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# Dilute oxalic acid pretreatment for biorefining giant reed (*Arundo donax* L.)

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## ABSTRACT

Biomass pretreatment is essential to overcome recalcitrance of lignocellulose for ethanol production. In the present study we pretreated giant reed (*Arundo donax* L.), a perennial, rhizomatous lignocellulosic grass with dilute oxalic acid. The effects of temperature (170–190 °C), acid loading (2–10% w/w) and reaction time (15–40 min) were handled as a single parameter, combined severity. We explored the change in hemicellulose, cellulose and lignin composition following pretreatment and glucan conversion after enzymatic hydrolysis of the solid residue. Two different yeast strains, *Scheffersomyces* (*Pichia*) *stipitis* CBS 6054, which is a native xylose and cellobiose fermenter, and *Saccharomyces carlsbergensis* FPL-450, which does not ferment xylose or cellobiose, were used along with commercial cellulolytic enzymes in simultaneous saccharification and fermentation (SSF). *S. carlsbergensis* attained a maximum ethanol concentration of 15.9 g/l after 48 h at pH 5.0, while *S. stipitis*, at the same condition, took 96 h to reach a similar ethanol value; increasing the pH to 6.0 reduced the *S. stipitis* lag phase and attained 18.0 g/l of ethanol within 72 h.

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## 1. Introduction

Lignocellulosic biomass includes agricultural residues, deciduous forestry species, municipal solid wastes, waste from pulp and paper industry, and dedicated energy crops. In almost all cases, their major component is cellulose, followed by hemicelluloses and lignin. Proteins, oils, pectins and ash make up the balance [1].

Cellulose and hemicelluloses, primary substrates for second generation bioethanol production, are the most abundant carbohydrates in nature and their conversion to fuel ethanol could have several benefits, including reductions in greenhouse gas emissions and fossil fuels dependence [2]. However, the natural resistance or recalcitrance to degradation of

lignocellulosic material is a major barrier to the economical development of biofuels and bioproducts [3]. Due to recalcitrance, a pretreatment step is necessary to hydrolyze hemicellulose, removing or rearranging lignin structure and, at the same time, altering the cellulose structure for the subsequent enzymatic hydrolysis [4,5]. Lignin plays a key role in the bioconversion of lignocellulosic material; it reduces the cellulose hydrolysis by forming a physical barrier and by non-productively binding cellulolytic enzymes [6]. Extensive pretreatment studies have been performed at temperatures above 150 °C in hot water, alkaline, ammonia, and dilute inorganic acids [4], but relatively few have been described for organic acids [7–9].

In the present study we pretreated giant reed (*Arundo donax* L.) with dilute oxalic acid. Previous studies had shown that

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dicarboxylic organic acids can hydrolyze  $\beta$ -(1,4)-bonds more selectively than sulfuric acid [7,10]. The basis for this is not really known, however, hemicellulolytic and cellulolytic enzymes catalyze hydrolysis through a general acid-base mechanism mediated by two carboxylic acids [7], and oxalic acid with its two pKa's might mimic this reaction through similar ion-pair mechanisms. This could result in more efficient catalysis of the  $\beta$ -(1–4) linkage while the relatively weaker ionization potential of oxalic relative to sulfuric acid could reduce subsequent dehydration reactions.

Giant reed (*Arundo donax* L.), a perennial, herbaceous rhizomatous crop of *Poaceae* family, which occurs over a wide range of climatic habitats, was used as substrate to test its suitability for second generation ethanol production. It is native in Asia and countries surrounding Mediterranean Sea; it has a C3 photosynthetic cycle, but its high rates of photosynthesis and productivity are similar to those of C4 species [11]. In several experimental studies carried out in Sicily, the above ground dry biomass yield of giant reed reached 30–40 t ha<sup>-1</sup> [12].

