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Physical fractionation of sweet sorghum and forage/energy sorghum for optimal processing in a biorefinery

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ABSTRACT

Sorghum offers enormous potential as a feedstock for the production of fuels and chemicals from both water-extractable sugars and the cell wall biopolymers, while its within-plant structural and compositional heterogeneity may allow for physical fractionations to tailor feedstock properties to a biorefining process. In this study, the stem internodes of two sorghum (*Sorghum bicolor* L. Moench) genotypes, a sweet sorghum ('Della') and a forage/energy sorghum ('TX08001'), were first subjected to fractionation by manual classification by stem anatomy and internode proximity to the ground to yield 18 fractions. These fractions exhibited substantial differences in cell wall morphology, composition, and recalcitrance to mild alkaline pretreatment and enzymatic hydrolysis. While the sweet sorghum cultivar held nearly 70% more water-extractable sugar (sucrose, glucose, fructose, starch) in the stems than the forage/energy sorghum hybrid, both cultivars exhibited comparable diversity of composition and these compositions were remarkably similar in similar tissues and stem regions between the two cultivars. The fractions isolated from the pith parenchyma were the least recalcitrant to mild alkaline pretreatment and enzymatic hydrolysis and contained less lignin than fractions isolated from the epidermis, outer and inner rind, and internal vascular bundles. The pith samples isolated from the lowest region of the stem from both cultivars exhibited near-theoretical sugar hydrolysis yields when no pretreatment was employed and exhibited the lowest lignin contents of any of the fractions. Next, a physical fractionation approach approximating a commercial "de-pithing" process utilizing wet disintegration and sieving was applied to the forage/energy sorghum. A pith-rich fraction representing approximately 20% of the extractives-free mass of the stem could be isolated with this approach and, relative to the other fractions, was low in lignin, high in ash, highly hygroscopic, and showed an improved response to mild alkaline pretreatment and enzymatic hydrolysis at low enzyme loadings. Overall, these results demonstrate how heterogeneity within sorghum stems can be exploited using physical fractionation approaches to yield fractions enriched in desired properties that may allow for more streamlined processing.

1. Introduction

Technologies for utilizing the polysaccharides in the cell walls of plants (*i.e.,* lignocellulose) as a feedstock for fermentation-derived ethanol have begun to be commercialized utilizing agricultural residues that include corn stover/cobs, wheat straw, and sugarcane bagasse (Schwab et al., 2016). However, this nascent cellulosic biofuels industry faces challenging economics derived from high cost of cellulosic sugars relative to starch and sucrose with contributions arising from the high capital and operating costs associated with large centralized lignocellulosic biorefineries (Dale, 2017). The adoption of biomass feedstocks that impart agronomic, logistical, and/or processing benefits offer one potential route for improving overall economics for lignocellulosic biomass to biofuels processes. Sorghum (*Sorghum bicolor* L.

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Moench) is considered a promising advanced feedstock suitable for cultivation in North America for the production of renewable biofuels and chemicals (Reddy et al., 2008; Rooney et al., 2007). Diverse sorghum varieties have been developed that include grain sorghum, sweet sorghum, and forage/energy sorghum that differ in the partitioning of carbon to optimize the yields of grain, stem sugar content, or lignocellulosic biomass, respectively (Kim and Day, 2011; Mullet et al., 2014). Sorghum can accumulate water-extractable sugars (sucrose, glucose, fructose, and starch) in stems as a substantial fraction of the overall plant mass (Calviño and Messing, 2012; McKinley et al., 2016), which represents a readily convertible source of carbon.

As with other graminaceous biomass feedstocks such as corn stover and sugarcane, sorghum exhibits substantial within-plant heterogeneity that can result in processing challenges. In graminaceous feedstocks, plant cell wall recalcitrance to either biological degradation or deconstruction and conversion in a biorefinery is known to be a function of the cell type, maturation stage, and composition (Costa et al., 2013; Crowe et al., 2017a; Jung and Casler, 2006). Physical fractionation technologies offer the opportunity to exploit these within-feedstock differences to potentially optimize processing in a biorefinery. Physical fractionation can be envisioned either as an integrated component of feedstock harvest (*e.g.*, selective collection of anatomical fractions) or at a conversion facility prior to deconstruction and conversion (*e.g.*, combinations of a comminution or mechanical disruption coupled to a physical fractionation by differences in particle properties).

