

Article

# Sugars Production from Municipal Forestry and Greening Wastes Pretreated by an Integrated Steam Explosion-Based Process

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Abstract: Increasing awareness of resource sustainability and waste management has led to the search for solutions while promoting circular economy principles. Among all kinds of lignocellulosic biomass available, one with growing interest is municipal forestry and greening waste (MFGW). MFGW makes up an important part of waste streams of municipal solid waste and is a potential feedstock for biological conversion in a lignocellulosic biorefinery. This work studied the fermentable sugars production from MFGW after steam explosion (SE) pretreatment combined with other pretreatments such as dilute acid, organosolv, and metal salts. A range of pretreatment conditions was evaluated according to different parameters: sugars recovery, degradation product generation, and enzymatic hydrolysis yield. At selected pretreatment conditions (diluted acid plus SE, 195 °C, 10 min, and 60 mg  $H_2SO_4/g$  MFGW), 77% of potential sugars content in MFGW was obtained. The effect of solids loading and enzyme dose on glucose release and glucose yield on enzymatic hydrolysis were also determined. Up to 70% of the main sugars in the MFGW were recovered for the coupled pretreatment and enzymatic hydrolysis (45 FPU/g glucan enzyme loading and 20% dry matter solid consistency), resulting in 80 g/L glucose that could be further utilized for ethanol production.

Keywords: municipal forestry and greening wastes; pretreatment; enzymatic hydrolysis

# 1. Introduction

Sustainable urban development requires an effective way to integrate the quality of life of people, environmental pollution reduction, and adequate waste management strategies. At present, about 50% of the human population lives in urban environments, and urban design changes, such as the introduction of green spaces, have been suggested to improve population health [1]. One of the underused lignocellulosic biomass resources, which significantly rises with rapid urbanization worldwide, is biowastes from cleaning and pruning parks and gardens in urban green spaces [2]. Although urban landscape management and municipal forestry and greening waste (MFGW) vary in size and quantity, they have the advantage that they occur continuously and locally [2]. The production of this waste could reach values of 1.5 kg/m<sup>2</sup> per year of the green area [3], and it is primarily formed by twigs, stems, stumps, branches, leaves, and grass clippings. Traditionally, pruning and garden cleaning wastes have ended up in landfill or intended for compost production or bio-stabilized material [4], which have a low added value and a small market. Therefore, an attractive alternative to using this waste, rich in organic matter, is biofuels production and bioproducts of high added value in a



biorefinery. The conversion of this lignocellulosic biowaste to energy production is a challenge because urban vegetation is of great diversity, and the composition of garden waste is not homogeneous. Different technologies for taking this waste are being investigated to produce biofuels [5–7] or high value-added products [8].

The composition of biomass permits the development of a multiproduct bioindustry that uses different components present in lignocellulose biomass and their intermediates, thus upgrading the value from the biomass feedstock. MFGW biomass may be used as raw material for biofuel production. The importance of the production of biofuels, such as bioethanol-based on lignocellulosic biomasses, is nowadays supported by the objectives of the recent European Energy Directive [9].

The most commonly used biochemical conversion pathway for biofuel production is the fermentation to ethanol by yeast. This conversion includes the following main steps: pretreatment, hydrolysis of structural carbohydrates (cellulose and hemicellulose) to sugars, fermentation of sugars, lignin extraction, and recovery and purification of fermentation products (ethanol) to meet biofuel technical specifications.

The recalcitrant structure of lignocellulose and its heterogeneous composition hamper the accessibility of enzymes to carbohydrates, limiting the hydrolysis of this biomass. Pretreatment has been recognized as one of the most expensive processing steps in cellulosic biomass-to-fermentable sugars conversion. The limited separation of cellulose and lignin that reduces cellulose accessibility and hence the effectiveness of subsequent hydrolysis, the generation of the by-products that inhibits downstream fermentation, the high use of chemicals and energy, high costs of enzymes, and high capital costs for pretreatment facilities are some of the technical barriers in the existing pretreatment processes [10]. Pretreatment is considered the critical step for setting up bioethanol production, which is the most capital intensive in a bioethanol plant and may represent between 30% and 50% of the total equipment cost [11]. To increase the accessible surface area, de-crystallize cellulose, achieve partial depolymerization of structural carbohydrates, maximize the enzymatic digestibility of the pretreated material, avoid the generation of sugar and lignin degradation by-products, limit energy demand, and minimize capital and operating costs are the primary challenges to address on the pretreatment step [12].

