EVALUATION OF FORESTRY BIOMASS QUALITY FOR THE PRODUCTION OF SECOND-GENERATION BIOFUELS

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Different classes of wooden material were analysed as to their chemical composition, especially as to the content of fermentable sugars: softwood and hardwood unbarked wood, SRC, VSRC and different grades of forest chips. High hexose contents were preferably found in unbarked softwoods, which presented up to 4% more C6 sugars than unbarked hardwoods. However, some hardwood species, such as eucalyptus and poplar clones, presented very high hexose content values. Short rotation coppices present just slightly lower average hexose content compared to mature wood, but very short rotation coppices are poorer in hexoses, by 3% on the average. The hexose content of forest chips is significantly affected by the non-wood fractions. On the average, the gap between mature unbarked wood and forest chips is of 5% for hardwoods and of 8% for softwoods. Finally, if upgrading of pentoses by fermentation into bioethanol is possible, hardwoods seem to be a good raw material, as the presence of C5 sugars is considerably more important than in softwoods.

Keywords: bioethanol, residual wood, SRC, VSRC, forest chips, hexoses, pentoses

INTRODUCTION

The EU transport sector accounts for more than 30% of the total energy consumption in the Community. This sector is by 98% dependent on fossil fuels and has contributed to more than 90% of the increase in CO_2 emissions between 1990 and 2010.[1] Moreover, the high share of oil among import expenses makes the transport sector extremely vulnerable to market disturbances, such as oil price fluctuations or any major economic crisis.

To reduce the economical and energetic dependence on oil and to fulfil the Kyoto targets concerning greenhouse gases, several actions have been undertaken by EU since the last decade, to favour the production of biofuels, such as The White Paper[2] on renewable energy, which announced a target to double the European Union's renewable energy share to 12% by 2010; the directives 2001/77/EC[3] and 2003/30/EC,[3] which set indicative 2010 targets for all member states and required actions to improve the growth, development and access of renewable energy; the national biomass action plans,[4] adopted in 2005 to focus attention on the specific needs for the Member States to develop Europe's biomass resources; the Renewable Energy Roadmap,[5] and the quite recent directive 2009/28/EC[3] covering all Renewable Energies.

The current production of liquid biofuels in the EU 25 is of about 2 Mtoe, which is less than 1% of the transport fuel market. Currently produced biofuels are called first-generation, *i.e.* the bioethanol produced from the juice directly extracted from sugar beet, or from sugars produced by the hydrolysis of starch and biodiesel produced from different oils.[6,7]

The second-generation biofuels appear as a most promising alternative to increase the yield of biofuels produced per hectare, as well as to enlarge the panel of biomass used for their production.[8] In this case, bioethanol is obtained by the hydrolysis of the polysaccharide fraction from the vegetal biomass in monosugars prior the fermentation.[9,10] The biodiesel can be obtained from a syngas produced by biomass gasification by Fischer-Tropsch synthesis.[11] This article evaluates the potential of wooden

biomass for the production of second-generation bioethanol from the fermentation of sugars released after the hydrolysis of polysaccharides. A brief description of the latter fractions and of the main steps of the process to convert biomass into bioethanol is also provided.

The major chemical organic components of wood can be classified as polysaccharides (cellulose, hemicelluloses and, sometimes, pectins), lignin and extractives. Inorganic species are also present and are often reported as "ash". The chemical compositions of the major wood species can be found in different textbooks.[12,13] Each of these components contributes to the properties of wood-based products.

Cellulose, the major chemical component of the fibre wall is composed of linear chains of D-glucose, a 6-carbon atom sugar (hexose), linked by β-1,4-glycosidic bonds with the degree of polymerization from 10,000 in native wood to 1,000 in bleached kraft pulps. Cellulose has a strong tendency to form intra- and inter-molecular hydrogen bonds by the hydroxyl groups on these linear cellulose chains, allowing the formation of different fibre structures and morphologies.[12,13] The presence of crystalline cellulose, with less ordered regions, and the size of the elementary fibrils work together to produce an interesting combination of contrary properties, such as stiffness and rigidity, on the one hand and flexibility, on the other.[14] Crystalline cellulose has very limited accessibility to water and chemicals. A chemical attack can be therefore expected to occur, primarily on amorphous cellulose and on the crystalline surface.

Unlike cellulose, hemicelluloses have a lower DP (50-300) with side groups on the chain molecule, and are essentially amorphous. Moreover, they are composed of different 5-carbon sugars or pentoses (β-D-xylose, α-L-arabinopyranose and α-L-arabinofuranose) and 6-carbon sugars or hexoses (β-D-glucose, α-D-galactose and β-D-mannose), as well as of some hexuronic acids (β-D-glucoronic, α-D-4-O-Methylglucuronic, β-D-galacturonic), and deoxy-hexoses (α-L-Rhamnose and α-L-fucose).[12,13] The main hemicelluloses present in softwood are galactoglucomannans and arabinoglucuronoxylan, while in hardwood the most important ones are the glucuronoxylans.

After polysaccharides, lignin is the most important macromolecular component of the vegetal world. However, lignin is unusual due to its aromatic chemical nature, heterogeneity and lack of a well-defined primary structure. Its most commonly noted function is the support through strengthening the wood (xylem cells) in trees and, by extension, the plant as a whole.[12,15] The lignin content in normal tissues varies between 15 and 35%, being dependent on several factors. However, lignin is particularly abundant in compression wood, but scarce in tension wood. Lignin fills the spaces in the cell wall between cellulose, hemicellulose and pectin components, especially in tracheids, sclereids and xylem. It is covalently linked to hemicellulose, forming the so-called lignin-carbohydrate complexes (LCC) and thereby it crosslinks different plant polysaccharides.[16,17] The most frequently suggested LCC linkages in native wood are benzyl ester, benzyl ether and glycosidic linkages.

