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# Sugar production from barley straw biomass pretreated by combined alkali and enzymatic extrusion

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## HIGHLIGHTS

- A novel pretreatment for the production of sugars from barley straw is presented.
- Chemicals and enzymes are combined with extrusion in two consecutive runs.
- Carbohydrate breakdown starts during extrusion with enzymes (bioextrusion).
- High sugar production by enzymatic hydrolysis of pretreated material (bioextrudate).

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## ABSTRACT

A pretreatment that combines a thermo-mechanical process (extrusion) with chemical and biological catalysts to produce fermentable sugars from barley straw (BS) biomass was investigated. BS was firstly extruded with alkali and then, the pretreated material (extrudate) was submitted to extrusion with hydrolytic enzymes (bioextrusion). The bioextrudate was found to have 35% (w/w dwb) of total solids in soluble form, partly coming from carbohydrate hydrolysis during bioextrusion. About 48% of soluble solids dry weight is comprised by sugars, mostly glucose and xylose. Further enzymatic hydrolysis of bioextrudate could be successfully carried out at high solid loading level of 30% (w/v), with sugar production yield of 32 g glucose and 18 g xylose/100 g bioextrudate at 72 h incubation (equivalent to 96 and 52 g/l concentration, respectively). These results, together with the high level of integration of the process, indicate a great potential of this pretreatment technology for sugar production from lignocellulosic substrates.



## 1. Introduction

Lignocellulosic biomass is the most promising feedstock to be used in biorefineries, as source for the production of biofuels, chemicals and other biomass-derived products (Uihlein and Schebek, 2009). This type of biomass has a heterogeneous composition, which includes cellulose, hemicellulose, starch, lignin, oils and proteins. Specific technologies need to be developed to achieve a complete fractionation of the material and the conversion of each fraction to high-value products (Menon and Rao, 2012).

Lignocellulosic biomass pretreatment technologies have been widely investigated over the last years as a first and crucial stage to breakdown fibre structure and make carbohydrates accessible to hydrolytic chemical or biological catalysts. Various biological, chemical, and physical pretreatment approaches have been proven to open the recalcitrant structure of lignocellulosic biomass and increase the susceptibility of cellulose to enzymatic attack. (Mosier et al., 2005; Tomás-Pejó et al., 2011). However, most of these pretreatments achieve these goals by solubilizing hemicelluloses and/or lignin that in turn can be degraded to compounds that can exert inhibitory effect on enzymes and microorganisms in the subsequent steps, due to the use of elevated temperatures and/or high concentration of chemicals such as solvents, acids, etc. On the contrary, twin-screw extrusion stands out as a technology that can effectively open the recalcitrant structure of lignocellulosic biomass into its constituents at mild temperature and chemicals conditions, preventing the formation of such inhibitory by-products (Duque et al., 2013). Other advantages are that it can be operated in continuous way and that produces little effluent, disposal and solid losses.

Extrusion process is based on the effect exerted by the tight rotation of a single or a twin-screw inside a stationary barrel equipped with temperature control. The most common equipment

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for the extrusion of biomass is the fully intermeshing co-rotating twin-screw extruder, whose configuration can be varied by the combination of different types of screw elements, which produce different effects, such as transport, mixing and shearing, along the extrusion process (Senturk-Ozer et al., 2011).

The application of extrusion in biomass pretreatment has been lately investigated as individual technique (Karunanithy and Muthukumarappan, 2010a,b, 2011a,c; Karunanithy et al., 2012; Yoo et al. 2011; Zhang et al., 2012), or in combination with chemicals, such as alkali, ammonia or ethylene glycol, in the so called "reactive extrusion" (Dale et al., 1999; Karunanithy and Muthukumarappan, 2011b,d, 2013; Lamsal et al., 2010; Eckard et al., 2011; Lee et al., 2010). Although the process has been successfully demonstrated without catalyst on a variety of substrates such as switch grass, prairie cord grass and corn stover (Karunanithy and Muthukumarappan, 2010a,b, 2011a), the integration of extrusion and chemical agents may result in more efficient biomass component fractionation. In the particular case of alkaline-extrusion, several approaches have been carried out by soaking the biomass with an alkaline solution prior to its introduction in the extruder. For instance, Karunanithy and Muthukumarappan (2011d) optimized the reactive extrusion conditions for switchgrass reaching a maximum sugar recovery after enzymatic hydrolysis of 86.8 for glucose and 84.5% for xylose, after soaking biomass for 30 min in 2% (w/v) alkali solution prior to extrusion. Zhang et al. (2012) also demonstrated the effectiveness of this sequential alkali-soaking/extrusion pretreatment in enhancing sugar release by subsequent enzymatic hydrolysis in corn stover.

