Research Article

Received: 18 December 2012 Revised: 8 March 2013 Accepted article published: 2 April 2013 Published online in Wiley Online Library: 8 May 2013

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6159

Chemical characteristics and biofuels potentials of various plant biomasses: influence of the harvesting date

Bruno Godin,[a,b]* Stéphane Lamaudière,[b] Richard Agneessens,[a] Thomas Schmit,[a] Jean-Pierre Goffart,[a] Didier Stilmant,[a] Patrick A Gerin[b] and Jérôme Delcarte[a]

Abstract

BACKGROUND: An optimal valorization of plant biomasses to produce biofuels requires a good knowledge of the available contents and molecular composition of the main chemical components, which changes with the harvesting date. Therefore, we assessed the influence of harvesting date on the chemical characteristics of various energy crops in the context of their conversion to biofuels.

RESULTS: We showed that the biomass chemical composition, enzymatic digestible organic matter, bioethanol and thermal energy production potential for each species are impacted by the harvesting date. The proportion of enzymatically digestible organic matter decreases as the harvesting date is delayed. This is related to the increase in cellulose and lignin contents. The suitability of the biomasses for bioethanol production increases with harvest stage, as the total carbohydrates content increases. The suitability of the biomasses as a source of thermal energy increases according to the harvesting date as the proportion of organic matter increases and the content of mineral compounds decreases. For all investigated energy conversions, the best harvesting period is autumn, because the significantly higher crop dry matter yield largely compensates for the sometimes slightly less favorable chemical characteristics.

CONCLUSION: While the biomass composition of energy crops changes with harvest stage, the dry biomass yield per unit area is the main factor that controls the total amount of chemical components, digestible organic matter, bioethanol and thermal energy that can be expected to be harvested per unit area. The biomass compositions presented in this paper are essential to investigate their suitability for bioenergy conversion.

Supporting information may be found in the online version of this article.

Keywords: plant maturity; fibrous biomasses; cellulose; hemicelluloses; renewable energy

INTRODUCTION

The main chemical components of plant biomasses (forage crops, agricultural residues and wood) are cellulose (linear homogeneous structural polysaccharide composed of D-glucose units), hemicelluloses (ramified heterogeneous structural polysaccharides composed of D-xylose, L-arabinose, D-mannose, D-galactose and D-glucose units), lignin (phenylpropanoid polymer composed of syringyl, guaiacyl and *p*-hydroxyphenyl units), pectins (ramified heterogeneous structural polysaccharides mainly composed of D-galacturonic acid units), soluble sugars (D-glucose, D-fructose, sucrose and fructans), starch (linear or ramified homogeneous non-structural polysaccharide mainly composed of D-glucose), proteins and mineral compounds.[1,2] The monosaccharidic composition of hemicelluloses, (D-xylose, L-arabinose, D-mannose, D-galactose and D-glucose) depends on the phylogenetic origins of the plant species. The hemicelluloses of commelinid monocotyledons have higher contents of xylan and arabinan, associated as arabinoxylan, and β-glucan.[1] The hemicelluloses of non-commelinid dicotyledons have higher contents of hemicellulosic glucan, in the form of xyloglucan, and mannan.[1]

The chemical composition of plant biomass changes as the latter matures. There is a thickening and lignification of the cell walls (increase of secondary cell walls) as the plant develops during the growing season. This causes an increase in the proportion of structural components (cellulose, hemicellulose and lignin) and a

* *Correspondence to: Bruno Godin, Biomass, Bioproducts and Energy Unit, Valorisation of Agricultural Products Department, Walloon Agricultural Research Center (CRA-W), Chaussée de Namur 146, B-5030 Gembloux, Belgium. E-mail: b.godin@cra.wallonie.be*

a *Biomass, Bioproducts and Energy Unit, Valorisation of Agricultural Products Department, Walloon Agricultural Research Center (CRA-W), B-5030, Gembloux, Belgium*

b *Bioengineering Group, Earth and Life Institute, Université catholique de Louvain, B-1348, Louvain-la-Neuve, Belgium*

decrease in the proportion of non-structural components (non-structural carbohydrates, proteins and mineral compounds) in the harvested biomass as growth is prolonged.[3] This has been shown for the structural and non-structural components of alfalfa, reed canary grass and switchgrass by Dien *et al.*[3] It has also been shown for the structural components of corn stems by Jung and Casler.[4] An optimal valorization of these biomasses to produce biofuels or green chemicals requires a good knowledge of the available contents and molecular composition of the main chemical components, which changes with the harvesting date.[5,6]

Solid (e.g. pellets), liquid (e.g. bioethanol) and gaseous (e.g. biogas) biofuels can be produced from biomasses.[7] These biofuels are usually further converted into three types of energy: thermal, electrical and mechanical.[8] In order to compare the energy value of biomasses, we decided to use the higher heating value (HHV), the methodology of Spatari *et al.*[9] and the enzymatically digestible organic matter (DOM)[10,11] as fast approaches to assess, respectively, the combustion potential,[5] the bioethanol potential and the potential anaerobic digestibility (biomethanation).

The aim of the present study was to assess the influence of harvesting date on the main chemical components of the biomasses (cellulose, hemicelluloses, lignin, total soluble sugars, starch, proteins, mineral compounds), the monosaccharidic components of hemicelluloses (xylan, arabinan, mannan, galactan, hemicellulosic glucan), DOM, the total bioethanol potential and the HHV of various crops (*Miscanthus*, switchgrass, fiber sorghum, fiber corn, spelt, tall fescue, cocksfoot, hemp and Jerusalem artichoke).