The production of ethanol from lignocellulosic raw materials can be summarized as follows: a) opening the ultra-structure of the cell wall to access the polymer chains of cellulose and hemicellulose by different pretreatments; b) hydrolysing the polysaccharides into monosugar syrup; c) fermenting the sugars to ethanol solution (mash) by microorganisms; d) distilling and dehydrating ethanol.

Different pretreatments used to expose the polysaccharides to the action of enzymatic or acidic hydrolysis have been recently reviewed by different authors.[18-20] The main classes of pretreatments are physical (comminution, irradiation, extrusion, expansion, etc), physico-chemical (hydrothermolysis, stream explosion, acids, alkali, gases, oxidant, polysaccharide solvents, delignification agents) or biological (fungi).[20]

Comprehensive reviews have been published by Taherezadeh and Keikhosro on the hydrolysis of polysaccharides either chemically[20] or enzymatically.[21] Chemical hydrolysis is mostly performed by sulphuric acid,[21,22] but other acids have also been used to this end (HCl, for example).[23] Enzymatic hydrolysis demands the combined action of different enzymes of the same family, the glycosylhydrolases. The hydrolysis of cellulose is achieved by the action of three major classes of enzymes: endo-gluconases,

exo-gluconases and β-glucosidases. The endo-gluconases attack the low-crystallinity regions of the cellulose fibre and create chain-ends. The exo-gluconases degrade the sugar chain until the dimeric unit of glucoses (cellobiose), which is finally converted into glucose. Hemicellulases are more complex and involve different enzymes, such as endo-1,4-β-D-xylanases, exo-1,4-β-D-xylosidases, endo-1,4-β-D-mannanases, β-mannosidases, acetyl xylan esterases, α-glucoronidases, α-glucoronidases, α-arabinofuranosidases, and α-galactosidases.[24] The main advantages of acid hydrolysis, compared to enzymes, refer to the absence of inhibition during hydrolysis, to the low cost of chemicals and short time of hydrolysis. On the other hand, enzymatic hydrolysis, performed under much milder conditions, gives higher hydrolysis yields, while avoiding the formation of fermentation inhibitory by-products.[22]

Once the monomeric sugars are released from the lignocellulosic matrix, hexoses (C6) can be straightforwardly fermented with *Saccharomyces* yeast or with *Zymomonas* bacterium.[9,11] The fermentation of pentoses (C5) challenges the industrial implementation of cellulosic bioethanol. On the one hand, the fermentation of pentoses is difficult to achieve at good rates and yields, yet it is needed for making the process economically feasible. There are two ways of fermenting C5: either by isolating then fermenting them with a naturally or GMO C5 fermenting microorganism, or by fermenting C5 and C6 together.[21,22] The latter pathway is much more challenging as two microorganisms acting together are necessary, therefore pentose fermentation must be introduced by genetic engineering in traditional microorganisms, such as *Saccharomyces*[25] or *Zymomonas*.[26]

The adequacy of the biomass raw material and of the industrial processes is one of the most challenging aspects for the future cellulosic bioethanol industry.[10] To be competitive with oil prices and to overpass the technical and economical performances, various first-generation biomass must be considered to supply the future second-generation biofuel industrial sites, while avoiding competition with current biomass-based and food industries. Concerning forest-based products, not only different wood species must be considered, but also parts of the trees not commonly used for the first conversion, such as small diameter logs, stems, brushwood, fines and different wood residuals resulting either from forest harvesting or from wood transformation. Moreover, as the fibrous structure of the wood tissues is not an important parameter for bioenergy purposes, new silvicultural strategies can be used in biomass production, such as short (or very short) rotation coppices.

Wood availability and quality are two subjects of special importance for establishing the plans of creating future biofuel plants. These aspects have been addressed in France through an ongoing 4-year national project named REGIX, making an inventory of quality data of both agricultural and forestry biomass. After a critical analysis of the existing data, the work was completed with two analytical campaigns on the biomass samples produced within this project. In the present investigation, the potential of different wooden raw materials for the production of bioethanol was studied. Different wood species, wood products including short and very short rotation coppices, and forest chips produced from different wood residuals were characterised in terms of chemical composition, particularly the amount of hexoses and pentoses present in each product.

EXPERIMENTAL

Vegetal material

Four series of wooden material were used in this work. The characteristics of the samples analyzed are presented in Table 1.

Analytical methods

The wood samples were received in different forms (logs, stems, chips, etc). The first step was to reduce their size to wood chips of less than 5 cm, which were then ground for the production of wood particles measuring less than 40 mesh (0.425 mm) for chemical analysis. Prior to the analysis of lignin and polysaccharides, the samples were extracted using an acetone/water sequence, with a high-pressure automatic extractor ASE 300 (Accelerated Solvent Extractor) from Dionex (USA). Extractions were performed at 1500 psi. The water extraction cycle included a heating period of 6 min, followed by a twofold, 10 min extraction in the static mode at 110 °C. The acetone extraction cycle consisted in a heating period of 5 min, followed by a twofold, 10 min extraction in the static mode, at 95 °C.