Steam explosion (SE) pretreatment is one of the most studied pretreatments for woody biomass. SE is a hydrothermal pretreatment in which biomass is quickly heated by introducing high-pressure steam in a reactor at high temperatures (>160 °C) and pressures (5–30 bar) for a defined residence time (ranging from seconds to several minutes). During the process at such high pressures, steam condensates and permeates the biomass initiating autohydrolysis reaction. SE combines both mechanical effects as a consequence of the sudden reduction of the pressure (explosive decompression) and the chemical effects due to hydrolysis of acetyl groups (autohydrolysis) that is present in hemicelluloses [13]. SE has been extensively described in the literature as suitable pretreatment for various materials, as well as SE combined with other treatments, e.g., diluted-acid or organosolv pretreatments [14].

Organosolv (OS) pretreatment uses organic solvents to remove lignin and favor hemicellulose fraction solubilization, thus increasing the cellulose accessibility. In general, OS pretreatment using ethanol as a solvent is carried out at temperatures ranging from 180 to 200 °C, solvent concentrations of 35–70% (w/w), and residence times ranging from 30 to 90 min. OS pretreatment can be applied to almost all kinds of raw materials, producing a highly digestible substrate and lignin residue with high-value utilization [10,15]. The combination of OS pretreatment with SE has recently been reported as an efficient fractionation method for birchwood biomass, producing a highly digestible cellulose substrate, even using low enzyme doses [16].

Meta salts (MS) have been studied as catalysts for hemicellulose solubilization in different biomasses such as switchgrass [17] or olive tree pruning [18]. Pretreatment with FeCl<sub>3</sub> has been reported to disrupt ester linkages between carbohydrates and lignin, while not affecting delignification, and removes the hemicellulose, thus enhancing the accessibility of cellulose to enzymes [19]. FeCl<sub>3</sub> pretreatment at high temperatures facilitates hydrolysis by increasing surface area [19].

This work aimed to study the effect of SE and the combined pretreatment of SE with other pretreatments, namely dilute acid, organosolv, and metal salt, on MFGW. As far as we know, there is scarce literature on the pretreatment of SE combined with other pretreatments for MFGW fractionation. For pretreatment evaluation, different parameters such as the composition of pretreated biomass, xylose and glucose recovery, degradation product generation, enzymatic hydrolysis yield, and overall xylose and glucose yields were used.

Besides, at selected pretreatment conditions, the effect of enzyme loading and solid consistency in enzymatic hydrolysis step was studied.

# 2. Materials and Methods

# 2.1. Raw Material

MFGW was locally collected from the Migas Calientes Composting Plant, located in Madrid (Spain). The material plant-based waste from the public gardens of Madrid was chopped and shredded in the plant. The shredded material was air-dried at room temperature to the equilibrium moisture content of less than 10% and milled using a centrifugal mill (Retsch ZM200, Retsch, Ins., Haan, Germany) to 2-mm particle size for chemical composition analysis and 10-mm particle size for pretreatment.

# 2.2. Pretreatment

Before pretreatment, 250 g (based on dry matter content) of MFGW was impregnated with water or catalyst solution (250 g) during 16 h. The moisture content of impregnated raw material was about 50%.

Pretreatments were carried out in bath unit equipped with 2-L steel reactor and sensors to control process parameters such as pressure, temperature, and reaction time. A scheme of steam unit is depicted in Figure S1. The pressure reactor was preheated at the set pretreatment temperature with saturated steam; after biomass addition into the reactor, it took less than 1 min to reach the working temperature. The following pretreatment conditions were tested:

- Steam explosion (SE) pretreatment: Temperatures 185, 205, and 220 °C for 20 min.
- Metal salt combined with SE pretreatment (MS-SE): Metal salt, FeCl<sub>3</sub> (0.1 M, 90 mg/g MFGW biomass); temperature, 180 and 195 °C; and residence time: 20 min.
- Organosolv combined with SE pretreatment (OS-SE): Solvent, ethanol (50%, *v*/*v*); temperature, 180 and 200 °C; and residence time, 20 min.
- Diluted-acid combined SE pretreatment (DA-SE): Acid, H<sub>2</sub>SO<sub>4</sub> (60 mg/g MFGW biomass); temperature, 180–215 °C; and residence time, 5–15 min.

