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COMPARISON OF AGRICULTURAL AND FOREST BIOMASS WITH REGARDS TO BIOLOGICAL PROCESSES FOR BIOETHANOL PRODUCTION OF SECOND GENERATION

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ABSTRACT: This study presents the comparison between agricultural and forest resources related to the needs of biochemical processes. Recommendations and exchanges with future users (IFP, ARD) were used in this study. 234 samples from two years (2007-2008 and 2008-2009) were selected and analysed. Various species were analysed for their chemical composition, especially the content of fermentable sugars. Then the results were shown for homogeneous species groups: annuals immature, annuals mature, forage grasses, perennials harvested green in the fall and dry in late winter, hardwood and softwoods forest woodchips, short and very short rotation coppice. Common methodologies in the analysis and a database of samples for agriculture and forestry were used. The levels of hemicellulose were in the same range for both agricultural and forest biomass between 15 and 25% d.m. Regarding cellulose content, three groups could be distinguished: summer annuals and forage grasses (25 to 30 %), perennials and forest hardwoods and softwoods chips (38 to 46 %) and finally SRC and VSRC (52 to 54 %). In general, forest biomass contained more lignin than agricultural biomass: 20 % d.m. to 30 % d.m. while for agricultural biomass, values ranged from 8 to 20 %. Perennial crops contained more lignin than annual, between 15 and 20 % d.m. The agricultural biomass contained more C_5 sugar and less C_6 sugar, than forest biomass with the exception of perennials harvested dry in late winter.

Keywords: bioethanol, agriculture, energy crops, short rotation forestry, forest residues, sugar

1 INTRODUCTION

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. In this case, bioethanol is obtained by the hydrolysis of the polysaccharides fraction from the vegetal biomass in monosaccharides prior the fermentation [1].

The major chemical organic components of biomass can be classified as polysaccharides (cellulose, hemicelluloses and, sometimes, pectins), lignin and extractives, and for some agricultural biomass, starch and soluble sugars. Inorganic species are also present and are often reported as "ash" content.

The production of ethanol from lignocellulosic raw materials can be summarized as follows [2]: a) opening the ultra-structure of the cell wall to access the polymer chains of cellulose and hemicellulose by different pre-treatments; b) hydrolysing the polysaccharides into sugar monomer syrup; c) fermenting the sugars to ethanol solution (mash) by microorganisms; d) distilling and dehydrating ethanol.

Different pre-treatments used to expose the polysaccharides to the action of enzymatic or acidic hydrolysis [3]: physical (comminution, irradiation, extrusion, expansion, etc), physico-chemical (hydrothermolysis, steam explosion, acids, alkali, gazes, oxidant, polysaccharides solvents, and delignification agents) or biological (fungi).

The first pilot productions of the second generation biofuels are currently being developed in France. However, there is a lack of references concerning the criteria of biomass quality.

Within the French project "REGIX" (2005-2009), an experimental network on both agriculture and forestry biomasses has been established. The biomass samples composition produced in this network was evaluated.

The objectives of REGIX were i) to establish a reference for the composition of different kind of biomass and ii) to identify the raw materials best suited to the production process of second-generation biofuels by thermochemical conversion.

This article evaluates the potential of different biomass for the production of second generation bioethanol through the fermentation of sugars released after hydrolysis of polysaccharides.

2 METHODOLOGY

2.1 Vegetal material

234 samples from the two campaigns analysis (2007-2008 and 2008-2009) were selected with 133 agricultural samples and 101 forestry samples. The distribution of samples per species is shown here below.

2.2.1 Agricultural resource

5 types of biomass have been selected due to their agronomic performance and the results of compositional analysis. Overall, the groups were adjusted with harvest stages including annuals and perennials:

- ✓ **annuals immature** composed of 27 samples of sorghum, maize and triticale (**Ai** noted later);
- ✓ **annuals mature** composed of 23 samples of sorghum, maize and triticale at a later stage with mostly the appearance of grain (denoted **Am** thereafter);
- ✓ **forage grasses** composed of 20 samples of fescue and brome (**Fg** noted later). Alfalfa, was the only dicotyledonous forage in the network but has an

atypical behaviour during chemical analysis (high nitrogen and ash). It was therefore decided to exclude comparative analysis with the forest resource.

- ✓ **perennials harvested green in the fall** with 23 samples of miscanthus, switchgrass and giant cane , Arundo donax L (**Pg** noted later);
- ✓ **perennials harvested dry in late winter** with 40 samples of miscanthus, switchgrass and giant cane, Arundo donax L (**Pd** noted later).