Table 1
Characteristics of the vegetal material analysed*

Wood species	Age	Origin	Characteristics	Fraction analysed	Number of samples analysed
			1st series - Hardwoods and Softwoods		
Beech, birch, chestnut *Eucalyptus globulus*, *Eucalyptus urograndis*, oak, poplar, Aleppo Pine Black Pine, Douglas-fir, Maritime Pine, Norway Spruce, Scots Pine	Undetermined	Undetermined	Logs arriving at pulp mill park yards Diameter: 15 to 25 cm	Unbarked wood	5 logs per wood species Total = 65 samples
			2nd series - Poplar and eucalyptus clones		
Poplar clones – *Ghoy, Blanc du Poitou, Dorskamp, I214, Robusta Raspalje, Beaupré*	23 years old	Cheffes (France)	1 m logs sampled at 2.5 and 4.5 m height	Unbarked wood	2 logs per tree 5 trees per clone Total = 80 samples
Poplar clone - *I45-51*	25 years old	Cheffes (France)			
E. urograndis - Clones A, B and C	7 years old	Brazil	Wood chips from the whole tree	Wood chips	5 trees per clone
			3rd series - Short rotation coppices (SRC) and very short rotation coppices (VSRC)		
Eucalyptus gunnii	12 years old	Longages (France)			6 trees
Poplar	11-13 years old	Auvillars, Cloyes sur Marne, and Maurupt (France)	Whole tree, tree top	Unbarked wood and boughs	8 trees
Locust	10-13 years old	St. Vitor de Melcape (France)			6 trees
Eucalyptus gunnii	1 year old	Longages (France)			25 stems
Poplar	1-2 years old	2 sites in France (Guéméné-Penfao and Charrey)	Whole stem	Unbarked stems	14 stems
Locust	5 years old	St. Vitor de Melcape (France)			9 stems
Willow	4 years old	St. Gilles (France)			5 stems

* The four series correspond to the sections described in Results and Discussion

The lignin content was measured by the Klason method, modified by Schwanninger and Hinterstoisser.[27] The cellulose and hemicellulose content was determined from ionic liquid chromatography analysis of monosaccharides after acidic hydrolysis, according to Puls *et al*.[28] Monosugar analysis was carried out after the two-step acidic hydrolysis of wood and pulps, by the ASTM method E1758-01(2007). The quantification of neutral monosugars was obtained on a DIONEX HPAE-PAD ion chromatograph equipped with a pulsed amperometric detector. From the analysis of monosugars, the corresponding polyoses were calculated, following the procedure described by Genco *et al*.[29] All data presented in this paper have been corrected as to the presence of ash, so they have an ash-free content

RESULTS AND DISCUSSION

Wood species variability

In recent years, an important pulp production delocalisation has been observed from the northern to the southern hemisphere. For this reason, pulpwood is to be considered in the future as a possible raw material for bioethanol production. Thirteen major wood species, seven hardwoods (beech, birch, chestnut, *Eucalyptus globulus*, *Eucalyptus urograndis*, oak and poplar) and six softwoods (Aleppo Pine, Black Pine, Douglas-fir, Maritime Pine, Norway Spruce and Scots Pine) were sampled for the first series. Representative samples of the wood currently available for supplying pulp mills in France were collected and analysed. Eucalyptus, the main wood species used in South America for pulp production, was also considered for the sake of comparison. Parameters, such as the age of trees, origin, soil quality, growth rate, etc., were not considered, although it is well-known that all these criteria can impact wood quality variability.[30,31] This vegetal material, composed only of unbarked wood, will be used for comparison with the three subsequent series of samples.

The average chemical composition of these wood species in terms of the main components (polysaccharides, lignin and extractives) is given in Table 2.

Table 2
Ash-free chemical composition of unbarked wood of different hardwoods and softwoods

Wood species	Lignin content (%)	Extractive content (%)	Polysaccharide content (%)	C6 content (%)	C5 content (%)
Hardwoods					
Beech	23.00	3.07	73.93	55.70	18.23
Birch	22.00	3.39	74.61	54.80	19.81
Chestnut	23.70	16.14	60.16	46.79	13.37
Eucalyptus globulus	15.94	3.65	80.41	62.80	17.61
Eucalyptus urograndis	27.43	2.46	70.11	59.37	10.74
Oak	23.50	13.26	63.24	49.48	13.76
Poplar	26.00	3.96	70.04	56.79	13.25
Softwoods					
Aleppo Pine	25.90	3.75	70.35	61.98	8.37
Black Pine	26.10	5.28	68.62	63.40	5.22
Douglas-fir	26.10	3.31	70.59	64.00	6.59
Maritime Pine	27.20	4.63	68.17	60.57	7.60
Norway Spruce	27.30	3.75	68.95	62.55	6.40
Scots Pine	26.90	4.82	68.28	62.32	5.96

The content of the fraction that is significant for biethanol production, the polysaccharides, varies from 60.2 for chestnut to 80.4% for *E. globulus*, among the hardwood species. The large difference observed for the wood species is governed by the content of non-polysaccharide components. Chestnut presents very high extractive and lignin contents, of 16.1 and 23.7%, respectively. Oak presents similar numbers: 13.3% for extractive content and 23.5% for lignin content. Chestnut and oak are known for their high extractive content, because of the presence of polyphenols.[32,33] *E. globulus* has a considerably lower lignin content (15.9%) than the values usually found for hardwoods, concomitantly with a relatively low extractive content (3.7%). Between these extremes, poplar and *E. urograndis* present a polysaccharide content

of around 70%, while birch and beech – around 74%. In both cases, the lignin content is the main controlling factor of polysaccharide availability, as the extractive content is similar for the 4 cited wood species.

