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## Steam explosion pretreatment of willow grown on phytomanaged soils for bioethanol production

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1 **Steam explosion pretreatment of willow grown on phytomanaged soils for**  
2 **bioethanol production**

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16

17

**1 Abstract**

2 A steam explosion (SE) process was evaluated as a pretreatment method to achieve  
3 simultaneously the pretreatment and the decontamination of trace elements (TE) from woody  
4 biomass for bioethanol applications. The willow biomass used in this study was obtained from  
5 short rotation coppice phyto-managed plots harvested on a TE-contaminated soils (Zn, Mn).  
6 The influence of the SE reaction severity on the composition of the cellulosic pulp and on the  
7 TE extraction in the water effluent was investigated. SE performed at 220°C after a 2 %  
8 sulfuric acid presoaking allowed an extraction of ~80 % of Mn and Zn in the water effluent.  
9 The enzymatic hydrolysis of the resulting pulps was examined. A cellulose-to-glucose  
10 conversion of ~80 % was obtained after 75 hr of incubation of the pulp obtained after a SE  
11 treatment performed at 180 °C. The subsequent fermentation into ethanol using  
12 *Saccharomyces cerevisiae* was successfully performed. No significant influence of TEs on the  
13 action of the biocatalysts (enzymes and yeast) was observed.

14

15 **Key words** : phytoremediation-borne biomass ; steam explosion ; bioethanol ; willow short  
16 rotation coppice ; trace element

17

## 1        **1. Introduction**

2        Soil pollution due to human activities is a world-wide issue with significant environmental  
3        impact and health risks (Gallego et al., 2015, Nagendran et al., 2006, Medina et al., 2015).

4        The presence of trace elements (TE) in high concentrations has been identified as one of the  
5        major threats to European soils. Recently, the utilization of plants and trees for the  
6        management of polluted land gained interest (Chalot et al., 2012, Suer and Andersson-Skold,  
7        2011, Witters et al., 2012). These phytotechnologies include plant phytoextraction using crops  
8        with TE extraction capacity or phytostabilization in order to limit TE dissemination (Gomez,  
9        2012). As a consequence, a subsequent utilization of the TE-enriched biomass seems to be  
10       suitable in order to limit the pollutant dissemination in the environment. It has been  
11       demonstrated from willow wood that a direct disposal of phytoextraction crops is associated  
12       with a high leachability of toxic TE (primarily Zn and Cd) and thus a high probability of  
13       return to the environment (Syc et al., 2012).

14       In this context, the utilization of contaminated land for growing industrial crops could be a  
15       promising approach not only for polluted land management but also for biomass production  
16       (Gomez, 2012). Willow (*Salix*) has been described to be a promising phytoremediation crop  
17       for TE extraction because of its capacity to accumulate in aboveground biomass  
18       compartments relatively high levels of TE such as Zn (up to ~1000 mg.kg<sup>-1</sup>) and Cd (~30  
19       mg.kg<sup>-1</sup>) (Syc et al., 2012, Migeon et al., 2009, Xu et al., 2018). The potential of Very Short  
20       Rotation Coppice (VSRC) of willow has been investigated for phytoremediation. The  
21       repeating harvesting of biomass (every 3-5 years) has been described to maximize the  
22       biomass production and to progressively reduce the TE content in the soil (Xu et al., 2015).  
23       Phytoremediation willow has been proposed as a potential source of biomass for bioenergy  
24       and various energy-recovery techniques such as combustion, gasification and pyrolysis  
25       (Migeon et al., 2009, Vervaeke et al., 2006, Delplanque et al., 2013). The mobilization of

1 phytoremediation willow VSRC could be profitable for the production of biofuels in order to  
2 reduce the impacts on the food markets. However, some important questions about the fate of  
3 the TE during the process and their content in biofuel have to be addressed.

4 In a recent paper, we described the pretreatment of TE-enriched woody (*Salix viminalis*) and  
5 non-woody biomass (*Nicotiana tabacum L.*) using dilute acid, alkali catalyzed and organosolv  
6 pretreatments for the production of bioethanol and biofuel (Asad et al., 2017). The  
7 distribution of TE in the pretreatment fractions (pulp, liquid effluent and lignin) was  
8 investigated. It was concluded that (1) the TE extractability was low in basic conditions, (2)  
9 using a dilute acid treatment TE were mainly recovered in the liquid effluent producing a  
10 clear pulp, (3) in organosolv treatment, TE extraction in liquid phase increased with the water  
11 content and the acidity but decreased with the temperature.

12 Steam explosion (SE) process is one of the most valuable and cost effective pretreatment  
13 technologies for cellulosic bioethanol production (Jacquet et al., 2015). This technology is  
14 currently developed at a commercial scale. During SE, biomass is treated with hot steam for  
15 short residence times (~ 2-30 min) at temperatures in the range of 180 to 210 °C, followed by  
16 explosive decompression. The effect of SE on biomass combines a chemical hydrolysis, a  
17 thermal effect during the steam treatment and a mechanical effect during the explosive  
18 decompression. SE induces (1) a partial breakdown of glucosidic and lignin-hemicelluloses  
19 linkages by hydrolysis and of intramolecular hydrogen bonds (2) a thermo-mechanical  
20 defibration due to the flash evaporation of water in the cell wall (Jacquet et al., 2015,  
21 Sauvageon et al., 2018). As a result, SE is described as an efficient way to decrease the  
22 particle size and to induce an extensive defibration of the biomass. This process has been the  
23 subject of many studies for decades for increasing the accessibility of carbohydrates to  
24 enzymes but to the best of our knowledge, the SE pretreatment of TE-contaminated biomass  
25 produced on phytomanaged sites has never been examined. The aim of this study was to

1 explore the potential of SE pretreatment of SRC willow biomass produced at TE-  
2 contaminated and phytomanaged sites, for the production of bioethanol with a sound  
3 management of TE to avoid their dissemination in the environment. The influence of the  
4 severity of the SE process on the composition in TE of the cellulosic residues was examined.  
5 The residual TE effect on the enzymatic hydrolysis of cellulose into glucose and on the  
6 fermentation step was also investigated.

