Full utilization of sweet sorghum for biofuel production

by

Nana Baah Appiah-Nkansah

B.S., Kwame Nkrumah University of Science and Technology, 2004
M.S., University of Tsukuba, 2009

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Biological and Agricultural Engineering
College of Engineering

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2017

Abstract

Sweet sorghum accumulates high concentrations of fermentable sugars in the stem, produces significant amount of starch in the grain (panicle) and has shown to be a promising energy feedstock. Sweet sorghum has a short growing season so adding it to the sugar cane system would be good. The overall goal of this dissertation is to enhance the attractiveness of biofuel production from sweet sorghum to fully utilize fermentable sugars in the juice, starch in the panicle and structural carbohydrates in the stalk for high efficiency and low-cost ethanol production.

Sweet sorghum juice was incorporated into the dry-grind process which increased ethanol yield by 28% increase of ethanol yield compared to the conventional ethanol method and decreased enzymatic hydrolysis time by 30 minutes. A very high gravity fermentation technique was applied using sweet sorghum juice and sorghum grain yielded 20.25% (v/v) of ethanol and 96% fermentation efficiency.

Response surface methodology was applied in order to optimize diffusion conditions and to explore effects of diffusion time, diffusion temperature, and ratio of sweet sorghum biomass to grain on starch-to-sugar efficiency and total sugar recovery from sweet sorghum. Starch hydrolysis efficiency and sugar recovery efficiency of 96 and 98.5% were achieved, respectively, at an optimized diffusion condition of 115 minutes, 95 °C, and 22% grain loading. Extraction kinetics based on the optimized diffusion parameters were developed to describe the mass transfer of sugars in sweet sorghum biomass during the diffusion process. Ethanol obtained from fermented extracted sugars treated with granular starch hydrolyzing enzyme and those with traditional enzymes were comparable (14.5 – 14.6% v/v). Ethanol efficiencies also ranged from 88.92 –92.02%.

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Acknowledgements

I would like to express my profound appreciation to my advisor, Dr. Donghai Wang, for giving me the opportunity to work under his guidance, encouragement, mentorship, advice, and financial support during my graduate program. I would like to thank Dr. Praveen Vadlani a member of my academic committee for his encouragements and the other members of my committee - Dr. Susan Sun and Dr. Lisa Wilken, for their time in serving on the supervisory committee and providing valuable suggestions. Many thanks also go to Dr. DeAnn Presley for willing to serve as my outside chair of my supervisory committee. I also want to express a special appreciation to my pastor, Apostle Lorenzo and Mrs. Mary Jackson as well as Pastors Roy Rawls and Karin Rawls for their fellowship and kind prayer support.

I would also like to thank my spouse, Hilda and daughter, Janet for their support and encouragement. I am also deeply grateful to Dr. Mary Rezac and Mr. Keith Rutlin of IGERT program and, for the financial support I received during the first and half two years.

I thank all the members in the bioprocessing group, Dr. Ningo Li, Dr. Ke Zhang, Ms. Yaritza Sanchez Gil, Ms Liman Liu, Ms Sarocha "Mimi" Pradyawong, Mr. Youjie Xu, Ms Kaelin Saul, Mr. Bairen Pang, and Mr. Xiwen Cao for making this process an enjoyable experience. Profound thanks to Dr. Joseph Harner III, Dr. Naiqian Zhang, Ms. Barb Moore, Mr. Randy Erickson, Mr. Ed Brokesh, Yong Wei and other faculty and staff in the Department of Biological and Agricultural Engineering for their help. I would also like to thank Ms Kerri Ebert for the several SARE travel support I received. I thank Kansas State University for providing a high level education with outstanding faculty, excellent facilities, and supportive staff.

Most importantly, I am deeply grateful to the Almighty God for his grace and wisdom.

Dedication

I dedicate this work to

my lovely daughter Janet,

spouse Hilda,

siblings Kojo, Kwame, Kofi, Fosuah, Safoah

mum Auntie Janet,

and dad Otis

Chapter 1 - Introduction

1.1 Background

The demand for liquid fuel for transporting people and goods is expected to increase by 70% within the same period (ExxonMobil, 2015); over half of petroleum is currently used for transportation (Wyman and Brethauer, 2010). However, the world's depleting petroleum resources, increasing and fluctuating gasoline prices, climate change, increasing emission of net CO_2, and other pollutants associated with the combustion of fossil fuels renders petroleum an unsustainable energy resource (EPA, 2015).

Environmental, social, and economic concerns regarding petroleum use have increased international interest in alternative energies and led governments, policymakers, and industries around the world to invest in research and development of a variety of alternative energy sources, such as solar, biomass, wind, geothermal, and hydro energy. For example, since 1975 Brazil has had a national biofuel program known as ProAlcool. ProAlcool is the most developed and integrated biofuel program in the world, intended to target high-scale alcohol production based on sugarcane as raw material (Mussatto et al., 2010; Sorda et al., 2010). As a result, Brazil is completely independent of fossil fuel importation. In comparison, 47% of crude oil processed in the United States in 2014 was imported (RFA, 2015). In 1997, the European Union, instituted an action plan known as "Energy for the Future" to pursue renewable sources of energy as substitutes for conventional fuels (Galbe and Zacchi, 2002). The U.S. government established the Energy Independence and Security Act (EISA) in 2005 and expanded it in 2007 to enhance national security, reduce dependence on foreign oil, increase domestic biofuel production, lower greenhouse gas emissions, and create new jobs. The EISA calls for annual production of 36 billion gal./yr of renewable fuels by 2022, meaning 15 billion gal./yr from corn-based ethanol, 16 billion

gal./yr from cellulosic-based ethanol, and 5 billion gal./yr from biodiesel and other advanced fuels (Eggeman and Atiyeh, 2010).

The production and demand of biofuel ethanol has been increasing consistently and significantly over the past 30 years, with corn starch being the major feedstock for ethanol production in the United States. Unfortunately, because of vast amounts of land, water, and fertilizer required to grow corn and the continuing food versus fuel debate, corn feedstock is not a sustainable choice for an alternative fuel. In 2000, over 90% of the U.S. corn crop was used to feed people and livestock, many in undeveloped countries, with less than 5% used to produce ethanol. In 2013, however, 40% of the U.S. corn crop was used for ethanol production, 45% was used to feed livestock, and only 15% was used for food and beverages. In 2014, over 130 billion gallons of gasoline were used in the United States and over 50 billion gallons of diesel were used (Conca, 2014). On average, one bushel of corn can produce approximately 3 gallons of ethanol (Albino, et al., 2012) . If all corn production in the United States was converted into ethanol, only 25% of the total 130 billion gallons of gasoline would be displaced (Conca, 2014). Sweet sorghum, a potential candidate for biofuel production, is considered to be a more efficient and cost-effective source of energy than corn because it requires less nitrogen and water. Sweet sorghum is a unique, versatile sugar crop that can be separated into starchy grains, soluble sugar in juice extracted from the stalk, and lignocellulose biomass (Rao et al., 2013; Blummel et al., 2009). All these components can be processed into ethanol (starch-based and cellulosic), syrup, animal feed, and electricity, as well as used as substrate for hydrogen and methane production (Antonopoulou et al., 2008; Gnansounou et al., 2005, Li et al., 2013). However, only three ethanol plants in the world are known to incorporate sweet sorghum crop in their facilities (Rainey and O'Hara, 2013) and no ethanol plants in the United States. In the current harvesting process of sweet sorghum for syrup production, the

crop is topped and the leaves are stripped before crushing the stalk for juice extraction (Regassa and Wartmann, 2014). The topped panicle composed of grain is currently left in the field; consequently, a significant amount of starch (60%–70%) that could be hydrolyzed and fermented into ethanol is lost. Moreover, the juice extraction process, achieved by pressing the stalk of the crop through a roller mill, is slow, labor intensive, and less efficient, with juice recovery below 50% (Regassa and Wartmann, 2014; Whitfield et al., 2012). Low juice extraction yield could be attributed to the relatively high fiber content of sweet sorghum stalk compared to sugarcane (Gnansounou et al., 2005). Another drawback associated with the milling process is sugar loss due to microbial activities (Wu et al., 2010; Whitfield, 2012). Wu et al. (2010) reported that up to 50% of total fermentable sugars in sweet sorghum is lost if the expressed juice is stored for one week at room temperature. This loss is a result of microorganisms that metabolize the sugars into organic acids (Wu et al., 2010).

The objective of this research is to use technological developments to enhance the economic attractiveness of ethanol production from sweet sorghum in order to fully utilize fermentable sugars in the juice, starch in the panicle, and structural carbohydrates in the stalk for high efficiency and low-cost ethanol production.

1.2 Research objectives

This research includes the following specific objectives:

1. To review sweet sorghum as a viable renewable bioenergy crop.
2. To incorporate sweet sorghum juice into current dry-grind ethanol fermentation process for improved yields.
3. To investigate ethanol production from mixtures of sweet sorghum juice and ground sorghum grain using a very high gravity fermentation technique with urea supplementation.

4. To develop model studies on extraction of fermentable sugars and nonstructural carbohydrates from sweet sorghum using a diffusion process
5. To study mass transfer kinetics of sugar extraction from sweet sorghum biomass via a diffusion process and SSF of extracted sugars

Chapter 2 - Sweet sorghum as a viable and renewable bioenergy crop: A review[1]

2.1 Abstract

Sweet sorghum (*Sorghum bicolor (L)* Moench), a C_4 plant, is known to be a unique, versatile, and a potential energy crop that can be separated into starchy grains, soluble sugar juice in the stem, and lignocellulose biomass. The fermentable sugars in the juice (53-85% sucrose, 9-33% glucose, and 6-21% fructose) can be directly converted into ethanol. The grain is primarily starch (62-75%), which can be hydrolyzed and fermented into ethanol. The bagasse, a dry fibrous lignocellulosic material, can be used for the production of cellulosic ethanol, heat and/or power co-generation. In this review, to potential to produce bioenergy (of various forms) using recently developed cultivars with improved agronomic performance is discussed. In addition, sweet sorghum is compared with other starch, sugar, and lignocellulosic feedstocks. Various studies conducted on alternatives pathways to convert whole sweet sorghum stalks and bagasse into bioenergy are presented. Finally, a techno-economic analyses of producing ethanol and other co-products from sweet sorghum is presented.

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[1] This chapter has been submitted for publication as a peer-reviewed research paper to the Journal of Industrial Crops and Products.

2.2 Introduction

The increasing global demand for petroleum fuel and its associated environmental concerns such as greenhouse gas emissions, global warming, global climate change, etc. has shifted worldwide attention to the production of sustainable, environmental friendly, and renewable fuel energy from energy crops. For instance, in 2015, the U.S. produced 14.7 billion gallons of biofuel ethanol, mostly from corn, and Brazil produced 7 billion gallons, mostly from sugarcane (RFA, 2016). Additionally, 1.3 billion gallons of ethanol was produced in 2013 in the EU countries from a combination of wheat, corn, barley rye, and sugar beet (RFA, 2014; Flach et al., 2013). Varying amounts of denatured ethanol were also produced from cassava, corn, wheat, sorghum, etc. in China, Canada, and in other regions of the world (RFA, 2014; Evans, 2013; Scott and Yang, 2013). Other crops known to produce ethanol, but not commonly used for industrial production are sweet potato, peal millet, oats, watermelon, and dates (Zabed et al., 2014).

Sweet sorghum (*Sorghum bicolor (L)* Moench), a C_4 plant, has been widely mentioned and tested as a bioenergy crop. Sorghums have been developed proportionally greater proportions of a specific carbohydrate; grain sorghums produce greater amounts of grain; forage sorghums are leafy with thinner stalks for animal palatability, and bioenergy sorghum types have maximized lignocellulosic biomass production. (Rooney et al., 2007). Sweet sorghum was developed primarily for significant quantities of soluble sugar in the stalk but the crop is unique in that it also produces significant quantities of starch and lignocellulosic biomass. The grain panicle is composed primarily of starch; the juicy stalk contains extractable juice high in sucrose, glucose and fructose; and the stalk bagasse is composed primarily of lignin, cellulose, and hemicellulose (Rao et al., 2013; Lau et al., 2006; Blummel et al., 2009).

Regardless of the type of sorghum, if it produces grain, the grain is located at the terminal growing point and is primarily starch (63-72%) and protein (11-15%), with smaller amounts of fiber (2-6%), ash (1-3%), and fat (1-4%). Historically, the grain has been used primarily as both human food and animal feed with applications for each, much like corn (Harrison and O'Hara, 2013; Reddy et al., 2006; Gnansounou et al., 2005). From a compositional standpoint, the grain from sweet sorghum is similar, if not identical, to typical grain sorghums.

Compared to other sorghum the unique characteristic of sweet sorghum is the accumulation of high concentrations of fermentable, soluble sugars in the stem. These sugars are extractable in the juice at concentrations of 12-18%. The composition of the sugar in the juice is primarily sucrose (53-85%), glucose (9-33%), and fructose (6-21%) with significant variation in these proportions due to both genotypic and environmental factors (Serna-Saldivar et al., 2012). Zabed et al. (2014) reported that the juice from sweet sorghum stalk contains 16.0-21.8% of free sugar compared to sugarcane juice (12.0-17.6%). Like sugarcane, the high concentration of fermentable sugar makes it suitable for ethanol fermentation (Rainey and O'Hara, 2013; Yu et al., 2012; Kim and Day, 2011; Wu et al., 2010; Reddy et al., 2005).

Historically, sweet sorghum was originally used as a natural sweetener in the form of a syrup which is produced when sweet sorghum juice is evaporated to high sugar concentrations. Other studies have shown that sweet sorghum juice can be processed into granulated sugar, syrup, animal feed, and used as substrate for hydrogen and methane production (O'Hara, 2013; Antonopoulou et al., 2008; Gnansounou et al., 2005). The use of sweet sorghum as an ethanol substrate is relatively recent, tracing back the late 1970's (Schaffert, 2007).

After juice extraction, the bagasse, which is a dry fibrous lignocellulosic material, can be used as animal fodder, cellulosic ethanol, butanol, wood plastic composites, biomass pellets,

polymers, soil fertilizer, and power co-generation (Rainey and O'Hara, 2013; Yu et al., 2012; Gnansounou et al., 2005; Sipos et al., 2009; Negro et al., 1999). Thus, interest in sorghum as a bioenergy crop is based on high productivity, stress tolerance, adaptation to the existing agricultural infrastructure and its flexibility in production as a starch, sugar or lignocellulosic crop that can produce a wide range of products.

This chapter reviews the agronomic requirement for cultivating sweet sorghum, the productivity of recently developed cultivars specifically for bioenergy production, and the various pathways of converting sweet sorghum crop into bioenergy as well as the technological and economic feasibility of producing biofuel from sweet sorghum.

2.3 Biology of sweet sorghum

Suitable sweet sorghum cultivars for biofuel production must possess several traits including; 1) high biomass yield, 2) thick, lodging tolerant stalks with and juicy internodes, 3) juice with high total soluble brix content, 4) high percentage of extractable juice, 5) a long period of industrial use (which extends the harvest season), and 6) a series of cultivars that differ in maturity of sweet sorghum cultivars to extend the harvest season over a period of months if the production system requires it. In addition to these characteristics, the requirements for traits such as abiotic and biotic stress tolerance and grain production vary from production system to production system. For example, significant grain yield is highly valued in the Indian production system while the Brazilian production model prefers that no grain be produced (Rao et al., 2013; Bitzer, 1997).

The initial development of sweet sorghum as an energy crop came in the mid 1970. Breeding programs began selecting sorghum cultivars with high biomass potential and this continued through the mid-1980s. (Rooney et al., 2007; Kovarik, 2013, Reddy et al., 2006). During

the same period, numerous sweet sorghum lines were identified among advance breeding progenies (Reddy et al., 2006). Shennong Tianza No. 2 was among the sweet sorghum hybrid bred to be used as feedstock for ethanol production in the early 1980s, which produced the highest yields of fermentable sugars (65% juice rate), grains (5.0 t/ha) and fresh stems (52 t/ha) (Gnansounou et al., 2005).

More recently, the detailed characterization of the genetic pathways for maturity have led to the production of both high biomass and sweet sorghum hybrids with dry or juicy stalks, low and high sugar content for various forms of biofuel production (Rooney et al., 2007). This has been made possible due to the existence of an extensive sorghum germplasm base that provided accessions relevant to bioenergy applications such as stalk sugar content (Reddy et al., 2006). Sweet sorghum improvement focuses on producing cultivars and hybrids with juicy stalks, high yield, high concentrations of fermentable sugar in the stalk and stability of sugar yields over a thirty day window (period of industrial use). In India, high quality grain yield is equally important while in other environments (Brazil) grain yield is not important and varieties with no grain yield are actually preferred (Rajvanshi and Nimbkar, 2008).

2.4 Recently developed varieties

Recent and popular developed sweet sorghum varieties are Della (mid-season variety, good disease resistance, early maturity), Dale (good standing ability, superior disease resistance), Sugar Drip (good late planting, susceptible to most diseases), M81E (similar to Dale in height, lodging resistance, and juice yield), Simon (high quality syrup, small stalk, low juice yields), and Theis and Brandes (late maturing, at least 2-3 weeks later than Dale). Ellet et al. (2013) assessed the soluble solids contents of eight sweet sorghum varieties in the Childers region of southeast Queensland, Australia. These were Rcv27751, Keller, Top-76-6, Dale, M81-E, Rio, Italian and

Wray. They found that total fermentable sugars contents ranged between 14% and 18%. Agronomic performance of the varieties was compared and showed that M81-E, Rio, and AFL Rcv27751 are varieties with the highest stalk. The field trials also revealed that Top 76-6, Dale, and AFL Rcv27751 had the highest quantities of total fermentable sugars under field conditions. The Top 76-6 variety had the best-combined traits for ethanol production from the stalk soluble sugar and higher nutritional value from the grain (Cifuentes et al., 2014). Table 2.1 shows nutritional analysis of common sweet sorghum cultivars among the globally recognized commercially available varieties. The four popular U.S. varieties, which have been identified, are M81-E, Keller, Top-76-6, and Dale (Ellet et al., 2013).

Table 2.1 Nutritional composition of some sweet sorghum grain varieties

Variety	**Moisture content (%)**	**Protein (%)**	**Fat (%)**	**Ash (%)**	**Crude fiber (%)**	**Oil (%)**
Dale[a]	6.14	12-13	3.6	1.71-2.2	1.67-2.2	3.72
Della[a]	7.02	12.6	-	1.67	2.12	3.6
M81-E[a]	7.36	12.1-13.48	3.1	1.9-2.16	2.7	
Sugar Drip[a]	6.77	12.65	-	1.85	2.55	3.51
Top76-6[a]	7.13	11.9-12.25	4.3	1.88-2.0	2.4-2.68	3.92
Umbrella[a]	7.34	13.29	-	1.66	4.26	4.07
Keller[b]	10.8	11.4	3.8	2.0	3.0	-
Rio[b]	11.2	12.8	3.1	1.6	1.7	-
Wray[b]	11.8	13.2	2.8	2.2	2.4	-
AFL[b] Rcv27751	10.8	12.4	0.8	3.0	6.2	-

[a]Wet basis [b]Dry weight basis. (Cifuentes et al., 2014; Harrison and O'Hara, 2013).

Adapted from Cifuentes et al. (2014) and Harison and O'hara (2013).

2.5 Sweet sorghum production

2.5.1 Growing location and planting time

Sorghum, domesticated in Africa, can be grown throughout tropical, subtropical, and some temperate regions of the world. Compared to most other C4 grasses, it has significantly better drought tolerance and is considered a low input crop. The actual productivity in any given region is a function of end use (biomass, grain or forage) and the climatic conditions as well as soil type and agronomic practices (Rooney et al., 2007; Grassi, 2001). In some tropical regions, sweet sorghum can be planted twice a year: first in the wet season (June-July) and second in the dry season (September-October) (Delta Farm Press, 2008; Ranolo, et al., 2007; Grassi, 2001). In temperate regions, planting is usually limited to once a year (Montross et al., 2009; Gnansounou et al., 2005; Steduto et al., 1997). Bonin et al. (2016) examined the impact of planting time, seeding rate and row spacing on biomass yield and ethanol potential from sweet sorghum (Top 76-6) in central Iowa, U.S. Planting was done in late May, early June, and late June at seeding rates of 4.5, 11.2 and 17.9 kg ha^{-1} with row spacing of 20, 38, and 76 cm with either 84 or 168 kg nitrogen application per hectare. Their results suggested that early planting of sweet sorghum (late May-early June) in 20-cm row widths significantly impacted the biomass yield (26-29 t ha^{-1}) with a theoretical ethanol potential of over 14,500 L ha^{-1}. Ethanol yield was estimated from total nonstructural carbohydrates, cellulose and hemicellulose components of the crop.

A major challenge associated with the production of sweet sorghum is the short harvest window in comparison with sugarcane (Burks et al., 2013). Sweet sorghum sugar concentrations and juice yields typically peak near physiological maturity of the grain; they then start to decline usually because the plants start to reallocate sugar to new vegetative growth (Parrella et al., , 2016). The limited harvest window of sweet sorghum limits its utilization as biofuel production. Two

approaches have been used to address the concentrations and stability of sugar in the stem. First, researchers in Brazil have evaluated different sweet sorghum varieties to determine their 'period of industrial use' (Schaffert, 2007). Genotypic variation was identified and now all sweet sorghums in Brazil are evaluated for period industrial use (Shaffert personal communication). In another approach to address the limited harvest window, Burks et al. (2013) developed a sweet sorghum production system that permits the continual harvesting of sweet sorghum hybrids over time (from late July through early November). To achieve the wide harvest window, the planting timing of the hybrids were proposed in April 15, May 15 and June 15 with different hybrids of variable maturity to ensure sweet sorghum production peaking for both sugar and yield every two weeks during the harvest season.

2.5.2 Soil, fertilizer and water requirements

While sweet sorghum can be produced in an array of different soils, the most productive soils are well drained and well-structured red or black clay loam soils (Ellet et al., 2013; Rao et al., 2013; Steduto et al., 1997). Sweet sorghum requires balanced fertilization to make a productive crop and the amount needed varies with available levels of N, P and K in the soil profile. Some studies have indicated that some nitrogen stress near maturity enhances sugar accumulation in the stalk, but the trend is not yet conclusive and needs further evaluation.

Although sweet sorghum is a drought resistant crop, water availability also plays a significant role on its productivity. The crop requires between 500 and 1,000 mm of rain/irrigation water to achieve desirable yields of 50-100 ton/ha total fresh above ground biomass and known to have a high radiation use efficiency (RUE: ~ 1.3-1.7 g MJ^{-1}). RUE is the amount of dry matter produced per unit of radiation intercepted (Monteith, 1993), and it's also an agronomic criterion for evaluating the crops performs in a new environment (Dercas and Liakata, 2007. In a 5-year

field trial, irrigated sweet sorghum ratoon crop performed well yielding an average height of over 3 m (9.84 ft.). Water application was done in mid-August (Ellet at al., 2013). Thus, that water availability and its timing are important to maximize productivity in sweet sorghum.

2.5.3 Yield and compositional changes during growth

The yield of sweet sorghum (20.6 t/ha) in the 3-yr rotation system with maize and soybean did not show a significant difference over the yield of sole-cropped sweet sorghum grown continuously on the same plots (21.8 t/ha) (Buxton et al., 1999). Sugar concentration of sweet sorghum generally increased with growth duration (Regassa and Wortmann, 2014; Zhao et al., 2009) and decreased with delayed planting (Regassa and Wortmann, 2014). Lueschen et al. (1991) reported 13% higher fermentable sugars and ethanol yields from cultivars planted earlier compared with later planting dates in the upper Midwest. At different growth stages, soluble sugars obtained from 19 improved sweet sorghum hybrids and varieties were found to be highest at post-physiological maturity with nearly 70%, 20%, and 10% for sucrose, glucose, and fructose, respectively (Kumar et al., 2010). Kumar et al. (2010) also observed that stalk weight, juice yield, Brix %, sugar yield, sucrose, glucose, and fructose contents, and pH showed considerable differences at the dough, physiological maturity and post-physiological maturity stages. Sucrose increased by 146% from dough stage to post-physiological maturity. In another study, cellulose and hemicellulose contents of five sweet sorghum hybrids and cultivars also increased significantly with time from 1.6 to 6.6 t/ha from anthesis to 40 days after anthesis (Zhao et al., 2009). Zhao et al. (2009) investigated changes in above ground dry weight of panicles, leaves, stem, and estimated ethanol yields from anthesis to 40 days after anthesis (DAA) of the five early maturity, middle maturity, and late maturity cultivars over a two year study period. The authors reported considerable increase of stem dry weight from 5.5–15.2 t/ha at anthesis to 9.0–20.2 t/ha

on 40 DAA for the two-year average. Total aboveground dry weight also increased from 8.6–22.2 t/ha at anthesis to 14.0–29.9 t/ha on 40 DAA for two-year average. In similar fashion, total soluble sugar content in the stems increased with time after anthesis, ranging between 203 and 476 g/kg during 0–40 DAA for the two-year average. Estimated ethanol yield from the carbohydrates (sugar, starch, cellulose and hemicellulose) also increased from 4867 to 13,032 L/ha over the same growth period. Total soluble sugar content and yield in stems of four sweet sorghum cultivars increased significantly from the physiological maturity dates to the frost date over a two-year study period (Zhao et al., 2012).

Harvesting of genotypes with 2 t/ha sugar yield at the physiological maturity was recommended by Kumar et al. (2010) to increase its desirability for industrial processing and to enhance the value chain of sweet sorghum. A wider window of harvest time of cultivars with a sustained sugar level from physiological maturity to post-physiological maturity was also suggested. Zhao et al. (2009) also recommended the harvest of sweet sorghum upon the early maturity of the cultivars from around 20 days after anthesis.

2.6 Sweet sorghum bioconversion to bioenergy

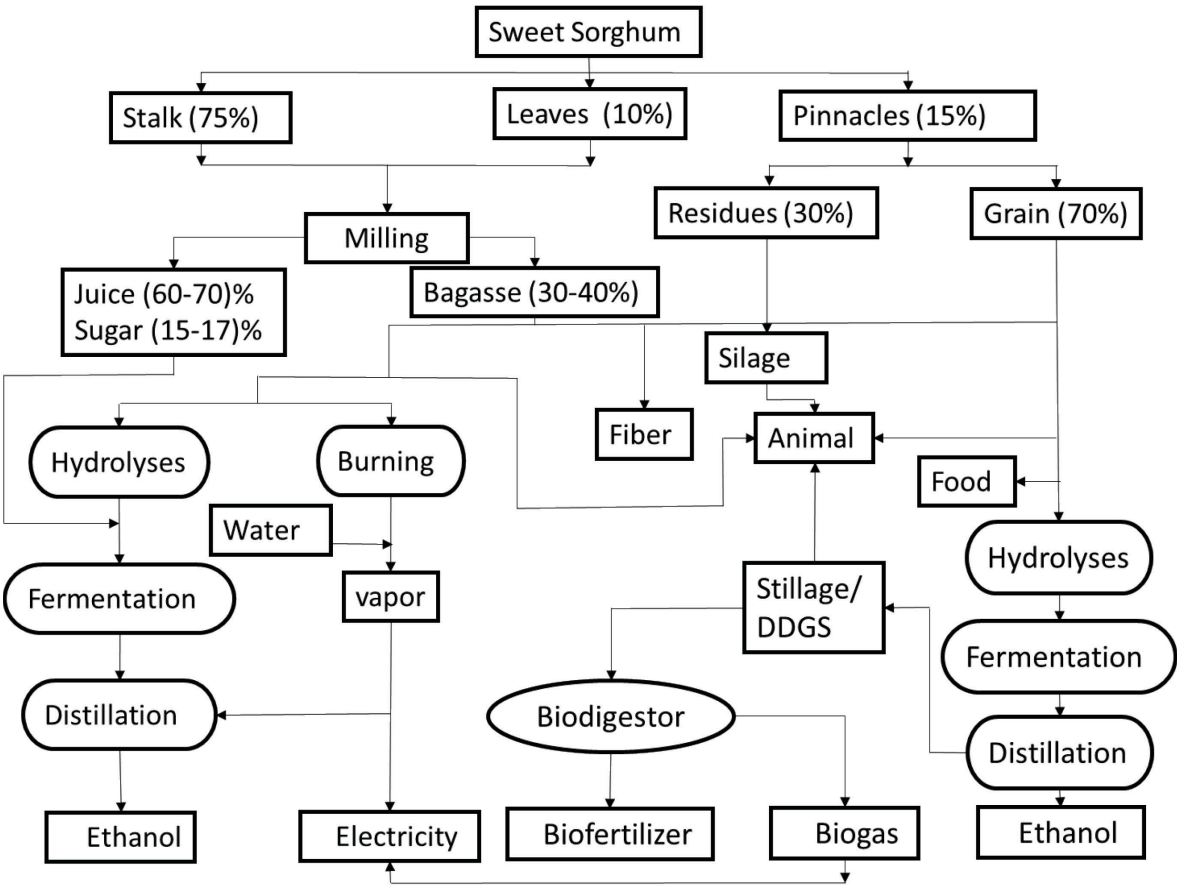
Historically, the extracted juice from the sweet sorghum stem was processed into syrup in the U.S., but several studies have indicated interest in sweet sorghum for ethanol production in the U.S. and elsewhere (Basavaraj et al., 2013; Rao et al., 2013; Ranola et al., 2007; Wu et al., 2010; Harrison and O'Hara, 2013; Bitzer, 1997). In the sweet sorghum juice-to-ethanol process, harvested stalks of sweet sorghum may be crushed on the field to separate the juice from the bagasse or transported from the farm to the processing plant for juice extraction. The obtained juice, which contains sucrose, glucose, and fructose, is usually supplemented with ammonium sulphate or micronutrients and fermented with microorganisms (typically, *Saccharomyces*

cerevisiae) into ethanol, which is blended with gasoline for transport fuel. (Zabed et al., 2014; Basavaraj et al., 2013). The residual bagasse may be utilized as a source of energy for the ethanol production process (Basavaraj et al., 2013).

2.6.1 Pathways of bioenergy production from sweet sorghum

Sweet sorghum can be utilized for bioenergy production via several processing pathways (Figure 2.1) These include: 1) enzymatic hydrolyses of the starch in sweet sorghum grain into simple sugar (glucose) and metabolizing the sugar by yeast fermentation into ethanol; 2) direct fermentation of extracted sweet sorghum juice (glucose, sucrose, and fructose) into ethanol; 3) enzymatic hydrolysis of pretreated lignocellulosic sweet sorghum biomass (leaves, bagasse, and pinnacle residuals) into glucose and xylose and fermentation of these sugars into ethanol; 4) burning of the biomass to produce heat and electricity; 5) anaerobic digestion of the stillage, a co-product from the grain starch fermentation process, for biogas; and 6) combusting the biogas to produce electricity (O'Hara, 2013; Rainey and O'Hara, 2013; Yu et al., 2012; Antonopoulou et al., 2006; Gnansounou et al., 2005; Negro et al., 1999; Sipos et al., 2009). For all of these conversion processes, the initial step after harvest is to extract the juice from the whole plant material creating both juice and bagasse streams.