After SE, the exploded material (slurry) was recovered into a cyclone connected to the reactor vessel and it was cooled and filtrated to separate liquid fraction (prehydrolysate) from solid fraction. Both solid and liquid fraction of all pretreated slurries were collected and evaluated. The solid fraction was washed with water (S/L ratio 1/10) and vacuum filtered for water-insoluble solids (WIS) fraction recovery. Mass yields of WIS (RY%) were calculated in relation to dry matter of MFGW biomass. The composition of both the WIS fraction and the liquid fraction were analyzed, as described below. The WIS fractions were employed in the enzymatic hydrolysis tests as substrate.

# 2.3. Enzymatic Hydrolysis Test

For pretreatment evaluation in terms of digestibility potentials, enzymatic hydrolysis trials were performed in Erlenmeyer flasks (100 mL) in an orbital shaker at 150 rpm (Infors AG, Bottmingen, Switzerland). Standard enzymatic hydrolysis assays at 5% (w/w) solid consistency were carried out using cellulase enzyme blend Cellic Ctec2 from Sigma. This enzyme blend contains cellulases,  $\beta$ -glucosidase, and hemicellulases. All assays were carried out in triplicate at 50 °C, pH 5 (0.05 M sodium citrate buffer), and 15 FPU/g substrate enzyme loading. Samples were taken at 72 h and centrifuged (10,000 g for 5 min) before HPLC analysis.

For the study of the effect of solids loading and enzyme dose on the enzymatic hydrolysis of the pretreated substrate under selected conditions, a random multilevel factorial experiment design in two blocks was performed. The substrate concentrations tested were 5%, 15%, or 20%; dry matter (DM) and the enzyme loading were also tested. A mixture of Cellic CTec2 (Sigma) and Cellic HTec2 (a xylanase preparation) (Novozymes, AS) was prepared in a ratio of 9:1 (v/v) and used in the enzymatic hydrolysis assays and added at different enzymes loading (15, 30, and 45 FPU per g of glucan). At 0, 24, 48, and 72 h, samples were withdrawn, and the sugar content was determined by HPLC, as described below.

## 2.4. Fermentation Assay

The resulting enzymatic hydrolysate obtained from WIS after selected pretreatment and enzymatic hydrolysis conditions were submitted to fermentation test.

Saccharomyces cerevisiae Ethanol red (Lesaffre, France) was used as fermentative microorganism. The inoculum was prepared by growing the yeast on an orbital shaker incubator at 150 rpm and 37 °C for 16 h, on a glucose-rich synthetic media containing: 30 g/L glucose, 2 g/L yeast extract, 1 g/L NH<sub>4</sub>Cl, 1 g/L KH<sub>2</sub>PO<sub>4</sub>, and 0.3 g/L SO<sub>4</sub>Mg·7H<sub>2</sub>O. After 16 h, cells were harvested by centrifugation at 5000 g for 5 min, washed once with 0.9% NaCl, and diluted with distilled water to obtain the desired inoculum concentration.

Ethanol fermentation of enzymatic hydrolysate was performed in triplicate in a 250 mL flask with a working volume of 50 mL. The enzymatic hydrolysate was supplemented with the same media as inoculum medium except for glucose. Incubation was carried out at 37 °C and 150 rpm for 30 h with 0.15 g yeast cells/L inoculum size.

## 2.5. Chemical Analysis

## 2.5.1. Extractives, Ash, Carbohydrate, and Lignin Content

NREL procedures for biomass composition analysis [20] were applied for the determination the chemical composition of both raw material (MFGW) and pretreated solid (WIS). Extractives content was only determined on the raw material by Dionex Accelerated Solvent Extractor System (ASE 200). After extraction, structural carbohydrates content was determined based on monomers content after a two-step sulfuric acid hydrolysis procedure. The sugars concentration was determined by high-performance liquid chromatography (HPLC).

#### 2.5.2. Oligomers, Monomers, and Degradation Products

Sugars concentration was determined by (HPLC) in a Waters 2695 liquid chromatograph with a refractive index detector. A CARBOSep CHO-682 LEAD column (Transgenomic, Omaha, NE, USA) operating at 75 °C with Milli-Q water (Millipore) as mobile-phase (0.5 mL/min) was used.

In prehydrolysate samples obtained after pretreatment, mild acid hydrolysis with 4%  $H_2SO_4$  (*w/w*) for 30 min at 121 °C was carried out to convert oligomers to monomers.