The aim of the present work was to study the effect of temperature, reaction time and dilute oxalic acid (OA) concentration during the steam-pretreatment of giant reed. We followed hemicellulose derived sugars and sugar degradation products released in the water soluble fraction, and the change in composition of the native hemicellulose, cellulose and lignin of the water insoluble fraction after pretreatment. Afterward, the susceptibility of residual cellulose and hemicelluloses to the enzymatic hydrolysis was evaluated employing a commercial cellulase enzyme complex, while two different yeast strains, *Scheffersomyces* (*Pichia*) *stipitis* CBS 6054, which is a native xylose and cellobiose fermenter, and *Saccharomyces carlsbergensis* FPL-450, which does not ferment xylose or cellobiose, were used along with commercial cellulase enzyme complex in simultaneous saccharification and fermentation (SSF).

## 2. Materials and methods

### 2.1. Raw material

Giant reed biomass (stems and leaves) was harvested in wild area, from a well-established cane-brake along the Tellaro torrent in Southern Italy, Sicily. The biomass was air-dried and size reduced with an electrical saw. Subsequently, leaves and stems moisture contents were stabilized at about 9.0%; the materials were homogenized and chipped to a particle size smaller than 2 cm (Wiley Mill model No 2, Philadelphia, USA), and stored at room temperature until compositional analysis and pretreatment step.

The raw material contained 57% (w/w) structural polysaccharides (35.7% glucan, 18.6% xylan, 1.6% arabinan, 0.6% galactan, 0.2% mannan and traces of rhamnan), 22.3% Klason lignin [13], 4.5% acetyl groups [14], 3.7% total ash of which 2.7% acid insoluble lignin ash [13].

### 2.2. Pretreatment

Giant reed chips and pretreatment solutions were placed in sealed stainless steel pressure vessels (approximately 1.1 L), which were mounted inside of a larger steam-tumbling

pretreatment unit and heated externally via steam while rotating at the speed of 2 rpm.

A solution of OA and water was loaded together with 100 g of material (dry weight basis) at a solvent/solid ratio of 4:1 (w/w) inside the stainless steel vessels.

Factors studied were: reaction temperature (150–190 °C), reaction time (15–40 min) and dilute-OA concentration (2–10% w/w); the three factors were combined through the combined severity parameter, hereinafter referred as CS, described by Chum et al. [15].

$$\text{Log} \left[ t \cdot \exp \left( \frac{T_p - T_{\text{ref}}}{14.75} \right) \right] - \text{pH}$$

where  $t$  is the time (min),  $T_p$  the pretreatment temperature (°C) and  $T_{\text{ref}}$  the reference temperature, usually set to 100 °C [16]. The pH was measured from the amount of oxalic acid added before reaction.

After pretreatment, the material was vacuum filtered in order to separate the water soluble from water insoluble fraction (hydrolyzate and solid fraction, respectively). The former was analyzed as reported in the Section 2.6, while the latter was washed at a liquid/solid of 4:1, weighed, and after analytical composition and pH adjustment used in the enzymatic hydrolysis and simultaneous saccharification and fermentation. Experimental conditions are reported in Table 1.

### 2.3. Enzymatic hydrolysis

Dilute-OA-pretreated giant reed was subjected to enzymatic hydrolysis in 125 ml Erlenmeyer flasks, each containing 2% (w/v) dry substrate in 50 ml (5 mM) sodium citrate buffer solution (pH 5.0). Hydrolysis was conducted at 50 °C using an orbital shaking incubator (New Brunswick Scientific, Innova 4400) at 200 rpm. A commercial preparation of Accellerase

**Table 1 – Experimental pretreatment conditions and relative combined severity factor [Log (R<sub>0</sub>) – pH].**

Run (#)	Temperature (°C)	Residence time (min)	[OA] (%w/w)	Log (R <sub>0</sub> ) – pH
1	170	25	3.00	2.06
2	170	25	5.00	2.22
3	170	25	10.00	2.36
4	190	25	3.00	2.65
5	190	25	5.00	2.76
6	190	25	7.00	2.86
7	170	40	3.00	2.26
8	170	40	5.00	2.37
9	170	40	7.00	2.47
10	180	25	3.00	2.35
11 <sup>a</sup>	180	25	5.00	2.46
12	180	25	7.00	2.59
13	190	40	8.00	3.11
14	190	40	3.00	2.68
15	180	15	5.00	2.24
16	180	15	2.00	1.96
17	180	40	3.00	2.56
18	180	40	5.00	2.67

<sup>a</sup> Average values of four determinations.

