Analytical Methods

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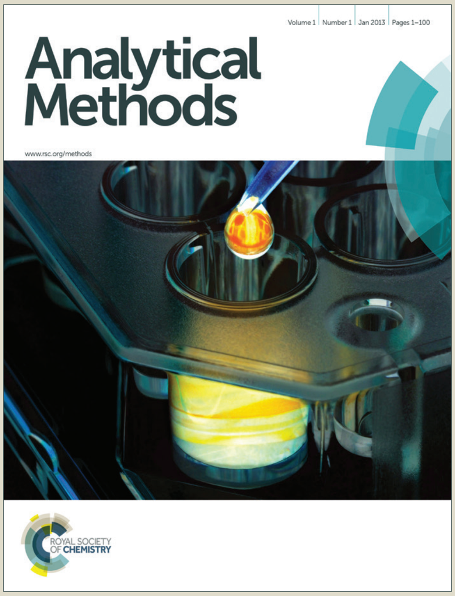

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Analytical Techniques for Chemical Analysis of Plant Biomass and their Products

Sílvio Vaz Jr.

Nowadays, the use of biomass as an alternative to non-renewable raw material for energy, materials and chemicals is a prominent theme for academy and industry. Many countries are spending financial resources and efforts to promote a green economy based on plant biomass. Chemical analyses are an important tool to ensure quality, reliability and the best usages for the economic potential of biomass. Analytical techniques can provide information about chemical composition, characterization of properties and determination of concentration for organic and inorganic species. This review discusses the main techniques and their application in chemical analysis of plant biomass and products, covering examples of application for industrial and scientific purposes. Furthermore, aspects of biorefinery, green chemistry, innovation and technological trends are considered.

Brazilian Agricultural Research Corporation (Embrapa); Embrapa Agroenergia - Parque Estação Biológica, Avenida W3 (final), CP 70770-901, Brasília-DF, Brazil. E-mail: silvio.vaz@embrapa.br

1. Introduction

The technological development of modern society has stimulated the need for control of products and processes, both to ensure that final products are consumed according to quality standards, besides to prevent negative impacts on the environment. The concern of the society demanding a sustainable supply became a point of strong commercial appeal to the productive sectors such as agribusiness, since the latter has been proposed in recent years a reduction in the greenhouse gases, increased productivity combined with lower tillage per area, decrease in agrochemicals and application of sustainable practices. One example in agribusiness is the bioenergy, here represented by ethanol and biodiesel, which seek to be seen as green fuels as a market strategy.

Modern chemistry plays a strong economic role in industrial activities, with an increasing trend in the importance of its application from the deployment of biorefineries and principles of green chemistry, which make use of the potential of biomass. In this context, analytical chemistry can contribute significantly to the productive chains of biomass, either vegetable or animal origin; but with the first offering the greatest challenges and opportunities for industrial exploitation from its chemical complexity.[1] It is worth mentioning that the chemical analyses are used for composition analysis, characterization of physical and chemical properties and to determine the concentration of chemical species, besides new applications in biomass chemistry.[2]

Techniques and analytical methods provide support for the implementation of laws applied on market and environment, to ensure the quality of raw materials and production processes, which enables the development of new materials and products that add value on biomass,[3] what can promote the bioeconomy and positive impact on chemical sciences.[4] Chemical analyses play an important role in the exploitation of biomass, as supporting technologies at all stages of agro-industrial chains as sugarcane, soybean, corn, forests, pulp and paper, waste and agricultural residues, among others sources of raw materials. We can observe in Fig. 1 a generic division of biomass by means their origin.

