



Combined alkali and hydrothermal pretreatments for oat straw valorization within a biorefinery concept



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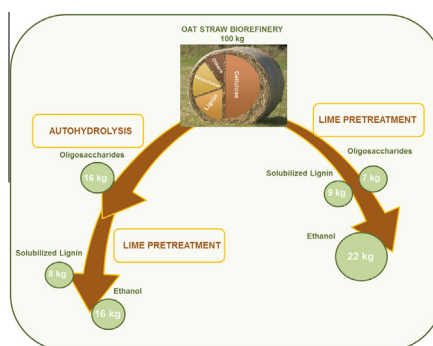
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HIGHLIGHTS

- Oat straw was revalorized following a biorefinery approach.
- Two configurations of process were developed: lime combined or not with autohydrolysis.
- 68% of hemicellulose was recovered as oligosaccharides using autohydrolysis.
- Ethanol concentration higher than 4% (w/w) was achieved in two different strategies.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of this work was the evaluation of lime pretreatment combined or not with previous step of autohydrolysis for oat straw valorization. Under selected conditions of lime pretreatment, 96% of glucan and 77% of xylan were recovered and 42% of delignification was achieved. Xylose fermentation to ethanol by metabolic engineered *Saccharomyces cerevisiae* (MEC1133) strain improved the ethanol production by 22% achieving 41 g/L. Alternatively, first step of autohydrolysis ($S_0 = 4.22$) allowed a high oligosaccharides recovery (68%) and subsequent lime pretreatment attained a 57% of delignification and 99% of glucan to glucose conversion. Oat straw processed by autohydrolysis and lime pretreatment reached the maximal ethanol concentration (50 g/L). Both strategies led to oat straw valorization into bioethanol, oligosaccharides and lignin indicating that these pretreatments are adequate as a first stage within an oat straw biorefinery.

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1. Introduction

Currently, biofuels from renewable sources as lignocellulosic materials are recognized as one of the possible alternatives to reduce the greenhouse emissions caused by the increasing oil-based fossil fuel consumption. A target of 136 billions liters of

renewable fuels (79 billion liters of the total volume obtained from lignocellulose biomass) by 2022 was established in the Energy Independence and Security Act of 2007 (USA). Nevertheless, the moderate development of cellulosic biofuel industry hinders a large-scale exploitation, being improbable to achieve this goal by 2022 (Chen and Önal, 2016). The scientific community is devoting great research efforts on improvement the techno-economic process of lignocellulose biofuels manufacturing which is reflected in the increasing published papers in this field (Akhtar et al., 2016).

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Processing of lignocellulosic biomass for bioethanol production requires at least one first stage pretreatment to alter its complex structure and improve the enzymatic saccharification of polysaccharides into fermentable sugars. Lignocellulosic pretreatment is considered the most expensive step involved in bioethanol production (Yang and Wyman, 2008). Therefore, the development of a cost-efficient pretreatment is one of the main challenges for lignocellulosic bioethanol commercialization. Only a few pretreatments have been identified as leading pretreatments (Wyman et al., 2011) and/or cost-effective processes, including steam explosion, liquid hot water or autohydrolysis, dilute acid and alkali pretreatments (Zu et al., 2014; Singh et al., 2015). A comparative study based on techno-economic analysis of several pretreatments for ethanol production showed that the minimum ethanol selling price obtained by hydrothermal and alkali lime processes was considerably reduced when two fractions (hemicellulose and cellulose) were employed in ethanol fermentation (Tao et al., 2011). Alkali treatment is recognized as an efficient delignification process to remove lignin and enhance enzymatic susceptibility of cellulose (Rabelo et al., 2013). Lime ($\text{Ca}(\text{OH})_2$) is used as alternative alkali for biomass processing at low temperature and pressure and has additional benefits compared to NaOH or KOH (low cost reagent, less safety requirements and easily recovered) (Mosier et al., 2005; Wang and Cheng, 2011). In addition, lower degradation of sugars (or inhibitors formation) during alkali process is produced compared to acid pretreatment (Jasaimut et al., 2013). Alternatively, lime processing can also be combined to previous pretreatment in order to improve the selective fractionation of lignocellulose biomass (into main components: cellulose, hemicellulose and lignin), achieving a more efficient process within a biorefinery scheme (Romaní et al., 2011; Ruiz et al., 2011). Hydrothermal treatment or liquid hot water is a recognized environmentally-friendly pretreatment used as first step of a biorefinery for extraction of hemicellulosic fraction into oligosaccharides (Garrote et al., 1999; Yañez et al., 2009) and it has been successfully combined with other treatment for improvement of ethanol production (Romaní et al., 2011; Akhtar et al., 2016).

