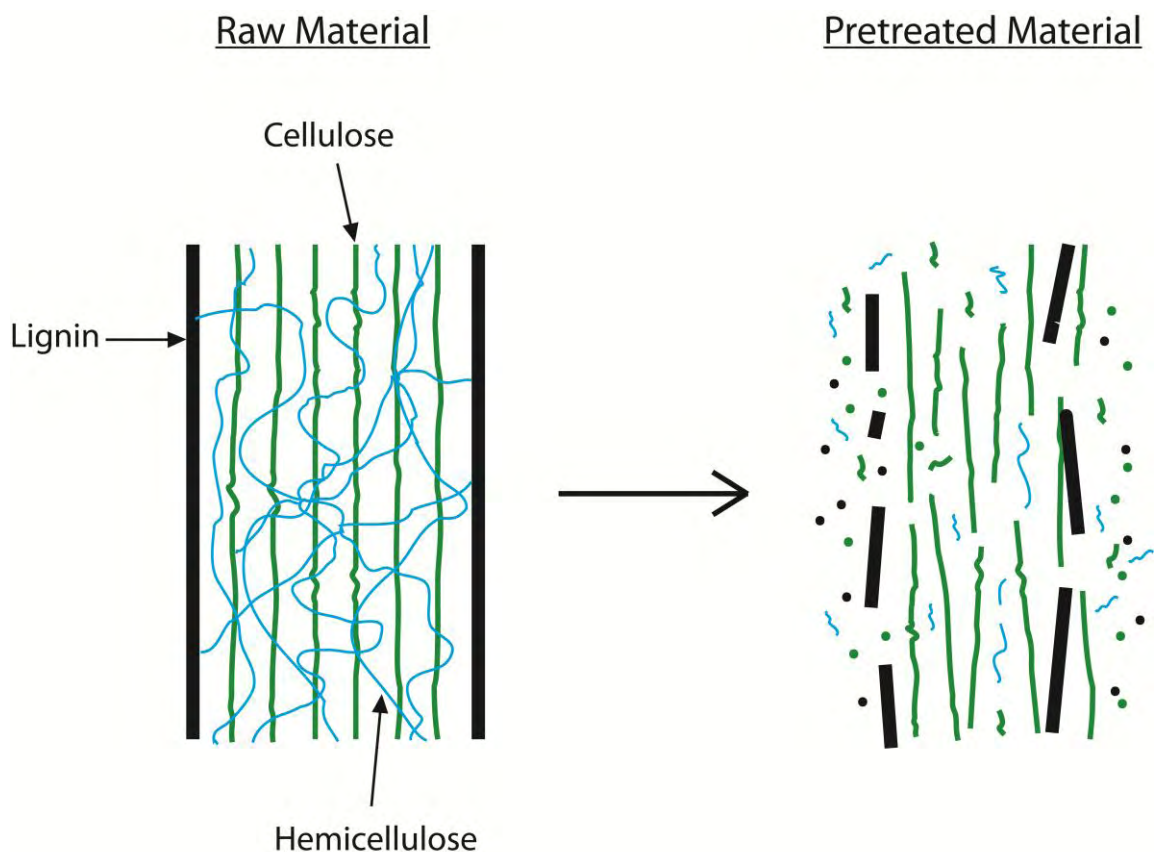
Figure 1-7: An effective pretreatment, redrawn from Zheng et al. and Hector et al. [64, 71].

It has been shown that without an effective and efficient pretreatment, the yield of cellulose hydrolysis by enzymes is less than 20%, while yields of over 90% are often exceeded with the aid of pretreatment, showing the importance of an effective pretreatment [72]. For

pretreatment, several processes have been proposed for the effective and efficient pretreatment of lignocellulose including physical, physio-chemical, chemical and biological processes [72]. These methods usually use a combination of different principles to achieve high yields, while at the same time reducing byproduct formation and high energy consumption [60].

Following pretreatment, hydrolysis of exposed cellulose and any remaining hemicelluloses can be carried out using chemical or biological methods. Chemical hydrolysis can usually be done simultaneously with chemical pretreatment, i.e. concentrated acid hydrolysis while biological hydrolysis must be carried out separately from pretreatment using enzymes in enzymatic hydrolysis. There are a number of other hydrolysis methods that do not employ chemicals or enzymes such as microwave, gamma-ray or electron beam irradiation but these have been found to be commercially unimportant and will therefore not be discussed in this review [52].

A number of pretreatment processes that are effective in producing hydrolysable products will be discussed in the following section.

1.4.2. Physical pretreatment

The physical pretreatment of lignocellulosic biomass can be achieved by a combination of grinding, chipping or milling. The aim of these specific processes is to increase the accessible surface area to enzymatic attack through decreasing the particle size, crystallinity and degree of polymerization [57]. However decreasing the particle size to that which is extremely small, often results in a high consumption of energy, which is undesirable. The initial moisture content and particle size of the respective raw material that is being processed has also been known to influence the energy consumption and performance of the following processing steps [60]. For example it was shown that the reduction of wheat straw from 0.8mm and 3.2mm with the aid of a hammer mill had a corresponding energy consumption of 51.6 and $11.4kW.h.t^{-1}$, which is much higher than the energy required to break up corn stover at a comparable moisture content [59]. An increased efficiency was reported in enzymatic hydrolysis from 17.7% for untreated material to 61.1% after 2 hours of ball milled pretreatment [73]. It is unlikely though that physical pretreatment alone will be a highly attractive pretreatment technology due to its high energy consumption and capital costs as well as its efficiency in increasing hydrolysis yields [72]. Previous studies on poplar

wood, switch grass, bagasse, ryegrass straw and corn fiber show that enzymatic digestibility is not affected by reducing the particle size below 40 mesh [74]. This should therefore be the limit of size reduction necessary to improve enzymatic digestibility using physical pretreatment.

1.4.3. Chemical pretreatment

A number of chemical compounds can be utilized for the efficient and effective pretreatment of lignocellulosic biomass. These include acids, alkalis, organic solvents and oxidizing agents [60, 72]. The use of these chemicals aids in disrupting the lignocellulosic structure through the removal of lignin and/or the solubilisation of hemicelluloses and cellulose type sugars.

1.4.3.1. Alkaline pretreatment

Alkaline pretreatment is a well studied and widely used chemical pretreatment technology. A number of bases are employed for this including sodium hydroxide, lime (calcium hydroxide), potassium hydroxide, ammonium hydroxide and aqueous ammonia [64].

Alkaline pretreatment is mainly a delignification process in which hemicelluloses sugars can be solubilized simultaneously or separately depending on the severity of the pretreatment [64]. The mechanism in which these sugars are solubilized and the material delignified is thought to be a saponification of the intermolecular ester bonds which are found crosslinking other components such as lignin to hemicelluloses [64, 74]. It has also been found the alkaline pretreatment can also efficiently remove both acetyl and various uronic acid substitutions present in hemicelluloses that can reduce the accessibility and effectiveness of enzymes to hemicelluloses and cellulose [74]. This type of pretreatment is also known to disrupt the lignin structure, which causes swelling of the polysaccharides leading to decreased crystallinity and degree of polymerization as well as the breaking of structural linkages between lignin and carbohydrates [64].

Factors that can be investigated with regards to alkaline pretreatment include time, temperature, type of catalyst and catalyst concentration/loading. Pretreatment with alkali can also be performed at room temperature for a couple of seconds up to a number of days. It has been found that alkali pretreatment causes less degradation of sugars to by-products

compared to acid hydrolysis due to the fact that alkaline pretreatment is primarily involved with delignification than hydrolysis of hemicelluloses and cellulose. This is a major benefit for subsequent fermentation steps [57]. Pretreatment with lime has many advantages over other alkalis such as cost, availability, safety issues and it is easily recoverable from the hydrolysate through reaction with CO_2 [66].

Wu et al. studied the alkaline pretreatment of two varieties of sweet sorghum to investigate the observed improvements in enzymatic hydrolysis yields [75]. In investigating the effects that different molar concentrations of NaOH have for different temperatures and time, they found that with increased temperatures, longer pretreatment times and with increased alkaline concentrations both the lignin and xylan removal rates where increased while only slight losses of glucan were observed. This had a correlating effect on enzymatic digestibility which increased with increasing pretreatment severity. The highest conversion (98.7%) of cellulose to glucose was observed when pretreating the bagasse at room temperature for 120min using 2.5M NaOH. The authors postulated that this digestibility was attributed mostly to the disruption of the hemicelluloses-lignin matrix due to the removal of lignin [75]. A similar result was observed by Gyalai-Korpos et al. in which lignin removals of between 2 and 7% with increasing pretreatment severity were seen [19]. Gyalai-Korpos et al. also found that the best pretreatment conditions for yields of sugars from enzymatic hydrolysis yields were different to those that gave the highest resulting ethanol yields. Although they did not give the exact reasons for this, they mentioned that acetic acid formation was detected in every sample which could potentially inhibit the fermentation yields, suggesting that different amount of byproducts could be present based on the respective pretreatment conditions [19]. This can further be confirmed comparing the pretreatment conditions which gave highest ethanol yields and that which gave highest hydrolysis yields as the highest ethanol yields were achieved at room temperature compared to high hydrolysis yield which were achieved at 121°C. And from Wu et al. we know that degradation rates of hemicellulosic sugars were increased with temperature resulting in more degradation products than for a pretreatment carried out at lower temperatures [75].

In another work in which the effectiveness of dilute ammonia pretreatment of sorghum was evaluated for its effectiveness on enzyme, a 54% increase in cellulose digestibility was observed upon using a dilute ammonia pretreatment compared to no pretreatment which

was attributed to the fact that the pretreatment removed 44% of the original lignin resulting in an increased surface area and increased porosity of the biomass [76].

Although alkaline pretreatments are effective in increasing the available surface area as well as the pore size through delignification of the biomass, a limitation of this type of pretreatment occurs with the conversion of some of the alkali to irrecoverable salts as well as incorporation of salts into the biomass during pretreatment reactions [66]. Alkaline pretreatments have also been found to be more effective for agricultural residues and herbaceous crops over woody materials and are therefore not suited for all feed stocks [58]. Added to this the reaction time for alkaline pretreatment ranges from hours to days which is substantially longer than other pretreatment methods require and costs associated with this pretreatment is substantially high [14, 51]. These disadvantages have so far prevented commercialization of lignocellulose to bio-ethanol process using alkaline pretreatment methods.

1.4.3.2. Pretreatment with ionic solvents

Recently pretreatment of cellulosic biomass with ionic liquids also known as "Green Solvents" has received a great deal of interest. Typically Ionic liquids are salts comprising large organic cations and small inorganic anions which are able to exist in the liquid state at relatively low temperatures [57]. The inherent characteristics of these solvents can be modified by adjusting the alkyl and anion constituents. Some of the interesting properties of Ionic solvents include chemical and thermal stability, non-flammability and low vapor pressures [57, 77].

It has been discovered that certain ionic liquids with anion activity can dissolve both carbohydrates and lignin simultaneously due to the formation of hydrogen bonds between the non-hydrated chloride ions of the ionic liquid and the sugar hydroxyl protons [57]. The main benefit of this has been described as the effective disruption of lignocellulose while minimizing the degradation product formation [57].

1.4.3.3. Dilute Acid pretreatment

Alternatively pretreatment can be performed using dilute acid which has been applied to broad range of feed stocks including softwoods, hardwoods, agricultural residues,

herbaceous crops, municipal solid wastes and paper sludges. Several different dilute acids have been studied including nitric acid, sulfuric acid, phosphoric acid, hydrochloric acid and peracetic acid [64]. Dilute acid has been viewed primarily as a pretreatment step which hydrolyses the hemicelluloses fraction making the cellulosic fraction amenable to enzymatic hydrolysis. Even though both hemicelluloses and cellulose can be hydrolysed with dilute acid, this is not preferred as the temperature required for hydrolysis of hemicelluloses and cellulose differs by about 50°C and the temperature required to hydrolyse cellulose leads to the formation of large amount of degradation products [28]. Although dilute acid pretreatment is involved mainly with hydrolyzing hemicelluloses into sugars, lignin is simultaneously degraded. At higher temperatures the hydrolysed lignin is known to recondense as pseudo-lignin onto the lignocellulose matrix during the cool-down after pretreatment and in some cases onto the surface of the fibre which can decrease the susceptibility of the biomass to enzymatic hydrolysis [78, 79]. The main advantages of dilute acid pretreatment compared to a concentrated acid process includes low acid consumption, the reduced equipment corrosion and reduced energy requirements to recover any remaining acid [28].

Many studies have been performed with dilute acid to maximize either xylose yields from pretreatment or glucose yields from subsequent enzymatic hydrolysis, but not many have been performed to maximize the yields from both simultaneously. Lloyd and Wyman investigated this and from their results it is evident that, upon increasing the severity of the pretreatment, the maximum xylose yields are reached first, followed by maximum total sugar yields and finally maximum glucose yields [80]. This makes sense as the best glucose yields will be achieved once all the xylose has been removed resulting in an increased accessible surface area for enzymatic action, but this comes at an excessive loss of xylose to degradation. It was also noted that for a particular H_2SO_4 concentration, the xylose release and degradation was accelerated as the temperature was increased [80]. In another study this was confirmed in which it was observed that the peak for xylose yield occurred at shorter and shorter pretreatment times as pretreatment temperature increased with a corresponding faster degradation result which suggests that temperature increases the aggressiveness of the acid [81]. This effect can be seen in the Figure 1-8 below.
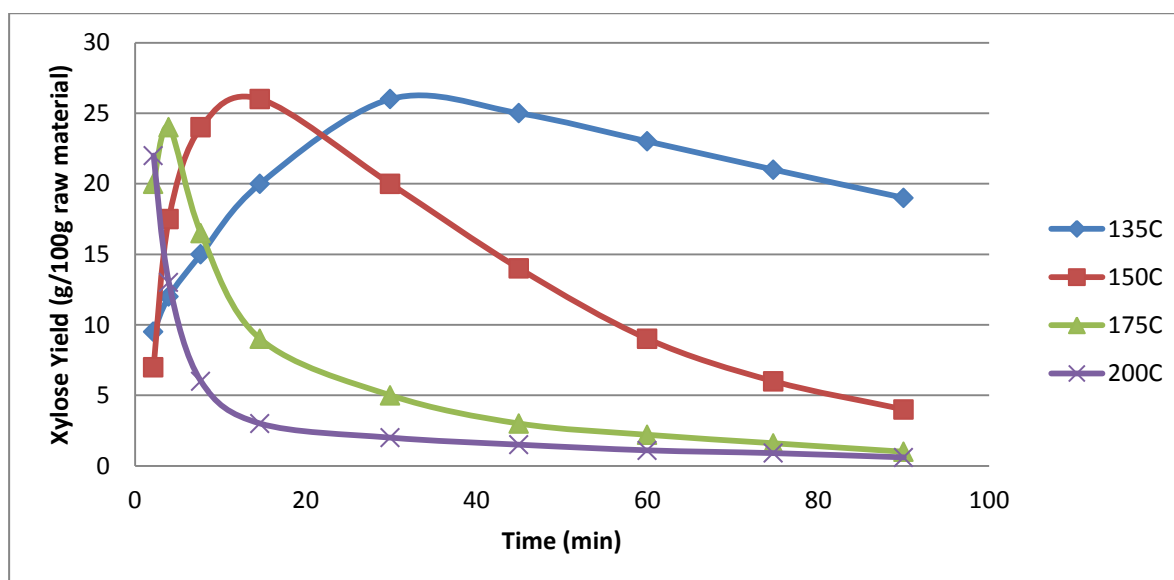
Figure 1-8: Xylan removal at different temperatures and times, redrawn from Lehinan et al. [81].

Lenihan et al. showed that increasing the acid concentration results in increasing the reaction rate at a specific temperature [81]. These two studies therefore suggest that the optimal sugar production can be achieved at a different combination of temperature, time and acid concentration which means that it must be decided beforehand which of these three one wants to minimise the most. This was also found by Jensen et al., who said that optimum conditions should be selected based on techno-economic and lifecycle environmental analyses [82]. Generally high acid concentrations in pretreatment are not desired as they result in high capital equipment costs to overcome corrosion problems. Similarly long pretreatment times are not desired as these result in lower productivity, which is not beneficial for the economics of an industrial plant [67, 82]. Processes that use higher temperatures up to a maximum of $250^{\circ}C$ are therefore desired as they don't result in such a large increase in capital expenditure but reduce the acid concentration and the pretreatment time [82].

In terms of the effect that chemical composition of biomass has specifically on dilute acid pretreatment, Castro et al. found that that different biomass types have different particular neutralising capacities as a result of cations present in the lignocellulosic matrix. They found that rapeseed straw could neutralise 19.7mg H_2SO_4/g dry biomass [83]. This is important to take into account when evaluating different varieties of a specific biomass such as sweet

sorghum in our case. The reason for this is evident as different sweet sorghum cultivars will most possibly have varying amounts neutralising agents such as ash and minerals which will in turn affect both the pretreatment and subsequent hydrolysis yields which could possibly be used to explain why under the same acid concentrations some varieties perform better than others. An increased neutralising capability of a feedstock will also increase its dependence on acid over temperature and time as was the case for switch grass compared to aspen and balsam wood [82].

Lignin, one of the main components of lignocellulose responsible for the recalcitrant nature of biomass has a major effect on the effectiveness of pretreatment [29]. Fu et. al explored this by genetically modifying switch grass to reduce the syringyl to guaiacyl ratio as well as the total lignin content and then subjected the biomass to a number of pretreatment conditions [29]. The main findings were that downgrading the biomass in terms of the lignin positively affected the final ethanol yields, with some conditions resulting in 38% more ethanol produced for the downgraded biomass compared to the control. Other interesting results were that less severe pretreatments and lower enzyme dosages (by 300 – 400%) were necessary for the same ethanol yield with the downgraded switch grass [84]. Furthermore Davison et. al looked at the effect that variation of both the S/G ratio and the lignin content have on the release of xylose with dilute acid hydrolysis [85]. They found that small incremental steps in the S/G ratio had a significant negative effect on the xylose yields, while increased lignin content, although not significant, showed decreased xylose yields as well. Similarly decreased lignin content in *sorghum bi.color* (L.Moench) resulted in higher glucose yields following dilute acid pretreatment [86]. Therefore, we can expect that the sweet sorghum cultivars with lower lignin content should respond well to pretreatment compared to sweet sorghum cultivars with higher lignin content. Lignin also responds differently to pretreatment based the conditions it is exposed to. At temperatures above its phase-transition point, it becomes fluid and exits through the cell wall matrix and into the pretreatment liquor and then depositing on the residual surface of the biomass upon cooling [87]. Alternatively at higher temperatures lignin has been known to break down into soluble compounds that then react with each other to from long chains that precipitate onto the lignocellulose matrix [88].

As dilute acid pretreatment is a process step in the lignocellulose to ethanol process, it is important that we understand the effect that pretreatment has on lignocellulose which will affect subsequent process steps.

In terms of comparing pretreatments performed in different reactors Castro et al. and Diaz et al. both suggested a modified severity factor to take into account the heat up and cool down time of the specific reactor [83, . This modified equation is seen below

$$R_0 = \int_0^t \exp\left(\frac{T(t) - 100}{14.75}\right) dt$$

Where R_o = severity factor,

             t = time,

             T   = temperature

This equation basically allows one to compare pretreatments in different reactors by calculating the R_0 of each pretreatment by assuming an instantaneous heat up and cool down time. For this specific research project this idea can be used to compare pretreatment in small scale reactors with pretreatment performed in the pilot plant steam explosion reactor that we have here at Stellenbosch. Although the above equation takes into account both the pretreatment time and temperature, it does not take into consideration the effect that acid has on the biomass. To include the effect of acid concentration the following equation can be expanded as follows:

$$CSF = \log R_0 - pH$$

where CSF stands for combined severity factor and pH is that of the resulting aqueous solution following pretreatment.

The kinetics of xylan hydrolysis during dilute acid pretreatment has generally been described by two models, namely the Saemon model and the two fraction model. Of the two, the two

fraction model has been found to fit the data better than the Saemon model for most studies [89]. The two fraction model proposes the following for the hydrolysis of xylan

$$\text{Xylan} \overset{k1}{\rightarrow} \text{xylose} \overset{k2}{\rightarrow} \text{degradation products}$$
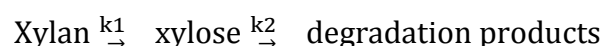
Where k1 is known as the rate constant (/min) associated with the release of xylose from xylan and k2 is the rate constant (/min) associated with the degradation of xylose. In most cases the release of xylose from xylan and the degradation of xylose to certain degradation products are considered to be first order reactions with the following equation holding [89].

$$\frac{M}{P_0} = \propto . \frac{k1}{k2 - k1} . \left(e^{-k1.t} - e^{-k2.t} \right)$$

where M and P_o are the concentration of xylose and xylan respectively, t is the hydrolysis time and α denotes the mass fraction of the susceptible xylan in the feedstock [89].

1.4.4. Physio-chemical pretreatment

Physio-chemical pretreatment refers to pretreatment in which both physical and chemical pretreatment is experienced by the feed stock simultaneously. A number of types of physio-chemical pretreatments have been developed including steam explosion, AFEX explosion and liquid hot water pretreatment. Of these steam explosion and ammonia fiber explosion will be discussed in the following sections.

1.4.4.1. Steam explosion

Steam explosion is the most extensively employed physio-chemical pretreatment for lignocellulosic biomass [51, 56, 60, 90]. This pretreatment combines both the chemical and mechanical effects of pretreatment into one. This form of pretreatment subjects the lignocellulosic biomass to pressurized steam for a certain period of time that can vary from seconds to minutes. At the end of the pretreatment, the reaction is explosively depressurized, disrupting the physical structure of the biomass. In uncatalyzed steam explosion elevated temperatures promote the degradation of the acetyl groups present in the hemicelluloses fraction into acetic acid in a process known as auto-hydrolysis.

Furthermore water has also been known to function as an acidic catalyst at elevated temperatures furthering the chemical interaction with the biomass [91]. Steam explosion utilizing water and steam alone is similar to dilute acid pretreatment in that it relies solely on the release of natural acids present within the hemicellulosic portion of the biomass, which then further hydrolyses the hemicellulosic fraction and has been found to release a maximum of 65% of the hemicellulose fraction [51]. Through use of an acid catalyst such as SO_2, H_2SO_4 or CO_2, the recovery of hemicelluloses can be increased [51, 92]. Of these, the weaker catalysts SO_2 or CO_2 are preferred as they result in a release of fewer inhibitory products [9]. As an added advantage for utilizing CO_2 it must be noted that this catalyst can be found in abundance on 1st generation ethanol plants due to the fact that *S. cerevisiae* releases this as a byproduct during ethanol production [92].

The characteristics of steam explosion that have made it a preferred pretreatment method include its reduced use of chemicals, reduced capital investment compared to other pretreatment methods, reduced energy consumption and its efficient biomass disruption characteristics [51, 56]. An added advantage is that steam explosion offers the possibility of using a large chip size increasing the energy efficiency of the pretreatment [57, 61]. It has also been reported that steam explosion further increases the crystallinity of the cellulose fraction by promoting crystallization of the amorphous region, it redistributes and disrupts the lignin fraction and easily hydrolyses the hemicelluloses fraction [51]. In spite of the many advantages to steam explosion, there are some limitations to steam explosion which are similar to dilute acid pretreatment which includes partial degradation of hemicellulose-sugars to inhibitory products as well as to solubilise and transform lignin compounds to chemicals which can be inhibitory to downstream processes [93]. Phenolic compounds and products are formed due to the breakdown of lignin based on the type of raw material being utilized which can have an effect on the subsequent hydrolysis and fermentation [57].

It has been found that the most important factors for steam explosion pretreatment include temperature, residence time, particle size, moisture content and the amount of acid catalyst present [57, 94]. For uncatalysed steam explosion the combined effect of residence time and temperature on the reaction can be described by the severity factor which can be seen in the following equation [57]. It was reported that for wheat straw the optimal conditions for uncatalysed steam explosion was for a severity factor of between 4 and 4.5 [61].

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

As mentioned above, particle size is an important factor and the use of small particle sizes in steam explosion is not favorable due to the increased economic cost of the entire process and the increased amount of energy that is necessary for communion of the raw material [57, 64, 95]. The effect of particle size on steam explosion was therefore evaluated on the herbaceous material *Brassica carinata* to determine how great an impact this can have on the results [96]. Of the three different particle sizes that Ballesteros et al. investigated in this study, namely 2 – 5mm, 5 – 8mm and 8 – 12mm it was found that the largest particle size was most beneficial in achieving good results [96]. The reason for this was that the largest particle size recovered the most cellulose in the WIS at the various conditions, while performing on par with the other particle sizes in terms of enzymatic hydrolysis. For example at the optimum conditions of 210°C and 8minutes, the cellulose recovery for the 8 – 12mm particle size was 88% compared to 70% and 57% for the 5-8mm and 2-5mm particle sizes respectively. This was the most notable difference as at all of the particle sizes similar quantities of hemicellulosic sugars in the pretreatment hydrolysate were recovered and had similar enzymatic hydrolysis yields. From this study it can therefore be seen that for this herbaceous material it is desirable to use larger chip sizes, which would also have benefits in terms of optimizing the process economy by reducing the milling power required for this particular process. Furthermore Cullis et al. have suggested that increasing the particle size of the raw material reduces the "relative severity" of the pretreatment resulting in a number of benefits, which includes a higher solids recovery as well as minimizing the condensation of residual recalcitrant lignin both of which result in a larger recovery of carbohydrates following enzymatic hydrolysis [97].

Some studies have been performed on a variety of raw materials to investigate the effect that moisture content has on steam explosion. Cullis et al. found that by increasing the moisture content from 12 – 30% in Douglas-Fir increased the hydrolyzability of the pretreated substrate [97]. A similar result was found for treating chopped poplar [98]. Ewanick and Bura found that for both uncatalysed and catalysed steam explosion of sugarcane bagasse, the hemicellulosic yield in the pretreated liquor was increased in both cases after increasing the moisture content of the raw material [25]. This same effect was not observed for the subsequent enzymatic hydrolysis in which soaking only had an effect on SO₂ catalysed steam explosion. Interestingly Ewanick and Bura also investigated the ethanol

production for uncatalysed and catalysed explosion in which they found that by increasing the moisture content for SO_2 catalysed explosion they were able to increase ethanol yield by 30% while for uncatalysed explosion there was no noticeable effect to the ethanol yield upon increasing the moisture content [25].

With regards to conditions that have usually been used in the pretreatment of agricultural residues by steam explosion, Sipos et al. found that two settings, 190°C and 10 minutes, and 210 and 5 minutes were sufficient to reach 85 – 95% yields of cellulose with a moisture content of 50% and an acid concentration of 2% SO_2 for sweet sorghum [99]. In a similar experiment for sugarcane bagasse, Carrasco et al. found that the highest combined sugar yield of 68g/100g of raw material (87%) was achieved at 190°C, 5 minutes and a 2% SO_2 concentration based on water content [23]. Ferreira-Leitao et al. has gone one step further by comparing the efficiency of CO_2 steam pretreatment to this regularly used SO_2 steam pretreatment [92]. As was expected CO_2 impregnation at the same pretreatment conditions resulted in a lower glucose yield of 52% compared to 86.3% for SO_2 impregnation due to the fact that CO_2 is a weaker acid. For better yields the pretreatment severity therefore had to be increased when using CO_2 and more favorable yields of 81.1% and 86.6% were realized at 205°C and 10 and 15 minutes respectively. The added benefit of being able to use more severe pretreatment temperature and times with CO_2 without increasing the formation of degradation products was described for CO_2 which is not possible when using SO_2. This factor as well as CO_2 being less toxic, cheaper, having a higher availability, lower corrositivity and lower occupational risk compared to SO_2 makes it a strong competitor to SO_2 catalysed steam explosion[92]. Table 1-6 below, compares steam explosion research that has been performed on sorghum and similar herbaceous material and agricultural residues.

Table 1-6: Previous steam explosion work performed on agricultural residues

Feedstock	Reactor size	Temp	Time (min)	Moisture content	Catalyst concentration	Solids loading (g DW/L)	Temp	pH	Time	Solids loading	Enzyme loading	% Xylan	% Glucan	Reference
			Pretreatment Conditions					**Enzymatic hydrolysis conditions**					**Yields**	
Sweet Sorghum	5000L	160°C	5 min	40%	-	40g/L	50°C	4.8	60h	10%	20FPU/g substrate	-	70%	Zhang et al.[18]
Sweet Sorghum	2L	190°C	10 min	0%	-	25g/L	50°C	4.8	72h	2%	20FPU/g glucan, 40CBU/g glucan	-	75%	
Sweet Sorghum	2L	190°C	10min	0%	5% SO₂	25g/L	50°C	4.8	72h	2%	20FPU/g glucan, 40CBU/g glucan		87%	Shen et al. [100]
Sweet Sorghum	2L	200°C	7.5min	0%	2.5% SO₂	25g/L	50°C	4.8	72h	2%	20FPU/g glucan, 40CBU/g glucan		88%	
Forage Sorghum	2L	210°C	6 min	-	-	75g/L	50°C	4.8	72h	5%	15FPU/g substrate	-	91%	Balesteros et al. [101]
Sweet Sorghum	10L	200°C	5 min	50%	2% SO₂		50°C	4.8	48h	2%	20FPU/g substrate	-	92%	Sipos et al. [99]
Forage Sorghum	1L	140°C	30 min	95%	2%H₂SO₄	27g/L	50°C	4.8	96h	10%	15FPU/g substrate	87%	79%	Corredor [90]
Sugarcane Bagasse	10L	216°C	5 min	50%	-	50g/L	38°C	5	48h	1.70%	25FPU/g substrate	55%	65%	Kaar et al. [102]

Substrate														Reference
Sugarcane Bagasse	10L	205ºC	10 min	50%	-	30g/L	40ºC	4.8	72h	2%	0.0046g 1.5L Cellulast/g substrate; 0.001g novozyme 188/g substrate	45	57%	Sendelius [103]
Sugarcane Bagasse	10L	190ºC	5min	50%	2% SO_2	30g/L	40ºC	4.8	72h	2%	0.0046g 1.5L Cellulast/g substrate; 0.001g novozyme 188/g substrate	52%	96.30%	Sendelius [103]
Sugarcane Bagasse	10L	190ºC	5min	30%	-	22.5g/L	40ºC	4.8	96h	2%	15FPU/g substrate	47%	45%	Ferriera – Leitao et al. [92]
Sugarcane Bagasse	10L	190ºC	5min	30%	3% SO_2	22.5g/L	40ºC	4.8	96h	2%	15FPU/g substrate	72%	80%	Ferriera – Leitao et al. [92]
Sugarcane Bagasse	10L	205ºC	15 min	30%	3% CO_2	22.5g/L	40ºC	4.8	96h	2%	15FPU/g substrate	51%	87%	Ferriera – Leitao et al. [92]
Wheat Straw	4.5L	198ºC	5 min	50%	-	44g/L	50ºC	4.8	72h	2%	44FPU/g substrate	80.00%	69%	Sun and Chen [104]
Switch Grass	1.5L	195ºC	7.5 min	-	-	33.3g/L	37ºC	5.5	10h	10%	10FPU/g substrate	-	52%	Ewanick and Bura [25]
Switch Grass	1.5L	195ºC	7.5 min	80%	-	33.3g/L	37ºC	5.5	10h	10%	10FPU/g substrate	-	50%	Ewanick and

Switch Grass	1.5L	195°C	7.5 min	80%	0.6% SO$_2$	33.3g/L	37°C	5.5	10h	10%	10FPU/g substrate	-	92%	Ewanick and Bura [25]
Paja Brava	10L	200°C	5 min	64%	2.50%	30g/L	40° C	4.8	72h	2%	15FPU/g substrate	71.70%	97%	Carrasco et al. [22]
Brassica Carinata	2L	210°C	8min	5%	-	50g/L	50°C	-	72h	2%	15FPU/g substrate	50%	98%	Ballesteros et al. [96]

1.4.4.2. Ammonia fiber explosion (AFEX)

The pretreatment of lignocellulosic biomass by ammonia fiber/freeze explosion involves both steam explosion and ammonia. Usually biomass is pre soaked to an initial moisture content of between 15 and 30% and placed with liquid ammonia in a pressure vessel at a loading of about 1 – 2 Kg NH_3/kg dry biomass under a minimum pressure of 12atm [51]. The advantage of AFEX is that it has a short processing time and is simple. During this process no sugars or other structural components are directly liberated from the biomass but rather opens up the chemical structure which exposes these polysaccharides to chemical attack in subsequent enzymatic hydrolysis [51]. A disadvantage of this process is that biomass types that have higher lignin contents such as aspen wood chips are not effectively pretreated using this process. Another problem encountered is the recovery of the ammonia used during the pretreatment which is extremely expensive [51].

1.4.5. Biological pretreatment

Biological pretreatment consists of using microorganisms/enzymes to break down the lignocellulose structure of biomass. Microorganisms that have been investigated include brown-, white- and soft rot fungi of which white-rot fungi have been seen to be the most effective in reducing the recalcitrant nature of lignocellulose [60]. The main disadvantage of this pretreatment is that degradation rates are slow and yields are low [51]. In addition, most lignolytic microorganisms consume and solubilise not only the lignin fraction but the hemicelluloses and cellulose fractions of the biomass which results in loss of sugars [64]

1.5. Impact of pretreatment on enzymatic hydrolysis:

As discussed previously the goal of pretreatment of lignocellulosic biomass is to effectively disrupt the lignocellulose matrix and/or remove the hemicelluloses fraction making the cellulose fraction accessible to enzymes [51]. Enzymes that are involved with the degradation of cellulose are known as cellulases and they are responsible for disruption of the β-1-4-glycosidic bond of glucan [105]. This group of enzymes can be broken up into three categories based on their respective function; endoglucanases, exoglucanases and β-glucosidases. The function of each of these is the following. Endoglucanases are responsible

for the reduction in the degree of depolymerisation of the substrate by attacking the interior in the amorphous portion of cellulose in a random manner [62]. Exoglucanases are then responsible for reducing the chain length of the glucan molecules by binding to the glucan ends and releasing the cellobiose units. Finally β-glucosidases are responsible for splitting each disaccharide cellobiose in two monomeric units of glucose [62]. There are a number of microorganisms that can be utilized to produce cellulose systems including aerobic filamentous fungi, aerobic actinomycetes, anaerobic hyperthermophylic bacteria and anaerobic fungi [62].

Usually enzymatic hydrolysis of native lignocellulose biomass is an inefficient process with low yields, typically around 20%, due to the structural features of biomass [72, 106]. Conventionally, the structural features that have an effect on the extent of enzymatic hydrolysis are divided into two main groups namely, chemical and physical. Features that are included in the chemical group are the composition of the cellulose, hemicelluloses, lignin and acetyl groups that are bound to hemicelluloses while pore volume, crystallinity, degree of polymerization, accessible surface area, biomass particle size and the physical distribution of lignin in the biomass matrix are included in the physical features of biomass which will affect the extent to which enzymatic hydrolysis will occur [106].

Enzymatic digestibility is a term used to describe the extent to which enzymatic hydrolysis has occurred. For example an enzymatic digestibility of 100% means that all of the available carbohydrate in the biomass has been hydrolysed to monomers through enzymatic hydrolysis. Correlations have been reported between three main structural features; lignin content, crystallinity and acetyl content; and the extent to which enzymatic digestibility occurs [74]. It was concluded that by just removing lignin, it is possible to produce a highly digestible biomass regardless of the acetyl content or crystallinity; that delignification and deacetylation remove parallel barriers that are prevent efficient enzymatic hydrolysis and that while decrystallisation significantly affects initial hydrolysis rates it has less effect on final monomer yields [74]. It has also been identified that delignification, decrystallisation and deacetylation impact on glucan and xylan digestibility differently, with delignification and deacetylation having a greater effect on xylan digestibility due to the fact that both

lignin and acetyl groups are attached to the hemicelluloses matrix while decrystallisation had the greatest impact on cellulose digestibility as cellulose is crystalline while hemicelluloses are not [74]. Others reported that lignin removal increased the effectiveness of enzymes by eliminating non-productive absorption sites and increasing accessibility to holocellulose (Hemicelluloses and cellulose) [107].

With regards to the effect that dilute acid pretreatment has on enzymatic hydrolysis, it has been observed that at higher temperature, i.e. above 170°C, the recondensation of lignin occurs which can counteract any high digestibility that would otherwise be achieved through the removal of lignin [82]. At the same time lignin undergoes a glass transition upon heating to temperatures above 120 - 160°C depending on the specific feedstock, which can cause increased digestibility by creating easier accessibility to the pores of the cellulose [82]. Higher lignin content in the raw material has also been shown to have a negative effect on enzymatic hydrolysis yields following dilute acid pretreatment [82]. This suggests that one should select cultivars with a lower lignin content as they should be more easily hydrolysable. Some of the structural changes following dilute acid pretreatment that are thought to enhance enzymatic hydrolysis include the restructuring of lignin complexes and the removal of xylan[82]. Increased xylan removal along with the restructuring and redistribution of lignin has been shown to result in increased yields of glucose attributed to increased pore volume and surface area in the pretreated solids [108]. To maximize glucose yields during enzymatic hydrolysis, xylose should therefore be completely removed at temperatures higher than the glass transition temperature of lignin. This will result in less than optimum yields of xylose though due to the fact that at higher temperatures xylose degrades into degradation byproducts simultaneously with the release of xylan from the lignocellulosic matrix [80]. A compromise in the pretreatment conditions required for optimal xylose and glucose yields will therefore need to be reached to achieve the highest combined sugar yield.

For high yields of glucose in enzymatic hydrolysis following auto catalysed steam explosion (no catalyst), previous studies have shown that optimal hemicelluloses solubilisation as well as an optimal enzymatic hydrolysis occurs at a temperature of around 190°C and 10 minutes [100]. Grous et al. also observed that pore size was significantly increased with steam explosion resulting in increased rates of enzymatic hydrolysis while drying of the pretreated material reduced the rate of hydrolysis [109]. Furthermore Grethlein et al. showed that

there was a linear relationship between a pore size of 5.1nm (the size of a cellulase enzyme) and the rate of enzymatic hydrolysis [110]. It has also been observed that the larger the surface area available for adsorption of proteins following pretreatment, the less amount of protein is required for efficient hydrolysis. This has the benefit of being cost effective and lowering the biomass to ethanol cost as the cost of enzymes makes up a large portion of the total cost [111].

A wide range of enzyme loadings have been employed in enzymatic hydrolysis, ranging from 5FPU/g cellulose to greater than 50FPU/g cellulose [51, 68, 109, 110]. Typical ranges though vary from 10FPU/g cellulose to 30FPU/g cellulose with a lower enzyme loading, resulting in a cheaper enzyme cost. Some studies report 5FPU/g cellulose as a sufficient loading but it is more frequently assumed that 10FPU/g cellulose will be the enzyme loading used industrially by 2020 [68].

1.6. Fermentation

The goal of any effective fermentation process is to efficiently convert a particular compound or compounds into the desired product through the use of a specific microorganism. In the producing bio-ethanol from lignocellulosic biomass, the fermentation process is particularly important as it converts available fermentable sugars, i.e. hexoses and pentoses, into ethanol. Without this process step, bio-ethanol production would be nearly impossible.

1.6.1. Organisms for fermentation

There are numerous organisms which can currently be used in fermentation of fermentable sugars to ethanol. These include yeast, bacteria and filamentous organisms. These organisms are able to use 6-carbon sugars as a food source, while producing ethanol as one of the byproducts of this process [51]. Some of the requirements that need to be met by a prospective organism is that it should withstand high ethanol concentrations (reduce distillation costs) and result in high ethanol yields [112]. Additionally the prospective organism should be able to tolerate inhibitory products such as furfural, 5-hydroxmethylfurfural, and acetic acid (formed during pretreatment of lignocellulose); be temperature tolerant; and have the ability to utilize multiple sugars as the carbon source if the organism is to be used in a simultaneous saccharification and fermentation process scheme [113].

Currently the most employed organism for ethanol production in fermentation is *saccharomyces cerevisiae* due to its good growth characteristics which includes it efficient ability to utilize hexoses (6-carbon sugars) in the production of ethanol (98% of $0.51g.g^{-1}$ at optimal conditions), its high tolerance to ethanol (up to 10%w/v in the fermentation medium) and other inhibitory products that could be present in the liquid hydrolysates of lignocellulose biomass [51, 60]. The main disadvantage of using a wild type *S. cerevisiae* is that it is unable to efficiently utilize pentoses such as xylose for ethanol production. This requires metabolic and genetic engineering of the microorganism to enhance the utilization of pentose sugars [60].

The most promising ethnologic bacteria currently, is *zymomonas mobilis* [114]*. Z. mobilis* has been widely recognized for its rapid and efficient bio-ethanol production (97% yield) from glucose feed stocks and its improved performance over traditional yeast fermentations (5% higher yields). Its main disadvantage is its inability to ferment pentose sugars, making it unsuitable for a biomass to ethanol process unless pentose fermenting capabilities can be introduced into its metabolism [51].

Other microorganisms for biomass to ethanol fermentation are known which can utilize pentose sugars such as xylose but these (i.e. *Pichia stipitis*, *candida shehatae*, and *pachysolen tannophilus*) have been characterized by less optimal ethanol yields and their tendency to re-assimilate ethanol [51].

In terms of pH, an organism must maintain a fairly constant balance to survive. In bacteria the pH range is generally between 6.5 – 7.5, while for yeasts and filamentous fungi this range is lower from 3.5 – 5. [51]

1.6.2 Fermentation inhibitors

Different pretreatment processes can aid in rendering the different carbohydrates of lignocellulosic biomass accessible and available for fermentation to ethanol. The resulting hydrolysate from pretreatment can contain varying inhibitory substances which negatively affect fermentation. The formation of these substances will depend on the method of pretreatment utilized as well as the type of biomass that is being pretreated [15].