2.1.2. Forestry resource

4 types of biomass have been identified:

- ✓ **hardwood forest woodchips** with 26 samples (denoted **Wh** thereafter);
- ✓ **coniferous forest woodchips** with 9 samples (denoted **Wc** thereafter);
- ✓ **short rotation coppice** (less than 5 years) with 18 samples (denoted **SRC** thereafter);
- ✓ **very short rotation coppice** with 48 samples (rated **VSRC** thereafter)

2.2 Analytical methods

The biomass samples were ground for the production of particles measuring less than 0.5 mm for the chemical analysis. Prior to lignin and polysaccharides analysis, the wooden 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 static mode at 110 °C. The acetone extraction cycle consisted of a heating period of 5 min, followed by a twofold, 10 min extraction in static mode, at 95 °C. Agricultural biomass samples were extracted using the first step of the NDF analytical method.

The lignin content was then measured by the Klason method. Monosaccharides content was determined from ionic liquid chromatography after acidic hydrolysis of the polysaccharides. Monosugar analysis was carried out after two-step acidic hydrolysis of wood and pulps, by the ASTM method E1758 - 01(2007). Quantification of neutral monosaccharides was obtained on a DIONEX HPAE-PAD ion chromatograph equipped with a pulsed amperometric detector. From monosugars (hexoses and pentoses) analysis, the corresponding polysaccharides were calculated, following the procedure described by Genco *et al*. [4] :

- ✓ Celluloses = (Glucan-Mannan/ b)/sample weight
  For hardwoods, b = 1.6, for softwoods b = 4.15
- ✓ Hemicelluloses = Total Sugar/sample weight – cellulose

For agricultural samples, no correction of glucan/mannan was made.

2.3. Graphical representation of the results: boxplots

The box plot, a translation of Box & Whiskers Plot, is an invention of Tukey (1977) to represent schematically the distribution of a variable. The box plot uses 5 values that summarize the data: the minimum, the 3 quartiles Q1, Q2 (median), Q3, and maximum.

Comparisons between agricultural and forest biomasses were performed by using this graphical representation and with the following criteria: lignin, cellulose, hemicellulose, sugars, C5 and C6.

3 RESULTS AND DISCUSSIONS

When considering biomass as feedstock for biochemical transformation, the most important quality criteria are:

- ✓ monosaccharide composition : the higher the hexoses content, the better for fermentation purposes. Because pentoses are hardly fermentable, their content is wanted to be the lowest possible
- ✓ Consistently with the hexose content, the higher is the cellulose content: the higher and more digestible fraction into fermentescible monosaccharides
- ✓ hemicelluloses content : the lowest, especially if rich in pentoses, because these sugars are hard to ferment, and to minimize the formation of fermentation inhibitors: intrinsic inhibitors (acetyl residues of hemicellulose precursor of acetic acid, pentoses may give rise to furfural)
- ✓ lignin content : low, but enough to furnish energy for the process. Lignin is however the main factor dictating pretreatment severity and inbihit polysaccharides deconstructing enzymes
- ✓ low content of components that increase the osmotic pressure (salts, amino acids, sugars unusable ...) and inhibitors of yeast: elicitors (SDN) , phytosanitary residues (antimicrobians ...), etc…

However determination of cellulose and digestibility of the raw biomass can not fully anticipate the quality of the biomass. The pre-treatment can greatly improve the accessibility of cellulose and several methods exist (physico-chemical, solvent, high pressure-high temperature extraction, acid catalysts, basic catalysts ...).

3.1. Comparison of C_5 and C_6 sugars content from different biomasses and adequacy with processes

The production of biofuels by biochemical processing of the sugars requires that biomass contains more C_6 sugars than C_5 (Figure 1). This observation is valid (a higher C_6 content than the content of C_5) for agricultural and forest biomass.

A general trend is that forestry biomass presents higher amount of C6 sugars than agricultural. The difference between forestry biomass and annual plants is of almost 30 % d.m.. However, perennial crops present the same level of hexoses than wooden biomass. Annual mature and immature plants and forage grasses present considerably low levels of hexoses, but they compensate this behaviour by the presence of starch and soluble sugars.

Concerning pentoses, agricultural crops present again higher content when compared to forestry biomass. Perennial cultures, especially harvest dried, are considerably rich in pentoses. For wooden biomass, softwoods chips present much lower content of pentoses when compared to hardwoods either harvested as (very) short rotation coppices or forest chips.

