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Switchgrass and Giant Miscanthus Biomass and Theoretical Ethanol Production from Reclaimed Mine Lands

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Abstract

Switchgrass (*Panicum virgatum* L.) and giant miscanthus (*Miscanthus x giganteus* Greef & Deuter ex Hodkinson & Renvoize) are productive on marginal lands in the eastern USA, but their productivity and composition have not been compared on mine lands. Our objectives were to compare biomass production, composition, and theoretical ethanol yield (TEY) and production (TEP) of these grasses on a reclaimed mined site. Following 25 years of herbaceous cover, vegetation was killed and plots of switchgrass cultivars Kanlow and BoMaster and miscanthus lines Illinois and MBX-002 were planted in five replications. Annual switchgrass and miscanthus yields averaged 5.8 and 8.9 Mg dry matter ha^{-1}, respectively, during 2011 to 2015. Cell wall carbohydrate composition was analyzed via near-infrared reflectance spectroscopy with models based on switchgrass or mixed herbaceous samples including switchgrass and miscanthus. Concentrations were higher for glucan and lower for xylan in miscanthus than in switchgrass but TEY did not differ (453 and 450 L Mg^{-1}, respectively). In response to biomass production, total ethanol production was greater for miscanthus than for switchgrass (5594 vs 3699 L ha^{-1}), did not differ between Kanlow and BoMaster switchgrass (3880 and 3517 L ha^{-1}, respectively), and was higher for MBX-002 than for Illinois miscanthus (6496 vs 4692 L ha^{-1}). Relative to the mixed feedstocks model, the switchgrass model slightly underpredicted glucan and slightly overpredicted xylan concentrations. Estimated TEY was slightly lower from the switchgrass model but both models distinguished genotype, year, and interaction effects similarly. Biomass productivity and TEP were similar to those from agricultural sites with marginal soils.

Keywords Cellulosic bioenergy feedstock · Mine reclamation · Near-infrared reflectance spectroscopy · Theoretical ethanol production · Theoretical ethanol yield

Abbreviations

ADL	Acid detergent lignin
ARA	Arabinan
GAL	Galactan
GLC1	Glucan from NIRSC model
GLC2	Glucan from NREL model
ASH1	Ash from NIRSC model
ASH2	Ash from NREL model
DM	Dry matter
EC	Electrical conductivity
LIG	Lignin from NREL model
aNDF	Amylase-treated neutral detergent fiber
MAN	Mannan
NIRS	Near-infrared reflectance spectroscopy
NIRSC	NIRS Forage and Feed Testing Consortium
NREL	National Renewable Energy Laboratory
TEP	Theoretical ethanol production
TEY	Theoretical ethanol yield
WV	West Virginia
XYL1	Xylan from NIRSC model
XYL2	Xylan from NREL model

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Introduction

Climate change awareness has prompted research into alternative energy sources to replace fossil fuels. Bioenergy crops can supply renewable feedstocks for ethanol production and other energy sources, reduce dependence on fossil fuel use [1], and offer economic returns to growers in rural areas. By 2022, fuel production from bioenergy crops is mandated to reach 136 billion liters (0.9 billion barrels) or about 15% of the total transportation fuel consumed in the USA [2]. The most widely produced biofuel is from maize (corn; *Zea mays* L.) grain conversion to ethanol. Increasing world food demands may constrain availability of agricultural land for bioenergy production from corn grain [3–6]. An annual crop with large soil nutrient requirements and risks of soil erosion and runoff that is cultivated primarily on productive agricultural lands, corn may be less well suited than herbaceous perennials for fuel production [7–11]. The use of corn stover for production of cellulosic ethanol increases ethanol production per land area, but growing corn for fuel still requires agricultural lands that could be used for food crops and can reduce contributions of valuable organic material to soil health [9, 12, 13].

Other crops that have been evaluated for bioenergy production capabilities have lower cultural inputs and management requirements. Switchgrass and giant miscanthus (hereafter, miscanthus) are warm-season (C_4) perennial grasses with promising biofuel production capabilities due to their productivity on marginal land such as reclaimed mine lands [14–20]. This is consistent with the emerging view that cellulosic bioenergy feedstock production should be based on perennials grown on marginal lands that are unsuitable for annual cropping [9, 15, 21, 22].

The Surface Mining Control and Reclamation Act (SMCRA) of 1977 requires coal mining companies in the USA to establish a post-mining land use that is acceptable to the land owner, will minimize impacts on the land and surrounding environment, and is compatible with surrounding unmined land uses. More than 80% of surface-mined land in the eastern USA coal mining region is reclaimed to agricultural uses such as pasture and hay production [23]. Another potential agricultural use for reclaimed lands in this region is the production of dedicated bioenergy feedstocks for transportation fuels and for direct combustion. Several studies have shown high yields for switchgrass grown on reclaimed mine lands in this region [14, 18, 19, 24]. Studies in Europe and the USA have shown miscanthus to be a promising cellulosic bioenergy crop candidate because of its capacity to produce yields double that of the current "model" biofuel crop, switchgrass [25–28]. Miscanthus may therefore have greater cellulosic ethanol production potential than switchgrass, making it a more attractive option for meeting current mandates. Little is known, however, of the biomass composition of miscanthus grown on mined lands.

