RESEARCH ARTICLE

The Impact of Particle Size on the Dilute Acid Hydrolysis of Giant Reed Biomass

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Received 07 August 2013; revision received 05 February 2014; accepted 06 February 2014 Published online 30 April 2014 (www.ejee.cl). DOI 10.7770/ejee-V2N1-art598
ISSN: 0719-269X

ABSTRACT Heterogeneous reaction systems, such as the dilute acid hydrolysis of lignocellulosic biomass for the recovery of hemicelluloses, are typically dependent of particle size which is considered as one of the most important variables that affect process yield and global production costs.

In this work, the effect of feedstock particle size on dilute acid hydrolysis is studied using Giant Reed (*Arundo donax*), a Mediterranean energy crop with a high potential for the production of bioenergy and added-value products, as a model feedstock. Five different particle sizes (ranging between < 0.25 to 4 mm) were studied using previously optimized dilute hydrolysis conditions.

Both the hydrolysates and the residual solids were chemically characterized. Hydrolysates always contained more than 32 g/L of total sugars (from which 93% are pentoses) and all the residual solids contained more than 50% glucan per dry weight of processed solids that corresponds to complete recovery of glucan in the solid phase. These results clearly show a selective hydrolysis of the hemicellulose regardless of the particle size fractions assayed. Actually, no statistically significant changes between fractions were identified, which is a further advantage for this raw material, as it enables efficient operation at higher particle size, positively affecting the energy costs and its upgrade within the biorefinery framework

KEYWORDS *Arundo donax*, Biomass pretreatment, heterogeneous catalysis, Hemicelluloses, Size reduction

Introduction

Giant Reed (*Arundo donax* L.) is an herbaceous plant common in southern Europe (Mediterranean region) and widely distributed in tropical, subtropical and temperate regions. It has a high agronomic productivity and the ability to be cultivated intensively in poor or marginal lands without much need for fertilizers [Lewandowski et al., 2003] , which makes it one of the preferred energy crops to be used as a feedstock for the biorefineries. In fact, it is one of the model raw materials to be transformed in the newly cellulosic ethanol European biorefinery [Chiaramonti and Giovannini, 2012]. Particularly interesting, it is the stem´s polysaccharide content (above 60%), from which near 40% of the total, is

hemicellulose [Shatalov and Pereira, 2012].

Hemicelluloses are typically less studied than cellulose and are more difficult to upgrade. Nevertheless, as in the biorefinery concept it is possible to derive products from all biomass components, hemicelluloses recovery has been receiving increasing attention.

Noteworthy, the use of dilute acid hydrolysis is usually the preferred method, as a significant number of advantageous traits can be identified, especially if free monosaccharides, and specially pentoses, are the target hemicellulose-derived products [Gírio et al., 2012]. Heterogeneous reaction systems, such as these, are typically dependent of particle size, which is considered as one of the most important variables that affects process yield and global production costs [Ballesteros et al.,2000; Vidal et al., 2011]. Generally, when larger chips are used, heat and mass transfer problems may result in over-hydrolysis of the outer side of the chips (with the associated formation of inhibitors) and incomplete hydrolysis in the inner side [Brownell et al., 1986]. On the other hand, smaller particles give a larger surface area but, are difficult to handle, more susceptible of being degraded during the hydrolysis process and make the overall process more expensive due to high energy requirements of the milling step [Ballesteros et al., 2000; Ballesteros et al., 2002] . Furthermore, studies conducted using diffusional models have demonstrated that sulfuric acid takes three times more time to penetrate 70% of the biomass when it is randomly cut into 10 and 30 mm fragments, than when ground between 0.85 and 1.20 mm [Kim and Lee, 2002].

The nature of the feedstock is the major factor influencing the efficiency of the milling process. To achieve the same particle size, it is necessary to resort to less than half the energy when treating herbaceous-type materials, as *A. donax*, compared to woods [Aden et al., 2002; Vidal et al., 2011]. As such, it would be of great practical interest to follow the economic feasibility improvement of *A. donax* dilute acid hydrolysis under conditions of reduced power consumption on material preparation during chopping and milling.

