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Multi-Step Exploitation of Raw *Arundo donax* L. for the Selective Synthesis of Second-Generation Sugars by Chemical and Biological Route

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Abstract: Lignocellulosic biomass represents one of the most important feedstocks for future biorefineries, being a precursor of valuable bio-products, obtainable through both chemical and biological conversion routes. Lignocellulosic biomass has a complex matrix, which requires the careful development of multi-step approaches for its complete exploitation to value-added compounds. Based on this perspective, the present work focuses on the valorization of hemicellulose and cellulose fractionsof giant reed (*Arundo donax* L.) to give second-generation sugars, minimizing the formation of reaction by-products. The conversion of hemicellulose to xylose was undertaken in the presence of the heterogeneous acid catalyst Amberlyst-70 under microwave irradiation. The effect of the main reaction parameters, such as temperature, reaction time, catalyst, and biomass loadings on sugars yield was studied, developing a high gravity approach. Under the optimised reaction conditions (17 wt% *Arundo donax* L. loading, 160 °C, Amberlyst-70/*Arundo donax* L. weight ratio 0.2 wt/wt), the xylose yield was 96.3 mol%. In the second step, the cellulose-rich solid residue was exploited through the chemical or enzymatic route, obtaining glucose yields of 32.5 and 56.2 mol%, respectively. This work proves the efficiency of this innovative combination of chemical and biological catalytic approaches, for the selective conversion of hemicellulose and cellulose fractions of *Arundo donax* L. to versatile platform products.

Keywords: *Arundo donax* L.; microwaves; Amberlyst-70; second-generation sugars; high gravity approach; chemical and enzymatic hydrolysis; cascade biomass exploitation

1. Introduction

The anticipated depletion of fossil resources in the nearest future together with their associated unfavorable environmental outcomes are driving research towards the exploration of promising alternative renewable resources. These alternative renewable resources include lignocellulosic biomass [1,2], animal manure and human sewage [3,4], meat processing waste [5,6] and aquatic biomass [7,8], which can be employed as biorefinery feedstocks. Among them, the lignocellulosic biomass represents a key feedstock, being abundant, safe, and cheap and it is mainly composed of three biopolymers (cellulose, hemicellulose and lignin), which are precursors of very valuable bio-products and biofuels [9,10]. For this reason, the selective fractionation of the lignocellulosic biomass is of paramount importance, in order to optimise the recovery and the valorisation of each biopolymer, adopting a multi-step approach. However, the conversion of this feedstock is

limited by its recalcitrant nature that makes indispensable the use of suitable pretreatment steps. They aimed at improving the efficiency of the hydrolysis of the polysaccharides [11–13] to release second-generation sugars (xylose and glucose), furfural, 5-hydroxymethylfurfural (HMF), levulinic acid (LA) and formic acid (FA) [14–21]. A large number of pretreatments were reported in the literature employing mineral acids [22–24], inorganic salts [25,26], alkaline solutions [23,27], ionic liquids [28,29], and organosolv [30,31], in addition to physico-chemical techniques, such as steam explosion [32]. The choice of suitable pretreatment strongly depends on the biomass fraction that should be exploited and on the target products. For instance, the organosolv treatment is efficient for the recovery of cellulose and lignin, but it is not suitable for that of hemicellulose [33]. On the other hand, the pretreatment with mineral acids, in particular H_2SO_4, is preferred for the conversion of both cellulose and hemicellulose fractions, because of its high efficiency to disrupt the lignocellulosic matrix, thus selectively releasing the monomeric sugars. However, low acid concentrations and mild reaction conditions must be employed, in order to limit the excessive degradation of the hemicellulose fraction in the liquid phase [22]. Therefore, the best hemicellulose pretreatment gives the highest xylose concentration in the hydrolysate and the lowest one of inhibitors, avoiding any additional detoxification step before fermentation [24,34]. At the end of the hemicellulose pretreatment, a cellulose-rich solid is recovered, which can be further exploited by a subsequent chemical or enzymatic hydrolysis step [25,35]. However, the employment of mineral acids has some disadvantages, such as the expensive catalyst recovery and the possible corrosion of the stainless steel equipment. About the last issue, it is reported that the use of H_2SO_4 concentration higher than 0.05 wt% can rapidly corrode a Type 316 stainless steel (widely used for the construction of autoclaves), even at moderate temperature and time, typical of the hydrothermal pretreatments [36]. Therefore, common stainless steels cannot be adopted for the acid hydrothermal treatments, which instead require special alloys, such as various grades of Hastelloy, zirconium or other corrosion-resistant alloys, which ensure excellent corrosion resistance to acids, even at high severity conditions [37]. These issues drove the research towards the employment of low-cost acid solid catalysts, such as styrene-based sulfonic acid resins (Amberlysts) [38–44]. The use of these catalysts can provide a large number of advantages over the traditional homogeneous acid catalysts: (i) better reaction control, (ii) reduced formation of by-products, (iii) easy and safe operation with minor corrosion problems compared to homogeneous catalysis, (iv) advantageous recovery of the catalyst with less waste disposal. In addition, the recyclability of these catalysts is a key requirement for the economics. In fact, despite the higher initial cost of the sulfonic acid resins compared to traditional sulfuric acid, if the acid resin catalyst maintains almost unaltered its starting catalytic performance (without desulfonation and leaching), it can be reused repeatedly in additional batch reactions. Therefore, its cost per batch operation can be progressively cut down, even below those of the traditional mineral acids, per unit of productivity. Lastly, depending on their thermo-chemical stability, resin catalysts can be used more advantageously in continuous systems, leading to significant economic advantages of productivity on an industrial scale. However, also heterogeneous catalysts still present some problems, in particular the inefficient solid-solid interaction between the biomass and the catalyst, which implies hampered hydrolysis. For this reason, the acid solid catalysts, such as Amberlyst-15, zeolites and ad hoc synthesized carbon-based solid acids, were used in combination with ionic liquids [41,43], which are able to dissolve cellulose and even raw lignocellulosic biomasses, or as catalysts for substrates pretreated by ball-milling [42] or acid/alkali solutions [40,44], in this last case not solving the corrosion problem. Different raw feedstocks and heterogeneous catalysts were reported in the literature for the hydrolysis of hemicellulose and cellulose, as summarized in Tables 1 and 2, respectively.

Table 1. Literature review of hemicellulose hydrolysis with heterogeneous catalysts.

Raw Material	Catalyst	Final Product	Yield	Process Conditions	Reference
Corn cob	HSO$_3$-ZSM-5	Xylose	28.7 wt%	120 °C, 6 h, S/L 1/20, Cat/sub 1:1 wt/wt	[38]
Corn cob	Carbon-based solid acid (C-SO$_3$H)	Xylose	78.1 mol%	140 °C, 6 h, S/L 1/100, Cat/sub 1:1 wt/wt	[39]
Giant reed	Amberlyst 35DRY	Total reducing sugars	43.0 wt%	IL pretreatment: 120 °C, 3 h, S/L 1/20 [C$_4$mim]Cl; acid hydrolysis: 120 °C, 1.5 h, S/L 1/20, Cat/sub 1:5 wt/wt	[41]
Rice straw	Carbon-based solid acid (–COOH)	Xylose	52.1 mol%	Alkali pretreatment: 120 °C, 4 h, KOH 6 wt%, S/L 1/10; ball-milling treatment: 4 h, 500 r·min^{-1}, Cat/sub 1:2 wt/wt; acid hydrolysis: 200 °C, 1 h, S/L 1/100, Cat/sub 1:2 wt/wt	[42]
Eucalyptus	Carbon-based solid acid (–COOH)	Xylose	83.0 mol%	215 °C, 0.5 h, S/L 1/130, Cat/sub 1:6.5 wt/wt	[44]

S/L = solid to liquid weight ratio; Cat/sub = catalyst to substrate weight ratio.

Table 2. Literature review of cellulose hydrolysis with heterogeneous catalysts.