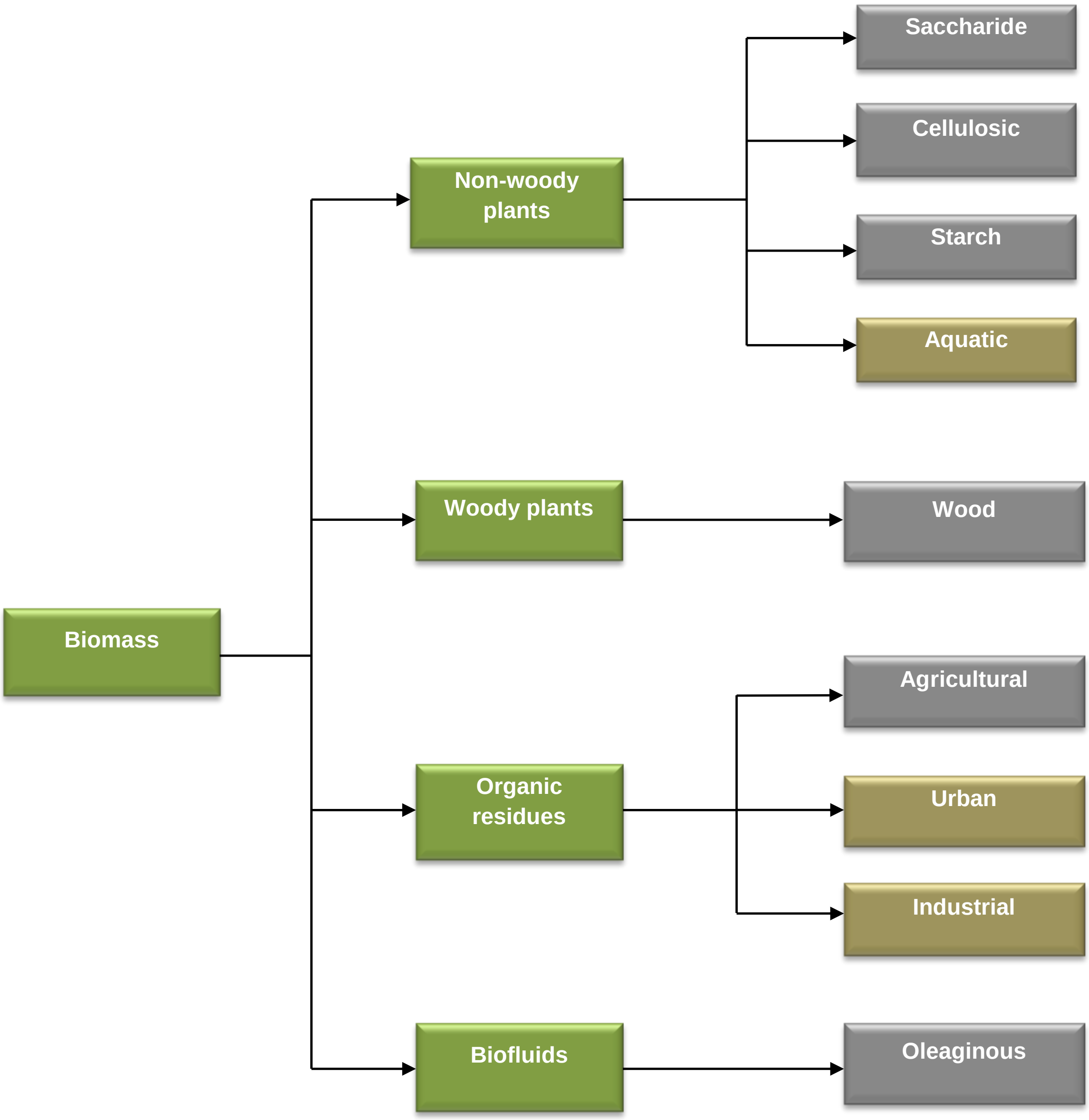

Fig. 1 Sources of biomass; gray boxes represent the most used biomass types for industrial and R&D activities

From the Fig. 1 we can consider three classes of biomass with large industrial and R&D uses as raw material for conversion processes (biochemical, chemical and thermochemical): (i) starch, cellulosic material and saccharide are sources of sugar (glucose); (ii) wood and agricultural residues are sources of cellulose, hemicellulose and lignin (the lignocellulosic material); (iii) oleaginous are sources of fatty acids and esters. Each one has their structural characteristics and chemical particularities, which are closely related to the analytical technology and approach to be applied for its analysis. Nowadays, we have an estimated worldwide production of renewable biomass of 210.7 million of tons/year to be used in biofuels, fibers and agriculture.[5] An exact value for this biomass production is not easy to obtain, because there is a large variation on the production from each country and difficulties to measure its quantity, but the Food and Agriculture Organization of the United Nations (FAO) works on this statistical compilation for the world food and agriculture production. However, is very clear to note the importance of the biomass on modern economy; a good example is the case of wood products generated:[5] a sawn wood production of 406.2 million of m^3, an wood based panels of 287.7 million of m^3, a wood pulp production of 173.3 million of tons and a paper and paperboard production of 403.2 million of tons. Table 1 shows a landscape of worldwide production of agro-industrial biomass.

Table 1 Production data of biomass for food and fiber uses; data obtained from FAO[5]

Biomass	**Production**
Cereal	2.5 billion of tons
Oil crop	170.3 million of tons
Root and tuber	747.7 million of tons
Vegetable	1.0 billion of tons
Fruit	608.9 million of tons
Fiber	28.1 million of tons

The biorefinery concept is a very important strategy for the development of biomass usages, where there is a productive biomass chain very similar to the petrochemical chain: fuels, energy, materials, bulk chemicals and fine chemicals.[6] Biorefineries uses a very large numbers of conversion processes due to biomass chemical diversity, high content of oxygen atom and water; these conversion processes are divided in three major families: (i) chemical processes (basically, catalytic synthetic routes), (ii) biochemical processes (fermentation and biocatalytic or enzymatic routes), (iii) thermochemical routes (gasification, pyrolysis, combustion, *etc*.).[6] Therefore, we need analytical chemistry to understand and to control these processes, their raw materials, products and residues.

For analytical purposes, the composition of vegetable biomass is described in the Table 2.

Table 2 Composition of biomass according Vassilev *et al.*[7]

Matter	Components
Organic matter	Structural components (cellulose, hemicellulose, lignin), extractives, others
	Organic minerals such as Ca-Mg-K-Na oxalates, others
Inorganic matter	Mineral species from different mineral classes (silicates, oxyhydroxides, sulphates, phosphates, carbonates, chlorides, nitrates, others)
	Poorly crystallized mineraloids of some silicates, phosphates, hydroxides, chlorides, others
	Amorphous phases such as various glasses, silicates, others
Fluid matter (mostly inorganic)	Moisture, gas and gas-liquid inclusions associated with both organic and inorganic matter

Therefore, a diverse analytical approach is desirable to understand composition and properties of biomass and their products from conversion processes, considering techniques for organic and inorganic species.

2. Analytical techniques for biomass and their products

A large variety of classical and analytical techniques may be applied to biomass analyses: titrimetry or volumetry; gravimetry; thermal analyses; electrochemical analyses; chromatography and electrophoresis; spectroscopy, spectrometry and spectrophotometry; mass spectrometry; and microscopy. There are good sources of detailed information about their principles in the analytical literature.[8-16] Table 3 presents some general uses of analytical techniques in chemical analysis of biomass and its products.