As an important contributor to within-plant heterogeneity, the parenchymatous tissue of the pith can comprise 20–30% of the mass of the stems of graminaceous feedstocks such as corn stover, sorghum, or sugarcane (Atchison, 1971). Relative to the other cell types in grasses, pith cells are known to be substantially less recalcitrant to biological degradation by rumen microbiota or cell wall-degrading enzymes due to their lower lignin content (Akin et al., 1977; Hansey et al., 2010; Jung and Allen, 1995). Furthermore, the pith is known for its extreme hygroscopicity, particularly after alkaline delignification, relative to other cell types (Maziero et al., 2013; Rainey, 2012) which can impart processing challenges in a biorefinery such as problems with pumpability, solid-liquid separations, and operation of reactors at high water-insoluble solids contents. Industrial "depithing" technologies have been developed and commercially implemented for the alkaline pulping of sugarcane bagasse (Lois-Correa, 1986, 2012). For these processes, the pith must be removed prior to alkaline pulping due to the poor mechanical strength of pith fibers, their poor drainability, and their higher silica content. An important variable for the feedstock response to comminution includes the moisture of the feedstock (Barakat et al., 2015; Lathrop et al., 1955) and "dry", "moist", or "wet" depithing processes have been developed (Atchison, 1971). Commercial depithing processes for sugarcane bagasse include a combination of moist-depithing (*i.e.*, hammer milling and sieving at 30–50% moisture) and wet-depithing (*i.e.*, disintegration or refining plus screening at 10–15% consistency), although wet-depithing processes have requirement for processing large volumes of water (Lathrop et al., 1955; Whittenmore et al., 1935). While sharing the process objective of physical separation of fiber fractions, there are differences in the process constraints. Namely, damage to fibers and generation of fines is a disadvantage for pulping and papermaking, while not necessarily so for a cellulosic biofuels process. As such, dry fractionation approaches, which are known to give low selectivity for pith separation due to the high level of fines formation (Lathrop et al., 1955), may not necessarily be unsuitable for biorefining applications.

The development and application of viable separation/fractionation strategies offers an opportunity to reduce heterogeneity in biomass feedstocks to enable optimal processing for example through parallel processing of fractions at different conditions or to allow for the production of diverse biomass-derived products with the potential to supply multiple markets. Notably, minimal work has been published on how feedstock fractionation may impact the operation of a cellulosic biorefinery employing sorghum as a feedstock. Motivated by this need, in the present work we investigate the diversity of composition within different anatomical fractions in two sorghum cultivars and determine the impact of this within-plant feedstock heterogeneity on the fraction responses to mild alkaline pretreatment and enzymatic hydrolysis. Finally, a fractionation approach employing comminution followed by sieving is employed to demonstrate how physical fractionation can result in streamlined processing in a biorefinery.

2. Material and methods

2.1. Biomass feedstock

Two sorghum cultivars were grown at the Texas A&M University Farm, College Station, Texas. The forage/energy sorghum TX08001 is a photoperiod-sensitive hybrid, resulting in an extended period of vegetative growth and consequently high biomass yields due to delayed anthesis (Gill et al., 2014; Olson et al., 2012) as well as exhibiting high nitrogen use efficiency (Olson et al., 2013). The sweet sorghum cultivar Della is an inbred with known characteristics of pathogen resistance, variable stalk height with susceptibility to lodging, and mid-season maturation/senescence (Bitzer, 2006). Stems from both cultivars were harvested in mid-August 2014, which for Della corresponded to 10–14 days post grain maturity and for TX08001 corresponded to the vegetative phase. After harvest, biomass was immediately frozen and stored at −20 °C.

2.2. Determination of water-extractable sugar content

Extractable sugar content was determined using 300–350 g of sorghum stems of known moisture content sampled from the lower stem nodes/internodes. These were mixed with 500 mL water and were mixed in a blender (Oster Simple Blend 100, Sunbeam Products, Inc., Boca Raton, FL) for 5.0 min at a power setting of "low" and this mixture was allowed to stand for 48 h at 4 °C. Following extraction, the liquid sample was analyzed for glucose, fructose, and sucrose by HPLC (Agilent 1100 series) equipped with a Bio-Rad Aminex HPX-87 P column using distilled water as the mobile phase at a flowrate of 0.6 mL/min and detection by refractive index (RI). Starch content in extracts was determined according to Sekhon et al. (2012). Extractions, moisture content, and sugar analysis were performed in triplicate, and using these results, the mass fraction of water-extractable sugars was determined on an unextracted dry biomass basis.

2.3. Manual physical fractionation by anatomy

Sorghum stems from both cultivars were first separated as "bottom", "middle", and "top", with each fraction representing 3–4 nodes/internodes (Fig. 1A). The stem internodes were next separated into different fractions using procedure modified from Wilson et al. (1993). For this, the epidermis + outer rind (EOR) containing a high density of vascular bundles was first separated. The inner rind was next removed by peeling until the light green tissue of the rind/epidermis was visible. Inner stem tissues were separated into vascular bundles and pith fractions. Next, the vascular bundles from the center of the stem and vascular bundle-rich inner rind were combined into a single fraction: vascular bundles + inner rind (VBIR). Finally, the different anatomical fractions were exhaustively extracted with distilled water to remove water-soluble extractives. After drying at 50 °C to ~5% moisture as determined by NREL/TP 510-42621 (Sluiter et al., 2008a), the manually classified fractions were individually ball milled (TissueLyser II, Qiagen, Hilden, Germany) for 3 cycles of 2 min with approximately two minutes of cooling of stainless balls and jars in liquid nitrogen between cycles. The separated tissues were stored in seal containers at ambient temperature until use.