The differences observed in the total polysaccharide content among the hardwood species are mainly caused by the C6 content. Chestnut and oak present the lowest hexose content, respectively 46.8 and 49.5%, while *E. globulus* has a value of 62.8%. The small differences in the pentose content brought beech, birch and poplar to a similar level of hexose content (54.8 to 56.8%), while for the two eucalyptus analysed (*E. globulus* and *E. urograndis*), despite a difference of 12% in the lignin content, a similar C6 content was observed, due to the huge difference in pentose content (17.6 for *E. globulus* and only 10.7% for *E. urograndis*).

As to softwoods, the differences are clearly less pronounced than those observed for hardwoods. The polysaccharide fraction of the wood species studied varies between 68.2 and 70.6%, resulting in a relative uniformity in lignin (25.9 to 27.3%) and extractive content (3.3 to 5.3%). These values, however, refer strictly to the samples studied in the project rather than demonstrating the intrinsic variability of these wood species.[31,32] The tendencies for the hexose and pentose content variability among softwoods are similar to those observed for polysaccharides, lignin and extractives. The range of C6 variation is from 60.6 to 64.0%, while the pentose content varies between 5.2 and 8.4%.

The composition of the polysaccharide fraction given in Table 3 reveals, unsurprisingly, that glucose is the most important sugar for all wood species. Although softwoods present a higher hexose content per mass unit of wood than hardwoods, the polysaccharides of the latter are richer in glucan. Softwood polysaccharides are composed of 69.4 to 73.9% glucose. For poplar and *E. urograndis*, the values reach 78.7 and 82.1%, respectively. The main difference for the hexose composition between softwoods and hardwoods is observed in mannose. It represents an important fraction of softwoods, varying from 13.6 (Douglas-fir) to 20.6% (Black Pine), although its content in hardwood polysaccharides does not exceed 4.4% (chestnut). Galactan also contributes to a high content of C6 in softwoods. It represents up to 4.1% of the polysaccharides present in the softwoods here studied while, for most hardwoods, it represents less than 2%. Concerning pentoses, the average xylan content of hardwoods is at least twice higher than that of softwoods. Most of the wood species present more than 20% xylans in their polysaccharides, the most important one being birch, with 26.1%. *E. urograndis* is the hardwood species with the lowest xylose content (15.1%), but this value is still considerably higher than those observed for softwoods, ranging between 7.2 (Scots Pine) and 10.1% (Aleppo Pine). The arabinose content is important neither in hardwoods nor in softwoods, usually representing less than 1% for most of the species, although it can reach 2% for Maritime Pine.

Short and very short rotation coppices

Short rotation coppices consist of densely planted, high-yielding varieties of different hardwood species. The establishment of SRC plantations has more in common with agricultural or horticultural crops than forestry. The rotation time, the harvesting mode and the plantation density distinguish SRC (short rotation coppices) from VSRC (very short rotation coppices). SRC is suitable for cutting as logs between 6 and 15 years old, as depending on the wood species. The typical densities are up to 1,500 plants per hectare. VSRC have been tested in the last years as energetic forest cultures with plantation density up to 10,000 plants per hectare, to be harvested by agriculture-like cropping machines between 1 and 4 years. In both cases, several rotations can be carried out if sprouting species are used.

Eucalyptus, locust tree, poplar and willow are the main wood species cultivated as V(SRC) for bioenergy purposes. In this second series, the chemical composition of the wood species cultivated as VSRC (1-3 year old trees) and SRC (10-13 year old trees) was studied (Table 4).

The lignin content of the different wood species was similar (19.1 to 20.8% for SRC and 18.9 to 19.9% for VSRC). However, for the other components, important differences appear when considering the difference of age. The extractive contents here measured are considerably higher than those obtained for the unbarked wood studied in the first series. Several explanations can be furnished

to these observations. The first concerns the age of the samples. It appears clearly that very young plants give higher extractive contents, as shown by the important differences observed between SRC (7.6 to 8.7%) and VSRC (8.4 to 14.9%). The intense physiological activity of the young plants, especially in the zones of xyleme, cambium and phloem, and especially the large ratio of these tissues, compared to the already formed wood, explain the high extractive content. The second explanation is the presence, in the V(SRC) samples, of all tree components (wood, bark, residual leaves, stems, twigs, etc). It is known that most of the non-wood components of the trees can have high extractive contents, compared to wood.[34] Finally, one might speculate that some lignin could not be extracted by acetone during extractive determination as, in the initial step of cell wall lignification, the lignin fragments present low molecular weight and are readily soluble in acetone. Acetone is indeed, a very good solvent for lignin, as already reported by different authors.[35-37]

Table 3
Sugar composition of the polysaccharide fraction of unbarked wood of different hardwoods and softwoods

Wood species	Glucose content (%)	Mannose content (%)	Galactose content (%)	Arabinose content (%)	Xylose content (%)
Hardwoods					
Beech	71.91	2.61	0.82	0.54	24.11
Birch	70.07	2.49	0.90	0.40	26.14
Chestnut	71.03	4.40	2.34	0.70	21.53
Eucalyptus globulus	74.50	2.28	1.32	0.60	21.31
Eucalyptus urograndis	82.08	1.50	1.10	0.23	15.09
Oak	74.73	1.53	1.98	0.90	20.86
Poplar	76.68	3.64	0.76	0.37	18.55
Softwoods					
Aleppo Pine	69.94	15.29	2.87	1.81	10.09
Black Pine	69.57	20.64	2.19	1.47	6.14
Douglas-fir	73.79	13.62	3.27	1.53	7.79
Maritime Pine	69.44	15.33	4.09	1.95	9.18
Norway Spruce	71.28	16.82	2.61	1.38	7.90
Scots Pine	69.48	18.79	3.00	1.58	7.15

