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Orange peel pretreatment in a novel lab-scale direct steam-injection apparatus for ethanol production

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ABSTRACT

Orange peel waste (OPW) was converted into ethanol by consecutive acid-catalysed steam-explosion (ACSE), enzymatic saccharification and fermentation with *Saccharomyces cerevisiae* F15. The first step was performed in the presence of 0.5% (volume fraction) sulphuric acid at a solid loading of 160 g L⁻¹ using a novel laboratory-scale Direct Steam-Injection Apparatus under different reaction temperatures and times. ACSE at 200 °C for 90 s (or 180 °C for 150 s) yielded the highest solubilization of pectin (~73%) and a positive effect on the subsequent enzymatic hydrolysis step, that was carried out at 50 °C with an enzyme loading of 6 FPU g⁻¹ cellulose. OPW, pretreated at 180 °C for 150 s, yielded the highest glucose solubilisation degree (~56%) at the end of the saccharification step, and the maximum ethanol yield coefficient (~0.495 g g⁻¹) and productivity (4.85 g L⁻¹ h⁻¹) at the end of the 3rd repeated fermentation batch in shaken-flasks.

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1. Introduction

Environmental, socio-economical benefits and high feedstock flexibility are expected from the production of second generation biofuels from several lignocellulosic feedstocks (e.g., agricultural, food and forest residues and organic fraction of municipal solid wastes). Even if these raw materials minimise competition for land and food with respect to sugar- and starch-based feedstocks, cellulosic ethanol production is by far a more complex task since it asks for chemical and biochemical processes to convert cellulose and/or hemicellulose into fermentable sugars as pretreatments steps to traditional fermentation and distillation [1,2].

Orange peel waste (OPW), the solid residue from the orange juice extraction process, might be a valuable lignocellulosic

feedstock for ethanol production. It consists of peel, rag (segment membranes and cores), juice sacs, and seeds and amounts to 50–70% of the fresh fruit mass [3]. The global citrus fruit production amounts to over 88 Tg per year [4], the about 55% of which being orange fruits. Thus, the annual world supply of OPW should be around 21 Tg, one third of which being easily available for further utilisation in the orange processing factories.

The use of OPW as feedstock for ethanol production has been so far largely assessed both at laboratory – [5,6] and pilot – [7] plant scales. The enzymatic saccharification of comminuted OPW was firstly studied by Grohmann and Baldwin [8], who showed that the concomitant presence of cellulase and pectolytic enzymes was required to attain high hydrolysis yields. Although fermentable sugars from the enzymatic

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hydrolysates of OPW were almost completely converted into ethanol by *Saccharomyces cerevisiae*, the economic viability of the process was reported to be hampered by the slow hydrolysis kinetics and by the small increase in the fermentable sugars derived from the enzymatic hydrolysis [9]. Thus, a variety of physico-chemical pretreatments of this waste was tested to either promote the selective removal of non-fermentable sugars and inhibitory compounds [6,10], or to increase the susceptibility of cellulose to hydrolysis. In this respect, although the dilute sulphuric acid hydrolysis of OPW failed to release sugars from cellulose and pectins, the resulting solid waste exhibited a high susceptibility to the subsequent enzymatic saccharification step [11]. Optimized conditions of diluted acid hydrolysis of OPW enabled the solubilization of about 0.42 g of total sugars per g of OPW [12]. A steam explosion pretreatment of OPW prior to simultaneous saccharification and fermentation significantly removed *D*-limonene from an initial mass fraction of about 0.7% [6,10] to as low as 0.07% [10], thus improving ethanol production.

Thus, according to most of these studies the dilute-acid hydrolysis and steam-explosion seem to be the best pretreatment options to increase OPW amenability to hydrolysis by cellulolytic enzymes, as well as to minimize the hydrolysate content in inhibitory compounds, such as *D*-limonene.

The main bottlenecks of bioethanol production from OPW are as follows: the process heat duty [1], the high contribution of the feed solid and pectin contents to the viscosity of the medium to be fermented and distilled [7], the current market price of cellulolytic and pectolytic enzymes, the availability of genetically modified microorganisms to ferment both hexoses and pentoses and thus maximise the ethanol produced, and finally the energy demand of the distillation step, that is highly dependent on the ethanol feed mass fraction, this being generally lower than 2.5% [1].