7

## 8 **2. Materials and methods**

9 EtOH, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> used in this study were purchased from Sigma Aldrich and  
10 used as received. The enzymes were obtained from Sigma Aldrich (St. Louis, MO).

11

### 12 ***2.1 Plants materials***

13 Willow (*Salix viminalis* W) was harvested on a TE-contaminated sediment disposal site  
14 located at Deûlémont (France) at an experimental plot described previously (Kidd et al.,  
15 2015). Because of their young age, willow samples contain bark. Willow stems with bark  
16 (stem diameter 1-3 cm) were milled to a powder using a Wiley mill and dried for 2–3 days at  
17 40 °C.

18

### 19 ***2.2 Steam explosion pretreatment***

20 Biomass was first impregnated during 15 hr at room temperature with a 0.9 % or 2 % (w/w  
21 based on wood dry matter content) dilute sulfuric acid solution (water/biomass ratio : 15/1).  
22 After pressing, the biomass was transferred into a 2 L pressure-resistant steam gun where 50 g  
23 (dry basis) of biomass were exposed to steam at varying temperatures and residence times  
24 (see Table 2 for experimental conditions, the framework of the steam explosion facility has  
25 been described in a previous publication, Simangunsong et al., 2018). After the residence

1 time, a pneumatic valve was open leading to the vapor phase exit from the reactor through a  
2 nozzle entraining the biomass. Both liquid (SE water effluent) and solid (pulp) fractions are  
3 then collected after SE pretreatment in the discharge tank. The severity factor, as shown in Eq.  
4 (1), describes the severity of the pretreatment as a function of residence time (min) and  
5 temperature (°C) (Jacquet et al., 2015). The severity factor can be extended to facilitate  
6 trading off among temperature, time, and acid concentration, using Eq. (1) (Chum et al.  
7 1990). Severity factor and combined severity factor values for this study are grouped in Table  
8 2.

9 Equation 1

$$10 \text{Log } R_0 = \log \left( t \exp \frac{(T - 100)}{14,75} \right)$$

11 Where Log  $R_0$  : severity factor, t : residence time (min), T : reaction temperature (°C)

12

13 Equation 2

$$14 \text{Log } C_s = \log R_0 - pH$$

15 Where Log  $C_s$  : combined severity factor, Log  $R_0$  : severity factor, pH : pH measurement of  
16 the acidic pretreated hydrolysate

### 17 **2.3 TE analysis**

18 SE pretreated willow pulps were dried overnight at 105 °C and then weighted aliquots (0.2 g  
19 dry mass) were placed in a 50 cm<sup>3</sup> Erlenmeyer flask respectively. Then 10 cm<sup>3</sup> of nitric acid  
20 (65 %) were added in the flask and placed on a graphite bed at 85 °C. The flask was heated  
21 until reddish-brown fumes no more and then 4 cm<sup>3</sup> of hydrogen peroxide was added  
22 dropwise. If some plant particles remained in the solution, an additional 4 cm<sup>3</sup> hydrogen  
23 peroxide was added for total dissolution of material. After cooling, the solution adjusted to  
24 200 cm<sup>3</sup> with distilled water. TE content was determined by Inductively Coupled Plasma-

1 Atomic Emission Spectrometry (ICP-AES, Radial ICAP 6500 Model, Thermo Fischer  
2 Scientific, Courtaboeuf, France), and all samples were analyzed in triplicate with certified  
3 reference materials (INCT-OBTL-5, LGC Promochem, Molsheim, France). The recovered  
4 percentages of Zn and Mn concentrations in the treated material were calculated based on the  
5 initial concentration of TEs in biomass.

6

#### 7 ***2.4 Klason lignin and sugars analysis***

8 Extractive-free samples were ground and their moisture content was measured (Kern MRS  
9 120-3 moisture analyzer). Approximately 0.175 g (exactly weighed) of dried samples was  
10 added to centrifuge tubes (50 cm<sup>3</sup> tubes from Corning®) to which 1.5 cm<sup>3</sup> of sulfuric acid (72  
11 %) were added. The mixture was stirred few times with a glass rod and placed in a water bath  
12 at 30°C for 1 hr. After adding 42 cm<sup>3</sup> of water, the tubes were autoclaved at 121 °C for 1 hr.  
13 The samples were then cooled and filtered with glass microfibers (particle retention 1.2 µm).  
14 The insoluble residue was washed with pure water, oven-dried at 105 °C and weighed.  
15 Monosaccharide contents of the soluble fraction were analyzed by HAPE-PAD (ICS-3000  
16 Dionex) equipped with a Dionex CarboPac™ PA-20 (3 x 150 mm) analytical column using a  
17 described method (Asad et al., 2017).

