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Comparative Study on Chemical Composition of Various Biomass Species

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In this study, the chemical composition of 32 samples coming from 29 different biomass species including a gymnosperm, 2 dicotyledonous angiosperms, 17 monocotyledonous angiosperms and 9 algae species was successfully determined using an established method applicable to analyze various biomass species. The obtained data allowed a direct comparison of the biomass in their chemical composition. It was, thus, revealed that although the chemical composition differed from one species to another, and even from different parts of the same plants, similar trends were found in the composition of biomass species belonging to the same taxonomic group. Based on those results, it was clarified that the chemical composition of a biomass sample is related to its taxonomy. Therefore, typical chemical composition for each taxonomic group was proposed and the potential of each group for different biorefinery platforms could be successfully established.

1. Introduction

Increasing energy security and mitigating climate change require the substitution of petroleum-based and nuclear-based productions to fulfill human needs for energy, chemicals and materials. One important supply of renewable resource for energy, chemicals and materials is biomass and it became a worldwide goal to develop biobased products through integrated biorefinery systems.

Biomass consists of all living organisms and their wastes. The world annual primary production of biomass was evaluated by Whittaker to be 1,855 (10^9) dried tons.[3] In 2009, the United Nations Environment Programme (UNEP) reported that 140 (10^9) tons of biomass is generated every year from agricultural land,[4] equivalent to approximately 50 billion tons of fossil oil.[5] The large quantities of available biomass all over the world can, therefore, be converted to a massive amount of energy and raw materials.

For the best use of those feedstocks, their chemical composition is known to be a key factor. Indeed, chemical composition is a unique fundamental code which characterizes and determines the properties, quality, potential applications and environmental problems related to any applications. Therefore, one of the fundamental aspects related to biomass use is to extend and improve the basic knowledge on composition and properties of biomass.[6]

Chemical composition of biomass is a formidable challenge due to the various origins, forms and complexity of the materials, as can be seen in Fig. 1 which shows a simplified evolution and diversity of various biomass species. Data on chemical composition for biomass varieties have been reported in almost all studies on biomass transformation. As a result, a huge amount of data exists and some similarities or differences in common chemical characteristics based on literature review were reported.[7] Additionally, a database gathering a multitude of studies which report chemical composition and properties of different feedstocks was settled by the US Department of Energy.[8] Despite such available information, comparison of the different results from literature review would be erroneous since the analytical methods and basis differ from an author to another and from a biomass species to another.
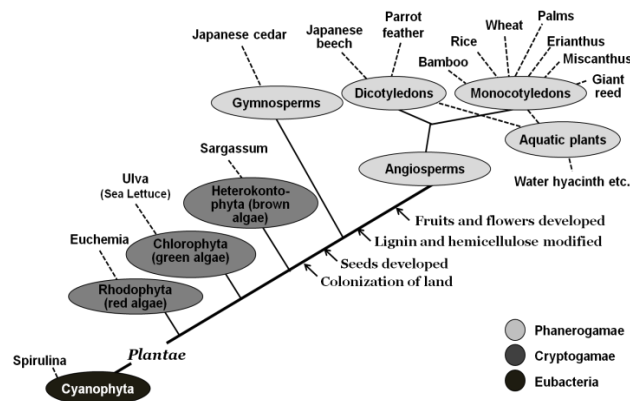

Fig. 1 Simplified evolution and diversity of various biomass species[1,2]

Woody biomass were analyzed according to wood analytical procedures,[9-11] whereas agricultural residues, grasses species[12] and algae have been mostly studied using feed and forage analytical procedures.[13-15] Terms and definition of the chemical components are also different between the methods: wood analytical method determines cellulose, hemicellulose and lignin, whereas feed and forage analytical procedures result in neutral detergent fiber, acid detergent fiber and crude fiber.

For those reasons, there exists a problem of consistency when comparing the data from the literature. Furthermore, direct comparisons of different biomass species such as wood, herbaceous, aquatic plants and marine algae in their chemical composition are quite few at present.

Such issues show that additional works on detailed comparisons of biomass in their chemical composition, under the same method and basis is absolutely needed. On this line of study, an analytical methodology applicable to characterize various biomass species in their chemical composition was established.[16] Thus, the purpose of the present work was to elucidate the

chemical composition of a wide range of biomass species, using the same method and basis. The chemical components were

