Ultrasound-assisted Dilute Acid Pretreatment of Coffee Silverskin for Biorefinery Applications

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Coffee silverskin (CSS) is an agro-food processing waste (AFW) largely produced in roasting factories at almost constant rate over the year. The CSS can be used as a source of fermentable sugars within a biorefinery approach for its exploitation. Pretreatment process and enzymatic hydrolysis are required to efficiently recovery monomeric fermentable sugars from AFWs such as CSS. Current research is focused on the selection of novel processes for biomass pretreatments able to provide effective lignin removal, to minimize the production of by-products that may inhibit the fermentation of the produced sugars, and to be cost saving with respect to conventional pretreatments.
The aim of the present study was to investigate the effect of the ultrasound (US) assisted dilute acid pretreatment on the effective separation of lignin and polysaccharides of CSS. The effects of two operating conditions – biomass loading and sulphuric acid concentration - were studied to assess their effects on the sugar yields after enzymatic hydrolysis. The production of typical species that may inhibit the fermentation of the hydrolysate was monitored. Inhibitors like furfural, 5-hydroxymethyl furfural (HMF), ferulic and p-coumaric acid were present at concentrations lower than 1 mg/L after enzymatic hydrolysis of the pretreated CSS.
As a result, the hydrolysate of US dilute acid pretreated CSS may be used for fermentation without detoxification step.

1. Introduction

The shift from fossil resources to renewable biomass-based resources is generally viewed as the key to the development of a sustainable industrial society and to the effective management of greenhouse gas emissions (Ragauskas et al., 2006). In particular, the recent trend of the development of eco-sustainable processes based on the biomass resources is the exploitation of biomass feedstock according to the biorefinery concept. The overall goal of the biorefinery production concept is the production of a variety of goods (fuels, chemicals, etc.) from biomass feedstocks by means of a combination of technology/processes aimed at the exploitation of all components of the biomass (Fitzpatrick et al., 2010). Biomass feedstocks include agro-food wastes (AFWs). Indeed, recent investigation points out that AFWs are a promising option for the production of marketable chemicals and/or biofuels, being economic and eco-friendly due to their unique chemical composition, large availability, sustainability and low costs (Sud et al., 2008). AFWs are mainly composed of organic materials such as carbohydrate polymers, proteins, lipids, lignin and organic acids (Ezejiofor et al., 2014).
Coffee Silverskin (CSS) may be classified as AFWs. CCS is the residual thin tegument wrapping the green coffee beans and it is produced during beans roasting (Esquivel and Jiménez, 2012). Typical composition of CSS is: 23.77 % cellulose; 16.68 % hemicellulose; 28.58 % lignin (dry matter) (Ballesteros et al., 2014). The global production of coffee beans is about 148 millions of 60 kg bags (ICO) and CSS is about 4.2 % (w/w) of coffee beans (Ballesteros et al., 2014). CCS is produced in roasting factories and it may be easily delivered as

renewable residues for biorefinery applications. The first step of CSS exploitation in a biorefinery is its pretreatment.

The pretreatment step is one of the main obstacles for the success of processes based on lignocellulosic biomass (Raganati et al., 2014). The main goals of the pretreatment process are to remove lignin (cross-linked polymers of phenolic monomers), to reduce the crystallinity of cellulose, and to increase the porosity of the lignocellulosic materials to enhance the efficiency of the enzymatic hydrolysis of cellulose and hemicellulose (Kumar et al., 2009). Conventional pretreatment strategies include acid, alkaline solvents, steam explosion, and wet oxidation (Velmurugan and Muthukumar, 2011). Conventional pretreatments are typically carried out at high temperature and produce compounds that may inhibit the fermentation step of hydrolyzed sugars. Recently, alternative pretreatments – e.g. lignin extraction by green solvent (ionic liquids/DES) pretreatment and ultrasound (US) assisted pretreatment - are investigated to increase the fraction of hydrolyzed sugars content and to reduce the inhibitor concentration (Procentese et al., 2015; Bussemaker and Zhang, 2013). US assisted pretreatment exploits high temperature (500−15000 K) and pressure (100−5000 atm) created for a period of microseconds by cavitation phenomena within the liquid phase to enhance physical and chemical disruption of the lignocellulosic biomass providing the separation of structural polymers (cellulose, hemicellulose and lignin) (Subhedar and Gogate, 2013). The use of aqueous solvents can enhance the chemical effects of the process (Luo et al., 2013). Enzymatic hydrolysis of pretreated biomass allows to release fermentable pentose and hexose sugars from carbohydrate polymers that can be used for the production of biofuels (e.g. butanol) (Procentese et al., 2014) and chemicals (e.g. succinic acid) (Ferone et al., 2016).