The main pretreatment variables which lead to the formation of inhibitory degradation products are temperature, pressure, time, pH, and the addition of certain catalysts [15]. There are three groups of inhibitory compounds that are formed during pretreatment of lignocellulosic biomass including furan derivates, weak acids and phenolic compounds [115]. The furan degradation products furfural (degradation product from pentoses) and 5-hydroxymethylfurfural (HMF; a degradation product of hexose) are formed in higher concentrations as pretreatment conditions become more severe [116]. Furans are known to decrease the volumetric ethanol productivity and yield, increase the lag phase time and/or slow the growth rate. These effects result and can be explained generally by the fact that yeast needs to redirect energy to cope with damage caused by the furans and by reduced NAD(P)H and ATP levels due to either enzymatic inhibition of regeneration and or consumption of cofactors [115]. The degradation path of a hexose sugar, glucose, can be seen in the following schematic.
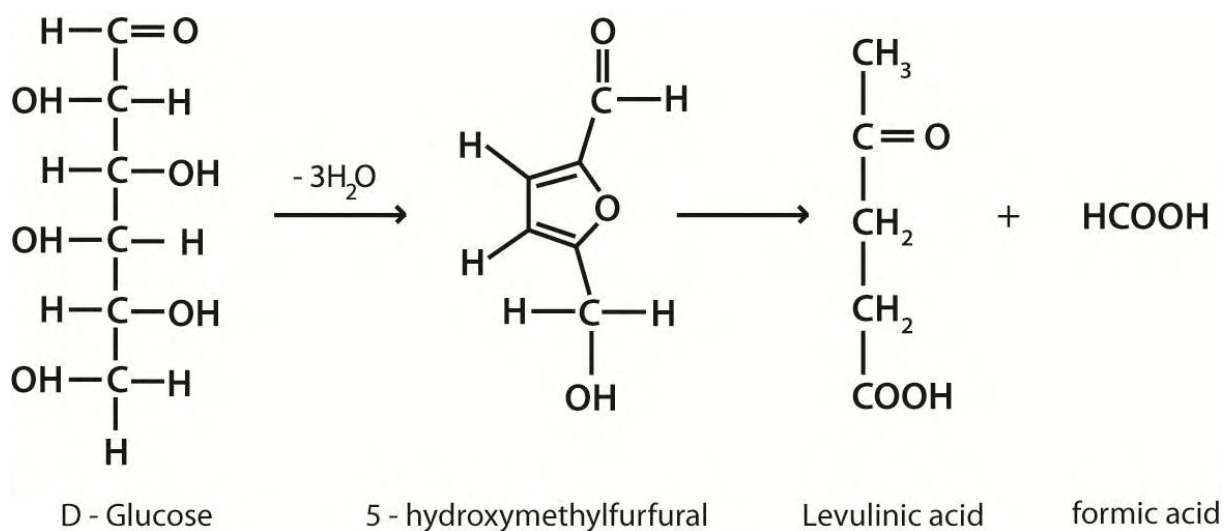
Figure 1-9: Degradation pathway of glucose, redrawn from Ulbricht et al. [117].

The common weak acids encountered in hydrolysates from pretreated lignocellulosic biomass includes acetic, formic and levulinic acid. Acetic acid is formed through de-acetylation of the hemicelluloses fraction of the lignocellulose while both formic and levulinic acid are formed during degradation of HMF. Formic acid can also possibly be formed from furfural during extended and elevated pretreatment of lignocellulose [15, 115]. Weak acids have been seen to inhibit both ethanol yields and biomass growth although for *S. cerevisiae* acetic acid, levulinic acid and formic acid all increase ethanol yields up to a

concentration of 100mmol l^{-1} for these acids. Concentrations higher than this resulted in a decreased ethanol yield due to cell deaths attributed to acidification of the cell cytoplasm's [118]. The lower biomass growth is related to the fact that the cell will try to negate the effect of the undissociated weak acids crossing the plasma membrane by ATPase pushing protons out of the cell instead of ATP hydrolysis [115].

The phenolic compounds formed will depend on the type of pretreatment and the ratio of H/G/S (4-hydroxybenzyl (H), guaiacyl (G) and syringyl (S) units) of lignin present in the biomass as well as the degree of methoxylation, internal bonding and association of lignin with hemicelluloses and cellulose [15, 115]. Currently there is a lack as to the exact mechanism of inhibition by phenolic compounds but the effect of these compounds is similar to that of furans, i.e. decreased biomass yield, growth, and ethanol productivity. High molecular phenolics have been observed to be more inhibitory while the position of the substituent also affects toxicity. Some possible mechanisms of inhibition that have been proposed include destruction of the electro-chemical gradient through transportation of protons back across the mitochondrial membranes and attacking the integrity biological membranes [115].

1.6.3 Separate hydrolysis and fermentation

In separate hydrolysis and fermentation (SHF), enzymatic hydrolysis and fermentation are performed separately as the name suggests in two separate vessels. This ensures that both the enzymatic hydrolysis and fermentation can occur at their optimum operation conditions with respect to temperature and pH. The main disadvantages include the high capital cost to set-up to different vessels as well as the fact that as the hydrolysis unfolds, accumulation of cellobiose and glucose inhibit activity of the cellulases [119]. It must also be noted that due to long processing periods, the chance of contamination is also high and must be monitored [120].

1.6.4 Simultaneous saccharification and fermentation

In reducing the cost of the lignocellulose to ethanol process it was proposed by Gauss et al. [121], that enzymatic hydrolysis and fermentation be combined in a single process step known as simultaneous saccharification and fermentation (SSF). The main and most

important reason for this is the fact that the glucose yield realized in a separate enzymatic hydrolysis step is low due to end product inhibition of glucose and cellobiose. This can be overcome by combining enzymatic hydrolysis with fermentation so that as the glucose is released it is utilized and converted to ethanol preventing end product inhibition of glucose [62]. Another added advantage is the reduction in the capital cost by 20% or more for the lignocellulose to ethanol process which is a major benefit as well as the fact that higher ethanol concentrations are possible which prevent contamination [14, 63, 122]. Other advantages of SSF over SHF include a lower required enzyme loading, a shorter process time and the fact that yeasts are also able to detoxify to some extent byproducts that are inhibitory to an efficient enzymatic hydrolysis resulting in higher sugar yields and in turn higher ethanol yields [51, 62, 122]. Loss of sugars is avoided in SSF as glucose does not need to be separated from lignin as it does prior to fermentation in the SHF process [62].

Although the benefits of SSF are most favorable, there are certain drawbacks as well. This includes the fact that the fermentation has to be run at less than optimal temperatures for enzymatic hydrolysis and higher than that which is optimal for yeast fermentation. Furthermore the yeast is not easily reused following fermentation due to the difficulty in separating lignin from the yeast which results in either extra costs for yeasts to be purchased from elsewhere or in yield losses of carbohydrates needed to grow up yeasts for the process [62]. Most of the work performed to date though has been involved with increasing the substrate loading, decreasing the enzyme and yeast concentration, and varying both the temperature and pH of the process to optimize and increase the ethanol production from SSF.

A high substrate loading is beneficial to the economy of the SSF process as a high substrate load would enable the process to achieve a high ethanol concentration. The main problem experienced with this is that when the substrate loading is increased in batch SHF and SSF, the ethanol yields tend to decrease and in practice it has been difficult to achieve a good ethanol yield with substrate loadings above 10%. To overcome this, a fed batch approach can be utilized which has the added benefit of keeping inhibitory product levels low, allowing for co-fermentation of xylose and glucose due to low glucose levels, and reduce end-product inhibition of ethanol on yeast [62].

In terms of the temperatures that should used for a SSF, a compromise has to be made between the optimal temperature for the yeast and cellulolytic enzymes needed. It has been suggested in numerous studies that this temperature is around 37°C since *S. cerevisiae* has an optimal temperature around 30°C and the maximum it can currently tolerate is 37°C while for cellulosic enzymes an optimal temperature of around 55°C is required [62, 123, 124].

1.7. Key questions and hypothesis

1.7.1. Key questions

Some of the key questions regarding the selection of Sweet Sorghum cultivars for bio-ethanol production are:

- Can one predict which cultivars will perform well based on their chemical composition?

- Can any of the chemical characteristics be used to predict which cultivars have the highest ethanol potential or highest combined sugar yields?

- Do different Sweet Sorghum cultivars differ greatly in terms of their response to dilute acid pretreatment, and maximum combined sugar yields achieved by pretreatment-hydrolysis?

- Do the optimal dilute acid pretreatment conditions for maximum combined sugar yields vary in terms of temperature, time and acid concentration for different Sweet Sorghum cultivars?

- Do some cultivars require reduced pretreatment severity and/or enzymatic hydrolysis (enzyme loading) requirements for the same overall sugar yield?

- What are the scale-up effects from small scale dilute acid pretreatment to pilot plant scale steam explosion pretreatment? I.e. can the same combined sugar yields be reached in both small scale and pilot plant scale pretreatment? And do the optimal yields occur at a similar pretreatment severity in both small scale and pilot plant scale? Similarly do the best performing cultivars from small scale correspond with those from pilot plant scale?

1.7.2. Hypothesis

It is hypothesized that there will be Sweet Sorghum cultivars which will perform better than others in terms of their response to pretreatment due to differences in chemical composition. It is also hypothesized that the optimum conditions for pretreatment will vary from cultivar to cultivar.

1.8. Aims, objectives, scope and deliverables

1.8.1. Aims and objectives

The work of this project centered round the optimization and selection of preferred sweet sorghum cultivars for bio-ethanol production. The first objective of this work was therefore to select three preferred sweet sorghum cultivars from an initial thirty-six cultivars selected from field trials based on biomass and non-structural sugar yields. In the first selection, ten cultivars were selected from the initial thirty-six followed by a second selection step in which five cultivars were selected. The first and second selection steps were done based on small scale dilute acid pretreatment. Following this, the top five preferred cultivars were optimized using small scale dilute acid pretreatment. Finally following on from the optimization step; the three best performing cultivars were pre-treated and optimized at a pilot plant level using steam explosion in a 19L steam explosion reactor. The small scale optimization was compared with the pilot plant pretreatment optimization for different cultivars looking for characteristics of individual cultivars which caused higher yields in some cultivars compared to others.

The second objective of this research was therefore to develop a pretreatment process for sweet sorghum by optimizing the different process parameters such as temperature, residence time, acid concentration and catalyst that resulted in the maximum recovery of fermentable sugars which could be utilized for ethanol production. It was investigated whether the same or a similar pretreatment can be used for different sweet sorghum cultivars, and to what extent pretreatment screening of cultivars could assist in development of new sweet sorghum cultivars for cellulosic ethanol production.

1.8.2. Scope

The scope of this work covered the selection of preferred sweet sorghum bagasse cultivars from thirty-six South African bred and grown cultivars using dilute acid pretreatment in small

scale tubular reactors. Following the selection of the preferred cultivars, they were optimized for combined sugar yields by varying temperature and time according to a statistical design of experiments. The three top performing cultivars were pretreated at a pilot plant scale level to evaluate the performance of these three cultivars against that observed at a small scale. The pretreatment at a pilot plant scale included pretreatment of sweet sorghum with and without the catalysts water and SO_2 and the effect of these three catalysts will be compared with regards to total fermentable sugar yields. Conclusions and recommendations were given based on the results observed for pretreatment of different sweet sorghum cultivars.

1.8.3. Deliverables

The deliverables from this project were:

- The selection of preferred sweet sorghum cultivars which are preferred for bio-ethanol production.
- A comparison of pretreatment performance in terms of estimated ethanol yields between the different cultivars.
- A comparison between small scale pretreatment and pilot plant pretreatment of Sweet Sorghum bagasse.
- Information of differences in pretreatment response of cultivars, as feedback to crop development efforts.

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Chapter 2: Selection of preferred sweet sorghum cultivars and their pretreatment optimization for bio-ethanol production.

2.1. Abstract

Sweet sorghum (*sorghum bi.color* (L.Moench)) is an adaptable and sugar rich crop which has characteristics making it desirable for the production of bio-ethanol. An initial thirty-six sweet sorghum cultivars grown in Kwa-Zulu Natal, South Africa were collected for evaluation of their potential bio-ethanol yields. The chemical composition of each cultivar was determined and subsequently each cultivar screened under the same conditions with dilute acid pretreatment and enzymatic hydrolysis. The pretreatment condition chosen for the initial screening was 170°C, 15 minutes, an acid concentration of 0.7% (w/w) H_2SO_4 and a solids loading 30% w/v. Enzymatic hydrolysis was carried out at an enzyme loading of 15FPU/g water insoluble solids, a pH of 4.8, a temperature of 50°C and a residence time of 72 hours. Major differences in pretreatment response were observed and it was found that higher in both lignin and ash content had significant negative effects on the pretreatment response. Total sugar yields with this initial screening of the thirty-six cultivars varied between 32.63 and 44.04g/100g raw material.

Combined with agronomic factors, plant breeder's criteria, pretreatment response and ethanol yield, the initial thirty-six sweet sorghum cultivars were reduced to ten for further screening. The selected cultivars from the initial screening were SS27, AS254, AS246, AS106, AS79, MSJH13, AS248, AS103, AP6 and AS245. Further screening was carried out to reduce the thirty-six cultivars to five and this was performed with two low severity pretreatments, namely 190°C, 5 minutes, 0.25% (w/w) H_2SO_4 at a solids loading of 30% (w/V) and 200°C, 5 minutes, 0.07% (w/w) H_2SO_4 at a 30% (w/V) solids loading. Coupled with the low severity pretreatments, two enzyme loadings of 3.75FPU/g WIS and 15FPU/g WIS were utilized in looking for cultivars which consistently performed well at these low severity pretreatment conditions. As was the case in the screening of the thirty-six sweet sorghum cultivars, it was statistically evident that increases in both lignin and ash contents had a negative effect on pretreatment response at these low severity pretreatments. Further it was found that

certain cultivars such as SS27 performed well for all of the conditions while other cultivars did not.

The five cultivars that proceeded to optimization with dilute sulfuric acid pretreatment were AP6, SS27, AS103, MSJH13 and AS246. As was with the selection of ten cultivars, these cultivars were selected based on acceptable agronomic criteria, pretreatment response and ethanol yields. Optimization of the pretreatment conditions for these preferred cultivars was achieved through the use of a central composite design with face centered star points. The factor ranges selected for the optimization were temperature at 180°C to 190°C, time at 5 to 15 minutes, and acid concentration at 0.25% (w/w) H_2SO_4, with a solids loading of 30% (w/V). Optimization resulted in total sugar yields varying for the preferred cultivars between 48.83 and 54.5g/100g raw material, which corresponded to a total sugar recovery between 75.81% and 84.98% of the sugars initially present in the raw material.

Of the five cultivars optimized at a small scale, three of the cultivars were further selected for optimization with steam explosion pretreatment. The three cultivars selected were SS27, AP6 and AS246 and each of these three cultivars were subjected to steam explosion with and without an acid catalyst. For steam explosion without an acid catalyst, air dried and water soaked material was evaluated, while for acid catalysed steam explosion, water soaked material was impregnated with SO_2. Steam explosion of the preferred cultivars was achieved with standard 2^2 factorial experimental designs in which temperature and time was varied. For both air dried and water soaked material the temperature was varied between 190°C and 205°C, while time was varied between 5 and 10 minutes. For SO_2 catalysed steam explosion, the temperature was varied between 185°C and 195°C while the time was varied between 5 and 10 minutes. It was possible to recover between 61.50% and 63.90% of the initial sugars present in the raw material with steam explosion of air dried material, while for steam explosion of water soaked material between 78.73% and 84.17% of the sugars present in the raw material could be recovered. The optimum pretreatment condition for both air dried and water soaked steam explosion occurred at a pretreatment temperature of 205°C and a residence time of 5 minutes. Combined sugar yields were much improved with SO_2 catalysed steam explosion. This resulted in combined sugar yields of between 61.06 and 64.03g/100g raw material or between 87.2 and 91.48% being recovered for the three selected sweet sorghum cultivars, AP6, SS27 and AS246, under SO_2 catalysed steam explosion. Estimated total ethanol yields that can be expected from the sweet sorghum crop

based on current agronomic yields show that the potential ethanol yield for these three cultivars is between 7131 and 8678 L/ha. Further increases in biomass yields will be needed to further increase total ethanol yields.

2.2. Introduction

Sorghum bi.color (L.Moench) is a plant that is adaptable to climatic conditions and high in soluble sugars. It is a C4 crop of the *andropogoneae* tribe of the family *poaceae* which includes grain, fiber and sweet sorghum [1, 2]. Between grain, fiber and sweet sorghum there are no observable taxonomical differences and both natural and cultivated selection results in the grain, fiber or sweet sorghum being produced [3]. Further *sorghum bi.color* (L.Moench) is drought resistant, has high biomass yields, has relatively low input requirements and can be grown over a wide range of climatic conditions [3, 4]. Compared to sugar cane, the cost of sweet sorghum cultivation has been found to be a third that of sugarcane due to its low water and fertilization requirements and it has been found that sweet sorghum will outperform sugarcane in terms of biomass yields over a short period of time [3, 5]. These factors make sorghum an ideal crop for production of both 1st and 2nd generation bio-ethanol.

1st generation bio-ethanol is produced primarily from sucrose and starch containing food crops by means of fermentation and subsequent distillation, while 2nd generation ethanol is produced from lignocellulosic sources such as agricultural residues, municipal green waste, and herbaceous material [6, 7]. While two thirds of lignocellulosic biomass based on oven dry mass is comprised of hemicelluloses and cellulose, which can be reduced to monomeric sugars and subsequently fermented to ethanol, the lignocellulose structure is highly resistant to attack by microbial organisms and a pretreatment step is required to overcome this recalcitrant nature of lignocellulosic biomass [8, 9]. Two widely used and investigated pretreatment methods that have been found to overcome the recalcitrance of lignocellulosic biomass are dilute acid pretreatment and steam explosion [10 - 16]. Dilute acid pretreatment has been applied on a broad range of feed stocks and with a number of different dilute acids such as nitric acid, sulfuric acid, phosphoric acid, hydrochloric acid, and peracetic acid [10]. Primarily dilute acid pretreatment has been utilized in hydrolyzing the hemicellulosic fraction present in lignocellulose into monosaccharides and degrading the lignin fraction, which render the cellulose fraction highly accessible to enzymes [7 - 11].

Steam explosion is a physio-chemical pretreatment which combines both a mechanical and chemical pretreatment into one. This pretreatment subjects the biomass to pressurized steam from a couple of seconds to a number of minutes before being explosively depressurized which subsequently disrupts the physical structure of the biomass. Steam explosion pretreatment can be performed with and without an acid catalyst. In the case of no added catalyst, acetyl groups present in the biomass are degraded and subsequently aid in hydrolyzing the hemicellulosic portion present in lignocellulose in what is known as auto hydrolysis. With an added catalyst such as SO_2, the hemicelluloses hydrolysis is much improved. Steam explosion is therefore preferred over other pretreatment methods due to its reduced use of chemicals, reduced capital investment, reduced energy consumption and efficient biomass disruption characteristics which improve enzymatic digestibility [15, 17].

Following an efficient pretreatment the remaining solids known as water insoluble solids (WIS), which are now highly digestible due to changes in physio-chemical properties such as pore volume, crystallinity, degree of polymerization, accessible surface area and biomass particle size, undergo enzymatic hydrolysis to reduce the cellulosic portion of lignocellulose to glucose [10 - 18]. Subsequently the monosaccharides resulting from both the pretreatment and enzymatic hydrolysis step can be fermented to ethanol. Currently two fermentation strategies are utilized, namely separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). In SHF, the water insoluble solids are hydrolysed with enzymes at optimal temperatures and the resulting monosaccharides are fed to a fermentation process at its optimal temperatures. In SSF, the water insoluble solids are fed simultaneously with the enzymes to a fermentation process in which the water insoluble solids are hydrolysed by the enzymes releasing sugars which are simultaneously fermented into ethanol at a temperature that is midway between that which is optimal for fermentation and enzymatic hydrolysis [19 - 23].

While dilute sulfuric acid pretreatment and steam explosion have been performed on sweet sorghum bagasse to a limited extent [24 - 30], comparisons on the affect of cultivar selection on sugar and ethanol yields have not been carried out. Further the variation of the chemical composition of sweet sorghum bagasse on pretreatment response has not been investigated. This study aimed to select preferred sweet sorghum cultivars for bio-ethanol production. Pretreatment response, agronomic characteristics and total ethanol yields formed the basis of selection. The affect of chemical composition on pretreatment response

was also investigated. Further pretreatment of preferred sweet sorghum cultivars with both dilute sulfuric acid pretreatment and steam explosion was investigated looking for optimal pretreatment conditions resulting in maximized combined sugar yields (g/100g raw material) and total ethanol yields (L/ha). The outcome of this study was the selection of three preferred sweet sorghum cultivars that can be used for bio-ethanol production as well as pretreatment conditions that resulted in optimal bio-ethanol yields.

2.3. Research design and methodology

Many of the analytical methods used in this study were taken from the National and Renewable Energy Laboratory in Golden, Colorado, US [31 - 34]

2.3.1 Raw material

2.3.1.1. Sample collection

Sweet sorghum (*Sorghum Bi.color* (L.Moench)) cultivars were collected from the Ukhulinga experimental research farm in Pietermaritzburg, Kwa-Zulu Natal, South Africa. Work on these cultivars began on the experimental farm in the 2007/2008 season in which the objectives were firstly screening of cultivars based on high non-structural sugar yield and secondly breeding of new lines of sweet sorghum that incorporated both high non-structural sugar content and improved biomass yields while having good agronomic characteristics such as disease resistance. Selected cultivars from two harvest seasons, namely the 2008/2009 (first) and 2010/2011 (second) harvest seasons, were processed by the Sugar Milling Research Institute (SMRI) at the University of Kwa-Zulu Natal, Durban, South Africa, to separate soluble sugars from the fibre. The processing of the fresh sweet sorghum was achieved through the use of a Jeffco cutter grinder (Jeffress Engineering, Dry Creek, Australia) for initial preparation followed by juice removal and subsequent dewatering in a Walkers 3 roller mill (Bundaberg Walkers, Queensland, Australia). Further the material was washed three times with warm water before being dried at 40°C. The soluble sugars were analysed for non-structural sugar content and the milled fibre, more commonly known as bagasse was transported to the University of Stellenbosch for further preparation, storage and process investigation. Agronomic data was collected for both harvest seasons which included stalk yield (ton/ha), dry matter content (%), brix (ton/ha), dry matter content (ton/ha) and fibre + ash (ton/ha). A list of the collected sweet sorghum cultivars for each season can be seen in Table 2-1 and Table 2-2.

Table 2-1: Sweet sorghum cultivars selected during 2008/2009 harvest season

AS018	AS072	AS079	AS082	AS103	AS106
AS240	AS241	AS242	AS244	AS245	AS246
AS247	AS248	AS249	AS250	AS251	AS253
AS254	AS255	AS256	AS258	AS259	AS263
MSJH5	MSJH5a	MSJH9	MSJH13	MSJH15	MSJH16
MSJH22	AP6	Cabin	SS27	HSS27	SS120

Table 2-2: Sweet sorghum cultivars selected during 2009/2010 harvest season

AP6	**SS27**	**MSJH13**
AS246	**AS103**	**-**

2.3.1.2. Sample preparation

Sample preparation varied for the various analyses and pretreatments. The aim of sample preparation was to ensure that a representative sample was selected, that the particle size of the sample was standardized and that the samples had as little variation between batches as possible.

For reduction of particle size a number of different machinery was used based on the raw material requirements, including an impact mill (Condux LV15M ,Netzch-Condux GmbH, Hanau, Germany) and an ultra centrifugal mill (Retch ZM 200, Monitoring and Control Laboratories, Parkhurst, RSA).

Similarly, sieving of material into different fractions was possible with two set-ups, the vibratory sieve shaker (Retch AS200 Basic, Monitoring and Control Laboratories, Parkhurst, RSA) and a pilot plant sieve shaker. The fraction collected between 420μm and 600μm from the vibratory sieve shaker was used for chemical composition analysis and small scale pretreatment, while the fraction collected between 680μm and 6.5mm from the pilot plant sieve shaker set-up was used for pretreatment in the steam explosion pilot plant.

To ensure as little variation as possible between samples, a quarter sampling method was used to sample material prior to the experiments. This ensured that variations between

samples did not occur during sampling. Once sampled, material was placed in correctly sized plastic bags and stored either in a shipping container with sufficient ventilation or in a conditioning room at a constant temperature of 24°C. Material utilized for pilot plant steam explosion was stored in the shipping container due to the large amounts of material needed for this pretreatment, while material utilized for small scale pretreatment and chemical composition was stored in a conditioning room as the material requirements for these was much less and space was available to store these.

2.3.1.3. Chemical composition analysis of the raw material and pretreated solids

The chemical composition of the sweet sorghum bagasse cultivars received in 2008/2009 and 2010/2011 were analysed using well documented methods described by:

- NREL laboratory analytical procedure (LAP) for determination of structural carbohydrates and lignin in biomass [31].
- NREL laboratory analytical procedure (LAP) for determination of extractives in Biomass [32].
- NREL laboratory analytical procedure (LAP) for determination of ash in biomass [33]
- NREL laboratory analytical procedure (LAP) for determination of total solids in biomass and total dissolved solids in liquid process fractions [35].

Results were calculated as described in the NREL methods and the number of replications was a minimum of four to obtain a sufficient standard deviation.

2.3.2. Pretreatment

Pretreatment is influenced by a number of factors including temperature, residence time, and chemical concentration. Pretreatment was investigated with regards to the yields of both soluble sugars and degradation products released during pretreatment and soluble sugar yields released during enzymatic hydrolysis. The composition of the WIS (Water insoluble solids) was also investigated in some situations to determine the effect of pretreatment on chemical composition of the WIS which can be used to calculate the digestibility of the WIS.

2.3.2.1. Small scale pretreatment

Small scale dilute sulfuric acid pretreatment was carried out in tubular reactors as was first described by Lloyd and Wyman [11]. The reactors were constructed of Hastelloy C276 tubing having an outside diameter of 1.27cm, a wall thickness of 0.0889cm and a length of 15.2cm. The internal volume was 14.6ml. The ends of the tubing were fitted with Teflon plugs and stainless steel Swagelok caps. For rapid heat up and temperature control of the tubular reactors two fluidized sand baths (Techne model SBL-2D, Action Instrument SA, Marshalltown, RSA) were utilized. One sand bath was set at 50°C above the pretreatment temperature and was used for rapid heat up of the tubular reactors, while the other was used for holding the reactors at the specific pretreatment temperature through the use of a PID control unit. Experiments are carried out in triplicate at minimum.

Prior to pretreatment a 1.5g biomass sample was weighed out and soaked overnight in the required H_2SO_4 acid solution at a solids loading of 5% (g acid solution/g dry biomass). Following soaking, the sample was vacuum filtered so as to increase the solids content to 30% (g acid solution/g dry biomass) before being placed in a tubular reactor. The tubular reactor was heated up and maintained at the required pretreatment temperature with the aid of two fluidized sand baths before being quenched in a cooling water bath at the end of the pretreatment residence time. Parameters investigated included temperature (^{o}C), pretreatment time (min), and H_2SO_4 acid concentration (% w/w). Following pretreatment, the product from pretreatment was vacuum filtered to separate the pretreated solids from the liquid. The liquid portion, also known as pretreatment liquor, was stored at -4°C until analysis for monomeric sugars and byproducts on an HPLC. Further the pretreatment liquor underwent post hydrolysis to determine the oligomeric sugar content of the liquor. The solids remaining from vacuum filtration were then washed to remove any byproduct and sugar residues using 50ml of de-ionized water. The washed solids also known as water insoluble solids (WIS) were dried at 40°C before being enzymatically hydrolysed.

2.3.2.2. Pilot plant pretreatment

A steam explosion pilot plant (IAP, GmBH, Graz, Austria) equipped with a 40 bar electrical boiler, a 19L reaction vessel and a cyclone collection tank was used for pretreatment of sweet sorghum bagasse. Automation of this pilot plant was through a control panel comprised of a PC based HMI/SCADA system with the associated PLC's and instrumentation. This pilot plant was used to evaluate the scale up effects on steam explosion pretreatment

compared to that observed in small scale dilute acid pretreatment as well as to compare optimal yields achieved with sweet sorghum bagasse cultivars. Parameters that were varied to optimize steam explosion include pretreatment temperature, pretreatment time and type of catalyst. The catalysts that were evaluated were air dried biomass (no catalyst), water soaked biomass (water impregnation) and SO_2 soaked biomass (SO_2 dissolved into water soaked biomass).

For dry steam explosion 600g air dried sweet sorghum bagasse was fed batch-wise into the pilot plant. For preparation of samples for both water and SO_2 impregnation, 600g air dried sweet sorghum bagasse was soaked in 12L of water overnight to ensure that the maximum possible absorption of water into the biomass. The soaked material was then spun in a AEG spin dryer to remove residual moisture that was not absorbed into the biomass during soaking. Following spin drying of the soaked material, for the water only impregnated material, the soaked biomass was then fed batch wise into the pilot plant for pretreatment. For SO_2 impregnation on the other hand, SO_2 was introduced into a sealed bag containing soaked biomass (previously spun down) from a SO_2 cylinder situated on a scale. The amount of catalyst added was by gram weight of dry biomass, i.e. 3% g SO_2/g biomass meant that 18g of SO_2 had to be added for 600g dry weight of biomass. The bag containing the SO_2 and biomass was then stored for 45 minutes to allow the gas to diffuse into the water soaked biomass. After the 45 minute impregnation time had passed the bag was opened allowing excess gas to escape after which the SO_2 impregnated material was fed batch wise into the pilot plant pretreatment reactor.

For each batch pretreated by steam explosion, prepared sweet sorghum bagasse was weighed and fed into the 19L reaction vessel; saturated steam at 30Bar was injected into the pressure reactor from an electric steam boiler capable of producing 40bar steam. The temperature of the 19L reaction vessel was controlled at the set-point through manipulation of the vessel pressure which was possible with two air actuated needle control valves (Samson AG, Frankfurt, Germany) capable of controlling the rate of steam injection from the boiler. Following the injection of steam, the 19L reaction vessel heated up in approximately 2 minutes, upon which the timing of the pretreatment commenced. At the end of the respective residence time, an air actuated ball valve capable of opening within less than 0.5s was automatically opened resulting in an explosive expansion/decompression of the biomass and steam out of the 19L reaction vessel into the cyclone collection tank. Excess

steam escaped to the atmosphere while the exploded biomass remained in the blow tank. The pretreated biomass sample was collected from the cyclone and further analysed according to the analysis scheme given in Figure 2-1 below.

The pretreatment slurry obtained from steam explosion was collected, weighed and stored in an appropriate container for further processing. The moisture content of the slurry was measured by placing 5g of the slurry, in duplicate, in a 105°C oven for 24 hours until a constant dry weight was reached. This measurement was used for calculating the solid recovery following pretreatment. Two 50g samples of the slurry were weighed out and pressed using a 4ton hydraulic press to separate the liquid fraction (also known as pre-hydrolysate or pretreatment liquor) from the solid fraction. The liquid fraction was stored for HPLC analysis of both the monomeric sugars and byproducts released during pretreatment as described in section 2.3.4.2. Following pressing of the 50g samples, the remaining solid fraction was washed in 500ml of reverse osmosis water and vacuum filtered to remove any sugar residues before being stored for enzymatic hydrolysis and chemical composition analysis. 4g samples of the washed solids (now known as water insoluble solids or WIS) were weighed out for moisture determinations and placed in a 105°C oven for 24 hours until constant dry weight was reached. The wash liquid was sent for analysis on the HPLC.

Total slurry weight (g)

Temperature of slurry (ºC)

Pretreated slurry

Moisture and total solids determination of slurry

Moisture content using 2-5g pretreated slurry in triplicate at 105ºC for 24hr

Take 3 representative samples of 50g each

Hydraulic Press

Solid

Liquid

HPLC: Sugars, byproducts

Volume (ml)

pH (important for **CSF**)

Post hydrolysis (5ml liquor for hydrolysis)

HPLC: Total Sugars

Vacuum filter Wash with 500ml deionised water (relation 1/10 slurry:water)

HPLC: Wash sent for HPLC analysis

Total Wet WIS weight (g)

Water insoluble solids (WIS)

Moisture at 105ºC and 24hr using 0.5 – 2g

Determination of WIS and WSS content of slurry

Dry at 30-40C enough material for chemical analysis

Take 1 g in duplicate/triplicate to be used in 50ml of prepared enzymatic solution

Milling at 5 mm mesh

Moisture at 105ºC and 24hr using 0.5 – 2g

Determination of digestibility

0.3g in duplicate/triplicate to be used for **NREL determination of structural carbohydrates and lignin**

Important for calculations (dry weight of the 0.3 g)

Figure 2-1: Analysis of pretreatment slurry following steam explosion [34]

2.3.3. Enzymatic hydrolysis

Enzymatic hydrolysis was evaluated in terms of the soluble sugars released from hydrolysis and in some cases the cellulose digestibility of the water insoluble solids (WIS).

2.3.3.1. Enzymatic hydrolysis of small scale pretreated material

Following small scale dilute acid pretreatment, washed solids, known as water insoluble solids (WIS) were enzymatically hydrolysed using a commercial cocktail of enzymes in a manner similar to that described by NREL's method for Enzymatic Saccharification of Lignocellulosic biomass [36] . The enzymatic hydrolysis was performed in 45 ml glass bottle tubes containing 2% (w/V) solids (WIS) in 10ml of prepared enzyme solution at a pH of 4.8. The enzyme solution was comprised of a 5.0 mM sodium citrate buffer containing the cellulase, Spezyme CP from Genenkor, and the β-glucosidases solution, Novozym 188 from Novozymes. The loading of enzymes was 15FPU of enzyme/g dry WIS where Spezyme CP had a filter paper activity of 60FPU/ml and Novozym 188 had an activity of 700FPU/ml. Enzyme activity was determined according to NREL's method for the Measurement of Cellulase Activity [37]. To prevent microbial contamination 0.02% sodium azide was added to the enzyme solution. The hydrolysis was carried out in a shaking water bath at a temperature of 50°C and a shaking speed of 100rpm for 72 hours. After the allotted time, samples were drawn and stored for analysis on an HPLC.

2.3.3.2. Enzymatic Hydrolysis of pilot plant pretreated material.

Following pretreatment of biomass with the pilot plant set-up, the water insoluble solids were enzymatically hydrolysed in a method similar to that employed in enzymatic hydrolysis of small scale pretreatment material. The only difference being that the enzyme hydrolysis was carried out in 250ml Erlenmeyer flasks with 50ml of enzyme solution. The makeup of the enzyme solution remained the same except for the fact that a new batch of Novozym 188 was utilized which had an activity of 929FPU/ml. All the other parameters for the enzyme hydrolysis as described in 2.3.3.1 were the same.

2.3.4. Analysis

The raw material, pretreatment residue (including the liquor and WIS) and liquor from enzymatic hydrolysis were analysed according to LAP methods described by NREL [31]–[34]. Different chemical components from these analyses are described in figure 2-2.
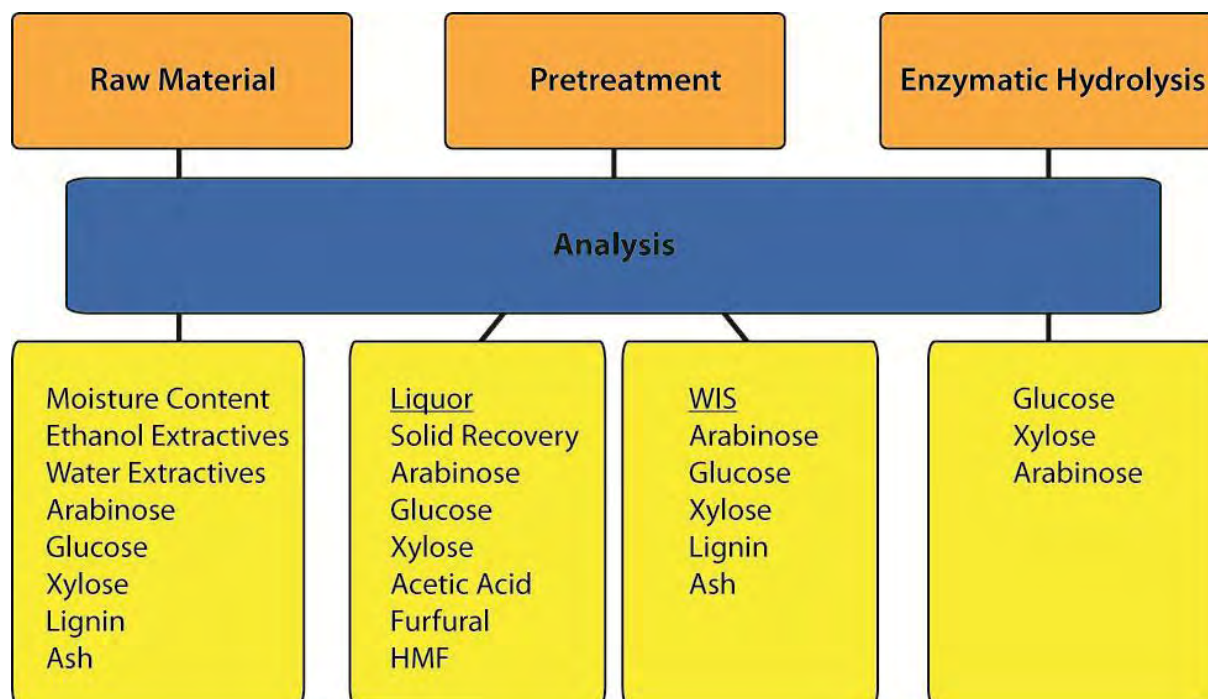
Figure 2-2: Chemical components analysed after different analysis [31]–[34]

2.3.4.1. Characterization of raw material and water insoluble solids (WIS)

A schematic diagram showing how both the raw material and WIS was characterized can be seen in

 2-3 below.
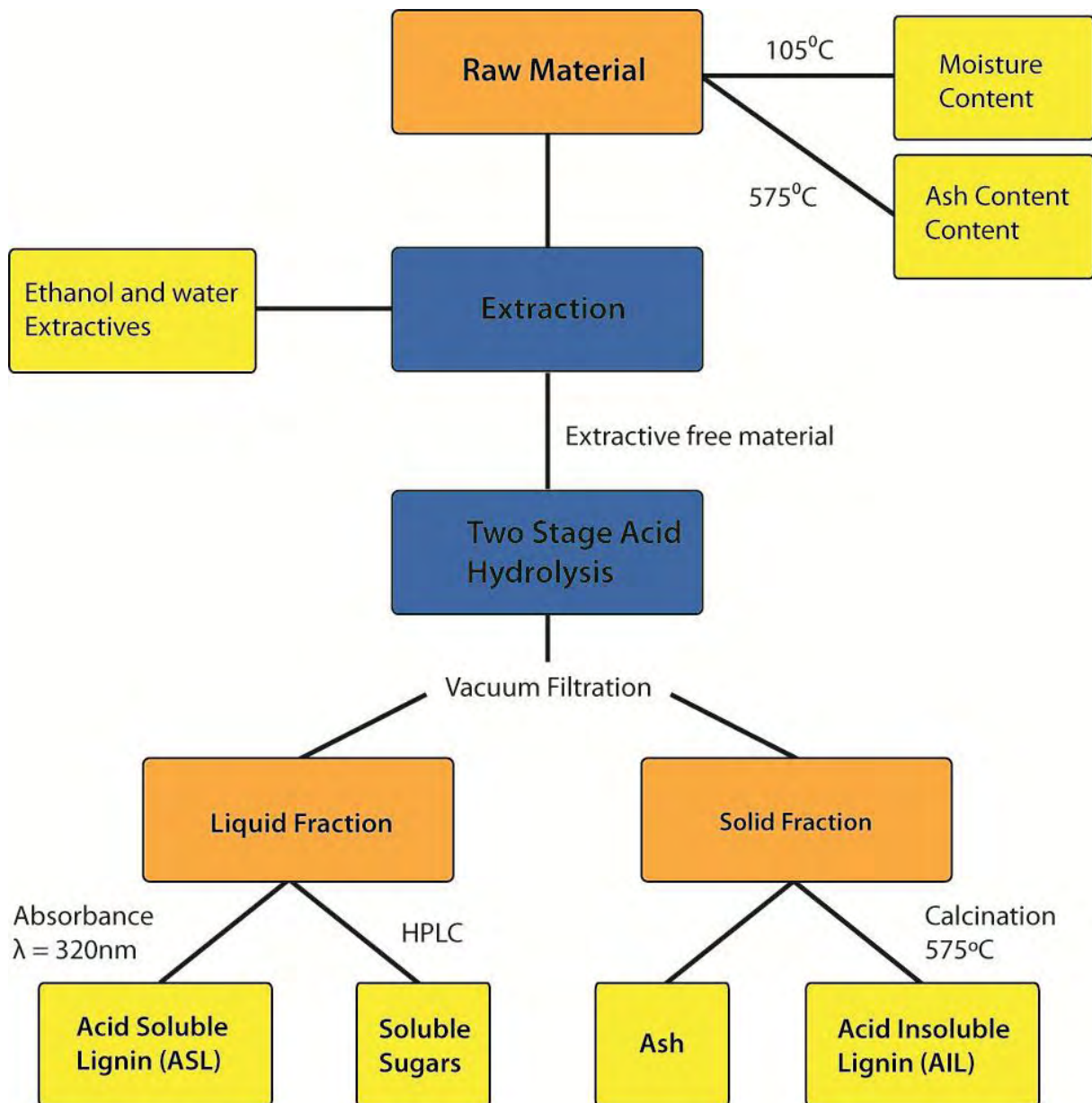
Figure 2-3: Methodology for characterization of raw material and water insoluble solids (WIS) [31]

Chemical composition was determined on an oven dry weight basis. To do so, the moisture content was determined by placing the samples in an oven at 105°C for approximately 24 hours until constant dry weight was reached. Once the moisture content had been determined, the biomass material was weighed out to correct for the moisture content and underwent a two-step extraction in which a water extraction was performed first followed by an ethanol extraction. Extractives such as tannins, waxes, soluble sugars, nitrogenous material and organic acids are present in the native biomass and have been known to influence the outcome of the acid hydrolysis and were therefore removed prior to hydrolysis [38]. Once the extraction was completed, the extractive free biomass underwent a two-step

acid hydrolysis. For this 0.3g of oven dry biomass was weighed out and mixed with 3ml of 72% H_2SO_4 after which it was placed in a water bath (Memmert Water bath WNB, Lasec, Ndabeni, Cape Town) for 1 hour at 30^0C. At the end of the first hydrolysis the product was diluted with 84ml of de-ionized water to bring the acid concentration down to 4% followed by which it was autoclaved for 1 hour at 121^0C. On completion of the autoclave cycle, the liquid fraction was separated from the solid fraction by vacuum filtration, and the liquid was analysed for soluble sugars on a HPLC as well as for acid soluble lignin (ASL) with a UV spectrometer at a wavelength of 320nm. The solid fraction was measured for ash and acid insoluble lignin (AIL) by calcinations at 575^oC in a furnace.