The main objective of this work was to evaluate the effect of particle size on the efficiency of dilute acid hydrolysis of the energy crop *A. donax*, over a relatively broad range of particles sizes.

Materials and Methods

Raw material

Giant Reed (*Arundo donax*) was sampled from a naturally growing crop population (Tapada da Ajuda, Instituto Superior de Agronomia, Lisbon). The air-dry reed stems, with diameters larger than 40 mm (Fig. 1) were manually stripped of leaves, chopped (Viking GE 35S, Austria) and then knife-milled (Fritsh, Germany) through the sieves of 1 mm or 4 mm. Milled material was further separated into five different fractions using a sieve shaker (see below).

Figure 1. A. donax dry stems after being stripped from their leaves clearly showing the empty kernel.

Diluted acid hydrolysis

The different particle size fractions selected were mixed, in 2 L glass flasks, with 1.27% (w/w) H_2SO_4 in a liquid-to-solid ratio of 7 (w/w), and soaked for 20 min at room temperature. The dilute acid hydrolysis was performed in an autoclave (AJC, Portugal) at 130ºC for 105 min, transposing previously optimized conditions [. After the reaction time had elapsed, the autoclave was slowly cooled down and the hydrolysate and solid phase were recovered using a hydraulic press (up to 200 bar) followed by filtration (Whatman® no. 1 filter paper). Each tested condition was performed at least in duplicate.

Analytical methods

Particle-size distribution

The quantification of the particle-size distribution was carried out based on the NREL protocol described in [Hames et al., 2005]. Milled samples (ca. 100 g) were screened, at least in triplicate, for 30 min through the series of selected sieves (Retsch, Germany) with different pore sizes, as shown in Table 1, using an appropriate sieve shaker (EVS1, Endecotts, England).

Table 1. Sieves used to characterize the particle size distribution of Arundo donax samples

Pore diameter, Φ (mm)	Sieve Number	Mesh
1	0.075	190*
2	0.150	100
3	0.250	63*
4	0.500	35
5	0.710	25
6	1.00	18
7	1.60	12*
8	2.36	8

*Estimated

Chemical characterization of liquid samples

Liquid samples were filtered through 0.45 ?m membranes (Pall Corporation, USA) and assayed by HPLC to quantify free monosaccharides (glucose, xylose and arabinose), aliphatic acids (acetic, formic and levulinic acids) and furan derivatives (furfural and 5-hydroxymethylfurfural, HMF). Quantifications were carried out by a HPLC (Agilent 1100 series, Waldbronn, Germany) using an Aminex HPX-87H column (BioRad) thermo stabilized at 50º C and using 5 mmol L^{-1} H_2SO_4 as mobile phase.To quantify the monosaccharides and aliphatic acids it was used a refractive index (RI) detector, and a DAD detector to quantify furans (using 280 nm data).

The total soluble phenolics were determined by the Folin-Ciocalteu method using Gallic acid as standard [and a spectrophotometer (Jasco model V530, Japan). All these determinations were carried out at least in duplicate.

Chemical characterization of liquid samples

Giant Reed biomass and processed solid residues samples were milled to particle size of less than 0.5 mm (Ika Werck MF10, Staufen; Germany), whenever necessary, or used as is for lower particle sizes. The moisture content of the sample was determined after, at least, 18 h at 105 ± 1^{o}C in a convection oven (Memmert, Germany). Ash content was quantified after, at least, 5 h at 550 ± 5 ^{o}C in a muffle furnace (Heraeus, Germany).

Chemical characterization of the solid samples was carried out according to a method based in NREL protocols [Sluiter et al., 2005; Sluiter et al., 2008] using a two-step acid hydrolysis, the first using with 72% (w/w) H2SO4 (30^{o} C, 60 min) followed by 4% (w/w) H2SO4 (121^{o} C, 60 min). The acid insoluble residue was recovered by filtration and considered as Klason lignin after correction for the ash content. Recovered filtrates were used to quantify glucan, xylan, arabinan and acetyl groups based on HPLC data, as described above. All the procedures were carried out at least in duplicate.