1000 enzymes (Genencor Inc., A Danisco division), at a loading of 0.50 ml/g cellulose was used. Endoglucanase activities correspond to 1000 units carboxymethylcellulose (CMC)/g cellulose, while  $\beta$ -glucosidase activities were 160 units para-nitrophenyl- $\beta$ -D-glucopyranoside (pNPG)/g cellulose. One CMC unit of activity liberates 1  $\mu$ mol/min of reducing sugars (expressed as glucose equivalents) under specific assay conditions of 50 °C and pH 4.8. One pNPG unit denotes 1  $\mu$ mol of nitrophenol liberated from para-nitrophenyl- $\beta$ -D-glucopyranoside in 10 min at 50 °C and pH 4.8 (Genencor Product Information).

The manufacturer states that some hemicellulase activities are present in the commercial preparation, but they do not provide quantities and these activities were not assessed in the present study.

#### 2.4. Microorganisms and growth conditions

*Schefferomyces (Pichia) stipitis* CBS 6054 [17], a native xylose- and cellobiose fermenting yeast, and *Saccharomyces carlsbergensis* FPL-450, hybrid of *Saccharomyces bayanus* and *Saccharomyces cerevisiae*, were used for simultaneous saccharification and fermentation of the solid fraction. Strains were maintained on YPD plates and stored at 4 °C. Cells were grown in 1000 ml Erlenmeyer flasks containing 400 ml of YPD (10 g/l yeast extract, 10 g/l peptone, 20 g/l glucose) in an orbital shaker incubator at 30 °C, shaken at 200 rpm. Following 24 h growth, cells were harvested by centrifugation (6708  $\times$  g, 10 min, 4 °C), washed with sterile deionized water and adjusted to a calculated concentration of 30 g/l dry cell weight (DCW) via standard curves relating 600 nm absorbance to DCW/l concentration (Agilent 8453, UV–Visible Spectroscopy system).

#### 2.5. Simultaneous saccharification and fermentation

Experiments were carried out in 125 ml Erlenmeyer flasks, each containing 10% (w/v) dry dilute-OA-pretreated giant reed substrate, 45 ml (5 mM) sodium citrate buffer solution at pH 5.0 and then adjusted with NaOH until stable value of 5.0 for *S. carlsbergensis*, pH 5.0 and 6.0 for *S. stipitis*, 0.50 ml/g cellulose of Accelerase 1000 enzymes and 5 ml of nutrients containing (g/l): 5 yeast extract, 5 urea, 0.5  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1  $\text{KH}_2\text{PO}_4$  for *S. stipitis* and 0.44  $(\text{NH}_4)_2\text{SO}_4$ , 0.5  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 1  $\text{KH}_2\text{PO}_4$  for *S. carlsbergensis*, respectively. The initial cells density used as inoculum was the same for both strains; 2 g/l. Erlenmeyer flasks were capped with cotton plug and incubated at 30 °C, 150 rpm for 96 h.

#### 2.6. Analytical methods

Carbohydrate compositions of the original and pretreated giant reed samples were measured by the Analytical Chemistry and Microscopy Laboratory (USDA Forest Products Laboratory) using an improved high-performance anion exchange chromatography (ICS-3000, Dionex, Sunnyvale, California) with pulsed amperometric detection (HPAEC-PAD), according to the method of Davis [13]. The solid materials were milled using a Wiley mill (Thomas Scientific, Swedesboro, New Jersey) before acid hydrolysis.

The hydrolysis conditions were acid concentration of 72%  $\text{H}_2\text{SO}_4$  (v/v) at 30 °C and 3.6%  $\text{H}_2\text{SO}_4$  (v/v) at 120 °C for the first and second stage, respectively. The supernatant of the acid hydrolyzate was directly used for ion chromatographic analysis. The measurement relative standard deviations (RSD) were reported based on internal regular quality assurance (QA) and quality control (QC).