The major difference between chemical composition determinations of the raw material and water insoluble solids (WIS) was the extractions performed on the raw material. The pretreatment step utilized in producing the WIS was considered to have the same effect as the extraction technique and therefore extraction was left out when analyzing the WIS for chemical composition. A more detailed methodology on how to perform the extractions and the chemical composition determination can be seen in the following two NREL procedures

- NREL Laboratory Analytical Procedure (LAP) for Determination of structural carbohydrates and lignin in biomass [31].
- NREL Laboratory Analytical Procedure (LAP) for Determination of extractives in Biomass [32].

2.3.4.2. Characterization of pretreatment liquor and enzymatic hydrolysis liquor

The liquid fraction collected after pretreatment and enzymatic hydrolysis was analysed according to the NREL procedure for the Determination of Sugars, Byproducts and Degradation products in Liquid Fraction Process Samples LAP 013, 014, 015 [34].

For analysis of the pretreatment liquor, the pH of the liquid samples was measured, and then prepared for analysis by High Performance Liquid Chromatography (HPLC). HPLC analysis was utilized in determining the monomeric sugars arabinose, glucose, xylose and the byproducts acetic acid, furfural, HMF and formic acid. As some of the pretreated liquor contained sugars in their oligomeric form, a procedure was needed to reduce these to their monomeric form for analysis. As described in the NREL procedure, this was achieved by taking 5ml of pretreatment liquor, adding a prescribed amount (determined from the pH) of 72% H_2SO_4 to bring the acid concentration up to 4% (w/w) H_2SO_4. The samples were

subsequently autoclaved for 30minutes at 121°C. Upon completion of the autoclave cycle the samples were removed and cooled on ice before being prepared for analysis on the HPLC.

For analysis of the enzymatic hydrolysis liquor a preparation step for deactivation and removal of the proteins was necessary to protect the columns. This was achieved with the aid of 35% perchloric acid (PCA). With this PCA method 2ml of sample was centrifuged and the supernatant was pipetted into a 2ml Eppendorf tube and diluted for HPLC analysis so that the final sample volume was 1.8ml. 109.8μl of 35% (v/v) PCA was added and the sample was incubated on ice for 15 minutes. Subsequently, 99μl of 7M KOH was added and the sample was further incubated on ice overnight. After the elapsed incubation time, the sample was filtered through 0.22 μl syringe filters into the appropriate HPLC vials for HPLC analysis.

Once the samples had been prepared, a dilution step was required, followed by analysis on the appropriate HPLC set-up. The set-up used for analysis of sugars present in the pretreatment liquor, post hydrolysis and enzymatic hydrolysis liquor as well as for the byproducts acetic acid and formic acid in the pretreatment liquor was an Aminex HPX-87H Column equipped with a Cation-H Micro-Guard Cartridge (Bio-Rad, Johannesburg, South Africa). Glucose, xylose, arabinose, acetic acid and formic acid were measure on this column with a RI detector (Shodex RI-101). The operating conditions for this set-up was a temperature of 65 °C, a flow rate of 0.6 ml/min and a mobile phase consisting of 5mM H_2SO_4.

For the byproduct determination of HMF and furfural, a Luna C18 (2) reversed phase column, equipped with a Luna C18 (2) pre-column (Phenomenex, Promolab, Randburg, South Africa) was utilized at a column temperature of 25°C and a flow rate of 0.7ml/min. The mobile phases used for the elution were 5 mM trifluoroacetic acid in water (A) and 5 mM trifluoroacetic acid in acetonitrile (B). Separation was carried out by gradient elution from 5% mobile phase B, increasing to 11% B over 14 minutes and then increasing to 40% B over 3 minutes. The mobile phase composition was held constant at 40% for 2 minutes, followed by a decrease to 5% B over 5 minutes and ending with a final step of constant composition at 5% B for 4 minutes in order to equilibrate. HMF and furfural concentration were measured with a Dionex ultimate 3000 diode array detector at 215nm and 285nm.

2.3.5. Experimental design

A number of experimental methods were utilized in evaluating and optimizing the sweet sorghum cultivars in this study. The methods are described below.

2.3.5.1. Screening of initial thirty six sweet sorghum bagasse cultivars

All thirty six sweet sorghum bagasse cultivars were pretreated under one set of pretreatment conditions to screen the cultivars for those that perform well based on agronomic factors, pretreatment response and ethanol yields with the aim of selecting cultivars with high total ethanol yields. At this stage the screening conditions were used to reduce the initial number of cultivars received to 10 and pretreatment was performed with a single pretreatment condition under small scale pretreatment as described in section 2.3.2.1. The pretreatment condition used was 170^oC, 15 minutes and an acid concentration of 0.7% (w/w) H_2SO_4 which was chosen from preliminary work performed on sweet sorghum bagasse at the University of Stellenbosch [39]. Out of the 16 different pretreatment conditions evaluated in this preliminary work, a pretreatment condition of 170^oC, 15 minutes and 0.7% H_2SO_4 resulted in the highest combined sugar yield for sweet sorghum. Therefore this condition was thought to be suitable in evaluating the initial number of sweet sorghum cultivars as it would highlight cultivars which would result in high combined sugar yields.

2.3.5.2. Reduction of ten cultivars to five

Once ten preferred cultivars had been selected, further small scale pretreatment work was carried out to screen the selected ten cultivars and reduce the number of cultivars for optimization to five. Two further pretreatment conditions were chosen for this second round of selection. These two pretreatment conditions were of low pretreatment severity to highlight cultivars that perform well under low severity conditions. The conditions were 190^oC, 5 minutes and an acid concentration of 0.25% (w/w) H_2SO_4 for the first condition and 200^oC, 5 minutes and an acid loading of 0.075% (w/w) H_2SO_4 for the second condition. Once again as this was a screening step, no specific experimental design was chosen.

2.3.5.3. Optimization of five preferred cultivars at small scale dilute acid pretreatment for two different harvest seasons

For small scale optimization, five preferred cultivars were selected. Each of the five preferred cultivars were optimized to identify differences in optimal pretreatment conditions between

cultivars as well as the response of these five selected cultivars to the pretreatment conditions with dilute sulfuric acid pretreatment. Pretreatment was optimized for combined sugar yields by varying pretreatment time, temperature and acid concentration through use of a central composite design, which evaluated a specific experimental region as described by Figure 2-4 [40]. The central composited design used was a 2^2 factorial with face centered star points. The factors evaluated where temperature and time and the high, low and centre levels can be seen in the following diagram, Figure 2-4.
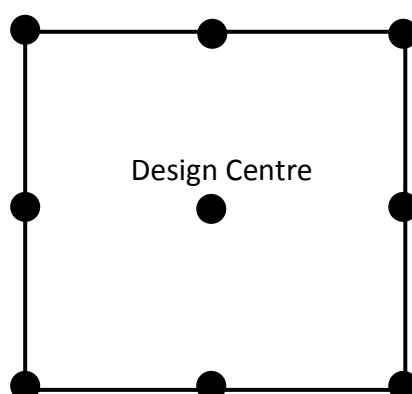
Figure 2-4: Experimental region represented by central composite design [40]

Table 2-3. Central composite design used in optimizing pretreatment at a small scale with 0.25% (w/w) H_2SO_4.

Factors	Range Levels		
	-1	0	+1
Temperature (°C)	180°C	185°C	190°C
Residence Time (min)	5	10	15

2.3.5.4. Optimization of three preferred cultivars using pilot plant steam explosion

Following the optimization of the five preferred cultivars using small scale dilute sulfuric acid pretreatment, three cultivars were selected to proceed to pretreatment at a pilot plant scale as described in section 2.3.2.2. The three preferred cultivars were pretreated under three different conditions looking at the effect that pretreatment had on air dried material (ADM), water soaked material (WSM) and water soaked material that had been impregnated with

sulfur dioxide. For each of these three pretreatment cases (i.e air dried, water soaked and SO$_2$ impregnated) a 2^2 factorial was carried out investigating the effect of temperature and time on pretreatment yield. The factorial designs can be seen in Table 2-4 and Table 2-5 below with the high, low and centre levels for the two factors. These factorial designs are aimed at covering a specific range (as indicated Figure 2-5) of temperature and time in which the optimum yields can be obtained.

Table 2-4: 2^2 Factorial design used in optimising air dried and water soaked steam explosion

Factors	Range Levels		
	-1	0	+1
Temperature (^{o}C)	190°C	197.5^{o}C	205°C
Residence Time (min)	5	7.5	10

Table 2-5: 2^2 Factorial design used in optimising SO$_2$ impregnated steam explosion

Factors	Range Levels		
	-1	0	+1
Temperature (^{o}C)	185°C	190°C	195°C
Residence Time (min)	2	5	8
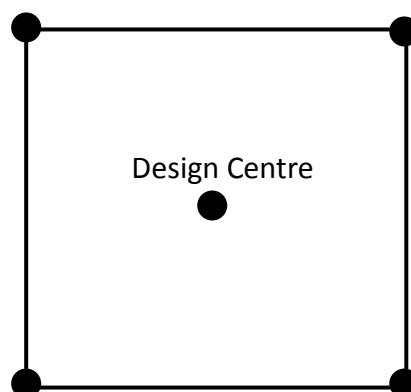
Figure 2-5: Experimental region of a 2^2 factorial [40]

2.3.6. Model fitting

Two experimental designs where used to investigate the effects of temperature and time on the pretreatment of different cultivars, namely a standard 2^2 factorial and a 2^2 central composite design. The standard 2^2 factorial was used in searching for the optimum sugar yields at a pilot plant scale while the 2^2 central composite designs were utilized in searching for the optimum sugar yields under small scale dilute sulfuric acid pretreatment. The central composited designs allowed the quadratic and linear main effects as well as the two-way interaction between effects to be investigated while the standard factorial designs only allowed for investigation of both the linear main effects and the two-way interaction between effects. Both of these designs reduce the required number of experiments in locating an optimum.

Models where fitted to both the standard factorial and central composite designs using a well know statistical program, Statistica 10 (Stat Soft Inc, Tulsa, USA). The general polynomial model that was for the standard factorial and central composite design can be seen in Equation 1 and Equation 2 respectively [40].

Equation 1 $y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \varepsilon$

Equation 2 $y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \varepsilon$

In the polynomial equations above y is the yield, β is the regression coefficient, x is the value of the effect and ε is the random error. The models were analysed with Statistica 10 (Stat Soft Inc, Tulsa, USA) using analysis of variance (ANOVA) to determine which effects were statistically significant. Further the Fischer statistical test (F-test) was utilized in evaluating the factors which significantly influenced the response variable. Subsequently the F-test was used to further refine the models if necessary and the ANOVA repeated for refined models. The results of the model fitting are discussed in subsequent sections.

2.3.7. Equations

2.3.7.1. Energy content of lignocellulose biomass

Jimenez and Gonzalez (1991) proposed an equation to estimate the higher heating value (HHV) of lignocellulose biomass based on the chemical composition [41][42]. Equation 3 can be seen below

Equation 3
$$HHV \left(\frac{Kj}{g} \right) = \left[1 - \frac{ash}{100 - ash} \right] (0.1739Ce + 0.2663L + 0.3219E)$$

Where   ash = Ash content in biomass (g/100g biomass)

Ce = Hemi - cellulose and cellulose content in biomass (g/100g biomass)

L = Lignin content in biomass (g/100g biomass)

E = Extractive content in biomass (g/100g biomass)

2.3.7.2 Pretreatment severity

Pretreatment severity is the severity of the pretreatment on the biomass being pretreated. It can be used to relate pretreatment parameters such as temperature, time and acid concentration to certain outcomes of pretreatment such as yields, digestibility and sugar recoveries. For pretreatment incorporating only temperature and time the severity factor can be seen in Equation 4 while for pretreatment incorporating acid catalysts the combined severity factor is given in Equation 5[11].

Equation 4
$$R_0 = t.\exp \left(\frac{T - 100}{14.75} \right)$$

Equation 5
$$CS = \log(R_0) - pH$$

Where R_o = pretreatment severity

t   = pretreatment residence time

T   = pretreatment temperature

CS = combined severity factor

pH = the pH of the pretreatment liquor

2.4. Results

2.4.1. Chemical composition of raw sweet sorghum bagasse cultivars

The raw material composition of the sweet sorghum bagasse (*Sorghum Bicolor* (L.Moench)) collected from the Ukhulinga experimental farm in Kwa-Zulu Natal during the 2008/2009 (first) and 2010/2011 (second) season was determined. The results of the chemical composition analysis can be seen in Table 2-6 for the thirty six cultivars harvested in the 2008/2009 season and in Table 2-9 for the six cultivars harvested in the 2010/2011 season. Determining the mean values for the chemical composition across the entire range of cultivars collected from the 2008/2009 season gave an ash, ethanol extractives, water extractive, lignin, glucose, xylose and arabinose content of 1.14, 1.62, 2.95, 17.88, 39.23, 22.15, and 1.53 g/100g raw material respectively.

In comparing the thirty six cultivars harvested in 2008/2009, substantial variation in chemical composition was observed between cultivars. For example, the xylose content, which represents around 90% of the hemicellulosic sugars in sweet sorghum bagasse, varied from 22.22 to 25.59 g/100g raw material, while the glucose content varied from 34.03 to 45.93 g/100g raw material and the lignin content varied from 14.29 to 21.23 g/100g raw material. Summing the available sugars, xylose, glucose and arabinose, the total available sugar content varied from 57.36 to 69.35 g/100g raw material. This indicates definite cultivars which would be favored for lignocellulose ethanol production based on total available sugars alone as cultivars with higher total available sugars should theoretically yield more ethanol per g raw material.

Differences in chemical composition components between cultivars selected for harvest in 2010/2011 with the same cultivars harvested in the 2008/2009 season were as follows. The ash and water extractive content increased for all six of the cultivars in the 2010/2011 season. The lignin, xylose and arabinose content generally decreased for the cultivars in the 2010/2011 season, which was observed by the fact that the median of the values for these cultivars decreased between the two harvest seasons, although not all of the cultivars indicated a decrease. In terms of the ethanol extractives, glucose and total available sugar content, a general increase was observed for the 2010/2011 harvest as the median for these values incorporating the preferred cultivars increased. In terms of sample variance, the chemical compositional components of the 2008/2009 cultivars had a much larger variance

compared to those of the 2010/2011 season. Glucose content was the component that had the highest difference in sample variance between the two seasons which subsequently meant that the total available sugar content also had a large difference in variance between the two seasons. The smaller sample variance observed for the chemical components of the 2010/2011 season meant that the cultivars harvested in this season where chemically much more similar to each other than the same six cultivars harvested in the 2008/2009 season.

A statistical analysis was carried out looking for significant differences in the chemical composition of the thirty six sweet sorghum cultivars collected during the 2008/2009 harvest season. This was achieved by means of a one way Anova, combined with the Fischer LSD and Bonferroni post-hoc test in Statistica 10 (Stat Soft Inc, Tulsa, USA). The above mentioned post-hoc tests looked for significant differences between samples. The mean, minimum, maximum and standard deviation for the chemical composition of the 2008/2009 sweet sorghum bagasse cultivars can be seen in Table 2-7. The chemical characteristics which showed the largest significant differences between cultivars were for the lignin and ash content while the least significant differences were observed for both the xylose and glucose content present in the raw material. This trend can be seen for both the Fischer LSD and Bonferroni post-hoc tests. For example with the Fischer LSD test 65.7% of the cultivars were significantly different ($p < 0.05$) from other cultivars with regards to their lignin content while with the more conservative Bonferroni test this value drops to 35.4%. Similarly with the Fischer LSD test 58% of the cultivars were significantly different from other cultivars with regards to their ash content while the more conservative Bonferroni test suggests that only 25.6% of the cultivars were significantly different from one another when it came to ash content.

In Table 2-8, the agronomic data of the cultivars from the 2008/2009 season can be seen. The cultivar having the highest dry matter yield was AP6 which yielded 19.6 tons/ha while the cultivar having the lowest yield was AS263, which had a dry matter yield of 5.0 tons/ha. One thing to note is that AP6 is a pearl millet which was planted earlier than the other sweet sorghum cultivars and so it can be expected that it will perform slightly different to the traditional sweet sorghum cultivars. Excluding the pearl millet cultivar AP6, the cultivar with the next highest yield was MSJH13, which had a dry matter yield of 14.3 tons/ha.

Comparing the agronomic data between the two different seasons, 2008/2009 and 2010/2011, a number of observations are observed. MSJH13 had a similar fresh stem, dry matter and fibre yield between the two seasons, but had a non-structural sugar yield in 2010/2011 half that obtained in 2008/2009. AP6 had the biggest observable change from season to season in terms of dry matter yield, which dropped from 12.33 tons/ha to 6.4 tons/ha. This change was attributed to the fact that in the 2008/2009 season AP6 was planted in September 2008, while in November 2010 it was planted at the same time as the sweet sorghum cultivars. The extra two months therefore accounted for the higher biomass yield that was observed in 2008/2009 with cultivar AP6. With regards to the other cultivars, fresh stem and dry matter yield decreased for MSJH16, SS27 and AS103 while it was found to increase for AS246. Non-structural sugar content increased for MSJH16, AS246 and SS27, but decreased for AS103. Lastly fibre and ash content increased for AS246, but decreased for MSJH16, SS27 and AS103.
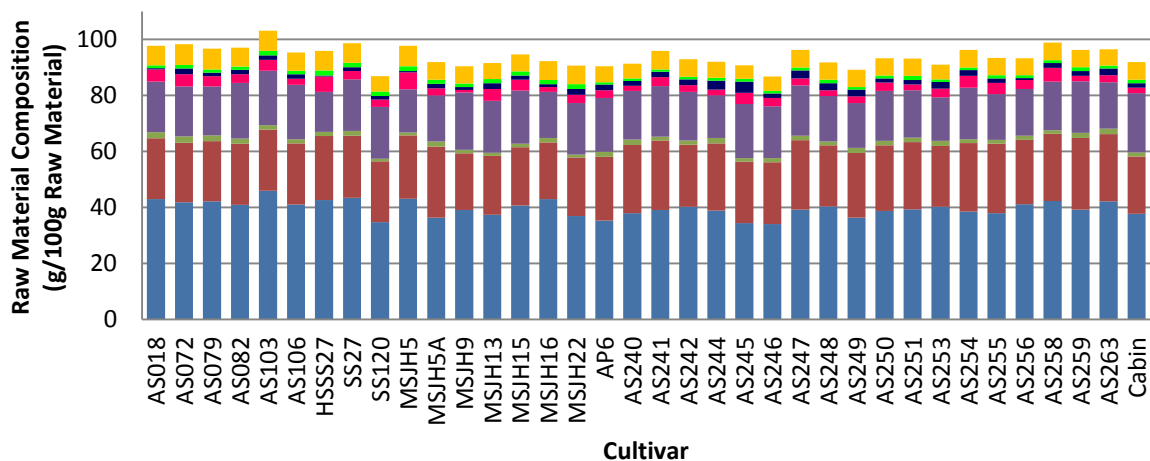
Figure 2-6: Chemical composition of the different sweet sorghum cultivars. ■ Glucose g/100g raw material, ■ Xylose g/100g raw material, ■ Arabinose g/100g raw material, ■ Lignin g/100g raw material, ■ Water extractives g/100g raw material, ■ Solvent extractives raw material, ■ Ash g/100g raw material, ■ Moisture g/100g raw material.

Table 2-6: Chemical composition of cultivars from the 2008/2009 field trials

Sweet Sorghum Cultivar	Chemical Components (g/100g raw material)								
	Moisture Content	Ash	Water Extractives	Solvent Extractives	Lignin	Glucose	Xylose	Arabinose	Total
AS018	7.02 ± 0.49	1.05 ± 0.13	4.30 ± 0.06	0.50 ± 0.04	18.01 ± 1.76	42.93 ± 2.83	21.70 ± 0.50	2.27 ± 0.00	99.52
AS072	7.40 ± 0.42	1.24 ± 0.37	4.31 ± 0.02	2.12 ± 0.07	17.89 ± 0.29	41.78 ± 1.75	21.27 ± 0.35	2.27 ± 0.09	100.07
AS079	7.42 ± 0.28	1.12 ± 0.17	3.68 ± 0.09	1.21 ± 0.01	17.52 ± 0.94	42.21 ± 2.77	21.45 ± 0.05	2.02 ± 0.04	98.23
AS082	6.75 ± 0.16	1.18 ± 0.16	3.05 ± 0.05	1.61 ± 0.05	19.93 ± 0.57	40.90 ± 0.69	21.89 ± 0.87	1.75 ± 0.17	98.64
AS103	7.16 ± 0.28	1.65 ± 0.04	3.88 ± 0.03	1.54 ± 0.45	19.49 ± 1.00	45.93 ± 0.60	21.82 ± 0.97	1.60 ± 0.33	104.63
AS106	6.49 ± 0.05	1.30 ± 0.10	2.10 ± 0.14	1.65 ± 0.09	19.49 ± 0.68	40.97 ± 2.57	21.90 ± 0.46	1.42 ± 0.21	96.75
HSS27	6.98 ± 0.54	1.88 ± 0.13	5.52 ± 0.14	0.32 ± 0.00	14.29 ± 0.21	42.67 ± 1.39	22.86 ± 0.00	1.38 ± 0.28	97.40
SS27	6.94 ± 0.70	1.55 ± 0.13	3.04 ± 0.13	1.40 ± 0.20	18.34 ± 0.68	43.42 ± 1.29	22.16 ± 0.68	1.74 ± 0.12	100.17
SS120	5.40 ± 0.93	1.59 ± 0.24	2.57 ± 0.19	1.35 ± 0.00	18.54 ± 0.38	34.70 ± 0.00	21.77 ± 0.19	0.89 ± 0.01	91.07
MSJH5	7.24 ± 0.09	1.71 ± 0.09	6.02 ± 0.23	0.52 ± 0.07	15.49 ± 1.00	43.13 ± 1.14	22.60 ± 1.39	0.97 ± 0.18	99.18
MSJH5A	6.26 ± 0.00	1.47 ± 0.37	2.47 ± 1.31	1.66 ± 1.49	16.53 ± 1.10	36.34 ± 1.56	25.41 ± 1.92	1.73 ± 0.30	95.78
MSJH9	6.12 ± 0.11	1.30 ± 0.25	0.96 ± 0.09	0.98 ± 0.04	20.45 ± 1.70	39.10 ± 2.27	20.22 ± 0.10	1.21 ± 0.08	92.74
MSJH13	5.64 ± 0.05	1.48 ± 0.04	4.30 ± 0.66	2.05 ± 0.12	18.53 ± 0.88	37.36 ± 0.90	21.07 ± 0.73	1.06 ± 0.05	95.81
MSJH15	6.02 ± 0.26	1.51 ± 0.18	3.87 ± 0.04	1.51 ± 0.28	18.94 ± 0.72	40.72 ± 2.27	20.74 ± 0.42	1.30 ± 0.00	96.41
MSJH16	6.73 ± 0.73	1.51 ± 0.14	1.72 ± 0.19	1.00 ± 0.05	16.51 ± 0.12	42.91 ± 1.99	20.24 ± 0.58	1.64 ± 0.12	95.51
MSJH22	6.73 ± 1.80	1.64 ± 0.10	3.03 ± 0.18	2.02 ± 0.37	18.39 ± 1.27	36.91 ± 0.48	20.87 ± 0.83	1.07 ± 0.11	94.82
AP6	5.72 ± 0.31	0.71 ± 0.10	2.75 ± 0.85	2.13 ± 0.35	19.11 ± 1.14	35.36 ± 2.80	22.68 ± 2.05	1.90 ± 0.25	94.66
AS240	5.22 ± 1.06	1.01 ± 0.16	1.83 ± 0.81	1.61 ± 1.15	17.45 ± 0.97	37.94 ± 0.40	24.33 ± 1.10	1.91 ± 0.11	94.32
AS241	6.64 ± 0.50	0.70 ± 0.08	3.26 ± 1.26	1.96 ± 0.08	18.02 ± 0.75	39.12 ± 0.83	24.76 ± 0.93	1.39 ± 0.13	98.81
AS242	6.26 ± 0.00	0.93 ± 0.05	2.42 ± 0.97	2.06 ± 0.18	17.36 ± 0.80	40.25 ± 0.90	22.13 ± 1.69	1.47 ± 0.35	92.88
AS244	5.76 ± 0.45	0.99 ± 0.01	2.12 ± 0.31	3.23 ± 0.04	15.12 ± 0.66	38.82 ± 1.14	24.03 ± 1.24	1.99 ± 0.15	95.04
AS245	4.75 ± 0.73	1.09 ± 0.08	4.01 ± 0.18	1.02 ± 0.32	19.27 ± 1.11	34.38 ± 2.89	21.93 ± 1.05	1.30 ± 0.19	92.17
AS246	5.12 ± 0.65	0.92 ± 0.11	2.91 ± 1.09	1.61 ± 0.30	18.60 ± 1.01	34.03 ± 1.03	22.02 ± 0.54	1.50 ± 0.30	91.62
AS247	6.26 ± 0.00	1.06 ± 0.13	2.52 ± 0.55	2.85 ± 1.08	17.90 ± 0.98	39.13 ± 1.51	24.89 ± 0.54	1.62 ± 0.08	99.39
AS248	6.26 ± 0.00	1.27 ± 0.05	2.14 ± 0.68	2.42 ± 0.51	16.16 ± 0.71	40.35 ± 1.99	21.77 ± 0.84	1.44 ± 0.00	91,82
AS249	6.19 ± 0.54	0.94 ± 0.01	2.46 ± 0.38	2.34 ± 0.22	15.97 ± 0.24	36.38 ± 0.00	23.02 ± 0.83	1.87 ± 0.00	89.18
AS250	6.26 ± 0.00	0.94 ± 0.17	2.89 ± 0.97	1.60 ± 0.65	17.86 ± 1.31	38.74 ± 1.04	23.48 ± 1.64	1.55 ± 0.18	96.43
AS251	6.26 ± 0.00	1.49 ± 0.07	2.05 ± 0.74	1.52 ± 0.07	16.93 ± 0.72	39.36 ± 2.32	23.98 ± 1.35	1.61 ± 0.06	96.50
AS253	5.20 ± 0.40	0.95 ± 0.13	2.99 ± 0.18	2.44 ± 0.38	15.62 ± 0.60	40.17 ± 2.02	21.75 ± 1.30	1.81 ± 0.00	94.04

AS254	6.26 ± 0.00	0.89 ± 0.04	4.32 ± 1.58	2.03 ± 0.31	18.38 ± 1.22	38.45 ± 0.54	24.53 ± 1.27	1.34 ± 0.38	99.20
AS255	6.26 ± 0.00	1.10 ± 0.03	4.06 ± 0.63	1.56 ± 0.06	16.39 ± 0.51	37.88 ± 0.75	24.81 ± 0.12	1.36 ± 0.19	96.65
AS256	6.00 ± 0.61	1.10 ± 0.13	3.20 ± 0.16	0.65 ± 0.37	16.66 ± 0.63	41.15 ± 1.45	22.99 ± 0.89	1.46 ± 0.44	93.21
AS258	6.26 ± 0.00	1.04 ± 0.03	4.86 ± 0.22	1.73 ± 0.10	17.41 ± 0.57	42.30 ± 2.04	23.92 ± 1.51	1.29 ± 0.00	98.81
AS259	6.26 ± 0.00	1.33 ± 0.03	2.00 ± 0.14	1.66 ± 0.10	18.39 ± 0.55	39.15 ± 1.02	25.59 ± 1.53	1.88 ± 0.07	99.51
AS263	5.77 ± 0.18	1.10 ± 0.02	2.51 ± 0.12	2.40 ± 0.17	16.58 ± 0.50	42.12 ± 1.17	23.99 ± 0.57	1.96 ± 0.00	99.56
Cabin	6.26 ± 0.00	1.23 ± 0.06	2.00 ± 0.46	1.61 ± 0.43	21.13 ± 0.74	37.66 ± 0.80	20.54 ± 0.77	1.44 ± 0.07	95.74
Minimum	**4.75**	**0.70**	**0.96**	**0.32**	**14.29**	**34.03**	**20.22**	**0.89**	
Maximum	**7.42**	**1.88**	**6.02**	**4.01**	**21.13**	**45.93**	**25.59**	**2.27**	
Median	**6.26**	**1.15**	**2.95**	**1.63**	**17.89**	**39.25**	**22.15**	**1.53**	

[a] Chemical components are given on an oven dry weight
[b] A minimum of four replicas were performed in characterizing the sweet sorghum cultivars

Table 2-7: Significant differences between chemical components in 2008/2009 sweet sorghum bagasse

	Chemical Component						
	Ash g/100g Raw material	Extractives Ethanol g/100g Raw material	Extractives Water g/100g Raw material	Lignin g/100g Raw material	Glucose g/100g Raw material	Xylose g/100g Raw material	Arabinose g/100g Raw material
Min	0.7	0.32	0.96	14.29	34.03	20.22	0.89
Max	1.88	4.01	6.02	21.13	45.93	25.59	2.27
Mean	1.15	1.63	2.95	17.89	39.25	22.15	1.53
Standard Deviation	0.29	0.67	1.12	1.52	2.79	1.48	0.34
	Significant Difference (%)						
Fischer LSD Test	58.0	40.6	46.8	65.7	44.9	36.5	46.6
Bonferroni Test	26.3	10.6	13.2	35.4	9.4	4.8	11.1

[a] A minimum of four replica's were performed in characterizing the mean and standard deviations shown in the table

Table 2-8: Agronomic characteristics of 2008/2009 sweet sorghum cultivars

Sweet Sorghum Cultivar	Fresh stem Yield	Dry content	Dry Matter	Stalk Non-structural sugar	Fibre+Ash
	Ton/ha	%	Ton/ha	Ton/ha	Ton/ha
AS018	39.5	25.1	9.9	5.6	4.4
AS072	32.8	22.6	7.4	4.4	3.0
AS079	36.3	29.3	10.6	5.8	4.9
AS082	33.2	28.2	9.4	5.0	4.4
AS103	45.1	28.5	12.9	6.0	6.9
AS106	34.7	32.2	11.2	5.3	5.9
HSS27	55.2	25.0	13.8	6.5	7.3
SS27	55.2	25.0	13.8	6.5	7.3
SS120	57.1	18.5	10.6	3.8	6.8
MSJH5	47.3	22.6	10.7	4.8	5.9
MSJH9	55.2	25.0	13.8	6.5	7.3
MSJH13	61.2	24.3	14.9	5.0	9.9
MSJH15	53.2	21.5	11.4	4.8	6.7
MSJH16	47.3	22.6	10.7	4.8	5.9
MSJH22	49.7	24.6	12.2	1.6	10.6
AP6	86.8	22.6	19.6	7.3	12.3
AS240	23.2	35.1	8.1	2.4	5.7
AS241	32.0	33.1	10.6	4.3	6.3
AS242	28.6	27.2	7.8	4.2	3.6
AS244	34.4	21.5	7.4	5.1	2.3
AS245	35.3	25.9	9.1	6.7	4.0
AS246	43.9	23.6	10.4	6.7	3.6
AS247	34.4	26.5	9.1	4.4	4.7
AS248	45.5	23.9	10.9	6.7	4.2
AS249	44.1	25.5	11.2	4.6	6.6
AS250	30.4	24.6	7.5	4.4	3.1
AS251	43.7	23.4	10.2	4.6	5.6
AS253	40.3	23.6	9.5	5.3	4.2
AS254	43.7	25.7	11.2	6.0	5.2
AS255	36.1	22.6	8.2	4.6	3.6
AS256	29.8	30.7	9.1	5.0	4.1
AS258	29.8	30.7	9.1	5.0	4.1
AS259	23.3	22.4	5.2	3.5	1.7
AS263	17.5	28.3	5.0	2.9	2.1
Minimum	**17.5**	**19.8**	**5.0**	**1.9**	**1.7**
Maximum	**86.8**	**35.1**	**19.6**	**7.3**	**12.3**
Median	**40.3**	**25.0**	**10.4**	**5.0**	**5.2**
Std.Deviation	**12.9**	**3.7**	**1.3**	**2.7**	**2.2**

[a] No agronomic data was collected for the sweet sorghum cultivars cabin

Table 2-9: Chemical Composition of cultivars from the 2010/2011 field trials

Sweet sorghum cultivar	Chemical component (g/100g raw material)							
	Moisture content	Ash	Water extractives	Solvent extractives	Lignin	Glucose	Xylose	Arabinose
MSJH 13	7.96 ± 0.45	1.94 ± 0.05	4.86 ± 0.67	2.06 ± 0.13	18.98 ± 0.03	39.88 ± 5.09	20.07 ± 2.69	1.17 ± 0.26
MSJH 16	7.39 ± 0.24	2.23 ± 0.07	5.77 ± 0.07	1.89 ± 0.08	18.26 ± 0.14	41.74 ± 0.05	21.05 ± 0.01	1.13 ± 0.19
SS27	7.41 ± 0.09	1.91 ± 0.03	5.16 ± 0.16	1.97 ± 0.02	18.36 ± 0.43	41.13 ± 0.10	21.45 ± 0.09	1.34 ± 0.19
AP6	6.45 ± 0.21	1.67 ± 0.09	5.74 ± 0.00	1.66 ± 0.12	17.81 ± 0.04	40.65 ± 0.49	20.57 ± 1.10	1.59 ± 0.06
AS103	6.89 ± 0.19	1.93 ± 0.04	6.05 ± 0.67	2.13 ± 0.03	17.84 ± 0.49	41.85 ± 0.87	21.10 ± 1.65	1.03 ± 0.06
AS246	7.54 ± 0.17	1.91 ± 0.67	5.53 ± 0.03	2.38 ± 0.17	18.10 ± 0.58	41.24 ± 0.00	21.57 ± 0.87	1.37 ± 1.65
Minimum	6.45	1.67	4.86	1.66	17.57	39.88	20.07	1.03
Maximum	7.96	2.23	6.05	2.38	18.98	41.85	21.57	1.59
Median	7.40	1.92	5.64	2.01	18.11	41.18	21.07	1.25

[a] Chemical components are given on an oven dry weight

Table 2-10: Agronomic characteristics of 2010/2011 sweet sorghum Cultivars

Sweet sorghum cultivars	Fresh stem yield	Dry content	Dry matter	Stalk non-structural sugar	Fibre+ash
	Ton/ha	%	Ton/ha	Ton/ha	Ton/ha
MSJH13	59.6	30.2	16.1	8.9	8.5
MSJH16	43.9	23.5	10.3	5.9	4.4
SS27	43.1	27.8	12	7.3	4.7
AP6	50.5	26.9	13.6	7.2	6.4
AS103	29.2	26.8	7.8	4.2	3.1
AS246	51.2	30.0	15.3	8.9	6.5
Minimum	**29.2**	**23.5**	**7.8**	**4.2**	**3.1**
Maximum	**59.6**	**30.2**	**16.1**	**8.9**	**8.5**
Median	**47.2**	**27.4**	**12.8**	**7.3**	**5.6**

2.4.2. Screening of initial sweet sorghum cultivars

2.4.2.1. Pretreatment of initial sweet sorghum cultivars

A number of sweet sorghum cultivars were screened for best pretreatment response and highest total ethanol yield. Dilute sulfuric acid pretreatment as described in section 2.3.2.1 was carried out on each of the cultivars at the same conditions for both pretreatment and enzymatic hydrolysis. The pretreatment condition selected for screening was a pretreatment temperature of 170°C, a residence time of 15 minutes and an acid concentration of 0.7% (w/w) H$_2$SO$_4$, while enzymatic hydrolysis was carried out in the standard manner as described in section 2.3.3.1. The results of the pretreatment and enzymatic hydrolysis screening can be seen in Table 2-11.

Combined sugar yield varied from 32.63 to 44.04 g/100g raw material. Furthermore the xylose yield obtained in the pretreatment liquor and the glucose yield obtained from the enzymatic hydrolysis step varied from 12.82 to 19.10 g/100g raw material and from 13.90 to 22.17 g/100g raw material respectively. These differences in pretreatment response indicated that some cultivars were better suited to pretreatment by dilute sulfuric acid than others. The best and worst performing cultivar in terms of combined sugar yields were AS072 and MSJH9 respectively, while for glucose yields obtained in enzymatic hydrolysis the best and worst cultivars were AS244 and AS248, respectively. The best and worst cultivars in terms of xylose released into the pretreatment liquor were AS249 and MSJH5 respectively. No direct correlation (i.e. the best xylose yield resulted in the best combined sugar yield) could be seen in comparing the best and worst cultivars for xylose yields from pretreatment, glucose yields from enzymatic hydrolysis and combined sugar yields, indicating that a number of factors and interactions between these factors were responsible for high combined sugar yields. Interestingly examining the variation in xylose yields obtained in the pretreatment liquor compared to the glucose yields obtained in enzymatic hydrolysis it was noticed that xylose yields obtained in the pretreatment liquor were quite similar for the majority of cultivars (i.e xylose yields differ by 1 – 3 g xylose/g raw material), while glucose yields from enzymatic hydrolysis vary consistently between cultivars (i.e. glucose yields from enzymatic hydrolysis vary between 1 – 6 g glucose/g raw material). At the specific dilute sulfuric acid pretreatment conditions utilized in this first screening step a similar response in xylose yield was observed while differences in cultivar were highlighted in the observed

enzymatic hydrolysis yields. This suggests that factors specific to individual cultivars will not enhance the xylose yields following pretreatment but rather the resulting digestibility of the substrate. It must be noted though that increasing or decreasing the pretreatment severity could easily shift this to result in enhanced xylose yields depending on substrate specific characteristics over enhanced enzymatic hydrolysis results and that the above mentioned observations will most probably only apply to the specific conditions used in the screening step.
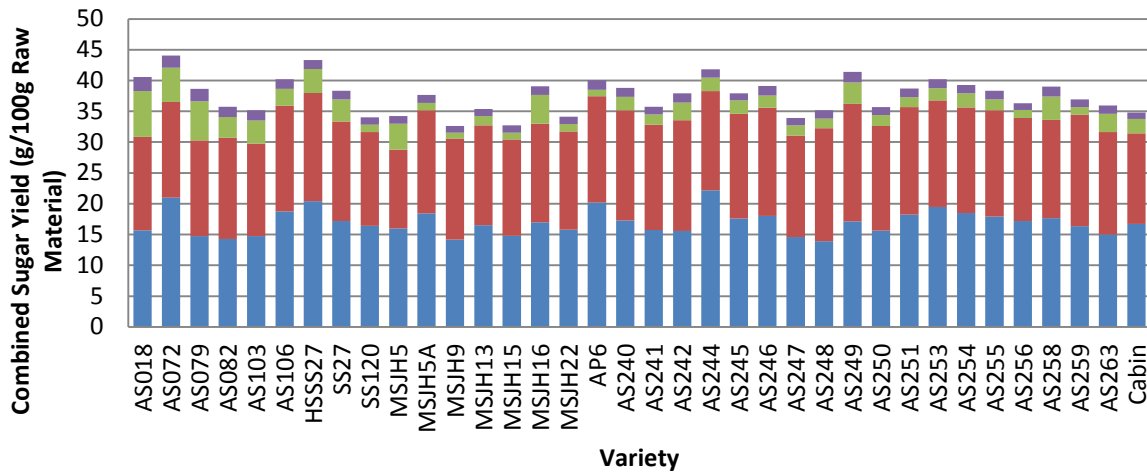
Figure 2-7 Response of initial cultivars to pretreatment by dilute sulfuric acid at 190⁰C for 15 minutes at an acid concentration of 0.7% (w/w) H2SO$_4$. ■ **Glucose yield from enzymatic hydrolysis (g glucose/g raw material),** ■ **xylose pretreatment liquor yield (g xylose/g raw material),** ■ **glucose pretreatment liquor yield (g glucose/g raw material),** ■ **arabinose pretreatment liquor yield (g arabinose/ g raw material)**

Table 2-11: Pretreatment response of individual sweet sorghum cultivars to the pretreatment conditions of 170°C, 15minutes and 0.7% (w/w) H$_2$SO$_4$

Sweet sorghum cultivar	Water insoluble solids Solid recovery %	Pretreatment liquor yield Arabinose g/100g raw material	Pretreatment liquor yield Glucose g/100g raw material	Pretreatment liquor yield Xylose g/100g raw material	Enzymatic hydrolysis yield Glucose g/100g raw material	Combined sugar yield g/100g raw material
AS018	52.20 ± 0.91	2.31 ± 0.13	7.40 ± 0.67	15.18 ± 1.64	15.70 ± 1.50	40.58
AS072	53.15 ± 1.28	1.97 ± 0.18	5.54 ± 1.16	15.55 ± 0.04	20.99 ± 0.45	44.04
AS079	54.10 ± 0.60	2.05 ± 0.15	6.34 ± 0.28	15.52 ± 1.32	14.76 ± 0.89	38.66
AS082	59.04 ± 0.93	1.63 ± 0.27	3.40 ± 1.30	16.44 ± 2.18	14.26 ± 1.68	35.73
AS103	58.34 ± 0.23	1.67 ± 0.14	3.74 ± 0.44	15.01 ± 0.98	14.73 ± 0.62	35.15
AS106	59.14 ± 0.88	1.53 ± 0.08	2.74 ± 0.05	17.14 ± 0.72	18.77 ± 1.48	40.18
HSS27	55.42 ± 0.16	1.47 ± 0.19	3.90 ± 1.22	17.53 ± 0.13	20.43 ± 4.78	43.33
SS27	58.87 ± 0.23	1.44 ± 0.11	3.55 ± 0.56	16.17 ± 0.32	17.19 ± 1.02	38.35
SS120	61.98 ± 1.16	1.23 ± 0.15	1.19 ± 0.31	15.18 ± 2.46	16.43 ± 1.19	34.03
MSJH5	58.03 ± 0.21	1.23 ± 0.13	4.18 ± 0.73	12.82 ± 1.78	15.98 ± 0.08	34.21
MSJH5A	60.99 ± 0.81	1.30 ± 0.24	1.20 ± 0.77	16.73 ± 2.04	18.43 ± 0.53	37.66
MSJH9	64.23 ± 0.39	1.09 ± 0.06	0.93 ± 0.13	16.41 ± 0.14	14.19 ± 1.54	32.63
MSJH13	64.19 ± 0.46	1.18 ± 0.09	1.50 ± 0.20	16.19 ± 0.02	16.54 ± 4.36	35.40
MSJH15	62.64 ± 0.57	1.19 ± 0.17	1.13 ± 0.30	15.63 ± 0.16	14.79 ± 0.21	32.75
MSJH16	58.53 ± 0.73	1.41 ± 0.07	4.70 ± 0.77	15.98 ± 0.11	16.99 ± 1.33	39.09
MSJH22	62.95 ± 1.43	1.15 ± 0.14	1.21 ± 0.26	15.95 ± 1.08	15.79 ± 1.64	34.10
AP6	62.50 ± 1.36	1.59 ± 0.23	1.05 ± 0.33	17.23 ± 1.71	20.20 ± 4.22	40.06
AS240	60.66 ± 0.67	1.47 ± 0.26	2.16 ± 0.26	17.88 ± 1.11	17.32 ± 2.29	38.83
AS241	62.25 ± 0.86	1.27 ± 0.24	1.64 ± 0.48	17.11 ± 0.55	15.74 ± 2.12	35.76
AS242	60.16 ± 1.54	1.54 ± 0.49	2.84 ± 1.01	17.98 ± 1.67	15.57 ± 1.59	37.94
AS244	60.68 ± 0.86	1.36 ± 0.25	2.17 ± 0.22	16.10 ± 1.27	22.17 ± 2.72	41.81
AS245	60.18 ± 1.37	1.19 ± 0.21	2.13 ± 0.63	17.03 ± 1.25	17.59 ± 3.41	37.94
AS246	60.65 ± 2.07	1.54 ± 0.29	1.99 ± 0.30	17.53 ± 1.04	18.04 ± 3.85	39.11
AS247	60.57 ± 1.47	1.16 ± 0.31	1.68 ± 0.41	16.47 ± 1.57	14.60 ± 2.65	33.91

AS248	60.89 ± 1.20	1.38 ± 0.03	1.57 ± 0.16	18.34 ± 0.32	13.90 ± 1.49	35.19
AS249	61.44 ± 0.25	1.69 ± 0.19	3.47 ± 0.52	19.10 ± 1.94	17.13 ± 0.65	41.39
AS250	60.30 ± 1.76	1.29 ± 0.10	1.75 ± 0.43	17.01 ± 0.50	15.61 ± 1.32	35.67
AS251	59.67 ± 0.89	1.39 ± 0.09	1.61 ± 0.19	17.46 ± 1.27	18.22 ± 2.54	38.68
AS253	61.40 ± 0.68	1.48 ± 0.10	1.98 ± 0.37	17.27 ± 0.65	19.48 ± 0.40	40.22
AS254	58.98 ± 1.41	1.35 ± 0.10	2.27 ± 0.38	17.17 ± 1.10	18.49 ± 2.95	39.28
AS255	61.01 ± 0.17	1.40 ± 0.19	1.76 ± 0.23	17.21 ± 0.44	17.93 ± 0.70	38.31
AS256	60.35 ± 2.19	1.09 ± 0.22	1.28 ± 0.45	16.78 ± 2.00	17.16 ± 3.77	36.31
AS258	58.13 ± 3.38	1.60 ± 0.10	3.82 ± 0.41	15.96 ± 2.07	17.64 ± 1.71	39.02
AS259	59.88 ± 0.40	1.23 ± 0.22	1.25 ± 0.08	18.09 ± 1.23	16.36 ± 0.04	36.93
AS263	58.48 ± 2.30	1.34 ± 0.30	2.93 ± 1.27	16.67 ± 2.58	14.98 ± 0.72	35.93
Cabin	66.35 ± 0.70	1.07 ± 0.15	2.31 ± 0.37	14.69 ± 1.81	16.74 ± 3.14	34.82
Min	52.20	1.07	0.93	12.82	13.90	32.63
Max	66.35	2.31	7.40	19.10	22.17	44.04
Median	60.33	1.38	2.15	16.70	16.87	37.94

[a] Combined sugar yield is the sum of sugars present in pretreatment liquor and enzymatic hydrolysis.