The aim of the present study was to investigate the application of US irradiation in the dilute acid pretreatment of CSS. Tests were carried out to assess the influence of operating parameters such as biomass loading and sulphuric acid concentration on sugar yield.

2. Materials and methods

2.1 Feedstock

Coffee silverskin was kindly supplied by Illycaffè SPA (Trieste, Italy) (Figure 1A). CSS was oven dried at 50 °C until constant weight was approached. The dry CSS was milled in a cutting mill (MF 10, IKA, Staufen, Germany) to a size lower than 0.5 cm (Figure 1B) and stored under controlled humidity. The raw moisture of CSS was 35%. Cellulose, hemicellulose and lignin content in CSS were assessed according to the US National Renewable Energy Laboratory protocols (Sluiter et al., 2008).

Figure 1: Raw CSS supplied by Illycaffè SpA (A); dried and milled CSS (B).

2.2 Pretreatment

Pretreatment tests were carried out in a 100 mL glass beaker. CSS powder was mixed in the beaker with a pre-fixed volume (40 mL) of H_2SO_4 solution. A probe type ultrasonic processor (VCX500, 20kHz, Sonics & Materials Inc, USA) equipped with an Epishear[TM] 6.4 mm probe (Active Motif, USA) was used for the US irradiation of the suspension. The beaker was housed in an ice bath to prevent the temperature increase of the suspension during sonication. The slurry produced after the sonication was washed with deionized water until the pH of supernatant increased up to 7. The biomass solid residue was recovered by centrifugation and it was oven dried at 50°C until a constant weight was reached. The recovered biomass solid was characterized in terms of cellulose, hemicellulose and lignin content.

2.3 Enzymatic hydrolysis

The enzymatic hydrolysis of the US pretreated CSS was carried out in 100 mL Pyrex® capped bottles: 1 g of US dilute acid pretreated biomass was incubated in 20 mL 0.1 M sodium citrate buffer pH 4.8 supplemented with 200 μL of 2% sodium azide. Hydrolysis was carried out using 0.015 $g_{enzyme}/g_{cellulose}$ of the cellulase cocktail Cellic CTec2 (Novozymes, Denmark) at 50 °C for 48 h. The bottles were housed on an orbital shaker operated at 180 rpm (SKI 4 Shaker Incubator, ARGO LAB, Italy). The hydrolysed sample was centrifuged to recovery the supernatant. The supernatant was filtered by means of a 0.2 μm filter (Millex, Ireland). The assessment of the reducing sugar in the filtered supernatant was carried out according to the 3,5-dinitrosalicylic acid method (Wood et al., 2012).

The enzymatic hydrolysis of pretreated samples was characterized in terms of ratio between the mass of the recovered sugars and the mass of sugars in the pretreated biomass (Y_S).

2.4 Operating conditions

Sample sonication lasted 8 min. The specific sonication power (energy for dry biomass unit) was 300 W/g_{DM}. Tests were carried out to assess the effects of biomass loading and acid concentration on ultrasound-assisted pretreatment of CSS. The biomass loading (mass of biomass for solution volume unit) ranged between 3 and 10% w/v. The concentrated H_2SO_4 (96%$_W$) was supplemented to the biomass suspension at dilution ranging between 0.5 and 2% v/v.

The table 1 list the set of operating conditions set in the test campaign. All the tests were carried out in duplicate and the average values were reported.