Results and discussion

Particle size distribution

Comminution is an essential step on biomass pre-treatment, as it enables to minimize potential heterogeneities and increase feedstock handling and overall process performance, nevertheless, as a time and energy consuming process, it must be carefully optimized. Unfortunately,

conversely to what occur for the enzymatic hydrolysis of cellulose [Alvira et al., 2010], the optimum particle size for biomass fractionation/pretreatment is seldom studied. In fact, it is also difficult to establish the "best" particle size as it depends on many factors such as the type/origin of biomass and on the processing conditions applied. For example, in the case of acid hydrolysis typical studied ranges vary from 0.85 to 3 mm [Kim and Lee, 2002], and for other treatments e.g. steam explosion, the typical studied ranges for particles sizes are rather higher (up to 12 mm). In the later, usually major recoveries were obtained when using particles with bigger sizes (between 8 to 12 mm), in detriment of smaller particles, 2 to 5 mm, [Ballesteros et al., 2002]. Nevertheless, the use of such larger particles is not adequate for classical acid hydrolysis, as conversely to steam-explosion, it lacks the physical effects on the biomass, derived from the rapid decompression. As such, for this study, it was considered a smaller range and two mill-sieves were chosen (1 and 4 mm). The particle size distribution obtained from the milling with the two different sieves tested is presented in Fig. 2.

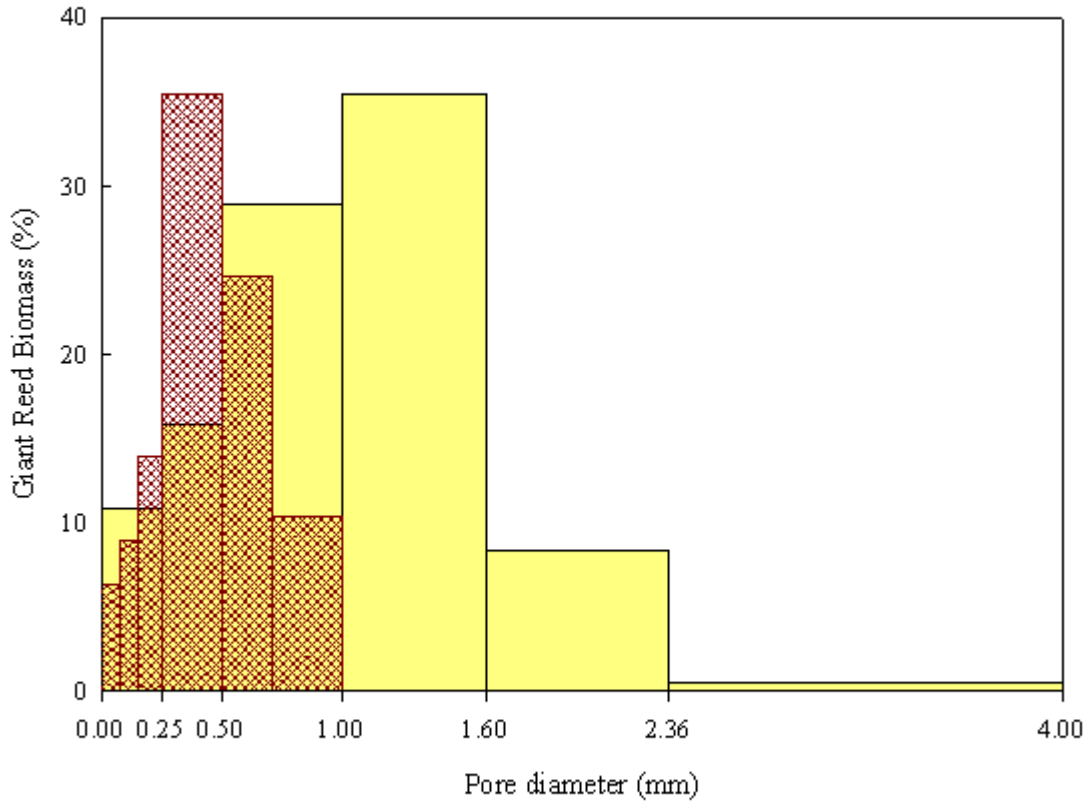

Figure 2. *A. donax* particle size distribution using 1mm (red/dotted) or 4 mm (yellow/filled) milling sieves.