MATERIALS AND METHODS

Biomass material

Miscanthus giganteus (*Miscanthus* × *giganteus* JM Greef & Deuter ex Hodk. & Renvoize; cv. Bical), switchgrass (*Panicum virgatum* L.; cv. Cave-in-Rock), fiber sorghum (*Sorghum bicolor* (L.) Moench; cv. H133), fiber corn (*Zea mays* L.; cv. Ronaldinio), spelt (*Triticum aestivum* L. ssp. *spelta* (L.) Thell.; cv. Cosmos), tall fescue (*Festuca arundinacea* Schreb.; cv. Kora), cocksfoot (*Dactylis glomerata* L.; cv. Terrano), hemp (*Cannabis sativa* L.; cv. Fedora 17) and Jerusalem artichoke (*Helianthus tuberosus* L.; aerial part; cv. Volkenroder spindel) were cropped in trials in 2009 and 2010 at Libramont (498 m above sea level; average annual temperature: 281.6 K ; average annual precipitation: 1260 mm ; 49° 55′ N, 05° 24′ E; Belgium). A plot between 9 and 24 m^2 of the whole above-ground biomass was harvested. The harvest of each biomass was realized in the same plot at different dates. The above-ground biomass was manually cut at 10 cm from the ground. For the last harvesting date of spelt, only the straw part was considered. The sorghum and corn cultivars investigated here have higher fiber contents (cellulose + hemicelluloses + lignin) than the usually cropped forage cultivars. Therefore, they are called fiber sorghum and fiber corn.

Immediately after the harvest, two representative subsamples of 750 g of each biomass were dried at 333 K for 72 h in a Memmert UFP800 oven (VWR, Heverlee, Belgium). After drying, the two subsamples were first milled with a 4 mm screen hammer mill (BOA, Waterleau, Herent, Belgium), followed by a second milling step with a 1 mm screen cyclone mill (Cyclotec, FOSS Benelux NV, Brussels, Belgium). The two subsamples were merged and stored in airtight bags at room temperature and protected from light in a dark box.

Chemical analyses

All chemicals were of analytical grade or equivalent and were purchased from VWR (Heverlee, Belgium) and Chem-Lab (Zeldelgem, Belgium). Technical duplicate aliquots were measured for each biomass sample of each harvesting date.

The xylan, arabinan, mannan, galactan and total glucan content were determined by a sulfuric acid hydrolysis method (SAH) after fractionation.[12] The hemicellulosic glucan content was determined by the SAH method, except that the cellulose solubilization step (incubation with H_2SO_4 12.2 mol L^{-1} for 1 h at 303 K) was skipped. The cellulosic glucan content was calculated as the difference between the total glucan and the hemicellulosic glucan content. The acid detergent lignin (ADL, which is considered as the lignin content) was determined by the Van Soest (VS) gravimetric method.[13] Total soluble sugars were determined by the Luff–Schoorl method.[14] Starch content was determined by the Ewers method.[14] Protein content was determined by the Kjeldahl method using 6.25 as conversion factor of nitrogen to protein.[15] Mineral content was determined by use of a muffle furnace set at 823 K for 3 h. The dry matter (DM) content was determined at 376 K for 4 h.

Soluble carbohydrates were extracted by mixing 500 mg dried sample with 9.5 mL deionized water in 15 mL polypropylene tubes. The obtained slurry was incubated at 343 K for 20 min by immersing the tubes in a water bath, with manual mixing of the samples every 5 min. The slurry was centrifuged (2700 × *g*, 10 min); the supernatant was collected with a 5 mL syringe and filtered through a 0.2 μm cellulose acetate filter unit (Sartorius Biolab Products, Vilvoorde, Belgium). The clear filtrate was analyzed by high-performance liquid chromatography using an Agilent 1200 series liquid chromatography (LC) system with a quaternary pump (Agilent, Wokingham, UK) connected to a 1200 series Agilent evaporative light-scattering detector (ELSD) (Agilent). Nitrogen (0.35 MPa) was used as nebulizer gas. The nebulizer tube temperature was set to 323 K and the ELSD gain was set to 9. Sample extracts were diluted (1:20 volume fraction to 1:100 volume fraction) in deionized water with 10% volume percentage of LC-grade acetonitrile and were injected (20 μL) and eluted in a Prevail Carbohydrates ES analytical LC column (250 mm × 4.6 mm i.d.; 5 μm particle size) (Grace, Lokeren, Belgium) with a Prevail Carbohydrates ES All-guard pre-column (12.5 mm × 4.6 mm i.d.; 5 μm particle size) (Grace). The mobile phases consisted of 95:5 (volume fraction) LC-grade acetonitrile–deionized water mixture (A) and 100% deionized water (B). The gradient condition was 0–15 min, 20–50% volume percentage of B; 15–25 min, 50–100% volume percentage of B; 25–30 min, 100% volume percentage of B; 30–32 min, 100–20% volume percentage of B; and 32–35 min, 20% volume percentage of B. The flow rate was set at 1 mL min^{-1} and the column temperature set at 298 K using an Agilent G1316A thermostated column compartment.

The ELSD response ((peak area) = a × (concentration)^b, with b set at 1.5) was calibrated for D-glucose, D-fructose, sucrose, raffinose and stachyose with five solutions of the authentic compounds, covering the range 0.6–3.0 g L^{-1}.

Biofuel potentials

Enzymatically digestible organic matter

The DOM of the biomass, as determined by the De Boever method,[10] was used to compare the suitability of the different biomasses for conversion by anaerobic digestion (biomethanation). For the purpose of our study, this method was