Raw Material	Catalyst	Final Product	Yield	Process Conditions	Reference
Corn cob	HSO$_3$-ZSM-5	Glucose	23.0 wt%	120 °C, 6 h, S/L 1/20, Cat/sub 1:1 wt/wt	[38]
Corn cob	Cellulase enzyme	Glucose	91.6 wt%	50 °C, 48 h, 40 FPU/g substrate	[39]
Rice straw	Amberlyst 15DRY	Total reducing sugars	26.8 wt%	Alkali pretreatment: 120 °C, 1 h, NaOH 2 wt%, S/L 1/7; acid hydrolysis: 140 °C, 4 h, S/L 1/20, Cat/sub 1:1.4 wt/wt	[40]
Rice straw	Carbon-based solid acid (–COOH)	Glucose	66.5 mol%	Alkali pretreatment: 120 °C, 4 h, KOH 6 wt%, S/L 1/10; ball-milling treatment: 4 h, 500 r·min^{-1}, Cat/sub 1:2 wt/wt; acid hydrolysis: 200 °C, 1 h, S/L 1/100, Cat/sub 1:2 wt/wt	[42]
Corn stover	PSSA/PIL	Glucose	20.0 wt%	105 °C, 7 h, S/L 1/20, Cat/sub 3:1 wt/wt, [EMIM]Cl/H$_2$O 4:1 wt/wt	[43]
Eucalyptus	Carbon-based solid acid (–COOH)	Glucose	31.0 mol%	215 °C, 0.5 h, S/L 1/130, Cat/sub 1:6.5 wt/wt	[44]

S/L = solid to liquid weight ratio; Cat/sub = catalyst to substrate weight ratio. PSSA/PIL = poly(styrene sulfonic acid)/poly(vinyl imidazolium chloride).

Among the lignocellulosic biomasses, giant reed (*Arundo donax* L.) is a suitable feedstock, being a perennial herbaceous plant with a high production yield (up to 30 t ha^{-1} year^{-1}), growing also in marginal lands. It is rich with structural C5 and C6 carbohydrates, which represent up to 60 wt% of the dry biomass [45]. For this reason, *Arundo donax* L. is widely used as a substrate for the synthesis of important platform-chemicals, biofuels, and second-generation sugars [14,16,25,27,35,46–48]. In

particular, the interest towards the synthesis of xylose and glucose is continuously growing, due to their promising applications in both chemical and biological processes to produce alcohols, acids, oils, hydrocarbons, hydrogen, and other valuable products [1,15,22,34,46,47,49–55]. However, up to now, only a few works discussed the employment of acid solid catalysts for the conversion of *Arundo donax* L. to value-added products, performing the reaction in the presence of ionic liquids as solvents, which have some criticisms related to their high viscosity, toxicity and cost, which strongly limit the sustainability of this approach. For example, You et al. carried out the hydrolysis of *Arundo donax* L. to second-generation sugars in 1–butyl–3–methylimidazolium chloride, adopting Amberlyst 35DRY as the catalyst. They carried out a pretreatment step without catalyst at 120 °C for 3 h employing the giant reed loading of 5 wt%. Subsequently, the Amberlyst 35DRY, with a catalyst/biomass weight ratio of 0.2 wt/wt, was added to the reaction mixture and the hydrolysis was performed at 120 °C for 1.5 h. Under these reaction conditions, the authors reported the highest yield to total reducing sugars of 43 wt%, based on the starting biomass amount [41]. Despite the good catalytic performance, the higher manufacturing cost for the Amberlyst-35, ascribed to the over-sulfonation treatment, and its thermal instability (desulfonation and deactivation) at temperatures higher than 150 °C shift the attention towards other types of Amberlyst resins. The present work proposes, for the first time, the employment of the acid resin Amberlyst-70 for the hydrolysis of hemicellulose and cellulose fractions of the untreated giant reed to xylose and glucose, respectively, working in water and under microwaves (MW) irradiation (Figure 1). In fact, monosulfonated Amberlyst-70, whose Brønsted acidity is due to the sulfonic groups present on its surface, shows acid site strength similar to that of the over-sulfonated Amberlysts-35 and -36 [56], but a higher thermal stability than most of the heterogeneous acid resins, which is a desirable requirement for the development of many biomass conversion processes [57].
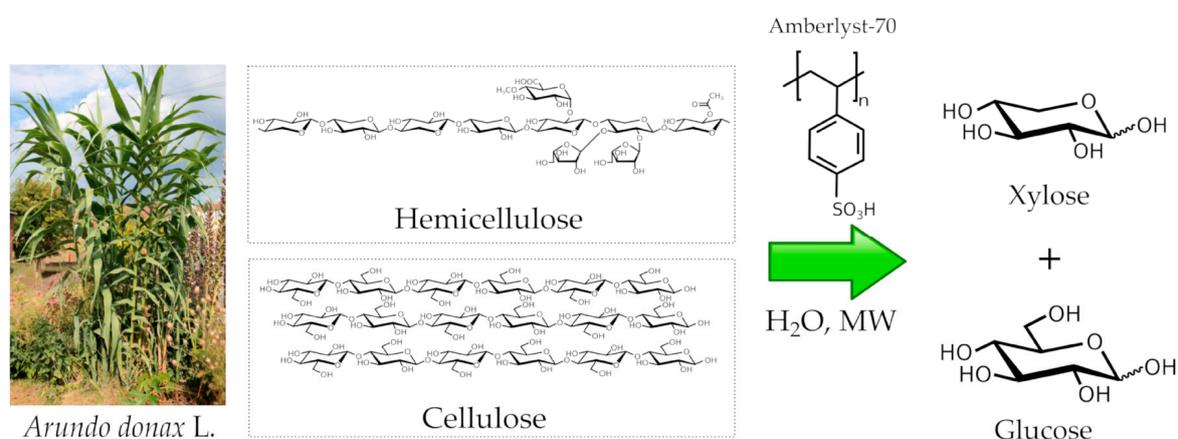
Figure 1. Amberlyst-70 catalysed conversion of the hemicellulose and cellulose fractions of the *Arundo donax* L. to xylose and glucose, respectively.

Moreover, this resin was already successfully employed by us in a previous research, where it was adopted as the acid catalyst for the conversion of fructose and inulin to HMF in water in the presence of microwave heating, resulting in an active and recyclable system [57]. From the perspective of the sustainability of the reaction, microwaves (MW) represent an important tool because they can reduce the reaction time and the energy consumption, thus improving the efficiency of the processes [58–62]. Moreover, MW play the key role of selectively breaking the hydrogen bonds during the biomass conversion, decreasing the stability of the lignocellulosic matrix [11,63]. In the first step of the proposed process, the xylose yield was optimised, while in the second one, the cellulose-rich solid residue was subsequently subjected to both chemical and enzymatic hydrolysis to give glucose. Regarding the chemical hydrolysis, the whole solid residue recovered at the end of the first step, containing embedded Amberlyst-70, was directly reprocessed under more severe reaction conditions, in order to convert the cellulose fraction and investigate the recyclability of the catalyst. For the enzymatic hydrolysis, the

unreacted *Arundo donax* L. was separated from the embedded Amberlyst-70 by sieving, and it was converted adopting the commercial cellulolytic enzymatic preparation Cellic® CTec2, which is effective for the hydrolysis of cellulose fraction of biomasses pretreated by alkaline and acid treatments [64–66]. The present work investigates a novel multi-step treatment, which consists of an integrated chemical and biological process. Lastly, the catalytic runs were carried out employing starting concentrations of substrate up to 17 wt%, a value higher than those reported in the literature, generally under 8 wt% [2,67]. The employment of high starting substrate concentrations is in agreement with the high gravity approach [68], which allows the production of more concentrated hydrolysates. This approach is particularly promising from the industrial perspective, because the higher concentrations of products lead to the increase of productivity and the reduction of capital and operating costs, requiring easier purification steps.

2. Results and Discussion

The multi-step hydrolysis approach is fundamental in order to exploit each fraction of the biomass. In the present study, as the first step, the MW-assisted hydrolysis of giant reed hemicellulose into xylose was investigated adopting Amberlyst-70 as heterogeneous catalyst and, subsequently, the recovered cellulose-rich residue (CRR) was employed as substrate for the conversion of its cellulose fraction into glucose, by means of the chemical or enzymatic hydrolysis treatment (Figure 2).
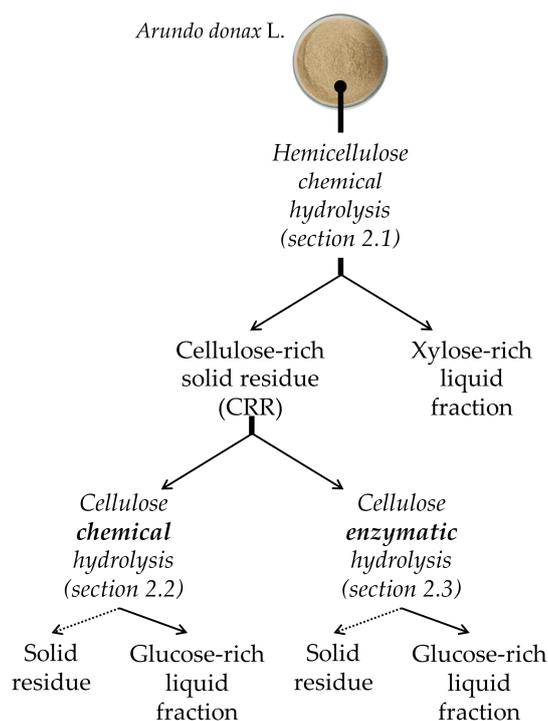
Figure 2. Diagram of the multi-step hydrolysis approach of *Arundo donax* L.