Table 3 Some examples of analytical techniques and their uses in chemical analyses of biomass

Technique	Principle of measurement	Example of use	Reference	Pros	Cons
Differential scanning calorimetry	Enthalpy changes	Determination of combustion properties of biomass (exothermic or endothermic)	17	Small quantity of sample; high sensitive; determines physico-chemical changes in materials impossible to determine by other technique	-
Capillary electrophoresis	Migration of ions or charged particles	High efficiency separation for polar compounds from biomass degradation	18	High separation efficiency	Limitation for non-polar compounds
Mass spectrometry	Molecular fragmentation	Structural identification and quantification of several organic compounds based	19	Identification and resolution of complex molecular structures	Necessity of separation techniques, as chromatography, for a better

		on m/z ratio			resolution
X-ray fluorescence spectroscopy	Emission of characteristic X-rays	Multielemental quantification in solid and liquid samples from biomass residues	20	Easy to handle; non-destructive	Chemical composition and morphology of the sample can affect the result
Infrared spectroscopy (near and medium)	Vibrational energy absorption	Structural identification of organic compounds and lignocellulosic components	21 22	Easy to handle, mainly for near infrared	Low resolution for compounds with same functional groups (sum of bands); however, the application of chemometrics can help to overcome this limitation
X-ray difractrometry	Intensity of X-rays diffracted	Determination of crystallinity and chemical composition of cellulose	23	Important physical information for natural fibers and polymers usages	Long acquisition time (hour or day) for process control
Scanning electron microscopy	Surface scanning with a primary electron beam	Surface and structural analysis of materials (*e.g.*, catalysts)	24	Important physical information for natural fibers and polymers usages	Long acquisition time (hour or day) for process control

Nuclear magnetic resonance (*e.g.*, ^{13}C in solid state)	Transition of nuclear spin inside an atomic nuclei; interactions between nuclei-nuclei and nuclei-surround electrons	Structural identification of organic compounds from biomass processing (*e.g.*, lignocellulosic and oleaginous)	25	Resolution of complex molecular structures	Long acquisition time (hour or day) for process control, except under a high concentration of the analyte (*e.g.*, fatty acids)
Voltammetry (*e.g.*, cyclic and square wave)	Changes in current as a function of potential	Chemical speciation and quantification of metals and non-metals (*e.g.*, catalysts for glycerin use), or verification of glucose or starchy oxidation processes	26	Rapid response	Search for the better electrolyte or voltammetric technique can expend time

From Table 3 and from the organic and inorganic composition of biomass (Table 2), we can notice several analytical technologies which enable an increase in the biomass knowledge of their properties and conversion processes. These technologies imply the observation of a large variety of chemical species, mainly, in aqueous medium or with water inside or adsorbed on their structures.

3. Application for biomass usages

Biomass chains typically require the application of chemical analyses that may encompass a large number of samples at a low cost - which are sought by the industrial segments. Such assays are not restricted only to manufacturing, but also in R&D; therefore, the analytical process follows pre-established steps to make suitable its application. Fig. 2 shows common steps in a biomass chain that make uses of chemical analyses and examples of application; we can notice thermal, spectroscopic, chromatographic and spectrometric methods involved.