Figure 2.1 The potential use of sweet sorghum for bioenergy (Schaffert, 2007).



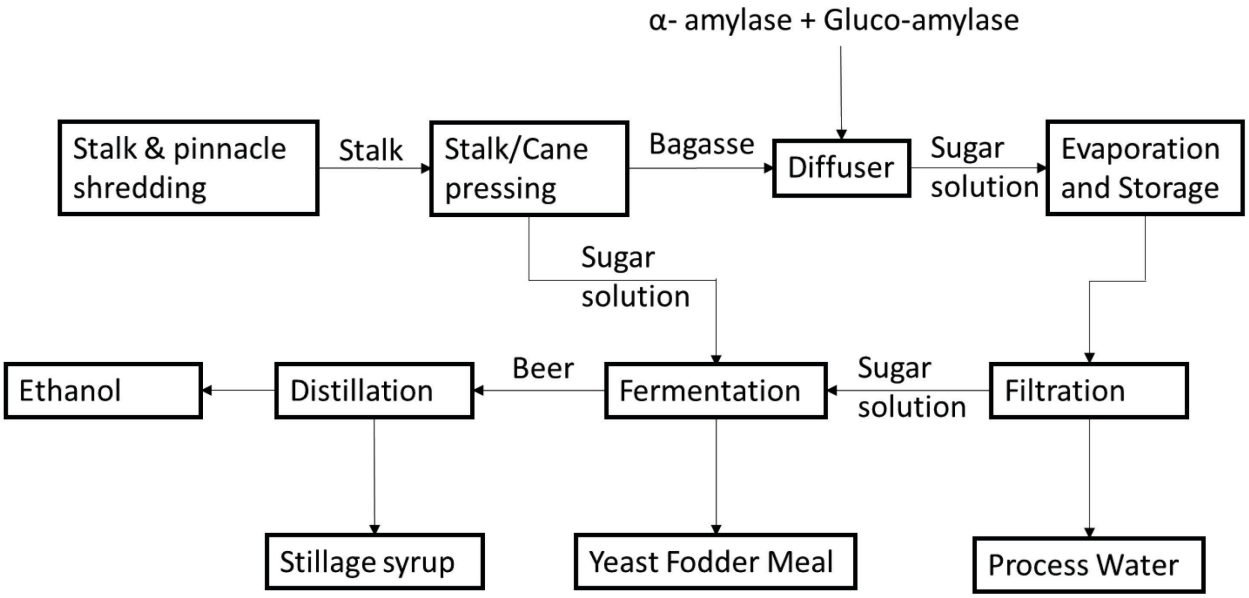
2.6.2 Juice extraction, treatment and storage

Traditionally, sweet sorghum juice is extracted by crushing the harvested stalks after the panicle is removed (either in the field or prior to crushing) through 2- or 3-roller mills in tandem or by using hydraulic press expression of juice from chipped stalks (Eggleston et al., 2013; Rao et al., 2013; Ranola et al., 2007). However, this extraction process is slow and labor-intensive with low juice recovery (Regassa and Wartmann, 2014; Whitfield et al., 2012). Press efficiency (juice extraction) of 45-55% has recently been reported (Caffery et al., 2014). Wu et al. (2009) also reported 58% juice recovery with roller mill, and 63-70% by screw press. Three crushing treatments for juice extraction from three cultivars (SSV 74, SPSSV 30 and CSH 22SS) have been used: 1) stalk only, 2) stalk plus leaf sheath, and 3) stalk and whole leaf (Rao et al., 2013). Juice

from the stalk was extracted using a power operated three-roller sugarcane machine miller without imbibition. The authors reported maximum juice recovery percentage of 43% for the stalks plus sheath crushing method, 41% for whole plant, and 40% for stalk alone. With regards to the sugarcane industry, sugar extraction from the cane is done with crushing the stalk or by diffusion process. It is possible to obtain 70 – 95% of the sugar from the cane in the large conventional mills as compared to extraction rate of less than 50% from small-scale roller mills (Rein, 2007; Reidenbach and Coble, 1985). Reidenbach and Coble (1985) compared juice extraction from sugarcane and sweet sorghum stalks using a three-roller mill with a 20.3 cm diameter top roller and 14.0 cm diameter bottom rollers. The length of chopped feedstocks used for the testing were 1, 2, 4 and 8 cm. Extraction rates based on the weight of the juice and the weight of the total weight of material processes were 54.9% for sugarcane and 54.6% for sweet sorghum, respectively.

In sugarcane, the diffusion process has improved extraction efficiency and this method was tested by Appiah-Nkansah et al. (2016) (Figure 2.2). The diffusion process extracts fermentable sugars and nonstructural carbohydrates from chopped sweet sorghum biomass and any grain in the system. Response surface methodology (RSM) was applied in order to optimize diffusion conditions and to explore effects of diffusion time, diffusion temperature, ratio of sweet sorghum biomass to grain on starch-to-sugar efficiency, and total sugar recovery from sweet sorghum. Starch hydrolysis efficiency and sugar recovery efficiency of 96.0 and 98.5% were achieved, respectively, at an optimized diffusion condition of 115 minutes, 95 °C, and 22% grain loading.

Figure 2.2. Sweet Sorghum biorefinery system which uses both soluble and non-structural sugars (Appiah-Nkansah et al., 2016).



It is a challenge to store the fresh juice without sugar degradation. Fermentable sugars extracted are not stable during storage. Up to 50% of the sugars may be lost when stored at room temperature for one week due to microbial activities. This loss is a result of microorganisms that metabolize the sugars into organic acids, ethanol, and carbon dioxide (Wu et al., 2009). A treatment technique to stabilize sweet sorghum juice for long-term storage was recently developed (Wu et al., 2015). Wu et al. (2015) showed that high-pressure homogenizing at 32 kpsi and high-level ozone treatment (600 mg ozone min^{-1}, 30 min treatment per 200 mL juice) can stabilize the juice for up to 23 weeks, with more than 90% of the original fermentable sugars retained. They found no indication of formation of lactic acid and ethanol with the 32 kpsi and ozone-treatment technique. Sodium benzoate (at 1,000 ppm concentration) was recently reported as suitable preservative to retain the quality of fresh sweet sorghum juice and extend the storage shelf life of juice up to 2 days at 37 ºC (Kumar et al., 2014). The storage shelf life of the sorghum juice was

also observed to be extended for 21 days with no effect on ethanol conversion efficiency when juice was pasteurized at 90 °C for 2 minutes (Kumar et al., 2013). On-field fermentation of extracted juice enabled 95% of the soluble sugars to be converted into ethanol under non-sterile conditions (Montross et al., 2009).

2.6.3 Direct fermentation of sweet sorghum juice

The production of ethanol from sweet sorghum juice via direct fermentation process is well-known. Ethanol production from six sweet sorghum varieties - Dale, Della, M81E, Top 76-6, Sugar Drip and Umbrella were recently investigated (Cifuentes et al., 2014). Top 76-6 variety had the highest ethanol productivity and the best grain nutritional characteristics. The average ethanol yield from the Top 76-6 variety was found to be nearly 220 g ethanol per kg of dry stem, which is equivalent to 2,465 L of ethanol per ha. Ethanol yields of 3380, 2780, 3000, 2950 and 2620 L/ha for Dale, M81E, Rio, Theis and Topper varieties respectively were also reported (Cifuentes et al., 2014). Wu et al. (2010) studied the features of sweet sorghum juice and their performance in ethanol fermentation. They reported that fermentation efficiencies of fresh juice, autoclaved juice, and concentrated juice with 20% sugar were higher than 93%.

Converting sugar from juice and sugar from starch could be an important approach where both are available. Appiah-Nkansah et al. (2015) incorporated sweet sorghum juice into the current dry-grind ethanol process to improve ethanol yield and water efficiency. The results showed that the ethanol yield from sweet sorghum juice with the optimum grain sorghum flour loading was about 28% higher than that from the conventional ethanol process. They also investigated ethanol fermentation performance from mashes of sweet sorghum juice and sorghum flour at low temperature hydrolysis using granular starch hydrolyzing enzyme and achieved 94.65% ethanol fermentation efficiency. Additionally, adding sweet sorghum juice could shorten enzymatic

hydrolysis time by 30 minutes which would reduce energy inputs. High fermentation efficiencies (94 – 95%) of fresh juice, autoclaved juice, and concentrated juice with 20% sugar have also been reported (Wu et al., 2010).

The most commonly used microorganism to metabolize fermentable sugars in the sweet sorghum juice into ethanol under anaerobic conditions is *Saccharomyces cerevisiae*. Several investigators have conducted studies on the application of response surface methodology technique to optimize ethanol production from sweet sorghum and to analyze the effects of individual process parameters on ethanol yield and final ethanol concentration using *Saccharomyces cerevisiae* (Luo et al., 2014; Yu et al., 2009; Wang et al., 2011; Phutela and Kuar, 2014). Wang et al. (2011) applied Box-Behnken central composite design of response surface methodology to optimize ethanol fermentation from sweet sorghum juice using brewing instant dry yeast fermentation parameters: temperature (25 - 35 °C), pH (4 - 6), and inoculum (1 - 5%) were analyzed. The optimum fermentation conditions for ethanol conversion from sweet sorghum were found at 27.7 °C, pH 5.4, 5% inoculation, and 9.5% ethanol yield. Phutela and Kaur (2014) also reported an ethanol yield of 8.83% (v/v) from sweet sorghum juice with fermentation efficiency of 87.33% using *Saccharomyces cerevisiae* NRRL Y-2034 under optimized conditions of temperature (30 °C), agitation rate (50 rpm), and inoculum size (7.5% v/v) by response surface methodology. Besides *Saccharomyces cerevisiae, Mucor hiemalis* microorganisms have shown to be good candidates for ethanol fermentation from sweet sorghum (Goshadrou et al., 2011).

2.6.4 Solid fermentation

The fermentation process that involves the growth and metabolism of microorganism on and inside of solid substrates under humidified conditions is known as a solid-fermentation (Song et al., 2014; Pandy, 2003). Compared with liquid-state fermentation, solid-state fermentation is

more environmentally friendly, produces less wastewater, and requires lower energy input (Song et al., 2014). Furthermore, high productivities, low production costs, extended product stability have been associated with the use of the solid-state fermentation technique. Solid-state fermentation is usually applied for food processing of rice, corn, wheat, barley and soybean products.

Solid-state fermentation for ethanol production from sweet sorghum has been explored because of the high sugar utilization and ethanol yield, low energy input and capital cost, and reduced water usage and wastewater output (Du et al., 2015; Li et al., 2013; Han et al., 2010). Several studies have been conducted on solid-state fermentation for ethanol production from sweet sorghum using *S. cerevisiae* (Shen and Liu, 2009; Bryan, 1990; Gibbons et al., 1986; Du et al., 2014), *S. cerevisiae* AF37X (Yu et al., 2008), *S. cerevisiae* TSH1 (Li et al., 2013), and thermotolerant yeast strain (Yu et al., 2008). Besides the traditional static solid state fermentation, other SSF such as gas stripping solid state technique has been explored for ethanol production from sweet sorghum.

The heterogeneous form of the media poses major technical challenges such as difficulties in maintain uniform moisture levels, substrate concentrates, and temperature controls with SSF (Shuler and Kargi, 2002; Holker and Lenz, 2005). Hence it is difficult to scale the process due to the associated engineering problems (Holker and Lenz, 2005). To address the control problems, a rotating drum fermenter is usually used for the mixing of the fermentation media in either a continuous or intermittent process (Shuler and Kargi, 2002). Li et al. (2013) demonstrated the use of advanced solid state fermentation (ASSF) using *S. cerevisiae* TSH1 strain on sweet sorghum stem. In their study, sweet sorghum stem was pulverized into particle sizes of 1-2 mm in diameter and 3-50 mm length, heated to 28 °C and combined with the TSH1 culture liquid into a continuous

rotary drum fermenter over a fermentation period of 2 weeks. The dosage rate was 1 – 2 mg dry cell weight/g dry sweet sorghum. Sweet sorghum, culture liquid and steam were constantly supplied to the fermenter. A total of 16 ton of sweet sorghum stems yielded 1 ton of ethanol (99.5%, v/v); at stem feed rate of 3.72 ton/h, the fermentation yielded 1.54 ton/h crude ethanol and finally purified to 99.5% v/v. The cost of fuel ethanol production was estimated as $615.4 per ton (49 cent/liter) on the premise that the price of the sweet sorghum stems is $30 per ton which is cost competitive compared with those of wheat-based fuel ethanol ($869.9 per ton), corn-based fuel ethanol ($841.7 per ton), and cassava-based fuel ethanol ($778.1 per ton). Du et al. (2014) isolated *S. cerevisiae* strains TSH1 from soil samples on which sweet sorghum stalks was stored. The strains were cultured with crushed feedstocks (96 tons) and ethanol fermentation process occurred in a 550 m^3 industrial rotary-drum fermenter at 30 °C for 21 hours. Ethanol fermentation process was completed at the 15 hour reaching a theoretical yield of about 88% at 10 g/kg/hr production rate. The cost of ethanol per ton was competitive in comparison to other ethanol production feedstocks such as corn and cassava. Their finding revealed strong solid state fermentation potential of TSH1 on sweet sorghum feedstocks at the industrial scale.

Other investigators have also demonstrated the application of low cost and high efficiency deep-bed solid state fermentation technology to produce ethanol from sweet sorghum stalks. Kwon et al. (2011) obtained the highest ethanol yield of 0.25 g-ethanol/g-dry stalk at 37 °C using 15–20 cm and 40 °C with 5-10 cm substrate particle size in the bioreactor with thermotolerant yeast, *Issatchenkia orientalis* IPE 100.

The production of single cell oil for biodiesel production has also been investigated. Economou et al. (2010) proposed an alternative method of producing single cell oil, using the oleaginous fungus *Mortierella isabellina* for biodiesel production by semi-solid state fermentation

of sweet sorghum biomass (with increased water content). In their work, *M. isabellina* was made to grow on 1–2 mm particle sizes of crushed sweet sorghum stalk under aerobic conditions of pH < 6 and NaOH solution for pH control at 28 °C for 8 days. They observed that the nitrogen and sugars contained in the sweet sorghum material were utilized by the microorganism to produce oil at a conversion rate about 10 g/100 g dry weight of substrate. The efficiency of oil production reached 0.88 g/100 g dry weight.

2.6.5 High gravity fermentation

Very high gravity (VHG) fermentation is defined as "the preparation and fermentation to completion of mashes containing 27 g or more dissolved solids per 100 g mash" (Wang et al., 2007). The theory of VHG was developed in the 1990s from the concept of high gravity (HG) fermentation (initiated in the 1980s) (Udeh and Kgatla, 2013; Puligundla et al., 2011; Ressull, 2003). VHG fermentation has shown to be environmentally friendly, versatile, and an emerging technology in both ethanol and beer production (Udeh and Kgatla, 2013; Yu et al., 2012; Puligundla et al., 2011; Nuanpeng et al., 2010; Laopaiboon et al., 2009). Other advantages of VHG include a potential reduction by 58% in process water requirement due to high solid mash preparation; low risks of bacterial contamination because bacteria cannot thrive under higher osmotic conditions; higher enzymatic activities due to decreased starch-to-water ratio; reduced fermentation process time; increased productivity and rate of fermentation in the product (Puligundla et al., 2011; Nuanpeng et al., 2010). The industrial application of VHG fermentation technology is known to reduce the energy cost associated to downstream processes such as distillation and stillage evaporation by 4% and capital and labor costs (Nuanpeng et al., 2010; Bai et al., 2008).

The results of VHG fermentation kinetic parameters (i.e. ethanol concentration, volumetric ethanol productivity, ethanol yield, and fermentation time) indicated that a very high ethanol concentration of 15% by volume could be obtained from sweet sorghum and this could potentially reduce energy used in the distillation process (Laopiaboon et al., 2009). Laopiaboon et al. (2009) compared the ethanol fermentation efficiency from sweet sorghum juice supplemented with sucrose or sugarcane molasses using *S.* cerevisiae NP01. Yeast extract and peptone or ammonium sulphate were used as nitrogen sources for ethanol production. The fermentation process was done in batch mode at 30 °C under static conditions. They achieved higher total soluble solids (up to of sweet sorghum juice to VHG levels with sucrose as adjunct as compared to using molasses (320 g/l). The results obtained was recently applied to develop kinetic models to demonstrate ethanol fermentation from sweet sorghum juice using VHG technique in the batch operation, continuous operation, and fed-batch operation modes (Thangprompan et al., 2013). Ethanol concentration of 90 g/l was obtained using a dilution rate of 0.01 /h from the continuous batch mode and 96 g/l from fed-batch production at quasi-steady state if the rate of change of their concentration are negligibly small compared to the overall rate of reaction over duration (Goeke and Walcher, 2013).

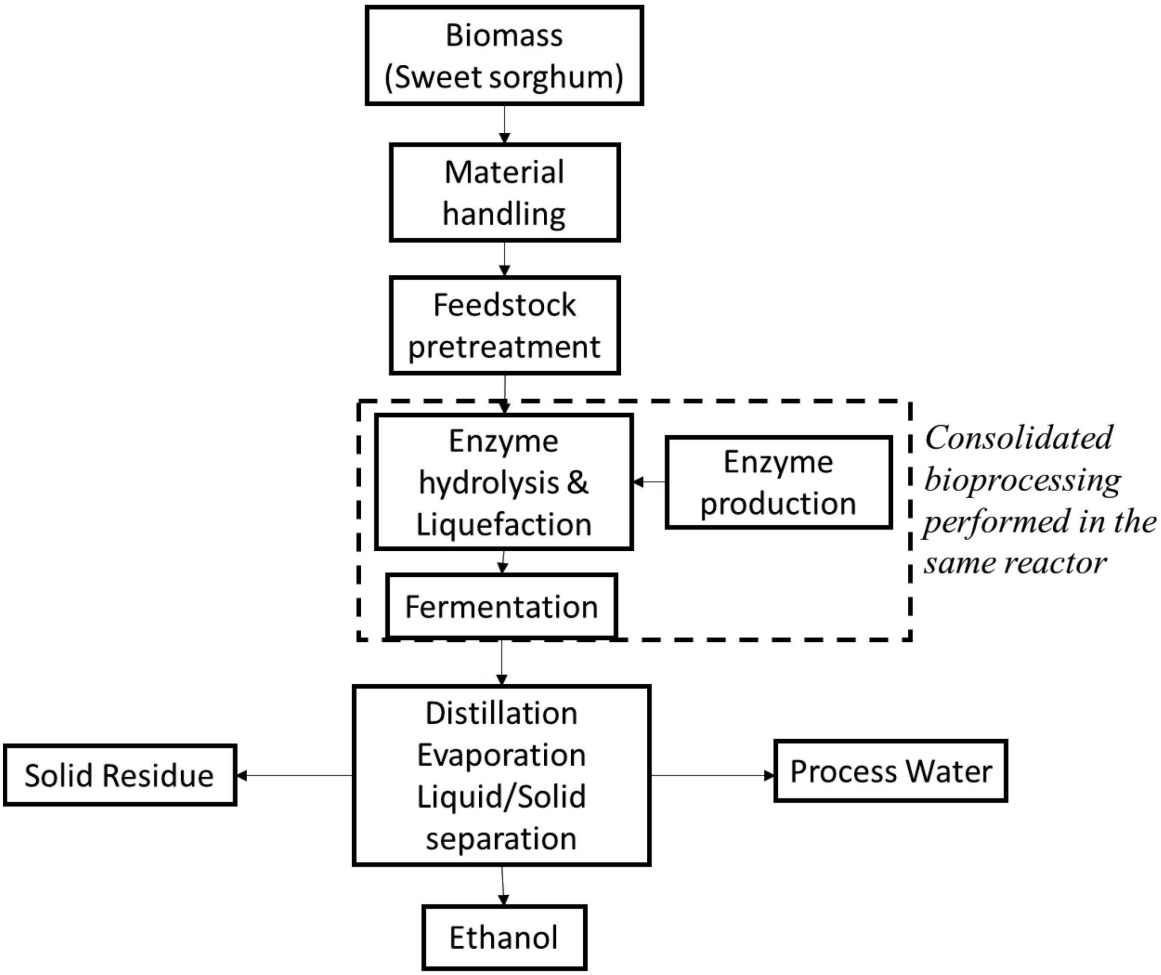
2.6.6 Sweet sorghum biomass (SSB) to biofuel

The production of biofuels from cellulosic biomass faces significant technical challenges. Success depends largely upon the physical and chemical properties of the biomass, pretreatment methods, effective enzyme systems, efficient fermentation microorganisms, and optimization of processing conditions. Pretreatment, enzymatic hydrolysis, and fermentation are the three major steps for ethanol production from lignocellulosic biomass. Pretreatment of lignocellulosic biomass is crucial before proceeding to hydrolysis (Brown and Brown, 2014; Cort, et al., 2010). The aim of pretreatment is to 1) separate lignin and hemicellulose from cellulose and make cellulose more

accessible for hydrolysis; 2) disrupts lignin and redistributes it, reducing its obstruction of cellulose and hemicellulose; and 3) increase surface area of cellulose and hemicellulose to enzyme treatment (Cort, et al., 2010; Kumar et al., 2009). Pretreatment technologies may be classified into: 1) biological, 2) physical (e.g. mechanical comminution, extrusion), 3) chemical (e.g. acid hydrolysis, alkaline pretreatment, ozone, ionic liquids, organic solvents), 4) physical-chemical (e.g. steam explosion, hot water pretreatment, thermo-hydrolysis, ammonia fiber explosion, and CO_2 explosion), 5) chemical-biochemical (e.g. enzymatic techniques microbial techniques), and 6) genetic engineering (Mussatto et al., 2010; Alvira et al., 2010; Mosier et al., 2005; Taherzadeh and Niklasson, 2004; Varga et al., 2004; Teymouri et al., 2004; Sun and Cheng, 2002; Fernandez-Bolaños, 2001; Kim and Hong, 2001; Zheng et al., 1998; Van Walsum et al., 1996).

Pretreatment process may be proceeded with enzymatic hydrolysis and fermentation in a single reactor. A cost effective way for cellulosic ethanol production is to combine enzyme production, enzymatic hydrolysis, and fermentation in one step in a process known as consolidated bioprocessing (CBP) (Figure 2.3). Cellulosic ethanol via enzymatic hydrolysis is another pathway where enzymes produced in a separate bioreactor are added to the hydrolysis and liquefaction stage (Figure 2.4) (Brown and Brown, 2013). These processes utilizes cellulose enzymes in place of acid or alkaline to breakdown cellulose into hexoses and pentose.

Figure 2.3 Cellulosic ethanol from sweet sorghum biomass via consolidated bioprocessing.

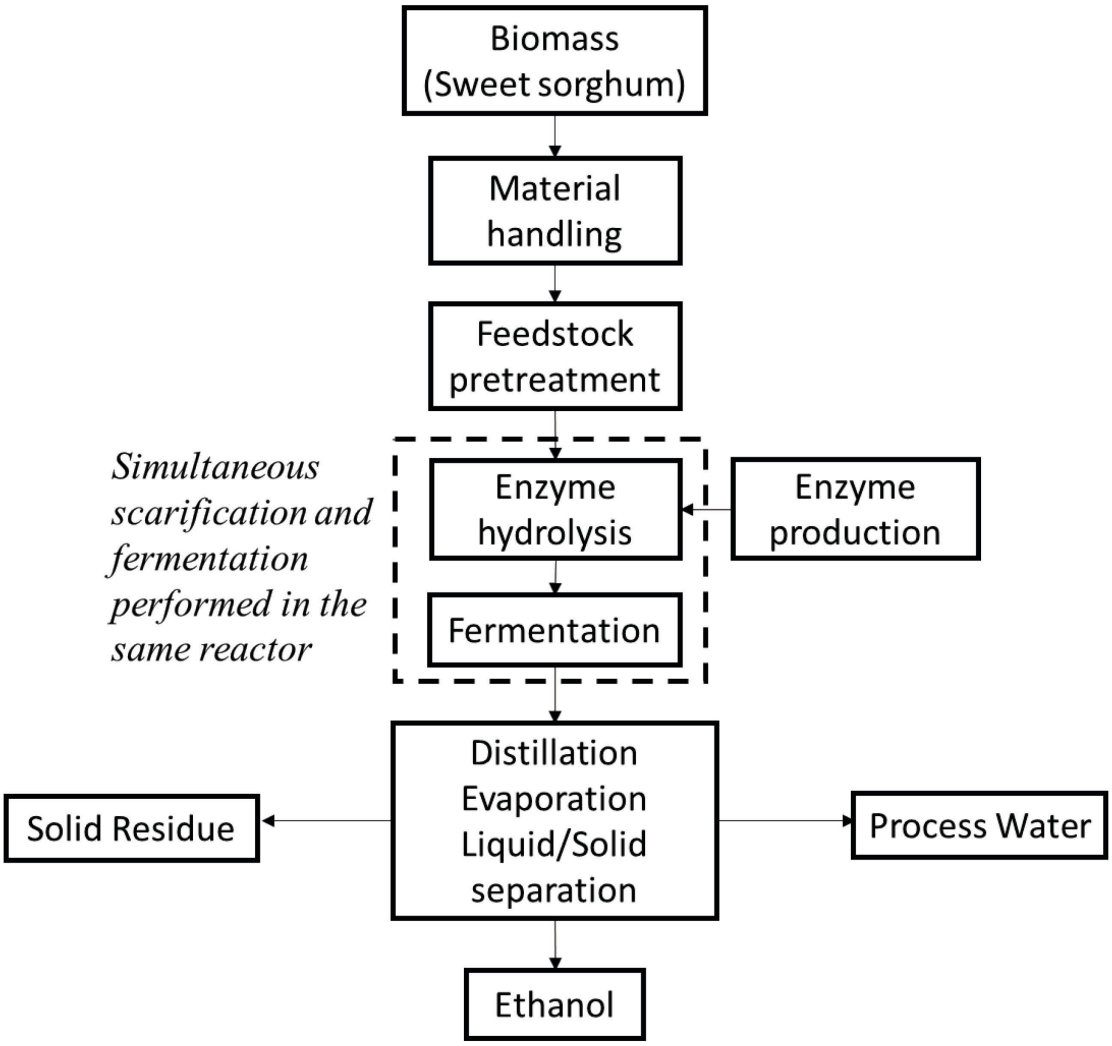


Zhang et al. (2010) compared four pretreatment techniques – dilute acid (1% w/v; at 180 °C for10 min), lime (1.5% w/v at 121 °C for 1 h), steam explosion (0.25 t/h high pressure, at 160 °C for 5 min) and ionic liquids (mixing of 5 g bagasse and 45 g [BMIM] Cl at 110°C and 120 rpm for 1 h) for enzymatic hydrolyses of sweet sorghum bagasse at 10% solid loading (w/v). Steam explosion method yielded the highest conversion efficiency with cellulose and glucose concentrations at 70% and 25g/L, respectively. The use of alkali ($Ca(OH)_2$ and NaOH) pretreatment to simultaneously optimize biomass conversion and lignin degradation from sweet sorghum bagasse was recently reported (Umagiliyage et al., 2015). The pretreatment was done by

adding NaOH and $Ca(OH)_2$ solutions (1.0 to 2.0% w/v) to ground SSB (5 to 11.7% loading) in 15 mL reactor tubes at 100 °C for 1 to 3.7 h. Optimal conditions for lime pretreatment was 1.7% (w/v) $Ca(OH)_2$ concentration, 6.0% (w/v) SSB loading, 2.4 h pretreatment time with predicted yields of 85.6% total biomass conversion and 35.5% lignin reduction. For sodium hydroxide pretreatment, 2% (w/v) NaOH, 6.8% SSB loading and 2.3 h duration were the optimal levels with predicted biomass conversion rate and lignin reduction of 92.9% and 50.0%, respectively. Other pretreatment and conversation methods have been investigated lately such as hydrothermal (Matsakas and Christakopoulos, 2013; Wang et al., 2012), extrusion (Heredia-Olea et al., 2015; Heredia-Olea et al., 2013), microwave (Choudhary et al., 2012), and ammonia fiber expansion (Li et al., 2010).

Several yeasts (*Candida, Pichia, Schizosaccharomyces*, and *Kluveromyce, Pachysole*), bacteria (*Clostridium, Bacillus, Bacteroides, Thermoanaerobacter*, and *Ervinia*), and filamentous fungi (*Fusarium, Mucor, Rhyzopus, Monilia*, and *Paecilomyces*) are capable of producing ethanol from the lignocellulos sweet sorghum bagasse (Taherzadeh and Karimi, 2007). Fungus *Mucor hiemalis* yielded 0.48 g/g of ethanol under anaerobic conditions with 81% efficiency following lime (12% (w/v) NaOH at 0 °C for 3 h.) pretreatment of 5% sweet sorghum bagasse (Goshadrou et al., 2011). In addition, genetically modified strains may include *S. cerevisiae, Zymomonas mobilis, Klebsiella oxytoca, Corynebacterium glutamicum*, and *Escherichia coli* could potentially produce cellulosic ethanol from the bagasse (Taherzadeh and Karimi, 2007).

Figure 2.4 Cellulosic ethanol from sweet sorghum biomass via enzymatic hydrolysis.



2.7 Comparison of sweet sorghum and other bioethanol feedstocks

Biomass feedstocks for producing ethanol can be categorized into three major groups: 1) soluble-sugar crops (e.g. sugar cane, sugar beet, sweet sorghum, and fruits); 2) starch-based conversion materials (e.g. corn, grain sorghum, sweet sorghum, wheat, rice, potatoes, cassava, sweet potatoes and barley); and 3) lignocellulosic biomass (e.g. woody crops, energy cane, straw, miscanthus, switchgrass, big bluestem, and crop residues) (Bonin et al., 2016; Zhang at al., 2015a; Zhang et al., 2015b; Capareda 2014; Zabed et al., 2014; Brown and Brown, 2014; Satya and Maiti,

2013; Sing et al., 2013; Balat and Balat, 2009; Wu et al., 2007; Geng et al., 1998.). Of all the bioenergy crops, sweet sorghum is the only feedstock which can be classified into all three types. Compared to other juice-containing-bioenergy crops such as sugarcane, energy cane, or sugar beet juice, ***sweets*** sorghum require less water, fertilizer and time to production (Table 2.2), Ethanol production process from sugarcane involves the clarification of the extracted juice where the juice is heated in stages in the presence of lime Ca(OH) to improve the juice quality (Quintero et al., 2008, Rein 2007). Additionally, the juice from sweet sorghum may contains amount of glucose (9-33%), sucrose (53%-85%), and fructose (6%-21%). Alternatively, sugarcane juice has higher amounts of sucrose (90-100% sucrose) and lower concentrations of glucose (0-4%) and and fructose (0-6%) fructose.