The degradation products generated during the SE treatment, such as 5-hydroxymethylfurfural (HMF), furfural, vanillin, hydroxybezoic acid, and syringaldehyde were determined by HPLC equipped with an Agilent 1200 series Diode-Array detector (Agilent Technologies, CA, USA). Acetic acid, formic acid, and levulinic acid were also quantified by HPLC equipped with a refractive index detector. Analysis conditions are described above [21].

# 2.5.3. Ethanol

Ethanol was analyzed by gas chromatography (GC) using a Varian CP-3800 System with an CP-8400 autosampler injector. The system was equipped with a flame ionization detector and a Carbowax 20 M column, operating at 85 °C. Injector and detector temperature were kept constant at 175 °C. The carrier gas (helium) was set at a flow of 15 mL/min.

Ethanol yield is presented as g ethanol produced per 100 g raw material. It was calculated using the following equation:

$$\frac{[\text{Ethanol produced from WIS during fermentation,  $\frac{g}{L}]}{[\text{dry solid WIS in the fermentation broth, }\frac{g}{L})]} \times \frac{\text{RY\%}}{100} \times 100$ (1)$$

#### 2.6. Statistical Analysis

Statistical analysis of the effect of solid loading and enzyme dose on enzymatic hydrolysis of the pretreated substrate on the enzymatic hydrolysis yield and glucose production was performed using Statgraphic Centurion XVII.I-X64 for Windows. Both linear and quadratic effects of the two variables under study were calculated, as well as their possible interactions, on the different responses studied. Their significance was evaluated by variance analysis (ANOVA). A randomized multilevel factorial experiment design was employed. The bases of the design were: number of experimental factors, 2; number of blocks, 2; number of responses, 2; number of trials, 18; and degrees of freedom, 11. The experimental factors studied were the solids load (10, 15, and 20%, w/w) and the enzyme dose (15, 30, and 45 FPU/g glucan).

## 3. Results

## 3.1. Raw Material Composition

Table 1 shows the composition results of MFGW. Lignin and carbohydrate analyzed constitute around 80% of the total solids in MFGW. The main component in MFGW is glucan (34.7%), followed by lignin (29.9%), hemicelluloses (14.0%), extractives (9.2%), and inorganic components (total ash, 3.7%). Extract compounds account for 9.2%, being the water extract 6.7% and organic extract 2.4%. MFGW contains 4.7% of acetyl groups. The acetyl groups content is in the range found in hardwoods [22], where the most significant pool acetyl groups in wood lignocellulose come from secondary cell walls, which constitute the bulk of biomass. However, acetyl groups are also found in hardwoods lignin, linked to  $\gamma$ -carbon of the aliphatic side chain of lignin syringyl and guaiacyl monomers [23].

Component	Percentage on Dry Weight Basis
Extracts	9.2
Glucan	$34.7 \pm 1.4$
Hemicelluloses	14.0
Xylan	$9.6 \pm 0.03$
Galactan	$2.2 \pm 0.12$
Arabinan	$1.3 \pm 0.02$
Mannan	$0.9 \pm 0.0$
Acetyl groups	$4.7 \pm 0.10$
Lignin	29.9
Whole Ash	$3.7 \pm 0.1$

Table 1. MFGW composition. Data are expressed as dry matter.

Structural carbohydrate content of MFGW, which accounts for about 48.7% of dry weight, makes this residue an attractive feedstock for bioethanol production.

As far as the urban greening waste composition is concerned, high variability is found in the literature. By comparing with the composition of urban greening wastes analyzed by other researchers, the raw material utilized in this research has higher carbohydrate and lignin content and less extract content. Raud et al. reported values of 22.96%, 6.89%, and 22.73% for cellulose, hemicellulose, and lignin content [24], respectively. Cubas et al. reported values of 24.8%, 15.7%, and 21.6% for cellulose, hemicellulose, and lignin content, respectively [25]. Values up to 18.9% extract content have also been reported [26]. The variability of reported composition is mainly due to the heterogeneity of the residue,

with varying proportions of grass, branches, hedge pruning, leaves, twigs, etc., which also depend both season and location [27].

### 3.2. Effect of Pretreatment Conditions on WIS and Prehydrolysates

Table 2 shows results of mass yields and the chemical composition of WIS obtained after different pretreatment conditions. SE alone and combined with other pretreatments tested (metal salt, organosolv, and diluted acid) provided mass yields (RY) ranging from 57.5% to 73.5%; lowest values were found in DA-SE pretreatment. This lower recovery of solids is associated with rises in hemicellulose solubilization, mainly xylan, extracts, and soluble ash. In general, RY percentage decreased as the temperature increases.

