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A comparison between SHF and SSSF processes from cardoon for ethanol production

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ABSTRACT

The purpose of the present work is the evaluation of *Cynara cardunculus* L. potential in terms of ethanol production from lignocellulosic feedstock for industrial purposes. After a steam explosion (SE) pretreatment at different severity conditions, to obtain the dissolution of hemicellulose and the separation between cellulose and lignin, two different processes were tested: separate hydrolysis and fermentation (SHF) and semi-simultaneous saccharification and fermentation (SSSF). The enzymatic hydrolysis was carried out with ™Ctec2 cellulase enzyme and fermentation with *Saccharomyces cerevisiae* yeast.

The results showed slightly better performances by SSSF process compared to SHF, especially at low severity conditions, with a maximum overall ethanol yield of 13.64 g of ethanol/100 g of raw material. For the SHF process the best overall ethanol yield achieved was 13.17 g of ethanol/100 g of raw materials. In addition, the SSSF process required at least 24 h less than the SHF to achieve the same final ethanol concentration for every severity condition tested.

1. Introduction

Second generation biofuels are obtained from non-food biomass such as residues from agriculture, forestry and industry, or from dedicated energy crops; considering the last field, cardoon (*Cynara cardunculus* L.) is a very promising herbaceous energy crop as raw material for industrial applications, with a low irrigation water requirement, high growth rate with significant dry matter accumulation and reduced fertilization level (Ierna and Mauromicale, 2010; Cravero et al., 2012; Ciancolini et al., 2013). Moreover, cardoon shows interesting yields from both lignocellulose (14–15 t/ha dry yield without irrigations) and grain (1.5–2 t/ha) widening the spectrum of potential applications (bioethanol, green forage, pharmacological compounds) (Foti et al., 1999; Fernandez et al., 2006; Angelini et al., 2009) and showing high potential to become in the future one of the key bioenergy crops in the Mediterranean environment (Ierna et al., 2012).

The use of biomass in many technological processes is crucial for the development of biorefineries in terms of energy, economic and environmental sustainability (Demirbas, 2009; Cherubini, 2010; Barakat et al., 2013). Among lignocellulosic biorefinery products, ethanol obtained from both cellulose and hemicellulose (Shatalov and Pereira, 2011; Hasunuma et al., 2013) could be used as a renewable fuel in the transportation sector or as a building block for the manufacturing of bio-based chemicals (diethyl ether, 1,3-butadiene, ethyl acetate, propylene and ethylene) (Posada et al., 2013).

Ethanol production from lignocellulosic biomass requires a pretreatment to reduce substrate recalcitrance and remove lignin; physical and chemical pretreatments improve polymers deconstruction but generate several inhibitors (furfural, hydroxymethylfurfural, acetic acid, formic acid, levulinic acid, phenolic compounds) that can obstacle the subsequent enzymatic hydrolysis and fermentation processes (Palmqvist and Hahn-Hagerdal, 2000; Phitsuwan et al., 2013).

Among pretreatment methods, hydrothermal processing is an interesting clean technology for the fractionation of lignocellulosic biomass. The main advantages, compared to chemical or biological pretreatments, are that hydrothermal processing does not use solvents or chemicals different from water, it reduces corrosion of equipment and the operational process is simple and economical. On the other hand, the main disadvantages are the degradation of part of the xylan fraction to volatile compounds, the incomplete disruption of the biomass bonds and the formation of inhibitors that influences the following steps. The process consists in treating the lignocellulosic biomass with water at high temperature and pressure which autoionizes into acid hydronium ions (H_3O^+), that act as catalyst, and basic hydroxide ions (OH^-). In addition, further H_3O^+

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are generated from acetic acid derived from acetyl groups present in lignocellulosic biomass. As a result, the cellulose crystallinity is reduced, the hemicellulose is depolymerized and solubilized and the enzymes accessibility to the biomass structure is enhanced through the re-localization of lignin and the increase in pore size.

The most important parameters that influence hydrothermal processes are temperature, residence time, particle size, moisture content and pH. In the SE process, the influence of temperature and time is described by the severity factor: low severity decreases economic costs and inhibitors formation, but at the same time, the biomass deconstruction is lower; high severity increases economic costs and the loss of hemicellulosic sugars, but the biomass deconstruction is improved as well as the enzyme accessibility and the digestibility of cellulose (Alvira et al., 2010; Pronyk et al., 2011; Chiaramonti et al., 2012; Ruiz et al., 2013).

After the pretreatment, ethanol can be obtained with different process configurations, such as separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and semi-simultaneous saccharification and fermentation (SSSF). The SHF process is divided into two steps, the first performed at the optimal conditions for the enzyme (50 °C and pH 5) and the second for the yeast (32 °C and pH 5), while in the SSF process the operational conditions are constant (37 °C and pH 5). SSSF consists of a pre-hydrolysis (4–24 h) followed by a SSF (Goncalves et al., 2014). Recent studies showed variable results of SHF and SSF in terms of ethanol yield, depending on the operational conditions, microorganism and biomass employed (Marques et al., 2008; Tomás-Pejó et al., 2008; Ask et al., 2012; Wirawan et al., 2012).