Table 4
Ash-free chemical composition of unbarked wood of 8 poplar and 3 eucalyptus clones

Wood species/clones	Lignin content (%)	Extractive content (%)	Polysaccharide content (%)	C6 content (%)	C5 content (%)
Poplar clones					
Ghoy	22.61	3.28	74.11	57.40	16.71
Blanc du Poitou	22.83	3.03	74.14	57.10	17.04
Dorskamp	22.13	3.17	74.70	54.70	20.00
I214	23.68	3.03	73.29	62.32	10.97
Robusta	21.87	2.94	75.19	66.71	8.48
Raspalje	20.91	2.93	76.16	63.41	12.75
Beaupré	21.23	3.25	75.52	63.03	12.49
I45-51	22.59	2.95	74.46	63.85	10.61
Eucalyptus clones					
E. urograndis - Clone A	27.66	3.06	69.28	57.62	11.66
E. urograndis - Clone B	26.98	1.84	71.18	59.96	11.22
E. urograndis - Clone C	27.66	2.47	69.87	60.52	9.35

As a consequence of the extractive content, the SRC samples are richer in polysaccharides than VSRC. The variations are between 71.2 and 74.1% for SRC and between 65.8 and 72.6%, respectively, for VSRC. For eucalyptus and poplar, the gap is

of approximately 7%, while no difference is observed for locust. However, these results must be carefully analysed as the difference of age between VSRC and SRC is not the same for all wood species. The increase of the total polysaccharide content from VSRC to SRC samples has different explanations for eucalyptus and poplar. For eucalyptus, only a small increase in C6 sugars is observed (3.3%), while the C5 sugar content increases up to 6.2%. For poplar, the C5 sugar content is the same, while 6.5% more C6 sugars were detected.

The analysis of the polysaccharide composition in individual monosugars (Table 5) also reveals differences between poplar and eucalyptus. While, for eucalyptus, the glucose content decreases from 75.6 (VSRC) to 72.7% (SRC), for poplar the values increase from 79.1 (VSRC) to 84.5% (SRC). Only minor changes are observed for the other C6 sugars. An important increase is observed for the xylose content of eucalyptus (15.6 for VSRC and 20.6% for SRC) while, for poplar, the trend is an opposite one (13.9 for VSRC and 11.1% for SRC). A slight increase in the arabinose content is also measured. These data suggest that the hemicellulose content changes during the growth of the trees, as already evidenced by several authors, along with their sugar composition.

As no samples of willow SRC were analysed, it is not possible to discuss the evolution of their chemical composition during growth. The only observation is that the SRC willow and poplar samples present very similar composition both in terms of their main component, as well as of the sugar composition of polysaccharides.

Intra-wood species variability: poplar, eucalyptus

Intra-wood species can be also an important source of variation in the hexose and pentose contents. To approach this subject, several poplar and eucalyptus clones were analysed (Tables 6 and 7).

The poplar clones studied here represent more than 80% of the poplar currently consumed in France. The trees of the clones studied here were cultivated on the same site and have the same age (23 years), except clone I214, which was 25 years old, but cultivated on a geographically close site with the same soil quality. The results listed in Table 6 show that, despite the low differences in lignin, polysaccharide and extractive contents among poplar clones, considerable variability exists in the C6/C5 balance. Indeed, the hexose content varies from 54.7 (Dorskamp) to 66.1% (Robusta), while the pentose content ranges from 8.5 to 20%. The fluctuations represent roughly 12% per unit of mass, while the difference between the minimum and maximum values of the polysaccharide content is less than 3%. These data demonstrate not only the importance of the poplar clone effect on the potential for bioethanol production, but also an interesting variability for future breeding programs of poplar, devoted to bioenergy. The polysaccharide composition in terms of monosugars is also quite interesting. The gap between the lowest (Dorskamp) and the highest (Robusta) glucose content is of about 24%. An interesting and intriguing aspect is the direct negative correlation between glucose and both galactose (C6 sugar) and xylose (C5 sugar) (Fig. 1), not observed in the previous series.

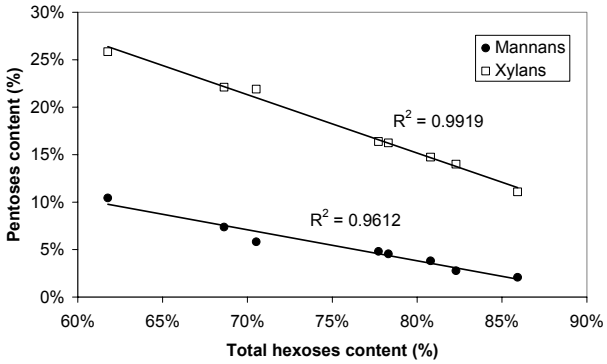

Figure 1: Correlations observed between the total content of hexoses and individual pentoses for the poplar clones (2nd series)

The same observations as to polysaccharide composition were made for 3 clones of *Eucalyptus urograndis*. However, the variation range is considerably lower than that observed for poplar. The explanations for the overall differences between eucalyptus and poplar concern the age of the trees, the origins of the controlled crossing, the small number of eucalyptus clones studied and the degree of maturity of the two breeding programs. Despite minor differences in lignin and total polysaccharide content, the hexose content variation reaches 3%. The glucose and xylan content balance in the polysaccharides is at the origin of all hexose and pentose variations.

