Industrial Crops and Products 95 (2017) 104–112

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Selective single-stage xylan-to-xylose hydrolysis and its effect on enzymatic digestibility of energy crops giant reed and cardoon for bioethanol production

Anatoly A. Shatalov[a,*], Ana Rita C. Morais[a], Luís C. Duarte[b], Florbela Carvalheiro[b]

[a] Universidade de Lisboa, Instituto Superior de Agronomia, Centro de Estudos Florestais (CEF), Tapada da Ajuda, 1349-017, Lisbon, Portugal
[b] LNEG-Laboratório Nacional de Energia e Geologia I. P., Unidade de Bioenergia, Estrada do Paço do Lumiar 22, 1649-038, Lisbon, Portugal

ARTICLE INFO

Article history:
Received 1 July 2016
Received in revised form
21 September 2016
Accepted 8 October 2016

Keywords:
Giant reed
Cardoon
Biorefinery
Dilute acid hydrolysis
Enzymatic digestibility
Xylose

ABSTRACT

The low temperature dilute sulfuric acid hydrolysis of two potential European energy crops – giant reed and cardoon have been studied for separation and valorization of total hemicellulosic carbohydrates prior to fractionation of cellulose and lignin, viewing as entry point to integrated biorefining scheme. The combined severity factor (CSF) was used to assess, on comparative basis, the selectivity and efficiency of xylan-to-xylose conversion and its effect on enzymatic digestibility of residual cellulose. The reliable quantitative correlations have been established for the first time between the CSF and the main outputs of acid and enzymatic hydrolysis. Xylose recovery of ca. 94% and 86% (as max.) was achieved at CSF 1.90 and 1.97, respectively for giant reed and cardoon, with formation of ca. 2.4% glucose, 1.1% furfural, 0.5% HMF and 3.2% acetic acid. Under these CSF levels, the enzymatic digestibility of residual cellulose was improved only up to 0.75 and 0.80 (75% and 80% total Glc recovery, respectively for giant reed and cardoon), vs. 0.09 and 0.19 for untreated biomass. The digestibility was further improved to 0.93 and 0.98 with increase in hydrolysis severity up to CSF 2.44, while substantial loss of xylose (ca. 40%) and furans formation (ca. 5% furfural) was found. The essential effect of cardoon stalk morphology on uniformity and modeling of biorefining separation processes was noted.

1. Introduction

Integrated conversion of lignocellulosic biomass into transportation fuel and chemicals within a multi-product biorefining concept (LCF biorefinery) is viewing as one of the more potential ways to ensure sustainable biorefining technology and smooth transmission from the petroleum-based to bio-economy (Fernando et al., 2006; Kamm and Kamm, 2007; FitzPatrick et al., 2010). The agro-based lignocellulosics, such as annual crops and perennial herbaceous species (grasses), represent abundant and low-cost biomass feedstocks for LCF biorefinery. Among a variety of herbaceous species evaluated as potential energy crops in both the US and Europe, four perennial rhizomatous grasses such as switchgrass, miscanthus, reed canary grass and giant reed showed the best potential for biomass production and were chosen for more extensive research programs (Lewandowski et al., 2003).

Giant reed (*Arundo donax* L.) and artichoke thistle cardoon (*Cynara cardunculus* L.) are abundant naturally growing perennial herbs native to the countries of the Mediterranean region. The high biomass productivity, annual harvesting period, easy adaptability to different soil and climatic conditions, ability to intensive cultivation and appropriate chemical composition (Perdue, 1958; Fernández, 1998; Piscioneri et al., 1999; Shatalov et al., 2001) made this herbs as the most promising energy crops for industrial utilization and particularly attractive lignocellulosic feedstocks for biorefinery schemes (Fernández et al., 2006; Williams and Biswas, 2010).

Similar to other lignocellulosics, giant reed and cardoon biomass consists mainly of three biopolymer components – cellulose, hemicelluloses and lignin, forming complex and rigid cell wall structure, resistant to chemical and enzymatic degradation. Development of effective and selective methods for primary fractionation of these biopolymers is a challenge of fundamental importance in LCF biorefinery. Polysaccharide portion of giant reed and cardoon accounts for 60–70% of dry biomass, where 25–30% falls on hemicellulosic polysaccharides (Shatalov and Pereira, 2011, 2012). The success of crop biorefining technology is therefore entirely dependent on

* Corresponding author.
E-mail address: anatoly@isa.ulisboa.pt (A.A. Shatalov).

http://dx.doi.org/10.1016/j.indcrop.2016.10.017
0926-6690/

effective access and conversion of carbohydrates. The hemicelluloses of giant reed and cardoon are composed by 90–95% of xylan polysaccharide, which can be used as a valuable source of xylose for many commercial technologies, such as biotechnological production of xylitol. The valorization of xylan by careful conversion to monomeric sugars prior to separation of cellulose and lignin can be an important first step (entry point) to integrated biorefining chain. Among a variety of reported approaches, the dilute sulfuric acid hydrolysis was proved to be a reliable and easily performed low-cost method for selective conversion of polymeric xylan to monomeric xylose (Sun and Cheng, 2002). It was shown that the yield of xylose after hydrolysis is strongly dependent on the type of feedstock used and the operation conditions applied (Rahman et al., 2007; Jeong et al., 2010; Akpinar et al., 2011). Under controlled (optimized) hydrolysis conditions the minimal cellulose degradation and by-products (e.g. furans) formation takes place, providing the high efficiency and selectivity of the overall process.