Table 1 Various biomass samples studied in this work

Kingdom	Subkingdom	Taxonomy			Scientific name	Common name	Sample No.	Sampling time	Sampling site
		Division	Class	Family					
Plantae	Phanerogamae	Gymnosperm		Cupressaceae	*Cryptomeria japonica*	Japanese cedar	1	12/2000	Kyoto, Japan
		Angiosperm	Dicotyledon	Fagaceae	*Fagus crenata*	Japanese beech	2	11/2000	Kyoto, Japan
				Haloragaceae	*Myriophyllum aquaticum* (aquatic plant)	Parrot feather	3	04/2010	Moriyama, Shiga, Japan
			Monocotyledon	Poaceae (Gramineae)	*Phyllostachys heterocycla*	Bamboo	4	06/2008	Kyoto, Japan
					Oryza sativa	Rice straw	5	10/2007	Aichi, Japan
						Rice husk	6		
					Triticum aestivum	Wheat straw	7	08/2007	Aichi, Japan
					Zea mays	Corn leaves	8	08/2010	Aomori, Japan
						Corn cob	9		
					Erianthus arundinaceus	Erianthus	10	09/2009	Nakhon Si Thammarat, Thailand
					Miscanthus sinensis	Miscanthus	11	09/2009	Kyoto, Japan
					Saccharum officinarum	Bagasse	12	04/2008	Okinawa, Japan
						Sugarcane leaves	13		
					Phragmites australis	Common reed	14	04/2011	Omihachiman, Shiga , Japan
					Arundo donax	Giant reed	15	05/2011	Seattle, USA
				Aracaceae (Palmae)	*Elaeis guineensis*	Oil palm trunk	16	08/2008	Johor Bahru, Malaysia
					Nypa fruticans	Nipa frond	17	04/2009	Nakhon Si Thammarat, Thailand
					Borassus flabellifer	Sugar palm frond	18	07/2009	Nakhon Si Thammarat, Thailand
				Pontederiaceae (aquatic plants)	*Eichhornia crassipes*	Water hyacinth	19	10/2011	Moriyama, Shiga, Japan
				Potamogetonaceae (aquatic plants)	*Potamogeton maackianus*	Sennin-mo	20	07/2010	Otsu, Shiga, Japan
				Hydrocharitaceae (aquatic plants)	*Egeria densa*	Okanada-mo	21	07/2010	Otsu, Shiga, Japan
					Hydrilla verticillata	Kuro-mo	22	07/2010	Moriyama, Shiga, Japan
					Elodea nuttallii	Kokanada-mo	23	07/2010	Moriyama, Shiga, Japan
	Cryptogamae	Heterokontophyta (brown algae)	Phaeophyceae	Sargassaceae	*Sargassum horneri*	Akamoku	24	07/2010	Kyoto, Japan
					Sargassum sp.	Sargassum	25	01/2011	Oki Island, Shimane, Japan
		Chlorophyta (green algae)	Ulvophyceae	Ulvaceae	*Ulva lactuca*	Sea lettuce	26	08/2010	Nagasaki, Japan
					Chladophora sp.	Chladophora	27	04/2011	Tuléar, Madagascar
			Bryopsidophyceae	Caulerpaceae	*Caulerpa taxifolia*	Caulerpa	28	12/2010	Okinawa, Japan
					Caulerpa lentillifera	Sea grape	29	08/2010	Okinawa, Japan
			Trebouxiophyceae	Chlorellaceae	*Chlorella vulgaris*	Chlorella	30	08/2010	Fukuoka, Japan
		Rhodophyta (red algae)	Rhodophyceae	Solieraceae	*Eucheumia* sp.	Euchemia	31	04/2011	Tuléar, Madagascar
Bacteria	Eubacteria	Cyanophyta (blue-green algae)	Cyanophyceae	Pseudanabaenaceae	*Spirulina* sp.	Spirulina	32	04/2011	Tuléar, Madagascar

quantitatively investigated for comparison among different biomass species.

2. Materials and methods

2.1 Plant materials

Table 1 shows the taxonomy of the biomass samples studied in this work, and their sampling details are also provided. As gymnosperm species, Japanese cedar (*Cryptomeria japonica*) was selected to be one of the representative one in Japan, while Japanese beech (*Fagus crenata*) was selected as one of the dicotyledonous angiosperms, together with parrot feather (*Myriophyllum aquaticum*). Furthermore, Gramineae monocotyledonous angiosperms were selected such as bamboo (*Phyllostachys pubescens*), rice straw and husk (*Oryza sativa*), wheat straw (*Triticum aestivum*), corn leaves and cob (*Zea mays*), erianthus (*Erianthus arundinaceus*), miscanthus (*Miscanthus sinensis*), sugarcane bagasse and leaves (*Saccharum officinarum*), common reed (*Phragmites australis*), giant reed (*Arundo donax*). In addition, palm monocotyledonous angiosperms such as oil palm (*Elaeis guineensis*), nipa palm (*Nypa fruticans*) and sugar palm (*Borassus flabellifer*) were studied.

Besides, monocotyledonous invasive aquatic plants such as water hyacinth (*Eichhornia crassipes*), sennin-mo (*Potamogeton maackianus*), okanada-mo (*Egeria densa*), kuro-mo (*Hydrilla verticillata*), as well as kokanada-mo (*Elodea nuttallii*) were collected from Lake Biwa in Japan. Also, algae species such as akamoku (*Sargassum horneri*), sargassum (*Sargassum* sp.), sea lettuce (*Ulva lactuca*), chladophora (*Chladophora* sp.), caulerpa (*Caulerpa taxifolia*), sea grape (*Caulerpa lentillifera*), chlorella (*Chlorella vulgaris*), euchemia (*Euchemia* sp.), spirulina (*Spirulina* sp.) were collected from different places all over the world as detailed in Table 1, to be analyzed and compared in their chemical composition.

2.2 Analytical methods for the determination of chemical composition and calorific value

These collected samples were air-dried, milled with a Wiley mill (1029-C, Yoshida Seisakusho Co., Ltd.), and sieved to retain particles of 150-500 µm in size (30-100 mesh).

The samples were, then, oven-dried and the summative chemical composition was determined according to the method detailed in Fig. 2.[16] In brief, ash was determined after incineration of the oven-dried samples at 600°C for 4h. For additional analyses, the samples were extracted with acetone. On the extractives-free samples, holocellulose and lignin were, respectively, determined by modified Wise method[17] and modified Klason method.[18] Both were then ash- and protein-corrected by subtracting ash and protein contents of the residues from the total residues yielded. Holocellulose was additionally corrected for its residual lignin content.[17] Cellulose content was determined as α-cellulose by extraction with 17.5 % aqueous sodium hydroxide of the holocellulose powder [19] and hemicellulose content was evaluated by difference between the corrected holocellulose and cellulose contents.

Additionally, monosaccharides composition was determined by a combined method. [20] Glucose was quantified as hydrolysate from the Klason lignin procedure with 72 % and subsequent 3%

H_2SO_4 treatments,[18] then analyzed with high-performance anion-exchange chromatography (HPEAC, Dionex ICS-3000 system) equipped with CarboPac PA-1 column (4mm x 250mm), whereas the other neutral sugars and the uronic acids by acid methanolysis method[21] with slight modifications. In brief, 2 ml of 2 M HCl in anhydrous methanol was poured to 10 mg of extractive-free sample for methanolysis at 100 °C for 3 h. Afterwards, the sample was cooled and 100 µl was pipetted into a 1 ml glass microtube which was, then, put into a desiccator and vacuumed overnight to remove the remaining methanol and HCl. When the sample was completely dry, 500 µl of pure deionized water was added. The resulting solution was diluted, if necessary, filtered with 0.45 µm Millipore filter, and finally analyzed with high-performance anion-exchange chromatography (HPAEC) using CarboPac PA1 as column. As for xylose, the higher value among the results from sulphuric acid hydrolysis and acid methanolysis was taken.