2.1. Microwave-Assisted Hydrolysis of Giant Reed Hemicellulose to Xylose Catalysed by Amberlyst-70

It is well-known that hemicellulose is more easily hydrolysable than cellulose, the two hydrolysis processes requiring different optimised reaction conditions. In the first step, the selective conversion of the hemicellulose into xylose was studied and optimised, in the presence of Amberlyst-70. Before the catalytic investigation, the composition of the starting raw *Arundo donax* L. was evaluated according to the NREL protocol [69–73], obtaining the following values (wt% on dry matter): glucan 36.3 ± 0.4, xylan 17.3 ± 0.2, arabinan 1.9 ± 0.1, ash 2.0 ± 0.0, Klason lignin (acid-insoluble residue) 22.0 ± 0.0, acid-soluble lignin 0.9 ± 0.1, other compounds 19.6 ± 0.9. The values derived from the mean of 3 replicates, ± standard deviation (SD).

The effects of the main process parameters, such as biomass loading, Amberlyst-70/*Arundo donax* L. weight ratio and temperature, on reducing sugars yield and by-product concentration, were investigated and the obtained results are reported in Tables 3 and 4, respectively. A preliminary investigation regarding the effect of the Amberlyst-70/*Arundo donax* L. weight ratio on the xylose recovery was carried out at 160 °C for 20 min, keeping constant the biomass loading (5 wt%) and ranging the Amberlyst-70/*Arundo donax* L. weight ratio between 0.1–0.3 wt/wt (runs 1–3, Tables 3 and 4). These reaction conditions were selected on the basis of previous literature research [40,41]. As reported in Table 3, the increase of the catalyst/substrate weight ratio from 0.1 to 0.3 wt/wt caused an increase of the biomass solubilisation, from 31.5 to 47.7 wt%, together with the improvement of sugars yields. In fact, the xylose yield, calculated respect to xylan content in the raw material, rose from 68.4 to 94.8 mol%, corresponding to the concentrations of 6.7 and 9.3 g/L, and the glucose yield, calculated respect to glucan content in the raw material, rose from 8.5 to 13.0 mol%, corresponding to the concentrations of 1.7 and 2.6 g/L. Regarding the xylose yield, the best result of 97.5 mol% was obtained adopting the catalyst/substrate weight ratio of 0.2 wt/wt (run 2, Table 3). These preliminary tests ensured the complete and selective hydrolysis of *Arundo donax* L. hemicellulose to xylose under mild reaction conditions, as confirmed also by the negligible by-product concentrations (run 2, Table 4). Among them, only acetic acid (AA), directly originated from the hydrolysis of hemicellulose [74], reached concentrations higher than 0.5 g/L. The formationof FA, LA, HMF and furfural, all deriving from the degradation pathways of glucose and xylose, was scarcely favored.

Table 3. Experimental set-up and results of hemicellulose hydrolysis. Reaction conditions: MW heating, 20 min.

Run	Biomass Loading (wt%)	Cat/Sub [1] (wt/wt)	Temperature (°C)	Biomass Solubilisation (wt%)	Xylose Yield [2] (mol%)	Glucose Yield [3] (mol%)
1	5	0.1	160	31.5	68.4	8.5
2	5	0.2	160	44.5	97.5	12.5
3	5	0.3	160	47.7	94.8	13.0
4	9	0.1	160	30.3	66.7	8.2
5	9	0.2	160	43.8	99.2	11.8
6	9	0.3	160	46.3	94.0	12.0
7	13	0.1	160	29.7	65.3	8.1
8	13	0.2	160	44.3	93.4	11.9
9	13	0.3	160	48.4	95.8	12.2
10	13	0.1	150	29.5	53.3	7.0
11	13	0.2	150	40.9	85.3	10.8
12	13	0.3	150	43.7	83.9	10.9
13	17	0.2	160	42.9	96.3	10.2

[1] Weight of Amberlyst-70/dry weight of starting giant reed; [2] Yield respect to moles of xylan in the starting giant reed; [3] Yield respect to moles of glucan in the starting giant reed.

To improve the sustainability of the process, the high gravity approach was adopted, testing the same experimental conditions (160 °C, 20 min, 0.1–0.3 wt/wt Amberlyst-70/*Arundo donax* L. weight ratio) at two higher biomass loadings, 9 wt% (runs 4–6, Tables 3 and 4) and 13 wt% (runs 7–9, Tables 3 and 4). It is important to underline that by keeping the Amberlyst-70/*Arundo donax* L. weight ratio constant and increasing the biomass loading, no significant variations of the biomass solubilisation and the sugars yield occurred, highlighting that these conditions are still favorable to the production of sugars, rather than to that of their degradation products [68]. Regarding the sugar concentrations, working with the Amberlyst-70/*Arundo donax* L. weight ratio of 0.2 wt/wt, these increased from 9.6 to 27.5 g/L for xylose and from 2.5 to 7.2 g/L for glucose, adopting 5 and 13 wt% biomass loadings, respectively. This positive trend is due to the very limited formation of by-products, even at these high biomass loadings. In fact, except for AA that derives from hemicellulose hydrolysis, each by-product (FA, LA, HMF and furfural) reached concentrations up to 1.3 g/L, which are lower than

those achieved with traditional mineral acids with the same biomass loadings [35,46]. Moreover, the Amberlyst-70/*Arundo donax* L. weight ratio of 0.2 wt/wt gave the best compromise between xylose yield and catalyst amount also working at 9 and 13 wt% of biomass loading, ensuring the very high xylose yields of 99.2 mol% and 93.4 mol%, respectively (runs 5 and 8, Table 3). To evaluate the possibility of reaching high xylose yields at milder reaction conditions, keeping high the biomass loading (13 wt%), the reaction was performed at 150 °C for 20 min with the same catalyst/biomass weight ratios (runs 10–12, Tables 3 and 4). Comparing runs 7–9 with runs 10–12, for each value of Amberlyst-70/*Arundo donax* L. weight ratio, the temperature of 150 °C led to lower sugars yields than those obtained at 160 °C and analogous by-product concentrations.

Table 4. Influence of the reaction conditions on by-product concentration formed during the hemicellulose hydrolysis. Reaction conditions: MW heating, 20 min.

Run	Biomass Loading (wt%)	Cat/Sub [1] (wt/wt)	Temperature (°C)	AA [2] (g/L)	FA [3] (g/L)	LA [4] (g/L)	HMF [5] (g/L)	Furfural (g/L)
1	5	0.1	160	0.8	0.2	0.0	0.1	0.0
2	5	0.2	160	0.9	0.2	0.0	0.1	0.0
3	5	0.3	160	1.1	0.3	0.0	0.2	0.0
4	9	0.1	160	1.6	0.1	0.1	0.4	0.0
5	9	0.2	160	2.1	0.7	0.3	0.7	0.0
6	9	0.3	160	2.8	0.7	0.5	0.7	0.1
7	13	0.1	160	2.9	0.2	0.2	0.8	0.4
8	13	0.2	160	4.1	1.1	0.5	1.1	0.7
9	13	0.3	160	4.2	1.1	0.6	1.0	0.7
10	13	0.1	150	1.0	0.0	0.0	0.3	0.1
11	13	0.2	150	2.9	0.7	0.3	0.9	0.5
12	13	0.3	150	3.1	0.7	0.4	0.8	0.5
13	17	0.2	160	4.7	1.0	0.5	1.3	1.0

[1] Weight of Amberlyst-70/dry weight of starting giant reed; [2] Acetic acid; [3] Formic acid; [4] Levulinic acid; [5] 5–Hydroxymethylfurfural.

For example, at the Amberlyst-70/*Arundo donax* L. weight ratio of 0.2 wt/wt, xylose and glucose yields at 150 °C were 85.3 and 10.8 mol%, respectively, whereas the corresponding data at 160 °C were 93.4 and 11.9 mol%, respectively.

The biomass loading was then further increased up to 17 wt% and the reaction was carried out at the previously optimised temperature of 160 °C, with the Amberlyst-70/*Arundo donax* L. weight ratio of 0.2 wt/wt, for 20 min (run 13, Tables 3 and 4). The xylose and glucose yields were kept almost constant to those achieved with lower biomass loadings (5, 9 and 13 wt%), significantly increasing the xylose and glucose concentrations up to 37.7 and 8.4 g/L, respectively. Moreover, the formation of by-products was limited, even working at 17 wt%, being their concentrations in the range 0.5–1.3 g/L, except for AA that reached 4.7 g/L. Although the fermentability of the hydrolysates was not tested in this work, the by-product concentrations were lower than the typical inhibition thresholds [75]. This is certainly a positive key aspect, enabling the direct biological conversion of these hydrolysates, without additional further relevant detoxification procedures [46,47]. In fact, in the fermentative route for sugars valorisation, the by-products act as strong inhibitors for microorganisms [55]. Therefore, the synthesized hydrolysate, characterized by a very low amount of by-products and a high quantity of reducing sugars (46.1 g/L), represents an ideal substrate for the production of value-added bio-chemicals through a subsequent biological route.

