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Effect of ozonolysis pretreatment parameters on the sugar release, ozone consumption and ethanol production from sugarcane bagasse

Rodolfo Travaini [a], Enrique Barrado [b], Silvia Bolado-Rodríguez [a,*]

[a] Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, University of Valladolid – UVa, Calle Doctor Mergelina, s/n, 47005 Valladolid, Spain
[b] Department of Analytical Chemistry, Faculty of Sciences, University of Valladolid – UVa, Paseo de Belén, 7, 47011 Valladolid, Spain

HIGHLIGHTS

- Ozone concentration is the fundamental parameter on sugar release yield from SCB.
- Moisture is the fundamental parameter on ozone consumption per gram of sugar released.
- Ozone consumption presented good linear fitting with sugars release yields.
- Degradation compounds inhibited *P. stipitis* but not fermentation by *S. cerevisiae.*
- Fermentation of glucose and xylose is necessary to achieve viable ethanol production.

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ABSTRACT

A $L_9(3)^4$ orthogonal array (OA) experimental design was applied to study the four parameters considered most important in the ozonolysis pretreatment (moisture content, ozone concentration, ozone/oxygen flow and particle size) on ethanol production from sugarcane bagasse (SCB). Statistical analysis highlighted ozone concentration as the highest influence parameter on reaction time and sugars release after enzymatic hydrolysis. The increase on reaction time when decreasing the ozone/oxygen flow resulted in small differences of ozone consumptions. Design optimization for sugars release provided a parameters combination close to the best experimental run, where 77.55% and 56.95% of glucose and xylose yields were obtained, respectively. When optimizing the grams of sugar released by gram of ozone, the highest influence parameter was moisture content, with a maximum yield of 2.98 $g_{SUGARS}/g\ O_3$. In experiments on hydrolysates fermentation, *Saccharomyces cerevisiae* provided ethanol yields around 80%, while *Pichia stipitis* was completely inhibited.

1. Introduction

With the aim of developing new environmental-friendly sources of fuels and chemical platforms, research groups around the world have focused their efforts on the development of procedures for production of these compounds from renewable sources. Within this context, and among the many production alternatives, the biorefinery concept has appeared. Like a traditional refinery, the core process of a biorefinery is the production of biofuels, but using biomass as raw material. The main biofuel being studied is bio-ethanol, produced from polymeric sugars present in lignocellulosic biomass. It has many advantages over other biofuels, such as the ability to blend with petroleum for its use on traditional engines; the use of its pure anhydrous form in dedicated engines; its high octane number and high vaporization heat; it is considered a clean, renewable and green combustible; etc (Thangavelu et al., 2016).

The production of second-generation ethanol requires a pretreatment step, to degrade lignin and liberate the polymeric sugars (Garcia-Cubero et al., 2010b). Among the different types of pretreatments, the ozonolysis has regained visibility in the last decade (Garcia-Cubero et al., 2010a; Kojima and Yoon, 2008; Mamleeva

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Abbreviations: AIL, acid insoluble lignin; ANOVA, analysis of variance; ASL, acid soluble lignin; OA, orthogonal array; SCB, sugarcane bagasse.

* Corresponding author.

E-mail addresses: rtravaini@gmail.com (R. Travaini), ebarrado@qa.uva.es (E. Barrado), silvia@iq.uva.es (S. Bolado-Rodríguez).

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et al., 2009). This chemical pretreatment consists in the use of ozone to oxidize, solubilize and degrade biomass lignin, generating a pretreated material with great characteristics for enzymatic hydrolysis. The main advantages that make ozonolysis a promising pretreatment include: low inhibitory compounds formation, generating mainly weak carboxylic acids; minimal effects on sugars polymers, by selective lignin degradation; operation at ambient pressure and temperature, with on-site ozone generation; and the development of new technologies for ozone generation, reducing production costs (Travaini et al., 2016). Plug flow are the reactors most commonly used for ozonolysis pretreatment, mainly as fixed beds, where moisturized lignocellulosic biomass is packed in a tubular reactor and ozone is fed in a gas flow. These dynamics, when applied with the optimal conditions, result in a process with minimal ozone waste (Bhattarai et al., 2015; Garcia-Cubero et al., 2012; Li et al., 2015).

Many works applying ozonolysis pretreatment have been reported with a wide variety of lignocellulosic materials, like agricultural residues, wood chips, municipal solid waste and microalgae (Travaini et al., 2015). Agricultural residues have focused the research, which has most commonly explored wheat straw, on studies about compositional changes, enzymatic hydrolysis yields, fermentation for biofuels and biogas production, etc.