Furthermore, in order to determine the phenylpropane units of lignin, alkaline nitrobenzene oxidation was performed on the extractives-free samples[22] with slight modifications, where the oxidized products were silylated using trimethylchlorosilane (TMCS), bis(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine in a volumetric ratio of 2:1:7 and analyzed by gas chromatography with veratraldehyde as an internal standard.

In addition, starch and protein determinations from extractives-free samples were, respectively, completed by perchloric acid method[23] and Kjeldahl nitrogen method by using a nitrogen factor of 6.25.[24]
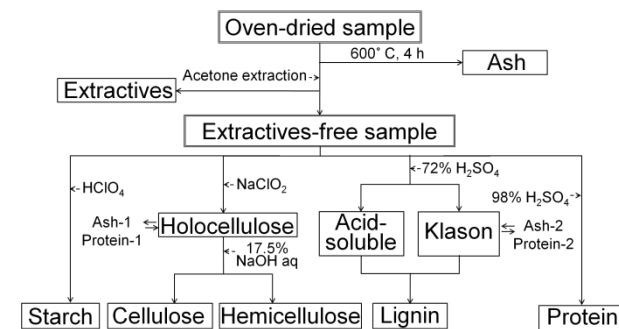

Fig. 2 Analytical method applied to various biomass samples to quantify their chemical composition[16]

The calorific values of the various biomass species were determined as their higher heating values (HHV) after complete combustion using an auto-calculating bomb calorimeter CA-4AJ, Shimadzu with a base temperature of 15°C.

2.3 Chemometrics

Principal component analysis method (PCA) was used to plot the 32 analyzed samples according to their 7 chemical components which are cellulose, hemicellulose, lignin, protein, extractives, starch and ash. The software used for such multivariate analysis was GNU Octave.

Table 2 Chemical composition and calorific value of the various biomass samples studied

Family	Sample No.	Biomass	Chemical composition (g/kg of the oven-dried biomass basis)									Calorific value (MJ/kg)
			Cellulose[a]	Hemi-cellulose[b]	Lignin		Protein	Extractives	Starch	Ash	Total	
					Klason	Acid-soluble						
Cupressaceae (softwood)	1	Japanese cedar	379	227	328	3	5	34	1	3	980	19.35
Fagaceae (hardwood)	2	Japanese beech	439	284	210	30	6	19	5	6	999	20.62
Haloragaceae	3	Parrot feather (aquatic plant)	263	194	146	29	162	53	2	112	961	18.28
Poaceae	4	Bamboo	394	311	193	13	18	38	11	12	990	20.32
(Gramineae)	5	Rice straw	345	218	184	18	47	45	9	133	999	16.74
	6	Rice husk	360	173	228	13	16	13	2	168	973	17.44
	7	Wheat straw	371	340	180	20	13	22	5	37	988	18.86
	8	Corn leaves	268	248	132	19	165	51	3	109	995	20.07
	9	Corn cob	343	328	151	29	58	28	19	35	991	19.10
	10	Erianthus	378	252	234	20	12	27	4	39	966	18.79
	11	Miscanthus	337	248	183	40	83	28	5	54	978	20.03
	12	Bagasse	383	309	209	15	17	35	2	20	990	-
	13	Sugarcane leaves	331	265	179	18	34	75	12	50	964	-
	14	Common reed	344	284	185	17	26	26	3	86	971	19.32
	15	Giant reed	416	242	225	24	11	33	4	32	987	20.06
Aracaceae (Palmae)	16	Oil palm trunk	306	284	243	39	6	36	29	41	984	19.11
	17	Nipa frond	324	291	179	17	23	19	8	105	966	19.79
	18	Sugar palm frond	317	313	191	18	30	45	10	50	974	18.27
Pontederiaceae (aquatic plants)	19	Water hyacinth	185	293	84	17	210	24	12	174	999	16.44
Potamogetonaceae (aquatic plants)	20	Sennin-mo	347	88	131	18	229	40	20	105	978	18.65
Hydrocharitaceae (aquatic plants)	21	Okanada-mo	262	181	54	17	226	26	16	201	983	16.44
	22	Kuro-mo	227	141	64	15	228	67	18	223	983	16.22
	23	Kokanada-mo	436	93	61	15	137	52	23	158	975	15.58
Sargassaceae	24	Akamoku	53	266	129	13	180	96	5	229	971	14.49
(brown algae)	25	Sargassum	203	428	60	13	96	19	1	171	991	14.02
Ulvaceae	26	Sea lettuce	80	421	30	3	120	41	7	257	959	15.88
(green algae)	27	Chladophora	49	339	0	0	108	2	71	402	971	11.46
Caulerpaceae	28	Caulerpa	102	354	29	8	261	65	8	167	994	16.28
(green algae)	29	Sea grape	56	207	21	5	146	54	3	500	992	8.47
Chlorellaceae (green algae)	30	Chlorella	50	424	0	0	423	9	1	76	983	23.48
Solieraceae (red algae)	31	Euchemia	60	660	11	7	75	10	10	150	983	12.50
Pseudanabaenaceae (blue-green algae)	32	Spirulina	0	296	0	0	502	30	1	144	973	19.52

[a] Cellulose = α-Cellulose
[b] Hemicellulose = Holocellulose - α-Cellulose   (Holocellulose was ash, lignin and protein-corrected)

3. Results and discussion

3.1 Chemical composition of various biomass species

Table 2 shows the chemical composition of various biomass species, based on g/kg of the oven-dried biomass basis. Chemical composition varies greatly among the analyzed biomass samples. Woody biomass samples ranked among the highest in the cellulose content with 379 g/kg for Japanese cedar (No.1) and 439 g/kg for Japanese beech (No.2). However, other non-woody biomass such as kokanada-mo (No.23), giant reed (No.15), bamboo (No.4), bagasse (No.12) and erianthus (No.10) had more or less similar cellulose contents to be, respectively, 436, 416, 394, 383 and 378 g/kg. Those lignocellulosic species have, therefore, high potential for cellulose applications. Cellulose in the Gramineae species (No.4-No.15) ranged from 268 to 416 g/kg, while it was between 306 and 324 g/kg in the palm species (No.16-No.18) studied. In the algae (No.24-No.32), cellulose was equal to or below 203 g/kg.