The carbohydrate (cellulose, hemicellulose, and lignin) provides structural support to the plants and resistance to biological attack. Sweet sorghum bagasse may contain 27-44% cellulose, 25.0-27.1% hemicellulose, and 11.0-24.7% lignin (Table 2.3). The bagasse is usually obtained after the juice has been extracted from the stripped sweet sorghum stalk, milled and dried to reduce the moisture content to about 10-15% (Umagiliyage et al., 2015; Serna-Saldívar et al., 2012). The chemical composition and calorific values of sweet sorghum bagasse is comparable with other lignocellulosic feedstocks (such as sugar cane, energy cane, corn stover, wheat straw, rice straw, switchgrass, miscanthus, and big bluestem) (Table 2.3).

Table 2.2 Comparison of sweet sorghum with other bioenergy feedstocks

Characteristics	Sweet sorghum *(Sorghum bicolor L.)*	Sugarcane *Saccharum officinarum L*	Sugar beet *Beta vulgaris L.*	Energy Cane *Saccharum spontaneum*	Corn *Zea mays L.*	Switchgrass *Panicum virgatum L*	Big bluestem *Andropogon gerardii*
Origin	Africa	New Guinea	Mediterranean	Asia	Mesoamerica	North America	North America
Classification	C4 warm season grass	C4 warm season grass	Biennial root crop	C4 warm season grass	C4 warm season grass	C4 warm season perennial grass	Perennial grass
Crop cycle	4-6 months	12–18 months	5–6 months	12-18 months	3–4 months	12 months	12 months
Crop planting season	All seasons	One season	One season	One season	All seasons	One season	One season
Propagation	Seed (8 kg ha^{-1})	Setts (40,000 ha^{-1})	Seed (3.6 kg ha^{-1}; pellet)		Seed (25 kg ha^{-1})	Seed (2 kg ha^{-1})	Seed
Water Management	Less water required (8000 m3 ha^{-1})	All year round (36,000 m^3 ha^{-1})	Requires water, 40–60% compared to sugarcane (18,500 m^3 ha^{-1})		Requires water (12,000 m^3 ha^{-1})	Low water required Drained soil	Low water required Drained soil
Fertilizer	Low nitrogen requirement 90N:40P:50K	Requires good management 250 to 400N: 125P:125K	Requires moderate management 120N:60P:60K	300N:150P: 150K	Requires good management 130N:60P:60K	Low nitrogen required	Low nitrogen required
Biomass/beet/ grain yield (t ha^{-1})	45-60	60–85	85–100	100	13[b]	10-25	3.2-11.4
Sugar content (%, w/w)	7-15	10–17.6[a]	15–21.8	9.8		-	-
Sugar yield (t ha^{-1})	3-7	5–12	11.25–18			-	-
Ethanol yield from juice (L ha^{-1})	5,000	4,350–7,000			5,506[b]	3,298	1,886

(Rao et al., 2013; Zabed et al., 2014;; Satya and Mait, 2013; Brown and brown, 2014; Fike and Parrish, 2009; Fike et al., 2006; Zhang et al., 2015a; Kim and Day, 2010 Reddy, 2007).
[a]Stalk yield for sugarcane and sweet sorghum; beet yield for sugarbeet; and grain yield for maize.

Table 2.3 Comparison of chemical composition of Sweet sorghum and other energy crops (dry basis).

Feedstock	Cellulose (%)	Hemicellulose (%)	Xylan (%)	Lignin (%)	Ash (%)	Calorific value (MJ/kg)
Sweet sorghum	27-44.6	25-27.1	21.5	11-24.7	0.4	18.32
Sugar cane	41.6	19-25.1	72.06-85	20.3-32	4.8	17.33
Energy cane	43.3	23.8	-	21.7	0.8	-
Corn stover	35-45.0	28.0	21.1	15-21	5-7	17.65
Wheat straw	44.2	27.3	-	19.3	3.0	-
Rice Straw	34.4-44.0	28.0	18-26	1-12	16-20.0	-
Switchgrass	36.0-43	31.6-36	25-27	17-22	5.73	18.4
Sugar beets	52.0	32.0	-	16	3.4	17.70
Miscanthus	46.0	29.0	24.1	15	2.6	17.1
Big bluestem	33.1-49.8	17.7-29.2	22.2-26	18	3.6	18.64

(Li et al., 2014; Jayapal et al, 2013; Serna-Saldívar et al., 2012; Albertson et al.,2014; Aden & NREL, 2012; Rossberg et al., 2014; Park et al., 2014; Satya and Mait, 2013; Byrt et al., 2013; Brown and Brown, 2014; Zhang et al., 2015a; Zhang et al., 2015b; Kim and Day, 2011).

Sweet sorghum grain is composed primarily of starch (~60-73%) which is comparable to that of corn and could be converted into simple sugars using enzymes and metabolized into bioethanol by microorganism (Appiah-Nkansah et al., 2016) (Table 2.4). Compared to grain sorghum, the grain yields in sweet sorghum are lower ranging from less than 1 MT/ha to over 3 MT/ha with significant differences in grain productivity between cultivars and hybrids (Corn, 2009). If this grain is fully converted to ethanol, the ethanol yields from sweet sorghum are substantially increased.

Table 2.4 Composition of cereal grains (dry basis)

Cereal Grains	Protein (%)	Fat (%)	Starch (%)	Fiber (%)	Ash (%)
Sweet sorghum grain	10-13	4	60-73	2.2	1.5
Grain sorghum	11.3	3.3	70.8	2.7	1.8
Wheat	12.2	1.9	71.9	1.9	1.7
Rye	11.6	1.7	71.9	1.9	2.0
Barley	10.9	2.3	73.5	4.3	2.4
Oats	11.3	5.8	55.5	10.9	3.2
Corn	9.1	4.4	73.4	2.3	1.4
Pearl millet	14.5	5.1	76.4	2.0	2.0
Rice	8.1	1.2	75.8	0.5	1.4

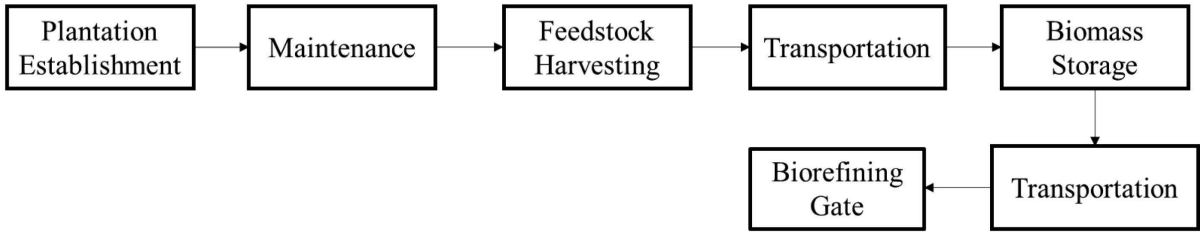
(Rainey and O'Hara, 2013, Appiah-Nkansah et al., 2016, Dendy, 1995; Wool and Sun, 2005).

2.8 Economic feasibility of production of sweet sorghum for biofuels

This section reviews several techno-economic analyses and multiple processing options, which have been conducted over the past decades to evaluate the viability of producing ethanol and co-products from sweet sorghum. These analyses were specifically focused on regional locations known for sweet sorghum cultivation such as, North-Central U.S. (Bennett and Anex, 2008), Midwest U.S. (Bennet and Anex, 2009), Southern U.S. (Daystar et al., 2014; Monge et al., 2014; Caffrey et al., 2014; Linton et al., 2011; Montross et al., 2009; Bennett et al., 2008; Lau et al., 2006), China (Liu et al., 2015; Guo et al., 2010; Gnansounou et al., 2005), India (Basavaraj et al., 2013; Rañola et al., 2007), and Australia (O'Hara, 2013). The economic viability of sweet sorghum as a bioenergy feedstock is dependent on several factors such as production cost, infrastructure cost, farm operation methods, transportation, market location, and co-products value (Bennet and Anex, 2008). Economic feasibility studies, as well, may include uncertainty factors

relating to crop yields, farm machinery operations, free sugar extraction efficiency, investments, milling machinery, boiler system and variables associated with transportation (Bennett and Anex, 2009). The sweet sorghum industrial system typically involves production, cultural practices/maintenance transportation, storage, milling, fermentation, and by-product utilization (Caffrey et al., 2014; Guo et al., 2010; Bennett and Anex, 2009). Figure 2.5 shows a supply system model considered in a techno-economic analysis of sweet sorghum as a viable bioenergy crop.

Figure 2.5. **Supply chain system for bioenergy crop – switchgrass and sweet sorghum (Daystar et al., 2014).**



Economic feasibility of ethanol production from sweet sorghum feedstock can be estimated by several economic approaches such as net present value (NPV), internal rate of return (IRR), and sensitivity analysis. NPV is an economic indicator, which is considered in making long-term investment decisions. A positive NPV shows value addition in discounted terms of the investment project whiles a negative value represents one that reduces value (Brown and Brown, 2014). Monte Carlo (stochastic variable) simulation is employed as a tool to determine probability distributions for NPV (Amigun et al., 2011). IRR is the annual compound rate of return when the NPV is set to zero. It is the average earned capacity of a project during its economic life (Basavarj et al., 2013). A viable project is one which the IRR is greater than the rate of interest. Sensitivity analysis may also be conducted to evaluate the impact of risk regarding the computation of the NPV or IRR (Perman et al., 2003). Parameters known to be highly sensitive are feedstock cost, process yield, and output market value (Brown and Brown, 2014).

A recently developed Monte Carlo probabilistic model showed a positive NPV determined that cellulosic ethanol conversion via hydrolysis (with NPV of $33 million and 67% economic feasibility) and pyrolysis (NPV $35 million and 41% economic feasibility) was economically feasible in the southern region of U.S. without any support from the government (Monge et al., 2014). The authors considered previous feedstock yields, biofuel and byproduct prices as stochastic variables. Feedstock was assumed to be harvested over a 30-mile radius and hauled as silage to the processing facilities. O'Hara (2013) conducted a techno-economic assessment which included sensitivity analysis of five bioenergy processing options of sweet sorghum feedstock in Eastern Australia: electricity production from fiber and lignin, ethanol production from juice and sugar, and fiber palletization into biomass pellets. Economic viability of the options was compared against each other using NPV and IRR. They reported positive NPVs which ranged from $289 to $483 million and IRRs of 30% to 34% which demonstrated the potential viability of the sweet sorghum biorefinery options. Bennet and Anex (2009) applied Monte Carlo simulations to model harvesting-transport-processing options for sweet sorghum as a bioethanol feedstock in the Midwest U.S. and concluded that it was cost competitive to produce fermentable carbohydrates from freshly harvested and seasonally processed sweet sorghum than from corn derived sugars. Based on a sensitivity analysis on four scenarios: 1) juice conversion to ethanol, 2) juice and bagasse conversion to ethanol, 3) processing juice to sugar, and 4) juice conversion to sugar and bagasse processing to ethanol. Gnansounou et al. (2005) suggested a flexible biorefining facility for serving both sugar and ethanol markets, and concluded that it was better to produce ethanol from bagasse than for the generation of electricity. They reported positive NPVs of all the four cases and ranged from $4.4 M to 208.7 M and IRR percentage of 8.3% to 14.8% with the option 4 yielding the highest NPV of 208.7 M and option 1 the highest IRR of 8.3%.

2.8.1 Production cost

The costs associated with the production of sweet sorghum include pre-harvest machinery (plowing, disking, planting, fertilizing, cultivating and spraying), seed, fertilizer, pesticides, crop insurance, interest on short-term loans, miscellaneous, harvest machinery, labor, land, and transportation (Table 2.5) (Brown and Brown, 2014; Linton et al., 2011). Farm production cost can also be affected by several uncertainties such management practices, soil type and fertility, topography, macroclimates, rainfall and temperature, percent of rented versus owned farmland and levels of crop insurance (Bennet and Anex, 2009)

Liu et al. (2015) conducted an economic cost and benefits analysis of sweet sorghum production as an energy crop in two locations in north east China (Wuyuan and Wudi). They considered the input including labor, machinery, diesel fuel, seeds, fertilizers, plastic film, pesticides, and water for irrigation and output including grain, fiber, and straw. Sweet sorghum production cost was low at Wudi due to lower external input (manpower (33%), machinery (16%), diesel (14%), fertilizers, seeds, plastic films, and insecticides), resulting in higher net returns and a benefit-cost ratio of 2.36. The cost of production was relatively high at Wuyuan due to high costs associated manual labor (64% of total production cost), machinery, diesel, and insecticide uses. Manual labor for both sites constituted planting, thinning, manual weeding, and harvesting activities. Bennett and Anex, (2008) applied Monte Carlo simulations and sensitivity analysis to estimate the cost of producing sweet sorghum as a viable ethanol feedstock in the North-central U.S. The authors considered uncertainties such as crop yields, machinery operations, and process efficiency in their cost estimation. Harvesting scenarios evaluated in their study included 1) 4-row self-propelled forage harvester and 2) sensitivity analysis reported indicated that uncertainties

associated with yield, harvest fields capacity had the greatest effect on net farm-gate production cost.

Table 2.5 Cost of production for sweet sorghum.

Item			Expenses		
			Variable ($/ha)	Fixed ($/ha)	Total
Pre-harvest machinery (hired labor included)			5.34[b] 6.68[c]	7.20[b] 7.33[c]	16.45[a] 12.54[b] 14.01[c]
17′ tandem disk, 105 hp tractor			4.58[b] 5.94[c]	4.47[b] 4.54[c]	12.54[b] 10.49[c]
21′ Field cultivator, 105 hp tractor			4.58[b] 11.28[c]	4.47[b] 12.22[c]	9.04[b] 23.50[c]
8-Row planter, 105 hp tractor			9.53[b] 6.20[c]	12.01[b] 5.60[c]	21.54[b] 11.79[c]
8-Row cultivator, 105 hp tractor			4.84[b] 1.45[c]	5.50[b] 1.62[c]	10.34[b] 3.07[c]
450 Sprayer (herbicide), 75 hp tractor			11.12[b] 11.86[c]		11.12[b] 11.86[c]
Liquid fertilizer application			6.68[c]		14.01[c]
Seeds, chemicals, etc.	$/Kg	Kg/ha			
Seed	11.02[a] 13.23[b,c]	3.62[a] 2.80[b,c]	37.07[b,c]		37.07[b,c]
Nitrogen/ammonium nitrate	0.62[a] 0.68[b] 1.01[c]	100.00[a] 44.8[b,c]	30.64[b] 45.47[c]		30.64[b] 45.47[c]
Phosphorus (P_2O_5)	1.01[a] 0.82[b] 1.10[c]	40.31[a] 67.2[b,c]	54.86[b] 74.13[c]		54.86[b] 74.13[c]
Potash (K_2O)	0.97[a] 0.51[b] 0.60[c]	20.00[a] 67.2[b,c]	34.10[b] 40.03[c]		34.10[b] 40.03[c]
Herbicides Atrazine 90DF	6.86[a] 0.60[c]	 2.24[a] 67.2[c]			
Lime			17.30[b,c]		17.30[b,c]
Insecticides Dipel ES					21.05[a]
Herbicide application			79.07[b,c]		79.07[b,c]
Crop insurance			17.30[b,c]		17.30[b,c]

Interest on pre-harvest variable costs		17.69[b]		17.69[b]
		20.19[c]		20.19[c]
Miscellaneous		24.71[b,c]		24.71[b,c]
Repairs and maintenance				
Harvest machinery				
implements				35.76[a]
Tractors				3.76[a]
Capital				331.28[a]
Total direct expenses				
Land rent				
% of rented land	55%[b,c]			
Cash rent equivalent	444.78[b,c]		244.63[b]	244.63[b]
			305.79[c]	305.79[c]
Transportation				
Total fixed expense			75.91[a]	
Total expense				407.19[a]
Production cost		275.40[b]	349.35[b]	624.74[b]
		398.66[c]	337.10[c]	735.76[c]

([a]Linton et al., 2011, [b]Bennet and Anex, 2008. [c]Bennet and Anex, 2009)

2.8.2 Transportation cost

Transportation involves hauling of feedstock from the field to the processing biorefining plant for ethanol production. It is known to have a major influence on the operations of a centralized biorefining industry and, thus, a reduction in transportation cost can significantly reduce the total cost of the system (Caffrey et al., 2014). Major factors that influence transportation cost may include loading and unloading costs, distance from the production field to the processing plant, truck load capacity/volume, and physical characteristics or form of the feedstock (i.e. moisture level, bulk density, etc.) (Caffrey et al., 2014; Bennet and Anex, 2009). The estimated transportation costs of fresh materials hauled to a large capacity plant (100 million gallons per year) for silage were found to be significantly high and ranged from $39 to $71/Mg for high moisture content (16% and 30%) and low plantation densities, respectively (2%). High transportation costs was due to high moisture (75% MC) and dry matter losses (Bennett and Anex,

2009). Transportation of on-farm ensiled materials to smaller plants (10 million gallons per year) was between $33 and $44/Mg for high (25.3% and 47.4%) and low plantation densities (3.2%) at a moisture content of 60%. Transportation cost per hour was assumed to be $100 for a maximum load weight of 36 Mg.

Budgetary projections, which estimates the costs, revenues, and profitability of an agricultural project, is known as enterprise budget (Bond, 2011). Using enterprise budget and on-site experimental yield data, Linton et al. (2011) estimated the cost of transporting wet sweet sorghum feedstock as a function of distance and its effect on profitability at two production sites in the South Eastern United States in three different scenarios. The cost of hauling fresh sweet sorghum feedstock over a distance of 64.37 km from the farm to processing point for feedstock crushing and juice expression was estimated to be $0.226 /t/km in the first estimate. The second estimated assumed the transportation of sugarcane which costed $1.10 /t of feedstock and $0.103 /t/km. In the third scenarios, the cost of transporting sweet sorghum was estimated at a rate of $0.696/t for first km then increased by $0.116/t/km for the next 34.12 km and finally to $5.96/t. Estimates in all the three scenarios incurred production losses which ranged from $419.27/ha to $1,002.83/ha. The haulage of the fresh biomass form from a yield estimate of 75 t/ha and 93.75 t/ha increased transportation costs and had a negative effect on the profit. Hence, the cost of transporting sweet sorghum feedstock to the processing facility for ethanol conversion can impact on the breakeven cost and play a key role in determining the economic feasibility of sweet sorghum biorefining. Transportation cost is therefore inversely proportional to the cost of production of sweet sorghum. An on-farm processing was found to be a cost-effective system (a decrease of production cost of $581/ha) for ethanol production from sweet sorghum which provides additional

income to the local farmers and contributes to the growth of the rural economy (Caffrey et al., 2014).

2.8.3 Milling cost

The objective of milling is to break up the grains into smaller particles and expose the starch granules for increased assess for enzyme hydrolysis. Milling is also applied to crush the stalk for juice extraction Bennett and Anex (2009) estimated milling equipment capital at $27 million for a 182 million liter per day ethanol capacity. Total estimated capital costs components (installation, contingency, and auxiliary facility cost) for 6,860 kW boiler for burning to 50% moisture content solid fuel were $1.6 million and a sizing exponent of 0.50. Larger milling and boiler equipment (9% annual increased plant capacity) was needed to reduce sweet sorghum fermentable carbohydrate cost by 50%.

2.8.4 Conversion cost

Caffery et al. (2014) modeled and analyzed the economic feasibility of five sweet sorghum biorefining scenarios in the Midwest US: 1) farm gate ethanol processing, bagasse used to produce heat, 2) on-farm fermentation of broth and transportation of broth to processing facility, bagasse utilized as animal feed, 3) juice-to-syrup production and transportation of the syrup to the biorefinery for rehydration and ethanol production, 4) on-farm ensiled biomass and transportation of ensiled biomass to the processing facility for cellulosic ethanol production, and 5) transportation of fresh biomass from farm to biorefinery as cellulosic feedstock. They assumed a transportation distance between the farm and processing facility to be 80.5 km. The total production cost ranged from $1,645 to 2,055/ha for ethanol production of 1,292-2,255 liters and bagasse values $38 to $63 per bone dry ton for five scenarios. The total production cost of scenario 1 was the least because of reduction in pump costs related to on-farm processing. Ethanol breakeven sales price

ranged from $0.54 to $1.07 per liter. Sensitivity analysis results could direct the path for research and development. Caffrey et al. (2014) conducted a sensitivity analysis to examine the impact of diesel fuel ($/L), fertilizer cost ($/ha), juice extraction efficiency (% weight removal), conversion efficiency (% of theoretical conversion rate), crop yield (BD ton/ha), and material losses (% weight or volume) on the breakeven sales price across all scenarios. Their results showed that further research and development on juice extraction, conversion efficiency and crop yield could impact on ethanol production costs. Decentralized ethanol conversion system was found to be economically attractive.

Basavaraj et al. (2003) assessed the economic viability of ethanol production from sweet sorghum as an alternative feedstock to sugarcane molasses in India. The motivation for their study was based on the Government of India's comprehensive National Policy on Biofuels established in 2009 which calls for the blending of at least 20% biofuels with diesel and petrol by 2017. The cost components used to estimate the total cost of ethanol production included distillery investment cost, sweet sorghum feedstock cost, distillery operations and maintenance costs, labor costs, chemical costs, power cost to operate the plant, marketing, and other related costs. Proceeds obtained from the sales of ethanol and by-products constituted the revenues. For ethanol conversation, a 40 kiloliter per day capacity plant operating for 180 days of an economic life span of 20 years was considered in their assessment. Major challenges associated with the plant included low ethanol yield due to over 24-hour delay in the sweet sorghum juice extraction and technical problems associated with processing equipment. Sensitivity analysis identified feedstock and ethanol costs as well as conversion rate (4.5%) as the key sensitive components. Based on a negative value of the NPV, the authors determined that economic feasibility of ethanol production

from sweet sorghum will require policy governmental support. Additionally, crop production and processing efficiency would also need to be improved.

2.9 Conclusion

Sweet sorghum is a unique energy crop that produces grain high in starch, stems with high juice quantity with soluble sugars and, and lignocellulose biomass. Crop improvement programs have enhanced sweet sorghum cultivars, producing hybrids with higher yields, higher sugar concentrations and increased periods of industrial use. Agronomic production systems are evolving and are unique to specific production environments. Additionally, the development of several processing pathways of producing bioenergy from sweet sorghum has been explored. Although it was economically viable to produce sweet sorghum in certain geographic regions, others places showed otherwise. On-farm processing was found to be a cost-effective system for ethanol production from sweet sorghum. Techno-economic feasibility of ethanol production of sweet sorghum may depend on improved crop production and process efficiency and governmental policy support in some places.

Chapter 3 - Incorporation of sweet sorghum juice into current dry-grind ethanol process for improved ethanol yields, energy savings and water efficiency[2]

3.1 Abstract

Sweet sorghum is a promising energy crop due to its low fertilizer and water requirements, short growth period, and high biomass yield. However, the challenge for sweet sorghum as a feedstock for ethanol production is its short harvest period and the extreme instability of its juice, both of which make achieving a year-round production process difficult. One way to solve this challenge and to meet the growing demand of bio-renewable ethanol is to incorporate sweet sorghum juice into the current dry-grind ethanol process. In the dry-grind process, the whole grain kernel is milled and fermented to produce ethanol. In this study, sweet sorghum juice with varying grain sorghum flour contents was liquefied, saccharified, fermented, and distilled to produce ethanol. Ethanol yield from sweet sorghum juice with the optimum grain sorghum flour loading was about 28% higher than that from the conventional ethanol process. Enzymatic hydrolysis with this process could be reduced by 30 min. The fermentation performance of sweet sorghum juice with grain flour using a raw starch hydrolyzing enzyme was also investigated, and ethanol yield was about 21% higher than that from the conventional process. This innovative technology enabling ethanol production from sweet sorghum juice could improve ethanol yield, save energy, and significantly decrease water use in the current dry-grind ethanol process.

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[2] Results have been published as a peer-review paper. Appiah-Nkansah N B, Saul K, Rooney W L, Wang D H. 2015. Adding sweet sorghum juice into the current dry-grind ethanol process for improving ethanol yields and water efficiency. Int J Agric & Biol Eng, 8(2): 97 − 103.

3.2 Introduction

According to the Renewable Fuels Association, the U.S. ethanol industry produced a total of 13.3 billion gallons of ethanol, representing 57% of the world's output in 2013. Over 98% of the renewable fuel produced in the same year was made from corn (RFA,2014). Ethanol production for blends such as E10, E15, E85, and mid-level blends is required to reach 36 billion gallons by 2022 according to the Renewable Fuel Standard (RFS) adopted by the U.S. Congress in 2005 and expanded in 2007 (RFA, 2014). To meet the growing demand for ethanol, potential energy crops such as wheat (George et l., 2014), hybrid poplar (Xue et al., 2014), and sweet sorghum could be integrated into current dry-grind ethanol production to help achieve the RFS target.

Sweet sorghum (*Sorghum bicolor L. Moench*) is a promising energy crop that has high water and nitrogen- use efficiency, short growing seasons (110 – 160 d), pest and disease tolerance, and high biomass productivity (45-80 t/hm^2), depending on variety and growing location (Geng et al., 1987; Rooney et al., 2007; Srinivasa et al., 2013). The thick stalk and juicy internodes maintain stem juiciness until maturity, and the plant has good residue digestibility when used for lignocellulosic ethanol production (Srinivasa et al., 2013). Fully matured stalks contain up to 70% water, and the remaining solid biomass is made of structural cellulose, hemicellulose, and non-structural carbohydrates (sucrose, glucose, and fructose) (Harrison and O'Hara, 2013). Unlike sugarcane, sweet sorghum also produces grain in the panicle and the grain represents 10%-30% of the total biomass. Sweet sorghum is not regarded as a food crop in the United States and can grow on diverse marginal lands. Sweet sorghum is drought-tolerant and can be cultivated in regions where other crops fail (Rao et al, 2013). Approximately 40 – 50% of sweet sorghum dry mass comprises fermentable sugars and starch (equivalent to corn yield of about 14 t/hm^2). If all of these

sugars and starches are converted to ethanol, potential ethanol yield could reach 5 600-6 000 L/hm^2 compared with corn ethanol yield from 4 000-4 300 L/hm^2 (Wu et al., 2013)

Sweet sorghum is considered a more efficient and cost-effective source of energy than corn because it requires less nitrogen and water (University of Kentucky, 2013). As a competitive biofuel feedstock source for ethanol production, sweet sorghum has been shown to be adaptable to environmentally friendly processing, resulting in ethanol-blended fuel with lower sulfur content and a high octane rating. In addition, an ethanol-gasoline mixture of up to 25% can be used without engine modification (Reddy et al., 2008; Reddy et al., 2005; Srinivasa et al., 2009).

The juice from sweet sorghum is extracted by mechanically crushing the stalk using roller mills or screw presses (Harrison et al., 2013; Gnansounou et al., 2005; Stevens et al., 2010). The typical composition of the fermentable juice in sweet sorghum is 53%-85% sucrose, 9%-33% glucose and 6%-21% fructose. Sugar cane juice, on the other hand, could contain 90% sucrose, 4% glucose and 6% fructose (Serna-Saldivar et al., 2010). Thus, sweet sorghum is a competitive feedstock for ethanol production. The bagasse obtained after juice extraction can be combusted to generate electricity, fodder for cattle, soil fertilizer or lignocellulosic ethanol feedstock (Stevens et al., 2010; Serna-Saldivar et al., 2010; Rohowsky et al., 2013). The greatest challenge in using sweet sorghum as a feedstock for ethanol production is its short harvest period and the extreme instability of the juice: up to 50% of total fermentable sugars in sweet sorghum juices would be lost if stored at room temperature for one week. This loss is due to the fact that microorganisms metabolize the sugars. This loss is due to the fact that microorganisms metabolize the sugars into organic acids and ethanol at room temperature (Wu et al., 2010). The lack of constant feedstock supply makes it difficult for the sweet-sorghum-based ethanol industry to achieve a year-round

production process, especially in temperate production environments. A possible solution to this problem is to incorporate sweet sorghum juice into the current dry-grind ethanol process.

The objective of this study is to develop a new processing technology for the current ethanol industry using sweet sorghum for ethanol production with improved energy, water efficiency and ethanol yield, and to meet the challenge of using sweet sorghum as an energy crop. Most ethanol plants require approximately 3 liter of water per liter of ethanol produced (RFA, 2014; NREL, 2012). Using sweet sorghum juice could significantly reduce the amount of water consumed per liter of ethanol produced and could lessen conflicts over water in the Midwest, where increasing water utilization by agricultural processing facilities, livestock operations, and urban areas heightens shortages.

In this study, the performance of ethanol fermentation by granular starch hydrolysis enzymes (GSHE) on sorghum grain flour is investigated as well. Granular starch hydrolysis, also described as native or raw starch hydrolysis, converts starch to fermentable sugars at lower starch gelatinization temperatures (Wang, 2009). Previous investigators have reported various studies on using GSHE to hydrolyze starch granules without prior cooking and liquefaction and simultaneous fermentation of sugars by yeast to produce ethanol (Wang, 2009; Robertson et al., 2006; Weller et al., 1984). It is also known that the granular starch hydrolysis process decreases energy input by 10%-20% (Robertson et al., 2006) may increase the capacity of conversion equipment because of lower slurry viscosity, and reduces the formation of undesirable Maillard reaction products (Kelsall and Lyons 2003).

3.3 Materials and methods

3.3.1 Materials

Sweet sorghum juice from sweet sorghum hybrid TX09052 was used in this study. TX09052 is an experimental sweet sorghum hybrid developed in the Texas A&M Agrilife Research sorghum breeding program. This hybrid was grown in College Station, Texas and at the soft dough stage of maturity; stalks were harvested and crushed using a three-roller mill (Ampro Sugar Cane Mill). Extracted juice was strained and immediately frozen at a temperature of -23°C. Prior to use, it was thawed to below room temperature. To separate remaining solid materials from the liquid, the juice was centrifuged by a Sorvall RC 6+ Centrifuge (Thermo Fisher Scientific, Asheville, NC) and concentrated to 18% sugar content by a vacuum evaporation process at room temperature. Cleaned grain sorghum samples were milled into flour through a 0.5 mm screen in an Udy cyclone mill (Udy Corp., Fort Collins, CO, USA) and used for ethanol fermentation.

3.3.2 Starch content analysis

The starch content of the sorghum grain was analyzed using a total starch kit (Megazyme International) following an accepted method (AACC, 2000).