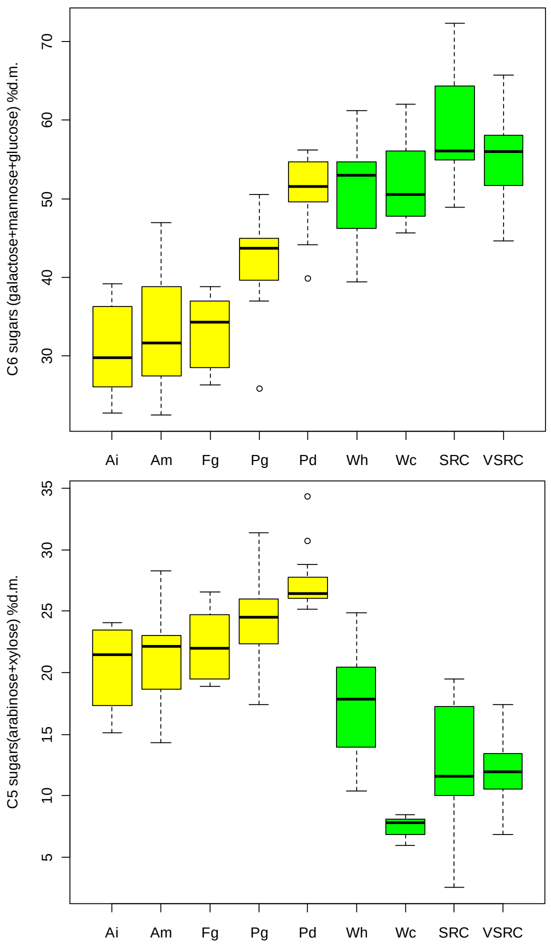


Figure 1 : Boxplot of the levels of C5 and C6 sugars (% total d.m.) for agricultural and forest biomass

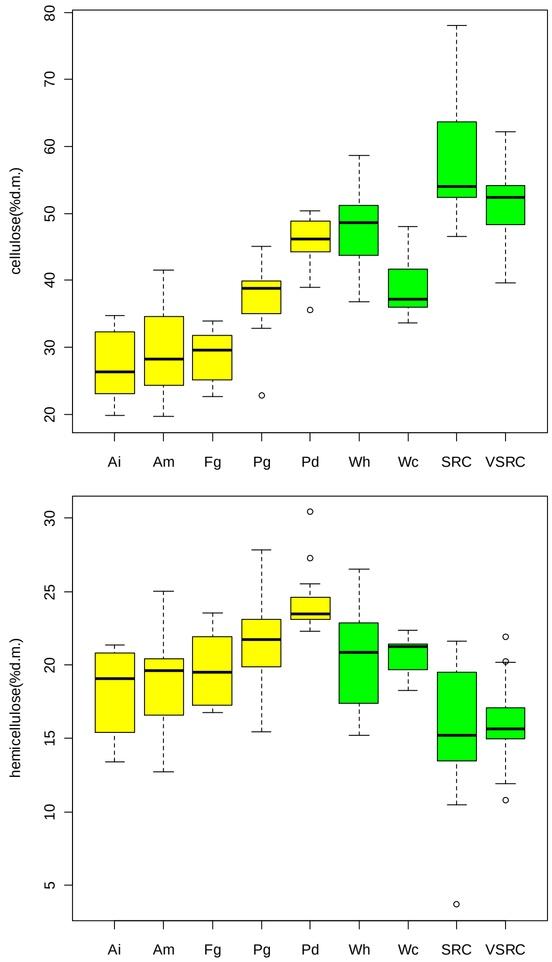


Figure 2 : Boxplot of the levels of cellulose and hemicellulose (% total d.m.) for agricultural and forest biomass

Using the conversion equations mentioned in the Methodology section, cellulose and hemicellulose content were calculated and are presented in Figure 2.

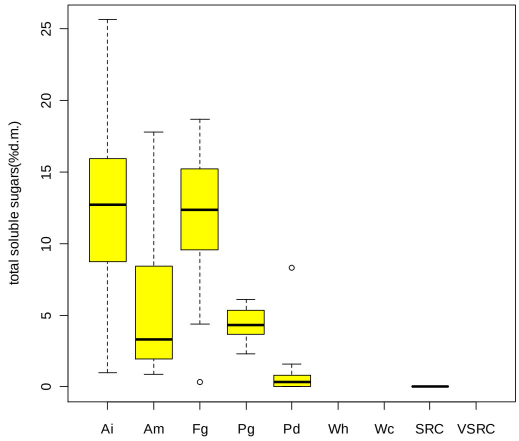
For cellulose, there are three distinct groups: summer annuals and grasses, perennials and woodchips forest (hardwood and coniferous) and finally the SRC and VSRC. The levels of hemicellulose are in the same range between agricultural and forest biomass between 15 and 25% d.m.