The objectives of this study were to determine (i) biomass yields of two cultivars of lowland switchgrass (Kanlow and BoMaster) and two lines of miscanthus (Illinois and MBX-002) on a reclaimed mine site; (ii) their biomass composition for ethanol conversion; and (iii) their theoretical ethanol yield (TEY, L Mg^{-1} of dry matter (DM)) and production (TEP, theoretical ethanol production, L ha^{-1}). Two near-infrared reflectance spectroscopy (NIRS) models were used to predict cell wall polysaccharide composition for calculation of TEY. These were developed from calibration sets containing switchgrass [29] or mixed herbaceous cellulosic feedstocks including switchgrass and miscanthus [30]. The switchgrass model is available to members of the NIRS Forage and Feed Testing Consortium (NIRSC, Hillsboro, WI), whereas the mixed feedstock model developed by the US Department of Energy National Renewable Energy Laboratory (NREL, Golden, CO) is not commercially available. We compared the suitability of the NIRSC switchgrass-based model for prediction of switchgrass and miscanthus composition to the NREL mixed feedstocks model, which included miscanthus samples in calibration and validation sets [30]. Biomass yields were multiplied by TEY to determine TEP at the ends of the fifth and sixth growing seasons.

Materials and Methods

Site Location and Treatment and Experimental Designs

Plots were established in spring 2010 at Alton, a 160-ha reclaimed surface mine located in Upshur County, WV (38.49° N, 80.11° W, 650 m elevation). This site was mined for Kittanning coal with truck-shovel equipment spreads. In 1985, the area was backfilled and reclaimed with a 15-cm depth of native forest topsoil (Gilpin silt loam; fine-loamy, mixed, active, mesic Typic Hapludults) placed over mixed sandstone-siltstone overburden. The reclaimed land was fertilized and limed according to regulations at the time and seeded with tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire), orchardgrass (*Dactylis glomerata* L.), birdsfoot trefoil (*Lotus corniculatus* L.), and clovers (*Trifolium* spp.). This site supported a nearly complete ground cover of herbaceous plants during the ensuing 25 years, which allowed for organic matter inputs and soil development. A 10-ha area with flat to slightly rolling topography was selected for this experiment. The ground cover was killed with glyphosate (*N*-(phosphonomethyl)glycine) at recommended rates the previous fall and again in the spring before seeding switchgrass and planting miscanthus rhizomes in 2010. No soil amendments were applied during the experiment.

Lowland switchgrass cultivars Kanlow and BoMaster and miscanthus lines Illinois and MBX-002 treatments were randomly assigned to 0.4-ha plots twice in one block and three times in a nearby (≤ 300 m) second block. Switchgrass seed

(Ernst Conservation Seeds, Meadville, PA 16335) was planted with a sod-seeding drill at 11 kg pure live seed ha^{-1}. Miscanthus rhizomes of 10 cm length (Mendel Biotechnology, Hayward, CA 94557) were planted at approximately 12,350 ha^{-1} (0.9-m spacing or about 4950 rhizomes $plot^{-1}$).

Soil and Biomass Collection and Analysis

In 2010 before the experiment was established, 10 soil samples were taken to a depth of 15 cm on a line crossing the two blocks. In 2015, soil samples were taken to a 15-cm depth from three random points in each plot and composited. All samples were analyzed for pH, electrical conductivity (EC), and selected nutrients and the 2010 samples were analyzed for organic matter concentration by loss on ignition at 550 °C. Soil samples were air-dried, weighed, and passed through a 2-mm sieve. Subsamples of the sieved fraction of each sample were taken using a riffle splitter and analyzed. For pH, 5 g soil was combined with 5 mL distilled deionized (DDI) water. The mixture was placed on an orbital shaker table and mixed for 15 min, then allowed to equilibrate for at least 1 h. A pH meter (Seven Easy, Mettler Toledo, Columbus, OH 43240) was used to measure pH. Electrical conductivity was determined by combining 5 g soil with 10 mL DDI water. The mixture was placed on an orbital shaker table and mixed for 15 min, then allowed to equilibrate for at least 1 h. A conductivity meter (Seven Compact S230, Mettler Toledo, Columbus, OH 43240) was used to measure EC.

Nutrients were extracted from soils with a Mehlich 1 solution (0.05 mol L^{-1} HCl and 0.025 mol L^{-1} H_2SO_4) [31]. For extraction, 25 mL Mehlich 1 solution was added to 5 g soil, mixed on an orbital shaker for 5 min, and allowed to equilibrate. Samples were filtered through Whatman No. 2 filter paper and an inductively coupled plasma emission spectrometer (Perkin Elmer, Boston, MA 02118) was used to analyze the filtrate for extractable P, K, Ca, and Mg.

From 2011 through 2015, biomass was sampled from six randomly placed quadrats (locations not repeated over years) $plot^{-1}$. Switchgrass was clipped from 0.21-m^2 quadrats at post-anthesis stage in early October (first freeze usually occurs in mid-November) to a stubble height of approximately 10 cm [32]. Miscanthus was clipped on the same dates at the same stage to the same stubble height, from 0.82-m^2 sampling areas centered over each initially planted rhizome. Samples were oven-dried at 60 °C to constant weight [33] to determine DM production. Plot biomass was not harvested annually and biomass from previous year's growth was < 5% of samples.

