STORAGE OF BIOMASS FEEDSTOCKS: RISKS AND OPPORTUNITIES

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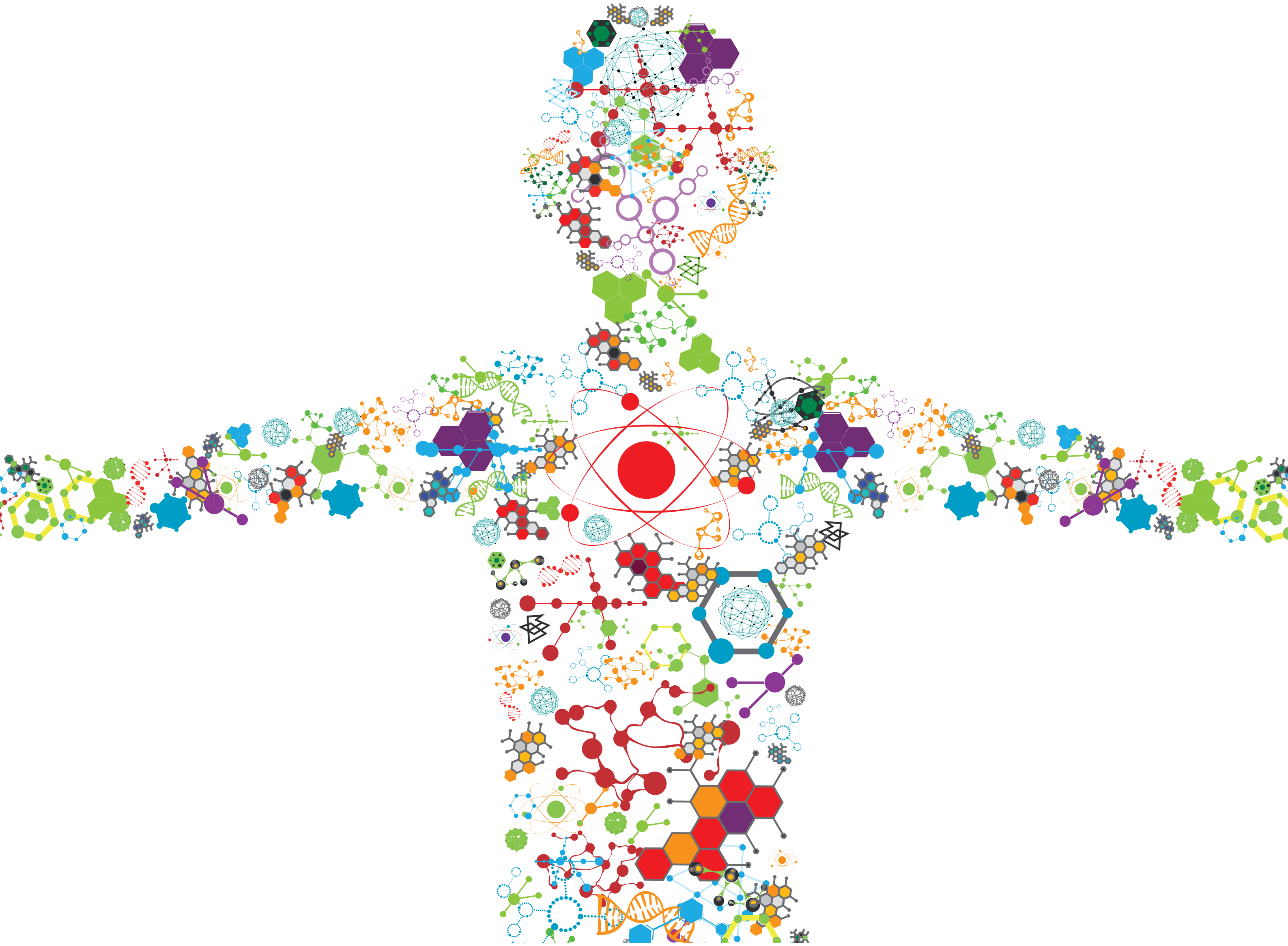

frontiers Research Topics

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STORAGE OF BIOMASS FEEDSTOCKS: RISKS AND OPPORTUNITIES

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Editorial: Storage of Biomass Feedstocks: Risks and Opportunities

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Editorial on the Research Topic

Storage of Biomass Feedstocks: Risks and Opportunities

Storage is a necessary unit operation in the biomass feedstock logistics supply chain, enabling biorefineries to run year-round despite daily, monthly, and seasonal variations in feedstock availability. For example, agricultural sources of biomass such as corn stover are harvested annually and require up to 9 months of storage to enable year-round operation (Darr and Shah, 2012). Industries that rely on forest resources, including the pulp and paper, pellet and bioenergy industries, often store biomass onsite at the processing center for days or weeks to ensure that sufficient material is available (Sahoo et al., 2018). There is much uncertainty about the effect of storage on different feedstocks and for differing utilization approaches. This Research Topic focused on the impact of storage of biomass prior to utilization for bioenergy and/or bio-based products.

At a minimum, effective storage approaches must preserve both the quantity and quality of biomass. Uncontrolled loss of biomass due to microbial degradation is common when storage conditions are not optimized. This can lead to physical and mechanical challenges with biomass handling, size reduction, preprocessing that have negatively impacted demonstration-scale integrated biorefineries (U.S. DOE, 2016). Degradation in storage can also result in biomass that is more recalcitrant to chemical and enzymatic approaches to depolymerization and ultimately results in lower product yields (Groenewold et al., 2020). Loss of feedstock to fires is also possible with dry, combustible feedstocks such as baled material.

The Research Topic was prefaced by a review from Wendt and Zhao that described the state of technology for dry and wet storage systems with a particular focus on improvements that have been observed in feedstocks destined for bioenergy utilization. The article pointed to improvements necessary in the area that can improve stability while maintaining cost competitiveness in comparison to fossil transportation fuels. Nguyen et al. proposed approaches to address this cost barrier by preconditioning biomass during anaerobic storage followed by a fractionation approach to isolate chemically distinct fractions that could have multiple product applications including biofuels, liquid plant biostimulants, and lignin-based phenolic resins for polymers. Such preprocessing is facilitated by biomass depots located near the field to minimize low density transportation costs.

Limiting dry matter loss is one of the most important considerations for storage system design. A study by Therasme et al. examined hot water extraction of wood chips and compared dry matter loss with freshly harvested chips under storage conditions of winter/summer storage, and followed dry matter loss over time and by location in the pile. Dry matter losses were higher during summer storage regardless of treatment. Fresh chips and extracted chips had similar dry matter losses in the initial storage, but extracted chips had much lower losses after 180 days of storage. A model was developed to predict dry matter loss over time in the pile. Quiroz-Arita et al. also developed a model

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to predict temperature response that results from heat produced during microbial respiration associated with dry matter loss. The model included contributions of conductive and convective heat transfer within a storage zone as well as evaporative heat loss to the environment and thermal capacitance of the biomass itself. Models such as this can be used to understand how temperature increases are indicative of storage losses under aerobic scenarios.

High moisture levels in aerobically stored biomass is directly correlated with dry matter loss due to microbial degradation. A study examining natural air drying with and without added heat was conducted by Mak et al. in western Canada on several types of stored woody biomass. The study demonstrated that positive energy gains could be made relative to the original energy content and that faster drying was possible by only drying during the most favorable conditions.

Smith et al. also investigated the relationship of moisture reduction and dry matter loss in corn stover as a function of aerobic storage in highly insulated storage reactors that mimic bale stacks. The study found that the rate and extent of degradation increased significantly above 36% moisture, wet basis. Stored induced changes were linked to chemical changes due to hemicellulose degradation as well structural changes including increased hydrophilicity, but conversion potential remained unchanged at the biorefinery gate.

Corn stover structural changes occurring in storage were also investigated by Nagle et al. Anaerobic storage through ensiling was utilized to preserve corn stover in long term storage. Minor structural losses in carbohydrates were observed compared to the non-ensiled control; however, bioconversion requirements remained constant. Ultrastructural changes of cell wall matrix removal and re-localization were shown using transmission electron microscopy in ensiled corn stover rind vascular bundles, suggesting that ensiling results in minor changes that may have structural integrity implications in further preprocessing.

Feedstocks applicable to bioenergy systems include agricultural residues (i.e., corn stover, wheat straw), herbaceous energy crops (switchgrass, miscanthus, energy cane, sweet sorghum), woody energy crops (hybrid poplar, coppice willow), forest products and residues, microalgae and macroalgae species, and fractions of municipal solid wastes. Wendt and Zhao suggest storage formats most commonly used for bioenergy resources potentially available in the United States. Wahlen et al. surveyed additional waste resources available in the southern United States that may be compatible with ensiling microalgae. The study then investigated blending grass clippings with microalgae, which preserved dry matter loss while lowering the nitrogen content for downstream thermochemical conversion through hydroprocessing.

A study by Müller and Hahn also investigated blending as a means to preserve biomass in anaerobic storage. Flower strips grown in Europe to enhance biodiversity offer a novel source of biomass available seasonally. The flower strips had modest ability to ensile by themselves but when combined with corn stover, the silage quality was much improved. Additionally, the flower strips contained high levels of nitrate which repressed *Clostridia* activity and preserved dry matter.

In summary, the research represented in the Research Topic exhibited the vast importance of stable storage for bioenergy crops as well as showing how storage-related effects may impact downstream conversion to biofuels or bio-based products through biological/biochemical and thermal/thermochemical and physical deconstruction. Many opportunities exist to use storage to begin to deconstruct the biomass, making it easier to depolymerize prior to conversion.

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LW and VT wrote the editorial with contributions from TV. All authors approved it for publication.

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Hot Water Extracted and Non-extracted Willow Biomass Storage Performance: Fuel Quality Changes and Dry Matter Losses

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Dry matter losses (DML) and fuel quality changes occurring in storage piles are important parameters for the management of any biomass supply system. This study evaluates the effect of a hot water extraction pretreatment, harvest season, depth in storage pile and initial moisture content on willow biomass fuel quality [moisture, ash, higher (HHV) heating value and lower (LHV) heating value] during storage, and models DML in storage piles based on experimental data. For the summer storage (SS) pile, mesh bags containing freshly harvested chips (FC) were inserted at 0.5–1 m deep in the pile. For the winter storage pile (WS), the mesh bags were filled with FC and hot water extracted chips (HC) with three different initial moisture contents inserted in the shell (<0.45 cm) and the core (1–1.5 m) of the pile. The ash contents through all sampling periods were in the range of 1.1–2.2% for FC and 0.6–2.1% for HC from both the shell and core of the WS pile. Higher ash contents, in the range of 2.1–3.4%, were observed in SS pile. Moisture contents of the storage piles had differing patterns over time. DML was the highest in the SS pile, reaching up to 33.6% after 140 days in storage; in contrast, there was no significant increase in DML over the first winter season. Although DML of FC and HC were in the same range during the initial storage period, DML of HC was 40% lower than FC after 180 days of storage. Higher DML was observed in the core (e.g., 17.3% for FC) compared to the shell (e.g., 12.1% for FC) at the end of the WS trial. There was no particular trend observed between initial moisture and DML. This study suggests that a linear model is sufficient to estimate DML, but a non-linear model may be needed for chips stored in SS piles for 6 months or longer. It also suggests that DML is reduced in storage piles created in winter, and that willow chips kept in SS should be utilized within 2 months for a DML below a 10% threshold.

Keywords: willow biomass, hot water extraction, bioenergy, storage, dry matter loss, fuel quality

INTRODUCTION

Short rotation woody crops (SRWC), such as shrub willow (*Salix* spp.) and hybrid poplar (*Populus* spp.), are being developed in North America and Europe for bioenergy (Mola-Yudego et al., 2016; Volk et al., 2016, 2018). In the Northeast United States, willow chips are currently mixed with other wood chips to generate heat and power (Volk et al., 2016). Storage is an essential component of the biomass supply system because harvesting windows are limited while demand for feedstock for power, biofuels and biobased chemicals is required year-round. Maintaining feedstock quality during storage is essential for end users and the success of the entire system.

Dry matter loss (DML) can occur at multiple places along the supply chain such as during harvesting, loading and unloading of vehicles, transportation, and storage. DML is an important factor as it can translate into loss of revenue (Routa et al., 2018; Therasme et al., 2019), waste of resources, higher greenhouse gas emissions per unit of energy delivered, and challenges at conversion facilities. A DML of 10% during the storage of willow biomass for heat production would result in 6% increase of greenhouse gas emissions per unit of delivered heat and 9% decrease of the net energy yield per unit of land (Whittaker et al., 2016).

DML in storage piles is caused by biological decomposition and chemical reaction (Krigstin and Wetzel, 2016) and is influenced by numerous factors, including composition of the material, particle size, cover system, storage duration, pile size, harvest season, species, oxygen availability, weather conditions, and moisture content (Manzone et al., 2013; Barontini et al., 2014; He et al., 2014; Hofmann et al., 2017; Pari et al., 2017; Pecenka et al., 2018; Whittaker et al., 2018). Additional questions remain to be addressed to understand the effect of biomass preprocessing on DML during storage and the variation of DML at different depth within a storage pile.

The season of harvest influences the initial quality of harvested biomass and will impact reactions in storage piles. Whether leaves are included in the harvested material or not has an influence on the quality of the material as well as potential implications for nutrient cycling at the site. During spring and summer, the harvested biomass dries naturally as a result of high air temperature. But, moisture content increases during autumn and winter storage (Filbakk et al., 2011). The moisture content of uncovered willow chip storage piles harvested in late spring with a moisture content of 46% decreased to 37–26% after 60 days of storage and increased thereafter until the end of the storage trial in mid-autumn (Therasme et al., 2019). However, Eisenbies et al. (2016) reported an increase of moisture from 42% at harvest in February to 49% in May and 44% in July. Bark and foliage have higher organic nitrogen and moisture content than wood and higher spore counts, both of which can stimulate bacterial and fungal growth (Krigstin and Wetzel, 2016).

Most recent SRWC storage studies in the United States do not report the amount of DML in outdoor storage pile of wood chips (Ergül and Ayrilmis, 2014; Lin and Pan, 2015; Eisenbies et al., 2016; Therasme et al., 2019). However, DML reported in European studies ranges from 0.9 to 4.5% per month and total DML can reach up to 47% after 18 months of storage in open outdoor piles (**Table 1**). While these studies contribute to a better understanding of the rate of DML and factors that influence it, the direct use of these values in modeling, such as techno-economic assessment or life cycle assessment of biomass storage systems, is only valid for storage under similar conditions. Also, while some studies show linear increase of DML over time others report positive but diminishing rate where DML approaches some asymptotic maximum (Mooney et al., 2012).

Hot water extraction (HWE) of wood chips is a preprocessing step that removes predominately hemicellulose along with smaller amounts of other compounds and ash. Hemicellulose can be hydrolyzed to generate fermentable C-5 sugars and organic acids, while leaving behind a solid residue. The solid residue that remains after HWE contains a higher fraction of lignin and cellulose than the non-extracted chips, resulting in an increase in the higher heating value (Therasme et al., 2018). The removal of a fraction of the original weight of the wood chips increases the porosity and pore size of the pulp fibers, which could affect water absorption and retention (Duarte et al., 2011). These changes in the structure and composition of the HWE wood chips raise questions about the dynamics of this material in storage piles and how DML is impacted.

The first objective of this study is to determine the dry matter loss, and changes in moisture, ash, and heating value of hot water extracted and non-extracted willow chips stored in a pile created at the start of winter and non-extracted willow chips in a pile created in the summer. The second objective is to evaluate the relationship between DML and storage time, moisture content, ash content, and harvest season of willow chips. Moisture content, ash content, and heating value are key parameters, especially for thermochemical conversion processes, because they influence the conversion efficiency and the cost of production. Conversion facilities will operate year-round so the changes in quality and DML associated with storage are important to understanding how feedstock systems for these facilities need to be designed and managed.

MATERIALS AND METHODS

To address the objectives of this study, we established two storage piles with freshly harvested willow biomass that were monitored on a regular basis. The first pile was constructed with leaf-on freshly harvested materials in summer 2017. A second pile was made with leaf-off materials harvested during the winter 6 months later. Bags of fresh chips (FC) and HWE chips (HC) with three levels of initial moisture contents were inserted in the core and shell of the winter pile. The bags were collected from the piles over the entire storage period to determine DML, moisture, ash, higher and lower heating values.

Summer Storage Pile

The summer storage (SS) trial took place in Solvay, NY (43°03′56.0′′N, 76°15′42.4′′W). The site was a former industrial site containing high level of calcium, sodium, and chloride ions (Effler, 1996). The area harvested for this experiment was planted in 2012 with the following willow cultivars: Fish Creek,

TABLE 1 | Reported values for dry matter loss (DML) for outdoor storage piles of willow and poplar chips.

Location	Species	Age of stand (years)	Storage length (months)	Harvest season	Total DML (%)	Monthly DML (%)	Cover	References
Germany	Poplar	3–4	6–8	Winter	15–27	2.5–3.4	Yes	Pecenka et al., 2014
Germany	Poplar	4–5	7	Winter	17–19	2.4–2.7	Yes	Pecenka et al., 2018
Germany	Poplar	4–5	9	Winter	21–22	2.3–2.4	Yes	Lenz et al., 2015
UK	Willow	3	6	Winter	18–22	3–3.7	No	Whittaker et al., 2018
Italy	Poplar	18	6	Spring	6–27	1–4.5	No	Barontini et al., 2014
Italy	Poplar	15	18	Spring	24.6–47.1	1.4–2.6	No	Pari et al., 2017
Italy	Poplar	6	5.7	Spring	5.1–9.8	0.9–1.7	Yes/No	Manzone et al., 2013
Poland	Willow	–	12	Winter	3.8–41	0.3–3.4	Yes/No	Krzyzaniak et al., 2016

SX61, Millbrook, Sherburne, SX64, and Canastota. They were coppiced after the 2012 growing season and harvested for the first time on 21–22 June 2017 with a New Holland FR9080 forage harvester equipped with a New Holland 130FB coppice header. The harvester was set to produce the largest chips size (33 mm). The leaf content determined by manual sorting of six random samples of harvested material was 7.4 ± 4.2%. The particle size distributions of the harvested chips are listed in **Table 2**.

The SS pile was constructed at the edge of the field on the same day that harvesting was conducted. Loads of willow chips were dumped in a rough linear pile on open ground and shaped to produce an even contour using a tractor equipped with a front loader. The pile was 21.8 m long, 8.8 m wide, and 2.6 m tall. A total of 68 mesh bags (45 × 55 cm) made of polypropylene and containing 1.2–2.2 kg of FC willow biomass were inserted (∼2 m intervals) at about 0.5–1 m from the surface of the storage pile. HC was not included in this trial because this material was not available at that time. The exact locations of the samples were identifiable with a colored rope that was tied to the bags and brought to the surface of the pile. The weight of wood chips inserted in each net bag was recorded. While filling the bags, a total of 38 samples of approximately 1 kg were collected for initial moisture content determination. Finally, up to 12 temperature sensors connected to three data loggers (HOBO U12-008) were placed at 0.5–1 m deep in the storage pile to record pile temperatures automatically every 30 min. Air temperature and precipitation for the site were extrapolated from PRISM (Parameter-elevation Regressions on Independent Slopes Model) climate data for the site[1].

Winter Storage Pile

The winter storage pile (WS) experiment took place in Tully, NY (42°47′50′′N, 76°07′09′′W). Both sites—Tully and Solvay—classify as "Dfb" (snow, fully humid, warm summer) under the Köppen–Geiger climate classification (Kottek et al., 2006). A mixture of different cultivars of 3-year-old aboveground stems was harvested with the same settings on the New Holland harvester after leaf fall. The harvesting operation for this trial occurred in 2017 at two separate dates; on December 17th to collect the materials for the HWE run and December 22nd for the pile construction.

TABLE 2 | Particle size distribution of fresh (FC) and hot water extracted (HC) willow chips that were placed in bags and inserted into storage piles.

Site	Chips	<3.5 mm	3.5–15.9 mm	15.9–31.8 mm	31.8–63 mm	>63 mm
		%	%	%	%	%
Winter	FC	3.5	10.2	60.1	23.7	2.6
Winter	HC	4.2	11.1	57.9	23.9	3.0
Summer	FC	5.9	20.4	34.2	35.5	3.9

A fraction of the FC biomass was preprocessed via HWE prior to being included in the storage piles. The extraction of FC equivalent to 263 kg OD was performed in a 1.8 m^3 digester for 2 h at 160°C. The liquid to wood ratio was 4.7:1. At the end of the process the liquor was drained and the HC were washed by adding water equivalent to the volume of liquor removed into the digester and re-cooking at 80°C for 15 min. After draining the remaining liquor, the HC were removed from the digester. The total weight of chips was recorded before and after HWE, and samples were taken prior and after the HWE for moisture content determination. The HWE process removed 23.5% of the dry weight of the starting biomass. Two thirds of the HC were sent to the drying room and the remaining fraction was kept as is with no further processing.

For the WS trial, the bags were filled with two types of chips—HC and FC—with three moisture levels each (**Table 3**). The groups of HC and FC with lower moisture content were obtained by drying. The drying occurred in a kiln connected with a computer for automatic control of the relative air humidity and drying temperatures. For each samples group, the chips were homogenized by mixing then transferred into net bags. There were 54 net bags per group of samples for a total of 324 net bags filled with leaf off HC and FC willow biomass.

The winter pile was constructed on an open area near the harvesting site (**Figure 1**). Chips collected in a dump wagon were deposited on the ground in a rough linear pile and then molded into a consistent contour using a tractor equipped with a front loader. The height of this pile was 2.5 m, the width was 5.4 m,

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[1] PRISM Climate Group, Oregon State University Data Explor. Time Ser. Values Individ. Locat. Available online at: http://prism.oregonstate.edu (accessed December 27, 2018).

TABLE 3 | Initial moisture content of freshly harvested and hot water extracted (HWE) willow chips that were inserted into storage piles in the summer (SS) with leaf material and in the winter (WS) when no leaves were present.

Chips	Pile label	Site	HWE pretreatment	Number of bags (samples)	Initial moisture (%)	Initial moisture category
FC[a]	SS[b]	Summer	No	68	50.8 (3.0)[c]	High
FC	WS	Winter	No	54	48.9 (0.5)	High
FC	WS	Winter	No	54	32.4 (0.8)	Medium
FC	WS	Winter	No	54	20.5 (1.1)	Low
HC	WS	Winter	Yes	54	70.6 (1.4)	High
HC	WS	Winter	Yes	54	61.3 (0.6)	Medium
HC	WS	Winter	Yes	54	21.6 (1.1)	Low

[a] *FC represents fresh chips, HC hot water extracted chips.*

[b] *SS and WS represent summer and winter storage piles, respectively.*

[c] *The value in parentheses is the standard deviation.*

and the length was 19.1 m. At each sampling point, three bags of each treatment were placed in the core (1–2 m from the surface) and the shell (<45 cm from the surface) of the pile. The exact locations of the samples in the pile were marked using colored flags for the shell samples and willow stems for the core samples. The sampling points were placed at 1.5–2.5 m apart of each other along the length of the pile. A total of eight temperatures probes connected to two data loggers (HOBO U12-008) were inserted in the shell and core of the piles following the description provided by Eisenbies et al. (2016) and Therasme et al. (2019). Data loggers inserted in this storage pile automatically recorded the pile temperature every 30 min.

Sample Collection and Laboratory Analysis

Samples were collected on a regular basis to monitor the loss of dry matter and changes of other characteristics of willow chips during storage. Up to four net bags were removed from the SS pile every 1–2 weeks starting on June 30th until November 11th 2017. Two additional sets of samples were removed months later to test if there would be additional increase of DML during the winter and summer seasons; one set of samples in April 2018 and another one in August 2018. For the WS pile, three samples from each group of bags were pulled out from the shell and the core of the pile on a monthly basis. Sampling started at the south end of the pile and at each sampling date we move to the next sample location to the north. This process was followed to minimize disturbances to bags that remained in the pile. During sampling, disturbance to the pile was minimized and localized because the location of the bags was marked. Hand tools were used to remove the samples and refilled the holes as soon as the bags were recovered.

The samples were taken to the lab immediately after being pulled out of the piles and dried at 65°C to a constant weight. The amount of DML was calculated based on the dry weight of samples before and after storage. Dried samples were split to reduce the size of the samples to about 200 g, then ground in a Wiley mill equipped with a 0.5-mm screen. The ash content was determined by combustion in a thermolyne muffle furnace (Model F30400) equipped with a ramping program in accordance with the National Renewable Energy Laboratory NREL/TP-510-42622 method (Sluiter et al., 2008). The results of ash contents are reported relative to the 105°C oven dry weight of the sample. The HHV was determined according to the ASTM method D5865-13: Standard test method for gross calorific value of coal and coke by using a Parr 6200 Oxygen bomb calorimeter (ASTM, 2013). The HHV results are reported on a dry basis. The LHV represents the maximum potential energy available in an as-received biomass fuel and was calculated using the formula described by Krigstin and Wetzel (2016).

Regression Analysis

The statistical analysis was conducted for DML by application of linear mixed models and non-linear regression models using Statistical Analysis System version 9.4 (SAS Institute, Cary, NC). The data for the SS and WS piles were combined together for this analysis. The data from the last two sampling points (284 and 417 days) of the SS pile were included only for the non-linear models. The non-linear models have the capability to represent curves with decreasing slope. However, the linear models apply for storage of biomass for a period of 1–6 months.

Linear Mixed Models

A full model (Equation 1) defines DML as a function of harvest season, initial moisture, moisture, ash, depth, HWE treatment and storage period. From the full model, three candidate models (Equations 2–4) were selected by using R^2 and Mallows Cp criteria. The MIXED procedure was used to fit the selected models to the data by considering harvest season, depth and HWE treatment as fixed effects while allowing random deviation from one sampling period to another. A first-order autoregressive AR(1) variance structure was chosen to take into account the correlation between measurements on bags from the same pile's depth at adjacent periods and an unstructured structure for the random terms. Since bags were not returned to the pile and the requirement for the levels of the repeated effect to be different for each observation within a subject, the averages of the replicates were used to fit the models. This step was necessary to avoid singularity of the variance of the unobserved random errors. Statistical significance for parameter coefficients was claimed for $p < 0.05$.

$$DML = \alpha_0 + \sum_{i=1}^{7} \alpha_i A_i + \sum_{i=1}^{7} \sum_{\substack{j=2 \\ i\neq j,\ j>i}}^{7} \beta_{ij} A_i A_j + \beta_{11} A_1^2 + \sum_{i=2}^{7} \gamma_i \, A_i A_1^2 \quad (1)$$

Where α_i, β_{ij}, and γ_i are regression model parameters, A_i and A_j are the covariates (i.e., harvest season, initial moisture, moisture, ash, depth, hot water extraction, and period), and A_1 is storage period.

Model 1:

$$DML = \alpha_0 + \alpha_1 \, Period + \alpha_2 \, Season \times Period + \alpha_3 \, Depth \times Period + \alpha_4 \, Extraction \times Period^2 \quad (2)$$

FIGURE 1 | A winter storage pile of willow chips created at an experimental field station in Tully, NY.

Model 2:

$$DML = \alpha_0 + \alpha_1\, Ash \times Period + \alpha_2\, Season \times Period \quad (3)$$

Model 3:

$$\begin{aligned} DML = {} & \alpha_0 + \alpha_1 Period + \alpha_2\, Season \times Period \\ & + \alpha_3\, Depth \times Period + \alpha_4\, Extraction \times Period \\ & + \alpha_5\, Inimoisture \times Period^2 \end{aligned} \quad (4)$$

Non-linear Mixed Models

Considering that DML increases at a decreasing rate and eventually approaches some asymptotic maximum, two non-linear models were fit using the NLMIXED procedure. Model 4 (Equation 5) is an exponential decay model that gives rise to a maximum value (k_0). However, model 5 (Equation 6) is based on a logistic function where the maximum DML (k_0) depends on harvest season.

Model 4:

$$DML = k_0 \times (1 - e^{(-k \times Period^c)}) \quad (5)$$

With $k_0 = \alpha_0 + \alpha_1\, Season + \alpha_2\, Depth + \alpha_3\, Extraction + \alpha_4\, Airtemp + \alpha_5\, Precipitation$, and $c = \gamma_0 + \gamma_1\, Season$.

Model 5:

$$DML = \frac{k_0}{1 + e^{(-k \times (t - t_m))}} \quad (6)$$

With $k_0 = \alpha_0 + \alpha_1 Season + \alpha_2\, Season \times Depth + \alpha_3\, Season \times Extraction$ and

$$t_m = \beta_0 + \beta_1\, Season. \quad (7)$$

Where:

Period: number of days in storage;
Season: dummy variable designating summer or winter storage pile (0, 1);
Depth: dummy variable designating samples from the shell or the core of a storage pile (0, 1);
Extraction: dummy variable designating storage of freshly harvested willow biomass or storage of HWE willow biomass (0, 1);
Inimoisture: initial moisture content prior to storage (%wb);
Airtemp: average daily air temperature of the current month of storage (°C);
Precipitation: average daily precipitation of the current month of storage (mm);
Ash: ash content of samples when pulled out of the storage pile (%).

RESULTS AND DISCUSSION

Weather Conditions and Pile Temperatures

For the SS, mean daily air temperature ranged from −19.1 to 24.4°C over the first 284 days, with an average air temperature of 7.5°C. During the first 2 months the mean daily air temperature was consistently above 15°C (**Figure 2**) creating favorable conditions for natural drying. Total precipitation was 913 mm over 284 days and the site received daily precipitation above 20 mm for a total of 9 days over the same period of time. The temperature in the SS pile increased rapidly to 47°C within 7 days and remained higher than the daily mean air temperature for 3 consecutive months; the pile temperature equilibrated with air temperature for the remaining of the SS trial.

For the WS pile the mean daily air temperature ranged from −19.8 to 26.4°C for a period of 207 days (**Figure 3**), with an average air temperature of 7.4°C. The air temperature during the WS pile trial was continuously below 15°C for more than 3 months. The site received a total of 585.5 mm of precipitation and a total of 5 days with daily precipitation exceeding 20 mm. Temperatures recorded in the shell and core of the WS pile showed differing patterns. The core temperature rose to 53°C in 3 weeks and remained higher than the air temperature for more than 3 months. However, the shell temperature followed the ambient air temperature for the entire storage trial and was below 0°C 65% of the time during the first 3 months.

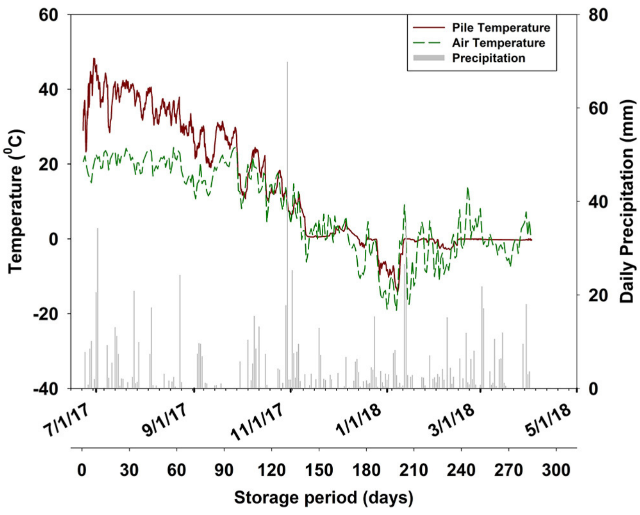


FIGURE 2 | Daily mean air temperature, precipitation, and pile temperature measured at 0.5–1 meter from the surface of a summer willow chips storage pile in Solvay, NY (the full dataset for air temperature is available at http://prism.oregonstate.edu/explorer/).

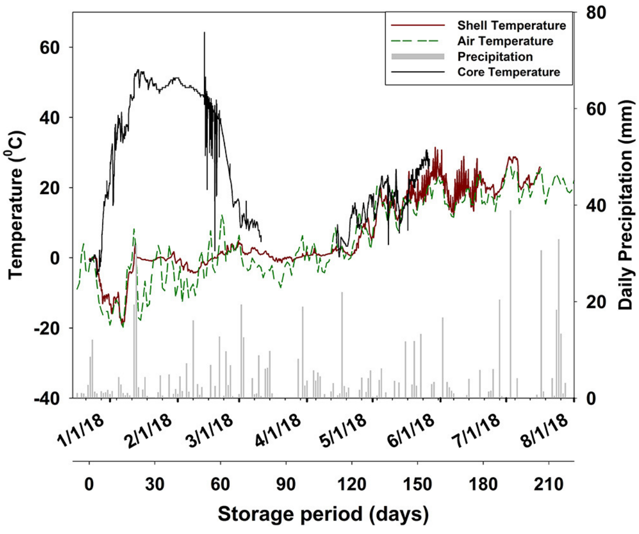


FIGURE 3 | Daily mean air temperature, precipitation, and temperature in the shell and core of a winter willow chips storage pile in Tully, NY.

The rapid increase of temperature immediately after the construction of chips storage piles was expected and has been reported previously in the literature (Jirjis, 1995; Krzyzaniak et al., 2016; Whittaker et al., 2018; Therasme et al., 2019). This increase of temperature could be associated with heat released through wood respiration and microbial activities. The season of the pile construction seems to play a role in the differing temperature patterns observed between the shell and the core of the storage piles. Because the air temperature was below zero degree celsius most of the time during the first 2 months of storage (**Figure 3**) in a chips pile created in winter, as opposed to a pile created in late spring or summer, the outside layer (shell) of the pile froze, thus inhibiting any microbial activity that would generate additional heat and cause an increase of temperature in the shell of the WS pile.

Dry Matter Loss and Quality Change During Storage

Initially DML in the SS pile was slow, reaching 5.6% by day 43 and then it increased over time and reached 33.6% after 141 days (**Table 4**, **Figure 4**). Determination of DML at two additional sampling periods at much later dates showed no additional loss of dry matter between storage days 141–284, which includes the winter season, and slight increase of DML at a rate of 0.7% per month for the remaining of the storage. The results from this trial are in line with previous findings that indicate a decline in the rate of DML after long storage time (Jirjis, 1995; Anerud et al., 2018). However, the DML found in the SS trial were significantly higher than the 7.3% reported for a 7 month storage of logging residues chips in a SS pile (Anerud et al., 2018). During the first 2 months, the moisture content in the SS pile was the same or slightly lower than the initial moisture content of the harvested FC, but after 2 months it was consistently higher and reached up to 80%. This increased moisture content later during the storage trial was consistent with data reported in the literature for storage pile studies conducted in the region (Eisenbies et al., 2016; Therasme et al., 2019). There were also small changes in ash content within and from one sampling period to another, with mean ash content by sampling period in the range of 2.1–3.4%. The mean HHV by period was consistent, varying from 18.7 to 19.4 MJ/kg. The higher end for both ash and HHV were recorded at 141 days and they were, respectively, 35 and 3% higher than the corresponding values at the beginning of the storage. Because the moisture content of the chips in the pile started to increase after 2 months, the LHV also declined and was as low as 3.3 MJ/kg after 141 days.

The results reported in **Tables 5**, **6** depict the variability of DML, moisture, ash and heating values of FC and HC in the shell and core of the WS pile. After 207 days, DML in FC bags across the three moisture treatments were 7.2–14.2% in the shell and 13.9–20.1% DML in the core. For HC bags, DML were in the range of 6.3–8.7% in the shell and 7.7–17.2% in the core. The DML reported here for WS pile correspond to a rate of 1.0–2.9% per month, and are within the range of reported DML from WS pile (see **Table 1**). But, they were lower than the rates of 3.0–3.7% per month that were found in storage trials of shrub willow in UK (Whittaker et al., 2018) and the rate of 3.4% per month from another trial conducted in Poland (Krzyzaniak et al., 2016). The lower rate of DML from the current study is mostly due, despite the difference in geographic locations and local climate conditions, to the fact that the pile for the current study was created in the beginning of the winter season (December) while the other trials from UK and Poland were started in mid to late winter (February and March). Therefore, the higher ambient air temperatures in the UK and Poland trials could foster more the microbial activities. On average the DML was higher in the core of the WS pile than the shell throughout the storage trial. Perhaps,

TABLE 4 | Changes (mean and standard deviation) in dry matter loss (DML), moisture, ash, and higher heating value (HHV) and lower heating value (LHV) in a leaf-on willow summer storage (SS) pile in Solvay, NY (first day is June, 22nd 2017).

Period Days	Moisture % w.b.	Cumulative DML %	Ash %	HHV MJ/kg	LHV MJ/kg
8	45.4 (4.1)[a]	2.1 (1.6)	2.5 (0.2)	18.9 (0.2)	9.2 (0.9)
15	39.8 (1.4)	0.3 (0.2)	3.2 (0.2)	–[b]	–
25	45.4 (6.7)	5.5 (2.1)	2.7 (0.4)	–	–
29	44.4 (2.4)	5.5 (1.3)	2.8 (0.8)	–	–
43	47.5 (2.7)	5.6 (2.1)	3.2 (0.5)	18.8 (0.1)	8.7 (0.6)
57	39.7 (9.2)	10.9 (7.0)	2.5 (0.3)	–	–
71	55 (10.1)	19.5 (10.1)	3.1 (1.4)	18.7 (0.3)	7.1 (2.3)
88	52.2 (10)	14 (8.3)	2.1 (0.9)	–	–
102	54.3 (4.3)	18.8 (5.0)	2.6 (0.3)	19 (0.1)	7.3 (0.9)
112	64.8 (2.8)	24.4 (6.3)	3.2 (0.4)	–	–
127	64.1 (11.6)	30.6 (2.9)	3.3 (0.6)	–	–
141	73.6 (3.6)	33.6 (7.0)	3.4 (0.2)	19.4 (0.8)	3.3 (0.9)
284	79.8 (3.5)	32.8 (4.6)	–	–	–
417	74.4 (2.2)	36.2 (5.6)	–	–	–

[a] *Value in parentheses is the standard deviations.*
[b] *Value not determined.*

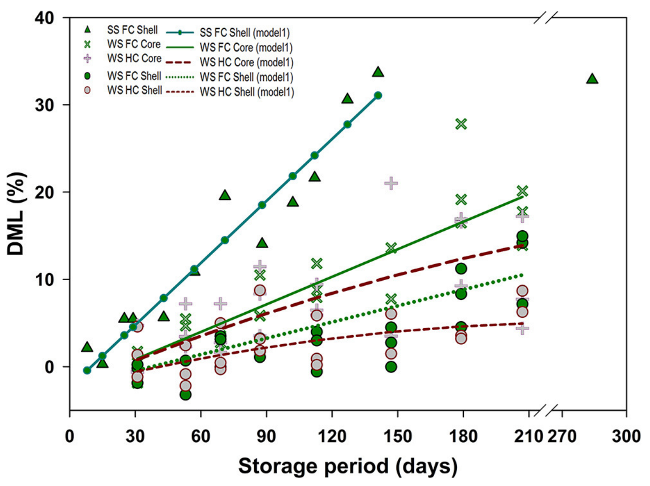


FIGURE 4 | Representation of predicted dry matter loss (DML) (model 1) and DML value for fresh (FC) and hot water extracted (HC) willow biomass chips that were stored at various initial moisture in outdoor piles created in the winter (WS) and summer (SS).

this may also be linked to the fact that the core of the WS pile was warmer than the shell during the first 2 months. However, the difference between FC and HC was relatively small during the first 150 days but was on average 6% higher in FC than HC at the end of the storage period. It is unclear why the difference between FC and HC become more evident only at the later stage of the storage, but it seems to be related with the structural and morphological changes of the wood chips during the extraction. HC has higher surface area than FC which may accelerate HC decays (Duarte et al., 2011). However, HC have higher proportion of lignin than FC (Therasme et al., 2018), therefore, preferential decomposition of carbohydrates may result in lower DML for HC in the long run.

In the absence of significant decay, it is possible to find negative but small values for DML as it was the case for some observations during the first 2 months in the shell of the WS pile. Similar observations were reported previously in the literature (Pecenka et al., 2014, 2018; Lenz et al., 2016). These results could be attributed to natural variations in the estimation of the true value.

The moisture content of FC (**Table 5**) and HC (**Table 6**) from the shell of the WS pile was constantly higher than the initial moisture (**Table 3**) for the entire storage period. The initial gradient of moisture among the different groups of bags that were inserted in the WS pile did not last for long in the shell of the WS pile. The low moisture FC bags, which had moisture differences at the beginning of the storage of 12% with medium moisture FC and 28% with high moisture FC, ended after 31 days of storage with moisture differences of only 4% with medium moisture FC and 15% with high moisture FC. For low moisture HC bags the differences were, respectively 12 and 17% with medium moisture HC and high moisture HC. At the end of the study period, the average moisture contents within all three initial moisture treatments were 57–61% for FC bags and 71–74% for HC bags from the shell of the pile. The higher moisture content observed for the HC could be explained in part by their increased cell wall porosity after hot water extraction (Duarte et al., 2011).

Similar to the shell, the moisture content of bags from the core of the WS pile was generally higher than the initial moisture content except for high moisture FC (**Table 5**) and high moisture HC (**Table 6**). The high moisture FC had up to 21% point decrease in moisture (113 days) while the highest decrease for low moisture HC was 7% after 207 days. These observations are the results of two processes: (1) dried and low moisture chips absorbing moisture from humid air and wetter chips in the surrounding, and (2) wetter chips releasing moisture as part of the natural drying process in the core of the pile. Both FC and HC from the core of the WS pile were drier than those from the shell. The moisture content of FC in the core was on average across the storage period 15–20% point lower than the shell, while it was 26% for low moisture HC and 7% for medium moisture HC and low moisture HC. At the end of the storage the moisture content in the core of the WS pile was 43–45% for all three FC groups. Differing moisture contents across different layers of the pile have been reported previously in the literature (Jirjis, 2005; Anerud et al., 2018; Therasme et al., 2019). For this trial, the differences between the moisture content in the shell and the core could be attributed to: (1) the high temperature recorded in the core of the WS during the first 2 months, thus facilitating the evaporation in the core, (2) low ambient air temperature limiting the evaporation in the outside layer (shell) of the pile, (3) addition of moisture to the shell of the pile through precipitation and exposure to air humidity.

Compared to FC from the SS pile, the FC and HC bags from the WS pile had lower ash content because of the absence of foliage in the winter and the removal of mineral elements during the HWE process. The ash content across all sampling

TABLE 5 | Changes in dry matter loss (DML), moisture, ash, higher (HHV), and lower (LHV) heating value for three initial moisture contents of fresh chips (FC) in the core and shell of a leaf-off willow winter storage pile (first day is December, 22nd 2017).

		Shell					Core				
Period (days)	**Initial Moisture (%)**	**DML (%)**	**Moisture (%)**	**Ash (%)**	**HHV (MJ/kg)**	**LHV (MJ/kg)**	**DML (%)**	**Moisture (%)**	**Ash (%)**	**HHV (MJ/kg)**	**LHV (MJ/kg)**
31	Low	−0.2 (0.7)[a]	39.1 (2.9)	1.5 (0.1)	18.8 (0)	10.5 (0.6)	0.4 (1.5)	25.2 (1.1)	1.6 (0)	18.6 (0.1)	13.3 (0.2)
31	Medium	0.2 (0.5)	43.4 (6.5)	1.5 (0.1)	18.6 (0.1)	9.4 (1.4)	1.7 (1)	32.7 (0.2)	1.4 (0.5)	18.7 (0.1)	11.8 (0.1)
31	High	−1.9 (1.6)	53.8 (3.3)	1.8 (0.5)	18.9 (0.1)	7.4 (0.7)	−1.9 (1.1)	42.9 (0.7)	1.6 (0.3)	18.8 (0.1)	9.7 (0.1)
53	Low	−0.9 (1.1)	36.6 (8)	1.7 (0.2)	–[b]	–	2.8 (1.1)	22.3 (1)	1.7 (0.1)	–	–
53	Medium	–	–	–	–	–	5.5 (1.1)	31.5 (3.2)	1.6 (0.1)	–	–
53	High	−3.2 (0.7)	48.6 (4.4)	1.7 (0.2)	–	–	5.6 (0.7)	53.3 (1)	1.7 (0.1)	–	–
69	Low	3.5 (0.5)	65.6 (5.4)	1.7 (0.1)	19 (0.1)	4.9 (1.1)	3.5 (0.8)	30.2 (8.1)	1.8 (0.1)	18.7 (0.4)	12.3 (1.5)
69	Medium	3.1 (2.3)	52.2 (9.8)	1.7 (0.1)	–	–	2.7 (0.5)	39.4 (16.2)	1.7 (0.1)	–	–
69	High	−0.1 (0.8)	61.9 (9.9)	1.8 (0.1)	18.9 (0.2)	5.7 (2.2)	3.3 (2.3)	38.9 (4.4)	1.9 (0.1)	18.8 (0.2)	10.5 (0.8)
87	Low	3.2 (0.9)	58.7 (1.2)	1.7 (0.1)	–	–	5.8 (1.9)	22.6 (0.7)	2.1 (0.4)	–	–
87	Medium	1.1 (0)[c]	57.7 (0)	1.8 (0)	–	–	5.9 (2.7)	27.9 (2.1)	1.7 (0.2)	–	–
87	High	1.8 (3.5)	64.5 (2.1)	1.8 (0.6)	–	–	11.8 (2)	36.2 (0.6)	1.5 (0.1)	–	–
113	Low	4 (1)	64.4 (3.7)	1.8 (0.3)	18.8 (0.1)	5.1 (0.8)	8.8 (0.1)	29.4 (6.9)	1.9 (0.1)	18.6 (0.3)	12.4 (1.6)
113	Medium	3 (1.1)	52.1 (3.7)	1.1 (0.7)	–	–	11.8 (0.7)	49 (3.8)	1.9 (0.1)	–	–
113	High	1.7 (2.7)	67.8 (1.6)	1.9 (0.2)	19.1 (0.3)	4.5 (0.5)	7.9 (2.2)	28.2 (4)	1.8 (0.2)	18.7 (0.2)	12.7 (0.9)
147	Low	4.5 (0.5)	63 (2.2)	1.9 (0.2)	–	–	13.6 (4.3)	52.2 (12.8)	2.2 (0.4)	–	–
147	Medium	2.7 (0)	62.5 (0)	1.9 (0)	–	–	7.8 (1)	42.3 (13.4)	1.8 (0.4)	–	–
147	High	0 (1.4)	60.1 (9.9)	2 (0.1)	–	–	–	–	–	–	–
179	Low	11.2 (1.4)	58.4 (7.2)	1.8 (0.3)	18.9 (0.2)	6.4 (1.6)	27.8 (8.2)	45.5 (9)	1.6 (0.8)	19 (0.1)	9.2 (1.9)
179	Medium	8.3 (0.2)	59.6 (3.4)	2.1 (0.2)	–	–	19.2 (4.6)	40.3 (18.8)	1.8 (0.3)	–	–
179	High	4.5 (1.6)	61.3 (6.2)	1.3 (0.7)	20.0 (1.0)	6.3 (1.8)	16.5 (7.6)	37.1 (16)	2 (0.9)	19 (0.3)	11.1 (3.4)
207	Low	14.2 (2.1)	57.3 (5)	2.2 (0.1)	–	–	13.9 (8.4)	43.5 (20.3)	2.1 (0.3)	–	–
207	Medium	14.9 (0)	61.3 (0)	2 (0)	–	–	20.1 (5.5)	55.1 (6.1)	2 (0.4)	–	–
207	High	7.2 (5.4)	58.2 (6.9)	1.6 (0.2)	–	–	17.8 (3.2)	46.3 (10.1)	2.1 (0.2)	–	–

[a] *The value in parentheses is the standard deviation.*
[b] *Value not determined.*
[c] *The number of replication is 1.*

periods was in the range of 1.1 to 2.2% for FC bags and 0.6 to 2.1 for HC from the shell and core of the WS pile. There was no clear trend for the HHV of the WS pile over time. However, the HC samples had slightly higher HHV than the FC samples. The HHV of FC samples for all the sampling periods ranged from 18.6 to 20 MJ/kg with an average of 18.9 MJ/kg. For HC, the HHV ranged from 19.2 to 20 MJ/kg with an average value of 19.6 MJ/kg. These differences can be explained by the structural changes that occurred on the chips during the HWE. When applied to bamboo, the HWE process decreases the oxygen/carbon ratio from 0.43 to 0.34 in the exterior and 0.37 in the interior surface (Ma et al., 2013). These observations corroborate with other findings that indicate a decrease of the percentage of hemicellulose, which has higher oxygen/carbon ratio and lower heating value than both lignin and cellulose, in HWE biomass (Demirbaş, 2001; Pu et al., 2011; Corbett et al., 2015; Therasme et al., 2018).

Linear and Non-linear Mixed Regression Models

Model 1 shows that harvest season and depth in the storage pile affect the slope of DML while HWE treatment affects the curvature of DML (**Table 7**). All the variables included in this model were significant. The coefficients of the equations to calculate the DML in the SS pile and WS pile (**Table 8**) were determined by replacing the dummy variables in model 1 by their assumed values. Model 1 predicts the highest rate of DML (7.1% per month) in SS piles. For WS pile, higher rate of DML is expected from the core (3.1% per month) than the shell (1.9% per month), and HC will end up with lower DML than FC at the end of the storage (**Figure 4**). The model predicts that after 3 months in storage, FC would lose 3.2% dry matter in the shell and 7.1% in the core while for HC it would be 2.2% in the shell and 6.1% in the core of a WS pile. DML of the pile can be obtained by the weighted average of DML in the shell and DML in the core. Considering a 45 cm shell (Eisenbies et al., 2016), the mean ratio between core and shell of the WS would be 60:40 which would result in 5.5% DML for FC after 3 months. The data for SS pile was not detailed enough to estimate the DML at different depth in the pile. However, it is expected that the predicted DML for SS piles represent the average DML. The bags in the SS piles were located at the interface of the core-shell line. Furthermore, it was previously found that the temperatures in core of unprotected piles were only 9.9°C higher than the shell (<45 cm) during the

TABLE 6 | Changes in dry matter loss (DML), moisture, ash, and higher (HHV) and lower (LHV) heating value for three initial moisture contents of hot water extracted chips (HC) in the core and shell of a leaf-off willow winter storage pile (first day is December, 22nd 2017).

		Shell					Core				
Period (days)	**Initial Moisture (%)**	**DML (%)**	**Moisture (%)**	**Ash (%)**	**HHV (MJ/kg)**	**LHV (MJ/kg)**	**DML (%)**	**Moisture (%)**	**Ash (%)**	**HHV (MJ/kg)**	**LHV (MJ/kg)**
31	Low	−1.2 (0.6)[a]	57 (3.8)	1.1 (0)	19.6 (0.1)	7.1 (0.9)	−0.5 (1)	24.9 (1.3)	1 (0.3)	19.6 (0.1)	14.1 (0.3)
31	Medium	1.4 (1.1)	69.1 (1.2)	1.2 (0.1)	19.7 (0.3)	4.4 (0.3)	−0.6 (1.2)	61.9 (1.1)	1 (0.3)	19.8 (0.1)	6 (0.3)
31	High	4.6 (1.9)	73.9 (2.1)	1 (0.4)	19.5 (0.4)	3.3 (0.4)	4.6 (0.6)	70 (0.2)	1.1 (0.1)	19.5 (0.1)	4.1 (0)
53	Low	−2.2 (2.7)	48 (13.3)	0.6 (0.4)	–[b]	–	3.5 (1.2)	32.7 (10.6)	0.9 (0.1)	–	–
53	Medium	−0.9 (0.1)	68.5 (6.9)	0.9 (0.2)	–	–	2.3 (0.9)	63.2(4)	1.2 (0.1)	–	–
53	High	2.4 (1.7)	72.5 (1.1)	0.7 (0.5)	–	–	7.1 (2.3)	69.5 (0.7)	0.7 (0.4)	–	–
69	Low	−0.3 (1.7)	63.2 (5.7)	1.4 (0.2)	19.3 (0.2)	5.6 (1.3)	2.7 (3.4)	37.9 (1.5)	1.2 (0.3)	19.5(0.4)	11.2(0.1)
69	Medium	0.5 (0.4)	71 (3.6)	1 (0.5)	–	–	1.2 (3.3)	61.6(2)	1.3 (0.1)	–	–
69	High	5 (0.4)	75 (2.1)	1.3 (0.2)	19.7 (0.3)	2.8 (0.2)	7.2 (1)	70(0.7)	1.3 (0.1)	19.2 (0.5)	4.1 (0.3)
87	Low	3.2 (1.8)	70.5 (1.8)	1.4 (0.2)	–	–	3.5 (1.7)	28.7 (0.3)	1.3 (0.1)	–	–
87	Medium	0.7 (0)[c]	71.7 (0.9)	1.1 (0.2)	–	–	8.3 (3.8)	62 (4.3)	1.4 (0.2)	–	–
87	High	8.7 (6.7)	79.9 (2.6)	1.4 (0.2)	–	–	11.5 (4.8)	64.5 (1.8)	1.6 (0.2)	–	–
113	Low	0.9 (3)	67.5 (5.4)	0.9 (0.3)	19.8 (0.2)	4.8 (1.2)	6.5 (1.5)	28.6 (11.8)	0.7 (0.5)	19.6 (0.3)	13.3 (2.7)
113	Medium	0.2 (2.2)	72.9 (0.3)	0.7 (0.4)	–	–	5.9 (1.3)	69.4 (7.5)	1.3 (0.2)	–	–
113	High	5.9 (1.1)	74.5 (3.7)	1 (0.2)	19.7(0.2)	3.2(0.8)	9.4 (2.8)	67.4 (1.4)	1.3 (0.4)	19.4 (0.4)	4.7 (0.4)
147	Low	1.5 (2)	69.6 (2)	1.2 (0.1)	–	–	3.5 (5.2)	55.5 (10.3)	1.5 (0.2)	–	–
147	Medium	2.4 (0.5)	72.9 (0.2)	1.5 (0.2)	–	–	21 (3.2)	61.1 (6.9)	1.7 (0.3)	–	–
147	High	6 (0.9)	73.7 (0.9)	1.4 (0.4)	–	–	–	–	–	–	–
179	Low	3.5 (2.5)	69.4 (1.8)	1 (0.5)	19.7 (0.2)	4.3 (0.3)	9.3 (1.2)	61.9 (4.8)	1.3 (0.3)	19.5 (0.2)	5.9 (1.1)
179	Medium	3.2 (1.8)	73.7 (0.5)	1.4 (0.2)	–	–	16.9 (4.8)	66.1 (7.5)	1.5 (0)	–	–
179	High	5.2 (0)	72.9 (0)	1.5 (0)	19.7(0)	3.6(0)	16.6 (7.9)	65.7 (2.4)	1.2 (0.6)	20 (0.1)	5.3 (0.5)
207	Low	8.7 (3.5)	71.1 (0.8)	1.5 (0.3)	–	–	7.7 (7.8)	38 (26.9)	1.5 (0.2)	–	–
207	Medium	6.3 (3.1)	73.6 (0.9)	1.4 (0.2)	–	–	17.2 (8.1)	67.3 (7.4)	2.1 (0.7)	–	–
207	High	–	–	–	–	–	11.2 (12.5)	63.6 (0.5)	1.5 (0.4)	–	–

[a] *The value in parentheses is the standard deviation.*

[b] *Value not determined.*

[c] *The number of replication is 1.*

first month of a storage trial of willow starting late spring to November (Therasme et al., 2019).

Model 2 shows the relationship between DML, number of days in storage and ash content (**Table 7**). At a given storage period higher ash content was related to higher DML. Although model 2 is defined by different parameters that model 1, it corroborates with the conclusion that was drawn from model 1. Ash content was 35% lower in harvested leaf-off willow than leaf-on willow. Also, HC has lower ash content than FC (**Tables 5**, **6**). Therefore, model 2 indicates lower rate of DML for HC than FC and lower rate for leaf-off chips than leaf-on. The increased proportion of ash alone could have been used to estimate the amount of DML, however preliminary screening using R^2 and CP Mallow criteria suggested that model 2 was a better option.