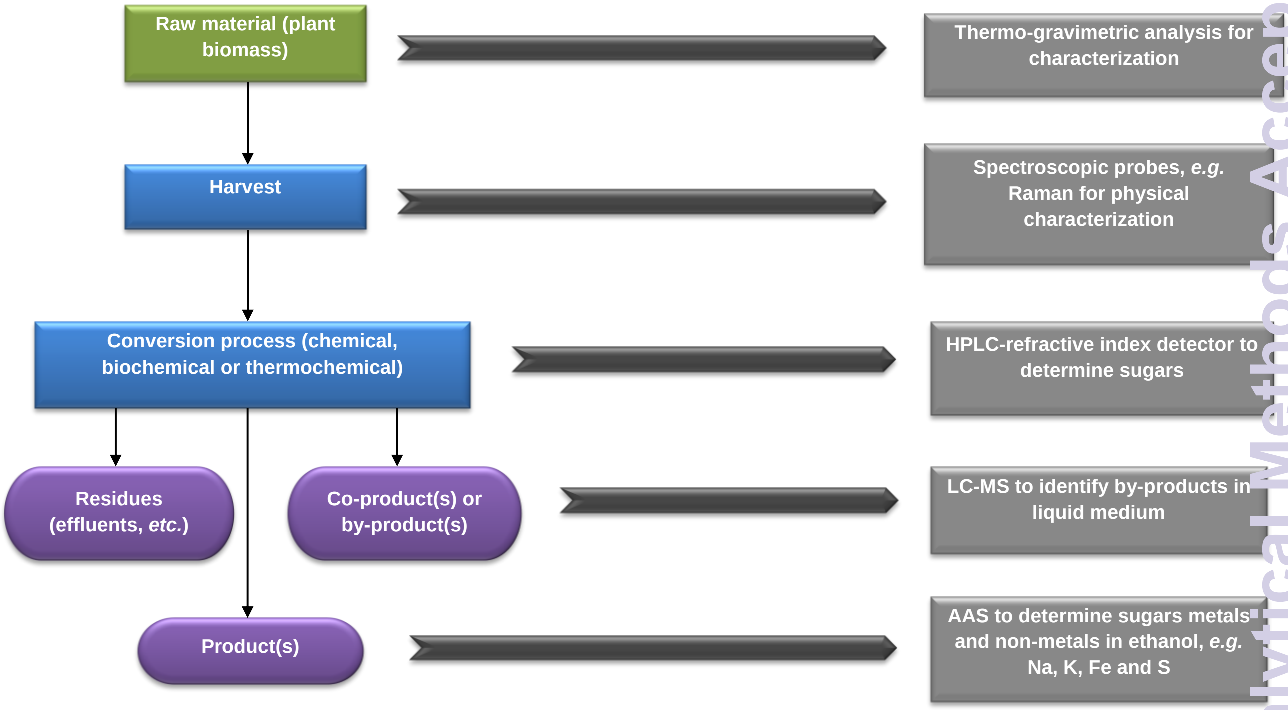

Fig. 2 Typical flowchart for an economic biomass chain (left). Chemical analyses could be involved in several steps, from raw material to products and residues (right)

Challenges to be overcome are basically related to a high heterogeneous chemical content - which becomes a characteristic of their products and by-products; methods of sample treatment cannot modify the structure of biomass components to be studied and produce only minimal modification of the molecules. Conversion processes need to be monitored in situ to provide reliable data. A good analytical approach must to cover: (i) composition of raw material, (ii) monitoring the conversion process, (iii) monitoring the effluent generated, (iv) composition of products and by-products; here we need to consider the cases specialties. A source of doubts for the analyst is the choice of technique and method within several possibilities. To the right choice is important to know the basis of various techniques, conditions in which these techniques are applied, the possible interferences, the desired accuracy, the amount of sample and the time and cost of analysis.

There is a set of points to be considered when planning an analytical strategy for biomass materials, comprising:

- Homogeneity of the sample;
- Understanding of the information required (*e.g.*, chemical composition, characterization of properties, *etc*.);
- Low cost and large number of samples;
- Standards for analysis and their variations across countries;
- Quality control *vs.* quality assurance;
- Interesting new area – renewable content requirements for laws in some countries; we need to access the analytical requirements for this.

Examples of applications of techniques for raw materials, quality control and quality assurance, R&D and real time analysis are treated in this item.

3.1. Determining composition of raw materials

What we need to know during the analysis of raw materials depends on the biomass usage. For instance, oleaginous to produce protein or biodiesel needs different analytical parameters than those for sugarcane to produce ethanol or saccharose. On the other hand, different techniques and methods could be used to obtain the information.

The analysis of the chemical composition of raw materials from biomass commonly includes analytical techniques that provide a rapid response (the shortest period of time between the beginning of the measure and the result), since the results will lead to the acceptance or not of the material for a production process, having direct financial implications for the early stages of production chains. Table 4 shows some examples of the use of analytical techniques in this step.

Despite the specificities of each technique, they usually have a system operating with a similarity level, which tends to facilitate intuitive application of these techniques by the professional who already has theoretical and practical knowledge of analytical chemistry. This has a direct influence on the solution of industrial and scientific challenges in an analytical laboratory, where methods must be validated and, in many cases, developed before anything.

Table 4 Examples of analytical techniques widely used in analyses of chemical composition of raw materials from biomass

Raw material	Parameter	Analytical technique	Reference	Pros	Cons
Sugarcane for ethanol production	Content of sugars	HPLC-refractive index detector	27	Methods established	Long acquisition time for chromatographic run (approximately 30 min)
Vegetable oils for biodiesel production	Content of fatty acids and esters	GC-flame ionization detector	28	Methods established	Necessity of organic solvent to extract the analyte
Bioenergy crops	Energetic characteristics	Near infrared spectroscopy	29	Rapid response and easy to handle	Low band resolution, which can be improved by chemometrics application
Residues for gasification	Energy content	Differential scanning calorimetry	30	Rapid response and easy to handle	-

The chemical composition of biomass is highly heterogeneous and demand robust methods of sample preparation. In the specific case of determining the content of cellulose, hemicellulose and lignin, the sample preparation takes place by drying, lyophilization, milling, acid treatment (preferably for cellulose and hemicellulose) and basic treatment with or without the presence of organic solvents (for lignin), followed by chromatographic analysis. Institutions such as the National Renewable Energy Laboratory (NREL), the International Lignin Institute (ILI) and the American Society for Testing and Materials (ASTM) are seeking for standardization and publication of preparation procedures, besides a complete analytical methodology.[31-33] Rocha *et al.* determined the composition of 50 samples of Brazilian bagasse related to different soil type and tillage for usage in the production of second-generation ethanol [34] (Table 5).

Table 5 Chemical composition determined for 50 samples of Brazilian bagasse[34]

Parameter	**Range of content (% m/m)**	**C.V. (%)**
Cellulose	40.54 – 46.17	3.5
Hemicellulose	18.90 – 26.90	7.5
Lignin	19.95 – 26.48	6.5
Extractives	1.96 – 13.29	55.6
Ash	1.01 – 5.50	43.8

3.2. Quality control of biofuels

Quality control (QC) of the final product requires a large number and variety of chemical analyses to compare with physical and chemical parameters of quality, often established by regulatory legislation. Parameters and their values depends on biofuel usage, physical state and properties and chemical composition; furthermore, we need to consider relevant aspects of national or international regulatory legislation – each country has its legal trade policy to be followed for bioenergy; there is a worldwide effort to unify parameters and methodologies.[35] Table 6 shows the Brazilian parameters for quality control of ethanol, an important biofuel for the energetic matrix. It may be noted a variety of analytical techniques applied to QC, positively reflecting on the quality of the final product; furthermore, techniques for this propose are easy to handle (volumetry, potentiometry and gravimetry) or have a high resolution (ion chromatography, gas chromatography and atomic absorption spectrometry).