#### 18 ***2.5 Enzymatic hydrolysis***

19 Enzymatic hydrolysis was performed on willow SE cellulosic pulp using standard literature  
20 conditions (Pan, 2008). The cellulosic pulp (1.0 g) was mixed with 50 cm<sup>3</sup> of 50 mM acetate  
21 buffer (pH 4.8) in a 100 cm<sup>3</sup> Erlenmeyer flask and then incubated by shaking for 30 min.  
22 After this preincubation step, the hydrolysis was initiated by adding 0.8 g.L<sup>-1</sup> of cellulase  
23 (*Trichoderma reesei*) and prolonged by shaking (175 rpm) at 40 °C for 30 hr. The glucose  
24 contents of the aqueous phase were quantified using a Shimadzu High Performance Liquid  
25 Chromatograph (HPLC), equipped with an evaporative light scattering (ELS) detector and a



1 Prevail Carbohydrates ES column. Acetonitrile/water (75 %, v/v) was used as the eluent.

2 Enzymatic hydrolysis data were reported as averages from duplicate experiments.

3

#### 4 ***2.6 Fermentation tests***

5 The aqueous phases from the enzymatic hydrolysis experiments were fermented using

6 Bakers' yeast (*Saccharomyces cerevisiae*). The method outlined in Söderstrom et al.

7 (Söderstroem et al., 2003) has been followed for these experiments.

8

#### 9 ***2.7 Detoxification of SE water effluents***

10 Phosphorylated kraft fibers are brought into contact with SE water effluent (0.1 w/w) at room  
11 temperature for 1 hr under stirring. TE content of the liquid effluent is measured before and  
12 after detoxification.

13

### 14 **3. Results and discussion**

15 The chemical composition of the willow samples, containing bark, examined in this study is

16 given in Table 1. Compared to literature reports on the composition of willow wood, willow

17 samples have a substantially lower polysaccharides content (~60 %) but significant amounts of

18 galactan and galacturonan (2.06 % and 3.57 % of the total sugars respectively). This can be

19 rationalized by the presence of bark, which contains high levels of pectins and phenolics,

20 lignin and tannins being recovered as acid-insoluble material using the Klason lignin method.

21 According to Han et al. (2013), willow RSC stem and bark contain ~70 % and 46 % of

22 polysaccharides respectively, bark retaining high content of lignin (24.1 %), water extracts

23 (24.7 %) and acetone extracts (5.1 %).

24

25 Table 1 – Composition of willow and trace element content (mg.kg<sup>-1</sup>)

1

2

3

<b>Klason lignin<sup>a</sup></b>	<b>37.4%</b>
<b>Cellulose<sup>a</sup></b>	<b>43.2%</b>
<b>Hemicellulose<sup>a</sup></b>	<b>16.0%</b>
<b>Extracts<sup>a</sup></b>	<b>7.75%</b>
<b>Zn<sup>b</sup></b>	<b>223.3±12.0</b>
<b>Mn<sup>b</sup></b>	<b>25.8±1.9</b>
<b>Cd<sup>b</sup></b>	<b>&lt;0.1</b>

11

12 <sup>a</sup> % of dry wood. <sup>b</sup> mg.kg<sup>-1</sup> determined ICP-AES

13

14 Willow is described to have a high biomass production and is one of the trees that  
 15 accumulates the most TE but at concentrations much lower than those found in  
 16 hyperaccumulator plants (Syc et al., 2012, Migeon et al., 2009, Xu et al., 2018). Significantly  
 17 lower TE concentrations were found in the stem compared with the leaves (Migeon et al.,  
 18 2009). The dominant TE concentrations in the studied willow SRC with bark samples of this  
 19 work is given in Table 1.

20 Steam explosion pretreatment performed on H<sub>2</sub>SO<sub>4</sub> impregnated milled willow wood has been  
 21 reported in a previous paper (Pan, 2008). The most favorable SE conditions regarding the  
 22 sugar yields were 200 °C for 4 or 8 min using dilute acid presoaking (0.25 % and 0.50 %  
 23 sulfuric acid w/w). The condition sets used in the present study were selected considering  
 24 these previously described results focusing on sugars yields but also, starting from a TE-  
 25 enriched wood, considering the TE extractability in the water effluent. In this context, higher  
 26 sulfuric acid concentrations were investigated in our study (0.9 % and 2 % w/w) for  
 27 promoting the TE extraction in the water effluents.

1 The chemical compositions of the pulp after SE treatment of willow according to  
 2 experimental conditions are given in Table 2. Fig.1 provides the effect of the combined  
 3 severity (Log Cs) on the pulp yield whereas the glucose and xylose contents in the pulp are  
 4 shown in Fig. 2. As seen in Fig. 1, an increase in severity leads to the solubilization of an  
 5 increasing amount of the initial biomass. As expected, it appears from Fig. 2 that the mass  
 6 loss was primarily due to the hemicelluloses solubilization (or degradation), resulting in a  
 7 decreasing of the xylan content. In agreement with the literature (Simangunsong et al., 2018),  
 8 an increasing proportion of xylan with the severity of the treatment was extracted from the  
 9 solid pulp and recovered in the aqueous effluent producing a glucan-rich pulp. Using harsh  
 10 conditions (220 °C, 8 min, 2 % H<sub>2</sub>SO<sub>4</sub>), a cellulose rich pulp containing approximately 97 %  
 11 of glucose among the total sugars in the treated wood was produced (Fig. 2).