Previously, a novel laboratory-scale direct steam-injection apparatus (DSIA) was developed to overcome the main drawback of the conventional batch-driven lab rigs, namely the long time needed to heat fibre slurry from room to reaction temperatures greater than 150 °C [13]. In this way, the process data collected can be more easily scaled up, especially if the batch heater is replaced by the continuous steam-injection heater, developed by ProSonix Co. (Milwaukee, WI, USA; <http://www.pro-sonix.com>).

As concerning the enzymatic hydrolysis of raw citrus peel wastes, several investigations have been so far carried out to maximize the recovery of monomeric sugars [8,9,14,15]. In particular, Santi [16] assessed the effect of different cellulase loadings in the range of (2–30) FPU g⁻¹ cellulose on the saccharification of OPW as such or after autoclaving at 130 °C under neutral or mild acidic conditions (0.5% volume fraction H₂SO₄) at a total solid content of 20 g L⁻¹. Whatever the enzyme loading applied, the glucose released from untreated OPW was less than 10% of the theoretical one; whereas the neutral or acidic pretreatment resulted in a higher solubilisation degree of about 30 or 45%, respectively, after 24-h incubation at an enzyme loading of 30 FPU g⁻¹. Since the glucose released did not significantly raise as the enzyme loading was increased from (6 to 30) FPU g⁻¹ cellulose, the lowest enzyme dosage was used to study the effect of solid loading in the range of (20–150) g L⁻¹. After 48-h incubation, as the solid

loading was set to (50, 100, or 150) g L⁻¹, the resulting glucose solubilisation was (52, 48 or 40) %, respectively. At solid loadings higher than 150 g L⁻¹, an insufficient mixing of the resulting OPW slurry was observed [16].

Main aims of this study were (i) to ameliorate the design and performance of the aforementioned lab-scale DSIA, (ii) to evaluate comparatively the impact of different acid-catalysed steam explosion (ACSE) pretreatments of OPW in DSIA on the susceptibility of its residual cellulose fraction to enzymatic saccharification under constant solid (150 g L⁻¹) and enzyme (6 FPU g⁻¹) loadings; and (iii) to maximise the ethanol yield and productivity per Mg of dry OPW. The selected combinations of temperature and residence time for the ACSE pretreatments were also investigated to assess whether the resulting sugars released and inhibitors formed might affect the extent of the subsequent steps of enzymatic hydrolysis and alcoholic fermentation.

2. Materials and methods

2.1. Raw materials

Orange peel waste (OPW) was kindly provided by Agrumi-Gel srl (Barcellona P.G., ME, Italy) in a powder form, its moisture mass fraction being (6.92 ± 0.32) %. Since this product was dried in rotary kilns at about 400 °C (http://www.agrumigel.it/agrumi-derivati_en.php?act=detail), the residual essential oils were practically nil. The raw material was ground using a lab-scale MF109 miller (IKA, Staufen, Germany) and sequentially sieved through a 1.0-mm screen. Its main chemical composition is shown in Table 1.

2.2. Equipment

The DSIA previously described [13] consisted of (i) a table cast-iron boiler type GVE 2 kW (Magnabosco Srl, Zugliano, Italy) to produce saturated steam up to 3 MPa; (ii) a 4-L stainless-steel reactor R equipped with an axial flow impeller and two rectangular baffles; (iii) an automatic piston electrovalve EV2 to flash suddenly the reaction medium after a reaction time of 5 s–30 min; (iv) a cono-cylindrical cyclone separator CS1 to separate residual particles and liquor from the vapour phase; (v) a filter FI to avoid dispersing the finest particles in the atmosphere.

The original design of the DSIA was modified in order to solve some critical aspects, such as (a) a low particle recovery efficiency by CS1 at solid contents of the order of 160 g L⁻¹ with the ensuing entrainment of finer particles in the vapour phase vented to the atmosphere; (b) a wide deviation band (±20 °C) with respect to prefixed reaction temperature (*T_R*) setpoints; and (c) low accuracy in the measurement of the injected steam mass (*m_S*).

To maximize the particle recovery after steam-explosion, another cyclone CS2 with the same geometric dimensions of CS1 was constructed and connected in series to CS1. This cyclone was equipped with an external jacket, where a cooling fluid consisting of ethylene glycol at –5 °C was circulated using an FL 300 Recirculating Cooler/Chiller (Julabo Labor-technik GmbH, Seelbach, Germany). In this way, most of the

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