According to model 3, the initial moisture of the chips did not have a significant contribution to the curvature of the DML curve. However, studies in Sweden reported that initial moisture correlates well with observed DML in wood chips storage; for initial moisture in the range of 20–58%, monthly DML ranges from 0.23–2.6%, with the highest monthly loss being associated with the highest initial moisture content (Wihersaari, 2005). Another storage trial on sweet sorghum bagasse, a non-woody biomass, showed that initial moisture strongly affected observed DML; 31% DML for storage at 26% moisture and 4% DML for storage at 12% moisture (Athmanathan et al., 2015). The differences of the findings with this study may be explained by the fact that the sweet sorghum biomass was stored indoor in a controlled environment, the bales with the 12% moisture were below the fiber saturation point, and that the moisture gradient in the willow chips between the different initial moisture treatments did not hold long enough to favor differing rate of DML.

The coefficients for model 4 and model 5 are reported in **Table 9**. Model 4 predicts a maximum DML of 33.4% after 140 days in the SS pile. The variables depth, harvest season, temperature and precipitation were all significant. Although model 4 predicts lower maximum DML for HC, the coefficient for the extraction term is not significant. This model suggests that for a given period higher air temperature and precipitation within the last 30 days leads to higher DML. Ambient air temperature regulates the temperature inside of the pile, which regulates the population of microorganisms inhabiting the pile. Suitable temperatures for most fungi ranges from 15 to 60°C;

TABLE 7 | Coefficients of three mixed model candidates for dry matter loss of willow biomass when stored in piles, and Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC).

	Model 1	Model 2	Model 3
AIC	581.6	584.0	591.6
BIC	584.5	585.7	593.3
p-value	0.02	<0.0001	0.0003
Effect			
Intercept	−2.3283 (0.02)[a]	−0.5266(0.46)	−2.3643 (0.006)
Period	0.2368 (<0.0001)	–	0.2460 (<0.0001)
Season*Period	−0.1748 (<0.0001)	−0.00499 (0.79)	−0.1836 (<0.0001)
Depth*Period	0.04321 (<0.0001)	–	0.04206 (0.001)
Extraction*Period^2	−0.00013 (0.01)	–	–
Initial moisture* Period^2	–	–	−1.43E-6 (<0.31)
Ash*Period	–	0.04068 (<0.001)	–

[a] *The value in parentheses is the p-value of the coefficient.*

TABLE 8 | Coefficients of model 1 to estimate the dry matter Loss (DML) in a summer storage (SS) pile and a winter storage (WS) pile of freshly harvested willow chips (FC) and hot water extracted willow chips (HC).

Pile	Extraction	Depth	α[a]	β	γ
SS	FC	Shell	−2.3283	0.2368	0
WS	FC	Shell	−2.3283	0.0620	0
WS	HC	Shell	−2.3283	0.0620	−0.00013
WS	FC	Core	−2.3283	0.1052	0
WS	HC	Core	−2.3283	0.1052	−0.00013

[a] $DML = \alpha + \beta\ period + \gamma\ period^2$.

TABLE 9 | Coefficients of two non-linear models that predict dry matter loss of hot water extracted chips (HC) and fresh chips (FC) when stored in outdoor summer and winter pile, and Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC).

	Model 4			Model 5		
AIC	466.6			562.8		
BIC	472.3			567.9		
	k_0	c	k	k_0	t_m	k
Intercept	22.1533 (0.0001)[a]	1.4765 (<0.0001)	0.00084 (0.09)	35.0193 (<0.0001)	88.338 (0.038)	0.031329 (<0.0001)
Season	−28.4006 (<0.0001)	–	–	−22.1005 (<0.0001)	37.6337 (0.369)	–
Depth	10.1050 (0.0003)	–	–	–	–	–
Season × Depth	–	–	–	7.15563 (<0.0001)	–	–
Extraction	−1.0940 (0.3801)	–	–	–	–	–
Extraction × Season	–	–	–	−5.67914 (<0.0001)	–	–
Air temperature	0.3705 (0.0014)	–	–	–	–	–
Precipitation	3.7489 (0.0025)	–	–	–	–	–

The parameters k_0, k, c, and t_m are defined in Equations (5) and (6).

[a] *The value in parentheses is the p-value of the coefficient.*

mesophilic fungi thrive at 20–30°C and thermophilic fungi show optimal growth at 40–50°C (Krigstin and Wetzel, 2016). However, the magnitude of the effect of temperature on DML declines after long storage period. For example, DML of SS pile increased rapidly during the first summer, but a year later (next spring/summer season) the rate of DML slowed down despite the recorded pile temperature was in the optimal range for microbial growth. This might be the result of low pile temperature during the winter season, so the fungi population did not survive, and maybe the initially available part of the wood for these microorganisms is already largely consumed and different group of microorganisms is needed to breakdown when the optimal temperature is reached again.

According to model 5, the rate of DML is maximal at the end of the third month of storage for SS pile and after the fourth month for WS pile. So, chips stored in winter pile could last longer in pile before they are being processed. It is unclear whether a storage pile constructed in early fall would show the same pattern observed for SS or WS pile or not, because after about 3 months in storage the difference between the pile temperature will be small, thus, the temperature inside a fall pile would not be favorable for microbial decays after three or more months in storage (during the winter season). However, one could hypothesize that a spring storage pile could still show the fastest mass loss after about 3–4 months because the pile temperature would be high enough for mesophilic fungi to thrive.

Scatter plots of actual DML vs. predicted DML (**Figure 5**) indicate that the selected models fit the data with high level of accuracy. The predicted DML from all the models, except model 2, have an overall 1:1 relationship (with $R^2 > 0.75$) with the actual DML. Also, the null likelihood ratio test is highly significant for models 1, 2, and 3, which indicates that the first order autoregressive structured covariance matrix is preferred to the diagonal matrix of the ordinary least squares null models. Model 1 has the lowest AIC and BIC among the linear models while the AIC and BIC for model 4 was lower than model 5 suggesting that model 1 would be the preferred linear model and model 4 the preferred non-linear model. Nevertheless, with an R-square of 0.76, model 5 can be very useful particularly when air temperature and precipitation data are not available. Simplified but accurate DML models are crucial for biomass supply chain logistics, techno-economic analysis, and life cycle assessment of bioenergy systems. For example, Mooney et al. (2012) used a DML model and storage cost of switchgrass bale to illustrate the breakeven prices for optimal outdoor storage. Another study (Routa et al., 2018) uses field experiment DML data, to evaluate the cost effects of DML of delimbed small diameter energy wood stems of pine during the storage in pile.

Predicted DML during storage can be used for the screening of optimal biomass storage pile size. For example, because DML in the shell differs from the core, one can vary the ratio between

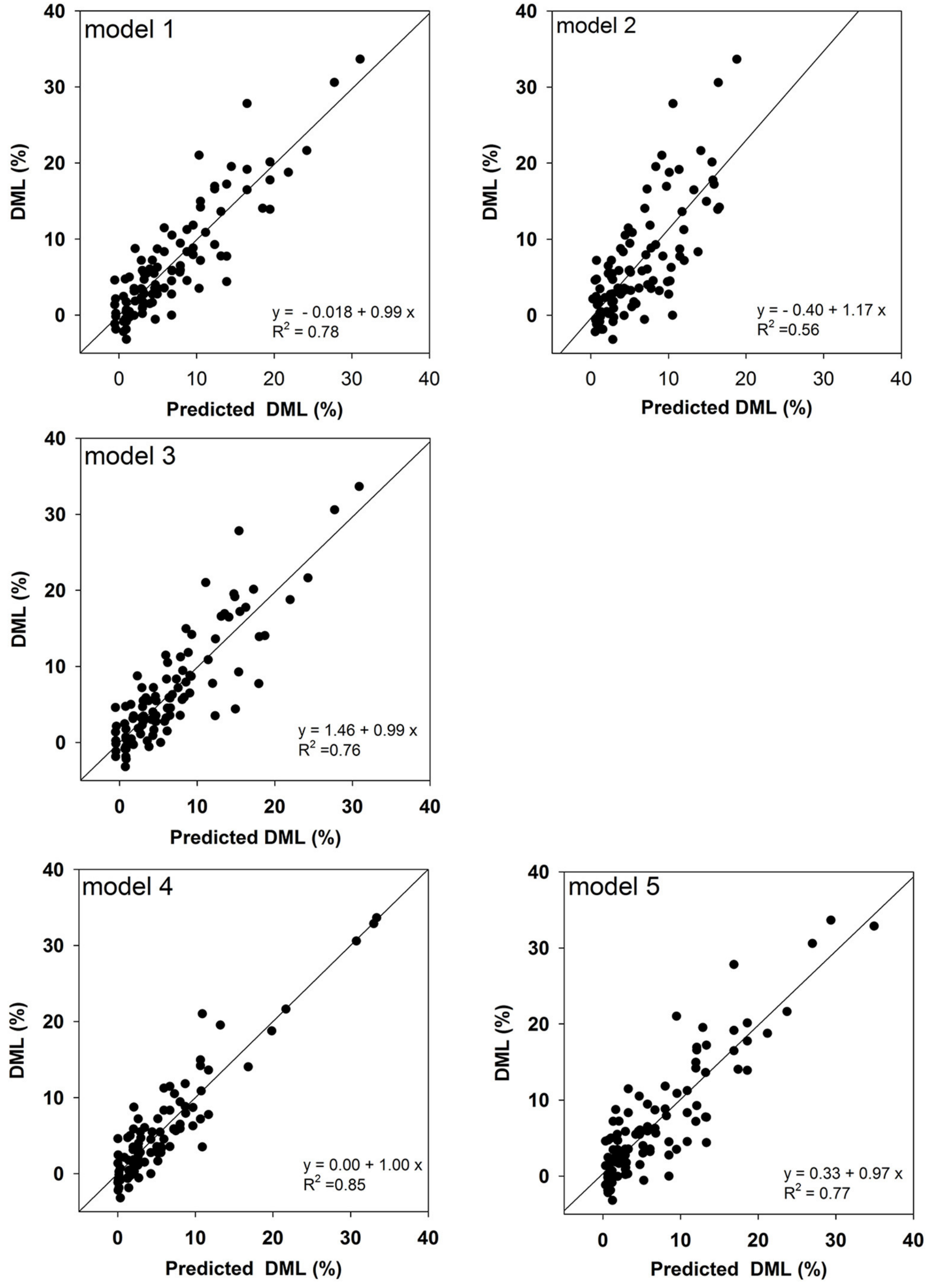
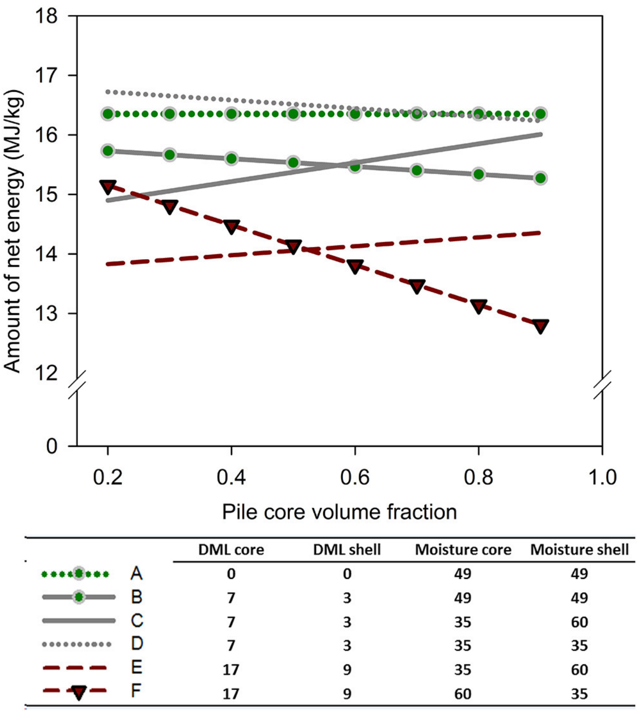


FIGURE 5 | Scatter plots and regression lines between dry matter loss (DML) and predicted DML from linear models 1, 2, and 3 and non-linear models 4 and 5 for willow chips stored in the shell and core of outdoor piles at different time of the year.

the volume of core and shell of the pile to minimize the overall DML for the entire pile. In the case of WS pile, it is evident that the ratio between core and shell should be kept low i.e., small size pile rather than large pile. However, for conversion pathways that are more dependent on the LHV of the fuel (e.g., biopower), the moisture content of the chips will also drive the selection of the optimal pile size. The change of the amount of net energy, i.e., excluding latent heat of water vaporization, on a dry basis, simulated for varying pile core volume ratio shows multiple patterns (**Figure 6**). Considering case A as a control, increased DML in the shell and core (case B) would result in reduced net energy per unit of initial mass of chips while a simultaneous increase of DML and decrease of moisture (case D) can show increased net energy. Considering the predicted



		DML core	DML shell	Moisture core	Moisture shell
••••●••••	A	0	0	49	49
—●—	B	7	3	49	49
——	C	7	3	35	60
••••••••••	D	7	3	35	35
– – –	E	17	9	35	60
–▼–	F	17	9	60	35

FIGURE 6 | Calculated net energy from 1 kg (oven dry) harvested willow chips for different pile core volume fractions for selected dry matter loss (DML) and moisture content. Case A represents the situation when the harvested biomass is combusted immediately after harvest with no DML. Cases B–F represent different hypothetical situations depicting different DML and moisture values for stored chips. The higher heating value (HHV) for this calculation is 18.7 MJ/kg.

DML and approximate moisture in the core and shell of a WS pile after 3 months (Case C) and 6 months (case E), the net energy increases when the fraction of the core volume increases. Therefore, there would be more advantages creating larger storage piles for scenario C and E.

End user facilities (e.g., biorefineries) will need biomass supplies all year round to be productive. This research study reports on storage piles created in the winter and summer seasons and provides useful insights on how the quality of willow biomass that is harvested at two different points and then delivered to an end user will change over time. This is important information to more accurately model willow biomass quality and costs for year-round supply. However, there are only two discrete harvesting events in this study and the occurred in 1 year so the variability of weather patterns is not captured. So, further storage trials with piles constructed at different points in fall and late summer and over different years are still needed to accurately reflect how DML will impact changes in both quality and quantity of biomass for year-round operations. While the discretization of the pile into shell and core in this study may suffice for relatively small piles created at the edge of the field, a rather more complex discretization method may be required for larger piles created at the end user facility or piles that have been submitted to compaction.

CONCLUSIONS

This study determined the changes in dry matter loss, moisture, ash, and heating value during the storage of shrub willow chips in piles built in summer and winter. Of the two storage pile experiments, it was shown that the rate of DML was higher in SS piles than WS piles; DML in a SS pile increased at a rate of 7.1% per month during the first summer and fall season of the storage, then decreased to a rate of 0.7% in the spring and the following summer while DML in a WS pile created with freshly harvested willow chips increased by only 1.0% per month in the shell and 2.6% in the core of the pile. This study presents three linear and two non-linear DML models that could be used in techno-economic analysis, environmental life cycle assessment, and supply planning of willow biomass for bioenergy applications in the northeast United States or other regions with similar climate patterns. The linear models apply to SS pile not exceeding 140–150 days as the slope of the DML in the SS pile will decrease and tend toward zero during the winter season. However, for a longer storage period, the non-linear models have the feature to capture the decreasing rate of DML over time. This study demonstrates also that while the DML of hot water extracted chips were in the same range with non-extracted chips in the early period of storage, the hot water extracted chips had lower DML at the end of the WS storage (207 days). Pile configuration (e.g., core/shell fraction) could be an important factor to consider in order to increase the net amount of energy from stored chips.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

AUTHOR CONTRIBUTIONS

OT, TV, and ME conceptualized and designed the experiments and revised the manuscript. OT, HS, and NU performed the experiments. OT performed the data analysis and drafted the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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An Assessment of Ambient and Heated Forced Air Drying Pre-treatments for Enhancing the Quality of Various Forest Biomass Feedstocks

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Maintaining high quality feedstock storage for sustained bioenergy production continues to be a challenge within the supply chain. Though forestry by-products have the potential to provide a carbon-neutral energy source that can be both economic and environmentally friendly, the heterogeneous nature of woody biomass makes the material challenging to store for long periods of time without material degradation, freezing, and fire concerns if not managed properly. The following study evaluated the scalable use of natural air drying (NAD) with and without supplemental heat on multiple woody biomass feedstocks (hog fuel, sawmill chips, and bark) to determine drying rates and feasibility in Western Canada. Test results demonstrated that NAD has the potential to dry and condition multiple types of woody biomass, while providing a positive net energy gain of 9–32% compared to the original biomass energy content. The use of supplemental heat resulted in an energy gain of 3 to −13% due to the increased heating energy consumption, but may be applicable in some circumstances when faster drying is required, weather conditions are unfavorable and/or alternative low-cost heating sources are available. Therefore, NAD shows promise in providing an alternative low-cost drying option for enhancing woody biomass feedstock in storage.

Keywords: natural air drying, woody biomass storage, supplemental heat, moisture content, bioenergy

INTRODUCTION

Low-value woody biomass is typically heterogeneous in size and high in moisture content (MC) (e.g., forest harvest residues; Acquah et al., 2016). Dry particulate wood fuels are preferred in many applications because of their relative ease of handling and storage, as well as their advantage in terms of burning efficiency in smaller, less expensive combustion systems. Much research has been devoted to increasing the value of heterogeneous forest biomass by encouraging a decrease in the moisture content during the storage period (**Table 1**). Since the supply of forest residues is not well-aligned with energy demand, storage is a necessary requirement in the supply chain. Tarps of

TABLE 1 | Summary of past literature studies displaying natural wind and air drying experimental results with and without supplemental heat.

Type	Biomass type	Experimental conditions	MC reduction	Energy	References
Natural wind drying	Wood chunks	Pile with a plastic cover on the top and sides	50–10% (w.b.) over 5 months	N/A	Gigler et al., 2004
	Woodchips	Cone shaped pile with a breathable tarp	60 to 13–20% (d.b.) over 1 year	N/A	Afzal et al., 2010
	Woodchips	Uncovered cone shaped pile	60 to 160–170% (d.b.) over 1 year	N/A	Afzal et al., 2010
	Woodchips	Uncovered cone shaped pile with a plastic bottom	60 to 120–160% (d.b.) over 1 year	N/A	Afzal et al., 2010
	Loose slash	Uncovered pile of loose slash	45–65% (d.b.) over 1 year	N/A	Afzal et al., 2010
	Woodchips	Uncovered windrow pile	56.4–44.4% (w.b.) over 6 months	N/A	Whittaker et al., 2018
	Woodchips	Uncovered piles	Outside MC 19.3% (w.b.) compared to 73.1% on the inside over 2 years of storage	N/A	Acquah et al., 2016
	Woodchips	Uncovered, plastic tarp, paper tarp	48.9–61.8% (w.b.)—no tarp, to 65.2% plastic tarp, to 52.4% paper tarp over 12 months	N/A	Wetzel et al., 2017
Natural air drying	Woodchips	Universal dryer with a solar collector system and a 11 kW axial blower	38.8–14.1% over 66 h	Energy gain of 4300 kWh with an input energy of 700 kWh	Tengesdal et al., 1988
	Woodchips	Covered barn with a drying floor with a 37 kW fan	52.2–48.3% (w.b.) over 24 h	Gain of 293 kWh at the cost of 665 kWh	Price, 2012
	Woodchips and chunks	Forced air drying tests	40–20%	Chips required 44–74% more energy than chunks	Arola et al., 1988
	Woodchips and chunks	Forced air drying in silos of 40–45 m^3	42 to 12–14% over 2 weeks (12 h/day)	Chips required 46% more energy than chunks	Mivell, 1988
	Woodchips	Forced air drying in 3 m deep piles	57–19.3% (w.b.) in 11.5 weeks	N/A	Jirjis, 1995
Natural air drying with heat	Woodchips	Warm air from 1 and 2.2 MW power plant funneled with a 4 kW radial fan	59.1–8.3 % (w.b.) over 6 days	2,500 kWh energy gain with 270–556 kWh energy input	Nordhagen, 2011
	Woodchips	Grain dryers with a 37 kW fan	34–7.5% (w.b.) over 25.5 h	4,033 kWh energy gain with ∼14,385 kWh energy input	McGovern, 2007
	Woodchips	Covered barn with a drying floor, boiler and a 37 kW fan	55.1–52.4% over 12 h	Gain of 104 kWh at the cost of 1,380 kWh	Price, 2012

several material types have been used to cover biomass piles, however the effect on the moisture content of stored biomass is variable (Gigler et al., 2004; Afzal et al., 2010; Wetzel et al., 2017). Numerous studies have shown that pre-treatment of the biomass by natural air drying (NAD) may be a possible alternative that can reduce the MC in green chips/chunks relatively quickly with and without supplemental heat (**Table 1**).

Studies on ambient air drying of biomass have included using natural wind, as well as forced air (with and without supplemental heat). Natural wind drying relies on the wind to naturally condition the material without any additional energy input. Gigler et al. (2004) investigated natural wind drying of willow chunks and found they could be dried from 50 to 10% MC w.b. over 5 months. They concluded that the factors such as air condition, particle size and pile dimensions all affected the rate of natural wind drying. Afzal et al. (2010) demonstrated that wind drying of white birch chip piles covered with a breathable tarp reduced moisture from 60 to 17% (d.b.) over a 1-year period. However, un-tarped piles in a 2018 study by Whittaker et al. and a 2010 study by Afzal et al. had divergent results, showing a loss of 12% MC (w.b.) to a gain of 110% MC (d.b.), respectively (Afzal et al., 2010; Whittaker et al., 2018). Wetzel et al. (2017) compared the condition of forest residue biomass piled over a 1-year period in New Brunswick, Canada. They found that tarping of piles with a plastic tarp significantly increased the moisture content (+33%) of the stored biomass, while tarping with a paper tarp had no effect on the moisture content. The un-tarped control pile, similar to the plastic tarped material, gained moisture (+25%). These experimental studies have demonstrated some potential for natural wind drying of woody biomass over time, however there are many variables which can influence its effectiveness. Therefore, it is difficult to prescribe conditions and get predictable results.

Biomass can also be dried by using forced ambient air or heated air. In Norway, farmers have adopted drying systems composed of a perforated floor that allows drying in shallow layers (Gislerud, 1990). The roof and walls are commonly used to redirect solar energy to improve the drying performance. The shallow-layer drying with forced air convection has a low airflow resistance, which allows simple axial fans to provide large air volumes with low energy inputs. Tengesdal et al. (1988) conducted a drying experiment that reduced the wood chip moisture content from 38.8 to 14.1% over 4 days, increasing the biomass energy content by 4,300 kWh at a cost of 700 kWh of electrical power, resulting in a net positive energy gain. Other drying trials have not shown such optimistic results but instead found that the energy expended on creating air flow was greater than the energy content increase in the biomass (Price, 2012). Price (2012) noted however that the rate of MC loss may be affected by the high RH during the drying trials.

The cost of drying chipped and chunk material with forced air are similar, and the management decision may well be dictated by the amount of time available between harvest and fuel delivery or fuel processing equipment availability. Various woodchip and/or chunk drying trials illustrated successful results in drying materials to 12–25% MC with various forced air set-ups. Generally, it was found that wood chunks dried faster than woodchips (Arola et al., 1988; Gjølsjø, 1988; Mivell, 1988; Nurmi, 1988; Sturos, 1988; Jirjis, 1995). The chunks, having a lower pressure drop when compared to the chipped material, required less energy for drying to the same MC level. The high airflow resistance of woodchips also results in longer drying times and higher energy costs compared to the lower pressure drop of chunks and lower fan energy requirements.

In some cases, forced air drying systems with supplemental heat can dry biomass faster and when conditions are unfavorable for drying (e.g., low temperature and/or high relative humidity). As an example, fuel chips could be dried to 12% MC during the March-October period in Sweden using unheated air, but would require additional heat to achieve satisfactory drying during the winter months (Gustafsson, 1988). Nordhagen (2011) reported that the gain in calorific value of the biomass with hydroelectricity was greater than the power used for fans, whereas other studies have shown a negative net energy when factoring in the additional heat energy used (McGovern, 2007; Price, 2012). McGovern and Price showed the energy input was 3–4 and 12 times higher when compared to the energy gain in the material. Rinne et al. (2014) and Atnaw et al. (2017) suggested that using solar energy is an alternative to add supplemental heat when drying biomass.

Price (2011) indicated that green wood residues can be dried relatively quickly (2–3 days) to 25–30% MC with minimal energy input using a fan and ambient air. Additional drying is possible with heated air, but several sources indicated that the energy used for drying exceeded the energy gained. The objective of this study was to determine drying rates for woody biomass found in Western Canada and to assess the feasibility of NAD to optimize the bioenergy supply chain. There is limited information available on the use of natural air drying of woody biomass in Canadian climates, specifically the use of supplemental heat, equipment and practices that would be most suitable at a farm- or small-scale enterprise.

MATERIALS AND METHODS

Test Apparatus

A small-scale test apparatus was developed in Portage la Prairie, Manitoba, consisting of six vertical silos, each suspended on a load cell (Vishay Revere 9363) and equipped with sensors at various heights for real-time weight, temperature and moisture monitoring (**Figure 1**). Each silo was equipped with a fan (EPM-Papst RG148/1200-3633) and an in-line heater (Omega AHF-14240; supplemental heat trial only) to provide constant airflow and supplemental heat to simulate one-dimensional flow through a pile/windrow of biomass up to 4 m high. The fan pushed the ambient air through the biomass from the bottom and released the air at the top.

A total of six fans and six heaters were connected to a variable-rate controller capable of independently varying the airflow rate and heat additions for each column. Each silo was 0.61 m in diameter and 4 m in height, holding ∼1.2 m^3 of material. Three temperature/humidity sensors (Measurement Specialties HTM2500LF) were used in each silo to monitor the biomass condition. The sensors were secured to a steel cable suspended from the top of the test apparatus and running vertically near the center of each silo. The sensors were vertically located ∼0.2, 2, and 3.8 m from the bottom of the silos in the 2017 trial and adjusted to 0.5, 2, and 3.5 m in the 2018 trial. An additional sensor was placed near the fan inlet to monitor ambient air conditions. Other design components included a removable roof to minimize

FIGURE 1 | Small-scale natural air drying with real-time monitoring capability, variable fan, and heater test apparatus for six runs.

the impact of precipitation and a sliding gate at the bottom of each silo to facilitate the material loading and unloading process.

The drying parameters collected included air temperature and relative humidity (RH), biomass temperature and moisture content, biomass weight, static pressure resistance, energy use, as well as airflow and heating rate. These measurements were used to assess the efficiency and viability of using NAD to dry forestry residues.

Natural Air Drying Trials

A total of four NAD trials were carried out between August to October 2017 and September to November 2018 to evaluate drying rates and energy requirements for three types of woody biomass: (i) hog fuel, (ii) sawmill woodchips, and (iii) bark. The aim of the NAD trials was to reach an average MC of 20–25% in each column, or dry for a maximum of 3 weeks, whichever occurred first. The trials were setup as a 2 × 3 factorial design without replications (**Table 2**). All three materials were tested with the "Design 1" factorial matrix, which compared starting MCs of 35–45% and 45–55% (w.b.), and airflow rates of 3.3, 13.4, and 26.8 $\text{L}\cdot\text{s}^{-1}\cdot\text{m}^{-3}$. The bark was retested to evaluate the effect of supplemental heat, "Design 2" which compared heat treatments of 0, 5, and 10°C above ambient and two airflow rates of 13.4 and 26.8 $\text{L}\cdot\text{s}^{-1}\cdot\text{m}^{-3}$.

Initial moisture content differences were attained by separating the material into two piles. One pile was left outside, unprotected from the environment, while the other was stored inside a well-ventilated building. Depending on the starting MC and the weather conditions, water was either added or removed from one pile to create the MC differential. The materials were mixed and sampled before loading the columns. The airflow rates were obtained by modulating the fans and verifying with a vane anemometer (Omega HHF143B), while the heat treatments were controlled by continuously measuring the temperature differential with two T-type thermocouples between the fan inlet and the heater outlet. The fan speeds (in rpm), weight of the columns, temperature, and RH measurements were logged and stored in the data acquisition system (eDAQ). In addition, the static pressure and energy consumption for the fans and heaters were measured with a manometer (Dwyer 477AV) and energy logger (On-set UX120-018) at the beginning and the end of each trial. The biomass was characterized to determine the initial physical properties in each trial, followed by a 3-week drying period and further characterization to determine any property variations.

Woody Biomass Characterization

The hog fuel consisted of mostly poplar but had varying amounts of spruce, balsam fir, and birch. The sawmill chips and bark were obtained from two different by-product streams when producing dimensional lumber from a pine log in the Manitoba Interlake Plain Region. The woody biomass materials were characterized to determine any change in their physical properties, which included MC, bulk density, porosity, and particle size distribution. Similarly, the bulk properties of the biomass columns were also measured, including the mass, height, *in situ* bulk density, static pressure, and airflow rate. Monitoring the column properties quantified the effect of NAD over 3 weeks on the biomass material when stacked to a height of 4 m.

Moisture content was measured following the methodology outlined in ISO 18134-1 and EN 14771-1:2009, and the compressed and uncompressed bulk density was obtained according to ISO 17828:2015 and EN 15103:2009. Standard ISO/DIS 18847 uses a buoyancy method; however, this method is only suitable for measuring the porosity of homogenous biofuels such as pellets and briquettes. As there is no confirmed standard for measuring the porosity of heterogeneous solid biofuels, the water pycnometry method from Annan and White (1998) was used. The method consisted of filling a round container with material and slowly adding water to displace the air bubbles between the solid particles. The porosity was determined by calculating the volume of water added against the total container volume. The particle size distribution was measured according to the Standards ISO/FDIS 17827-1, EN15149-1:2010, with recommended sieve sizes from Agnew and Landry (2016).

Equilibrium Moisture Content

The MC of wood depends on the temperature and RH of the surrounding air. Therefore, the biomass MC was determined based on the equilibrium moisture content (EMC) proposed by Simpson (1998). EMC is achieved when the MC of the wood reaches an equilibrium point (with constant relative humidity and temperature over an extended time period). Equations (1)–(5) were used to calculate the EMC of the wood surrounding the individual sensors, where T is temperature (°C), h is relative humidity (decimal), EMC (decimal), and W, K, K_1, and K_2 are

TABLE 2 | NAD factorial design matrix.

		Design 1		**Design 2**		
		MC_L (35–45% MC)	**MC_H (45–55% MC)**	**H_0 (0°C above ambient)**	**H_Low (5°C above ambient)**	**H_High (10°C above ambient)**
Airflow rate	A_Low ($3.3\ \text{L}\cdot\text{s}^{-1}\cdot\text{m}^{-3}$)	Treatment 1	Treatment 4	–	–	–
	A_Med ($13.4\ \text{L}\cdot\text{s}^{-1}\cdot\text{m}^{-3}$)	Treatment 2	Treatment 5	Treatment 1	Treatment 3	Treatment 5
	A_High ($26.8\ \text{L}\cdot\text{s}^{-1}\cdot\text{m}^{-3}$)	Treatment 3	Treatment 6	Treatment 2	Treatment 4	Treatment 6

coefficients of an adsorption model by Hailwood and Horrobin (1946).

$$EMC = \frac{1800}{W} * \left(\frac{Kh}{1 - Kh} + \frac{K_1 Kh + 2 K_1 K_2 K^2 h^2}{1 + K_1 Kh + K_1 K_2 K^2 h^2} \right) \tag{1}$$

$$W = 349 + 1.29T + 0.0135T^2 \tag{2}$$

$$K = 0.805 + 0.000736T - 0.00000273T^2 \tag{3}$$

$$K_1 = 6.27 - 0.00938T - 0.000303T^2 \tag{4}$$

$$K_2 = 1.91 + 0.0407T - 0.000293T^2 \tag{5}$$

Drying Performance

The drying performances were measured using two methods. For the first method, the weight of the columns was continually monitored, and drying was determined by calculating the weight loss before and after drying. The second method used the individual sensors within the columns to determine the drying conditions within the column. These sensors monitored a small amount of surrounding biomass and provided an indication of the internal temperature and MC (based on EMC). The NAD energy balance was estimated by calculating the total gain (ΔE_{Total}) between the final (E_F) and starting (E_S) biomass energy in Equations (6)–(8) without considering the equipment energy consumption. Equations (9) and (10) shows the gain ratio ($Gain_{ratio}$) after deducting the equipment consumption (E_C) to illustrate the potential energy gain/loss with respect to the initial energy content. The biomass net calorific value (NCV) was calculated based on the estimated moisture of the material within the column, while the energy consumption was based on the equipment current draw. Theoretically, drying woody biomass will result in a material with a higher energy content since the drier material is closer to the theoretical higher heating value of woody biomass. The weight loss measured within the column was assumed to be equal to the water loss.

$$\Delta E_{Total} = E_F - E_S \tag{6}$$

$$E_{F/S} = NCV_i * WT_i * 0.2778 \tag{7}$$

Where E = energy (kWh), NCV = net calorific value (MJ/kg), WT = weight (kg), 0.2778 is the conversion factor for MJ to kWh.

$$NCV_M = \frac{NCV_0\, x\ (100 - M) - 2.44M}{100} \tag{8}$$

Where NCV_M = net calorific value at moisture, M (MJ/kg), NCV_0 = net calorific value at 0% moisture (19 MJ/kg), and M = Moisture content (w.b.%) (Francescato et al., 2008)

$$Gain_{ratio} = \frac{E_F - E_C}{E_S} - 1 \tag{9}$$

$$E_C = V * I * T \tag{10}$$

Where E_C = equipment energy consumption, V = volt (V), I = current (A) and T = time (hours).

RESULTS AND DISCUSSION

Biomass Properties

Three types of biomass materials (hog fuel, sawmill woodchips, and bark) were characterized for MC, uncompressed and compressed bulk density, as well as porosity (**Table 3**). Depending on the type of biomass and MC, the uncompressed and compressed bulk density ranges were 180–340 kg·m^{-3} and 260–400 kg·m^{-3} respectively, and the uncompressed and compressed porosity ranges were 63–71% and 55–66%, respectively. The hog fuel had the lowest compressed to uncompressed bulk density (17–18%), followed by sawmill woodchips at 15–23%, and bark at 36–51%. Since the bark material could pack together more tightly, this may have had a more negative influence on air flow. The bark's ability to compress under load as compared to the other two materials can be seen in **Figure 2**.

The average particle size distribution for the hog fuel, sawmill woodchips, and bark are presented in **Table 4**. The distribution curve indicated that the hog fuel contained the highest amount of fines, followed by bark and sawmill woodchips. The hog fuel and bark had a more normally distributed particle size distribution over the measured size range as compared to the sawmill woodchips, where over 90% (by weight) were found in the 7.5–44.8 mm size range.

Biomass Column Characterization

The mass, height, static pressure, and airflow rate of each biomass filled column was measured to determine the drying parameters. **Figure 3** shows the static pressure of the drying system plotted against the airflow rate for the four NAD treatments. For the first design matrix (hog fuel, sawmill, and bark-trial 1), the materials have a different resistance to airflow due to their physical properties. At lower airflow rates, the static pressure

TABLE 3 | Summary of woody biomass physical properties before NAD (sample size of 3 for averaged data).

	Hog fuel		Sawmill woodchips		Bark		Bark–2nd trial
MC, *n* = 3 (%)	44.5 ± 0.4	61.7 ± 0.5	44.7 ± 0.9	54.5 ± 0.4	38.5 ± 2	42.8 ± 3	42.2 ± 2
Uncompressed bulk density (kg·m^{-3}) w.b.	260 ± 6	337 ± 7	211 ± 8	268 ± 8	184 ± 22	202 ± 28	188 ± 4
Compressed bulk density (kg·m^{-3}) w.b.	304 ± 14	396 ± 6	259 ± 3	309 ± 4	261 ± 32	275 ± 28	283 ± 4
Compressed-Uncompressed density increase (%)	17	18	23	15	42	36	51
In situ bulk density (kg·m^{-3}) w.b.	252 ± 20	363 ± 11	253 ± 4	296 ± 16	284 ± 6	305 ± 14	288 ± 6*
Uncompressed porosity (%)	63 ± 1	61 ± 1	68 ± 1	67 ± 0.3	71 ± 0.4	71 ± 1.4	69 ± 1
Compressed porosity (%)	58 ± 2	55 ± 1	58 ± 6	61 ± 1	65 ± 2.1	63 ± 0.1	66 ± 2

**Sample size of 6.*

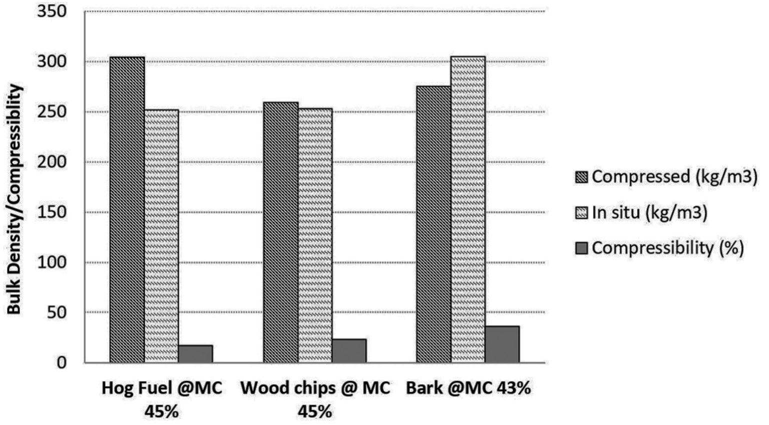

FIGURE 2 | Bulk density and compressibility ratios of green hog fuel, woodchips, and bark with respective moisture contents of 45, 45, and 43%.

TABLE 4 | Average particle size distribution for the different woody biomass materials.

	Proportion of the sample retained on each sieve (%)							**Median size (mm)**
	Particle size range (mm)							
	<1	**1–4**	**4–7.5**	**7.5–15.5**	**15.5–44.8**	**44.8–66.5**	**>66.5**	
Hog fuel, *n* = 4	6	10	16	38	29	0	0	5.6
Sawmill woodchips, *n* = 4	0	1	6	38	53	1	1	8.1
Bark, *n* = 6	5	8	12	24	45	4	3	7.8

differences between the materials are almost negligible. At higher airflow rates, the larger particle sizes in the sawmill chips resulted in a lower airflow resistance when providing the same airflow rate, which is in agreement with Arola et al. (1988). This indicates that the material properties will have an impact on how much air can be economically used to dry woodchips.

The additional in-line heaters, in "Bark—Trial 2 with heat," saw an increase in the static pressure compared to the other trials. This indicated that the fan required more energy to provide the same amount of airflow during the heated trials. This is due to the increased distance the air has to travel and airflow restriction from the heater coils. Similarly, this shows that the design of the air duct system will also have an impact on how much energy will be expended to dry woodchips.

Ambient Air Conditions

NAD relies on the drying capacity of the ambient air. The temperature, EMC range and averages for each time period are presented in **Table 5** to illustrate the potential drying capacity. The EMC is calculated with Equations (1)–(5), which is based on the ambient temperature, relative humidity, and coefficients of an adsorption model by Hailwood and Horrobin (1946). A lower EMC indicates that the air has a higher capacity to dry biomass due to its ability to absorb more water.

Drying Analysis

The drying performances of the four trials are shown in **Figure 4**, which displays the biomass MC and ambient air EMC over 3 weeks. The biomass MC was based on the initial MC samples as well as the continuous weight measurements of each silo. The EMC curve in each figure illustrates the potential drying capability of the ambient air if the biomass was exposed to those conditions for an extended period of time. If the EMC curve is below the biomass MC, the air provided will have a drying effect on the woodchips. The one exception is during the heated trials where the heat added lowered the EMC curve based on the temperature increase.

Figure 4 illustrates that the NAD trial could successfully dry a variety of biomass materials during the summer and fall months to a moisture level of 25% or below during a 3-week period. All treatments provided some reduction in MC, with the higher air flow trials delivering the best results with moisture levels close to or below 20% in many cases. The average ambient EMC during this period was 12.1% and ranged between 5 and 23% depending on the hourly weather changes. Therefore, the biomass would be continuously dried if it's MC was over 23%. Once the MC fell below 23%, the biomass either gained or lost moisture depending on the ambient conditions. This was observed near the end of the trial (∼day 16 with the hog fuel) where the biomass material increased in MC due to an extended high humidity period. To overcome this limitation of the equipment, there is potential to use a control system to maximize the effect of NAD, while

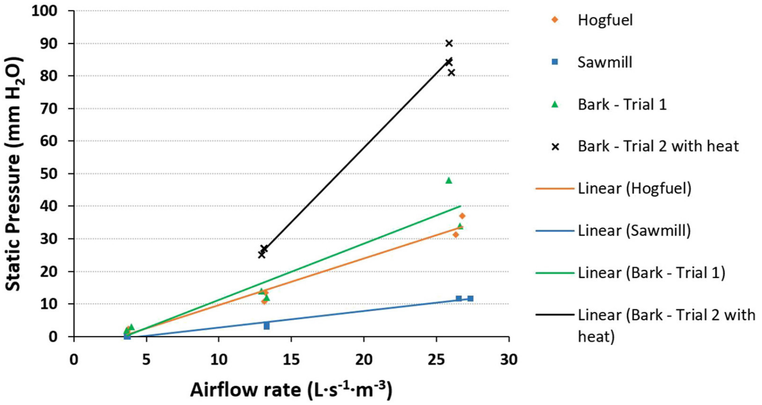

FIGURE 3 | Changes in static pressure with varying airflow rates (3, 13, 27 L $s^{-1} \cdot m^{-3}$). Displaying results for each natural air drying trial (hog fuel, sawmill woodchips, and bark with and without supplemental heat).

TABLE 5 | Ambient air temperature, equilibrium moisture content (EMC), and drying potential at various time periods.

	Hog fuel	Sawmill woodchips	Bark	Bark–with heat
Trial dates	28 August to 19 September 2017	23 September to 16 October 2017	07 September to 01 October 2018	10 October to 02 November 2018
Temperature range (°C)	6 to 35	0 to 23	−0.5 to 26	−10 to 25
Avg. temperature (°C)	18.7	10.9	12.0	4.4
EMC range (%)	5 to 23	5 to 22	5 to 26	5 to 25
Average EMC (%)	12.1	13.5	16.1	15.2

minimizing the energy consumption of operating a fan when conditions are unfavorable. This observation was similar to the findings from Price (2012) who indicated that it is common to use hygrometers to trigger grain drying when relative humidity falls below a given threshold in order to improve efficiency.

When comparing the drying rates obtained for the various treatments with hog fuel (**Figure 4A**), the starting moisture content (MC_L & MC_H) had a smaller effect compared to the airflow rates (A_{Low}, A_{Med}, and A_{High}). The low airflow treatments (A_{Low}) had the lowest average drying performance at around 15% difference, followed by medium airflow (A_{Med}) at 30%, and high airflow (A_{High}) at 37% over 22 days. The higher airflow rates indicated a higher drying rate over the entire trial, but the drying rate (% day^{-1}) gradually decreased as the differential between the biomass MC and the ambient EMC decreased. The higher airflow rates (A_{Med} and A_{High}) showed a visible diurnal variation in MC, which indicated a different drying potential depending on the day/night. The slope on the drying curve was greater during the warmer temperatures around mid-afternoon and was lower during the night. It is difficult to compare the effect that the individual biomass materials had on the drying rates, due to the various seasonal periods and associated EMC's across the trials.

In summary, the low airflow treatment (A_{Low}, 3.3 $Ls^{-1}m^{-3}$) in the first three trials with hog fuel, sawmill chips, and bark indicated a low average drying rate of 15, 9, and 9% over 3 weeks, respectively. The medium airflow treatment (A_{Med}, 13.4 $Ls^{-1}m^{-3}$) resulted in 30, 24, and 17% drying performance where the high airflow treatment (A_{High}, 26.8 $Ls^{-1}m^{-3}$) was 37, 34, and 23%, respectively. The low airflow rates were unable to reach the target MC after a 3-week duration. The "Medium" and "High" airflow rates may therefore be recommended as a starting point to scale the NAD application in a three-dimensional storage system if a similar time-frame and dry-down performance is desired. Secondly, these drying treatment curves could be manipulated (with limitations) to estimate the required airflow and duration during different months to obtain a particular dry-down performance. These drying rates are a snapshot of the potential drying performance of NAD and should not be compared since these trials are influenced by the drying factors (material and weather conditions) suggested by Gigler et al. (2004).

The NAD results with three biomass types indicated that the medium (A_{Med}) and high airflow treatments (A_{High}) were capable of removing about 17–30% MC and 23–37% MC over 3 weeks, respectively. When comparing these results with the NAD studies in **Table 1**, the performance is very similar to the study by Mivell (1988) where the material MC dried about 30% over a 2-week period. The other studies display variable drying periods, as low as 1 day and up to 12 weeks. It is therefore not practical to compare drying performances when the duration is drastically different.

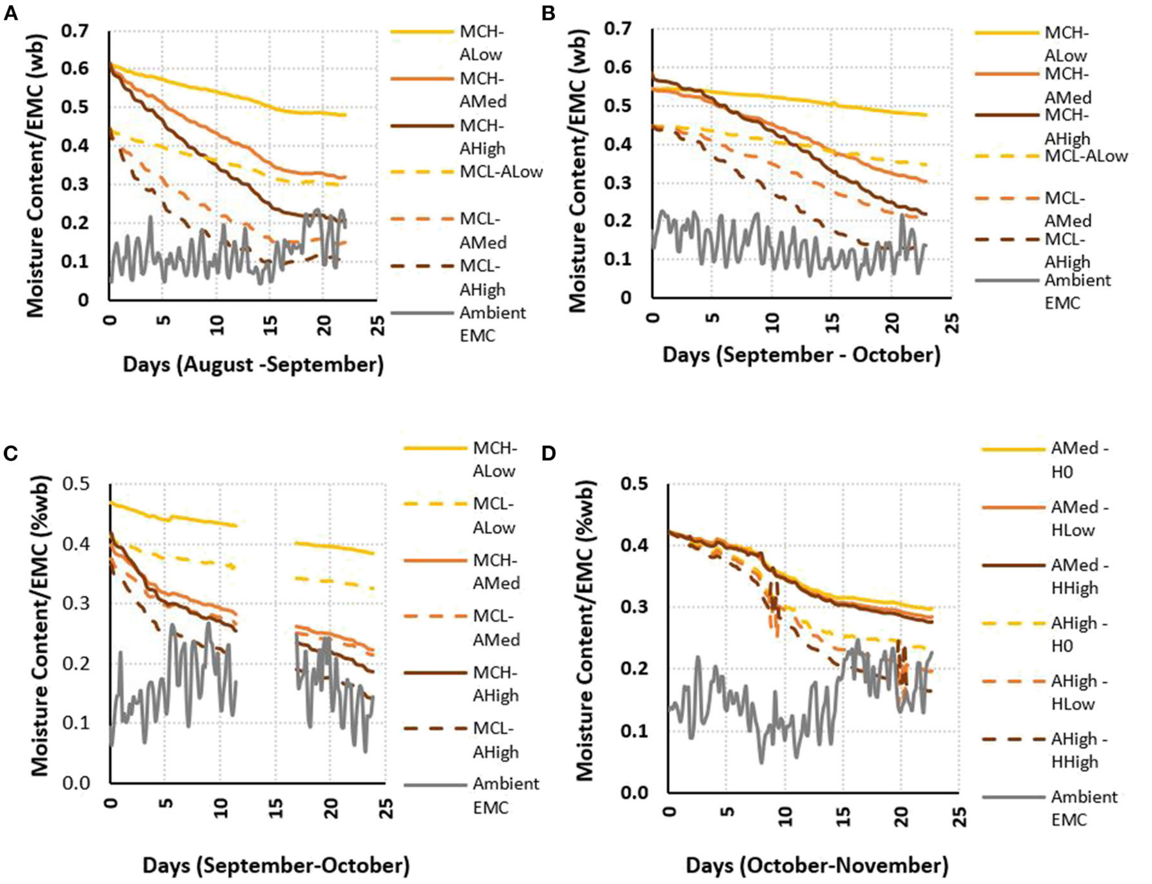

FIGURE 4 | Average biomass drying performance over time for each feedstock material/ trial compared with local ambient equilibrium moisture content (EMC): **(A)** hog fuel, **(B)** sawmill woodchips, **(C)** bark without supplemental heat, and **(D)** bark with supplemental heat ($MC_{L/H}$, low/high moisture content; $A_{Low/Med/High}$, low/medium/high airflow; $H_{0/Low/High}$, no/low/high heat settings).

The fourth trial, using bark material, and supplemental heat with two airflow rates, was performed to evaluate if drying could occur when ambient air conditions were not optimal. The medium airflow (A_{Med}) drying rates for no, low, and high heat (H_0, H_{Low}, H_{High}) were 13, 14, and 15%, respectively. Similarly, the high airflow (A_{High}) drying rates were 19, 23, and 26%, respectively. **Figure 4D** showed a noticeable drying performance difference between the two airflow rates with a smaller effect from the supplemental heat. Each additional heat treatment (5°C above ambient air) increased the drying performance by about 1% for the medium airflow and increased ∼3% for the high airflow. This was expected since the heater controls were designed to provide more heat until a target temperature differential was obtained. Therefore, the heaters proportionately added more heat to a higher airflow compared to a lower airflow, thus improving the drying performance.

Preliminary Energy Assessment

For the energy assessment of the trials, the changes in the energy value (kWh) of the biomass before and after drying are shown for each trial without considering the cost of operating the fans and heaters (Equation 6). The net energy gain (%) provided an indication of the gain or loss compared to the initial energy content after deducting the input energy (Equation 9). Overall, a higher positive net balance demonstrated superior treatment performance (**Figures 5, 6**). These energy calculations were based on a limited number of samples and do not account for all the factors that affect the useful energy content of the materials (i.e., ash content, combustion efficiency of system, etc.).

Figure 5 summarizes the potential energy savings for the hog fuel, woodchips, and bark materials when no supplement heat was added. These trials measured an energy gain (ΔE) of 80–230 kWh for each treatment without considering the input energy of 7–35 kWh, while measuring an overall net energy gain ratio of 8–32% when compared to the original biomass energy content. The higher airflow rates led to higher total energy gains in all three materials due to increased water removal. However, the net energy gain ratio showed that the difference between the high and medium air flow rates were minimal after considering the extra energy consumed by the fan operating at a faster speed. This suggested that the highest air flow rate, 26.8 $L \cdot s^{-1} \cdot m^{-3}$,

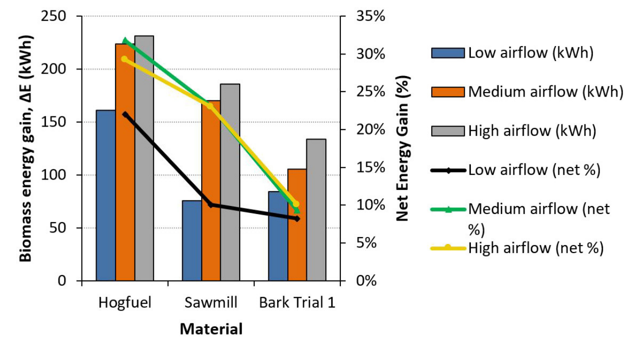

FIGURE 5 | Energy gains (ΔE) (kWh) from natural air drying of biomass without supplemental heat, along with net energy gain (%) (including equipment energy consumption) for hog fuel, woodchips, and bark trials.

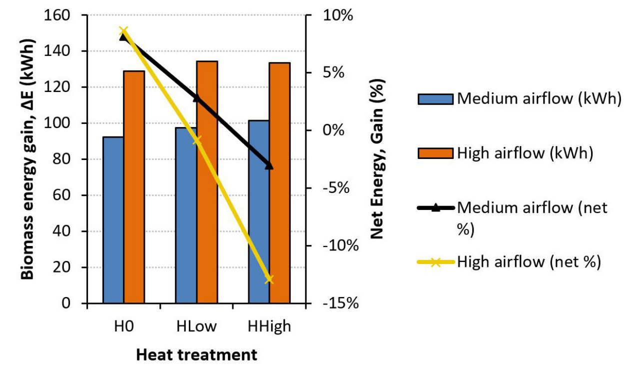

FIGURE 6 | Energy gains (ΔE) (kWh) from natural air drying of bark material with and without supplemental heat along with net energy gain (%) when factoring in equipment energy consumption.

has the potential to remove more water only if the increase in energy use is justified. The lowest air flow rate, 3.3 $L{\cdot}s^{-1} \cdot m^{-3}$, provided the lowest energy gain between 8 and 22%, but still managed to provide a positive net energy gain. There is potential to optimize the energy use through controlling the fan activity to a water loss set point. McGovern (2007) reported that there is potential to reduce drying energy by turning off the fans when the drying rate slows or stops. Regardless of the optimum return, these results demonstrate the potential of NAD as an energy-effective method to dry biomass due to the positive net return for all trials.

In comparable drying reports by Tengesdal et al. (1988) and Price (2012) the magnitude of scale, drying duration, climate, material and set-up varied from our study. Tengesdal et al. showed an energy gain of 4,300 kWh with an input energy of 700 kWh, while Price showed a gain of 293 kWh with an input energy of 665 kWh. Price (2012) and Tengesdal et al. (1988) performed the drying analysis over 1 and 3 days, respectively, compared to 3 weeks in this study. Tengesdal et al. also dried woodchips at a shallower depth of 0.75 m compared to the 4 m depth in our study. These differences highlight the importance of optimizing NAD for various user storage requirements where results can be very dependent on equipment setup, biomass volume and protocol design. Supplemental heat could potentially improve drying when ambient air conditions are not adequate for water removal. **Figure 6** compares the energy balance when supplemental heat is added to the air flow for drying the bark material. Similar to the trials without supplemental heat, the higher air flow rate had a higher energy gain (ΔE) of 129–134 kWh compared to the medium air flow rate of 92–102 kWh. However, the energy increase to power the fans and heaters of 18–256 kWh eliminated the biomass energy gains and resulted in a 3 to −13% energy gain ($\text{Gain}_{\text{ratio}}$) when compared to the original biomass energy content. These results showed lower losses compared to McGovern (2007) and Price (2012), while less gains were found compared to Nordhagen (2011). McGovern (2007), Price (2012), and Nordhagen (2011) reported an energy gain and input energy of 4,033 kWh with 14,385 kWh energy use, 104 kWh with 1,380 kWh energy use, and 2,500 kWh with 270–556 kWh energy use, respectively. Due to differences in experimental setup and conditions, the multitude of variables makes it difficult to directly compare these results. For example, McGovern used a grain dryer which used 60°C hot air for 1 day, while Price used a biomass boiler providing 30°C ambient air for half a day. Nordhagen (2011) used the surplus heat from hydroelectric plants to raise the dryer temperature to 15–26°C and was only required to power a fan over a maximum of 6 days. Nonetheless, the trials without supplemental heat treatment provided the highest energy gain ratio of ∼8–9% even in the colder climates between October and November in Western Canada. However, the material did not reach the target of 25% MC within a 3-week duration. Heat recovery/self-heat recuperation technology will make the use of supplemental heat much more favorable by minimizing energy losses along with the benefit of decreased drying time. Liu et al. (2014) found that self-heat recuperation dryers may have the potential to reduce energy consumption by 75–85.7% compared to conventional heat dryers. Such improvements to drying systems will greatly decrease the energy costs associated with heat drying but will likely take time to be vastly implemented (especially by smaller bioenergy/biomass storage operations).

Summary

This study found that the energy input without supplemental heat during warmer months in Western Canada can result in an energy gain of 9–32% compared to the original biomass energy content over 3 weeks. The supplemental heat trials indicated an energy gain of 3 to −13% over a 3 week duration due to the additional energy required. Waste heat recuperation would therefore be necessary to make supplemental heat more favorable.

Table 6 displays the total time and energy used for each treatment to either dry to 25% MC or the lowest MC calculated by the end of the 3 week trial. It can be seen that several treatments were able to dry the material to the target goal of 25% MC much faster than others, where drying time could

TABLE 6 | Total time and energy used to bring the material to an average of 25% MC or the energy used over the total drying period.