The hemicelluloses can be easily extracted during pre-treatment but will be difficult to convert into ethanol because of weak fermentescibility of pentoses. The residual cellulose is difficult to hydrolyze.

3.2. Soluble sugars and starch

Only agricultural biomasses contain soluble sugars (up to 13 % d.m. for annuals immature and forage grasses) and starch (up to 27 % d.m. for annuals mature).

Starch and soluble sugars were very dependent on the stage of crop plant (Figure 3).



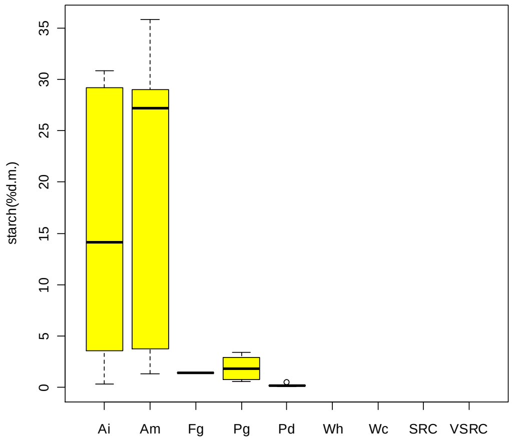


Figure 3 : Boxplot of the levels of total soluble sugars and starch (% total d.m.) for agricultural and forest biomass

3.3. Comparison of lignin content from different biomasses

The lignin content of agricultural and forest biomass is shown in Figure 4. Forest biomass contains more lignin: 20 % d.m. to about 30 % d.m. for woodchips coniferous forest. For agricultural biomass, perennial plants contain more lignin whose content ranged 15 - 20 % d.m.

For biochemical processes, the presence of lignin is problematic Lignin restricts the accessibility of enzymes to cellulose. In addition, pretreatment that aim at delignification may generate lignin degradation products which can inhibit enzymes action and fermentation On the other hand, the energy content of lignin is important for the process, mainly for the concentration of ethanol after fermentation. In case of overproduction or lignin, others ways of adding value can be considered: especially as source of molecules of new materials.

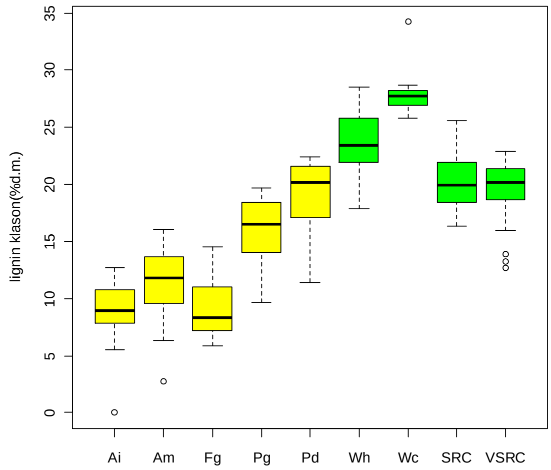


Figure 4 : Boxplot of the lignin content (% total d.m.) for agricultural and forest biomass

4 CONCLUSIONS

This work allowed improving our knowledge on the composition of a large range of biomass which could be grown in France for bioethanol production**.** The sugar content and composition significantly vary according to the species.

The highest contents in the most easily fermentable components (C6 monomers) were observed in the forestry products, (very) short rotation coppices ranking the best, and then perennial crops harvested in late winter. C5 monomers are predominantly present in agricultural biomass, but important content is also observed for hardwoods.

High amounts of lignin, especially in the woody biomass, could be problematic due to the reduction of the accessibility of enzymes to cellulose and lignin degradation products during the physicochemical pretreatments stages.

Finally, the high content of ash of some biomasses would have also to be taken into account because it could have an impact into the fermentation steps. To conclude, the biomass composition will be a key point to be considered while building the supply chain of the future 2G bioethanol plants in France but it will have to be integrated with other crucial aspects such as the production performances and the environmental impact of the potential biomasses.

5 ACKNOWLEDGEMENTS

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