Once optimised the biomass loading (17 wt%), the temperature (160 °C) and the Amberlyst-70/*Arundo donax* L. weight ratio (0.2 wt/wt), a kinetic study was performed and the obtained results are reported in Figure 3. The xylose concentration ranged from 30.6 to 32.6 g/L prolonging the reaction time from 5 to 30 min, reaching the maximum value of 37.7 g/L, corresponding to the xylose yield of 96.3 mol%, after 20 min. Differently, glucose concentration linearly increased

prolonging the reaction time, from 4.2 g/L to 10.5 g/L, this last corresponding to the glucose yield of 13.0 mol%. Regarding the by-product concentrations, they showed analogous trends of that found for glucose, except for HMF, which was converted into LA and FA after 20 min. These trends can be rationalized on the basis of the process severity, which takes into account the combined effect of temperature and time [25]. In fact, the process severity increased with time, promoting not only the xylan and glucan depolymerisation but also the formation of by-products. In conclusion, the reaction time of 20 min ensured the highest xylose yield of 96.3 mol%, corresponding to the ponderal yield of 18.9 wt%, calculated respect to the amount of the starting *Arundo donax* L.
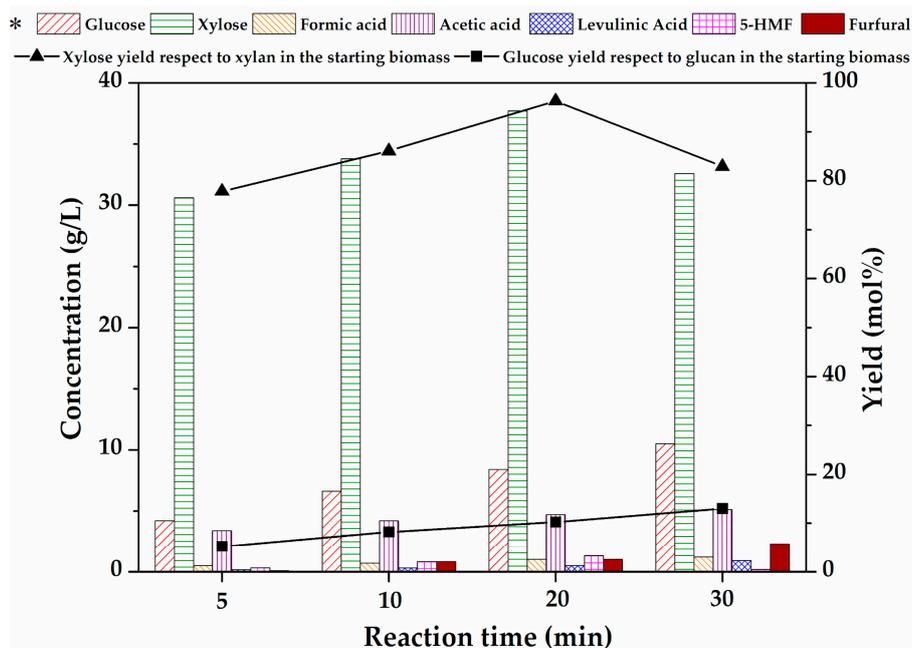
Figure 3. Effect of reaction time on xylose and glucose yields (mol%) and on by-product concentration (g/L). Reaction conditions: 17 wt% biomass loading, 160 °C, Amberlyst-70/*Arundo donax* L. weight ratio 0.2 wt/wt (* Compounds concentration).

The cellulose-rich residue (CRR) recovered at the end of the optimised reaction (run 13) represented the 57.1 wt% of the starting raw *Arundo donax* L. and its chemical composition was the following (wt% on dry matter): glucan 52.5 ± 0.4, xylan 0.4 ± 0.1, arabinan 0.2 ± 0.0, ash 2.1 ± 0.0, Klason lignin (acid-insoluble residue) 35.9 ± 0.8, acid-soluble lignin 0.4 ± 0.1, other compounds 8.5 ± 1.4. The values represent the mean of 3 replicates, ± standard deviation (SD). Therefore, these data confirm the complete hydrolysis of the hemicellulose fraction.

The CRR was also characterized by FT-IR spectroscopy and its spectrum is reported in Figure 4, together with that of the raw *Arundo donax* L. The comparison between the two spectra shows, in the case of the CRR, the decrease of the intensity of band at 1731 cm^{-1}, assigned to the C=O stretching of the acetyl groups, confirming the occurred depolymerisation of the hemicellulose fraction [14,76]. Moreover, the absorption bands at 1508 and 1456 cm^{-1}, due to the C=C stretching of benzene rings of lignin and the bending vibration of the CH$_2$ groups of cellulose [14,76], respectively, were more intense in the case of CRR. Also the bands at 1052 and 1031 cm^{-1}, assigned to C–O–C stretching of the pyranose ring in cellulose and to the C–O stretching of hydroxyl and ether groups of cellulose, were more intense in the CRR [76]. All these observations confirmed that the quantitative removal of hemicellulose from *Arundo donax* L. led to the enrichment in cellulose and lignin in the CRR.
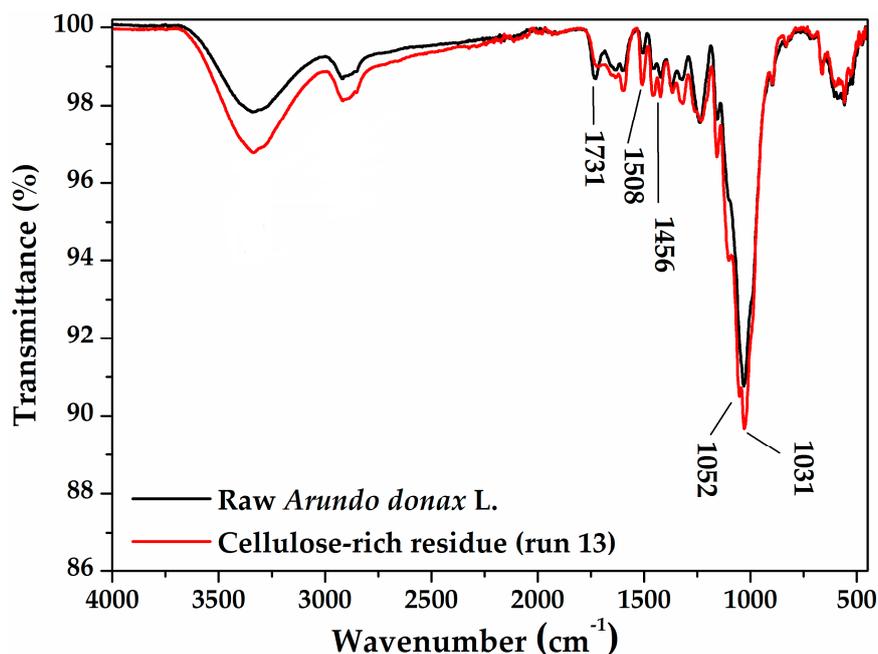
Figure 4. FT-IR spectra of the starting raw *Arundo donax* L. (black) and the cellulose-rich residue recovered from run 13 (red).

To investigate the crystallinity index (CrI) of biomass before and after the hydrolysis of hemicellulose, the solids were characterized by X-ray Diffraction (XRD) analysis. Figure 5 reports the XRD diffractograms of the raw biomass (A) and CRR after the selective hemicellulose dissolution (B). The CrI of the samples was estimated by the deconvolution method. In particular, five Gaussian curves, each corresponding to Miller indices of the crystalline cellulose (1 0 1), (1 0 −1), (2 0 1), (0 0 2) and (0 4 0) were used. In addition, a sixth Gaussian curve for the amorphous phase, due to the presence of other amorphous components, such as hemicellulose and disordered cellulose, was included [77,78]. The calculated CrI value of raw *Arundo donax* L. was 53.8%, in agreement with data already reported in the literature for this kind of biomass. [78]. After complete hemicellulose hydrolysis under the optimised reaction conditions (run 13), the CrI increased up to 66.7%. Also this result is in agreement with the literature, highlighting that the mild treatment increased the CrI of biomass, by removing the hemicellulose and the amorphous phase of cellulose, not involving the crystalline one [78–80]. The XRD results confirmed that the first step of hydrolysis was selective towards hemicellulose and non-crystalline cellulose fractions. Amberlyst-70 resulted in an effective and selective heterogeneous catalyst for the complete removal of hemicellulose under the adopted reaction conditions, allowing us to achieve concentrated xylose hydrolysates, thanks also to the combined effect of MW irradiation and the high biomass loading.