2.4.2.2 Statistical analysis of thirty six pretreated sweet sorghum cultivars

Statistical differences in chemical composition were evident for the initial sweet sorghum bagasse cultivars which can be seen in section 2.4.1. To evaluate whether chemical composition affected pretreatment response, cultivars analysed statistically to look for differences in chemical composition that resulted in a difference in pretreatment response. Using STATISTICA 10 (Stat soft Inc., Tulsa, USA), a correlation matrix was drawn up investigating the effect of chemical composition on pretreatment response. The correlation matrix between chemical composition and different components of the pretreatment response can be seen in Table 2-12 below. Correlations that were statistically significant are highlighted in bold in Table 2-12. Further in Figure 2-8, values that are higher than R* Critical are significant and graphically portray which chemical components had a significant effect on pretreatment response. Negative effects indicate that with an increase in a particular chemical component there will be a decrease in the associated pretreatment response while a positive effect indicates that with an increase in a particular component there will be a corresponding increase in the pretreatment response.

Table 2-12: Correlation matrix showing significant correlations between chemical components and pretreatment response

Chemical composition	Pretreatment response		
	Xylose pretreatment yield	Glucose enzymatic yield	Combined sugar yield
Ash	*-0.43*	-0.11	0.23
Water extractives	*-0.41*	0.14	0.18
Ethanol Extractives	*0.33*	0.17	0.06
Lignin	-0.20	*-0.35*	*-0.43*
Glucose	*-0.37*	-0.20	0.07
Xylose	*0.38*	0.21	0.15
Arabinose	0.20	0.25	*0.58*

Chemical components had the greatest number of effects on the release of xylose during pretreatment under the selected conditions. The significant negative effects on xylose yield where the ash, water extractives and glucose content in the raw material while the significant positive effects on xylose yield where the ethanol extractives and xylose content of the raw material. For release of glucose during enzymatic hydrolysis, only lignin was found to have a significant negative effect and no positive significant correlations where found with respect to glucose enzymatic yields. In terms of the effect of chemical composition on

the combined sugar yield, lignin had a significant negative effect while arabinose content had a significant positive effect.
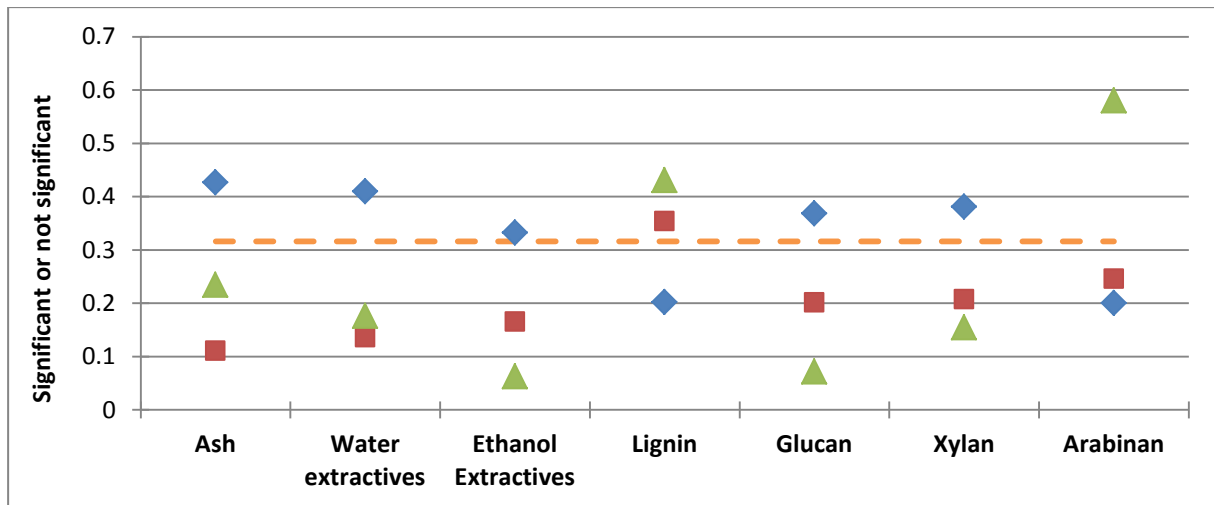
Figure 2-8: Raw material characteristics significantly influencing pretreatment, ♦ Xylose pretreatment liquor yield, ■ Glucose enzymatic hydrolysis yields, ▲ Combined sugar yield, - - - R* Crit

2.4.2.3 Ethanol yields of pretreated cultivars

The potential ethanol yields for the sweet sorghum cultivars as listed in Table 2-13 for the 2008/2009 harvest season can be seen in Figure 2-9. For ethanol from the sweet juice the yields were calculated based on a 100% conversion of sugars present to ethanol which assumes production of 0.51g EtOH/g sugar consumed [43]. Calculation of the ethanol yields from the lignocellulose where similarly calculated based on the combined sugar yield (g/100g raw material) resulting from dilute sulfuric acid pretreatment and the agronomic fibre yield (ton/ha) for individual cultivars given in Table 2-11 and Table 2-10 with a theoretical maximum conversion of sugar to ethanol (i.e. 0.51g of EtOH/g of sugar). This resulted in total ethanol yields, which was the sum of ethanol yields calculated for both the sweet juice and the lignocellulose fraction, varying between 7904L/ha and 2341 L/ha for the sweet sorghum cultivars. Of the total ethanol yield, ethanol yields from the sweet juice varied between 4712 L/ha and 125 L/ha, while ethanol from the lignocellulose varied between 3192 L/ha and 412 L/ha. Interestingly a high combined sugar yield resulting from pretreatment did not result in a cultivar necessarily having a high total ethanol yield.

Table 2-13 Theoretical ethanol yields of individual sweet sorghum cultivars based on measured pretreatment response

Sweet sorghum cultivar	Combined sugar recovery	Lignocellulosic ethanol yield[a]	Juice ethanol yield[b]	Total ethanol yield[c]	Potential ethanol yield[d]
	%	L/ha	L/ha	L/ha	L/ha
AS018	60.7	1140	3600	4740	5483
AS072	67.4	850	2857	3707	4120
AS079	58.9	1216	3730	4945	5799
AS082	55.4	1012	3225	4238	5057
AS103	50.7	1568	3852	5420	6952
AS106	62.5	1523	3432	4955	5874
HSS27	64.8	2056	4176	6232	7357
SS27	57.0	1820	4176	5996	7377
SS120	59.3	1436	3576	5012	6001
MSJH5	51.3	1297	3116	4413	5649
MSJH5A	59.3	1428	3116	4543	5527
MSJH9	53.9	1517	1254	2771	4073
MSJH13	59.5	2268	3206	5474	7026
MSJH15	52.2	1676	1616	3292	4835
MSJH16	60.3	1680	3096	4776	5887
MSJH22	57.9	1848	1924	3772	5121
AP6	66.9	3192	4712	7904	9496
AS240	60.5	1432	1577	3010	3950
AS241	54.8	1450	2799	4249	5450
AS242	59.4	877	2715	3592	4193
AS244	64.5	632	3271	3903	4253
AS245	65.9	970	4324	5294	5799
AS246	67.9	921	4344	5265	5702
AS247	51.7	1026	2863	3889	4853
AS248	55.4	952	4324	5276	6048

AS249	67.5	1770	2993	4763	5618
AS250	55.9	715	2831	3546	4112
AS251	59.6	1409	2960	4370	5332
AS253	63.1	1090	3439	4528	5169
AS254	61.1	1320	3898	5218	6064
AS255	59.8	894	2941	3835	4439
AS256	55.4	972	3238	4211	4998
AS258	57.8	1045	3238	4283	5050
AS259	55.4	412	2262	2674	3006
AS263	52.8	480	1862	2341	2772
Min	50.7	412	1254	2341	2772
Max	67.9	3192	4712	7904	9496
Median	59.3	1297	3206	4413	5450

[a] Lignocellulose ethanol yields calculated assuming 0.51g EtOH/g sugar consumed, where available sugar is that released from pretreatment response in terms of combined sugar yields. Further the agronomic fibre yield (ton/ha) taken into account to calculated ethanol in ton/ha.

[b] Sweet juice ethanol yield calculated assuming 0.51g EtOH/g sugar consumed, where available sugar is that present in the juice found in the stem.

[c] Total ethanol yield is the sum of the calculated lignocellulose and sweet juice ethanol.

[d] Potential ethanol is the sum of the calculated lignocellulose and sweet juice ethanol, assuming 100% recovery of sugars available in the lignocellulose.

This could be seen by the fact that AS072 had the second highest combined sugar recovery from fibre of 67.%, but had one of the lowest total ethanol yields with 3707L ethanol/ha while AS103 had the lowest combined sugar recovery from fibre of 50.68% but one of the highest total ethanol yields with 5420L ethanol/ha. This indicates that high ethanol yields are dependent on a number of factors of which sugar recovery is not the most important.
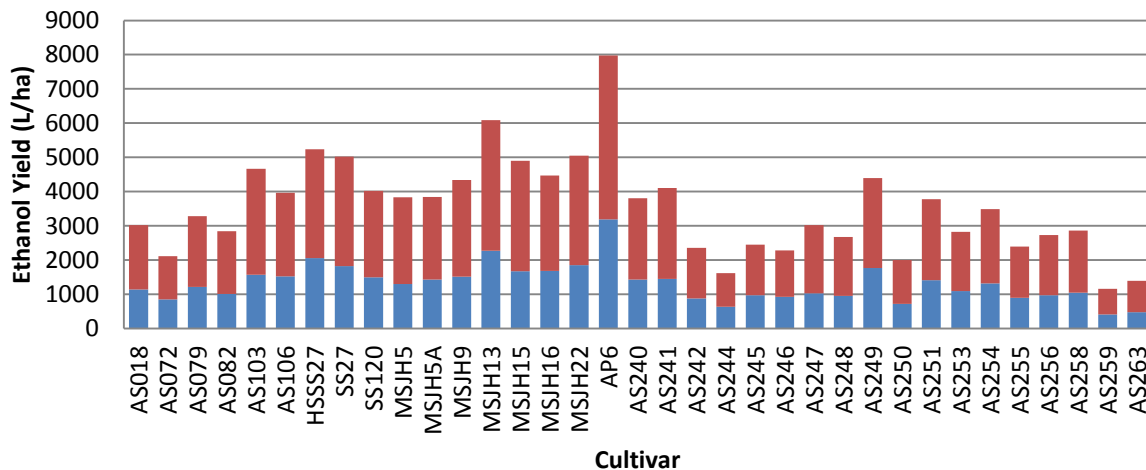
Figure 2-9: Estimated ethanol yields of sweet sorghum cultivars based on agronomic data and pretreatment at a single dilute acid pretreatment condition of 170°C, 15 minutes and 0.7% (w/w) H_2SO_4 and enzymatic hydrolysis of the WIS at a 2% solids loading, cellulase loading of 15 FPU/g WIS at a pH of 4.8 ■ Ethanol from sweet juice. ■ Ethanol produced from lignocellulose. Note: no agronomic data collected for sweet sorghum cultivar cabin which meant that ethanol yields could not be calculated for this cultivar.

Instead, the factors directly influencing the total ethanol yields included dry matter yield, which comprised both the fibre and non-structural sugars yield (measured by BRIX value), and the combined sugar yield from dilute sulfuric acid pretreatment and enzymatic hydrolysis. Cultivars that gave high ethanol yields were found to have a high biomass yield (dry matter yield), without compromising the release of sugar by pretreatment-hydrolysis. This was highlighted by the fact that AP6 which had a dry matter yield of 19.6 tons/ha produced almost 8000L/ha of total ethanol compared to the next highest ethanol producing cultivar SS27, which had a corresponding total ethanol yield of around 6000L/ha at a dry matter yield of 13.8tons/ha. Furthermore the lowest ethanol yield was observed for AS263 which had the lowest dry matter yield of 5.0 tons/ha. As total ethanol yields were also comprised of both ethanol from the juice and ethanol from the lignocellulose (i.e fibre or bagasse) it was important that both of these were maximized to ensure that the highest possible ethanol yields were obtained. It must be noted that ethanol from the juice will

always be favored over ethanol from lignocellulose due to the economic costs associated with producing ethanol from lignocellulose compared to that from the juice. Therefore, selection of preferred cultivars for further optimization was limited to those that show acceptable levels of ethanol production from juice.

As can be noted in Table 2-13, in maximizing ethanol from the lignocellulose portion of a cultivar, it was evident that high fibre yields per hectare were necessary. This was substantiated by the fact that with similar sugar recoveries over 3000L ethanol/ha could potentially be produced from the lignocellulose of a number of cultivars compared to cultivar AS246 with which only 1000L of lignocellulosic ethanol/ha was possible. Furthermore the ability of AP6 to produce higher quantities of ethanol was attributed to the fact that 12.33tons/ha of fibre were produced from it during the 2008/2009 growing season compared to only 3.6 tons/ha of fibre from AS246.

2.4.2.4. Comparing the energy content of the different sweet sorghum cultivars

The ethanol yield possible from the lignocellulose portion of each of the sweet sorghum cultivars was compared with each of the cultivars associated lignocellulose energy content. The energy contents were calculated using an equation for the HHV of biomass given in section 2.3.7.1 from which some interesting observations were noticed [41, 42]. Firstly there is a correlation with a R^2 value of 0.9451 between the ethanol yields of individual cultivars and the energy content of these cultivars. Secondly it can be seen that some cultivars yielded more ethanol per hectare than was predicted by the correlation, while other cultivars yielded less than predicted. As a single pretreatment condition was chosen for this first round of screening, the observations seen in Figure 2-10 suggest that the dilute sulfuric acid pretreatment condition selected was favorable for certain cultivars while for others it was not. An example of this was SS27, which gave a much higher ethanol yield of 2056L/ha instead of the predicted ethanol value of 1763L/ha. Compare this to AS103 which gave an ethanol yield of 1568L/ha instead of the predicted ethanol yield of 1821L/ha. In the case of these two cultivars with similar energy content per ha, it was noticed that sugar recovery was extremely important in realizing high lignocellulose ethanol yields and that a cultivar which does not perform well in pretreatment will not be preferred for lignocellulose ethanol production alone, due to a loss in potential ethanol. Therefore if cultivars are to be selected based solely on their lignocellulosic ethanol yields it is important that cultivars have high combined sugar recoveries.
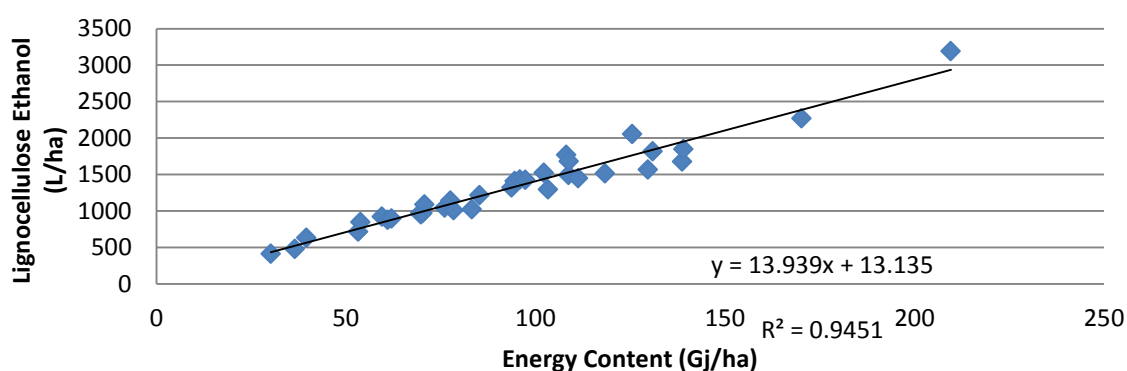
Figure 2-10: Lignocellulosic ethanol yield (L/ha) plotted against energy content of sweet sorghum bagasse (Gj/ha).

2.4.3. Selection of ten cultivars

The desired outcome of this research project was to select three preferred sweet sorghum cultivars that could be used for ethanol production. In selecting these three cultivars it was therefore necessary that the initial thirty six sweet sorghum cultivars were reduced in a number of stages. The first stage in the selection incorporated agronomic factors, pretreatment response, ethanol yields from both the sweet juice and lignocellulose and recommendations from the plant breeders (University of Kwa-Zulu Natal), all of which were deemed important. The selection criteria for this selection step as well as for the other selection steps can be seen in Table 2-15.

The parameter chosen to take all of the above mentioned factors into consideration was the total ethanol yield on a liters per hectare basis. Total ethanol included the ethanol produced from both the sweet juice and the lignocellulose fraction of sweet sorghum. This parameter was chosen as it allowed yield to be evaluated on a per hectare basis rather than on a per ton basis thereby including agronomic factors such as fresh stem yield which were of importance to the plant breeders. As discussed previously ethanol from the lignocellulose is deemed to be more expensive to produce than ethanol from the sweet juice which was taken into consideration when selecting preferred cultivars.

In Table 2-14, the total ethanol yields for the sweet sorghum cultivars are ranked from highest to lowest and the plant breeders top ten cultivars are listed next to these. Of the top ten sweet sorghum cultivars based on total ethanol yields, seven of these formed part of the

plant breeder's selection of sweet sorghum cultivars which they desired to advance to the next round of selection.

Table 2-14: Ranking of top ten sweet sorghum cultivars

Sweet sorghum cultivar	Lignocellulosic ethanol[a]	Juice ethanol[b]	Total ethanol[c]	Rank total ethanol	Plant breeders choice
	L/ha	L/ha	L/ha		
AP6	3198	4712	7910	1	10
SS27	1942	4176	6118	2	2
MSJH13	2273	3206	5479	3	8
AS103	1571	3852	5423	4	4
AS245	972	4324	5296	5	
AS248	954	4324	5278	6	5
AS246	923	4344	5267	7	1
AS254	1323	3898	5221	8	
AS106	1526	3432	4958	9	
AS079	1218	3730	4948	10	3
MSJH16	1683	3096	4780	11	9
AS249	1773	2993	4766	12	
AS018	1142	3600	4742	13	
MSJH5A	1431	3116	4546	14	
AS253	1092	3439	4531	15	
MSJH5	1300	3116	4415	16	
AS251	1412	2960	4373	17	7
AS258	1047	3238	4285	18	
AS241	1453	2799	4251	19	6
AS082	1014	3225	4240	20	
AS256	974	3238	4212	21	
SS120	1498	2437	3935	22	
AS244	633	3271	3904	23	
AS247	1028	2863	3891	24	
AS255	896	2941	3837	25	
MSJH22	1852	1924	3776	26	
AS072	851	2857	3708	27	
AS242	878	2715	3593	28	
AS250	716	2831	3547	29	
MSJH15	1680	1616	3296	30	
AS240	1435	1577	3012	31	
MSJH9	1520	1254	2774	32	
AS259	412	2262	2675	33	
AS263	481	1862	2342	34	

[a] Lignocellulose ethanol yields calculated assuming 0.51g EtOH/g sugar consumed, where available sugar is that released from pretreatment response in terms of combined sugar .yields. Further the agronomic fibre yield (ton/ha) taken into account to calculated ethanol in ton/ha.
[b] Sweet juice ethanol yield calculated assuming 0.51g EtOH/g sugar consumed, where available sugar is that present in the juice found in the stem.
[c] Total ethanol yield is the sum of the calculated lignocellulose and sweet juice ethanol.

The ethanol yields reflected in Table 2-14 have previously been shown in Table 2-13 where the results were discussed. The cultivar which ranked as the top of the selection was AP6 with a total ethanol yield of 7910L/ha. AP6 produced the most ethanol compared to all of the other cultivars from both the sweet juice and the lignocellulose. The lowest ranked cultivar was AS263 with a total ethanol yield of 2342L/ha. Interestingly the range in combined sugar recovery following pretreatment and enzymatic hydrolysis, experienced by the top ten cultivars, varied from 50.7% for AS103 to 67.9% for AS246 of which these two cultivars were respectively both the worst performing and best performing cultivars in terms of combined sugar recovery. Note that in Table 2-14 only seven of the top ten cultivars that the plant breeders chose were included in the selection. The main reason for this was that the plant breeders selected cultivars based on high soluble sugar content, improved biomass yields and agronomic characteristics such as disease resistance while ignoring the potential ethanol from the lignocellulose fraction, which was used in conjunction with the potential sweet juice ethanol to rank the varieties in terms of the total ethanol yield. Having a closer look at the plant breeders choices, the three cultivars AS241, AS251 and MSJH16 were therefore not selected in the overall top ten due to the fact that they had substantially lower total ethanol yields. Furthermore MSJH16, AS251 and AS241 had total ethanol yields of 200L/ha, 600L/ha and 700L/ha less than the worst of the selected top ten cultivars.

Table 2-15: Selection criteria involved with each selection step

Selection step	Selection criteria
36 – 10	Highest total potential ethanol yield (L/ha), which is calculated based on agronomic factors and pretreatment response yields. Plant breeders recommendations
10 – 5	Highest average total potential ethanol yields (L/ha) calculated for two pretreatment conditions and two enzyme loadings for each. Plant breeders recommendation, i.e. availability of material
5 - 3	Highest average total potential ethanol yields (L/ha) calculated at the optimum condition of each cultivars. Average includes yields between two harvest seasons. Availability of material for pilot plant steam explosion

2.4.4. Selection of five preferred cultivars

Selection of five preferred cultivars from the previously selected ten was needed to reduce the number of cultivars proceeding to optimization. In doing so combined severity factors were investigated to locate suitable pretreatment conditions. Subsequently, the suitable pretreatment conditions were used to evaluate performance of individual cultivars at numerous conditions and selection was carried out with the criteria listed in table 2-15. Cultivars that performed well were selected on this basis.

2.4.4.1 Combined severity factor investigations

Preliminary work was carried to investigate how sweet sorghum cultivars responded to pretreatment at different pretreatment severities. The range of pretreatment conditions studied in investigating the combined severity factor included temperatures ranging between 150°C and 210°C, times between 5 and 60 minutes, and acid concentration between 0 and 0.96% (w/w) H_2SO_4. A number of cultivars were included in evaluating the combined severity factor including MSJH5, MSJH9, MSJH13, MSJH22, AS106, HSS27, AS245, AS246, AP6 and mixtures of cultivars. These cultivars were chosen at random so that the results of the combined severity factor investigations were representative of a number of cultivars. The response to pretreatment that was of interest when investigating pretreatment severity was the combined sugar recovery. Combined severity factor ranges or values in which the combined severity factor gave consistently high combined sugar recoveries were therefore important. Furthermore, combined sugar recovery (that resulting from a combination of the recoveries from pretreatment and enzymatic hydrolysis) was preferred over sugar recovery obtained from pretreatment or enzymatic hydrolysis alone due to the fact that the pretreatment severity needed for good recoveries of sugars in pretreatment and in enzymatic hydrolysis differed. The results of the combined severity factor work can be seen in appendix I. In Figure 2-11, the combined sugar recoveries resulting from pretreatment across a wide range of combined severity factors can be observed. Combined sugar recovery is the recovery of sugar as a percentage of that which was initially present in the raw material.
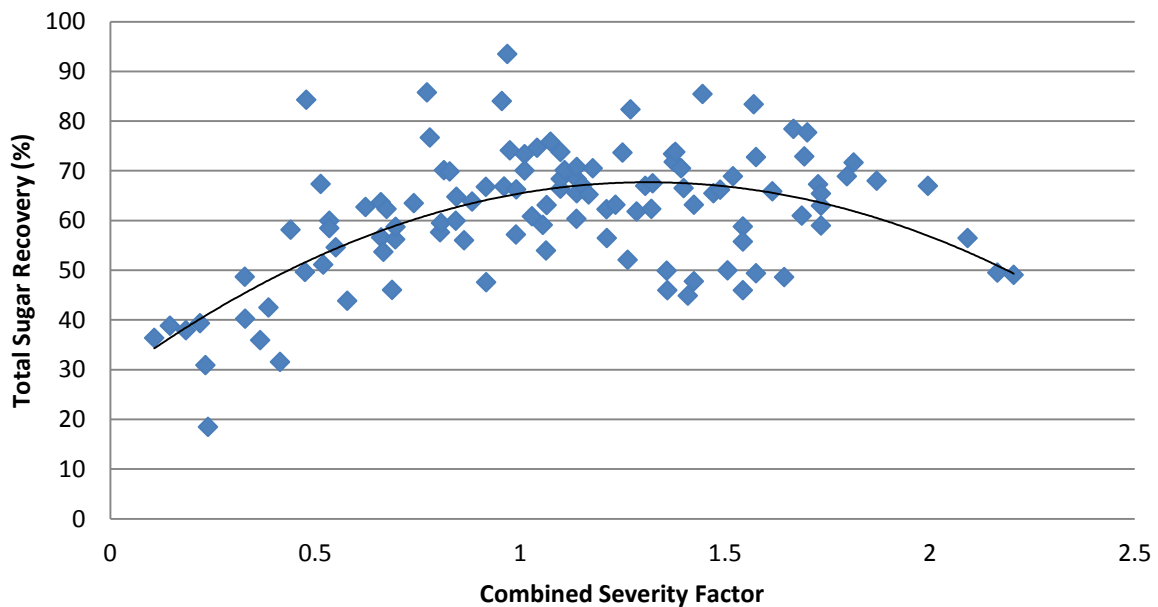
Figure 2-11: Combined sugar recovery versus the combined severity factor

As expected, at low combined severity factors, i.e. 0.5 and less, the pretreatment was not severe enough to release high yields of sugars and generally the combined sugar recoveries were less than 50% of that available in the raw material. Between a combined severity factor of 0.5 and 1.75, combined sugar recovery was between 50% and 80%. At combined severity factors higher than 1.75 an insufficient amount of combined severity factor values were investigated to interpret the combined severity factor range higher 1.75. A general trend of decreasing combined sugar recovery can be observed which above combined severity factors above 1.75 and would most probably be due to degradation of sugars at increased severities [11, 44]. As lower combined severity factors were of interest due to the associated economic benefits, further work at combined severity factors above 1.75 was not carried out to substantiate the above mentioned observations. Some observations regarding the work at different pretreatment severities were that at the same or similar combined severity factors, depending on the pretreatment conditions, the combined sugar recoveries varied greatly. This was substantiated by the spread of data points in Figure 2-11. Furthermore at various combined severity factors multiple data points were collected due to the fact that combined severity factor was calculated with the three factors, temperature, time and pH resulting in the possibility of two different sets of pretreatment conditions having the same pretreatment severity. One such combined severity factor value was 0.96 in which two different pretreatment conditions resulted in the same value, namely 190°C, 15 minutes, 0.25% (w/w) H_2SO_4 and 205°C, 10 minutes, 0.07% (w/w) H_2SO_4. These two sets of

pretreatment conditions gave completely different results. The first set of pretreatment conditions at 190°C, 15 minutes, 0.25% (w/w) H_2SO_4 resulted in a combined sugar recovery of 84.06%, while the second set at 205°C, 10 minutes, 0.07% (w/w) H_2SO_4 resulted in a combined sugar recovery of 66.85%. As different cultivars were used, namely AS245 and HSS27, the difference could have been as a result of differences in composition between the two cultivars. Although different cultivars were utilized for the two different sets of conditions, the 20% difference in sugar recovery seems to indicate that the combined severity factor is not an accurate predictor of response to pretreatment when comparing two pretreatments that are not related, i.e. different temperatures, times and acid concentration and with different cultivars. Rather it seems to indicate in which combined severity factor ranges, good pretreatment response could be expected.

Although the combined severity factor was not an accurate predictor of determining pretreatment response, the work performed in this section was useful in selecting pretreatment conditions that resulted in high yields at low severity. Two such conditions were 190°C, 5 minutes, 0.25% (w/w) H_2SO_4 and 200°C, 5 minutes and 0.07% (w/w) H_2SO_4 which had combined severity factors of 0.48 and 0.51 respectively.

2.4.4.2. Pretreatment at two conditions and two enzyme loadings

Two pretreatment conditions with two enzyme loadings were chosen for use in selection of five preferred sweet sorghum cultivars from the ten previously selected cultivars. The pretreatment conditions chosen were 190°C, 5minutes, 0.25% (w/w) H_2SO_4 and 200°C, 5 minutes and 0.07% (w/w) H_2SO_4, while the two enzyme loadings chosen for these pretreatment conditions were 3.75 FPU/g WIS and 15 FPU/g WIS . The above mentioned conditions which were chosen for the selection step were taken from preliminary work evaluating combined sugar recoveries at varying combined severity factors ranges using a number of cultivars. This work can be seen in section 2.4.4.1. The pretreatment conditions chosen gave high combined sugar recoveries at low pretreatment severity. This was desired to ensure that cultivars performing well at these low severity conditions were selected. The thinking behind this method was that low pretreatment severity would reduce the operating costs of a prospective second generation bio-ethanol plant and therefore cultivars that perform well under these conditions would be favored. Two sets of conditions at low severity were chosen to ensure that selection of cultivars was based on performance at different low pretreatment severities to introduce robustness into selected cultivars, as

cultivars that performed well consistently at different conditions are important. Furthermore, coupled with two low severity pretreatments, enzymatic hydrolysis at both a low enzyme loading of 3.75 FPU/g WIS and the usual enzyme loading of 15 FPU/g WIS were incorporated in selecting preferred cultivars. Utilising both a low enzyme loading and the usual enzyme loading meant that cultivars were selected based on their ability to reduce the amount of enzyme required for sugar release by hydrolysis, which was considered favorable due to the fact that the enzyme costs associated with second generation bio-ethanol production currently represents a large fraction of total operating costs [45]. Hence, reduction in the necessary enzyme loading based on using a particular cultivar over another was a beneficial characteristic to select for.

The results of these two pretreatment conditions and the subsequent enzymatic hydrolysis at the two enzyme loadings can be seen in Table 2-16 and Table 2-17. At a pretreatment condition of 190°C, 5 minutes and 0.25% (w/w) H_2SO_4, SS27 performed the best at both enzyme loadings giving a combined sugar yield of 38.7 and 49.86 g/100g raw material for the low and high enzyme loading respectively. AS103 gave the lowest combined sugar yield of 28.54g/100g raw material with an enzyme loading of 3.75 FPU/g WIS, while MSJH13 gave the lowest combined sugar yield of 42.67g/100g raw material at 15 FPU/g WIS. At a pretreatment condition of 200°C, 5 minutes and 0.07% (w/w) H_2SO_4, SS27 gave the highest combined sugar yield of 29.53 g/100g raw material at an enzyme loading of 3.75 FPU/g WIS while AS254 gave the highest combined sugar yield of 42.01 g/100g raw material at an enzyme loading of 15 FPU/g WIS. At 200°C, 5minutes and 0.07% (w/w) H_2SO_4, the lowest combined sugar yield was observed with AP6 with 20.05 and 31.64g/100g raw material being released for the low and high enzyme loadings respectively.

In terms of the xylose yields obtained for a pretreatment condition of 190°C, 5minutes and 0.25% (w/w) H_2SO_4, all of the cultivars performed consistently well except for AS103 which performed poorly with a xylose yield of 11.04g/100g raw material compared to the other cultivars which achieved xylose yields between 15 and 17g/100g raw material. The major differences between cultivars could be seen in the yields of glucose from enzymatic hydrolysis, where for both enzyme loadings at each pretreatment condition the glucose yields varied greatly. For 190°C, 5minutes and 0.25% (w/w) H_2SO_4, the lowest glucose yield following enzymatic hydrolysis at a loading of 15 FPU/g WIS was 19.29g/100g raw material while the highest was 28.24g/100g raw material. Similarly for an enzyme loading of 3.75

FPU/g WIS at this pretreatment condition, as well as for both enzyme loadings at 200°C, 5minutes and 0.07% (w/w) H_2SO_4, a large variation in the glucose yield following enzymatic hydrolysis can be observed which was between 5 – 8g/100g raw material.

In selecting five cultivars to progress to the next round of optimization from the ten chosen in section 2.4.3, cultivars that consistently gave high ethanol and combined sugar yields at low pretreatment severity and at low enzyme loadings were preferred. Furthermore cultivars producing high yields at low pretreatment severity and low enzyme loadings will be cost effective in terms of both capital and operating costs and cultivars that can consistently do this over a range of conditions are thought to be robust. For this reason the combined sugar and ethanol yields of individual cultivars at each of the pretreatment conditions at two enzyme loadings were calculated and then averaged as can be seen in Table 2-19 and Table 2-18. The average yields from the two pretreatment conditions with the two enzyme loadings were ranked from highest to lowest to show which of the cultivars consistently produced high ethanol yields as well as high sugar yields at the different conditions. In Table 2-19, it can be seen that AP6 had the highest average ethanol yield for the different conditions, while AS106 had the worst average ethanol yield. Interestingly, only two cultivars, AP6 and SS27, ranked similarly for all conditions while the remaining cultivars changed position slightly from condition to condition. For the collective sugar yields in Table 2-18, SS27 had the highest average sugar yield while AS103 had the lowest average sugar yield for the two pretreatment conditions and enzyme loadings.

Table 2-16: Pretreatment at 190°C, 5minutes, 0.25% (w/w) H_2SO_4

Sweet sorghum cultivar	Water insoluble solids	Pretreatment liquor yield			Enzymatic hydrolysis yield		Combined sugar yield	
	Solid recovery	Arabinose	Glucose	Xylose	Glucose @ 3.75 FPU/g WIS	Glucose @ 15 FPU/g WIS	@ 3.75 FPU/g WIS	@ 15 FPU/g WIS
	%	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material
AP6	65.1 ± 1.5	1.94 ± 0.21	0.95 ± 0.42	15.72 ± 0.60	13.26 ± 1.57	28.24 ± 1.77	32.50	47.48
SS27	57.7 ± 0.9	1.96 ± 0.10	3.13 ± 0.96	17.04 ± 1.21	15.50 ± 2.14	26.66 ± 5.50	38.70	49.86
MSJH13	65.2 ± 0.1	1.47 ± 0.02	1.63 ± 0.53	15.89 ± 0.70	11.98 ± 0.59	19.29 ± 0.23	31.53	38.84
AS103	59.9 ± 1.9	1.52 ± 0.05	2.37 ± 0.91	11.04 ± 2.74	12.66 ± 1.75	26.79 ± 1.65	28.54	42.67
AS245	63.8 ± 1.5	1.45 ± 0.20	1.99 ± 0.62	17.12 ± 0.33	10.28 ± 0.34	22.98 ± 1.37	31.66	44.35
AS246	62.2 ± 1.2	1.66 ± 0.09	1.70 ± 0.62	15.48 ± 0.02	14.43 ± 0.06	22.13 ± 2.66	33.99	41.69
AS254	63.6 ± 2.0	1.51 ± 0.13	1.83 ± 0.12	15.08 ± 0.16	12.44 ± 1.89	25.74 ± 4.15	31.11	44.41
AS248	63.3 ± 1.3	1.64 ± 0.09	1.78 ± 0.15	16.72 ± 0.31	14.07 ± 1.56	26.02 ± 0.55	34.46	46.41
AS106	59.4 ± 3.7	1.51 ± 0.07	2.57 ± 0.09	15.81 1.45	11.83 ± 1.41	24.45 ± 2.39	31.88	44.50
AS79	58.2 ± 3.5	1.94 ± 0.33	4.29 ± 1.56	15.71 ± 1.43	14.52 ± 1.95	25.00 ± 4.10	38.35	48.83
Min	57.7	1.45	0.95	11.04	10.28	19.29	28.54	38.84
Median	62.8	1.58	1.91	15.76	12.96	25.37	32.19	44.46
Max	65.2	1.96	4.29	17.12	15.50	28.24	38.70	49.86

Table 2-17: Pretreatment at 200°C, 5 minutes, 0.07% (w/w) H₂SO₄

| Sweet sorghum cultivar | Water insoluble solids | Pretreatment liquor yield | | | Enzymatic hydrolysis yield | | Combined sugar yield | |
| | Solid recovery | Arabinose | Glucose | Xylose | Glucose @ 3.75 FPU/g WIS | Glucose @ 15 FPU/g WIS | @ 3.75 FPU/g WIS | @ 15 FPU/g WIS |
	%	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material
AP6	70.6 ± 1.2	1.29 ± 0.00	0.91 ± 0.55	4.52 ± 0.04	12.78 ± 1.64	24.37 ± 1.06	20.05	31.63
SS27	63.9 ± 1.8	1.44 ± 0.04	1.45 ± 0.06	7.58 ± 0.23	18.95 ± 0.10	30.06 ± 0.68	29.53	40.63
MSJH13	69.6 ± 1.9	1.12 ± 0.10	0.79 ± 0.05	7.21 ± 0.08	14.47 ± 0.06	29.42 ± 0.71	23.73	38.68
AS103	66.0 ± 2.4	1.09 ± 0.06	1.13 ± 0.12	7.58 ± 0.81	15.46 ± 0.99	26.00 ± 0.76	25.45	35.98
AS245	67.7 ± 1.3	1.00 ± 0.14	1.10 ± 0.31	8.07 ± 0.97	10.38 ± 0.07	23.34 ± 0.72	20.99	33.95
AS246	64.9 ± 2.9	1.05 ± 0.29	1.02 ± 0.34	6.88 ± 1.49	14.34 ± 0.03	24.40 ± 0.63	23.92	33.98
AS254	66.5 ± 1.6	1.14 ± 0.10	1.03 ± 0.09	9.59 ± 0.53	12.75 ± 0.41	30.05 ± 1.50	24.71	42.01
AS248	67.0 ± 2.0	1.18 ± 0.07	1.01 ± 0.24	7.36 ± 2.78	15.26 ± 0.10	31.78 ± 0.22	25.13	41.64
AS106	67.0 ± 2.5	1.05 ± 0.11	0.99 ± 0.02	8.16 ± 2.70	12.90 ± 0.81	26.18 ± 0.68	23.23	36.50
AS79	63.2 ± 1.6	1.31 ± 0.15	1.25 ± 0.11	4.78 ± 1.45	14.36 ± 0.04	24.40 ± 2.32	21.95	31.99
Min	63.16	1.00	0.79	4.52	10.38	23.34	20.05	31.63
Median	66.73	1.13	1.03	7.47	14.35	26.09	23.83	36.24
Max	70.6	1.44	1.45	9.59	18.95	31.78	29.53	42.01

Table 2-18: Combined sugar yield across two different pretreatment conditions and enzyme loadings

Sweet sorghum cultivars	Combined sugar yield (g/100g raw material)				Sum of combined sugar yield at different conditions (g/100g raw material)	Rank sum of combined sugar yield
	200°C, 5min, 0.07% (w/w) H_2SO_4, 3.75 FPU/g WIS	200°C, 5min, 0.07% (w/w) H_2SO_4, 15 FPU/g WIS	190°C, 5min, 0.25% (w/w) H_2SO_4, 3.75 FPU/g WIS	190°C, 5min, 0.25% (w/w) H_2SO_4, 15 FPU/g WIS		
SS27	29.5	40.6	38.7	49.9	39.7	1
AS254	25.1	41.6	34.5	46.4	36.9	2
AS246	24.7	42.0	31.1	44.4	35.6	3
AS106	22.0	32.0	38.3	48.8	35.3	4
AS79	23.2	36.5	31.9	44.5	34.0	5
MSJH13	23.9	34.0	34.0	41.7	33.4	6
AS248	23.7	38.7	31.5	38.8	33.2	7
AS103	25.4	36.0	28.5	42.7	33.2	8
AP6	20.0	31.6	32.5	47.5	32.9	9
AS245	21.0	34.0	31.7	44.4	32.7	10

Table 2-19: Estimated ethanol yield of cultivars for two different pretreatment conditions and enzyme loadings

Sweet sorghum cultivars	Estimated ethanol [a] (L/ha)				Average ethanol [b] (L/ha)	Rank average
	200°C, 5min, 0.07% (w/w) H$_2$SO$_4$, 3.75 FPU/g WIS	200°C, 5min, 0.07% (w/w) H$_2$SO$_4$, 15 FPU/g WIS	190°C, 5min, 0.25% (w/w) H$_2$SO$_4$, 3.75 FPU/g WIS	190°C, 5min, 0.25% (w/w) H$_2$SO$_4$, 15 FPU/g WIS		
AP6	6090	6886	6946	7976	6975	1
SS27	5385	5839	5760	6217	5800	2
AS248	4911	5296	5129	5408	5186	3
AS103	4832	5237	4951	5494	5128	4
AS245	4787	5073	5022	5302	5046	5
MSJH13	4518	5345	4949	5354	5042	6
AS246	4830	5034	5034	5191	5022	7
AS254	4614	5116	4800	5186	4929	8
AS79	4325	4597	4770	5054	4687	9
AS106	4192	4626	4475	4888	4545	10

[a] Estimated ethanol yield is the sum of the calculated lignocellulose and sweet juice ethanol as previously calculated where 0.51g EtOH is produced/g sugar consumed in which for the lignocellulose portion the g sugar is the combined sugar yield at the respective pretreatment condition.