Monomeric sugar concentrations in the hydrolyzate fraction were determined by HPLC (Gilson 307 system, Villiers-le-Bel, France) equipped with a refraction index detector (Hitachi High Technologies Corporation model L-2490, Japan), using an organic acid column (Bio-rad Laboratories Inc., Hercules, CA) HPX-87H (300  $\times$  7.8 mm) operating at 55 °C, 5 mM  $\text{H}_2\text{SO}_4$  as mobile phase and 0.3 ml/min as flow rate. Acetic acid and ethanol concentrations were measured by the same method. A mild post-acid hydrolysis (4%  $\text{H}_2\text{SO}_4$ , 121 °C, 60 min) of the hydrolyzate fraction was performed to estimate the oligomeric forms, and the resultant solution analyzed as above.

Hydroxymethylfurfural (HMF) and furfural in the hydrolyzate fraction were measured by HPLC (HP, 1090 Series II, Hewlett–Packard, Now Agilent Technologies, Palo Alto, CA) with a Phenomenex C18(2) column (250  $\times$  4.6 mm) and acetonitrile (ACN), water and acetic acid 1% as mobile phase at a flow rate of 0.8 ml/min. All samples were properly diluted and filtered through 0.22  $\mu$ m spin-filter before analysis to remove the particle size.

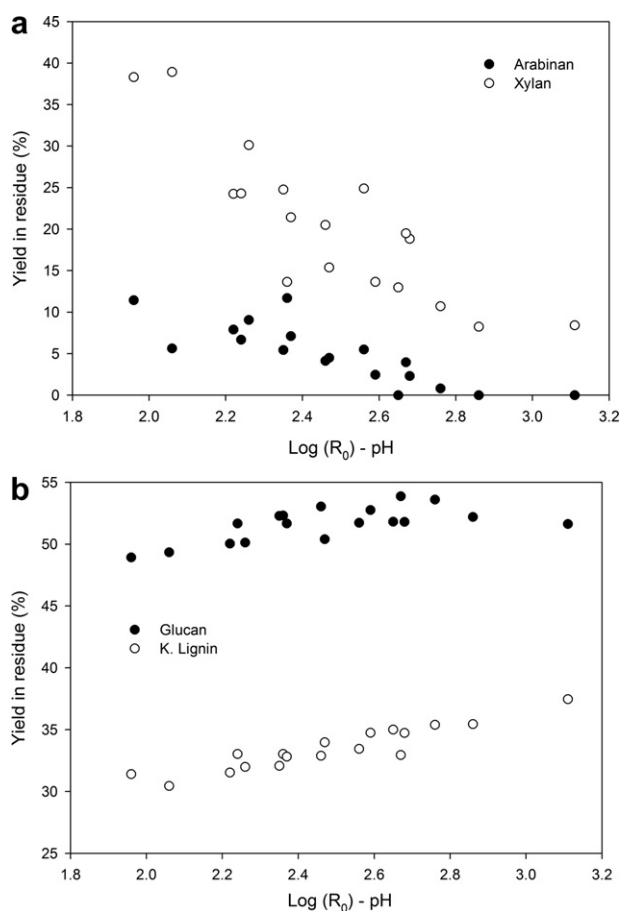
### 3. Results and discussion

Dilute-OA-pretreatment is an effective vector for selective removal of hemicelluloses fraction from giant reed substrate. The combined severity (CS) factor, which describes the effect of pretreatment conditions (reaction temperature, reaction time and OA concentration), has been plotted against the residual hemicellulosic and cellulosic polymers, and the lignin still present in the solid residue after pretreatment (Fig. 1a and b). Yields were calculated based on original, untreated giant reed substrate.

For this purpose, only xylan and arabinan were considered as hemicellulosic polymers because galactan, mannan and rhamnan were completely removed following almost all conditions tested of dilute-OA-pretreatment; only small traces of galactan and mannan were detected at the lowest CS, with a yield in the residue of 5 and 9% of the original untreated giant reed material, respectively (data not shown).

Fig. 1a shows that the higher the CS the less hemicellulosic polymers, namely xylan and arabinan, remained in the residue. At the lowest CS (1.96) about 62% of xylan and 89% of arabinan have been selectively removed after dilute-OA-pretreatment; these values linearly increased till 92 and 100% respectively for xylan and arabinan at the highest CSs (from 2.86 to 3.11). Similar results have been reported with oxalic acid pretreatment of different feedstocks [8,9].