The recovered CRR represented a good starting feedstock for the cascade valorisation of the cellulose fraction via chemical or enzymatic routes.
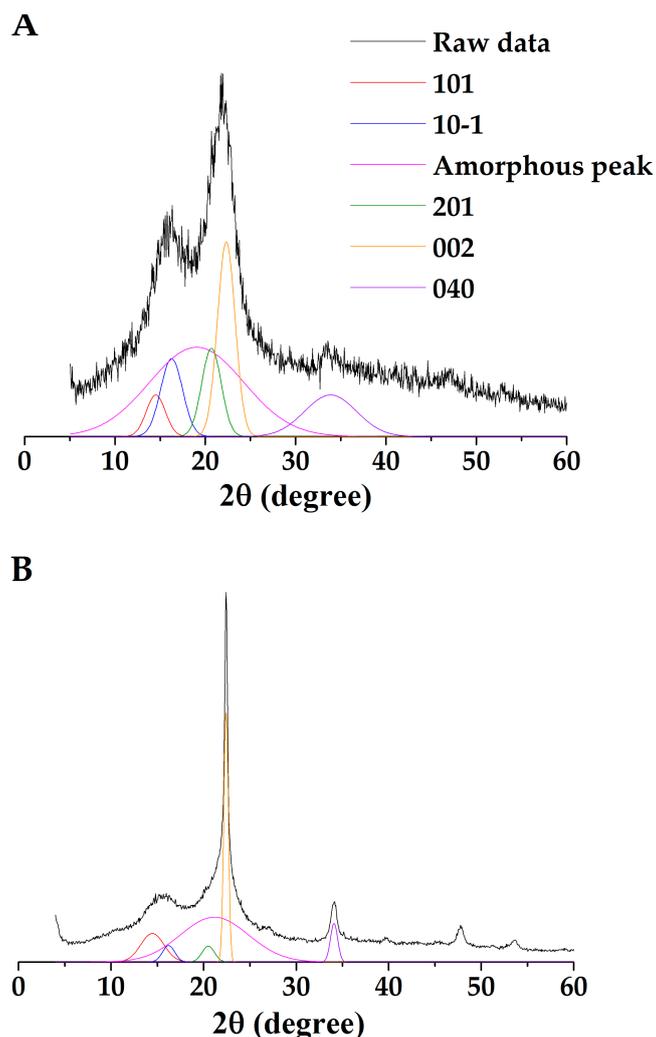
Figure 5. XRD diffractograms of the starting raw *Arundo donax* L. (**A**) and the CRR recovered from run 13 (**B**).

2.2. Chemical Hydrolysis of Cellulose-Rich Residue (CRR) to Glucose

The whole solid residue recovered at the end of the optimised first step of hydrolysis (run 13), which included both CRR and embedded Amberlyst-70, was further processed, in order to investigate the conversion of cellulose to glucose by chemical hydrolysis catalysed by the same heterogeneous catalyst. However, the formation of a solid by-product, named "humins", can take place during the synthesis of sugars, as a consequence of degradation pathways [25]. Humins could be present on the CRR and Amberlyst-70 surfaces, contributing to hamper the conversion of cellulose into glucose. To partially remove humins, the whole solid recovered from run 13 was washed with acetone, which is efficient for this purpose [57,81], and subsequently dried under vacuum at 50 °C and reprocessed. The reaction was carried out with the CRR loading of 17 wt%, analogously to the conditions of the first step. On the basis of the *Arundo donax* L. solubilisation achieved in the previous step, equal to 42.9 wt%, the embedded Amberlyst-70/CRR weight ratio was 0.35 wt/wt. The obtained results are reported in Table 5. Harsher reaction conditions than those adopted for the hemicellulose conversion were necessary for this second hydrolysis step, due to the higher recalcitrance of cellulose towards the hydrolysis. On this basis, the preliminary run was carried out at 180 °C for 60 min (run 14, Table 5), achieving low biomass solubilisation and glucose yield, due to the mild reaction conditions, not appropriate to promote the hydrolysis.

Table 5. Experimental set-up and results of cellulose hydrolysis. Reaction conditions: MW heating, CRR loading 17 wt%, Amberlyst-70/CRR weight ratio 0.35 wt/wt.

Run	Time (min)	Temperature (°C)	AA [1] (g/L)	FA [2] (g/L)	LA [3] (g/L)	HMF [4] (g/L)	Furfural (g/L)	Biomass Solubilisation (wt%)	Glucose Yield [5] (mol%)
14	60	180	2.5	1.5	1.9	0.8	0.6	17.2	3.9
15	60	190	2.5	1.1	3.2	1.9	0.8	35.9	26.4
16	20	190	2.2	1.5	1.6	0.6	0.5	27.3	32.5

[1] Acetic acid; [2] Formic acid; [3] Levulinic acid; [4] 5-Hydroxymethylfurfural; [5] Yield respect to moles of glucan in the CRR solid.

For this reason, the temperature increased to 190 °C (run 15, Table 5), which strongly improved the biomass solubilisation and glucose yield, up to 35.9 wt% and 26.4 mol%, respectively. However, under these reaction conditions, also the by-product concentrations, in particular those of LA and HMF, increased, proving that glucose degradation was promoted. The decrease of the reaction time from 60 to 20 min (run 16, Table 5) limited the formation of by-products and, despite the biomass solubilisation decreased, a high glucose yield of 32.5 mol% was achieved, corresponding to the concentration of 38.7 g/L and the ponderal yield of 18.9 wt% respect to CRR. These are the best results obtained in the present work for the chemical hydrolysis of CRR and they are better than those reported in the literature. In fact, this glucose ponderal yield was higher than the total reducing sugars yield reported by Meena et al., equal to 15.1 wt%, for the conversion of alkali pretreated rice straw with recycled Amberlyst-15 [40]. It is important to underline that the authors reached this yield working with the biomass loading of 7 wt% and the Amberlyst-15/biomass weight ratio of 1 wt/wt, reaction conditions more favorable towards the hydrolysis than those adopted in the present investigation (17 wt% and 0.35 wt/wt). However, in our case, a higher biomass loading and fewer catalysts were employed, resulting in more sustainability from an applicative point of view. In addition, the biomass adopted by Meena et al. was previously pretreated with an alkaline solution, which favors the delignification process, promoting the subsequent hydrolysis, while in the present research, Amberlyst-70 was employed as catalyst for the multi-step hydrolysis of unpretreated *Arundo donax* L.

The chemical composition of the solid recovered from the optimised chemical hydrolysis (190 °C and 20 min) of CRR confirmed the exploitation of the *Arundo donax* L. cellulose. In fact, the final residue contained (wt% on dry matter): glucan 44.3 ± 0.3, ash 2.7 ± 0.2, Klason lignin (acid-insoluble residue) 49.2 ± 0.9, acid-soluble lignin 0.2 ± 0.0, other compounds 3.8 ± 0.5. The values represent the mean of 3 replicates, ± standard deviation (SD). The mass balance flow diagram of xylan, glucan, lignin and ash for the optimised multi-step chemical process is reported in Figure 6.
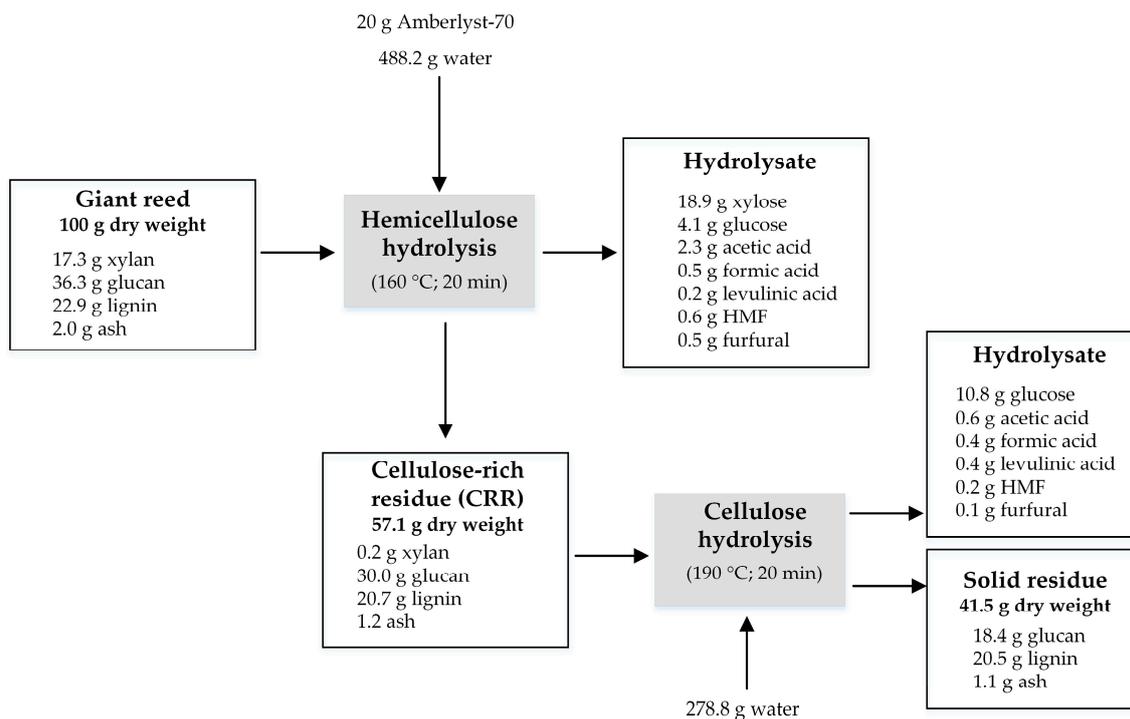
Figure 6. Mass balance flow diagram under the optimised reaction conditions for the two-step chemical hydrolysis of both raw giant reed and CRR.

