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Biorefinery of Energy Crop Cardoon (*Cynara cardunculus l.*) - Hydrolytic Xylose Production as Entry Point to Complex Fractionation Scheme

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Abstract

Response surface methodology (RSM) was employed for statistical modeling and optimization of low temperature dilute sulfuric acid hydrolysis of hemicellulose fraction of energy agro-crop cardoon (*Cynara cardunculus L.*), as an entry point to complex biorefinery scheme. The 2^3 central composite rotatable design (CCRD) was used to assess the effect of the principal independent process variables (reaction time, temperature and acid concentration) on efficiency and selectivity of heteroxylan conversion to xylose. The second-order polynomial model was fitted to experimental data to find optimal reaction conditions of xylan-to-xylose hydrolysis by multiple regression analysis. The effect of acid concentration (linear and quadratic) was found as a more significant (p=0.001-0.007) for monomeric xylose recovery in solution. The maximal xylose yield of ca. 86% (18.08 g /100 g biomass) was achieved after cardoon hydrolysis at 138.5ºC in 1.28% sulfuric acid solution for 52 min, vs. 87% predicted by model. The resulting xylose-enriched substrate revealed low concentration of toxic substances (1.04% furfural, 0.33% 5-hydroxymethylfurfural, 2.03% glucose), providing required quality for subsequent xylose (bio)conversion to final products (e.g. to xylitol). The enzymatic saccharification/digestibility of insoluble residue after hemicellulose removal was improved in four times, resulting in cellulose conversion to fermentable glucose by 76% vs. 19% for unhydrolyzed cardoon.

Keywords: Xylan; Xylose; Hydrolysis; Statistical modeling; Optimization; Cardoon

Introduction

The biorefinery concept of biomass treatment is an area of much current scientific interest and research activity. The multi-step processing (fractionation) of different biomass feedstocks into a wide range of value-added products through combination of physical, chemical and biological approaches is viewed as a more potential way to guarantee the sustainability of the future bio-based economy [1,2]. Of all existing the large-scale bioconversion schemes, the particular attention has been given to biorefinery of lignocellulosic biomass (LCF, or Lignocellulosic Feedstock Biorefinery), as a more diverse and valuable source of chemicals [3,4]. Lignocellulosics (wood, straw, reeds, grasses, agro-crop and forest residues, paper and cellulosic municipal wastes, etc.) basically consist of three major chemical constituents: cellulose, hemicelluloses and lignin. Assuming that carbohydrate fraction account for up to 80% (and more) of lignocellulosic raw materials [1], the efficient access and conversion of carbohydrates to chemical bulk products (industrial intermediates) and the corresponding final products is a key factor for successful commercialization of LCF biorefinery [5]. The so-called "sugar platform" of biomass fractionation was developed based on biochemical conversion processes with focus on the fermentation of sugars extracted from biomass feedstocks to produce fuel, such as ethanol, or other building block chemicals [6].

The agro-based lignocellulosics, such as annual crops and perennial herbaceous species (grasses), represent abundant and low-cost feedstocks for LCF biorefinery. Some perennial rhizomatous herbs, i.e., switchgrass, alfalfa, miscanthus, canarygrass and giant reed, were indicated as the more potential energy crops for bioenergy production in both the US and Europe [7]. *Cynara cardunculus L.* (cardoon or artichoke thistle) is an abundant naturally growing perennial herb native to the Mediterranean region. For biomass production, cardoon can be cultivated as a perennial field crop in dry farming with annual harvest of the whole aboveground biomass (yielding up to 30 t of dry matter per ha [8] for several years [9]. As a raw material for industrial applications, the use of cardoon biomass as a solid fuel or for production of seed oil, biodiesel, paper pulp, green forage and pharmacologically active compounds was reviewed [10]. The high biomass productivity and appropriate chemical composition makes cardoon as a very attractive lignocellulosic feedstock for LCF biorefinery schemes. Hemicelluloses are the second largest (after cellulose) constituent of cardoon biomass accounting for about 25-30% of the stalk-wall material [9]. Similar to other agro-crop species, the hemicellulosic fraction of cardoon consists almost entirely of xylan heteropolysaccharide [9], representing a valuable source of xylose for subsequent (bio) chemical conversion to final products, e.g., xylitol. The careful xylan isolation and depolymerization to monomeric sugars can therefore be an important first step (entry point) in the complex scheme of cardoon fractionation (biorefinery) to individual chemical components. The most widely used and tested approach of xylan conversion to monosaccharides is based on hydrolysis reaction [11,12]. Particularly, the dilute sulfuric acid hydrolysis under moderate temperatures was proved to be a reliable and easily performed low cost method for quantitative conversion of xylan to xylose and reactivity (accessibility) improvement of cellulose and lignin in solid residue [13-17]. The yield of xylose after hydrolysis is strongly dependent on the type of raw material used and operation conditions applied. Under controlled (optimized) hydrolysis conditions the minimal cellulose

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degradation and by-products (basically furans and acetic acid) formation takes place, providing the high efficiency and selectivity of the overall process.