12

13 Table 2 – Experimental conditions for the SE pretreatment. Compositions of the solid residues  
 14 after pretreatment.

15

Glc: glucose, Xyl : xylose.

Table 2 – Experimental conditions for the SE pretreatment. Compositions of the solid residues after pretreatment. Glc: glucose, Xyl : xylose.								
T <sup>o</sup> C	T	PS <sup>a</sup>	Log	Glc <sup>a</sup>	Xyl <sup>b</sup> %	Other	Zn <sup>c</sup>	Mn <sup>c</sup>
	(mi n)	%	Cs	%		sugars <sup>b</sup> %		
180	2	0.9	0.0	70.4 9	22.06	7.45	55.06 ±3,43	48.29±1 3,13
180	5	0.9	0.4	79.3 1	15.57	5.11	38.97 ±2,61	37.13±1 ,13
180	8	0.9	0.6	80.3 9	15.50	4.11	30.43 ±1,48	40.83 ±1,14
200	2	0.9	0.5	81.9 7	13.93	4.10	34.00 ±4,40	22.92 ± 9,85
200	5	0.9	0.9	82.5 5	12.92	4.54	26.74 ±2,09	34.75 ±0,98
200	8	0.9	1.1	87.4 0	9.59	3.01	30.24 ±0,58	31.32 ±0,07
220	2	0.9	1.1	87.3 2	9.17	3.51	26.74 ±2,09	34.75 ±0,98

220	5	0.9	1.5	92.8				
				6	5.04	2.11	28.43	27.97
							±5,13	±3,39
220	8	0.9	1.7	94.1	4.36		30.13	52.89
				8		146	±0,57	±0,51
180	2	2	0.3	72.9			38.97±	37.13±1
				4	20.33	6.73	2.61	.13
180	5	2	0.7	78.1	16.91	4.93	34.00±	34.37±0
				6			4.40	.09
180	8	2	0.9	83.6	13.18	3.14	30.24±	31.32±0
				7			0.85	.07
200	2	2	0.8	81.9	13.45	4.62	28.43±	27.97±3
				4			5.12	.39
200	5	2	1.2	89.3	8.15	2.47	24.16±	2378±1.
				8			1.49	84
200	8	2	1.4	93.4	4.77	1.76	21.52±	24.49±1
				7			3.75	.48
220	2	2	1.4	93.5	4.68	1.79	24.11±	22.25±0
				3			1.46	.49
220	5	2	1.8	93.5	4.50	1.95	16.07±	17.15±1
				6			2.22	.49
220	8	2	2.0	97.3	2.31	0.37	14.84±	16.25±1
				2			2.97	.36

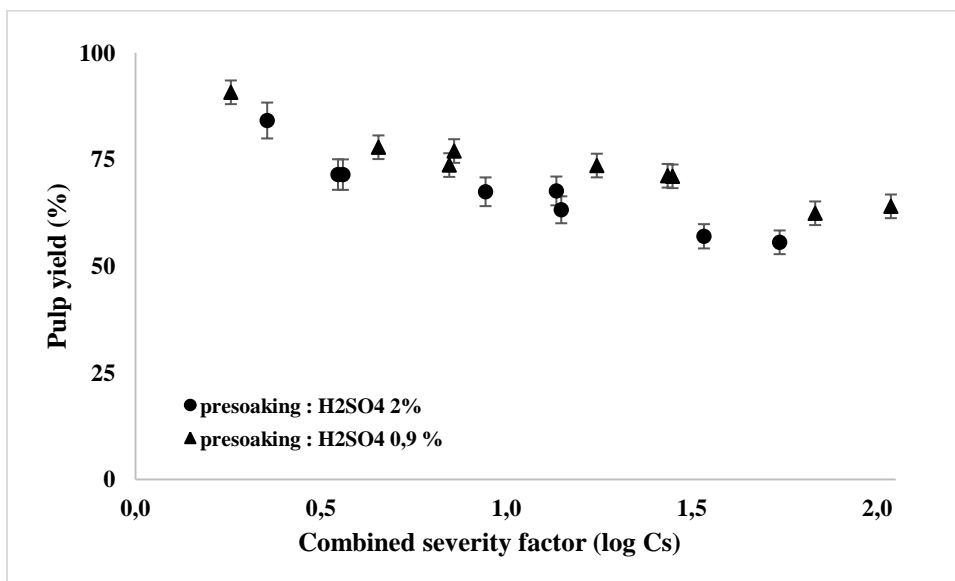
11

12 <sup>a</sup> Concentration of H<sub>2</sub>SO<sub>4</sub> w/w during the presoaking step. <sup>b</sup> % w/w based on the sugars content. <sup>c</sup>  
 13 mg.kg<sup>-1</sup> determined ICP-AES