Table 6 Some analytical parameters of quality for the Brazilian ethanol (anhydrous and hydrated) for fuel usage[36]

Parameter	**Unity**	**Specification**		**Method**	**Technique**
		Anhydrous	**Hydrated**		
Acidity (max.)	mg/L	30	30	ASTM* D7795	Volumetry
pH	-	-	6 – 8	ASTM D6423	Electrochemistry (direct potentiometry)
Residues (max.)	mg/100mL	5	5	ASTM E1690-08	Gravimetry
Chloride content (max.)	mg/kg	1	1	ASTM D7328	Ion chromatography
Ethanol content (min.)	% v/v	98	94.5	ASTM D5501	Gas chromatography-flame ionization detector
Sulphate content (max.)	mg/kg	4	4	ASTM D7328	Ion chromatography

Iron content (máx.)	mg/kg	5	5	ASTM D6647	Atomic absorption spectrometry

*ASTM = American Society for Testing and Materials

For a quality assurance (QA) of analytical methods and results we can apply procedures from the document Principles on Good Laboratory Practice (GLP) from the Organisation for Economic Co-operation and Development (OECD).[37] These procedures comprise:

- Responsibilities of the quality assurance personnel;
- Test systems;
- Receipt of samples, handling, sampling and storage;
- Standard operation procedures;
- Performance of the study;
- Reporting of study results.

QC and QA tools must be applied together to obtain the best confidence for biomass products like a biofuel.

3.3. Research and development of products and processes

Research can be conceptualized as an activity focused on problem solving, by employing scientific processes, including problem formulation and application of a scientific method to obtain the solution.[38] Allied to this concept, R&D activities are aimed, among other goals, to give practical application to the results generated in the preliminary stage of research, or search for the solution, either in laboratory or industrial scale.

Generally, in this step the use of a technique depends on the analytical needs arisen during the experimental work (*e.g.*, identification of products and by-products of an innovative process). It may be mentioned in this context a research aiming to produce second-generation biofuels, biomass gasification, materials and chemicals from lignin or lignocellulosic sugars.

It is worth noting that currently the increase in use of hyphenated techniques has shown gain in separation and detection.[39] These techniques are characterized by the union of two or more analytical techniques (*e.g.*, solid phase microextraction-GC-mass spectrometry, SPME-GC-MS), which can optimize the sample preparation, time and costs involved. Certainly, such techniques are also very useful in research of biomass and might have more uses following the emergence of new challenges for production processes and analyses.

The recent approach of innovative multidimensional separation techniques for complex chemical mixtures could be extended to biomass. Liquid-phase coupled to gas-phase can generate LC-GC and LC-GCxGC; supercritical fluid-phase coupled to gas-phase generates SCF-GCxGC; and liquid-phase coupled to liquid-phase generates LCxLC. Hyphenation with MS could generate GCxGC-MS and LCxLC-MS. Their advantages rises from possibility to work with different selectivity and distinct retention profile from each one, what could improve the number of molecules detected by mean high resolution separations; however, a chemometric data treatment is required to obtain a consistent result.[40] Good examples are the use of GCxGC-FID and GCxGC-MS for quantitative analysis of crude and stabilized bio-oil.[41] Similarly, LCxLC is growing as a high separation tool that should be taken in account for complexes products from biomass conversion, as lignin derivatives. Table 7 describes improvements in analytical methodology from hyphenated techniques when compared against more conventional techniques.

Table 7 Comparative differences between some new hyphenated techniques and conventional techniques

Hyphenated techniques	**Conventional techniques**	**Improvements**	**Sample**	**Reference**
GCxGC-FID	GC-FID	Separation efficiency Sensitivity for complex samples	Lignin derivatives in aqueous medium (phenols and hydrocarbons)	42
LC-MS^n	LC-MS	Sensitivity	Hydroxycinnamates from leaves (plant secondary metabolites)	43
Py-SPME-CG-MS	Py-GC-MS	Time (without laborious sample treatment) Costs	Anhydrosugars produced by pyrolysis of hexoses, pentoses and deoxyhexoses from natural gums	44
SFC-GCxGC-FID	GCxGC-FID	Time Costs Safe	Mixtures containing alkanes, aromatics, PAHs,	45

	nitrogenated organics, and sulfur-containing organics

Furthermore, direct spectrometric techniques as DESI-MS (desorption electrospray ionization-mass spectrometry) and DART-MS (direct analysis in real time-mass spectrometry) could be used to study biomass components, as saccharides and oils, in a short period of time.[46, 47] LIBS (laser induced breakdown spectroscopy) rises also as a possibility of direct technic to determine elemental composition and polymer constitution.[48, 49] NMR and NIR compact devices are has been increasingly used for rapid measurements in fields to determine oil content in seeds,[50] and biomass content above ground in crop harvest,[51] what can be applied for sugarcane and corn straw management.