Table 1: Operating conditions of tests

Trial number	Biomass loading (%w/v)	H_2SO_4 conc. (%v/v)
1	3	2
2	3	0.5
3	10	0.5
4	10	2
Unpretreated	0	0

2.5 Inhibitor analysis

The concentrations of furfural, HMF, ferulic acid and p-coumaric acid were measured in the liquid phase of the CSS-pretreated and hydrolysed. The liquid samples were centrifuged, filtered by means of a 0.2 μm cut-off filter (Millex, Ireland), and analysed by means of a HPLC Agilent 1100 system (Agilent Technology, Palo Alto, USA). Inhibitors were separated by means of a Phenomenex Luna C18(2) column (250 mm × 4.6 mm, 5 μm) at room temperature. The mobile phase was 0.1% formic acid aqueous solution (A) and pure methanol (B). The gradient of the mobile phase was: 0-20 min 5% A/95% B-30% A/70% B; 20-40 min 30% A/70% B; 40-45 min 30% A/70% B; after 45 min 5% A/95% B. The volumetric flow rate was: 1.2 mL/min from 0 to 20 min; increase up to 1.5 mL/min between 20 to 21 min; 1.5 mL/min from 21 to 40 min; decrease down to 1.2 mL/min from 40 to 45 min. The injection volume was set at 10 μL. Separated products were detected by means of a photodiode array detector at 276 nm (Agilent Technologies, model G1365B). The concentration of inhibitors was measured with respect to standards.

3. Results and discussion

3.1 Composition of raw and pretreated CSS

Raw CSS and pretreated CSS were analysed according to the procedure reported in §2.1 in terms of cellulose, hemicellulose and lignin content. The composition is reported in Table 2. The total fraction of cellulose-hemicellulose-lignin in the pretreated biomass was higher than that assessed in the raw material: the pretreatment reduces the fraction of components such as ashes, proteins, and lipids. The lignin content seems not to be affected by the pretreatment according to potential dissolution and re-polymerization reactions

promoted by ultrasound (Bussemaker and Zhang, 2013). The fraction of cellulose/hemicellulose reduced with respect to the lignin for the samples 3 and 4 indicating a likely loss of sugars in the pretreatment solvent.

Table 2: Biomass composition after pretreatment

Trial number	Cellulose (%$_{DM}$)	Hemicellulose (%$_{DM}$)	Lignin (%$_{DM}$)	Tot analysed (%$_{DM}$)
1	25 ± 0.7	8.5 ± 0.8	34 ± 0.3	67.5 ± 2.1
2	24.5 ± 1.4	9 ± 0.7	34 ± 0.7	67.5 ± 2.8
3	24 ± 0.4	9 ± 0.6	35 ± 0.3	68 ± 1.3
4	23.5 ± 0.8	9 ± 1.1	36 ± 1.4	68.5 ± 3.3
Raw dry biomass	23.5 ± 1	7.5 ± 0.7	31 ± 0.8	62 ± 2.5

