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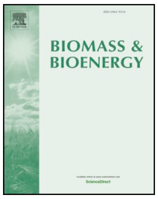

Research paper

Furfural, 5-HMF, acid-soluble lignin and sugar contents in *C. ladanifer* and *E. arborea* lignocellulosic biomass hydrolysates obtained from microwave-assisted treatments in different solvents

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A B S T R A C T

Cistus ladanifer L. and *Erica arborea* L. are the two most representative shrub species from the Iberian Peninsula. With a view to their valorization, their biomass hydrolysate components, obtained from microwave-assisted treatments with choline chloride/urea - HNO_3 10%, *N,N*-dimethylacetamide/$NaHCO_3$ and *N,N*-dimethylacetamide/CH_3OK as solvents, have been measured using a spectrophotometric method. Concentrations of furfural and 5-(hydroxymethyl)furfural (5-HMF) in the filtrate have been determined after reduction with $NaBH_4$. The production of total sugars, reducing sugars and non-reducing sugars has also been assessed. The obtained results support the choice of microwave-assisted choline chloride/urea deep eutectic solvent in acid media as the preferred method (over the polar aprotic solvent-based alternatives) for the extraction of lignin, furfural, 5-HMF and sugars from *C. ladanifer* and *E. arborea* biomass, attaining the best production yields for 60 min exposure times. Another is the case if the aim of the treatments is to recovery sugars from both shrubs for subsequent enzymatic saccharification: the very low 5-HMF contents resulting from the dimetylacetamide systems (especially is association with CH_3OK) make them highly advantageous as compared to the traditional method using NaOH.

1. Introduction

Lignin, interlaced with cellulose and hemicellulose, forms a complex crystal structure called lignocellulose that provides support and protection to plant cells [1]. This matrix is difficult to degrade, requiring treatments that break down its structure, hydrolyze the hemicellulose and increase the exposed surface to favor the enzymatic hydrolysis of cellulose [2]. For this purpose, different approaches may be used: physical processes, such as grinding or heating; chemical methods, such as the addition of acids or bases; physical-chemical treatments, such as self-hydrolysis or thermo-hydrolysis; and biological ones, such as the use of enzymes capable of degrading lignin (ligninases or lignin-modifying enzymes, LMEs).

In conventional biomass treatments, thermochemical pretreatments are generally carried out at high temperatures or high operating pressures in order to achieve high cellulose conversion. An alternative to conventional heating is the application of microwave (MW) radiation [3–5], in which the direct contact between the product and the electromagnetic field generated by the microwaves produces a volumetric heating that causes an instantaneous temperature increase [6,7], resulting in an acceleration of the process and higher yields under milder reaction conditions with significant energy-savings [8].

The composition of the liquid phase of the treatments includes organic acids (mainly acetic acid, formic acid and levulinic acid), furan derivatives (2-furfuraldehyde, furfural and 5-(hydroxymethyl)-2-furaldehyde), and phenolic compounds (mainly coumaric acid, syringaldehyde and vanillin) [9,10]. Under acidic conditions, and especially at high temperatures, furfural is readily produced from pentoses and 5-HMF is formed from hexoses. Because both furfural and 5-HMF are formed from carbohydrates, they interfere with the accuracy of sugar analysis of any biomass materials. Furthermore, both are harmful to the fermentation of sugars if their concentrations exceed certain thresholds [11].

In the study presented herein, the suitability of various MW-assisted

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treatments in different eco-friendly reaction media has been investigated with a view to breaking the intricate structure of the lignocellulosic biomass obtained from two Mediterranean shrubs (viz. *C. ladanifer* and *E. arborea*). One of the proposed treatments involves an innovative solvent category: the so-called deep eutectic solvents (DESs), which consist of a hydrogen bond donor and a hydrogen bond acceptor, associated with each other by means of hydrogen bond interactions, resulting in a eutectic mixture with a melting temperature much lower than that of its constituents. DESs have advantages over conventional ionic liquids (ILs), characterized by the formation of strong ionic bonds, since the later are more expensive and toxic [12]. The other two assayed solvents have been mixtures of a polar aprotic solvent (*N,N*-dimethylacetamide, DMAc) with weak and strong bases (namely sodium bicarbonate ($NaHCO_3$) and potassium methoxide (CH_3OK), respectively). Both categories of solvents can be used as environmentally friendly replacements of conventional solvents and processes with a view to optimizing biorefineries, aiming at a greener and more sustainable industry. The efficiencies of these three reaction media (a DES mixture of choline chloride:urea and HNO_3 10%, DMAc/$NaHCO_3$ and DMAc/CH_3OK) have been compared in terms of lignin, furfural, 5-HMF and sugars extraction.

2. Materials and methods

2.1. Samples and reagents

The study was carried out on a plot located in the municipality of Ayoó de Vidriales (42° 07′ 10″ N, 6° 06′ 59″ W), in the province of Zamora, Castilla y León, Spain. The chosen area (> 1.2 ha) is a mixed shrubland in which the dominant shrub species are *Erica arborea* L. subsp. *angustifolius* (Daveau) Sennen & Pau, and *Cistus ladanifer* L. cultivar 'Spanish Lime'. Details on the sampling procedure, analogous to that described by Ruiz-Peinado et al. [13], have been reported in a previous paper [14]. There were no size fractionation processes, which are common in annual and perennial species. Selected samples corresponded to healthy individuals and featured similar characteristics to the rest of the population.