3.3.3 Ethanol fermentation of varying grain sorghum loadings with sweet sorghum juice

Samples of grain sorghum flour (30.0 g dry base db) were weighed into a clean 250 mL Erlenmeyer flask and mixed with 100 mL of preheated (about 60°C) enzyme solution containing 0.1 g of KH_2PO_4 and 20 μL of Liquozyme (alpha-amylase, Novozymes, Franklinton, NC) to form an evenly suspended slurry. Additional samples of grain sorghum flour (6.0 g, 9.0 g, 12.0 g, and 15.0 g) were also weighed into clean 250 mL Erlenmeyer flasks and mixed with 100 mL of preheated (60°C to 70°C) sweet sorghum juice; each flask contained 0.1 g of KH_2PO_4, and 20 μL

of Liquozyme (240 KNU/g, about 1.15 g/mL) (alpha-amylase, Novozymes, Franklinton, NC). One hundred milliliters of sweet sorghum juice was measured into another clean 250 mL Erlenmeyer flask and mixed with 0.1 g of KH_2PO_4. For starch liquefaction, the flasks were transferred to a 70°C water-bath shaker operating at about 180 r/min. The temperature of the water bath was gradually increased from 70°C to 90°C in a 30 min period, kept at 90°C for a few minutes, and then, lowered to 85°C; liquefaction continued for 60 min. Flasks were then removed from the water bath, and materials sticking on the inner surface of the flasks were pushed back into the mashes with a spatula. The spatula and inner surface of the flasks were rinsed with 3-5 mL of distilled water. After cooling to room temperature (25°C to 30°C), the pH of the mashes was adjusted to around 4.2 with 2 mol/L HCl.

3.3.4 Preparation of Inoculum

Dry yeast was activated by adding 1.0 g of active dry yeast into 19 mL of preculture broth (containing 20 g of glucose, 5.0 g of peptone, 3.0 g of yeast extracts, 1.0 g of KH_2PO_4 and 0.5 g of $MgSO_4 \cdot 7H_2O$ per liter) and incubated at 38°C for 30 min in an incubator operating at 200 r/min. The activated yeast culture had a cell concentration of 1×10^9 cells/mL.

3.3.5 Simultaneous saccharification and fermentation (SSF)

The SSF process started with the addition of 1.0 mL of activated yeast culture, 100 μL of Spirizyme, (750 AGU/g, about 1.15 g/mL) (Novozymes, Franklinton, NC), and 0.30 g of yeast extract into mashes in each flask. Flasks were sealed with an S-airlock with mineral oil. Fermentation was conducted at 30°C for 72 h in an incubator shaker operating at 150 r/min. Fermentation performance was monitored by weighing the fermentation flasks over the 3 d incubation period at 4, 8, 18, 24, 32, 44, 56 and 72 h of fermentation. The weight loss was due to the evolution of CO_2 during the fermentation process ($C_6H_{12}O_6 \rightarrow 2C_2H_6O + 2CO_2\uparrow$).

3.3.6 Distillation

After the fermentation process (72 h), the finished mash was transferred to a 500 mL distillation flask. The Erlenmeyer flask was washed several times with 100 mL of distilled water. Two drops of antifoam agent were added to the distillation flask before the flask was placed on a heating unit to prevent foaming during distillation. Distillates were collected into a 100 mL volumetric flask immersed in ice water. When distillates in the volumetric flask approached the 100 mL mark (about 99 mL), the volumetric flask was removed from the distillation unit. Distillates in the volumetric flask were equilibrated for a few hours in a 25°C water bath. The ethanol concentration was determined by HPLC following the method described by Wu et al. (Wu et al., 2007). Fermentation efficiencies were calculated as the actual ethanol yield divided by the theoretical ethanol yield. The theoretical ethanol yield was determined using the total starch contents in the samples, assuming 0.511 g ethanol from 1 g of starch (Thomas et al., 1996).

3.4 Ethanol fermentation with granular starch hydrolyzing enzyme (GSHE)

Samples of grain sorghum flour (6.0, 9.0, 12.0 and 15.0 g) were weighed into clean 250 mL Erlenmeyer flasks. One hundred milliliters of sweet sorghum juice was also measured into another clean 250 mL Erlenmeyer flask. Flasks containing sorghum grain flour were mixed with warm sweet sorghum juice (60°C to 70°C) to hydrate the starch granules. Samples were treated with 60 μL granular starch hydrolyzing enzyme (Stargen 002, Novozymes, Franklinton, NC, USA), and pH was adjusted to 4.2 by 2 mol/L HCl. Flasks were then set in a water bath at 48°C for 2 h. The SSF process started with the addition of 1.0 mL of activated yeast culture and 0.30 g of yeast extract in each flask. Fermentation was conducted following the procedure mentioned above.

3.4.1 Statistical analysis

All experiments were performed at least in duplicate. The tabular results presented were the mean values of repeated experimental data. Regression analysis was conducted in Microsoft Excel with the linear regression function.

3.5 Results and discussion

3.5.1 Ethanol fermentation of sweet sorghum with varying sorghum grain loading

Figure 3.1 shows the comparison of ethanol yields of sweet sorghum juice with varying grain sorghum loadings. Fermentation of the juice-only sample was completed after 32 h of fermentation and yielded 11.33 % (v/v) ethanol, with a high conversion efficiency of 93.15%. Sweet sorghum juice containing 6.0 g of grain sorghum flour and the control, 32.0 g flour and water (instead of juice) had similar ethanol performance and offered comparable ethanol yields of 14.36% and 14.05% (v/v) after the 72 h, respectively (Table 3.1).

Although fermentation of the control was complete after about 65 h, the process continued for 12.0 and 15.0 g samples through 72 h. Among the grain sorghum flour samples, the 15.0 g loading showed the highest yield (18.05% (v/v)) and the lowest conversion efficiency (90.93%) (Table 3.1). Fermentation results showed that ethanol fermentation efficiency decreased as flour loading increased, corroborating the results obtained by previous investigators (Liu et al., 2013). Samples with lower starch loading would give higher fermentation efficiency, if the same amount of yeast were used for the ethanol conversion from sugar (Liu et al., 2013). Decreasing efficiencies may be attributed to higher viscosity with increasing starch content (Wu et al., 2007 Liu et al., 2013; Wang et al., 2008; Zhoa et al., 2008). Sweet sorghum juice was found to be viscous and exhibited pseudoplastic behavior (Akbulut and Özcan, 2008), ground grain sorghum mash is also known to be viscous (Zhao et al., 2008).

Figure 3.1 Comparison of ethanol yields of sweet sorghum juice (100 mL) with varying grain sorghum flour loadings

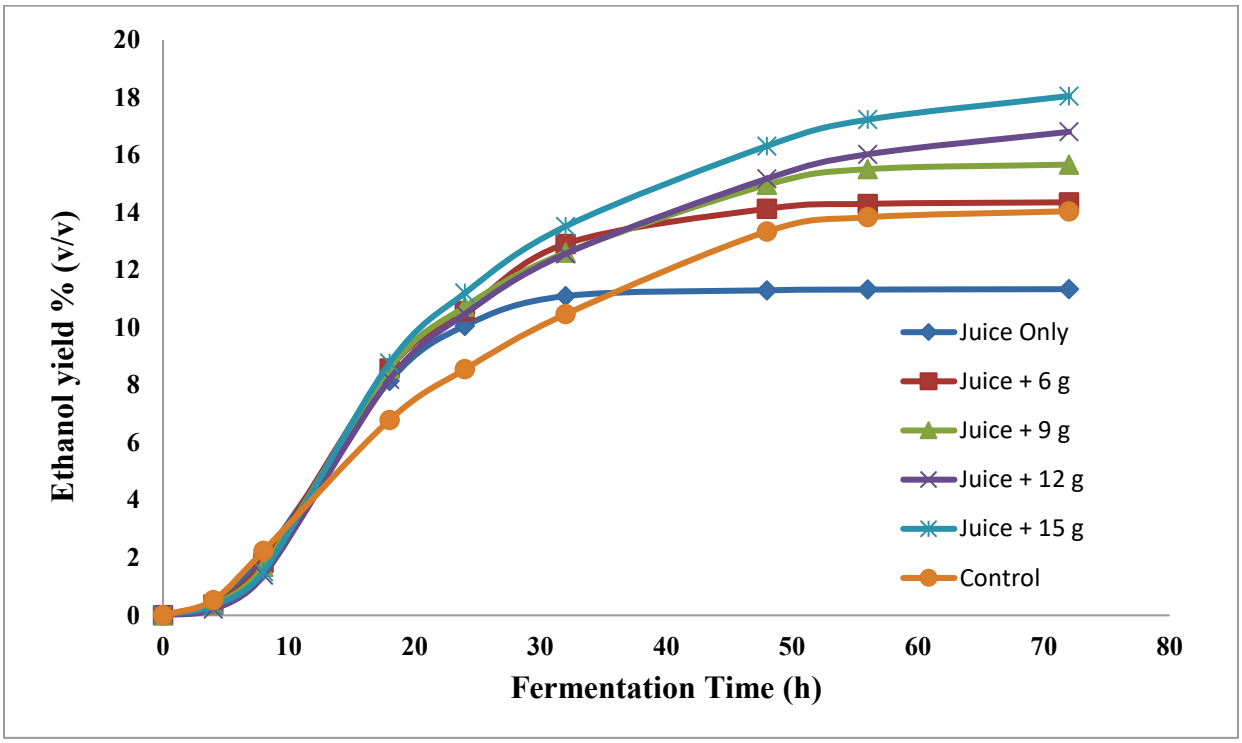

Table 3.1 Table 1 Ethanol yields and fermentation efficiencies of sweet sorghum juice with varying grain sorghum loading

	Juice sugar content %	Flour starch content	Theoretical ethanol yield % (v/v)	Actual ethanol Yield % (v/v)	Ethanol fermentation efficiency %
Juice only	18.89	0	12.12	11.29[a]	93.15[b]
Juice + 6.0 g flour	18.89	71.57	15.21	14.36[b]	94.41[a]
Juice + 9.0 g flour	18.89	71.57	16.75	15.67[c]	93.55[b]
Juice+ 12.0 g four	18.89	71.57	18.29	16.81[d]	91.91[c]
Juice + 15.0 g flour	18.89	71.57	19.95	18.05[e]	90.48[d]
Control- 30.0 g flour (db)	0	71.70	15.48	14.05[b]	90.75[d]

Means in the same column followed by different superscript letters indicate significant differences ($P \leq 0.05$)

In this study, the sample with 15.0 g of grain sorghum displayed the highest ethanol yield of 18.05% (v/v), a 28.47% increase compared with the control (14.05% (v/v)), greater than average yield from highly irrigated sorghum (14.10% (v/v)) (Liu et al., 2013), and greater than average

ethanol yield (14.44% (v/v)) from 70 sorghum genotypes and elite hybrids (Wu et al., 2007) Samples with high yields also had high conversion efficiency, which agreed with previous studies of ethanol fermentation from grain starch. The highest yield found in this study was greater than the results obtain from modified and conversional dry-grind processes using four different corn types, as published by Kullar et al., (2009). They reported the highest final ethanol yields of 15.7% (v/v) for wet fractionation, 15.0% (v/v) for dry fractionation, and 14.1% (v/v) for the conventional process. Results from this research showed that incorporating sweet sorghum juice into dry-grind ethanol production allows high gravity fermentation and therefore, results in high ethanol yield.

3.5.2 Ethanol fermentation with varying enzymatic hydrolysis times

Based on the results obtained from the above study, the optimal ethanol fermentation of sorghum mashes from sweet sorghum juice by altering starch enzymatic hydrolysis time was investigated. Four flasks consisting of homogenous slurries of 15.0 g grain sorghum flour and 100 mL sweet sorghum juice were liquefied, saccharified, and fermented by *S. cerevisiae* to produce ethanol following the above procedure. Starch enzymatic hydrolysis among the flasks was conducted for periods of 30, 45, 60 and 90 min. The ethanol yields of the samples after the 72 h fermentation period are displayed in Figure 3.2 and Table 3.2compares the yields and efficiencies. As shown in Figure 2, no significant difference in ethanol yields occurred among the four samples. Ethanol yields were comparable and ranged from 17.84% (v/v) for the 30 min hydrolysis sample to 18.05% (v/v) for the 90 min sample (Table 3.2), which corresponded to similar efficiencies of 89.12% to 90.93%, respectively. In this section, the hydrolysis time of 60 min was as the control. The difference in ethanol yields between the 30 min sample and the 60 min sample was 0.49%, and the change in yield between the 45 min and 60 min samples was 0.48%. Similar to the

graphical representation, the conversion efficiencies in Table 3.2 also demonstrated little difference among the samples.

Figure 3.2 Effect of hydrolysis time on ethanol yield from mixture of sweet sorghum juice (100 mL) and grain sorghum flour (15.0 g)

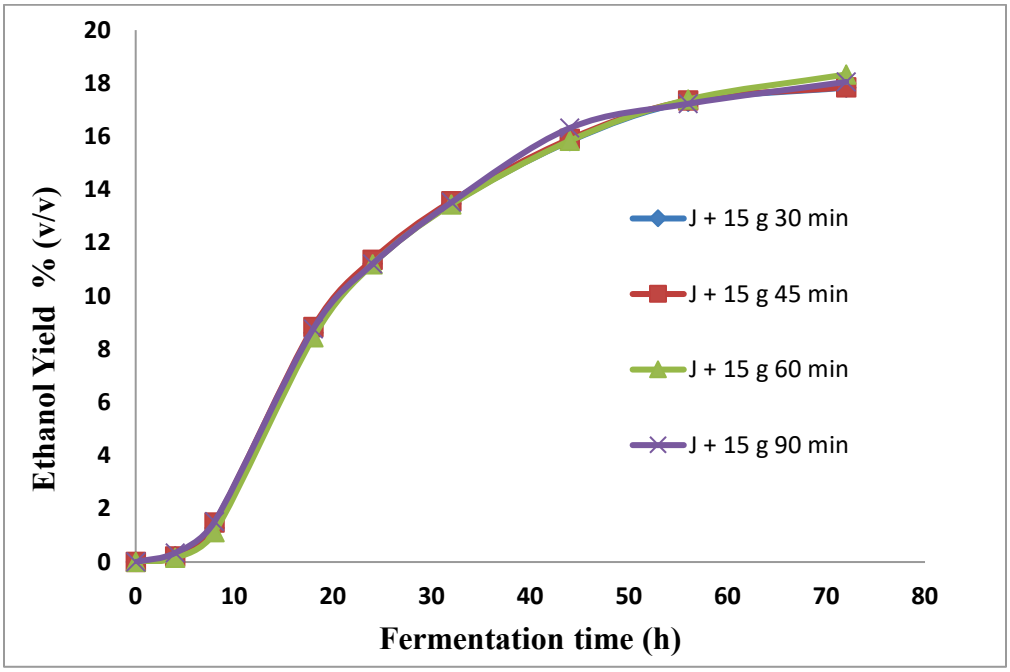

Table 3.2 Ethanol yields and fermentation efficiencies of mixture of sweet sorghum juice (100 mL) and grain sorghum flour (15.0 g) with varying hydrolysis times

Hydrolysis time/min	Juice sugar content %	Flour starch content %	Theoretical ethanol yield % (v/v)	Actual ethanol yield%	Ethanol fermentation efficiency %
30	18.89	71.57	19.95	17.84[a]	89.42[c]
45	18.89	71.57	19.95	17.85[a]	89.47[c]
60	18.89	71.57	19.95	18.33[a]	91.88[a]
90	18.89	71.57	19.95	18.05[a]	90.48[b]

Means in the same column followed by different superscript letters indicate significant differences ($P \leq 0.05$)

Results indicate that enzymatic hydrolysis for ethanol production from sweet sorghum juice with grain sorghum starch can be shortened to 30 min to save time and conserve energy in the dry-grind ethanol fermentation process.

3.5.3 Ethanol fermentation by GSHE

Ethanol yield performances of sweet sorghum juice and grain sorghum flour by the granular starch- hydrolyzing enzyme, Stargen 002, are presented in Figure 3.3. Samples had similar yield performance until after 18 h of fermentation, when differences in ethanol yields emerged. Significant differences in ethanol yields among the samples were noticed at the end of the fermentation process (72 h) and varied from 10.73% to 16.97% (v/v). Conversion efficiencies also ranged from 87.66% to 94.65% (Table 3.3). However, samples that contained 9.0 g and 15.0 g of grain sorghum flour loading showed comparable yield performance throughout the entire fermentation process. From observing the yield curves, it can be concluded that fermentation of the juice-only sample was completed in approximately 24 h and produced the lowest ethanol yield (10.73%, v/v), but the highest conversion efficiency (94.85%). The high conversion efficiency of the juice alone can be attributed to the lesser amount of sugars available for the same amount of yeast conversion to ethanol compared with the other samples. The 15.0 g loading showed the highest ethanol yield of 16.97% (v/v), representing a yield increase of 20.78% compared with the control

Results indicated that sorghum starch content had a significant effect on ethanol yield. Ethanol concentration increased with increasing sorghum flour loading. The yield obtained from this study also was slightly higher the ethanol yield produced from the modified and conversional dry-grind process reported by Kullar et al (2009).

Figure 3.3 Comparison of ethanol yield performances from sweet sorghum juice (100 mL) with varying grain sorghum flour loadings by granular starch hydrolysis enzymes

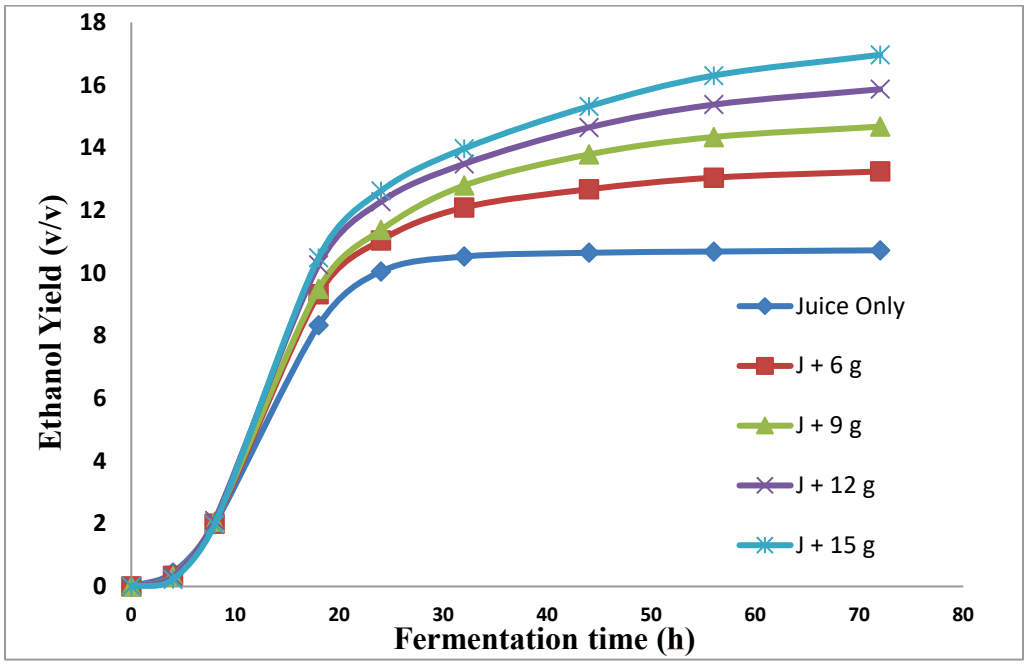

Table 3.3. Comparison of ethanol yields and fermentation efficiencies of sweet sorghum juice (100 mL) with varying grain sorghum loading by GSHE

	Juice sugar content %	Flour starch content %	Theoretical ethanol yield % (v/v)	Actual ethanol yield % (v/v)	Ethanol fermentation efficiency %
Juice only	17.5	0	11.33	10.73[a]	94.65[a]
Juice + 6.0 g flour	17.5	71.57	14.42	13.24[b]	91.82[b]
Juice + 9.0 g flour	17.5	71.57	15.96	14.67[c]	91.92[b]
Juice + 12.0 g flour	17.5	71.57	17.51	15.87[d]	90.63[c]
Juice + 15.0 g flour	17.5	71.57	19.05	16.70[e]	87.66[d]

Means in the same column followed by different superscript letters indicate significant differences ($P \leq 0.05$)

3.6 Conclusions

Results showed incorporating sweet sorghum juice into the current dry-grind ethanol process can improve ethanol yield. A potential saving of energy and increase water efficiency is expected as well. High-gravity fermentation can be applied when using sweet sorghum juice instead of water for ethanol fermentation. Ethanol yield from the mixture of sweet sorghum juice and sorghum flour was about 28% higher than from the conventional method, and ethanol yield increased as flour loading increased. The results of this study also showed that the enzymatic hydrolysis time could be reduced by 30 min, which will help conserve water and energy. In addition, sweet sorghum juice enhances the potential for ethanol production from starch-based materials by granular starch-hydrolyzing enzymes.

Chapter 4 - Ethanol production from mixtures of sweet sorghum juice and sorghum starch using very high gravity fermentation with urea supplementation[3]

4.1 Abstract

Very high gravity (VHG) fermentation, a recently developed technology for higher bioethanol productivity has shown to be eco-friendly, and economical in both ethanol and beer production. The objective of this research was to study the ethanol production dynamics using mixtures of grain sorghum flour and sweet sorghum juice at VHG fermentation. The effect of the ratio of grain sorghum and sweet sorghum juice and sugar concentrations on ethanol fermentation performance with and without urea supplementation was studied. Results showed that 20.25% (v/v) of ethanol and up to 96% fermentation efficiency could be obtained from ≈ 33% (w/v) dissolved solids. The results also showed that the optimum sugar ratio of grain sorghum and sweet sorghum juice (18% sugar) for VHG fermentation is 1 to 1 (sugar to sugar).

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[3] This chapter has been submitted for publication as a peer-reviewed research paper to the Journal of Industrial Crops and Products.

4.2 Introduction

Biofuel processing technologies capable of increasing ethanol productivity, cost-effectiveness, energy saving, and water efficiency in the current dry-grind ethanol processes would contribute significantly in meeting the growing demand for fuel for commercial transportation. Very high gravity (VHG) fermentation, an evolving fermentation process, has shown to be environmentally friendly, high yielding, and cost-effective in both ethanol and beer production, (Udeh and Kgatla, 2013; Yu et al., 2012; Puligundla et al., 2011; Nuanpeng et al., 2010; Laopiaboon et al., 2009). VHG fermentation is ethanol production using mashes containing 27 g or more of dissolved solids per 100 g mash (Wang et al., 2007). The theory of VHG fermentation was developed in the 1990s from the concept of high gravity (HG) fermentation (initiated in the 1980s) (Puligundla et al., 2011; Udeh and Kgatla, 2013; Russell, 2003). The advantages of VHG fermentation include a potential reduction by over 50% in process water requirement due to high solid mash preparation; low risks of bacterial contamination because bacteria cannot thrive under higher osmotic conditions; higher enzymatic activities due to decreased starch-to-water ratio; and increased productivity and rate of fermentation in the product (Nuanpeng et al., 2010; Puligundla et al., 2011). Industrial application of VHG fermentation technology is known to reduce the energy cost associated with downstream processes such as distillation and stillage evaporation by 4% as well as capital and labor costs (Nuanpeng et al., 2010; Bai et al., 2008). It was also been recently suggested that concentrating the sugar content in sweet sorghum juice to about 110 g/L could save energy in distillation process (Nghiem et al., 2016).

A major challenge associated with VHG fermentation, however, is the long hours involved to complete the process. This is often referred to as stuck or sluggish fermentation (Russell, 2003; Peralta-Contreras et al., 2013). Sluggish fermentation may arise due to inadequate yeast metabolic

activity needed to completely catalyze sugars into alcohol. The high initial sugar concentrations (without nutrients), deficient amino nitrogen, low water activity, and higher ethanol concentration, result in high osmotic pressure causing high intracellular ethanol accumulation; therefore, creating stress to the yeast cells. This situation negatively impacts the yeast dynamics and physiological fitness resulting in ineffective fermentation process (Kawa-Rygielska and Pietrzak, 2014; Udeh and Kgatla 2013; Pradeep et al., 2011; Russell, 2003; Thomas et al., 1994). Long VHG fermentation duration (12 days) for wheat mash containing 36.5 g of dissolved solids per 100 mL at 17 °C using *Saccharomyces cerevisiae* has been reported (Jones and Ingledew, 1994). In another study, less than 50% of total sugars from wheat mash was fermented within 9 days (Thomas et al., 1994). Wang et al. (1998) also reported VHG fermentation duration of 120-114 hours for rye and triticale mashes containing 28.5 g dissolved solids per 100 mL at 20 °C.

Previous studies on VHG ethanol fermentation using different carbohydrates and supplementation sources have been conducted recently. Kawa-Rygielska and Pietrazk (2014) investigated ethanol productivity from very gravity maize mashes supplemented with spent brewer's yeast as a nutrient source. They achieved ethanol yield up of 142 g/dm^3 with spent brewer's yeast supplementation after 96 hours of fermentation. Pradeep et al. (2012) studied the optimization of ethanol production from finger millet mash by VHG process using *Saccharomyces bayanus.* Reddy and Reddy (2005) reported that the supplementation of 4% horse gram flour increased the final concentration to 14% ethanol, and 15% (v/v) in malted horse gram flour-supplemented medium using yeast *Saccharomyces cerevisiae* with VHG fermentation compared with the yield 11% (v/v) in the conventional process. Maximum ethanol yield ranging from 21.5 – 23.8 % (v/v) and 97.6% fermentation efficiency from >300 g/L wheat mashes has also been reported (Thomas et al., 1993; Thomas and Ingledew, 1992). It is also important to note that the

range of ethanol yield obtained in most plants around the world is 10-12% (v/v) (Puligundla et al., 2011).

Several VHG ethanol fermentation studies have been done using sweet sorghum juice (Khongsay et al., 2012; Bvochora et al., 2000; Nuanpeng et al., 2010; Laopaiboon et al., 2009), sorghum grains (Chang et al., 2011;), sweet potato mash (Zhang et al.,2010), cassava starch (Yingling et al.,2011), and finger millet mash (Pradeep et al., 2010). However, there are very limited studies on VHG co-fermenting grain sorghum starch with sweet sorghum juice. Recent studies, however, have shown that ethanol yields from sorghum grain starch co-fermented with sweet sorghum juice could be about 28% higher than the conventional method (Appiah-Nkansah et al., 2015). Grain sorghum has a comparable starch content (64.3 – 73.8%, db) to corn and it is usually blended with corn in some commercial ethanol plants in the U.S. (Nghiem et al., 2016). Sweet sorghum juice may consists of 13.7 – 15.89% sugars (Wu et al., 2010) and can directly be fermented by yeast *Saccharomyces cerevisiae* into ethanol. The juice is normally obtained by mechanically crushing harvested stalks using roller mills or screw presses (Harrison and O'Hara, 2013). In addition, extraction of the fermentable sugars and nonstructural carbohydrates from sweet sorghum biomass and grains by the diffusion method has recently been proposed (Appiah-Nkansah et al., 2016).

In VHG bioethanol production from cereal, the grain is ground, and mixed with water to form mash, which is cooked in the presence of enzyme alpha-amylase, saccharified with glucoamylase and fermented with *Saccharomyces cerevisiae* to produce ethanol. During the starch-to-sugar convention process, also known as mashing or liquefaction process, complex nitrogenous compounds and nutrients are released. However, these nitrogenous materials would need to be digested into amino acids, peptides, and free amino nitrogen (FAN), before they can be

useable by the microorganism (Peralta-Contreras et al., 2013; Thomas and Ingledew, 1990). FAN is an essential nutrient for growth and production of the microorganism (Djameh et al., 2014). Hence, mash supplementation with nitrogenous nutrients such as urea, yeast extract, and ammonium salts, under very high gravity would stimulate increased ethanol productivity conditions (Pradeep et al., 2012). Besides nitrogenous nutrients, mineral elements, which are required in very little amount–such as potassium, magnesium, zinc, calcium, and manganese–are known to be vital for growth the yeast (Udeh and Kgatla, 2013).

The objective of this research was to investigate ethanol production from mixtures of sweet sorghum juice and ground sorghum grain using *Saccharomyces cerevisiae* under VHG fermentation technique. Urea as a nitrogenous supplement was used in this study due to its cost-effectiveness as yeast nutrient in ethanol production (Jones and Ingledew, 1994). The ideal ratio of the starch and juice required for high possible ethanol yield and fermentation efficiency was studied. FAN consumption dynamics by the yeast and their effect on ethanol fermentation yield and efficiency were also studied.

4.3 Material and methods

4.3.1 Materials

Sweet sorghum juice from sweet sorghum juice provided by Texas A&M Agrilife Research Sorghum Breeding Program and regular grain sorghum provided by Kansas State University Agricultural Research Farm were used in this study. Moisture content of the ground grain sorghum was determined using American Association of Cereal Chemists and National Renewable Energy Laboratory (NREL) standard methods (AACC, 2000; Sluiter et al., 2008.) The grain flour were stored in sealed plastic bags at room temperature. To obtain the juice, stalks were harvested and crushed using a three-roller mill (Ampro Sugar Cane Mill). Extracted juice was strained and

immediately frozen at a temperature of -23 °C. Prior to use, it was thawed to below room temperature. The juice was concentrated from about 11% sugars to 14%, 16%, and 18% sugar contents by a vacuum evaporation process at room temperature using a Ratovapor (Büchi Labortechnik AG, Flawil, Switzerland).

4.3.2 Starch and sugar content analysis

Total starch content of the materials was analyzed using a total starch kit (Megazyme International) in adherence to the AACC standard method (AACC, 2000). Sugar content of sweet sorghum juice was analyzed using HPLC with a Rezex RCM Monosaccharide (300 × 7.80 mm) column and a Refractive Index Detector RID—G1362A (Agilent Technologies, Santa Clara, CA) following the method described for ethanol in section 2.7.

4.3.3 Protein content analysis

The crude protein analysis of the DDGS were completed using LECO TruMac N (St. Joseph, MI, USA) analyzer. Initially, the instrument was prepared for operation as described in the TruMac operator's instruction manual. The system was then conditioned by analyzing 3 to 5 blanks. The calibration standard used was 0.5g of EDTA. A crucible was used to weigh 0.5 g of the sample, and the sample mass and sample identification was entered into the software. The samples were run in duplicates and the percent crude protein was recorded.

4.3.4 Free amino nitrogen (FAN)

FAN in the in all the mashes were analyzed by ninhydrin-based method as described in the modified International Ninhydrin Method (The Brewery Analysis software LZV936, 2014). FAN is an important chemical property in starch fermentation which significantly correlates to fermentation efficiency.