Significantly, a step towards the integration of the alkali and extrusion pretreatment was taken in a previous work carried out by the authors (Duque et al., 2013), by introducing the alkaline solution directly into the extruder and running the whole process in a continuous way. In that work, NaOH and water were fed into the extruder and after extrusion, extrudate was collected, washed and used as substrate in enzymatic hydrolysis tests. The yields of enzymatic hydrolysis of the washed extruded material in the best conditions (6% NaOH/DM ratio and 68 °C) were 88.9% for glucose and 71.3% for xylose (values in % of the maximum theoretically achievable if all glucan and xylan contained in extrudate were transformed to sugars), in experiments at 5% solids and 27 FPU/g glucan enzyme load. However, it was necessary to extensively wash the extruded material before the subsequent step of enzymatic hydrolysis to remove catalyst and neutralize for enzymatic hydrolysis that implied high water consumption. Recently, Um et al. (2013) have also reported on the effect of chemicals, such as alkali, in the extrusion of rapeseed straw in a continuous twin-screw reactor, by feeding directly the catalyst into the extruder at a similar NaOH/DM ratio, but at higher temperature of 170 °C. The positive effect of alkali addition is shown by a 2.4-fold increase in enzymatic digestibility in comparison to untreated material, reaching a maximum of 60% in experiments at 2% solids loading. The authors claim an important role of particle size reduction during extrusion in the effectiveness of the process, based on significant improvement of enzymatic digestibility also in experiments conducted by feeding hot water instead of alkali.

The first objective of the present work is to develop an advanced integrated alkaline extrusion process that includes a neutralization step into the extruder. This advanced process configuration allows avoiding the washing step of extrudate and thus, the water consumption at this stage. Moreover, this integrated process allows generating a pretreated material at high solid loading level, which can be used directly in a subsequent step for sugar production. The alkaline-extrusion process of the present work aims at enhancing the action of the alkaline agent, which promotes glucan conversion by cleavage of $H_2$ bonds of cellulosic structure and as a consequence, swelling of cellulose (Carrillo et al., 2005). Moreover, the work addresses an additional goal by performing a second extrusion run on the alkaline extruded biomass with hydrolytic enzymes to provide a good interaction enzyme-substrate at the high solids conditions in which process is carried out. Thus, a "bioextrudate" is produced, which can be directly incubated for sugar release from carbohydrates by enzyme action, without any additional downstream operation. The composition and characteristics of extruded materials and the sugar production of bioextrudate in subsequent enzymatic hydrolysis incubation at high solid loading is investigated.

## 2. Methods

### 2.1. Raw material

Barley straw (BS) (6% moisture content) was provided by Centre for the Development of Renewable Energy Sources (CEDER), (Soria, Spain). Biomass was coarsely crushed to about 5 mm particle size using a laboratory hammer mill (Retsch), homogenised and stored until used.

### 2.2. Twin-screw extrusion pretreatment

#### 2.2.1. Twin-screw extruder

Extrusion experiments were carried out in a twin-screw extruder (Clextral Processing Platform Evolum® 25 A110, Clextral, France), composed of 6 modular barrels of 100 mm length. In each barrel, different screws of 25 mm diameter are fitted to provide transport, mixing and shearing effects. A computer into the extruder controls the extruder barrel temperature and the screw speed. A volumetric feeder KMV KT20 (Ktron) connected to the extruder is used to provide continuous biomass feeding.

#### 2.2.2. Alkaline pretreatment

The operational parameters for alkaline extrusion of BS were selected based on results from a previous optimization study described elsewhere (Duque et al., 2013). Accordingly, extrusion of BS was carried out at an even temperature of 68 °C and at a ratio of NaOH/BS dry weight (w/w) of 7.5%. The screw profile used in this step is shown in Fig. 1A. Alkaline pretreatment was run by continuously feeding BS biomass through the feeder at a feeding rate of 0.6 kg/h fresh weight and a NaOH solution (10% w/v) through a metering pump connected to the extruder in module #2. The present work integrates a neutralization step by feeding a solution of diluted phosphoric acid (1% w/v) in module #4. The aim is to get the extrudate at the output with a pH value from 5 to 5.5. Both NaOH and phosphoric acid solution flows were adjusted to provide a liquid flow to solid input ratio (L/S) inside the extruder of 12/1. This value had been previously shown to provide a proper operation during this stage.

Regarding screw profile (Fig. 1A), in the first two barrels conveying elements were installed to transport the biomass forward, while in the third barrel a mixing section was included to achieve a good mixture between the biomass and the catalyst. Two reverse screws, one in module #4 and the other immediately after module #5, were set to increment the pressure and shearing effect in the extruder. A filtration unit was set up in module #5 to separate a liquid fraction or filtrate before extruded material output. The residence time of the biomass in contact with the soda can be estimated in less than 1 min, considering the total time of the extrusion run (about 2 min) and the sequence of the different steps (material feeding, alkaline treatment, neutralization and filtration) during the run.

After extrusion, a portion of the extrudate was dried, milled and analysed for major components as described below. Likewise, the