14

15

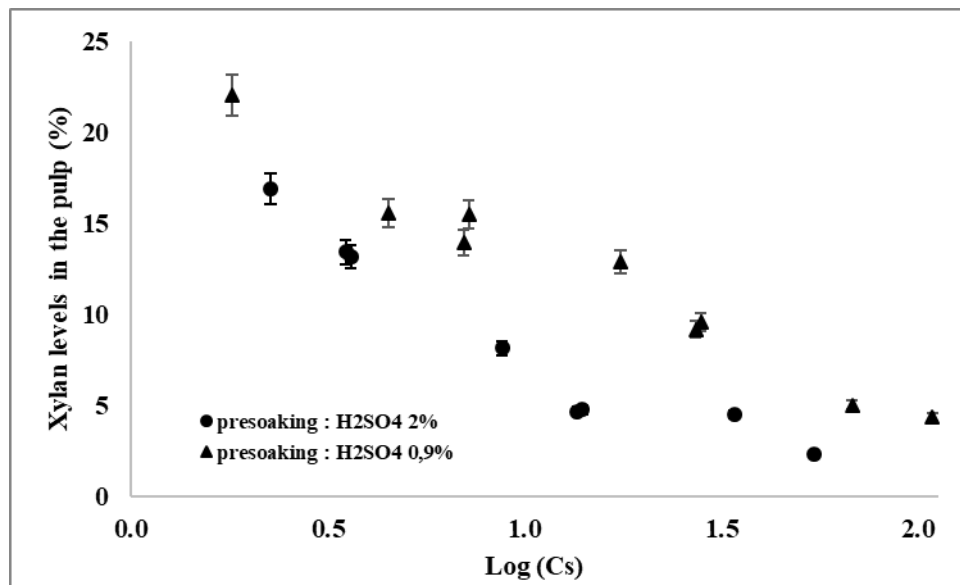
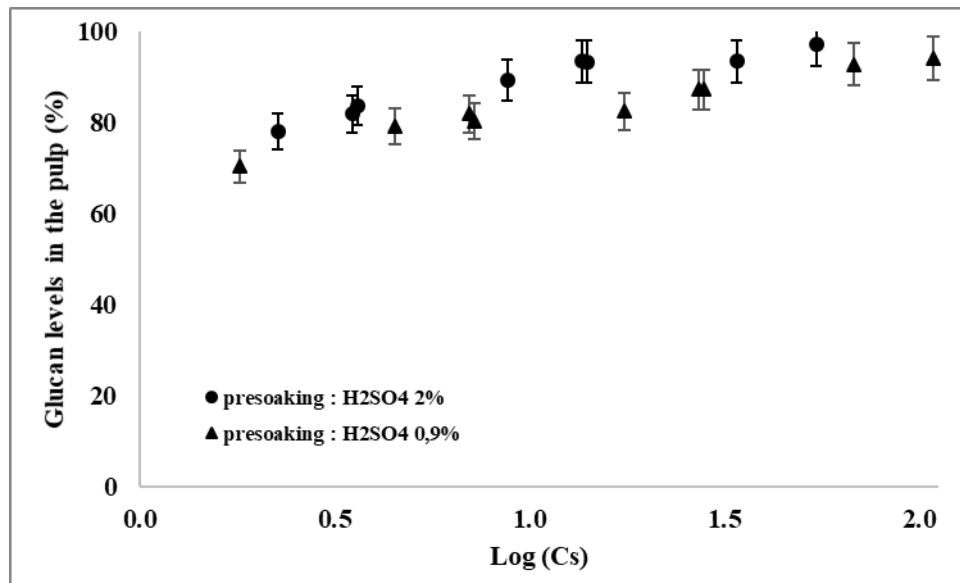
16 Fig. 1. Pulp yield after SE as a function of the combined severity (log Cs) for the two  
 17 presoaking conditions performed.



18

19

1 Fig.2. Glucan (A) and xylan (B) levels (% based on the sugar contents) in the solid residues  
 2 after SE as a function of the combined severity (Log Cs) for the two presoaking conditions.



5 The influence of the SE severity on TE content (Mn and Zn) remaining in the residual pulp is  
 6 given in Fig. 3. It can be seen (Fig 3A and 3B) that the TE extraction was affected by the pre-  
 7 soaking conditions (H<sub>2</sub>SO<sub>4</sub> %). In agreement with previous papers regarding acid-catalyzed  
 8 pretreatment of TE-enriched biomass, an increase in the acid concentration and in residence  
 9 time of the treatment enhanced the TE extraction in the water effluent (Asad et al, 2017,  
 10 Sassner et al., 2007). The lignocellulosic matrix has been described to have affinity with  
 11 divalent metal ions, which may be bound primarily to non-cellulosic polysaccharide