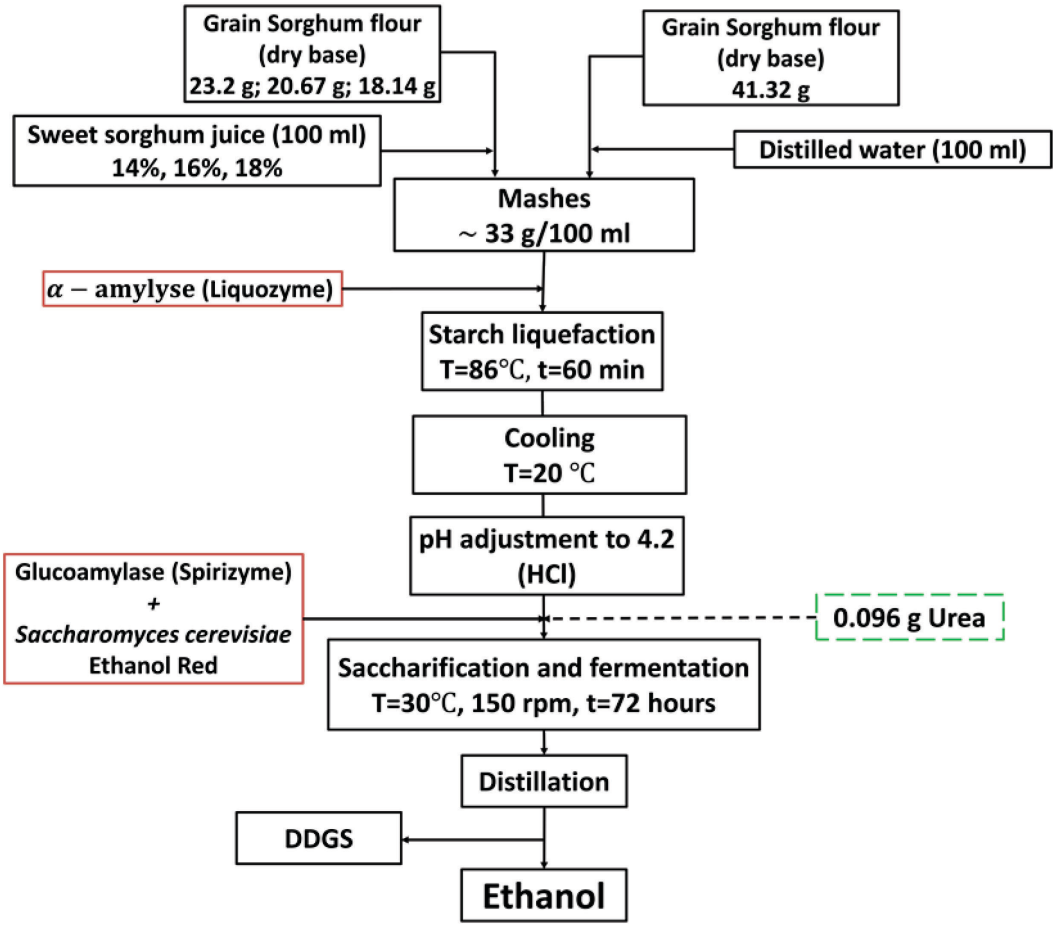
4.3.5 Mashing and liquefaction of sorghum with sweet sorghum juice

A detailed flowchart of the mashing, liquefaction, and saccharification process is shown in Figure 4.1. For mashing, sorghum grain flour 23.2 g (db), 20.67 g, (db), 18.14 g (db), and 41.32 g (db) were mixed respectively with 100 mL sweet sorghum juice containing 14%, 16%, 18%, 0% (distilled water) in 250 mL Erlenmeyer flasks to form ≈ 33% (w/v) mashes. The flour were gently dispersed in preheated juice (≈ 60-70 ℃) in 100 mL Erlenmeyer flasks, and then 27.55 µL of Liquozyme®SC DS (α—amylase 267 KNU/g, 1.266 g/mL; Novozyme Inc., Franklinton, NC) and 0.14 g of KH_2PO_4 were added. The flasks were carefully transferred into a 70 °C water-bath shaker and set to operate at 180 rpm. The temperature of the water bath was gradually increased from 70 °C to 90 °C in a 30 min period, kept at 90 °C for a few minutes, and then lowered to 86 °C. Liquefaction continued for 60 min. Flasks were then removed from the water bath, and materials sticking on the inner surface of the flasks were pushed back into the mashes with a spatula. The spatula and inner surface of the flasks were rinsed with 3-5 mL of distilled water and pH of the mashes were adjusted to ≈ 4.2 by 2 molar HCl after cooling to room temperature.

4.3.6 Microorganism and inoculum preparation

The microorganism used in this study was *Saccharomyces cerevisiae* (Lesaffre Yeast Coperation, Milwankee, WI). Dry yeast was activated by dispersing 1.0 g of active dry yeast in 19 mL of pre-cultured broth (containing 20 g of glucose, 5.0 g of peptone, 3.0 g of yeast extracts, 1.0 g of KH_2PO_4, and 0.5 g of $MgSO_4 \cdot 7H_2O$ per liter) and incubated at 38 °C for 30 min in a 12400 Incubator Shaker (New Brunswick Scientific Co. Inc., Edison, NJ) operating at 200 rpm. The activated yeast culture had a cell concentration of about 1×10^9 viable cells/mL.

Figure 4.1. Flowchart of experimental procedure for mashing, liquefaction, and SSF process.



4.3.7 Simultaneous saccharification and fermentation (SSF)

Aliquot of 1.38 mL of the activated yeast culture, 137.7 μL of Spirizyme, (750 AGU/g, about 1.15 g/mL) (Novozymes, Franklinton, NC), and 0.41 g of yeast extract into mashes in each flask. Flasks were sealed with an S-airlock with mineral oil. Another batch of samples were prepared in the same procedure, this time with the addition of 0.096 g of urea to form 16 mM urea solution per 100 mL in each flask. Fermentation was conducted at 30 °C for 72 h in a 12400 Incubator Shaker (New Brunswick Scientific Co. Inc., Edison, NJ) operating at 150 rpm. Fermentation performance was monitored by weighing the fermentation flasks over the 3-day

incubation period at 4, 8, 16, 24, 32, 40, 48, 56, 64, and 72 h of fermentation, respectively. The weight loss was due to the evolution of CO_2 during the fermentation process ($C_6H_{12}O_6 \rightarrow 2C_2H_6O + 2CO_2\uparrow$).

4.3.8 Distillation

After the fermentation process of 72 h, the finished mash was transferred to a 500 mL distillation flask. The Erlenmeyer flask was washed several times with 100 mL of distilled water. Two drops of antifoam agent were added to the distillation flask before the flask was placed on a heating unit to prevent foaming during distillation. Distillates were collected into a 100 mL volumetric flask immersed in ice water. When distillates in the volumetric flask approached the 100 mL mark (about 99 mL), the volumetric flask was removed from the distillation unit.

4.3.9 Ethanol, glycerol, and organic acid analysis

Ethanol and glycerol concentrations were analyzed by a high pressure liquid chromatograph (HPLC) with a Rezex RCM Monosaccharide (300 × 7.80 mm) column and a Refractive Index Detector RID—G1362A (Agilent Technologies, Santa Clara, CA). Twenty microliters of the sample is injected with the mobile phase which is HPLC-grade deionized water. The mobile phase with the analyte is pumped was pumped at a high pressure of ≈33-42 bar into a column packed with monosaccharides calcium ions. The elution rate was maintained at 0.6 mL/min and a column temperature of 80℃. The components (i.e. ethanol or glycerol) separated in the column were detected with the refractive index detector and quantified. The theoretical ethanol yield was determined using the total starch contents in the samples, assuming 0.511 g ethanol from 1 g of starch (Thomas et al., 1996). Fermentation efficiencies were calculated as the actual ethanol yield divided by the theoretical ethanol yield.

Organic acid concentrations were analyzed by high pressure liquid chromatograph (HPLC) with Rezec ROA-Organic acid H^+ column using 5mM H_2SO_4 solution as the mobile phase.

4.3.10 Statistical analysis

Statistical analysis were performed by using Microsoft® Excel® 2013.

4.4 Results

4.4.1 Effect of grain starch and juice ratios on ethanol production

The ethanol fermentation profile and fermentation efficiencies of mashes with and without urea supplementation are represented in Figure 4.2 and Figure 4.3, respectively. Ethanol production was estimated based on the weight loss of samples during the 72 hours due to the release of CO_2. The plateau of the fermentation performance curve (Figure 4.2) suggest that ethanol production of all the supplemented mashes were completed within the first 48 h and yields ranged from 19.92 to 20.25% (v/v). Supplemented mash containing 18% sugars produced the highest ethanol yield of 20.25% (v/v) and about 96% conversion efficiency (Figure 4.3). It can also be observed (Figure 4.2) that sample containing 0% sugar concentration (i.e. no sweet sorghum juice) completed faster than the other counterparts and produced 97% of the total ethanol within the first 40 h of fermentation.

As expected, mashes not supplemented with urea generally displayed slow fermentation process rates with the final ethanol yield after 72 hours ranging from 16.59 – 19.47% (v/v). The mash containing no juice was higher amount of ethanol (19.49 % (v/v)) with 91.11% fermentation efficiency compared to the other non-supplemented counterparts.

Figure 4.2. Effect of the ratios of grain sorghum flour and sweet sorghum juice on ethanol yield (with and without urea supplementation) during VHG fermentation (33% mashes, v/v).

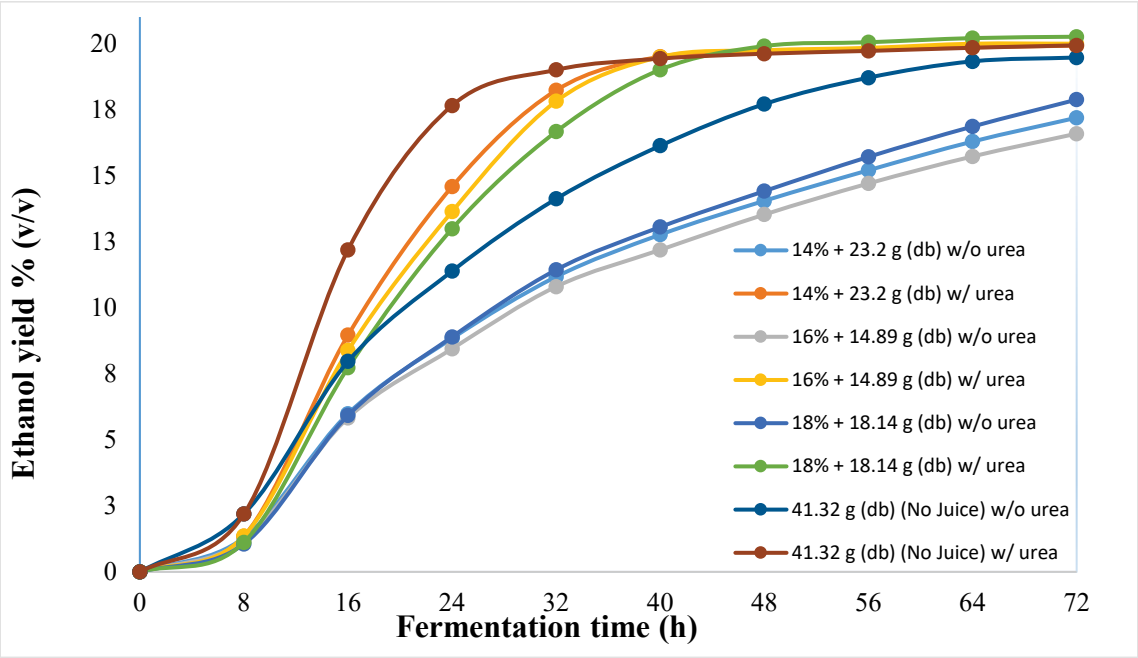
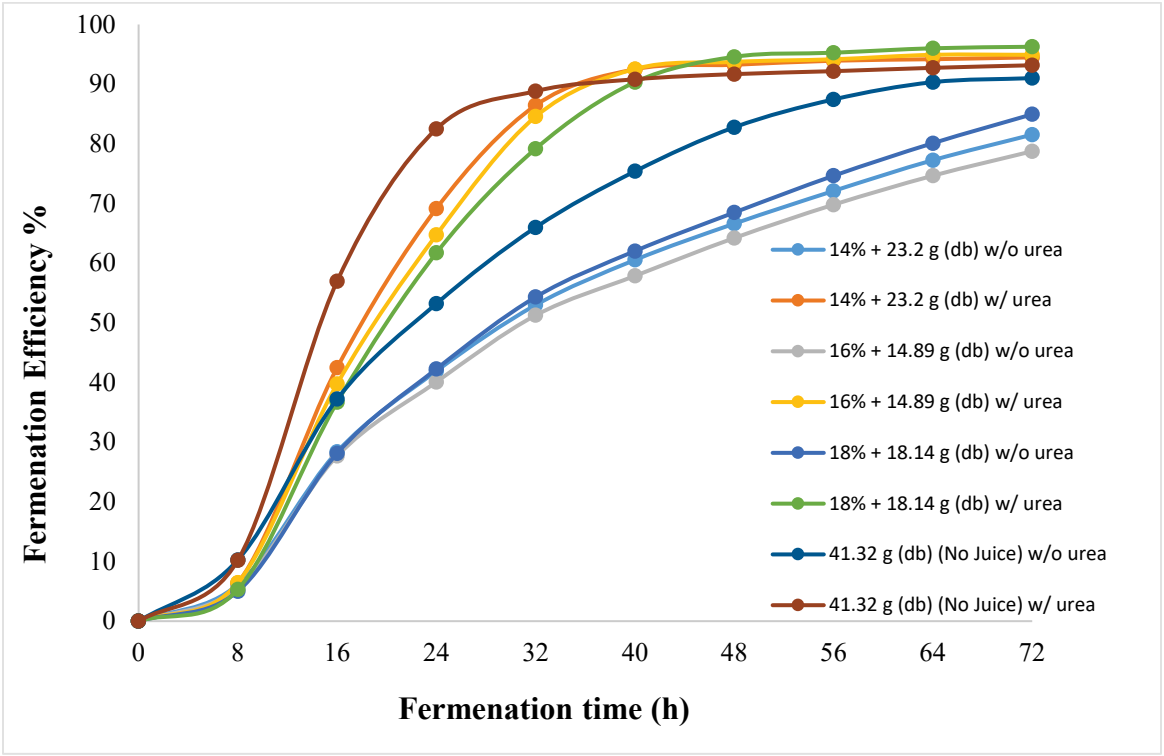


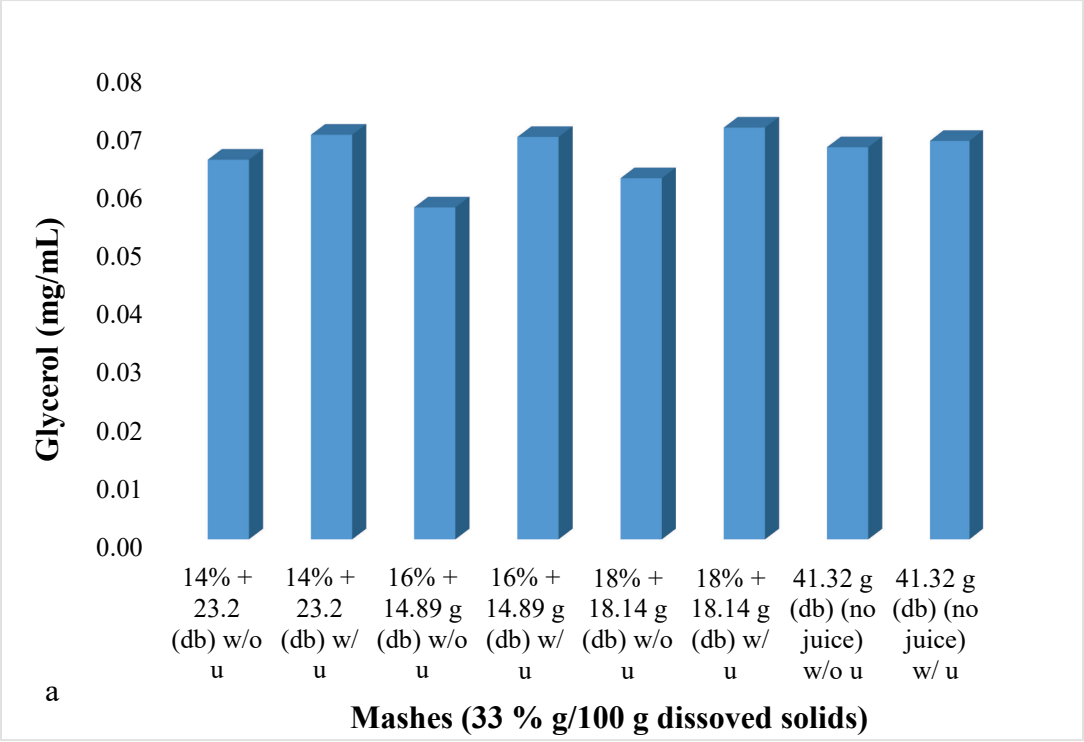
Figure 4.3. Effect of the ratios of grain sorghum flour and sweet sorghum juice on ethanol efficiency (with and without urea supplementation) during VHG fermentation (33% mashes, v/v).



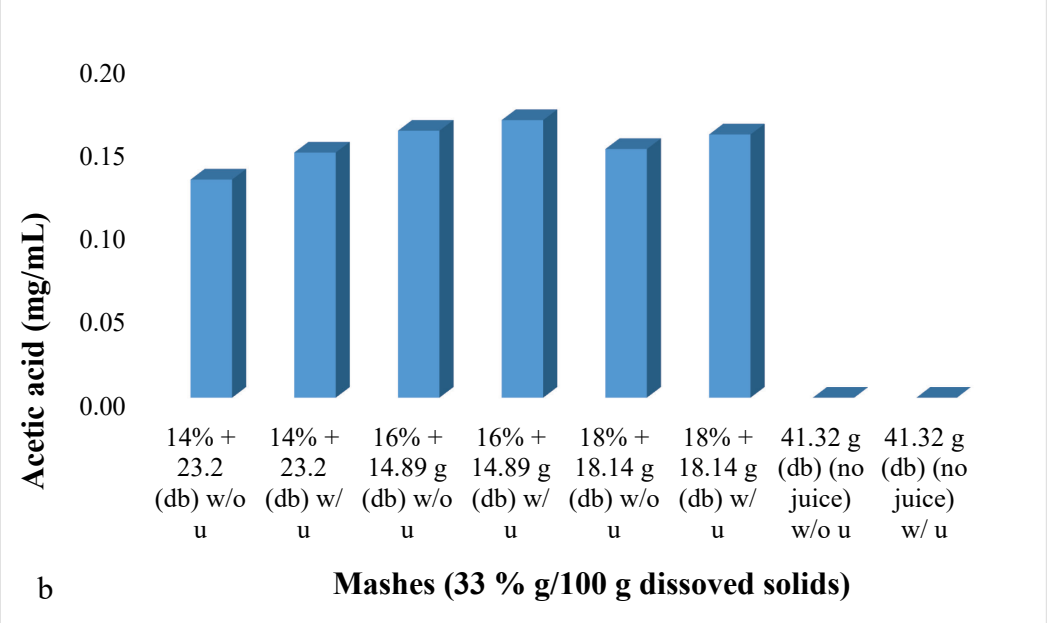
4.4.2 Co-products of yeast metabolism

The glycerol and acetic acid produced at the end of the fermentation period ranged from 0.05 – 0.07 mg/mL and 0.00 to 0.16 mg/mL, respectively and represented in Figure 4.4 It can be observed that supplemented mashes produced slightly higher amounts of co-products than non-supplemented ones.

Figure 4.4 Glycerol and acetic acid as co-products generated during VHG fermentation of grain sorghum flour and sweet sorghum juice at various ratios, a-glycerol and b-acetic acid.



a



b

4.5 Discussions

4.5.1 Ethanol yields and fermentation efficiencies

In this study, ethanol fermentation process performance under VHG from grain sorghum starch mixed with sweet sorghum juice at various ratios and different juice sugar concentrations (14%, 16%, 18%, and 0%) with and without urea supplementation (16mM) was investigated. All samples contained mashes made up with sugar concentrations of about 33 g of dissolved carbohydrates per 100 ml. Increased ethanol fermentation production was achieved by adding urea to fermentation slurries. The mashes prepared with 18% sugar concentration yielded the highest ethanol yield and fermentation efficiency for the mashes with urea supplement. It is important to note that after 48 hours of fermentation, 98% of the total ethanol was produced by supplemented sample containing 18% sugars. Ethanol yields from supplemented mashes were remarkably higher than the non-supplemented ones. After 72 hours, ethanol yield from the 18% sugar content was about 13% higher than its non-supplemented counterpart. Besides higher ethanol yields,

supplementing the sweet sorghum and juice mashes also reduced the fermentation time. On the average, fermentation process for the supplemented mashes was completed within the first 40 hours. Hence, maximum ethanol yields (19.93 – 20.25% (v/v)) were obtained in less than two days of fermentation with high ethanol efficiency (93.17 – 96.26%). In a previous study, 16 mM urea supplementation of rye and triticale mashes (28.5 g dissolved solids/100mL) accelerated sugar uptake and shortened fermentation time by 48 hours for both cereals (Wang et al., 1998). Wang et al., achieved ethanol yields of 409 L/tonne and 417-435 L/tonne for rye and triticale grains respectively. Ethanol efficiency ranged from 89 to 90% with urea supplementation. In this present work, the rate of sugar consumption was faster within the first 24 h in urea-supplemented mash with 0% sugar content. This might be due to the higher assimilable nitrogen content of the grain sorghum and further explained in section 4.3.3. The addition of 0.96 g/L urea during VGH fermentation (300 g glucose/L) was also found to have played an important role of increasing the overall metabolic heat growth and protecting the yeast from osmotic stress due to higher carbohydrate concentrations in the medium, and enhanced the production of ethanol (Theerarattananoon et al., 2008). The high efficiency obtained in this work suggested that more sugars were consumed in a shorter period. Thus, for maximum ethanol production from VHG grain sorghum and sweet sorghum juice, a ratio of 50% grain sorghum to 50% sweet sorghum juice (18%) could be considered. The sweet sorghum juice may be concentrated by the addition of syrup as suggested by Nghiem et al., (2016).

The sluggish fermentation performance as observed in the non-supplemented mashes could be attributed to inhibition of yeast growth and development of osmotic stress conditions. The ethanol fermentation profile curve suggested that the process had not been completed would have continued after the 72 hours. Among the non-supplemented mashes, that which contained no juice

yielded the highest amount of ethanol (19.47% (v/v)), which was 9% higher than mashes with 18% sugar concentration. The high yield obtained from the whole grain sorghum flour mash could be attributed to the grain protein digestibility as further explained in section 4.5.3. It has been already established that besides starch content, protein digestibility was found to positively affect ethanol production from grain sorghum (Wang et al., 2008, Wu et al., 2007).

4.5.2 Co-products of yeast metabolism

Glycerol, an important co-product of yeast alcoholic fermentation helps in the maintenance of cells redox balance and also serves as an osmoprotectant thus helps in maintaining high viability of yeast cells under VHG conditions and is usually produced in small amounts (Russell, 2003). It is synthesized in the cells in response to the osmotic stress and released into the media (Udeh and Kgatla, 2013). Glycerol and organic acids (acetic acids) amounts determined in this study showed that supplemented mashes produced slightly higher amount of these co-products compared to the non-supplemented ones. Kawa-Rygielska and Pietrzak, (2014) described that during ethanol fermentation of VHG maize mashes supplemented with spent brewer's yeast, more glycerol and organic acids are produced. Similar results with the production of glycerol and organic acids were also observed during the VHG fermentation of wine musts supplemented with ammonium sulfate (Bely et al., 2003). In this work, small concentrations of acetic acid (0.13-17 mg/mL) were observed at the end of the fermentation of studied mashes.

4.5.3 Impact of different factors on production of ethanol

Different factors such as inoculation size, temperature, and free amino nitrogen (FAN) in combination with the supplementation impacted ethanol fermentation process.

4.5.3.1. Inoculation size

Inoculum concentration also known as pitching (cells/mL or cells/g) is known to affect sugar consumption rate and ethanol productivity (Zabed et al., 2014). In our work, the activated yeast culture had an initial inoculation size of 10^9 cells/mL, which may have contributed to the shortened ethanol fermentation duration of 16 mM urea-supplemented mashes by facilitating the rapid consumption of sugars as suggested Figure 4.2. In a recent study, a 6.0% increase in yeast density reduced the fermentation time from 72 to 48 h due to intense cell multiplication (Zabed, et al., 2014). In other research, lower pitching (15 or 30 $x10^6$ cells/mL) prolonged ethanol fermentation process (>168 hours) whiles obtaining 17.4% (v/v) ethanol yield from VHG wheat mashes (35% dissolved solids w/v) without nutrient supplementation. However, when the pitching rate was increased to 75 x 10^7 cells/mL, an increase yield of 21.5% (v/v) with 97.6% ethanol fermentation efficiency was achieved under VHG condition (Thomas and Ingledew, 1992). Other studies have reported longer fermentation duration (>150 hours) at a pitching size of 10^6 cells/mg from VHG wheat mashes and 120 hours with 50 $x10^6$ cells/g with urea supplementation (Thomas and Ingledew, 1993; Wang et al., 1998). Yeast cells are generally considered to be the heart of any fermentation process, thus the growth and development of the cells at the highest possible cell concentration is a prerequisite for maximum ethanol productivity over a short period (Russell, 2003). Yeast cell growth and development were not monitored in this study, however, the relative sharp rise of the ethanol yields by the urea supplemented mashes suggested a very high multiplications yeast cells within the first 40 h.

Figure 4.2 suggested that pitching and the urea addition promoted cell growth and development in the media within the first 40 h and that stimulated the fermentation process. Thus, the higher the inoculation size, the more rapidly cells grow and multiply, and the faster the free sugars are metabolized into alcohol resulting in increased ethanol yield and higher conversion efficiency over a relatively short period of time. High pitching, along with the urea supplementation, contributed to the reduction of fermentation time. On the other hand, the decrease in the fermentation rates of the mashes not supplemented with urea as observed in this study suggested slow growth and multiplication of yeast cells due to osmotic stress under VHG conditions.

4.5.3.2 Temperature

Fermentation temperature is an important factor that impacts fermentation process and ethanol production. Increasing fermentation temperature (from 17 ºC to 33 ºC) reduced the process time from 120 to 40 hours of wheat mash under VHG (Jones and Ingledew, 1993). The fermentation temperature in this study was maintained at 30 °C, which is known to be the optimum temperature for *S. cerevisiae* (Zabed et al., 2014). As observed in Figure. 1, a sharp rise of the fermentation profile curve represents the higher viability of cells, which could be attributed to the operation temperature. Puligundla et al. (2011) obtained 20% (v/v) after 55 hours from wheat mashes (36.5 g/100 ml dissolved solids) with urea supplementation over a fermentation temperature of 27-30 °C. Jones and Ingledew (1993) also reported a 20.6% v/v from corn wheat mash (36.5% (w/v)) using *S. cerevisiae* at 27 °C with 55 h with 16 mM urea supplementation. Low fermentation temperature slows down the fermentation process. According to Jones et al. (1994), 44.0% of sugars were utilized in more than 240 hours producing the lowest ethanol yield at a fermentation temperature of 15 ºC. Previous investigators who applied VHG technique at low

temperatures of 20 ºC obtained yields from 11.0% (v/v) to 23.8% (v/v) on wheat, rye, and triticale grain over 48 to 230 hours and with or without yeast extract supplementation. (Thomas and Ingledew, 1990; Thomas et al., 1994; Thomas et al., 1993). It is therefore clear that keeping the fermentation temperature at 30 ºC in this present study maximized the metabolic activities of the yeast and shortened the fermentation time with urea as nutrient supplement.

4.5.3.2. Free amino nitrogen

Free amino nitrogen (FAN) is a soluble protein that has been further digested into free amino acids and small peptides which is essential for yeast development and metabolism (Djameh et al., 2014; Goldammer, 2008). In general, the amount of useable nitrogen in media is function of FAN levels. It has been suggested that the required FAN levels for VHG fermentation is 150 g/mL (Dlamini et al., 2015; Thomas and Ingledew, 1990). A wide FAN variation levels of 31-139 mg/L in wort of different sorghum grain has also been reported (Dlamini et al., 2015). The urea with the higher nitrogen content (46%) contributed to yeast growth and development. In the present work, a separate experiment was conducted to study the production and the consumption kinetics of FAN and represented in Figure 4.5. It must be noted that FAN analysis was done following the liquefaction stage (i.e 0 hours before SSF) at 24, 48 and 72 hours of the fermentation. With the exception of the mash containing 0% sugars, FAN uptake for other unsupplemented mashes occurred in first 50 hours into the fermentation Figure 4.5a The crude protein and nitrogen contents of the raw ground grain in this study were 13.07% and 2.09%, respectively and those for the DDGS were 37.12% and 5.94%, respectively Table 4.1. Hence the nitrogen content after the liquefaction and SSF process increased by about 184%. Therefore, it is clear that initial FAN level (83.71 mg/L) and rapid decline of the FAN profile of the 0% sugars non-supplemented mash is due to grain sorghum protein digestibility and the release of useable nitrogen into the media during liquefaction

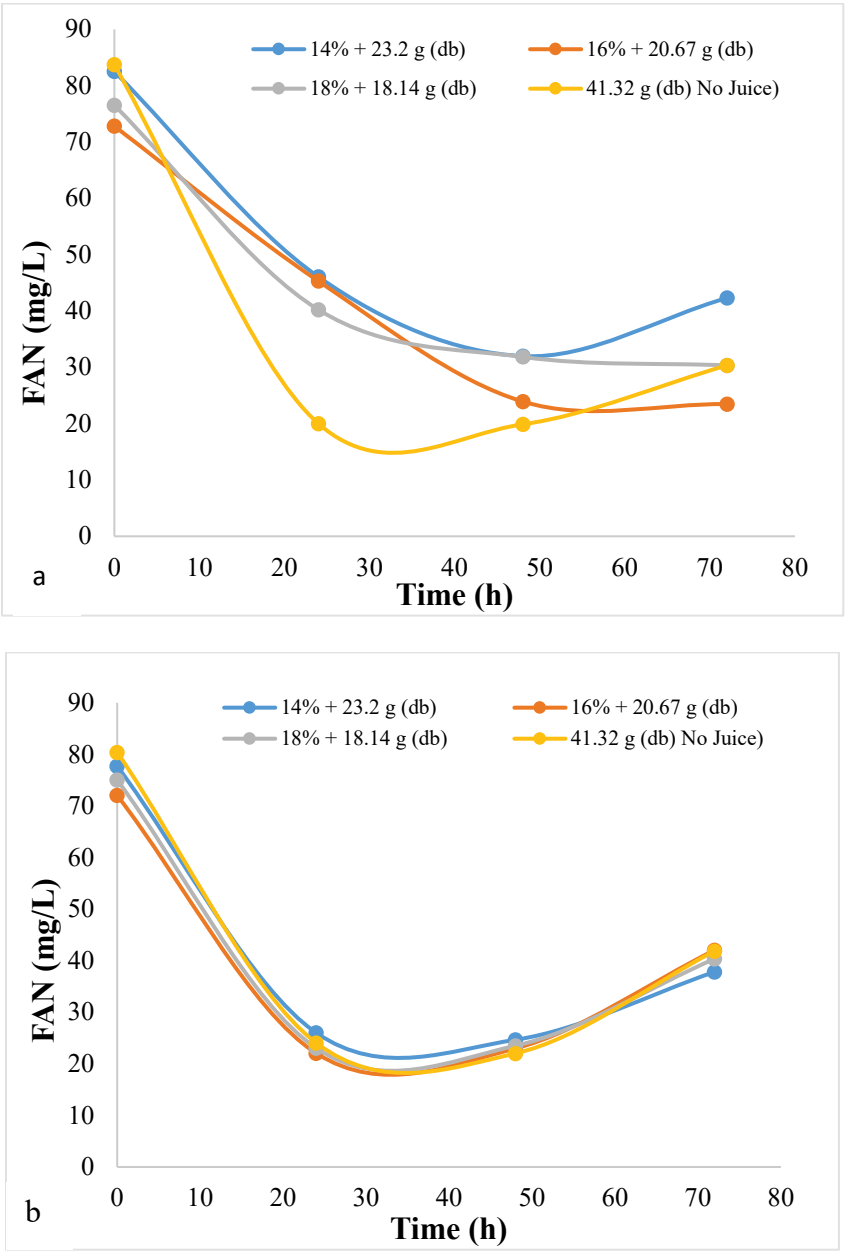
and fermentation process. This facilitated the higher yeast metabolism and high fermentation efficiency of about 91% and yield of 19.47% (v/v). High protein digestibility resulted in higher levels of FAN for yeast metabolism (Nghiem et al., 2016).