**Table 2.** Chemical composition of pretreated solids (WIS) under various pretreatment conditions. Data are expressed as dry matter.

Brotrootmont	Time (min)	Temperature (°C)	RY (%)	C	Contents (%	»)	Recovered	Removed	Removed
rietreatment				Glucan	Xylan	AIL	Glucan	Xylan	AIL *
SE		185	73.5	39.6	8.1	38.6	81.8	37.7	5.1
	20	205	71.4	40.1	6.0	38.8	81.1	55.9	7.3
		220	72.8	42.3	4.3	40.7	87.1	67.3	5.6
MS-SE	20	180	69.7	37.3	5.9	39.1	73.5	56.8	8.8
	10	195	69.0	37.7	6.0	38.1	73.6	57.1	12.1
OS-SE	20	180	73.4	37.0	9.5	36.1	76.8	27.2	11.5
	20	200	69.4	38.5	6.9	36.5	75.6	49.7	15.3
DA-SE	F	195	64.8	41.4	3.3	43.8	75.8	77.8	5.1
	3	205	57.5	41.6	1.5	47.6	67.8	90.7	8.5

\* AIL, acid-insoluble lignin.

The glucan content in WIS after pretreatments ranged from 37.0% to 42.3%. Those values are slightly higher than the glucan content of the raw material; the enrichment in glucan is attributable to hemicellulose solubilization. Glucan recovery in WIS fraction varied from 67.8% to 87%. In SE pretreatment carried out by the authors with other wastes such as wood [28] and herbaceous wastes [29], higher glucan recovery values in WIS fraction were found, ranging 82–99% in steam-exploded rapeseed straw and 86.4–93.3% in steam-exploded eucalyptus wood. Due to the high heterogeneity of MFGW (the waste is made up of herbaceous biomass such as grass clippings and waste trimmings from hardwood and softwood species) and considering that the glucan content in MFGW was determined by total hydrolysis with sulfuric acid, the glucose values determined include not only the glucose from the cellulose fraction but also glucose from other polysaccharides that could be present in the lignocellulosic waste such as hemicelluloses fraction and starch. These fractions can be solubilized during pretreatment, thus explaining the glucan recovery values in the WIS found.

In general, xylan removal rises as temperature increases, thus it depends on the pretreatment conditions. For instance, results obtained at 180 °C indicate that the MS-ES pretreatment presents a higher capacity of xylan removal in comparison with OS-ES pretreatment, which generates about 57% removal comparing with about 27% for OS-ES combinational pretreatment. According to available studies, the role of the metal salts such as FeCl<sub>3</sub> in the transformation of biomass is related to Lewis's acid character, that is, the ability to attract pairs of electrons. Pretreatment with metal salts is considered as a low pH reaction system with higher catalytic activity than inorganic acids [30], but, in this work, the highest values of xylan solubilization were obtained with DA-SE pretreatment, where xylan solubilization reached 91% at 205 °C. The addition of H<sub>2</sub>SO<sub>4</sub> as catalyst is known to produce a high solubilization and hydrolysis of hemicellulose fraction. Ballesteros et al. reported an 85% of hemicellulosic-sugars recovery in the liquid fraction on pretreated wheat straw at 180 °C for 10 min using sulfuric acid as catalyst [31].

Although organosolvent pretreatments are considered effective methods for extracting the lignin fraction, it is worth mentioning the low delignification obtained with OS-SE pretreatment, in which

less than 10% lignin solubilization was obtained. It may be that the reaction time was not sufficient in the case of MFGW pretreatment, but, in the literature [16], delignification values of 77% have been reported when birchwood was pretreated with ethanol (60%, v/v) at 200 °C for 15 min. These conditions are quite similar to the condition utilized in this work.