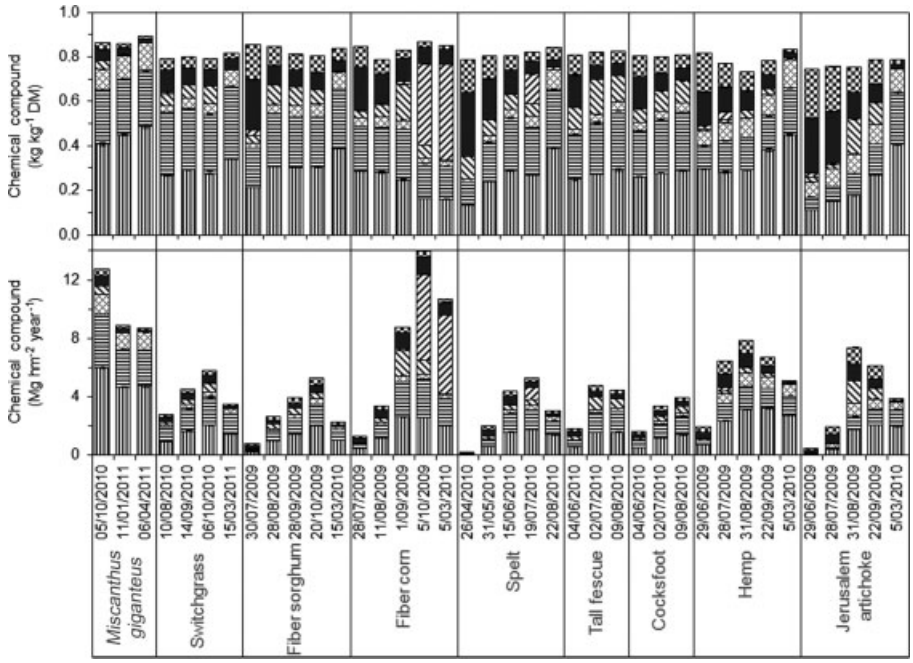

Figure 1. Main chemical composition (top) and yield (bottom) of the different investigated biomasses according to the harvesting date. Error bars correspond to standard deviation of the technical duplicate analysis for each biomass sample. The figures of these standard deviations can be found in supporting information Table A1.

considered much faster than determining the real biomethane potential, while the results can be correlated with anaerobic digestibility of the biomass without any pretreatment.[10,16] We considered that the DOM corresponds to the *minimum* level of anaerobic digestibility of the biomass without any pretreatment. Indeed, microbial digestion is expected to produce more enzymes *in situ*, with a broader substrate spectrum than the enzyme cocktail used in the assay. Microbial digestion can also progress for longer periods of time than the enzyme assay. Briefly, biomass samples were incubated, chronologically, with pepsin in 0.1 mol L^{-1} HCl for 24 h at 313 K, with 0.1 mol L^{-1} HCl for 45 min at 353 K and with cellulase in an acetate buffer at pH 4.8 for 24 h at 313 K. Solubilization of the biomass was determined gravimetrically.

Bioethanol

Bioethanol potential was calculated as the ethanol that can be expected from the fermentation of the directly available soluble sugars and starch, and also from cellulose and hemicelluloses after biomass hydrolysis. The bioethanol potential of carbohydrates was assessed by following the methodology of Spatari *et al.*,[9] on the basis of (i) the monomers expected from the hydrolysis yields of cellulose, hemicelluloses (hydrolysis yields of respectively 91% and 81% with a liquid hot-water pretreatment)[17] and starch (hydrolysis yield of 96%);[18] (ii) the stoichiometric ethanol fermentation yields of monosaccharides (92.5% for glucose and fructose, and 86% for xylose, arabinose, galactose and mannose);[19] and (iii) the ethanol recovery yield (99.5%).[9]

Thermal energy

Thermal energy was assessed on the basis of the higher heating value (HHV). HHV was determined by the method using a Parr controlled oxygen bomb calorimeter.[20] The sample was ground, pelletized and dried overnight at 376 K. The higher calorific value was then measured using a Parr 6200 calorimeter.

Crop yields

The yield per hectare (hm^2) of each plant biomass component and expected biofuel were calculated by multiplying the specific biomass characteristics by the biomass DM yield per hectare.

RESULTS AND DISCUSSION

Biomass chemical composition

The progress of the chemical composition of the analyzed biomasses is shown in Fig. 1 as a function of the harvesting date (details can be found in supporting information Table A1). The biomasses with the highest and lowest content of structural components (cellulose, hemicelluloses and lignin) are *Miscanthus* and Jerusalem artichoke, respectively. The biomass with the highest starch content is fiber corn. For all crops, the content of proteins and mineral compounds decreases with aging, while the content of structural components increases (Fig. 1 top). The progress of these chemical components according to the harvesting date can be explained by the fact that, during plant development, the leaf part decreases whereas the stem part increases. The leaves have less lignified cell walls (poor in secondary cell walls) and more metabolically active cells with a high content of non-structural components.[21,22] The stems have lignified cell walls with a high content of structural components to stand erect. The lignification (increase of structural components; increase of secondary cell walls) of cell walls increases with the plant's development.[21,22] A peak in the content of total soluble sugars (sum of the soluble monosaccharides, sucrose and fructans) is generally observed in the summer (Fig. 1, top). It corresponds to the anthesis period of the plant. It is known that after anthesis the plant will rapidly increase its contents of cellulose, hemicelluloses and lignin and rapidly decrease its content of soluble sugars.[21] This corresponds to lignification of the cell walls.[21] Late winter-harvested biomasses have higher content of structural components and lower content of non-structural

components compared to the same biomass harvested during autumn (Fig. 1, top). This can be explained by nutrient translocation to the rhizomes, solubilization and the leaching of the non-structural components during the winter.[23]

The progress over time of the main chemical components in fiber corn is different because of the increase of the starch content, which dilutes the other main chemical components (Fig. 1, top). Starch accumulation is also observed in spelt, but to a lesser extent; only the straw was analyzed for the last harvesting date and no remaining starch was found (Fig. 1, top).