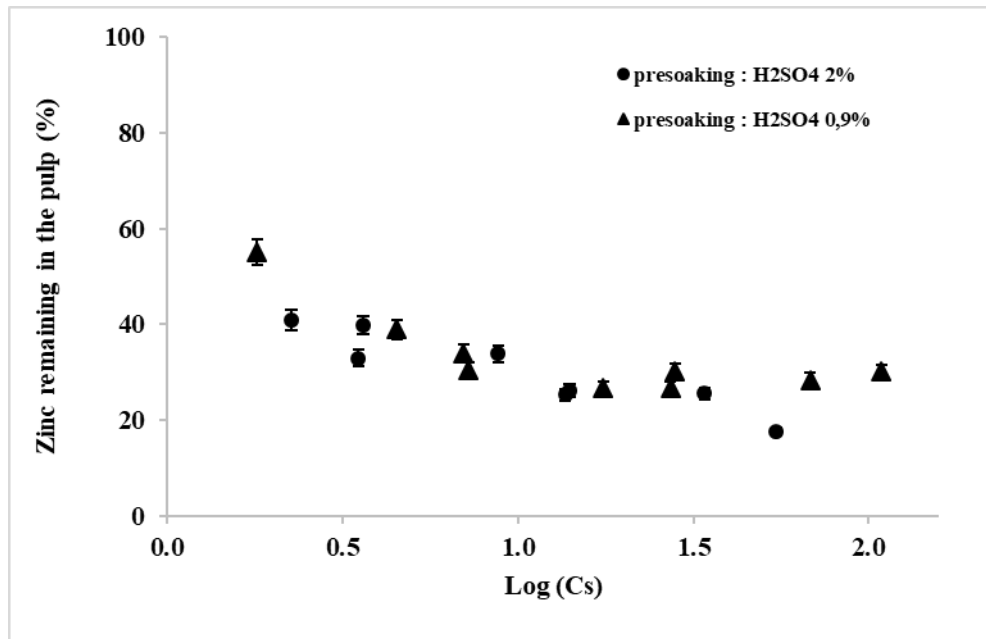
1 components (Asad et al, 2017). As a result, the acid-catalyzed removal of the hemicellulosic  
2 fraction during the steaming step of SE previously mentioned could partly explain the  
3 concomitant sugars and TE extraction in the liquid stream of the process. The results recently  
4 published starting from the same biomass and using dilute acid pretreatment (similar  
5 treatment but without explosive decompression), showed that an increase in temperature  
6 resulted in a decrease of the TE extraction from the pulp (Asad et al, 2017). Interestingly, an  
7 opposite trend was observed in the present study using SE, the higher the operating  
8 temperature, the higher the TE extraction in the water effluents. Indeed, using comparable  
9 conditions at 200°C and 5 min residence time, ~ 37 % of Zn was recovered in the pulp using  
10 dilute acid treatment compared to ~ 21 % for SE. In the SE process, the temperature is  
11 correlated to pressure which has a direct impact on the shearing forces applied to the  
12 lignocellulosic fibers during the explosive release. Pielhop et al. (2016) showed that  
13 increasing the pressure difference of the explosion led to more defibration and to smaller  
14 particle size, increasing the digestibility. As a result, at high operating temperature an  
15 extensive disruption of the fibrils during SE with a strong reduction of the particle size could  
16 increase the accessibility of the TE in the cell wall tissue facilitating their extraction in  
17 aqueous phase.

18

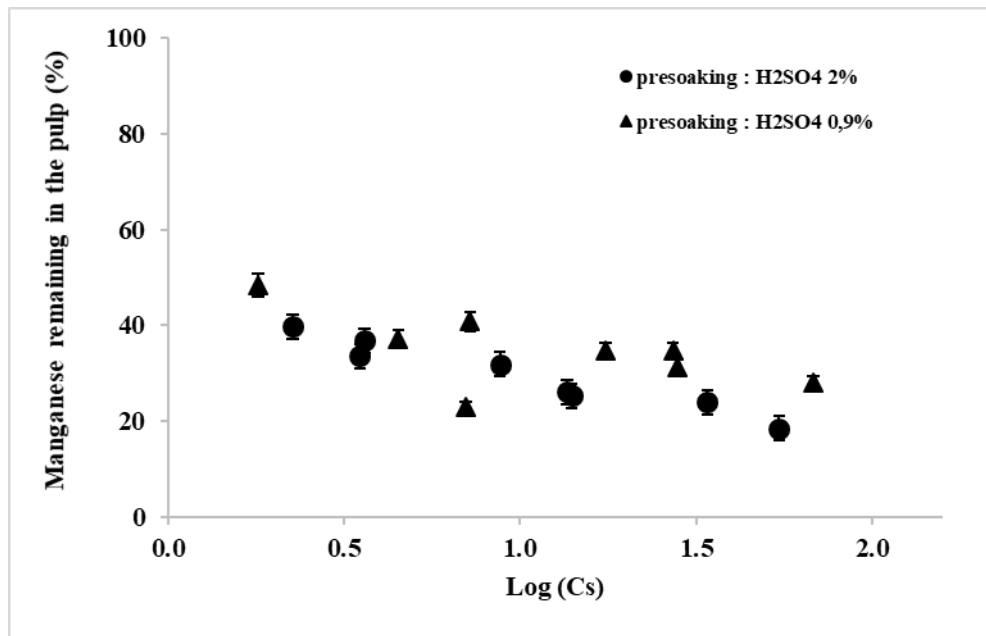
19 Fig. 3. Trace elements (expressed as percent of the total initial content of the raw material)  
20 recovery in the SE wood pulp vs combined severity.