Near-infrared Reflectance Spectroscopy

Switchgrass and miscanthus samples from 2014 and 2015 were analyzed via NIRS with two spectrophotometers with corresponding chemometric prediction models developed with partial least squares regression. Eight constituents comprising set A (Table 1) were predicted with a scanning monochromator (SpectraStar 2400 RTW and UCal 3.0 software, Unity Scientific, Brookfield, CT) and two compositional prediction models available to members of the NIRSC. These

Table 1 Codes for biomass compositional traits predicted with three NIRS models and used in theoretical ethanol calculations for switchgrass and miscanthus

	Prediction model[a]		
	A) NIRSC		B) NREL
Compositional variable	Grass hay[b]	Switchgrass bioenergy[c]	Mixed feedstocks bioenergy[d]
Ash	ASH1		ASH2
Acid detergent or NREL lignin	ADL		LIG
Amylase-treated neutral detergent fiber	aNDF		
Cell wall carbohydrates			
Arabinan		ARA	
Xylan		XYL1	XYL2
Galactan		GAL	
Glucan		GLC1	GLC2
Mannan		MAN	

[a] *NIRSC*, NIRS Forage and Feed Testing Consortium; *NREL*, National Renewable Energy Laboratory

[b] Summary statistics for 11ghu24_0811.prd: For ADL, $n = 134$, calibration range = 0.2–10.6% of DM, standard error of cross-validation (SECV) = 0.94, r^2 of CV = 0.74. For aNDF, $n = 779$, calibration range = 28.8–84.5% of DM, SECV = 3.03, r^2 of CV = 0.94. For ASH1, $n = 293$, calibration range = 3.0–25.5% of DM, SECV = 1.30, r^2 of CV = 0.88

[c] Vogel et al. [29]

[d] Payne and Wolfrum [30]. LIG is according to NREL Laboratory Analytical Procedures [33, 41]

were a switchgrass bioenergy model based on 129 samples grown on agricultural sites in the Great Plains region of the USA representing diverse cultivars, locations, and harvesting techniques [29]; and a grass hay model (11ghu24_0811.prd) developed from 134 to 779 cool- and warm-season grass samples, depending on the constituent reported here [34]. Four constituents comprising set B (Table 1) were predicted with a scanning Fourier-transform monochromator (Thermo Antaris II FT-NIR, Thermo Fisher Scientific, Waltham, MA) and NREL mixed cellulosic feedstocks model based on 232 calibration samples including 16 of switchgrass and 30 of miscanthus [29]. The NREL mixed feedstocks model was validated with an external set of 25 samples including two of switchgrass and eight of miscanthus, and we therefore considered it to be a standard against which to judge the suitability of the NIRSC switchgrass model for analysis of both species. All samples were ground to pass a 2-mm screen of a cutting mill (Wiley Laboratory Mill, Mod. 4, Thomas Scientific, Swedesboro, NJ). Samples for set A were riffle split and re-ground through a cyclone mill (Tecator Cyclotec, FOSS North America, Eden Prairie, MN) to pass a 1-mm screen. Ground samples were packed into ISI (Infrasoft International LLC, State College, PA) sample cups for set A and borosilicate scintillation vials for set B to a consistent packing density.

Spectra from set A were recorded as the reciprocal log of reflectance (log 1/R) at 1-nm increments over a wavelength range of 1250–2348 nm and standardized to a master instrument (FOSS Model 6500, FOSS North America, Eden Prairie, MN) managed by the NIRSC. The switchgrass bioenergy model was used to predict concentrations of cell wall carbohydrates arabinan (ARA), galactan (GAL), glucan (GLC1), mannan (MAN), and xylan (XYL1), and the grass hay model was used for prediction of ash (ASH1), acid detergent lignin (ADL), and amylase-treated neutral detergent fiber (aNDF, Table 1), as commonly used in analysis of forage nutritional value [35]. The latter three constituents were included because aNDF serves as an index of overall cell wall concentration reflecting sample maturity stage and extent of weathering [36] and because of their utility in simplified approaches to calculation of TEY [10, 37–39]. Summary statistics for these models are provided in Vogel et al. [29] and Table 1. Reference wet chemical methods for NIRSC bioenergy and grass hay calibration samples are described in Vogel et al. [29] and AOAC International [40] methods 2002.04, 973.18, and 942.05 for aNDF, ADL, and ash, respectively. Data for spectral outliers defined by mean global and neighborhood distances ≥ 4.5 and ≥ 1.7, respectively, were not used. This resulted in rejection of at least one cell wall carbohydrate from six samples out of 40 across 2014–2015.

Samples in set B were each scanned 128 times over a wavelength range of 1111–2500 nm and glucan (GLC2), xylan (XYL2), lignin (LIG), and ash (ASH2) concentrations were predicted with the NREL mixed feedstocks model [30; Table 1]. Reference wet chemical methods for NREL model samples are described in online Laboratory Analytical Procedures for determination of structural carbohydrates, lignin, and ash [41] and reviewed in Sluiter et al. [33]. Although ADL and LIG are both Klason lignin procedures involving 72% sulfuric acid hydrolysis at room temperature, the NREL LIG procedure involves an additional acid hydrolysis step at 121 °C. Prediction uncertainty was determined using Martens' uncertainty algorithm [42]. All predicted values fell within an acceptance range of ≤ 2.5 times the root mean square standard error of calibration of the analyte. All calculations were performed in the R programming language.

Theoretical Ethanol Yield and Production

Theoretical ethanol yield and production were calculated from compositional and biomass data according to two methods based on five or two cell wall polysaccharides predicted with NIRSC or NREL feedstock models (Table 2). The switchgrass and mixed feedstocks models expressed carbohydrates in polymeric form (i.e., arabinan, galactan, glucan, mannan, and xylan) and assumed 100% ethanol conversion [29]. *Method 1* included hexoses glucan, galactan, and mannan and pentoses xylan and arabinan for analysis based on a full profile of cell wall carbohydrates [29, 43]. Method 1 TEY predictions were from the NIRSC switchgrass model since it was the sole model that included all five carbohydrates. *Method 2* involved only the primary cell wall carbohydrates glucan and xylan [43] as predicted by NIRSC and NREL bioenergy models. Concentrations of five and two carbohydrates from the NIRSC switchgrass model were used to calculate TEY1 and TEY2A, respectively (Table 2). These were compared to determine the proportion of TEY1 calculated from five polysaccharides to that accounted for by TEY2A based only on glucan and xylan. Predictions of GLC2 and XYL2 from the NREL mixed feedstocks model were considered standards against which to assess predictions of switchgrass and miscanthus GLC1 and XYL1 from the NIRSC switchgrass model, since both species were included in development and validation of the NREL model. Similarly, while TEP1 for both species was based on five carbohydrates predicted by the NIRSC switchgrass model, TEP2B based on glucan and xylan predicted by the NREL model was considered a standard for comparison because both species were represented in the NREL model.