2.3. Enzymatic Hydrolysis of Cellulose-Rich Residue (CRR) to Glucose

Enzymatic hydrolysis represents one of the most important approaches for the selective production of second-generation sugars. Enzyme activity is significantly affected by the biomass structure and by the presence of degradation by-products [82]. In fact, the biomass structure can influence the accessibility of enzymes to the polysaccharides, which is a crucial step for hydrolysis [32]. In particular, the crystallinity of cellulose, the porosity/size of fibers of pretreated biomass and the presence of lignin certainly influence the hydrolysis reaction [83]. Lignin inactivates enzymes by forming lignin-enzyme complexes and hampers the specific adsorption of enzymes onto the polysaccharides, reducing the efficiency of the hydrolysis [84]. In the present work, a preliminary study on enzymatic conversion of the recovered CRR to glucose was performed, adopting the commercial cellulolytic enzymatic preparation Cellic® CTec2. To study the performance of enzymatic hydrolysis of CRR under different reaction conditions, the embedded Amberlyst-70 was previously separated from CRR by sieving. The results of the enzymatic hydrolysis are reported in Figure 7. The effect of biomass loading on enzymatic digestibility was investigated, comparing two different loadings of CRR (2 and 9 wt%) and using an enzyme dosage of 15 FPU/g glucan, typically lower than those adopted in the literature for the pretreated *Arundo donax* L. hydrolysis (>60 FPU/g glucan) [35,85,86].
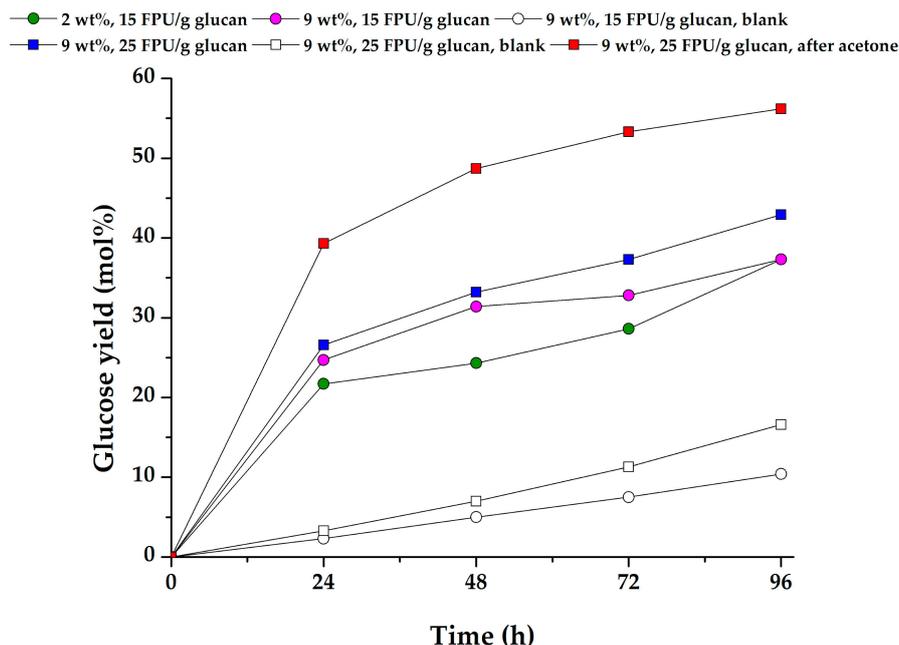
Figure 7. Kinetics of enzymatic hydrolysis of the CRR recovered from run 13 in Tables 1 and 2 (coloured curves) and raw *Arundo donax* L. as blank (white curves).

In both tests, the glucose yield increased during the entire time range, achieving the same glucose yield of 37.3 mol%, corresponding to the glucose concentration of 21.5 g/L, at the end of the reaction. The implementation of high biomass loading in the enzymatic hydrolysis favors the scale-up of the process and its economic sustainability from an industrial point of view, thanks to the increase of sugars concentration and the decrease of the costs related to the downstream workup. The increase of biomass loading did not negatively affect the enzymatic digestibility, thus the higher CRR amount (9 wt%) was adopted in the following tests. To demonstrate the beneficial role of the first hydrolytic step on the subsequent enzymatic hydrolysis in this proposed cascade approach, a blank test was performed under the same reaction conditions (9 wt%, 15 FPU/g glucan) adopting the raw *Arundo donax* L. as substrate. As reported in Figure 7, a linear profile was observed, reaching the glucose yield of 10.4 mol%, corresponding to the glucose concentration of 4.2 g/L, after 96 h, which was lower than that achieved starting from the CRR. Subsequently, on the basis of the promising results ascertained working with 15 FPU/g glucan, the enzyme concentration increased to 25 FPU/g glucan, which represents the typical dosage adopted in the literature [32,81,87,88], and the hydrolysis of both CRR and raw *Arundo donax* L. (blank test) were performed, maintaining the substrate loading at 9 wt%. The glucose yield increased up to 42.9 mol%, corresponding to the glucose concentration of 24.7 g/L, in the enzymatic hydrolysis of the CRR, and up to 16.6 mol%, corresponding to the glucose concentration of 6.7 g/L, in the respective blank test. These values were higher than those achieved working with the enzyme concentration of 15 FPU/g glucan, proving that the increase of enzyme concentration promoted the hydrolysis. However, it is recognized that enzyme performance is reduced during lignocellulose hydrolysis by interaction with lignin or lignin-carbohydrate complex. In particular, the mechanism of enzyme inhibition may involve both the adsorption of the enzyme on the insoluble lignin and interactions with the solubilised low-molecular lignin. Both these interactions can cause the non-specific adsorption of the enzyme onto the polysaccharides of the substrate [84]. Therefore, the high lignin content of the CRR (36.3 wt%) hampered the complete enzymatic hydrolysis. Moreover, as previously proposed, the conversion of cellulose into glucose could be limited also by the presence of humins on the CRR surface, which could hamper the contact between enzymes and cellulose, thus acting as an inhibitor of the binding between them [89]. On this basis, the CRR was washed with acetone, dried in an oven, and employed as the substrate for the enzymatic hydrolysis (biomass loading of 9 wt% and the

enzyme concentration of 25 FPU/g glucan). Under these reaction conditions, the glucose yield was further increased to 56.2 mol%, corresponding to the concentration of 32.8 g/L, after 96 h, confirming the beneficial role of the washing step. These results agree with those obtained from the enzymatic hydrolysis of the steam-pretreated giant reed performed under the same reaction conditions, being the yield of glucose equal to about 56 mol% [32]. Moreover, the obtained glucose concentration of 32.8 g/L was comparable with the value of 30.9 g/L reported by Aliberti et al. [85], who worked with the same steam-pretreated giant reed loading (9 wt%), but with higher enzymatic concentration (69.6 FPU/g glucan) than that adopted in the present study. In addition, unlike this cited work, our approach provided the valorisation of the Amberlyst-70 catalysed hydrolysis of the hemicellulose fraction, thus developing better the biorefinery concept. Cellulose conversion analogous to those reported in the literature for steam-pretreated giant reed was reached, leading to similar glucose yields, thus proving the efficiency of the multi-step approach proposed in this work.