During the pretreatment conditions tested, xylan, arabinan, galactan and mannan were reduced drastically, while glucan and lignin contents increased (Fig. 1b). This could be related to the higher recalcitrance of glucan and lignin under the pretreatment conditions used in the experiments. However, glucan and lignin contents increased mainly due to



**Fig. 1 – a) Yield of hemicellulose (% of xylan and arabinan), b) cellulose and lignin present in the residue after dilute-OA-pretreatment of giant reed as a function of the combined severity factor  $[\text{Log}(R_0) - \text{pH}]$ . Yields were calculated based on original, untreated giant reed substrate.**

hydrolysis of hemicellulosic fraction by dilute-OA-pretreatment; this is also confirmed by the solid recovery after pretreatment that was significantly affected by the pretreatment severity. Its yield ranged from 74.6% (CS 1.96) to 55.1% (CS 3.11) based on starting solid loading of 100 g original untreated giant reed material (Table 2).

Glucan ranged from 48% (w/w) at the lowest CS (1.96) to a maximum of 53% at 2.76. The amount decreased at the most severe CSs (2.86 to 3.11) because of some hydrolysis and degradation of cellulose occurred at these harsher conditions. Lignin increased linearly from the lowest to the highest CS (from 30 to 37% w/w, respectively) mainly because of almost complete hydrolysis of the hemicellulosic fraction. Moreover, during pretreatment at high temperatures, lignin breaks down and forms soluble compounds that reacting themselves and with hemicellulose derived and degraded sugars, form longer insoluble chains that, through condensation reactions, precipitate on the fiber surface [18,19].

The effects of the CS on the composition of the hydrolyzate fraction are shown in Fig. 2.

At the lowest CS (1.96) xylan as xylose and arabinan as arabinose were collected in the hydrolyzate at a yield of about

32 and 40%, respectively; yields are based on all available xylan and arabinan present in the raw material after taking water of hydration into account. Their amounts increased linearly up to a yield of 55 and 58% respectively for xylose and arabinose at a range of CS between 2.22 and 2.47. Contextually, the yield of acetic acid released from the acetyl groups present in the hemicelluloses increased from the lowest to the highest CS (51% at 1.96 and 100% at 3.11, respectively). Acetic acid is an inhibitor of yeasts, and it is a particularly severe inhibitor of xylose fermentations. Therefore its presence is undesirable because at the optimal pH for fermentation ( $\approx \text{pH}$  4.5–5) acetic acid is largely un-dissociated. According to current hypotheses, this permits diffusion or transport of acetic into the cell cytoplasm, where it dissociates and decreases the intracellular pH [20]. Subsequently, the acetyl moiety must be pumped out of the cells by processes that consume metabolic energy. This results in decreased capacity to take up nutrients, a long lag phase and – at concentrations exceeding  $\approx 5\text{--}10\text{ g/l}$  – the death of cells [20,21]. At higher CSs, the yield of xylose and arabinose in the hydrolyzate fraction was not accounting for the amount of xylan and arabinan released from the residue. This could be explained by high temperature and long residence time (high CSs) resulting in the decomposition of pentoses and hexoses to furfural and HMF respectively, which in turn can degrade to formic and levulinic acids [22].

In order to evaluate the effectiveness of dilute-OA-pretreatment on monomer formations, a mild post-acid hydrolysis (4%  $\text{H}_2\text{SO}_4$ , 121 °C, 60 min) of the hydrolyzate fraction was carried out. This fraction was calculated for both pentoses (xylose and arabinose) and glucose (Table 2). It ranges from 79 to 100% for xylose (with 100% monomers in 14 of the 18 conditions tested), from 68 to 100% for arabinose (with 100% monomers in 15 conditions) and from 40 to 100% for glucose (with 100% monomers in only 4 conditions). The lowest concentration of monomers for xylose and arabinose was detected at the lowest CSs (from 1.96 to 2.26), while for glucose it was observed that more severe conditions are needed for monomers release. The low pH found in almost all conditions tested ( $\approx 2.0$ ), can aid the depolymerization of oligomeric to monomeric forms of pentoses, as it has been reported for several raw materials pretreated with organic and inorganic acids [9,23]. Thus, dilute-OA-pretreatment can hydrolyze hemicelluloses to monosaccharides in a single stage pretreatment. This might be a disadvantage under severe conditions since monomer accumulation leads to furfural or HMF production [22].