NMR has an important role in the study of the biomass components, mainly for the lignin structure. For instance, 2D HSQC NMR (two dimensional – heteronuclear single-quantum coherence) with ^{1}H and ^{13}C heteronuclear couplings can be applied to identify monomeric and dimeric structures present in lignin.[52] Furthermore, ^{31}P NMR can be used as a marker for labeling hydroxyl groups to determine lignin composition and to understand their degradation mechanisms, especially during wood liquefaction.[53]

3.4. Monitoring of conversion processes in real time

The need for monitoring conversion processes in real time led to the creation of the term *process analytical chemistry* or PAC, often also called process analytical technology or PAT. PAC/PAT - as an area of research, innovation and application - favors the use of techniques and robust methods, preferably in real time and direct mode (on-line), with analyses performed directly in the reactor, rather than analyses in laboratory.[54, 55] A main advantage of this approach is that analyses *in situ* provide faster result for taking corrective action, and consequent adjustment on the process development or production. An example is the monitoring of variables such as temperature, pH, pressure, formation of products and by-products, including others, to ensure the quality of the final product.

Nevertheless, the need to have robust analytical instrumentation, such as electrochemical sensors for simple and automated use, limited the number of analytical parameters to be analyzed; values for limits of detection (LOD) and quantification (LOQ) hardly reach laboratory values. However, the continuing development of new analytical technologies and new materials will certainly increase the chances of obtaining best results, by accepting greater variation in physiochemical conditions of the reactive medium, which allows a better identification of chemical species. In the latter case, can be used detectors of absorption in the ultraviolet (UV), and near (NIR) and medium infrared (MIR) with Fourier transform (FTIR).[55]

Applications of the approach PAC/PAT by the use of methods of flow analysis for chemical and biochemical processes can be noticed.[56] Flow analysis is a class of instrumental technique that allows analyses *in situ*, and their methods can serve well to the concept presented here. A good example is presented in the Fig. 3, where a conversion process for sugar is accompanied by PAC based on FIA system.

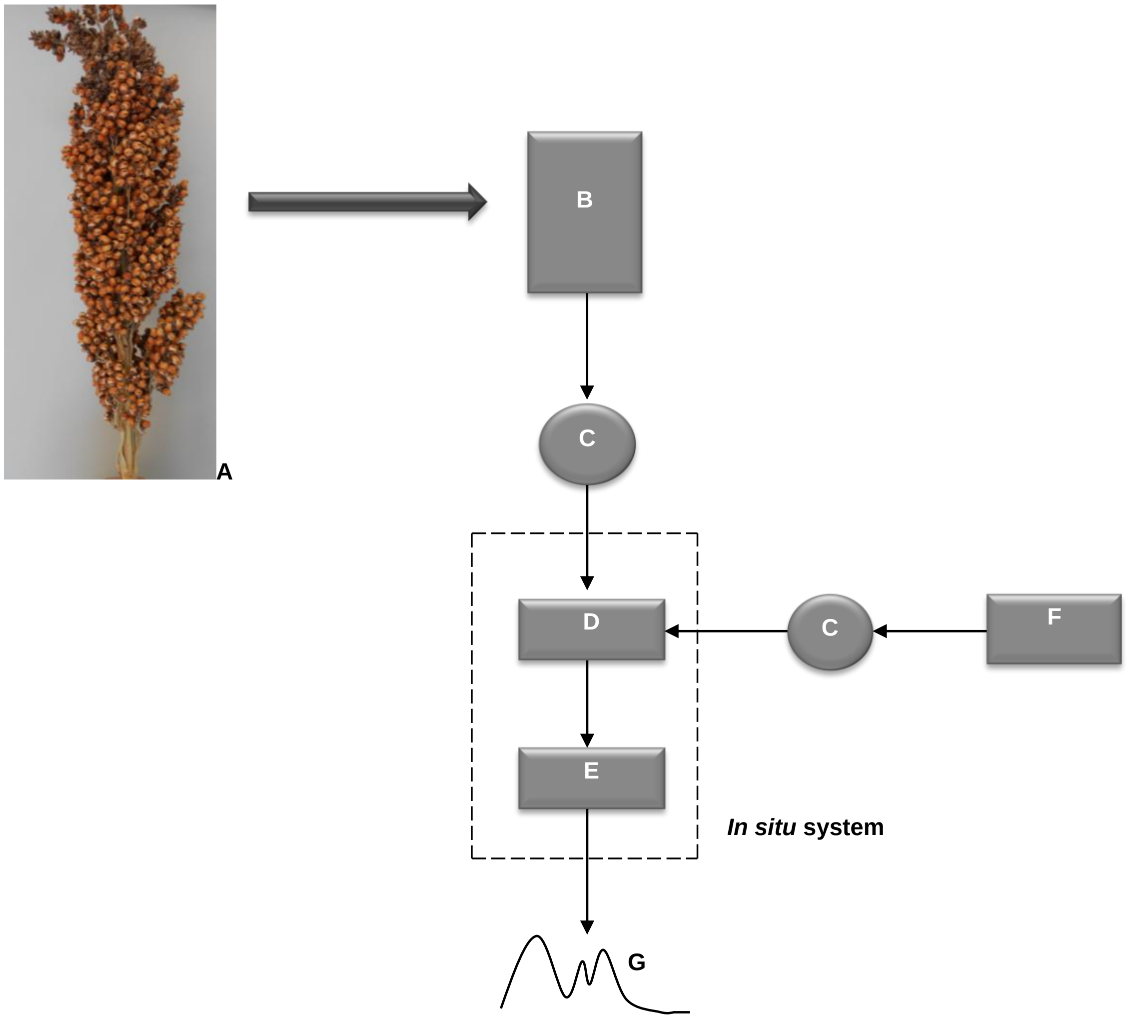

Fig. 3 PAC concept based on FIA technology, where: (A) is the biomass (sorghum); (B) reactor for conversion process; (C) pump; (D) microreactor for analytical reaction; (E) UV detector; (F) reagent vessel; and (G) is the produced signal

3.5. Alternative methodologies for treatment of biomass samples

Plant biomass has an intrinsic heterogeneity due to its chemical constitution, as a result of the different molecular structures of the main components (cellulose, hemicellulose, and lignin) and others (proteins, oils, *etc*.), which may vary depending upon the plant species, climate, soil type and tillage. Its heterogeneity is reflected in the products, co-products, by-products, waste and liquid effluents from conversion processes.