Statistical Analysis

Soil chemical data from 2015 were analyzed using a mixed model ANOVA (PROC GLIMMIX) with SAS Version 9.4 [44], to test fixed effects of species and genotype within species (hereafter cultivar (species)), accounting for a random effect of block. Biomass was analyzed using PROC

Table 2 Carbohydrate constituents and calculations used in two methods to predict theoretical ethanol yield (TEY) and theoretical ethanol production (TEP) of switchgrass and miscanthus at Alton, WV

Method and model[a]	*n* cell wall sugars[b]	Constituents and conversions[c]	Unit
		Carbohydrates (g kg^{-1} DM)	
Method 1: NIRSC bioenergy prediction model			
HEX1	3	(GLC1 + GAL + MAN) × 0.57 × 1.267	L Mg^{-1}
PEN1	2	(XYL1 + ARA) × 0.579 × 1.267	L Mg^{-1}
TEY1	5	HEX1 + PEN1	L Mg^{-1}
TEP1	5	TEY1 × biomass yield (Mg DM ha^{-1})	L ha^{-1}
Method 2: NIRSC (A) and NREL (B) bioenergy prediction models			
HEX2A	1	GLC1 × 0.57 × 1.267	L Mg^{-1}
PEN2A	1	XYL1 × 0.579 × 1.267	L Mg^{-1}
HEX2B	1	GLC2 × 0.57 × 1.267	L Mg^{-1}
PEN2B	1	XYL2 × 0.579 × 1.267	L Mg^{-1}
TEY2A	2	HEX2A + PEN2A	L Mg^{-1}
TEY2B	2	HEX2B + PEN2B	L Mg^{-1}
TEP2B	2	TEY2B × biomass yield (Mg DM ha^{-1})	L ha^{-1}

[a] *NIRSC*, NIRS Forage and Feed Testing Consortium; *NREL*, National Renewable Energy Laboratory

[b] Number of cell wall hexose (HEX) and pentose (PEN) polysaccharides in the near-infrared reflectance spectroscopy model for prediction of biomass composition

[c] After Dien et al. [43] and Vogel et al. [29]. Codes for polysaccharides are in Table 1

GLIMMIX with year as a repeated variable and autoregressive covariance structure. Fixed effects were species, cultivar (species), year, and year × species and year × cultivar (species) interactions, accounting for a random effect of block. The significance criterion for all tests was alpha = 0.05. When cultivar (species) was a significant effect, the SLICE option in LSMEANS was utilized to compare genotypes within each species. Compositional and ethanol production variables were analyzed like biomass, except that year was not a repeated effect. Pearson correlation coefficients for pairs of selected compositional and ethanol yield variables were determined with JMP Pro Version 12.2 [45]. Variables lacking normal distribution were transformed as needed.

Results and Discussion

Environmental Conditions and Soil Characteristics

Precipitation and temperature data from weather stations near the study site did not appear to explain annual patterns of biomass production or composition (Table 3 and Fig. 1). Monthly precipitation was below normal in June 2012, September 2013 and 2014, October 2013 and 2015, and May and August 2015. While monthly precipitation varied widely among years, total precipitation during each 7-month growing season from 2011 to 2015 was within 5% of the long-term average of 847 mm, except for 12% higher precipitation in 2011. Lower precipitation in September–October 2013 and August and October 2015 in particular may have reduced productivity (Fig. 1). Monthly mean temperatures did not depart greatly from long-term averages and if anything were higher, especially for April and October.

Before the experiment was established in 2010, soil pH ranged from 7.0 to 7.7 and all nutrients were at medium to high levels suitable for plant growth (Table 4). Organic matter ranged from 1.8 to 4.0% with a mean of 2.6% (data not shown). In 2015, soil pH averaged 7.0 to 7.2 across treatments and these mine soils had medium to high levels of P, K, Ca, and Mg (Table 4). Treatment effects were found only for P but the small difference associated with species would not be expected to alter biomass yield or composition. The nutrient concentrations in mine soils in 2010 appear to be higher than those measured in 2015, so it is possible that the uptake of nutrients by these biomass crops are reducing the concentrations in the soils. Mine soils are quite variable both within and across reclaimed areas [46, 47] and the 25 years of forage growth with incorporation of organic matter and soil development should have helped to buffer potential differences in soil chemical properties at this site. Bioenergy crops like switchgrass and miscanthus have demonstrated biomass yields of > 5 Mg ha^{-1} on marginal soils at soil pH and nutrient levels comparable to those in the present study [20, 32, 36, 48–52].