Furfural formation is undesirable because it is toxic to the yeasts used in the subsequent fermentation step and its formation reduces the sugar yield. Furfural concentration was strongly influenced by the severity of the pretreatment; it ranged from 1.84 g/l to 6.96 g/l at 1.96 and 2.86 CS, respectively (Table 2). At the highest CS (3.11), furfural concentration slightly decreased probably because it broke down to formic acid, which is even more toxic than either furfural or acetic acid [24]. HMF concentration was relatively low, even at the highest CSs, indicating that dilute-OA-pretreatment virtually leaves hexoses unaffected. Its concentration ranged from 0.21 to 0.96 g/l at the lowest and highest CS, respectively (Table 2).

**Table 2 – Solid recovery, xylose, arabinose and glucose monomer forms, xylan to lignin ratio, furfural and 5 hydroxymethylfurfural (HMF) as a function of combined severity factor [ $\text{Log}(R_0) - \text{pH}$ ].**

$\text{Log}(R_0) - \text{pH}$	Solid recovery (%w/w)	Xylose monomers ratio (%)	Arabinose monomers ratio (%)	Glucose monomers ratio (%)	Xylan/Lignin ratio	Furfural (g/l)	HMF (g/l)
2.06	73.14	85.02	89.97	75.00	0.24	2.57	0.26
2.22	73.29	100	100	78.26	0.14	3.03	0.34
2.36	69.94	100	100	79.32	0.11	4.19	0.37
2.65	65.40	100	100	77.78	0.07	5.64	0.44
2.76	58.94	100	100	100	0.06	6.55	0.18
2.86	56.06	100	100	100	0.07	6.96	0.59
2.26	68.20	98.92	100	87.50	0.18	4.03	0.46
2.37	67.55	100	100	85.71	0.12	4.22	0.39
2.47	65.07	100	100	91.67	0.10	4.74	0.56
2.35	68.08	89.35	93.21	76.92	0.14	4.50	0.76
2.46 <sup>a</sup>	68.14	100	100	79.16	0.11	3.18	0.24
2.59	64.45	100	100	100	0.07	4.47	0.41
3.11	55.15	100	100	100	0.10	5.58	0.96
2.68	61.00	100	100	44.44	0.08	6.28	0.51
2.24	62.98	100	100	80.00	0.14	4.17	0.41
1.96	74.63	79.07	83.65	40.00	0.23	1.84	0.21
2.56	64.01	100	100	61.54	0.14	4.47	0.58
2.67	63.75	100	100	80.00	0.11	4.01	0.35

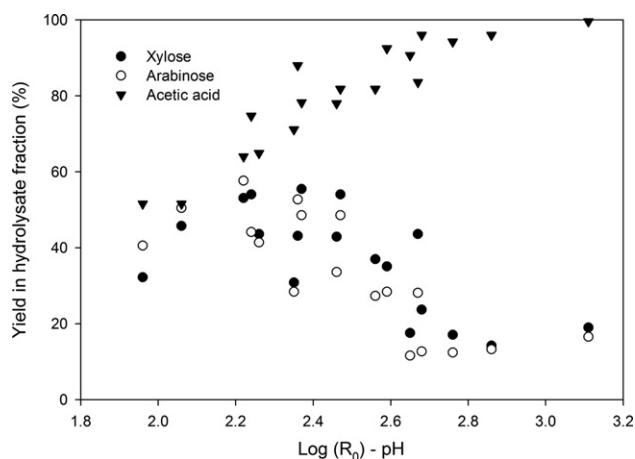
a Average values of four determinations.

The giant reed solid fraction from dilute-OA-pretreatment was subjected to enzymatic hydrolysis at a solid loading of 2% (w/v) using a commercial cellulase preparation. Glucan conversion after 96 h of enzymatic hydrolysis increased with the CS, reaching a maximum of 68% at CS 2.76 (Fig. 3). Glucan conversion was calculated based on measured glucose concentration in the enzymatic hydrolyzate taking the volume of the media into account, the initial solid consistency and the relative glucan content of the pretreated giant reed sample and the percentage of solid recovered after pretreatment.