When comparing the results of *E. urograndis* with those of eucalyptus, from the previous sections, huge differences in the polysaccharide content are observed. *E. urograndis* presents roughly 10% less polysaccharides than *E. globulus* at comparable age, although the hexose contents of the two wood species are in the same range (59.4% for *E. globulus* and 57.6 to 60.5% for *E. urograndis*). Therefore, differences are caused by the higher content of pentoses of *E. globulus* (17.6%) compared to *E. urograndis* (9.4 to 11.7%). In terms of individual sugars, the glucose content of *E. urograndis* (Table 5) is 5 to 8% higher than that of *E. globulus* (Table 3). On the contrary, the xylose content is 5 to 8% higher for *E. globulus*, compared to *E. urograndis.* The mannose, galactose and arabinose contents are approximately the same for the two eucalyptus species. SRC *E. gunnii* samples (10 years old) present a 2-4% total polysaccharide and hexose content higher than *E. urograndis* (Tables 4 and 6). The sugar composition of polysaccharides reveals that *E. urograndis* is richer in glucose (80 to 84.5%) than *E. gunnii* (72.7%). On the other hand, *E. gunnii* presents higher contents of galactose, arabinose and xylose (Tables 5 and 7).

Table 5
Sugar composition of the polysaccharide fraction of unbarked wood of 8 poplar and 3 eucalyptus clones

Wood species	Glucose content (%)	Mannose content (%)	Galactose content (%)	Arabinose content (%)	Xylose content (%)
Poplar clones					
Ghoy	70.52	5.82	1.10	0.64	21.91
Blanc du Poitou	68.62	7.37	1.03	0.87	22.11
Dorskamp	61.77	10.44	1.02	0.91	25.85
I214	80.79	3.82	0.41	0.22	14.74
Robusta	85.92	2.08	0.72	0.19	11.08
Raspalje	77.72	4.82	0.72	0.35	16.39
Beaupré	78.30	4.56	0.59	0.29	16.25
I45-51	82.29	2.78	0.68	0.26	14.00
Eucalyptus clones					
E. urograndis – Clone A	80.03	2.14	0.99	0.24	16.60
E. urograndis – Clone B	81.65	1.30	1.29	0.21	15.55
E. urograndis – Clone C	84.53	1.06	1.03	0.25	13.13

Table 6
Ash-free chemical composition of different hardwoods species cultivated as short rotation coppices (SRC) or very short rotation coppices (VSRC)

Wood species/clones	Lignin content (%)	Extractive content (%)	Polysaccharide content (%)	C6 content (%)	C5 content (%)
SRC					
Eucalyptus gunnii	19.08	7.86	73.06	56.38	17.60
Poplar	20.83	7.63	74.09	62.72	11.37
Locust	20.13	8.71	71.17	54.91	16.26
VSRC					
Eucalyptus gunnii	19.33	14.85	65.82	53.06	12.72
Poplar	19.94	12.80	67.33	56.24	11.01
Locust	18.91	8.47	72.62	55.74	16.88
Willow	19.78	12.94	67.28	56.59	10.69

Table 7
Sugars composition of polysaccharides fraction of different hardwood species cultivated as short rotation coppices (SRC) or very short rotation coppices (VSRC)

Wood species	Glucose content (%)	Mannose content (%)	Galactose content (%)	Arabinose content (%)	Xylose content (%)
SRC					
Eucalyptus gunnii	72.73	1.25	3.18	2.20	20.64
Poplar	84.56	1.85	1.25	1.20	11.13
Robinier	74.83	1.24	1.10	1.84	20.99
VSRC					
Eucalyptus gunnii	75.64	1.19	3.82	3.73	15.62
Poplar	79.15	2.35	2.13	2.45	13.92
Locust	73.49	2.22	1.05	2.24	21.00
Willow	79.91	2.25	1.95	2.35	13.54

Table 8
Ash-free chemical composition of different hardwood and softwood forest chips

Wood species/clones	Lignin content (%)	Extractive content (%)	Polysaccharide content (%)	C6 content (%)	C5 content (%)
Hardwoods					
Hornbean	20.4	9.28	70.4	48.0	22.3
Mixed hardwoods 1	24.9	9.3	65.9	54.7	11.1
Mixed hardwoods 2	22.0	3.98	74.0	53.6	20.5
Oak	25.3	12.39	62.3	42.8	19.5
Oak/chestnut 1	21.9	10.1	68.0	54.0	14.0
Oak/chestnut2	23.5	10.7	65.9	51.4	14.5
Poplar 1	23.5	4.0	72.5	61.0	11.5
Poplar 2	25.9	4.41	69.7	52.3	17.4
Poplar 3	20.9	3.04	76.1	61.2	14.9
Poplar 4	24.5	4.12	71.4	54.4	17.0
Softwoods					
Douglas-fir	27.7	4.38	67.9	62.0	5.9
Maritime Pine	27.1	17.4	55.5	48.0	7.5
Mixed softwoods 1	34.2	6.76	59.0	50.6	8.5
Mixed softwoods 2	27.7	3.06	69.3	61.4	7.8
Mixed softwoods 3	28.3	11.98	59.7	52.3	7.5

The results of this series support the observation that the hemicellulose content and composition play a determinant role in the potential of fermentable sugars, even at the intra-wood species level.