21

A) Zinc      B) Manganese



1



2

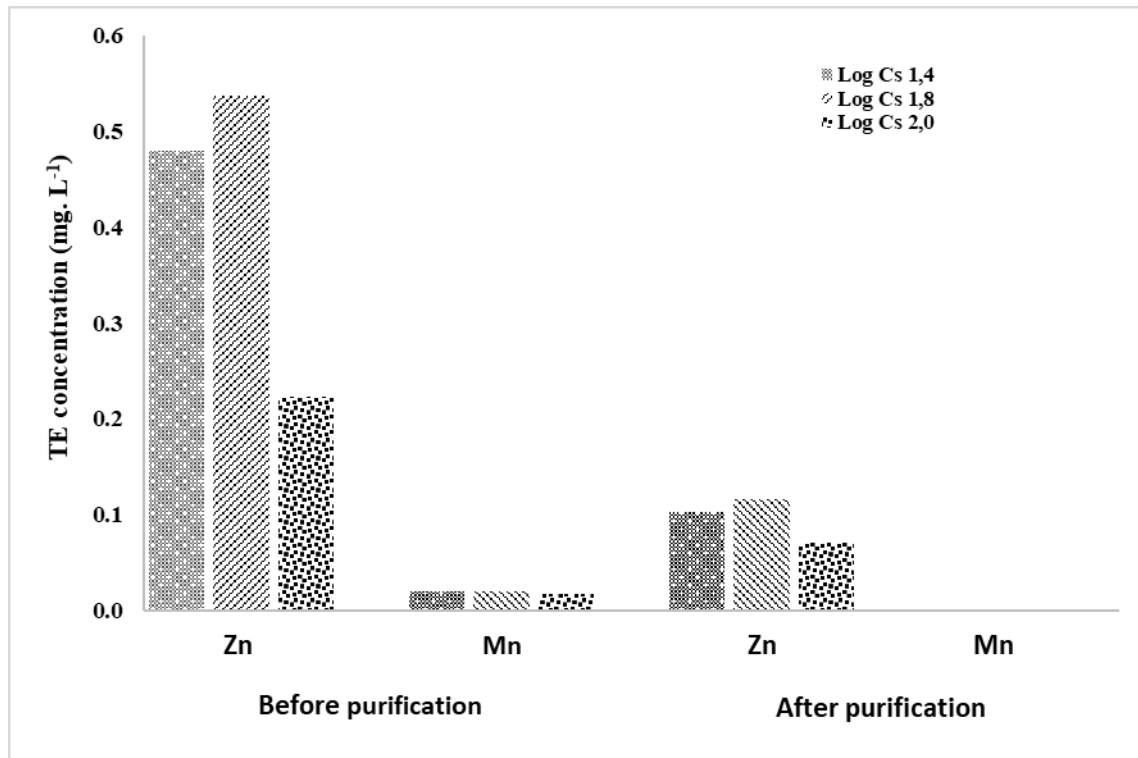
3 In a previous study performed on willow wood (Asad et al, 2017), we showed that in a basic  
 4 pretreatment, TE were mostly recovered in the pulp while in an organosolv pulping ~ 30 % -  
 5 40 % of Mn and Zn were solvent-extracted. Syc et al. (2012) studied the composting of  
 6 willow contaminated with Zn and Cd. According to these authors, 24 – 30 % of the total Zn  
 7 amount and 8 – 12 % of the total Cd were leachable at weakly acid pH with strong effect of  
 8 the pH, the more acidic medium, the higher Cd extraction in water. Using a SE pretreatment  
 9 performed at 220 °C during 5 min after a 2 % w/w H<sub>2</sub>SO<sub>4</sub> impregnation, ~ 80 % of Zn and

1 Mn were removed from wood and recovered in the water effluent. Thus, steam explosion  
2 appears to be an efficient method for the water extraction of TE from phytoremediation wood.  
3 In this context, TE removal from the water stream (water condensed in the discharge tank of  
4 the SE facility) has to be considered carefully to minimize TE dissemination and negative  
5 environmental impacts of the whole process. In the literature, efficient TE adsorbents  
6 exhibiting a high adsorption efficiency and short equilibrium time have been described via  
7 coordination and ion exchange. Graphene, zeolite, chitosane derivatives have been  
8 successfully used to remove heavy metal ions from wastewater (Zhang et al., 2018). In the  
9 present study, a phosphorylated cellulosic material (Belosinschi et al., 2017) was  
10 experimented as TE adsorbent for the detoxification of water effluents recovered after SE.  
11 Fig. 4 gives the Zn and Mn concentrations before and after a resin treatment (0.1 % of resin  
12 w/w, 1 hr, 20 °C). After resin adsorption, a reduction of ~ 80 % of Zn was observed and Mn  
13 concentration was under the limit of detection. Thus in this preliminary experiment, we  
14 demonstrated that the detoxification of the SE liquid effluents could be possible using an ion  
15 exchange resin.

16

17 Fig. 4. Zn and Mn concentrations before and after detoxification  
18 by resin treatment (0.2 % of resin w/w, 1 hr, 20 °C)