Material	Trial	Initial MC (%)	Final MC (%)	Total drying time* (days)	Total energy used* (kWh)
Hog fuel	MC_L-A_{Low}	45	30	22	9
	MC_L-A_{Med}	45	25	8	6
	MC_L-A_{High}	45	25	5	7
	MC_H-A_{Low}	62	48	22	9
	MC_H-A_{Med}	62	32	22	16
	MC_H-A_{High}	62	25	15	22
Sawmill woodchips	MC_L-A_{Low}	45	35	23	10
	MC_L-A_{Med}	45	25	17	13
	MC_L-A_{High}	45	25	11	16
	MC_H-A_{Low}	55	48	23	10
	MC_H-A_{Med}	55	30	23	17
	MC_H-A_{High}	58	25	20	29
Bark	MC_L-A_{Low}	42	33	24	7
	MC_L-A_{Med}	37	25	17	10
	MC_L-A_{High}	37	25	5	7
	$MC_{H-}A_{Low}$	47	39	24	7
	$MC_{H-}A_{Med}$	40	25	20	12
	MC_H-A_{High}	42	25**	13	19
Bark with heat	A_{Med}-H_0	42	30	23	18
	A_{Med}-H_{Low}	42	29	23	71
	A_{Med}-H_{High}	42	28	23	129
	A_{High}-H_0	42	25	15	34
	A_{High}-H_{Low}	42	25	13	80
	A_{High}-H_{High}	42	25	11	122

**Total drying time and energy does not include any time/energy after the material reached the target MC.*

***Linear interpolation due to lost data.*

be significantly affected by simple modifications. For example, the hog fuel material (MC_L-A_{Med}, MC_L-A_{High}) showed that the higher airflow was able to shorten the drying time by 3 days with an increase of 1 kWh (**Table 6**). Thus, in situations where time saving benefits outweigh increased energy costs, higher airflow might be favored (considering energy used will still vary with other factors such as air-drying capacity). Future NAD studies should include a more detailed sensitivity analysis to highlight overall costs and benefits for several woody biomass materials, drying parameters, environmental factors, as well as desired economic and temporal targets.

CONCLUSIONS

The experimental trials in this study determined that natural air drying (NAD) of woody biomass with and without supplemental heat resulted in net energy gains of 3 to −13% and 9–32%, respectively. NAD was shown to have high potential as a pre-treatment strategy for improving woody biomass storage, however seasonality, forced air speed, and equilibrium moisture content will be important factors to consider when determining possible energy gains. Optimization must also take user storage requirements into consideration since faster drying times may occasionally be desired at a higher energy cost (e.g., increased fan speed). The use of supplemental heat should be carefully evaluated on a case-by-case basis due to the increased energy requirement. Though it has the potential to increase the drying performance of woody biomass, heat recuperation technology will be necessary to increase net energy gains.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to qualified researchers.

AUTHOR CONTRIBUTIONS

JA, SM, SK, and SW: conceptualization. LG, JA, SW, and SM: funding acquisition. LG, SK, and JA: supervision. JM, HL, LG, JA, and CH: research and investigation. JM, HL, and LG: writing (original draft). CH, JM, and SK: visualization and writing (review and editing). LG, JA, SM, and SV: resources.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Ensilability of Biomass From Effloresced Flower Strips as Co-substrate in Bioenergy Production

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Flower strips are grown to an increasing degree in order to enhance the ecological value of agricultural landscapes. Depending on their profitable life span and the crop sequence, the strips' biomass must be mulched after flowering to enable repeated tillage. A promising alternative is the use of the flower strips' biomass as a co-substrate for biomethanisation – thereby contributing to the climate-friendly generation of energy. This potential bioenergy substrate occurs only seasonally and is commonly produced only in limited quantities at a farm scale. To realize the additional benefit of flower strips as energy suppliers, stock piling of the strips' biomass is required. However, information about the ensilability of flower strip biomass is still rare. We conducted a 2-year study to analyze the ensilability of pure biomass from effloresced flower strips and mixtures of flower strip biomass with 33 and 67% whole crop maize, respectively. Ensiling took place in 3 l model silos at laboratory scale after chopping the substrate. Before ensiling several chemical characteristics of the biomass stock were determined to assess the substrate's biochemical ensilability potential (dry matter content, water-soluble carbohydrates, buffering capacity, nitrate content). The process-engineered ensiling success after 90 days was determined based on fermentation patterns. The ensilability potential of the pure flower strip substrates reached modest levels (fermentability coefficients according to Weißbach vary around the threshold of 45). Nevertheless, acceptable silage qualities were achieved under the laboratory conditions (pH ranging from 4.2 to 4.7). Compared to pure flower strip biomass, the addition of maize noticeably improved both the substrate's biochemical ensilability potential and the quality of real fermented silage. We conclude that a mixture of 33% biomass from flower strips with 67% whole crop maize can be regarded as a recommendable ratio if proper ensiling technology is applied.

Keywords: ensiling, biomass, field margins, buffer strips, preservation success, substrate composition, fermentation pattern, biomethanisation

INTRODUCTION

Two developments characterize the current situation in the agricultural sector: the increasing demand for food (Davis et al., 2016) and the growing importance of bio-based energy production (Hennig et al., 2016). Both developments are linked via their respective land requirements and are held responsible for the negative effects of intensive land use on biodiversity (Robertson et al., 2012; Tilman and Clark, 2015). To counteract these adverse tendencies and to enhance the ecological value of agricultural landscapes, buffer strips along field margins (Mante and Gerowitt, 2006; Fritch et al., 2011) and vulnerable waterbody zones (Buckley et al., 2012) are growing in importance. For ecological and esthetic reasons, these buffer strips mostly contain a broad mixture of flowering annuals, biennials (Jacot et al., 2007) and perennials (Carlsson et al., 2017). In Europe, the support measures under the so-called second pillar of the EU Common Agricultural Policy (CAP) framework have led to a significantly increasing area of flowering strips in many regions (Haaland et al., 2011) recently.

Depending on their profitable life span and the crop sequence in which they are integrated, the strips' biomass must be mulched after flowering in late summer in order to enable repeated tillage in early autumn. Since many species, such as mallows, can form enormous biomasses, mulching, and tilling are associated with a great deal of effort. A promising alternative is the use of the flower strips' biomass as a source of renewable bioenergy (Christen and Dalgaard, 2013; Golkowska et al., 2016). This kind of biomass is especially appreciated as it does not compete with food production (Dauber et al., 2012; Gelfand et al., 2013) and has numerous ecological benefits, e.g., providing habitats for insects and birds. Although other conversion routes of tall herb biomass to energy like combustion (Ciesielczuk et al., 2016) are conceivable, biomethanisation is of the greatest importance (van Meerbeek et al., 2015). This technology does not require expensive drying and is most widespread in European rural areas (Capodaglio et al., 2016).

At farm scale, the biomass from effloresced flower strips crops up only seasonally and in limited quantities (Ferrarini et al., 2017). Therefore, stock piling is required if the strips' biomass is supposed to be used as a substrate for the production of bioenergy. A well-founded knowledge of the storage capability of the biomass is essential for several reasons: (1) to avoid energy losses (Einfalt, 2017; Towey et al., 2019), (2) to prevent the entry of substances that interfere with the conversion processes, e.g., ammonia N (Poggi-Varaldo et al., 1997), (3) to make targeted use of the advantages of any preliminary conversion effects, e.g., ensiling as methane potential booster before anaerobic digestion (Teixeira Franco et al., 2016), caused by degradation processes and an increase in volatile fatty acids (VFA) (Corno et al., 2016), and thus, to design an economically efficient storage process. Expertise in the storage capability of flower strip biomass would not only be useful for the ensiling and energetic use of the flower strips, but also for harvests from perennial wild flower stands, as found in increasing numbers in restoration projects across Europe (von Cossel and Lewandowski, 2016) and North America (Voigt et al., 2012).

However, information about the ensilability of flower strip biomass is still rare. Despite an extensive literature research, only one peer-reviewed source (Oh et al., 2010) on the topic could be found. Further information stems from gray literature such as conference contributions and non-peer-reviewed technical contributions, e.g., Kalzendorf (2011). In addition, a wide range of possible seed mixtures and varieties makes it actually impossible to assume a generalizable composition of the flower strip biomass and thus, of the substrate for ensiling. Multispecies mixtures containing effloresced dicots that were neither bred nor intended for the purpose of biomass utilization and stock piling may hold some surprises regarding their carbohydrate composition, their secondary metabolites, their epiphytic population and further factors that potentially influence the ensiling success significantly.

Against the background of scarce knowledge, it seemed reasonable to determine the ensilability of effloresced flower strip biomass using an approach based on the biochemical characteristics of the biomass stock. From the substrate properties of the flower strip substrates, we intended to calculate estimates of their ensiling capability based on known biochemical principles of fermentation and to check these estimates in laboratory experiments. With this approach, we aimed for conclusions that potentially could be applied to ensiling of a wide range of wild flower substrates.

In detail, we wanted to answer the following questions:

i. What are the substrate characteristics of the biomass from effloresced flower strips? Are there peculiarities compared to well-known forage substrates?
ii. Does the standing year play a role in the substrate characteristics?
iii. How to evaluate the substrate characteristics with regard to ensilability?
iv. Are the results of characteristic-based ensilability assessments reflected by measured qualities of corresponding silages?
v. Is a mix of flower strip biomass with whole crop maize a contribution to the ensiling success?

MATERIALS AND METHODS

Substrates

The flower mix substrates originated from plots of a field trial in Rostock (Germany, 54°04′04.1″ N 12°04′55.7″ E). The perennial flower mixture used, "BG 70" (Saaten Zeller GmbH & Co. KG), was developed especially for the use as biomass substrate in biogas plants and contains 23 species. The first sowing took place in 2014. In 2015, the experiment was repeatedly established at the same location. In this way, comparable variants could be sampled in 2015 both from the first and second main standing year after establishment. The mixed flower stands received no fertilizer. Further details on the field experiment, the seed mixture and their botanical development are given in (de Mol et al., 2018).

The growths from the effloresced flower mixture were mowed with a Haldrup parcel harvester on September 12, 2014 (first standing year after establishment) and September 16, 2015 (first and second standing years) at a stubble height of 8–10 cm. With increasing population age, we observed the tendency of the dominance of individual competing species such as melilot (*Melilotus* ssp.). Since melilot is recommended as a biogas substrate (Bull, 2014), we included a representative of the genus *Melilotus*, yellow sweet clover (*Melilotus officinalis*), in our investigation in 2015. Nearly pure stands of yellow sweet clover from field plots of the same project in Malchow (Germany, 53°59′08.8′′ N 11°28′22.1′′ E) were used for this purpose on September 18, 2015.

Immediately before the harvest, the yield shares of the main species components were estimated. The estimates were validated using three subsamples per variant, divided by species and weighed separately. The degree of senescence was also estimated and validated in the same way. Botanical compositions and selected field characteristics of the evaluated substrate variants are shown in **Table 1**.

As a reference substrate, fresh chopped whole crop maize from a neighboring field (variety "Ronaldinho," breeder KWS®) harvested at early silage ripening stage was used. With the help of the BBCH scale (Weber and Bleiholder, 1990), the harvest stages were specified in terms of developmental physiology to BBCH 82 in 2014 and BBCH 87 in 2015. The maize biomass was used to prepare different mixtures with the flower strip biomass for ensiling (see section "Ensiling Procedure").

Ensiling Procedure

The harvested biomasses from the flower strip mixtures and from the yellow sweet clover were chopped to a length of 2–4 cm. The chopping length of the whole crop maize was 0.5–1.5 cm. All substrates were used for ensiling as pure substrates (100% flower mix substrate = FM100; 100% maize = ZM100; 99% yellow sweet clover = YSC99; see **Table 1**). In addition, mixed substrates from the flower strip's biomasses with maize were prepared. The mixing ratios were 1:2 (33% flower mix, 67% maize = FM33) and 2:1 (67% flower mix, 33% maize = FM67). Proportions are based on fresh weights immediately before ensiling. In terms of dry matter, this would correspond to a flower biomass:maize – mixing ratio of 2.9:1 in 2014 and 3.6:1 in 2015 for FM67, and a ratio of 0.7:1 (2014) and 0.9:1 (2015) for FM33, respectively.

The feedstock substrates were ensiled in at least three replicates in 3 l glass jars. The jars were washed and sterilized (180°C, 8 h) before the substrates were filled in and compressed in layers by hand. The resulting final packing densities ranged from 0.35 to 0.60 g cm^{-3} DM. The filled jars were closed airtight with a rubber-lined lid that was fixed by clips. Glass jars of all treatments were stored in a dark, tempered room (16°C) for 90 days. After ensiling the silages were removed from the glass jars, sealed airtight in plastic bags and stored at −40°C prior to the analyses of fermentation profiles.

Furthermore, subsamples from each substrate (ca. 500 g FM) were dried in a temperature-controlled range of <45°C and thereafter grounded to a sieve mesh of 1 mm wide. The four field repetitions were reduced to two test repetitions for lab capacity reasons using a sample splitter. This pooled material was used for the determination of the substrate's biochemical properties in both test years.

Biochemical Analyses

Several biochemical parameters which are suitable to estimate the ensilability and the fermentation success were determined from the substrates immediately before ensiling and from the fermented substrates after ensiling, respectively. In the study period 2015, the analysis spectrum could be extended to nitrate, buffering capacity (BC) and NDF (see subsection "Parameters Characterizing Substrate's Ensilability").

Parameters Characterizing Substrate's Ensilability

DM content of the feedstock immediately before ensiling was determined by oven drying at 45°C to a constant weight. BC was analyzed by titration with lactic acid (0.1 mol l^{-1}) to a pH of 4.0 according to (Weißbach, 1992). We analyzed the sum of water-soluble carbohydrates (WSCH) and the enzyme-insoluble organic matter (EULOS) by Near Infrared Reflectance Spectroscopy (NIRS, Bruker® MPA, Bruker, Germany) with the photometrical Anthron method according to Naumann and Bassler (2012) as the reference for WSCH and the enzymatic method according to de Boever (de Boever et al., 1986) as the reference for EULOS. Dry combustion technique (Elementar® Analyzer, Vario Max CNS, Elementar, Germany) has been adapted to determine crude protein contents (CP, N × 6.25). Nitrates were analyzed by continuous-flow analysis (CFA Analyzer AA3, Seal®, Germany). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and crude fibre (CF) were determined by wet chemical analyses using a Fibretherm, Gerhardt®, Germany. Hemicellulose contents have been estimated as the difference between NDF and ADF concentrations.

In order to characterize fermentability in a more holistic manner, the two parameters DM and WSCH/BC were combined to the fermentability coefficient (FC) according to Weißbach and Honig (1996):

$$FC = DM[\%] + 8WSCH/BC \tag{1}$$

Feedstocks with FC < 35 are considered as "difficult-to-ensile," whereas those with FC > 45 are referred to as "easy-to-ensile."

Fermentation Characteristics of Ensiled Substrates

After thawing of the frozen silage samples at room temperature, silage extracts were prepared from 50 g silage and 200 mL deionized water. The pH values of these extracts were measured potentiometrically by a calibrated pH analyzer (precision 0.01). Between each measurement of pH, a cleaning of the probe was carried out with distilled water. Fermentation products were analyzed in the filtrated extracts thereafter. Lactic acid was determined by HPLC (Aminex HPX-87H, Bio-rad®, United States) with a flow rate of 0.60 ml min^{-1} at the UV detector. Short-chain fatty acids and ethanol

TABLE 1 | Main species composition and field characteristics of the flowering mixture's substrate stocks to be ensiled.

Substrate	Standing age (year of harvest)	Main species	Percentage share %	Senescent biomass in % FM	Harvest DM content in % FM
Flower mix	1 (2014)	*Chenopodium album*	26	14.2	40.2 (1.90)
		Malva ssp.	24		
		Tanacetum vulgare	17		
		Artemisia vulgaris	13		
		Other species	20		
Flower mix	1 (2015)	*Malva* ssp.	28	16.8	42.1 (6.19)
		Chenopodium album	23		
		Tanacetum vulgare	18		
		Centaurea nigra	13		
		Other species	18		
Flower mix	2 (2015)	*Tanacetum vulgare*	23	17.8	42.8 (3.40)
		Artemisia vulgaris	20		
		Malva ssp.	18		
		Melilotus ssp.	16		
		Other species	23		
Yellow sweet clover	2 (2015)	*Melilotus officinalis*	99	0.5	25.0 (0.46)

Dry matter (DM) contents are presented as means with standard deviation of the mean in brackets.

were quantitatively separated by gas chromatography (GC-14A, CLASS-VP, Shimadzu®, Kyoto, Japan). The ammonium content in the silage extracts was determined according to the method of Voigt and Steger (1967). Silage DM was determined by drying to a constant weight (105°C, 24 h) and was corrected for the loss of volatiles during drying as described by Weißbach and Strubelt (2008a,b). Ashing followed after drying at 600°C for at least 4 h in a muffle furnace until obtaining a light gray ash color and led to the parameter crude ash content (CA).

Potential Biogas Yield Estimation

The potential for methane formation was estimated using practice-proven estimation equations based on biochemical parameters of the substrates before ensiling (Weißbach, 2009).

$$\text{ZM100}: VS = 984 - (CA) - 0.47(CF) - 0.00104(CF)^2 \quad (2)$$

$$\text{FM100, YSC99}: VS = 1000 - (CA) - 0.62(EULOS) - 0.000221(EULOS)^2 \quad (3)$$

The substrate's amount of fermentable organic substances (VS g kg^{-1} DM) was estimated for pure maize using Eq. (2) and for all other pure substrates using Eq. (3). Mixed substrates were assessed by weighted means of (2) and (3) according to the mass proportion of the single substrates.

Substrate-specific biogas (BGY) and methane (CH_4Y) yield potentials of the tested feedstock substrates were derived from VS as follows:

$$BGY = 0.80(VS) \quad (4)$$

$$CH_4Y = 0.42(VS) \quad (5)$$

BGY and CH_4Y are given in norm liter per kg (Nl kg DM^{-1}) and are corrected of VFA.

Data Analysis

Biochemical composition data are presented as averages and standard deviation of the mean (s_d) with $n = 2$ replicates. In the absence of real local repetitions, the effects of the standing age on substrate's biochemical properties were analyzed including the flower-maize-mixtures FM67 and FM33 as replicates. The parameters whose values were below the detection limit ("not detected") in most samples were not included in studying the differences in the biochemical compositions of the silages.

All evaluation-relevant data records were first tested for normal distribution using the Shapiro–Wilk test. For a given normal distribution, analysis of variance (ANOVA) was applied to investigate the effects of the factors "substrate" (2014 and 2015) and "standing age" (2015 only). If the values were not normally distributed and neither log nor sqrt transformations achieved a normal distribution, mixed linear models were applied with "substrate" and "standing age" as a fixed factors and "year" as random variable. Modeled parameters were estimated with an ANOVA of type III and a Satterthwaite's adjustment.

The substrate specific patterns of the fermentation products were visualized with non-metric multidimensional scaling (NMDS) based on Bray–Curtis distances. The influence of substrate properties on fermentation profile was additionally tested with a goodness-of-fit permutation test using the squared correlation coefficient as test statistics.

All statistical analyses were performed by scripts using the R environment version 3.3.2 (R Development Core Team, 2016). The R-package "lme4" was used to calculate the mixed linear models (Bates et al., 2015), and the "vegan" package to perform NMDS (Oksanen et al., 2018).

RESULTS

Substrates' Biochemical Properties

The substrates' properties with a known or reasonably suspected influence on the ensiling capability were determined from substrates immediately before ensiling in 2015 (**Table 2**). With more than 40%, the dry matter content was highest in the pure flower strip mixture substrate (FM100). The lowest DM content was found in the silage maize, which was not yet fully silage-ripened. The blends of flower strip mixture and maize reached intermediate values. No trend in DM content could be discerned with regard to the feedstocks of different standing ages. The ash contents of the flower mixture substrates were very low (<7% DM). However, it should be noted that the mixtures were harvested using plot technology.

Pure substrate from effloresced flower mixtures were characterized by very high crude fiber contents (>45% DM) and low crude protein contents (<8% DM) reflecting the late ontogenetic state of the dominating plant species at the harvest time. In contrast to this, yellow sweet clover (YSC99), which has the potential to dominate perennial flower strips after several years of use, had CF (25.6% DM) and CP (22% DM) values that resemble legume forage plants such as alfalfa. The nitrate content was the only characteristic that has been significantly influenced by the age of the flower strips stand ($F = 7.78$; $P = 0.049$). In the second year after the establishment of the mixtures, the nitrate content of the harvested biomass decreased by 1.4 to only 0.1 g per kg DM. A tendency toward higher WSCH values could not be statistically confirmed.

Substrates' Ensilability Assessment

The FC of the pure flower mix substrate was not significantly influenced by its standing age ($F = 0.216$; $P = 0.666$). This fact allowed us to average the FC values over the levels of this factor (**Figure 1**) and find a significant effect of the substrate type on the FC (ANOVA, $F = 17.98$; $P = 0.020^*$).

The substrate-specific characteristics of FC in the study are shown in **Figure 1**. All substrates containing maize as a component clearly exceeded the FC > 45 threshold and thus indicate good conditions for low-loss preservation. The unusually high values of the maize-dominated test variants FM33 and ZM100 are due to their very high content of WSCH, which is also reflected in high WSCH/BC-ratios (see also **Figure 2**). In contrast to mixtures with maize, the two pure flower mix substrates FM100 and YSC99 had FCs that are within the limits of good conservation suitability.

Below 28% DM, an increasing risk of leachate from the feedstock must be expected. However, the effloresced stands had sufficiently high (>30%) contents of DM without wilting efforts (**Figure 2**). This finding does not apply to the dominant stocks of yellow sweet clover (YSC99) whose biomass was still vital at the time of harvest and contained little senescent material. On the other hand, dry matter contents of the pure flower mixture in the first cropping year exceeded the recommended DM-range of 30–40% and reached a level that is only suited as a metabolic substrate for very osmotolerant lactic acid producers.

Realized Silage Quality

Silage fermentation patterns varied according to substrate, year, and standing age. ANOVA after fitting GLMM models revealed significant effects of substrate types on silage characteristics for most of the main fermentation products (**Table 3**), namely pH, lactic acid, acetic acid, and ethanol. The only exceptions were butyric acid and propionic acid, since their contents were partly below the detectability threshold and thus escaped the biostatistical model estimations.

Despite trends in feedstock analysis before ensiling (see "Substrates' Ensilability Assessment"), standing age caused only minor variation in the main silage characteristics leading to non-significant effects in the mixed models.

Only lab-silages containing maize fell below the pH value threshold of four (**Table 3**). Undesirable butyric acid was found only in the variants of the pure flower mix substrates with DM contents of more than 40% in the harvested substrate. In order to allow a better comparison of the silage with the properties of the harvested substrate, which was investigated only in 2015, relevant fermentation parameters of the results from 2015 are shown separately in **Figure 3**.

When comparing the amount of lactic acid formed (**Figure 3A**) with the corresponding pH values (**Figure 3B**), it is noticeable that yellow sweet clover did not follow the common trend of decreasing pH values at higher lactic acid concentrations. Since acetic acid and ethanol are metabolites of the same bacterial group (coli-erogenic), their contents in the laboratory silos were compared (**Figures 3C,D**). The comparison revealed that during ensiling of effloresced flower mixture biomass, less alcohol was formed in relation to acetic acid.

Relationship Between Substrate Properties and Fermentation Profiles

In order to make relationships between substrate biochemical characteristics and fermentation patterns visible, a complex multivariate analysis was carried out. We applied a NMDS which allowed us to include the whole range of characteristics in the analysis and to represent them graphically (**Figure 4**). The goodness of fitting the multidimensional data to the reduced dimensioned NMDS was god (see **Supplementary Figure A1** for details).

The plot contains a table presenting the results of the vector fitting procedure additionally. The data on the expression of the substrate characteristics before ensiling served as vectors. The substrate characteristics of this figure-integrated tabular list were arranged according to the closeness to the matrix of fermentation characteristics, expressed by the squared correlation coefficient. These are also the vectors with a relatively high gradient length, which can be seen from the length of the arrows.

On the one hand, it is noticeable that the individual substrates always form well-defined clusters if they are 1-year stocks. On the other hand, there is a trend toward splitting into subgroups, as in

TABLE 2 | Chemical characterization of the tested feedstock variants before ensiling (experimental year 2015, means from two laboratory repetitions with standard deviations in parentheses).

Type of feedstock substrate[1]	FM 100		FM 67		FM 33		ZM 100	YSC 99
Standing year	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**1**
Parameter[2]								
Dry matter content (g kg^{-1})	426.7 (8.0)	400.9 (1.1)	363.8 (6.4)	380.7 (2.2)	325.5 (2.2)	315.0 (1.9)	266.6 (3.8)	268.6 (4.0)
Crude ash (g kg^{-1} DM)	63.8 (1.7)	65.3 (0.9)	66.0 (1.7)	62.0 (0.5)	53.8 (0.4)	52.0 (1.2)	32.6 (0.4)	89.3 (0.8)
Crude protein (g kg^{-1} DM)	61.1 (6.3)	55.3 (3.9)	69.2 (4.2)	69.0 (2.2)	75.2 (0.4)	81.2 (2.5)	74.1 (11.2)	213.7 (5.1)
Crude fiber (g kg^{-1} DM)	460.7 (39.9)	426.6 (23.7)	399.7 (25.6)	388.1 (49.3)	330.3 (50.1)	282.2 (50.2)	222.5 (4.4)	242.9 (13.0)
Hemicellulose (NDF-ADF, g kg^{-1} DM)	160.6 (4.1)	215.4 (2.5)	184.9 (1.3)	215.5 (1.8)	182.2 (2.5)	187.0 (1.8)	215.2 (2.7)	110.6 (1.6)
Water-soluble carbohydrates (g kg^{-1} DM)	3.2 (0.6)	9.9 (0.3)	15.1 (0.4)	36.1 (0.2)	85.1 (0.3)	115.5 (3.4)	182.9 (1.5)	49.9 (0.8)
Nitrate content (g kg^{-1} DM)	1.5 (0.01)	0.1 (0.06)	0.7 (0.21)	0.1 (0.05)	0.6 (0.16)	0.2 (0.07)	0.3 (0.04)	0.3 (0.05)
Buffering capacity (g LA kg^{-1} DM)	9.2 (0.14)	6.8 (0.10)	8.5 (0.11)	8.0 (0.01)	6.8 (0.16)	6.9 (0.05)	10.4 (0.65)	22.4 (0.28)

[1] FM100 – 100% flower mix; FM67 – 67% flower mix, 33% maize; FM33 – 33% flower mix, 67% maize; ZM100 – 100% maize; YSC99 – 99% yellow sweet clover. [2] DM, dry matter; LA, lactic acid; WSCH, water-soluble carbohydrates.

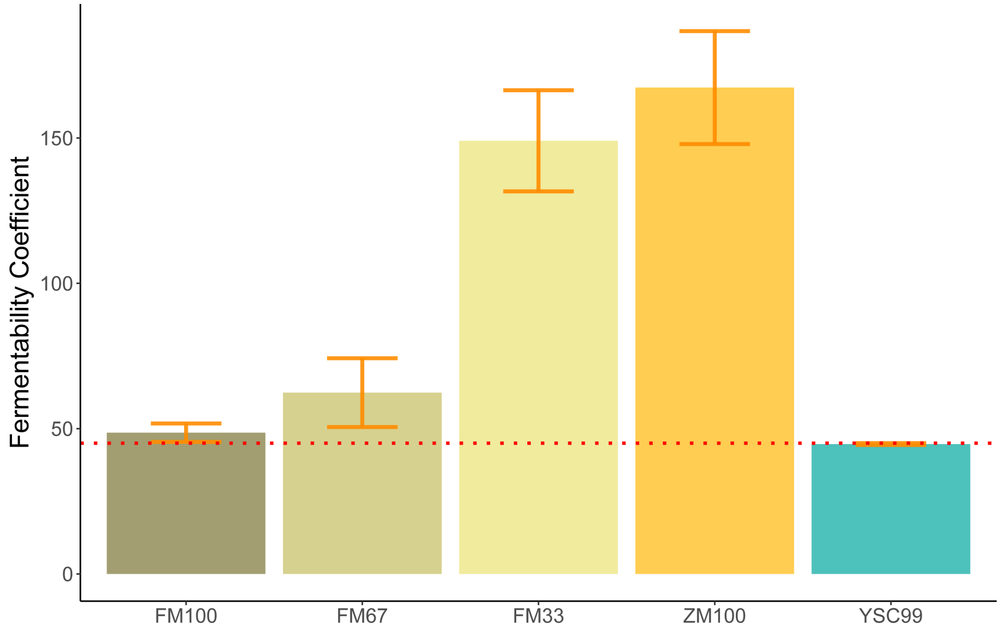


FIGURE 1 | Mean Fermentability Coefficients (FC) of the tested feedstock substrates. Error bars indicate standard deviations of the mean. (Sample size: FM100, FM67, FM33 *n* = 4; ZM100, YSC99 *n* = 2). The red dotted line indicates the FC threshold according to Weißbach and Honig (1996).

the case of the pure flower mixture variant FM100, shown on the left side of the plot.

DISCUSSION

Substrate Characteristics and Fermentation Patterns

To our knowledge, this study is the first exploring the ensilability of effloresced flower strip's biomass. Regarding the scarcity of data concerning biomasses from wildflower mixtures, we consider the description of the substrate characteristics valuable as well; especially since the botanical composition of the stock is known and adequately described. With the inclusion of melilot, the 2-year study shows quite a wide range of possible substrate compositions despite limited numbers of variants.

The high fiber contents found in the growths of the flowering strips together with the high percentages of senescent foliage, low protein and sugar contents are characteristics of fast-growing, high-flowering dicotyledons with a low tendency to vegetative regeneration and persistence. Such substrate constellations offer poor conditions for successful ensiling due to a lack of readily available sugars for the lactic acid formation (Pitt, 1990) and a high stock of harmful molds and yeasts (Dunière et al., 2013). Consequently, a low lactic acid content of only 4 g kg^{-1} was formed in the pure flowering mixture silage (FM100). Nevertheless, this was sufficient to lower the pH value to below

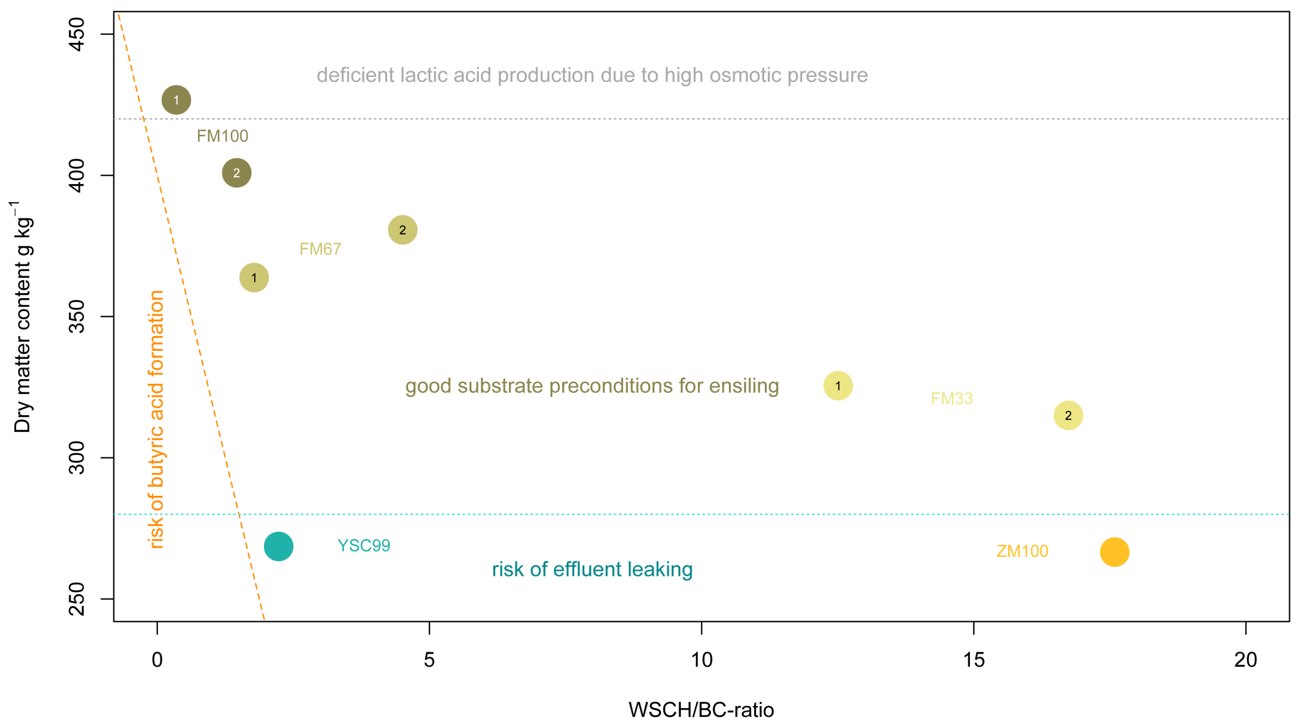


FIGURE 2 | Arrangement of the tested feedstocks in the estimation frame according to Weißbach and Honig (1996). The digits 1 and 2 within the location points indicate the standing year. The dashed orange line reflects the critical dry matter content as a function of the WSCH/BC-ratio. The dotted light-gray line shows approximately the beginning of the range of limited metabolic activity of natural epiphytic lactic acid bacteria population due to forced osmotic pressure.

TABLE 3 | Main fermentation products of the tested lab-scale ensiled feedstock after a storage period of 90 days (2 year means with standard deviations in parentheses).

Feedstock Substrate[1]	Standing age (years)	pH	Lactic acid (g kg^{-1} DM)	Acetic acid (g kg^{-1} DM)	Butyric acid (g kg^{-1} DM)	Propionic acid (g kg^{-1} DM)	Ethanol (g kg^{-1} DM)
FM100	1	4.54 (0.09)	4.57 (1.69)	1.45 (0.23)	0.62 (0.217)	0.09 (0.006)	0.38 (0.17)
	2	4.30 (0.24)	4.09 (0.84)	1.07 (0.12)	0.02 (0.003)	0.04 (0.004)	0.11 (0.05)
FM67	1	3.92 (0.06)	6.22 (0.53)	1.57 (0.29)	n.d.	0.01 (0.002)	0.75 (0.55)
	2	3.86 (0.02)	6.69 (0.32	1.34 (0.24)	n.d.	0.01 (0.002)	0.70 (0.26)
FM33	1	3.90 (0.23)	6.60 (0.32)	1.80 (0.49)	n.d.	n.d.	0.51 (0.07)
	2	3.72 (0.02)	6.84 (0.21)	2.30 (0.44)	n.d.	n.d.	0.64 (0.06)
ZM100	1	3.64 (0.09)	7.80 (0.43)	2.22 (0.14)	n.d.	0.12 (0.015)	1.43 (0.44)
YSC99	1	4.61 (0.03)	9.66 (1.27)	1.88 (0.32)	n.d.	0.03 (*n.f.*)	1.25 (0.25)
ANOVA results (*F*; *P*)							
	Substrate	$F = 112.69$; $P < 0.001$	$F = 33.81$; $P < 0.01$	$F = 10.24$; $P < 0.01$	n.f.	n.f.	$F = 19.09$; $P < 0.01$
	Standing age	$F = 3.90$; $P = 0.055$	$F = 0.59$; $P = 0.446$	$F = 0.28$; $P = 0.599$	n.f.	n.f.	$F = 0.17$; $P = 0.684$

n.d., not detectable; n.f., not feasible. [1]FM100 – 100% flower mix; FM67 – 67% flower mix, 33% maize; FM33 – 33% flower mix, 67% maize; ZM100 – 100% maize; YSC99 – 99% yellow sweet clover.

4.7, which is necessary for stable storage at a dry matter content of 40% (Kalač, 2011). The occurrence of butyric acid indicates that the reduction of the pH value was slow, so that the preservative acidification effect was not yet present in the initial storage phase. A certain contribution of fiber degradation to low molecular saccharides could also have contributed to continued lactic acid formation. Unfortunately, the fiber fractions of the silages after fermentation were not analyzed again, which could have helped to verify this thesis by comparing pre-ensiling with post-ensiling results. If we recall the ordination (**Figure 4**, left pointing arrows), we can see that the characteristics CF, DM, and NO_3 have the greatest influence on the fermentation patterns of pure FM100-silages. However, it is not very likely that the contribution of crude fiber to the explanation of fermentation profiles is related to the carbohydrate donors. If that was the case the NDF arrow would rather point in the direction of the FM100 positions. Instead, it seems to be the effect of an intercorrelation with the dry matter content: the older the plants in the stand, the drier and more fibrous they become. It is therefore obvious to assume that the ontogenetic development of the flower mixture stands is the

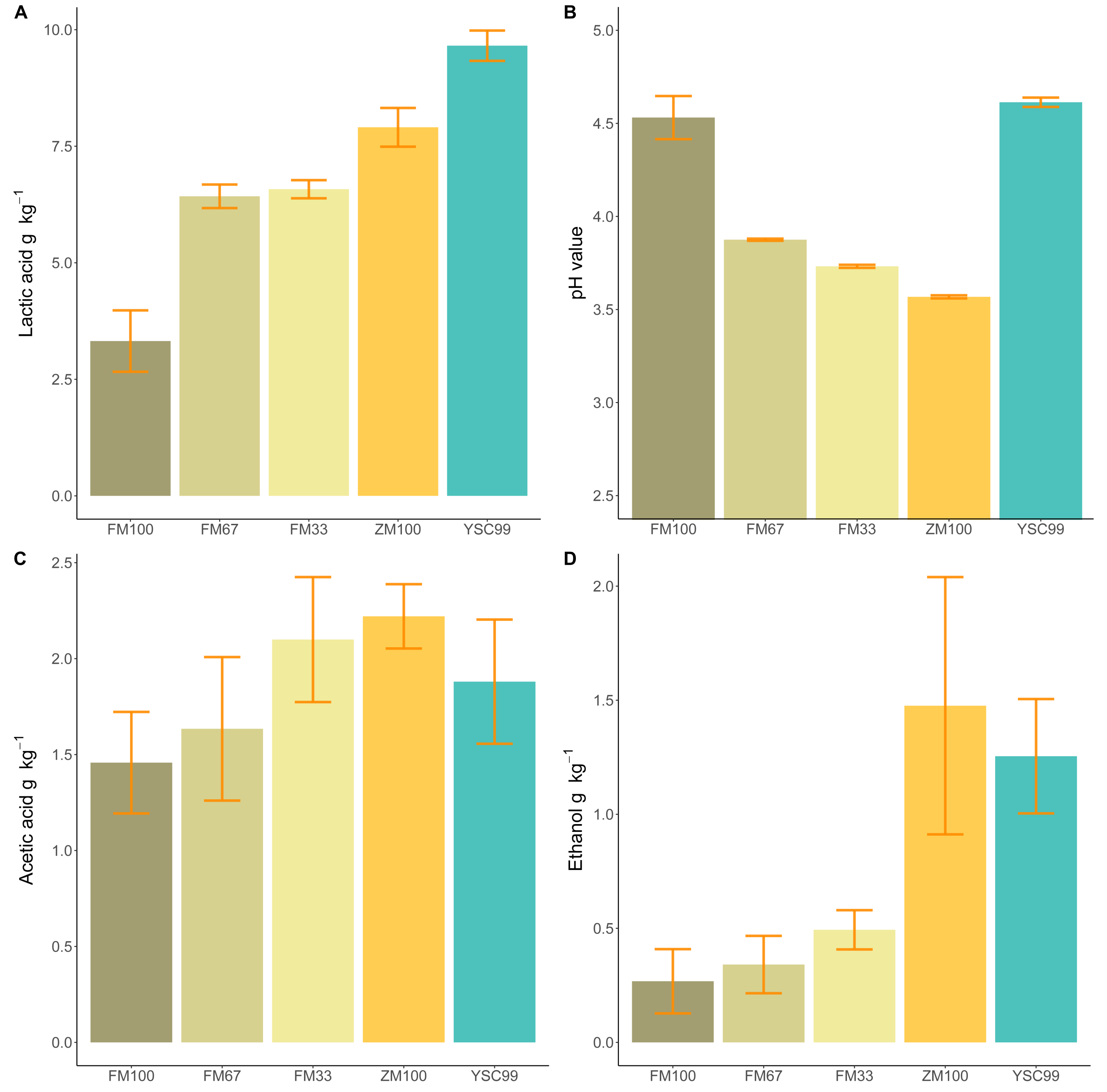


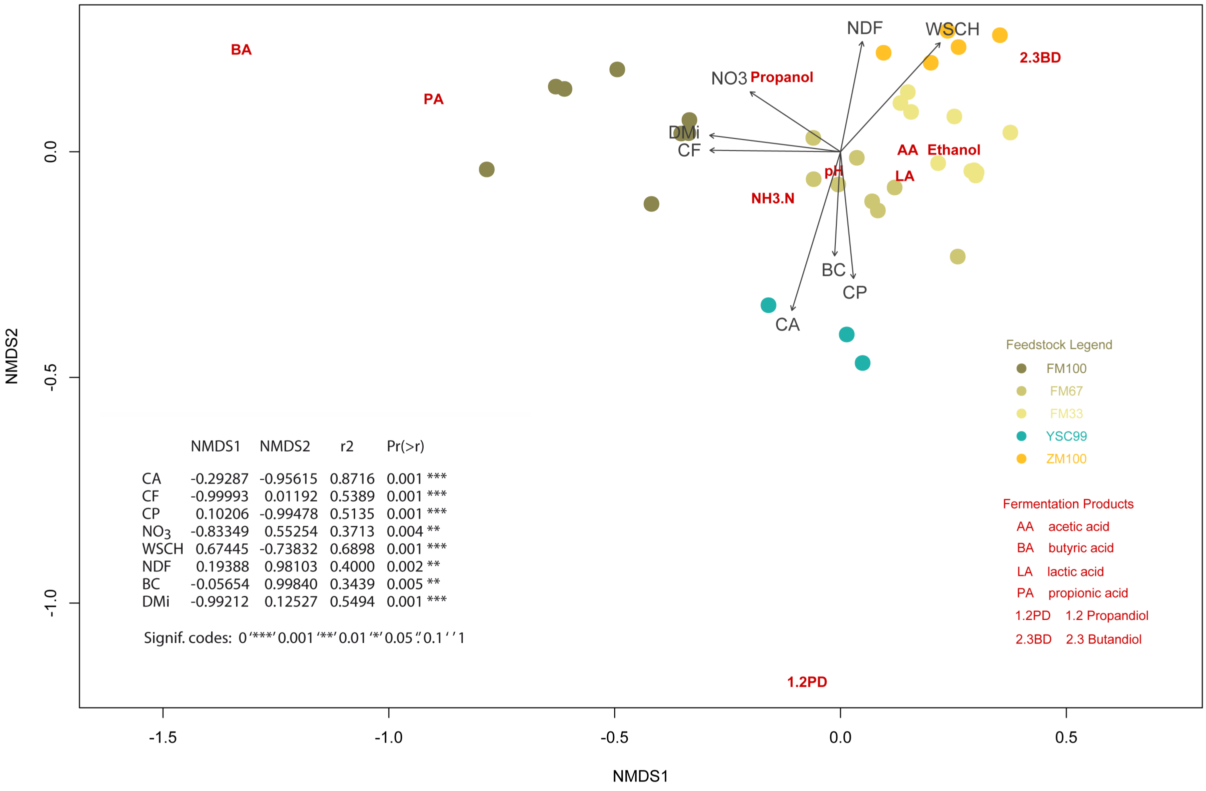
FIGURE 3 | Mean fermentation products of the tested feedstock substrates in 2015. **(A)** Lactic acid content, **(B)** pH value, **(C)** acetic acid content, **(D)** ethanol content. Error bars indicate standard deviations of the mean (Sample size: FM100 *n* = 4, FM67 *n* = 4, FM33 *n* = 5; ZM100 *n* = 5, YSC99 *n* = 3).

significant background-variable and responsible for variations in silage quality. Obviously, the known Clostridia-suppressive effect of nitrate (Kaiser et al., 1999) is rather important in the limit range of fermentability.

For the fermentation acid patterns of the maize-dominated silages, the height of the WSCH and the NDF fraction played an important role (**Figure 4**, right pointing arrows), although there was no lack of easily fermentable saccharides. Nonetheless, the ratio of the fermentation products lactic acid, acetic acid, ethanol and 2,3-butanediol might have been influenced by these ingredients in a way which has not been recognized as random.

Substrate's Ensilability Assessment

The prediction of ensiling success on the basis of the substrates' biochemical properties is both a promising and a difficult undertaking, as not only biochemical, but also physical and microbiological processes are involved (Müller and Bauer, 2006). Assuming a proper ensiling technology and an average lactic



	NMDS1	NMDS2	r2	Pr(>r)
CA	-0.29287	-0.95615	0.8716	0.001 ***
CF	-0.99993	0.01192	0.5389	0.001 ***
CP	0.10206	-0.99478	0.5135	0.001 ***
NO_3	-0.83349	0.55254	0.3713	0.004 **
WSCH	0.67445	-0.73832	0.6898	0.001 ***
NDF	0.19388	0.98103	0.4000	0.002 **
BC	-0.05654	0.99840	0.3439	0.005 **
DMi	-0.99212	0.12527	0.5494	0.001 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

FIGURE 4 | Non-metric multidimensional scaling ordination plot showing the position of the fermentation characteristics (dark red colored abbreviations) in relation to the initial biochemical substrate properties (darkgray colored arrows including abbreviations). The location of the corresponding substrates is additionally point-plotted and explained in a legend. Nomenclature of the biochemical characteristics: CA, crude ash; CF, crude fiber; CP, crude protein; NDF, neutral detergent fiber; NO_3, nitrate; WSCH, water soluble carbohydrates; BC, buffering capacity; DM_i, initial dry matter content of the substrates (before ensiling).

acid bacteria (LAB) stocking on the phyllosphere is given, the existing estimation framework can be successfully applied for the major forage crops (Weißbach and Honig, 1996). The few authors dealing with the fermentability of herbs or herb-rich growths (Daniel and Opitz von Boberfeld, 1997) found that some of these species oppose specific effects on fermentation processes and attribute this to secondary metabolites (Weißbach, 1998). Consequently, the conservation results could not be reliably predicted with the existing substrate-based estimation frameworks. In our study, the flower strip mixtures also contained plants with notable amounts of antimicrobially active secondary metabolites like *Melilotus* (coumarins) or *Tanacetum* (flavonoids, terpenes, coumarins). Nevertheless, we can state that the results of the ensilability classifications prior to ensiling (**Figures 1**, **2**) are sufficiently consistent with the fermentation profiles of the silages obtained from them. Therefore, our results do not argue against the application of the existing estimation frames (developed for forages) for the ensiling of flower strip mixtures. However, one should be aware that particularly high concentrations of antimicrobial active metabolites, similar to variations in nitrate contents in the feedstock, could modify the ensiling success. In order to expand the still rare knowledge in this respect, further targeted investigations are necessary, both on the laboratory level and in practice.

The Effect of Standing Age on Ensilability of Biomass From Flowering Strips

In our analyses, the factor "standing age" proved to be of little influence on ensilability. However, this was also partly due to differences in the degrees of freedom (one degree for factor "standing age" against five degrees for the factor "substrate") and thus, due to the study design. The short rotation type of flower stripe examined here represent the most frequently occurring option of buffers in European arable landscapes due to designated support schemes and administrative regulations. The effect of the year of use on ensilability has two aspects: the changes in the soil nutrient pool and the botanical shifts in the mixed stands. In the comparison of the first year with the second standing year, both processes left their imprints on the biochemical characteristics of the grown substrates. The significant decrease in the nitrate content is a sign of N-limitation that is already beginning in the second year after establishment. Although there were no serious shifts in the abundance of the dominating species, higher contents of WSCH and lower CF concentrations indicate physiologically younger plant material in the second standing year. This finding could also be explained by more restrained growth due to N-depletion. From the point of view of ensilability assessment, this results in advantages for the availability of monosaccharides for lactic acid formation, but also in disadvantages for butyric acid inhibition with increasing standing age. According to Kaiser et al. (1997), a minimum content of 1.5% NO_3 should be targeted in order to achieve sufficient safety against butyric acid formation under field conditions. In our experiment, the advantages and disadvantages of the age-affected substrate pattern apparently compensated each other, so that there were no significant deviations with regard to the fermentation profiles.

In the case of perennial flowering mixtures, experience has shown that the age of the crop stand can have a major influence on substrate characteristics, especially if there is a stronger shift from annuals to perennials (de Cauwer et al., 2006). Under humid climate conditions, grass coverage increases with increasing standing age (de Cauwer et al., 2005). This stand development can lead to an improvement in ensilability if at least two cuts are made. However, this development reduces many ecosystem services of flowering strips. In addition, nitrogen fertilization would also be required to maintain a level of biomass production, which justifies mowing and transport.

Further Implications for the Storage of Biomass From Flowering Strips

In accordance with Teixeira Franco et al. (2016), we consider classical measures of production engineering measures such as short chopping lengths and good compaction to be more important than additives in order to ensure a low-loss storage of biomass – also from flower mixtures designed for energy recovery. However, the use of additives to increase storage safety or energy yield (Herrmann et al., 2011) is widespread. Based on our investigations of the substrate composition, it seems that if an application of additives was considered for late harvested flower strips, enzyme application would be more promising than inoculation with LAB. Generally, late summer growths have a high content of natural epiphytes (Filya et al., 2007), including LAB, so that LAB-inoculations are not necessary to guarantee the desired lactic acid formation. In fact, there is a risk that the inoculant LAB are overwhelmed by the natural epiphytes and do not affect fermentation significantly (Muck, 1989). Moreover, contents of less than one percent WSCH are not sufficient for an economically justifiable LAB-application (Bolsen et al., 1996). On the other hand, hemicellulose contents up to 21% DM are a promising pool for successful depolymerization by suitable enzyme products (Schimpf et al., 2013) that have the potential to enhance biogas yield if biomethanisation was chosen as conversion path for biomass from flower stripes.

Gravimetrically determined mass losses of laboratory silos like jars are not really suitable to describe the storage losses of biomass to be expected under real conditions of a field storage pile (Wendt et al., 2018). The individual weighings carried out as part of our study showed losses on the order of 0.5% and essentially reflected the fermentation activity as a whole. The latter, in turn, is strongly dependent on the DM content. Therefore, approaches such as those of Goeser et al. (2015) to draw conclusions about the expected losses under practical conditions from the fermentation patterns appear more successful. Following this logic, the mixture FM33 has to be recommended, since the proportion of fresh maize is sufficient to form an adequate amount of lactic acid for butyric acid-free storage, but the advantage of the higher dry matter content from the flowering strip biomass – another precondition of low storage loss – is still evident.

Technological Aspects of Realizing the Bioenergy Potential of Biomass From Flowering Strips

For the energetic utilization of biomasses rich in lignocellulose, such as that of flower strips, a number of conversion routes are possible, e.g., combustion (van Meerbeek et al., 2015), ethanol (Chen et al., 2011) or biogas production (Vollrath et al., 2016). For the latter two techniques, ensiling is an important component of the production process (Chen et al., 2007) facilitating storage (Emery et al., 2015) and pre-treatment of the substrate (Essien and Richard, 2018).

Economically and logistically, the way of utilization is to be preferred, that not only copes best with the substrate's qualities but also enables short routes of transport. Regarding the routes of transport biomethanisation is the preferred way to process biomass from flowering strips in the rural areas of Europe due to the large number of decentralized biogas plants (Capodaglio et al., 2016). The question of the substrate quality, however, cannot be answered independently of the specific type of biogas plant. Certainly, very few plant operators would rely on a substrate that delivers significantly lower methane yields than maize. In the present study it became obvious that using maize as a co-substrate is essential to realize the bioenergy potential of flower strip biomass. On the one hand maize proved to be an excellent mixing substrate to ensure low-loss ensiling of the flower strip biomass, especially in the case of the variant FM33. On the other hand, the mixing with maize optimized the specific methane yield of the flower strip biomass. The pure flower mixture (variant FM100) only had a specific methane yield of approx. 180 Nl CH_4 kg^{-1}, while the mixed substrate (FM33) with 67% fresh maize content yielded approx. 300 Nl CH_4 kg^{-1} representing nearly 90% of the reference yield of pure maize (see **Supplementary Figure A2**). Thus, the FM33 variant did not only have the best storage properties, but also promises high acceptance as substrate by the operators of biogas plants.

The production of a mixed substrate, however, remains a challenge at the commercial scale. The optimum crop for mixing would be maize that has not yet matured too far with DM contents of 22–28% on a whole plant basis; in particular if the biomass of the effloresced flower mixture is no longer vital and exceeds DM contents of more than 40%. The maize would supply the substrate with high contents of WSCH and moisture to lower the osmotic pressure and to enhance the compactability of the feedstock during ensiling. In practice silage maize is harvested at DM contents of 30–36%. Therefore, it may be a good idea to apply the widespread practice to use maize from the field edges and from hunting corridors as an early mixing substrate that is harvested before the actual silage maize campaign starts.

CONCLUSION

The use of increasing amounts of flower strips' biomass as a source of renewable bioenergy is a promising option to reconcile economic and environmental concerns. A primary challenge associated with the realization of this alternative is to store the feedstock in a way that losses are minimized. Due to the similarity of the biomass with delayed harvested forage, ensiling offers a cost-effective form of storage. Since there is little experience with the ensiling capability of flower strip mixture's substrates, we studied the ensilability of botanically classified and composition-related described feedstock from late harvested flower strips as pure substrate or blended with whole-crop maize. This study showed that existing frameworks developed for roughages could be successfully applied to predict the ensiling success on the base of the substrates' biochemical properties. This knowledge is important in order to make the right preparations and process-related decisions that lead to low-loss storage of this largely unknown feedstock. Pure biomass from effloresced flowering strips is set on a certain risk of misfermentation if not blended with a favorable feedstock like maize. We conclude that a mixture of 33% biomass from flower strips with 67% whole crop maize can be regarded as a recommendable ratio for low-loss storage. In addition, the multivariate approach used in this study to uncover the relationship between characteristics of the initial substrate and the fermentation pattern seems applicable for further investigations of substrate storage as a basis for the production of bioenergy.

DATA AVAILABILITY STATEMENT

All relevant data is contained within the manuscript. In addition, raw data from processed data will be made available by the authors, without undue reservation, to any qualified researcher on request.

AUTHOR CONTRIBUTIONS

JM and JH contributed to the conception and the design of the study; JH organized and administrated the project; JM wrote the first draft of the manuscript; JH supplemented and improved the manuscript; JM performed the statistical analysis in coordination with JH. Both authors contributed to manuscript revision, read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2020.00014/full#supplementary-material

FIGURE A1 | Shepard plot showing scatter around the regression between the interpoint distances in the final NMDS configuration against their original dissimilarities.

FIGURE A2 | Substrate-specific biogas **(a)** and methane **(b)** yield potentials of the tested feedstock substrates calculated according to Weißbach (2009). Calculations are based on ash content, crude fiber content, and enzyme solubility of harvested substrates before ensiling. Numbers in the bar indicate the standing age of the biomass stock. Substrates nomenclature: FM100 = pure biomass from flowering stripes, FM67 = mixture of 67% flower stripe's biomass and 33% silage maize, FM33 = 33% flower stripe's biomass and 67% silage maize, ZM100 = pure silage maize, YSC99 = 99% yellow sweet clover.