The composition of main sugars and degradation products determined in liquid fraction (hydrolysate) obtained after MFGW biomass pretreatment is given in Table 3. The main sugars were xylose and glucose, accounting for 67–78% of total sugars analyzed. Sugars were presented in a considerable proportion (>70% glucose and >80% xylose) as oligomers in SE and OS-SE pretreatments. As expected and due to the acid condition DA-SE pretreatment, more than 80% sugars were in monomeric form. In contrast, in MS-SE pretreatment, less than 50% were in monomeric form. The total sugars (glucose, xylose, arabinose, and galactose and) production ranged from 6.8 to 17.4 g per 100 g of dry MFGW, with lower values using MS-SE (6.8 and 8.3 g per 100 g raw material for 180 and 200 °C, respectively) and OS-SE pretreatments (7.4 and 8.8 g per 100 g raw material for 180 and 200 °C, respectively). The highest values of sugars production were obtained at the lowest temperatures with SE (17.4 g per 100 g raw material) and DA-SE (16.0 g per 100 g raw material) combinational pretreatment.

**Table 3.** Composition of liquid fraction (prehydrolyzated). Data are expressed as g per 100 g dry MFGW, except for vanillin, hydroxybezoic acid, and syringaldehyde, which data are expressed as mg per 100 g dry MFGW.

Pretreatment		SE			MS-SE		OS-SE		DA-SE	
Temperature (°C)		185	205	220	180	195	180	200	195	205
Time (min)		20		20	10	20		5		
Main sugars	Monomeric glucose	0.57	0.75	0.7	1.21	1.26	0.75	0.83	4.68	4.62
	Oligomeric Glucose	2.06	2.57	1.85	1.58	1.33	2.21	2.43	0.98	0.01
	Monomeric Xylose	0.25	0.58	0.77	0.78	0.76	0.15	0.38	6.42	6.41
	Oligomeric Xylose	3.42	4.05	3.75	2.44	1.51	2.48	3.17	0.48	0.52
Degradation products	Furfural	0.07	0.25	0.51	0.21	0.16	0.03	0.16	0.39	0.61
	HMF	0.04	0.1	0.2	0.12	0.11	0.03	0.08	0.36	0.42
	Acetic acid	0.68	1.36	1.92	1.2	1.24	0.46	1.01	2.24	2.42
	Formic acid	0.28	0.45	0.47	0.63	0.64	0.24	0.33	0.54	0.4
	Levulinic acid	0.02	0.03	0	0.03	0.03	0.02	0.02	0.01	0.09
	Vanillin	3	8	15	3	2	3	4	4	7
	Hydroxybenzoic acid	20	40	74	20	17	21	31	36	< 0.5
	Syringaldehyde	ND	6	15	3	3	2	4	7	10

It should be noted that the objective was to achieve high sugar content from the hemicellulose fraction and low content in degradation products. The highest sugar content was obtained after DA-SE pretreatment. Acetic acid ranged from 0.68 to 2.42 g/100 g, getting the higher values with the DA-SE pretreatment; these results are in line with the elevated xylan removal found in WIS fraction. With respect to the degradation products, the vanillin content achieved was from 2 to 15 mg/100 g dry MFGW, and hydroxybenzoic acid content was from less than 0.5 to 74 mg/100 g dry MFGW.

The effect of different pretreatment on xylan fraction is illustrated in Figure 1. DA-SE- pretreatment produced the highest xylan-derived sugars in liquid fraction, reaching values over 60%.



Monomeric xylose in liquid fraction Oligomeric xylose in liquid fraction Xylan in WIS residue Furfural in liquid fraction Losses

Figure 1. Xylan distribution in MFGW pretreated with SE, MS-SE, OS-SE, and DA-SE pretreatments.

# 3.3. Evaluation of Potential Digestibility

One of the parameters utilized for pretreatment evaluation in this research was the digestibility using enzymatic hydrolysis assays. Enzymatic hydrolysis test was performed at 5% (w/v) solid consistency and 15 FPU/g substrate enzyme loading. Figure 2 depicts the enzymatic hydrolysis yields for glucan (EHYg) and xylan (EHYx) expressed as a percentage of the theoretical yield. The results obtained on the digestibility of pretreated MFGW show a strong correlation with pretreatment type. the best results were achieved in the DA-SE pretreated samples, reaching yields of between 71.6% and 76.8% at 195 and 201 °C, respectively. The lower yields were under 36%, which were obtained in OS-SE and MS-SE pretreated samples. Enzymatic hydrolysis yields increase as temperature rises. SE pretreatment achieved 24%, 44%, and 57% yields at 185, 205 and 220 °C, respectively. Enzymatic hydrolysis yield referred to potential glucose/xylose in WIS fraction is a useful tool to evaluate the efficiency of pretreatment on saccharification performance. However, it is also valuable to know overall glucose and xylose produced per gram of MFGW. These values include sugars (glucose and xylose) obtained after both pretreatment and enzymatic hydrolysis steps. Based on the raw material composition (34.7% glucan and 9.6% xylan), the maximum potential yield was 38.9 g glucose/100 g of dry MFGW, including also the glucose present in extractives fraction, and 10.8 g xylose/100 g dry MFGW, giving a total of 49.7 g sugars (glucose + xylose)/100 g dry MFGW. Thus, glucose could reach up to 78.2% and xylose 21.7% of the total of recovered sugars. These data denote the more significant potential contribution of glucose to the total yield, showing the importance to recover the potential glucan. The results of overall glucose (OGY) and xylose (OXY) yields are shown in Figure 2.