1

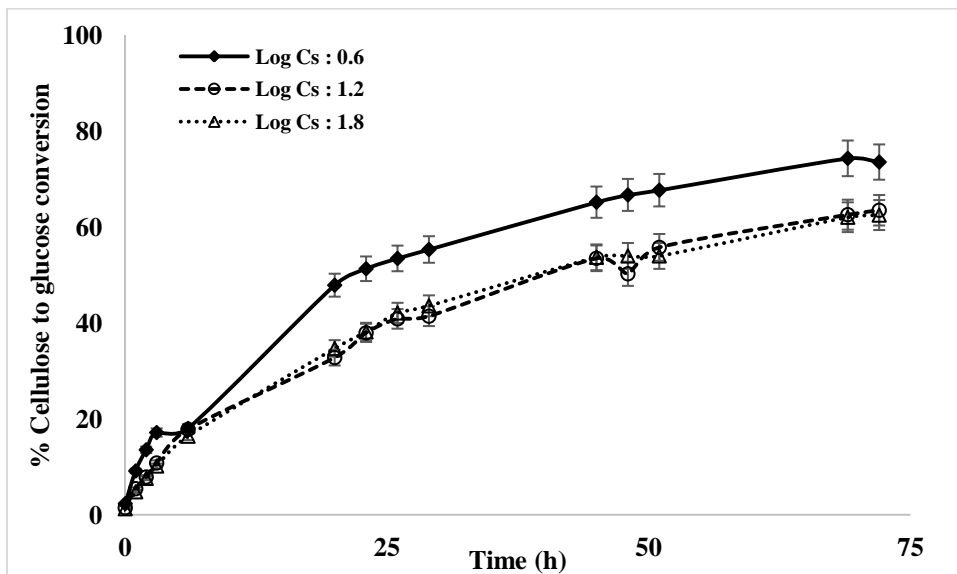
2 The results of enzymatic saccharification of phytoremediation SRC willow wood pretreated  
3 by steam explosion performed during 5 min at three different temperatures (180 °C, 200 °C  
4 and 220 °C), corresponding respectively to combined severity factors of 0.6, 1.2, 1.8, are  
5 shown in Figure 5A. A cellulose-to-glucose conversion of ~ 80 % was obtained after 75 hr of  
6 incubation of the pulp obtained after a steam explosion treatment performed at 180 °C. This  
7 value is close to that previously reported starting from pretreated willow pulps (Huijgen et al.,  
8 2011). The presence of TE and the relatively high Klason lignin content in willow wood do  
9 not negatively impact the enzymatic hydrolysability of lignocellulosic substrate. At higher  
10 pretreatment temperatures (200 °C and 220 °C) slightly lower cellulose-to-glucose  
11 conversions were observed (~ 75 %). Using these more severe conditions, the degradation  
12 reactions gave rise to furfural and HMF (Sassner et al., 2007), which inhibited the enzymatic  
13 digestion. The sugar solutions resulting from the enzymatic hydrolysis were fermented using  
14 *Saccharomyces cerevisiae* to investigate their fermentability. Figure 5B shows ethanol yields  
15 after 12 hr of fermentation of the enzymatic hydrolysates derived from SE-pulps containing  
16 respectively Zn (88 mg.kg<sup>-1</sup>, 87 mg. kg<sup>-1</sup>, 71 mg. kg<sup>-1</sup>) and Mn (11 mg. kg<sup>-1</sup>, 9 mg. kg<sup>-1</sup>, 8

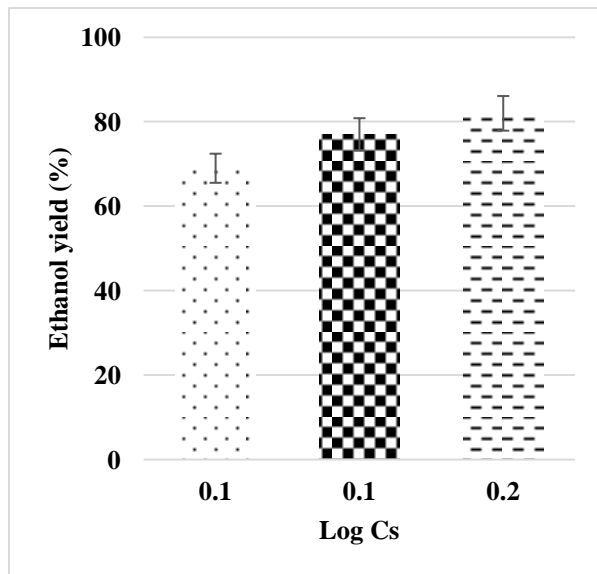
1 mg. kg<sup>-1</sup>). No significant difference was observed in the ethanol yields (~ 65 %) demonstrating the fermentability of the liquors. It has been described that TE can exhibit a toxic effect toward microorganisms and decrease the fermentation efficiency (Yang et al, 2017, Gomez et al., 2002). However, *Saccharomyces cerevisiae* is recognized to exhibit a relatively high tolerance to inhibitors and the toxicity was primarily reported in the presence of Cd, Hg and Pb (Buijs et al., 2013). In the present study, the relatively low concentrations of TE (< 2 mg.L<sup>-1</sup>) had no significant impact on the yeast productivity.

Fig. 5. Enzymatic saccharification of phytoremediation SRC willow wood.

A) Cellulose-to-glucose conversion (%) at different points during enzymatic hydrolysis of willow1 (W1) SE pulps.

B) Ethanol yield after 12 hr of fermentation





1

## 2 **Conclusion**

3 In this study we have examined the SE pretreatment of willow grown on phytomanaged soils  
4 and contaminated with moderate to low levels of TE (Zn, Mn) for bioethanol production. SE  
5 was evaluated and optimized for the decontamination of TE from woody biomass and the  
6 recovery of heavy metals in the water effluents of the process. It has been shown that the  
7 explosive decompression step in the SE process increased the extractability of TE from the  
8 cell wall tissue. The enzymatic saccharification of the resulting cellulosic pulps followed by  
9 yeast fermentation have been examined and no significant inhibitory effect of the residual TE  
10 on the biocatalysts was observed. Thus we have demonstrated the possibility to combine  
11 phytomanagement approaches for decontamination of polluted soils with utilizations of the  
12 biomass produced such as bioethanol production.

13

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