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Preservation of Microalgae, Lignocellulosic Biomass Blends by Ensiling to Enable Consistent Year-Round Feedstock Supply for Thermochemical Conversion to Biofuels

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Seasonal variation in microalgae productivity is a significant barrier to economical production of algae biofuels and chemicals. Summer production can be 3–5 times higher than in the winter resulting in uneven feedstock supplies at algae biorefineries. A portion of the summer production must be preserved for conversion in the winter in order to maintain a biorefinery running at capacity. Ensiling, a preservation process that utilizes lactic acid fermentation to limit microbial degradation, has been demonstrated to successfully stabilize algae biomass (20% solids) and algae-lignocellulosic blends (40% algae-60% lignocellulosic biomass, dry basis) for over 6 months, resulting in fuel production cost savings with fewer emissions. Preservation of algae as blends could be beneficial to biorefineries that utilize thermochemical approaches to fuel production as co-processing of algae and lignocellulosic biomass has been observed to enhance biocrude yield and improve oil quality. This study conducts a resource assessment of biomass residues in the southern United States to identify materials available during peak algae productivity and in sufficient quantity to meet the algae storage needs of an algae biofuel industry. Eight feedstocks met the quantity threshold but only three, distillers grains, haylage, and yard waste, were also available in season. Storage experiments utilizing both freshwater and marine strains of microalgae – *Scenedesmus acutus*, *Chlorella vulgaris*, *Chlorella zofingiensis*, *Nannochloropsis gaditana*, and *Porphyridium purpureum* – and yard waste were conducted for 30 days. Storage losses were less than 10% in all but one case, and the pH of all but one blend was reduced to less than 4.7, indicating that yard waste is a suitable feedstock for blending with algae prior to storage. To better understand whether the benefits to conversion realized by processing blends might be affected by storage, elemental analysis and bomb calorimetry of pre- and post-storage algae-yard waste blends were conducted to characterize changes occurring during storage. Storing algae biomass as blends with lignocellulosic biomass could be an effective method of mitigating seasonal variability in algae biomass production while retaining the synergistic effect of co-processing algae blends in thermochemical conversion.

Keywords: microalgae, biofuels, ensiling, hydrothermal liquefaction, resource assessment, preservation

INTRODUCTION

Microalgae are a promising feedstock for biofuel production due to their high energy content relative to other feedstocks, their rapid growth rate, and ability to be cultivated on marginal lands using non-potable water (e.g., brackish water and seawater) (Wijffels and Barbosa, 2010; Wijffels et al., 2010; Williams and Laurens, 2010). Yet, despite these benefits, challenges to commercialization of algae for fuel production remain (Greenwell et al., 2010; Bull and Collins, 2012; Day et al., 2012). Providing a consistent year-round supply to an algae biorefinery is a recognized barrier to economically produced algae biofuels (Davis R. et al., 2014). Like most crops, algae biomass production varies seasonally with maximum yields occurring during the summer months (June–August), where production can be 3–5 times greater than that achieved in the winter (Davis R. E. et al., 2014).

Variability in algae productivity complicates the sizing of downstream conversion facilities (Davis R. et al., 2014) since biorefineries sized to accommodate summer productivity would be underutilized in the winter. Design cases for the production of biofuels from algae biomass sponsored by the United States Department of Energy Bioenergy Technologies Office (DOE-BETO) mitigate for seasonal variability in algae production by designing conversion facilities to accommodate spring biomass production rates, requiring the preservation of excess algal biomass produced in the summer for conversion in the winter (Davis R. et al., 2014; Jones et al., 2014). In this manner conversion facilities can operate at capacity year-round. Long-term preservation of algae biomass, however, is challenging due to the high moisture content (80%, wet basis) of harvested algae biomass. Drying is a common approach to preserving high moisture plant material but the algae biomass (20% solids) rheology and high moisture content (80%, wet basis) make this both technically challenging and costly (Wahlen et al., 2017). Harvested microalgae biomass is also susceptible to microbial degradation and requires active storage solutions to limit biomass loss (Wendt et al., 2017a).

Ensiling is an alternative preservation strategy that does not require drying. Herbaceous biomass is regularly preserved through ensiling for the forage industry and can be used to stabilize high-moisture feedstock destined for bioenergy production (Wilkinson et al., 2003; Wendt et al., 2018). Oxygen-limited conditions in ensiling enable the fermentation of soluble sugars to organic acids, resulting in a lower pH that inhibits microbial activity (Rooke and Hatfield, 2003). Utilizing ensiling instead of drying as a preservation strategy for microalgal biomass can reduce the cost of fuel production by $0.32 per gallon of gasoline equivalent (GGE) (Wendt et al., 2019).

Thermochemical conversion of algae blended with lignocellulosic biomass to fuels by hydrothermal liquefaction (HTL) has many benefits that could serve to reduce the cost of producing fuel from microalgae biomass (Jarvis et al., 2018). Jarvis et al. (2018) noted that bio-oil resulting from HTL processing of algae-herbaceous blends had novel compounds that were not present in either feedstock processed alone. Blend bio-oil also contained less N and less O than the biocrudes derived from algae and lignocellulosic biomass, respectively. Co-processing algae and lignocellulosic biomass also had operational benefits. When processing lignocellulosic material, a buffer such as Na_2CO_3 is typically co-fed to neutralize acidic products and obtain a neutral bio-oil. When lignocellulosic biomass was processed along with algae, a neutral bio-oil was produced that did not require addition of the buffer. Surprisingly, the authors of this study also noted that processing algae-lignocellulosic blends had a synergistic effect on biocrude yield; more biocrude was produced from blends than from either feedstock by itself (Jarvis et al., 2018). Preservation of algae blended with lignocellulosic biomass will be essential to ensuring that the benefits of algae blends to HTL conversion are realized year-round.

Ensiling has been shown to be an effective approach to stabilizing microalgae blended with corn stover (Wendt et al., 2017a). When blends containing 40% microalgae biomass and 60% corn stover (dry basis) were inoculated with *Lactobacillus acidophilus* storage losses were limited to <8% dry matter after 35 days in storage (Wendt et al., 2017a). Furthermore, ensiling was estimated to be only 65% of the cost of drying while requiring 10% as much energy and reducing greenhouse gas emissions by as much as 75% (Wendt et al., 2017b). Ensiling microalgae-lignocellulosic biomass blends could be an effective approach to preserving material for the year-round operation of an HTL facility.

Although algae blended with corn stover has been shown to be stably preserved through ensiling for extended periods of time, corn stover is not an ideal herbaceous blendstock because the two crops do not overlap in their season of production in the majority of the United States. Corn stover is available in the fall, when algae biomass is expected to be utilized in conversion processes as it is produced. Therefore, an alternative crop residue, available during the summer months, is needed to enable storage of excess algae as blends with herbaceous biomass. In this study, a resource assessment of biomass residues available in the southern United States was conducted to identify underutilized biomass that is widely available during the precise time when it is needed and yard waste was identified as a likely candidate. Storage studies were then conducted with multiple strains of algae blended with yard waste to determine the suitability of this approach. Stored algae/yard waste blends were then further characterized to determine how compositional changes occurring in storage might affect HTL conversion of algae-yard waste blends.

MATERIALS AND METHODS

Materials

Algae cultivation of *Scenedesmus acutus*, *Nannochloropsis gaditana*, *Chlorella zofingiensis*, *Chlorella vulgaris*, and *Porphyridium purpureum* was performed at the Arizona Center for Algae Technology and Innovation in Mesa, AZ, in a containment greenhouse. *S. acutus* LRB0401 was inoculated at 0.05 g/L and grown in BG-11 medium. Algae was cultured in 110 L vertical flat panel photobioreactors with a 2-in. light path using natural lighting (natural diurnal light and dark periods). High temperatures averaged 20°C and low temperatures averaged 7°C during both batch runs. Each batch culture was grown over a 3-week period and harvested when culture density reached 3 g/L. The algae biomass was dewatered at 1800 × g through Lavin 20–1160 V Centrifuges (AML Industries, Inc., Warren, OH, United States) with a flow rate of approximately 2 L/min. Dewatered algae were placed into Ziploc® bags, stored in a cooler on ice, and shipped overnight to Idaho National Laboratory. The other strains were grown in a similar manner. Media for *N. gaditana* and *P. purpureum* was adjusted to 35 g/L salt using Oceanic Sea Salt. Yard waste (grass clippings and leaves) was collected fresh and frozen prior to size reduction with a Wiley mill (model 4, Thomas, Swedesboro, NJ, United States) to pass through 6 mm screen. Yard waste remained frozen during size reduction.

Resource Assessment

This resource assessment provides an estimate of the feedstock inventories for the southeastern and southwestern regions of the United States. The purpose of the assessment is to provide insight into the types of feedstocks that may be available in each region but does not make assertions about availability or prices needed to divert the feedstocks from current uses. The data for the crops and crop residues came from the 2012 Census of Agriculture (Vilsack, 2014). When feedstock information was not directly available from the source, a residue-to-product ratio was used to estimate the quantity of residues available based on the primary product yield (Koopmans and Koppejan, 1997). Distiller's grains inventories were estimated based on ethanol plant location and production; the locations and production of currently operation ethanol plants were taken from Ethanol Producer Magazine (Ethanol Producer Magazine, 2016) with a factor of 17 dry tons of distillers grain per gallon of production. The production of MSW yard waste is based on population. The average value of yard waste produced per person per year was defined from a sample of published location specific waste generation reports (cited in **Supplementary Material**). The average value was then multiplied by the county population to estimate the inventory of yard waste. The quantity of each feedstock was then georeferenced to a county in ArcGis 10.2.3 to produce spatial coverages for the feedstocks (**Supplementary Figures 1–18**).

Storage Experiments

Storage studies were conducted in 4 oz (118 mL) or 16 oz (473 mL) air-tight mason jars (Ball Mason Jars, Newell Brands, Atlanta Georgia). Gas collection was accommodated by fitting standard canning lids (Ball Mason Jars, Newell Brands, Atlanta Georgia) with bulkhead fittings (P/N SS-400-61, Swagelok, Solon, OH, United States). Rubber gasket material and stainless-steel washers were used to seal the bulkhead fitting to the lid. A quarter-turn plug valve (P/N B-4P4T, Swagelok, Solon, OH, United States) was connected to the bulkhead fitting with 1/4″ OD stainless steel tubing to facilitate reactor headspace gas exchange with nitrogen at the beginning of storage studies. Fermentation gas was collected in foil gas collection bags (FlexFoil, P/N 262-01, SKC, Inc., Eighty Four, PA, United States) connected to the plug valve with either silicon tubing (P/N EW-96410-16, Cole-Parmer, Vernon Hills, IL, United States) or C-flex ULTRA tubing (P/N EW-06434-16, Cole-Parmer, Vernon Hills, IL, United States). Microalgae biomass (20% solids) and yard waste was mixed together using a handheld kitchen blender for approximately 5 min. Algae-yard waste blended material was then packed into pre-weighed jars, pre-weighed lids were tightened and the assembled jar containing biomass was weighed again. The biomass loading varied with experiment depending on the amount of algae available from 16 to 36 g (dry basis) in 118 mL jars and 106 g (dry basis) in 473 mL jars. Jars were then made anaerobic by subjecting the headspace to vacuum and then nitrogen gas, repeating the process three times. Once anaerobic, jars were fitted with a gas collection bag and placed in the dark at room temperature for 30 days.

At the conclusion of the storage period jars with and without lids were weighed again. Moisture content of initial and stored material was determined gravimetrically after drying at 105°C until reaching a constant weight. Dry matter loss for each storage replicate is reported as a percentage of the initial material according to Eq. (1):

$$\% \, dry \, matter \, loss = \left[\frac{(Initial \, dry \, material \, (g) - Final \, dry \, material \, (g))}{Initial \, dry \, material \, (g)} \right] * 100 \quad (1)$$

Stored biomass was removed from each jar, sampled for moisture and organic acid content and frozen at −20°C until used for further analysis.

Analysis of Fermentation Products

Gases and organic acids produced during the ensiling process were collected and analyzed as previously described (Wendt et al., 2017a). Briefly, the total volume of gases collected in gas sampling bags over the course of the storage period was measured and the composition of the gas (CH_4, CO, H_2, N_2, O_2, and CO_2) was determined by gas chromatography as previously described (Wendt et al., 2017a). The quantity of nine organic acids (succinic acid, lactic acid, formic acid, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid) from each storage replicate were measured by HPLC as previously described (Wendt et al., 2017a). The HPLC detector was calibrated with standards at five concentration levels (P/N 95917, Absolute Standards, Inc., Hamden, CT, United States). Duplicate samples from each storage replicate were measured in duplicate by HPLC.

Elemental Analysis

Biomass from the larger-scale (∼500 mL) storage study of *S. acutus* microalgae biomass blended with yard waste was analyzed for C, H, N, O, and S content and for energy density (i.e., calorimetry). This was done for both initial materials (yard waste, *S. acutus* biomass, the unstored blend) and each 30-day storage replicate. The yard waste, initial blend and stored blends were prepared for analysis by first drying at 105°C followed by size reduction to a top size of 0.2 mm in a Retsch ultra centrifugal mill (Retsch, Haan, Germany). The *S. acutus* initial staring material was first freeze-dried and then ground to a fine powder by mortar and pestle. Elemental analysis (C, H, N, and S) was accomplished using a LECO TruSpec CHN with S add-on module (LECO, St. Joseph, MI, United States) following ASTM D5373-10 (CHN) and ASTM D 4239-10 (S) (ASTM, 2010a,b). Oxygen was determined by difference. Samples were analyzed in triplicate.

RESULTS AND DISCUSSION

Resource Assessment

The United States DOE-BETO has established a milestone within their Multi-Year Program Plan (MYPP) to model the sustainable supply of 20,000,000 tonnes (22,046,000 United States ton) of algal biomass annually by 2022 (DOE-BETO, 2016). Based on current design cases, a portion of algal biomass, which amounts to ∼ 6.5% of the total annual algal biomass production, will be produced in excess of conversion capacity during productive summer months and will need to be preserved for use later in the year (Davis R. et al., 2014). This equates to 1,300,000 tonnes (1,143,000 United States ton) of algal biomass. To achieve a blending ratio of 40% algae and 60% lignocellulosic biomass for preservation through ensiling, 1,950,000 tonnes (2,149,507 United States ton) of wet herbaceous biomass needs to be identified to preserve excess biomass from 20,000,000 tonnes algal biomass produced annually.

A resource assessment of crops and crop residues suitable for blending with algae and ensiling was conducted in the southern United States, an area expected to be productive for microalgae cultivation (Wigmosta et al., 2011). A total of eight feedstocks were identified that are currently being produced across the southern United States in sufficient quantity to be blended with 1,300,000 tonnes of algal biomass and preserved through ensiling (**Table 1**). Corn stover, energy/sugar cane and rice straw are the most abundant crop residues identified in this assessment, however, their availability does not overlap with the most productive months for algae (June–August). Yard waste, haylage and distillers grains are each available in sufficient quantity for blending and storing in the season required. Both haylage [\$110–\$220 ton^{-1} (USDA, 2018)] and distiller's grains [\$130-\$175 ton^{-1} (USDA, 2020)] have high feedstock costs because of their value as livestock feed. Yard waste, however, has limited utility and its disposal is often accompanied by a tipping fee, leading to low feedstock cost [\$64 ton^{-1} (Roni et al., 2019)]. Therefore, yard waste was selected as a representative feedstock for storage studies to understand how storage might impact the thermochemical conversion of the blend. **Figures 1**, **2** display county level annual availability of yard waste. Maps (**Supplementary Figures 1–17**) and feedstock-specific information are available in the **Supplementary Material** for each feedstock included in the resource assessment.

The quality of the feedstocks included in the resource assessment and their suitability for HTL conversion varied from one feedstock to another. Elemental analysis of feedstocks measuring the carbon, hydrogen, nitrogen, sulfur, and oxygen content can be a good indicator of the quality of a material for thermochemical conversion. Feedstocks with higher amounts of carbon and hydrogen will have greater energy content, while the presence of nitrogen and oxygen decrease the energy content. The elemental analysis of feedstocks included in the resource assessment are listed in **Table 2**, with the exception of haylage and peanut hay. Cotton stalks had the highest carbon content of any of the herbaceous feedstocks followed by distiller's grains (49.3% and 48.8%, respectively). The energy content of distiller's grains, however, was higher (21.2 MJ/kg vs. 18.4 MJ/kg) due to its lower oxygen content compared to cottons stalks (34% vs. 43%) and was the highest of any of the feedstocks.

Yard waste, another feedstock whose season of availability coincided with peak algae production, had an energy content (19.1 MJ/kg) higher than many of the other feedstocks in the resource assessment. In addition, the low cost of yard waste makes it an attractive feedstock to blend with algae prior to storage. The disposal of yard waste often carries a tipping fee to the landfill or disposal site, causing the cost of obtaining this biomass to be very low (Roni et al., 2019). However, one drawback of yard waste as a feedstock is the ash content, which represents a fraction of the biomass that cannot contribute to biofuel production and can affect the operation of a biorefinery (Lacey et al., 2018). Blending has been previously shown to be an effective approach to reducing the ash content of a feedstock, such as yard waste (Thompson et al., 2019). The anticipated amount of algae produced in excess of conversion capacity during the summer amounts to only 6.5% of the total annual algae production. Therefore, when stored algae-lignocellulosic blends are needed to fill gaps in algae production, they will be blended with freshly harvested algae and other seasonally available biomass residues, effectively diluting the amount of ash contributed by yard waste. To take advantage of its low cost, in-season availability, and higher energy content, yard waste was selected as the blending agent for storage experiments.

Storage Performance

Storage studies of algae-yard waste blends were conducted with biomass from multiple strains of algae for a period of 30 days in anaerobic conditions. Both freshwater (*S. acutus*, *C. zofingiensis*, and *C. vulgaris*) and saltwater (*N. gaditana* and *P. purpureum*)

TABLE 1 | Resource assessment of biomass residues available annually in the southern United States.

	Southwestern United States		Southeastern United States	
Resource	**Period of availability**	**Estimated annual inventory (ton)**	**Period of availability**	**Estimated annual inventory (ton)**
Corn Stover	August–November	11,187,082	July–October	27,958,773
Cotton stalks	October–December	4,064,226	September–November	4,174,541
Peanut hay	October–November	462,923	September–October	4,383,210
Rice straw	August–September	2,756,610	August–October	7,157,144
Sorghum	August–October	1,256,652	August–October	776,201
Haylage	April–September	2,866,954	April–September	934,803
Distillers grains	Continuous	2,496,000	Continuous	2,064,000
Sugar cane/energy cane*	November–March	1,401,926	October–March	27,380,199
Yard waste	April–September	2,588,903	April–September	2,439,955

**Annual inventory considers the bagasse of sugar cane/energy cane.*

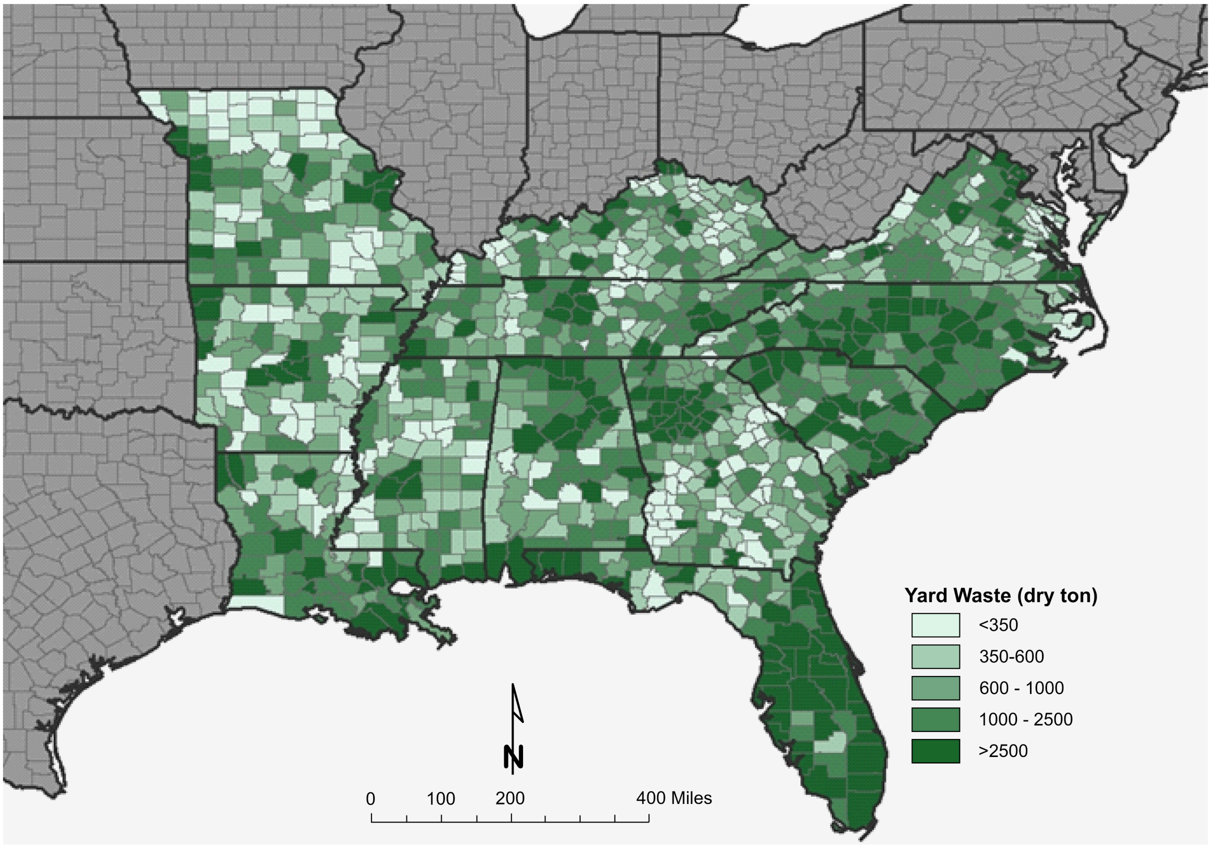

FIGURE 1 | County-level resolution of annual inventory of yard waste in the Southeastern United States.

strains were mixed with yard waste and evaluated for stability in storage. Dry matter loss, a measurement of how much material is consumed in storage by biological processes, ranged from a low of 4.0% (dry basis, db) in the case of *S. acutus* at the 500 mL scale to a high of 12.8% (db) occurring in stored *N. gaditana*-yard waste blends (**Table 3**). All but *N. gaditana* resulted in losses lower than 10% (db). The final pH of stored biomass ranged from 3.90 to 7.05. Generally, pH below 4.5 was indicative of low dry matter loss, the exception being *C. vulgaris*-yard waste blend, which achieved the lowest pH (3.90) but had the second highest dry matter loss (9.7). One explanation could be the large total organic acid production occurring in *C. vulgaris*-yard waste blends, which produced the most organic acids in storage (see Section "Organic Acid Production"). The formation of some organic acids is accompanied by CO_2 production and therefore loss of biomass. Lactic acid fermentation, where there are two pathways for production, is a good example. Homolactic fermentation produces only lactic acid with no loss of carbon,

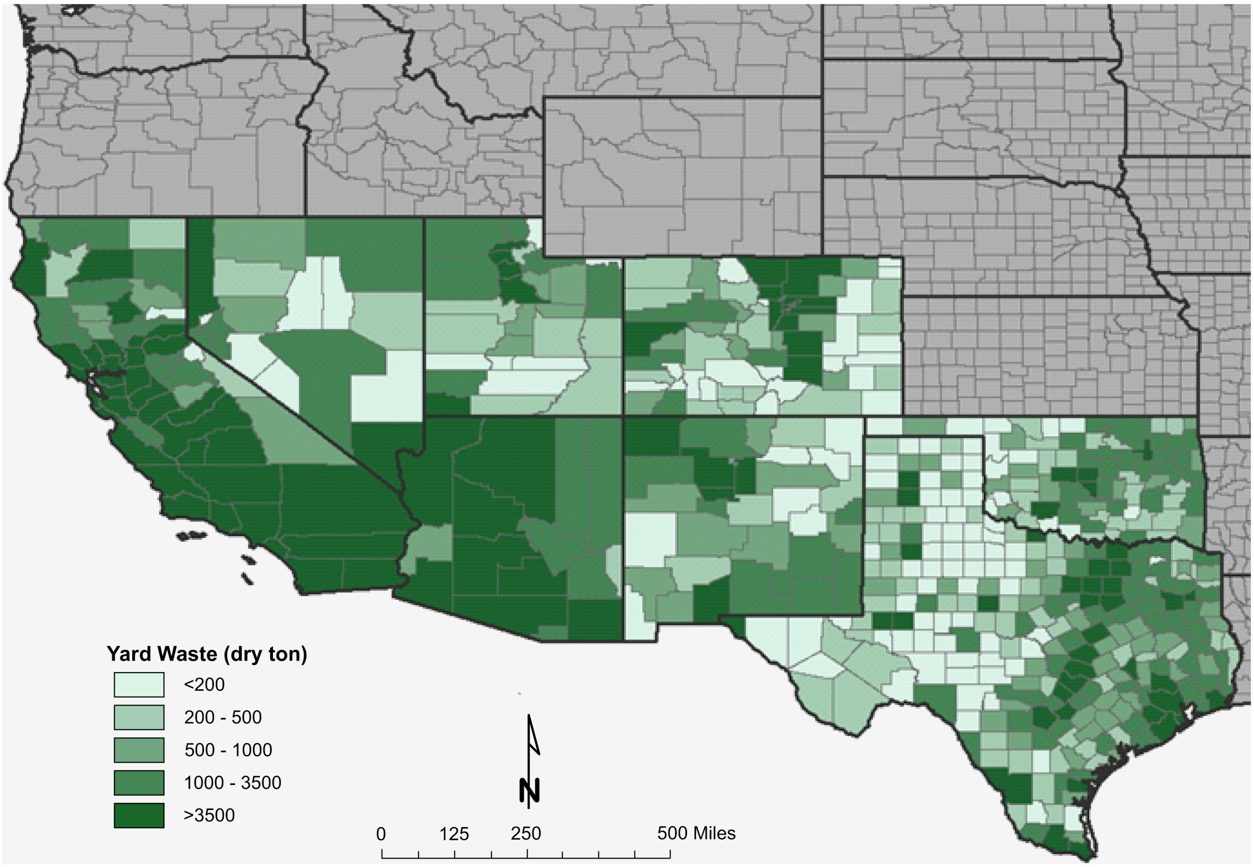

FIGURE 2 | County-level resolution of annual inventory of yard waste in the Southwestern United States.

TABLE 2 | Elemental composition of herbaceous feedstocks included in geographical resource assessment.

Material	Ash (%, db)	C (%, db)	H (%, db)	N (%, db)	O (%, db)	S (%, db)	HHV (MJ/kg)	References
Corn stover	4.7	47.9	5.9	1.7	38.6	0.18	19.8*	Wendt et al., 2017a
Cotton stalks	2.7	49.3	6.3	0.8	43.5	ND	18.4	Fu et al., 2012
Peanut Hay	–	–	–	–	–	–	–	Data not available
Rice straw	13.9	44.2	6.2	0.8	48.8	ND	17.4*	Worasuwannarak et al., 2007
Sorghum	4.6	41.3	5.4	1.3	52.0	ND	16.3	Yue et al., 2018
Haylage	–	–	–	–	–	–	–	Data not available
Distillers grains	ND	48.8	6.6	5.4	34.1	ND	21.2*	Wang and Brown, 2014
Sugar cane bagasse	1.6	45.5	5.6	0.8	48.1	ND	17.5*	Waheed and Williams, 2013
Yard waste	9.7	45.2	5.9	3.5	32.7	0.23	19.1	This study

*db, dry basis. *Higher heating value (HHV) was calculated by the method of Channiwala and Parikh (2002). ND, not determined.*

TABLE 3 | Storage performance of algae blended with yard waste (40% algae:60% yard waste).

Organism	Scale (mL)	Dry matter loss (%, db)	pH	Lactic acid (%, db)	Organic acid (%, db)	CO_2 (g/kg, db)
Scenedesmus acutus	100	4.8 ± 0.8	3.98 ± 0.01	8.9 ± 0.3	16.3 ± 0.5	4.6 ± 0.4
Scenedesmus acutus	500	4.0 ± 0.3	3.98 ± 0.3	9.9 ± 0.1	20.8 ± 0.4	5.4 ± 0.4
Nannochloropsis gaditana	100	12.8 ± 1.2	7.05 ± 0.13	5.6 ± 1.1	15.4 ± 0.8	41.0 ± 2.5
Chlorella zofingiensis	100	5.6 ± 0.7	4.09 ± 0.04	10.8 ± 0.6	19.9 ± 1.1	0.9 ± 0.8
Chlorella vulgaris	100	9.7 ± 0.6	3.90 ± 0.01	11.6 ± 0.4	21.2 ± 0.0	0
Porphyridium purpureum	100	6.5 ± 2.6	4.74 ± 0.04	8.8 ± 0.3	19.8 ± 1.7	13.4 ± 4.1

db, dry basis. values are the average of triplicate measurements and the variation is the standard deviation.

TABLE 4 | Elemental composition of yard waste, algae and algae-yard waste blends before anaerobic storage and algae-yard waste blends after 30 days anaerobic storage.

Material	Time stored (days)	Ash (%, db)	C (%, db)	H (%, db)	N (%, db)	O (%, db)	S (%, db)	HHV (MJ/kg)
Yard Waste	0	9.74 ± 0.03	45.25 ± 0.09	5.95 ± 0.10	3.52 ± 0.02	35.31 ± 0.21	0.23 ± 0.01	19.1 ± 0.1
S. acutus, 20% solids	0	4.46 ± 0.00	52.14 ± 0.02	7.27 ± 0.02	8.42 ± 0.05	27.43 ± 0.18	0.29 ± 0.04	23.9 ± 0.0
S. acutus-yard waste blend	0	6.96 ± 0.16	48.08 ± 0.23	6.55 ± 0.16	5.19 ± 0.08*	33.02 ± 0.45	0.19 ± 0.01	21.2 ± 0.1
S. acutus-yard waste blend	30	7.03 ± 0.01	48.14 ± 0.03	6.64 ± 0.12	5.30 ± 0.02*	32.70 ± 0.06	0.19 ± 0.02	21.2 ± 0.2

db, dry basis. S. acutus-yard waste blends contained 40% algae-60% yard waste on a dry basis. Values are the average of triplicate measurements and the variation is the standard deviation. Storage experiments were conducted in triplicate. Post-storage material was combined to form a composite sample which was then measured in triplicate. Differences between the material properties of each material was determined by One Way ANOVA with pairwise comparison by the Holm–Sidak method ($p < 0.05$). Each property of the initial starting material (yard waste, S. acutus and S. acutus-yard waste blends) except sulfur content was different from one another. The lone difference between the S. acutus blend caused by storage is marked with an asterisk.

whereas heterolactic fermentation produces acetic acid and CO_2 in addition to lactic acid (McGechen, 1990).

CO_2 measurement in algae-yard waste storage experiments has proved to be challenging. The volume of total gas evolution was measured for each storage replicate and carbon dioxide was quantified. The highest measured production of CO_2 occurred in the *N. gaditana* blends (41 ± 2.5) where the greatest dry matter loss was also observed. Measured carbon dioxide evolution alone does not account for total dry matter loss. For *N. gaditana* the dry matter loss experienced was 12.8% of the initial dry matter, while CO_2 evolution accounted for only 32% of that loss. The ratio of CO_2 to total loss was highest in *N. gaditana*. For the *C. vulgaris*-yard waste blend no gas evolution was measured at all, despite having the second greatest dry matter loss. It has been noted in other silage studies that CO_2 measurement is difficult in laboratory-scale silos and often results in large variation among replicates (El Hag et al., 1982; Weinberg et al., 1995). In the present study, we have observed algae-yard waste blends expand in storage due to gas production, causing some reactors to buckle and fail. The algae-yard waste blends tend to trap gases rather than allowing them to release. In some experiments a greater headspace was left to accommodate this expansion, and nitrogen gas-vacuum cycles were used to purge the jars of oxygen and establish an anaerobic environment. The addition of nitrogen gas to laboratory reactors further complicates the measurement of CO_2.

Organic Acid Production

The content and composition of organic acids present in stored algae-yard waste blends varied among the different strains of microalgae evaluated (**Table 3** and **Figure 3**). The *C. vulgaris*-yard waste blend generated the most organic acids in storage (21.2%, db) and *N. gaditana* the lowest (15.4%, db). Lactic acid was the primary component of total organic acids in each stored blend and was greater than 50% of total organic acids in all but three cases (*N. gaditana* 36%, *P. purpureum* 44%, and *S. acutus*, 500 mL, 49%). Butyric acid was present in each stored blend at less than 1% of total organic acids except for stored the *N. gaditana*-yard waste blend where it made up 8% of total organic acids. Succinic and acetic acids also comprised substantial proportions of total organic acids (7–19% and 3–15%, respectively).

The presence of organic acids in the post-storage biomass could have positive benefits in HTL conversion. Ross et al. (2010) explored the use of alkali and organic acids as catalysts in the HTL processing of microalgae. They found that biocrude yield was increased with organic acid catalysts relative to the alkali. Organic acid catalysts also affected the quality of the biocrude by increasing the size distribution of biocrude molecules with greater lower molecular weight compounds than observed with the alkali catalysts. This resulted in a biocrude with a lower boiling point and improved flow properties (Ross et al., 2010). Although Ross et al. (2010) performed their study with formic and acetic acids, the presence of longer chain organic acids (e.g., lactic acid, propionic acid) in the stored algae-yard waste blends could similarly benefit biocrude yield and quality.

Elemental Composition

Often proximate and ultimate analysis is used to determine the suitability of materials for thermochemical conversion to oils in processes such as HTL. The blend of *S. acutus* and yard waste was selected as a representative algae-yard waste blend to determine whether these types of blends would be suitable for HTL conversion and what impacts anaerobic storage might have on the suitability of this material. To accommodate the analysis, this particular blend was prepared in sufficient quantity and stored at a greater scale compared to the other algae-yard waste blends evaluated in storage only. **Table 4** lists the ash content, elemental composition and energy density for pre-storage yard waste, *S. acutus* biomass and the two blended together at a 60:40 ratio (yard waste: *S. acutus*) and for the post-storage blend.

Initial starting materials differed from one another in nearly every aspect. The ash content of the yard waste was 9.74% (db) compared to 4.46% (db) for algae biomass. Ash content of algae is likely to be highly variable between different species and different cultivation methods for a given species. Marine strains have higher ash content than freshwater strains due to the greater salt content of seawater. Strains cultivated in open raceways are likely to have higher ash content than strains cultivated in photobioreactors due to concentration of salts caused by evaporation and from soil particles entering from the external environment.

Algae typically contain higher proportions of protein and lipid than herbaceous biomass and less carbohydrates. This is reflected in the elemental composition of yard waste and *S. acutus* biomass. *S. acutus* biomass is more carbon (52%) and hydrogen (7%) rich

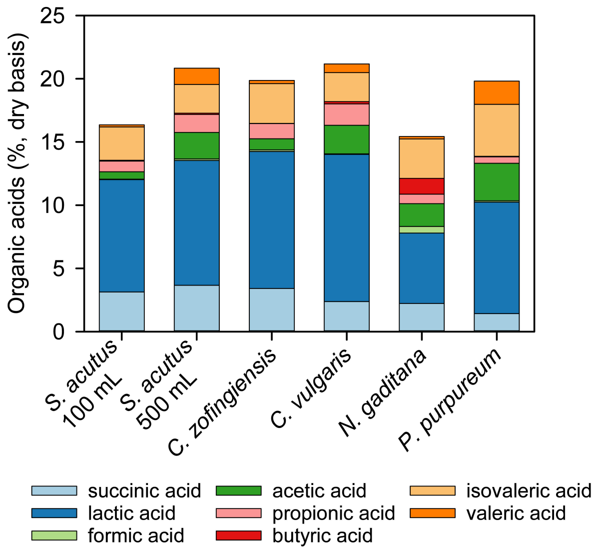

FIGURE 3 | Composition of organic acids produced during wet anaerobic storage of algae-yard waste blends. Yard waste was blended with microalgae biomass from multiple species and stored anaerobically for 30 days in 100 mL volumes unless otherwise indicated. All blends contained 60% yard waste and 40% algae biomass on a dry material basis.

compared with yard waste (45% and 6%, respectively) due to the lipid content of microalgae, and yard waste has a greater oxygen content due to its structural sugar content (cellulose and hemicellulose). Algae nitrogen content is substantially higher than that found in the yard waste (8.4% vs. 3.5%), likely due to differing concentrations of protein in the two materials. The blend of yard waste and algae (60:40) resulted in a material with properties consistent with the composition of the initial materials and their proportion in the final blend.

The energy content of the two initial unblended materials is consistent with their respective elemental compositions. The relationship between elemental composition and energy content is described by several equations (Channiwala and Parikh, 2002). Generally, carbon and hydrogen content correlate positively with energy content, while oxygen correlates negatively. The energy content reported in **Table 4** is measured as described in the materials and methods and not calculated. Yard waste has a lower energy content than algae biomass (19.1 MJ/kg vs. 23.9 MJ/kg). Blending of the two materials results in a blend with energy content that is intermediate to the two initial feedstocks.

Post-storage algae-yard waste blends did not differ in elemental composition. A one-way ANOVA analysis did not find any differences in ash, C, H, O, or S content ($p < 0.05$). There was a small statistically significant difference in nitrogen content with the stored blend having a slightly higher nitrogen content. Though significant, this difference is likely inconsequential. As one would expect based on the correlation between elemental composition and energy content, calorimetry did not find any significant difference in the energy content of stored and unstored algae-yard waste blends.

CONCLUSION

This study has identified eight crops or crop residues in the southern United States that could support the preservation needs of 20,000,000 metric tonnes of algal biomass annually. Although only distiller's grains, haylage and yard waste were available when algae biomass production is maximal. Storage studies conducted with yard waste and several freshwater and marine strains of algae were successfully preserved over 30 days with all but one experiencing less than 10% dry matter loss and a final pH of less than 4.7. Elemental analysis of stored *S. acutus* blends demonstrated that the elemental composition and the higher heating values do not change significantly due to storage. This raises the possibility that fuel yield in HTL may also be unaffected by storage. The production of organic acids in storage significantly increases their presence in the biomass and their ultimate effect on HTL processing, biocrude yield and processing, is uncertain. Direct HTL processing of stored algae-lignocellulosic blends is needed to accurately assess the impact of storage on the yield of biocrude and the quality of final fuel.

DATA AVAILABILITY STATEMENT

The resource assessment for each feedstock and its source information is included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

BW and LW designed the study. BW, LW, and AM conducted the storage experiments. VT and DH conducted the resource assessment. TD and HG provided algae biomass. BW drafted the manuscript with contributions from each co-author. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2020.00316/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
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Review on Bioenergy Storage Systems for Preserving and Improving Feedstock Value

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Long-term storage is a necessary unit operation in the biomass feedstock logistics supply chain, enabling biorefineries to run year-round despite daily, monthly, and seasonal variations in feedstock availability. At a minimum, effective storage approaches must preserve biomass. Uncontrolled loss of biomass due to microbial degradation is common when storage conditions are not optimized. This can lead to physical and mechanical challenges with biomass handling, size reduction, preprocessing, and ultimately conversion. This review summarizes the unit operations of dry and wet storage and how they may contribute to preserving or even improving feedstock value for biorefineries.

Keywords: biomass, biofuels, feedstock logistics, long-term storage, recalcitrance

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INTRODUCTION

The utilization of renewable biomass feedstocks for fuel and energy production offers the potential to displace a significant portion of petroleum-based transportation fuels and related greenhouse gas emissions. The transportation sector utilizes one third of all energy and 70% of all petroleum consumed in the United States (Davis and Boundy, 2019). Electrification of the grid with renewable energy sources, such as wind and solar power, will contribute to reducing carbon-based fuels in the light-duty vehicle fleet. However, the need for sustainably-produced, liquid transportation fuels will remain since aviation fuel use is projected to double in the next 20 years (International Air Transport Association, 2018) and heavy-duty vehicles and marine vessels will likely require carbon-based fuels (U.S. Department of Energy and Bioenergy Technologies Office, 2016). Furthermore, bio-derived fuel and chemical production can result in the carbon negative technologies that are necessary to counteract the global warming of 1.5°C above pre-industrial levels (First, 2019).

Renewable biomass feedstocks include non-food material such as corn stover, herbaceous and woody energy crops, forest product residues, algae, and municipal solid waste. Estimates suggest that over 1 billion tons of these feedstocks are available annually for sustainable utilization in bioenergy production systems (Langholtz et al., 2016). This bioeconomy has the potential to create over 1 million jobs and $260 billion in U.S. revenue, displace 30% of liquid transportation fuels, and reduce 50% of greenhouse gases compared to petroleum (U.S. Department of Energy and Bioenergy Technologies Office, 2016).

Major unit operations in the conversion of biomass feedstocks to fuels include supply and logistics operations including harvest, collection, transport, storage, and formatting followed by biochemical conversion of carbohydrates to fuels and chemicals (**Figure 1**). Feedstock supply and logistics unit operations generally begin with the harvest of a crop or a portion of the crop that is cultivated either on an annual basis (e.g., corn, wheat, sorghum, etc.), on a perennial basis (e.g., switchgrass, miscanthus, etc.), or a multi-year basis (e.g., willow, pine, etc.). In the case of agricultural residues including corn stover, commonly accepted practices are based on dry, baled logistics systems. Harvesting of the grain fraction of the plant is performed simultaneously or just preceding harvesting of the biomass residue (Birrell et al., 2014). Formation of windrows occurs either during harvest or by a windrower followed by drying in-field to facilitate stable storage conditions and collection of the biomass from windrows into bales (Hess et al., 2007; Shah and Darr, 2016). Bales are stored either field-side or at a centralized location until further use (Darr and Shah, 2012). Size reduction to meet biorefinery particle size specifications is performed either at the biorefinery gate (Hess et al., 2009a) or at a biomass feedstock depot (Hess et al., 2009b). Depot concepts have been proposed to facilitate densification of biomass into low-moisture pellets for stable storage and low transportation costs. The cost and performance of these logistics systems and associated unit operations have been well-documented (Hess et al., 2007, 2009a,b), and estimates in 2018 suggest that delivered cost of corn stover to a refinery is estimated at $84/US ton depending on the harvest method and the draw ratio of the biorefinery (Roni et al., 2018). These costs are low compared to the forage industry but are necessary to be competitive with fossil-based fuels of approximately $3/gallon.

Multiple approaches to convert biomass resources to energy sources exist and are generally characterized as either biochemical or thermochemical. Each conversion technology has advantages and disadvantages in terms of their flexibility to feedstock source and related chemical composition as well as regarding the product generated from that feedstock. These diverse conversion approaches facilitate utilization of geographically localized biomass feedstocks. For example, agricultural residues are concentrated in the middle and eastern portion of the U.S., while woody biomass and forest thinnings are concentrated in the southeast and western portions of the U.S. (Langholtz et al., 2016). All these conversion approaches have a role in the formation of a stable bioeconomy and reducing the dependence on fossil-fuel based resources (U.S. Department of Energy and Bioenergy Technologies Office, 2016).

Biochemical conversion of lignocellulosic biomass including corn stover has been facing technical challenges during scale up despite significant investment by three commercial-scale integrated cellulosic-based biorefineries in the U.S. last decade. All these biorefineries have struggled to make biofuels a reality. Dale summarized two primary challenges that were faced including the lack of understanding of how to stably store biomass for long durations and the difficulty to chemical deconstruction in biomass during pretreatment operations (Dale, 2017). The first challenge is a result of the susceptibility of biomass to microbial or physical loss when not stored in a stable manner, and the later issue stems from the variations and complexities in corn stover and associated challenges of converting this feedstock into fuels (Richard, 2010; Dale, 2017). Understanding lignocellulosic biomass and overcoming the associated recalcitrance is key to addressing the challenges for biochemical conversion. Therefore, the focus of this review article is aligned closely with biochemical conversion approaches for corn stover but may have applicability toward thermochemical conversion and other lignocellulosic biomass as well. This review will highlight the impact of long-term storage on conversion operations with the focus of how storage systems may be used to overcome both the challenge of stable storage for bioenergy systems and be complementary to pretreatment systems.

LIGNOCELLULOSIC BIOMASS STRUCTURE AND ASSOCIATED RECALCITRANCE

A fundamental understanding of the structure of lignocellulosic biomass is necessary for the prediction of how biomass may be affected during each unit operation between harvest and conversion. Lignocellulosic biomass, such as corn stover, consists of an intricate combination of cellulose, hemicellulose, and lignin, that provide strength to the plant cell walls (Cosgrove, 2005; Cosgrove and Jarvis, 2012). Plant walls (**Figure 2**) consist of a primary wall, which is composed of cellulose, xyloglucans, and pectin as well as 10–20% protein (Himmel et al., 2007). Secondary walls contain cellulose, xylans, glucomannans and lignin and are separated into S1, S2, and S3 layers (Mellerowicz and Sundberg, 2008). A thin layer, termed the middle lamella, connects plant cells to each other and is rich is pectin (Iwai et al., 2002). These cell wall components are multi-functional, supporting nutrient transport during growth while providing strength to the plant such that it can withstand environmental factors including wind, moisture, and physical impact. However, the complex nature of biomass tissues and their chemical makeup presents a challenge for a biorefinery. The term recalcitrance describes the resistance of lignocellulosic biomass to biological, chemical, and thermal methods of deconstruction. Each plant tissue and cell wall layer are built of unique chemical signatures increasing this recalcitrance to deconstruction, and an understanding of the chemical makeup and bonds holding them together is essential in order to effectively deconstruct and depolymerize lignocellulosic biomass.

Cellulose microfibrils are the main component of the primary and secondary cell wall in plants. Microfibrils are composed of multiple glucose chains arranged in parallel in a crystalline fashion, with individual glucose chains linked internally and to each other through hydrogen bonds (Himmel et al., 2007). Individual glucan chains and are comprised of 500–14,000 repeating D-glucose units; two D-glucose molecules are linked in the β-1,4 position and rotated 180 degrees from each other, forming a cellobiose unit as shown in **Figure 3** (Mohnen et al., 2009). Himmel has proposed that cellulose microfibrils are arranged into 36 glucan chains arranged in a radial fashion (Himmel et al., 2007), whereas Fernandes has proposed 18–24 glucan chains in sheets are present in each microfibril (Fernandes et al., 2011). Primary cell walls contain only three to four layers of the microfibrils, while the secondary cell walls are thought to contain hundreds of microfibrils (McCann et al., 1990). One distinct attribute of secondary cells walls is the varied orientation of cellulose microfibrils in the S1, S2, and

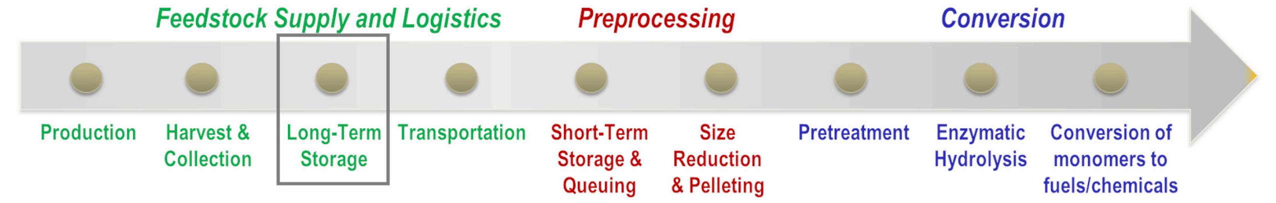

FIGURE 1 | Unit operations in the conversion of lignocellulosic biomass to fuels and chemicals through a biochemical conversion approach. This review will describe the impact of long-term storage (gray box) on conversion operations.

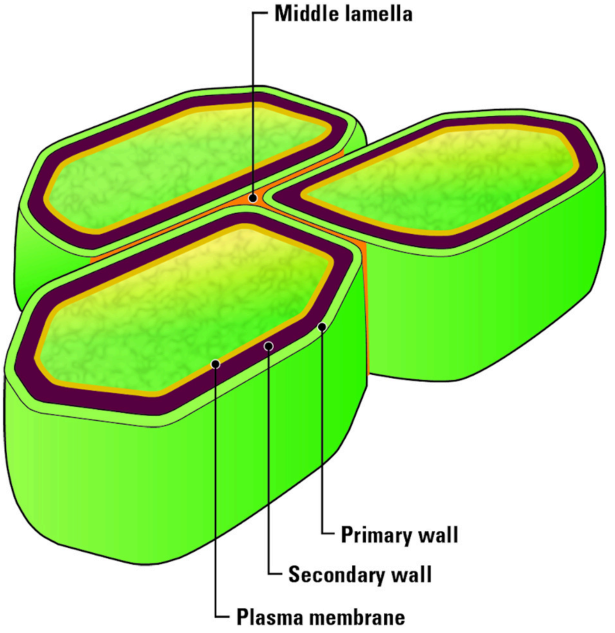

FIGURE 2 | Biomass plant cell wall structure. Photo credit: U.S. Department of Energy Genomic Science Program. https://genomicscience.energy.gov.

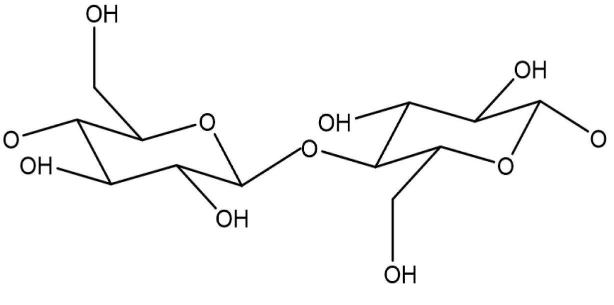

FIGURE 3 | Cellulose backbone consisting of D-glucose molecules linked in the β-1,4 position and rotated 180 degrees from each other.

S3 layers, which contributes to the strength of the plant tissues (Zhong and Ye, 2014).

Hemicellulose is comprised of a complex matrix of polysaccharides generally consisting of long chains with a β-1,4-backbone and multiple side chains. Hemicellulose surrounds cellulose microfibrils and associates with them through hydrogen bonds (Busse-Wicher et al., 2014), helping to strengthen the plant's primary and secondary cell walls (Marriott et al., 2016). The composition and complexity of hemicellulose has been extensively reviewed (Mohnen et al., 2009; Marriott et al., 2016). Xyloglucan has a -1,4-glucan backbone with xylose side chains. Xylans have a -1,4-xylose backbone and can contain other polysaccharide side chains including arabinan and glucuronic acid. Mixed-linkage glucans are linked at both -1,3 and -1,4 positions. Gluco- and galactomannans consist of a -1,4 mannan backbone that can be substituted with glucan and galactan, respectfully. Acetyls and phenolic acids, such as ferulic acid, are common side chains linked to the hemicellulose (Harris and Picataggio, 2008), and these have been shown to reduce the accessibility of cellulose to enzymatic attack (Selig et al., 2015). Therefore, the association of hemicellulose and cellulose is a key factor in reducing biomass recalcitrance.

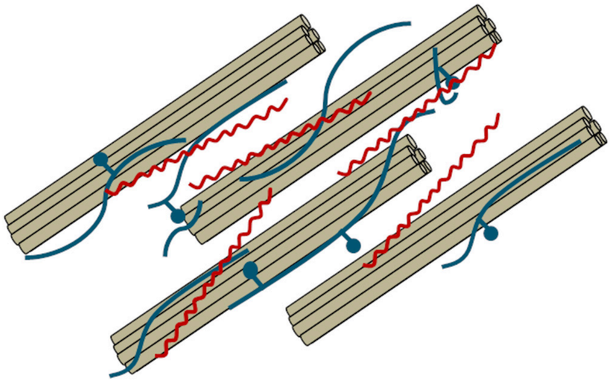

FIGURE 4 | Graphical description of cellulose microbifibrils (brown) surrounded by hemicellulose (blue) and pectin (red). Adapted from Cosgrove, 2005.

Pectin is a 1,4-linked galacturonic acid-based polysaccharide that is principally located in the middle lamella and primary cell wall of lignocellulosic biomass (O'Neill et al., 1990; Mohnen et al., 2009). Pectin is generally not located in the secondary cell wall but can be present in the outer secondary cell wall layers. Pectin is proposed to form covalent bonds with hemicellulose and increases the strength of the cell wall (Popper and Fry, 2008). A graphical depiction of the interactions between pectin (red) with hemicellulose (blue) and cellulose (brown) is shown

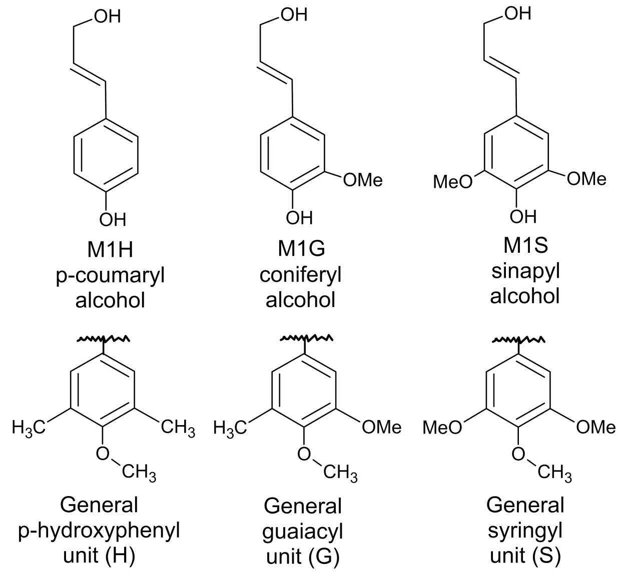

FIGURE 5 | Lignin alcohol precursors and resulting monolignins.

in **Figure 4**. Pectin content is generally highest in dicots but is also present in monocots (Jarvis et al., 1988). Pectin can act as a barrier against enzymatic attack and therefore is an important component when considering the conversion of lignocellulosic biomass to biofuels.

Lignin is a complex molecule that is made up of hundreds of monomers. Lignin concentrations are highest in middle lamella and primary cell walls (Donaldson et al., 2001), yet these components are of low concentration in the cell wall compared to secondary cell walls. Lignin is also present in the cellulose microfibril-rich secondary cells walls (Freudenberg and Neish, 1968) of which the S2 layer is the largest fraction (Mellerowicz and Sundberg, 2008). Lignin fills the space between cellulose, hemicellulose, and pectin and thus serves to strengthen the cell wall. Lignin is hydrophobic and can protect the cells from enzymatic attack and resulting degradation. Monolignins are the building blocks of lignin; they are synthesized from phenylalanine in the cytosol through a complex set of enzymatic reactions and are characterized by their number of methoxy side chains (Boerjan et al., 2003). *p*-coumaryl, coniferyl, and sinapyl alcohols have zero, one, and two methoxy side chains, respectfully (Freudenberg and Neish, 1968). These monolignins are transported into the cell wall, where they are then polymerized oxidatively to another monolignin or a growing lignin chain, likely as a result of a peroxidase or laccase that results in the formation of a free radical (Boerjan et al., 2003; Ralph et al., 2004). Therefore, *p*-coumaryl, coniferyl, and sinapyl alcohols result in the formation of p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units within a lignin molecule (**Figure 5**). Linkages between cellulose, hemicellulose, pectin, and lignin can be ester or ether and can either directly link these molecules or use acid bridges such as ferulic acid or hydroxycinnamin acid (Harris and Picataggio, 2008; Marriott et al., 2016).

The complex nature of the composition and associated bonds between cellulose microfibrils, hemicellulose, pectin, and lignin and resulting heterogeneity of plant tissues present a challenge for conversion of their respective monomers to fuels and chemicals (Himmel et al., 2007; Marriott et al., 2016). Additionally, factors such as the presence of waxes, the abundance of sclerenchyma and associated tissue strength, and inhibitors to fermentation (i.e., acetic acid, ferulic acid) contribute to biomass recalcitrance (Himmel et al., 2007).

LIGNOCELLULOSIC BIOMASS CONVERSION APPROACHES

Effective biochemical-based strategies for converting the biomass into fuels and chemicals generally involve the utilization of chemicals, heat, and enzymes to break down the lignocellulosic biomass into monomers followed by conversion to fuels through approaches including fermentation (Foust et al., 2009). Recalcitrance is a significant challenge for biochemical-based conversion approaches as the cellulose microfibril is not accessible to enzymatic attack until hemicellulose and lignin have been decoupled from the matrix (Himmel and Picataggio, 2009). Enzymatic action on cellulose microfibrils is further complicated by the strong hydrogen bonding within cellulose sheets in the microfibrils (Nishiyama et al., 2002) as well as the hydrophobic layer on the outside of the sheets that reduce the effectiveness of acid attack (Matthews et al., 2006).