[b] Average ethanol is the average ethanol yield at all pretreatment conditions and enzyme loadings.

2.4.4.3 Statistical analysis of the effect of chemical composition for two different pretreatment conditions and enzyme loadings

A statistical analysis was carried out looking for significant correlations between the chemical components of the ten selected cultivars and their pretreatment at two pretreatment conditions with two enzyme loadings. STATISTICA 10 (Stat soft Inc., Tulsa, USA) was used to determine the correlation matrix between the chemical components in the raw material and the pretreatment response. Table 2-20 shows the correlation matrix for pretreatment at 190°C, 5 minutes and 0.25% (w/w) H_2SO_4 with the two enzyme loadings of 3.75FPU/g.WIS and 15FPU/g.WIS as well as the combined sugar yield corresponding to an enzyme loading of 3.75 FPU/g WIS and 15 FPU/g WIS. Table 2-21 shows the correlation matrix for pretreatment at 200°C, 5 minutes and 0.07% (w/w) H_2SO_4 with the two enzyme loadings of 3.75 FPU/g WIS and 15 FPU/g WIS as well as the combined sugar yield corresponding to an enzyme loading of 3.75 FPU/g WIS and 15 FPU/g WIS. Correlations that were found to be statistically significant are highlighted in bold.

Table 2-20: Correlation matrix showing significant correlations between chemical components and pretreatment response for 190°C, 5minutes and 0.25% (w/w) H_2SO_4 with two enzyme loadings

Chemical component	Pretreatment and enzymatic hydrolysis response				
	Xylose pretreatment yield	Glucose enzymatic yield		Combined sugar yield	
		3.75 FPU/g WIS	15 FPU/g WIS	3.75 FPU/g WIS	15 FPU/g WIS
Ash	-0.16	0.20	-0.04	0.16	0.02
Water extractives	-0.001	0.11	-0.08	0.21	0.09
Ethanol extractives	-0.17	-0.22	-0.06	-0.50	-0.42
Lignin	-0.47	**-0.74***	-0.24	**-0.79***	**-0.59****
Glucose	-0.51	0.07	0.43	0.11	0.30
Xylose	0.013	0.07	0.48	-0.06	0.28
Arabinose	-0.22	0.42	**0.60****	0.33	**0.59****

*Statistically significant at P < 0.05; **statistically significant at P < 0.1

For pretreatment at 190°C, 5 minutes and 0.25% (w/w) H_2SO_4, significant negative correlations were found to be present between lignin and the release of glucose during enzymatic hydrolysis at an enzyme loading of 3.75 FPU/g WIS as well as between lignin and the combined sugar yield at both enzyme loadings. Further significant positive correlations were found between arabinan and the release of glucose during enzymatic hydrolysis upon using an enzyme loading of 15 FPU/g WIS and the combined sugar yield upon using an enzyme loading of 15 FPU/g WIS.

Table 2-21: Correlation matrix showing significant correlations between chemical components and pretreatment response for 200°C, 5minutes and 0.07% (w/w) H_2SO_4 with two enzyme loadings

Chemical component in raw material	Pretreatment and enzymatic response, significant effect				
	Xylose pretreatment yield	Glucose enzymatic yield		Combined sugar yield	
		3.75 FPU/g WIS	15 FPU/g WIS	3.75 FPU/g WIS	15 FPU/g WIS
Ash	0.26	*0.73**	0.43	*0.76**	0.44
Water extractives	0.24	0.39	0.21	0.50	0.27
Ethanol extractives	-0.03	-0.35	0.24	-0.38	0.14
Lignin	0.07	*-0.78**	*-0.62***	*-0.71**	-0.52
Glucose	0.08	*0.60***	0.32	*0.56***	0.28
Xylose	0.43	-0.05	0.27	0.23	0.39
Arabinose	*-0.77**	-0.00	-0.51	-0.38	*-0.65**

*Statistically significant at P < 0.05; **Statistically significant at P < 0.1

For pretreatment at 200°C, 5 minutes and 0.07% (w/w) H_2SO_4 significant positive correlations were found between ash and the release of glucose for an enzyme loading of 3.75 FPU/g WIS as well as for combined sugar yield upon using an enzyme loading of 3.75 FPU/g WIS. Similarly to pretreatment at 190°C, 5 minutes and 0.25% (w/w) H_2SO_4, significant negative correlations were found for lignin upon pretreating the selected cultivars at 200°C, 5 minutes and 0.07% (w/w) H_2SO_4. At this condition significant negative correlations were observed between lignin and the release of glucose for both enzymes loadings and further between lignin and the combined sugar yield when using an enzyme loading of 3.75 FPU/g WIS. Unlike pretreatment at 190°C, 5 minutes and 0.25% (w/w) H_2SO_4 significant positive correlations were determined between glucose and the release of glucose with an enzyme loading of 3.75 FPU/g WIS and the combined sugar yield when an enzyme loading of 3.75 FPU/g WIS was utilized when the pretreatment conditions were 200°C, 5 minutes and 0.07% (w/w) H_2SO_4. With pretreatment 200°C, 5 minutes and 0.07% (w/w) H_2SO_4 significant negative correlations were determined between arabinose and xylose pretreatment yield as well as for combined sugar yield upon using an enzyme loading of 15 FPU/g WIS.

2.4.5. Optimization of five preferred cultivars with small scale dilute acid pretreatment

Five preferred sweet sorghum cultivars were selected for further optimization based on the highest average total potential ethanol yields (L/ha), which can be seen in Table 2-19. Selection criteria for this step are further defined in Table 2-15. Unfortunately during the

2011 growing season cultivar AS248 experienced lodging and cultivar AS245 failed to germinate resulting in these cultivars having to be excluded from further optimization. With these two cultivars out of any further optimization the cultivars that were selected based on their average combined ethanol yields at different pretreatment conditions as given in Table 2-19 for further optimization were AP6, SS27, AS103, MSJH13 and AS246.

Pretreatment optimization was performed through the use of a central composite design [40]. The experimental design points can be observed in Table 2-3. Results for the central composite design are summarized in Table 2-22, Table 2-23, Table 2-24, Table 2-25 and Table 2-26 for the five preferred cultivars AS103, AP6, SS27, AS246 and MSJH13 respectively. The relative contribution of the total arabinose, total xylose and total glucose yield for individual pretreatment conditions can be observed in Figure 2-12, Figure 2-16, Figure 2-20, Figure 2-24, and Figure 2-28 for AS103, AP6, SS27, AS246, and MSJH13 respectively.

AS103 had the highest maximum combined sugar yield of 54.5g/100g raw material while SS27 had the lowest maximum combined sugar yield of 48.83g/100g raw material for the experimental region of study. The maximum combined sugar yields for AS103 and SS27 occurred at 190°C, 5minutes and 0.25% (w/w) H_2SO_4 while the maximum combined sugar yields for AS246 and MSJH13 were 50.23 and 50.29g/100g raw material respectively and occurred at 185°C, 10 minutes and 0.25% (w/w) H_2SO_4. The maximum combined sugar yield for AP6 was 53.45 g/100g raw material and occurred at 180°C, 10 minutes and 0.25% (w/w) H_2SO_4. Except for SS27, the maximum xylose yield was released during pretreatment for each of the preferred cultivars at the same condition that resulted in maximum combined sugar yields. In descending order the maximum xylose yields were 17.92g xylose/100g raw material, 17.52g xylose/100g raw material, 17.16g xylose/100g raw material, 16.44g xylose/100g raw material and 15.63g xylose/ 100g raw material for AS246, AP6, AS103, SS27 and MSJH13 respectively. In descending order the combined sugar recovery was 84.98%, 84.42%, 82.47%, 78.11%, and 75.81% for AP6, AS103, MSJH13, AS246 and SS27 respectively. Combined sugar recovery (%) and sugar yield (g/100g raw material) varied by 10% between the best performing and worst performing cultivar showing that selection of cultivar is critical in obtaining maximum combined sugar yields.

The data for each of the cultivars was fitted to a quadratic model with two way interactions using STATISTICA 10 (Stat soft Inc., Tulsa, USA) as described in section 2.3.6. The fitted

surfaces for each cultivar along with their R^2 value can be seen for each of the 5 preferred cultivars below, namely AS103, AP6, SS27, AS246 and MSJH13. Furthermore the fitted models are described below.

For cultivar AS103, the accuracy of the fitted models for xylose, glucose and combined sugar recovery was poor with the highest accountability of data represented by the combined sugar yield surface plot which had an R^2 of 64.2%. The fitted models for xylose, glucose and combined sugar yield respectively can be observed by Equation 6, Equation 7, and Equation 8. Of the models fitted for cultivar AS103, only the model for combined sugar yield could be improved by ignoring the quadratic effect of temperature according to the ANOVA (F-test). While all of the models for AS103 where poor, they indicated that the optimum condition was located in the region of 190°C, 5min and 0.25% (w/w) H_2SO_4 which is confirmed by looking at the pretreatment data for AS103. The models indicate that potentially the yield of AS103 could be increased slightly but this is not certain as the combined sugar yield starts leveling off in the region of 190°C, 5min and 0.25% (w/w) H_2SO_4.

Equation 6	$Y_{xylose\ (g/100g\ raw\ material)}$ = -211.166 + 1.203T + 15.504t – 0.045t^2 – 0.08Tt	**R^2 = 59.4%**
Equation 7	$Y_{glucose\ (g/100g\ raw\ material)}$ = -137.693 + 0.882T + 10.898t – 0.050t^2 – 0.053Tt	**R^2 = 54.9%**
Equation 8	$Y_{Combined\ Sugar\ (g/100g\ raw\ material)}$ = -371.958 + 2.212T + 28.326t – 0.105t^2 – 0.142Tt	**R^2 = 64.2%**

Although the models for AS103 were not that accurate, they did follow trends observed by experimental data points and generally the yields predicted by the model were within 2g of the experimental data points. With further experimental work the models could be further improved. With AP6 the accuracy of the fitted models for xylose, glucose and combined sugar yield was poor for xylose yield at 60.1%, excellent for glucose yield at 94.0% and reasonably good for combined sugar yield at 78.19%. For xylose yield the model was refined by ignoring the quadratic effect of temperature and the interaction between temperature and time. The model for xylose yield is indicated by Equation 9. For the glucose yield model the effects that were ignored were the linear effect of temperature and the linear interaction between temperature and time. The model for glucose yield is shown by Equation 10. For combined sugar yield the model was refined effectively according to the F-test by ignoring the quadratic effect of temperature and the interaction between temperature and time. Equation 11 shows the model for combined sugar yield for cultivar

Equation 9 $Y_{\text{xylose (g/100g raw material)}}$ $= 70.83181 - 0.35119T + 2.06069t - 0.11443t^2$ **R^2 = 61.0%**

Equation 10 $Y_{\text{glucose (g/100g raw material)}}$ $= 19.21356 + 3.14367t - 0.13648t^2$ **R^2 = 94.0%**

Equation 11 $Y_{\text{Combined sugar (g/100g raw material)}} = 100.8948 - 0.4318T + 5.5110t - 0.2672t^2$ **R^2 = 78.2%**

Equation 9 indicates that xylose yield is influenced by the linear effect of temperature and the linear and quadratic effect of time while xylose yield was not influenced by interaction between temperature and time. As the xylose model could only account for 61% of the data, the model does not fit the data adequately. Equation 10 indicates that the glucose yield for cultivar AP6 was influence by time alone. This model fitted the experimental data for glucose yield well and in the temperature range of 180°C - 190°C this equation should predict the data well. Equation 11 fits the data reasonably well and the model for combined sugar yield follows trends observable in the data. At the longer time point of 15 minutes, the model predictions deviated from data points while still following trends observed.

Similarly to cultivar AS103, cultivar SS27 showed poor accuracy for the models fitted to xylose, glucose and combined sugar yield.   The model for xylose, glucose and combined sugar yield are given by Equation 12, Equation 13 and Equatino 14. Only the model for glucose yield (Equation 13) could be improved by ignoring the linear interaction between temperature and time and the quadratic effect of temperature.

Equatino 12 $Y_{\text{xylose yield (g/100g raw material)}} = -1695.20 + 17.86T - 0.05T^2 + 13.44t - 0.03t^2 - 0.07Tt$ **R^2 = 45.3%**

Equation 13 $Y_{\text{glucose yield (g/100g raw material)}} = -23.3744 + 0.2576T + 1.1177t - 0.0504t^2$ **R^2 = 59.3%**

Equatino 14 $Y_{\text{combined sugars (g/100g raw material)}} = -1549.02 + 16.07T - 0.04T^2 + 19.21t - 0.09t^2 - 0.09Tt$ **R^2 = 40.1%**

Due to the low accuracy of the model for xylose yield and combined sugar yields, these models could not be used to describe trends in the experimental data. The fitted model for glucose yield was slightly better in that some of the data points were predicted by the model while others were way off.

For cultivar AS246, the model for xylose yield had a high accuracy with all effects included while with refining glucose yield and combined sugar yield had extremely low accuracies. The models fitted for cultivar AS246 with regards to xylose, glucose and combined sugar yield are given by Equation 15, Equation 16 and Equation 17 respectively. The model for glucose yield couldn't be improved by ignoring effects according to the F-test while combined sugar yield could be improved slightly by ignoring the linear effect of time on the response variable.

Equation 15 $Y_{xylose\ yield\ (g/100g\ raw\ material)} = -2479.07 + 26.96T - 0.07T^2 + 5.14t - 0.19t^2 - 0.01Tt$ **$R^2 = 99.5\%$**

Equation 16 $Y_{glucose\ yield\ (g/100g\ raw\ material)} = -487.863 + 5.199T - 0.013T^2 + 3.068t + 0.033T - 0.020Tt$ **$R^2 = 11.4\%$**

Equation 17 $Y_{combined\ sugar\ yield\ (g/100g\ raw\ material)} = -3039.09 + 33.55T - 0.09T^2 - 0.17t^2 + 0.02Tt$ **$R^2 = 45.4\%$**

The xylose model for AS246 while being statistically accurate (as determined by STATISTICA 10) did not follow trends observable in the data and was in fact out a factor of 10. Furthermore the model for combined sugar yield was also inaccurate. The model for glucose yield although being the least accurate represented what was observed by the data points for dilute acid pretreatment of AS246.

For cultivar MSJH13, the models fitted for xylose, glucose and combined sugar yield were reasonably well fitted with an accuracy of around 70 – 84%. The models fitted for xylose, glucose and combined sugar yield for MSJH13 are given by Equation 18, Equation 19 and Equation 20 respectively.

Equation 18 $Y_{xylose\ yield\ (g/100g\ raw\ material)} = -4235.15 + 46.13T - 0.13T^2 - 0.03t^2$ **$R^2 = 71.8\%$**

Equation 19 $Y_{glucose\ yield\ (g/100g\ raw\ material)} = -5140.22 + 54.97T - 0.15T^2 + 12.10t - 0.06Tt$ **$R^2 = 83.2\%$**

Equation 20 $Y_{combined\ sugar\ yield\ (g/100g\ raw\ material)} = -9895.83 + 106.49T - 0.29T^2 + 17.11t - 0.09Tt$ **$R^2 = 75.7\%$**

None of the fitted models for xylose, glucose or combined sugar yield followed trends observed in the pretreatment data for MSJH13. Further pretreatment data is needed to improve the models for MSJH13.

Table 2-22: Small scale dilute acid optimization of sweet sorghum cultivar AS103

Pretreatment condition			Water insoluble solids	Pretreatment liquor yield			Enzymatic hydrolysis		Combined sugar yield	
Temperature	Time	Acid	Solid recovery	Arabinose g/100g raw material	Glucose g/100g raw material	Xylose g/100g raw material	Xylose g/100g raw material	Glucose g/100g raw material	g/100g raw material	% Recovery
°C	min	% (w/w)	(%)							
180	5	0.25	71.15 ± 0.73	0.63 ± 0.02	0.92 ± 0.02	8.77 ± 0.39	2.29 ± 0.07	26.17 ± 0.66	38.79	60.62
180	10	0.25	65.52 ± 1.11	0.99 ± 0.15	1.82 ± 0.04	8.99 ± 1.99	1.23 ± 0.05	27.00 ± 0.02	40.02	62.55
180	15	0.25	66.59 ± 0.74	1.09 ± 0.40	1.61 ± 0.40	12.69 ± 3.60	1.07 ± 0.17	28.40 ± 2.27	44.85	70.11
185	5	0.25	66.19 ± 0.29	1.30 ± 0.55	2.53 ± 0.78	10.72 ± 4.61	1.22 ± 0.06	25.03 ± 1.30	40.81	63.78
185	10	0.25	67.10 ± 0.38	1.30 ± 0.13	1.52 ± 0.27	12.68 ± 0.69	1.23 ± 0.17	31.15 ± 0.77	47.88	74.83
185	15	0.25	63.09 ± 0.41	0.98 ± 0.19	2.12 ± 0.09	8.39 ± 1.78	0.73 ± 0.03	27.44 ± 0.36	39.66	61.99
190	5	0.25	66.24 ± 0.85	1.35 ± 0.09	1.49 ± 0.15	17.16 ± 0.71	1.47 ± 0.04	32.93 ± 1.49	54.40	85.02
190	10	0.25	64.50 ± 0.41	1.49 ± 0.02	3.37 ± 0.51	14.46 ± 0.41	0.75 ± 0.03	26.65 ± 0.08	46.72	73.03
190	15	0.25	64.98 ± 0.11	0.86 ± 0.10	1.63 ± 0.10	12.48 ± 0.97	0.85 ± 0.05	30.40 ± 0.48	46.23	72.26

Figure 2-12: Small scale optimization of sweet sorghum cultivar AS103, ■ **Arabinose recovery,** ■ **Xylose recovery,** ■ **Glucose recovery,** ■ **Combined Sugar recovery**

Figure 2-13: Xylose yield surface plot for sweet sorghum cultivar AS103, R^2 = 59.4%

Figure 2-14: Glucose yield surface plot for sweet sorghum cultivar AS103, R^2 = 54.9%
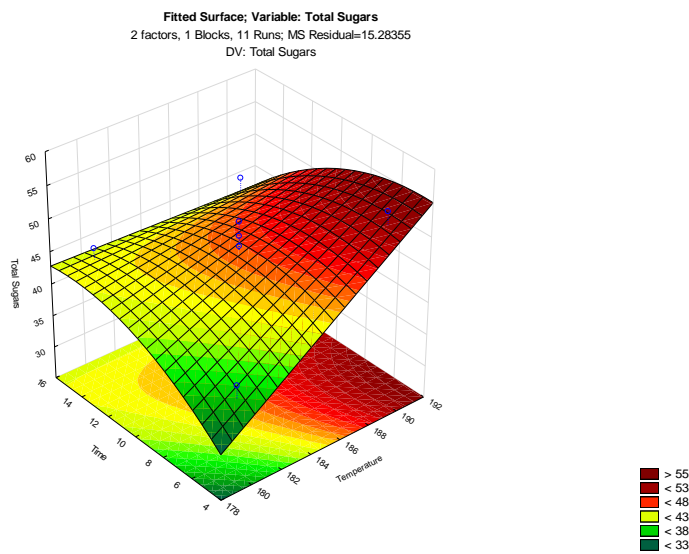
Figure 2-15: Combined sugar recovery surface plot for sweet sorghum cultivar AS103, R^2 = 64.2%

Table 2-23: Small scale dilute acid optimization of sweet sorghum cultivar AP6

Pretreatment condition			Water insoluble solids	Pretreatment liquor yield			Enzymatic hydrolysis		Combined sugar yield	
Temperature	Time	Acid	Solid Recovery	Arabinose	Glucose	Xylose	Xylose	Glucose	g/100g raw material	% Recovery
°C	min	% (w/w)	(%)	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material		
180	5	0.25	71.59 ± 2.12	1.31 ± 0.19	1.21 ± 0.18	12.03 ± 1.64	2.21 ± 0.53	26.55 ± 5.72	43.32	68.97
180	10	0.25	67.44 ± 0.43	1.67 ± 0.05	2.81 ± 0.19	17.52 ± 0.39	0.64 ± 0.16	30.81 ± 1.80	53.45	85.09
180	15	0.25	67.86 ± 0.50	0.97 ± 0.19	0.99 ± 0.40	8.86 ± 1.50	1.66 ± 0.06	30.70 ± 2.02	43.18	68.75
185	5	0.25	69.39 ± 1.01	1.39 ± 0.40	1.94 ± 0.11	12.29 ± 3.34	1.36 ± 0.47	24.78 ± 0.95	41.75	66.48
185	10	0.25	67.47 ± 0.44	1.50 ± 0.05	1.49 ± 0.00	13.20 ± 0.04	1.33 ± 0.11	31.90 ± 1.67	49.42	78.68
185	15	0.25	67.12 ± 0.37	1.55 ± 0.13	1.99 ± 0.01	14.54 ± 1.96	0.63 ± 0.04	29.36 ± 0.23	48.07	76.53
190	5	0.25	67.32 ± 0.41	0.94 ± 0.29	0.71 ± 0.23	10.33 ± 2.63	1.69 ± 0.03	26.92 ± 0.49	40.59	64.63
190	10	0.25	65.81 ± 1.07	1.42 ± 0.21	3.79 ± 0.24	12.61 ± 1.07	0.37 ± 0.10	28.78 ± 0.40	46.98	74.79
190	15	0.25	65.89 ± 0.60	0.54 ± 0.04	0.77 ± 0.02	6.23 ± 0.08	1.15 ± 0.02	30.72 ± 0.40	39.43	62.77

Figure 2-16: Small Scale optimization of sweet sorghum cultivar AP6, ■ Arabinose recovery, ■ Xylose recovery, ■ Glucose recovery, ■ Combined Sugar recovery.

Figure 2-17: Xylose yield recovery plot of sweet sorghum cultivar AP6, R^2 = 60.1%

Figure 2-18: Glucose yield recovery plot of sweet sorghum cultivar AP6, R^2 = 94.0%

Figure 2-19: Combined sugar yield surface plot of sweet sorghum cultivar AP6, R^2 = 78.19%

Table 2-24: Small scale dilute acid sweet sorghum cultivar SS27

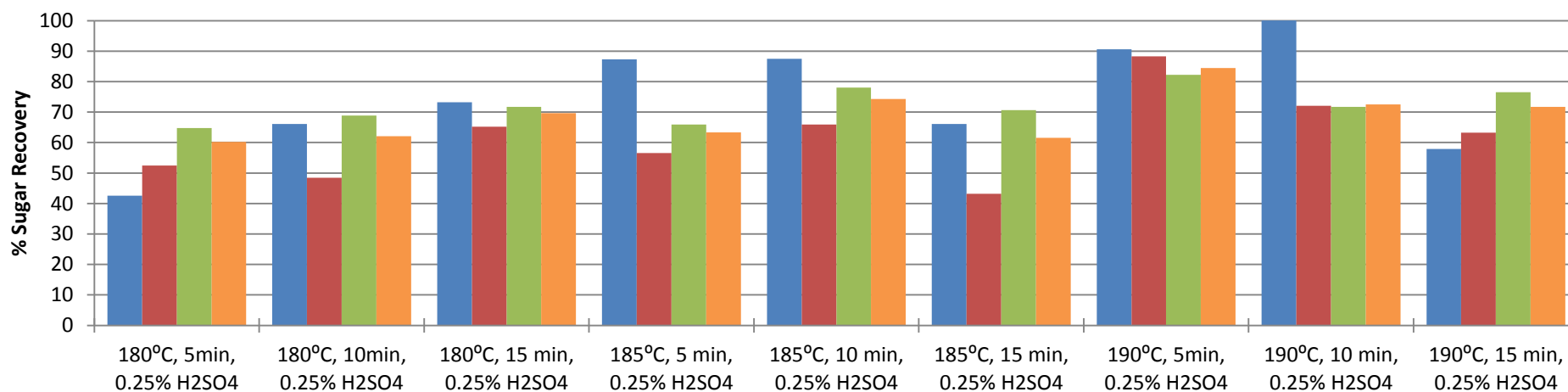| Pretreatment condition			Water insoluble solids	Pretreatment liquor yield			Enzymatic hydrolysis		Combined sugar yield	
Temperature	Time	Acid	Solid recovery	Arabinose g/100g raw material	Glucose g/100g raw material	Xylose g/100g raw material	Xylose g/100g raw material	Glucose g/100g raw material	g/100g raw material	% Recovery
°C	min	% (w/w)	(%)							
180	5	0.25	68.73 ± 0.16	0.91 ± 0.41	1.64 ± 0.57	10.41 ± 3.84	1.95 ± 0.08	24.95 ± 0.89	39.86	62.35
180	10	0.25	62.19 ± 2.16	1.60 ± 0.24	3.21 ± 0.09	15.40 ± 1.65	1.13 ± 0.15	26.31 ± 1.18	47.66	74.56
180	15	0.25	65.88 ± 0.33	1.30 ± 0.22	1.87 ± 0.14	14.51 ± 1.15	1.29 ± 0.08	27.43 ± 0.37	46.40	72.59
185	5	0.25	63.49 ± 0.34	1.79 ± 0.10	3.24 ± 0.74	16.44 ± 1.36	0.91 ± 0.13	25.09 ± 0.57	47.48	74.28
185	10	0.25	65.34 ± 1.87	1.38 ± 0.27	2.42 ± 0.62	15.22 ± 2.24	1.18 ± 0.37	28.21 ± 0.60	48.41	75.73
185	15	0.25	61.28 ± 0.05	1.34 ± 0.48	2.68 ± 1.11	11.89 ± 4.54	0.56 ± 0.08	25.26 ± 0.69	41.72	65.27
190	5	0.25	65.44 ± 0.01	1.22 ± 0.09	1.96 ± 0.19	15.15 ± 0.88	1.56 ± 0.03	28.95 ± 0.78	48.83	76.39
190	10	0.25	61.39 ± 1.12	1.20 ± 0.45	2.77 ± 1.15	11.56 ± 4.20	0.96 ± 0.81	27.55 ± 2.77	44.04	68.89
190	15	0.25	63.97 ± 1.17	0.87 ± 0.22	1.89 ± 0.49	12.29 ± 3.36	0.85 ± 0.03	30.02 ± 2.37	45.92	71.84
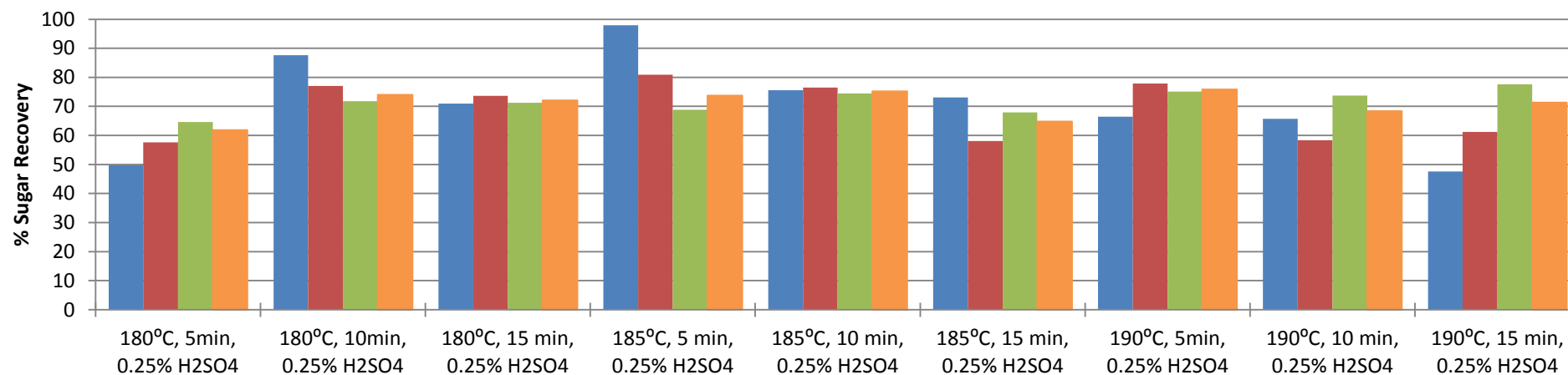
Figure 2-20: Small scale optimization of sweet sorghum cultivar SS27, ■ Arabinose recovery, ■ Xylose recovery, ■ Glucose recovery, ■ Combined Sugar recovery.

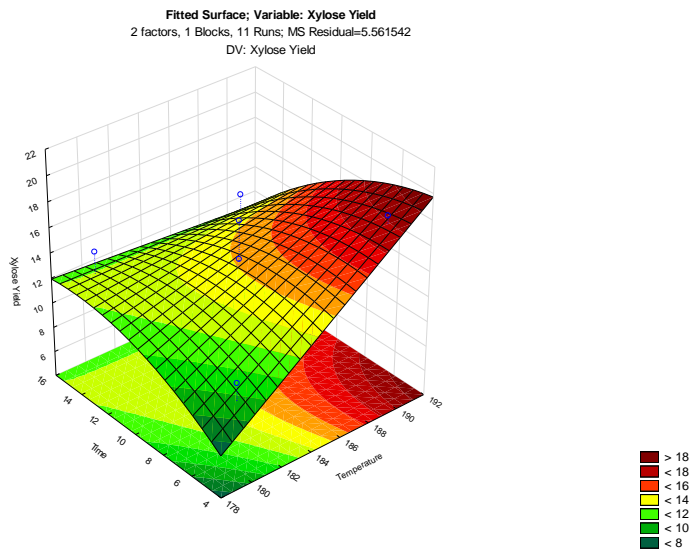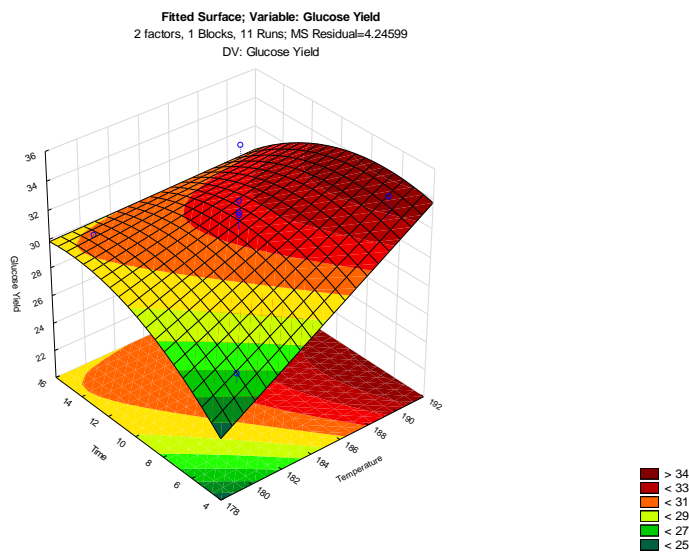**Figure 2-21: Xylose yield surface plot sweet sorghum cultivar SS27, R^2 = 45.3%**

**Figure 2-22: Glucose yield surface plot sweet sorghum cultivar SS27, R^2 = 59.3%**
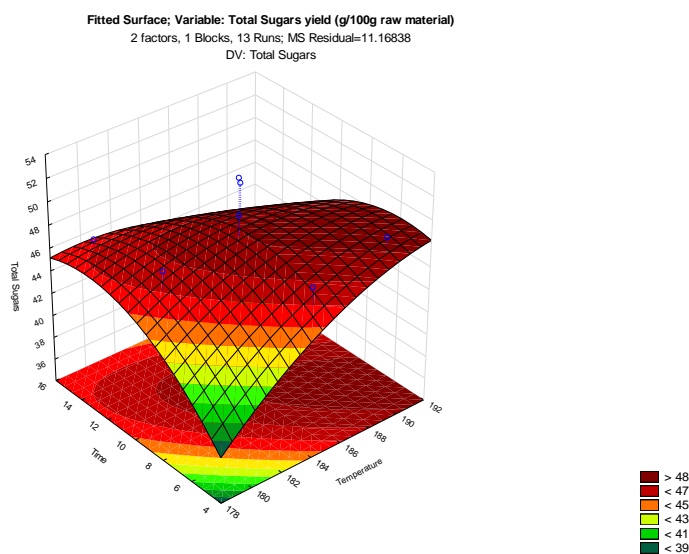
Figure 2-23: Combined Sugar yield plot for sweet sorghum cultivar SS27, R^2 = 40.1%

Table 2-25: Small scale dilute acid optimization of sweet sorghum cultivar AS246

Pretreatment condition			Water insoluble solids	Pretreatment liquor yield			Enzymatic hydrolysis		Combined sugar yield	
Temperature °C	Time min	Acid % (w/w)	Solid Recovery (%)	Arabinose g/100g raw material	Glucose g/100g raw material	Xylose g/100g raw material	Xylose g/100g raw material	Glucose g/100g raw material	g/100g raw material	% Recovery
180	5	0.25	67.38 ± 0.18	1.54 ± 0.16	1.70 ± 0.41	11.92 ± 4.35	1.64 ± 0.22	25.95 ± 1.05	42.75	66.61
180	10	0.25	66.97 ± 0.58	1.55 ± 0.21	1.95 ± 0.27	17.73 ± 0.60	0.85 ± 0.18	20.15 ± 0.80	42.22	65.91
180	15	0.25	66.28 ± 0.82	1.27 ± 0.07	1.67 ± 0.35	12.11 ± 0.52	1.19 ± 0.11	28.00 ± 0.21	44.24	69.05
185	5	0.25	67.27 ± 0.18	1.24 ± 0.40	1.46 ± 0.28	13.54 ± 4.60	0.81 ± 0.07	23.04 ± 0.89	40.09	62.47
185	10	0.25	66.37 ± 0.71	1.36 ± 0.22	1.91 ± 1.23	17.92 ± 0.49	1.11 ± 0.46	27.93 ± 1.14	50.23	78.40
185	15	0.25	65.61 ± 0.33	0.92 ± 0.08	1.97 ± 0.30	12.63 ± 0.90	0.54 ± 0.00	23.12 ± 0.49	39.19	61.17
190	5	0.25	66.35 ± 0.20	0.81 ± 0.42	0.81 ± 0.38	9.36 ± 4.29	1.40 ± 0.08	29.28 ± 0.44	41.66	65.03
190	10	0.25	64.92 ± 0.21	1.18 ± 0.13	2.91 ± 0.49	14.56 ± 0.95	0.44 ± 0.00	22.27 ± 0.02	41.37	64.58
190	15	0.25	64.64 ± 0.17	0.74 ± 0.07	1.07 ± 0.16	8.91 ± 1.10	0.88 ± 0.05	29.05 ± 1.44	40.65	63.46
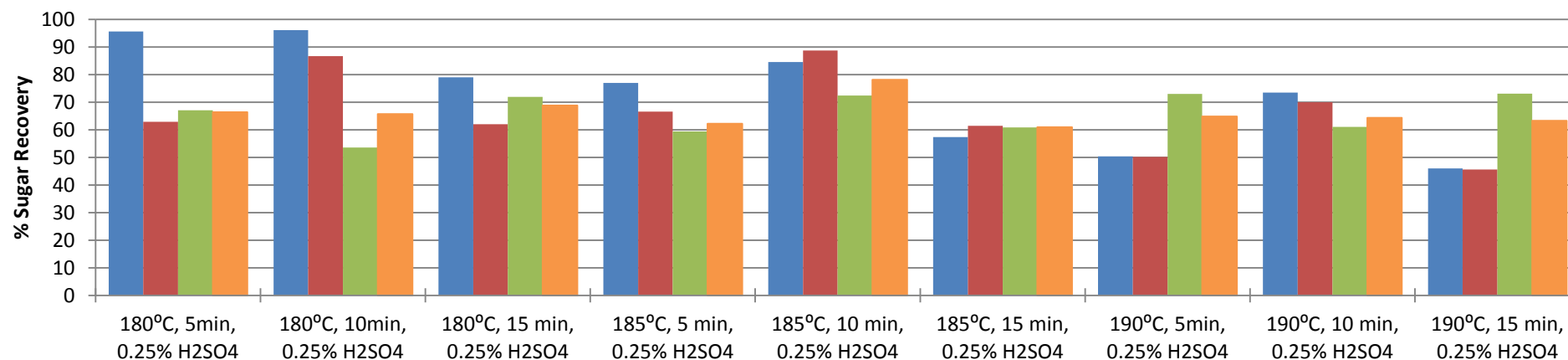
Figure 2-24: Small scale optimization of sweet sorghum cultivar AS246, ■ **Arabinose recovery,** ■ **Xylose recovery,** ■ **Glucose recovery,** ■ **Combined Sugar recovery.**
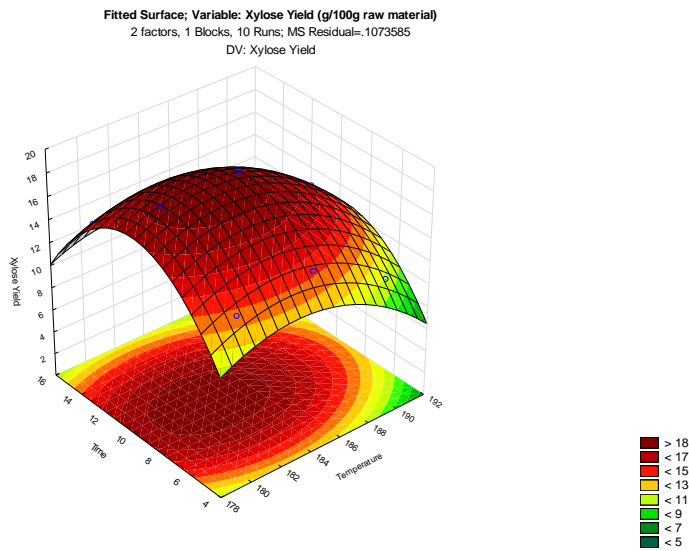
Figure 2-25: Xylose yield surface plot of sweet sorghum cultivar AS246, R^2 = 99.5%
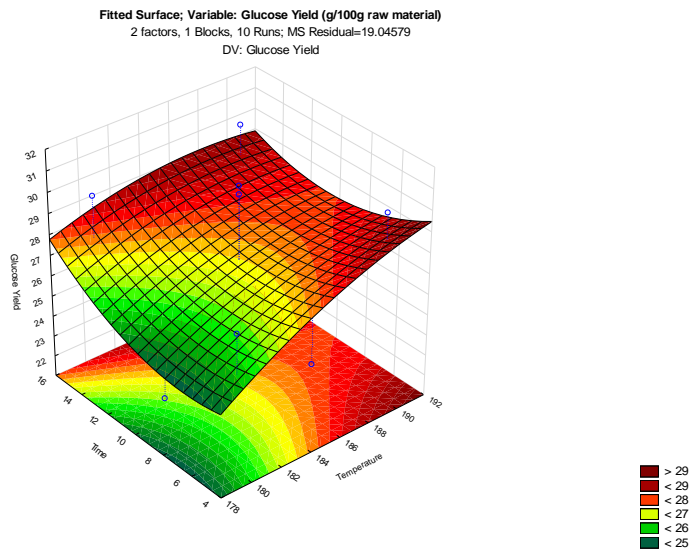
Figure 2-26: Glucose yield surface plot of sweet sorghum cultivar AS246, R^2 = 11.4%
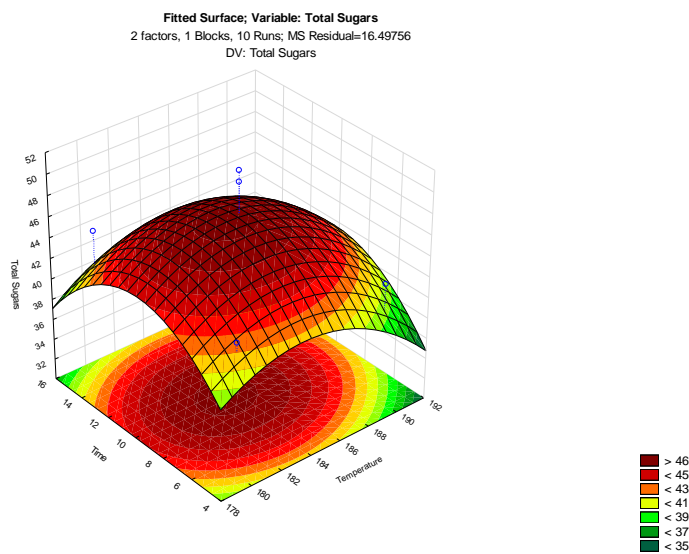
Figure 2-27: Combined sugar yield surface plot of sweet sorghum cultivar AS246, R^2 = 48.5%