Biomass Production

Switchgrass and miscanthus stands were well established by the end of the second growing season, after which density continued to increase. Switchgrass produced a uniform plant cover and miscanthus had spread to densities of as many as

Table 3 Monthly precipitation totals and temperature means during growing seasons of 2011–2015 and 30-year (1981–2010) normals for same months at Rock Cave, WV, 11 km from the Alton, WV mine site

Year	April	May	June	July	August	September	October	Total or mean
	Monthly total precipitation[a] (mm)							
2011	200	131	91	107	97	156	170	951
2012	78	169	39	190	116	129	177	897
2013	62	107	139	173	233	61	76	853
2014	64	131	101	157	145	62	188	850
2015	164	69	245	158	58	107	47	848
Mean	101	115	123	149	130	102	132	852
30-year	110	142	137	144	128	93	93	847
	Monthly mean temperature (°C)							
2011	13.4	16.1	19.6	22.6	20.6	17.4	10.4	17.2
2012	10.3	17.9	19.3	23.2	20.4	16.8	11.4	17.0
2013	12.0	15.7	19.8	21.5	19.4	16.7	12.1	16.7
2014	11.8	16.1	20.6	20.1	19.7	17.1	11.6	16.7
2015	11.6	18.3	21.2	21.8	20.1	18.9	11.6	17.7
Mean	11.8	16.8	20.1	21.8	20.1	17.4	11.4	17.0
30-year	10.5	15.1	19.4	21.4	20.7	17.1	11.0	16.5

[a] Precipitation data for May 2011, July 2012, May 2014, April 2015, and Sept. 2015 were missing at Rock Cave and were substituted from Sutton Lake, WV, 29 km farther from Alton than Rock Cave. From Northeast Regional Climate Center CLIMOD 2 data

25–100 tillers 0.82-m^2 area centered over each rhizome planting point at the end of the third growing season [24]. It is characteristic of switchgrass and miscanthus to reach full stand establishment by the end of the third year [32, 53, 54].

There were no treatment × year interactions for biomass production, which was higher for miscanthus than for switchgrass across genotypes and years (8.9 vs 5.8 Mg ha^{-1}, Fig. 1). Multi-year mean yields for Kanlow and BoMaster switchgrass

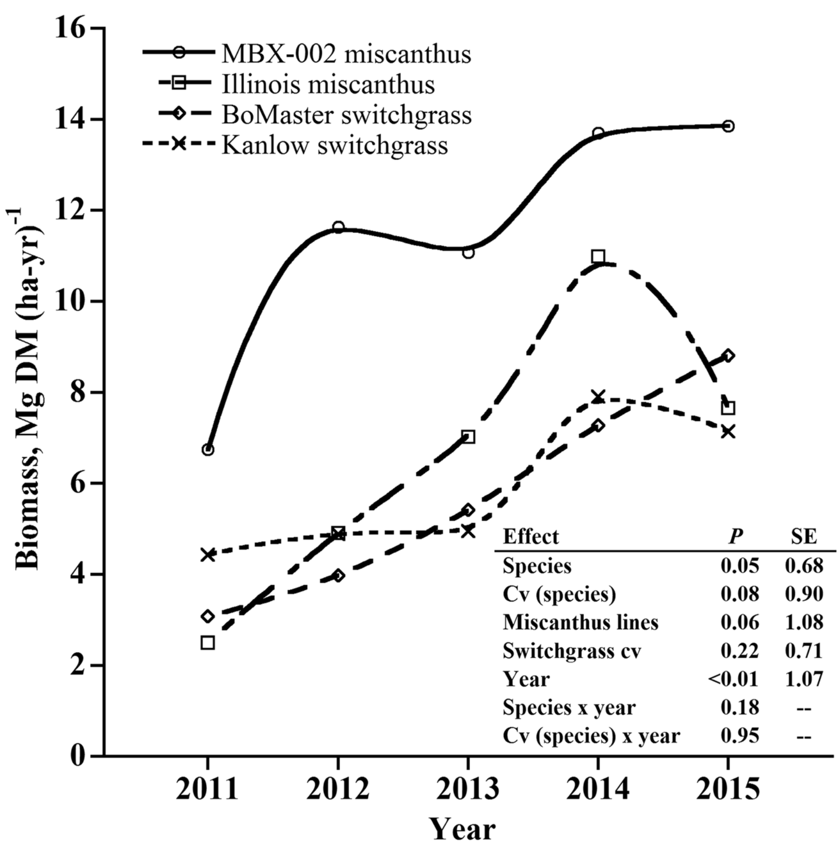

Fig. 1 Biomass harvested in early October from five growing seasons (2011–2015) at Alton, WV

Table 4 Chemical properties of reclaimed mine soil at Alton, WV, in 2010 before the experiment was started and in 2015 where switchgrass and miscanthus were established

Year and effect	Soil characteristic					
	pH	EC (μS cm^{-1})	P (mg kg^{-1})	K ($cmol^{+}$ kg^{-1})	Mg ($cmol^{+}$ kg^{-1})	Ca ($cmol^{+}$ kg^{-1})
2010 samples						
Range before planting	7.0–7.7	NA	1.8–7.1	0.3–0.5	0.2–0.9	1.5–4.0
Mean	7.4	NA	4.0	0.4	0.6	3.2
2015 samples						
Species, *P* value	0.51	0.28	0.03	0.13	0.80	0.41
Miscanthus	7.0	107	3.3	0.10	0.20	2.3
Switchgrass	7.2	130	2.1	0.10	0.21	1.9
SE	0.4	28.1	0.4	0.005	0.08	0.4
Cultivar (species), *P* value	0.48	0.46	0.28	0.12	0.44	0.48
Miscanthus, *P* value	0.33	0.41	0.39	0.08	0.97	0.35
Switchgrass, *P* value	0.47	0.36	0.18	0.27	0.21	0.44

[a] *EC*, electrical conductivity; *P*, phosphorus; *K*, potassium; *Mg*, magnesium; *Ca*, calcium

did not differ (5.8 and 5.7 Mg ha^{-1}, respectively), while Illinois was less productive than MBX-002 miscanthus (6.4 vs 11.4 Mg ha^{-1}). Kanlow and BoMaster switchgrass biomass yields increased from 3.9 to 7.5 Mg ha^{-1} between 2011 and 2015 (Fig. 1). Kanlow was 20–40% more productive than BoMaster during 2011and 2012. Illinois miscanthus yields increased steadily from 2.4 to 10.4 Mg ha^{-1} in 2011 to 2014, but declined to 7.0 Mg ha^{-1} in 2015. The MBX-002 line showed much greater biomass production than Illinois, ranging from 6.5 Mg ha^{-1} in 2011 to 11.5 Mg ha^{-1} in 2012, but stabilized at approximately 13.8 Mg ha^{-1} in 2014 and 2015. The MBX-002 line approached miscanthus yields of 15–20 Mg ha $year^{-1}$ on agricultural lands with 25 kg N ha^{-1} during the establishment year and none thereafter [27].