In this study, polysaccharides other than cellulose were considered as hemicellulose and its content in the biomass species studied varied from 88 to 660 g/kg. Euchemia (No.31), Sargassum (No.25) and chlorella (No.30) presented the highest values with, respectively, 660, 428 and 424 g/kg. Although cellulose is the principal carbohydrate in higher plants, it was not the case in algae species (No.24-No.32) where hemicellulose, varying from 207 to 606 g/kg, was the predominant carbohydrate. The major part of the hemicellulosic saccharides in algae might probably be alginate as well as sulfated polysaccharides such as agar and carageenan, as they were reported to be the major structural polysaccharide of algae, for instance, brown and red algae.[25,26] The lowest hemicellulose contents were found in the aquatic plants, for instance, sennin-mo (No.20) and kokanada-mo (No.23) with, respectively, 88 and 93 g/kg. The detailed monosaccharides composition will be discussed later.

As a carbohydrate, starch was a minor component in all the biomass samples studied. The lowest value was found in Japanese cedar (No.1) with 1 g/kg and the highest in oil palm trunk (No.16) to be 29 g/kg. A similar content of starch in oil palm to be 17 g/kg was previously reported.[27]

Lignin as the sum of Klason lignin and acid-soluble lignin was the highest in softwood Japanese cedar (No.1) with 331 g/kg, whereas it was inexistent in 3 of the algae species studied, namely spirulina (No.32), chlorella (No.30) and chladophora (No.27). In the other aquatic samples, lignin ranged from 18 for euchemia (No.31) to 175 g/kg for parrot feather (No.3), whereas in the terrestrial ones, it varied from 151 for corn leaves (No.8) to 331g/kg for Japanese cedar (No.1). Among the biomass species studied, lignin content was, therefore, lower in the aquatic samples and particularly in the marine algae as compared to the terrestrial ones. Since the roles of hydrophobic lignin are mostly to provide mechanical strength to the cell walls and to conduct water without its leaking from the cell walls, such properties are mostly important for terrestrial plants, but not for aquatic plants and algae. This might be the reason of the difference in the lignin content.

It was historically considered that algae and seaweeds did not contain lignin but recent publications questioned such belief and lignin distribution in plant kingdom is currently under reevaluation.[28,29] First, "lignin-like compounds" have been identified in primitive green algae.[30-31] Later on, an anatomical and structural study on the red alga Calliarthron revealed the presence of secondary walls and true lignin in that species, suggesting a convergent evolution of cell wall structure or a deeply conserved evolutionary history of these traits.[32] Evidence was also provided to prove that the red alga contained guaiacyl propane, *p*-hydroxyphenylpropane as well as syringyl propane units.[32] Other studies reported some lignin contents in the green alga sea lettuce to be 15 g/kg[33] and in brown algae including sargassum species to be between 141 and 175 g/kg.[34] In this study, Klason lignin contents of brown algae, namely akamoku (No.24) and sargassum (No.25), as well as green algae, specifically sea lettuce (No.26), caulerpa (No.28) and sea grape (No.29), in addition to euchemia (No.31) as a red alga were, respectively, 142, 73, 33, 37, 26 and 18 g/kg, after ash and protein corrections, as in Table 2. Further evidence on the existence of lignin in these samples will be discussed later. Such finding on the lignin content of algae can, therefore, be a meaningfully supportive data on the study of cell wall evolution of plants.

The determination of protein content of biomass is important since this constituent can be considered as a substrate for protein-based value-added products but it might also affect the choice of a biomass for a given application. Depending on the applications, protein in the feedstock might be desirable or not. Whilst protein was very high in spirulina (No.32) and chlorella (No.30) with respectively 502 and 423 g/kg, the samples analyzed with green leaves such as aquatic plants growing in lakes (Nos.3,19-23), brown and green algae (No.24-No.30) and corn leaves (No.8) also presented high amounts of protein. High protein content in these samples was probably due to the presence of photosynthetic pigment-protein complex present in the biomass leaves. The other biomass studied had their protein contents equal to or below 261 g/kg. However, protein content greatly varied among samples taken from different parts of the same plant, for instance, protein in corn leaves (No.8) to be 165 g/kg was much higher than that of the corn cob (No.9) of the same corn plant to be 58 g/kg. Similarly, rice straw (No.5) had higher protein than rice husk (No.6) of the same rice plant. Those results suggest that protein might be concentrated in the leaves of those photosynthetic organisms.

Extractives mostly consist of low molecular compounds soluble in liquids of low polarity. Extractives from woods were found to present undesirable effects on pulp and paper-making.[35,36] Similar trends are also expected in other biorefinery applications. Thus, acetone extractives were also studied in the biomass. Chladophora (No.27) and chlorella (No.30) presented the least acetone extractives, while akamoku (No.24) had the highest one to be 96 g/kg. The amounts of extractives varied among the biomass species studied and even within different parts of the same plant. As an example, rice straw (No.5) presented 45 g/kg of extractives, whereas rice husk (No.6) from the same plant had 13 g/kg. A similar result was found for corn leaves and cob (Nos.8 and 9) with respectively 51 and 28 g/kg. The other biomass species studied had their extractives ranged between 10 and 75 g/kg.

The inorganics present in the biomass were analyzed as ash.

The values fluctuated greatly among the samples studied. While Japanese cedar (No.1) had the lowest ash content with only 3 g/kg, the highest values were found in aquatic species such as sea grape (No.29) with 500 g/kg, followed by chladophora (No.27), sea lettuce (No.26), akamoku (No.24) and kuro-mo (No.22) with, respectively, 402, 257, 229 and 223 g/kg. Sargassum and some aquatic plants were proved to have the capacity to intake minerals and heavy metals from the water they grow in,[37,38] such a property might explain their high ash contents. Among the terrestrial plants studied, rice husk (No.6) had the highest ash content with 168 g/kg. In the literature, ash contents in rice husk vary from 143 to 170 g/kg and such high ash contents were reported to be due to high silica content.[39-41]

Overall, the summative results comprised between 959 and 999 g/kg. The majority of all the biomass constituents were, therefore, identified. Although the chemical composition of the biomass species differed from a species to another, similarities were found in the composition of samples belonging to the same taxonomic group.