Table 2-26: Small scale dilute acid optimization of sweet sorghum cultivar MSJH13

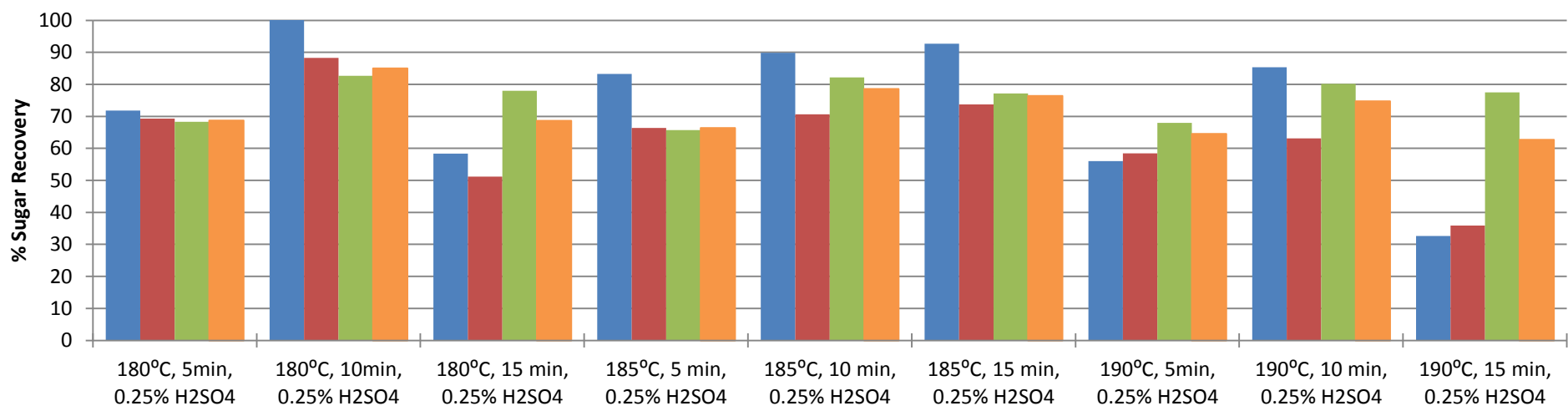| Pretreatment condition			Water insoluble solids	Pretreatment liquor yield			Enzymatic hydrolysis		Combined sugar yield	
Temperature	Time	Acid	Solid recovery	Arabinose g/100g raw material	Glucose g/100g raw material	Xylose g/100g raw material	Xylose g/100g raw material	Glucose g/100g raw material	g/100g raw material	% Recovery
ºC	min	% (w/w)	(%)							
180	5	0.25	70.43 ± 1.09	0.86 ± 0.26	1.20 ± 0.23	9.84 ± 1.54	1.79 ± 0.09	21.37 ± 3.62	35.06	58.16
180	10	0.25	71.18 ± 0.36	0.95 ± 0.09	2.05 ± 0.16	14.16 ± 2.04	1.06 ± 0.00	25.68 ± 0.16	43.90	72.22
180	15	0.25	68.28 ± 0.11	0.93 ± 0.32	1.42 ± 0.52	11.97 ± 4.64	1.20 ± 0.03	28.15 ± 0.88	43.68	72.84
185	5	0.25	70.33 ± 0.03	1.04 ± 0.49	1.51 ± 0.56	13.70 ± 5.69	0.88 ± 0.04	27.78 ± 0.92	44.92	75.20
185	10	0.25	67.08 ± 0.36	1.32 ± 0.03	1.90 ± 0.23	15.63 ± 0.48	0.86 ± 0.06	30.57 ± 0.70	50.29	82.71
185	15	0.25	68.19 ± 0.49	1.09 ± 0.10	2.26 ± 0.32	14.25 ± 1.43	0.54 ± 0.01	29.21 ± 0.93	47.36	78.18
190	5	0.25	68.79 ± 0.61	0.82 ± 0.21	0.93 ± 0.20	11.73 ± 2.92	1.40 ± 0.26	29.13 ± 0.99	44.01	72.67
190	10	0.25	68.57 ± 0.29	0.66 ± 0.07	2.02 ± 0.30	9.07 ± 0.91	0.43 ± 0.02	25.26 ± 0.78	37.44	61.86
190	15	0.25	66.77 ± 0.50	0.70 ± 0.28	1.40 ± 0.50	11.28 ± 4.42	0.86 ± 0.06	29.31 ± 1.81	43.55	72.52
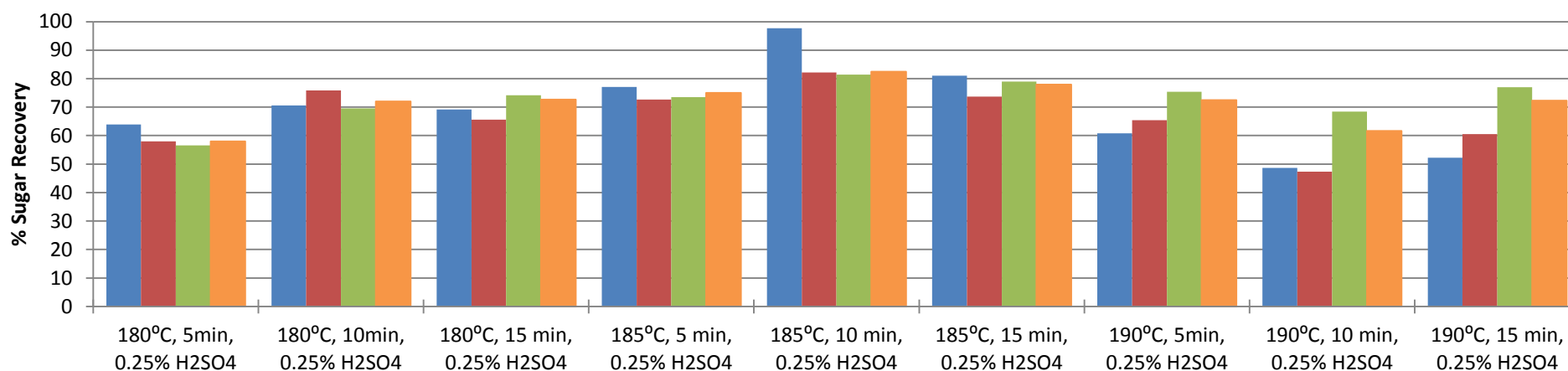
Figure 2-28: Small scale optimization of sweet sorghum cultivar MSJH13, ■ Arabinose recovery, ■ Xylose recovery, ■ Glucose recovery, ■ Combined Sugar recovery.
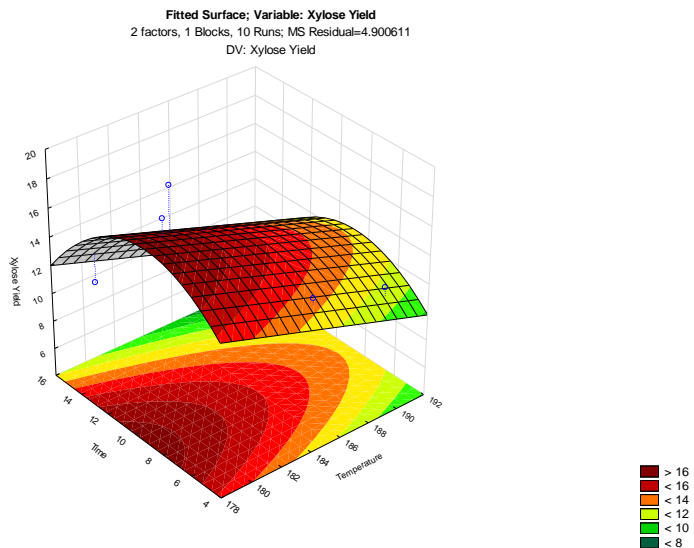
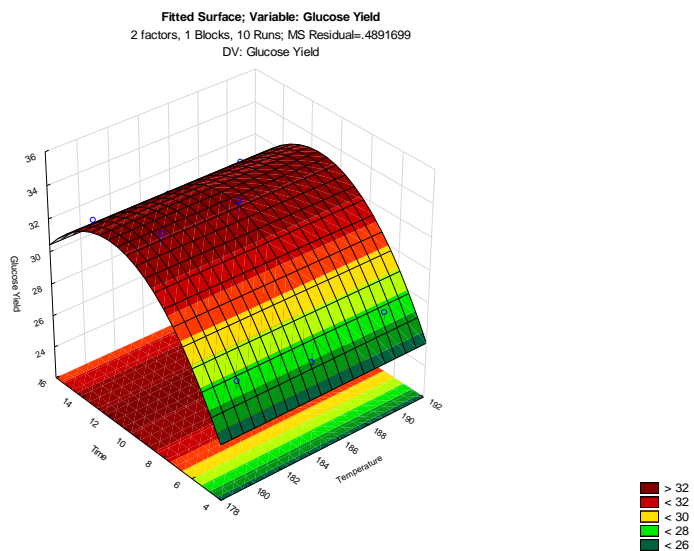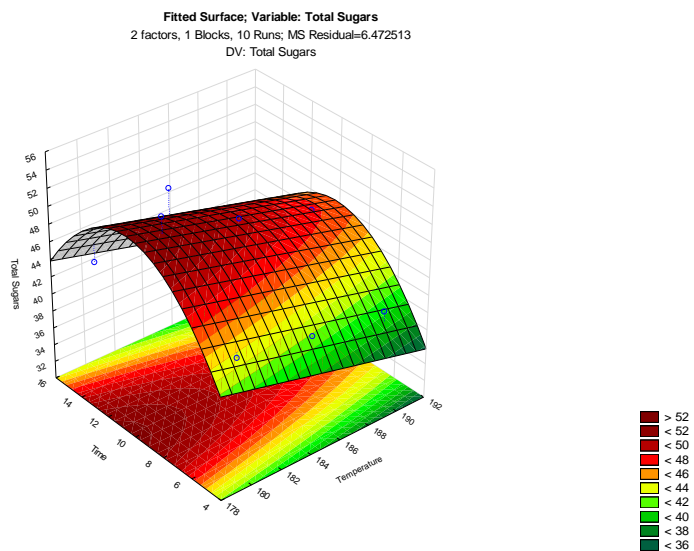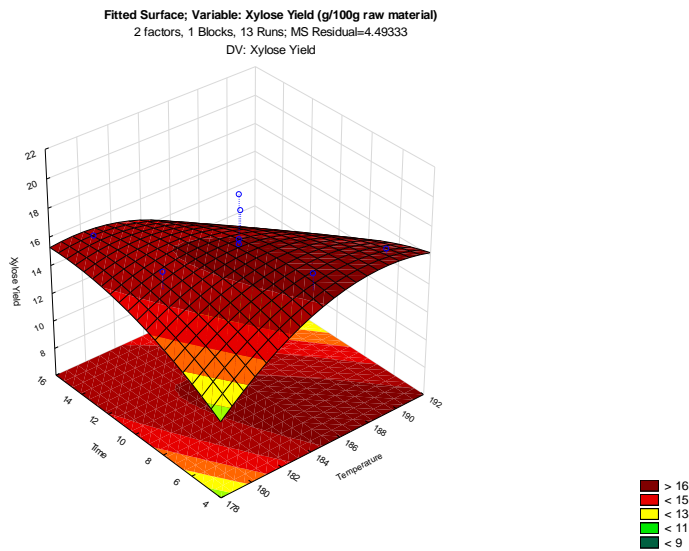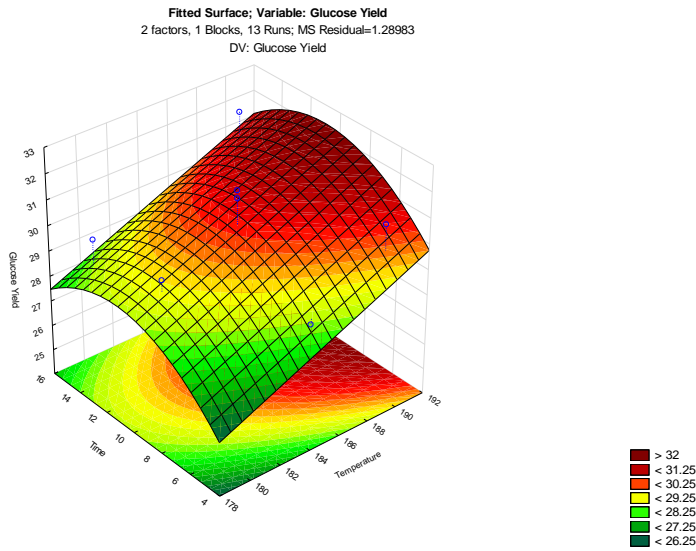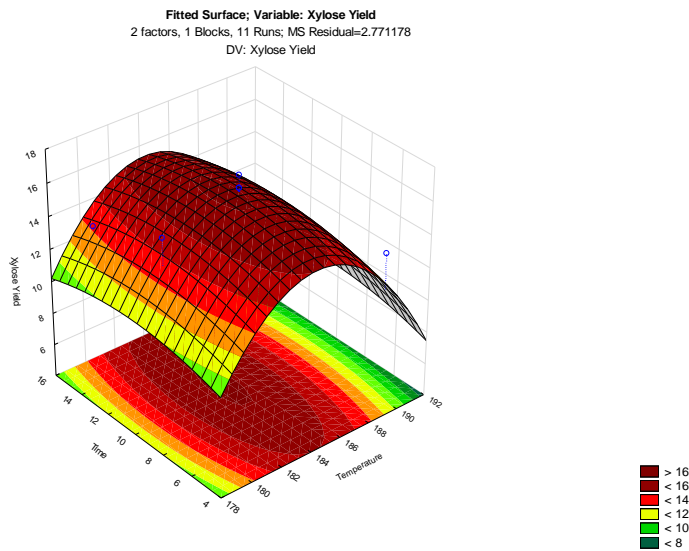**Figure 2-29: Xylose yield surface plot of sweet sorghum cultivar MSJH13, R^2 = 71.8%**
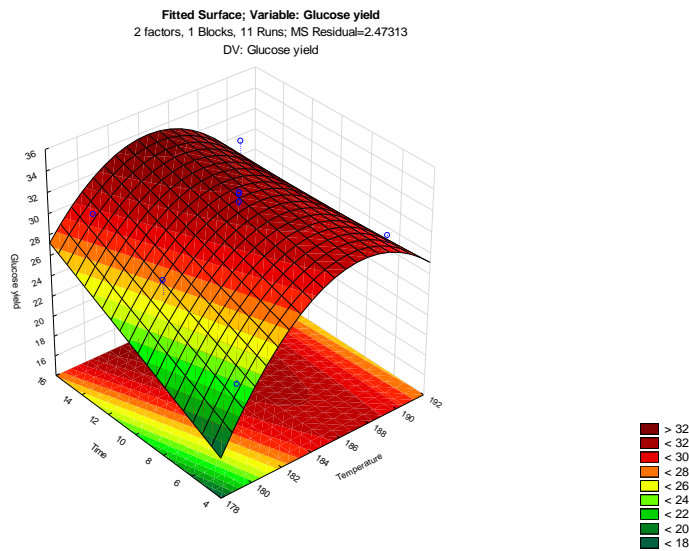
Figure 2-30: Glucose yield surface plot of sweet sorghum cultivar MSJH13, R^2 = 83.2%
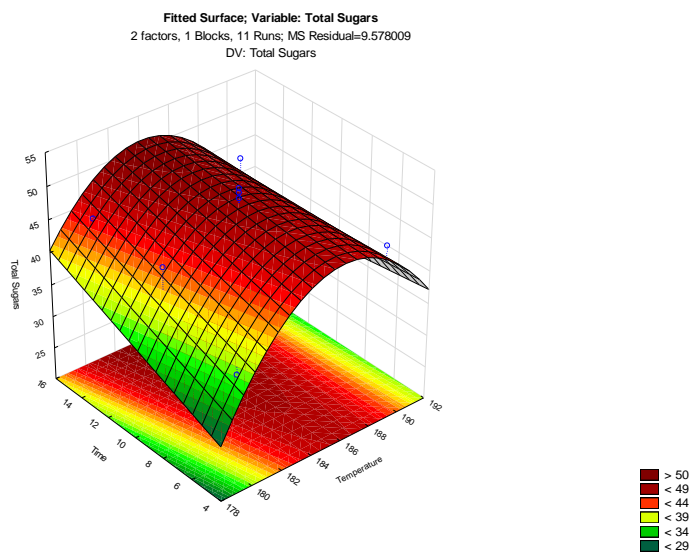
Figure 2-31: Combined sugar yield surface plot of sweet sorghum cultivar MSJH13, 75.7%

Table 2-27: Byproduct concentration in pretreatment liquor of sweet sorghum cultivar AS103

Pretreatment condition			Byproduct concentration		
Temperature	Time	Acid	Acetic Acid	HMF	Furfural
°C	min	% (w/w)	g/L	g/L	g/L
180	5	0.25	0.53 ± 0.25	0.01 ± 0.00	0.19 ± 0.09
180	10	0.25	2.17 ± 0.45	0.08 ± 0.01	0.75 ± 0.18
180	15	0.25	3.38 ± 0.77	0.10 ± 0.02	1.98 ± 0.28
185	5	0.25	1.93 ± 0.82	0.07 ± 0.03	0.57 ± 0.21
185	10	0.25	3.31 ± 0.74	0.11 ± 0.02	1.88 ± 0.57
185	15	0.25	2.50 ± 0.55	0.16 ± 0.02	1.32 ± 0.23
190	5	0.25	2.56 ± 0.30	0.07 ± 0.02	1.34 ± 0.28
190	10	0.25	4.77 ± 0.05	0.29 ± 0.00	2.61 ± 0.06
190	15	0.25	4.33 ± 0.09	0.21 ± 0.00	3.31 ± 0.08

Table 2-28: Byproduct concentration in pretreatment liquor of sweet sorghum cultivar AP6

Pretreatment condition			Byproduct concentration		
Temperature	Time	Acid	Acetic Acid	HMF	Furfural
°C	min	% (w/w)	g/L	g/L	g/L
180	5	0.25	0.82 ± 0.14	0.03 ± 0.01	0.57 ± 0.12
180	10	0.25	5.36 ± 0.05	0.12 ± 0.02	2.28 ± 0.38
180	15	0.25	1.97 ± 0.39	0.11 ± 0.05	1.86 ± 0.51
185	5	0.25	2.65 ± 0.56	0.04 ± 0.01	0.79 ± 0.06
185	10	0.25	2.74 ± 0.07	0.13 ± 0.00	2.52 ± 0.07
185	15	0.25	4.84 ± 0.61	0.14 ± 0.01	2.79 ± 0.41
190	5	0.25	0.90 ± 0.22	0.04 ± 0.01	0.68 ± 0.19
190	10	0.25	5.03 ± 0.40	0.26 ± 0.01	4.02 ± 0.25
190	15	0.25	2.19 ± 0.26	0.15 ± 0.02	2.52 ± 0.40

Table 2-29: Byproduct concentration in pretreatment liquor of sweet sorghum cultivar SS27

Pretreatment condition			Byproduct concentration		
Temperature	Time	Acid	Acetic acid	HMF	Furfural
°C	min	% (w/w)	g/L	g/L	g/L
180	5	0.25	1.00 ± 0.33	0.03 ± 0.01	0.41 ± 0.07
180	10	0.25	3.84 ± 0.35	0.12 ± 0.01	1.08 ± 0.07
180	15	0.25	3.59 ± 0.25	0.16 ± 0.03	2.09 ± 0.33
185	5	0.25	3.71 ± 0.76	0.12 ± 0.02	0.90 ± 0.25
185	10	0.25	3.49 ± 1.30	0.14 ± 0.05	1.57 ± 0.41
185	15	0.25	3.98 ± 1.64	0.22 ± 0.11	2.29 ± 1.13
190	5	0.25	1.83 ± 0.01	0.07 ± 0.00	0.91 ± 0.03
190	10	0.25	4.05 ± 1.21	0.22 ± 0.08	2.41 ± 0.74
190	15	0.25	4.06 ± 1.25	0.23 ± 0.08	3.00 ± 0.97

Table 2-30: Byproduct concentration in pretreatment liquor of sweet sorghum cultivar AS246

Pretreatment Condition			Byproduct concentration		
Temperature	Time	Acid	Acetic Acid	HMF	Furfural
°C	min	% (w/w)	g/L	g/L	g/L
180	5	0.25	1.46 ± 0.61	0.06 ± 0.02	0.75 ± 0.37
180	10	0.25	4.79 ± 0.33	0.11 ± 0.02	1.58 ± 0.19
180	15	0.25	3.44 ± 0.21	0.14 ± 0.01	2.20 ± 0.25
185	5	0.25	3.11 ± 1.14	0.06 ± 0.02	0.86 ± 0.27
185	10	0.25	3.64 ± 1.47	0.11 ± 0.04	1.73 ± 0.51
185	15	0.25	4.43 ± 0.48	0.15 ± 0.02	2.40 ± 0.22
190	5	0.25	1.39 ± 0.65	0.04 ± 0.02	0.67 ± 0.24
190	10	0.25	4.70 ± 0.41	0.17 ± 0.02	2.47 ± 0.38
190	15	0.25	3.24 ± 0.54	0.17 ± 0.01	2.40 ± 0.42

Table 2-31: Byproduct concentration in pretreatment liquor of sweet sorghum cultivar MSJH13

Pretreatment condition			Byproduct concentration		
Temperature	Time	Acid	Acetic acid	HMF	Furfural
°C	min	% (w/w)	g/L	g/L	g/L
180	5	0.25	0.93 ± 0.43	0.03 ± 0.02	0.42 ± 0.24
180	10	0.25	3.73 ± 0.71	0.05 ± 0.01	1.07 ± 0.28
180	15	0.25	3.04 ± 1.18	0.10 ± 0.04	1.78 ± 0.63
185	5	0.25	3.15 ± 1.12	0.05 ± 0.02	0.86 ± 0.17
185	10	0.25	4.23 ± 0.08	0.17 ± 0.01	2.88 ± 0.06
185	15	0.25	4.71 ± 0.41	0.14 ± 0.02	2.64 ± 0.05
190	5	0.25	1.84 ± 0.31	0.05 ± 0.01	0.99 ± 0.16
190	10	0.25	3.21 ± 0.64	0.08 ± 0.01	1.79 ± 0.25
190	15	0.25	3.95 ± 1.26	0.17 ± 0.05	2.84 ± 0.82

The byproduct concentrations for the small scale dilute sulfuric acid pretreatment optimizations can be seen in Table 2-27 to Table 2-31. Comparing the byproduct concentrations at the optimum conditions, one sees that the byproduct concentrations varied between cultivars. SS27 had the lowest byproduct concentration at its optimum condition with 1.83g/L acetic acid, 0.07g/L HMF and 0.91g/L Furfural. The next lowest byproduct concentration at its optimum pretreatment condition was AS103 with 2.56g/L acetic acid, 0.07g/L HMF and 1.34 g/L Furfural. Following this was AS246 with 3.64 g/L acetic acid, 0.11 g/L HMF and 1.73 g/L Furfural and MSJH13 with 4.23 g/L acetic acid, 0.17 g/L HMF and 2.88 g/L furfural. The lowest byproduct concentration was observed for AP6 at its optimum pretreatment condition with 5.36 g/L acetic acid, 0.12 g/L HMF and 2.28 g/L

Furfural. Higher optimum pretreatment conditions were not associated with high byproduct concentrations and rather it was longer pretreatment times that resulted in higher byproduct concentrations at the optimum pretreatment conditions. A pretreatment time of 5 minutes is therefore desired to reduce the formation of inhibitory byproducts. Further from the byproduct data it can be seen that choice of cultivar will impact on byproduct concentrations which will subsequently effect fermentations that will be exposed to the byproducts.
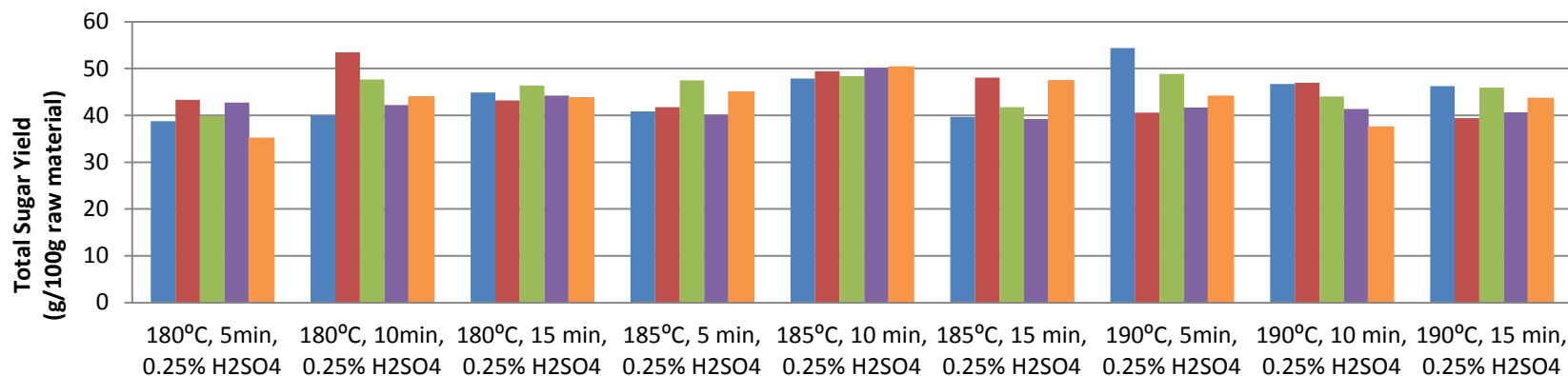
Figure 2-32: Combined sugar yield (g/100g raw material) of sweet sorghum cultivars for different experimental points in the central composite design. ■ Sweet sorghum cultivar AS103, ■ Sweet sorghum cultivar AP6, ■ Sweet sorghum cultivar SS27, ■ Sweet sorghum cultivar AS246, ■ Sweet sorghum cultivar MSJH13.

Table 2-32: Estimate ethanol yields based on optimized pretreatment response of sweet sorghum cultivars

Sweet Sorghum Cultivar	2009 Harvest			2011 Harvest			
	Lignocellulose Ethanol[a] (L/ha)	Juice Ethanol[b] (L/ha)	Total Ethanol[c] (L/Ha)	Lignocellulose Ethanol[a] (L/ha)	Juice Ethanol[b] (L/ha)	Total Ethanol[c] (L/Ha)	Average Total Ethanol yield[d] (L/Ha)
AS103	2426	3878	6304	1090	2715	3805	5055
AP6	4249	4719	8968	2211	4654	6865	7916
SS27	2304	4202	6506	1483	4719	6202	6354
AS246	1169	4331	5500	2110	5753	7863	6681
MSJH13	3218	3232	6450	2763	5753	8516	7483

[a] Lignocellulose ethanol yields calculated assuming 0.51g EtOH/g sugar consumed, where available sugar is that released from pretreatment response in terms of combined sugar yields. Further the agronomic fibre yield (ton/ha) taken into account to calculated ethanol in ton/ha.
[b] Sweet juice ethanol yield calculated assuming 0.51g EtOH/g sugar consumed, where available sugar is that present in the juice found in the stem.
[c] Total ethanol yield is the sum of the calculated lignocellulose and sweet juice ethanol.
[d] Average ethanol is the estimated average ethanol yield for the two harvest seasons.

In Figure 2-32, the combined sugar yield (g/100g raw material) for each of the sweet sorghum cultivars is compared at each of the central composite design points. At 185°C, 10 minutes and 0.25% (w/w) H_2SO_4 and at 180°C, 15 minutes and 0.25% (w/w) H_2SO_4 the yields are most similar and between the best and worst performing cultivar there is a difference in yield of 5.0% and 7.5% respectively. At 190°C, 5minutes and 0.25% (w/w) H_2SO_4 the difference between the best and worst performing cultivar was 34.0% showing that the choice of cultivar can result in significant differences in combined sugar yield. It is therefore important that one takes this into account when selecting cultivars.

Coupled with differences in pretreatment yields as described previously, the agronomic yields from year to year can have a major effect on the ethanol yields per hectare. In Table 2-32, the effect of agronomics can be observed for AS103 in which total ethanol yield (L/ha), which includes ethanol produced from the sweet juice and lignocellulose, decreased between 6304 and 3805L/ha between the 2008/2009 and 2010/2011 harvest. This decrease was due to the stalk yield which decreased from 45.1 to 29.2tons/ha. It is important therefore that cultivars which consistently give good agronomic yields over a number of seasons are selected to ensure that ethanol yields will consistently be high. Furthermore cultivars with high biomass yields should be preferred, due to the potentially higher ethanol yields which can be produced from the lignocellulose fraction, without compromising ethanol yields from the sweet juice.

2.4.6. Optimization of three preferred cultivars with pilot plant steam explosion

The three preferred cultivars optimized in pilot plant steam explosion were AP6, SS27 and AS246. These three cultivars were chosen based on good agronomic yields between seasons and high total ethanol yields and included the widest range of pretreatment response to ensure that the effects of cultivar on pretreatment could be evaluated. These selection criteria are listed in table 2-15. This meant that although AS103 had the highest combined sugar yield in small scale dilute acid pretreatment it couldn't be included for further optimization due to its poor agronomic performance between harvests. As AP6 had the next highest combined sugar yield in small scale dilute acid, only lower than AS103 by 2%, it was included in the selection. Furthermore AP6 also had the highest average total ethanol yield over two harvest seasons making it a preferred cultivar. As a control in the pilot plant

optimization, SS27 was included as it had the lowest maximum combined sugar yield for the small scale dilute acid pretreatment conditions evaluated previously, enabling the effect of cultivar on yields to be evaluated in pilot plant steam explosion. The final preferred cultivar to be included in the selection was AS246 as insufficient biomass was collected for MSJH13. Fortunately cultivar MSJH13 and cultivar AS246 had similar optima for dilute acid pretreatment response, both of which occurred at the same pretreatment conditions.

Each of these three cultivars, AP6, SS27 and AS246 were steam exploded at the same conditions to evaluate differences in pretreatment response between the 3 cultivars at pilot plant scale. Different pretreatment strategies, including dry, water soaked and SO_2 catalysed steam explosion were evaluated for the three preferred cultivars. In Table 2-4 and Table 2-5 the experimental design points for the standard designs used to evaluate the three preferred cultivars for the different pretreatment strategies can be found. Table 2-33 until Table 2-42 shows the pretreatment liquor, enzymatic hydrolysis; byproducts and water insoluble solids (WIS) data with air dried steam explosion for AP6, SS27 and AS246 respectively while Table 2-43 until Table 2-51 shows the pretreatment and water insoluble solids (WIS) data with water soaked stream explosion for AP6, SS27 and AS246 respectively.

2.4.6.1 Air dried steam explosion of three preferred sweet sorghum cultivars

The combined sugar yields for air dried steam explosion showed that the three preferred cultivars performed similarly with the highest combined sugar yield for each of the cultivars occurring at a pretreatment temperature of 205°C and a pretreatment time of 5minutes. Of the three cultivars, SS27 achieved the highest combined sugar yield of 44.73g/100g raw material followed by AS246 with a combined sugar yield of 43.20g/100g raw material and AP6 with a combined sugar yields of 43.07g/100g raw material. In terms of sugar recovery 63.90%, 61.99% and 61.50% of the initial sugars present were recovered for SS27, AS246 and AP6 respectively. From the results it could be seen that a more severe pretreatment was required to maximize the glucose yields released during enzymatic hydrolysis compared to that which was necessary for the maximum combined sugar yields. It was found that at 205°C and 10 minutes glucose yields of 36.65/100g raw material, 35.98 and 35.91g/100g raw material were released during enzymatic hydrolysis for AP6, AS246 and SS27 respectively while at 205°C and 5 minutes the glucose released during enzymatic hydrolysis was only 33.45, 33.74 and 31.98g/100g raw material for AP6, SS27 and AS246 respectively.

While the data for glucose released during enzymatic hydrolysis pointed to increasing the pretreatment severity further, trends in the xylose yields indicated that this would not be beneficial for improving both the xylose and combined sugar yields. The increase in pretreatment severity would only cause further degradation of the xylose released during pretreatment and result in a combined sugar yield which would not be maximized. This is substantiated by low xylose yields of between 2 and 3 g/100g raw material at a pretreatment condition of 205°C and 10minutes. Xylose yields peaked in air dried steam explosion at a pretreatment condition of 197.5^{o}C and 7.5 minutes, which were still low with a maximum of around 50% of the xylose being recovered at this pretreatment condition for the three preferred cultivars. Further the xylose released during pretreatment was found to be predominantly in oligomeric form with xylose yields following pretreatment consisting of 80% oligomers with the remained being monomers. These results show that air dried steam explosion should not be preferred for maximizing combined sugar yields as the conditions required for maximum glucose release during enzymatic hydrolysis are too severe for those required for xylose release during pretreatment. While the glucose yields released at a pretreatment temperature of 205° and a pretreatment time of 10 minutes were high, xylose yields during pretreatment at all of the observed pretreatment conditions was poor suggesting that steam explosion of air dried material is only beneficial in recovering glucose and not for the recovery of xylose. Further low yield of xylose following pretreatment suggests that degradation of xylose during air dried steam explosion into degradation byproducts occurred rapidly to explain the low yields of xylose during pretreatment.

The chemical composition of the water insoluble solids (WIS) for the preferred cultivars pretreated with air dried steam explosion reveals that both lignin and glucose content increased while arabinose and xylose content decreased with increasing pretreatment severity. Further the digestibility of the glucose present in the WIS increased with increasing severity. The highest glucose digestibility was 88.82% for cultivar SS27 at a pretreatment temperature of 205°C and 5 minutes, which corresponded to the highest yield of glucose during enzymatic hydrolysis. The trend for xylose content in the WIS combined with the xylose yields in the pretreatment liquor further showed that while xylose was released with increasing pretreatment severity, its degradation to furfural was rapid resulting in a low recovery of xylose in the pretreatment liquor. Comparing the chemical composition between

the preferred cultivars at the different pretreatment conditions showed that the cultivars responded similarly to pretreatment under air dried steam explosion.

As expected the concentrations of the byproducts acetic acid, formic acid, furfural and HMF increased with increasing pretreatment severity for air dried steam explosion for all of the preferred sweet sorghum cultivars which corresponded to the release of xylose following pretreatment. AP6 had the lowest furfural and HMF concentrations at the optimal pretreatment condition (205°C, 5 minutes) with 1.4g/L furfural and 0.46 g/L HMF, while SS27 had the highest furfural and HMF concentrations at the optimal pretreatment condition with 2.12 g/L furfural and 0.95 g/L HMF. For acetic acid AP6 had the lowest concentration at the optimal pretreatment condition of 12.02g/L while SS27 had the highest acetic acid content of 13.17 g/L. Further AS246 had the lowest formic acid concentration of 4.97 g/L at the optimum pretreatment condition while AP6 had the highest formic acid concentration of 5.73g/L. These differences in byproduct concentrations for the preferred cultivars show that choice of cultivar can potentially affect the ethanol yields in fermentation due to the varied byproduct concentration and the associated fermentation inhibition.

Table 2-33: Air dried steam explosion of cultivar AP6, pretreatment and enzymatic hydrolysis yields

Pretreatment condition			Water insoluble solids	Pretreatment Liquor yield			Enzymatic hydrolysis		Combined sugar yield	
Temperature °C	Time min	Catalyst %	Solid recovery (%)	Arabinose g/100g raw material	Glucose g/100g raw material	Xylose g/100g raw material	Xylose g/100g raw material	Glucose g/100g raw material	g/100g raw material	% Recovery
190	5	None	81.48 ± 0.54	0.54 ± 0.04	0.72 ± 0.07	4.65 ± 0.58	6.08 ± 0.22	15.58 ± 0.49	27.57	39.38%
190	10	None	74.19 ± 0.83	0.45 ± 0.03	1.08 ± 0.01	7.50 ± 0.23	4.84 ± 0.28	21.39 ± 1.30	35.25	50.34%
197.5	7.5	None	71.98 ± 0.91	0.34 ± 0.03	1.04 ± 0.03	7.44 ± 0.09	4.04 ± 0.06	27.94 ± 0.38	40.81	58.28%
197.5	7.5	None	69.49 ± 0.45	0.34 ± 0.05	1.22 ± 0.15	7.03 ± 1.03	4.17 ± 0.34	27.74 ± 1.27	40.51	57.85%
205	5	None	66.93 ± 0.88	0.23 ± 0.03	0.92 ± 0.03	5.12 ± 0.30	3.35 ± 0.09	33.45 ± 0.45	43.07	61.50%
205	10	None	66.74 ± 2.42	0.11 ± 0.05	0.92 ± 0.03	2.42 ± 0.20	1.92 ± 0.05	36.65 ± 0.75	42.01	59.99%

Table 2-34: Air dried steam explosion of cultivar AP6, pretreatment liquor byproduct concentration and fraction of sugars that are in oligomeric form

Pretreatment condition		Oligomeric fraction in pretreatment liquor			Byproducts			
Temperature °C	Time min	Arabinose % Oligomer	Xylose % Oligomer	Glucose % Oligomer	Acetic Acid g/L	Formic Acid g/L	Furfural g/L	HMF g/L
190	5	54.44	94.17	93.31	5.90 ± 0.06	2.35 ± 0.02	0.18 ± 0.01	0.09 ± 0.00
190	10	44.52	89.47	90.60	4.30 ± 0.01	1.84 ± 0.00	0.32 ± 0.00	0.10 ± 0.01
197.5	7.5	34.30	93.31	83.55	7.43 ± 0.02	3.40 ± 0.04	0.85 ± 0.01	0.26 ± 0.00
197.5	7.5	33.57	94.25	83.60	7.24 ± 0.13	3.25 ± 0.07	0.83 ± 0.04	0.27 ± 0.02
205	5	22.53	90.86	72.78	12.02 ± 0.18	5.73 ± 0.12	1.40 ± 0.01	0.46 ± 0.00
205	10	10.62	81.73	53.70	10.41 ± 0.24	4.76 ± 0.54	1.51 ± 0.00	0.57 ± 0.00

Table 2-35: Air dried steam explosion of cultivar AP6, WIS composition and digestibility

Pretreatment condition			WIS chemical composition				Digestibility	
Temperature	Time	Catalyst	Lignin	Arabinose	Glucose	Xylose	Glucose	Xylose
°C	min	%	%	%	%	%	%	%
190	5	None	25.82 ± 0.11	1.28 ± 0.39	51.55 ± 1.25	19.46 ± 0.43	37.09 ± 1.17	38.31 ± 1.40
190	10	None	28.51 ± 0.27	0.46 ± 0.20	60.41 ± 0.65	14.64 ± 0.04	47.72 ± 2.91	44.57 ± 2.56
197.5	7.5	None	32.25 ± 0.10	0.14 ± 0.03	54.16 ± 7.31	8.12 ± 1.09	71.67 ± 0.96	69.11 ± 1.07
197.5	7.5	None	32.58 ± 0.23	0.15 ± 0.01	52.00 ± 1.24	8.15 ± 0.18	76.77 ± 3.52	73.70 ± 5.92
205	5	None	33.61 ± 0.33	0.16 ± 0.01	62.75 ± 0.90	6.82 ± 0.19	79.64 ± 1.08	73.29 ± 2.04
205	10	None	36.70 ± 0.53	0.06 ± 0.02	62.73 ± 0.24	3.8b4 ± 0.00	87.53 ± 1.79	74.83 ± 1.85

Table 2-36: Air dried steam explosion of cultivar SS27, Pretreatment and enzymatic hydrolysis yield

Pretreatment Condition			Water Insoluble Solids	Pretreatment Liquor yield			Enzymatic Hydrolysis		Combined Sugar yield	
Temperature	Time	Catalyst	Solid Recovery	Arabinose g/100g raw material	Glucose g/100g raw material	Xylose g/100g raw material	Xylose g/100g raw material	Glucose g/100g raw material	g/100g raw material	% Recovery
°C	min	%	(%)							
190	5	None	79.40 ± 0.55	0.43 ± 0.04	2.26 ± 0.19	6.27 ± 0.51	4.94 ± 0.08	13.96 ± 0.39	27.85	39.79%
190	10	None	75.26 ± 1.08	0.37 ± 0.02	1.92 ± 0.07	7.36 ± 0.29	4.40 ± 0.61	20.76 ± 2.85	34.81	49.74%
197.5	7.5	None	65.01 ± 0.62	0.31 ± 0.01	2.22 ± 0.17	7.62 ± 0.65	3.41 ± 0.08	26.90 ± 1.78	40.48	57.83%
197.5	7.5	None	67.46 ± 1.51	0.35 ± 0.03	2.29 ± 0.02	8.14 ± 0.37	3.71 ± 0.29	26.73 ± 1.81	41.22	58.89%
205	5	None	63.95 ± 1.03	0.24 ± 0.02	1.86 ± 0.25	5.92 ± 0.63	2.97 ± 0.13	33.74 ± 0.50	44.73	63.90%
205	10	None	65.25 ± 3.09	0.09 ± 0.05	1.22 ± 0.20	2.15 ± 0.28	1.67 ± 0.08	35.91 ± 0.74	41.03	58.61%

Table 2-37: Air dried steam explosion of SS27, Pretreatment liquor byproduct concentration and fraction of sugars in oligomeric form