Switchgrass and miscanthus productivity were comparable with those on other marginal and reclaimed lands under annual applications of 0–60 kg N ha^{-1} [15, 36, 48, 51, 55]. Annual biomass yields of these species on agricultural soils under limited N rates have ranged from 2.5 to 16.0 Mg DM ha^{-1} for switchgrass [6, 15, 27, 56–59] and from 11.9 to 34.6 Mg DM ha^{-1} for miscanthus [5, 27, 59]. On marginal land, annual yields with < 67 kg N ha^{-1} have ranged from 2.0 to 19.0 Mg DM ha^{-1} for switchgrass [5, 6, 11, 15, 16, 49, 54, 55]. On fertile WV mine soils, DM production in the seventh year of growth was 10.0 and 19.0 Mg DM ha^{-1}, respectively, for switchgrass cultivars Shawnee and Cave-In-Rock [14]. No published studies have reported miscanthus yields on reclaimed mine land in the eastern USA.

Biomass Composition According to Differing NIRS Prediction Models

Species × year interactions affected treatment responses for ASH2 and aNDF, and interactions of cultivar (species) × year affected treatment patterns for aNDF (Table 5). For ASH2, switchgrass had higher levels than miscanthus, MBX-002 miscanthus had higher levels than Illinois, and ASH2 was higher in 2015 than in 2014. Small interaction effects were largely due to greater annual variability among switchgrass than miscanthus genotypes. For ASH2, miscanthus was similar among years while switchgrass was higher in 2015 than in 2014. For aNDF, species did not differ in 2014 but switchgrass was less productive than miscanthus in 2015, and BoMaster switchgrass was more productive in 2014 than in 2015 while Kanlow switchgrass was similar among years. The major effect for aNDF was year, with 2014 being higher than 2015. Ash concentration expressed as ASH1 from the NIRSC model was slightly higher, and not different among species, compared with ASH2 from the NREL model, which was higher for switchgrass. Among miscanthus lines, ASH1 and ASH2 differed in opposite directions to a minor extent. Across treatments, lignin concentration was approximately threefold higher for LIG from the NREL model than for ADL from the NIRSC model. Differences in accuracy of reference wet chemical procedures used to obtain ADL and LIG, and in the magnitude of their concentrations, have been observed by others [29, 60–62]. While both are Klason lignin procedures, LIG determination involves an additional acid hydrolysis step in an autoclave and the ADL procedure may not retain all lignin components [63]. There were no differences between switchgrass cultivars for biomass compositional traits (Table 5), but miscanthus lines differed in concentrations of both forms of ash and lignin. Miscanthus biomass was slightly higher than switchgrass in ADL, but not LIG, concentration, and Illinois had higher ADL and LIG concentrations than MBX-002 miscanthus. While the effect was small, aNDF was higher in miscanthus than in switchgrass (87.5 vs 86.2%). Concentrations of ASH2, ADL, LIG, and aNDF also differed among years when species were averaged together. Brown et al. [64] compared NIRS-predicted composition of Cave-In-Rock, Carthage, and Shawnee switchgrass biomass grown on mine

Table 5 Biomass compositional traits for switchgrass and miscanthus during 2014–2015 at Alton, WV

Effect	Biomass compositional trait[a] (% of DM)				
	ASH1	ASH2	ADL	LIG	aNDF
Species, *P* value	0.93	< 0.01	0.05	0.66	0.01
Miscanthus	4.54	1.88	5.46	18.78	87.48
Switchgrass	4.51	2.91	5.06	18.62	86.20
SE	0.22	0.14	0.19	0.26	0.34
Cultivar (species), *P* value	0.11	< 0.01	< 0.01	< 0.01	0.63
Miscanthus, *P* value	0.05	< 0.01	< 0.01	< 0.01	0.69
MBX-002	4.06	2.28	4.89	17.83	87.62
Illinois	5.02	1.48	6.03	19.74	87.34
SE	0.33	0.18	0.24	0.36	0.50
Switchgrass, *P* value	0.51	0.06	0.96	0.37	0.39
BoMaster	4.65	2.68	5.07	18.39	85.92
Kanlow	4.36	3.13	5.05	18.85	86.49
SE	0.30	0.18	0.23	0.36	0.46
Year, *P* value	0.69	< 0.01	< 0.01	0.05	< 0.01
2014	4.46	1.56	4.90	19.07	88.30
2015	4.59	3.23	5.62	18.34	85.38
SE	0.22	0.14	0.19	0.26	0.34
Species × year, *P* value	0.58	< 0.01	0.09	0.29	< 0.01
Cultivar (species) × year, *P* value	0.24	0.11	0.10	0.39	< 0.01

[a] Codes for compositional constituents are in Table 1

soils and found slightly higher ASH1 values of 5.8% compared to the 4.5% reported here for Kanlow and BoMaster, and aNDF values of 79% were lower than values of 86% reported here. Vogel et al. [29] reported NIRS-predicted values of 5.8% for ASH1 and 73.7% for aNDF for Kanlow switchgrass. No published information is available for ash, lignin, and aNDF concentrations of miscanthus grown on mined lands.