Apart from the chemical composition, the calorific values were studied. As a result, the calorific values of all the studied biomass species varied from 8.47 MJ/kg as in sea grape (No.29), to 23.48 MJ/kg as in chlorella (No.30). The higher plants (No.1-No.23) presented similar trends in their calorific values which were accounted between 15.58 and 20.62 MJ/kg, while for lower species (Nos.24-29, 31), the values were from 8.47 to 16.28 MJ/kg. Such low value could be explained by their high inorganic contents, determined as ash content in Table 2. Chlorella (No.30) was an exception among the Cryptogamae algae species since it presented the highest calorific value among all the studied samples. Such high calorific value is also reflected in its low ash content as shown in Table 2. The Eubacteria spirulina (No.32) also showed rather high calorific value as compared to the other algae species. From these lines of results, it became clear that the calorific value is also related to the taxonomy of the studied biomass.

3.2 Monosaccharides composition of various biomass species

Carbohydrates in biomass mainly consist of cellulose and hemicellulose which further comprise hexoses, pentoses and uronic acids. The detailed monosaccharides composition of biomass is necessary to determine their most suitable use, to evaluate the theoretical yields and to monitor the corresponding processes.

Therefore, hexoses, pentoses and uronic acid contents were studied in the biomass and the obtained results are shown in Table 3 which represents the monosaccharides composition of the biomass samples studied. Japanese cedar (No.1), representative of softwoods presented the highest glucose content among the assayed biomass species with 447 g/kg, followed by kokanada-mo (No.23) with 422 g/kg, then by Japanese beech (No.2) and giant reed (No.15), both containing 417 g/kg glucose and then erianthus (No.10) with 411 g/kg. Bagasse (No.12), wheat straw (No.7) and bamboo (No.4) presented similar glucose contents to be, respectively, 407, 406 and 402 g/kg. The other biomass species had their glucose contents equal to or below 392 g/kg. The glucose content is actually reflected in the cellulose content, as reported in Table 2.

Types and proportion of the other various hemicellulosic monosaccharides showed a wide variation range among the biomass studied. Mannose was the highest in softwood Japanese cedar (No.1) to be 57 g/kg, most probably coming from its mannan. Parrot feather (No.3) and kokanada-mo (No.23) had mannose as their predominant hemicellulosic saccharides, while the other samples had their mannose content lower than 20 g/kg.

Galactose was particularly high in the red alga euchemia (No.31) to be 337 g/kg, most probably derived from agar which is a sulfated polysaccharide, typical for red algae, and composed of agarose and agaropectin. Agarose is the principal component of agar and consists of a repeating monomeric unit of agarobiose which is a disaccharide composed of D-galactose and 3,6-anhydro-L-galactopyranose.[42,43] In general, agaropectin contains similar sugars, but in addition has sulfate bound in ester linkage to the number of carbon six of the galactopyranose units and carboxylic acid groups resulting from the attachment of pyruvic acid groups via *0*-glycosidic linkages. D-Glucuronic acid has also been reported to be present.[42] Another sulfated polysaccharide present in red alga cell wall is carageenan, a galactan composed majoritarily of D-galactose units.[43] Thus, the high galactose content in euchemia (No.31) may reflect its content in agar and carageenan. Among the other species studied, chlorella (No.30) had quite high galactose content as well, most likely coming from its galactan.[44] The other species had their galactose equal to or below 120 g/kg.

Sea lettuce (No.26) showed rhamnose as its predominant neutral monosaccharide with 124 g/kg. A similar range was also reported elsewhere for another species of sea lettuce (*Ulva pertusa*) to be 137g/kg.[45] Such high rhamnose might be coming from the polysaccharide ulvan which is specific for green algae species and known to be composed mainly of rhamnose.[46] Other green algae such as chladophora (No.27) and caulerpa (No.28) also had relatively high rhamnose content as compared to the rest of the biomass samples studied, while terrestrial monocotyledonous angiosperm species (No.4-No.18) had quite low rhamnose content to be equal to or lower than 5 g/kg. Fructose could be detected only in aquatic plants that are parrot feather (No.3), sennin-mo (No.20), okanada-mo (No.21) and kuro-mo (No.22) with 22, 3, 1 and 1 g/kg, respectively.

Corn cob and bagasse (Nos. 8 and 9) were remarkable for their high xylose content to be, respectively, 312 and 260 g/kg. Such a high xylose content could also be observed elsewhere for corn cob.[39] However, not only corn cob and bagasse but all the monocotyledonous species studied, belonging to Poaceae family, as known as Gramineae (No.4-No.15), had their xylose relatively higher as compared to the lower plants, most probably due to their high xylan content.[47] Species belonging to the Aracaceae family, usually known as Palmae family (No.16-No.18) also showed relatively high xylose contents, similar to the ones of Gramineae. Conversely, the aquatic monocotyledonous species, belonging to Pontederiaceae (No.19), Potamogetonaceae (No.20) and
Hydrocharitaceae (No.21) families showed rather low xylose content to be between 21 and 58 g/kg.

Dicotyledonous Japanese beech (No.2) had its xylose content in similar range as the ones of the Poaceae monocotyledonous species (No.4-No.15), while dicotyledonous

parrot feather (No.3), which is an aquatic plant, had very low xylose content to be 32 g/kg, similar to the monocotyledonous aquatic plants (No.20-No.23). From those results, it seems that the aquatic plants belonging to the Phanerogamae (Nos.3,19-23) were similar in their xylose content. Similarly, the terrestrial angiosperm in Phanerogamae also showed comparable ranges of xylose content.