Pretreatment Condition		Oligomeric Fraction in Pretreatment liquor			Byproducts			
Temperature	Time	Arabinose	Xylose	Glucose	Acetic Acid	Formic Acid	Furfural	HMF
°C	min	% Oligomer	% Oligomer	% Oligomer	g/L	g/L	g/L	g/L
190	5	42.56	93.24	90.32	4.07 ± 0.08	0.28 ± 0.00	0.28 ± 0.00	0.19 ± 0.00
190	10	37.05	91.67	83.91	3.58 ± 0.00	0.62 ± 0.01	0.62 ± 0.01	0.21 ± 0.01
197.5	7.5	31.37	88.44	75.12	8.85 ± 0.25	1.31 ± 0.07	1.31 ± 0.07	0.61 ± 0.05
197.5	7.5	34.95	90.23	79.33	6.44 ± 0.03	1.05 ± 0.01	1.05 ± 0.01	0.41 ± 0.01
205	5	24.22	82.00	65.83	13.17 ± 0.20	2.12 ± 0.03	2.12 ± 0.03	0.95 ± 0.05
205	10	8.84	54.68	28.79	14.23 ± 0.06	2.50 ± 0.04	2.50 ± 0.04	1.33 ± 0.05

Table 2-38: Air dried steam explosion of cultivar SS27, WIS chemical composition and Digestibility

Pretreatment Condition			WIS chemical Composition				Digestibility	
Temperature	Time	Catalyst	Lignin	Arabinose	Glucose	Xylose	Glucose	Xylose
°C	min	%	%	%	%	%	%	%
190	5	None	25.84 ± 0.79	0.87 ± 0.02	54.67 ± 1.77	19.23 ± 0.88	32.16 ± 0.90	32.32 ± 3.45
190	10	None	28.13 ± 0.80	0.56 ± 0.06	57.12 ± 0.26	13.13 ± 0.13	48.30 ± 6.64	44.53 ± 6.18
197.5	7.5	None	32.27 ± 0.46	0.15 ± 0.00	54.21 ± 1.38	7.49 ± 0.13	76.34 ± 5.04	70.09 ± 1.67
197.5	7.5	None	31.55 ± 0.89	0.18 ± 0.03	54.67 ± 5.73	7.97 ± 0.93	72.49 ± 4.92	63.51 ± 7.23
205	5	None	33.69 ± 0.59	0.19 ± 0.06	63.31 ± 1.08	6.22 ± 0.08	83.34 ± 1.24	74.61 ± 3.19
205	10	None	38.26 ± 0.44	0.09 ± 0.01	61.96 ± 0.33	3.47 ± 0.03	88.82 ± 1.84	73.72 ± 3.45

Table 2-39: Air dried steam explosion of cultivar AS246, pretreatment and enzymatic hydrolysis yields

Pretreatment condition			Water insoluble solids	Pretreatment liquor yield			Enzymatic hydrolysis		Combined sugar yield	
Temperature	Time	Catalyst	Solid recovery	Arabinose	Glucose	Xylose	Xylose	Glucose	g/100g raw material	% Recovery
°C	min	%	(%)	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material		
190	5	None	79.82 ± 6.30	0.68 ± 0.01	1.84 ± 0.01	6.96 ± 0.23	5.15 ± 0.26	13.57 ± 0.41	28.20	40.47%
190	10	None	73.97 ± 3.31	0.42 ± 0.04	1.48 ± 0.10	7.14 ± 0.71	4.49 ± 0.32	20.09 ± 1.27	33.61	48.24%
197.5	7.5	None	72.65 ± 0.83	0.40 ± 0.02	2.00 ± 0.17	8.96 ± 1.03	3.83 ± 0.06	27.41 ± 0.57	42.60	61.14%
197.5	7.5	None	72.70 ± 0.96	0.37 ± 0.05	1.80 ± 0.13	8.05 ± 0.97	3.67 ± 0.21	25.06 ± 1.04	38.96	55.90%
205	5	None	68.49 ± 1.16	0.22 ± 0.08	1.37 ± 0.15	6.24 ± 0.00	3.38 ± 0.09	31.98 ± 0.58	43.20	61.99%
205	10	None	68.63 ± 0.34	0.12 ± 0.02	1.02 ± 0.11	2.42 ± 0.16	1.81 ± 0.06	35.98 ± 0.91	41.35	59.34%

Table 2-40: Air dried steam explosion of AS246, pretreatment liquor byproduct concentration and fraction of sugar in oligomeric form

Pretreatment Condition		Oligomeric Fraction in Pretreatment liquor			Byproducts			
Temperature	Time	Arabinose	Xylose	Glucose	Acetic Acid	Formic Acid	Furfural	HMF
°C	min	% Oligomer	% Oligomer	% Oligomer	g/L	g/L	g/L	g/L
190	5	67.78	94.16	92.29	4.07 ± 0.08	1.68 ± 0.03	0.24 ± 0.00	0.15 ± 0.02
190	10	41.50	91.69	84.89	3.58 ± 0.00	1.42 ± 0.00	0.67 ± 0.01	0.28 ± 0.00
197.5	7.5	39.67	91.22	80.24	8.85 ± 0.25	3.51 ± 0.07	1.18 ± 0.01	0.45 ± 0.02
197.5	7.5	36.80	90.81	79.98	6.44 ± 0.03	2.33 ± 0.01	1.04 ± 0.00	0.37 ± 0.01
205	5	21.81	90.60	78.98	13.17 ± 0.20	5.06 ± 0.09	1.86 ± 0.03	0.69 ± 0.00
205	10	12.37	60.38	36.54	14.23 ± 0.06	4.81 ± 0.02	1.97 ± 0.06	0.76 ± 0.02

Table 2-41: Air dried steam explosion of AS246, WIS chemical composition and digestibility

Pretreatment condition			WIS chemical composition				Digestibility	
Temperature	Time	Catalyst	Lignin	Arabinose	Glucose	Xylose	Glucose	Xylose
°C	min	%	%	%	%	%	%	%
190	5	None	26.98 ± 0.40	1.05 ± 0.11	55.32 ± 1.33	20.37 ± 0.47	30.74 ± 0.93	31.65 ± 1.61
190	10	None	30.69 ± 0.47	0.53 ± 0.05	56.88 ± 1.40	13.81 ± 0.44	47.74 ± 3.02	43.95 ± 3.15
197.5	7.5	None	33.09 ± 0.12	0.17 ± 0.00	52.10 ± 0.51	7.81 ± 0.09	72.42 ± 1.51	67.55 ± 1.01
197.5	7.5	None	33.35 ± 0.79	0.20 ± 0.02	55.14 ± 2.79	8.70 ± 0.41	62.52 ± 2.59	58.12 ± 3.39
205	5	None	33.82 ± 0.13	0.28 ± 0.00	61.89 ± 2.68	7.14 ± 0.25	75.45 ± 1.37	69.10 ± 1.82
205	10	None	37.99 ± 0.26	0.05 ± 0.02	61.63 ± 2.36	3.60 ± 0.13	85.05 ± 2.15	73.23 ± 2.44

Table 2-42: Estimated total ethanol yields, based on pretreatment response to air dried steam explosion

Sweet sorghum cultivar	2009 Harvest			2011 Harvest			Average total ethanol yield [d] (L/Ha)
	Lignocellulosic ethanol [a] (L/ha)	Juice ethanol [b] (L/ha)	Total ethanol [c] (L/Ha)	Lignocellulosic ethanol [a] (L/ha)	Juice ethanol [b] (L/ha)	Total ethanol [c] (L/Ha)	
AP6	1782	4654	6436	3424	4719	8143	7289
SS27	1359	4719	6078	2111	4202	6312	6195
AS246	1815	5753	7568	1005	4331	5336	6452

[a] Lignocellulose ethanol yields calculated assuming 0.51g EtOH/g sugar consumed, where available sugar is that released from pretreatment response in terms of combined sugar yields. Further the agronomic fibre yield (ton/ha) taken into account to calculated ethanol in ton/ha.

[b] Sweet juice ethanol yield calculated assuming 0.51g EtOH/g sugar consumed, where available sugar is that present in the juice found in the stem.

[c] Total ethanol yield is the sum of the calculated lignocellulose and sweet juice ethanol.

[d] Average ethanol is the estimated average ethanol yield for the two harvest seasons

2.4.6.2 Water soaked steam explosion of three preferred sweet sorghum cultivars

Similarly to dry steam explosion, the preferred cultivars achieved the highest combined sugar yields for water soaked steam explosion at a pretreatment temperature of 205°C and a pretreatment time of 5 minutes. The results for water soaked steam explosion are given in Table 2-43 until Table 2-51. AS246 achieved the highest combined sugar yield of 58.65g/100g raw material followed by SS27 with a combined sugar yield of 55.39g/100g raw material and AP6 with a combined sugar yield of 55.13g/100g raw material. These combined sugar yields corresponded to combined sugar recoveries of 84.17%, 79.13% and 78.73% for AS246, SS27 and AP6 respectively. Further xylose pretreatment yields peaked at 205°C and 5 minutes with AS246 achieving a xylose yield of 14.23g/100g raw material, SS27 achieving a xylose yield of 13.28g/100g raw material and AP6 achieving a xylose yield of 12.82g/100g raw material. The yields of glucose released during enzymatic hydrolysis were also maximized at 205°C and 5 minutes with 39.29, 36.42 and 37.43g/100g raw material for AS246, SS27 and AP6 respectively.

Trends in the data for the water soaked steam explosion pointed to the fact that both the xylose released during pretreatment and the glucose released during enzymatic hydrolysis increased with increasing severity up until 205°C and 5 minutes. Further increasing the time to 10 minutes resulted in both degradation and loss of xylose in the pretreatment liquor while increasing the time to 10 minutes resulted in a decrease in the glucose yields following enzymatic hydrolysis for cultivars SS27 and AS246, while only slightly increasing the glucose yields for cultivar AP6 by 2g/100g raw material. The optimum region for maximum combined sugar yields is therefore around 205°C and 5 minutes for water soaked steam explosion. This is substantiated by the high yields of xylose following pretreatment and high yields of glucose at this condition which result in a high combined sugar yield.

The chemical composition analyses of the water insoluble solids for bagasse that had under gone water soaked steam explosion showed that lignin content, glucose content and enzymatic digestibility increased with increasing severity. Further to that both arabinose and xylose content in the WIS decreased with increasing pretreatment severity. These observations result from hydrolysis of the hemicellulose sugaric arabinose and xylose during pretreatment which enhances the fraction of both lignin and glucose in the remaining water insoluble solids. Further the remaining solids are more digestible due to disruption of the lignocellulose matrix. The highest enzymatic digestibility of glucan to glucose was observed

for AP6 at the optimum pretreatment conditions of 205 ᵒC and 5 minutes with digestibility of 89.98%. This was followed by SS27 and AS246 with corresponding enzymatic digestibilities of 87.29% and 82.26% respectively.

The results observed for the preferred cultivars at the pretreatment condition which gave the maximum combined sugar yield for both dry and water soaked steam explosion indicated that the optimum occurred at the same pretreatment conditions for both types of steam explosion. While the same condition resulted in the maximum combined sugar yields for both pretreatment methods water soaked steam explosion should be preferred over dry steam explosion due to the much higher recovery of xylose in the pretreatment liquor which was double that possible with dry steam explosion. A higher recovery of xylose results in a higher recovery of the available sugars which is important in second generation bio-ethanol production.

Similarly to steam explosion of air dried material the byproduct concentrations increased with increasing pretreatment severity for steam explosion of water soaked material. The byproduct concentration at the optimal pretreatment condition for water soaked steam explosion consisted of 2.76 g/L to 3.55 g/L of acetic acid, 0.64 g/L to 0.73 g/L formic acid, 0.57 g/L to 0.76 g/L of furfural and 0.13 g/L to 0.19 g/L HMF for the three preferred cultivars. Of the three preferred cultivar, the lowest byproduct concentration was observed for AP6 followed by SS27 and subsequently AS246. Compared to air dried steam explosion the byproduct concentrations were substantially lower for furfural and HMF with water soaked steam explosion. This gives water soaked steam explosion a substantial benefit over air dried steam explosion which will manifest in an improved fermentation of hydrolysates coming from water soaked explosion.

Table 2-43: Water soaked steam explosion of AP6, Pretreatment and enzymatic hydrolysis yields

Pretreatment condition			Water insoluble solids	Pretreatment liquor yield			Enzymatic hydrolysis		Combined sugar yield	
Temperature	Time	Moisture content	Solid recovery	Arabinose	Glucose	Xylose	Xylose	Glucose	g/100g raw material	% Recovery
°C	min	%	(%)	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material		
190	5	70%	75.21 ± 0.66	0.80 ± 0.10	0.54 ± 0.01	4.94 ± 0.01	5.68 ± 0.46	15.25 ± 1.00	27.22	38.87
190	10	70%	74.94 ± 0.74	0.84 ± 0.09	0.68 ± 0.10	8.32 ± 0.10	5.26 ± 0.08	26.00 ± 0.03	41.11	58.70
197.5	7.5	70%	65.18 ± 0.72	0.66 ± 0.18	0.55 ± 0.01	5.94 ± 0.01	4.25 ± 0.06	36.29 ± 1.42	47.70	68.11
197.5	7.5	70%	63.18 ± 2.33	0.67 ± 0.02	0.81 ± 0.04	6.19 ± 0.04	4.01 ± 0.09	33.74 ± 0.84	45.42	64.86
205	5	70%	58.94 ± 1.22	0.81 ± 0.05	1.22 ± 0.23	12.82 ± 0.23	3.25 ± 0.25	37.43 ± 2.38	55.13	78.73
205	10	70%	68.79 ± 1.77	0.51 ± 0.02	1.33 ± 0.06	9.61 ± 0.06	2.53 ± 0.04	39.00 ± 1.72	52.98	75.66

Table 2-44: Water soaked steam explosion of AP6, pretreatment liquor, byproduct concentration and fraction of sugars that were in oligomeric form

Pretreatment condition			Oligomeric fraction in pretreatment liquor			Byproducts			
Temperature	Time	Moisture content	Arabinose	Xylose	Glucose	Acetic Acid	Formic Acid	Furfural	HMF
°C	min	%	% Oligomer	% Oligomer	% Oligomer	g/L	g/L	g/L	g/L
190	5	70%	5.12	92.98	94.45	1.03 ± 0.02	0.30 ± 0.01	0.07 ± 0.02	0.02 ± 0.00
190	10	70%	9.70	88.16	94.22	1.62 ± 0.00	0.43 ± 0.00	0.20 ± 0.01	0.05 ± 0.00
197.5	7.5	70%	5.13	67.24	86.41	1.91 ± 0.73	0.39 ± 0.16	0.37 ± 0.14	0.07 ± 0.03
197.5	7.5	70%	0.00	69.36	90.40	2.16 ± 0.07	0.47 ± 0.01	0.43 ± 0.01	0.09 ± 0.00
205	5	70%	24.19	78.62	91.76	2.76 ± 0.23	0.65 ± 0.05	0.57 ± 0.00	0.13 ± 0.00
205	10	70%	13.11	51.20	80.30	3.18 ± 0.80	0.68 ± 0.15	0.96 ± 0.25	0.23 ± 0.07

Table 2-45: Water soaked steam explosion of AP6, WIS chemical composition and digestibility

Pretreatment condition			WIS chemical composition				Digestibility	
Temperature	Time	Moisture content	Lignin	Arabinose	Glucose	Xylose	Glucose	Xylose
°C	min	%	%	%	%	%	%	%
190	5	70%	24.27 ± 0.04	1.24 ± 0.02	46.87 ± 1.92	8.73 ± 0.78	43.26 ± 2.84	86.57 ± 7.06
190	10	70%	25.68 ± 0.26	0.42 ± 0.21	42.79 ± 4.06	7.98 ± 0.58	81.08 ± 0.11	88.04 ± 1.31
197.5	7.5	70%	34.20 ± 2.44	0.36 ± 0.02	71.77 ± 0.60	8.90 ± 0.08	77.59 ± 3.03	73.34 ± 0.97
197.5	7.5	70%	32.41 ± 0.52	0.32 ± 0.02	69.89 ± 2.42	8.79 ± 0.44	76.43 ± 1.90	72.09 ± 1.63
205	5	70%	30.03 ± 0.93	0.32 ± 0.01	70.38 ± 0.30	7.50 ± 0.01	89.98 ± 0.00	73.51 ± 5.73
205	10	70%	30.02 ± 0.56	0.39 ± 0.05	70.39 ± 0.15	5.06 ± 0.70	80.53 ± 3.56	72.80 ± 1.24

Table 2-46: Water soaked steam explosion of SS27, pretreatment and enzymatic hydrolysis yields

Pretreatment condition			Water insoluble solids	Pretreatment liquor yield			Enzymatic hydrolysis		Combined sugar yield	
Temperature	Time	Moisture content	Solid recovery	Arabinose	Glucose	Xylose	Xylose	Glucose	g/100g raw material	% Recovery
°C	min	%	(%)	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material		
190	5	70%	78.02 ± 1.44	0.78 ± 0.06	1.15 ± 0.13	5.61 ± 0.83	6.22 ± 0.47	17.47 ± 1.00	31.23	44.61
190	10	70%	68.20 ± 4.74	0.77 ± 0.03	1.56 ± 0.13	8.97 ± 0.60	4.69 ± 0.48	24.91 ± 3.54	40.90	58.43
197.5	7.5	70%	67.26 ± 0.27	0.80 ± 0.01	1.23 ± 0.14	6.34 ± 0.22	4.26 ± 0.13	36.32 ± 1.97	48.96	69.95
197.5	7.5	70%	64.64 ± 2.38	0.61 ± 0.12	1.64 ± 0.18	8.12 ± 1.37	3.98 ± 0.10	34.67 ± 0.74	49.03	70.04
205	5	70%	60.78 ± 0.11	0.77 ± 0.06	2.32 ± 0.15	13.28 ± 0.73	3.16 ± 0.01	36.42 ± 1.00	55.39	79.13
205	10	70%	62.62 ± 0.47	0.52 ± 0.04	2.35 ± 0.13	9.30 ± 1.11	2.30 ± 0.00	35.51 ± 1.05	49.98	71.40

Table 2-47: Water soaked steam explosion of SS27, pretreatment liquor, byproduct concentration and fraction of sugars in oligomeric form

Pretreatment condition			Oligomeric fraction in pretreatment liquor			Byproducts			
Temperature	Time	Moisture content	Arabinose	Xylose	Glucose	Acetic acid	Formic acid	Furfural	HMF
°C	min	%	% Oligomer	% Oligomer	% Oligomer	g/L	g/L	g/L	g/L
190	5	70%	0.00	90.54	95.40	1.00 ± 0.02	0.28 ± 0.00	0.08 ± 0.00	0.03 ± 0.00
190	10	70%	0.75	84.49	94.81	1.74 ± 0.01	0.41 ± 0.00	0.28 ± 0.01	0.07 ± 0.00
197.5	7.5	70%	0.00	52.48	82.67	2.48 ± 0.03	0.41 ± 0.01	0.50 ± 0.00	0.12 ± 0.00
197.5	7.5	70%	27.37	80.81	92.79	1.24 ± 0.07	0.22 ± 0.02	0.28 ± 0.00	0.06 ± 0.00
205	5	70%	22.05	71.12	88.95	3.26 ± 0.08	0.64 ± 0.02	0.76 ± 0.06	0.19 ± 0.01
205	10	70%	3.30	39.83	66.71	4.49 ± 0.16	0.78 ± 0.03	1.50 ± 0.03	0.41 ± 0.01

Table 2-48: Water soaked steam explosion of SS27, WIS chemical composition and digestibility

Pretreatment condition			WIS chemical composition				Digestibility	
Temperature	Time	Moisture content	Lignin	Arabinose	Glucose	Xylose	Glucose	Xylose
°C	min	%	%	%	%	%	%	%
190	5	70%	23.88 ± 0.40	1.12 ± 0.01	47.35 ± 2.61	7.66 ± 1.00	47.29 ± 2.71	104.03 ± 7.82
190	10	70%	27.03 ± 0.09	0.64 ± 0.01	48.25 ± 2.66	7.51 ± 0.25	75.68 ± 10.74	91.60 ± 9.43
197.5	7.5	70%	30.19 ± 0.04	0.37 ± 0.10	72.64 ± 0.36	8.64 ± 0.36	74.34 ± 4.02	73.37 ± 2.30
197.5	7.5	70%	29.05 ± 0.17	0.40 ± 0.02	71.18 ± 0.37	8.70 ± 0.03	75.36 ± 1.62	70.81 ± 1.86
205	5	70%	29.71 ± 0.79	0.32 ± 0.14	69.74 ± 0.78	6.95 ± 0.14	87.29 ± 0.81	74.96 ± 0.18
205	10	70%	33.14 ± 0.62	0.45 ± 0.00	66.58 ± 0.82	4.49 ± 0.02	85.16 ± 2.51	81.58 ± 0.07

Table 2-49: Water soaked steam explosion of AS246, pretreatment and enzymatic hydrolysis yields

Pretreatment condition			Water insoluble solids	Pretreatment liquor yield			Enzymatic hydrolysis		Combined sugar yield	
Temperature	Time	Moisture content	Solid Recovery	Arabinose	Glucose	Xylose	Xylose	Glucose	g/100g raw material	% Recovery
°C	min	%	(%)	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material		
190	5	70%	74.49 ± 1.60	0.93 ± 0.02	1.02 ± 0.05	6.62 ± 0.77	6.15 ± 0.10	17.79 ± 0.18	32.51	46.65
190	10	70%	68.43 ± 1.42	0.81 ± 0.04	0.94 ± 0.07	7.44 ± 0.81	4.53 ± 0.39	25.10 ± 2.06	38.82	55.70
197.5	7.5	70%	62.28 ± 4.00	0.89 ± 0.04	0.96 ± 0.02	6.26 ± 0.03	3.68 ± 0.02	33.94 ± 1.97	45.72	65.62
197.5	7.5	70%	65.85 ± 1.59	0.47 ± 0.04	0.93 ± 0.16	5.55 ± 0.52	4.25 ± 0.29	37.23 ± 0.69	48.42	69.49
205	5	70%	67.14 ± 1.79	0.88 ± 0.00	1.96 ± 0.08	14.23 ± 0.07	2.83 ± 0.00	39.28 ± 1.51	58.65	84.17
205	10	70%	62.90 ± 1.56	0.59 ± 0.03	1.96 ± 0.17	9.89 ± 0.30	2.14 ± 0.13	34.31 ± 1.29	48.90	70.17

Table 2-50: Water soaked steam explosion of AS246, pretreatment liquor byproduct concentration and fraction of sugars in oligomeric form

Pretreatment condition			Oligomeric fraction in pretreatment liquor			Byproducts			
Temperature	Time	Moisture content	Arabinose	Xylose	Glucose	Acetic Acid	Formic Acid	Furfural	HMF
°C	Min	%	% Oligomer	% Oligomer	% Oligomer	g/L	g/L	g/L	g/L
190	5	70%	3.59	90.85	95.88	1.32 ± 0.01	0.38 ± 0.00	0.12 ± 0.00	0.03 ± 0.00
190	10	70%	0.00	80.78	92.31	1.57 ± 0.01	0.38 ± 0.00	0.26 ± 0.00	0.06 ± 0.00
197.5	7.5	70%	0.00	48.41	80.80	2.64 ± 0.03	0.43 ± 0.00	0.61± 0.01	0.12 ± 0.00
197.5	7.5	70%	0.00	69.91	89.79	1.36 ± 0.13	0.23 ± 0.03	0.36 ± 0.00	0.08 ± 0.00
205	5	70%	20.23	72.14	89.05	3.55 ± 0.45	0.73 ± 0.11	0.71 ± 0.10	0.19 ± 0.03
205	10	70%	19.89	51.59	72.88	4.19 ± 0.78	0.72 ± 0.11	1.41 ± 0.29	0.34 ± 0.07

Table 2-51: Water soaked steam explosion of AS246, WIS chemical composition and digestibility

Pretreatment condition			WIS chemical composition				Digestibility	
Temperature	Time	Moisture content	Lignin	Arabinose	Glucose	Xylose	Glucose	Xylose
°C	min	%	%	%	%	%	%	%
190	5	70%	25.22 ± 0.97	1.11 ± 0.01	44.79 ± 2.50	7.44 ± 0.23	53.33 ± 0.55	111.01 ± 1.84
190	10	70%	27.18 ± 0.14	0.65 ± 0.01	48.92 ± 3.89	7.26 ± 0.01	74.98 ± 6.14	91.14 ± 7.84
197.5	7.5	70%	29.91 ± 2.06	0.41 ± 0.01	70.17 ± 0.39	8.41 ± 0.31	77.67 ± 4.50	70.18 ± 0.30
197.5	7.5	70%	27.01 ± 1.20	0.46 ± 0.06	69.80 ± 0.28	9.25 ± 1.02	81.24 ± 1.51	69.73 ± 4.68
205	5	70%	30.02 ± 0.33	0.37 ± 0.00	69.27 ± 0.38	6.23 ± 0.17	82.26 ± 0.00	67.74 ± 0.00
205	10	70%	34.07 ± 1.09	0.30 ± 0.05	69.65 ± 0.80	4.54 ± 0.06	78.32 ± 2.94	74.85 ± 4.48

Table 2-52: Estimated total ethanol yields, based on pretreatment response to water soaked steam explosion

Sweet sorghum cultivar	2009 harvest			2011 harvest			Average total ethanol [d] yield (L/Ha)
	Lignocellulosic ethanol [a] (L/ha)	Juice ethanol [b] (L/ha)	Total ethanol [c] (L/Ha)	Lignocellulosic ethanol [a] (L/ha)	Juice ethanol [b] (L/ha)	Total ethanol [c] (L/Ha)	
AP6	2281	4654	6935	4383	4719	9102	8018
SS27	1683	4719	6401	2614	4202	6815	6608
AS246	2464	5753	8217	1365	4331	5696	6956

[a] Lignocellulose ethanol yields calculated assuming 0.51g EtOH/g sugar consumed, where available sugar is that released from pretreatment response in terms of combined sugar yields. Further the agronomic fibre yield (ton/ha) taken into account to calculated ethanol in ton/ha.

[b] Sweet juice ethanol yield calculated assuming 0.51g EtOH/g sugar consumed, where available sugar is that present in the juice found in the stem.

[c] Total ethanol yield is the sum of the calculated lignocellulose and sweet juice ethanol.

[d] Average ethanol is the estimated average ethanol yield for the two harvest seasons

2.4.6.3. SO$_2$ catalysed steam explosion of three preferred sweet sorghum cultivars

The pretreatment conditions utilized for SO$_2$ catalysed steam explosion where less severe in terms of the pretreatment temperatures and times compared to both air dried and water soaked steam explosion. Results for SO$_2$ catalysed steam explosion are given in Table 2-53 to Table 2-58. The experimental points are described in Table 2-5. Unlike dry and water soaked steam explosion, the response of cultivars to SO$_2$ catalysed pretreatment differed from cultivar to cultivar. SS27 had the highest combined sugar yield with 64.03g/100g raw material followed by AS246 with a combined sugar yield of 63.74/100g raw material and AP6 with a combined sugar yield of 61.06/100g raw material. Both SS27 and AS246 achieved this combined sugar yield at 185°C and 8 minutes while AP6 achieved its highest combined sugar yields at 195°C and 2 minutes. In terms of glucose released during enzymatic hydrolysis, these were similar with SS27 having the highest glucose yield of 36.80g/100g raw material followed by AS246 with 35.05g/100g raw material and AP6 with 34.85g/100g raw material. In terms of xylose yields, the highest release of xylose during pretreatment was 18.11g/100g raw material for SS27, followed by AS246 with a maximum xylose release of 17.70g/100g raw material and AP6 with a maximum xylose of 16.69g/100g raw material. Further xylose yields resulting from SO$_2$ catalysed experiments showed a higher fraction of monomers compared to steam explosion of both air dried and water soaked material. Generally the fraction of xylose in oligomeric form was less than 45% with SO$_2$ catalysed steam explosion while for air dried steam explosion the fraction of xylose in oligomeric form was generally over 90% while for water soaked steam explosion the fraction xylose in the oligomeric form was generally between 70% and 90%.

Byproduct concentrations at the optimum conditions were highest for cultivar AS246 with 6.27 g/L of acetic acid, 0.37 g/L formic acid, 0.37 g/L furfural and 0.12 g/L HMF. Cultivar SS27 had the next highest byproduct concentration of 5.88 g/L acetic acid, 0.34 g/L of formic acid, 0.36 g/L of furfural and 0.13 g/L of HMF. AP6 had the lowest byproduct concentration of 3.56 g/L of acetic acid, 0.23 g/L of formic acid, 0.14 g/L of furfural and 0.07 g/L of HMF. The lower byproduct concentration for cultivar AP6 can be attributed to the lower pretreatment times used of 2 minutes compared to the pretreatment time of 8 minutes in the case of cultivar SS27 and AS246. Compared to air dried steam explosion and water soaked steam explosion the byproduct concentrations for SO$_2$ catalysed steam explosion were most similar to water soaked steam explosion as opposed to air dried steam explosion. Acetic acid

concentrations were generally higher for SO_2 catalysed steam explosion compared to water soaked steam explosion while furfural and HMF concentrations were generally lower for SO_2 catalysed steam explosion as compared to water soaked steam explosion.

Investigating the data, it can be seen that at both the least and most severe condition the yields of glucose during enzymatic hydrolysis was poorer than observed at the other conditions. This indicates that the range of condition chosen were sufficient in locating the optimum. Increasing the severity beyond 195°C and 8 minutes with SO_2 catalysed steam explosion would only result in a further loss/degradation of glucose as is evident in the trend of decreasing glucose yields, from 39.44 to 30.67g/100g raw material, which is observed when increasing the time from 2 to 8 minutes at 195°C. Similarly decreasing the pretreatment severity below 185°C and 2 minutes when performing SO_2 catalysed steam explosion will result in poor enzymatic hydrolysis yields as can already be seen in the decreasing trend when reducing the time from 8 minutes to 2 minutes at a pretreatment temperature of 185°C.

Table 2-53: 3% SO$_2$ catalysed steam explosion of AP6, pretreatment and enzymatic hydrolysis yields

Pretreatment condition			Water insoluble solids	Pretreatment liquor yield			Enzymatic hydrolysis		Combined sugar yield	
Temperature	Time	Moisture content	Solid recovery	Arabinose	Glucose	Xylose	Xylose	Glucose	g/100g raw material	%
°C	min	%	(%)	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material		
185	2	70%	57.75 ± 1.16	2.25 ± 0.08	2.07 ± 0.04	15.45 ± 0.84	3.27 ± 0.23	27.35 ± 1.01	50.40	71.97
185	8	70%	54.60 ± 1.58	2.35 ± 0.02	3.17 ± 0.05	15.91 ± 0.03	2.64 ± 0.04	29.06 ± 1.66	53.12	75.86
190	5	70%	59.75 ± 1.31	1.84 ± 0.11	2.66 ± 0.44	15.55 ± 1.78	3.65 ± 0.10	34.27 ± 2.53	57.98	82.80
190	5	70%	62.55 ± 1.39	1.69 ± 0.44	2.63 ± 0.21	15.95 ± 1.01	4.55 ± 0.11	33.15 ± 0.71	57.97	82.78
195	2	70%	66.97 ± 0.98	2.32 ± 0.15	2.89 ± 0.15	16.69 ± 0.97	4.31 ± 0.29	34.85 ± 1.89	61.07	87.20
195	8	70%	54.98 ± 0.01	1.71 ± 0.04	4.97 ± 0.22	13.62 ± 0.29	1.83 ± 0.30	30.67 ± 5.75	52.80	75.40

Table 2-54: 3% SO$_2$ catalysed steam explosion of AP6, byproduct concentration in pretreatment liquor and fraction sugars in oligomeric form

Pretreatment condition			Oligomeric fraction in pretreatment liquor			Byproducts			
Temperature	Time	Moisture content	Arabinose	Xylose	Glucose	Acetic acid	Formic acid	Furfural	HMF
°C	min	%	% Oligomer	% Oligomer	% Oligomer	g/L	g/L	g/L	g/L
185	2	70%	9.63	29.92	35.87	3.23 ± 0.02	0.14 ± 0.01	0.12 ± 0.01	0.03 ± 0.00
185	8	70%	11.09	33.84	27.49	3.06 ± 0.06	0.23 ± 0.01	0.18 ± 0.00	0.05 ± 0.00
190	5	70%	4.53	33.64	20.26	4.41 ± 0.02	0.25 ± 0.01	0.21 ± 0.00	0.07 ± 0.00
190	5	70%	9.36	48.67	40.53	3.68 ± 0.13	0.24 ± 0.00	0.16 ± 0.00	0.06 ± 0.00
195	2	70%	14.94	42.62	35.39	3.56 ± 0.17	0.23 ± 0.02	0.14 ± 0.02	0.07 ± 0.02
195	8	70%	1.87	21.05	20.18	5.52 ± 0.03	0.34 ± 0.01	0.42 ± 0.00	0.16 ± 0.00

Table 2-55: 3% SO₂ catalysed steam explosion of SS27, pretreatment and enzymatic hydrolysis yields

Pretreatment condition			Water insoluble solids	Pretreatment liquor yield			Enzymatic hydrolysis		Combined sugar yield	
Temperature	Time	Moisture content	Solid Recovery	Arabinose	Glucose	Xylose	Xylose	Glucose		
°C	min	%	(%)	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	%
185	2	70%	61.72 ± 1.17	2.00 ± 0.00	3.64 ± 0.15	18.00 ± 0.18	3.36 ± 0.24	30.76 ± 2.03	57.79	82.57
185	8	70%	62.88 ± 2.98	2.63 ± 0.07	6.21 ± 0.75	15.71 ± 1.05	2.68 ± 0.16	36.80 ± 0.07	64.03	91.48
190	5	70%	63.59 ± 2.29	1.76 ± 0.29	3.98 ± 0.05	18.11 ± 0.11	2.98 ± 0.49	34.32 ± 5.62	61.15	87.36
190	5	70%	62.05 ± 1.11	1.47 ± 0.21	3.84 ± 0.20	17.03 ± 0.22	3.85 ± 0.24	35.18 ± 0.87	61.37	87.67
195	2	70%	58.99 ± 1.50	1.86 ± 0.01	4.19 ± 0.59	14.95 ± 0.37	3.95 ± 0.69	33.02 ± 4.84	57.80	82.80
195	8	70%	51.48 ± 1.34	1.43 ± 0.07	7.17 ± 0.47	12.95 ± 0.40	1.97 ± 0.28	31.04 ± 0.93	54.56	77.95

Table 2-56: 3% SO₂ catalysed steam explosion of SS27, byproduct concentration in pretreatment liquor and fraction of sugars in oligomeric form

Pretreatment condition			Oligomeric fraction in pretreatment liquor			Byproducts			
Temperature	Time	Moisture content	Arabinose	Xylose	Glucose	Acetic acid	Formic acid	Furfural	HMF
°C	min	%	% Oligomer	% Oligomer	% Oligomer	g/L	g/L	g/L	g/L
185	2	70%	6.64	25.19	32.87	4.96 ± 0.44	0.15 ± 0.01	0.14 ± 0.00	0.05 ± 0.00
185	8	70%	2.10	23.77	20.91	5.88 ± 0.52	0.34 ± 0.02	0.34 ± 0.02	0.13 ± 0.01
190	5	70%	10.88	38.40	30.70	4.99 ± 0.02	0.29 ± 0.00	0.22 ± 0.01	0.10 ± 0.00
190	5	70%	0.00	38.72	36.98	4.55 ± 0.06	0.25 ± 0.00	0.19 ± 0.01	0.08 ± 0.00
195	2	70%	11.11	35.27	25.46	4.79 ± 0.21	0.26 ± 0.03	0.21 ± 0.00	0.09 ± 0.01
195	8	70%	19.82	24.78	18.40	5.63 ± 0.10	0.65 ± 0.04	0.40 ± 0.00	0.28 ± 0.01

Table 2-57: 3% SO₂ catalysed steam explosion of AS246, pretreatment and enzymatic hydrolysis yields

Pretreatment condition			Water insoluble solids	Pretreatment liquor yield			Enzymatic hydrolysis		Combined sugar yield	
Temperature	Time	Moisture content	Solid recovery	Arabinose	Glucose	Xylose	Xylose	Glucose	g/100g raw material	%
°C	min	%	(%)	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material	g/100g raw material		
185	2	70%	64.61 ± 4.21	2.01 ± 0.06	2.37 ± 0.10	14.46 ± 0.11	4.35 ± 0.45	26.77 ± 3.10	50.10	71.89
185	8	70%	61.59 ± 0.48	2.25 ± 0.13	6.02 ± 0.49	16.96 ± 0.69	2.73 ± 0.07	35.05 ± 1.21	63.00	91.48
190	5	70%	59.70 ± 0.35	2.02 ± 0.06	3.03 ± 0.20	16.22 ± 0.31	3.75 ± 0.47	31.95 ± 0.68	56.98	81.76
190	5	70%	60.96 ± 1.32	1.87 ± 0.08	3.28 ± 0.29	16.09 ± 1.14	3.41 ± 0.16	32.82 ± 4.21	56.77	82.46
195	2	70%	61.04 ± 2.93	1.85 ± 0.19	3.90 ± 0.19	15.47 ± 0.85	4.42 ± 1.16	30.30 ± 4.56	55.93	80.26
195	8	70%	44.45 ± 4.47	1.45 ± 0.07	7.18 ± 0.40	10.89 ± 0.74	1.50 ± 0.10	33.87 ± 1.69	54.88	78.76

Table 2-58: 3% SO₂ catalysed steam explosion of AS246, byproduct concentration of pretreatment liquor and fraction sugars in oligomeric form

Pretreatment vondition			Oligomeric fraction in pretreatment liquor			Byproducts			
Temperature	Time	Moisture content	Arabinose	Xylose	Glucose	Acetic acid	Formic acid	Furfural	HMF
°C	min	%	% Oligomer	% Oligomer	% Oligomer	g/L	g/L	g/L	g/L
185	2	70%	11.39	39.96	44.66	3.38 ± 0.27	0.11 ± 0.01	0.10 ± 0.00	0.03 ± 0.00
185	8	70%	1.20	18.50	16.87	6.27 ± 0.34	0.37 ± 0.02	0.36 ± 0.02	0.12 ± 0.00
190	5	70%	14.70	44.50	31.77	4.25 ± 0.16	0.26 ± 0.01	0.19 ± 0.00	0.08 ± 0.00
190	5	70%	4.19	39.54	29.52	4.22 ± 0.07	0.26 ± 0.01	0.20 ± 0.00	0.08 ± 0.00
195	2	70%	3.99	41.51	33.26	4.67 ± 0.10	0.29 ± 0.02	0.20 ± 0.00	0.09 ± 0.00
195	8	70%	19.36	24.92	15.27	5.24 ± 0.05	0.70 ± 0.01	0.37 ± 0.01	0.29 ± 0.01

Table 2-59: Estimated total ethanol yield, based on pretreatment response to SO2 catalysed steam explosion

Sweet sorghum cultivar	2009 harvest			2011 harvest			Average total ethanol yield [d] (L/Ha)
	Lignocellulosic ethanol [a] (L/ha)	Juice ethanol [b] (L/ha)	Total ethanol [c] (L/Ha)	Lignocellulosic ethanol [a] (L/ha)	Juice ethanol [b] (L/ha)	Total ethanol [c] (L/Ha)	
AP6	2732	4654	7386	5251	4719	9970	8678
SS27	2143	4719	6862	3329	4202	7531	7196
AS246	2689	5753	8442	1489	4331	5820	7131

[a] Lignocellulose ethanol yields calculated assuming 0.51g EtOH/g sugar consumed, where available sugar is that released from pretreatment response in terms of combined sugar yields. Further the agronomic fibre yield (ton/ha) taken into account to calculated ethanol in ton/ha.

[b] Sweet juice ethanol yield calculated assuming 0.51g EtOH/g sugar consumed, where available sugar is that present in the juice found in the stem.

[c] Total ethanol yield is the sum of the calculated lignocellulose and sweet juice ethanol.

[d] Average ethanol is the estimated average ethanol yield for the two harvest seasons

2.5. Discussion

2.5.1. Composition of raw sweet sorghum bagasse

Second generation bio-ethanol can be produced from the hemicelluloses and cellulose fraction of sweet sorghum bagasse. To maximize yield, the maximum amount of fermentable sugars should be recovered from this feedstock. High sugar content present in the lignocellulose matrix should increase the ethanol potential of a cultivar. The fermentable sugars contained within the chemical structure of sweet sorghum bagasse as complex carbohydrates include arabinose and xylose present in the hemicellulose fraction while glucose is present in the cellulose fraction [26 - 30, 46, 47]. The fermentable sugar content for the sweet Ssrghum bagasse cultivars evaluated in the 2008/2009 and 2010/2011 season varied from 57.36 to 69.35 % (Table 2-6, Table 2-9). This range of fermentable sugar content falls within the range reported elsewhere in literature for sweet sorghum Bagasse. Mehmood et al. reports combined fermentable sugar content from the hemicelluloses and cellulose portion of the sweet sorghum bagasse as 59.41% while Zhang et al. reports fermentable sugar content as 71.6% [26, 30]. Higher fermentable sugar content is desired and cultivars with higher sugar contents should be preferred due to higher potential ethanol yields per ton of biomass.

The chemical compositions of the sweet sorghum cultivars were typical for that of herbaceous type materials which have been reported to have chemical compositions comprised of between 24 and 40% cellulose, 12 and 38% hemicellulose and 6 and 29% lignin [48]. Furthermore the sweet sorghum cultivars had chemical compositions similar to those more specific for sweet sorghum. In other studies with sweet sorghum cellulose content varied between 34 – 41.3%, xylose content between 17.4 – 27.1% and lignin content between 15.2 – 25.4% [26, 29, 30, 46, 47, 49]. The lignin content of the sweet sorghum cultivars in this study varied between 14.29% and 21.13%, cellulose varied between 34.03% and 45.93%, and the hemicelluloses content (consisting of arabinose and xylose) varied between 21.42% and 27.47% (Table 2-6). These variations in carbohydrate content point to the fact that to obtain maximal yields, some cultivars should be selected over others based on their structural carbohydrate content. Therefore, in selecting cultivars it is equally as important that one understands why some cultivars were richer in structural carbohydrates than others. This would aid in increasing the structural carbohydrate content of preferred

sweet sorghum cultivars in subsequent breeding programs. A number of reasons have been suggested to explain variations in chemical composition of cultivars grown during the same season including plant maturity at harvest, crop cycle length and how many days after anthesis (flowering) cultivars are harvested [50]. It was found that cellulose and hemicellulose content decreased with time after anthesis while cellulose, hemicellulose and lignin increased with crop cycle length. Furthermore, late maturity sweet sorghum cultivars exhibited higher cellulose and hemicellulose yields compared to early maturity sweet sorghum cultivars [50, 51]. While all of these factors (plant maturity, crop cycle length, date of harvest following anthesis) could have been at play in the sweet sorghum cultivars evaluated in this study, the differences in chemical composition are most likely to be due to genetic makeup as the cultivars where harvested at a similar stage in their growth. Furthermore a number of breeding lines were grown in the 2008/2009 season including lines from India, Australia and others lines developed at the University of Kwa-Zulu Natal. Therefore detailed work entailing studies of plant maturity, crop cycle length and different harvest dates following anthesis would result in further increases in combined sugar content of the sweet sorghum bagasse cultivars evaluated in this study.