Forest chips

The last series analysed in this paper concerns the forest chips produced for bioenergy purposes in different harvesting situations. Fifteen different softwood and hardwood forest chips (listed in Table 1) were analysed as to their chemical composition (Table 8), in particular polysaccharide content and sugar composition (Table 9). The lignin content of forest chips is rather uniform within their main botanic groups (hardwoods and softwoods). For hardwoods, the lignin content varies from 20.4 to 25.9%, which is a normal variation range. For softwoods, the four samples analysed show a lignin content between 27.1 and 28.3%, only one sample of residuals from the final harvesting of softwoods presenting a value of 34.2%. However, as to the extractive content, the situation is quite different.

A common observation for both softwood and hardwood forest chips was the relatively higher than usual extractive content in several samples. In most cases, the wood species can explain these values, for example for oak, chestnut or horbean, while, for the only softwoods presenting a very high

extractive content (Maritime Pine), the explanation is that this vegetal material was only 2 years old and, consequently, it presented the same characteristics as those of hardwood S(VRC).

Table 9
Sugar composition of the polysaccharide fraction of different hardwood and softwood forest chips

Wood species	Glucose content (%)	Mannose content (%)	Galactose content (%)	Arabinose content (%)	Xylose content (%)
Hardwoods					
Hornbean	65.47	1.04	1.77	4.45	27.28
Mixed hardwoods 1	77.16	3.71	2.24	2.06	14.82
Mixed hardwoods 2	69.87	1.00	1.53	1.23	26.39
Oak	65.65	0.89	2.15	3.66	27.65
Oak/chestnut 1	75.91	1.97	1.56	0.47	20.09
Oak/chestnut 2	74.40	1.94	1.70	0.99	20.97
Poplar 1	80.52	2.65	0.92	0.81	15.10
Poplar 2	71.42	2.24	1.39	1.28	23.67
Poplar 3	78.00	1.45	1.03	1.07	18.46
Poplar 4	73.05	2.14	0.99	1.43	22.38
Softwoods					
Douglas-fir	70.82	15.62	4.81	1.72	7.02
Maritime Pine	67.64	13.78	5.08	4.79	8.70
Mixed softwoods 1	67.10	13.23	5.32	3.78	10.56
Mixed softwoods 2	72.60	13.70	2.38	1.66	9.66
Mixed softwoods 3	68.90	13.99	4.62	3.63	8.85

The consequence of lignin and extractive content fluctuations is the variation in the polysaccharide content between 62.3 and 74.0% for hardwood, and between 55.5 and 67.9% for the softwood forest chips here analysed. The gap between the extreme values of hexose content for softwoods and hardwoods is also impressive. For hardwoods, the variation is from 42.8 (whole tree oak) to 61.2% (poplar, final harvesting). The glucose content varies from 65.5% for hornbeam and whole tree oak chips to 8% for chips produced from the final harvesting of poplar top trees. For softwoods, the range of hexose variation lies between 48 (2 y/o Maritime Pine) to 62% (Douglas-fir). The main difference here is caused by an important presence of mannose, the content of which is up to 15.6% for Douglas-fir forest chips, besides glucose. This latter sugar presents a relatively uniform variation (67.1 to 72.6%). Generally speaking, the maximum values for the hexose content of both softwoods and hardwoods appear at the same level of the vegetal material analysed in the previous sections. However, the minimum values are considerably lower than those observed for all samples used in this study, which suggests that non-wood components – such as bark or leaves – or even accidental soil contamination that may occur during the production of forest chips reduces the potential of hexoses released for bioethanol production.

The pentose content is also an important variable, in particular for hardwoods: from 11.1 for the whole tree mixed hardwoods to 22.3% for the whole tree hornbeam. The most important sugar is xylose with proportions reaching 27.0% for two samples (hornbeam and oak). These two samples also present high values of the arabinose content, suggesting an important presence of arabinoxylan-type hemicelluloses in these wood species. Softwood forest chips present a considerably lower pentose content than the hardwood ones, confirming the observations of the first series. These results underline the needs of upgrading pentoses for bioethanol production, especially from hardwoods.

Overview of forestry biomass quality for bioethanol production

For the sake of comparison, the different series here analysed were pooled in six groups: softwood unbarked wood, hardwood unbarked wood, SRC, VSRC, softwood forest chips and hardwood forest chips. The average, minimum and maximum values, as well as the standard deviation are graphically presented in Figure 2 – for lignin and

extractive content – and in Figure 3 for hexose and pentose contents of different classes.

On the average, the unbarked wood of softwood and hardwood species presents similar values for the extractive content. However, a much higher scattering among hardwood data was observed, as indicated by the high standard error bars and by the extreme values. Some wood species, in particular those containing high polyphenolic substances in their extractives, such as chestnut and oak, contribute to this behaviour. On the other hand, when other non-wood components are present, as in the case of the forest wood chips, the average and standard errors of the extractive content increase remarkably, as due, in part, to the presence of easily extractible organic materials, such as bark, leaves or needles. Some soil contamination is probably also responsible for the high extractive content, especially for residual wood. SRC and VSRC present a very high content of extractives, compared to mature wood or even forest chips. Data scattering is more important for VSRC, as shown by the standard error bars and extreme values. The high proportion of non-wood to wood fraction and the important physiological activity during the first years of growth are the main hypotheses explaining this behaviour.