With regards to the supplemented mashes, most of the FAN uptake occurred during the first 30 hours into the fermentation for the supplemented mashes Figure 4.5b followed by a gradual rise in the production of FAN after the first 30 h. This could be attributed to the secretion of peptide enzymes from yeast cells into the media due to partial autolysis of cells (Peralta-Contreras et al., 2013; Wang et al., 1998; Thomas et al., 1993) or the release of FAN due to protein degradation inside the cell with urea supplementation (Wang et al, 1998). Additionally, the increased nitrogen and protein contents of the DDGS from the initial 13.07% and 2.09% (Table 2.1) suggested the release of nitrogen into the media for enhanced yeast activities and rapid glucose uptake resulting in the shortening of the fermentation time with improved fermentation yields and efficiencies. Similar findings were also reported by Peralta-Contreras et al. (2013).

Table 4.1DDGS Nitrogen and Protein contents (%) with and without urea supplement

Samples	With supplement		Without supplement	
	Nitrogen (%)	Protein (%)	Nitrogen (%)	Protein (%)
14% sugar + 23.2 g (db)	5.77	36.06	4.97	31.07
16% sugar + 20.67 g (db)	5.65	35.32	4.45	27.82
18% sugar + 18.14 g (db)	5.50	34.39	4.37	27.33
41.32 g (db) (No juice)	6.17	38.59	5.94	37.12

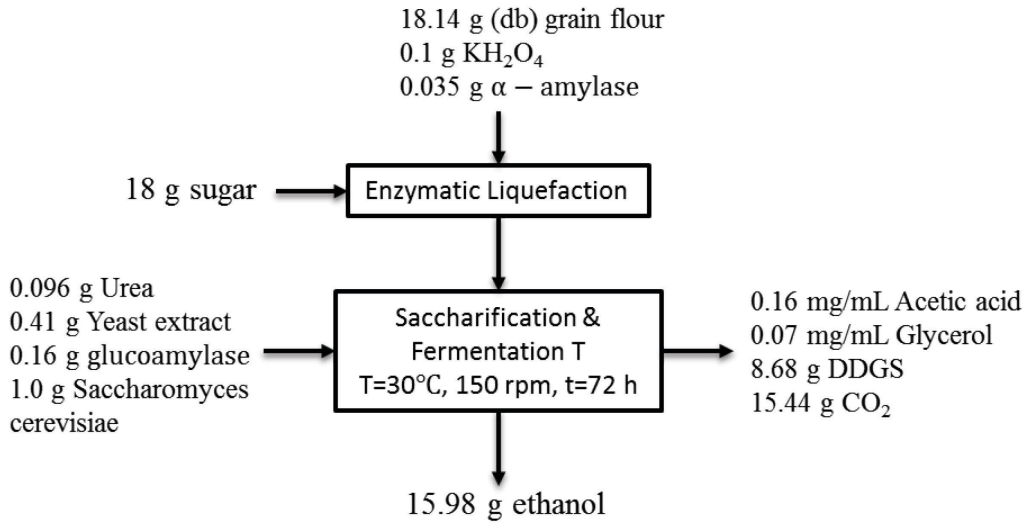
Figure 4.5. Free amino nitrogen content changes as function of fermentation time during VHG fermentation, a-with supplementation and b-without supplementation.



4.5.4 Mass balance

A mass balance was developed for the conversion of the 18.48 g grain sorghum starch and 18% sugar content sweet sorghum juice with urea supplementation as shown in Figure 4.6. About 16 g ethanol, 9 g DDGS and 15 g CO_2 were produced from 18.48 g grain sorghum and 18 g sugar content of sweet sorghum juice. 15.44 g

Figure 4.6. Mass balance of VHG fermentation with substrates containing18.48 g (db) grain sorghum and 100 mL sweet sorghum (18% sugar) with supplementation.



4.6 Conclusion

In this work, ethanol yield and ethanol fermentation efficiency from VHG mashes of ground sorghum grain and sweet sorghum juice at various ratios were investigated. The highest ethanol yield was produced by mashes prepared from sweet sorghum juice with 18% sugar content. In other words, by using VHG grain sorghum prepared with sweet sorghum juice at a ratio of 50% grain sorghum to 50% sweet sorghum juice (18% sugars) produced higher ethanol yields. Additionally, factors such as inoculum size, temperature, FAN in combination with nutrient supplementation influenced ethanol yield and ethanol fermentation efficiency. Improving the production process of bioethanol to make it more efficient using sweet sorghum juice and grain sorghum will results in better utilization of the feedstock. This will translate into cost reduction, which will make the sorghum industry more profitable and more attractive.

Chapter 5 - Model study on extraction of fermentable sugars and nonstructural carbohydrate from sweet sorghum using diffusion process

5.1 Abstract[4]

Sweet sorghum stores a high concentration of soluble sugars in its stalk and produces grain in the panicle. This grain represents a significant amount of starch. The ethanol industry currently uses sugarcane processing methods for sweet sorghum; however, sweet sorghum differs from sugarcane in that sweet sorghum produces significant quantities of grain which is predominantly starch. The objective of this research was to increase ethanol production from sweet sorghum by fully utilizing all fermentable sugars which include starch in the grain and nonstructural carbohydrates in the stalk. The diffusion process was utilized to extract fermentable sugars and nonstructural carbohydrates from chopped sweet sorghum biomass and grains. Response surface methodology (RSM) was applied in order to optimize diffusion conditions and to explore effects of diffusion time, diffusion temperature, ratio of sweet sorghum grain to total biomass on starch-to-sugar efficiency, and total sugar recovery from sweet sorghum. RSM results showed that starch conversion efficiency and sugar recovery efficiency of 96% and 98.5%, respectively, were achieved at an optimized time of 114.9 min, temperature of 95 °C, and 22% grain loading.

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[4] Results have been published as a peer-review paper. Appiah-Nkansah, N.B., Zhang, K., Rooney, W. and Wang, D., 2016. Model study on extraction of fermentable sugars and nonstructural carbohydrate from sweet sorghum using diffusion process. Industrial Crops and Products, 83, pp.654-662

5.2. Introduction

Sweet sorghum (*Sorghum bicolor* (L.) Moench), a C_4 plant, is a unique and versatile sugar crop that can be separated into starchy grains, soluble sugar in juice extracted from the stalk, and lignocellulose biomass (Rao et al., 2013 and Blummel et al., 2009). All these components can be processed into ethanol (starch-based and cellulosic), syrup, animal feed, and electricity, as well as used as substrate for hydrogen and methane production (Antonopoulou et al., 2008, Gnansounou et al., 2005 and Li et al., 2013). The juice extracted from sweet sorghum stalks contains water-soluble nonstructural carbohydrates (sucrose, glucose, and fructose) and structural carbohydrates (cellulose and hemicellulose) (Li et al., 2013 and Serna-Saldívar et al., 2012a). The juice from the stalk may contain 20 to 50% of dry matter of the entire plant (Whitfield et al., 2012). Using modelling, Rainey and O'Hara (2013) reported that a total ethanol yield of 8130 L/ha could be achieved from sweet sorghum, assuming a total productivity of 60 t/ha of sweet sorghum consisting of 3 t/ha of grain (73% starch), 50 t/ha of stalk (15% total sugars and 15% dry fiber), and 7 t/ha of leaves (40% dry fiber). The grain yield is typically 3–7 t/ha (Rainey and O'Hara, 2013 and Rao et al., 2013), and stalk yield per hectare is 45–65 ton (Rao et al., 2013). Recent studies have shown that sweet sorghum juice could be incorporated into the current starch-based ethanol process in order to conserve water and achieve yields of 28% higher than the conventional process (Appiah-Nkansah et al., 2015). Ethanol fermentation efficiencies of sweet sorghum from juice could range from 85 to 93% (Appiah-Nkansah et al., 2015 and Serna-Saldívar et al., 2012b).

Fermentable sugar composition of sweet sorghum feedstock range from 16 to 22% as compared to sugarcane juice (12–17.6%), sugar beet juice (16%), and watermelon juice (7–10%) (Zabed et al., 2014). In addition, sugar yields from sweet sorghum have been reported to be 4–10 t/ha as compared to sugarcane (5–12 t/ha) and sugar beet (11.25–18 t/ha) (Rao et al., 2013; Regassa

and Wartmann, 2014). Sweet sorghum grain, which consists of 60–70% starch, can be hydrolyzed and saccharified into glucose and subsequently fermented to produce biofuels (O'Hara, 2013 and Rainey and O'Hara, 2013). Based on these numbers, sweet sorghum is a competitive bioethanol feedstock that could be integrated into existing sugarcane ethanol-processing plants. However, only three sugarcane plants are known to incorporate sweet sorghum crop into their facilities—Mossman Central Factory in Australia, the Triangular Sugar Mills in Zimbabwe, and U.S. Department of Agriculture pilot plant in Texas (Rainey and O'Hara, 2013, Smith et al., 1973 and Woods, 2000).

Sweet sorghum has a similar physiological structure to sugarcane, thereby allowing use of the same mechanical harvesting approach. Sweet sorghum can also be manually harvested and the stalk can be expressed in the field. In the manual harvesting process, the crop is topped and the leaves are stripped before crushing the stalk for juice extraction (Regassa and Wartmann, 2014). The topped panicle is composed of grain that is left in the field. Consequently, a significant amount of starch (60–70%) that could be hydrolyzed is left in the field.

Traditionally, juice extraction is achieved by pressing the stalk of the crop through a roller mill, but this process is slow, labor intensive, and less efficient, with juice recovery below 50% (Regassa and Wortmann, 2014 and Whitfield et al., 2012). Low juice extraction yield could be attributed to the relatively high fiber content of sweet sorghum stalk compared to sugarcane (Gnansounou et al., 2005). Another drawback associated with the milling process is sugar loss due to microbial activities (Wu et al., 2010; Whitfield, 2012). Wu et al. (2010) reported that up to 50% of total fermentable sugars in sweet sorghum is lost if the expressed juice is stored for one week at room temperature. This loss is a result of microorganisms that metabolize the sugars at room

temperature (≈25 °C), under the low pH (≈4.7) and anaerobic conditions into organic acids (lactic acid, formic acid, acetic acid), carbon dioxide, and ethanol.

Diffusion is an alternative to juice extraction from the stalk. In this process, biomass is hammer-milled to uniform particle sizes and then passed through a series of continuous hot water flushes in which the concentration of solute is continuously reduced (Rein, 1995). Thus, liquid extraction recovers the sugars from cane tissues, while the conventional milling process employs mechanical juice expression. The diffusion method is the more effective of the two methods because it can achieve very high sucrose extraction (pol/sucrose ratio of 0.988) (Rein, 1995). The diffusion system is also energy efficient and requires lower maintenance and capital costs because of lack of excessive pressure and shear forces of the roller mills (Cotlear, 2004). Typically diffusion plants include dewatering mills which utilizes approximately half of the power required in energy-intensive hammer mill (Rein, 2007).

The objective of this research was to enhance the economic attractiveness of ethanol production from sweet sorghum using technological developments in order to fully utilize fermentable sugars, starch in the panicle, and nonstructural carbohydrates in the stalk for high efficiency and low-cost ethanol production. In this work, response surface methodology (RSM) was applied in order to study the interactive effect of diffusion time, diffusion temperature, and grain loading on sugar extraction from sweet sorghum feedstock.

5.3 Material and methods

5.3.1 Materials

Sweet sorghum grain and dried bagasse were obtained from Texas A&M University, College Station, Texas, for this research. The sweet sorghum was harvested just after physiological maturity of the grain in the panicle. At this stage of growth the grain is fully developed, has

approximately 30% moisture content and stalk sugar content has peaked and will start to reduce with increased maturity. The bagasse was carefully screened to remove grain kernels using a Seedburo seed blower (Seedburo Equipment Co., Des Plaines, IL). The grain and bagasse, separately milled through a 3.99 mm screen in a Schutte Buffalo hummer mill (Schutte-Buffalo Hammermill, LLC, Buffalo, NY), were used for the diffusion test and analysis. Moisture content of the materials was determined as bagasse (4.88%) and grain flour (11.68%) using standard American Association of Cereal Chemists (AACC) and National Renewable Energy Laboratory (NREL) methods (AOAC, 2000 and Sluiter et al., 2008.) The bagasse and grain flour were stored in sealed plastic bags at room temperature, and starch content of the sorghum grain was analyzed using a total starch kit (Megazyme International) in adherence to the AACC standard method (AACC, 2000).

5.3.2 Sugar extraction by starch hydrolyses and diffusion process

To determine starch-to-sugar conversion efficiency and sugar recovery efficiency, initial fermentable sugars in the sweet sorghum bagasse were used as the control. For sugar extraction, 40 g of biomass (grain + bagasse) was weighed in a 500 mL beaker. Approximately 750 mL of distilled water was preheated in a microwave for 5 min, and 500 mL of the preheated water was measured. Approximately 400 mL of the preheated water was poured into the 1 L reaction vessel of a Parr pressure reactor (Parr Instrument Co., Moline, IL), and the biomass sample was added. The reactor is equipped with impeller mixers and a controlled heating system. The remaining ~100 mL was used to thoroughly rinse the beaker into the reaction vessel. Thirty microliters of Liquozyme® SC DS (α—amylase 267 KNU/g, 1.266 g/mL; Novozyme Inc., Franklinton, NC) was added to the content. The reaction vessel was then coupled to the Parr reactor assembly and set to run for 120 min at a set temperature of 85 °C and an impeller speed of 100 revolution per minutes

(rpm). The heating source was removed and the reactor was cooled to a temperature below 50 °C. The vessel was disengaged and 150 µL of Spirizyme® Achieve (glucoamylase >900 AGU/g, 1.161 g/mL; Novozyme, Inc.) was added to the mixture. The vessel was fixed again to the Parr reactor system and set to run for 40 min at a temperature of 60 °C; then the slurry was recovered. The described process was carried out with bagasse added together with the grain before starch hydrolysis and under varying conditions according to the experimental design presented in Table 5.1. The diffusion process was based on hot water extraction of residual sugars from the bagasse. All removed slurry was centrifuged in order to separate out the solid portions, which was filtered through a 0.2 µm membrane for sugar analysis.

Table 5.1. Experimental design with RSM

Run	**Coded variables**			**Actual experimental variables**			**Results**	
	Time (X1)	Temp (X2)	Grain (X2)	Time (min) (X1)	Temp °C (X2)	Grain (%) (X3)	Starch efficiency[b] YSE (%)	Sugar recovery efficiency[c] YSRE (%)
1	-1	0	-1	60	85	15	77.85	94.46
2	+1	0	-1	120	85	15	84.36	96.09
3	-1	0	+1	60	85	25	78.74	91.78
4	+1	0	+1	120	85	25	87.88	95.31
5	-1	-1	0	60	75	20	71.65	90.90
6	+1	-1	0	120	75	20	78.12	92.98
7	-1	+1	0	60	95	20	87.04	95.84
8	+1	+1	0	120	95	20	95.23	98.47
9	0	-1	-1	90	75	15	72.57	93.14
10	0	-1	+1	90	75	25	76.32	90.85
11	0	+1	-1	90	95	15	89.81	97.45
12	0	+1	+1	90	95	25	94.82	98.00
13	0	0	0	90	85	20	84.37	94.98
14	0	0	0	90	85	20	86.67	95.72
15	0	0	0	90	85	20	85.97	95.50

[a]Grain (%) is percentage of grain loading.

[b]Y_{SE} is the starch conversion efficiency.

[c]Y_{SRE} is the sugar recovery efficiency.

5.3.3 Sugar analysis

A high pressure liquid chromatograph (HPLC) with a Rezex RCM Monosaccharide (300 × 7.80 mm) and a Refractive Index Detector RID—G1362A (Agilent Technologies, Santa Clara, CA) were used to analyze sugars at 40 °C and HPLC water as mobile phase at a flow rate of 0.6 mL/min. Chromatograph temperature was maintained at 80 °C.

5.3.4 Experimental design and statistics analysis

RSM was applied in order to optimize diffusion conditions and explore the effect of diffusion time, diffusion temperature, ratio of sweet sorghum grain to biomass on starch-to-sugar efficiency, and total sugar recovery from sweet sorghum. The ratio of grain to biomass, also known as the harvest index, represent the biomass yield or carbohydrate that could be considered for processing (Field et al., 2008). Optimum diffusion conditions for sugar extraction were analyzed using the Box–Behnken design. Three coded levels were used for design factors: time, temperature, and grain loading. The experimental design is described by the following equation:

$$Y_i = \beta_0 + \Sigma\, \beta_i X_i + \Sigma\, \beta_{ij} X_i X_j + \Sigma\, \beta_{ii} X_{i\,2} \quad (1)$$

where Y_i is the predicted response by the response surface model, whereby Y_{SE} is starch-to-sugar conversion efficiency; Y_{SRE} is sugar recovery efficiency; β_0 is the intercept; β_i, β_{ij}, and β_{ii} represents partial regression coefficients for each linear term, interaction term, and quadratic term, respectively; and X_i represents design factors or condition parameters—time (minutes) (X_1), temperature (°C) (X_2), and grain loading (%) (X_3). Design factors were in the range of 60–120 min, 75–95 °C, and 15–25% (g grain per gram total biomass) for time, temperature, and grain loading, respectively. Table 1 shows the experiment design and corresponding values for starch conversion efficiency (%) and sugar recovery efficiency (%).

Starch-to-sugar conversion efficiency, and sugar recovery efficiency, Y_{SR}, are defined in the following calculations:

$$Y_{SE} = \frac{MG}{M_{GS} \times 0.69 \times 1.11}\ x100\% \quad (2)$$

$$Y_{SR} = \frac{S_T}{M_B + M_{GS} \times 0.69 \times 1.11} \times 100\% \quad (3)$$

where S_T is total sugars obtained after diffusion (g), M_B is mass of sugar from biomass (g), M_B used was determined based on the results of a preliminary experiment conducted using only the bagasse at 85 °C over 120 min), M_G is mass of sugar from grain (g), M_{GS} is mass of grain solid (g), 0.69 (g/g) is the starch content, and 1.11 is the mass coefficient of starch to sugar (g/g). Considering a total biomass content (grain + bagasse) of 40 g used in the experiment, the mass of grain stated also indicated the ratio of bagasse used. Hence the mass of bagasse was not included in (3).

Data obtained from the experiments (Table 1) were analyzed using analysis of variance (ANOVA), and RSM results were analyzed using Design-Expert 9.0.3.1 (Stat-Ease, Inc., Minneapolis, MN). Mean values from the experiments were reported. Global *F*-Test was used to determine statistical significance of the developed models. Adjusted multiple coefficient of determination, R_{2adj}, was computed in order to determine how well the regression model fits the data (Mendenhall and Sincich, 2012). Statistical diagnostics in Design-Expert software was used to verify model assumptions of the residuals. Independent variables were coded according to the following equation (Zhang et al., 2012):

$$x_i = \frac{(X_i - X_i^*)}{\Delta X_i} \quad (5)$$

where x_i is the coded value of the i_{th} independent variable, X_i is the actual value of the i_{th} independent variable, X_{i*} is the actual i_{th} independent variable at the center point, and ΔX_i is the step change value.

5.4 Results and discussion

5.4.1 RSM optimization of starch conversion efficiency

RSM linear and quadratic regression models were developed to investigate the effects of diffusion time, diffusion temperature, and grain loading and their interactive effect on starch-to-sugar conversion efficiency. Because the quadratic regression model fit the data better than the linear model, only the quadratic model is reported. ANOVA results for the second-order response model are shown in Table 5.2. ANOVA of the full quadratic regression model showed that the fitted model was highly significant, with Fishers' test (F-value) of 62.75 and the corresponding P-value of 0.0001 at 95% level of confidence ($P < 0.05$). Because the computed F-statistic value (62.75) exceeded the F-critical value (3.48), we rejected the null hypothesis (H_0) in favor of the alternative (H_1) based on a type I error rate (the error of rejecting a null hypothesis when it is actually true) of 5%; therefore, we concluded that a significant relationship exists between starch conversion efficiency and diffusion condition parameters. The coefficient of determination of the regression analysis, R^2 value, was 0.9912, meaning that approximately 99% of the variability in starch conversion efficiency was explained by the model. As an alternative measure of model adequacy, the adjusted multiple coefficient of determination (adjusted R^2) was 0.9754 meaning that after adjusting the sample size and number of parameters, approximately 98% of total variation in starch conversion efficiency was explained by the model which suggests a suitable fitness of the model.

Table 5.2. ANOVA of full model and reduced model for starch conversion efficiency

. Source	Sum of Squares	df	Mean Square	*F Value*	*p-value*
Model	743.22	9	82.58	62.75	0.0001
Residual	6.58		5	1.32	
Lack of Fit	3.80	3	1.27	0.91	0.5611
Pure Error	2.78		2	1.39	
Cor Total		749.80		14	
R2 = 0.9912, adjusted R2 = 0.9754					
ANOVA for the regression of the reduced model					
Model	738.31	5	147.66	115.68	<0.0001
Residual	11.49		9	1.28	
Lack of Fit	8.71	7	1.24	0.89	0.6208
Pure Error	2.78		2	1.39	
Cor Total		749.80		14	
R2 = 0.9847, adjusted R2 = 0.9762					

RSM developed a corresponding quadratic regression model for starch-to-sugar conversion efficiency, as shown in the following equation obtained for the coded factors:

$$Y_{SE} = 85.67 + 3.79X_1 + 8.53X_2 + 1.65X_3 + 0.43X_1X_2 + 0.66X_1X_3 + 0.31X_2X_3 - 1.92X_1^2 - 0.74X_2^2 - 1.55X_3^2 \quad (5)$$

Table 5.3 shows regression coefficients of processing variables in the model for starch-to-sugar conversion efficiency. As shown in Table 5.3, linear effects of time (minutes) (X1), temperature (°C) (X2), and grain loading (%) (X3), as well as quadratic effects of time (X12) and grain loading (X32) significantly impacted starch conversion efficiency ($P < 0.05$). The temperature term (X2) had the most significant effect on starch conversion efficiency, with $P < 0.0001$, indicating a stronger linear effect on the response variable (YSE). Based on F-values and P-values, the interactive terms of time and temperature (X_1X_2), time and grain loading (X_1X_3), and temperature and grain loading (X_2X_3) had a negligible interactive effect on the response ($P > 0.1$). Additionally, the quadratic term of temperature (X^2_2) had no significant effect on starch conversion efficiency ($P > 0.1$). Because interactive factors and the quadratic term of temperature were found to be insignificant, the full quadratic model was simplified and reduced to include only significant linear

terms and second-order terms, as shown in Eq. (6). The P-value (<0.0001) and corresponding F-value of 115.68 for the reduced model (Table 5.2) shows that the model is highly significant. The "Lack of Fit F-value" of 0.89 implies that "Lack of Fit" is not significant relative to the pure error.

$$Y_{SE} = 85.21 + 3.79\ X_1 + 8.53\ X_2 + 1.65\ X_3 - 1.86X_1^2 - 1.49\ X_3^2 \quad (6)$$

Table 5.3 Regression coefficients and their significance in the quadratic model for starch conversion efficiency.

Term	Coefficient Estimate	Standard Error	Mean Square	***F***-Value	***P***-Value
Intercept	85.67	0.66	82.58	62.75	0.0001
X_1	3.79	0.41	114.84	87.26	0.0002
X_2	8.53	0.41	582.09	442.31	< 0.0001
X_3	1.65	0.41	21.68	16.47	0.0097
X_1X_2	0.43	0.57	0.74	0.56	0.4872
X_1X_3	0.66	0.57	1.73	1.31	0.3036
X_2X_3	0.31	0.57	0.40	0.30	0.6065
X_1^2	-1.92	0.60	13.56	10.30	0.0237
X_2^2	-0.74	0.60	2.04	1.55	0.2680
X_3^2	-1.55	0.60	8.83	6.71	0.0488

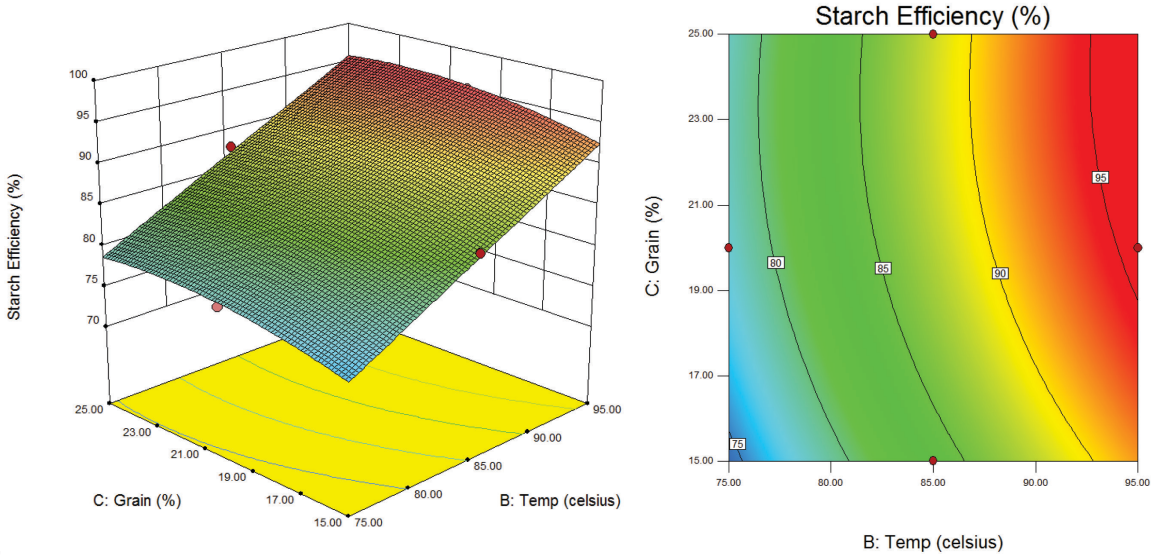
Eq. (6) is based on the coded factors and it is useful for identifying the relative impact of factors by comparing regression coefficients of individual parameters. Consequently, temperature had the highest impact on starch conversion efficiency, followed by time and grain loading, similar to full model analysis.

Figure 5.1 and Figure 5.2 represent 3D response graphs and corresponding 2D contour plots based on the fitted model for the interaction between grain loading (%) and temperature (°C), temperature (°C) and time (minutes), and time (minutes) and grain loading (%) on sugar extraction from sweet sorghum bagasse and grain. The graphs were obtained by holding three variables constant while the two variables of interest varied within their experimental range. The response surface graph was used to determine optimum levels of diffusion parameters for maximum observed response at the highest point of the surface. In the 2D contour plots, color intensity

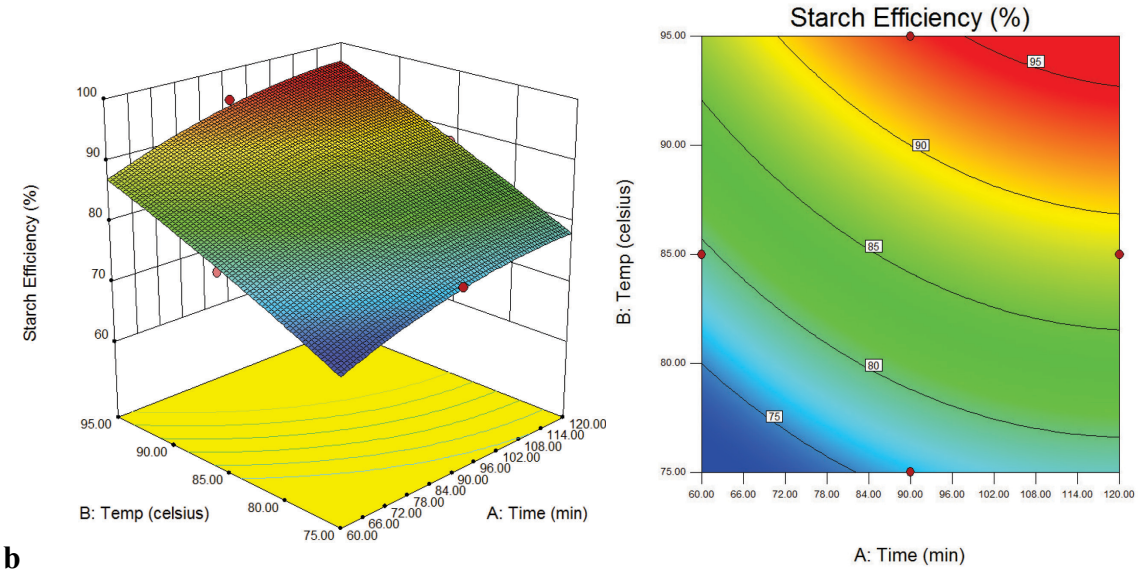
represents the observed response (y); intense color indicates the region of maximum response that was restricted in the smallest eclipse. The 3D response graph and contour plot of starch conversion efficiency as a function of grain loading (%) and temperature (°C), and temperature (°C) and time (minutes) is shown in Figure 5.2, indicating that starch hydrolysis efficiency increased as time and temperature increased. High conversion efficiency of 95% was observed at 95 °C with grain loading of approximately 22–23% at 115 min. Heating at temperatures above 90 °C caused moisture absorption and swelling of starch granules, and shear-induced disruption with stirring caused rupture, thereby exposing granules to enzymatic hydrolysis. Amylopectin crystallites may have melted, causing amylose to leach out at 95 °C (BeMiller, 2007 and Sun, 2005a) and enhancing increased enzymatic access to the granules. The 3D response surface graph and contour plot indicating the effect of grain loading and time are represented in

Figure 5.1c, in which the starch conversion efficiency was shown to increase from 71.65% to 95.23% as temperature and grain loading increased. However, high starch conversion was not observed by increasing the amount of grain loading, possibly due to increased viscosity at high starch loading. Intensity of the interactive effect was reflected by the shape of the contour lines. High temperature cooking and stirring increases starch hydrolysis (Wu et al., 2006); therefore, starch efficiency increases with increasing temperature, time, and grain loading.