Species × year interaction affected treatment response patterns for both expressions of glucan and for XYL2, and cultivar (species) × year interaction also affected response patterns for both expressions of glucan (Table 6). Small interaction effects resulted from greater variability among years for BoMaster switchgrass than for other genotypes. For GLC1, GLC2, and XYL2, miscanthus was similar among years while switchgrass was higher in 2014 than in 2015, particularly for BoMaster. For both NIRS prediction models, hexose and pentose polysaccharides differed between species (Table 6), with slightly higher levels of ARA, GAL, MAN, XYL1, and XYL2 in switchgrass and slightly higher levels of GLC1 and GLC2 in miscanthus. Switchgrass and miscanthus are both warm-season perennial grasses that differed by up to 2.9 percentage units in concentrations of all cell wall carbohydrates. For the two most abundant sugars, GLC2 was lower in switchgrass than in miscanthus (37.4 vs 40.4%), while XYL2 was higher in switchgrass than in miscanthus (24.5 vs 22.0%). Although values were higher in the present study, relative differences among miscanthus and switchgrass glucan and xylan values were similar to those in Sanford et al. [59]. Switchgrass glucan and xylan values were higher than those in Schmer et al. [11] and Vogel et al. [29], and similar to those in Dien et al. [43, 60] and Adler et al. [56], with approximately the same proportions as in the present study in all cases. Switchgrass cultivars did not differ in cell wall polysaccharide levels except for GAL and MAN, consistent with findings by Brown et al. [64] of only a few differences in cell wall carbohydrate levels among Cave-In-Rock, Carthage, and Shawnee switchgrass. Illinois and MBX-002 miscanthus also showed differences in GAL and MAN. Concentrations predicted by the NIRSC switchgrass model averaged 3.9 percentage units higher for glucan, and 2.6 percentage units lower for xylan than values from the NREL mixed feedstocks model, but magnitudes and directions of treatment effects were very similar among models. This suggests the spectral and chemical similarity of switchgrass and miscanthus calibration samples and the possible validity of the NIRSC switchgrass bioenergy model for prediction of the cell wall composition of both species under the conditions of this study.

Theoretical Ethanol Yield and Production According to Differing Methods of Calculation

Differences in cell wall polysaccharide concentrations were reflected in correspondingly higher TEY1 levels (based on five carbohydrate constituents) for switchgrass than for miscanthus (480 vs 465 L Mg^{-1}), but no species differences for TEY2 (based on glucan and xylan) according to NIRSC or NREL models

Table 6 Cell wall composition for switchgrass and miscanthus during 2014–2015 at Alton, WV

Effect	Cell wall carbohydrate[a] (% of DM)						
	ARA	GAL	GLC1	GLC2	MAN	XYL1	XYL2
Species, *P* value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Miscanthus	2.96	0.80	35.44	40.38	0.12	25.14	22.03
Switchgrass	3.40	0.99	34.64	37.44	0.34	26.58	24.46
SE	0.05	0.03	0.16	0.45	0.03	0.20	0.17
Cultivar (species), *P* value	0.06	0.09	0.84	0.03	0.77	0.07	0.02
Miscanthus, *P* value	0.04	0.04	0.56	0.02	0.55	0.03	< 0.01
MBX-002	3.07	0.84	35.35	39.83	0.11	25.62	22.43
Illinois	2.86	0.76	35.54	40.92	0.13	24.67	21.63
SE	0.07	0.03	0.24	0.51	0.04	0.29	0.22
Switchgrass, *P* value	0.22	0.45	0.97	0.19	0.69	0.42	0.25
BoMaster	3.35	0.98	34.65	37.74	0.34	26.42	24.29
Kanlow	3.46	1.00	34.64	37.13	0.33	26.73	24.62
SE	0.07	0.03	0.22	0.51	0.03	0.27	0.22
Year, *P* value	0.52	0.23	< 0.01	< 0.01	0.03	0.17	< 0.01
2014	3.21	0.88	35.64	39.61	0.20	26.06	23.94
2015	3.16	0.91	34.44	38.20	0.25	25.66	22.55
SE	0.05	0.03	0.16	0.45	0.03	0.20	0.17
Species × year, *P* value	0.49	0.51	< 0.01	< 0.01	0.34	0.49	0.03
Cultivar (species) × year, value	0.81	0.70	< 0.01	0.02	0.31	0.36	0.06

[a] Codes for cell wall polysaccharides are in Table 1

Table 7 Theoretical ethanol yield and theoretical ethanol production calculated according to Methods 1 and 2 for switchgrass and miscanthus biomass from 2014 to 2015 at Alton, WV

Effect	Theoretical ethanol yield and production[a]				
	TEY1 (L Mg^{-1})	TEY2A (L Mg^{-1})	TEY2B (L Mg^{-1})	TEP1 (L ha^{-1})	TEP2B (L ha^{-1})
Species, *P* value	< 0.01	0.10	0.14	< 0.01	< 0.01
Miscanthus	465	440	453	5654	5594
Switchgrass	480	446	450	3807	3699
SE	2.6	2.2	2.4	1303	1346
Cultivar (species), *P* value	0.18	0.60	0.67	0.25	0.03
Miscanthus, *P* value	0.08	0.38	0.52	0.10	0.01
MBX-002	470	442	452	6384	6496
Illinois	460	438	454	4925	4692
SE	4.1	3.3	2.9	1383	1387
Switchgrass, *P* value	0.57	0.61	0.54	0.85	0.59
BoMaster	479	444	451	3871	3880
Kanlow	482	447	449	3744	3517
SE	3.2	3.0	2.9	1336	1385
Year, *P* value	0.06	< 0.01	< 0.01	0.39	0.20
2014	476	448	462	4970	4950
2015	469	437	441	4491	4342
SE	2.6	2.2	2.4	1085	1346
Species × year, *P* value	< 0.01	0.04	< 0.01	0.31	0.38
Cultivar (species) × year, *P* value	0.03	0.04	< 0.01	0.20	0.24