Table 3 Monosaccharides composition of the biomass samples studied (g/kg of the original oven-dried biomass basis)

Family	Sample No.	Biomass	Hexoses					Pentoses		Uronic acid
			Glc	Man	Gal	Rhm	Fru	Xyl	Ara	
Cupressaceae (softwood)	1	Japanese cedar	447	57	12	2	0	64	6	9
Fagaceae (hardwood)	2	Japanese beech	417	14	36	23	0	213	9	20
Haloragaceae	3	Parrot feather (aquatic plant)	289	41	24	13	22	32	37	16
Poaceae (Gramineae)	4	Bamboo	402	5	32	3	0	234	42	9
	5	Rice straw	355	3	12	3	0	216	30	7
	6	Rice husk	349	2	17	3	0	178	21	4
	7	Wheat straw	406	1	7	2	0	179	25	27
	8	Corn leaves	268	1	8	5	0	169	27	8
	9	Corn cob	344	1	14	2	0	312	54	13
	10	Erianthus	411	1	5	1	0	177	20	3
	11	Miscanthus	382	1	6	2	0	190	29	4
	12	Bagasse	407	0	5	1	0	260	15	13
	13	Sugarcane leaves	335	3	7	1	0	210	23	9
	14	Common reed	360	0	4	2	0	244	24	6
	15	Giant reed	417	0	2	1	0	226	16	5
Aracaceae (Palmae)	16	Oil palm trunk	338	11	8	4	0	220	38	17
	17	Nipa frond	392	10	19	2	0	175	27	33
	18	Sugar palm frond	392	1	6	3	0	182	23	14
Pontederiaceae (aquatic plants)	19	Water hyacinth	163	0	98	13	0	44	40	131
Potamogetonaceae (aquatic plants)	20	Sennin-mo	343	6	5	8	3	58	26	19
Hydrocharitaceae (aquatic plants)	21	Okanada-mo	267	19	59	16	1	51	16	24
	22	Kuro-mo	241	1	44	12	1	29	24	11
	23	Kokanada-mo	422	31	26	8	0	25	14	26
Sargassaceae (brown algae)	24	Akamoku	55	15	25	0	0	6	0	203
	25	Sargassum	230	4	120	2	0	85	0	230
Ulvaceae (green algae)	26	Sea lettuce	78	0	1	124	0	59	0	100
	27	Chladophora	46	0	15	61	0	63	33	122
Caulerpaceae (green algae)	28	Caulerpa	90	0	67	47	0	207	22	4
	29	Sea grape	66	14	30	2	0	129	0	7
Chlorellaceae (green algae)	30	Chlorella	43	0	141	39	0	65	19	113
Solieraceae (red algae)	31	Euchemia	64	0	337	21	0	20	0	350
Pseudanabaenaceae (blue-green algae)	32	Spirulina	80	0	29	14	0	57	13	90

Glc : Glucose, Man : Mannose, Gal : Galactose, Rhm : Rhamnose, Fru : Fructose, Xyl : Xylose, Ara : Arabinose

Table 4 Molar ratio of syringaldehyde and *p*-hydroxybenzaldehyde to vanillin in the biomass samples studied as determined by alkaline nitrobenzene oxidation

Family	Sample number	Species	Lignin (g/kg)	Vanillin	Syringaldehyde	*p*-Hydroxybenzaldehyde
Cupressaceae (softwood)	1	Japanese cedar	331	1	0	0.05
Fagaceae (hardwood)	2	Japanese beech	240	1	2.00	0
Haloragaceae	3	Parrot feather (aquatic plant)	175	1	1.00	0.76
Poaceae (Gramineae)	4	Bamboo	206	1	1.28	0.45
	5	Rice straw	202	1	0.67	0.52
	6	Rice husk	241	1	0.15	0.23
	7	Wheat straw	200	1	0.49	0.11
	8	Corn leaves	151	1	0.65	0.56
	9	Corn cob	180	1	0.64	0.77
	10	Erianthus	254	1	0.66	0.13
	11	Miscanthus	223	1	0.63	0.42
	12	Bagasse	224	1	0.80	0.19
	13	Sugarcane leaves	197	1	0.74	0.09
	14	Common reed	202	1	1.03	0.14
	15	Giant reed	249	1	1.09	0.23
Aracaceae (Palmae)	16	Oil palm trunk	282	1	3.50	0.00
	17	Nipa frond	196	1	1.20	0.03
	18	Sugar palm frond	209	1	1.56	0.13
Pontederiaceae (aquatic plants)	19	Water Hyacinth	101	1	0.84	0.67
Potamogetonaceae (aquatic plants)	20	Sennin-mo	149	1	1.43	2.47
Hydrocharitaceae (aquatic plants)	21	Okanada-mo	71	1	1.15	2.01
	22	Kuro-mo	79	1	1.26	1.76
	23	Kokanada-mo	76	1	0.70	0.93
Sargassaceae (brown algae)	24	Akamoku	142	1	0.23	2.70
	25	Sargassum	73	1	0.44	2.40
Ulvaceae (green algae)	26	Sea lettuce	33	1	1.36	0.11
	27	Chladophora	0	0	0	0
Caulerpaceae (green algae)	28	Caulerpa	37	1	1.25	1.95
	29	Sea grape	26	1	1.13	1.78
Chlorellaceae (green algae)	30	Chlorella	0	0	0	0
Solieraceae (red algae)	31	Eucheumia	18	1	1.24	0.90
Pseudanabaenaceae (blue-green algae)	32	Spirulina	0	0	0	0

As for the lower plants, their xylose content was equal or inferior to 85 g/kg, except for caulerpa (No.28) and sea grape (No.29) which showed relatively high xylose content, to be 207 and 129 g/kg, respectively.

Arabinose varied from 0 to 54 g/kg for all the biomass samples studied. The highest content was found in corn cob (No.9), while arabinose was absent in the brown algae (Nos.24 and 25) studied and some green algae, namely sea lettuce (No.26) and sea grape (No.29) as well as in the red alga euchemia (No.31). Among the higher plants, the Poaceae species (No.4-No.15) studied presented relatively higher arabinose content as compared to wood species (Nos.1 and 2). Since xylose is also high in Poaceae, the arabinose might come from the arabinoxylan, known to be the major hemicellulose in that family.[48]

The algae species had significantly high uronic acid content, up to 350 g/kg for the red alga euchemia (No.31), followed by the brown algae sargassum (No.25) and akamoku (No.24), with 230 and 203 g/kg, respectively. For the red alga euchemia (No.31), such high uronic acid content might be due to the agaropectin,[42] while in the brown algae sargassum (No.25) and akamoku (No.24), such high uronic acid might come from their alginate which is a glycuronan consisting of residues of D-mannuronic acid and L-guluronic acid.[49] The other biomass species studied had their uronic acid content lower or equal to 131 g/kg.