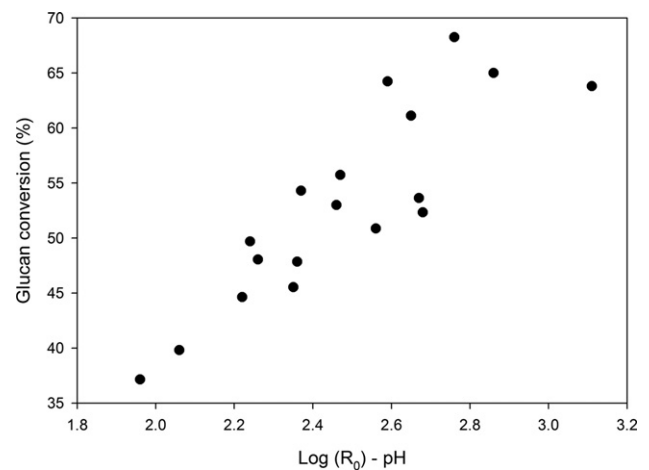
The lower xylan content observed in the solid fraction that was pretreated at higher severities as compared to lower severities, might have contributed to the higher saccharification rate, since it has been shown that the percentage of

residual xylan appears to be a good inverse indicator of cellulose digestibility [25]. Our results also agree with those of Lee et al. [26], who showed that the lower the xylan/lignin ratio the higher the glucan conversion (Table 2).

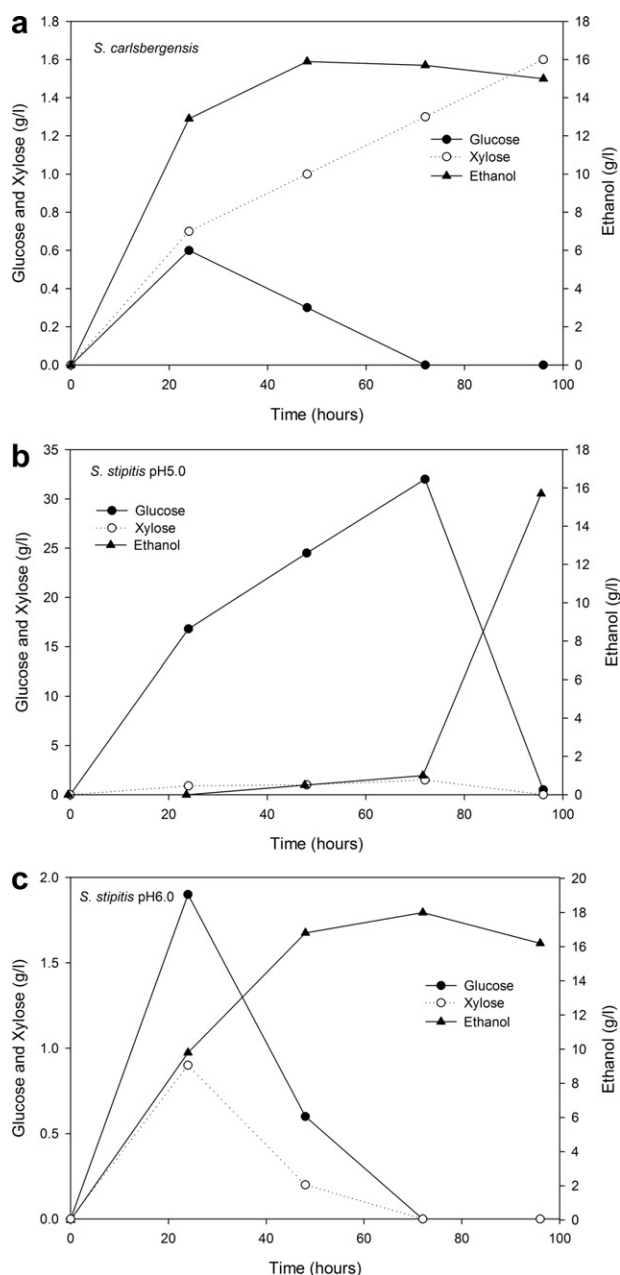
Pretreatment at CS 2.76 was chosen for simultaneous saccharification and fermentation process because it resulted in highest glucan conversion after enzymatic hydrolysis among all conditions tested. Solid pretreated giant reed substrate, loaded at 10% (w/v), was simultaneously saccharified and fermented by Accelerase 1000 enzymes, *S. stipitis* CBS 6054 or *S. carlsbergensis* FPL-450, respectively. In the simultaneous saccharification and fermentation process sugars released from enzymatic saccharification are simultaneously fermented to ethanol by yeasts avoiding sugar accumulation and the possible inhibition to the enzymes [27].