Sugarcane is used in many countries for alimentary sugar and first-generation ethanol production, and sugarcane bagasse (SCB) is its main residue. In Brazil, for example, 200 million tons of SCB were generated in the 2014–2015 harvest, producing 27 billion liters of ethanol and 37 millions tons of sugar (UNICA, 2015). It presents important advantages over other lignocellulosic residues for second-generation ethanol production, highlighting its low ash content, high sugar polymer content, low cost, high viability and the possibility to integrate second-generation ethanol production into the first generation factories (Soccol et al., 2010). Some studies on SCB ozonolysis pretreatment have recently been carried out (Barros et al., 2013; Souza-Correa et al., 2014, 2013; Travaini et al., 2013); nevertheless, they are just exploratory studies. In the first study about SCB ozonolysis, Travaini et al. (2013) observed how the pretreatment can affect the structural composition of the pretreated biomass and increase enzymatic hydrolysis efficiency, when compared with the raw material. Barros et al. (2013) studied the use of SCB ozonolysis pretreatment combined with wet disk milling, and found that the association provided better results than the individual pretreatments. The works of Souza-Correa et al. (2014,2013) studied SCB ozonolysis pretreatment process parameters one factor at a time, applying a NaOH wash as a detoxification step that can influence the results and interpretations. To this day, no systematical and statistical studies about SCB ozonolysis process parameters and their optimization are available; neither has the fermentability of hydrolysates from ozonated SCB been studied, and there is just one study about the fermentation of wash-detoxified ozone pretreated SCB (de Cassia Pereira et al., 2016).

In this work, an OA $L_9(3)^4$ experimental design was performed in order to study the main ozonolysis process parameters (moisture content, ozone concentration, ozone/oxygen flow and SCB particle size). The applied orthogonal array of this experimental design enables the study of, firstly, the way that process parameters individually affect sugar release yields after enzymatic hydrolysis, ozone consumption and time of reaction, and also, the influence of the interactions between process parameters. The results obtained were model-fitted to predict the combination of parameters that optimizes sugar release yields and the ozone consumption. The most concentrated hydrolysates were fermented for ethanol production by the yeasts *Saccharomyces cerevisiae* bakery's strain and *Pichia stipitis* DSM 3651. Finally, a preliminary energy consumption assessment was made.

2. Material and methods

2.1. Sugarcane bagasse

SCB was kindly donated by Usina Virgolino de Oliveira S/A Açúcar e Álcool, José Bonifácio, São Paulo State, Brazil. It was washed with distilled water to remove sugar residues and particulate material, dried in a ventilated oven at 37 °C and stored in hermetic plastic bags until use.

The washed and dried SCB was sieved manually using, initially, a n° 4 mesh (4.76 mm) sieve to remove extra-large fibers that can interfere in pretreatment, and top particles were discarded (particle size P1). A portion of the resulting sieved SCB was used for experiments (particle size P2); while another portion of SCB particle size P2 was sieved again with a n° 120 mesh (0.125 mm) sieve, and both top (particle size P3) and bottom (particle size P4) sieved materials were used for experiments. The percent yield of sieving and the chemical composition (acid insoluble lignin (AIL), acid soluble lignin (ASL), cellulose, xylan and ash) of each particle size sample are shown in Table 1.

2.2. Ozonolysis pretreatment

Ozonolysis pretreatment experiments were conducted in a fixed bed reactor (glass column 50 cm in height and 2.7 cm in diameter), as described before by Travaini et al. (2013), but with some modifications as indicated below.

Before each experimental run, the reactor was filled with 24 ± 1 g (dry basis) of SCB previously moisturized with distillated water to the required value and gently mixed to homogenize the mixture. The ozone generator was fed with industrial grade oxygen. Before each test, the inlet oxygen flow was measured with an oxygen rotameter, the ozone production was controlled by energy supply and the ozone concentration in the generated gas flow was determined using iodometric titration (APHA-AWWA-WEF, 2005). The ozone/oxygen stream was saturated with water passing through a scrubber bottle before entering the reactor in order to minimize moisture losses. Outlet reactor gas flow was passed through 2% KI solution, and the absorbance periodically determined each 30 s, after color appearance, by spectrophotometry at 464 nm. Ozonolysis pretreatment experiments were stopped 15 min after KI solution reached an absorbance of 0.800 (corresponding to 0.028 g of O_3 escaped). The ozone concentration and ozone/oxygen flow in each experiment were used for calculating the ozone expended during the reaction time. The quantity of non-reacted ozone leaving the reactor during the additional 15 min was iodometrically measured (APHA-AWWA-WEF, 2005) and subtracted from the expended ozone to calculate the grams of ozone reacted in each experiment.

The resulting ozone pretreated SCB in the reactor was divided into four parts within the length of the reactor, and the one just before the gas outlet was discharged in order to avoid non-homogeneous pretreated samples (García-Cubero et al., 2009). The other three quarters were gently mixed, the moisture content measured by gravimetry, and stored hermetically in a freezer until enzymatic hydrolysis occurred.

2.3. Enzymatic hydrolysis

Ozone pretreated SCB was enzymatically hydrolyzed in Erlenmeyer flasks (6% w/w, dry basis) in sodium citrate buffer pH 4.80 (0.05 M), with 10 FPU and 30 CBU per gram of cellulose. Enzyme dosages were calculated based on the raw material's cellulose content, using the commercial enzyme cocktails Celluclast 1.5 L as cellulase and Novozym 188 as β-glucosidase sources, both from