Altogether, the differences and similarities in the monosaccharides composition of biomass samples studied reflected well their taxonomy.

### 3.3 Phenylpropane units of lignin

The chemical structure of lignin in the biomass samples was examined and the obtained results are shown in Table 4 which reveals the molar ratio of syringaldehyde and *p*-hydroxybenzaldehyde to vanillin in the studied biomass species as determined by alkaline nitrobenzene oxidation where vanillin, syringaldehyde and *p*-hydroxybenzaldehyde correspond respectively to the guaiacyl type lignin unit (G), syringyl type lignin unit (S) and to the *p*-hydroxyphenyl propane unit (P).

As expected, Japanese cedar (No.1), representing softwood showed mainly G moiety, with small ratio of the P one, while dicotyledonous hardwood Japanese beech (No.2) presented G and S moieties only. Gramineae species yielded all 3 moieties in fluctuating molar ratios, which is in accordance with previous findings suggesting that P unit might be the distinguishing characteristics of monocotyledon.[50] Interestingly, as an aquatic dicotyledonous species, parrot feather (No.3) also showed all 3 aldehydes, implying that the chemical structure of its lignin would be more similar to the one of monocotyledonous species, rather than the one of wood, which is the most representative of dicotyledons.

Among the monocotyledonous palm species, nipa frond (No.17) and sugar palm frond (No.18) presented the 3 moieties, with very small quantity of P, whereas oil palm trunk (No.16) yielded only G and S units. The relatively large occurrence of S moiety to range from molar ratios of 1.2 to 3.5 in the palm species suggests that palm lignin could be similar to hardwood lignin. However, the presence of P in nipa frond and sugar palm frond suggests that their lignin structure would be more similar to the ones of Graminae. Other researchers showed that P from palm

species can be detected in small quantities or not detected at all, and, thus, would not be a distinctive factor.[51-54]

Concerning aquatic species, the samples containing lignin could yield all 3 moieties in different molar ratios. *p*-Hydroxybenzaldehyde was even predominant in sennin-mo (No.20), okanada-mo (No.21), kuromo (No.22), akamoku (No.24), sargassum (No.25), caulerpa (No.28) and sea grape (No.29). It was reported for herbaceous samples that a large proportion of *p*-hydroxybenzaldehyde and vanillin produced after the alkaline nitrobenzene oxidation could be formed respectively from *p*-coumaric acid and ferulic acid esterified or etherified with lignin, and not from P and G moieties in the lignin polymer itself.[22]
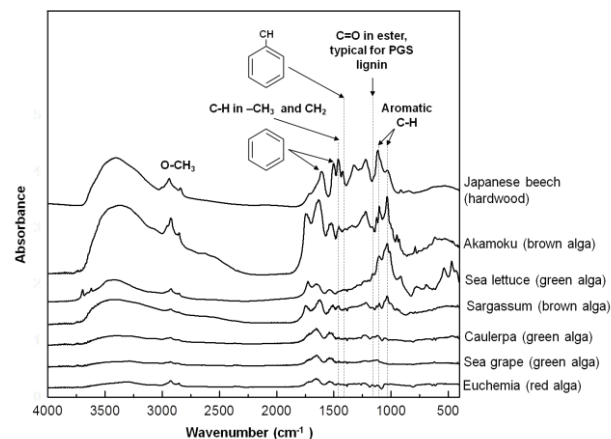
Fig. 3 FT-IR spectra of crude Klason lignins from algae species as compared to the one from Japanese beech (*Fagus crenata*)

In order to confirm the presence of the 3 moieties in those algae species, FT-IR spectra of the crude Klason lignin of 2 brown algae that are akamoku (No.24) and sargassum (No.25), 3 green algae which are sea lettuce (No.26), caulerpa (No.28) and sea grape (No.29) as well as a red alga named euchemia (No.31) were studied.

As a result, Fig. 3 shows the FT-IR spectra of crude Klason lignins from the algae species as compared to the one from Japanese beech (No.2).

The absorption spectra for the algae studied, except for akamoku, showed quite lower intensity, most probably due to their low lignin content but also to the remaining inorganics and protein
in the crude Klason lignin residues. However, the algae species, together with Japanese beech showed absorption bands between 2939-2842 cm^{-1}, which correspond to C-H stretch[55] as well as methoxyl group bands between 2965-2945 cm^{-1}.[56] They also presented absorption bands in the regions 1593-1605 cm^{-1} and 1515-1505 cm^{-1}, both of which coming from aromatic skeletal vibrations, typical for lignin[55] and positive peaks at 1460-1470 cm^{-1} assigned to -CH deformations in -CH$_3$ and -CH$_2$- . A band in the range 1422-1430 cm^{-1}, proof of the presence of aromatic skeletal vibration combined with -CH in plane deformation[55] is also present in the algae as well as Japanese beech.

Finally, although in rather low intensities, absorption bands at 1160-1166 cm^{-1}, representing -C=O in ester, typical for P, G and S type lignin[55], were also present in spectra of the algae studied but missing in the one of Japanese beech. Such lines of evidence confirm that these 6 algae studied presented lignin of P, G and S types. Therefore, the results on the lignin properties of algae from this work complete those of Martone *et al.*[32] for instance concerning the presence of S type lignin in some algae species, which is quite a noticeable breakthrough. Such findings are surely important for further studies on lignin distribution among living organisms but also for further understanding of the cell wall evolution over the plant kingdom.