[a] Models and methods used to calculate alternative theoretical ethanol yield (TEY) and production (TEP) values are defined in Table 2

Table 8 Significance and Pearson correlation coefficients among alternative NIRS-predicted constituents and calculations of theoretical ethanol yield for switchgrass and miscanthus biomass from 2014 to 2015 at Alton, WV

Comparison[a]	*n*	*P* value	*r*
ASH1 vs. ASH2	38	0.49	0.12
ADL vs. LIG	37	0.04	0.34
XYL1 vs. XYL2	37	< 0.01	0.75
GLC1 vs. GLC2	38	< 0.01	0.72
TEY1 vs. TEY2A	32	< 0.01	0.98
TEY1 vs. TEY2B	32	< 0.01	0.51
TEY2A vs. TEY2B	37	< 0.01	0.62

[a] Codes for compositional constituents and alternative theoretical ethanol yield (TEY) and production (TEP) variables are in Tables 1 and 2

(models A and B, respectively; Table 7). There were no cultivar (species) effects for any TEY variable, but species × year interactions for TEY1, TEY2A, and TEY2B reflected previously mentioned compositional interactions, suggesting greater variability of BoMaster switchgrass than other genotypes among years. Theoretical ethanol yield (TEY2) calculated with GLC1 and XYL1 from the NIRSC model (Method 2) averaged 94% of TEY1 (443 vs 472 L mg^{-1}) calculated with all five cell wall polysaccharides from the same model (Method 1), and both expressions of TEY were highly correlated ($r = 0.98$; Table 8). While less strongly correlated ($r = 0.62$), TEY2A calculated from GLC1 and XYL1 concentrations from the NIRSC switchgrass model averaged 98% of TEY2B (443 vs 452 L Mg^{-1}) calculated from GLC2 and XYL2 concentrations from the NREL mixed feedstocks model (both Method 2). There were no treatment × year interactions for TEP based on methods of calculation and NIRS models. Although species had little to no variation in TEY, TEP was higher for miscanthus using both methods because of higher miscanthus biomass (Table 7 and Fig. 1), which effect has also been reported by Sanford et al. [59] and Nichols et al. [10]. While TEY calculated from GLC and XYL was slightly higher in 2014 (448 vs 437 TEY2A and 462 vs 441 L Mg^{-1} TEY2B), average biomasses of 9.92 and 9.49 Mg ha^{-1} in 2014 and 2015, respectively, resulted in no differences among years in TEP. While Method 1 calculations indicated no species difference in TEP, Method 2 indicated higher TEP2B for MBX-002 than for Illinois miscanthus lines (6496 vs 4692 L Mg^{-1}) and no differences among switchgrass cultivars BoMaster and Kanlow (3880 and 3517 L Mg^{-1}, respectively).

Relative to the mixed feedstocks NREL model predicting two polysaccharides in Method 2, the NIRSC switchgrass model predicting five polysaccharides in Method 1 resulted in slight overprediction of TEP (means of 4730 and 4646 L ha^{-1} for TEP1 and TEP2B, respectively). While species differed for both TEP variables, only TEP2B from the NREL model discriminated among miscanthus lines. As with cell wall polysaccharide and TEY determinations, these results suggest the possible validity of the NIRSC switchgrass bioenergy model for prediction of TEP of both species under the conditions of this study. This is further supported by the strength of correlations among glucan and xylan concentrations predicted by both models (Table 8). While NIRSC and NREL model predictions are poorly correlated for ash and lignin, these variables are not included in TEY and TEP calculations.

Conclusions

Biomass and ethanol yields from switchgrass and miscanthus grown on mine soils compared favorably with those from other studies under little to no N fertilization on marginal sites and at other reclaimed lands with good quality mine soils in this region. After 6 years of growth at the Alton reclaimed mine site, biomass yields were 6–8 and 7–13 Mg DM ha^{-1} among genotypes of switchgrass and miscanthus, respectively. Greater levels of cell wall xylan and lower levels of glucan in switchgrass, relative to miscanthus, translated into similar TEY between species when predicted by two-polysaccharide models. Between species and among miscanthus lines, concentrations of all cell wall polysaccharides differed according to the five-polysaccharide NIRSC switchgrass bioenergy prediction model, resulting in higher TEY for switchgrass but no differences among cultivars. Based on the two-polysaccharide prediction approaches with both models, species and cultivars did not differ in TEY. When greater biomass yields of miscanthus were combined with similar or only slightly different TEY among species according to two- and five-carbohydrate prediction approaches, miscanthus had higher TEP values than switchgrass (5654 vs 3807 for TEP1 and 5594 vs 3699 L ha^{-1} for TEP2B). The growth, biomass yields, and cell wall composition of these species make them promising candidates for bioenergy feedstock production on reclaimed mined lands and for potential ethanol conversion. These theoretical ethanol yield and production estimates do not factor in large-scale commercial harvesting and management at ethanol plants, or issues associated with conversion rates and efficiency. While expanding the NIRSC switchgrass bioenergy model to include miscanthus and other cellulosic bioenergy species is desirable, similarities of TEY estimates and sensitivity to species effects among the NIRSC and NREL mixed herbaceous feedstock models suggest possible applications of the NIRSC model to analysis of miscanthus composition under the conditions of this study.

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