Agricultural residues (as straw from crops) are considered as one of the most important potential sources of renewable lignocellulosic biomass for bioethanol production (Kim and Dale, 2004). In addition, the use of agricultural wastes is a less controversial solution than the use of whole energy crop (usually employed as food crops) (Gómez-Tovar et al., 2012). Straw from crops such as barley, oat, rice, sorghum and wheat are distinguishable by its high carbohydrate content (54–70%) that implies a potential ethanol production ranging from 0.26 to 0.31 L/kg of dry biomass (Kim and Dale, 2004). The fermentation of all sugars present in lignocellulose biomass (hemicellulosic sugars such as xylose and glucose from saccharification of cellulose) is another of identified challenge for ethanol production since the main strain used for ethanol production (*Saccharomyces cerevisiae*) is not able to consume xylose, naturally. Recent advances in this field reveal that the suitable selection of host strain for metabolic engineering xylose pathway is of the utmost importance since the strain background could improve the lignocellulose-to-bioethanol processes (Jin et al., 2013; Wimalasena et al., 2014). Industrial environments have been identified as a promising source of natural robust strains (Pereira et al., 2014) suitable for metabolic engineering in order to develop more efficient strains for lignocellulosic processes (Romaní et al., 2015; Cunha et al., 2015).

Oat is one of the major crops produced annually (Kim and Dale, 2004) with an annual production of 21 M tonnes being Russia the highest producer (FAO). Oat straw has not been as extensively studied as wheat straw, corn cob and sugarcane. Scientific researches about valorization of oat straw residues include the manufacturing of functional food (as prebiotics) (Berger et al.,

2014) and its bioconversion to biofuel energy as bioethanol, biogas and methane (Dereire et al., 2010; Sapci, 2013; Gómez-Tovar et al., 2012). On the other hand, a suitable process configuration for an integral use of oat straw fractions could lead to multi-products in a biorefinery scheme which would fulfill the requirements for a sustainable growth.

The objective of this work was the development of oat straw-based biorefinery by cost-effective pretreatment technologies (lime treatment and autohydrolysis). Two possible configurations were followed: lime pretreatment and sequential stages of autohydrolysis and lime treatment. Overall mass balance of the two configurations was performed in order to compare recovery of main components (cellulose, hemicellulose and lignin) and evaluate the efficiency of pretreatments on oat straw fractionation. In addition, susceptible pretreated oat straw samples were subjected to simultaneous saccharification and fermentation for bioethanol production.

2. Materials and methods

2.1. Raw material and analysis composition

Oat (*Avena sativa L*) straw, raw material used in this work, was kindly provided by a local company in the southern of Spain. Oat straw was milled to pass an 8 mm screen, homogenized in a single lot and stored in a dry and dark place until its use. Oat straw was analyzed for aqueous and ethanol extractives, ashes, carbohydrate and lignin following NREL protocols (NREL/TP-510-42618-42622-42618). Oat straw samples were Soxhlet extracted with distilled water (1 g of oat straw per 50 g of distilled water) for 8 h at 100 °C in order to determinate the aqueous extractives. After that, 1 g of oat straw (without aqueous extractives) was Soxhlet extracted with 50 g of 80% of ethanol for 8 h at 80 °C to quantify the ethanol extractives. Oat straw was subjected to quantitative acid hydrolysis with 72% sulphuric acid for polysaccharides determination (measured as monosaccharides by HPLC using an index detector, Biorad Aminex HPX-87H column, eluted with 0.006 N H_2SO_4 , flow rate of 0.6 mL/min, oven temperature of 50 °C). Klason lignin content was gravimetrically determined.

2.2. Oat straw processing

2.2.1. Lime pretreatment

Samples of oat straw (10 g) were mixed with 100 g of water, placed in a 250 mL flask and subjected to lime pretreatment under conditions listed in Table 1 (run 1–12) in which temperature, time and $\text{Ca}(\text{OH})_2$ loading were evaluated. Experiments carried out at 121 and 134 °C were autoclaved (miniclave of 12 L) and for experiments at 90 °C, it was employed a thermostatic oil bath. After treatment, pretreated samples were washed with abundant water until pH = 7, weighted for solid yield (SY) determination and analyzed for glucan, xylan and Klason lignin content (as it was described in Section 2.1). For an easier interpretation of results, the recovery of glucan, xylan and Klason lignin were calculated by the followed equation:

$$R_{Gn \text{ or } Xn \text{ or } KL} = SY \cdot \frac{Gn \text{ or } Xn \text{ or } KL}{Gn_{RM} \text{ or } Xn_{RM} \text{ or } KL_{RM}} \quad (1)$$

where, subscripts Gn, Xn and KL are referred to recovery (R) of glucan, xylan and Klason lignin, respectively and RM to raw material (oat straw). SY is solid yield (g of lime pretreated oat straw/100 g of oat straw).

Percentage of delignification was also calculated and employed for the discussion of results using the following equation:

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