Soluble polysaccharides (such as pectins), acid-soluble lignin, organic acids, alcohols, pigments and lipids compose most probably the non-identified fraction of each biomass (Fig. 1 top), as also suggested by Hames[24] for other biomasses. Hemp and Jerusalem artichoke have a higher non-identified fraction compared to the other biomasses. This can be explained by their botanical differences. Hemp and Jerusalem artichoke are non-commelinid dicotyledons that are known to have a higher pectin content and a lower hemicellulose content as compared to the other biomasses that are commelinid monocotyledons.[1,12]

Figure 1 (bottom) shows the biomass chemical components that can be harvested per unit area as a function of the harvesting date. The crop DM yields and DM contents at each harvest time are shown in Table 1. It appears that the crop DM yield is the parameter that influences most the amount of chemical components that can be collected per unit area. Therefore, lower yields of fibers (cellulose, hemicelluloses and lignin) are actually observed for late winter harvest, while this harvesting date presents the highest specific contents of structural components (cellulose, hemicelluloses and lignin) (Fig. 1, top). The loss of leaf biomass during the winter can explain this yield decrease.[23] The nutrient translocation from the stems and/or leaves to the rhizomes can also explain this decrease for perennial crops.[23]

Fiber corn has a low specific content of structural components but it has a high yield of structural components per unit area because of its high biomass yield unit area. This is also observed for hemp, which has an intermediate specific content of structural components that ends up as a relatively high yield per unit area owing to the high total dry matter produced.

Perennial crops such as *Miscanthus* and switchgrass that are harvested in autumn are expected to need a higher fertilization level, as compared to the same crop harvested during the winter, because such an early harvest disables the crop to translocate its nutrients down to its rhizomes.[23] If fertilization is supplied as mineral fertilizer instead of recycled digestate or biomass residues, the amount of life cycle greenhouse gas production would also increase due to the manufacture and spreading of increased amounts of mineral fertilizers.[23] The crop DM yield per unit area is highly dependent on the area where it is grown.[25]

Table 1. Crop yield (as dry matter) and dry matter content of the different investigated biomasses according to harvesting date

	Harvest period	Crop DM yield (Mg hm^{-2} yr^{-1})	Dry matter content (kg kg^{-1} FM)
Miscanthus giganteus	05/10/2010	14.8	0.364
	11/01/2011	10.4	0.340
	06/04/2011	9.7	0.797
Switchgrass	10/08/2010	3.5	0.288
	14/09/2010	5.7	0.284
	06/10/2010	7.4	0.323
	15/03/2011	4.3	0.933
Fiber sorghum	30/07/2009	0.9	0.111
	28/08/2009	3.1	0.157
	28/09/2009	4.8	0.207
	20/10/2009	6.6	0.338
	15/03/2010	2.7	0.289
Fiber corn	28/07/2009	1.5	0.111
	11/08/2009	4.3	0.133
	1/09/2009	10.6	0.185
	5/10/2009	16.1	0.379
	5/03/2010	12.5	0.587
Spelt	26/04/2010	0.2	0.188
	31/05/2010	2.5	0.153
	15/06/2010	5.5	0.202
	19/07/2010	6.4	0.436
Spelt straw	22/08/2010	3.6	0.721
Tall fescue	04/06/2010	2.3	0.204
	02/07/2010	5.8	0.344
	09/08/2010	5.4	0.359
Cocksfoot	04/06/2010	2.0	0.183
	02/07/2010	4.2	0.334
	09/08/2010	4.9	0.333
Hemp	29/06/2009	2.4	0.092
	28/07/2009	8.4	0.216
	31/08/2009	10.8	0.341
	22/09/2009	8.5	0.339
	5/03/2010	6.1	0.821
Jerusalem artichoke	29/06/2009	0.6	0.097
	28/07/2009	2.5	0.090
	31/08/2009	9.8	0.172
	22/09/2009	7.7	0.201
	5/03/2010	4.9	0.759

Monosaccharidic composition of hemicelluloses

Figure 2 presents the composition of the hemicelluloses as a function of the harvesting date (details can be found in supporting information Table A2). The biomasses with the highest and lowest absolute content of hemicelluloses are switchgrass and Jerusalem artichoke, respectively. Xylan is the major absolute and relative hemicellulosic component. The last harvesting date of *Miscanthus* has the highest relative content of xylan, whereas the last harvesting date of switchgrass has the highest absolute content of xylan. The absolute and relative contents of xylan and arabinan, at a similar harvest date, are higher in the commelinid monocotyledons as compared to the non-commelinid dicotyledons (hemp and Jerusalem artichoke). The opposite is observed for the absolute and relative content of mannan and the relative content of galactan. This is consistent with the fact that commelinid monocotyledons are known to have hemicelluloses with higher contents of arabinoxylan and β-glucan, and lower contents of xyloglucan and mannan, as compared to non-commelinid dicotyledons.[1,12] Figure 2 shows that, in general, an increase of the total hemicellulose content is mainly due to an increase of the xylan content, while the relative contents of arabinan and hemicellulosic glucan decrease. This can be associated with cell wall lignification (increase of secondary cell walls), decrease in the leaf part of the plant and the corresponding increase in the stem part of the plant.[21,22]

For the commelinid monocotyledons, at the same harvest date, the absolute and relative hemicellulosic components are generally