Biochemical approaches to the conversion of lignocellulose begin to overcome this recalcitrance in a pretreatment step that utilizes the combination of temperature, caustic, and time to increase the digestibility of lignocellulosic biomass. The particle size necessary for biochemical conversion depends on pretreatment chemistry (Vidal et al., 2011), but a nominal 6 mm size is often recommended to minimize the cost of size reduction while increasing the surface area for pretreatment (Foust et al., 2009; Humbird et al., 2011). Dilute acid pretreatment generally occurs between temperatures of 140 and 200°C, and hemicellulose hydrolysis is the primary mode in which this pretreatment chemistry makes cellulose more accessible to enzymatic attack (Torget et al., 1991). Alkali treatments include applying sodium hydroxide (Grohmann et al., 1989) as well as lime (Kim and Holtzapple, 2005) to remove acetyl groups from xylan and remove lignin through oxidation (Katahira et al., 2016). Steam explosion can be used to increase the surface area though defibrillation and is catalyzed by the removal of acetyl groups from hemicellulose (Saddler et al., 1993). Ammonia-based pretreatment such as ammonia fiber explosion (AFEX) impregnates plant cells during a pressure change, which results in both deacetylation as well as reduced crystallinity of cellulose (Gollapalli et al., 2002). Ionic liquids solubilize cellulose, hemicellulose, and lignin, which are then selectively precipitated to isolate these components (Heinze et al., 2005; Shill et al., 2011). The commonality of these pretreatment methods is that they target specific biomass components with the goal to make others more accessible to subsequent enzymatic attack.

Enzymatic hydrolysis succeeding pretreatment is performed using glycosidases including cellulases or mixtures of enzymes that attack components in hemicellulose (e.g., xylanases, mannanases, arabionfuranosidases, and pectin lyases) (Bayer et al., 2004). Upon release of carbohydrate monomers, fermentation can proceed by yeast or bacteria. Ethanol fermentation was one of the first commercialized approaches for fuel generation from lignocellulosic biomass (Humbird et al., 2011) and is based on the technology of the grain ethanol industry. Additional fermentation approaches that have gained recent attention include production of carboxylic acids including butyric acid (Nelson et al., 2017; Saboe et al., 2018) or propionic acid (Wang et al., 2017) that can be upgraded catalytically to hydrocarbon fuels (Cortright et al., 2002). Succinic acid is also a produced through fermentation (Song and Lee, 2006; Salvachúa et al., 2016) and is a valuable chemical building block (Song and Lee, 2006; Nikolau et al., 2008). The commonality between all these approaches is the production and subsequent utilization of carbohydrate monomers to higher-value fuels and chemicals.

Recent attention has also been focused on lignin utilization to increase the economics of biorefineries. Combustion for process heat was the original use of lignin in cellulosic biorefinery models (Humbird et al., 2011). However, the pressure for lignocellulose-derived fuels to be cost competitive with fossil-based transportation fuels require either lower conversion costs or higher value end uses of the conversion products. Lignin can be depolymerized by chemicals and enzymes and utilized for high value products (Ragauskas et al., 2014). Multiple fermentation pathways exist for lignin monomers including adipic acid (Vardon et al., 2015) and muconic acid (Salvachúa et al., 2018). Improvements in biomass recalcitrance reduction are also necessary to further advance this field given the complexity of lignin molecules.

Thermochemical approaches for biomass conversion utilize heat and/or catalysts to create either heat through combustion, into liquids such as bio-oils through pyrolysis of liquefaction, or into combustible gases through gasification (McKendry, 2002). Thermochemical conversion approaches have been extensively reviewed elsewhere. Thermochemical approaches require biomass to be at a small particle size to increase surface area, typically less than 2 mm. Thermochemical conversion is often favorable for soft and hardwood biomass feedstock due to their elevated lignin level compared to herbaceous biomass feedstocks since lignin has a higher calorific value compared to carbohydrates. Thermochemical conversion approaches also can be used to generate combustible gases from low value feedstocks such as municipal solid wastes. Biomass recalcitrance in relation to thermochemical conversion but is gaining attention in order to understand mechanisms that improve fuel yield (McCann and Carpita, 2015). For example, Kim et al. reported on the application of partial-oxidative pyrolysis to depolymerize lignin and thus allow for increased conversion of cellulose to levoglucosan in bio-oil (Kim et al., 2014). Similarly, a low temperature pyrolysis method combined with two-dimensional gas chromatography coupled with mass spectrometry has been shown to identify storage related changes in cellulose, hemicellulose, and lignin-based pyrolysis products (Groenewold et al., 2019). Advancements in the understanding of biomass recalcitrance and related yield in thermochemical conversion systems is necessary to further predict approaches to increase fuel yield.

FIGURE 6 | Corn stover bale stack at a satellite storage location.

BIOMASS STORAGE SYSTEMS

Seasonal variation is a challenge for most agricultural products, necessitating storage in order to provide a biorefinery with year-round access to the product. Agricultural residues, such as corn stover, are typically available during a 1–2-months window and are dependent on the harvest of the primary product. Energy crops are also harvested seasonally but have a more flexible harvest window since it is the primary product as opposed to residues that are reliant on a commodity crop. Engineered storage systems offer the opportunity to minimize the seasonal variation of biomass availability and allow a biorefinery to operate year-round with a consistent feedstock supply. Long term storage also allows for a biorefinery to be sized at the appropriate scale such that down-time is minimized, and this reduces costly capital expenditures.

Dry Storage Systems

The primary goal in storage is to preserve the reducing equivalents in biomass, and dry storage systems are one solution for stably storing biomass over long periods of time. Bale stacks are the state of technology for field-side storage of agricultural residues (Shah et al., 2011), and a corn stover bale stack is shown in **Figure 6**. Bales are generally covered with tarps to reduce moisture accumulation from precipitation, while improved surfaces are recommended to prevent wicking of soil moisture by the bottom bales. Smith et al. described the moisture distribution of tarped and untarped corn stover bales entering storage at the same moisture content (22% wet basis); after 5 and 9 months moisture had redistributed to levels up to 65% just under the surface of the tarp as well as in the bottom bales where moisture adequate drainage was not present (Smith et al., 2013). Overall, bale-based storage can effectively preserve biomass when under ideal conditions but must be managed carefully to maintain stable conditions.

Biomass stored in dry systems is particularly susceptible to microbial degradation if conditions conducive to enzymatic activity or microorganism growth are present. Water activity (a_w), which describes the ability of water to react chemically and biologically, drives the storage stability of a range of industrially-relevant nutritional products for human and livestock consumption (Beuchat, 1981). Water activity ranges between 0 and 1 and corresponds with no water being available for utilization and all water being available, respectfully. Water activity can be calculated by determining the relative humidity of air in a sample in equilibrium, and moisture sorption isotherms are used to determine the relationship of water activity and moisture content for a given material (Beuchat, 1981). Water activity is also impacted by temperature, which is one reason refrigeration is an effective preservation method. The relationship between water activity and microbial stability is well-documented, with bacteria growth prevalent when $a_w > 0.85$, yeasts prevalent between a_w values of 0.80–0.90, and mold growth dominant when a_w value is 0.85–0.60. Only enzymes are considered active at $a_w < 0.60$. Athmanathan et al. (2015) related water activity to dry matter loss in switchgrass and demonstrated no appreciable loss at $a_w > 0.85$, which corresponded to a moisture content of approximately 16% (wet basis). The relationship between biomass source, chemical composition, free versus bound water, and environmental conditions such as temperature can be used relate moisture content and water activity, and an enhanced understanding of these parameters can be used to positively impact biomass storage stability.

A recent study suggests that an average of only 36% of corn stover harvested in the U.S. is capable of entering long-term bale storage at moisture levels that result in stable storage (Oyedeji et al., 2017), which makes corn stover a particularly challenging feedstock to store using dry approaches. Similarly, a moisture content of 20% or less has been recommended for stable corn stover in baled storage (Darr and Shah, 2012). Significant losses of dry matter have been reported in field-side storage of corn stover that exceeds this moisture threshold due to microbial degradation (Shinners et al., 2007; Smith et al., 2013). Microbial degradation of aerobically stored biomass materials can be characterized in terms of CO_2 production, microbial heat generation and resulting temperature increase, and dry matter loss (McGechan, 1990; Wendt et al., 2014). Aerobic microbial degradation by bacteria, yeast, and fungi consumes valuable carbohydrates and produces CO_2 as a byproduct, leaving behind material enriched in non-fermentable biomass components such as ash. This degradation has been documented to begin with hydrolysis of acetyl groups and reduction in hemicellulose, which has been measured by wet chemical analysis, such that the microorganisms can access cellulose (Wendt et al., 2014, 2018a). Hemicellulose modification has also been documented in corn stover that suffered severe aerobic degradation during storage using a pyrolysis/two-dimensional gas chromatography/mass spectrometry (Py-GCxGC-MS) approach (Groenewold et al., 2019). In this study, formation of acetic acid and furfural, which correlate to acetyl and C5 sugar degradation, was increased in corn stover samples that suffered severe degradation compared to samples that suffered only mild degradation. Understanding how microbial degradation might be used as a partial pretreatment is a topic that has not been widely reported, and this moisture management approach may have applicability in bioenergy systems that rely on dry storage approaches.

Bale storage systems can be cost prohibitive in many industrial settings because the shear amount of combustible material present must be managed safely. Corn stover bales are at risk of loss due to fires (Webb et al., 2018), necessitating significant land use to create a physical barrier to protect a burning stack from igniting other stacks. Additional insight into how dry storage systems can be managed and/or configured to reduce this risk in a cost-effective manner will support bioconversion designs by protecting the valuable asset of biomass in the logistics supply chain.

Wet Storage Systems

An alternative approach to feedstock supply logistics systems that rely on baling biomass is to adopt the commonplace practices of the forage industry. Wet, anaerobic storage systems (i.e., ensiling) are an alternative to dry storage and have consistently and successfully demonstrated biomass preservation in long term storage for livestock feed and forage. Wet biomass logistics systems have been proposed for corn stover, primarily to address the concern of catastrophic loss of corn stover stacks to fires (Wendt et al., 2018a,b). Wet logistics systems are based on forage chopping herbaceous biomass in the field at moisture contents between 40 and 65% (wet basis), transporting the chopped biomass in silage trucks, and utilizing anaerobic storage systems including silage bags, bunkers, or drive-over piles to limit oxygen and preserve biomass (Ferraretto et al., 2018). **Figures 7–9** show the harvest, transport and unloading, and resulting anaerobic

FIGURE 7 | Collection of corn stover with forage chopper into a walking floor trailer.

FIGURE 8 | Simultaneous formation and compaction of a drive over storage pile with corn stover unloaded from walking floor trailers.

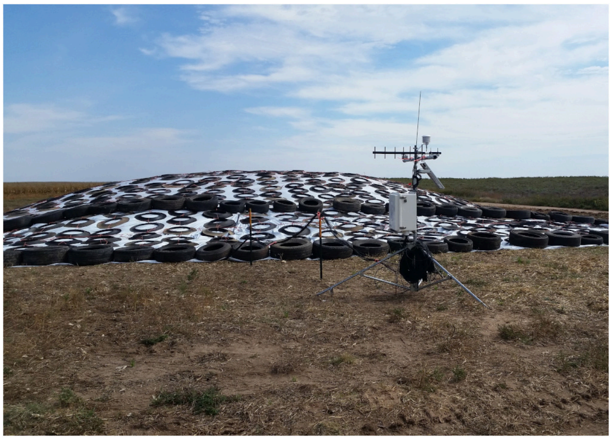

FIGURE 9 | Covered drive over storage pile.

storage pile described in Wendt et al. (2018a). Ensiling is a common practice for corn and grasses in humid climates of the world including parts of the United States and in Europe (Muck and Kung, 2007). Over 121 million tons of corn silage were harvested in 2018 in the United States and stored for livestock forage using this approach (U.S. Department of Agriculture, 2018). Ensiled biomass can be stable for months to years if anaerobic conditions are maintained. Expected dry matter losses under best management practices range from 6 to 15% depending on storage structure, with losses as low as 3% possible (Muck and Kung, 2007; Borreani et al., 2018).

The success of ensiling relies on mechanical exclusion of air through compaction, utilization of oxygen present through respiration, and fermentation to produce organic acids and a corresponding reduction in pH (McDonald et al., 1991). Obligate aerobic microorganisms are primarily responsible for the initial consumption of oxygen through respiration, although plant respiration also plays a role (Pahlow et al., 2003). Once this oxygen has been consumed then lactic acid bacteria proliferate and produce organic acids (Pitt et al., 1985). Soluble sugars, which are commonly referred to as water soluble carbohydrates in the forage literature, serve as the energy and carbon source for the initial fermentation as well as sustained but reduced growth of lactic acid bacteria during the stable storage stage (Pahlow et al., 2003). The combination of anaerobic conditions and the presence of organic acids and corresponding low pH serve to reduce overall microbial activity in ensiled systems, and Leistner (2000) described this combination of factors to promote stability as the hurdle concept.

The soluble sugars in biomass can constitute a significant portion of biomass, and their presence is important for successful ensiling. These sugars are transported through actively growing plants, forming structural sugars as the plant grows (Cosgrove, 2005). Corn stover can contain between 4 and 12% of these soluble carbohydrates depending on the growth phase of the plant (Chen et al., 2007). Forages grasses can have a wide range of soluble carbohydrates with anywhere from 5 to 30% (McDonald et al., 1991), and up to 16.3% soluble carbohydrates have been documented in switchgrass (Dien et al., 2006). Sweet sorghum can contain up to 20% soluble carbohydrates (Rains et al., 1990). The stage of growth often determines the level of soluble carbohydrate reserves in the plant, with the levels decreasing after anthesis and as the plant sends carbohydrate reserves to the roots for wintering. Soluble carbohydrate levels in grasses have been shown to vary between 10 and 35% depending on the environmental conditions and the stage of growth (Wulfes et al., 1999). Similarly, soluble carbohydrate levels in corn stover as low as 2.5% of total mass have been present at the time of harvest and still resulted in successful preservation in ensiling (Wendt et al., 2018a). Ensuring that sufficient fermentable soluble sugars are present at the time of ensiling is necessary to support organic acid production and pH reduction. Low cost additives such as molasses or chemicals can be applied when sufficient soluble sugars are not available, as discussed in the following sections.

Dry matter loss and final pH during the ensiling process is related to the type of lactic acid bacteria present and their fermentation pathway. Lactic acid formation by homofermentative lactic acid bacteria during ensiling results from the direct conversion of glucose to lactic acid, whereas heterofermentative lactic acid bacteria convert glucose to lactic acid, acetic acid, ethanol, and CO_2 (McDonald et al., 1991). Therefore, homolactic acid fermentation results in lowest losses of carbon and associated dry matter and is preferred during ensiling. However, acetic and propionic acids have been shown to inhibit spoilage microorganisms during aerobic exposure at the time of utilization of silage (Kung et al., 1998). Therefore, a mixture of acids is commonly desirable in ensiled biomass.

The protective effect of organic acids during preservation is based on inhibition of unwanted microorganisms. Lambert and Stratford describe the mechanism by which undissociated weak acids permeate across microbial plasma membranes and then dissociate into protonated hydrogen molecules and deprotonated hydroxyl groups (Lambert and Stratford, 1999). This is followed by proton pumping out of the cell, which leaves the hydroxyl group in the cytochrome and thus lowers the internal cell pH

(Lambert and Stratford, 1999). The low pK_a of lactic acid (3.78) makes this the preferred organic acid for stability compared to acetic acid (pK_a = 4.75) or butyric acid (pK_a = 4.82). Lactic acid dominated silages tend to have a pH near 3.7–3.9, and thus there is an overall increase in the level of undissociated acids outside of cell walls at lower pH values.

Degradation as a result of oxygen exposure in ensiling is a significant risk for these storage systems. Oxygen exposure is present during the formation and deconstruction of anaerobic storage piles. Delayed sealing or covering in ensiling has been shown to encourage the consumption of soluble carbohydrates by aerobic bacteria, yeast, and fungi (Henderson and McDonald, 1975; Pahlow et al., 2003). This results in not only less of this carbon source being available for lactic acid bacteria but also competition between lactic acid bacteria and clostridia. Clostridia produce butyric acid in silage, which is associated with higher dry matter loss in storage and lower consumption of the forage by ruminants (Muck and Kung, 2007). Clostridia spores can be passed into milk and can lead to contamination in milk and the products that are made from milk including cheese (Drouin and Lafrenière, 2012). This issue is of lower concern for bioenergy systems because pretreatment generally occurs at temperatures that can deactivate spores such that they are not passed into the fermentation process. However, the higher dry matter loss as a result of oxygen exposure is a concern for bioenergy systems due to the loss of convertible carbon to the atmosphere.

Corn stover for bioenergy production is available at the time of grain harvest and accordingly contains lower initial moisture contents and lower soluble sugars compared to feedstock dedicated for forage (Pordesimo et al., 2004, 2005). This presents a challenge when ensiling corn stover because the reduction of water corresponding increases the interstitial oxygen that must be either mechanically removed or biologically consumed in order to establish conditions that favor fermentation. Similarly, insufficient soluble carbohydrates for fermentation ultimately result in lower organic acid production. Despite these challenges, Shinners et al. and Wendt et al. both demonstrated that low-moisture ensiling (∼40% moisture, wet basis) was possible, with <5% loss was experienced over 6 months in covered, drive-over storage piles (Shinners et al., 2011; Wendt et al., 2018a). Similarly, ensiled corn stover has demonstrated slight pretreatment in ensiled storage conditions (Darku et al., 2010; Essien and Richard, 2018). Therefore, ensiling provides a solution for biomass to be stored in a stable format and utilized in bioenergy conversion systems throughout a calendar year notwithstanding the biomass being seasonally available.

Long-term wet, anaerobic storage has been shown not only to stabilize biomass but can also provide an environment to begin depolymerization of structural components, such as lignin and hemicellulose, a benefit that could help to lower conversion costs for high moisture feedstock. The high moisture environment provides an environment that enables biological and chemical reactions to occur. The pH of typical ensiled material is in the range of 3.5–4.5, depending on the fermentation pathway. This pH range inhibits most growth by obligate aerobic bacteria, yeast, and fungi, and even lactic acid bacteria have reduced activity at pH levels below 4 (Venkatesh et al., 1993). However, organisms that are active may be producing enzymes that can liberate the carbohydrates from the biomass and support their growth. Fructan hydrolases produced from the ensiled plants themselves (Ould-Ahmed et al., 2017) or by select lactic acid bacteria strains that can create fermentable sugar monomers from polysaccharides (Merry et al., 1995; Muck and Kung, 2007). This may occur in anaerobic storage systems even with low degradation rates. Gusovius et al. (2019) correlated the reduction of fiber size in hemp to dissolution of the middle lamella by microbial activity in anaerobic storage. Similarly, delamination in the middle lamella in pine has also been documented as a result of fungal treatment (Goodell et al., 2017). Further investigation is necessary to understand the role of long-term storage to influence cell walls and related structural integrity of biomass.

Despite the multiple benefits of wet anaerobic systems for corn stover in promoting stability in long term storage, prior research has been unable to close the cost gap between wet systems and their lower cost dry counterparts. The primary drawback of wet systems for corn stover is that the moisture in the biomass as well as the bulk, chopped format makes handling this biomass more costly than handling dry, baled biomass. For example, prior research has shown that transportation costs double for chopped corn stover compared to baled stover as a result of reduced bulk density compared to baled biomass (Wendt et al., 2018b). However, the size reduction that can be accomplished during forage chopping that is used in wet logistics systems can reduce both harvest and collection costs as well as the cost of further size reduction during preprocessing. Harvest and collection costs were reduced from \$21 Mg^{-1} in a bale-based logistics system to less than \$16 Mg^{-1} in a wet logistics system (Wendt et al., 2018b). Likewise, size reduction during forage chopping is capable of reducing particle size geometric mean to 5–10 mm (Lisowski et al., 2017), whereas baled logistics systems for corn stover rely on one to two steps of size reduction with a 6 mm screen during preprocessing. However, wet anaerobic storage costs are higher more than its baled counterpart. Field-side storage costs for baled corn stover are estimated to range between \$5 and \$18 Mg^{-1}, while anaerobic storage of corn stover in piles is estimated to cost between \$15 and \$22 Mg^{-1} (2015 US dollars, Vadas and Digman, 2013; Wendt et al., 2017, 2018b). Additional research is necessary to identify approaches that willo address the cost barrier of wet anaerobic storage compared to baled storage.

Storage Selection Based on Feedstock Type

Feedstock type and harvest scenario both impact the most suitable long-term storage approach. **Table 1** lists the herbaceous crop residues and energy crops identified in the Billion Ton report (Langholtz et al., 2016) and the most common storage approach utilized for them. Residues that are harvested based on timing of the grain harvest are generally lower moisture content and compatible with baled storage; these include the straws

TABLE 1 | Herbaceous crop residues and energy crops identified in the Billion Ton study linked to their common storage method.

Biomass type	Dry storage	Wet storage
Barley straw	x	
Corn stover	x	x
Energy cane	x	x
Grain sorghum stubble	x	
Miscanthus	x	x
Rice straw	x	
Sugarcane bagasse		x
Switchgrass	x	x
Wheat straw	x	

and grain sorghum stubble. Energy crops including switchgrass and miscanthus are generally harvested after senescence and subsequently stored in baled formats. However, harvest of these plants is not dependent on a primary commodity crop and the timing can be flexible such that anaerobic wet storage could be compatible with these crops. Crops that are high moisture at the time of storage including energy cane and sugarcane bagasse are best suited for wet storage systems. As discussed previously, corn stover is often stored in dry, baled formats, but challenges with achieving the desired moisture content for stability are inherent to this crop and provide an opportunity for wet storage to address this challenge. However, long-term wet storage operation is one of the unit operations in the feedstock logistics operations that can be used to improve the quality of the corn stover with the aim of reducing downstream processing requirements for conversion to fuels and chemicals. The following sections describe approaches that have or could be used to facilitate this reduction in recalcitrance.

Storage Amendments

The application of amendments to biomass to promote stability prior to anaerobic storage is commonplace in the forage industry. The goal of these amendments is to promote the fastidious formation of a low pH environment that result in stable storage and maintain desirable qualities for forage (Muck et al., 2018). Amendments may include acids or alkali applied directly to the biomass or microbial amendments to encourage a specific fermentation pathway, and either of these can be effective at reducing storage losses. Storage amendments are so commonplace that forage choppers are often equipped with sprayers that can apply liquid inoculants during harvest. The following section describes some of the primary amendments that have been used over the last century for forage silage and may have applicability for bioenergy systems.

Microbial Amendments

Lactic acid bacteria are commonly added to silage during harvesting to promote the proliferation of these organisms and thus more rapid fermentation during ensiling (Muck and Kung, 2007). Homofermentative lactic acid bacteria that produce primarily lactic acid have demonstrated reduced aerobic stability upon removal from storage compared to the acetic acid containing biomass produced by heterofermentative lactic acid bacteria (Muck and Kung, 2007; Muck et al., 2018). A wide range of microbial inoculants are available commercially, and they generally contain a mixture of bacterial species to improve the palatability of the feedstock for livestock (Muck et al., 2018). Anaerobic storage with microbial inoculants has been suggested to positively influence performance in bioenergy conversion systems. The combination of high-moisture storage with bacterial inoculants have been demonstrated to increase sugar release in wheat and rice straw, corn stover and corn silage, and forage sorghum (Linden and Murphy, 1990; Henk and Linden, 1996; Oleskowicz-Popiel et al., 2011).

Enzymes have also been added to silage in order to increase the level of soluble carbohydrates for consumption by lactic acid bacteria (Kung et al., 1991; Kung, 1998). Common enzymes include cellulases, xylanases, and pectinases, and most are applied in combination with a lactic acid bacteria inoculant that can utilize the sugars released enzymatically (Muck et al., 2018). Organisms that produce ferulic acid esterase have also been added to silage with mixed success in improving digestibility of livestock feed (Lynch et al., 2015). Enzymes also have a role in bioenergy conversion systems, where depolymerization of structural hemicellulose in long-term storage could be utilized to reduce pretreatment severity at the biorefinery. Low-moisture corn stover (∼20%, wet basis) amended with xylanase increased recovery of hemicellulose-related sugars by 10% over untreated controls during long-term storage (Smith et al., 2009). A common concern when adding enzymes during long-term storage is the excessive hydrolysis of carbohydrates (Kung and Muck, 2015), which results in elevated substrate for fermentation in anaerobic storage or excessive loss upon aerobic exposure. This balance must be carefully managed based on feedstock type and utilization strategy.

Acidic Amendments

Organic and mineral acids have been used extensively in silage to rapidly decrease pH and preserve the nutrient content of the biomass. Virtanen used a blend of hydrochloric and sulfuric acids to preserve silage, and this work demonstrated that a pH of 4.0 was necessary to inhibit soluble carbohydrate and protein degradation along with butyric acid formation (Virtanen, 1933). Virtanen received a Nobel Prize in Chemistry in 1945 for this and delete Contribution To The Field (The Nobel Prize in Chemistry, 1945). Sulfuric acid is a strong acid and is used specifically to reduce pH, however, Virtanen recommended that it not be applied alone due to poor digestibility by rumen. Formic acid is common silage additive that is considered to reduce pH rapidly as well as provide antimicrobial effects. Formic acid is proposed to disrupt the electron transport chain by inhibiting cytochrome oxidase (Keyhani and Keyhani, 1980). While this may be desired for the suppression of spoilage microorganisms, this same mechanism has resulted in histotoxic hypoxia in farmers exposed to vapors while making silage (Liesivuori and Kettunen, 1983). It has also been noted that yeasts have a higher tolerance to formic acid treated silages than lactic acid bacteria, such that the aerobic stability of formic acid treated silages is poor (Henderson et al., 1972). Formic acid is still used as a silage additive, particularly in European countries due to the ban on antibiotics in livestock feed. However, its use is limited in the United States because it traditionally is a higher cost acid. Approaches to produce lower-cost formic acid are necessary to enable additional utilization of this acid in forage and bioenergy storage systems.

Propionic acid is a low-cost additive often used in the United States, particularly in haylage (Knapp et al., 1976). Propionic acid additives have demonstrated to reduce yeast proliferation upon removal of ensiled biomass from storage, thus increasing the aerobic stability of the biomass (Woolford, 1975). Similarly, numerous acid and acid salt combinations have been described for their preservation effect on silage during storage and upon exposure to oxygen (Muck et al., 2018). Nadaeu et al. demonstrated an improvement in aerobic stability of corn silage from 5.7 to 11.8 days for biomass that entered storage after treatment with a combination of formic, propionic, benzoic, and sorbic acids (Nadeau et al., 2011). Acid salt combinations including potassium sorbate, sodium benzoate, and sodium nitrite have also shown to increase aerobic stability in corn silage (Da Silva et al., 2015). Perennial grasses, including switchgrass, have been successfully preserved in high-moisture storage amended with mineral acid and experienced up to 17% improvement in cellulose conversion to ethanol (Williams and Shinners, 2012). In summary, acids have demonstrated as effectiveness as a direct approach in improve ensiling performance and aerobic stability of biomass upon utilization. Further knowledge on the effect of these treatments to improve performance in bioconversion to fuels and chemicals will increase their utilization in commercial biorefineries.

Alkaline Amendments

Alkaline treatments have been used for stabilizing wet harvested biomass by creating a basic environment which can restrict unwanted fermentation. Anhydrous ammonia has been applied to forage for over 50 years to improve nitrogen levels and prevent proteolysis and deamination in forage, which improves the quality of the biomass for livestock feed (Huber and Santana, 1972; Huber et al., 1979). Anhydrous ammonia has been demonstrated to raise pH and decrease lactic acid formation during the initial days of ensiling as well as decrease protein degradation in long-term anaerobic storage (Johnson et al., 1982).

Calcium oxide, or lime, has been used as an additive for biomass with the dual aim of improving storage stability as well as to impact thermochemical conversion performance (Xiong et al., 2017; Bozaghian et al., 2018). Calcium oxide (CaO) reacts with water to produce calcium dihydroxide [$Ca(OH)_2$], which then reacts with CO_2 to form calcium carbonate ($CaCO_3$). Calcium carbonate is understood to act as a sorbent and reacts with other inorganics including silica and sulfur during thermochemical conversion (Wang et al., 2016), which increases the melting temperature of the resulting inorganic complex and thus reduces undesirable slagging on reactor surfaces and catalysts (Bozaghian et al., 2018). Calcium oxide treatment of reed canary grass was shown to increase pH to greater than 9 in biomass containing 35–65% moisture, which is desirable to reduce proteolytic organisms but not sufficiently high such that protein degradation occurred. In this study the 35% moisture content biomass exhibited stable aerobic storage over 90 days due to the combined effect of initial increased pH and reduction of moisture through drying (Xiong et al., 2017), however, higher moisture contents levels resulted in storage losses up to 30% and the subsequent reduction of pH levels to 8–9 likely as a result of liberation of acetyl side chains from the hemicellulose. Similarly, lime has been applied to poplar over a 12 week period to enhance the solubilization of lignin though oxidation and deacetylation of hemicellulose through hydrolysis in order to improve the digestibility of wood in enzymatic hydrolysis (Rocio et al., 2011).

Sodium hydroxide has been assessed for use during storage to reduce biomass recalcitrance, and the advantage of this alkali above lime is that it is readily soluble. Sodium hydroxide has been used to improve the digestibility of wheat and barley straws for livestock feed by reducing lignin content (Chesson, 1981; Lindberg et al., 1984). Sodium hydroxide treatment during 1–3 days of storage has also been applied to corn stover at 80% moisture content (wet basis) in order to increase biogas yields in anaerobic digestion, and these studies have indicated that hemicellulose is most susceptible to short-term sodium hydroxide exposure as a result of removal of acetyl groups (Pang et al., 2008; Zheng et al., 2009; Zhu et al., 2010). Cui et al. investigated the use of sodium hydroxide treatment during 90-day ensiling of corn stover in plastic bags at moisture contents ranging from 45 to 75% moisture (wet basis) (Cui et al., 2012). This study showed that lignin and cellulose degradation was complete within 5 days of storage but that xylan degradation continued over the 90-day storage period; however, significant dry matter loss of 13–21% occurred during the storage period. An increase in acetic acid levels was observed during the first 15 days of storage, and subsequent reduction of structural acetate after this period is consistent with the dry matter loss experienced. Similarly, glucose and xylose yields were reduced in samples that experienced 90 days of storage compared to 5 and 12 days of storage. This study shows the importance of maintaining stable storage conditions when combining sodium hydroxide with long term storage.

Alkali treatments have shown to reduce chemical recalcitrance of biomass to deconstruction and are the state-of-the-technology for cost-competitive biochemical conversion of carbohydrate and lignin monomers to biofuels (Chen et al., 2016; Davis et al., 2018). However, these high-severity treatments require significant alkali loading during short thermal residence times in order to be efficient at the biorefinery scale. Anaerobic storage offers the opportunity to allow the deacetylation reactions to occur over a longer residence time with the added benefit of protecting biomass from uncontrolled dry matter loss. As discussed in this section, alkali treatment has demonstrated reduced recalcitrance in terms of improved digestibility for rumen. However, the combination of well-preserved biomass resulting from anaerobic storage and alkali treatment have not yet been applied in relation to both physical and chemical preprocessing to form convertible carbohydrate monomers for bioenergy systems.

Storage Systems Linked to Conversion

The impacts of long-term storage are an important variable to consider in the conversion of biomass resources to fuels and chemicals. The conditions experienced during storage and resulting biochemical changes in cells can positively or negatively impact conversion potential. For example, corn stover that had experienced significant aerobic degradation (30% loss of dry matter) was shown to have a significant shift in structural to soluble xylan but no change in structural glucan (Wendt et al., 2018a). However, after either dilute acid or dilute alkaline treatment the efficiency of enzymatic hydrolysis to depolymerize glucan was increased in the aerobically degraded biomass; this suggests that the loss of hemicellulose in storage resulted a slight pretreatment effect. Dilute acid and dilute alkaline pretreatments have been applied to anaerobically stored biomass as well with success (Wendt et al., 2018a), and in this case dilute alkali treatment was effective in showing an increase in carbohydrate release after anaerobic storage. Limited data on deacetylation pretreatment is available for corn stover, but alkali groups in hemicellulose hydrolyzed during storage should positively impact deacetylation. Additionally, organic acids produced during anaerobic storage may serve as catalyzing agents during pretreatment including during steam explosion (Liu et al., 2013) or hot water extraction (Ambye-Jensen et al., 2018; Essien and Richard, 2018). However, ammonia fiber expansion pretreatment is primarily performed prior to long term storage because it results in a shelf-stable format. Additional insight is needed to understand how long-term storage can be used to enhance deconstruction based on each biomass type and each pretreatment chemistry.

CONCLUSION AND FUTURE DIRECTIONS

Long-term storage of biomass is a reality for any agricultural system and is a key unit operation for bioenergy systems. However, the costs necessary to produce stable storage conditions are often misaligned with the pressures of producing biofuels that are competitive with their fossil counterparts. Focus on multiple research directions can address this cost disparity and should include (1) understand how baled biomass systems can provide protection from moisture and related physical and microbial losses, (2) application of how wet, anaerobic systems commonly used in forage might be used to overcome the cost barrier that currently makes them less attractive for bioenergy systems, and (3) an enhanced understanding of how these storage systems may affect biomass recalcitrance and subsequent conversion to fuels or chemicals. There is also potential to shift the focus of long-term storage from a cost center to a value-added operation such that bioconversion, energy balances, and sustainability are positively impacted. Securing the storage operation of the feedstock logistics and supply chain will be a key component to making the bioeconomy a reality.

AUTHOR CONTRIBUTIONS

LW drafted the manuscript with contributions from HZ. LW and HZ revised the manuscript and prepared figures. All authors approved the manuscript for publication.

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Total and Sustainable Utilization of Biomass Resources: A Perspective

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Feedstock cost is a major variable cost component in conversion to biofuels and chemicals. Consistent feedstock quality is critically important to achieve high product yield and maximum onstream time. Traditionally, raw biomass materials are delivered directly to the biorefineries where they are preprocessed to feedstock prior to being converted to products. Since many types of biomass materials—including agricultural residues, energy crops, and logging residues—are harvested according to growth cycles and optimal harvesting time, just-in-time steady supply of raw biomass to the biorefineries is not possible. Instead, biomass materials are stored, then delivered to the biorefineries as needed. Experience to date indicates that this approach has caused many issues related to logistics, biomass losses due to microbial degradation and fire, and inconsistent feedstock quality due to variability in the properties of as-delivered biomass. These factors have led to high feedstock cost, low throughput, and low product yield for the biorefineries. Idaho National Laboratory has developed a new strategy to address the problems encountered in the traditional approach in biomass feedstock supply, storage, and preprocessing mentioned above. The key components of this strategy are (1) preservation and preconditioning of biomass during storage, (2) utilization of all the biomass, including minor components that are normally considered wastes or contaminants, and (3) maximization of the value of each component. This new approach can be accomplished using feedstock preprocessing depots located near the biomass-production sources.

Keywords: biomass, feedstock, preprocessing, co-products, conversion-ready feedstock, biorefinery, depot, corn stover fractionation

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INTRODUCTION

This paper focuses primarily on utilization of agricultural residues, specifically corn stover, and herbaceous energy crops. Feedstock cost is the largest manufacturing-cost component in cellulosic biofuel production. The National Renewable Energy Laboratory (NREL) has projected that, to meet the DOE fuel selling price target of \$2.50/GGE by 2030, the price of biomass feedstock delivered to the reactor throat for a biomass-to-hydrocarbon fuels biochemical conversion facility must be <\$71.3/dry short ton (2016 U.S. dollars) (Davis et al., 2018). Based on experience at pioneer biorefineries, this target price would be very difficult to meet using current technology and only applicable in certain locations where low-cost biomass is available. In addition to obtaining low-cost feedstock, another major issue with the conventional approach is that preprocessing of raw biomass materials (especially baled agricultural residues) is difficult and often leads to low equipment uptime. The major challenges identified by industry include biomass-feedstock flowability, variability in feedstock properties, lack of equipment-performance data, and lack of standard feedstock specifications (US DOE, 2016). Additionally, integrating feedstock preprocessing with biofuel conversion in a single facility lowers plant productivity as operational issues in the preprocessing area often cause shutdown of downstream conversion-unit operations.

One approach in reducing the cost of delivered biomass feedstock is to blend high-carbohydrate biomass (e.g., two-pass corn stover or switchgrass) and low-cost biomass (e.g., grass clippings) to achieve a projected cost of $79.1/dry short ton (2016 U.S. dollars) by 2022 (Roni et al., 2018). The blended biomass materials are pelleted to facilitate high-density storage and shipping as well as improved handling characteristics at the biorefinery. However, the blending approach is restricted to areas where the low-cost and high-carbohydrate-content biomass materials are available. Another issue with supplying biomass feedstock with highly variable component (carbohydrates and lignin) concentrations is that a biorefinery capable of converting these major components to biofuels and high-value coproducts would be complex and require high capital investment, which is a significant barrier to commercialization, especially for new technologies. The capital cost of a 50 million annual gallons cellulosic ethanol plant is estimated at $4.30 (1999 dollars) per annual gallon, compared to about $1.25 per annual gallon for a dry-grind corn-ethanol plant (McAloon et al., 2000).

Idaho National Laboratory is investigating a new approach through which advanced feedstock depots preserve biomass and convert raw biomass into several conversion-ready feedstocks, targeting a wide range of markets, including biofuels, bioproducts, animal feed, and agriculture. In this way, the potential value of preprocessed biomass material is higher than for single-use and, with a larger customer base, the financial risk of such feedstock depots would be reduced. The conversion-ready feedstocks can also be tailored to end users' specifications. This approach combines in-storage preconditioning of biomass to minimize microbial degradation of carbohydrates with fractionation to produce high-value products. The following sections provide insights into key components of an advanced feedstock preprocessing depot that would produce many benefits: simplified biomass supply logistics, in-storage preconditioning, and product fractionation and recovery.

BIOMASS SUPPLY AND LOGISTICS

Corn stover has been identified as the most abundantly available agricultural residue suitable for conversion to biofuels and chemicals (Langholtz et al., 2016). In the agriculture sector, the two most common methods of storing agricultural residue are (1) square or round bales and (2) ensiled piles or bunkers. Assuming an average dry mass per large square bale is 500 kg, a 2,000-metric ton/day facility will consume 4,000 bales per day, not counting dry matter losses during storage and preprocessing. The normal inventory of a biorefinery is 5 days. For a 2,000 metric tons/day facility, the storage area for a 5-day inventory is at least 5.9 hectares (14.5 acres), assuming each 2,000-bale stack is 7-bale high and spaced 60 m apart to minimize the risk of fire spreading from one stack to another (Webb et al., 2018). Because the harvesting period for corn stover averages 4 months, storing an 8-month supply of bales in satellite storage requires an area of at least 256 hectares (633 acres). Handling and transporting bales from field to satellite storage then to biorefineries significantly add to the cost of feedstock. Storage of biomass bales (e.g., corn stover) has many problems that lead to high dry matter loss, variability in feedstock properties, and high cost. A 2,000-bale stack (7 bales high) requires up to 59% of the bales to have at least one side exposed either to the ground or external air if the stack is not covered. If the top of the stack is covered, up to 45% of the bales are exposed. Moisture movement in exposed bales leads to higher degradation and variable properties (Smith et al., 2013). Moisture and ash were identified as the major properties that have significant impact on the operability of feedstock-preprocessing equipment (US DOE, 2016). High moisture and multiple layers (flakes) of corn stover bales reduce the throughput of bale grinders, cause surge flows, and result in variable particle size (Nguyen, 2019). Multipass baling logistics lead to high extrinsic ash content due to soil contamination (Bonner et al., 2014). Another issue with bale logistics is the large amount of polypropylene twine (for square bales) and polyethylene bale net wrap (for round bales) requiring disposal. It is estimated that a 2,000-metric ton/day biorefinery using square bales generates about 8.4 million pieces of 6.7 m-long twine every year of operation. Use or recycling this waste could be problematic for many rural locations. Most large square balers leave several pieces of twine (tailings), each 2–4 cm long, on the bales. These contaminants are difficult to detect and remove. Removal of bale twine and net wrap using current mechanical technology is <100% successful. Twine and net-wrap contaminants can cause clogging of piping and equipment (Sluska and Bushong, 2019).

The heterogenous components of corn stover (stalk, leaf, husk, cob) and softwood logging residuals (white wood, bark, twigs, needles) lead to variable physical and mechanical properties and chemical compositions. These variabilities result in material-handling and operational problems, lower throughput and product yields in pioneer biorefineries (US DOE, 2016). Fractionating biomass into major components and converting them to conversion-ready feedstock is one way to address the material-handling and conversion-yield issues.

High-moisture (40–65% wet weight basis), anaerobic storage of feedstock has many advantages over bale storage, including lower dry matter loss (Wendt et al., 2018), much lower fire risk, and lower cost of handling. It also provides the opportunity to carry out leaching or microbial or chemical preconditioning during storage. Compared to bale storage, pile storage requires a significantly smaller storage area: a 5-day inventory pile for a 2,000 metric ton/day facility only needs about 0.64 hectare (1.58 acre) assuming a recommended average compacted bulk density of 240 kg/m^3 (15 lb/ft^3) dry basis to achieve low dry matter loss (Holmes and Muck, 2007). The pile can be located next to the biorefinery, and the feedstock can be conveyed from the storage area into the plant. This results in lower handling costs. Biomass-chopping logistics (Mann et al., 2019), as opposed to baling

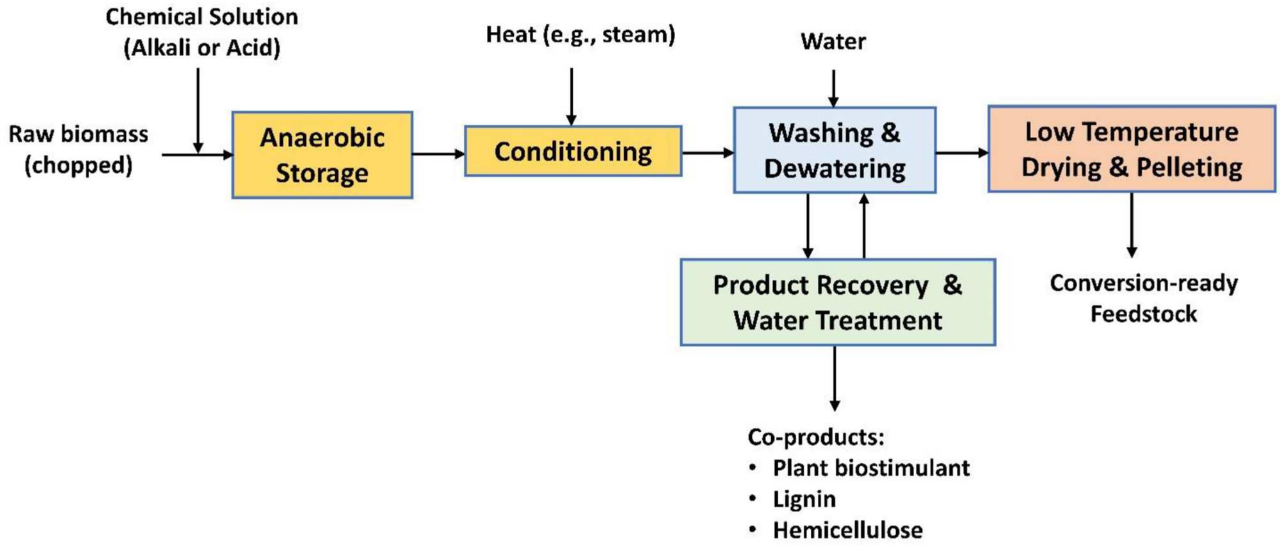

FIGURE 1 | High-moisture anaerobic storage and fractionation of biomass.

logistics, is more suitable for high-moisture, anaerobic storage, especially for herbaceous energy crops. Chopped corn stover can be compacted using a 0.3-m-diameter auger to a relaxed bulk density of about 208 kg/m^3 (13 lb/ft^3) dry basis or higher (Franz, 2007). As a comparison, the bulk density of corn stover square bales is about 177 kg/m^3 (11 lb/ft^3), and about 141 kg/m^3 (8.8 lb/ft^3) dry basis for round bales. Mobile screw compactors can be used to compact chopped biomass into transporters in the field (Gruithuis et al., 2007; Jiskra et al., 2017). The compacted biomass can then be transferred to depots for preprocessing to conversion-ready densified feedstock. A possible configuration of chopped biomass logistic is shown in **Figure 1**. A one-pass harvester blows chopped corn stover into mobile forage wagons in the field. The wagons are pulled to the side of the field, where the chopped biomass is compacted in transporters or driven directly to nearby preprocessing depots. The forage wagons can precompact chopped biomass to 112–139 kg/m^3 dry basis (Suokannas and Nysand, 2009). The net cost of transporting and handling of chopped corn stover has potential to be lower than that for baled corn stover because of the shorter total travel distance to the local depot and the elimination of intermediate bale-storage and handling steps. **Table 1** lists the main advantages of the chopped biomass logistics over the baling logistics.

Logging residues have also been identified in the billion-ton report as promising low-cost woody biomass. Softwood logging residues comprise mainly branches and treetops. With proper storage and preprocessing, the logging residues can be turned into a suitable feedstock for thermochemical conversion to biofuels. To minimize contamination by soil and facilitate in-field preprocessing, the logging residues should be stored in piles, and not scattered on the ground. The residues should be seasoned for about 1 year or longer to partly lower the moisture content to less than about 25% (wet basis) and facilitate defoliation (Nilsson, 2016). Pile drying to below 25% moisture content may not be practical in regions with high yearly precipitation. Generally, in the Southeastern U.S., it takes 1 year for the moisture content of logging residues to lower to about 30%, and this time requirement is longer for the Pacific Northwest U.S. Drying logging residues, however, may result in lower bark removal efficiency because the wood-to-bark adhesion strength is significantly higher at moisture content below about 40% (Chow and Obermajer, 2004). Logging residues can be sorted, screened and chipped in the field, compacted in transporters, then transferred to preprocessing depots.

BIOMASS PREPROCESSING AND FRACTIONATION

The goal of biomass preprocessing is to produce consistent feedstock that meets conversion specifications. The heterogeneous makeup and properties of corn stover and softwood logging residues have proven to be difficult barriers to overcome using traditional preprocessing techniques, including milling, air classifying, and screening. The shear and impact forces required to break the various anatomical fractions of corn stover (rind, pith, leaf, husk, and cob) differ by tissue type (Anazodo, 1980; Zhang et al., 2016, 2017; Workiye and Woldsenbet, 2019). Applying sufficiently high impact and shear force to fracture tough components such as cob, husk and rind will pulverize the more fragile components such as pith and leaf, which leads to wide particle-size distribution with a high proportion of fines. Wide particle-size distribution has the potential to cause uneven mass flow, heat, and mass transfer in continuous high-solid pretreatment reactors. Furthermore, corn stover rind is more recalcitrant than the pith and leaf fractions (Crofcheck and Montross, 2004; Duguid et al., 2009; Zeng et al., 2012; Li et al., 2014); therefore, mild pretreatment (such as hot

TABLE 1 | Qualitative comparison between baling and chopping logistics for herbaceous biomass.

Attributes	Baling Logistics	Chopping Logistics
Agricultural residues harvest and collection logistics	Common	Uncommon for most uses but common for producing silage
Dry matter loss during storage outdoors for weeks & months	10–20% depending on the moisture content of the bales and the weather conditions	5–6% under anaerobic storage condition
Biomass properties	Significant variability in properties: moisture, ash, fiber integrity, particle size, chemical composition	More consistent properties than baled biomass
Fire risk	High fire risks from lighting, self-combustion and arson	Very low fire risk because of >45% moisture and anaerobic conditions
Cost of storage and handling	High because the bale stacks take a lot of space and must be stored far apart to prevent fire from spreading. The storage area for bales is approximately 9 times that for piles, plus there are many satellite bale storage sites.	Storage piles can be located next to the feedstock depots so the biomass can be conveyed into the preprocessing area. The modular depots are strategically located near the biomass sources to lower the cost transportation.
Facilitating production of multiple products including conversion-ready feedstocks?	No, as it would be very expensive.	Yes, biological and chemical treatment can be readily incorporated into the high- moisture biomass storage operation.
Impact on feedstock depot and biorefinery operations	Low operational reliability and product yield because of the high variability in biomass properties. Higher capital and operating costs for biorefineries.	Improved operational reliability and lower capital and operating costs.
Impacts by weather	Wet and cold weather may prevent field drying and baling operations. As a result, the sources of biomass supply are generally limited to dry weather areas.	Weather has less impact on harvesting and collection compared to baling operation.
Waste streams	Improper disposal of bale twines and net wrap can cause negative impacts on the environment and wide life.	No waste bale twines and net wrap. Lower environmental impact and carbon footprint.

water and dilute sodium hydroxide pretreatment) of corn stover likely results in a compromised enzymatic-hydrolysis sugar yield.

One method to achieve high (>90% theoretical) total sugar yield after pretreatment and enzymatic hydrolysis of corn stover is to apply high-temperature, short-residence-time dilute sulfuric acid steam explosion pretreatment (Tucker et al., 2003). This pretreatment method is effective in deconstructing the recalcitrant rind fibers but does not significantly degrade more labile components such as pith, leaf and husk. This approach is analogous to the increased milling forces discussed above; increased processing intensity is used to overcome biomass heterogeneities. However, dilute acid pretreatment releases organic acids, lignin and carbohydrate degradation products that are inhibitory to enzyme and fermenting organisms (Casey et al., 2010; Jonsson et al., 2013; Qin et al., 2016).

An alternate method is to fractionate corn stover into the major anatomical components, then process them separately. This method could be economically viable if high-value uses of one or more components could be developed. As an example, bleached soda pulp can be produced from corn-stover stalks, but the presence of pith causes low yield and poor drainage (Byrd and Hurter, 2014). Depithed corn stalks are expected to improve drainage and make corn-stover pulp production more feasible. In this method, fractionation of corn stover could be performed during harvesting, before all the components are mixed and compacted together (such as in a bale); entanglement of these anatomical components makes it much more difficult to separate them. During harvest, it is possible to separate corn plants into a fraction comprising stalks and leaves and a fraction comprising husk and cobs (Shinners et al., 2009). Using screening and air classification, the leaves can be readily separated from the stalks, and the husk separated from the cobs. The separation could be performed in the field before compacting the various fractions into transporters. The stalks can potentially be de-pithed at the preprocessing depots using similar technology for de-pithing sugarcane bagasse or industrial hemp (Ren et al., 2016; Chen and Qu, 2017).

Alternately, or in addition to anatomical fractionation of biomass, chemical fractionation is possible. One fractionation option produces conversion-ready feedstock which, depending on the starting raw biomass, can be used in biochemical or thermochemical conversion processes (**Figure 1**). Alkali treatment, combined with mechanical deconstruction of fibers, can significantly improve the enzymatic cellulose digestibility of lignocellulosic biomass (Chen et al., 2014; Yuan et al., 2017). More than 40% of the lignin content can be extracted from corn stover to produce a conversion-ready, carbohydrate-rich fiber fraction and a lignin-rich liquor. Lignin extraction can be accomplished via alkali pre-impregnation during storage (Wendt, 2019) followed by conditioning and washing. Conditioning may include high temperatures (>50°C) and/or additional chemical treatment (e.g., with peroxide) to improve delignification (Saha and Cotta, 2014; Mittal et al., 2017). The partially delignified fiber is then washed to recover solubilized products, mechanically dewatered, deconstructed, air-dried, and then pelletized to produce a conversion-ready feedstock. The liquid fraction, a coproduct, contains solubilized carbohydrates, lignin, organic acids, minerals, and other extractives.

PRODUCT APPLICATIONS

Pioneer biorefineries based on enzymatic hydrolysis technology generally utilized lignin remaining after fermentation as fuel in a biomass boiler. This lignin-utilization method is of low value because of the low heat content of high-moisture (about 50% wet weight basis) lignin cake. The extractives, organic acids, phenolics, and inorganics require remediation or waste treatment before disposal, which results in increased operational complexity and costs.

Feedstock preprocessing depots utilizing fractionation processes can produce multiple diverse products serving a wide customer base including biofuel producers, biochemical, biomass powers, agriculture, horticulture, and animal feed. Conversion-ready feedstocks in flowable pellet form will improve the operational reliability, reduce capital, and operating costs of biorefineries. Feedstock depots generate no waste stream of bale polypropylene twine or polyethylene net wrap when using chopped biomass logistics.

The liquid stream can be further fractionated to recover a lignin powder product (via acid precipitation and filtration) and a liquid product containing other soluble components (carbohydrates, organic acids, phenolics, extractives, and inorganics). The liquid product could potentially be used as a biostimulant to promote plant growth.

Alkali lignin can be used as substitute for phenol in lignin-based phenolic-resin applications for manufacturing of composite wood products (Ghorbani et al., 2016; Zafar et al., 2019), lignin-based polymers (Naskar and Tran, 2017; Ganewatta et al., 2019), or as antioxidant and antimicrobial agents (Spiridon, 2018) or converted to fuels and chemicals (Beckham, 2019; Ha et al., 2019).

Resin and fatty acids can be extracted from low-value fractions of logging residues such as needles, bark, and small branches (Eriksson et al., 2018).

CONCLUSION

The current agricultural practice of multipass harvesting, collection, and baling of herbaceous biomass is not suitable for providing feedstock that meets the required specification of biorefineries without expensive and complex preprocessing methods to produce consistent quality feedstock from raw biomass with high variability of properties. Single-pass chopped biomass logistics with in-field compaction, combined with chemically treated anaerobic storage, will not only minimize soil contamination and eliminate bale twine and net wrap contaminants, but also lead to viable options for fractionating biomass to useful products and minimize waste streams. Furthermore, a preprocessing depot can be flexibly configured to produce conversion-ready feedstocks that meet specific qualities for individual conversion technologies. It is envisioned that feedstock depots can supply conversion-ready feedstocks to a variety of conversion technologies and create a wide range of coproducts so that these depots would operate as profitable businesses, not dependent on a single biorefinery. This approach has the potential to lower technical and economic barriers to growing a biobased economy. Early adopters of multiproduct feedstock depots include biomass-feedstock integrators and suppliers, feed aggregators, biomass-pellet producers, and wood-mulch producers. They are already in the business of biomass preprocessing and serve well-established industries, such as wood products, pulp and paper, biomass power plants, feed lots, and horticultural sectors. It would be an incentive for these biomass-feedstock producers to expand their product portfolio into higher-value products such as conversion-ready feedstocks, lignin, and extractives.

AUTHOR CONTRIBUTIONS

QN, WS, BW, and LW prepared this manuscript. QN and LW contributed conception and design of the study. QN wrote the first draft of the manuscript. WS contributed to the Introduction and Biomass Supply and Logistics section. BW and LW contributed to the Biomass Preprocessing and Fractionation section. All authors contributed to the Product Applications section and manuscript revision, reading and approving the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Microbial Heat and Organic Matter Loss in an Aerobic Corn Stover Storage Reactor: A Model Validation and Prediction Approach Using Lumped-Parameter Dynamical Formulation

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Corn stover dry matter loss effects variability for biofuel conversion facility and technology sustainability. This research seeks to understand the dynamic mechanisms of the thermal system, organic matter loss, and microbial heat generation in corn stover storage operations through system dynamics, a mathematical modeling approach, and response analysis to improve the system performance. This study considers epistemic uncertainties including cardinal temperatures of microbial respiratory activity, specific degradation rate, heat evolution per unit substrate degraded, and thermal conductivity in corn stover storage reactors. These uncertainties were managed through calibration, a process of improving the agreement between the computational and benchmark experimental results by adjusting the parameters of the model. Model calibration successfully predicted the temperature of the system as quantified by the mean absolute error, 0.6°C, relative to the experimental work. The model and experimental dry matter loss after 30 days of storage were 5.1% and 4.9 ± 0.28%. The model was further validated using additional experimental results to ensure that the model accurately represented the system. Model validation obtained a temperature mean absolute relative error of 0.9 ± 0.3°C and dry matter loss relative error of 3.1 ± 1.5%. This study presents a robust prediction of corn stover storage temperature and demonstrates that an understanding of carbon sources, microbial communities, and lag-phase evolution in bi-phasic growth are essential for the prediction of organic matter preservation in corn stover storage systems under environment's variation.