3.2 Hydrolysis of US-assisted pretreated CSS

The hydrolysis of raw CSS was characterized by sugar yield (*Ys*) of about 0.103 g/g. Figure 2 reports the results of sugar yields as a function of the biomass loading (BL) and of the sulphuric acid concentration (Ac). The analysis of the *Ys* suggests that the sugar yield of the pretreated biomass was larger than that assessed for the raw CSS. Although the fraction of lignin increased in the pretreated CSS, the US-assisted acid pretreatment was successful whatever the operating conditions set.
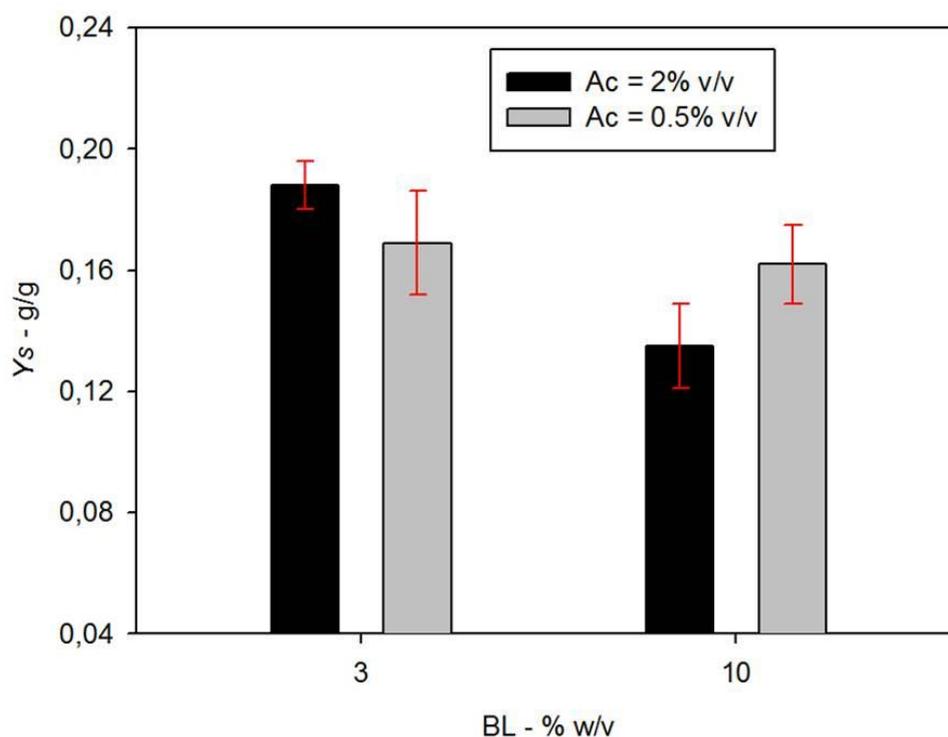
Figure 2: Sugar yield as function of biomass loading (BL) and acid concentration (Ac).

The effect of the biomass loading on sugar yield is presented in Figure 2. At the largest acid concentration investigated, the increase of biomass loading adversely affected the sugar yield. At low acid concentration effect of biomass loading on sugar yield seems to be negligible. It is possible to infer that at high biomass loading the accessibility of the biomass to pretreatment agents (acid molecules, radicals and other chemical species produced during cavitation phenomena) is reduced. In heterogeneous systems (slurry), the cavitation

activity is enhanced by the presence of the solids but at large concentration of solids the viscosity increases and the mixing reduces with the mass transfer and heat transfer (Rehman et al., 2013). The negative effect of large biomass loading on pretreatment of lignocellulosic biomass is in agreement with previous study reported by Noureddini and Byun (2010) for dilute acid pretreatment of distiller's grain and corn fibre without US.

Effect of acid concentration on sugar yield is also presented in Figure 2. At low level of biomass loading, the sugar yield slightly increased with the acid concentration: pretreatment occurring at low biomass loading allows much effective dispersion of the chemical species in the solution so that their interaction with the lignocelluloses provides much effective biomass modification.

Increase in acid concentration allows larger lignin conversion. At fixed level of lignin conversion, increase in biomass loading leads to increase of the concentration of products from lignin depolymerisation. These product can locally reach values large enough to promote lignin recondensation in polymeric species (Bussemaker and Zhang, 2013). This phenomenon can hinder effective dissolution of lignin at both large biomass and acid concentrations so that the composition and structure of pretreated biomass provides decrease in sugar yield.

3.3 Analysis of inhibitors

The analysis of the liquid phases of hydrolysis pointed out that the fermentation inhibitors - furfural, HMF, ferulic acid and p-coumaric acid - were present at concentrations lower than 1 mg/L. The concentration was lower than the toxic levels known for various *Clostridium* sp. (Ezeji and Blaschek, 2008).

4. Conclusions

In this work the effect of biomass loading and acid concentration on ultrasound-assisted pretreatment of CSS were assessed. The decrease of biomass loading and the increase of acid concentration increased the sugar yield. The main inhibitors for acetone-butanol-ethanol fermentation (furfural, HMF, ferulic acid and p-coumaric acid) were present at concentration lower than the toxic limit for various *Clostridium* sp.

Acknowledgments

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