3.4 Cluster analysis of biomass

All 32 biomass samples of different origin were used for a cluster analysis by principal component analysis (PCA). The 7 features used were the contents of cellulose, hemicellulose, lignin, protein, extractives, starch and ash.
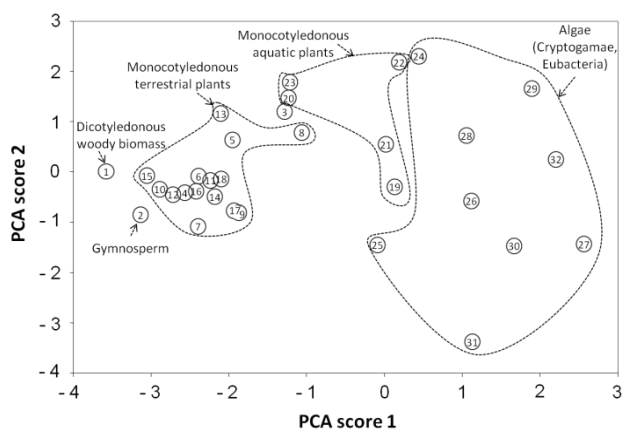

Fig. 4 PCA score plot for the 32 biomass samples characterized by their contents in cellulose, hemicellulose, lignin, protein, extractives, starch and ash.

Figure 4 shows the resulting scatter plot using the scores of the first 2 principal components, preserving 44.6 and 23.6 % of the total variance, respectively. Woody biomass represented by Japanese cedar (No. 1) and Japanese beech (No.2) were relatively close in the clustering but appeared to be respectively quite apart from the other samples. The monocotyledonous terrestrial plants including Gramineae (No.4-No.15) and palm species (No.16-No.18) built a relative compact cluster, while the other samples were rather diverse. The monocotyledonous aquatic plants (No.20-No.23) formed an intermediate cluster between the higher plants and the lower ones. The algae species represented by Cryptogamae (No.24-No.31) and Eubacteria (No.32) samples formed a different cluster on the right, reflecting their different composition from the other plant materials.

The results from the cluster analysis demonstrate that the chemical composition of the biomass species is related to the taxonomic classification.

3.5 Chemical composition of various biomass species in relation with their taxonomic classification and their potential for different biorefinery platforms

As discussed previously, although the chemical composition of the biomass species differed from a species to another, similarities were found in the composition of samples belonging to the same taxonomic group. Therefore, representatives of each predominant taxonomic group were selected and represented in Fig. 5 which shows the chemical composition of the biomass species studied according to their taxonomic classification.
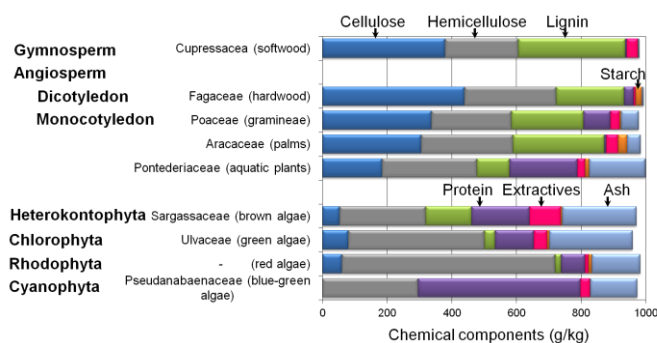
Fig. 5 Chemical composition of various biomass species in relation with their taxonomic classification

From Fig. 5, it became more obvious that chemical composition of the lower plants is very different from the higher ones. In the lower plants including Heterokontophyta, Chlorophyta, Rhodophyta and Cyanophyta, the carbohydrates are characterized by the inexistence or low range of cellulose but high hemicellulose content with acid sugars. Such a pattern changes in the higher plants where cellulose content is high. Lignin is also inexistent or low in the lower plants and became higher over the evolution.

Such differences in the chemical composition of the lower plants and higher ones reflect the taxonomy of the samples as well as the evolution theory presented in Fig. 1 as lignin and hemicellulose were modified over the evolution.

From the revealed classification in the chemical composition of the various biomass species and their calorific values, it is possible to determine the potential of each taxonomic group for various biorefinery platforms. Table 5 shows the potential of the diverse taxonomic groups of biomass for combustion and for different biorefinery platforms which were determined by Werpy *et al* in the model of biobased product flowchart. [57]

According to the chemical composition, gymnosperm species showed very high potential for syngas and lignin as well as high potential for sugar platform and for combustion. A similar pattern can be seen for dicotyledonous hardwood, with very high potential for sugar platform. Gramineae and palm species showed similar potential for syngas, sugar and lignin platforms as well as for combustion but the potential of the Gramineae for protein platform was superior to the one of palm species. Finally, the monocotyledonous aquatic plants showed more or less similar trends with the algae groups in their potential for biorefinery applications.

Table 5 Potential of the various taxonomic groups of biomass for different biorefinery platforms and for combustion

Taxonomy	Groups	Biorefinery platforms				Combustion
		Syngas	Sugar	Lignin	Protein	
Gymnosperm	Softwood	+++++	++++	+++++	-	++++
Angiosperm						
Dicotyledon	Hardwood	+++++	+++++	++++	-	++++
Monocotyledon	Gramineae	++++	++++	++++	+++	++++
	Palms	++++	++++	++++	+	++++
	Aquatic plants	++++	+++	+	++++	+++
Heterekontophyta	Brown algae	+++	+++	+	++++	++
Chlorophyta	Green algae	+++	+++	+	++++	++ *
Rhodophyta	Red algae	+++	+++	+	++++	++
Cyanophyta	Blue-green algae	+++	++	-	+++++	++++

+++++ : very high potential   ++ : low potential

++++ : high potential   + : existing potential if treated in a high scale production

+++ : medium potential

- : no potential

* Except for Chlorellaceae which had high potential

4   Conclusions

The chemical composition of 32 biomass samples belonging to 29 species could be revealed in this work. The results showed that the chemical composition of the examined samples differed from a species to another but also from different parts of the same plant. However, similarities were found in species belonging to the same family, allowing the establishment of typical chemical composition of the significant taxonomic groups of plants and algae species to be potentially used for green chemistry and biorefinery. Conclusively, this characterization study is a very important tool and basis to develop efficient biorefinery strategies.

Acknowledgment

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