The material milled with the 1 mm sieve can be divided into 3 main fractions: size less than 0.250 mm (29.2%), size between 0.250 mm (inclusive) and 0.500 mm (35.5%) and larger than 0.500 mm (35%). As expected, the milling with 4 mm sieve showed different distribution profile, covering a more wide range of particle sizes. In this case, 56% of the material has a size below 1 mm, 35.7% presents dimensions between 1 mm (inclusive) and 1.6 mm, and

only ca. 9% of the particles obtained had a size higher than 1.6 mm. Given the percentages obtained, it seems more advantageous to use a 4 mm screen in the milling process as it will enable to reduce energy costs, with a significant decrease of particle size.

In order to further validate this, and based on this particle size distribution, the milled material was separated into five different fractions (Table 2).

Table 2. Fractions used to study the effect of particle size on hydrolysis efficiency

Fraction	Minimum Size mm	Maximum Size mm
A	-	<0.250
B	0.250	<0.500
C	0.500	<1.000
D	1.000	<1.600
E	1.600	4.000

The chemical compositions of these different fractions were determined showing that no significant statistical differences could be found between them (Table 3). This is due to the fact that the material itself is rather homogenous, not presenting differential resistances to milling, nor different chemical compositions induced by different tissues as it is common for other materials.

Table 3. Average chemical composition (g/100 g dry matter) for the fractions with the different particle size and the respective standard deviations

	Average	Standard deviation
Glucan	37.36	1.72
Xylan	19.72	1.46
Arabinan	2.15	0.15
Acetyl groups	3.70	0.49
Klason Lignin	23.01	0.59
Ash	4.96	0.45
Others	6.94	0.45

Furthermore it is important to note that it has a high content of polysaccharides (approx. 63%), similar to other herbaceous feedstocks, which further supports its use as a model raw material. Its hemicellulose is mainly constituted by pentoses, especially xylose that accounts for the larger part of all hemicellulosic sugars and also as for other herbaceous feedstocks, its lignin content is low. It is also highly acetylated, presenting one of the major degree of acetylation described in the literature for similar herbaceous feedstocks [Gírio et al., 2012]. It also has an high ash content, but similar, for instances to corn straw [Moniz et al., 2013].

As such, these material seems perfectly adequate to evaluate the impact of particle size on the hydrolysis efficiency, as any other potential factors related to chemical composition that may interfere on the results are absent.

Effect of particle size on the hydrolysate composition

The chemical composition of the hydrolysates produced under optimized dilute acid hydrolysis conditions using the different particle size fractions are shown in Table 4.

Table 4. Chemical composition (g/L) of the acid hydrolysates obtained for the different particle size

Compounds (gL)	Fractions A	B	C	D	E	Average	Standard deviation
Glucose	2.30	2.39	2.74	2.08	2.57	2.42	0.25
Xylose	28.71	28.34	28.11	28.14	31.05	28.87	1.24
Arabinose	2.34	2.26	2.38	1.78	2.34	2.22	0.25
Formic acid	1.12	1.13	0.92	0.58	0.65	0.88	0.26
Acetic acid	5.83	5.76	5.61	6.57	6.86	6.13	0.55
Levulinic acid	0.10	0.11	0.10	0.11	0.13	0.11	0.01
HMF	0.06	0.06	0.07	0.07	0.07	0.07	0.01
Furfural	1.26	1.41	1.44	2.05	1.37	1.51	0.31
Phenolics compounds	1.61	1.60	1.77	1.51	1.49	1.60	0.11

Comparing the composition of the different hydrolysates obtained from the different fractions, it can be observed that only the hemicellulosic fraction was hydrolysed into the liquid fraction. Furthermore, no statistically significant differences were found for the different compounds present/produced, as the variation coefficient, defined as the ratio of standard deviation to the average, is typically close to 10%, with minor unrelated exceptions for formic acid and furfural, clearly pointing out to random effects and not to a defined trend.