Keywords: microbial heat, organic matter loss, corn stover, bi-phasic growth, microbial respiratory activity, storage reactor, model calibration, model validation

INTRODUCTION

Corn stover has long been recognized as a bioresource to reduce the United States' (U.S.) dependence on foreign oil (Graham et al., 2007) and the primary feedstock for ethanol and other potential biofuels such as butanol (Qureshi et al., 2010; Green, 2011). One of the significant challenges of corn stover-derived biofuel is the variability of the feedstock, particularly in the carbohydrate content of biomass, with consequences in the biofuel yields and economics (Kenney et al., 2013). For instance, moisture content beyond 25% can contribute to dry matter losses equal to or greater than 20% due to microbial degradation of carbohydrates in storage (Kenney et al., 2013; Wendt et al., 2018). Moisture contents from 15–20%, on the other hand, have lower dry matter losses effects in biomass storage and reduce safety risks such as self-ignition (Rentizelas et al., 2009). This feedstock variability of corn stover has been demonstrated to be highly sensitive in metrics of sustainability such as life-cycle net energies, carbon dioxide emissions, and the cost of biofuels (Kim and Dale, 2005; Spatari et al., 2005; Baral et al., 2017, 2018). The effects of environmental factors in the corn stover properties such as moisture, temperature, and dry matter loss have been researched in field and laboratory studies (Wendt et al., 2014, 2018; Essien and Richard, 2018; Wang et al., 2019). These previous research efforts have demonstrated that the microbial heat resulting from degradation of carbohydrates plays a role in the corn stover thermal system and organic matter losses. However, the dynamic mechanisms between changes in the environment and the microbial kinetics in corn stover are not understood.

Many researchers have investigated microbial kinetics in composting processes (Rosso et al., 1993; Hamelers, 2004; Kulcu and Yaldiz, 2004; Richard and Walker, 2006; Richard et al., 2006; De Guardia et al., 2008; Lin et al., 2008). Others have studied kinetics in anaerobic digestion of corn stover and microbial heat evolution from glucose degradation in soil (Kimura and Takahashi, 1985; Li et al., 2016). Calorimetric research of soil microbes showed that changes in microbial growth, glucose depletion as an energy source, and the evolution of heat are proportional and can all be described as a sigmoidal curve characteristic of Monod equation (Monod, 1949; Kimura and Takahashi, 1985). As a result, the microbial heat evolution curve can express the maximum specific growth rate or specific degradation rate of the substrate because of microbial respiratory activity. Experimental results in soil, for instance, determined an average heat evolution of 1287 ± 52 KJ.mol glucose^{-1} (Kimura and Takahashi, 1985). External sources of temperature, oxygen, and moisture content, however, have been demonstrated to control the maximum specific growth rate of microorganisms (Hamelers, 2004). One of the most comprehensive studies is a cardinal temperature model with inflection that describes the mathematical representation of maximum specific growth rates in the optimal and suboptimal range of temperatures from various thermophilic, mesophilic, and psychrophilic strains grown in different media (Rosso et al., 1993; Richard and Walker, 2006). Based on the cardinal temperature model with inflection model, the cardinal temperatures for *Escherichia coli* are a minimum temperature of 4.9°C, an optimum temperature of 41.3°C, and a maximum temperature of 47.5°C. Likewise, multiple linear regression has been used to describe the mathematical representation of the half-saturation coefficient of oxygen as a function of temperature and moisture in composting, ranging from −0.67 to 1.74% O_2 expressed in a volume percentage (v/v%) (Richard et al., 2006). Lastly, hydrolysis kinetic constants of corn stover (1-mm sieve material) in anaerobic digestion is reported at values from 0.04 to 0.17 d^{-1}. The cardinal temperatures of microbial growth, moisture in suboptimal conditions, and hydrolysis and heat evolution per unit substrate kinetics have not been researched in aerobic corn stover storage environments.

State-of-the-art kinetic models of composting are mostly inductive, governed by a data-oriented approach, including first-order kinetic reactions and multiplicative environmental factors that change growth and microbial respiratory activity using composting rates (Hamelers, 2004). Heat transfer and water vapor transfer models used to predict temperature and moisture in biomass, on the other hand, are deductive or mechanistic, relying not only on data but also on the laws of physics (Hamelers, 2004; Bedane et al., 2011, 2016). These existing heat transfer and water vapor transfer models have successfully represented the physics in biomass, ignoring the connections such as the dynamic response of input heat in the growth of microorganisms. For instance, a two-dimensional model based on Fick's diffusion equation, and the governing heat balance equation have been demonstrated to predict heat and moisture in woody biomass (Bedane et al., 2011). Furthermore, water vapor transport has been effectively predicted in a model with pore and surface diffusion as a lumped parameter at a variety of relative humidity percentages, from 10 to 90% (Bedane et al., 2016). Experiments at laboratory and field scales have illustrated the heat in corn stover storage systems' biological and physical processes, including microbial heat, conductive, convective, and radiative heat transfer (Wendt et al., 2014, 2018). The individual heat evolution processes, both microbial and physical, and coupling mechanisms of heat in the thermal system of corn stover, are still unknown.

This research seeks to understand the dynamic mechanisms of the thermal system, organic matter loss, and microbial heat generation in corn stover storage through system dynamics, a mathematical modeling approach of systems, and response analysis to improve the system performance (Ogata, 1998). Aleatory and epistemic uncertainties must be considered and differentiated in the construction of the mathematical model (Oberkampf et al., 2004a). This study finds epistemic uncertainties, including the specific degradation rate, cardinal temperatures of microbial growth, thermal conductivity, and heat evolution per unit substrate degraded. We deal with these uncertainties through calibration, a process of improving the agreement between the computational and benchmark experimental results by adjusting the parameters of the model (Trucano et al., 2006). To assess that the model accurately represents the system, we measure the agreement between computational and a variety of experimental results through validation (Oberkampf and Barone, 2006). This study systematically assesses the predictive capability of a system dynamic corn stover storage reactor through model calibration and validation.

EXPERIMENTAL AND COMPUTATIONAL METHODOLOGIES

To understand the dynamic mechanisms of microbial heat in aerobic corn stover storage, we must evaluate the predictive capability of the dynamic lumped thermal system following a systematic validation and calibration of the computational model with the experimental data from the corn stover storage reactors. **Figure 1** illustrates the validation, calibration, and prediction approach (Oberkampf and Barone, 2006) applied to the corn stover thermal model. In this approach, first, we obtain the input quantities to develop the computational model from the experimental work in the corn storage reactors. Second, we compare the validation metric, which is system temperature and substrate, of the computational and experimental results to measure the accuracy of the model. Third, we establish an engineering decision based on the expected accuracy of the model, where a feedback loop is taken for additional calibration to reduce the error of the model relative to the experimental data. Lastly, we evaluate the predictive capability of the model with a blind computational prediction of additional corn stover storage reactor operating conditions. For an extensive study of validation, calibration, and prediction approach, see references: (Oberkampf et al., 2004a; Oberkampf and Barone, 2006). The next sections describe the methodologies of the experimental work in the corn storage reactors, computational dynamic lumped thermal system, and the validation, calibration, and prediction process.

Corn Stover Storage Reactor Experiments

The laboratory-scale corn stover storage reactors studied in this research are located at Idaho National Laboratory, loaded with corn stover harvested in Hardin County, Iowa, in October 2018. The storage reactors consist of four replicates, and each reactor has a total volume of 100-L and a 76-L working volume. A complete description of the design and operation of the storage reactors can be found in: (Wendt et al., 2014; Bonner et al., 2015) and is illustrated in **Figure 2**.

The loaded biomass was compressed at 3.9 kPa at five 300 s intervals. Moisture content was determined by collecting five representative samples and drying at 105°C for 24 h in a Shel Lab forced air oven (Sheldon Manufacturing, Cornelius, OR, United States). Additionally, water exiting the reactors was collected and measured using a condensing column cooled with a solution of water and propylene glycol. To allow biomass drying during storage, we controlled the airflow by mass flow controllers (Brooks Instruments, Hatfield, PA, United States). The airflow rates in reactors 1 and 2 were 0.25 standard liters per minute (slpm) and 1.0 slpm in reactors 3 and 4. Airflow rates of 0.25 and 1.0 slpm were selected because they demonstrated significant differences in the microbial activity in corn stover storage systems (Wendt et al., 2014). Corn stover biomass was stored for 34 days in reactors 1 and 2 and 11 days in reactors 3 and 4.

Each reactor contained four resistance temperature detectors (RTDs) and 15 K-type thermocouple wires (Omega Engineering, Norwalk, CT, United States) placed throughout the biomass to measure temperature. Circulating water surrounded each reactor jacked set to offset the internal temperature by −0.5°C, controlled through a feedback loop between a Labview (National Instruments, Austin, TX, United States) control interface and the RTDs. Corn stover and water jacket temperatures data were collected and exported to a text file every minute from the RTDs and every 5 minutes from the thermocouples.

Gas chromatography was used to measure the concentration of O_2, N_2, and CO_2 in the reactors' off-gas with an Agilent 490 Micro GC (Santa Clara, CA, United States). Gas samples were initially collected each hour along with a sample of ambient air, and the data were exported to a spreadsheet, where they could be analyzed daily. As the biomass degradation rate decreased, reaching a quasi-steady CO_2 production, sample frequency was decreased from one to 6 h. We assumed glucose oxidation is a suitable representation in these experiments, calculated from the CO_2 data and the empirical formula (Porges et al., 1956) to estimate dry matter loss as follows:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Heat}$$

$$CH_2O + O_2 \rightarrow CO_2 + H_2O + \text{Heat}$$

The corn stover temperatures measured in storage reactors and substrate degradation calculated from CO_2 data were used for validation and calibration of the system dynamic

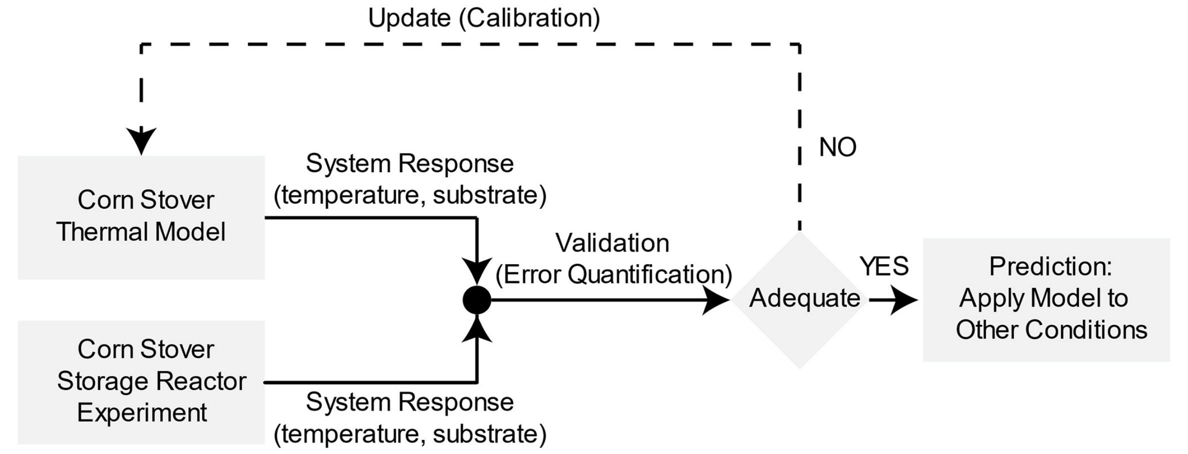

FIGURE 1 | Validation, calibration, and prediction of corn stover storage reactor dynamic thermal system. Adapted from Oberkampf and Barone (2006).

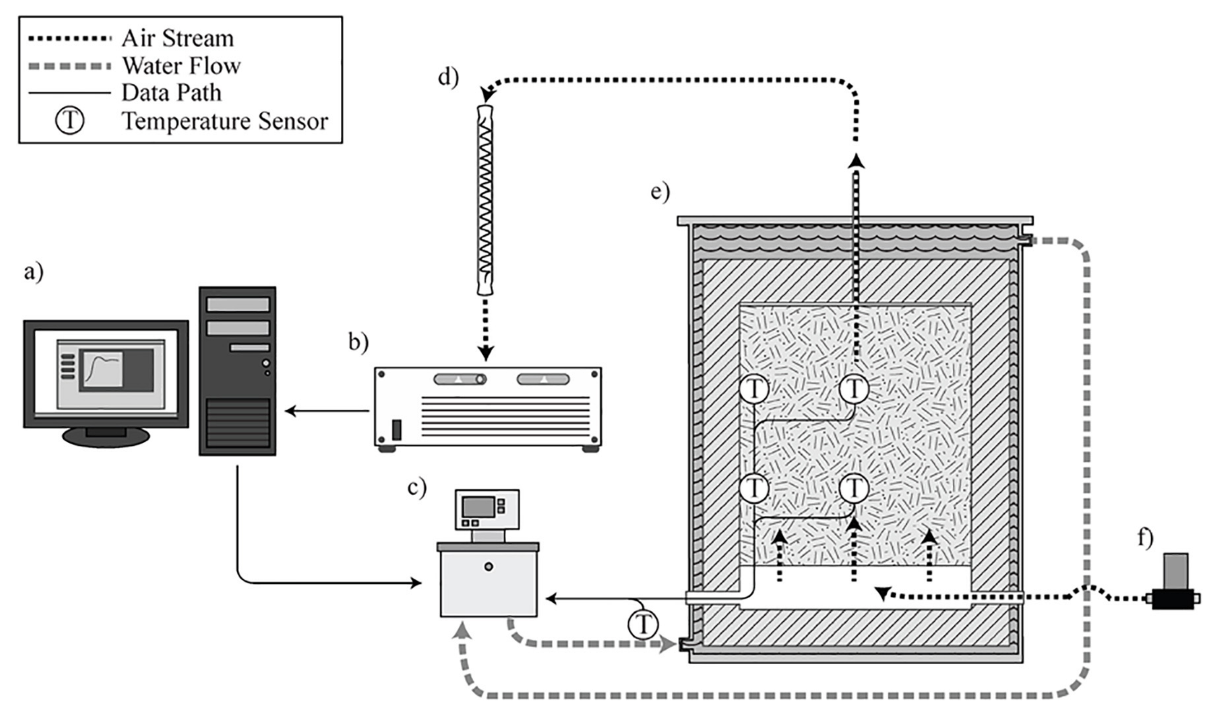

FIGURE 2 | Operational illustration of the laboratory reactor system. **(a)** LabView control interface and data logging. **(b)** Gas chromatograph. **(c)** Heated water circulator. **(d)** Vapor condenser. **(e)** Reactor loaded with biomass in operation. **(f)** Mass flow controlled gas supply (Wendt et al., 2014; Bonner et al., 2015).

model. For modeling purposes, the corn stover temperature, ambient temperature, loading dry matter mass, airflow rates, and moisture uncertainties are small and are treated as deterministic. The initial conditions of these model inputs are summarized in **Table 1**.

TABLE 1 | Model inputs for dynamical formulation.

Parameter	Reactor 1	Reactor 2	Reactor 3	Reactor 4
Initial corn stover temperature (°C)	10.6	12.9	13.8	13.5
Ambient temperature (°C)	24.2	24.3	24.0	23.5
Loading dry matter (g)	6456	7080	7071	7290
Air flow rate ($cm^3.min^{-1}$)	365	347	1265	1209
Initial moisture content (%)	30.86	29.86	28.99	29.15
Final moisture content (%)	15.46	16.93	18.01	18.38
Storage time (days)	34.7	34.1	10.7	10.7

System Dynamics Formulation

The system temperature of the corn stover storage reactor is an essential metric to the model because of its effects on the respiration of the microorganisms and in the organic matter degradation (Kimura and Takahashi, 1985; Rosso et al., 1993; Richard and Walker, 2006; Richard et al., 2006). To understand the thermal parameters that influence thermal conditions and, therefore, microbial respiratory activity, we developed a lumped thermal system model (Palm, 1983; Ogata, 1998; Incropera et al., 2007; Quiroz-Arita et al., 2020). The model of the corn stover storage reactor considers microbial heat evolution, conductive heat transfer, convective heat transfer, evaporation, and the bulk thermal capacitance of the corn stover biomass. An energy balance was carried out using a single thermal node, assuming a thermally homogeneous reactor, and the resulting ordinary differential equation was solved numerically. This dynamic thermal model is described in the following sections.

Microbial Heat Evolution

Heat evolution is associated with an increase in biomass growth and substrate depletion (Kimura and Takahashi, 1985). As described in section "Corn Stover Storage Reactor Experiments," we calculated substrate degradation from the experimentally measured CO_2 and the empirical formula of glucose used for validation and calibration of the system dynamics model. Substrate degradation modeling has been proposed as a first-order differential equation for composting processes, including multiplicative environmental factors that change the biological response (Hamelers, 2004):

$$\frac{dS}{dt} = -k_s \cdot f(T) \cdot f(M) \cdot (S_0 - S) \tag{1}$$

Where S is the substrate, S_0 the initial substrate conditions, k_s the substrate decay rate, $f(T)$ the temperature factor, and $f(M)$ the moisture factor. $f(T)$ was computed from Eq. (2) (Rosso et al., 1993; Richard and Walker, 2006), describing the substrate decay rates in the optimal (T_{opt}) and suboptimal (T_{max}, T_{min}) range of temperatures (T) of the growth phases in the stored corn stover. T_{opt}, T_{max}, and T_{min} were treated as epistemic uncertainties, as described in section "Validation, Calibration, and Prediction." $f(M)$ was computed from the Monod Eq. (3), assuming a linear drying rate of corn stover moisture content (M) presented in **Table 1** during storage. The 25% value in Eq. (3) corresponds to M at half the maximum specific growth rate, determined from dry matter loss experiments conducted at 20, 25, 30, 36, and 50%.

$$f(T) = \frac{((\mathrm{T} - \mathrm{T_{max}}) \cdot (\mathrm{T} - \mathrm{T_{min}})^2)}{((\mathrm{T_{opt}} - \mathrm{T_{min}}) \cdot ((\mathrm{T_{opt}} - \mathrm{T_{min}}) \cdot (\mathrm{T} - \mathrm{T_{opt}}) - (\mathrm{T_{opt}} - \mathrm{T_{max}}) \cdot (\mathrm{T_{opt}} + \mathrm{T_{min}} - 2 * \mathrm{T})))} \quad (2)$$

$$f(M) = \frac{\mathrm{M}}{(0.25 + \mathrm{M})} \quad (3)$$

Substrate degradation, microbial growth, and heat can be described as a sigmoidal curve characteristic of Monod equation (Monod, 1949; Kimura and Takahashi, 1985). Therefore, CO_2 and microbial heat are proportional to the substrate degraded in the corn stover storage reactor. Anaerobic digestion modeling strategies have described hydrolysis and biogas as a first-order differential equation, including a conversion coefficient from the substrate to product (Vavilin et al., 2008; Quiroz-Arita et al., 2019). CO_2 and microbial heat (Q_m), therefore, were computed from Eqs. (4) and (5). The conversion coefficient (y_{CO2}) from the substrate to CO_2 is 1.44 g CO_2/S, calculated from the experiments described in section "Corn Stover Storage Reactor Experiments." The conversion coefficient from the substrate to microbial heat (y_m), was treated as an epistemic uncertainty described in section "Validation, Calibration, and Prediction."

$$\frac{dCO_2}{dt} = y_{CO2} \cdot S \quad (4)$$

$$\frac{dQ_m}{dt} = y_m \cdot S \quad (5)$$

Conductive Heat Transfer

Thermal conductivity governs the rate of heat dissipation in the corn stover storage (Karki et al., 2015). Heat transport by conduction was experimentally performed with a feedback loop through a water jacket in the corn stover storage reactor, as described in section "Corn Stover Storage Reactor Experiments" Conductive heat transfer (Q_k) is modeled as a function of the thermal conductivity (K), the characteristic length (L), the heat flux area (A), and the net temperature difference between the corn stover and water jacket ($T_2 - T_1$) following Eq. (6) (Incropera et al., 2007). The spatially distributed reactor's temperatures obtained from the 15 K-type thermocouple wires (section "Corn Stover Storage Reactor Experiments") demonstrate that the heat is diffusing faster near the top flange of the reactor, suggesting heat losses through the stainless-steel parts of the reactor as illustrated in **Supplementary Material**. K and A, therefore, are treated as epistemic sources of uncertainty, as explained in section "Validation, Calibration, and Prediction." The value of L is 0.08 m. The water jacket temperature was obtained from the experimental work as described in section "Corn Stover Storage Reactor Experiments." and the corn stover temperature is numerically solved, as described in section "Thermal Capacitance."

$$Q_k = -\frac{K}{L} \cdot \mathrm{A} \cdot (T_2 - T_1) \quad (6)$$

Convective Heat Transfer

Heat is also transported from the corn stover to the local atmosphere through convective heat transfer (Palm, 1983; Bergman et al., 2011). Convective heat transfer (Q_h) is modeled as a function of the net temperature difference between the corn stover and the ambient temperature ($T_2 - T_1$) and a heat transfer coefficient (h_i) (Bergman et al., 2011):

$$Q_h = -h_i \cdot \mathrm{A} \cdot (T_2 - T_1) \quad (7)$$

h_i (8) is estimated from the Nusselt number (N_{ux}) (9), the air thermal conductivity (k), and L. The Nusselt number is a function of the Reynolds number (Re) and the Prandtl Number (Pr) (10). The Prandtl number is a function of kinematic viscosity (ν), thermal diffusivity (α) (11), thermal conductivity (k), fluid density (ρ), and fluid specific heat (C_p). The Reynolds number (12) is a function of the fluid velocity (u), L, and the kinematic viscosity (ν) (Bergman et al., 2011).

$$h_i = \frac{N_{ux} \cdot k}{L} \quad (8)$$

$$N_{ux} = 0.0296 \cdot Re^{4/5} \cdot Pr^{1/3} \quad (9)$$

$$P_r = \frac{\nu}{\alpha} \quad (10)$$

$$\alpha = \frac{k}{\rho \cdot C_p} \quad (11)$$

$$Re = \frac{u \cdot L}{\nu} \quad (12)$$

Evaporation Heat Loss

Thermal energy can be lost from the system through evaporation (Incropera et al., 2007). For the case of the corn stover storage reactor, evaporation losses were measured daily, and the rate (E) was computed as the derivative of the condensate volume ($\frac{\Delta V}{\Delta t}$) as described in section "Corn Stover Storage Reactor Experiments." The specific enthalpy (h) due to evaporation was used in the heat balance, 2257 kJ kg^{-1} evaporated water, to compute the thermal energy loss:

$$E = -h \cdot \frac{\Delta V}{\Delta t} \quad (13)$$

Thermal Capacitance

The thermal capacitance (C_{th}) of the corn stover biomass is defined as the capacity of the system to store thermal energy (Palm, 1983; Ogata, 1998). This characteristic is a function of thermal properties of the system including density (ρ), volume (V), and the specific heat ($c(wet)$):

$$C_{th} = \rho \cdot V \cdot c(wet) \quad (14)$$

Corn stover density at sieve materials sizes of 2 mm, 4 mm, and 8 mm are reported at 942, 954, and 832 $kg.m^{-3}$, respectively

(Karki et al., 2015). The uncertainty of density is assumed negligible in the model. The volume of the corn stover storage reactor is 0.074 m^3. Previous authors have demonstrated that the specific heat of woody biomass depends on temperature (T) and M (Ragland et al., 1991). This dependence has not been researched for corn stover. Therefore, we used the relationship for dry wood biomass [$c(dry) = KJ\ kg^{-1}\ K^{-1}$] as given by Eq. (15) (TenWolde et al., 1988; Ragland et al., 1991). A correction factor term for the specific heat of wet wood biomass [$c(wet) = KJ.kg^{-1}.K^{-1}$] is recommended to account for the energy absorbed by the wood-water bonds as given by Eq. (16) (TenWolde et al., 1988; Ragland et al., 1991).

$$c(dry) = 0.1031 + 0.00386 \cdot T \tag{15}$$

$$c(wet) = [c(dry) + 4.19 \cdot M]/(1 + M) + (0.02355 \cdot T - 1.32 \cdot M - 6.191) \cdot M \tag{16}$$

Lastly, the thermal capacitance of stainless steel was considered in the total thermal capacitance by assuming a stainless-steel density of 7750 kg.m^{-3} and a specific heat of 480 J kg^{-1} K^{-1}. The stainless-steel volume (V_{ss}) was treated as an epistemic uncertainty.

Energy Balance and Dynamic Thermal Simulation

The heat balance (q_{th}) was computed by considering Q_m, Q_k, Q_h, and E following Eq. (17). The time history of the corn stover storage temperature (18) is numerically calculated using the Dormand–Prince (RKDP) method in Matlab® at a variable time step for reactors 1 through 4. The theory of the RKDP numerical analysis method can be reviewed in Prince and Dormand (1981).

$$q_{th} = Q_m + Q_k + Q_h + \mathrm{E} \tag{17}$$

$$\frac{dT}{dt} = \frac{1}{C_{th}} \cdot q_{th} \tag{18}$$

Validation, Calibration, and Prediction

The predictive capability of the system dynamics model is evaluated using the dataset gathered at the corn stover storage reactor, as described in section "Corn Stover Storage Reactor Experiments." The temperatures of the corn stover and dry matter losses were used to quantify the error between model and experiment for the system dynamics model. The error of the system dynamics model, temperature and substrate, is quantified as the difference between each experimental data point (Y_i) and the value of the model at each time step [$f(x)_i$] (19) (Oberkampf et al., 2004b). The dynamic thermal model error was quantified by the mean absolute relative error (20). The predicted dry matter loss relative error is quantified from Eq. (21) at quasi-steady state.

$$error = f(x)_i - Y_i \tag{19}$$

$$Mean\ Absolute\ Relative\ Error = 1/n \cdot \sum_{i=1}^{n} \left| f(x)_i - Y_i \right| \tag{20}$$

$$Relative\ error = \left(\left| Y - f(X) \right| / Y \right) \cdot 100 \tag{21}$$

The parameters of the storage reactor system are calibrated using the data from reactor 2. Calibration was performed to estimate epistemic uncertainties, including the cardinal temperatures of microbial growth, the heat evolution per unit substrate degraded, substrate decay rate, heat flux area, and stainless-steel volume. These parameters, the baseline, and bounds values are summarized in **Table 2**. The parameters were simultaneously calibrated by minimizing the error of the model concerning the experimental data based on a cost function (22) using the Levenberg–Marquardt algorithm to solve the non-linear least-square problem with a parameter tolerance of $1e^{-6}$ in Matlab. For an extensive theory of the Levenberg–Marquardt algorithm, see reference: (Moré, 1978).

$$Cost\ Function = \sum error^2 \tag{22}$$

These calibrated parameters were then used for model validation using the model input data (**Table 1**) from reactors 1, 3, and 4 (Oberkampf et al., 2004b; Ferson et al., 2008; Roy and Oberkampf, 2011). The propagated uncertainty in the overall system includes the uncertainty in inputs from the validated system dynamics model (Roy and Oberkampf, 2011).

TABLE 2 | Parameters, baseline, and bounds for the system dynamics model calibration.

Parameter	Baseline	Lower bound	Upper bound
T_{opt} (°C)	41.3	35	55
T_{min} (°C)	4.9	0	20
T_{max} (°C)	65	55	75
y_m ($J.g^{-1}$)	10	0	Infinite
k_d (s^{-1})	$8.9e^{-6}$	0	Infinite
K ($W.m^{-1}.K^{-1}$)	9	6	14
A (m^2)	0.6	0.29	0.975
V_{ss} (m^3)	0.01	0	Infinite

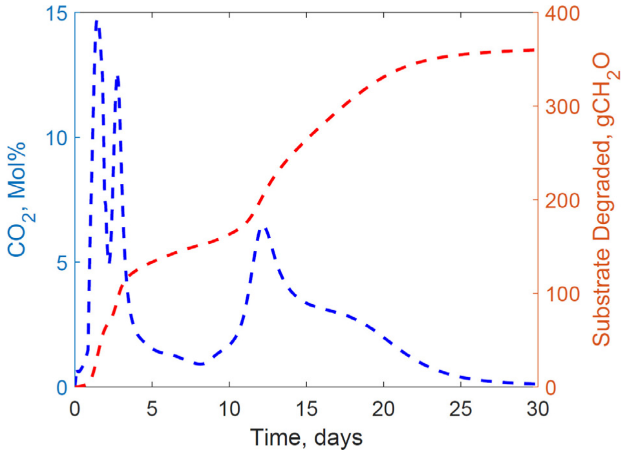

FIGURE 3 | Reactor 2 experimental carbon dioxide and calculated cumulative substrate degraded calculated from empirical glucose formula demonstrating a bi-phasic growth curve in the system.

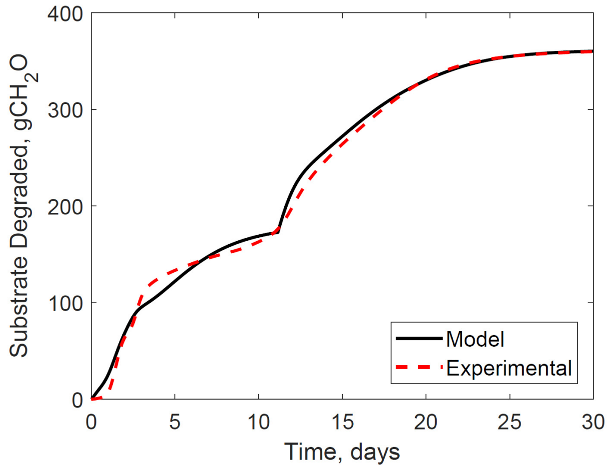

FIGURE 4 | Reactor 2 substrate degraded calibration of system dynamics model. The model inputs are 347 cc.min^{-1} airflow rates, 30% initial moisture content, 17% final moisture content, 24°C ambient temperature. The initial model condition is 7080 g. The relative error is 2.0%.

RESULTS AND DISCUSSION

The results of this research are synthesized into three components. First, we present the results of the epistemic uncertainties calibration in the microbial system dynamics model, compare the substrate degraded results gathered from the experiments in the storage reactor 2, and discuss the relevance of understanding the cardinal temperatures in the bi-phasic growth in corn stover. Second, we present the results of the epistemic uncertainties calibration in the dynamic thermal model, compare the thermal results gathered from the experiments in the storage reactor 2, and discuss the implications of the laboratory-scale model in the development of field-scale storage models. Lastly, we validate the system dynamics model to evaluate predictive capability under different operating conditions. The temperature and substrate degraded error of the model is assessed for the storage reactors 1, 3, and 4. We discuss the importance of developing a better understanding of the microbial communities and biomass characteristics in the development of system dynamics models.

Model Calibration Estimated the Epistemic Uncertainties in Bi-Phasic Microbial Substrate Degradation and Heat Generation in Corn Stover Storage

This section presents the calibration results of the substrate degradation model using the dataset gathered from reactor 2. **Figure 3** illustrates the experimental CO_2 measured through gas chromatography and the cumulative substrate degraded, calculated from the empirical glucose formula. Corn stover

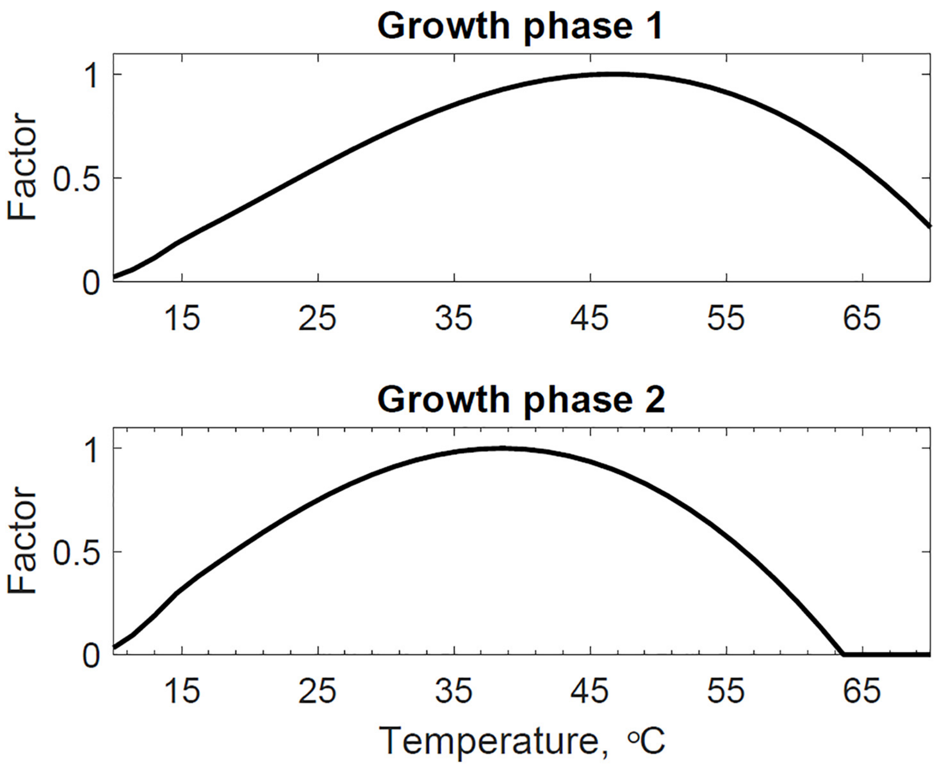

FIGURE 5 | Reactor 2 calibrated cardinal temperatures for two growth phases. The first growth phase optimum, minimum, and maximum temperatures are 46.7, 4.4, and 73.6°C. The second growth phase optimum, minimum, and maximum temperatures are 38.5, 5.1, and 63.6°C.

storage in reactor 2 exhibited a bi-phasic microbial growth, demonstrated through CO_2 spikes at 0 and 11 days, and the bi-phasic exponential curve in the substrate degraded. Bi-phasic or diauxic growth was defined by Monod (1949) and Chu and Barnes (2016) as a bi-phasic exponential growth and intermitted lag-phase in cultivating media with two carbon sources. Monod, for instance, identified this bi-phasic growth in *E. coli* cultures with glucose and lactose media, where he observed the strain utilized lactose as a secondary carbon source after complete glucose depletion. The identification of the microbial communities, chemical characterization, and fractionation in the corn stover used in our experiments is beyond the scope of this study. While kinetics is widely researched in the literature for composting, our research is the first calibrating microbial kinetics parameters of two growth phases in corn stover storage, including the cardinal temperatures, decay rate, and heat evolution per unit glucose degraded through a system dynamics model.

Figure 4 presents the calibrated substrate degraded predicted by the model and compared to the experimental dataset in the storage reactor 2. Model calibration estimated 50.1% of the substrate degraded by the first growth phase, and the remaining 49.9% by the second growth phase. The calibrated lag stage of the second growth phase is 11.2 days. The model results are consistent with the bi-phasic growth curve demonstrated in the experiments, supported by Monod and Chu and Barnes (Monod, 1949; Chu and Barnes, 2016). To accomplish the agreement between model and experimental results, we assumed moisture and temperature are the environmental factors controlling the microbial respiratory activity and substrate decay rate as previously studied in composting processes by others, including Hamelers (2004); Richard and Walker (2006), and Richard et al. (2006). This modeling strategy using dimensionless environmental factors that control microbial growth in dynamic thermal and biomass systems were successfully demonstrated in predictive algal biomass models by Quiroz-Arita et al. (2020). We computed the dimensionless moisture factor from experiments at moisture content varying from 20 to 50% and Monod equation (**Supplementary Figure S1**), where values of one represent ideal conditions for biological activity that increase dry matter losses, and values of zero representing inhibition of biological activity, thus reducing dry matter losses. The cardinal temperatures for the dimensionless temperature factor are epistemic uncertainties in our model. Rosso L. identified a minimum temperature of 4.9°C, an optimum temperature of 41.3°C, and a maximum temperature of 47.5°C temperatures for *Escherichia coli* (Rosso et al., 1993). We used these temperatures as the baseline values for calibration, except for the maximum temperature assuming a baseline temperature of 65°C for thermophiles in an uncertain range from 55 to 75°C as supported in extensive studies of these microorganisms by Brock, T.D. (Brock, 2012). **Figure 5** illustrates the calibrated cardinal temperatures for the two growth phases, one representing best microbial respiratory activity and dry matter loss conditions and zero representing inhibition.

The dimensionless temperature factor demonstrates that the system temperature dynamically controls microbial respiratory activity and substrate decay rate during storage. **Figure 6** illustrates the dynamic response of the temperature factor in reactor 2. At time zero corn stover is at 13°C, near suboptimal temperatures for the microbial respiratory activity, 4 and 5°C in this study for the first and second growth phases, respectively, resulting in slow respiration and substrate decay rates. Microbial respiration results in substrate oxidation and heat, elevating the temperature in the system near-optimal conditions, 47 and 39°C in this study, increasing microbial respiration rates, substrate decay rates, and heat. As additional microbial heat elevates the system temperature, we approach inhibiting conditions, 74 and 64°C in this study, which results in a reduction of the system temperature. **Table 3** presents the calibrated cardinal temperatures and substrate decay rates for the two growth phases in the corn stover storage reactor 2. The verification of these calibrated microbial kinetics parameters is beyond the scope of this research and can change under different environmental conditions. **Figure 7** shows that the cumulative dry matter loss and microbial heat generation curves are consistent with sigmoidal growth curves, as demonstrated by Kimura and Takahashi (1985) in calorimetric studies of soil microbes.

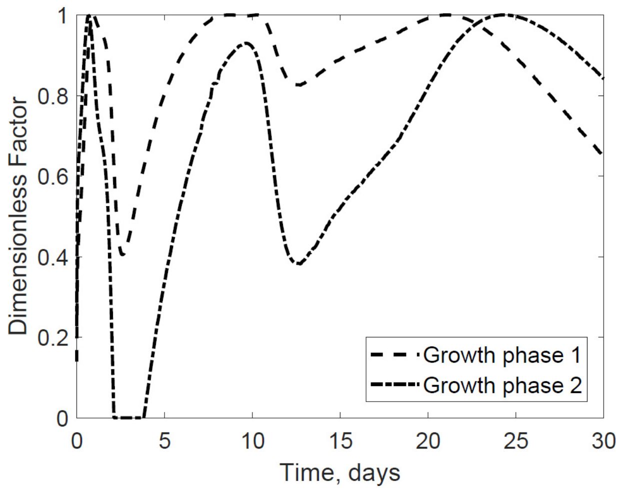

FIGURE 6 | Dynamic response of microbial activity due to variations in the thermal system. The environmental temperature (dimensionless) factor represent ideal conditions for growth and organic matter loss as a value of 1. Values of zero represent inhibiting conditions that reduce microbial growth and dry matter loss. The model describes how environmental factors change through time.

TABLE 3 | Calibrated parameters for two growth phases.

Parameter	Growth phase 1	Growth phase 2
T_{opt} (°C)	46.7	38.5
T_{min} (°C)	4.4	5.1
T_{max} (°C)	73.6	63.6
k_d (s^{-1})	$7.6e^{-6}$	$8.3e^{-6}$
y_m (J g^{-1})	9.7	9.7
K (W m^{-1} K^{-1})	10.1	10.1
A (m^2)	0.6	0.6
V_{ss} (m^3)	0.01	0.01

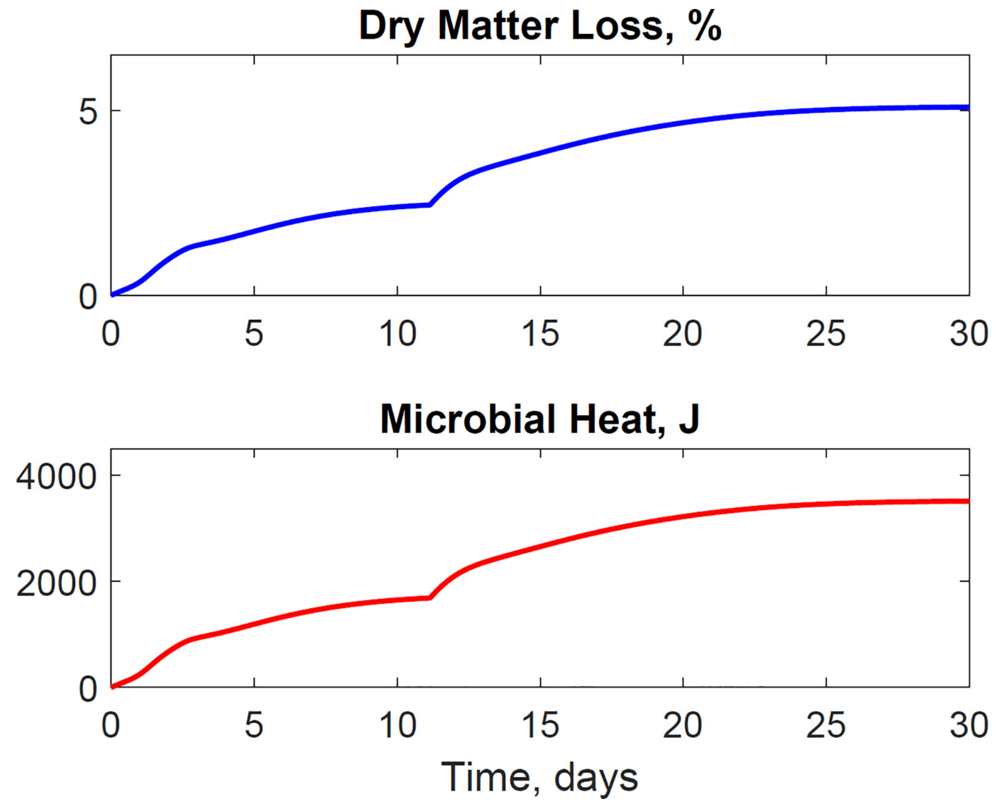

FIGURE 7 | The model illustrates the dynamics of dry matter loss and microbial heat evolution. The sigmoidal microbial heat governs the temperature rise in the system. Microbial activity and heat itself are dynamically controlled for the system temperature.

Experimental and model dry matter losses are 4.9 ± 0.28% and 5.1% as calculated from the fraction of substrate degraded of the original corn stover. Heat evolution per unit substrate degraded is an epistemic uncertainty in our model. Heat per unit substrate with other epistemic microbial kinetic parameters is calibrated to minimize the integrated error by comparison of the model to experiment substrate degraded. Our calibration process provided a heat evolution per unit substrate degraded value of 9.7 $J.g^{-1}$, three orders of magnitude lower than values obtained by Kimura and Takahashi (1985) in soil with a glucose substrate. Environmental factors can control heat evolution, including moisture, carbon sources, and microbial and engineered feedback temperature itself. A better understanding of the initial and final organic matter characteristics and rigorous data collection of water vapor with CO_2 can improve the validation of microbial heat in future upgrades of our model.

Model Calibration Estimated the Epistemic Uncertainties of the Corn Stover Storage Thermal System

The thermal system consists of the microbial heat evolution results discussed in section "Model Calibration Estimated the Epistemic Uncertainties in Bi-phasic Microbial," conductive heat transfer, convective heat transfer, evaporation heat loss, and capacitance of the system. System dynamics has been widely researched for thermal systems, our research, however, is the first applied to understand the dynamic mechanisms between the physical environment and microbial kinetics in corn stover

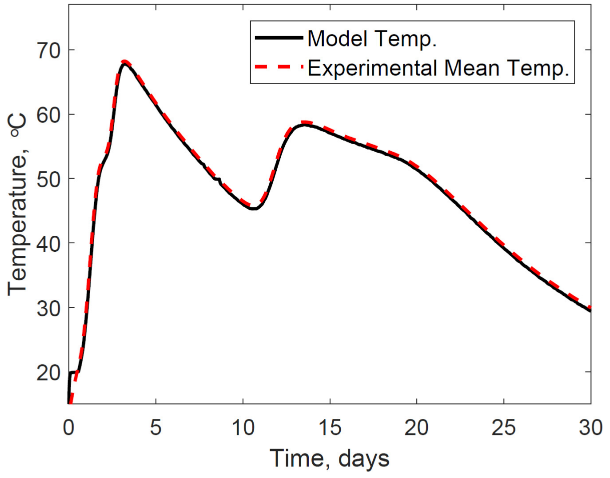

FIGURE 8 | Reactor 2 temperature calibration of the system dynamics model. The model inputs are 347 $cc.min^{-1}$ airflow rates, 30% initial moisture content, 17% final moisture content, 24°C ambient temperature. The initial model condition is 12.9°C. The mean absolute error is 0.6°C.

TABLE 4 | Model temperature and dry matter loss error.

Parameter	Reactor 1	Reactor 2	Reactor 3	Reactor 4	Mean
Mean absolute relative error (°C)	1.2	0.6	1.1	0.8	0.9 ± 0.3
Experimental dry matter loss (%)	4.6	5.0	3.5	3.8	4.2 ± 0.7
Model dry matter loss (%)	4.7	5.1	3.4	3.6	4.2 ± 0.8
Dry matter loss relative error (%)	2.2	2.0	2.9	5.3	3.1 ± 1.5

storage reactors, calibrating epistemic parameters for this specific experiment. **Figure 8** presents the calibrated system temperature predicted by the model and compared to the experimental dataset in the storage reactor 2. The system temperature demonstrates the effects of the bi-phasic microbial heat generation and substrate respiration rates discussed in section "Model Calibration Estimated the Epistemic Uncertainties in Bi-phasic Microbial."

Epistemic microbial kinetic and physical parameters in the system are calibrated to minimize the integrated error by comparison of the model to experimental temperature. The reactor's spatially distributed temperatures demonstrated that the heat is diffusing faster near the top flange of the reactor, suggesting heat losses through the stainless-steel parts of the reactor, convective heat transfer, and evaporation as illustrated in **Supplementary Multimedia Material**. **Table 3** shows the calibrated thermal parameters, including thermal conductivity (K) and heat flux area (A) used in the conductive heat transfer, and stainless-steel volume (V_{ss}) used in the thermal capacitance. Our calibration process estimated K, A, and V_{ss} of 10.1 W m^{-1} K^{-1}, consistent with values reported in the literature for stainless steel (Incropera et al., 2007), and 0.6 m^2 and 0.01 m^3, physically possible for the storage reactor dimensions. **Supplementary Figures S6**, **S7** illustrate other means of heat loss than heat diffusion, including the convective heat transfer and the evaporation heat loss. We obtained an experimental heat transfer coefficient of 2.2 W m^{-2} K^{-1}, in agreement with values used in natural convection of gases reported in the literature, 2–25 W m^{-2} K^{-1} (Incropera et al., 2007). Evaporation heat loss was experimentally computed from the derivative of the condensate volume and the specific enthalpy. Lastly, **Supplementary Figure S8** illustrates the specific heat of biomass. The values obtained in the model are consistent with dry wood values, 1200–1500 J kg^{-1} K^{-1} (Ragland et al., 1991), 20% moisture content wood, 1700–2300 J kg^{-1} K^{-1} (Ragland et al., 1991), and dry corn stover, 1395–1610 J kg^{-1} K^{-1} (Dupont et al., 2014). The biomass

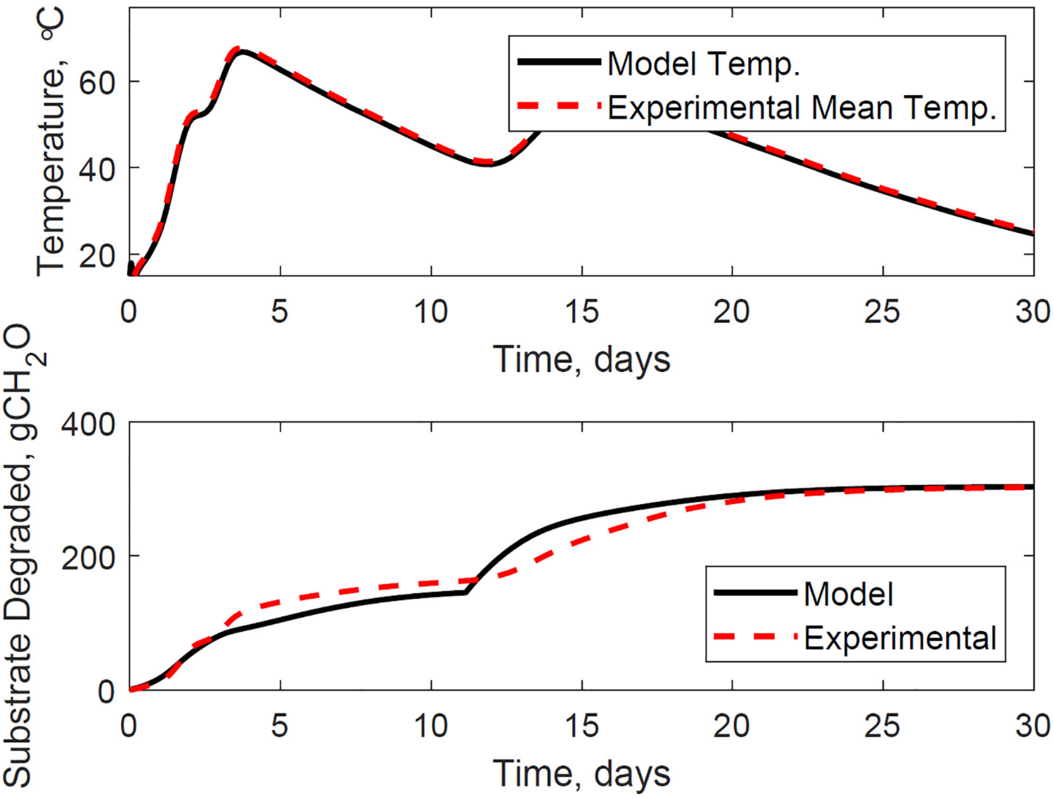

FIGURE 9 | Reactor 1 temperature **(top)** and substrate degraded **(bottom)** validation of system dynamics model. The model inputs are 365 cc.min^{-1} airflow rates, 31% initial moisture content, 15% final moisture content, 24°C ambient temperature. The initial model conditions are 10.6°C and 6456 g. The temperature mean absolute error is 1.2°C, and the dry matter loss relative error is 2.2%.

specific heat governs the capacity of corn stover to store heat in the system.

Model calibration of the thermal system model reduced the mean absolute relative error to 0.6°C, calculated from the transient error (**Supplementary Figure S2**). Each reactor was surrounded with a circulating water jacket set to offset the internal temperature by −0.5°C, therefore, predicted temperatures below this value could have negative implications in thermal conductivity calculations. **Supplementary Figures S3, S4**, for instance, illustrate time intervals during storage where corn stover temperature is above the water jacket temperature, expecting thermal diffusion from the corn stover to the surrounding water jacket. However, model corn stover temperatures in such time intervals that mispredict values below the water jacket governed the diffuse of heat from the water jacket to the corn stover. **Supplementary Figure S5** illustrates these implications in the calculations of conductive heat transfer, where positive values represent the sources of errors in our model. The closed-loop feedback system controller starts at temperatures above 20°C. Therefore, heat diffusion from the water jacket to the corn stover is expected during the lag phase of microbial respiration, and heat diffusion from the corn stover to the water jacket is expected in the log phase of microbial respiration. The constraints of our model are because of the temperature control strategy in the surrounding water jacket. Under field storage conditions, however, such controlling strategies are absent, and a better prediction of conductive heat transfer between the ambient air and corn stover is expected.

Model Validation Demonstrated the Predictive Capability of the Storage Reactor System

The performance of the system dynamics calibrated model is evaluated using a dataset not used for calibration, including reactors 1, 3, and 4. **Table 1** includes the model inputs, and **Table 3** presents the calibrated parameters used in model validation. We quantified the error of the system responses, temperature, and substrate degraded against the dataset gathered from experiments in reactors 1, 3, and 4. **Table 4** synthesizes the system temperature and dry matter loss error quantification of the model for reactor 2, used for calibration, and reactors 1,3, and 4 used for validation. The mean absolute relative error quantifies the system temperature error, and the dry matter loss relative error quantifies the system substrate degraded error.

Figure 9 illustrates the evaluation of the model using the dataset of reactor 1, which was not used for calibration. The calibrated model successfully predicted the temperature and substrate degraded of the system, where the mean absolute relative error is 1.2°C, and the dry matter loss relative error is 2.2%. Reactor 1 presents the bi-phasic growth characteristics of Reactor 2. Therefore, the substrate degraded fraction for each

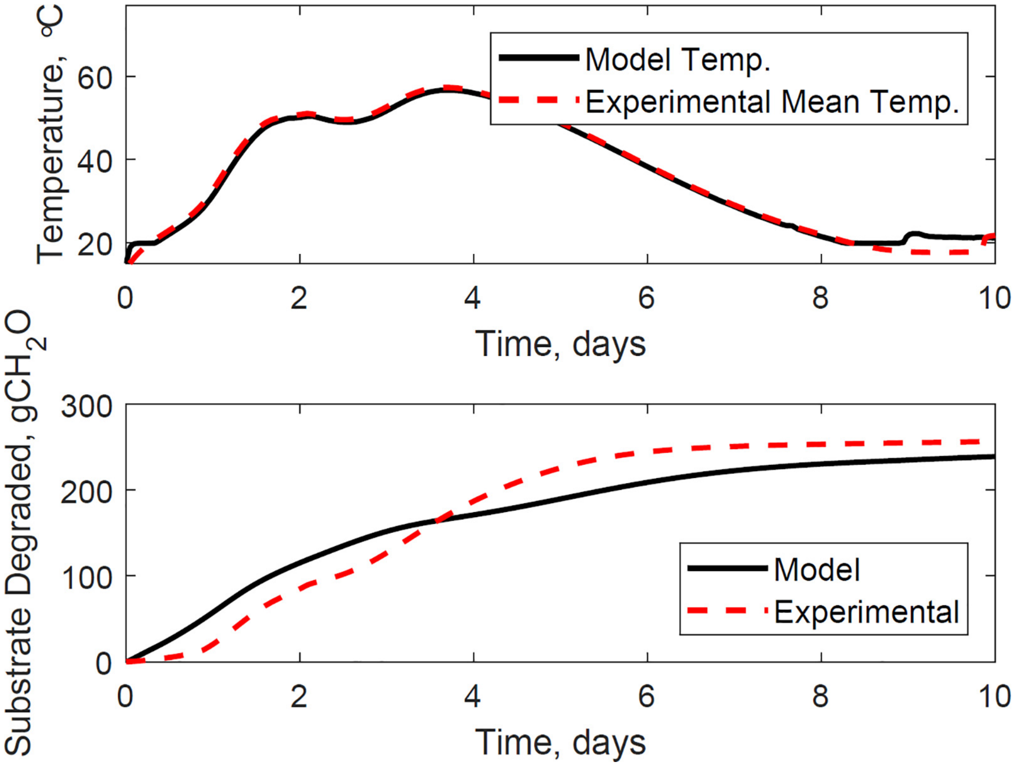

FIGURE 10 | Reactor 3 temperature **(top)** and substrate degraded **(bottom)** validation of system dynamics model. The model inputs are 1265 cc.min^{-1} airflow rates, 29% initial moisture content, 18% final moisture content, 24°C ambient temperature. The initial model conditions are 13.8°C and 7071 g. The temperature mean absolute error is 1.1°C, and the dry matter loss relative error is 2.9%.