Agronomically the sweet sorghum cultivars grown in the 2008/2009 season performed poorly compared to that observed in Louisiana, USA, which negatively affected the stalk non-structural sugar yields and fibre yields [52]. Dry matter yields for the sweet sorghum bagasse cultivars evaluated in this study were typically between 5.0 – 14.9 Tons/ha and stalk non-structural sugar yields were typically between 3.6 – 6.3 tons/ha, both of which were lower than reported elsewhere where dry matter yields of up to 22.3 tons/ha and stalk non-structural sugar yields of up to 10.1 tons/ha were possible [52]. This shows that there is significant room for increasing both the fresh stem yields and stalk non-structural sugar yields. While yields of sweet sorghum cultivars bred under South African conditions were low, this is most probably due to cultivars being bred for the sweet juice rather than the high biomass yields. Further stalk non-structural sugar values have been found to vary between sweet sorghum cultivars at maturity [53], while plant height and the corresponding dry matter weight of cultivars have been found to increase with number of days to anthesis. Therefore further studies on sweet sorghum cultivated under South African conditions should focus on increasing the biomass yield in terms of dry matter yield (tons/ha) by looking at crop cycles and number of days until harvest and anthesis. It is thought that this

will increase the biomass yield of sweet sorghum cultivars and in turn increase the potential ethanol possible per hectare.

2.5.2. Screening of Sweet Sorghum Cultivars

High recovery of fermentable sugars from the lignocellulose matrix in sweet sorghum bagasse is necessary to realize high ethanol yields. For this reason, sweet sorghum cultivars from which high yields of fermentable sugars are possible are preferred for bio-ethanol production. The pretreatment conditions chosen for screening the initial sweet sorghum cultivars resulted in an average of 73.0 and 50.5% of xylose and glucose present in the raw material being recovered respectively. Note that the yields above are medians of the xylose and glucose recoveries of the data set in Table 2-11. According to Lloyd and Wyman, a higher recovery of xylose compared to glucose in dilute acid pretreatment suggests that pretreatment severity is low due to the fact that during pretreatment xylose yields are maximized first followed by combined sugars and finally glucose yields [11]. Sweet sorghum Cultivars that had higher combined sugar yields compared to other cultivars are therefore preferred as they respond well to low pretreatment severity.

In evaluating the effect of dilute acid pretreatment on cultivar it was noted that cultivar affected the yields of sugars from pretreatment and enzymatic hydrolysis as well as the combined sugar yields. This is substantiated by the variation in pretreatment response in which combined sugar yield varied between 32.63 and 44.04g/100g raw material. While it was difficult to predict the response from the chemical composition of a cultivar, it is postulated that the lignin and ash content of specific sweet sorghum cultivars were the main components responsible for the observed differences in pretreatment response [38, 54, , 55]. This is substantiated by the observation in section 2.4.1 in which the above two chemical components, lignin and ash, were significantly different ($p < 0.05$) for 65.7% and 58.0% of the sweet sorghum cultivars respectively and were the components that showed the highest number of significant differences between cultivars. Furthermore it has previously been stated that both ash and lignin content affect pretreatment response. Ash acts as a neutrilising agent and reduces the effective acid concentration lowering the severity of the pretreatment while increased lignin content as well as an Syringyl/guaiacyl ratio negatively affect pretreatment response [38, 54, 56, 55]. While no direct correlation could be found to describe the effect of chemical composition on pretreatment response for

the chosen pretreatment conditions during screening some statistically significant effects were found between the chemical composition and pretreatment response.

The chemical components as shown in section 2.4.2.2 which significantly affected the release of xylose during pretreatment were the ash, water extractives, ethanol extractives, glucan and xylan content. Ash content negatively affected pretreatment which is due to its neutrilising capacity as described and observed in other studies [38, 51, 57]. Increased neutrilising capacity with increased ash content therefore results in a decrease in the release of xylose during pretreatment which has been reported elsewhere [38, 54, 57] . Minimizing ash content within sweet sorghum cultivars would therefore be beneficial to increase xylose yields during pretreatment and therefore combined sugar yield.

While water extractives have been known to form insoluble lignin-like compounds during hydrolysis which would be likely to further inhibit enzymatic hydrolysis of cellulose even at more severe pretreatment conditions [58], it is unsure of the mechanisms that would have a significant negative effect on the release of xylose during pretreatment. As water extractives have been known to contain components such as alditols, aliphatic acids, inorganic ions, oligomeric sugars and a number of different length oligomers cautiously identified as being derived from phenolic glycosides [59], it is postulated that some of these components interact with the dilute acid in such a manner so that the acid catalyst is inactivated. Further work in future studies should be done in determining the chemical components of the water extractives, so as to determine the mechanism involved with this significant effect performed for a more conclusive understanding.

The effect of the ethanol extractives on the xylose released during pretreatment was shown to be positively significant. While ethanol extractives usually contain hydrophilic and waxy components it was not clear what exactly these were comprised of and without much literature on the interaction of the ethanol extractives with the acid catalyst during dilute acid pretreatment it is hard to say what the mechanism effecting xylose release positively was. Future work will be necessary to accurately describe the components of ethanol extractives which have an interaction on pretreatment.

Lignin content in the raw material was found to be inversely correlated with both glucose yield and combined sugar yield and this effect was significant as shown in Table 2-12. Similarly Chang and Holtzapple [60] observed an inverse correlation between lignin content

and enzymatic digestibility and Lindedam et al [57] found a negative correlation between lignin content and combined sugar release. Furthermore Chen and Dixon [61] found a strong negative correlation between lignin and the sugar released during enzymatic hydrolysis. The effect of lignin content on the yields of glucose release during enzymatic hydrolysis can be attributed to the fact that hydrolytic enzymes have been known to adsorb onto lignin [8]. Furthermore cellulose is embedded in the complex lignocellulose matrix containing lignin which obstructs the access of hydrolytic enzymes to the cellulose [8]. It is therefore beneficial to develop and select cultivars with low lignin content but care must be taken when doing so, so as not to introduce negative characteristics into the prospective cultivars such as lodging.

As cultivars that have lower ash content would result in potentially less acid being used to achieve the same pretreatment yields, one would imagine that a potential cost benefit of requiring less acid depending on cultivar could arise. Furthermore as reduced lignin content potentially results in higher glucose and combined sugar yield, one could expect that a lower enzyme loading would be necessary if the appropriate cultivar were selected.

Arabinan content was found to have a significant positive effect on the combined sugar yield obtained from pretreatment and enzymatic hydrolysis. Previously it has been reported that arabinose was the hemicellulosic sugar released most rapidly, which has been attributed to the arabinosyl linkages which are highly susceptible to dilute acid pretreatment and the heat labile glucosidic linkages to arabinosyl substitutions that are easily hydrolysed during pretreatment [62, 63]. It is postulated therefore that due to the high susceptibility of arabinosyl linkages to pretreatment by dilute acid and high temperatures, a cultivar with a higher percentage of arabinan will be more easily hydrolysed due to the fact that there will be more linkages between arabinan, the xylan backbone and lignin. It is thought that disruption of these linkages will result in a higher fragmentation and solubilisation of the cell wall components resulting in improved enzymatic hydrolysis and higher combined sugar yields from pretreatment and enzymatic hydrolysis. Cultivars with high arabinan content should therefore be favored.

While high sugar recovery in terms of combined sugar yield is important, cultivars that produce high ethanol yields on a per hectare basis will be more valuable. Combined ethanol yields of the sweet sorghum cultivars evaluated in this study ranged from 2772 to 9496 L/ha

showing that selection of high ethanol yielding cultivars is important. Furthermore it was shown that by producing ethanol from the lignocellulose fraction of sweet sorghum the possible ethanol production could be doubled. Comparing ethanol yields to that reported elsewhere, the highest ethanol yield observed in this study was 4712L/ha from the sweet juice and 3192L/ha from the lignocellulose of AP6 which was substantially lower to that reported to that by Tew et al. in which 7680L/ha of ethanol was possible from the sweet juice and combined ethanol yield of 15 121L/ha was possible from the sweet juice and lignocellulose [52, 64]. The low ethanol yields obtained with the sweet sorghum cultivars evaluated in this study can be attributed to the lower dry matter yields observed. The plant breeding program from which the sweet sorghum collected for this study evaluated the applicability of different sweet sorghum cultivars under South African conditions; shifting the focus of plant breeding strategies from non-structural sugar production to high biomass production will result in an increase in total ethanol yields.

Comparing total ethanol yields (Table 2-13) with combined sugar yields (Table 2-11); it is evident that while high sugar recoveries are important for maximizing the ethanol potential of a particular cultivar, the most important factor for high total ethanol yields is fresh stem yield. Increasing the fresh stem yields by cultivating high biomass sorghums will translate into increased sweet juice ethanol and lignocellulose ethanol [65]. Sweet sorghum cultivars should therefore be bred to increase biomass yields. Furthermore this is substantiated by the correlation between lignocellulose ethanol and energy content (Figure 2-10) indicating that potential lignocellulose ethanol (L/ha) from sweet sorghum increases with energy content, i.e biomass (tons/ha).

2.5.3 Response of cultivar to pretreatment and enzymatic hydrolysis.

Selection of pretreatment conditions affected the combined sugar yield from pretreatment-hydrolysis of various cultivars. This was evident by the fact that in the case of using two different set of pretreatment conditions, namely 190°C, 5 minutes and 0.25% (w/w) H_2SO_4 and 200°C, 5 minutes and 0.07% (w/w) H_2SO_4, certain cultivars showed improved performance over other cultivars for 190°C, 5 minutes and 0.25% (w/w) H_2SO_4 while at 200°C, 5 minutes and 0.07% (w/w) H_2SO_4 the opposite was the case. Similarly performance of enzymes was affected by both pretreatment conditions and cultivar. This was evident by

the fact that the sugar yield from enzymatic hydrolysis increased by 223% for cultivar AS245 while only increasing by 161% for cultivar MSJH13 when the enzyme loading was increased for 3.75 FPU/g WIS to 15 FPU/g WIS with pretreatment at 190°C, 5 minutes and 0.25%. Similarly the glucose released from enzymatic hydrolysis increased by 235% for AS254 while only increasing by 158% for SS27 when increasing the enzyme loading from 3.75 FPU/g WIS to 15 FPU/g WIS with pretreatment at 200°C, 5minutes and 0.07% (w/w) H_2SO_4. Additionally it could be seen that certain cultivars such as SS27 performed consistently well at different conditions of pretreatment and enzyme loading while other cultivars such as AS246 only performed well at certain conditions. Selection of cultivars that perform well at different pretreatment conditions and enzyme loadings should therefore be preferred as they could potentially outperform other cultivars at a number of conditions.

Looking statistically at the data for the pretreatment of the ten selected cultivars at two difference pretreatment conditions and two enzyme loadings one can make some conclusions on why certain cultivars performed well compared to others. Lignin was found to be inversely correlated ($p < 0.05$) with the release of glucose during enzymatic hydrolysis at 3.75FPU for both pretreatment conditions while only inversely correlated ($p < 0.1$) with the release of glucose at an enzyme loading of 15FPU for a pretreatment condition of 200°C, 5 minutes and 0.07% (w/w) H_2SO_4. This inverse correlation between lignin and the release of glucose can be attributed to unproductive binding of enzymes on lignin [8]. This unproductive binding of enzymes can be observed nicely in the data as for the low enzyme loading of 3.75FPUg^{-1}DM the correlation between lignin and an enzyme loading of 3.75 FPU/g WIS was highly correlated at $p < 0.05$ for both pretreatment conditions while at an enzyme loading of 15 FPU/g WIS the correlation was only significant at $p < 0.1$ for pretreatment at 200°C, 5 minutes and 0.07% (w/w) H_2SO_4. The lignin therefore had the greatest effect on the enzymatic hydrolysis when the enzyme loadings was low and when pretreatment was less severe as in the case of the significant correlation for pretreatment at 200°C, 5 minutes and 0.07% (w/w) H_2SO_4. These results suggest two things, firstly low lignin content is desired in sweet sorghum cultivars to achieve high sugar yields and secondly one can reduce the difference in combined sugar yields between cultivars with different lignin contents by either increasing the enzyme loading or increasing the severity of the pretreatment although increasing either the enzyme loading or severity of pretreatment will increase operational costs in a second generation bio-ethanol plant. These results agree with

studies elsewhere in which lignin content was found to increase the swelling and accessibility of enzymes to cellulose at a low enzyme dosage (5FPU/g cellulose) while upon using higher enzyme dosages lignin had less of an effect on the yields resulting from enzymatic hydrolysis in which the enzymes were found to overcome the limitations lignin imposed [66]. Likewise rice straw was found to yield twice as much glucose at an enzyme loading of 6.5 FPU/g WIS compared to sugarcane bagasse and silver grass which was attributed to rice straws low lignin content [67].

As a reduced enzyme loading has a significant effect on the cost of ethanol production in which it has been shown that the cost of enzymes is the most expensive cost following capital and feedstock cost [68], it is important to select sweet sorghum cultivars with low lignin content as they both require less enzymes and a lower pretreatment severity. An example of this can be observed with sweet sorghum cultivar SS27 which had the lowest lignin content of 14.29g/100g raw material which resulted in it outperformed the other cultivars in combined sugar yields at all of the pretreatment conditions and enzymatic loadings except for 200^oC, 5 minutes and an enzyme loading of $15FPUg^{-1}DM^{-1}$ where it was the third best cultivar amongst the ten preferred cultivars.

While ash content has normally been described to have a significantly negative correlation with pretreatment response variable it was found to be positively correlated with the glucose released from enzymatic hydrolysis and the combined sugar yield under an enzyme loading of $3.75FPUg^{-1}DM^{-1}$ and pretreatment of 200^oC, 5 minutes and 0.07% (w/w) H_2SO_4. Possibly this is due to the low acid concentration of this pretreatment meaning that the acid catalyst had a limited role in the pretreatment step and the pretreatment was mainly due to the high temperatures involved. This is further indicated by the fact that 190^oC, 5 minutes and 0.25% (w/w) H_2SO_4 did not show this significant correlation and one must attribute the effects of ash observed for 200°, 5 minutes and 0.07% (w/w) H_2SO_4 as temperature related effects and that the enzymatic hydrolysis is improved by the ash content.

Arabinan was found to be negatively correlated ($p < 0.05$) for xylose pretreatment yields and combined sugar yields under an enzyme loading of $15FPUg^{-1}DM^{-1}$ with pretreatment at 200^oC, 5 minutes and 0.07% (w/w) H_2SO_4 while being positively correlated ($p < 0.1$) for the release of glucose during enzymatic hydrolysis and combined sugar yields under an enzyme loading of $15FPUg^{-1}DM^{-1}$ with pretreatment at 190^oC, 5 minutes and 0.25% (w/w) H_2SO_4. As

arabinan was found previously to be positively correlated with combined sugar yields (section 2.5.2), it was no surprise that this was the case for 190°C, 5 minutes and 0.25% (w/w) H_2SO_4. For 200°C, 5 minutes and 0.07% (w/w) H_2SO_4 this was not this case and it is postulated that the arabinosyl linkages were incompletely hydrolysed during pretreatment which is thought to have had a negative effect on the xylose yields during pretreatment and the subsequent release of glucose during enzymatic hydrolysis. This is the most plausible reason as the arabinosyl linkages have previously been shown to be susceptible to hydrolysis with dilute acid hydrolysis [62, 63]. While the glucosidic linkages to arabinosyl substitutions are heat labile and it must be assumed that these are hydrolysis by the high temperatures for both 190°C, 5 minutes and 0.25% (w/w) H_2SO_4 and 200°C, 5 minutes and 0.07% (w/w) H_2SO_4, it can be seen that even at a similar severity the arabinosyl linkages are not completely hydrolysed with the low acid concentration present in 200°C, 5 minutes and 0.07% (w/w) H_2SO_4.

2.5.4. Optimization of five preferred sweet sorghum bagasse cultivars with dilute acid pretreatment

Five preferred cultivars were optimized at a small scale with dilute sulfuric acid pretreatment. The cultivars selected included AP6, SS27, AS103, MSJH13 and AS246. Combined sugar yield for these five preferred sweet sorghum cultivars ranged between 48.83 and 54.5g/100g raw material corresponding to a combined sugar recovery between 75.81% and 84.98% of the sugars present in the raw material. This was similar to results of a previous study in which a recovery of 85.8% combined sugar yield was achieved for dilute sulfuric acid pretreatment of s*orghum bicolor* (L.Moench) at pretreatment conditions of 121°C, 1% (w/w) H_2SO_4, 60 minutes and 20% solids loading [26]. Similarly Vancov et al. recovered 81.1% of combined sugars at a pretreatment condition of 121°C, 1% (w/w) H_2SO_4, 90 minutes and 10% solids loading [69]. While the yield in the study by Mehmood et al. of 85.8% was slightly higher it was achieved with a lower solids loading of 20% compared to the solid loading of 30% used in the present study. Upon increasing the solids loading at the optimal condition in the study by Mehmood et al. it was found that the yield dropped substantially [26]. In another study optimizing dilute acid pretreatment of sweet sorghum bagasse with a number of different acids a maximum combined sugar yield of around 57%

was possible for both sulfuric and hydrochloric acid [28]. The sugar yield of 57% observed by Heredia-Oleo et. al [28] was low and possibly could be attributed to choice of pretreatment conditions evaluated in the optimization. The recoveries therefore realized in this study are comparable with the optimal recoveries of combined sugars for sweet sorghum bagasse.

Xylose recovery for the preferred cultivars varied between 77.87% and 88.69% of the initial xylose present in the raw material under the optimal pretreatment condition of each cultivar. Except in the case of cultivar SS27 the xylose recovery was the highest at the optimum pretreatment condition. Glucose recovery for the preferred cultivars varied between 73.06% and 82.70% of the initial glucose present in the raw material. This compared with a recent study on the dilute sulfuric acid pretreatment of photoperiod sensitive Sorghum in which a maximum recovery of glucose was 80.3% at pretreatment conditions of 160^oC, 40 minutes and 1% (w/w) H_2SO_4 [70]. Much improved recovery of glucose above 80% under dilute sulfuric acid pretreatment is thought to be difficult when using an enzyme loading of 15FPU/g WIS as increases in pretreatment severity will result in degradation of both hemicellulosic and cellulosic sugars present in the pre-hydrolysate and water insoluble solid fraction [70]. Improvements in glucose recovery may be improved by increasing the loading of enzymes but this will have an associated negative production cost on the production of ethanol. In studies involving other agricultural residues recovery of glucose from the raw material was 65% for rape seed straw [54]

While recoveries of combined sugars were similar to optimal recoveries in other studies, not all of the fitted models were highly accurate. Therefore only the models showing good fit for the experimental data should be used to locate optimum regions for pretreatment. Interestingly the fitted models showed different significant main effects and interactions between the factors temperature and time for the five preferred sweet sorghum cultivars. This showed that raw material composition had a substantial effect on the pretreatment of the preferred pretreatment conditions. Pretreatment areas of interest in which yield could possibly be slightly improved are therefore around 190^oC and 5 minutes for cultivar AS103, around 180^oC and 10 minutes for cultivar AP6, between 185^oC - 190^oC and 5 minutes for cultivar SS27 and around 185^oC and 10 minutes for both cultivar AS246 and cultivar MSJH13. All of the above areas of interest are at a sulfuric acid concentration of 0.25% (w/w) H_2SO_4. While the maximum combined sugar yield of 54.4g/100g raw material was obtained at 190^oC, 5 minutes and 0.25% H_2SO_4 for sweet sorghum cultivar AS103. It was found that a

single pretreatment condition, namely 185°C, 10 minutes and 0.25% H_2SO_4 could be used to achieve combined sugar yields of between 88% - 92% of the maximum combined sugar yield recorded for all five of the preferred sweet sorghum cultivars. Therefore utilizing this single pretreatment condition, sweet sorghum cultivars could potentially be combined before pretreatment in a single pretreatment strategy rather than separate conditions for each cultivar.

2.5.5 Optimization of three preferred cultivars with pilot plant steam explosion

Three cultivars were pretreated with steam explosion. The chosen cultivars were AP6, SS27 and AS246. Each of these three cultivars was pretreated with dry, water soaked and SO_2 catalysed steam explosion. Comparing the dry matter contents of the different Steam explosion strategies showed that the air dried steam explosion slurry following pretreatment had dry matter content of between 25 and 40% while for steam explosion of water soaked and SO_2 catalysed steam explosion the dry matter content was 15 and 20%. Leibbrandt suggested that for dilute acid pretreatment, a minimum solids concentration following pretreatment of 35% would be required for the process to be energy efficient [71]. While this holds for dilute acid pretreatment, no reference was given for steam explosion but assuming that this is the case one would expect therefore that a dewatering step would be necessary to increase the solids content above 35% and closer to 50%.

2.5.5.1. Air Dried Steam explosion

With air dried steam explosion of the three preferred sweet sorghum bagasse cultivars it was possible in this study to recover between 61.50% and 63.90% of the initial sugars present in the raw material at the optimum pretreatment condition of 205°C and 5 minutes. This consisted mainly of glucose with a glucose yield of around 74.5% to 79.03% of the initial glucose present in the raw material being recovered. While no other study could be found to compare data for sweet sorghum bagasse at the optimal pretreatment condition for dry steam explosion it was found in other studies that 71% of the glucose could be recovered at a pretreatment temperature of 190°C and a time of 10 minutes or that 91% of the initial glucose could be recovered at a pretreatment temperature of 210°C and a time of 6 minutes [24, 25]. As in the case of Ballesteros et al [24], the high glucose recovery was found to be at the expense of xylose recovery which deemed this pretreatment condition too severe to

obtain a maximum combined sugar yield. Statistically no correlation could be found between the chemical composition of the three preferred sweet sorghum cultivars and the pretreatment response as the sample size was too small for this analysis. Major differences were observed between cultivars in small scale dilute acid pretreatment (11% difference in combined sugar recovery between best and worst performing cultivar) while with air dried steam explosion using the same cultivars the differences were negligible (Maximum of 2.4% difference in combined sugar recovery). Pretreatment response differences are therefore thought to be minimized with air dried steam explosion due to the fact that no acid catalyst was used which would favor physical pretreatment mechanisms rather than chemical pretreatment mechanisms. As steam explosion was carried out on a pilot plant scale, without an acid catalyst, these physical mechanisms are thought to overcome the chemical characteristics of the 3 preferred cultivars that were seen to affect the cultivars at a small scale. Therefore the use air dried steam explosion over other steam explosion methods could minimise the need for sweet sorghum cultivar selection as cultivars performed similarly.

Unfortunately for steam explosion of air dried material in this study the xylose recovery was poor for the optimal pretreatment condition which resulted in the highest combined sugar yield. At this condition a yield of 36.93% to 41.10% of the initial xylose present in the raw material could be recovered for the three preferred sweet sorghum cultivars. As not much work has looked at steam explosion of air dried sweet sorghum, no sweet sorghum data was found to compare with the xylose recovery observed in this work. In another study carried out by Kaar involving sugarcane bagasse it was found that 55% of the initial xylose in the raw material could be recovered at a pretreatment temperature of 216°C and time of 5 minutes while with a study on *brassica carinata* a xylose recovery of 50% could be achieved at 210°C and 8 minutes [72]. Further most studies involving steam explosion of agricultural residues that had been air dried prior to steam explosion only reported glucose recoveries and did not specify the recovery of xylose [24, 25, 30] in their findings presumably due to poor data and or low recoveries of xylose. It was found in this study that the condition required to maximize combined sugar yields in steam explosion of air dried sweet sorghum bagasse favored degradation of hemicelluloses into their degradation products at a faster kinetic rate than the hydrolysis of xylan into xylose. This can be attributed to the higher temperatures necessary, around 205°C, for maximizing sugar yield from enzymatic hydrolysis with air dried

material. Other studies have shown a similar effect with dilute acid pretreatment at temperatures around 200°C in which the kinetic rate for degradation of xylose into its degradation product is only slightly slower than the kinetic rate for hydrolysis of xylan into xylose [11, 67]. Furthermore it was seen that as time increases so the kinetic rate for degradation overtakes that for xylan to xylose and a reduction in xylose yields is seen [11, 67]. This could be observed in this study in which 80% of the xylan had been hydrolysed at 205°C and 5 minutes while only 20% of xylan could be recovered in the pretreatment liquor as xylose. At this optimal temperature for pretreatment an increase in pretreatment time from 5 to 10 minutes at 205°C with air dried steam explosion, corresponded to an increased byproduct yield revealing that at this temperature the kinetic rate for degradation become faster than that for xylan in xylose. This makes air dried steam explosion unsuitable for maximizing the conversion of available sugars into ethanol and suggests that air dried steam explosion should be optimized rather for conversion of hemicellulose into byproducts such as furfural with conversion of cellulose into ethanol.

Byproduct concentrations in the pretreatment liquor for air dried steam explosion at the pretreatment conditions which resulted in the maximum combined sugar yield were between 0.58 g/L to 1.49 g/L of Acetic acid, 0.03 g/L to 0.05 g/L of formic acid, 1.4g/L to 2.12g/L of furfural and 0.46g/L to 0.95g/L of HMF. Previously it has been shown that a hydrolysate mixture consisting of a furfural concentration of 2.6g/L and a HMF concentration of 4.2g/L was high enough to limit ethanol production of a thermo tolerant yeast by 50% [73]. This points to two things, firstly as much of the pretreatment liquor/hydrolysate as possible needs to either be removed prior to a C6 fermentation either through a washing or pressing step for efficient fermentation or organisms need to be genetically engineered that can ferment both the C5 and C6 sugars while resisting the byproduct inhibition.

2.5.5.2. Water soaked steam explosion

Water soaked steam explosion of the three preferred sweet sorghum bagasse cultivars at 205°C and 5 minutes was sufficient to recover between 78.73% and 84.17% of the sugars present in the native raw sweet sorghum bagasse. Further the yield of xylose was between 67.56% and 70.63% of the xylose initially present in the raw material while the yield of glucose was between 85.28% and 92.09% of that initially present in the raw material at a pretreatment condition of 205°C and 5 minutes. These yields of glucose were substantially

improved over results in another study in which the highest glucose yield for water soaked steam explosion of sweet sorghum bagasse was 70% at a pretreatment temperature of 160°C and 5 minutes [30]. The reason for this is that in the only other study to date on water soaked steam explosion of sweet sorghum bagasse a single non optimal pretreatment condition was utilized to compare a number of pretreatment technologies. Further than this studies on sorghum bagasse have either employed SO_2 as a catalyst with and without overnight soaking in water or as air dried material without a catalyst [24, 25, 27, 74] and so no comparative data could be used to directly evaluate water soaked steam explosion results observed in this study. Looking therefore to other agricultural residues for comparison steam explosion of water soaked material has shown glucose yields of up to 65%, 69% and 50% for Sugar cane bagasse [72, 75, 76], Wheat straw [77] and Switch grass [78] respectively. These lower yields can be attributed to the fact that these previous studies focused on SO_2 catalysed steam explosion and so the pretreatment conditions chosen were not optimized for water soaked steam explosion as was the case in this study. Therefore optimizing water soaked steam explosion of sweet sorghum resulted in glucose yields that were comparable to that which was possible with SO_2 catalysed steam explosion as shown by Sipos et al. [27] who achieved 85 – 95% conversion of initial glucose present in the raw material at pretreatment conditions of either 190°C and 10 minutes or 210°C and 5 minutes with a moisture content of 50% and a SO_2 concentration of 2%. Differences in combined sugar recoveries (around 6.5% at optimum conditions) between the three preferred cultivars revealed that the chemical pretreatment effect was more prominent with water soaked steam explosion over air dried steam explosion. Previously water has been described to act as an acid catalyst in steam explosion and this was evident in this study [13]. While increased differences in pretreatment response were observed, optimum conditions for each of the cultivars were the same. This points to the fact that water alone was not sufficiently able to overcome the physical mechanisms of pilot plant steam explosion and enhance the pretreatment response due to individual characteristics of the three preferred cultivars.

Xylose released during pretreatment in water soaked steam explosion was found to exist predominantly in oligomeric form as was to be expected from previous studies. At 205°C and 5 minutes xylose released for the three cultivars contained between 71.12% and 78.62% oligomers. Overall at 205°C and 5 minutes the yield of xylose which included both monomeric and oligomeric sugars consisted of between 67.56% and 70.63% of the original

xylose present in the raw material. Sassner et. al also found that for uncatalysed steam explosion of s*alix* optimal yields of xylose were around 80% and that the majority of the xylose was in oligomeric form [79]. This was attributed to the longer residence times necessary to achieve a good recovery of glucose which in turn resulted in substantial degradation of hydrolysed xylose. Compared to other agricultural residues undergoing water soaked steam explosion pretreatment the xylose yield release for air dried steam explosion of sweet sorghum bagasse in this study were higher than the 55% xylose yield recorded for sugarcane bagasse [72] but lower than the 80% xylose yield recorded for wheat straw [77].

Byproduct concentrations observed for water soaked steam explosion were substantially lower than observed for air dried steam explosion. At the same pretreatment condition, namely 205°C and 5 minutes, the byproducts concentration for water soaked material was between 60% and 65% lower for furfural and 70% to 80% lower for HMF than was found for air dried steam explosion. This will have a profound impact on the ethanol yields in subsequent fermentation steps of hydrolysates that contain the inhibitors as it has been shown that increasing the inhibitor concentration has a negative effect on the ethanol production [73].

2.5.5.3. SO$_2$ catalysed steam explosion

SO$_2$ catalysed steam explosion of sweet sorghum bagasse resulted in a combined sugar yield of 61.06 to 64.03 g/100g raw material for the three preferred cultivars AP6, SS27 and AS246 which was much improved over both water soaked and air dried steam explosion in this study. The high yields observed in this study which recovered 87.2% to 91.48% compare well with results observed by Sipos et al. who achieved 85 – 90% conversion with the two settings 190°C and 10 minutes or 205°C and 5 minutes [27].

Xylose yields observed in SO$_2$ catalysed steam explosion were substantially improved over air dried and water soaked steam explosion with around 90% of xylose possible with SO$_2$ catalysed steam explosion compared to a xylose recovery of 70% for water soaked steam explosion and 60% for air dried steam explosion. Other authors have shown that the presence of SO$_2$ in the steam explosion has in fact protected liberated pentoses from degradation to inhibitory byproducts compared to steam explosion with water soaked steam explosion which is possibly what has been observed in this study involving pretreatment of sweet sorghum bagasse that has been air dried, water soaked or SO$_2$ impregnated [25].

Further Morjanoff and Gray showed that with SO_2 steam explosion of sugarcane bagasse a 98% recovery of xylose was possible, once again highlighting that hemicelluloses degradation is limited with the use of SO_2 as a catalyst [80]. Furthermore, Corredor et al. realized a pentose recovery of 94% with forage sorghum using a "modified" steam explosion with 2% Sulfuric acid at 140^oC and 30 minutes [81]. For optimal xylose recoveries it is therefore important to utilize SO_2 as a catalyst in steam explosion. Further the percentage of xylose in monomeric form over oligomeric form was much greater for SO_2 catalysed steam explosion than for either air dried steam explosion or water soaked steam explosion. Oligomers comprised up to 45% of xylose recovered with SO_2 catalysed steam explosion compared to 70 – 90% with water soaked steam explosion and 90% for air dried steam explosion. Similarly this effect on the fraction of xylose in oligomeric form was observed by Sassner et al. [79]. It is hypothesized that the kinetics of xylose oligomer degradation to xylose monomers was sped up with a catalyst such as SO_2 over the degradation of xylose monomers to inhibitory products but much more detailed work would need to be done to prove this. Lloyd et al. have previously shown though that the fraction of xylan oligomers has decreased with increasing catalyst concentration [11].

Glucose yields observed in SO_2 catalysed steam explosion were also improved over steam explosion of air dried material and water soaked material of sweet sorghum bagasse in this study. Recovery of glucose was between 83% - 95% with SO_2 catalysed steam explosion which compared to water soaked steam explosion results in this study. In a recent work by Shen et al. , a glucan to glucose conversion of 88% was recorded at their optimum condition of 200^oC, .5 minutes and 2.5% SO_2 while a conversion of 100% was recorded at a more severe pretreatment condition, namely 210^oC, 10 minutes and 5% SO_2 showing that near theoretical recoveries recorded here are in line with results recorded elsewhere [25]. Similarly to previous observations regarding xylose yields, it has been found that the pretreatment mechanisms involved with SO_2 catalysed steam explosion prevent degradation of hexose type sugars such as glucose [25]. This would explain the higher glucose recoveries for sweet sorghum under SO_2 catalysed conditions. Further in this study involving sweet sorghum bagasse using an acid catalyst resulted in high glucose yields at a wide range of conditions which was not the case with either air dried or water soaked steam explosion. This was also observed by Sassner et al. who found that with SO_2 catalysed steam explosion, the range of pretreatment conditions resulting in high glucose recoveries was much larger

than for uncatalysed steam explosion. Further byproduct concentrations for SO_2 catalysed steam explosions did not follow trends observed in this study for air dried and water soaked steam explosion. Rather than increasing with increasing pretreatment severity, the byproduct concentrations were similar across the range of pretreatment conditions studied for SO_2 catalysed steam explosion. While the acetic acid concentrations in the pretreatment liquor were slightly higher than observed for water soaked steam explosion, furfural and HMF concentrations were lower for SO_2 catalysed steam explosion compared to water soaked steam explosion.

Interestingly the effect of chemical composition was more pronounced on the preferred cultivars when employing SO_2 steam explosion versus air dried and water soaked steam explosion. This was evident in the fact that the optimum condition for AP6 was different to the optimum pretreatment conditions required for SS27 and AS246. Further differences in combined sugar yields were more pronounced between each of the cultivars at the pretreatment conditions investigated. Statistically no correlations could be observed between the chemical composition of the preferred cultivars and their combined sugar yields due to the small sample size as well as no observable trends in the data between chemical composition and pretreatment response which could explain the difference between cultivars. Even so SO_2 has been known to more efficiently and aggressively hydrolyze and cleave bonds during pretreatment due to improved chemical interaction with the biomass compared to water soaked steam explosion which relies on auto-hydrolysis mechanisms alone which could possibly explain the difference observed with SO_2 as a catalyst [16]. Further work is required with a larger sample size to properly investigate the chemical interaction of sweet sorghum cultivars with SO_2 catalysed steam explosion as well as to include physical properties of each cultivar such as pore size, crystallinity, types of bonds present and so on.

2.6. Conclusions

The aim of this study was to select preferred sweet sorghum cultivars for further development as dedicated bio-ethanol feed stocks for use in the South African energy industry. In evaluating a number of sweet sorghum bagasse cultivars as potential bio-ethanol feed stocks it was found that large differences in chemical composition were evident and that statistically the chemical components which were most influential on dilute acid pretreatment response were lignin and ash content of sweet sorghum cultivars. Furthermore, sweet sorghum cultivars which are characteristically low in lignin and ash content are desired as bio-ethanol feed stocks due to their improved pretreatment performance. This meant that large variations in lignin content of between 14.29 and 21.14g/100g raw material and ash content variation between 0.7 and 1.88g/100g raw material resulted in combined sugar yields varying between 32.63 and 44.0g/100g raw material with dilute acid pretreatment at 170°C, 15 minutes and 0.7% (w/w) H_2SO_4. Furthermore it was found that in evaluating a smaller subset of the initial number of sweet sorghum cultivars that while certain cultivars such as SS27 were able to produce consistently good results over a number of pretreatment conditions, other cultivars AS246 were sensitive to changes in pretreatment condition. While the mechanics of this could not be fully explained in this study, it was evident that by increasing the pretreatment severity and enzyme loading, the effect of cultivar on pretreatment response could be reduced. This highlights that there will be a positive economic impact which could be gained in correctly selecting preferred sweet sorghum cultivars due to chemical characteristics such as low lignin and ash content

In estimating ethanol yields for the lignocellulose portion of the initial sweet sorghum cultivars it was found that while the effect of chemical characteristics on pretreatment response was most important in maximizing lignocellulosic ethanol yields on a kg per ton of biomass received basis, it was equally if not more important to utilize high biomass producing cultivars in maximizing ethanol yields on a ton/ha basis. This was evident in that the range of lignocellulosic ethanol yields estimated were between 412L/ha and 3192L/ha for the initial cultivars pretreated at a specific condition showing that differences in cultivar selection was more pronounced on a L EtOH/ha basis than on a L EtOH/ton bagasse basis due to inclusion of both good and poor agronomic performers. In selecting cultivars for the bio-ethanol industry as much emphasis must be placed on breeding high biomass cultivars as

in selecting cultivars that respond well to pretreatment to obtain sweet sorghum cultivars which are engineered specifically for 2nd generation bio-ethanol production. This will have a benefit for farmers who look to maximize profit per hectare and industry who will look to maximize conversion efficiencies. Optimization of 5 preferred sweet sorghum cultivars, AP6, AS103, SS27, MSJH13 and AS246 revealed that high conversions are possible for this feedstock with dilute sulfuric acid pretreatment resulting in combined sugar yields of between 48.83 and 54.5g/100g raw material at different optimum conditions. A 10% difference in combined sugar yield was observed between the best and the worst of the preferred cultivars with dilute sulfuric acid pretreatment. It was found that a single pretreatment condition, namely 185°C, 10 minutes and 0.25% H$_2$SO$_4$ could be utilized to achieve combined sugar yields that were 88% - 92% of the maximum recorded combined sugar yield. This shows that a single pretreatment condition could be used to effectively pretreat a number of different sweet sorghum cultivars if multiple pretreatment conditions are not economically viable.

Air dried steam explosion was found to be unsuited for maximizing total ethanol yields from lignocellulose in that the pretreatment condition required for successfully liberate the cellulose fraction, namely 205°C and 5 minutes, resulted in a high byproduct yield from the hemicellulose fraction with a xylose yield that only worsened over time. This suggests that air dried steam explosion should not be utilized for ethanol production alone and would be better suited for use in production of both furfural and ethanol. Water soaked steam explosion resulted in a substantial improvement over air dried steam explosion. This was evident in an increase in combined sugar yield from 44.73 to 55.39g/100g raw material through use of the water soaked steam explosion method. These improved yields coupled with a reduction of the acetic concentration in the pretreatment liquor from 14 to 3.5g/L shows that water soaked steam explosion is preferred over air dried steam explosion of sweet sorghum bagasse for ethanol production. Further use of an experimental design in this study improved the enzymatic hydrolysis yield to 95% compared to the only other known study with water soaked steam explosion of sweet sorghum bagasse in which a yield of 70% was achieved. Of the three steam explosion strategies evaluated in this study it was shown that SO$_2$ catalysed steam explosion resulted in near theoretical yields of sugars which gave combined sugar yields of between 64.00 and 70.55 g/100g raw material with the top 3 selected sweet sorghum cultivars AP6, AS246 and SS27. SO$_2$ catalysed steam explosion

should therefore be preferred over both air dried and water soaked steam explosion. Similarly to optimization with dilute sulfuric acid pretreatment, optimization with SO_2 steam explosion resulted in a 9% difference in combined sugar yields between the top and worst performer selected highlighting the potential economic benefit of selecting the correct sweet sorghum cultivars to be employed as dedicated bio-energy or bio-ethanol feed stocks. At these near theoretical conversions the top three preferred cultivars AP6, AS246 and SS27 had a total estimated ethanol yield of between 7131 L/ha and 8678 L/ha over two harvest seasons indicating the importance in selecting high biomass crops for bio-ethanol production.

2.7. Recommendations

Based on the results and conclusions of this study, research in the following areas is recommended to further improve sweet sorghum as a dedicated bio-ethanol feedstock:

1. The influence of crop cycle length, days after antithesis and other agronomic factors should be investigated as to their influence both on chemical composition and typed of steam explosion yields for sweet sorghum cultivars.

2. For steam explosion, only a single catalyst concentration of 3% SO_2 was utilized. Therefore it could be worthwhile to investigate different SO_2 concentrations to both increase the xylose monomer content and reduce the catalyst concentration.

3. Other factors affecting response of sweet sorghum bagasse to steam explosion should be investigated such as particle size and biomass loading. As in this study the particle size of sweet sorghum bagasse was reduced prior to steam explosion, reducing the amount of communition required to reduce the particle size will have an associate reduction in processing costs. Further if increasing the amount of biomass loaded to the steamgun reactor is possible without negatively affecting the combined sugar yields, there will also be an associated reduction in processing cost.

4. It was found that the two chemical components, lignin and ash, influenced the small scale dilute sulfuric acid pretreatment. Therefore it is recommended that the influence of chemical composition on steam explosion pretreatment yields of sweet sorghum bagasse

should be investigated. A number of sweet sorghum cultivars could be steam exploded at the same pretreatment conditions with differing enzyme loadings to investigate this. This will further evaluate the effect that non-preferred and preferred sweet sorghum cultivars, based on chemical composition, have on ethanol yields. In performing this though, only high biomass producing cultivars should be selected to focus evaluating the effect that chemical composition has on steam explosion.

5. As it has been found that a catalyst such as SO_2 is required to reach theoretical sugar yields, further green catalysts such as CO_2 should be evaluated for steam explosion of sweet sorghum bagasse. CO_2 will be readily available on 2nd generation ethanol plants and when used in steam explosion, will produce carbonic acid.

6. It was found that both agronomic factors and chemical composition of sweet sorghum varied over the two harvests. Therefore pretreatment comparisons (both dilute sulfuric acid and steam explosion) should be performed to investigate the average yields over a number of harvest seasons.

7. After dilute sulfuric acid and steam explosion pretreatments, fairly standard enzyme cocktails were investigated. Therefore it is recommended that a number of new cocktails be investigated at the optimum pretreatment conditions to evaluate the minimum enzyme loading necessary.

2.8. References

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