Figure 5.1.3D response surface of starch conversion efficiency in relation to (a) temperature and grain loading with constant time; (b) temperature and time with constant grain loading; (c) time and grain and their corresponding 2D contour plot.



a



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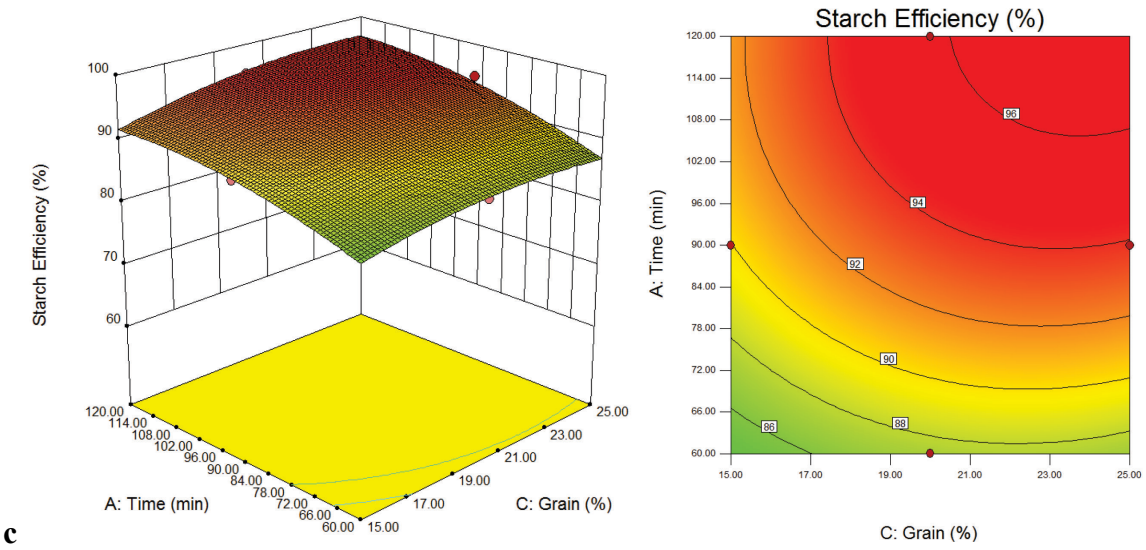
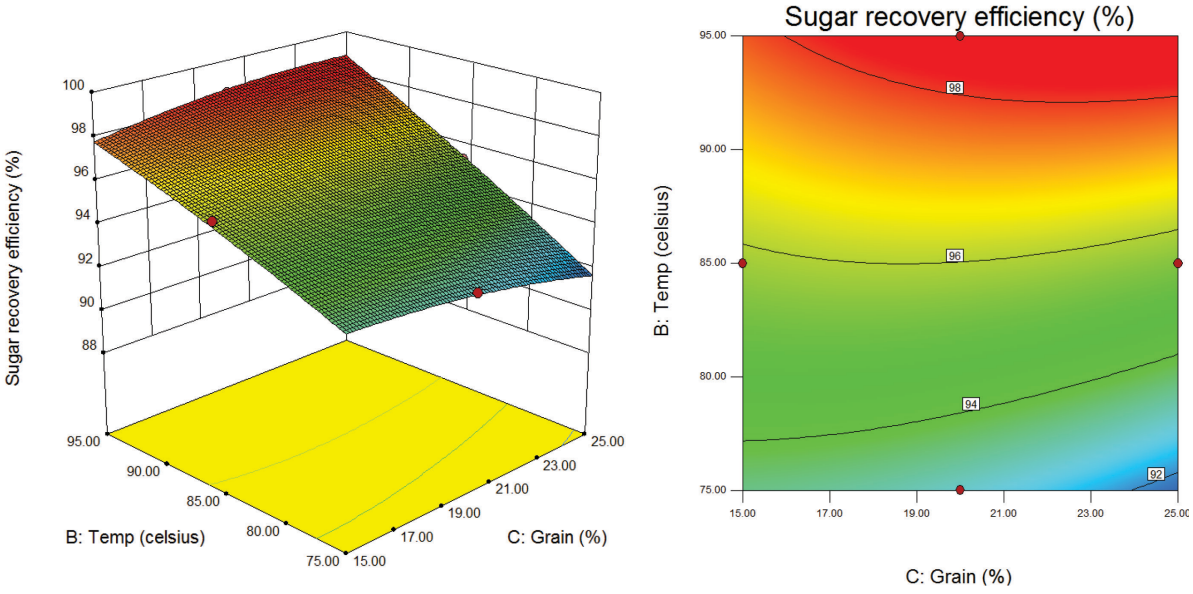


c

Figure 5.2 3D response surface of sugar recovery efficiency in relation to (a) temperature and grain loading; (b) temperature and time; (c) time and grain and their corresponding 2D contour plots.



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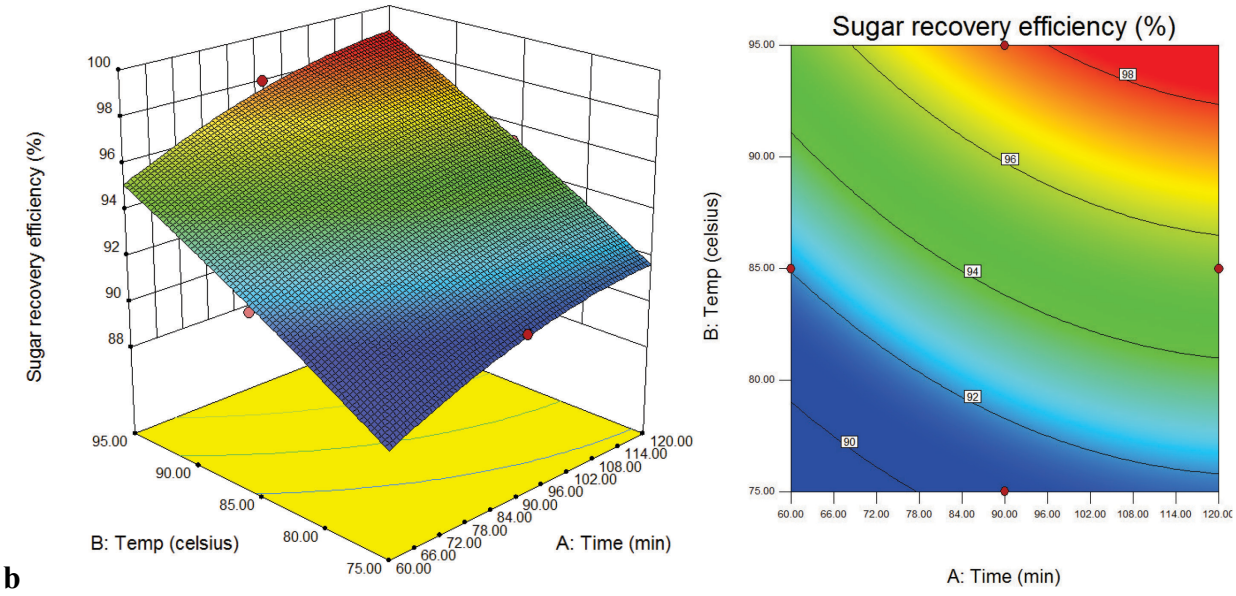

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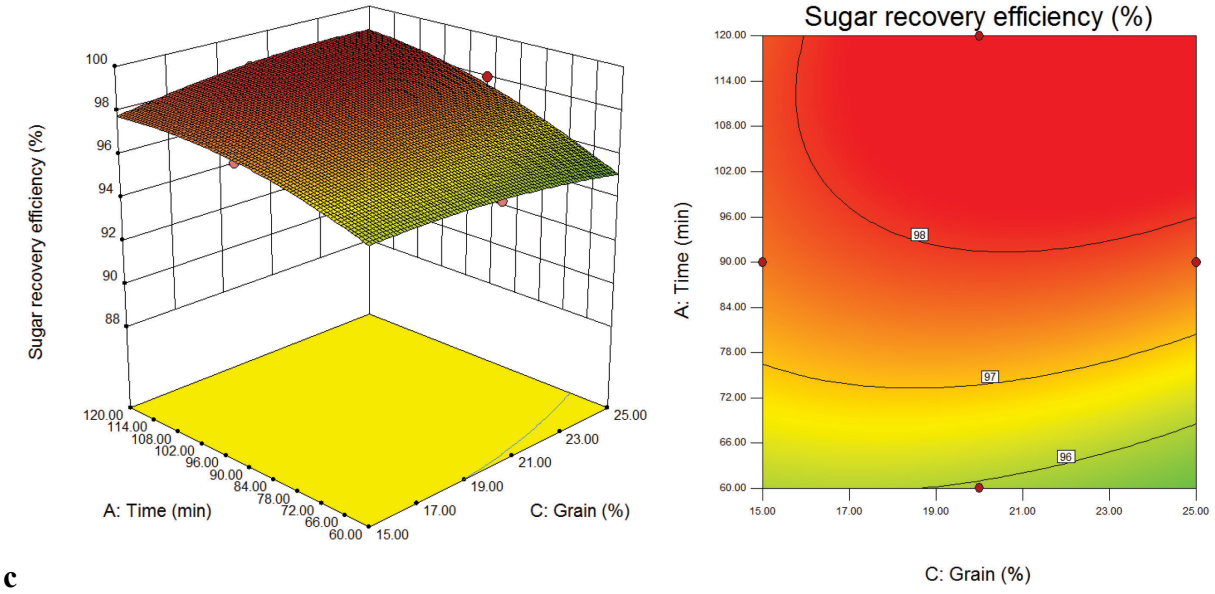


c

Diagnostic tests were conducted to validate model assumptions and check model accuracy. Diagnostic plots (not reported) established by Design-Expert software revealed no gross violation in model assumptions. A linear trend on the normal probability plot suggested that the normality assumption of the model was most likely satisfied. Studentized residual plots also showed no

presence of an outlier since observed residuals lie within the range of ±3 standard deviations of their mean of zero. Therefore, the assumption that residuals have a mean of zero was most likely satisfied. Residuals were evenly dispersed around the mean zero line in the residual plot, suggesting a lack of heterogeneity of residuals and indicating that the assumption of constant variances was most likely satisfied. Therefore, the model can be used to make predictions.

5.5. RSM optimization of sugar recovery efficiency

ANOVA results for second-order response surface models for sugar recovery efficiency are presented in Figure 5.2. ANOVA of the quadratic regression model also showed that the fitted model was highly significant, with *F*-value of 55.20 and corresponding *P*-value of 0.0002 at 95% level of confidence ($P < 0.05$). The computed *F*-statistic value (55.20) exceeded the *F*-critical value (3.48) so the null hypothesis (H_0) was rejected in favor of the alternative (H_1) at a type I error rate of 5%. Based on this evidence, a combined effect of the independent variables (X_i) significantly contributed to sugar recovery efficiency. The coefficient of determination of the regression analysis, R^2 value, was 0.9900, indicating that approximately 99% of sugar recovery efficiency variability could be accounted for by the model, suggesting model adequacy. The adjusted multiple coefficient of determination (adjusted R^2) was 0.9721. As mentioned, after adjusting the sample size and number of parameters, the model explained approximately 97% of total variation in sugar recovery efficiency.

Table 5.4. ANOVA of full and reduced regression equation model for sugar recovery efficiency.

Source	Sum of Squares	df	Mean Square	F-Value	p-value
Model	80.42	9	8.94	55.20	0.0002
Residual	0.81	5	0.16		
Lack of Fit	0.52	3	0.17	1.22	0.4806
Pure Error	0.29	2	0.14		
Cor Total	81.23	14			
R^2 = 0.9900, adjusted R^2=0.9721					
		ANOVA for the regression of the reduced model			
Model	78.89	5	15.78	60.82	<0.0001
Residual	2.34	9	0.26		
Lack of Fit	2.05	7	0.29	2.04	0.3675
Pure Error	0.29	2	0.14		
Cor Total	81.23	14			
R^2 = 0.9713, adjusted R^2=0.9553					

Regression coefficients of processing variable in the model for sugar recovery efficiency are shown in Table 5.5. The corresponding second-order regression model for sugar recovery efficiency established by RSM is shown in Eq. (7).

$$Y_{SRE}=95.4+1.23X_1+2.74X_2-0.65X_3+0.41X_1X_2+0.48X_1X_3+0.71X_2X_3-0.65X_1^2-0.20X_2^2-0.34X_3^2 \quad (7)$$

Table 5.5 Regression coefficients and their significance for quadratic model for sugar recovery efficiency.

Source	**Coefficient Estimate**	**Standard Error**	**Mean Square**	**F-Value**	**p-value**
Intercept	95.40	0.23	8.94	55.20	0.0002
X_1	1.23	0.14	12.17	75.19	0.0003
X_2	2.74	0.14	59.94	370.24	< 0.0001
X_3	-0.65	0.14	3.37	20.84	0.0060
X_1X_2	0.14	0.20	0.076	0.47	0.5232
X_1X_3	0.48	0.20	0.91	5.60	0.0642
X_2X_3	0.71	0.20	2.01	12.45	0.0168

$\mathbf{X_1^2}$	-0.65	0.21	1.56	9.66	0.0266
$\mathbf{X_2^2}$	-0.20	0.21	0.15	0.94	0.3765
$\mathbf{X_3^2}$	-0.34	0.21	0.43	2.63	0.1657

Results of experimental analysis by RSM indicated that linear terms of time (X_1), temperature (X_2), and grain loading (X_3), as well as the interactive term of temperature and grain loading (X_2X_3), the quadratic term of time (X_1^2), and grain loading significantly affected sugar recovery efficiency ($P < 0.05$), as shown in Table 5.5. Eq. (7) is also useful for identifying relative impact of the terms by comparing factor coefficients. Again, the linear term of temperature (X_2) had the most significant effect on the response, with $P < 0.0001$, followed by time ($P = 0.0003$) and percentage grain loading ($P = 0.006$). Results also showed that interactive terms of time and temperature (X_1X_2) and time and grain loading (X_1X_3), as well as quadratic terms of temperature (X_2^2), grain loading (X_3^2), and temperature had no significant effect on the response ($P \geq 0.1$). The full quadratic model was simplified and reduced in order to include only significant linear terms, interactive terms, and quadratic terms shown in Eq. (8). The equation shows that temperature had the highest impact on starch conversion efficiency, followed by time. The negative coefficient for the single factor, grain (X_3) variable, denotes the effect of decreased grain loading on sugar recovery efficiency. In addition, the equation demonstrates that the interaction between temperature and grain significantly impacted starch efficiency. The *P*-value (<0.0001) and corresponding *F*-value of 60.82 (Table 5.4) shows that the reduced model is highly significant. A "Lack of Fit *F*-value" of 2.04 implies that the "Lack of Fit" was not significant relative to the pure error which is good.

$$Y_{SRE}=95.05+1.23X_1+2.74X_2-0.65X_3+0.71X_2X_3-0.61X_1^2 \quad (8)$$

In addition, diagnostic plots (not reported) did not indicate any gross violation in model assumptions.Figure 5.2 represents the 3D response of a maximum sugar recovery efficiency of 98.47% and a minimum efficiency of 90.85% in relation to temperature and grain loading (at a constant time of 120 min), temperature and time (at constant grain loading of 25%), and time and grain loading (at a constant temperature of 95 °C).

5.6. Effect of time, temperature, and grain on sugar extraction

This study investigated sugar extraction from sweet sorghum bagasse and sweet sorghum ground grain with average particle size of approximately 4 mm. Sugar recovery from bagasse was achieved through the process of diffusion in which dissolved sugar molecules in solution were separated by the difference in concentration in the biomass and the solvent due to concentration gradient. Cane resident time is a significant design parameter in sugarcane extraction (Rein, 2007). Temperature also stimulates the rate of mass transfer (Jia et al., 2013). Rein (1974) estimated that a 5 °C increase of temperature from 75 °C to 80 °C could yield a 2% increase in sugar extraction from sugar cane. In this work, high sugar recovery efficiency greater than 98% was achieved at an increased temperature of 90 °C and a diffusion time of 114 min (Figure 5.2). Therefore, the longer the diffusion time at an increased temperature, the higher the sugars extracted from the bagasse. High temperature increased molecule movement rate, thereby speeding up the rate of diffusion, as asserted in previous studies. Cotlear (2004) reported that sugarcane extraction was influenced by higher concentration differences between the interior cell and the extracting solvent. In addition, increased temperatures stimulated an increased rate of mass transfer of sugar molecules from feedstock cells by increasing molecular mobility. Because plant protein denatures at increased temperatures (Sun, 2005b and Harrison et al., 2003), the permeability of sugar molecules from the sweet sorghum cell may have been enhanced as well due to denaturing of the protein lining at the

stalk cell walls. Protein denaturing of cell lining (Rein, 2007) causes the release of sugar-containing cells, consequently promoting sugar extraction from these cells. Organic acid-producing organisms are active at room temperatures up to 70 °C (Wu et al., 2010, Rein, 1995 and Rein, 2007); therefore, maintaining a diffusion temperature well over 90 °C may have prevented considerable sugar losses because microbial activities were controlled. Because sugar extraction is a mass transfer process, it can be assumed that the agitation by stirring might have caused increased molecular velocity, potentially facilitating the release and transfer of sugar molecules in raptured cells into extraction solvent (Rein, 2007).

5.7. Optimization conditions

Optimum diffusion conditions obtained by Box–Behnken design in RSM are summarized in Table 2.1. The table also includes responses at grain loading of 10, 15, 20, 25, and 30%. According to our designed model, at a 95% confidence interval, the highest starch efficiency and sugar recovery efficiency were obtained at 96.03% and 98.45%, respectively. Optimized diffusion parameters were diffusion temperature of 95 °C, diffusion time of 114.93 min, and grain loading of 22.04%.

Table 5.6 Optimized diffusion conditions.

Response	Time (min)	Temp (°C)	Grain flour loading (%)	Starch efficiency (%)	Sugar recovery efficiency (%)
1	120.00	95.00	10.00	86.42	98.33
2	115.75	95.00	15.00	92.49	98.38
3	114.82	95.00	20.00	95.60	98.43
4	115.52	95.00	25.00	95.78	98.49
5	120.00	95.00	30.00	93.01	98.56
Optimized factors	114.936	95.000	22.04	96.03	98.45

5.8 Conclusion

In this study, a regression model was developed for the extraction of fermentable sugars and starch from sweet sorghum using response surface models among diffusion time, diffusion temperature, and grain loading. The models predicted sugar extraction from sweet sorghum. The interaction of time, temperature, and grain loading significantly affected starch-to-sugar conversion efficiency and sugar recovery efficiency. At an optimized time of 114.9 min temperature of 95 °C, and 22% grain loading, starch efficiency and sugar recovery efficiency of 96% and 98.5%, were obtained, respectively.

Chapter 6 - Study on mass transfer kinetics of sugar extraction from sweet sorghum biomass via diffusion process and SSF of sugars

6.1 Abstract

Sweet sorghum juice, a potential bioethanol feedstock, can be incorporated in the dry-grind ethanol process to improve yields. The juice is normally obtained by pressing the stalk through roller mills in tandem. Juice extraction by this process is known to be less efficient, labor intensive, and susceptible to considerable fermentable sugar loss due to microbial activities when stored under room temperature. Fermentable sugar extraction from the sweet sorghum juice using response surface methodology with higher sugar recovery efficiency via diffusion has recently been proposed. In this study, extraction kinetics based on the optimized diffusion parameters (time, temperature and grain loading) were developed to describe the mass transfer of sugars in sweet sorghum biomass during the diffusion process. Diffusion parameters obtained from previous studies were also used to extract free sugars and convert them into ethanol using granular starch hydrolyzing enzymes and conventional enzymes. Ethanol yields at 72 h of fermentation mashes treated with granular starch hydrolyzing enzyme (GSHE) and those with traditional enzymes were comparable (14.49 – 14.56% v/v). Ethanol efficiencies also ranged from 88.92 –92.02%.

6.2 Introduction

Sweet sorghum juice can be directly converted into bioethanol via anaerobic fermentation by yeast *Saccharomyces cerevisiae* (Du et al., 2014; Phutela and Kaur, 2014; Wu et al., 2015; Wu et al., 2010). The fermentable sugars in the juice can also be utilized as raw material for the industrial production of lactic acid by *Lactobacillus sp*. (Hetényi et al., 2010), acetone-butanol by *Clostriduim acetobutylicum* (Cheng et al., 2008), and the production of other organic acids (Whitefield et al., 2012). A recent study, however, showed that a combination of juice and starch from the grains could improve ethanol yield by nearly 30% while reducing the starch enzymatic hydrolysis time by 30 minutes (Appiah-Nkansah et al., 2015). Another study also reported that co-fermenting corn with sweet sorghum juice could results in a 37% reduction in the quantity of corn required in the dry-grind process(Nghiem et al, 2016). Ethanol fermentation efficiencies from sweet sorghum juice may also range from 85 to 93% (Appiah-Nkansah et al., 2015, Serna-Saldívar, 2012; Wu et al., 2010).

The juice from the stem is normally extracted by 2- or 3-roller mills in tandem following the harvesting and stripping off the leaves (Eggleston et al., 2013; Rao et al., 2013; Rañola et al., 2007). During the harvesting process, the pinnacle is chopped and left on the field and thus significant amount of starch (63-73%) that could be processed into ethanol are lost. The juice extraction with this process is usually less efficient, butcould be up to 54.6% (Regassa and Wortmann, 2014; Rein, 2007; Reidenbach and Coble, 1985; Whitefield et al., 2012). Low juice extraction yield could be attributed to the relatively high fiber content of sweet sorghum juice. Additionally, juice obtained is not stable because about 50% of the fermentable sugars could be lost when stored at room temperature due to microbial activities (Wu et al., 2010).

Diffusion process has been recently shown as an alternative and innovative method of obtaining both nonstructural and fermentable sugars from sweet sorghum feedstock (Appiah-Nkansah, et al., 2016). Diffusion is defined as "the net transfer of matter from a region of high concentration to that of low concentration which" which is due to thermal molecular movement until state of equilibrium is reached (i.e. state of uniform concentration) (Crank et al., 1981). In the sugarcane industry, diffusers have been operational since before the 19th century (Rein, 2007). In this process, the feedstock is hammer-milled to uniform particle sizes and then passed through a series of continuous hot water flushes in which the concentration of solute is continuously reduced (Rein, 1995). Sweet sorghum has similar physiological structure to sugarcane and could allow the use of the same harvesting and transportation equipment used in the sugarcane infrastructure (Viator et al., 2009, Wood, 2000). Furthermore, sweet sorghum has a very short season and therefore can be grown during the fallow period between stands in sugarcane production. Moreover, since the harvest periods of both crops overlap, sweet sorghum could be integrated in the existing sugarcane industry (Kim and Day, 2011). The integration of the sweet sorghum starch in the diffusion process via enzymatic hydrolysis could, as well, improve the overall fermentable sugar extraction from the energy crop.

In fact, few studies have been reported on the kinetics of extraction of sugars from sugar crops. El-Belghiti and Vorobiev (2004) investigated the extraction of sugar from slices of sugar beet (30 mm in diameter and 8.5 mm in thickness) by applying pulsed electric field treatment at room temperature. Jemai and Vorobiev, (2002) also reported a study on the effect of moderate electric field pulse on the diffusion coefficient of sugar from apple slices. The mass transfer kinetics of sugar from chopped sweet sorghum stalks (2 – 16 mm) for solid-state fermentation process has recently been published. (Mao et al., 2015). However, no study has been done on the

mass transfer kinetics of fermentable sugars from ground sweet sorghum biomass for ethanol production.

A previous model study of fermentable sugar extraction and starch hydrolysis from sweet sorghum bagasse and grain flour via diffusion process showed increase of sugar diffusivity from the sweet sorghum feedstock (Appiah-Nkansah et al., 2016), but the mass transfer kinetics of the sugars molecules were not studied. Appiah-Nkansah et al., (2016), applied the Box-Behnken design in response surface methodology (RSM) to optimize diffusion conditions to achieve the highest fermentable sugar extraction from sweet sorghum. RSM is a set of statistical and optimization techniques aimed at optimizing quality characteristics of a production process and product (Myers et a., 2016).

In this research, the kinetic sugar transfer of sugar extraction from sweet sorghum biomass based on the RSM optimized parameters from previous study is investigated. Additionally, the optimized diffusion conditions obtained in the previous study are also applied to extract sugars and nonstructural carbohydrates from sweet sorghum feedstock. This is followed by a study of the ethanol fermentation performance using traditional enzymes and granular starch hydrolysis enzyme (GSHE), Stargen 002. GSHE consist of both alpha-alymase and gluco-amylase that has a synergistic effect on the hydrolysis of granular starch to glucose. The utilization of GSHE in the ethanol process can eliminate need for high temperature (>80 ℃) cooking during the starch hydrolysis and liquefaction stage thus reducing energy input ethanol yield (Appiah-Nkansah, 2015; Li et al., 2014; Wang, 2009,)

6.3 Materials and methods

6.3.1 Materials

Sweet sorghum grain and dried bagasse were obtained from Texas A&M Agrilife Research sorghum breeding program, Texas A&M University, College Station, Texas. The feedstock was prepared following the method described in Appiah-Nkansah et al., (2016). Granular starch hydrolyzing enzyme, (Stargen™ 002) was obtained from Genencor International (Palo Alto, CA). Alpha-amylase (Liquozyme® SC DS) and gluco-amylase (Spirizyme) were obtained from Novozymes, Franklinton, NC. The GSHE was Stargen 002 enzyme with an activity of 570 GAU/g (where GAU = one glucose unit) and specific gravity of 1.13 - 1.16 g/ml. Liquozyme® SC DS (alpha – amylase) has an activity of 267 KNU/g, (where KNU = kilo novo α-amylase units) and specific gravity of 1.266 g/mL. Spirizyme® Achieve (gluco-amylase) has an activity of >900 AGU/g, and specific gravity of 1.161 g/mL.

6.3.2 Starch content and moisture content analysis

The starch content of the sorghum grain was analyzed using a total starch kit (Megazyme International) following an accepted method (AACC, 2000). Moisture content of the materials was determined using standard American Association of Cereal Chemists (AACC) and National Renewable Energy Laboratory (NREL) methods (AOAC, 2000; Sluiter et al., 2008.)

6.3.3 Kinetic model study

Extraction of sugar molecules from the biomass is considered to be a mass transfer process of sugar solutes migrating from the inside of the fractured cell by diffusion into bulk solution. The following first-order kinetic equation (Mao et al, 2015; Belghiti and Vorobiev, 2004) was applied to study the kinetics of sugar transfer from sweet sorghum biomass:

$$C^* = C_d^*(1 - e^{-k_d t}) \quad (1)$$

Where $C^* = \frac{C}{C_\infty}$, C is the solute concentration in the solution at any time during the extraction process, C_∞ is the equilibrium solute concentration, $C_d^* = C_d/C_\infty$, C_d is the final solute due to diffusion, k_d (min^{-1}) is the rate constant for the diffusion stage.

6.3.4 Sugar extraction

Batch extractions were performed in a Parr reactor (Parr Instrument Co., Moline, IL) operating in a horizontal mode Figure 6.1. For each experiment, 40 g of biomass (grain + bagasse) was used. An amount of 8.8 g of sweet sorghum grain flour representing 22.02% of biomass and 31.2 g of sweet sorghum bagasse (77.98% of the biomass) was weighed in a 500 mL beaker. Approximately 750 mL of distilled water was preheated in a microwave for 5 min and 500 mL of the preheated water was measured. Approximately 400 mL of the preheated water was poured into the 1 L reaction vessel of a Parr pressure reactor, and the biomass sample were added. The reactor is equipped with impeller mixers and a controlled heating system. The remaining ~100 mL was used to thoroughly rinse the beaker into the reaction vessel. Thirty microliters of Liquozyme® SC DS (alpha – amylase 267 KNU/g, 1.266 g/mL) was added to the content. The reaction vessel was then coupled to the Parr reactor assembly, oriented horizontal position (Figure 1), and set to run for 120 min at a set temperature of 85°C and an impeller speed of 100 revolutions per minute (rpm). The heating source was removed and the reactor was cooled to a temperature below 50°C. The vessel was disengaged and 150 L of Spirizyme® Achieve (glucoamylase >900 AGU/g, 1.161 g/mL) was added to the mixture. The vessel was fixed again to the Parr reactor system, set to run for 40 min at a temperature of 60°C, and then the slurry was recovered. Recovered slurries were centrifuged in order to separate out the solid portions using by a Sorvall RC 6+ Centrifuge (Thermo Fisher Scientific, Asheville, NC). The liquid potion which contained dissolved free sugars were utilized as solvent for the next batch process as described above for 4 addition runs.

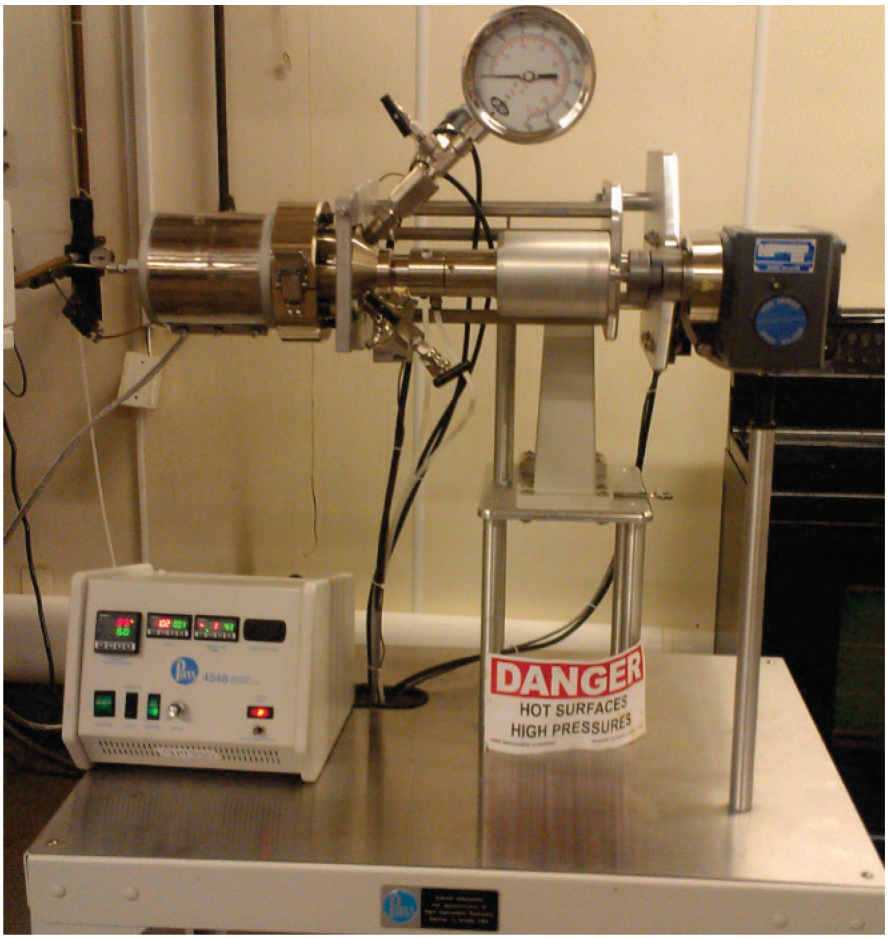


Figure 6.1. Stirred Parr reactor operating in horizontal position

6.3.5 Sugar and ethanol analysis

Components were analyzed by a high pressure liquid chromatograph (HPLC) with a Rezex RCM Monosaccharide (300 × 7.80 mm) and a Refractive Index Detector RID—G1362A (Agilent Technologies, Santa Clara, CA). Twenty microliters of the sample was injected with the mobile phase (HPLC-grade deionized water). The mobile phase with the analyte was pumped at a high pressure of ≈33-42 bar into a column packed with monosaccharides calcium ions. The elution rate was maintained at 0.6 mL/min and a column temperature of 80 ℃. Components (i.e. sugars, ethanol, organic acids) separated in the column were detected with the refractive index detector and quantified. The theoretical ethanol yield was determined using the total starch contents in the

samples, assuming 0.511 g ethanol from 1 g of starch (Thomas et al., 1996). Fermentation efficiencies were calculated as the actual ethanol yield divided by the theoretical ethanol yield.