Similar results were described for a similar biomass, rice straw [Karimi et al., 2006]. Comparing the recovery of lignocellulosic components before and after size reduction, for the particles with sizes between 0.295 and 0.833 mm and 2 mm to 50 mm no effect was determined on hemicellulosic sugars or their derived products (e.g. furans). Actually, only glucose showed a higher yield (a 44% increase) when using the smaller dimensions, conversely to observe in this work, where also glucan hydrolysis where also not increased. Works on switchgrass, and wood species [Yat et al.,2008] presented the same results in the range <0.599 mm to 1.68 mm, that we now extend up to <4 mm for Giant Reed.

Regardless particle size, under optimal conditions, *A. donax* hydrolysate contains about 28 gL^{-1} of xylose (93% of the total pentoses). Furthermore, glucose was found in relative low amounts and similar to arabinose concentration (approx. 2.5 gL^{-1}), rendering this hydrolysate quite interesting

for studying pentose upgrade due to its high pentose/total sugars ratio. Most noteworthy is also the low impact that particle size had on furans production, especially furfural, clearly indicating that the studied particle size range did not significantly influence mass transfer phenomena during the acid hydrolysis. Actually, the amounts of potential microbial inhibitors, furfural, 5-hydroxymethylfurfural and phenolic compounds together with aliphatic acids content (mainly acetic acid) are relevant, as expected both from the composition of the initial raw material and from the high solid loading used. In fact, the majority of the works dealing with batch dilute acid hydrolysis of straws use liquid-to-solid ratios in the range of 10-20 w/w [Mussatto and Roberto et al., 2004; Ballesteros et al., 2007; Vázquez et al., 2007; et al., 2007; Shatalov et al., 2011; Shatalov and Pereira et al.,2012], producing hydrolysates with lower concentrations of inhibitors and sugars.

Effect of particle size on the processed solids composition

As expected from the results described above, the composition of the processed solids was also fairly constant (Table 5). All residual solids contained more than 50% glucan that corresponds to a complete recovery of glucan in the solid phase. A similar trend was also observed for lignin, whose content is also increased as compared to the initial feedstock. These results clearly show a selective hydrolysis of the hemicellulose regardless of the particle size fractions assayed.

As for the liquid composition, the variation coefficient for the solids constituents is typically close to 10%, with minor unrelated exceptions for the hemicellulosic components, but again no correlation could be obtained based on composition and average particle size. Previous results have shown a similar lack of trend [Chen et al., 2011; Vidal et al., 2011] .

Concerning the processed solids composition and size it is important to note that although differences in particle size does not seem to exert significant influence towards the efficacy of acid pretreatment, special attention should be given on potential effects on subsequent cellulose upgrade throughout an enzymatic hydrolysis, a process also strongly influenced by particle size [Alvira et al., 2010; Vidal et al., 2011] .

Table 5. Average chemical composition (g/100 g of processed dry matter) for the fractions with the different particle size and the respective standard deviations

Compounds %	Average	Standard deviation
Glucan	54.83	2.68
Xylan	4.27	1.48
Arabinan	0.37	0.16
Acetyl groups	0.40	0.56
Klason Lignin	32.60	1.67
Ash	2.34	0.84

Conclusions

For the studied range, the particle size of Giant Reed had no significant impact on the dilute acid hydrolysis process, as no significant differences were found for both the hydrolysates and the pretreated biomass. This is an advantage of this raw material, as it enables an efficient operation at relatively higher particle sizes than the most commonly described for acid hydrolysis, which will positively affect milling costs, that typically account for almost one third of the power requirement of the entire upgrade process [Hamelinck et al., 2005]. This trait should be further explore and its impact on further biomass processing e.g. on a possible cellulose upgrade based on an enzymatic hydrolysis step will give further insight on the effect of size reduction on Giant Reed overall conversion within the biorefinery.

Under optimal hydrolysis conditions it was possible to selectively fractionate hemicelluloses with a high recovery of monosaccharides yielding a hydrolysate with a chemical composition very favorable for the production of pentose derived compounds, e.g. xylitol.

Acknowledgments

This work has been supported by Fundação para a Ciência e a Tecnologia (FCT, Portugal) within research contract PTDC/AGRCFL/103840/2008. Ivone Torrado gratefully acknowledges the grant funded by FCT within the same project. The authors thank Céu Penedo, and Belina Ribeiro for their support.

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