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Effects of enzymatic hydrolysis and ultrasounds pretreatments on corn cob and vine trimming shoots for biogas production

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HIGHLIGHTS

- The enzymatic hydrolysis enhanced the transformation of VTS and corn cob into biogas.
- The ultrasounds pretreatment harmed the biogas generation from both materials.
- The US + H pretreatment improved the biogas production from corn cob but not from VTS.
- The impact of pretreatments for biogas generation was more noticeable for VTS.

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ABSTRACT

Due to their lignocellulosic nature, corn cob and vine trimming shoots (VTS) could be valorized by anaerobic digestion for biogas production. To enhance the digestibility of substrates, pretreatments of lignocellulosic materials are recommended. The effect of enzymatic hydrolysis, ultrasounds pretreatments (US) and the combination of both was assayed in lignocellulosic composition, methane, and biogas yields. The pretreatments leaded to a reduction in lignin and an increase in neutral detergent soluble compounds making corn cob and VTS more amendable for biogas conversion. The US were negative for biogas production from both substrates and in particular strongly detrimental for VTS. On the opposite side, the enzymatic hydrolysis was certainly beneficial increasing 59.8% and 14.6% the methane production from VTS and corn cob, respectively. The prior application of US did not potentiate (or not sufficiently) the improvement in the methane production reflected by the enzymatic hydrolysis pretreatment of VTS and corn cob.

1. Introduction

The use of conventional non-renewable sources of energy involves several drawbacks highlighting reserves depletion or global warming, so today's challenge is to provide an environmentally sustainable alternative (Bessou et al., 2011). Biogas, a mixture formed mainly by methane and carbon dioxide (Liguori et al., 2013), represents a green energy source that can be an option for meeting partial energy needs. Biogas could be generated by the anaerobic digestion of biodegradable materials. Hence, characteristics of corn cob and vine trimming shoots, which are agricultural wastes with a lignocellulosic nature, make them suitable for the sustainable production of biogas. Nevertheless, crystalline and recalcitrant composition of lignocellulose hinders enzymatic and microbial accessibility to disrupt the biomass structure and therefore to convert agricultural wastes into biogas (Zheng et al., 2014). For the optimal lignocellulose utilization in anaerobic digestion, is recommended to submit materials to different pretreatment technologies (Monlau et al., 2012; Zheng et al., 2014).

Enzymatic hydrolysis of lignocellulosic matrix can help to promote the anaerobic digestion performance (Divya et al., 2015; Schroyen et al., 2015; Ziemiński et al., 2012) in an efficient and environmentally friendly mode (Ziemiński et al., 2012). The enzymatic hydrolysis breakdowns polymers providing monomers easily assimilable by the anaerobic microbiota for their further conversion into methane (Cesaro and Belgiorno, 2014), and solubilizes phenolic compounds avoiding theirs inhibitory effect on the biogas production (Schroyen et al., 2014). Moreover, enzymatic

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hydrolysis overcomes drawbacks associated with conventional chemical pretreatments (Bessou et al., 2011) such as the generation of side products (furfural, 5-hydoxymethylfurfural, levulinic acid or formic acid) that frequently disturb or inhibit fermentations (Vanholme et al., 2013). Despite the benefits of enzymatic pretreatment, the implicit characteristics, and highly ordered structure of native lignocellulosic materials make them resistant to enzymatic attack (Kratky and Jirout, 2011).

The goodness of ultrasounds pretreatments (US) to ameliorate enzymatic hydrolysis has been already suggested (Bussemaker and Zhang, 2013; Subhedar and Gogate, 2013). US allow relocalization, disruption or partial removal of lignin (Iskalieva et al., 2012), destruction of wax layers and silica bodies deposited onto the surface of lignocellulose (Luo et al., 2013), and fragmentation of biomass components, increasing the surface area for reactions (Iskalieva et al., 2012; Luo et al., 2013). As a result, US increase the lignocellulose degradability reducing the structural rigidity of lignocellulose, increasing enzymatic accessibility (Iskalieva et al., 2012) and improving mass transfer (Subhedar and Gogate, 2013).

The objective of this study was to analyze the effect of enzymatic hydrolysis, ultrasounds and the combination of ultrasounds and enzymatic hydrolysis as pretreatments of corn cob and vine trimming shoots (hereinafter VTS) for the anaerobic production of biogas. As far as we know, it is the first time that VTS are employed for this purpose. Consequently, the present work will contribute to the knowledge of the aptitude of VTS for methane and biogas production before and after pretreatments.

2. Materials and methods

2.1. Substrates

Corn cob and VTS are lignocellulosic by-products of agro-industrial processing which were the focus materials for this study. Corn cobs were collected from Mondariz (Pontevedra, Spain), while VTS were gently provided from EVEGA (Viticulture and Enology Station of Galicia, Ourense, Spain). These substrates were dried at room temperature, grinded in an electric shredder MTD 220E (Saarbrücken, Germany), then milled in an IKA® Werke universal mill model M 20 (Staufen, Germany) and sieved to get a particle size below 2 mm.