After selective hydrolysis and valorization of hemicellulosic polysaccharides, the residual cellulose can be used as a source of cellulosic fibers for production of high purity dissolving grade pulps for chemical synthesis (Sixta, 2006; Shatalov and Pereira, 2013, 2014). Since hemicellulose removal is presumed to destroy the lignin-carbohydrate matrix shielding cellulose microfibrils in the cell wall material (Zhang, 2008; Leu and Zhu, 2013), the dilute acid hydrolysis can significantly improve accessibility and therefore enzymatic digestibility (saccharification) of cellulose portion in pre-hydrolyzed biomass, providing a valuable source of fermentable sugars (glucose) for bioethanol production and being therefore an effective pre-treatment method (Sun and Cheng, 2002; Martin et al., 2007; Castro et al., 2011; Shafiei et al., 2015; Cotana et al., 2015; Gaur et al., 2016). However, the high temperatures (up to 200–230 °C), typically used in acidic pre-treatments of lignocellulosic biomass do not provide a required quality of sugar (xylose) hydrolysate for further biochemical conversion, due to the low hydrolysis selectivity under severe reaction conditions.

As an integral part of multidisciplinary research project on biorefinery of energy agro-crops, the low-temperature dilute sulfuric acid hydrolysis has been designed for selective single-stage conversion of total polymeric xylan of giant reed and cardoon biomass to monomeric xylose, viewing as an entry point to complex biorefining scheme with complete fractionation and utilization of the main chemical constituents (hemicelluloses, cellulose and lignin). The process severity concept was used as a basic model to assess (on comparative basis for both crops) the selectivity and efficiency of total xylan-to-xylose conversion and its effect on enzymatic digestibility of residual cellulose. For the first time, the reliable quantitative correlations have been established between the process severity conditions and the main outputs of acid and enzymatic hydrolysis, using two very potential European energy crops (giant reed and cardoon) as the representative models of low-cost agro-based lignocellulosic biomass. The principal results of this study are discussed in the present paper.

2. Materials and methods

2.1. Materials

The cardoon (*Cynara cardunculus* L.) and giant reed (*Arundo donax* L.) biomass was sampled, respectively, from the university experimental plantation field (School of Agronomy, University of Lisbon) and from the naturally growing crop population (Tapada da Ajuda, Lisbon). The air-dry whole stems of both crops were manually stripped of leaves, milled and screened to uniform particle size of 40–60 mesh and stored in the sealed plastic bags at room temperature until use. The moisture content of material was determined according to NREL standards (Sluiter et al., 2008a). Chemical composition of giant reed and cardoon stem biomass is shown in Table 1.

Table 1
Chemical composition of giant reed and cardoon biomass (% on oven-dry material).

Component	Giant reed	Cardoon
Ash	5.04	5.44
Silica SiO_2	2.24	0.08
Extractives	12.22	7.36
Dichloromethane	0.36	0.41
Ethanol	4.99	3.25
Water	6.88	3.70
Lignin	24.02	18.19
Acid-insoluble	21.85	15.46
(Klason)	2.17	2.73
Acid-soluble	59.46	69.87
Holocellulose	33.85	39.28
α-Cellulose	0.18	0.75
Rha	1.94	1.17
Ara	23.47	21.49
Xyl	0.38	1.10
Man	0.64	1.35
Gal	40.02	52.27
Glc		

Commercial enzyme preparations Celluclast 1.5L (cellulases from *Trichoderma reesei*) and Novozyme 188 (β-glucosidases from *Aspergillus niger*) were purchased by Sigma Co. Enzymatic activity (FPU/mL and pNPGU/mL, respectively) was determined before enzyme application (Ghose, 1987; Berghem and Pettersson, 1974).

All other chemicals used in this study were of analytical grade purity and purchased by Sigma, Aldrich, Fluka and Riedel-de Haën companies.

2.2. Acid hydrolysis

Dilute sulfuric acid hydrolysis was performed in stainless steel digesters rotated in an oil bath, as detailed elsewhere (Shatalov and Pereira, 2011, 2012). The process variables were reaction time (20–70 min), reaction temperature (120–150 °C) and acid concentration (0.2–1.8%). Hydrolysis experiments were replicated for each condition set to obtain reproducible analytical data. Insoluble solids after hydrolysis were separated by filtration under vacuum, carefully washed with deionized water to delete any free acid and kept moist (or frozen) until further analysis and enzymatic processing. The collected hydrolysate, combined with washing waters, was analyzed on degree of monosaccharide recovery and degradation.

2.3. Enzymatic saccharification

Enzymatic digestibility of insoluble residues after acid hydrolysis has been carried out using standard NREL procedure (Selig et al., 2008). Solid residue (0.15 g on dry basis) was mixed with commercial cellulases (60 FPU/g cellulose) and β-glucosidases (64 pNPGU/g cellulose) in 50 mM sodium citrate buffer (pH 4.8) and incubated at 50 °C for 72 h under 70 rpm rotation. Sodium azide (2% solution) was added as antibiotic to prevent any microbial infection during digesting. The release of soluble sugars (basically glucose and xylose) was monitored by HPLC and corrected by blank tests on substrate and enzymes. The enzymatic digestibility (saccharification) was defined as a ratio of cellulose digested to cellulose loaded (g/g).

2.4. Analytical methods

Residual lignin in process solids was determined as acid-insoluble (Klason) lignin after two-step acid hydrolysis, according to NREL standard (Sluiter et al., 2008b).