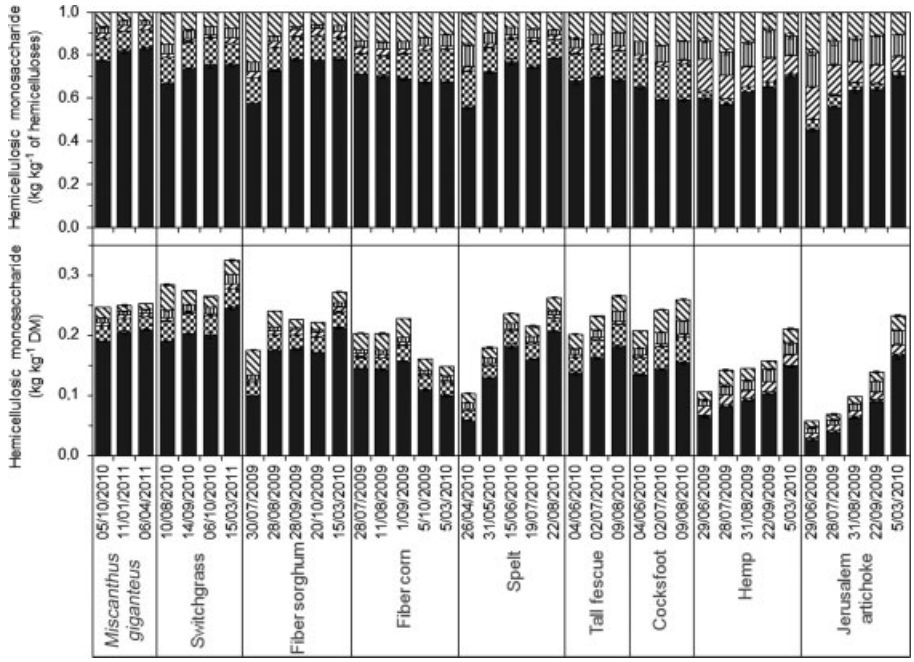

Figure 2. Relative (top) and absolute (bottom) monosaccharidic composition of hemicelluloses of the different investigated biomasses according to the harvesting date. Error bars correspond to standard deviation of the technical duplicate analysis for each biomass sample. The figures of these standard deviations can be found in supporting information Table A2.

(in decreasing order of importance) xylan, arabinan, hemicellulosic glucan, galactan and mannan. During growth, the absolute and relative contents of xylan generally increase, whereas there is a decrease in the relative content of hemicellulosic glucose (Fig. 2). The progress over time of the hemicellulosic content in the fiber corn dry matter is different (Fig. 2) due to the contribution of starch, as discussed above. This different progress is not observed with the last harvest stage of spelt, as discussed above (Fig. 1, top).

For the non-commelinid dicotyledon biomasses, at the same harvest date, the absolute and relative hemicellulosic components are generally (in order of decreasing importance) xylan, hemicellulosic glucan, galactan, mannan and arabinan. Non-commelinid dicotyledons are indeed known to have hemicelluloses with higher contents of xyloglucan and mannan, as compared to commelinid monocotyledons.[26,27] Figure 2 shows that, in general, the absolute content of xylan, mannan, galactan and hemicellulosic glucan and the relative content of xylan increase during growth, whereas the relative contents of mannan and hemicellulosic glucan decrease.

Arabinoxylan, xyloglucan (which also contains galactan and hemicellulosic glucan), β-glucan and mannan (which also contains galactan and hemicellulosic glucan) are the four main types of structural polysaccharides of hemicelluloses.[26,27] The progress over time of the relative contents of these hemicellulosic polysaccharides according to the harvesting date can be explained by the increasing degree of lignification (increase of secondary cell walls) of the cell walls during its development. Lignified cell walls have a higher content of arabinoxylan and lower contents of xyloglucan, β-glucan and mannan compared to less lignified cell walls.[26,27]

It has been mentioned by Carpita and McCann[1] that arabinoxylan is more substituted by arabinan in commelinid monocotyledons, as compared to non-commelinid dicotyledons. The fact that the lignin of commelinid monocotyledons is highly linked to the arabinan residue of arabinoxylan by ferulate residues explains the high degree of substitution of arabinoxylan by arabinan.[1]

Soluble and reserve carbohydrates

The mono-, di- and oligosaccharides that contribute to the total soluble carbohydrates were further characterized for the various biomasses. Figure 3 presents the total concentration of easily fermentable carbohydrates that can be collected when harvesting the different biomasses under the specified conditions. In late winter, no soluble carbohydrates were detected. In Jerusalem artichoke, oligosaccharides up to a degree of polymerization of 25–30 were detected (data not shown). They were considered as oligofructans.[28] The oligosaccharidess appeared in July; their concentration was maximal in August, then decreased in autumn. Oligosaccharides were also detected in spelt, autumn fiber sorghum, August cocksfoot–alfalfa and tall fescue. In tall fescue, the oligosaccharides were present particularly in July and decreased in October.

Biomass characteristics as guide in the selection of transformation technology

Enzymatically digestible organic matter

DOM was used to assess the suitability of the analyzed biomasses for anaerobic digestion (biomethanation). The progress of DOM of the analyzed biomasses according to the harvesting date is shown in Fig. 4 (details are given in supporting information Table A3). For the purpose of our study, this method was considered much faster than determining the real biomethane potential, while the results can be correlated with anaerobic digestibility of biomasses without any pretreatment.[10,16] The biomasses with the highest DOM per kilogram of the dry organic matter are fiber corn, immature rye, cocksfoot, tall fescue and Jerusalem artichoke. This can be explained be their high total soluble sugars, starch and/or protein content which are a part of the DOM.[10] It is generally

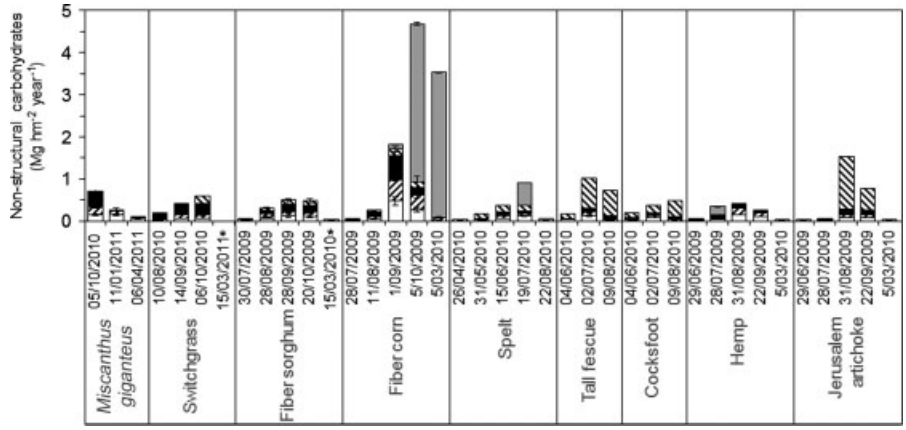

Figure 3. Identified saccharides that contribute to the total soluble carbohydrates: glucose, fructose, sucrose, other unidentified soluble carbohydrates and starch according to the harvesting date. Error bars correspond to standard deviation of the technical duplicate analysis for each biomass sample. *, not determined.