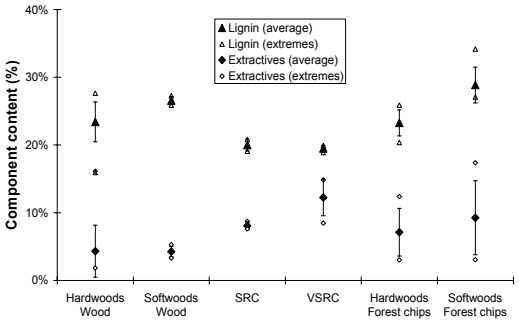

Figure 2: Variability of lignin and extractive contents for different classes of wood samples analysed in this work. Average, minimum and maximum values, and standard deviation are presented

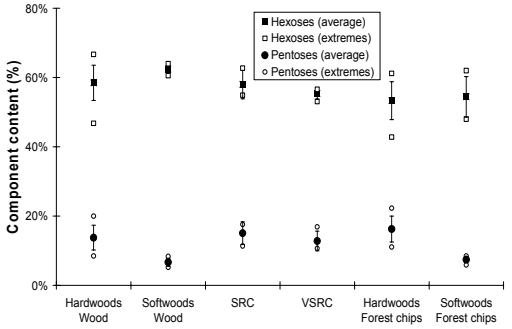

Figure 3: Variability of hexose and pentose contents for different classes of wood samples analysed in this work. Average, minimum and maximum values and standard deviation are presented

The average lignin content of the unbarked wood of softwood species is 3% higher than that of hardwoods, as described for some wood species.[12,13] The observations for standard deviation and extreme values are similar to those already mentioned for the extractive content, *i.e*. a much higher scattering was observed for hardwood than for softwood species. For forest wood chips, the average values are only slightly affected when non-wood fractions are present, but the values are very spread, especially for softwoods. Finally, SRC and VRSC are surprisingly less lignified than mature wood, presenting relatively low scattered data.

The balance between hexoses and pentoses is affected by the more or less important presence of non-polysaccharide fractions, wood species and age. Thus, unbarked softwood is by far the best type of wooden material here studied as to its hexose content. On the average, it is 4% higher than in unbarked hardwood species. However, individually, some hardwood species could have higher hexose values, as shown by both high standard deviation and extreme values. Some eucalyptus and poplar clones are the main candidates for the production of high hexose content wooden materials. SRC presents only a slightly lower average hexose content compared to mature wood. For VSRC, the differences are more remarkable. On the average, VRSC is by 3% less rich in hexoses, compared to SRC and mature wood, but still more significantly if considered individually (- 6% for poplar, for example). Finally, the gap between mature unbarked wood and forest chips is, on the average, 5% for hardwoods and 8% for softwoods.

Hardwoods contain at least twice the pentose content of softwoods. The unbarked wood of hardwood species present, on the

average, around 14% pentoses, yet standard deviation is quite high (± 3.5%). The average values for SRC and forest chips are, respectively, 2 and 3% higher than those of mature wood, while VRSC presents 1% lower weight. One may thus assume that the non-wood fractions present in SRC and forest chips contain different types of hemicelluloses and contribute to increasing the C5 content. Beyond the important difference observed in comparison with hardwoods, unbarked softwoods present, on the average, an almost 2% lower pentose content than that of softwood forest chips.

CONCLUSIONS

Second-generation bioethanol is produced from the fermentation of sugars released by the hydrolysis of biomass polysaccharides. The hexoses and pentoses released can be transformed into bioethanol by different fermentation pathways. Thus, the ratio of the different sugars present in wood is a very important quality parameter for such an utilisation. The intrinsic variability is a major factor to be taken into account for the wood supply of second-generation bioethanol processes.

Different sources of variability in the hexose and pentose contents were studied, and six classes of wooden material were analysed: unbarked softwoods, unbarked hardwoods, SRC, VSRC, hardwood forest chips and softwood forest chips.

High hexose contents are found mainly in unbarked softwood, which presented up to 4% more C6 sugars than the unbarked hardwoods. However, individually, some hardwood species present very higher hexose content values, for example some eucalyptus and poplar clones. Short rotation coppices present only a slightly lower average content of hexoses compared to mature wood, while very short rotation coppices are less rich is hexoses, by 3% on the average. The hexose content of forest chips is considerably affected by the non-wood fractions. On the average, the gap between mature unbarked wood and forest chips is of 5% for hardwoods, and of 8%, respectively, for softwoods.

If the upgrading of pentoses through fermentation is possible, hardwoods seem to present a higher potential, as the presence of C5 sugars is much more important than in softwoods.

An important part of the differences in the hexose and pentose contents between the different wood samples here analysed can be explained by the lignin and extractive contents. Unbarked softwoods are more lignified than hardwoods, by 3% on the average. This difference is still more important for forest chips. SRC and VSRC samples presented by 3% less lignin than their corresponding mature wood. On the other hand, a high extractive content was obtained for forest chips, SRC and VSRC samples. Also, some particular wood species present a very high extractive content even for unbarked wood, such as chestnut and oak.

Obviously, the amount of bioethanol effectively produced from different wooden materials does not depend only on their original content of hexoses or pentoses. Other factors, not studied here, such as the fibre ultrastructure of different wood species and tissues, cellulose crystallinity, type of hemicelluloses and their behaviour during hydrolysis, the content of fermentation inhibiting compounds, among others, contribute to the different steps of bioethanol production. This work needs to be completed with a more systematic approach coupling the intrinsic variability of wood with different steps of the processes, to properly establish the potential of wooden biomass for bioethanol production.

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