Cardoon as a biomass feedstock has interesting potential for ethanol production in terms of high polysaccharides content and low lignin content (Gominho et al., 2001; Ballesteros et al., 2007); previous studies investigated *C. cardunculus* L. with chemical pretreatment (acid hydrolysis) (Del Campo et al., 2006; Ballesteros et al., 2008), but the use of steam explosion process with cardoon feedstock is an interesting way to avoid external catalyst addition (Negro et al., 2003).

The present experimentation carried out a campaign of tests for ethanol production, comparing the SHF and SSSF processes. The cardoon underwent the following steps: raw material size reduction and physical pretreatment, enzymatic hydrolysis and ethanol fermentation by yeasts. The steam explosion pretreatment was tested at different severity factors (varying temperature and residence time) in order to decompose the lignocellulosic biomass structure and make cellulose and hemicellulose more accessible during the hydrolysis step.

The results are presented in terms of water insoluble substrate (WIS) recovery and ethanol yield both on 100 g of cellulose and on 100 g of raw material (RM).

2. Materials and methods

2.1. Biomass recovery

The raw material used in the tests was collected in the cardoon experimental fields property of the University of Perugia, located near Perugia (Umbria region in central Italy). The biomass was collected after the blossoming stage in September. It was then air dried and chopped to obtain chips in the range of 2–3 cm.

2.2. Steam explosion pretreatment

The raw material was subjected to a SE pretreatment to deconstruct the biomass and obtain a fractionation of cellulose, hemicellulose and lignin.

Table 1
Main conditions in the steam explosion experimentations.

Pretreatment test	Moisture (%)	T (°C)	t (min)	Log R_0
1	11.69	200	8	3.85
2	13.43	200	12	4.02
3	14.01	210	11	4.28
4	13.43	220	10	4.53

The SE pretreatment plant used was described elsewhere (Cotana et al., 2014a), while the pretreatment conditions are described by the severity factor log R_0, (Overend et al., 1987) according to Eq. (1):

$$R_0 = te^{[T-100/14.75]} \quad (1)$$

where t is the time (minutes) and T is the temperature (°C).

Each pretreatment was carried out with 500 g of biomass with a moisture content lower than 15%. The reactor was pre-heated to the selected temperature with saturated steam, before introducing the biomass. The final heat up time was, for all the tests, less than 20 s. After the SE, the material exploded into the cyclone was immediately recovered and washed with cold water and, at the end, filtered for the separation of the solid and liquid fraction. In this layout, the heat up and cool down times can be considered negligible.

The experimental campaign included 4 tests at different severity factors, and each test was replicated 6 times; the steam explosion conditions are reported in Table 1. Each test generated the samples A1,A2,A3,B1,B2,B3,C1,C2,C3,D1,D2,D3 employed in the SHF tests and E1,E2,E3,F1,F2,F3,G1,G2,G3,H1,H2,H3 employed in the SSSF tests.

After the pretreatment a WIS rich in cellulose and lignin and a pretreatment liquor rich in pentose sugars (xylose and arabinose) derived from hemicellulose were collected. Since the natural yeast (*Saccharomyces cerevisiae*) is not naturally able to ferment pentose sugars, the pretreatment liquor was not employed in the fermentation.

For each log R_0 value 3 samples of WIS were processed by SHF and 3 by SSSF. Before processing, the WIS was washed and the water was separated by a press.

2.3. Saccharification and fermentation

Both Saccharification and fermentation of WIS were performed in semi-sterile conditions in a 5 L automated reactor (Biostat® A-Plus – Sartorius) for 96 h and filled to a final weight of 3100 g (WIS + water). The WIS was diluted with deionized water resulting in a final dry matter (DM) concentration of 8% w/w and sterilized at 80 °C for 30 min into the automated reactor. All the trials were carried out at pH 5 with the same enzyme and yeast dosage.

An enzyme ([TM]Ctec2) with an activity of 120 FPU/mL was employed in all experimentations. Enzyme loading of 30 FPU/g glucan into WIS was used, equivalent to 15 FPU/g of WIS approximately, as suggested by the provider Novozymes in order to determine the maximum enzymatically accessible cellulose content.

Ethanol red® (Fermentis) yeast was employed for fermenting the sugar into solution (direct pitching) at a suggested dosage level 0.07% w/w (g yeast/g final weight). Before yeast inoculation, 23.8 g of urea solution (400 g/L) were added as a nitrogen source.

2.4. Separate hydrolysis and fermentation

The process was divided into two separate steps: pure enzymatic hydrolysis and pure fermentation. In the first step, the cellulose was hydrolyzed for 48 h at 50 °C and 350 rpm. In the second step, the yeast was inoculated after the temperature and the