**Fig. 2 – Yield of xylose, arabinose and acetic acid recovered in the hydrolysate fraction after dilute-OA-pretreatment of giant reed as a function of the combined severity factor [ $\text{Log}(R_0) - \text{pH}$ ]. Yields were calculated based on original, untreated giant reed substrate.**



**Fig. 3 – Glucan conversion after 96 h enzymatic hydrolysis (%) as a function of the combined severity factor [ $\text{Log}(R_0) - \text{pH}$ ].**



**Fig. 4 – Simultaneous saccharification and fermentation of dilute-OA-pretreated giant reed solid substrate using a) *Saccharomyces carlsbergensis* FPL-450 at pH 5.0 and *Schefferomyces (Pichia) stipitis* CBS 6054 at b) pH 5.0 and c) pH 6.0 as a function of the combined severity factor [Log (R<sub>0</sub>) – pH].**

Complete fermentation of glucose and maximum ethanol production of 15.9 g/l was reached after 48 h incubation with *S. carlsbergensis*, while 15.7 g/l with *S. stipitis* after 96 h, both at pH 5.0 (Fig. 4a and b). *S. carlsbergensis* did not consume xylose while *S. stipitis* consumed all available xylose; despite the fact that xylose was consumed by *S. stipitis*, maximum ethanol concentration was slightly lower with doubled fermentation time than what observed with *S. carlsbergensis*. The ethanol yield, calculated from the glucan present in the pretreated giant reed solid fraction for *S. carlsbergensis* and according to

the glucan and xylan for *S. stipitis*, was 52 and 49% of the theoretical value, respectively.

*S. carlsbergensis*, belonging to the *Saccharomyces* genus, is generally more tolerant to low pH and acid than is *S. stipitis* [28], and this is particularly true when the latter is fermenting xylose; the long lag phase observed with *S. stipitis* before sugars consumption could, therefore, be attributed to acetic acid and other inhibitory components of the non-detoxified pretreated giant reed substrate. Acetic acid is more toxic at lower pH, and as noted, incubation pH was increased to 6.0; at this condition 18.0 g/l of ethanol concentration was reached after 72 h without any sugar accumulation (Fig. 4c). The % theoretical ethanol yield increased to 57, based on all available xylan and glucan present in the pretreated giant reed solid.

The higher ethanol concentration obtained with *S. stipitis*, at pH 6.0, also could be attributed in part to its numerous genes for endoglucanases and  $\beta$ -glucosidases [8], along with xylanase, activities [29,30] and because *S. carlsbergensis* yeast strain used in this study was not able to ferment xylose.

## 4. Conclusion

High yielding, non-food crop and relatively high carbohydrate composition makes giant reed a promising feedstock for an ethanol biorefinery concept. Dilute oxalic acid pretreatment can remove, at high combined severities, hemicelluloses and prepare the solid residue for enzymatic hydrolysis and simultaneous saccharification and fermentation. Xylose and arabinose, released from hemicellulose hydrolysis, were found as monomers in almost all conditions while for glucose it was observed that more severe conditions are needed for monomers release. However, increasing the oxalic acid concentration or the temperature and reaction time can induce formation of sugar degradation products. Theoretical ethanol yield after simultaneous saccharification and fermentation of the solid fraction was higher with *Schefferomyces (Pichia) stipitis* than *S. carlsbergensis*, when using pH 6.0. An ethanol yield of 57% of the theoretical is relatively low for industrial process, but this could be improved with further strain selection and development. Furthermore, utilization of hemicellulosic derived sugars as a fermentation substrate, alone or in co-fermentation with the solid fraction, would improve the overall bioconversion of giant reed biomass into bioethanol.

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