The solid recovered from the optimised enzymatic hydrolysis (9 wt%, 25 FPU/g glucan) of acetone-washed CRR was 67.5 wt% of the CRR and 38.5 wt% of initial raw *Arundo donax* L. and its chemical composition confirmed the occurred fractionation of the *Arundo donax* L. cellulose in this innovative process scheme. In fact, the final solid residue contained (wt% on dry matter): glucan 32.7 ± 0.4, ash 3.1 ± 0.2, Klason lignin (acid-insoluble residue) 53.4 ± 0.8, acid-soluble lignin 0.1 ± 0.0, other compounds 10.7 ± 1.2. The values represent the mean of 3 replicates, ± standard deviation (SD). The mass balance flow diagram of xylan, glucan, lignin, and ash for the optimised multi-step process involving the chemical and biological routes is reported in Figure 8.
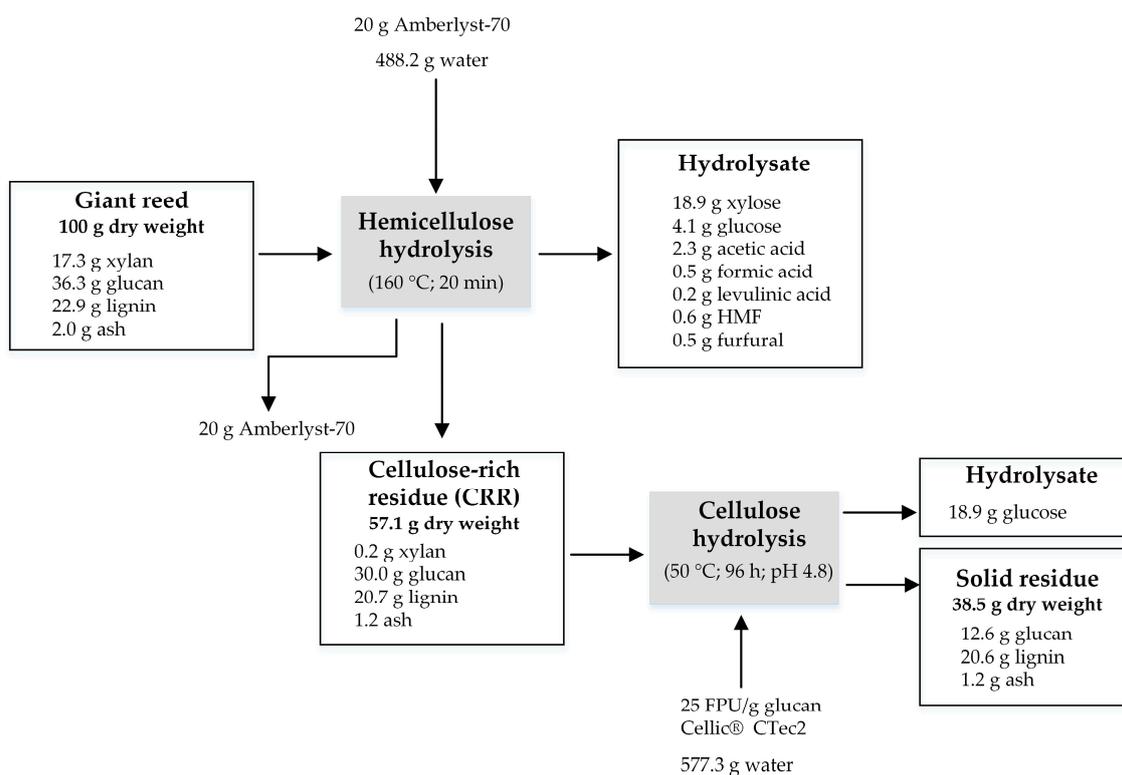
Figure 8. Mass balance flow diagram for chemical hydrolysis of raw giant reed and enzymatic hydrolysis of CRR, both of them under the optimised reaction conditions.

Regarding the possible exploitation of the final solid residue, many strategies are available, and the choice must be careful, applying the sustainability criteria and considering its chemical composition. In fact, the final solid residue is a hydrochar, more similar to lignin than to the starting lignocellulosic feedstock and, as such, it could be used in energy and environmental fields, including the applications as adsorbents, precursor of catalysts, soil amendment, anaerobic digestion, composting

and electrochemical energy storage materials [90]. In this work, a significant residual fraction of glucan is still present together with lignin. Therefore, the lignin exploitation must be synergistically integrated with that of glucan. This last one could be ideally converted into HMF and/or LA, but the catalytic performances of Amberlyst-70 are weak, due to the presence of the recalcitrant lignin, which hampers complete glucan solubilisation and conversion in the water medium. In addition, the syntheses of HMF or LA require harsher reaction conditions, which are not suitable for the catalyst stability. Instead, more advantageously, in order to overcome the recalcitrance of lignin, a very smart approach was proposed by Antonetti et al. [91], which used butanol as liquefaction solvent for the conversion of sugars into butyl levulinate, allowing the direct production of the value-added and marketable levulinates and, downstream of the treatment, a more useful hydrochar. On the other hand, in the field of bioconversion, Liu et al. [92] demonstrated that the co-presence of glucose facilitates both the lignin solubilisation and the subsequent polyhydroxyalkanoate (PHA) production, carried out in the presence of *Pseudomonas putida* KT2440. More than 70% of the residual sugars were released from the residue, producing the soluble lignin stream that contains both lignin and residual sugars for synergistic bioconversion. By this way, the integrated biorefinery increased the fermentable sugar yield and improved the PHA production, thus enhancing the carbon use efficiency.

3. Materials and Methods

3.1. Materials

Arundo donax L. was provided by the Institute of Life Sciences of Scuola Superiore Sant'Anna (Pisa, Italy). It was harvested from long term field trials carried out at the Enrico Avanzi Interdepartmental Centre for Agro-Environmental Research (CIRAA) of the University of Pisa (San Piero a Grado, Pisa, Italy) (latitude 43°68' N, longitude 10°35' E). The whole raw biomass was ground in 1.0 mm average size particles and dried at 105 °C in the oven until constant weight. Amberlyst-70 (concentration of acid sites: 2.55 meq/g; surface area: 36 m^2/g; particle size: 0.5 mm; average pore diameter: 22 nm; divinylbenzene content: 8 wt%; maximum temperature: 190 °C) was kindly provided by Rohm and Haas (Rohm and Haas, Philadelphia, PA, USA) and used as received. 5–hydroxymethylfurfural (95%) was purchased from AVA-Biochem (AVA Biochem AG, Zug, Switzerland). Xylose (>99%), glucose (>99%), formic acid (99.8%), acetic acid (>99%), levulinic acid (98%), furfural (99%) were provided by Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA) and employed as received. The enzymatic preparation Cellic® CTec2 was kindly provided by Novozymes (Novozymes, Bagsvaerd, Denmark) and employed as received.

3.2. Chemical Hydrolysis of Arundo donax L.

The hydrolysis of *Arundo donax* L. was carried out in the single-mode microwave (MW) reactor Discover S-class System produced by CEM Corporation (Matthews, NC, USA). This equipment is able to work up to the maximum temperature of 300 °C and up to the maximum pressure of 300 psi. The reactor is able to adjust the power of the emitted radiation in order to reach the set temperature and keep it constant during the reaction, providing the maximum power of 300 W. In a standard reaction, the proper amounts of biomass and Amberlyst-70 were weighed and charged in the MW vessel together with 20 mL of water. The vessel was placed in the MW reactor and heated at the desired temperature for the selected time under magnetic stirring. At the end of the reaction, the vessel was rapidly cooled at room temperature through external air flow. The mixture was filtered on funnel Buckner and the solid fraction was dried in an oven over the night and weighted. The liquid fraction was filtered through a syringe filter 0.45 μm PTFE (Whatman, Maidstone, UK) and analysed as such by High Performance Liquid Chromatography (HPLC) instrument (Jasco, Easton, PA, USA). Each experiment was replicated three times with an error of less than 5%.

The same protocol was also adopted for the chemical hydrolysis of the CRR recovered by the hydrolysis of *Arundo donax* L. in the presence of the embedded Amberlyst-70 as the catalyst, employing a previous washing of the whole solid with acetone (three times, weight ratio of 1:3 solid/acetone).

3.3. High Performance Liquid Chromatography (HPLC)

The HPLC Jasco LC-2000 (Jasco, Easton, PA, USA) was employed to analyse the liquid samples. The instrument was equipped with the column Benson 2000-0 BP-OA (Benson Polymeric Inc., Reno, NV, USA) (300 mm × 7.8 mm × 10 μm) kept at 60 °C and the 0.005 M H_2SO_4 aqueous solution was used as mobile phase with the flow-rate of 0.6 mL/min. The components concentration was evaluated on the basis of the calibration curves obtained from the analysis of standard solutions. At least three replicates for each concentration of standards and samples were done and the reproducibility of this technique was within 3%.