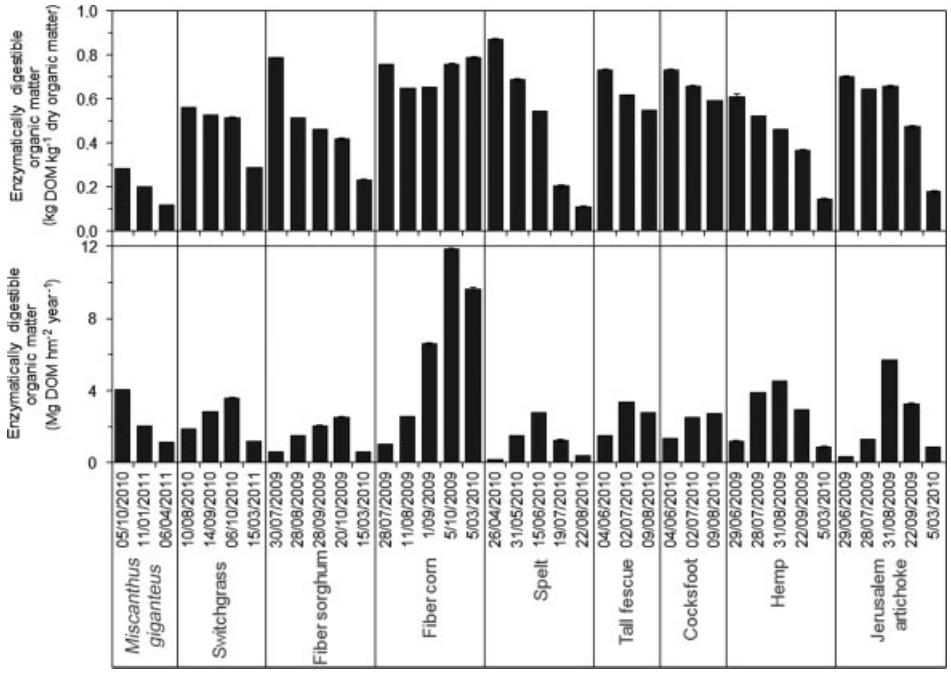

Figure 4. Enzymatically digestible organic matter (top) and yield (bottom) of the different investigated biomasses according to the harvesting date. Error bars correspond to standard deviation of the technical duplicate analysis for each biomass sample. The figures of these standard deviations can be found in supporting information Table A3.

observed that, during growth, DOM content decreases (Fig. 4, top). This can mainly be explained by the increase of cellulose and lignin contents during plant aging (Fig. 1, top) due to cell wall thickening and lignification, decrease of the leaf part and increase of the stem part of the plant, as mentioned above.[21,22] The progress over time of the enzymatic degradability in fiber corn is different, mainly because of the important increase in starch content which is a part of DOM (Fig. 4).[10] This different progress over time is not observed with the last harvest stage of spelt because only the straw part is considered (Fig. 1, top).

Figure 4 (bottom) shows the progress of yields of DOM per unit crop area according to harvesting date. The amount of DOM unit crop area depends mainly on the biomass yield and only to a secondary extent on its degradability. The highest quantity of DOM per unit crop area is therefore observed for the autumn harvest.

Bioethanol

The calculated ethanol production expected from the analyzed biomasses is shown in Fig. 5 (details in supporting information Table A3). Soluble carbohydrates and starch/fructanes can be readily fermented to bioethanol with current technologies, whereas fermentation of biomass cellulose and hemicelluloses requires a hydrolysis pretreatment. Without any pretreatment, only 20% of the ethanol production from cellulose and hemicelluloses can be attained.[29] Fiber corn and *Miscanthus* are the biomasses with the highest total bioethanol yield per kilogram of DM. Late winter *Miscanthus* and hemp are the biomasses with the highest bioethanol potential coming from structural carbohydrates (Fig. 5, top). Fiber corn is the biomass with the highest bioethanol potential coming from non-structural carbohydrates (Fig. 5, top). For most substrates, the main sources of ethanol are structural carbohydrates, except for fiber corn, Jerusalem artichoke and

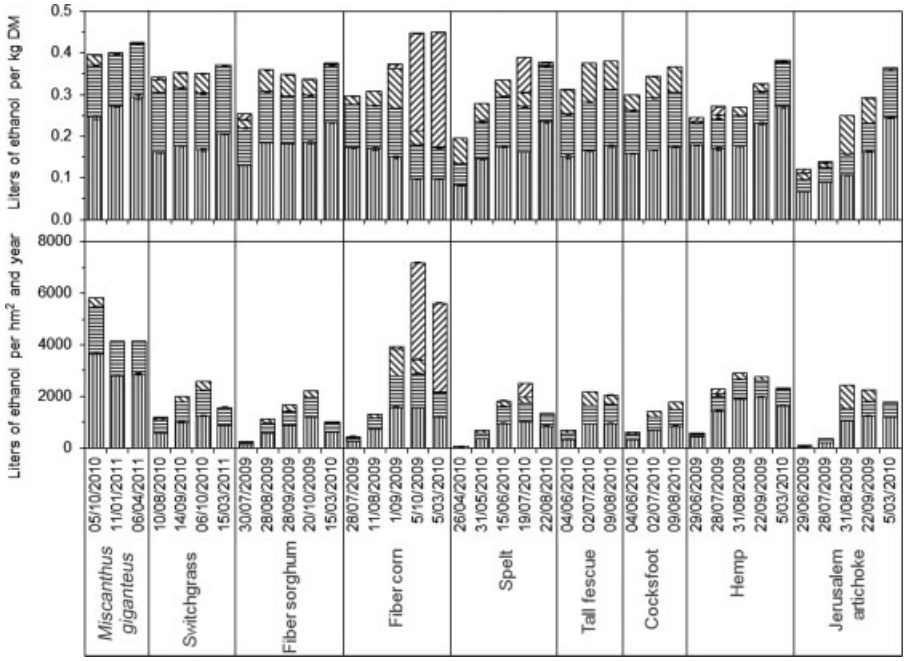

Figure 5. Bioethanol potential (top) and bioethanol yield (bottom) of the different investigated biomasses according to the harvesting date. Error bars correspond to standard deviation of the technical duplicate analysis for each biomass sample. The figures of these standard deviations can be found in supporting information Table A3.