6.3.6 Inoculum preparation

Dry yeast was activated by adding 1.0 g of active dry yeast into 19 mL of preculture broth (containing 20 g of glucose, 5.0 g of peptone, 3.0 g of yeast extracts, 1.0 g of KH_2PO_4 and 0.5 g of $MgSO_4 \cdot 7H_2O$ per liter) and incubated at 38°C for 30 min in an incubator operating at 200 rpm The activated yeast culture had a cell concentration of 1×10^9 cells/mL.

6.3.7 Ethanol fermentation

Three treatments of the extracted solutions were prepared for ethanol fermentation process – extracted solution only ('Juice only'); extracted solutions combined with grain sorghum flour using conventional enzymes ('Traditional'); and extracted solution combined with grain flour using GSHE ('GSHE'). Cleaned grain sorghum samples were milled into flour through a 0.5 mm screen in an Udy cyclone mill (Udy Corp., Fort Collins, CO, USA) and used for ethanol fermentation.

One hundred milliliters of extracted sugar solutions were weighted into 250-mL Erlenmeyer flasks and supplemented with 0.3 g of yeast extract per flask. After adjusting pH values to 4.2 with 2N hydrochloric acid, the sample was incubated with 1.0 mL freshly activated dry yeast (Ethanol Red) and 100 μL of Spirizyme at 30 ℃ for 72 hours in a 12400 Incubator Shaker (New Brunswick Scientific Co. Inc., Edison, NJ) operating at 150 rpm. For conventional enzyme treatment Fifteen grams (dry base) of ground sorghum (60% starch content) was mixed with 100 mL of preheated (about 60°C) extracted juice containing 0.1 g of KH_2PO_4 and 20 μL of Liquozyme (alpha-amylase, Novozymes, Franklinton, NC) to form an evenly suspended mash. For starch liquefaction, the flask was transferred to a 70°C water-bath shaker operating at about 180 rpm.

The temperature of the water bath was gradually increased from 70°C to 90°C in a 30 min period, kept at 90°C for a few minutes, and then, lowered to 85°C; liquefaction continued for 60 min. The flask was then removed from the water bath, and materials sticking on the inner surface of the flask were pushed back into the mash with a spatula. The spatula and inner surface of the flasks were rinsed with 3-5 mL of distilled water. After cooling to room temperature (25°C to 30°C), the pH of the mashes was adjusted to around 4.2 with 2M HCl for the fermentation process. For the granular starch hydrolyzing enzyme treatment, samples of grain sorghum flour 15 g db were weighed into clean 250 mL Erlenmeyer flask. One hundred milliliters of warm (about 60°C) extracted sugar solution was also poured into the flasks and mixed to form an evenly suspended mash. The mash was then treated with 60 μL granular starch Stargen 002) carefully transferred into a 48 °C water-bath shaker and set to operate at 180 rpm for 2 h.. The pH was adjusted to 4.2 by the same procedure described above.

The SSF process started with the addition of 1.0 mL of the activated yeast culture, 100 μL of Spirizyme, (750 AGU/g, about 1.15 g/mL) (Novozymes, Franklinton, NC), and 0.30 g of yeast extract into mashes in each flask. Flasks were sealed with an S-airlock with mineral oil. Fermentation was conducted at 30°C for 72 h in an incubator shaker operating at 150 r/min. Fermentation performance was monitored by weighing the fermentation flasks for 3 day incubation period at 4, 8, 18, 24, 32, 44, 56 and 72 h of fermentation. The weight loss was due to the evolution of CO_2 during the fermentation process ($C_6H_{12}O_6 \rightarrow 2C_2H_6O + 2CO_2\uparrow$).

6.3.8 Statistical analysis

Statistical analysis were performed by using Microsoft® Excel® 2013. Matlab R2013a was used to develop extraction kinetic model graph. Final ethanol yields and efficiencies were

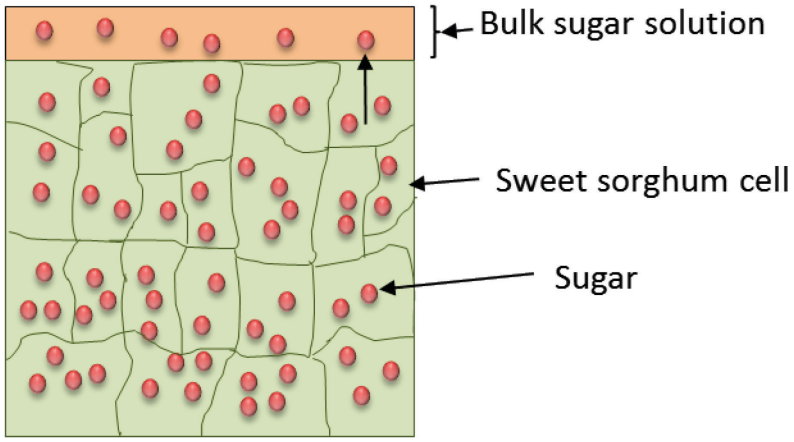
compared using analysis of variance in SAS 9.4 (SAS Institute, Cary, NC) with 0.05 ($P<0.05$) statistical significance. Fermentation experiments were performed at least in duplicate

6.4 Results and discussion

6.4.1 Kinetic Study of sugar transfer from sweet sorghum biomass via diffusion

Figure 6.2 presents an illustration of sugar transfer by thermal movement from the interior of the ruptured cells and through the thin cell membrane into the bulk solution. The transfer of sugar molecules is driven by the concentration gradient from the high concentrated region into the low concentration region according to Fick's law of diffusion (Belghiti and Vorobiev, 2004; Crank et al., 1981; Doran, 2013). The purpose of the kinetic study is to describe parameters such as solid/solvent ratio, temperature and hydration level for predicting the entire mass transfer curve to aid in process design and also for assessing the feasibility of the process in the industry (Toda el al., 2016).

Figure 6.2 A schematic diagram of the structure of sweet sorghum indicating the transfer of sugar from the interior cell plant cell into the bulk solution.



In this work, the extraction kinetics of sugar from the sweet sorghum biomass are analyzed based on the RSM model (Equation (2)), from the previous study (Appiah-Nkansah et al., 2016).

The effects of process variables such as temperature, solid/solvent ratio, etc, on the rate of sugar extraction are known to be important for kinetic study (Mao et al, 2015; Toda el al., 2016).

The parameters of time, temperature and grain/biomass loading ratio, significantly influenced sugar extraction in the previous study. These optimized parameters developed are used to predict the entire mass transfer curve of sugar extraction and to understand the delineation of the sugar transfer process via diffusion. The developed model is transformed into the first-order kinetic equation expressed in Equation (1): The RSM model is described in coded form in equation (2).

$$Y = 95.05 + 1.23x_1 + 2.74x_2 - 0.65x_3 + 0.71x_2x_3 - 0.61x_1^2 \quad (R_{Adj}^2 = 0.96) \qquad (2)$$

This can be converted into an equation using the natural or actual experimental variables by substitution based on the relation;

$$x_i = \frac{(X_i - X_i^*)}{\Delta X_i} \qquad (3)$$

where x_i is the coded value of the i^{th} independent variable, X_i is the actual value of the i^{th} independent variable, X_i^* is the actual i^{th} independent variable at the center point, and ΔX_i is the step change value. The equation in terms of the actual factors becomes

$$Y = 95.05 + 1.23\ \frac{(X_1-90)}{30}x_1 + 2.74\frac{(X_2-85)}{10}x_2 - 0.65\frac{(X_3-20)}{5}x_3 + 0.71\frac{(X_2-85)}{10}.\frac{(X_3-20)}{5} -$$

$$0.61\left[\frac{(X_1-90)}{30}\right]^2 \qquad (4)$$

The simplified equation then becomes:

$$Y = 89.35 + 0.163x_1 - 0.01x_2 - 1.34x_3 + 0.014x_2x_3 - 0.00068x_1^2 \qquad (5)$$

When $x_1 = 120$; $x_2 = 95$; and $x_3 = 25$

$$Y = 89.35 + 0.163(120) - 0.01(95) - 1.34(25) + 0.014(95)(25) - 0.00068(120)^2$$

$$Y = 97.918 = C_d^*(1 - e^{-k_d.120}) \qquad (6)$$

When $x_1 = 60$; x_2= 75; and $x_3 = 15$

$Y = 89.35 + 0.163(60) - 0.01(75) - 1.34(15) + 0.014(75)(15) - 0.00068(60)^2$

$Y = 91.582 = C_d^*(1 - e^{-k_d.60})$ (7)

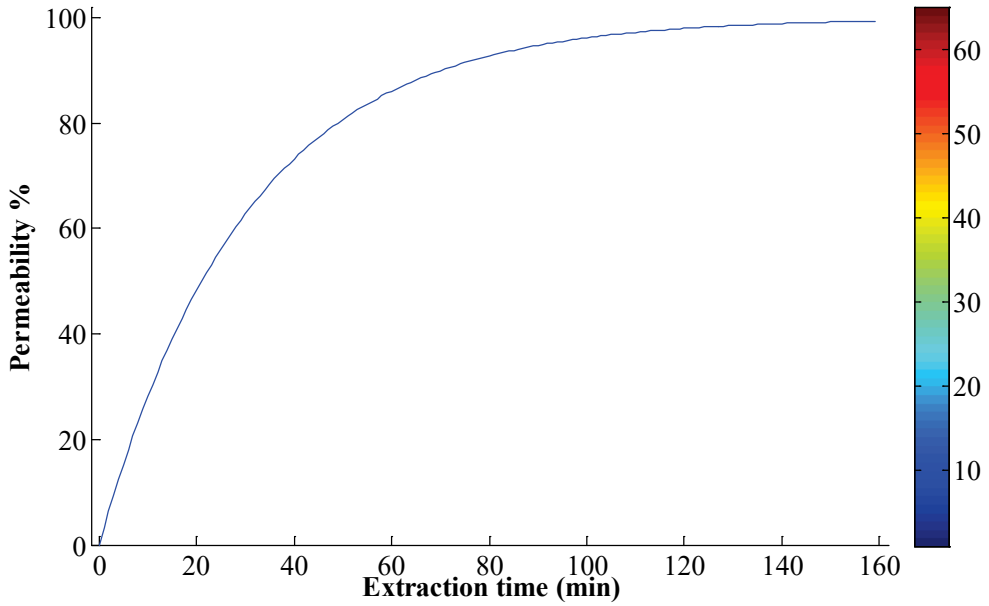
Based on (6) and (7); k = 0.033 min^{-1}, C = 99.82

Then $Y = 99.82(1 - e^{-0.033t})$

The kinetic model describing the mass transfer of sugar molecules from biomass into the solution represented by the following equation and is:

$C^* = 99.82(1 - e^{-0.033t})$

Figure 6.3. Kinetics of extraction of sugar from sweet sorghum biomass



The kinetic model curve which displays the mass transfer dynamics of sugar molecules during diffusion is shown in Figure 6.3. It can be observed that, the concentration of the sugar molecules changes with time during the sugar extraction process. The profile of the graph shows rapid transfer of sugar solutes from the biomass into the solvent occurred at the beginning of the process and during the first 80 min. Sugar extraction rate then gradually decreased over the

following 20 min until drastically slowing down and becoming constant, corresponding to maximum yield and reaching equilibrium after about 115 minutes. The quick sugar transfer of sugar at the initial stage is attributed to high concentration gradient at the beginning of the process. A similar extraction kinetics behavior of rapid transfer of solutes at the beginning of the process with time were observed during the extraction of olive oil (Meziane and Kadi, 2008), soybean oil (Dagostin et al., 2015; Toda et al., 2016) using ethanol as solvent; and canola oil, sunflower oil, jojoba oil, using hexane (Allawzi et al., 2005; Baümler et al., 2010, Fernández et al., 2012; Perez et al., 2011;). Toda et al., (2016) examined the kinetics of soybean oil and free fatty acids extraction from soybean collets at varying temperature levels. Oil transfer from the soybean into the solvent was faster at the initial stages, and then decreased until equilibrium was reached. Mao et al., (2015) recently, studied the mass transfer of sugar in sweet sorghum stalks of different particle sizes – 2, 4, 6, 12, 16 mm over a temperature range of 20 – 60 ℃ for solid state fermentation. Sugar transfer in smaller particle reached equilibrium faster because shorter time was needed due to the increased surface area available for extraction and decreased mass transfer distance.

Additionally, increasing temperature also enhanced the rate of extraction. Sugar extraction from 2 mm particle sizes at 60 ℃ reached equilibrium after 150 minutes into the extraction process. In this study, the migration of sugar sweet sorghum feedstock (milled through 3.99 mm at screen) at 95 ℃ reached equilibrium at a relatively faster rate (after 115 minutes) and that could be attributed to the higher extraction temperature. Toda et al, (2016) also observed an increased rate of oil transfer from the solid matrix with increased temperature. Higher mass transfer may also be achieved by the convective currents of the bulk fluid in motion (Doran, 2013). Increasing stirring speed facilitated the rate of extraction (Mao et al., 2015). In the present model study, stirring speed was maintained at 100 rpm with the Parr reactor tilted to the horizontal potion (Figure 6.1),

ensuring rapid transfer of sugar from the solid matric into the bulk solution. Thus, high temperature plus stirring increases the random movement and solubility of sugar molecules in diffusion.

6.5 Sugar yields

It is important to note that the sweet sorghum bagasse used in this work had been previously stored in a container at room temperature for over a year hence it is expected that some amount of free sugars might have been lost over the storage period. Nevertheless, we investigated the amount of sugars that could be extracted from the feedstock by applying the diffusion method. In this work, centrifuged sugar solution obtained from the first run was utilized as the extraction solvent for second run and this process was repeated for the subsequent runs. Potions of centrifuged solutions after each run was taken for HPLC analysis.

Figure 6.4 and Figure 6.5 represents the total sugars extracted from the sweet sorghum feedstock and the efficiencies, respectively. The results showed a strong quadratic relationship between total sugars and the number of runs and fitted the data very well with R^2 being greater than 0.99 ($R^2 = 0.9987$). Sugar released ranged from 3.56 to 14.24 g/L over with corresponding efficiencies of 91.1-64.4%. In this work, extracted sugar solutions were used as solvents for subsequent runs resulting in increasing sugar concentrations in the subsequent runs which might have reduced the rate of mass transfer of sugar molecules from the sugar cells into the solvent, hence the decreasing extraction efficiencies observed. Based on the outcome, the total sugars and the extraction efficiency could be related to number of runs by the following second order equations:

$$Y_{TS} = -0.03X_n^2 + 2.8X_n + 0.88 \ (R^2 = 0.9987) \quad (8)$$

$$Y_{Eff} = 0.07X_n^2 + 7.0X_n + 97.8 \ (R^2 = 0.9987) \quad (9)$$

where Y_{TS}, Y_{Eff}, and X_n represents the total sugars, extraction efficiency and number of runs respectively.

Figure 6.4. Total sugars extraction

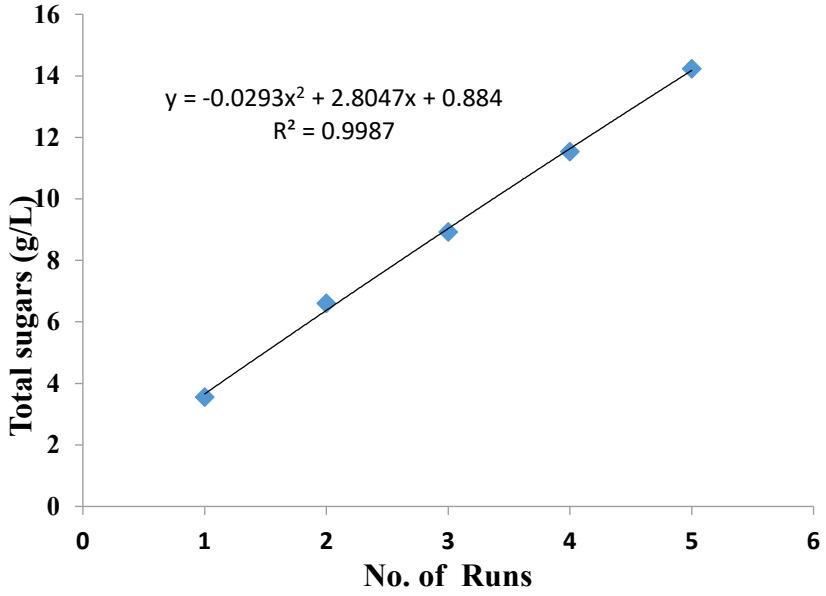
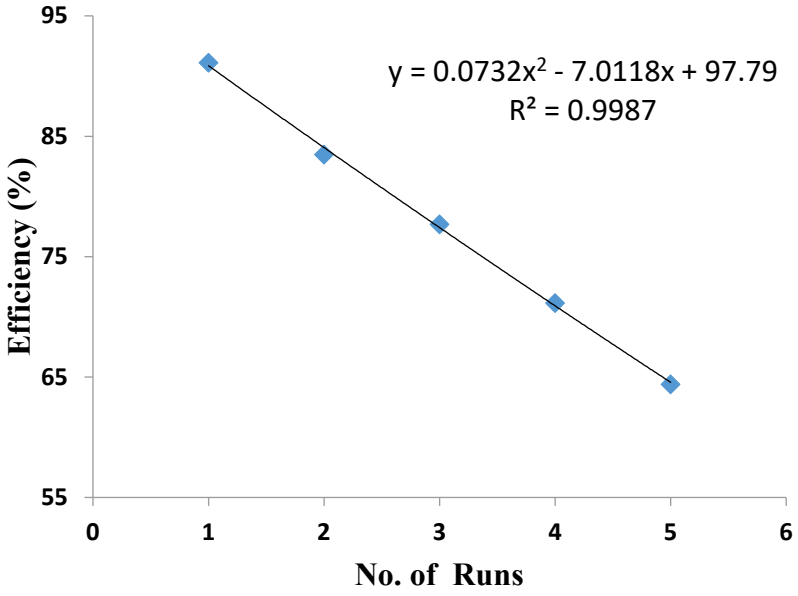


Figure 6.5 Extraction efficiency



6.6 Ethanol fermentation yields and efficiencies

Ethanol fermentation profile among the treatment was comparable during the first 40 h into the process. Ethanol yield for 'Juice only' was complete around 40 h and remained constant towards the end. From 32 to 68 h, ethanol concentrations of 'GSHE' were slightly higher compared to 'Traditional'. The final ethanol yield at 72 h of the 'Traditional' was 14.56 % (v/v) and that of 'GSHE' was 14.49 % (v/v) (Figure 6.6) It is important to note that the granular starch hydrolyzing enzyme used in this study contains *Aspergillus kawachi* alpha-amylase expressed in *Trichoderma reesei* and a gluco-amylase from *Trichoderma reesei.* Gohel and Duan (2012) examined low temperature hydrolysis for (25% dry solids) Indian broken rice (68.45% starch content) and pearl millet (60% starch content) using GSHE, (Stargen 002) along with acid fungal protease (Fermgen) under yeast fermentation conditions. The acid fungal protease was utilized to digest protein in the grains into amino acids, peptides, and free amino nitrogen for yeast vitality. They obtained 11-12% (v/v) and 9-10% (v/v) from Indian broken rice and Indian pearl millet respectively. Fermentation efficiencies also ranged from 97 to 98% in both feedstocks compared to 81-90% fermentation efficiency observed in conventional process. Pin et al., (2007) also compared the fermentation process from corn mashes (25% dry solids content) of using, Stargen 001, a raw starch hydrolyzing enzyme with conventional enzymes and reported similar ethanol yields of 14.1-14.2% (v/v). Ethanol fermentation profile of samples treated with the Stargen 001 were also comparable to those treated with conventional enzymes. Genencor International (Palo Alto CA), the company that manufactures, Stargen 002 reported, slightly above10 % (v/v) of ethanol from corn (28% dry solids) from Stargen 002 (DuPont, 2012). No difference ($P < 0.05$) in the final ethanol yields and efficiencies observed among the two enzyme treatments. The conversion efficiencies obtained in this study ranged from 88.92 – 92.02 % (Figure 6.7). The high ethanol

fermentation efficiency of the 'Juice only' (92.02%) obtained in the work, was anticipated because broths with lower sugar concentrations would have higher fermentation efficiency if the same amount of yeast and nutrients were used for ethanol conversion from sugars (Liu et al., 2013; Appiah-Nkansah, 2015). Ethanol yields and efficiencies reported by Appiah-Nkansah et al. (2015), from mashes of sweet sorghum juice with varying grain sorghum flour samples using Stargen 002 ranged from 10.73 – 16.70% v/v and 87.66 – 94.65% respectively.

Figure 6.6 Ethanol fermentation yield.

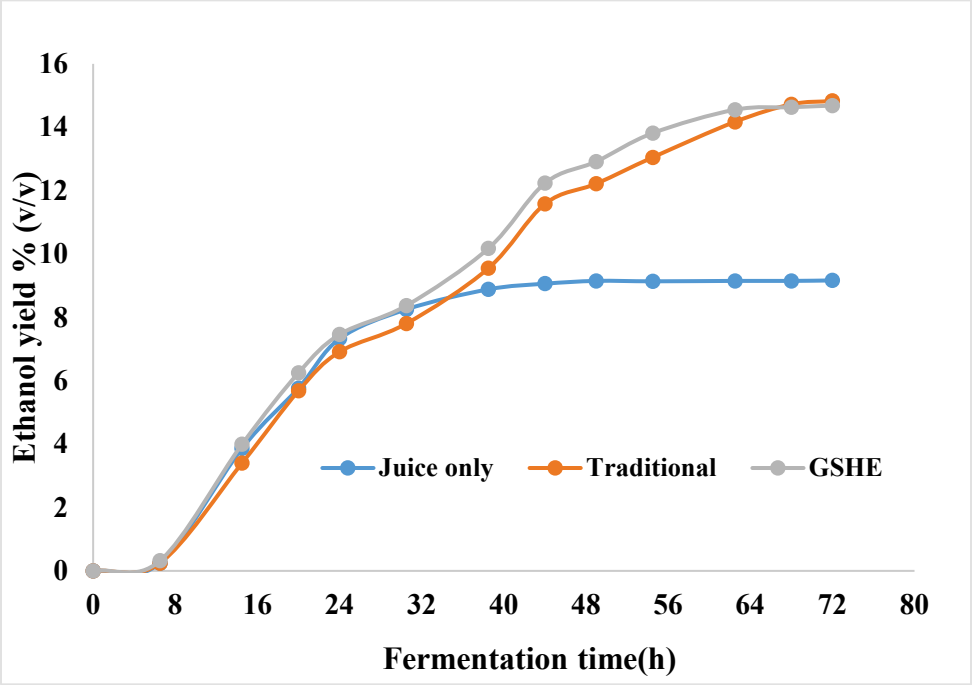
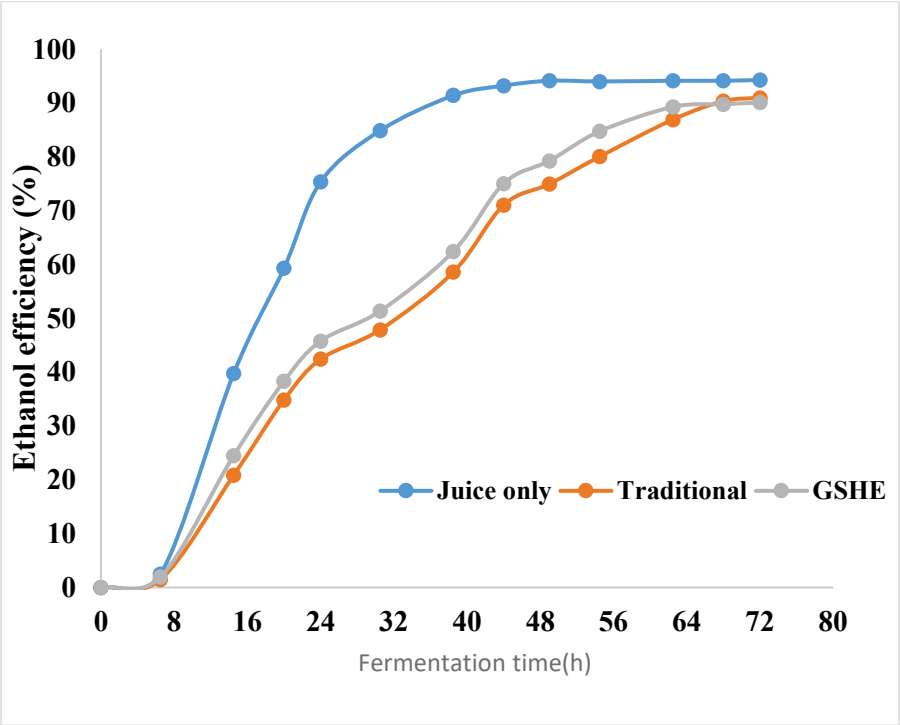


Figure 6.7. Ethanol conversion efficiency



6.7 Conclusion

In this study, a model for describing the kinetics of sugar transfer from sweet sorghum biomass via diffusion was developed. The diffusion conditional parameters were applied to extract free sugars and non-structural carbohydrates from sweet sorghum feedstock. Sugar transfer from sweet sorghum biomass reached equilibrium at a faster rate with higher extraction temperature of 95℃. There were strong linear relationship between total diffusion runs and sugars extracted as well as extraction efficiencies. Ethanol fermentation process using GSHE was compared with conventional enzymes for extracted sugars combined with grain sorghum flour. This work demonstrates that granular starch hydrolyzing enzyme could be the utilized as an energy conserving alternative process for bioethanol conversion of sweet sorghum juice feedstock.

Chapter 7 - Conclusion and future work

7.1 Conclusions

Sweet sorghum is a unique energy crop that produces grain high in starch, stems with high juice quantity with soluble sugars, and lignocellulosic biomass. Crop improvement programs have enhanced sweet sorghum cultivars, producing hybrids with higher yields, higher sugar concentrations and increased periods of industrial use. Agronomic production systems are evolving and are unique to specific production environments. Additionally, the development of several processing pathways of producing bioenergy from sweet sorghum has been explored. Although it was economically viable to produce sweet sorghum in certain geographic regions, others places showed otherwise. On-farm processing was found to be a cost-effective system for ethanol production from sweet sorghum. Techno-economic feasibility of ethanol production of sweet sorghum may depend on improved crop production and process efficiency and governmental policy support in some places.

Incorporating sweet sorghum juice into the current dry-grind ethanol process can improve ethanol yield, save energy, and increase water efficiency. High-gravity fermentation can be applied when using sweet sorghum juice instead of water for ethanol fermentation. Ethanol yield from the mixture of sweet sorghum juice and sorghum flour was about 28% higher than that from the conventional method and ethanol yields increased as flour loading increased. The results of this study also showed that the enzymatic hydrolysis time could be reduced by 30 min, which will help conserve water and energy. In addition, sweet sorghum juice enhances the potential for ethanol production from starch-based materials by granular starch-hydrolyzing enzymes.

The highest ethanol yield from VHG mashes of ground sorghum grain and sweet sorghum juice at various ratios was produced by mashes prepared from sweet sorghum juice with 18% sugar content. Hence, the use of VHG grain sorghum mashed with sweet sorghum juice a ratio of 50% grain sorghum to 50% sweet sorghum juice containing 18% sugar concentration would produce higher ethanol production. Inoculum size, temperature, FAN and nutrient supplementation influenced ethanol yield and ethanol fermentation efficiency. Improving the production process of bioethanol to make it more efficient using sweet sorghum juice and grain sorghum will results in better utilization of the feedstock. This will translate into cost reduction, which will make the sorghum industry more profitable and more attractive

A regression model developed predicted fermentable sugar extraction and starch from sweet sorghum. Response surface methodology was applied to optimize the diffusion time, diffusion temperature, and grain loading. The interaction of time, temperature, and grain loading significantly affected starch-to-sugar conversion efficiency and sugar recovery efficiency. At an optimized time of 114.9 min temperature of 95 °C, and 22% grain loading, starch efficiency and sugar recovery efficiency of 96% and 98.5%, were obtained, respectively.

A mode for describing the extraction kinetics of sugar from sweet sorghum biomass via diffusion was developed. Diffusion conditional parameters were applied to extract free sugars and non-structural carbohydrates from sweet sorghum feedstock. There were strong linear relationship between total diffusion runs and sugars extracted as well as extraction efficiencies. Ethanol fermentation process using GSHE was compared with conventional enzymes for extracted sugars combined with grain sorghum flour.

7.2 Recommendations for future work

Few studies have been reported on the co-fermenting of sweet sorghum juice with corn and with grain sorghum. Very high gravity fermentation technique could be further explored to examine ethanol yield performance of sweet sorghum juice combined together with both grain sorghum and corn grains and to assess the optimum ratio for achieving higher ethanol yields and conversion efficiencies with traditional enzymes and granular starch hydrolyzing enzymes. The use of other nitrogenous nutrient supplement could also be optimized.

Cellulosic ethanol production from sweet sorghum biomass could be examined following optimized lignocellulosic pretreatment methods. The use of non-corrosive and environmentally friendly pretreatment methods such as ionic liquids, liquid hot water, could be optimized in order to increase enzyme efficiency and improve the yield of monomeric sugars from sweet sorghum lignocellulose.

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