3.4. Compositional Analysis of the Feedstocks

The chemical composition of the feedstocks, e.g., starting *Arundo donax* L., the recovered CRR and final solid residues, was evaluated through the standard NREL protocols [69–73]. Briefly, the analytical procedure involves two hydrolysis steps: the first hydrolysis with 72 wt% sulfuric acid at 30 °C for 1 h, followed by the second hydrolysis of the slurry with 4 wt% sulfuric acid at 121 °C for 1 h. The slurry was filtered through a ceramic crucible and the filtered liquid phase was analysed by HPLC in order to get the compositional data related to the C5 and C6 carbohydrates. Furthermore, this liquid phase was analysed also by UV-Vis spectroscopy (Jasco, Easton, PA, USA) for the quantification of the acid-soluble lignin. Regarding the insoluble residue recovered after the 2nd hydrolysis step, it was dried up to constant weight, and its gravimetric quantification gave the Klason (acid-insoluble) lignin content. Lastly, ash content was determined as the percentage of residue remaining after dry oxidation of the starting biomass at 575 °C for 24 h.

3.5. X-ray Diffraction Method (XRD) Analysis

X-ray powder diffraction was performed using a Bruker D2 Phaser diffractometer (30 kV, 10 mA) (Bruker, Billerica, MA, USA) operating in Bragg-Brentano geometry (θ − θ scan mode) and equipped with a 1-dimensional Lynxeye detector. Ni-filtered Cu Kα radiation was used. Data were collected in the scan range 4–65° in 2θ, with a scan step of 0.02° and counting times of 0.1 s/step. Data were processed through the software Diffrac.Eva (Bruker AXS, Karlsruhe, Germany) and the peak fitting was performed using the software PeakFit (Systat Software Inc., San Jose, CA, USA). Crystallinity index (CrI) was evaluated according to the peak fitting method, considering five diffraction peaks for the crystalline phase at 2θ = 14.4°, 16.2°, 20.5°, 22.4°, and 33.9°, corresponding respectively to the Miller indices (1 0 1), (1 0 −1), (2 0 1), (0 0 2) and (0 4 0), and the peak at approximately 2θ = 19°, for the amorphous phase [64]. The R^2 values for the fitted peaks were always greater than 0.95. The peak fitting allows the evaluation of the area of the peaks and, on this basis, the CrI was determined through the following equation:

$$CrI = [1 - (A_{AM}/ A_{TOT})] \times 100 \tag{1}$$

where A_{AM} is the area of the peak corresponding to the amorphous cellulose, and A_{TOT} is the total area of all peaks.

3.6. Enzymatic Hydrolysis of Arundo donax L.

Before carrying out the enzymatic hydrolysis of raw *Arundo donax* L. and the CRR, the enzymatic activity of commercial preparation Cellic® CTec2 was quantified through the standard NREL protocol [93]. 0.5 mL of a diluted sample containing enzymes was incubated with 50 mg of Whatman No.1 filter paper strip (Whatman, Maidstone, UK) and 1.0 mL of sodium citrate buffer at 50 °C for 1 h. The enzymatic reaction was terminated by adding 3.0 mL of 3,5-dinitrosalicylic (DNS) agent

at 95 °C for 5 min. Subsequently, the amount of released glucose was measured as reducing sugar at 540 nm using Varian Cary 300 Scan UV-Visible Spectrophotometer (Varian Inc., Palo Alto, CA, USA). Glucose standards were prepared and analysed with the samples to obtain a standard curve. One unit of cellulase (FPU/mL) was defined as the amount of enzyme releasing 2.0 mg of glucose in 1 h. The enzymatic activity of commercial preparation Cellic® CTec2 was equal to 134.5 FPU/mL. The enzymatic hydrolysis of raw *Arundo donax* L. and the CRR recovered by the hydrolysis of *Arundo donax* L., previously separated from the embedded Amberlyst-70 by sieving, was conducted at pH = 4.8 and 50 °C in a 150 mL flask employing 50 mL of the 0.05 M citrate buffer solution and the enzyme Cellic® CTec2, shaking at 160 rpm. Different substrate loadings and dosages of enzymes were investigated in the present study, in agreement with the ranges reported in the literature [32,79,81]. Every 24 h, samples of 2 mL were withdrawn, cooled in ice in order to stop the enzymatic activities, centrifuged and analysed by HPLC for determining the glucose concentration. Both hydrolysis and analytical determinations were carried out in triplicate and the reproducibility of the reactions was within 5%.

The same protocol was also adopted for the enzymatic hydrolysis of the CRR recovered by the hydrolysis of *Arundo donax* L., which was previously washed with acetone (three times, weight ratio of 1:3 biomass/acetone).

3.7. Fourier Transformation Infrared Spectroscopy (FT-IR)

The Perkin–Elmer Spectrum Two spectrophotometer (Perkin–Elmer, Waltham, MA, USA), equipped with an Attenuated Total Reflectance (ATR) apparatus, was used to analyse the *Arundo donax* L. and the CRR. The acquisition of each spectrum was provided by 12 scans, with a resolution of 8 cm^{-1}, in the wavenumber range between 4000–450 cm^{-1}.

3.8. Definitions

The substrate loading adopted in all the runs was defined as follows:

$$\text{Substrate loading (wt\%)} = [m_{substrate}/(m_{substrate} + m_{water})] \times 100 \tag{2}$$

where $m_{substrate}$ is the weight (g) of the starting substrate and m_{water} is the weight (g) of water adopted as solvent of the reaction.

In all experiments, the masses (m_i) of the compounds in the hydrolysate were calculated using the equation:

$$m_i = C_i \times V \tag{3}$$

where C_i(g/L) is the concentration of the compound and V (L) is the volume of the hydrolysate.

The ponderal yield (wt%) of each product (xylose and glucose) respect to the dry weight (g) of the starting substrate ($m_{substrate}$) was calculated according to the following equation:

$$\text{Product yield (wt\%)} = (m_{product}/m_{substrate}) \times 100 \tag{4}$$

The molar yield of glucose and xylose respect to the moles of the respective units in the polysaccharides (glucan, xylan) in the starting substrate ($m_{substrate}$) was also determined, according to the following equations [94]:

$$\text{Glu. yield (mol\%)} = (m_{glucose} \times 0.90)/(m_{substrate} \times G_f) \times 100 \tag{5}$$

$$\text{Xyl. yield (mol\%)} = (m_{xylose} \times 0.88)/(m_{substrate} \times X_f) \times 100 \tag{6}$$

where the numbers 0.90 and 0.88 take into account the stoichiometry and the molecular weights in the hydrolysis of cellulose and hemicellulose, respectively to glucose and xylose; whereas G_f and X_f represent the percentage (wt%) of glucan and xylan in the composition of the starting substrate, respectively.

4. Conclusions

The proposed cascade biomass exploitation represents a novel promising strategy to maximize the conversion of *Arundo donax* L. structural carbohydrates into simple sugars, such as xylose and glucose, which are key intermediates in several biorefinery processes. From the sustainability and green chemistry perspective, in the first step, for the first time, the heterogeneous acid catalyst Amberlyst-70 was adopted for the preliminary hydrolysis of hemicellulose fraction in combination with MW irradiation and the high gravity approach. The optimised reaction conditions resulted 160 °C, 20 min, Amberlyst-70/*Arundo donax* L. weight ratio of 0.2 wt/wt with the initial biomass loading of 17 wt%, which ensured the highest sugars concentration of 46.1 g/L, the xylose yield of 96.3 mol%, the glucose yield of 10.2 mol% and very low concentrations of by-products, thus underlining the high selectivity of the process. In the second step, the exploitation of cellulose fraction in the recovered CRR was performed through the chemical or biological route. Regarding the chemical conversion, the present study proved the feasibility of the cellulose conversion in the presence of the embedded Amberlyst-70 obtaining, after only 20 min, glucose yields up to 32.5 mol%, corresponding to the concentration of 38.7 g/L. The obtained glucose yield was promising and the employment of the same catalyst for the two steps allowed the reduction of the process cost; however, better results in terms of cellulose exploitation were obtained adopting the enzymatic hydrolysis. In fact, the biological route confirmed the efficiency of the performed preliminary hydrolysis for the successive exploitation of the CRR through the enzymatic conversion carried out with high-solids loading and with low enzymatic dosage. In fact, the best process reaction conditions (Cellic$^{®}$ CTec2 25 FPU/g glucan, 96 h, biomass loading of 9 wt%) ensured the glucose yield of 56.2 mol%, corresponding to the concentration of 32.8 g/L, without the co-production of by-products. The proposed approach allowed us to avoid traditional pretreatments, which were replaced by the first reaction step that enabled the complete xylose recovery. In conclusion, the multi-step approach resulted promising for the valorisation of hemicellulose and cellulose fractions in raw *Arundo donax* L. to xylose and glucose, underlining that both routes, chemical and biological, were very valuable.

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