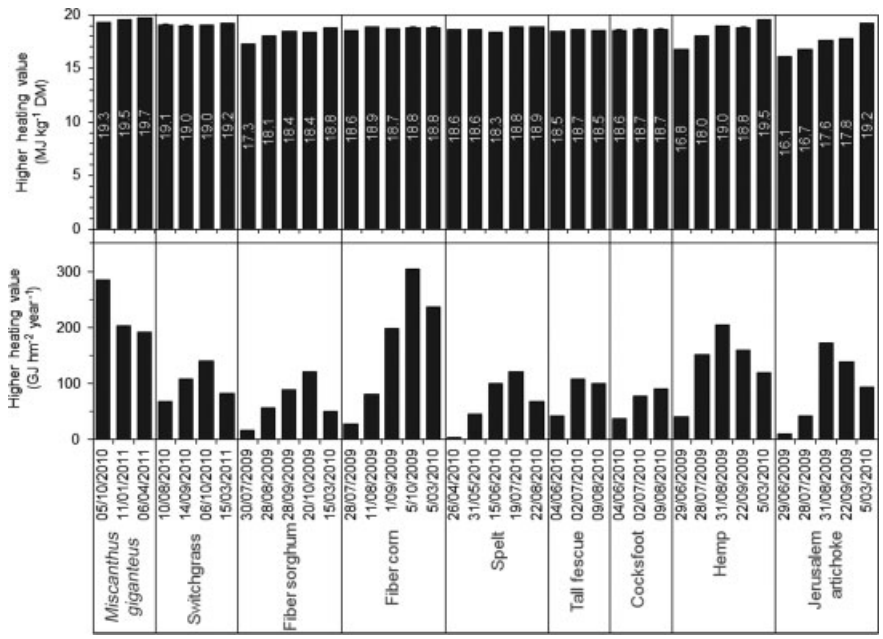

Figure 6. Higher heating value (top) and thermal energy yield (bottom) of the different investigated biomasses in dry state, according to the harvesting date. Error bars correspond to standard deviation of the technical duplicate analysis for each biomass sample. The figures of these standard deviations can be found in supporting information Table A3.

tall fescue, where a significant contribution is brought by non-structural carbohydrates (Fig. 5, top). The increase in bioethanol potential from structural carbohydrates, according to harvesting date, is linked to the increasing content of structural components (Fig. 1, top, and Fig. 5, top). Fiber corn shows a different pattern because the increasing starch content dilutes the structural carbohydrates (Fig. 1, top, and Fig. 5, top). This different progress is not observed with the last harvest stage of spelt because only the straw part is considered (Fig. 1 top).

A peak of bioethanol potential from non-structural carbohydrates can be observed in the summer (Fig. 5, top), as a consequence of the above-mentioned peak of total soluble sugars for the same period (Fig. 1, top).

The total bioethanol potential per unit crop area that can be expected for the analyzed biomasses is shown in Fig. 5 (bottom). The total bioethanol potential per unit crop area is mostly affected by the crop DM yield. The highest total bioethanol potential per unit crop area is therefore observed for the autumn harvest.

Thermal energy

HHV was used to assess the suitability of the biomasses for conversion to thermal energy by combustion, independently of their humidity (which can be adjusted by drying, if required). Figure 6 presents the progress of biomass HHV according to the harvesting date (details in supporting information Table A3). Late winter-harvested *Miscanthus* is the biomass with the highest HHV per kilogram of DM. HHV increases very slightly with plant aging (Fig. 6, top). This can mainly be explained by the increase in organic matter content (complement of the mineral content, which decreases) and by the slightly increasing lignin content during plant aging (Fig. 1, top).

The thermal energy that can be expected per unit crop area from combustion of harvested biomass is shown in Fig. 6 (bottom). HHV per unit area is mainly affected by the crop DM yield. The highest thermal energy production potential per unit crop area is therefore observed for the autumn harvest. Nevertheless, in real combustion, the biomass water content reduces the net useful energy yield and its mineral content can lead to clinker formation.[30] Table 1 and Fig. 1 (top) show that a winter harvest or harvest as straw is more appropriate because the biomass presents higher DM and lower mineral contents, as compared to the autumn harvest.

CONCLUSIONS

The research presented here shows that the chemical characteristics of various fibrous energy crops change with plant aging, with a significant increase in the content of structural components, a decrease in the content of non-structural components and a decrease in enzymatic digestibility. In the hemicellulosic components, there is an increase in the relative content of xylan and a decrease in the relative content of hemicellulosic glucan. DOM, used to predict anaerobic digestibility, decreases according to the harvesting date and is related to the increase in cellulose and lignin content (decrease in non-structural components). The increase in total bioethanol yield per biomass DM with harvesting date can be explained by the increase in total carbohydrates content, mainly cellulose and hemicelluloses. The biomass heating energy value increases with the harvesting date as a consequence of the increasing proportion of total organic matter, and the corresponding decrease in mineral content. Based on the chemical characteristics, the last harvesting date, where the biomass is the most fibrous, offers the best substrates for combustion and bioethanol production; whereas the first harvesting date, where the biomass is the less fibrous, offers the best substrates for anaerobic digestion, except for fiber corn. Fiber corn has the highest anaerobic potential at the last harvesting date because, at that date, it has an important content of starch which is a part of the DOM.

The amount of harvestable biomass increases during the growth season, but decreases during winter. For all investigated energy conversions, the best harvesting period is autumn, because the significantly higher crop DM yield largely compensates for the sometimes slightly less favorable chemical characteristics.