Most of the preparatory methods for samples was developed using concentrated acids for aggressive attack in order to release the analyte from the matrix. However, the use of basic reagents have enabled the application of preparation procedures such as extractions and digestions,[57] that can reduce costs, negative impacts on environment and occupational hazards. Furthermore, these alternative strategies can contribute to the establishment of green chemistry principles for analytical chemistry (Fig. 4, item 4).

An example of basic digestion is the determination of Ca, Fe, Mg, Mn and Zn in lignocellulosic biomass by atomic absorption spectrometry with good accuracy and precision, where the sample is treated for the extraction of analytes with carbonate and sodium hydroxide and ethylenediaminetetraacetic acid (EDTA),[58] which can be extended to another spectrometric atomic techniques, as graphite furnace absorption (GFAAS) and inductively coupled plasma-optical emission (ICP-OES). Determination of these elements is important: in catalytic processes of biomass conversion[3] such metals could influence on the catalyst performance and alter the reaction kinetics. Na, K, Ca and Mg can be determined in biodiesel using the same analytical technique, but with a sample preparation through formation of microemulsions, providing an increase in the stability of the signal.[59]

An example of time optimization is the determination of glucose for second-generation ethanol production from lignocellulosic biomass, by using Raman spectroscopy. In this case, it requires little sample preparation, with this being only filtrated.[60] Thus, the extent of the preparation procedures will depend directly on the physical characteristics of the analytical instrument, as well as the phenomenon that allows the taking of the measurement.

Typically, methods and preparation procedures are the focus of constant improvement and optimization, aiming to achieve higher effectiveness in determining the real concentration of an analyte, or higher accuracy. A technology that permits hyphenation mode (preparation-separation-detection), as solid-phase extraction (SPE) and microextraction (SPME), *etc.*,[61] provides an advance on biomass knowledge and use.

Ionic liquids (IL) have drawn attention in recent years due to their special properties, which can be used advantageously in analytical chemistry as an alternative for organic solvents for preparative step. Their properties as high thermal stability, negligible vapor pressure, and non-flammability, in addition to varying viscosities, conductivity, and miscibility in different solvents can be used to dissolve samples for analyses by means chromatographic, electrophoretic and electrochemical techniques.[62] Besides, IL can promote a green analytical process and improve the extraction efficiency,[63] reducing time and solvent consumption.

3.6. Relevant considerations

It is vital to have a management plan for the analytical process to be applied. As considered in the item 3.2, the plan should be structured according to procedures from GLP, including studies on environmental impacts of chemical species and data control to determine reliability and reproducibility.[37] In some cases, it is necessary also to follow the norm ISO 17025,[64] which establishes the criteria and procedures for accreditation of the analytical laboratory.

The use of chemometrics for planning and data treatment can be seen as a powerful mathematic tool in cases where only analytical technique is not enough to provide qualitative or quantitative information. This is very common when a large amount of variables is present (*e.g.*, chemical composition, concentration, wavelength, absorption intensity, *etc*.) requiring a multivariate analysis. Some examples are the use of partial least square (PLS) regression method in the MID analysis of biodiesel[65] and the use of principal component analysis (PCA) model in the NIR analysis to determine chemical composition of biomass based on exploratory analysis.[66]

In respect to the most recent technological development for time otimization, miniaturization techniques as lab-on-a-chip and microfabrication offer personalized analytical systems, lower energy demands, ultra-fast analysis, and high throughput; but this is not completely ready to use[67] and overcoming of technical challenges related to fabrication will determine their applications for biomass and other complex samples. However, this is a good opportunity to improve separation sciences, and to access data in real time and mobile mode. Additionally, the use of smaller columns and ultra-high-pressure pumps for ultra-high performance liquid chromatography (UHPLC) has promoted liquid chromatography achieves separation efficiency near to GC,[61] what can help in time optimization, mainly, for QC.

4. Green analyses for a sustainable analytical chemistry

Armenta and colleagues discussed broadly the creation of the term green analytical chemistry, its milestones and examples of application, namely: (i) sample treatment; (ii) oriented scanning methodologies; (iii) alternatives to toxic reagents; (iv) waste minimization; (v) recovery of reagents; (vi) on-line decontamination of wastes; and

(vii) reagent-free methodologies.[61] Thus, it should be considered that the analysis of biomass should be based on the 12 principles of green chemistry,[68] since the context of their application is reflected in the sustainability of raw materials and processes. For instance, the application of 7 from the most representative principles for analytical chemistry will contribute to achieve a more sustainable analytical methodology, what is presented in Fig. 4.