Samples of biomass (mainly from the stem) were dried in a stove and crushed in a knife mill. Their chemical composition (elemental analysis, summative constituent analysis, and moisture content) can be found in Table 1:

Furfural (CAS No. 98-01-1), 5-HMF (CAS No. 67-47-0), lignin (CAS No. 8068-05-1) and D-(+)-glucose analytical standards (CAS No. 50-99-7) were purchased from Sigma-Aldrich Química SL (Madrid, Spain). The standard solutions were prepared with deionized water. Sodium borohydride (CAS No. 16940-66-2), 3-amino-5-nitrosalicylic acid (DNS, CAS No. 831-51-6), phenol (CAS No. 108-95-2), choline chloride (ChCl, CAS No. 67-48-1), urea (CAS No. 57-13-6), titanium dioxide (CAS No. 13463-67-7), *N,N′*-dimethylacetamide (DMAc, CAS No. 127-19-5), sodium bicarbonate (CAS No. 144-55-8), potassium methoxide (CAS No. 865-33-8), sodium hydroxide (CAS No. 1310-73-2) and potassium sodium tartrate (CAS No. 6381-59-5) were also supplied by Sigma Aldrich.

Table 1
Overall chemical composition of *E. arborea* and *C. ladanifer* [14,36]. Values are given as an average of 25 repetitions, followed by the minimum and maximum values in brackets.

	Erica arborea	*Cistus ladanifer*
Elemental analysis:		
C (%)	51.0 (49.3–52.8)	47.8 (47.5–50.1)
H (%)	6.2 (6.0–6.4)	6.4 (6.0–6.8)
N (%)	1.0 (0.3–1.1)	0.8 (0.3–1.9)
O (by diff., %)	~41.8	~45.0
Vegetal components:		
Cellulose (%)	40.0 (37.3–41.1)	55.0 (54.9–55.7)[a]
Lignin (%)	39.5 (39.3–40.1)	25.3 (24.5–34.2)
Hemi-cellulose (%)	11.0 (9.7–13.8)[b]	10.2 (10.1–10.9)[b]
Extractive (%)	9.5 (5.7–11.0)	9.5 (9.4–9.6)
Moisture (wt.%)	26.0	26.8

[a] This cellulose content is higher than that of most woods, which is usually in the 35–50% range.

[b] These hemicellulose contents are lower than those of most woods, which usually range from 20% to 30%.

2.2. Methods

2.2.1. Microwave-assisted deep eutectic solvent system

A deep eutectic solvent system based on choline chloride-urea (Fig. 1) was assessed for the hydrolysis of *C. ladanifer* and *E. arborea* biomass. Choline chloride/urea DES was prepared by stirring the mixture of choline chloride and urea (mole ratio 1:2) at 80 °C until a homogeneous colorless liquid was formed, which was then stored in a vacuum dryer. Subsequently, biomass samples (200 mg) were treated by a mixture (1 cm^3) of choline chloride/urea and HNO_3 10%, with TiO_2 (20 mg) as a catalyst, in a microwave digestion system –a Milestone (Sorisole, BG, Italy) Ethos-One microwave oven equipped with a magnetic stirrer system– at 120 °C for an “effective time” (isothermal treatment time) between 1 and of 60 min (*viz.*, 1, 5, 10, 20, 30, 40, 50 and 60 min), plus the heating and cooling ramps, which also contributed to the thermal budget. The heating up to 120 °C started with a ramp set to 19 $°C{\cdot}min^{-1}$ during the first 5 min, followed by a second ramp at a rate of 2.5 $°C{\cdot}min^{-1}$ for 10 min. The cooling down to room temperature took 25 min, at a rate of ~4.8 $°C{\cdot}min^{-1}$. The DES was finally removed by washing with water and was recovered by crystallization.

2.2.2. Microwave-assisted DMAc-sodium bicarbonate system

Alternatively to the DES-based method, 8 cm^3 of a colorless, water-miscible, high boiling liquid –viz. *N,N′*-dimethylacetamide (DMAc) with formula $CH_3C(O)N(CH_3)_2$– was used, in the presence of 40 mg of sodium hydrogen carbonate ($NaHCO_3$), as a treatment agent for *C. ladanifer* and *E. arborea* wooden samples (200 mg of biomass samples). The same procedure explained above for the microwave-assisted DES treatment was followed for the polar aprotic solvent-based treatment. DMAc was removed by washing with water and recovered by distillation.

2.2.3. Microwave-assisted DMAc-potassium methoxide system

The third approach investigated for the hydrolytic treatment of *C. ladanifer* and *E. arborea* biomass (200 mg) was based on a mixture of 8 cm^3 of DMAc with 40 mg of potassium methoxide (commonly used as a catalyst for transesterification in the production of biodiesel). The solution was then treated as in the previously discussed methods.

2.2.4. Alkaline treatment

Solutions were prepared with 200 mg of each sample and 2 cm^3 of NaOH (4 $kg\,m^{-3}$), which were stirred for 24 h. From these solutions, 0.3 cm^3 of each sample were isolated and then diluted to 25 cm^3 (to keep the concentration within the spectrophotometer measurement range and to avoid absorption flattening due to saturation). When necessary, HCl was used to keep a neutral pH.

2.2.5. Acid-soluble lignin, furfural and 5-HMF contents

The acid-soluble lignin (ASL), furfural and 5-(hydroxymethyl)-furfural contents were determined according to the methodology proposed by Chi et al. [15], based on the measurement of their respective maximum absorbance at 205 nm, 277 nm and 285 nm, and which makes use of the effect of the reduction with borohydride on the furfural and 5-HMF maxima mentioned above. For these latter two chemical species, their initial absorbance in the UV–vis spectrum was measured and, after the addition of 30 mg of sodium borohydride to eliminate the