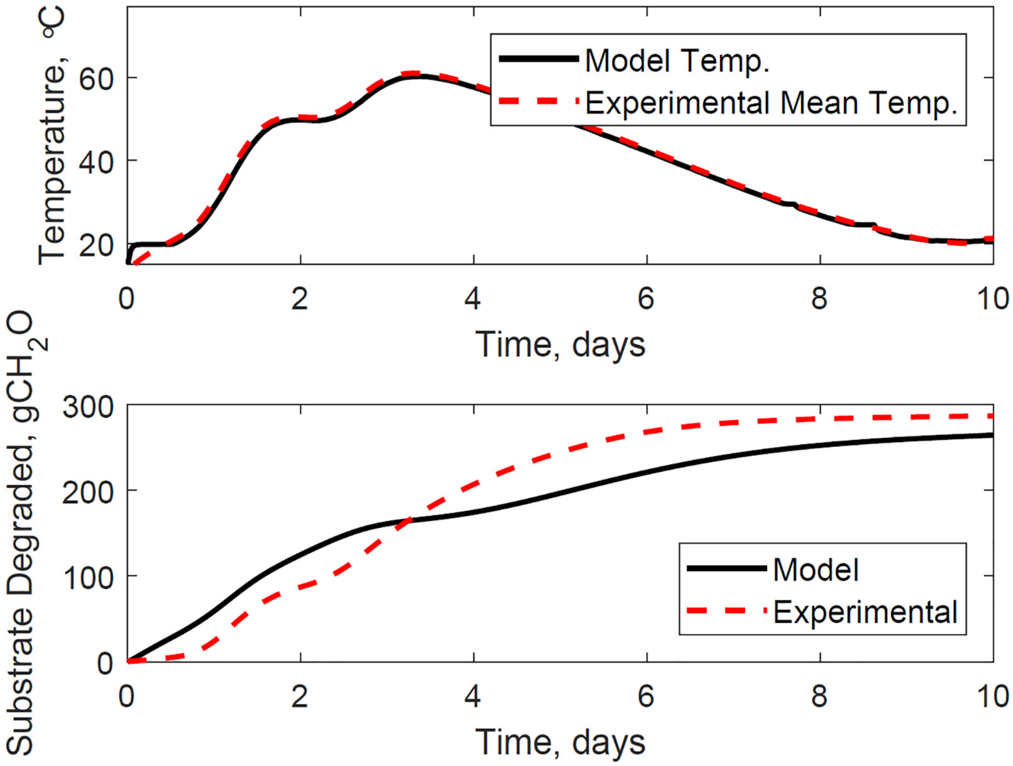

FIGURE 11 | Reactor 4 temperature **(top)** and substrate degraded **(bottom)** validation of system dynamics model. The model inputs are 1209 $cc.min^{-1}$ airflow rates, 29% initial moisture content, 18% final moisture content, 24°C ambient temperature. The initial model conditions are 13.5°C and 7290 g. The temperature mean absolute error is 0.8°C, and the dry matter loss relative error is 5.3%.

growth phase, and the lag stage of the second growth phase estimated for reactor 2 is valid for reactor 1. This validation was expected as both reactors 1 and 2 have similar initial conditions and model inputs, and operated airflows at 356 ± 13 $cm^3.min^{-1}$. **Figures 10**, **11** illustrate the evaluation of the model using the dataset of reactors 3 and 4, with operated airflows at 1237 ± 40 $cm^3.min^{-1}$. Reactor 3 temperature mean absolute error is 1.1°C, and the dry matter loss relative error is 2.9%. Reactor 4 temperature mean absolute error is 0.8°C, and the dry matter loss relative error is 5.3%. The calibrated model successfully predicted the system temperature of reactors 3 and 4. However, the calibrated model has constraints to represent the bi-phasic growth in the substrate degraded, which reached a steady state in a shorter residence time than reactors 1 and 2. As a result, the substrate degraded fractions for the two growth phases, and the lag stage for the second growth phase obtained through calibration in reactor 2, are not valid under higher airflow rates used for reactors 3 and 4.

Although a robust predictive capability of the calibrated model is demonstrated for the system temperature in reactors 1, 3, and 4, a higher degree of uncertainty in the substrate degraded is observed under different environments in reactors 3 and 4. We assumed in our model two growth phases represented by two differential equations, and the calibrated kinetic parameters predicted the substrate degraded for reactors 1 and 2. For reactors 3 and 4, a single differential equation and the kinetic parameters calibrated for the second phase predicted the substrate degraded with relative errors of 2.9 and 5.3% but cannot accurately represent the bi-phasic growth observed in the experimental data. The studies of the carbon sources and microbial communities existing in the experiments are beyond the scope of this research. Additionally, a better understanding of aerobic corn stover storage systems requires a more comprehensive study of the microbial response to electron acceptor variation (O_2) and lag-phase evolution. For instance, a recent study of Chu and Barnes (2016) demonstrated tradeoffs between adaptation and high growth rates in bi-phasic growth, with longer lag-phase in environments where switching carbon sources in less frequent and shorter lag-phase in environments where switching carbon sources is more frequent. These findings and the constraints observed in our system dynamics model highlight the need to refine the model inputs, including the existing carbon sources and microbial strains, and develop a better understanding of lag-phase adaptation in corn stover storage systems.

CONCLUSION

Calibration and validation of an aerobic storage reactor system demonstrated an average predictive temperature mean absolute relative error of 0.9 ± 0.3°C and dry matter loss relative error of 3.1 ± 1.5%. The thermal and substrate degraded models were calibrated using data set from reactor 2, and the predictive capability was demonstrated using data sets from reactors 1, 3, and 4. These models show that lumped-parameters assumptions for thermal and substrate degraded in corn stover storage reactors are well-founded. The constraints of our model indicate the importance of developing a better understanding of the initial and final carbon sources, and rigorous data collection of water vapor with CO_2 to validate microbial heat. Additionally, model development under field storage conditions is expected to contribute to a better prediction of conductive heat transfer between the ambient air and corn stover. Lastly, a comprehensive characterization of carbon sources and microbial communities, and lag-phase study in corn stover storage systems will expand the predictive capability of the model under other spectrums in the environment. This contribution will allow us to scale the model to field conditions incorporating seepage, convective heat transfer under wind and bale orientation, precipitation, evaporation, and radiation. Future model development under field conditions will contribute to engineering strategies to control microbial activity, minimize dry matter loss, reduce variability for biofuel conversion facility, and improve technology sustainability.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

CQ-A was the lead author, developed the system dynamics model, performed the model calibration and validation, and drafted the manuscript. JM and WS performed the storage reactors operation and data acquisition. CQ-A, JM, MP, LW, and WS performed the data analysis. LW and WS revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2020.00777/full#supplementary-material

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Effects of Storage Moisture Content on Corn Stover Biomass Stability, Composition, and Conversion Efficacy

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Variable moisture content of biomass during storage is known to compromise feedstock stability, yet a great deal of uncertainty remains on how to manage or mitigate the issue. While moisture contents above 20% risk unacceptable losses in aerobic feed and forage storage, no quantitative relationship exists between corn stover moisture content and rates or extents of degradation for bioenergy use. This work quantifies the relationship between initial moisture content of aerobically stored corn (*Zea mays* L.) stover biomass and dry matter loss through time. Corn stover with 20% to 52% moisture was stored under aerobic conditions in laboratory reactors while dry matter loss was measured in real time, reaching extents of 8% to 28% by the end of storage. Rates and extents of degradation were proportional to moisture content but were not linearly related. A moisture content "threshold" exists between 36% and 52% above which rates and extents of degradation increase rapidly. Compositional changes included glucan and lignin enrichment resulting from hemicellulose component (xylan and acetyl) biodegradation. Moisture desorption characteristics of the post-storage materials suggest chemical and/or physical changes that increase biomass hydrophilicity. Monomerization of carbohydrates though dilute acid pretreatment and enzymatic hydrolysis resulted in only minor changes, suggesting that degradation does not negatively influence conversion potential of the remaining biomass. Total dry matter preservation remains one of the most significant challenges for a biorefinery.

Keywords: biomass, dry matter loss, aerobic storage, feedstock logistics, composition, sugar release

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INTRODUCTION

Corn (*Zea mays* L.) stover has been targeted as an early adoption feedstock for pioneer biofuel production in the United States because of its current availability and low cost (Hess et al., 2009). However, use of agricultural residues challenges the ability to control biomass quality as harvest timing and operations are dictated by the primary crop. Because stover biomass is not the highest priority during grain harvest, variations in biomass conditions, specifically moisture content, can be large within a given year and between years (Kenney et al., 2013). Corn stover moisture contents at harvest are predicted to exceed 40% (wet basis) nationwide over approximately one third of the United States in an average year (Oyedeji et al., 2017). Moisture contents >20% threaten the stability of aerobically stored feedstock (Darr and Shah, 2014). For a year-round conversion facility dependent on a seasonally available feedstock, uncertainty of losses throughout long term storage can have serious consequences relative to operational efficiency and costs at the biorefinery (Rentizelas et al., 2009; Darr and Shah, 2014).

Numerous research efforts have demonstrated the volatility of dry matter loss of biomass stored in various configurations and across a range of moisture contents (McGechan, 1990; Shinners et al., 2007, 2010, 2011; Shah et al., 2011; Smith et al., 2013). However, because of the inherent challenges related to environmental control, sampling, and replication of field-scale storage studies, no conclusive relationships have been developed to describe the rates and extents of dry matter loss in a way that enables proactive decision making throughout a year-long storage period. Without a functional understanding of biomass losses during storage it is difficult to accurately assess the quantity of feedstock that must be procured and how that supply should be handled throughout the year. To combat this, analyses of feedstock logistics systems typically assume users will over-purchase to ensure that an adequate supply of biomass is on hand to cover a generalized – or assumed "average" – dry matter loss (Rentizelas et al., 2009). While this method is effective for "average" years, it does not account for instances beyond the norm. Considering the severe droughts and flooding events that have impacted corn production in the United States Midwest over the past several years, an understanding of "abnormal" conditions on feedstock logistics is prudent and necessary.

The costs for uncertain losses in storage impact a range of stakeholders within a bioenergy production system. In cases where on-farm storage is employed and payment for the biomass is conducted at the time of delivery to a biorefinery, the farmer incurs the direct financial consequence of storage losses. In this case, the farmer invested in the harvest, collection, and storage of an initial mass of material, but was only able to deliver and be paid for the original mass less dry matter losses, effectively inflating their production costs and reducing their profit. Two primary concerns arise in this scenario: (1) how does this reduced return impact grower satisfaction and continued participation in biomass production, and (2) is the end-user prepared to source additional material (presumably from greater distances and at greater costs) to offset the losses suffered in storage? On the other hand, if centralized or satellite storage is used in a way where the refinery owns the biomass immediately after harvest, losses during storage effectively increase the end-users realized feedstock price. For example, if feedstock was purchased at a farm-gate price of 40 \$ tonne^{-1} and the material suffered 10% loss in storage before being used, the as-recovered material is being consumed at price of 44.4 \$ tonne^{-1}. This represents a 10% loss in revenue for the producer/farmer. For end-users processing hundreds of thousands of tonnes per year, such cost increases and uncertainties in available inventory can be large, the number of contracts necessary to provide enough material can increase, the supply radius required to source the materials will grow, and final fuel selling prices may be negatively impacted (Cafferty et al., 2013). Regardless of who owns the biomass during the period of degradation, the consequences of material loss and quality changes in storage will negatively impact both the producer—less/lower quality material delivered—and the biorefinery—lower quality material and a need to purchase additional material to replace the lost/degraded biomass. Because of this, we must understand how biomass supply systems can operate in the face of uncertain conditions, how these systems can adapt to natural variation, and ultimately how this variability impacts the costs of procuring biomass and producing renewable fuels.

Since high moisture corn stover (>30% at grain harvest) will occur in areas that typically produce "dry" stable biomass (Oyedeji et al., 2017), methods of mitigating the risks associated with storage losses are needed. As discussed by Darr and Shah (2014), a number of different storage methods may be employed to improve the stability of biomass in storage, with anaerobic methods being the common and trusted option for stabilizing feedstocks of exceedingly high moistures, and protection from precipitation being the go-to for ensuring stability of dry materials. However, even when following conventional best management practices, risk and uncertainty in storage remain, as demonstrated by Smith et al. (2013), who showed plastic wrapped high moisture bales of energy sorghum to reach over 40% dry matter loss by 9 months in storage and tarped low moisture sorghum to suffer 25% dry matter loss over the same time. Rentizelas et al. (2009) proposed an alternative management solution utilizing multiple feedstocks harvested at different points throughout the year to minimize the total time spent in storage. While the researchers concluded that a multi-feedstock approach was effective, differences in the cost and benefit of various storage methods failed to outcompete low cost storage solutions for low value biomass. As a result, pioneer biorefineries have had to struggle with feedstock storage related challenges such as variations in moisture, material composition, and yield (Lamers et al., 2015).

Dry matter losses in stored biomass occur when microorganisms use available carbohydrates for growth and energy. Losses not only result in a reduction of biomass quantity, they also result in biomass that is compositionally altered because the microorganisms consume both soluble and structural sugars, leaving behind the more recalcitrant structural sugars as well as enriching the biomass in lignin and ash (Shinners et al., 2011; Smith et al., 2013). Previous research has shown a relative decrease in xylan and increase in glucan percentages in corn stover that experienced high levels of dry matter loss as a result of preferential hemicellulose degradation (Wendt et al., 2014, 2018). While the combined structural sugar content of the recovered dry matter remains high, the reactivity of the remaining structural sugars relative to the starting material is uncertain without additional conversion testing. Few comparative studies exist that show the impact of storage losses on conversion performance of herbaceous biomass (Agblevor et al., 1994, 1996) and none to date describe the impacts of dry matter loss on conversion performance in dilute-acid pretreatment and enzymatic hydrolysis.

The microbial activity that drives carbohydrate loss under aerobic conditions is primarily controlled by reducing moisture content in storage (Shinners et al., 2007). However, the water activity rather than the water content of the material directly affects the rates and extents of biodegradation (Beuchat, 1983). Water activity (a_w) can be defined functionally as the relative humidity of the air around a material that is at its equilibrium moisture content at any given temperature. A water activity between 0.6 and 0.7 represents the lower bound at which most bacterial and fungal activity may occur (Beuchat, 1981), thus represents a biologically stable storage state. Water contents (wet basis; abbreviated w.b.) for corn stover biomass fractions at 0.6 to 0.7 a_w range from 12% to 14% (Igathinathane et al., 2005). Rates and extents of biodegradation increase as water activities go from 0.6 (osmophilic yeasts), through 0.8 (most molds), and beyond 0.9 (most bacteria) (Beuchat, 1981). However, the relationship between water activity and biodegradation is not necessarily linear nor easily estimated since biodegradation is also dependent on substrate availability, temperature, and the composition and functional abilities of the microbial communities initially present on the substrate. For complex substrates such as corn stover, each plant tissue type may have its own water content/water activity relationship (Igathinathane et al., 2007). The net result is dependent on the sum of the parts, thus will vary as the tissue composition changes as result of what tissues are there and how they change as a result of biodegradation.

The objectives of this work are to: (1) quantify the rates and extents of corn stover biodegradation occurring at a range of fixed moisture contents, (2) measure the change in biomass chemical components (structural and soluble sugars, lignin, and ash) resulting from dry matter loss, (3) measure the change in reactivity to pretreatment and enzymatic hydrolysis resulting from dry matter loss, and (4) begin to develop the parameters needed to predict biomass storage stability for aerobically stored corn stover. This work uses laboratory reactors to monitor corn stover biomass under aerobic conditions and at 20%, 25%, 30%, 36%, and 52% moisture contents (w.b) and measure dry matter loss in real-time. Rates and extents of biodegradation by native microflora are compared for each condition along with the corresponding compositional changes. Reactivity, measured by sugar yield after pretreatment and subsequent enzymatic hydrolysis, was evaluated to understand the impact on biorefinery conversion potential. Finally, adsorption isotherms of the stored materials were used to evaluate the water content and water activity relationships between native and degraded materials. The data generated by this work provides the foundation for understanding the relationship between biomass moisture content in storage, storage stability, and the resultant impact of biodegradation on the as-delivered biomass composition. These relationships are needed to predict biomass storage performance relative to harvest timing/moisture content, storage duration/delivery scheduling, and material blending to reduce day-to-day quality variations.

MATERIALS AND METHODS

Biomass and Material Preparation

Corn stover was harvested using an AGCO LB34B single-pass baler in Stevens County, KS, at a moisture content of 55%. A portion of the biomass was immediately packed into 208 L drums, transported to Idaho National Laboratory (INL), Idaho Falls ID, and stored at −20°C. Prior to initiating the experiments, each drum of material (approximately 50 kg of wet biomass) was spread into a thin-layer to thaw and dry to a specified moisture content at ambient room temperature (17° to 23°C). A total of five drums of material was used to provide material at 20%, 25%, 30%, 36%, and 52% moisture (**Table 1**). Each lot of material was homogenized and split by hand before being loaded into duplicate reactors for storage.

Laboratory Storage

The design and function of the laboratory scale storage reactors used in this work have been described by Bonner et al. (2015) and applied to corn stover storage research by Wendt et al. (2014). Each reactor consists of a 100 L inner chamber (76 L usable) for housing a biomass sample (approximately 8 to 10 kg dry matter) surrounded by a temperature-controlled water jacket. A feedback loop between a LabVIEW (Version 11.0.1, National Instruments, Austin, TX, United States) control interface and temperature sensors in the biomass column is used to control the jacket temperature to 0.5°C below that of the biomass in the center of the reactor, allowing natural biological self-heating to drive the storage temperature with minimal heat loss to the chamber walls. Atmospheric air is supplied via mass flow controller (Brooks Inst. Model 5850E, Hatfield, PA, United States) at 1 L min^{-1} into the bottom of the reactor where it is heated and humidified by bubbling through a 7.6 cm layer of water before flowing upward through the biomass column and out a single port at the top of the reactor. Gas exiting the reactor is then routed through a glycol chilled condensation coil to remove moisture prior to automated sampling and delivery to a gas chromatograph (Agilent MicroGC300, Santa Clara, CA, United States) for oxygen, nitrogen, and carbon dioxide analyses every 4 h.

Corn stover at each of the five moisture contents was tested in duplicate, requiring a total of ten reactor runs. Biomass was packed into the reactors by hand at a dry matter density of 90 ± 10 kg m^3 (**Table 1**), which is similar to other reported studies (Wendt et al., 2014, 2018; Bonner et al., 2015).

Once in operation, reactors were allowed to self-heat naturally while the temperature of the column and the composition of the gas exiting each reactor were recorded. Reactor trials were

TABLE 1 | Corn stover moisture content, load mass (dry mass), and dry matter density for each of the ten storage reactors.

Sample ID	Moisture Content,% wb	Initial Load Mass, kg	Dry Bulk Density, kg/m^3
20A	20.1	7.3	76.3
20B	20.1	8.3	87.5
25A	25.9	7.9	76.9
25B	25.9	9.3	90.3
30A	30.6	8.5	77.9
30B	30.6	9.8	89.8
36A	36.5	11.0	91.9
36B	36.5	12.1	100.8
52A	52.2	15.9	99.7
52B	52.2	16.9	105.9

terminated once both duplicates had returned to and stabilized at ambient room temperatures (23° to 25°C), which corresponded to a bulk respiration rate of <0.4 g CO_2 (kg DM remaining)$^{-1}$ d^{-1} and resulted in several days difference between completion times for individual reactors, which ran from 55 to 85 days. The biomass from each reactor was unloaded individually, homogenized by mixing in a new 3-mil food-grade super-sack liner (BAG Corp, Richardson, TX, United States) for each reactor. Material was spread to a depth of 5 to 10 cm within the sack liner. Composite samples of 100 to 125 g (fresh) mass were randomly collected ($n = 3$) and used for analyses.

Material Analysis

Moisture content of the biomass before and after storage was measured by drying a subsample at 105°C for 24 h. Dry matter loss of each reactor was determined by utilizing the CO_2 concentration measured in the off-gas during storage to calculate the consumption of carbohydrate (CH_2O) though aerobic respiration using a molar ratio of 1:1 (McGechan, 1989), such that:

$$\text{Dry Matter Loss (\%)} = \frac{\sum CH_2O}{DM_i} \cdot 100 \qquad (1)$$

Where the cumulative mass of CH_2O at any time in storage is related to the initial dry mass, DM_i to calculate dry matter loss over the entire storage period.

Chemical compositional analysis of unstored and stored corn stover composite samples was performed in duplicate according to standard biomass procedures developed by the National Renewable Energy Laboratory (NREL) (Sluiter and Sluiter, 2011). Extractives from water and ethanol were determined using an ASE 350 (Dionex, Sunnyvale, CA, United States) (Sluiter et al., 2008b). The extracted biomass was subjected to a two-stage acid hydrolysis (Sluiter et al., 2008a). The liquor from the acid hydrolysis was analyzed using HPLC with a refractive index detector for monomeric sugars and UV-VIS (210 nm) sugar degradation products (Agilent, Santa Clara, CA, United States) and an Aminex HPX 87P and 87H columns (Bio-Rad, 300 × 7.8 mm, Hercules, CA, United States). The solids were used to determine lignin and ash (Sluiter et al., 2008a). Acid-soluble lignin fractions were analyzed using a Varian Cary 50 ultraviolet-visible spectrometer (Agilent, Santa Clara, CA, United States) (Sluiter et al., 2008b). The compositional analysis of unstored corn stover was performed on a sample from each moisture content and averaged ($n = 5$), while duplicate reactors were averaged for each moisture condition.

Organic acids were extracted from the unstored and stored corn stover composite samples in duplicate using a 1:10 ratio of wet biomass (50 g) to 18 MΩ-cm nanopure water. Samples equilibrated at 4°C for 72 h. An aliquot was filtered to 0.2 μm and acidified to a pH of 4 with sulfuric acid. Organic acids were analyzed using high performance liquid chromatography (HPLC) with a refractive index detector (Waters, Milford, MA, United States) and an Aminex HPX 87H ion exclusion column (Bio-Rad, 300 × 7.8 mm, Hercules, CA, United States).

Pretreatment and Enzymatic Hydrolysis

Dilute acid pretreatment was performed using a Dionex ASE 350 Accelerated Solvent Extractor (Dionex Corporation, Sunnyvale, CA, United States) at 10% (w/w) solids loading by adding 30 mL of 1% sulfuric acid (w/w) in 66-mL Dionium cells, as described previously (Wolfrum et al., 2013). Briefly, reaction conditions included a 360 s ramp in temperature to 130°C followed by a 420 s incubation (severity factor = 1.73), which were determined to be optimal for corn stover by Wolfrum et al. (2013). A 150 mL rinse was then performed at 100°C to neutralize the biomass. The pretreatment liquor was analyzed for monomeric and polymeric sugars using HPLC with the HPX-87P column, as described above. Fermentation inhibitors including acetate, furfural, 5-hydroxymethylfurfural, and levulinic acid were measured using HPLC with the HPX-87H column, as described above. Yields were calculated based on glucan and xylan levels in the initial biomass sample compared to glucan and xylan released during pretreatment. For both pretreatment and enzymatic hydrolysis experiments, all feedstock composition, and hydrolyte liquors organic acid, monomeric sugar, and total sugar concentrations were determined using appropriate National Renewable Energy Laboratory (NREL) laboratory analytical procedures (LAPs) (Sluiter et al., 2008a,b; Sluiter and Sluiter, 2011), which include the yield calculations. Triplicate pretreatment experiments were conducted on duplicate samples for each storage treatment, and the results were combined ($n = 6$).

Enzymatic hydrolysis was performed in triplicate on non-pretreated and pretreated, washed biomass. Briefly 0.15 g (dry basis) of biomass was added to a 20 ml scintillation vial at 1.5% (w/w) solids loading and 50 mM sodium citrate buffer, pH 4.8, based on methods from Selig et al. (2008) and used in our laboratory by Hoover et al. (2018). Final reaction volume was 10 mL. Cellic® CTec2 and HTec2 enzyme complexes (Novozymes; Franklinton, NC, United States) were added at loading rates of 20 mg protein and 2 mg protein per g dry mass biomass, respectively. Sodium citrate buffer was supplemented with 0.02% NaN_3 in the biomass slurry to prevent microbial contamination. Enzyme and substrate blanks were prepared as controls. After an incubation period of 120 h at 50°C (New Brunswick Innova 4080, Enfield, CT, United States), aliquots of liquor were removed, filtered through a 0.2 μm filter, and analyzed for monomeric sugars using Megazyme assay kits (D-Glucose GOPOD Format Kit for glucose, D-Xylose Assay Kit for xylose; Bray, Ireland). Sugar yields were calculated by dividing the sugar released in dilute-acid pretreatment and enzymatic hydrolysis liquors by the initial sugar content in the biomass sample. Reactivity in pretreatment, enzymatic hydrolysis, and combined pretreatment and enzymatic hydrolysis was calculated by dividing the sum of released and xylan released based on the total glucan and xylan present in the hydrolysis product from the initial materials' compositional analyses.

MATERIAL ISOTHERMS

Water activity isotherms were generated using a Decagon Devices Inc., AquaSorp Isotherm Generator (Pullman, WA, United States). The instrument uses a dynamic dew-point isotherm method, which unlike traditional salt-slurry isotherm methods, automatically records the sample's mass and water activity over time as it is exposed to desiccant dried or water saturated air, causing the sample to undergo desorption or adsorption, respectively (Schmidt and Lee, 2012). The instrument operates at a fixed temperature (0.1°C) with an internal micro-balance (0.1 mg) and chilled mirror dew-point sensor (0.005 a_w), eliminating the need to manually handle the sample and disrupt the testing conditions. Cycling isotherms consisting of an initial desorption, adsorption, and second desorption were recorded from 0.05 a_w to 0.85 a_w (or 5% to 85% equilibrium relative humidity or *e.m.c*). The instrument's sample cup was loaded with 250 mg to 500 mg of material ground to pass a 2 mm screen (Thomas Model 4 Wiley mill, Thomas Scientific, Swedesboro, NJ, United States) and equilibrated to the test temperatures within the sealed sample chamber before desorption began.

Tests were performed at 25°C with triplicate samples of the starting material and duplicate samples of the stored materials (one composite sample from each reactor). The instrument's airflow over the sample material was set to 60 mL/min. Completed samples were dried at 105°C for 24 h to determine dry mass for calculating moisture content.

Isotherms were fit to the temperature-independent GAB model [Guggenheim-Anderson-de Boer; van den Berg and Bruin (1981)] using Decagon SorpTrac software (v. 1.14; Decagon Devices Inc., Pullman, WA, United States). Model calculations are presented in equation 2, below. The GAB model is widely used in the food industry and its parameters relate to material specific properties, where C is a heat (energy) constant, k is a material specific drying parameter, and M_0 is the monolayer moisture content (expressed in the dry basis).

$$e.m.c = \frac{M_0 \cdot k \cdot C \cdot a_w}{\left[\left(1 - k \cdot a_w\right)\left(1 - k \cdot a_w + C \cdot k \cdot a_w\right)\right]} \tag{2}$$

The monolayer moisture content is the moisture content at which all hydrophilic groups present in a material are associated with a water molecule. Water molecules and their solutes are assumed to be mobile and available to enter into chemical reactions above this point (Labuza and Altunakar, 2007). Since the calculations used in the GAB model use dry mass basis, sorption isotherms will be discussed in the dry basis—like compositional data—rather than wet basis, which is used to discuss moisture content.

DATA ANALYSIS

Data from the laboratory storage reactors was analyzed and modeled using Excel 2011 (Microsoft Corp., Redmond, WA, United States) and JMP software (SAS Institute Inc., Cary, NC, United States). For compositional analysis (n = 4) and sugar release experiments (n = 6), single-factor one-way analysis of variance (ANOVA) was performed in SigmaPlot (version 13.0) to identify significant differences, and Tukey's honest significant difference (HSD) test was performed if the ANOVA was significant at $p < 0.05$ for a multiple-level comparison of statistical equivalency. Sorption isotherm model parameters (C, k, and M_0) were compared using the Student's t-test in SigmaPlot ($p < 0.05$) to identify significant differences between the initial and stored materials' adsorption and desorption characteristics of the GAB isotherms. When tests for normal distribution or equal variance failed a Mann-Whitney U test for difference between medians was used.

RESULTS AND DISCUSSION

Storage Performance

Final bulk moisture contents remained ±5% (absolute) of the initial moisture contents. Moisture tended to migrate upwards resulting in a moisture decrease in the lower quarter of the reactors and a moisture increase in the upper quarter based on grab samples from the top and bottom (data not shown). The respiration profiles measured during biomass storage, determined by quantifying the CO_2 released from each reactor, were proportional to the biomass moisture contents (**Figure 1**). **Figure 2** shows that within 6 days of storage sharp CO_2 concentration spikes can be seen in the off gas of the biomass stored between 25% and 52% moisture content. The magnitude of the respiration spike is larger and occurs earlier with increased moisture content, though the duration of the spike is similar among moistures >25%. However, the timing of the respiration pattern of the 20% moisture material differs from that of the higher moisture contents. Maximum respiration rates begin nearly three weeks later and are nearly an order of magnitude less than that of the 52% moisture conditions. Notwithstanding, the pattern of early peak respiration rate followed by lower sustained respiration rate before eventually tapering off is common among all the tested conditions. This three phase "peak, shoulder, and decline" respiration pattern is described in greater detail in Bonner et al. (2015) for both woody and herbaceous biomass storage. In summary, storage stability is greatly affected by moisture content within the first two weeks of storage. Losses are minimized at moisture contents of 20% or less. Above this level biological activity is almost immediate and is proportional to moisture content. The reasons for this delay are not clear but are likely related to the impact of water activity, as discussed below.

Figure 3 shows the average accumulated dry matter loss over time for duplicate storage conditions. The initial loss rates (slopes) and final extents (maximum values) increase with increasing moisture content but are not linearly proportional. Onset of dry matter loss and maximum loss rates are similar for moisture contents between 25% and 52%. Onset is delayed and maximum DML rates are much less at 20% moisture. This indicates that a threshold exists between 20% and 25% moisture, above which appreciably more microbial activity occurs early in storage. Igathinathane et al. (2008) has previously shown that fungal growth on biomass is largely dependent on water activity (a_w), with a precipitous increase in growth beginning at a_w values greater than 0.89. Based on their sorption experiments, corn stover at 20% moisture would have a water activity near 0.85, while stover at 25% moisture would reach >0.9, potentially

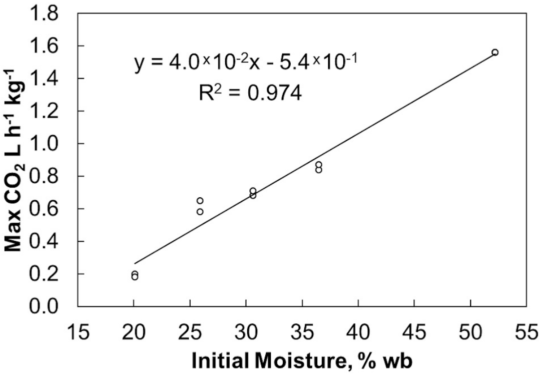

FIGURE 1 | CO_2 peaks measured in the off gas over the moisture contents used in this study.

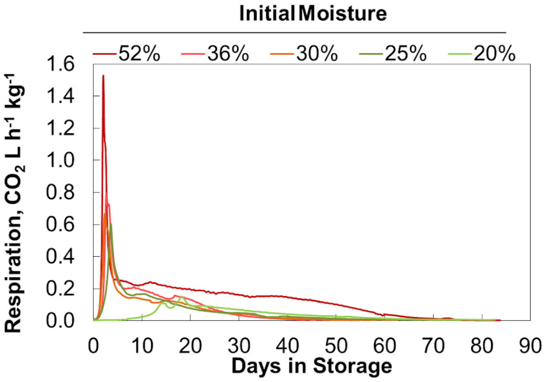

FIGURE 2 | CO_2 concentration measured in the off gas. Averages of 2 reactors at each moisture content shown.

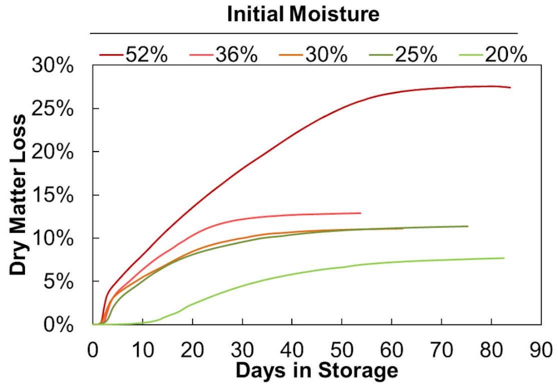

FIGURE 3 | Total dry matter loss over time. Averages of 2 reactors at each moisture content shown.

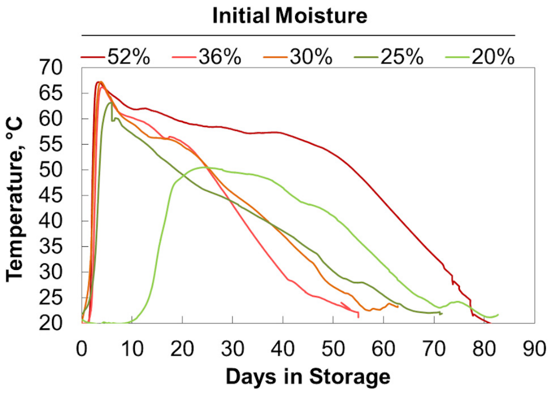

FIGURE 4 | Temperature during storage. Averages of 2 reactors at each moisture content shown.

traversing this critical range for microbial activity. Corn stover stored at 25%, 30%, and 36% moisture behaved similarly, both in terms of dry matter loss rate and total extent of dry matter loss. Again, this aligns well with previous work that has shown the water activity to equilibrium moisture content relationship approaches a vertical asymptote, meaning this 10% span in moisture has little effect on the water activity of the material (e.g., a_w 0.9 to 0.95) and presence of mold growth (Igathinathane et al., 2008). There appears to be another threshold between materials stored at 36% and 52% moisture. The 52% moisture material exhibited both a higher initial CO_2 spike and a greater sustained rate of dry matter loss over the storage period, culminating in substantially greater total dry matter loss. However, the lack of data between 36% and 52% moisture prevents any inferences from being made as to the specific moisture content where degradation increase rapidly as a result of increasing moisture. Nevertheless, these results demonstrate that moisture reductions in the range of 36% to 25% can have a strong positive impact on aerobic storage stability.

Initial moisture content similarly affected self-heating due to microbial respiration (**Figure 4**). As a result of the delay in respiration seen in the 20% moisture material, these reactors exhibited delayed self-heating. After this delay, the 20% moisture samples heated to a lower maximum temperature than the other material. The 25%, 30%, and 36% moisture samples heated to similar maximum temperatures (with the 25% moisture samples having a slightly lower maximum temperature) before cooling at similar rates. Again, this matches the microbial respiration rates shown in **Figure 2**. The 52% moisture reactors had a heating rate and maximum temperature like those of the material ranging from 25% to 36% moisture. However, sustained microbial respiration at 52% moisture resulted in higher temperatures for a longer duration of time. Since respiration rates drive both the measured temperature and the dry matter loss, we explored the relationship between temperature, time, and extents of dry matter loss. High Degree Days (HDD) is the product of the time spent (days or fraction thereof) above a selected temperature (in this case, 45°C) and the difference between the elevated temperature and 45°C. It is a method employed by Shinners et al. (2011) for field storage and produces results in degree days above a specific temperature and indicates the severity of respiratory biodegradation in stored biomass. Plotting dry matter loss by HDD (**Figure 5**) shows a linear

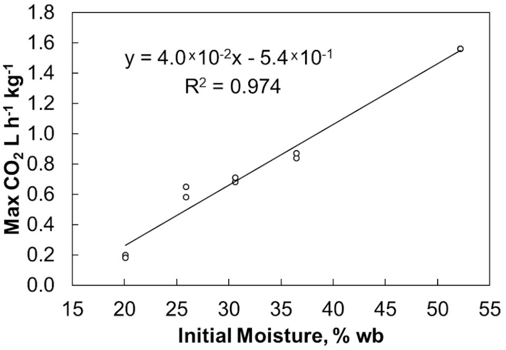

FIGURE 5 | Relationship of dry matter loss to time spent at elevated temperatures. HDD represents the number of days stored material spent above 45°C.

increase in accumulated dry matter loss with time spent at temperatures greater than 45°C ($r^2 = 0.95$). This relationship suggests that internal stack temperature, which can be monitored relatively easily, may be useful as a "real-time" indicator of storage stability. Specifically, internal stack temperature, which may easily be measured accurately in a number of locations, can be used to alert feedstock producers, aggregators, and end users of material instabilities that could lead to compositional changes and material losses as a result of biodegradation. This information could be used to identify lots of materials that require additional protection from moisture exposure during storage or to schedule delivery of "at-risk" materials before their conversion value is lost.

Composition Changes

For each moisture content analyzed in the storage reactors, compositional analyses were assessed for the corn stover before and after storage. Average values are shown in **Table 2**. Total extractives declined in the 25%, 30%, and 35% moisture storage conditions, but they subsequently increased in the high dry matter loss conditions of the 52% reactors. Soluble glucose concentration decreased in the stored reactors relative to the initial conditions. It remained low in every tested storage condition suggesting a rapid and irreversible loss of soluble glucose, likely a result of the microbial activity occurring over time. Polymeric glucan concentration was significantly enriched in storage as noted by the *P*-value of 0.037, yet the pairwise comparisons did not detect significant differences in the means of the unstored and stored samples. The apparent increase in as-recovered glucan shown in **Table 2** is a direct result of the selective loss of the hemicellulose components xylan and acetic acid (acetyl), which decrease significantly (ANOVA, $p < 0.05$ shown by superscript letters) with increasing dry matter loss. Soluble xylose increased in conditions greater than 20% moisture content, while structural xylan was significantly reduced, most notably during storage at 52% moisture. Galactan was enriched during 52% moisture storage while arabinan was relatively constant across the range of measured degradation. Acetic acid, a measure of acetyl groups within the hemicellulose, decreased because of biodegradation during storage, with over 50% reduction in the 52% moisture condition. Lignin was significantly enriched because of storage at 36% and 52% moisture, while ash was not statistically impacted as a result of storage. The changes in chemical composition resulting from dry matter loss provide insight into the impact of storage at a range of moisture contents. The enrichment of glucan at the expense of xylan resulting from dry matter loss suggests that high-moisture storage conditions lead to systematic compositional changes that may be important in conversion, especially if the process economics rely on the presence of a critical concentration of C-5 carbohydrates.

Pretreatment and Enzymatic Hydrolysis

Sugar recovery after combined dilute-acid pretreatment and enzymatic hydrolysis ranged from 79% to 86% of available glucose and 88% to 94% xylose being released across the range of moisture contents examined. No statistical difference in glucose or xylose yield was observed between any of the corn stover samples regardless of dry matter loss (**Figure 6**). The relative high severity of the dilute acid pretreatment assay used may have masked any subtle yield differences in wet harvested corn stover. Similar results have been reported in both aerobically and anaerobically stored corn stover (Wendt et al., 2018). Likewise, no difference was seen in feedstock reactivity—defined as the monomeric sugar yield relative to the

TABLE 2 | Composition (% of dry matter) of corn stover before and after reactor storage as affected by initial moisture.

Storage conditions	Total extractives	Soluble glucose	Soluble xylose	Glucan	Xylan	Galactan	Arabinan	Acetic acid	Total lignin	Ash
Unstored	10.9 (1.3)^{a}	2.2 (0.1)^{a}	0.3 (0.0)^{a}	34.4 (0.6)	25.3 (0.7)^{a}	1.5 (0.2)^{a}	4.0 (0.2)	4.2 (0.0)^{a}	11.2 (0.3)^{a}	4.0 (0.4)
20%	8.7 (0.3)^{a,b}	0.6 (0.0)^{b}	0.2 (0.0)^{a,b}	36.5 (0.9)	26.3 (0.1)^{a,b}	2.1 (0.0)^{a}	4.0 (0.3)	3.3 (0.3)^{b}	12.3 (0.6)^{a,b}	4.2 (0.5)
25%	7.7 (0.3)^{b}	0.7 (0.0)^{b}	0.3 (0.0)^{a,b,c}	36.7 (0.8)	25.2 (0.3)^{a,b,c}	2.2 (0.0)^{a}	4.2 (0.1)	3.1 (0.3)^{b,c}	11.9 (0.2)^{a,b,c}	4.0 (0.4)
30%	7.9 (0.5)^{b}	0.7 (0.1)^{b}	0.4 (0.1)^{a,b,c}	36.9 (0.7)	25.9 (0.7)^{a,b,c,d}	1.8 (0.0)^{a,b}	4.3 (0.1)	2.8 (0.0)^{b,c,d}	11.7 (0.1)^{a,b,c,d}	4.9 (0.3)
36%	7.3 (1.0)^{b}	0.5 (0.2)^{b}	0.5 (0.0)^{a,c,d}	36.7 (1.4)	23.8 (0.2)^{a,c,d,e}	1.8 (0.0)^{a,b}	4.0 (0.2)	2.5 (0.3)^{c,d,e}	12.7 (0.7)^{b,c,d,e}	5.0 (0.1)
52%	9.5 (1.0)^{a,b}	0.5 (0.2)^{b}	0.6 (0.0)^{d}	36.5 (0.3)	22.9 (1.3)^{c,e}	3.7 (0.2)^{c}	3.8 (0.1)	1.9 (0.0)^{e}	14.0 (0.2)^{b,e}	4.8 (0.0)
*P-value**	0.010	<0.001	0.002	0.037	0.005	<0.001	0.178	<0.001	0.001	0.036

Values in the parenthesis represent the standard deviation; letters represent significantly different groups based on Tukey's tests following results of an ANOVA ($p < 0.05$).
**Results of ANOVA; results significant if $P < 0.05$.*

total structural and soluble sugars before pretreatment—in any of the corn stover samples after combined pretreatment and enzymatic hydrolysis (**Figure 7**). However, statistically significant differences were seen between the sugar yields of the individual steps of dilute acid pretreatment and enzymatic hydrolysis alone. Pretreatment yields were higher for the untreated and 20% moisture content stover, intermediate for the 25% and 30% moistures, and lower for the 36% and 52% moistures, likely a result of respiratory loss of soluble sugar monomers and oligomers. A concomitant increase in structural sugar yield in enzymatic hydrolysis in the 36% and 52% moisture samples was sufficient to balance the reduction of pretreatment yields, resulting in no net change in sugar release in the combined pretreatment and enzymatic hydrolysis. These results suggest that the combined effect of self-heating and degradation increased accessibility of enzymatic attack on the remaining cellulose-rich fraction of the biomass. Degradation of hemicellulose observed in this study and elsewhere (Wendt et al., 2014, 2018) confirm these results. In summary, the differences that were exhibited in mild dilute acid pretreatment and in enzymatic hydrolysis are indicative of the minor but significant changes in structural composition as a result of high-moisture aerobic storage and associated degradation.

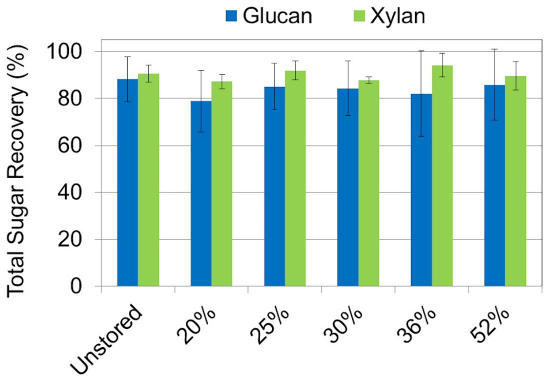

FIGURE 6 | Total sugar recovery of glucan and xylan based on carbohydrate concentrations after dilute acid pretreatment, but before enzymatic hydrolysis. Results show that total glucan and xylan recovery after pretreatment were the same among all storage moistures.

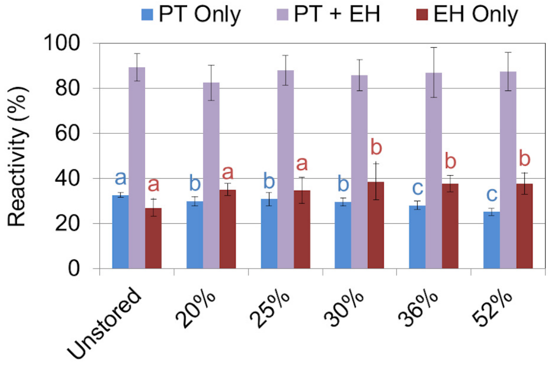

FIGURE 7 | Biomass reactivity—defined as monomeric sugar released relative to total sugar initially present—as a result of dilute acid pretreatment (PT), enzymatic hydrolysis (EH), and the combination of PT and EH. Letters indicate significant differences (ANOVA, $p < 0.05$) in reactivity between the storage moistures after pretreatment (blue) and enzymatic hydrolysis (red). The combined effect of pretreatment and enzymatic hydrolysis overcame any differences seen in the individual steps alone.

Sorption Isotherms

Moisture sorption isotherms follow the sigmoidal shape of type II isotherms typical of porous biological media and other agricultural products (Igathinathane et al., 2005; Labuza and Altunakar, 2007). All isotherms showed a distinct hysteresis loop between the adsorption and desorption isotherms. The initial adsorption isotherms (Adsorption #1) were divergent from subsequent second (and greater) adsorptions (**Figure 8**) and because of this divergence only the second sorption cycles were used in the analyses. One representative example of each storage moisture isotherm is shown in **Figure 9**. Each point represents one discrete measurement of water activity at a specific weight during the test. The topmost grouping of points shows the second

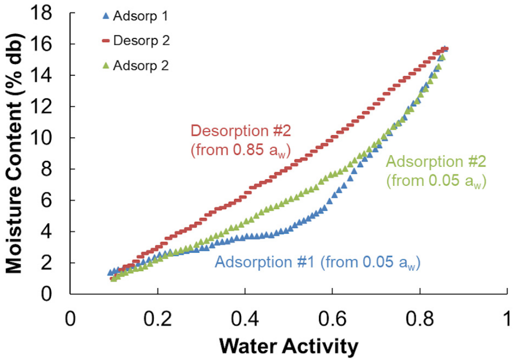

FIGURE 8 | Adsorption and desorption isotherms for corn stover stored at 20% moisture content that shows the hysteresis loop used in the GAB isotherm models (Adsorption and Desorption #2) and the initial divergent adsorption (Adsorption #1).

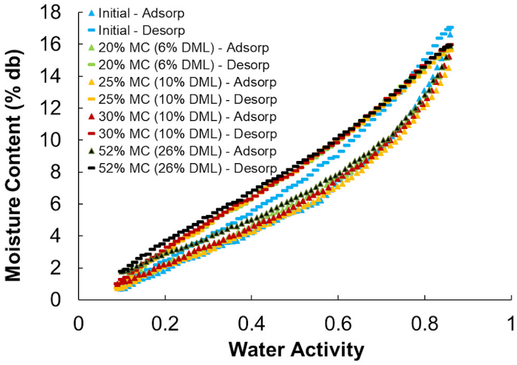

FIGURE 9 | Adsorption and desorption isotherms for representative corn stover samples before (Initial) and after storage at various moisture contents.

TABLE 3 | Average GAB isotherm model parameters (1-SD) for 40°C dried (∼5% mc, wb) corn stover before and after storage.

	Desorption				Adsorption			
	C	***k***	***M*₀**	***n***	***C***	***k***	***M*₀**	***n***
Unstored	2.5 (0.20)	0.77 (0.01)	7.0 (0.32)	4	3.3 (0.37)	0.9 (0.01)	3.9 (0.03)	4
After-storage	3.5 (0.46)	0.67 (0.05)	8.5 (1.05)	5	3.9 (1.2)	0.8 (0.04)	5.1 (0.74)	8
P-value	0.006	0.005	0.03		0.004*	0.004*	0.01	

*Significant differences resulting from storage were evaluated using Students t-test (2-tailed) or Mann-Whitney U (for samples with unequal variances). Parameters for stored and unstored stover are significantly different for both desorption and adsorption models. Monolayer moisture contents (dry basis) are significantly higher after storage and subsequent dry matter loss. *Failed equal variance test—used Mann-Whitney U; results significant if P < 0.05.*

desorption phase. The bottommost grouping of points shows the second adsorption phase. Between them is the second desorption of the unstored material, which plots outside of the group of stored samples below it. This indicates that the unstored materials have a lower water activity (more tightly bound water; less water available for biochemical reactions) than do the stored materials at any given moisture content. For reference, 1% to 18% dry basis is equivalent to 1% to 15% wet basis and spans a range of 0.05 to 0.85 a_w for these corn stover samples.

Results from the individual adsorption and desorption isotherms were fit to the GAB model and the model parameters of C, k, and M_0 were compared between unstored (initial) and the combined stored materials. This model was used as it has a viable theoretical background, is used widely in the food, forest product, and agricultural product industries, and its parameters have physical meaning in terms of the sorption process (Labuza and Altunakar, 2007). Duplicate moisture content samples were insufficient replication to evaluate more than "before" and "after" storage effects. Four initial samples were taken randomly from the original materials that went into the reactors resulting in unequal numbers of initial (n = 4) and after-storage (n = 5 and n = 8 for desorption and adsorption, respectively) samples tested. Insufficient sample existed to test materials from the 36% moisture reactors and three after-storage desorption isotherms were rejected as a result of analytical errors. **Table 3** shows the average GAB desorption and adsorption isotherm model parameters for initial and after-storage corn stover samples. Heat constants (C), material drying parameters (k), and monolayer moisture contents (M_0) were significantly altered because of storage, dry matter loss, and compositional changes noted above. Material specific drying parameters were reduced, and monolayer moisture contents were increased because of the changes that occurred during storage. The increase monolayer moisture content indicates that at the point that all sorption sites are "wetted" there is more water present in the after-storage samples than the initial samples. Possible causes for this include (1) a greater number of available sorption sites available after biodegradation, (2) the presence of more or "stronger" (more hydrophilic) sorption sites after compositional changes, and/or (3) the presence of fewer or "weaker" (less hydrophilic) hydrophobic sites blocking adsorption after compositional changes (van den Berg and Bruin, 1981). Physical and chemical changes resulting from biodegradation have the potential to open more pore spaces (loss of structural integrity), create more surface area (pitting and increased surface roughness), consume or expose biomass chemical components with different hydrophilic tendencies (van den Berg and Bruin, 1981).

CONCLUSION

Variable moisture content of herbaceous crop residues at harvest impacts material stability in storage and ultimately feedstock logistics and processing performance. Despite this recognized variability, many logistics case studies rely on low moisture baled feedstock to reduce handling costs and preserve dry matter. While moisture contents above 20% risk unacceptable losses in aerobic feed and forage storage, no quantitative relationship exists between corn stover moisture content and rates or extents of degradation for bioenergy use. Without such a relationship the cost of high moisture aerobic storage, both to the producer and refinery, cannot be reliably estimated. In this work corn stover was stored using laboratory storage reactors at a range of initial moisture contents (20%, 25%, 30%, 36%, and 52%) to evaluate differences in self-heating, dry matter loss, chemical composition, sugar yield, and moisture sorption characteristics. The use of intermediate sized laboratory-scale storage reactors improved environmental control, provided high-fidelity dry matter loss measurement, and improved sampling efficiency, which reduced or eliminated some of the uncertainties associated with field and bale scale tests. Effective control, sampling, and measurements allows us to quantify the rates and extents of dry matter loss and link those losses to chemical and physical changes. The results of this study describe how storage behavior is dramatically impacted by moisture content and the resultant microbial activity, with dry matter losses ranging from 8% to 28% across the measured moisture content range. The chemical composition of these materials differed proportionately to the extent of dry matter loss, though even the most severe cases yielded quantities of total sugars comparable to fresh material when processed through dilute acid pretreatment and enzymatic hydrolysis. While the total structural carbohydrate content in the as-delivered dry matter remained relatively constant, the ratio of glucan to xylan increased significantly because of dry matter loss. When calculated on an as-harvested basis assuming a stover yield of 4.5 Mg ha^{-1} (2 tn ac^{-1}) and a starting total glucan plus xylan content of 62% (2,800 kg ha^{-1}), 28 kg of available sugars are lost per 1% dry matter loss occurring in storage.

Moisture sorption isotherms show that physical and/or compositional changes that occur during high moisture storage change the wetting and drying characteristics of the materials significantly. Results of this study show that short-term stability exists for corn stover stored at <35% moisture, but that above this threshold degradation is rapid and extensive. Losses occur primarily within the hemicellulose components, which result in higher as-received glucan and lignin concentrations. Dry matter loss and compositional changes measured under these controlled conditions provides the basis for predicting storage stability within a supply system that provides corn stover to a biorefinery or processing depot. Understanding biomass storage stability as a function of storage environment is necessary to develop management strategies to deliver consistent corn stover feedstock to end users. Future work will explore the details and mechanisms of the physical and chemical changes resulting from dry matter loss and examine the role that microbial communities play in these storage-related losses.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

LW, IB, WS, and JM performed the storage experiments and data analysis. All authors drafted and revised the manuscript and approved the final version of the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Chemical and Structural Changes in Corn Stover After Ensiling: Influence on Bioconversion

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Production of biofuels, bioproducts, and bioenergy requires a well-characterized, stable, and reasonably uniform biomass supply and well-established supply chains for shipping biomass from farm fields to biorefineries, while achieving year-round production targets. Preserving and stabilizing biomass feedstock during storage is a necessity for cost-effective and sustainable biofuel production. Ensiling is a common storage method used to preserve and even improve forage quality; however, the impact of ensiling on biomass physical and chemical properties that influence bioconversion processes has been variable. Our objective in this work was to determine the effects of ensiling on lignocellulosic feedstock physicochemical properties and how that influences bioconversion requirements. We observed statistically significant decreases ($p < 0.05$) in the content of two major structural carbohydrates (glucan and xylan) of 5 and 8%, respectively, between the ensiled and non-ensiled materials. We were unable to detect differences in sugar yields from structural carbohydrates after pretreatment and enzymatic hydrolysis of the ensiled materials compared to non-ensiled controls. Based on this work, we conclude that ensiling the corn stover did not change the bioconversion requirements compared to the control samples and incurred losses of structural carbohydrates. At the light microscopy level, ensiled corn stover exhibited little structural change or relocation of cell wall components as detected by immunocytochemistry. However, more subtle structural changes were revealed by electron microscopy, as ensiled cell walls exhibit ultrastructural characteristics such as wall delimitation intermediate between non-ensiled and dilute-acid-pretreated cell walls. These findings suggest that alternative methods of conversion, such as deacetylation and mechanical refining, could take advantage of lamellar defects and may be more effective than dilute acid or hot water pretreatment for biomass conversion of ensiled materials.

Keywords: feedstock logistics, ensiling, bioconversion, pretreatment, ethanol, bioproducts

INTRODUCTION

The United States Department of Energy's *2016 Billion-Ton Report: Advancing Domestic Resources for a Thriving Bioeconomy* (Langholtz et al., 2016) projects that 1.0 billion tons of biomass will be available by 2030 and 1.2 billion tons by 2040. Conversion of the 1.2 billion metric tons of biomass could result in the production of 50 billion gallons of biofuels, 50 billion pounds of bio-based chemicals and bioproducts, and 85 billion kilowatt-hours of electricity to power 7 million households (Rogers et al., 2016). This resource would contribute 1.1 million jobs to the U.S. economy and keep $260 billion in the United States. Additionally, the collection, conversion, and utilization of the 2030 biomass volume targets "could displace 9.5% of fossil energy consumption and avoid as much as 446 million tons of CO_2 equivalent emissions annually" (Rogers et al., 2016). Secondary impacts include bolstering rural economies, creating jobs, and improving both soil and water quality through application of advanced agronomic practices (U.S. Department of Energy Office of Energy Efficiency and Renewable Energy, 2015; Bonner et al., 2016). Achieving the needed economics and logistics required not only improved biomass conversion processes, but more importantly, biomass supply chains that reduce risk and allow for commodity processing of agricultural residues, energy crops, and lower-value waste feedstocks. Some of the largest barriers for cellulosic-based fuels and chemicals do not reside so much in the biorefinery as in the upstream operations of biomass harvesting, handling, storage, transport, and pretreatment/preprocessing (Dale, 2017; Rivers, 2018). Biomass storage is one of these critical upstream challenges (Darr and Shah, 2012; Ebadian et al., 2017). Inman et al. (2010), Rogers et al. (2016) reported that in order to achieve the 2022 Renewable Fuel Standard (RSF) production target of 21 billion gallons of biofuel, over 254 billion metric tons of biomass would be required. The majority of the tonnage would have to be stored and stabilized to provide a consistent year-round supply. The inventory level needed to achieve this degree of consistent, stable biomass supply would require a storage area of 1.37 billion m^3, which is over twice the 0.62 billion m^3 available for grain storage in 2014 (Darr and Shah, 2012). While grain bins and other well-established agricultural infrastructures have been used to reduce grain losses by minimizing dry-matter loss and exposure to moisture (Darr and Shah, 2012; Smith et al., 2013; Wendt et al., 2018b), the fibrous nature and low density of lignocellulosic biomass—primarily agricultural residues—increase the challenges for biomass storage and transport compared to grain (Dale, 2017). Currently, different variations of dry bale storage systems exist, including different ground covers, top covers, and storage shelters. Regardless, the most common practice is to aggregate biomass prior to delivery to the biorefinery. These practices have shown dry-matter losses (DML) of <10%, increasing with increased moisture content, and have been previously described in some detail (Darr and Shah, 2012; U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office, 2016; Dale, 2017; Wendt et al., 2018a,b). Drying time prior to baling varies seasonally, and incomplete drying can lead to biological degradation and self-heating, increasing DML and the risk of spontaneous combustion (Smith et al., 2013; Wendt et al., 2018a; Webb and Chambers, 2019). Fires in stacked bale storage systems lead to major losses in stored biomass, have proven to be difficult to control, and potentially create health risks from smoke and small particle inhalation. Fires in biomass stack yards have occurred in several of the large cellulosic ethanol plants (U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office, 2016; Webb et al., 2018). Reducing fire risk has focused on alternative bale stacking methods and providing more space between stacks to reduce fire size and spread, but fire risk has not been eliminated (U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office, 2016).