**Figure 2.** Enzymatic hydrolysis glucan (EHYg) and xylan (EHYx) yields<sup>1</sup> and overall glucose (OGY) and xylose (OXY) yields<sup>2</sup> from WIS obtained at different pretreatment conditions. <sup>1</sup> Data are expressed as a percentage of potential glucose (or xylose) on the pretreated MFGW. <sup>2</sup> Data are expressed as a percentage of potential glucose (or xylose) on untreated MFGW.

With respect to the overall yield of glucose, higher values were reached by DA-SE pretreatment (25.6 and 22.2 g/100 g dry MFGW at 195 and 205 °C, respectively, which is equivalent to 65.9% and 57.1% of potential glucose). These results improve on those obtained by Raud et al. who reported 67% enzymatic hydrolysis yield and 15.45 g glucose/100 g wastes from urban greening with diluted-acid pretreatment (130 °C, 60 min, 1% H<sub>2</sub>SO<sub>4</sub>) [24]. Besides, from an economic point of view, it is required to consider all sugars of the MFGW biomass and not only the glucose from cellulose. Xylan is the major component found in hemicellulose, and it can also ferment into ethanol by pentose fermenting yeasts. DA-SE pretreatment at 195 °C achieved the highest xylose yield, 8.3 g/100 g MFGW, equivalent to 76.4% of theoretical.

## 3.4. DA-SE Pretreatment Selection

Based on the results shown above, DA-SE pretreatment was selected for further experimentation. Temperatures and residence time conditions ranging 185–215 °C and 5–15 min, respectively, were studied. The results from the solid recovery (RY%) and composition of WIS after DA-SE pretreatment are given in Table S1. Solid recovery yields ranged from 57.7% to 64.8%. Glucan content in WIS ranged 39.3–45.9%, xylan content 1.5–3.3%, and acid-insoluble lignin (AIL) 43.3–47.6%. Glucan content raised as residence time increased. As expected, high xylan removal was obtained. Glucan content in WIS fraction decreased slightly as temperature increased.

Table S2 illustrates the composition on sugars and products degradation determined in hydrolysates, whereas Figure S2 illustrates the effect of temperature and time on xylan fraction after DA-SE pretreatment.

The effect of temperature and residence time on enzymatic hydrolysis yield and overall sugars yield are depicted in Figure 3. The highest enzymatic hydrolysis yield from glucan (EHYg), around 73%, was achieved at 195 °C/10 min and 205 °C/5 min. The highest OGY and OXY were for the

pretreatment at 195 °C with a sulfuric acid concentration of 60 mg/g dry MFGW and a reaction time of 10 min, achieving 28.3 g glucose/100 g dry MFGW and 8.2 g xylose/100 g dry MFGW; the overall sugars reached 36.5 g/100 g dry MFGW, equivalent to 73.4% total potential main sugar content in raw material.



**Figure 3.** Enzymatic hydrolysis glucan (EHYg) and xylan (EHYx) yields and overall glucose (OGY) and xylose (OXY) yields from WIS obtained at different DA-SE pretreatment conditions.

Based on different parameters evaluated, DA-SE combinational pretreatment carried out at a temperature of 195 °C for 10 min was selected for MFGW biomass for the study of the effect of solid content and enzyme loading in enzymatic hydrolysis step.