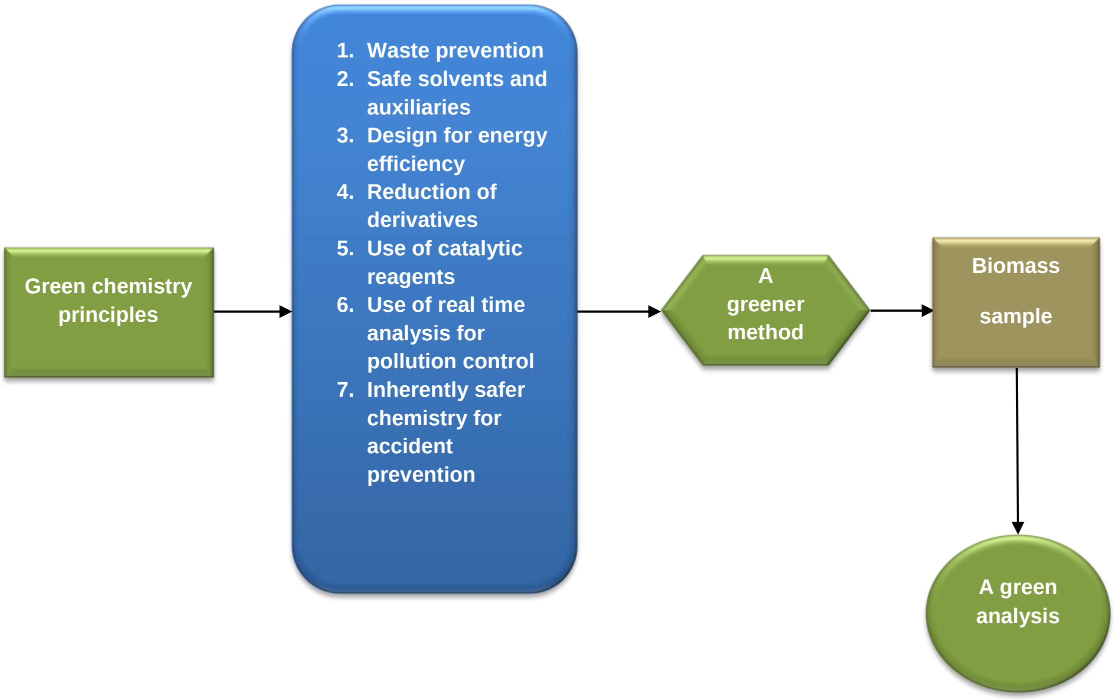

Fig. 4 Application of 7 most representative principles from 12 green chemistry principles to develop a green analysis of biomass

Waste prevention, safe solvents and auxiliaries, energy efficiency and inherently safer chemistry for accident prevention are obvious for all chemical operations. Safer chemicals, reduction of derivatives, and use of catalysts should be taken in account for each analysis because each analytical process has its technical particularities; the use of real time analysis for pollution control is a good opportunity for technology development in analytical chemistry, by means an *in situ* systems for effluent analyses (gaseous and liquids). In a large number of cases is not possible to apply all of these principles due to sample or medium particularities, but is very important to consider one by one in an analytical process. This exercise will ensure the “greener” of the analysis.

As a practical guidance, De la Guardia and Garrigues established the main objectives to be considered for a green analytical chemistry:[69] (i) simplification; (ii) reagents selection to avoid based on toxicity, renewability or degradability data; (iii) maximization of information; (iv) minimization of consumes, considering number of samples, volumes or masses of reagents, energy consumption; and (v) detoxification of wastes. These objects will define the best strategy to be applied, as a result of the principles presented in the Fig. 4. We can consider as an example based on these green objectives the supercritical-fluid chromatography in the analysis of fatty acid ethyl esters,[70] where a supercritical fluid is used as the mobile phase to reduce time of analysis, solvent quantity and effluent generation.

5. Conclusions and trends

Chemical analysis of biomass is an enthusiastic branch of analytical chemistry because it can provide information about constitution of raw material, conversion processes, products, by-products and residues. Then, this can be applied on a whole production chain to solve many technical, scientific and economic problems.

Currently, we have a lot of sophisticated techniques and methods and the understanding of their principles is very necessary to take all potential for a better application. On the other hand, process analytical chemistry enables monitoring processes in real time, what can promote a gain in time for information collection in industrial processes. In both cases, is fundamental to establish a management plan to ensure the data reliability, besides to consider the use of chemometrics for multivariate analysis.

Sample preparation in a state that permits its analysis can bring difficulties to the analyst, because biomass is highly heterogeneous. So, the analyst needs to see the sample as a challenge to be attacked with strategies that favors a use of greener reagents, little volume of solvents, and hyphenation or automation possibilities.

Green chemistry and sustainability of processes and products are themes that passed from academic discussion to industrial usage. Then, analytical chemistry as part of chemical sciences should follow this current trend, what can contribute for a bioeconomy based on biomass usage instead non-renewable raw sources, as the oil, contributing to reduce negative environmental impacts from the modern society.

Regarding to trends for chemical analyses of biomass, some points can be highlighted:

- ✓ Increasing in the use of spectroscopic probes to monitoring conversion processes, as Raman and FTIR; these techniques can reduce time and costs without previous treatment or with minimal sample processing;
- ✓ Decreasing in invasive techniques, due to the necessity to study raw material components without modification on their chemical structure -

this is the case of lignin; here is a good business opportunity for compact systems (*e.g.*, NMR and NIR);

- ✓ Increasing in the use of hyphenated techniques for complex samples, to possibility a better detection and quantification of products and by-products from conversion processes and raw material;
- ✓ Increasing in the miniaturization and automation of analytical systems, to achieve larger analytical capacity in laboratories; an automated laboratory can run analyses 24 hours per day;
- ✓ Establishment of recognized worldwide methodology for the quality control of raw material and products, as oleaginous biomass and biodiesel or sugarcane and ethanol; this is very important for a biobased global economy;
- ✓ Increasing in the use of green methods, to reduce negative impacts on environment and health.

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