Aliquots from the homogenized lot were submitted to different pretreatments (as described below). Raw or pretreated corn cob and VTS were then used as starting materials for anaerobic digestion.

2.2. Van Soest fiber analysis

Total solids (TS) and volatile solids (VS) of substrates and inoculum were determined gravimetrically following standard methods.

The Van Soest fiber analysis (Van Soest et al., 1991) allows the organic matter fractionation into soluble components, hemicellulose, cellulose and lignin by sequential extraction with neutral and acid detergents, followed by strong acid extraction. Therefore, substrates composition was determined with the help of a Gerhardt Fibrebag system (Les Essart Le Roi, France). Changes in fiber composition induced by enzymatic, ultrasounds and combined ultrasounds-enzymatic pretreatments on corn cob and VTS were investigated by analyzing the separated solid fraction.

The sample (1 g) was placed into a polypropylene bag. Bags were boiled in one standard fiber apparatus using a specially designed rack following the sequence: 360 mL of neutral detergent solution for 1 h to determine neutral detergent fiber (NDF); 360 mL of acid detergent solution for 1 h to determine acid detergent fiber (ADF), and 40 mL of H_2SO_4 72% (v/v) for 3 h to determine acid detergent lignin (ADL). After each step, samples and bags as a whole were dried at 105 °C and weighted. Correction for ash was made.

The different lignocellulosic constituents were calculated by the difference of weights: hemicellulose (NDF–ADF), cellulose (ADF–ADL), lignin (ADL), and neutral detergent soluble compounds (NDS) (100%-NDF).

2.3. Pretreatments

2.3.1. Ultrasounds conditions for pretreatment

Ultrasounds pretreatments (US) were applied using an ultrasonic processor series Autotune model 750 W (Fisher Bioblock Scientific, Cedex, France). The system consisted of a sounding line of 13 mm diameter coupled to a generator of low frequency (20 kHz), and a microprocessor for control. A continuous ultrasonic power of 150, 450 or 750 W (equivalent to 20, 60 or 100% of power, respectively) for 62.5 s was used for pretreatment of substrates. The amount of substrate treated in each test was 2.5 g (dry basis). The samples were sonicated in 30 mL polypropylene bottles with 20.25 mL of sodium-phosphate buffer (50 mM pH 6.0).

After US, the volume of the bottles was completed up to 25 mL until reach a final solid:liquid proportion of 1:10 (w/v) by adding 4.75 mL of the sodium-phosphate buffer (for US alone), or with the enzyme Ultraflo® L (for the hydrolyzed samples) according to enzymatic hydrolysis conditions.

2.3.2. Enzymatic hydrolysis

Raw or US samples were submitted to the enzymatic hydrolysis. Ultraflo® L gently provided by Novozymes (Bagsværd, Denmark) was employed for the hydrolysis. Ultraflo® L is an enzyme preparation with *endo*-1,3(4)-β-glucanase, and collateral cellulose, xylanase and feruloyl esterase activities among others. The hydrolysis started by adding Ultraflo® L in an amount equivalent to 0.2 U feruloyl esterase per gram of dry milled corn cob or VTS. The enzymatic hydrolysis was performed in 30 mL polypropylene bottles with screw cap, containing 2.5 g of substrate, Ultraflo® L and the required volume of sodium-phosphate buffer (50 mM pH 6.0) until a final solid:liquid proportion of 1:10 (w/v) was reached. The bottles were transferred into a shaker incubator where the hydrolysis was carried out at 150 rpm and at 40 °C for 3 h.

After hydrolysis, the samples were boiled (100 °C for 10 min) for thermal inactivation of enzymes, and centrifuged at 10,000 rpm for 15 min. Finally, supernatant (liquid fraction) was separated and solid residue was used as substrate for anaerobic digestion to produce biogas.

2.4. Phenolic acids determination in liquid fraction

High Performance Liquid Chromatograph (HPLC) (Agilent, model 1200, Palo Alto, CA, USA) equipped with an Agilent Zorbax SB-Aq C18 column (4.6 × 150 mm, 5 μm particles) and with an Agilent Zorbax SB-Aq analytical guard column (4.6 ID × 12.5 mm, 5 μm) was employed to determine coumaric and ferulic acids. Injection volume of each sample was 20 μL and these phenolic acids were separated at 35 °C using a gradient elution program at a flow rate of 1 mL/min. The gradient elution program was from 0 to 48% of solvent B (0–35 min), from 48 to 100% of solvent B (35–40 min), 100% of solvent B (40–56 min), from 100 to 0% of solvent B (56–60 min) and 0% of solvent B (60–65 min). The diode array detector (UV detector) was set at 276 nm. Coumaric acid (*trans*-4-hydroxycinnamic acid) and ferulic acid (*trans*-4-hydroxy-3-methoxycinnamic acid) were the standards.