# 3.5. Study of the Effect of Solid Content and Enzyme Loading in Enzymatic Hydrolysis Step

To enhance the economic feasibility of industrial sugar production using lignocellulosic biomass, the amount of biomass in the enzymatic hydrolysis step needs to be increased, which is known as "high-solids" enzymatic hydrolysis (i.e., with over 15% DM solids (w/w) in the reaction mixture) [32]. However, this approach still poses several technical difficulties that reduce the process efficiency, generally known as the "high-solids effect" (i.e., the decrease in glucan conversion yields as solids load increases) [33,34]

The effect of enzyme loading at different levels of DM solids consistency on glucose production and enzymatic hydrolysis yield is illustrated in Figure 4. Glucose concentration highly depends on the solids loading used. The higher is the solids consistency, the higher is the glucose concentration obtained. In addition, the enzyme dose affects glucose production, so that the concentration increases as the enzyme loading rises, and its effect is more significant than the solids loading increase.



**Figure 4.** Enzymatic Hydrolysis yield (%) during the DA-SE MFUW pretreated at 15, 30, and 45 FPU/g glucan: (a) 10% DM solid consistency; (b) 15% DM solid consistency; and (c) 20% DM solid consistency.

A maximum enzymatic hydrolysis yields of 74% was obtained with the lowest solid consistency tested (10% DM, *w/w*) and the highest enzyme dose. The increase in solid consistency to 20% DM (*w/w*) allowed achieving a 68% enzymatic hydrolysis yield. Besides, the enzyme dose effect on enzymatic hydrolysis yields is more noticeable as the solids' content increases. With 10% solid consistency loading, the HE yields achieved with the different enzyme doses ranged from 65% to 74%, while, with 20% solid consistency, EH yield ranged from 4%1 to 68%. Enzymatic hydrolysis yield dropped 12% when the enzyme loading was reduced from 45 to 15 FPU/g glucan at 10% solid consistency. Time is also a parameter to consider, particularly at higher solid consistency loading, for which longer hydrolysis times are required. This effect is also observed with the enzyme dose but is less noticeable.

The multiple response optimization of Statgraphic Centurion was used to conjugate the response surface of each response variable and the determination of values which render the optimal point to both enzymatic hydrolysis yield (EHYg) (%) and glucose production (g/L). Figure 5 shows the superposition of two response variables. The optima point (desirability function = 0.87) (45 FPU/g glucan and 20% DM solid consistency) predicts a glucose production of 80 g/L and an enzymatic yield of 68.8%.

The resulting enzymatic hydrolysate obtained at 72 h from WIS after selected pretreatment (DA-SE, 195 °C, 10 min) and enzymatic hydrolysis conditions (20% DM solid consistency, 45 FPU/g glucan) were submitted to fermentation test using *S. cerevisiae* Ethanol Red. After 24-h fermentation, a concentration up to 39 g/L was obtained, being the ethanol concentration above the 4% (*w/w*) reported for economically feasible distillation [32]. An overall ethanol yield of 9.0 g bioethanol/100 g untreated MFGW could be obtained at selected pretreatment conditions. This value is slightly higher than those reported in the literature. Raud et al. [5] obtained 8.6 g/100 g mixed urban greening wastes when N<sub>2</sub> explosion pretreatment was used.



**Figure 5.** Response surface for desirability function using DM solid consistency and enzyme loading as controlled variables.

# 4. Conclusions

Urban forestry and greening wastes are rich in lignocellulosic materials and can be used as raw material in a biorefinery approach.

The results obtained in this study show that up to 70% of the main sugars present in urban forest and greening wastes were recovered for the coupled DA-SE pretreatment (195 °C for 10 min and 60 mg  $H_2SO_4/g$  raw material) and enzymatic hydrolysis steps (run at 45 FPU/g glucan). The maximum yield declined to about 65% when the enzyme loading was decreased to 30 FPU/g glucan and further to 54% for an enzyme loading of 15 FPU/g glucan.

An ethanol yield of 9.0 g bioethanol/100 g untreated MFGW could be obtained from insoluble solid obtained at selected pretreatment conditions. Despite the high ethanol concentration (39 g/L), some enhancements are expected, and hence additional work is required for valorization of the pentoses sugars (as xylose) fraction, utilizing yeast able to convert pentoses into ethanol.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/1996-1073/13/17/4432/s1, Figure S1. Scheme of SE unit. Figure S2: Xylan distribution in MFGW pretreated with DA-SE pretreatment. Table S1: Chemical composition of pretreated solids under DA-SE pretreatment. Table S2: Composition of liquid fraction (prehydrolyzated) obtained after DA-SE pretreatment. Data are expressed as g/100 dry MFGW except for phenols, for which data are expressed as mg/100 g dry MFGW.

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