An alternative to dry storage in silos or stacked bale yards is a wet storage system, also known as ensiling, has been employed to preserve feedstock for livestock forage. Ensiling is achieved through fermentation by anaerobic bacteria, primarily heterofermentative and homofermentative lactic acid strains. These microbes ferment free sugars, lower the pH by producing carboxylic acids such as acetic, butyric, propionic, and lactic acids, while further reducing the oxygen content, thus creating an anaerobic environment (Chen et al., 2007; Smith et al., 2013; Essien et al., 2018). These final low-pH and low-O_2 conditions reduce microbial activity and preserve biomass.

The potential benefits of ensiling in the context of a biorefinery supply system include reduced dependence on seasonality for biomass harvesting thus allowing for a wider harvest window; reduced DML; and reduced fire risk. Ensiling may provide additional benefits by reducing handling and preprocessing challenges, such as size reduction prior to ensiling, negating the need for additional preprocessing at the biorefinery (Wendt et al., 2018b). Reduction in pretreatment severity required for bioconversion of ensiled biomass has been reported by some groups (Essien et al., 2018), but the overall results in the bioconversion between field and lab studies have been variable (Wendt et al., 2018a). Variables that can influence the impact of ensiling on bioconversion include the type and variety of the feedstock; ensiling methodology and use of additives; chemical treatments such as alkaline, dilute acid, or wet oxidation; type of bioconversion process and/or enzymatic hydrolysis; and, finally, the scale of the studies (Wendt et al., 2018a).

Techno-economic analysis using corn stover, comparing a field chopped logistics system incorporating ensiling to a bale-based logistics system, reported that cost per dry ton for the chopped logistics system was slightly higher compared to the bale logistics system: $137.86 and $125.70, respectively (Wendt et al., 2018b). Additional benefits such as enhanced fermentation of ensiled materials for carboxylic acid fermentation (Lin et al., 2016; Nelson et al., 2017) may increase production amounts for both fuels and chemicals. Achieving reduction of pretreatment requirements resulting from wet storage may be more challenging; inconsistent results for wet storage when accounting for differences in variation in the feedstock, harvesting and collection practices, and storage regimes overshadow smaller improvements in reducing pretreatment requirements when operating at larger production scales.

Results from smaller-scale studies (Chen et al., 2007) have evaluated the enzymatic hydrolysis of materials ensiled both with and without enzyme addition, comparing the sugar release and holocellulose (cellulose and hemicellulose) losses after enzymatically hydrolyzing untreated and ensiled feedstocks. Ensiling significantly ($p < 0.05$) increased the sugar release and holocellulose loss in cotton stalks, wheat straw, and barley compared to untreated feedstocks. However, Chen et al. were unable to detect changes in sugar release or holocellulose loss upon adding enzymes at the start of the ensiling process ($p < 0.05$). In a companion study (Chen et al., 2007), these researchers compared chemically pretreated feedstocks to enzyme-assisted ensiled feedstocks. The sugar and ethanol yields from the pretreated feedstocks were higher than yields from the enzyme-assisted ensiled wheat straw and triticale; yields from the enzyme-assisted ensiled hay materials were comparable to yields from the chemically treated materials.

Wet oxidation has been successfully used to pretreat whole-crop maize (Thomsen et al., 2008; Xu et al., 2010; Essien et al., 2018). The combined pretreatment and enzymatic hydrolysis of the ensiled green maize, described previously, resulted in up to >90% and >80% yields from glucan and xylan hydrolysis, respectively, depending on pretreatment condition. However, these studies were not compared to a non-ensiled sample to understand the potential change in recalcitrance reduction that occurred as a result of storage. Simultaneous saccharification and fermentation (SSF) of the pretreated ensiled maize residues showed no inhibition and, in several cases, achieved 95% to 98% of theoretical ethanol yields (Thomsen et al., 2008). Oleskowicz-Popiel et al. (2011) reported that ethanol yields from SSF were highest in all three of the ensiled and hydrothermally treated maize, rye, and clover biomass, reaching 80% of theoretical yield when compared to ethanol yields from the conversion of maize, rye, clover, and non-ensiled biomass using hydrothermal pretreatment. Anaerobic digestion of ensiled herbaceous feedstocks such as grasses and corn stover has shown increased biogas production (Janke et al., 2019), with 71% of methane potential achieved from ensiled sugar cane combined with molasses, an ensiling additive. However, no increase in methane potential was observed when materials were ensiled without additives.

Previous studies have explored some of the range of variables highlighted above, and most were performed at a smaller scale (Wendt et al., 2018a), making comparisons to larger-scale performance difficult. The specific objectives of the work presented here were to (1) compare the effects of two pretreatment processes on the digestibility of ensiled and non-ensiled corn stover, with and without ensiling additives; (2) characterize the yields from both conversion processes using compositional analysis of both materials; and (3) examine the microscale structure of both the ensiled and non-ensiled corn stover to determine the level of structural changes occurring from ensiling to gain a better understanding of mechanisms of preservation and conversion.

MATERIALS AND METHODS

Corn Stover Ensiling

Corn stover was collected from study plots at the Iowa State University Agronomy and Agricultural Engineering Farm in Ames, Iowa. The harvesting and ensiling setup was conducted using the whole (100% removal) cut fraction. The corn stover and grain were harvested at the time of grain maturity at approximately 49% total solids (w/w). A single-pass harvester simultaneously harvested the corn grain and separated it from the stover. The chopped stover (approximately 52 kg) was blown into a wagon pulled behind the harvester. The wagon was then unloaded onto a 30-ft by 50-ft tarp (**Figure 1**). Randomized representative subsamples from the stover were collected and either stored at −20°C or dried at 45°C and then stored at ambient temperature. Approximately 5 kg (10%–12%) of the collected stover was either frozen or field dried, leaving approximately 47 kg of stover available for ensiling.

The remaining corn stover was divided into two groups: one was ensiled with additives, the other without. The materials ensiled with additives were prepared using Silamax 50 WS (from Chemorse in Des Moines, Iowa), which contains *Enterococcus faecium*, *Lactobacillus plantarum*, and *Pediococcus acidilactici* bacteria. The additive also contains cellulase, fungal and bacterial amylases, proteases, and beta-glucanase enzymes. A subsample of corn stover was taken from the initial stover pile and mixed, using shovels, with Silamax 50 WS, representing an application rate of 1.75 kg/t fresh stover. One liter of water was sprayed on the pile during mixing to wet the additive. The ensiling reactors consisted of 19-liter buckets with gas seals attached to the lids, $n = 3$ for corn stover ensiled with and without additives. The reactors were filled to the top with either untreated or additive-treated corn stover, at a packing density of approximately 74.4 kg Dry Material (DM)/m^3. The buckets with the ensiled corn stover were stored in an environmental chamber at 37°C to simulate the center of a silage pile.

Prior to analysis, samples (frozen, dried, and ensiled with and without additives) were subsampled using the Pierre Gy method (Pitard, 1993) and reduced in size to 6.25 mm using a Wiley knife mill (from Thomas Scientific in Swedesboro, NJ, United States). Subsamples of the material were further milled to 2 mm and used for all compositional analysis. All samples were oven dried at 60°C prior to compositional analysis. A schematic diagram of samples showing both the collection and ensiling of the corn stover is shown in **Figure 1**.

Corn Stover Analysis

The chemical composition for all dried, frozen, and ensiled feedstock samples was determined using the National Renewable Energy Laboratory (NREL's) standard laboratory analytical procedures (LAPs) (National Renewable Energy Laboratory [NREL], 2013). Briefly, biomass is first extracted sequentially with water and ethanol using an Accelerated Solvent Extractor (ASE Dionex CA, model 200). Solvent was removed using a rotary evaporator, then dried and weighed. Structural carbohydrates were determined using a two-step acid hydrolysis to fractionate

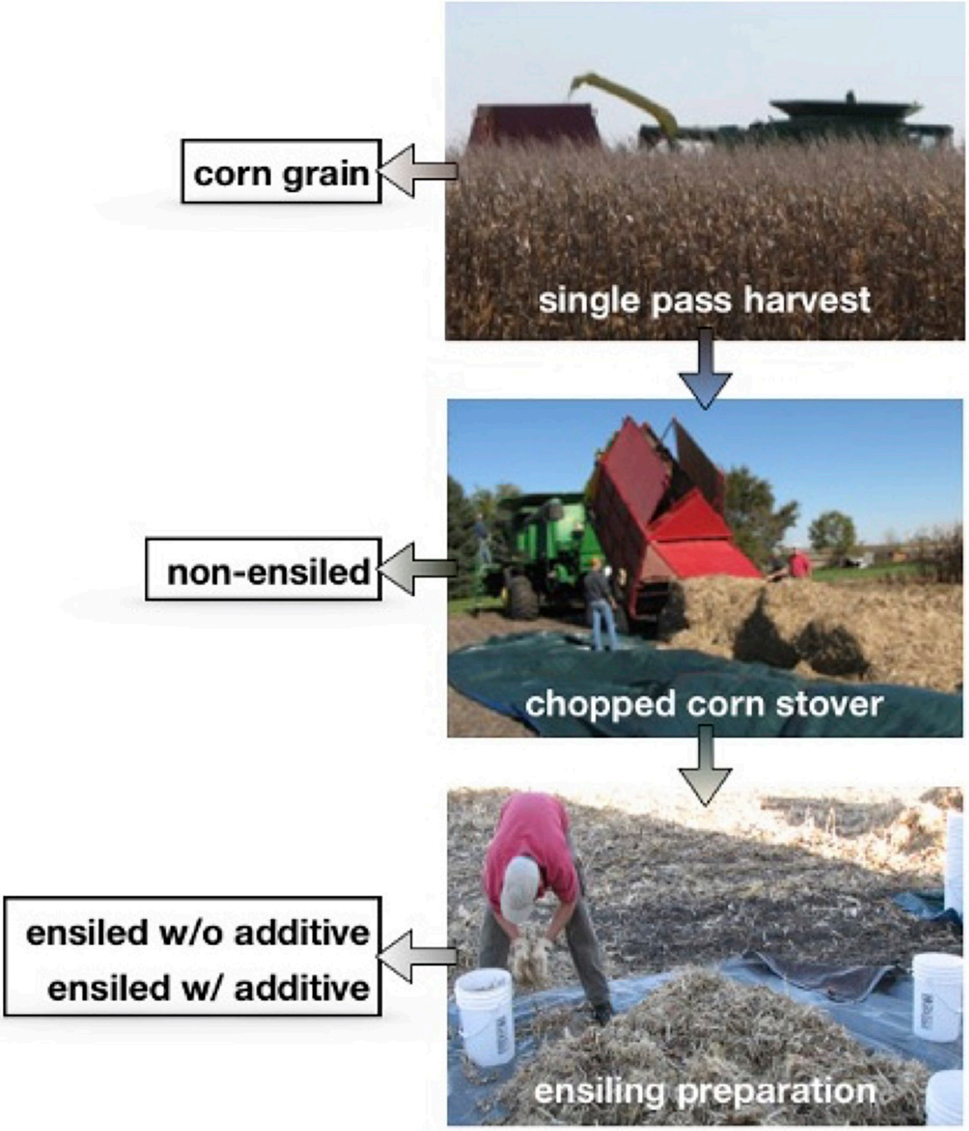

FIGURE 1 | Schematic diagram of the sampling scheme used in this work. All samples were taken from a single tarp containing approximately 50 kg of chopped corn stover collected with a single-pass harvester. Ensiling was set up with and without additives the same day as the harvest. Samples of the stover from the tarp were frozen and dried in a 45°C oven, also on the same day as the harvest.

the biomass into forms that are more easily quantified. The extracted biomass was digested using 72% (w/w) sulfuric acid, then diluted to 4% sulfuric acid and further digested at 121°C for 60 min. Samples were neutralized and filtered prior to analysis using Agilent Technologies (Santa Clara, CA, United States) model 1100 HPLC and the BioRad Aminex HPX-87P column (Hercules, CA, United States). Acid-insoluble lignin is determined by rinsing the filtered solids with deionized water, followed by drying and then weighing to determine the amount of remaining lignin.

Starch was determined from the Association of Official Agricultural Chemists [AOAC] (1998) method 996.11 (Megazyme, 2014). Extracted and non-extracted biomass was analyzed for starch using a two-enzyme digestion of the solid biomass. A 100-mg sample was first treated with 190-proof ethanol, followed by an addition of dimethyl sulfoxide, and then immersed in boiling water for 5 min. Three hundred units of α-amylase (from Megazyme in Wicklow, Ireland) were then added and incubated in boiling water for 6 min. The samples were then incubated in a 50°C water bath, followed by the addition of 0.1 mL (20 units) amyloglucosidase (from Megazyme), and incubated for 30 min at 50°C. Samples were centrifuged, filtered, and analyzed for glucose by high-performance liquid chromatography (HPLC).

The pretreated samples were analyzed, focusing on the liquor for monomeric and oligomeric sugars, acetate, lignin, furfural, and hydroxymethylfurfural (HMF) using the NREL LAPs (National Renewable Energy Laboratory [NREL], 2013). Percentage of total solids (%) was calculated on the whole pretreated slurry to determine the mass of remaining solids. The remaining cellulose was calculated by subtracting the mass of the solubilized glucose in the liquor phase from the initial cellulose content in the sample. The fraction of remaining cellulose was then multiplied by the mass of remaining solids to determine the mass of cellulose in the solids. This number was used to determine the cellulase loading.

Organic Acid Analysis

Samples were homogenized and aqueous analytes were extracted from the solids using a modification of the method described in Carr et al. (1984). Solids were ground (1:10 w/v) for 60 s in a laboratory blender (Waring model 3390D25) and filtered through a syringe filter (Whatman GD/X; 0.2 μm, non-sterile). Organic acids were analyzed by HPLC using a Bio-Rad Aminex HPX-87H column.

Pretreatment Experiments

All corn stover samples were pretreated using a MultiClave 10X Reactor (Autoclave Engineers in Erie, PA, United States): 170°C reaction temperature, 6.5-min residence time, 0.07 g/g H_2SO_4 (wt. acid/wt. biomass); and 200°C reaction temperature, 20-min residence time, no acid addition. A 5% solids loading was used for all samples, resulting in a total mass per well of 25 g. Each pretreatment condition was done in triplicate. Thus, a total of 48 experiment samples were generated: 8 samples (3 ensiled samples with additives, 3 ensiled samples without additives, 1 field-dried sample, and 1 frozen sample), each pretreated under two conditions (dilute sulfuric acid and water-only) with three replicates. A control corn stover material (Pioneer 33A14 variety) was included in one of the pretreatment wells, totaling six additional samples, to serve as a method validation standard for the pretreatment.

The MultiClave reactor was heated using two sand baths (Techne Inc., Cambridge, United Kingdom) for the pretreatment experiments. The larger industrial bath (Model IFB-121) was set at 230°C to accelerate the heat-up period, and the smaller sand bath was set at the desired reaction temperature. When the internal reactor temperature was within 10°C of the target temperature, the reactor was transferred from the larger sand bath into the smaller sand bath to maintain reaction temperature for the duration of the experiment. Temperature was monitored throughout the experiment. Immersing the reactor into a bucket of ice quenched the reaction.

Pretreated slurries were removed from the reactor and separated into solid and liquid fractions using a vacuum flask and glass-fiber filters. The liquors were analyzed for monomeric and total sugars, organic acids, and solids content. The pretreated solids were washed with deionized water, and the solids content and total weight were recorded. These data were used to determine the amount of sugars hydrolyzed during pretreatment as well as the cellulose content of the pretreated biomass required for enzyme loading.

Enzymatic Hydrolysis Experiments

All corn stover samples (ensiled and non-ensiled) were enzymatically hydrolyzed using a modified protocol by Dowe (2009). A commercial cellulase (GC220 from Genencor in Rochester, NY, United States), was loaded at 40 mg protein per gram cellulose (24 Filter Paper Units (FPU)per gram cellulose) with a beta-glucosidase (β-G)activity of 232 μmol/min/mL. The Genencor enzyme loading was based on the residual cellulose remaining in the pretreated material. Samples were incubated for 5 days at 48°C on a shaking incubator set to 100 rpm. The liquor from the post-enzymatic hydrolysis slurry was separated using a syringe filter (0.45 μm) and analyzed for monomeric sugars, based on NREL LAPs (National Renewable Energy Laboratory [NREL], 2013).

Yield Calculations

The structural carbohydrate yield from cellulosic materials is a critical measure of the pretreatment and enzymatic hydrolysis performance. It is defined as the fraction of a given carbohydrate mass recovered from the aqueous biomass slurry in either monomeric or oligomeric form. This occurs after either pretreatment alone or after a sequential pretreatment and enzymatic hydrolysis. In this work, we calculated both the glucan and xylan yield for both the dilute acid and the hot water pretreatment processes along with enzymatic hydrolysis of the pretreated solids. We did not include degradation products from pretreatment, such as furfural and hydroxy-methyl furfural, in these yield calculations. The combined sugar release from both pretreatment and enzymatic hydrolysis, weighted by their initial mass fraction, is defined as feedstock total carbohydrate yield and is calculated using the following equation:

$$\text{Total Carbohydrate Yield} = \frac{\begin{array}{c}\text{Xylan}_{\text{Pretreatment}} + \text{Xylan}_{\text{EnzymaticHydrolysis}} \\ + \text{Glucan}_{\text{Pretreatment}} + \text{Glucan}_{\text{Enzymatic Hydrolysis}}\end{array}}{\text{Xylan}_{\text{Total}} + \text{Glucan}_{\text{Total}}}$$

The overall feedstock total carbohydrate yield thus accounts for the release of both hexose and pentose sugars from the corn stover and is used as a basis of comparison between the two pretreatment processes and impact on enzymatic hydrolysis processes between the ensiled and non-ensiled corn stover.

Statistical Analyses

All statistical analyses were performed in the open-source language R (R Development Core Team, 2020). Important statistical tests were performed using analysis of variance (ANOVA) and statistical equivalency tests were performed using the Student's *t*-test for two-level comparisons, or the Tukey Honest Significant Difference (HSD) test for multiple-level comparisons. All significance tests were at $p = 0.05$.

Stereomicroscopy

Whole chopped pieces of various tissue fractions of frozen, ensiled, or pretreated corn stover were examined without further processing. Images were captured on a Nikon SMZ1500 stereomicroscope with a digital camera.

Sample Preparation for Optical and Electron Microscopy

Three-millimeter samples of ensiled corn stover stalk rind tissue were fixed and embedded using microwave processing. Samples were fixed 2 × 6 min (2 on, 2 off, 2 on) in 2.5% glutaraldehyde buffered in 0.1 M sodium cacodylate buffer pH 7.2 (from EMS in Hatfield, Pennsylvania) under vacuum. The samples were dehydrated by treating with increasing concentrations of ethanol for 1 min at each dilution (30%, 60%, 90%, and 3 × 100% ethanol). After dehydration, the samples were infiltrated with LR White resin (from EMS in Hatfield, Pennsylvania) for 3 min with one step at room temperature (RT) overnight in increasing concentrations of resin (10%, 30%, 60%, 90%, 3 × 100% resin, diluted in ethanol). Infiltrated samples were transferred to flat-bottomed TAAB capsules and polymerized in a nitrogen-purged vacuum oven at 60°C for 24 h. LR White-embedded samples were sectioned on a Leica EM UTC ultramicrotome (from Leica in Wetzlar, Germany) with a DiATOME diamond knife.

Immunolabeling

Sections of embedded corn stover rind were placed on ProbeOn Plus (from Fisher Scientific, Pittsburgh, PA, United States) microscope slides and incubated in a 5% non-fat dry milk w/v phosphate-buffered saline (PBS), 0.1% Tween 20 (milk/PBST)-blocking solution for 30 min at 25°C. Primary probes—PentaHIS-CBM3 (40 μg/mL milk/PBST), rat α-pectin JIM5 (1:5 v/v milk/PBST dilution) (from Carbosource, Athens, GA, United States), and 4 min, 6-diamidino-2-phenylindole (DAPI, 10 μg/mL milk/PBST) (from Molecular Probes, Eugene, OR, United States)—were applied on sections for 1.5 h at 25°C and then rinsed three times with PBST. Secondary probes – α-PentaHIS: Alexa555 (against CBM3, 1:50 milk/PBST dilution) (Qiagen, Hilden, Germany) and goat α-rat IgM:Alexa488 (against JIM5, 1:200 milk/PBST dilution) (Molecular Probes, Eugene, OR, United States) were applied on sections for 1.5 h and then rinsed 3X with PBST. Sections were dried overnight at 4°C in the dark.

Confocal Scanning Laser Microscopy (CSLM)

Images were captured using a Nikon C1 Plus microscope (from Nikon in Tokyo, Japan), equipped with the Nikon C1 confocal system and four lasers (403 nm, 561 nm, 643 nm, and Argon tunable 458/477/488/515 nm), and operated via Nikon's EZ-C1 software. The 435–465-nm filter was also used to detect autofluorescence. Each optical section of each channel series was scanned twice using Nikon EZ-C1 Average. For all images shown, a series of optical sections was collected, and a subset of this series was used to project the images using either Nikon EZ-C1's Volume Render, Maximum function or ImageJ's 3D Projection, Max function. ImageJ (from the National Institutes of Health in Bethesda, MD, United States) was used to open projected images, separate and combine color channels, and adjust contrast and brightness of images.

Transmission Electron Microscopy (TEM)

LR White-embedded ultra-thin sections were collected on 0.35% Formvar-coated copper slot grids (from SPI Supplies in West Chester, PA, United States). Grids were post stained for 3 min with 2% aqueous uranyl acetate and 3 min with 1% $KMnO_4$. Images were taken with a 4-megapixel Gatan UltraScan 1000 camera (from Gatan in Pleasanton, CA, United States) on a FEI Tecnai G2 20 Twin 200 kV LaB6 TEM (from FEI in Hillsboro, OR, United States) operating at 200 kV.

RESULTS AND DISCUSSION

Evidence of Expected Eight-Week Ensiling Conditions With and Without Additives

The ensiling storage reactors were monitored for the duration of the study and showed no evidence of gas-seal failure on the reactor bodies and no indication of spoilage, however, this biomass was not included in the conversion study. At the end of the 8-week study, the moisture content of the ensiled materials was 0.51 g/g dry biomass. Organic acid concentrations in the Silamax-additive and standard- ensiled materials were 33.8 g/kg dry wt. and 35 g/kg dry wt., respectively. The measured pHs in the Silamax-additive and non-additive-ensiled materials taken at the end of the ensiling study were 4.17 and 3.95, respectively. The slight variation in organic acid levels and pHs in these two conditions is likely due to differences in between the native microbial consortia present and the consortia added with Silamax. However, these results suggest that both the ensiling preservation process and the effect of the additives occurred as expected.

Total Structural Carbohydrates Decreased in the Ensiled Samples

The compositional analysis results of the ensiled and dried materials are shown in **Table 1**. All values are weight% on a dry-weight basis. The starch content of the frozen non-ensiled sample was determined to be 2.13%, but starch analysis was not performed on the dried sample. No starch was detected in either ensiled sample likely due to microbial consumption during the ensiling process.

The two non-ensiled samples (stover frozen after harvest or dried at 45°C after harvest) collectively represent the non-ensiled materials. The compositional data for each of these samples is the average of two replicate analyses. The structural carbohydrate fractions for both non-ensiled corn stover materials were similar. The sucrose concentration was slightly lower in the frozen sample than in the dried fractions. Except for the sucrose measurement, the component values for both non-ensiled samples are within the precision of the wet chemical analysis methods (Megazyme, 2014). Thus, we treat both non-ensiled samples as from the same population. The average and standard deviation of the composition of these two samples (n = 4) are also shown in **Table 1**. The ensiled data represent duplicate analyses of triplicate samples, for a total of six measurements per condition.

TABLE 1 | Compositional analysis of non-ensiled and ensiled corn stover using NREL Standard Laboratory Analytical Procedures (LAPs).

Constituent (% w/w)	Non-ensiled				Ensiled w/o additives		Ensiled with additives	
	Frozen	Dried	Mean	SD	Mean	SD		SD
Sucrose	1.0	4.9	2.9	2.4	1.8	0.2	1.8	0.2
Extractives	9.0	11.8	10.4	2.0	18.1	0.4	16.8	0.3
Glucan	37.9	37.0	37.5	0.8	35.3	0.4	36.0	0.1
Xylan	21.1	21.6	21.4	0.8	19.4	0.6	19.7	0.1
Galactan	2.4	2.5	2.5	0.5	0.9	0.1	0.9	0.1
Arabinan	3.4	3.9	3.6	0.9	2.0	0.2	2.1	0.1
Lignin	16.9	15.3	16.1	0.9	16.1	0.1	16.2	0.2
Ash	3.4	4.0	3.7	0.4	3.4	0.2	3.6	0.3
Total	97.8	100.0	98.9	2.0	96.3	0.5	96.4	0.4

Data are presented on a dry matter% w/w basis.

The data in **Table 1** shows large differences between the composition of the non-ensiled and ensiled materials. The total extractives (the sum of water and ethanol extractives) increased from 10% in the non-ensiled materials to 18% in the samples ensiled without an additive and to 17% in the samples ensiled with an additive. The glucan mass fraction decreased by an absolute average of 1.8% as a result of ensiling (all non-ensiled minus all ensiled samples), representing an average relative loss of 4.8% of the initial glucan content. Xylan content decreased by an absolute average of 1.8%, or an average relative loss of 8.4% of the initial xylan content, while galactan and arabinan content showed small relative reductions. Differences in the glucan, xylan, and water extractives content between the non-ensiled and ensiled materials were significant ($p < 0.05$). Total structural carbohydrates (the sum of glucan, xylan, galactan, and arabinan) decreased in the ensiled samples by an average of 6.7%, representing a relative loss of structural carbohydrates of 10.3%.

Differences in Pretreatment and Enzymatic Hydrolysis Yields Between Ensiled and Non-ensiled Samples for Xylose, but Not for Glucose

Total carbohydrate yield results from the pretreatment and enzymatic hydrolysis experiments are summarized in **Figures 2**, **3** for dilute-acid and hot-water pretreatments, respectively. These experiments did not reveal significant differences in overall feedstock carbohydrate yield or total glucose yield between the non-ensiled corn stover and either set of ensiled samples for either dilute-acid or hot-water pretreatment. The ensiled samples showed small but statistically significant increases in xylose yield after dilute-acid pretreatment and enzymatic hydrolysis compared to the non-ensiled samples, suggesting that the ensiling process used in this study modestly improved hemicellulose conversion. However, because the overall feedstock total carbohydrate yield calculation weights the glucose yield more heavily, these small differences did not translate to changes in overall feedstock reactivity. No differences in xylose yield were seen for the hot-water-pretreated samples.

Severe pretreatment conditions can lead to degradation of the primary sugars to unwanted byproducts (furfural from xylose degradation, HMF from glucose degradation). The samples subjected to hot-water pretreatment showed significantly higher production of HMF and furfural compared to the dilute-acid-pretreatment samples due to the longer residence time and higher temperature required for the hot-water pretreatment. Furfural concentration was higher in the hot-water-pretreated samples an average of 4.95 g/L for frozen and dried samples compared to the ensiled materials, which averaged 7.85 g/L. Furfural concentration in the acid-pretreated materials averaged 1.50 g/L for the frozen and dried samples, compared to 1.69 g/L in the ensiled materials.

Figure 4 provides another way to examine the results for this work, showing the xylose and glucose yields for both dilute-acid and hot-water pretreatment followed by saccharification. For both the non-ensiled and ensiled samples, the majority of glucose was released during enzymatic hydrolysis of the pretreated solids rather than during pretreatment, while xylose release (both oligomeric and monomeric) occurred primarily during pretreatment. Hot-water pretreatment resulted in the release of much higher levels of oligomeric rather than monomeric xylose, which is consistent with previous experiments (Mosier et al., 2005). As before, there are significant differences between the ensiled and non-ensiled samples for xylose yield, but not for glucose yield. Pretreating ensiled materials at process-relevant conditions may impact sugar yields and incur additional costs due to the entrained water potentially affecting catalyst impregnation and heating requirements from the additional water. However, the water that enters with ensiled materials can reduce the water footprint required at the biorefinery and could have positive sustainability impacts. This relationship is out of the scope of this study but has been described elsewhere (Wendt et al., 2018b).

Removal or Re-localization of Cellulose or Pectin by Ensiling Was Not Detected by Immunocytochemistry

To examine whether ensiling caused any redistribution of cell wall components or changes in cell wall structure, we utilized labeling of cellulose and pectic polysaccharides with a carbohydrate-binding module (CBM) probe and specific

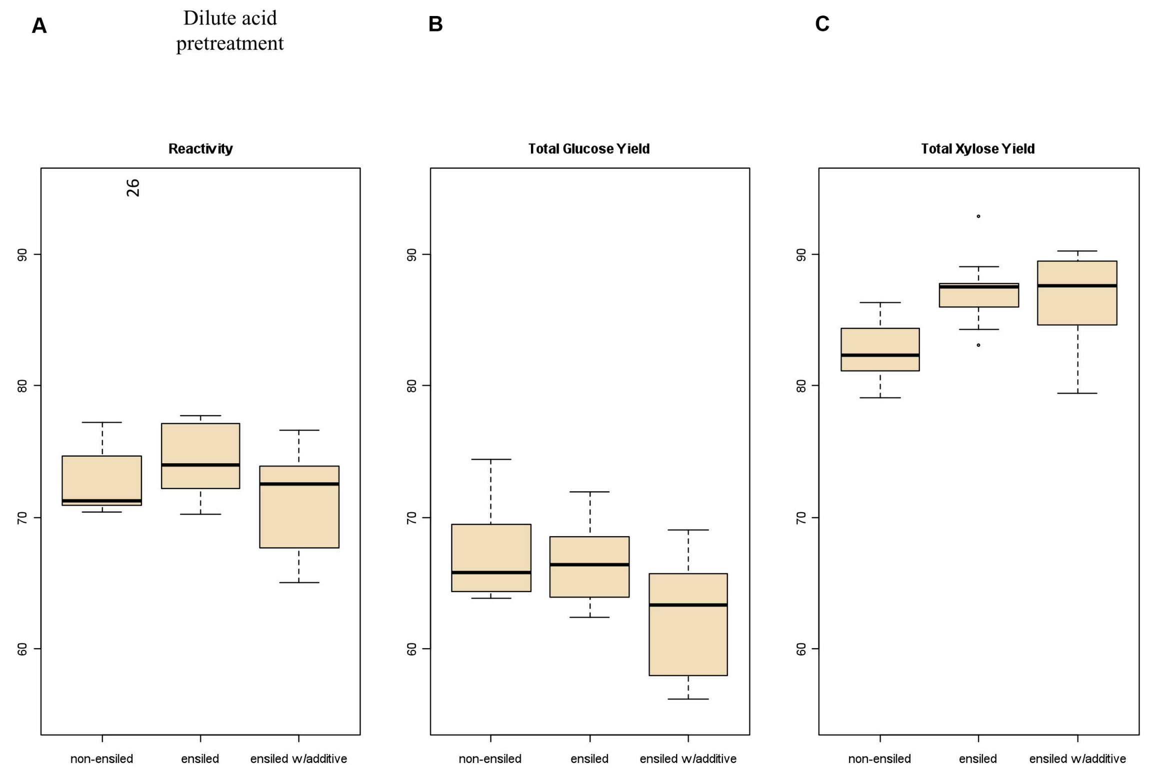

FIGURE 2 | Box plots of **(A)** overall feedstock total carbohydrate yield, **(B)** total glucose yield, and **(C)** total xylose yield for dilute-acid-pretreated corn stover samples both ensiled (with and without additives) and non-ensiled. No statistically significant differences in the mean overall feedstock total carbohydrate yield or total glucose yield between ensiled and non-ensiled materials were measured.

antibodies in plastic-embedded sections of ensiled and non-ensiled corn stover rind. These probes were visualized by fluorescence CSLM. **Figure 5** shows representative CSLM light micrographs of non-ensiled (a–b), ensiled (c–d), and dilute-acid-pretreated (e–f) corn stover cell walls. The images were collected from samples of the same cell and tissue types to allow comparison. **Figures 5A,C,E** are oblique transverse sections through corn stalk rind parenchyma cells. These cells have mature primary cell walls. CBM3:Alexa555 (red) and JIM5:Alexa488 (green) were used to probe cellulose and pectin accessibility, respectively. With these cell wall component probes, the ensiled tissue displays a cleaner, more evenly distributed fluorescent signal with clearer cellulose/pectin signal differentiation at the cell corners. **Figures 5B,D,F** are of oblique longitudinal sections through corn stalk rind sclerenchyma cells. These cells have mature primary and secondary cell walls. With this cell type, a similar pattern of less, but more discretely localized pectin is seen in the ensiled sample. The dominant features of the pretreated cell walls are a stronger cellulose-only signal, minimal pectin signal, and coalesced/redistributed cell wall material adhered to the cell wall surfaces (**Figures 5E,F** arrows).

Overall, the images of both ensiled corn stover parenchyma and fiber cells appear similar to those same cell types in non-ensiled corn stover (**Figure 5**). Ensiled parenchyma images show cell walls of uniform thickness compared with those of neighboring cells, as well as distinct pectin-rich middle lamellae and cell corners. Ensiled sclerenchyma images show cell walls of regular thickness as well as distinct pectic-rich middle lamellae and cellulose-rich secondary walls. Cells of ensiled stover rind, regardless of cell type, do not appear to be collapsed, nor do cell wall layers appear to be delaminated at the resolution of optical microscopy.

Ensiled Cell Walls Exhibit Ultrastructural Characteristics Intermediate Between Non-ensiled and Dilute-Acid-Pretreated Cell Walls

At the light microscopy level ensiled corn stover exhibited little structural change or relocation of cell wall components as detected by immunocytochemistry. However, ultra-thin sections of the same samples by TEM were analyzed to determine if more subtle structural changes were present at the ultrastructural level (**Figure 6**). At first glance, the ensiled cell walls again did not appear dramatically different from non-ensiled samples. There was no dramatic loss of cell wall integrity leading to collapse of cell lumen or extensive cell wall delamination, nor was there evidence of a severely degraded cell wall as seen in previous work (Donohoe et al., 2009). A more careful analysis at higher magnification, however, did reveal some ultrastructural changes

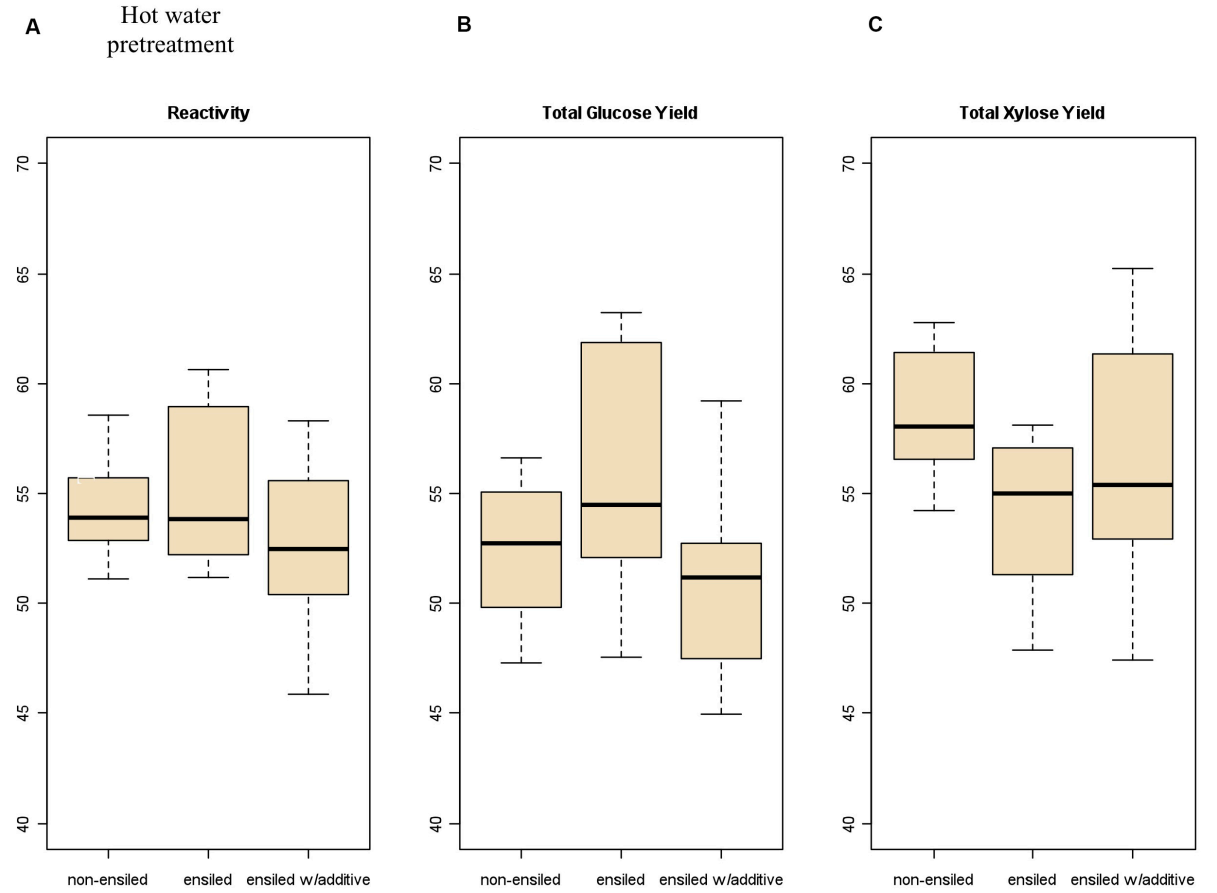

FIGURE 3 | Box plots of **(A)** overall feedstock total carbohydrate yield, **(B)** total glucose yield, and **(C)** total xylose yield for hot-water-pretreated corn stover samples both ensiled (with and without additives) and non-ensiled. No statistically significant differences in overall feedstock total carbohydrate yield, total glucose yield, or total xylose yield between ensiled and non-ensiled materials were measured.

that could have an impact on the further pretreatment and saccharification of ensiled biomass.

Figure 6 displays TEM micrographs of fiber cell walls from near corn stalk rind vascular bundles. These cells have mature lignified compound middle lamella (ml) and secondary (2°) cell walls. Micrographs a, c, and e display a cell corner region. Panels b, d, and f how a more magnified view of secondary cell walls from each condition where the texture of the cell wall provides evidence of cell wall matrix removal and re-localization. The ensiled cell walls display some structural differences compared to non-ensiled controls. The individual lamella within the secondary cell wall are more distinct and the staining pattern within the secondary cell wall is more granular in appearance suggesting some removal or re-localization of cell wall components. However, as expected, the extent of removal and re-localization in ensiled walls is not as extensive as in dilute acid pretreatment.

The appearance of the fine lamellar structure of the secondary cell wall in ensiled compared to non-ensiled walls implies that some of the inter lamellar connections have weakened or been partially removed (**Figure 6**). Similar lamellar separation patterns enhanced by pretreatments have been observed in previous studies (Donohoe et al., 2009). At higher resolution the subtle differences among non-ensiled, ensiled and pretreated cell walls are revealed. The fine pattern of distribution of low and high densely staining material in the non-ensiled sample is typical of the natural density and distribution of the hemicellulose and lignin in native cell walls. In a dilute acid pretreated sample, the pattern of dense material has become coarser. In previous work, we have shown that this pattern is partly due to the extraction of hemicellulose and the coalescence and migration of lignin within the cell wall (Donohoe et al., 2008). While ensiled cell walls do not exhibit the same coalescence pattern as the dilute acid pretreated material, they do have a different structure and coarser staining pattern than the control. This pattern is consistent with some extraction and reorganization of the cell wall matrix components and a partial loosening of the wall structure evidenced by a lower overall electron density and regions of visibly increased porosity. These ultrastructural observations also suggest that alternative conversion methods, such as deacetylation and

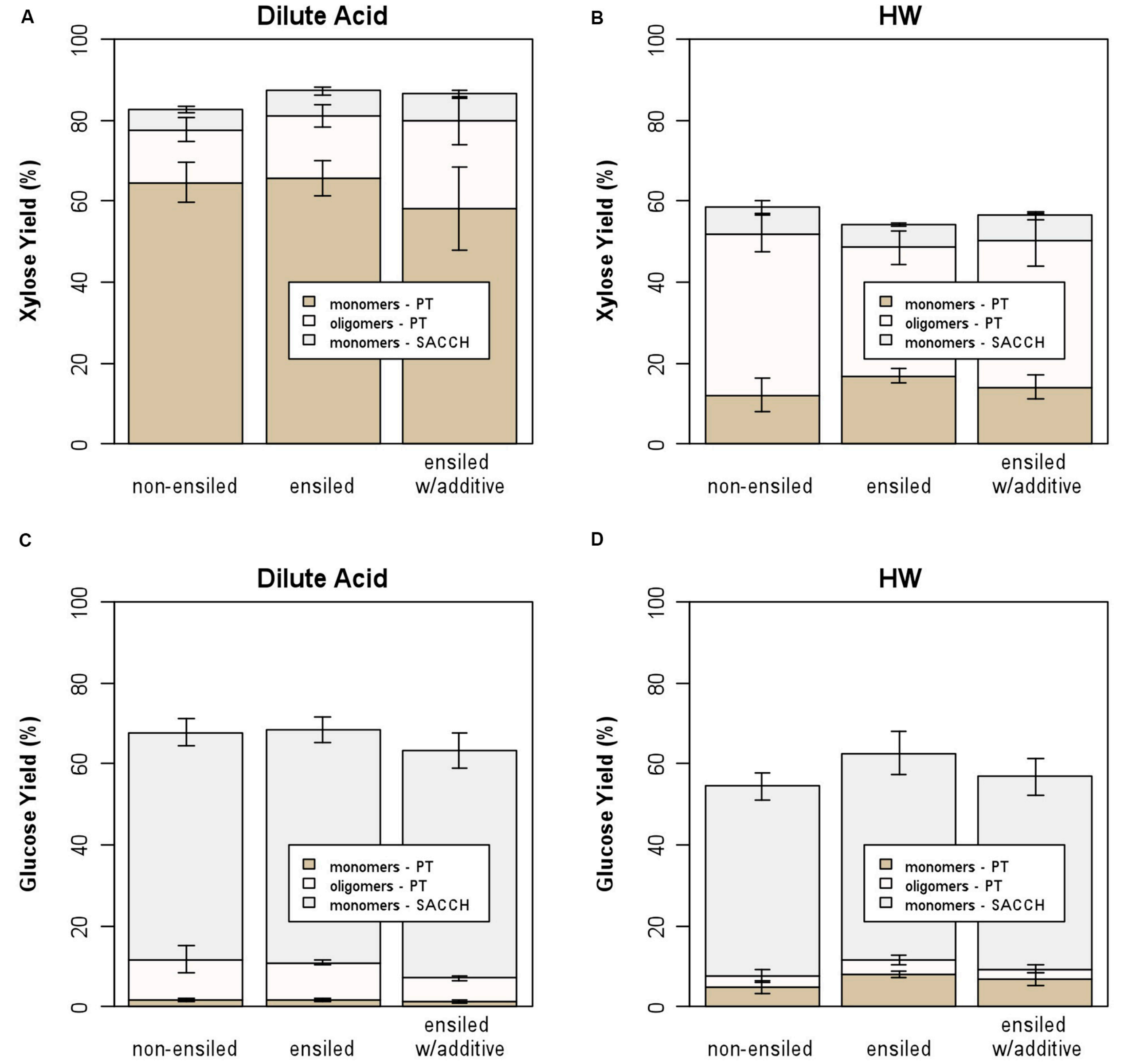

FIGURE 4 | Stacked bar plot showing contribution of oligomeric and monomeric xylose and glucose for hot-water and dilute-acid pretreatment and by saccharification of the pretreated solids; **(A)** xylose yield from dilute-acid pretreatment, **(B)** xylose yield from hot-water pretreatment, **(C)** glucose yield from dilute-acid pretreatment, and **(D)** glucose yield from hot-water pretreatment. The majority of xylose is released during pretreatment, while most glucose is released from saccharification.

mechanical refining (DMR), that could take advantage of the lamellar defects and may be more effective than dilute acid or hot water pretreatment for biomass conversion of ensiled materials.

CONCLUSION

Providing year-round feedstock supply having consistent quality, quantity, cost, and stability is a major challenge to future biorefineries. Traditional bale systems, while representing the current preferred method for storage of agricultural residues, are limited due to dry-matter losses, requirements for in-field drying, and fire risk. Wet storage by ensiling agricultural residues offers the potential for reducing these risks. The high moisture content coupled with an anaerobic environment, at low pH reduces material losses providing more options for collection and storage. Ensiling and ensiling with additives did not reduce the bioconversion requirements of the carbohydrates from either dilute acid or hot water pretreatment of corn stover. Additional technoeconomic analysis is necessary to determine if the cost/benefit of ensiling offsets the loss of biomass

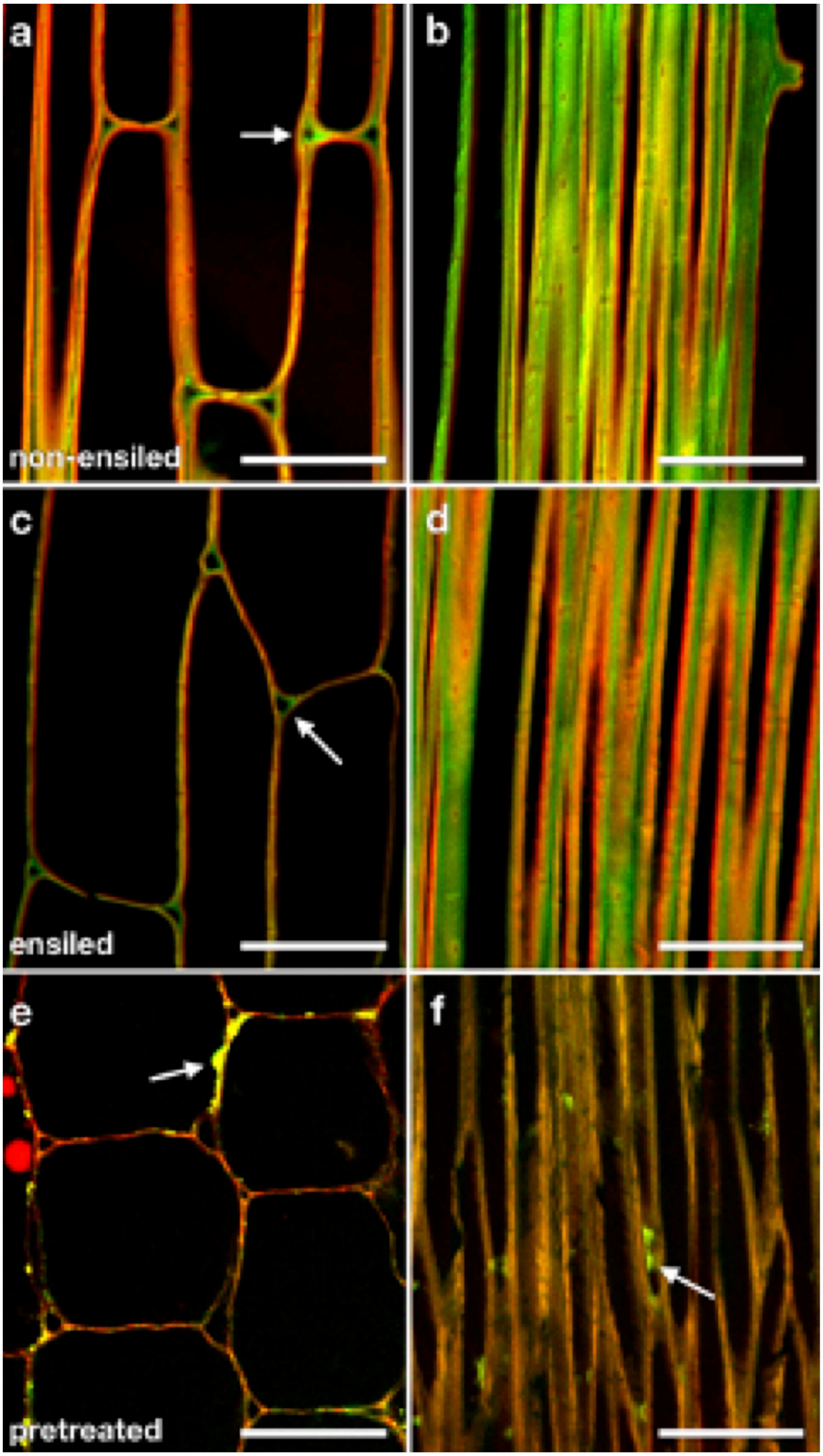

FIGURE 5 | CSLM light micrographs of non-ensiled **(a,b)**, ensiled **(c,d)**, and dilute-acid pretreated **(e,f)** corn stover cell walls. Panels **(a,c,e)** are oblique transverse sections through corn stalk rind parenchyma cells. These cells have mature primary cell walls. CBM3:Alexa555 (red) and JIM5:Alexa488 (green) were used to probe cellulose and pectin accessibility, respectively. Note that when using identical probe concentrations, ensiled stover displays a cleaner, more evenly distributed fluorescent signal with clearer cellulose (red)/pectin (green) signal differentiation at the cell corners [**(a,c)** arrows]. Panels **(b,d,f)** are oblique longitudinal sections through corn stalk rind sclerenchyma cells. These cells have mature primary and secondary cell walls. Again, a pattern of less, but more discretely localized pectin is seen in the ensiled sample. The dominant features of the pretreated cell walls are a stronger cellulose-only signal, minimal pectin signal, and coalesced/redistributed cell wall material adhered to the cell wall surfaces [**(e,f)**, arrows]. Scale bars = 50 μm.

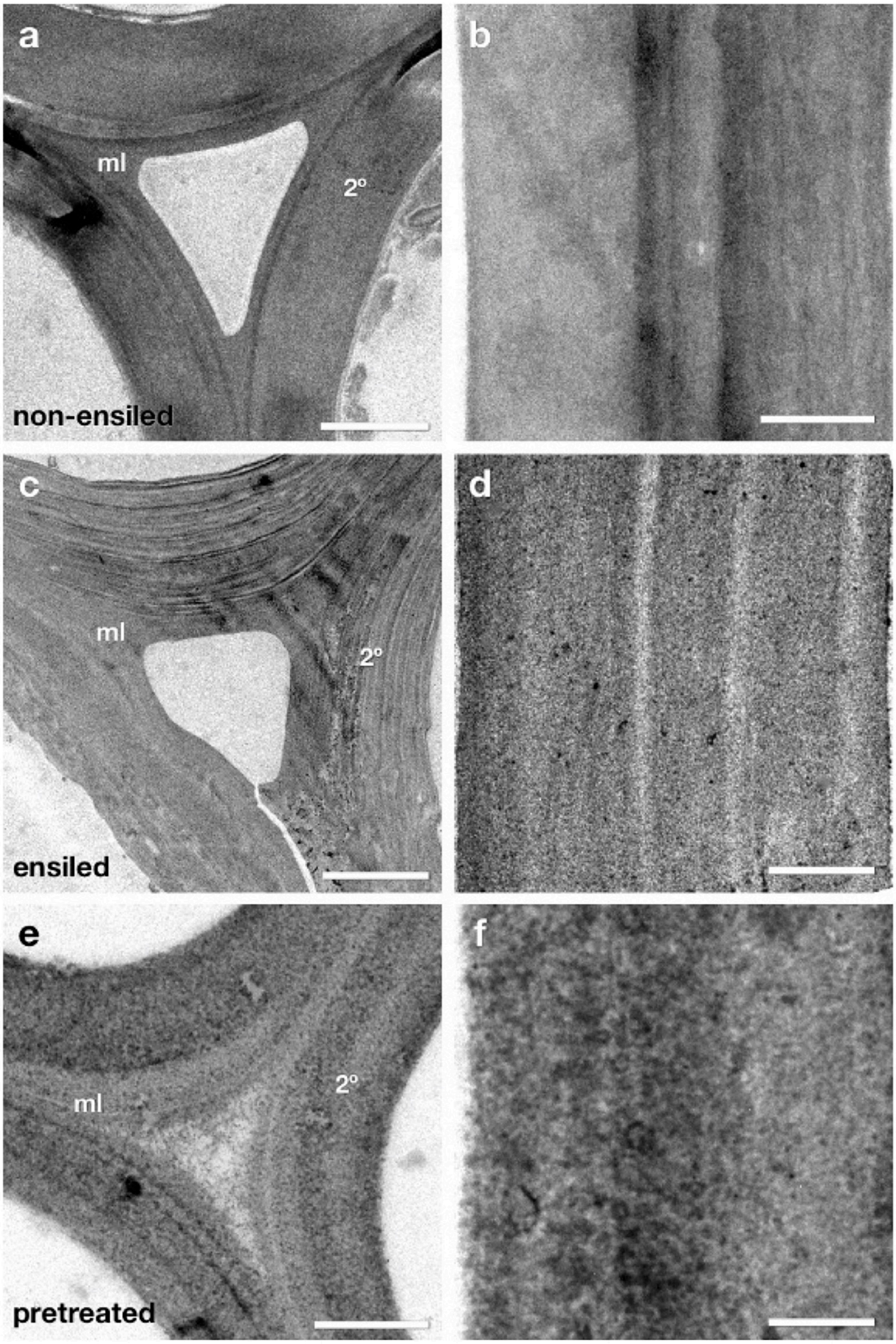

FIGURE 6 | TEM micrographs of fiber cell walls from near corn stalk rind vascular bundles. These cells have mature lignified compound middle lamella (ml) and secondary (2°) cell walls. Micrographs **(a,c,e)** display a cell corner region where the curvature of the wall is greatest and the middle lamella (ml) contact between adjacent cells can be seen. Panels **(b,d,f)** show a more magnified view of secondary cell walls from each condition where the texture of the cell wall provides evidence of cell wall matrix removal and re-localization. The ensiled cell walls display some structural differences compared to non-ensiled controls. The individual lamella within the secondary cell wall are more distinct **(c)** and the staining pattern within the secondary cell wall **(d)** more granular in appearance, suggesting some removal or re-localization of cell wall components. However, as expected, the extent of removal and re-localization in ensiled walls is not as extensive as in dilute acid pretreatment **(e,f)**. Scale bars: **(a,c,e)** = 2 μm; **(b,d,f)** = 0.5 μm.

carbohydrates and the additional transportation cost from the entrained water associated with wet storage.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

NN and EW planned the experiments, analyzed the conversion results, and wrote the manuscript. EW conducted statistical analysis of the conversion results. BD designed the microscopy and image analysis and wrote the manuscript. NW and EK conducted pretreatment and enzymatic hydrolysis experiments used in this study and contributed to the manuscript. TH performed the microscopy and image analysis. All authors read, edited, and approved the final draft of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2020.00739/full#supplementary-material

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Conflict of Interest: CR was employed by the company Verd Company.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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