ACKNOWLEDGEMENTS

This research was funded by the Walloon Agricultural Research Center (CRA-W) with the support of the Belgian Science Policy and by the ENERBIOM project (ENERBIOM Project No.14GR23024 of the European territorial co-operation program in the context of INTERREG IV A 'Grande Région' 2007–2013 No. CCI2007CB1 63PO064, co-financed by the FEDER-EU funds). The authors are grateful to the technicians of the BIOETHA2 and the ENERBIOM projects for their technical support.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Carpita N, McCann M, The cell wall, in *Biochemistry and Molecular Biology of Plants*, ed. by Buchanan B, Gruissem W and Jones R. American Society of Plant Physiologists, Rockville, MD, pp. 52–108 (2000).
2 Pauly M and Keegstra K, Cell-wall carbohydrates and their modification as a resource for biofuels. *Plant J* **54**:559–568 (2008).
3 Dien B, Jung H-J, Vogel K, Casler M, Lamb J, Iten L, et al, Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. *Biomass Bioenerg* **30**:880–891 (2006).
4 Jung H and Casler M, Maize stem tissues: impact of development on cell wall degradability. *Crop Sci* **46**:1801–1809 (2006).
5 McKendry P, Energy production from biomass. Part 1: Overview of biomass. *Bioresour Technol* **83**:37–46 (2002).
6 Kamm B and Kamm M, Principles of biorefineries. *Appl Microbiol Biot* **64**:137–145 (2004).
7 Bessou C, Ferchaud F, Gabrielle B and Mary B, Biofuels, greenhouse gases and climate change: a review. *Agron Sustain Dev* **31**:1–79 (2011).
8 Ghysel F, Godin B, Flamin C, Delfosse P, Delcarte J and Stilmant D, Valorisation énergétique des fourrages: comparaison de trois filières, enjeux et opportunités. *Fourrages* **203**:163–173 (2010).
9 Spatari S, Bagley D and MacLean H, Life cycle evaluation of emerging lignocellulosic ethanol conversion technologies. *Bioresour Technol* **101**:654–667 (2010).
10 De Boever J, Cottyn B, Andries J, Buysse F and Vanacker J, The use of a cellulase technique to predict digestibility, metabolizable and net energy of forages. *Anim Feed Sci Tech* **19**:247–260 (1988).
11 De Boever J, Cottyn B, Buysse F and Vanacker J, The use of an enzymatic technique to predict digestibility, metabolisable and net energy of compound feedstuffs for ruminants. *Anim Feed Sci Tech* **14**:203–214 (1986).
12 Godin B, Agneessens R, Gerin P and Delcarte J, Composition of structural carbohydrates in biomass: precision of a liquid chromatography method using a neutral detergent extraction and a charged aerosol detector. *Talanta* **85**:2014–2026 (2011).
13 Van Soest P, Collaborative study of acid-detergent fiber and lignin. *J AOAC* **56**:781–784 (1973).
14 European Union, Commission Regulation No 152/2009. *Offic J EU* **L54**:1–130 (2009).
15 AOAC, *Official Methods of Analysis* (15th edn). Association of Official Analytical Chemists, Washington, DC (1990).
16 Lesteur M, Latrille E, Maurel V, Roger J, Gonzalez C, Junqua G, et al, First step towards a fast analytical method for the determination of biochemical methane potential of solid wastes by near infrared spectroscopy. *Bioresour Technol* **102**:2280–2288 (2011).
17 da Costa Sousa L, Chundawat S, Balan V and Dale B, 'Cradle-to-grave' assessment of existing lignocellulose pretreatment technologies. *Curr Opin Plant Biol* **20**:1–9 (2009).
18 Buchholz K and Seibel J, Industrial carbohydrate biotransformations. *Carbohyd Res* **343**:1966–1979 (2008).
19 Hamelinck C, van Hooijdonk G and Faaij A, Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. *Biomass and Bioenerg* **28**: 384–410 (2005).
20 European Committee for Standardization, *CEN/TS 14918: Solid Biofuels – Method for the Determination of Calorific Value*. European Committee for Standardization, Brussels (2005).
21 Hatfield R, Jung H, Broderick G and Jenkins T, Nutritional chemistry of forages, in *The Science of Grassland Agriculture*, Vol. **2**, ed. by Barnes R, Nelson C, Moore K and Collins M. American Society of Agronomy, Madison, WI, pp. 467–485 (2007).
22 Nizami A-S, Korres N and Murphy J, Review of the integrated process for the production of grass biomethane. *Environ Sci Technol* **43**:8496–8508 (2009).

23 Cadoux S, Preudhomme M, Machet J, Mary B, Fourdinier E, Ferchaud F, *et al.* Productivity and environmental impacts of energy crops: first results of the long term experiment, in *Biomass and Environment: 17th European Biomass Conference and Exhibition*, Hamburg (2009).
24 Hames B, Biomass compositional analysis for energy applications, in *Methods in Molecular Biology*, Vol. 581, ed. by *Mielenz J*. Biofuels: Methods and Protocols. Humana Press, New York, pp. 145–183 (2009).
25 Cadoux S, Briand S, Chabbert B, Besnard A, Félix I, Savouré M, *et al.* Biomass productivity of different energy crops under French conditions: results of the 'Regix' experimental network, in *18th European Biomass Conference and Exhibition*, Lyon (2010).
26 Ishii T, Structure and functions of feruloylated polysaccharides. *Plant Sci* **127**:111–127 (1997).
27 Vogel J, Unique aspects of the grass cell wall. *Cur Opin Plant Biol* **11**:301–307 (2008).
28 Downes K and Terry L, A new acetonitrile-free mobile phase method for LC–ELSD quantification of fructooligosaccharides in onion (*Allium cepa* L.). *Talanta* **82**:118–124 (2010).
29 Balat M, Balat H and Oz C, Progress in bioethanol processing. *Prog Energ Combust* **34**:551–573 (2008).
30 Saidur R, Abdelaziz E, Demirbas A, Hossain M and Mekhilef S, Review on biomass as a fuel for boilers. *Renew Sust Energ Rev* **15**:2262–2289 (2011).