Only limited published data related to acid hydrolysis of cardoon are available [18-20], basically reporting biomass (pre)treatment under drastic reaction conditions for the purpose to improve the enzymatic accessibility/reactivity of cellulose for ethanol production. The high temperatures used at these studies (up to 200-230°C) led to incomplete xylan conversion to monomeric sugars (xylose) during auto-hydrolysis process (when no mineral acid was used as catalyst), or caused substantial monosaccharide (basically xylose) decomposition (up to 4.2% of furfural) as well as significant cellulose degradation (by 10-18%) in the presence of 0.1-0.2% of sulfuric acid as catalyst, thereby providing unacceptable quality of sugar hydrolyzate for xylose utilization.

The low temperature (below 150°C) dilute sulfuric acid hydrolysis of energy crop cardoon (*Cynara cardunculus* L.) has been examined to produce a high quality xylose-rich substrate ready for (bio)chemical conversion to final products and reactive hemicellulose-free solid residue ready for subsequent fractionation within complex biorefinery scheme. The response surface methodology (RSM) was used for hydrolysis modeling and optimization with the aim to maximize efficiency and selectivity of xylan polysaccharide conversion to monomeric units within one-step reaction. The principal results of this study are reported in the present paper.

Materials and Methods

Materials

The cardoon (*Cynara cardunculus L.*) used in this study was sampled from the university experimental plantation field (Institute of Agronomy, Technical University of Lisbon). The air dried stalks were manually stripped of leaves, milled and screened to particle size of 40-60 mesh and stored in sealed plastic bags at room temperature until using for chemical analysis and hydrolysis experiments. The moisture content of prepared material was determined according to TAPPI standards.

All chemicals used were of analytical grade purity and purchased from Sigma, Ardrich and Fluka Chemical Co.

Acid hydrolysis: For optimization study, 5 g (on oven-dry basis) of cardoon sawdust were hydrolyzed with 75 ml of diluted (0.5-1.5%) sulfuric acid in the stainless steel autoclaves (ca. 100 cm^3 capacity) rotated in an oil bath at 130-150°C for 30-60 min, with replication of each experimental conditions set. After heating-up period (ca. 2min), the loaded autoclaves were held at desired temperature for pre-determined residence time. Reaction was terminated by immediate autoclave plunging into an iced-water bath. The solid residue was separated by filtration, thoroughly washed by deionized water and kept frozen for subsequent analysis and treatment. The hemicellulosic hydrolyzate, combined with washing water, was collected in the volumetric flask and analyzed on degree of monosaccharide recovery and degradation after hydrolysis.

Enzymatic hydrolysis: The enzymatic digestibility of insoluble lignocellulosic residue after acidic hydrolysis was checked by NREL standard procedure [21]. Residue (0.15 g) was mixed with commercial preparations of cellulases (60 FPU/g cellulose) and β-glucosidases (64 *p*NPGU/g cellulose) in 50mM sodium citrate buffer (pH 4.8) and incubated at 50°C for 24-72 hours under 150 rpm rotation. Sodium azide (2% solution) was added as antibiotic to prevent any microbial infection during digesting. The release of soluble sugars (basically glucose) was determined by HPLC and corrected by blank tests on substrate and enzymes. The enzymatic digestibility (saccharification) was defined as a ratio of cellulose digested (g) to cellulose added (g).

Analytical methods

Extractives were determined gravimetrically after successive Soxhlet extraction by dichloromethane, ethanol and water. Ash, silica (as SiO_2), acid-insoluble (Klason) and acid-soluble lignin were determined according to TAPPI standards. Holocellulose was isolated by modified chlorite method in the presence of sodium acetate buffer [22]. Hemicelluloses were successively extracted from holocellulose by 5% and 24% potassium hydroxide solutions and recovered by ethanol addition, giving, respectively, fractions A and B. The insoluble residue after alkaline extraction of holocellulose was accepted as α-cellulose [22].

The monosaccharide composition of holocellulose and hemicelluloses was determined by GC as alditol-acetate derivatives using the following conditions: FID, SP-2330 column, injector temperature 240°C; detector temperature 250°C; initial column temperature 230°C; final column temperature 240°C; 2°C min^{-1} rate; 2-deoxy-D-glucose as an internal standard.

The total content of reducing sugars and furans in hemicellulosic hydrolysates after the preliminary tests was determined by DNS method and UV-spectroscopy, respectively [23,24].

The concentration of xylose, glucose, acetic acid, furfural and hydroxymethylfurfural (HMF) in hydrolysates was analyzed by HPLC using Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) operating at 50°C, in combination with a cation H^+-guard column (Bio-Rad, Hercules, CA, USA) in a Waters (Waters, Milford, USA) HPLC system. A RI detector (Waters 2410) was used for monosaccharides and aliphatic acids quantification whereas a UV-Vis detector (Waters 486) set at 280 nm was used for furfural and HMF measurement. The mobile phase was 5mM sulfuric acid and the flow rate 0.4 ml min^{-1}.

Xylan conversion after hydrolysis (Y_1) was defined as a ratio of xylose content in hydrolysate to xylose content in cardoon. Hydrolysis selectivity (Y_2) was defined as a ratio of xylose to glucose in hydrolysate.

Experimental design and statistical treatment: Response Surface Methodology (RSM) was employed for statistical data treatment and optimization of hydrolysis conditions by multiple regression analysis using Statistica 6.0 (Statsoft, USA) software. The 2^3 central composite rotatable design (CCRD) with three independent variables at five different levels, six star (axial) points and five central points (total 19 runs) was adopted to find linear, quadratic and interaction effects of independent process variables on experimental responses. A second-order polynomial model was fitted to each set of experimental data to predict optimal reaction conditions by following equation:

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i<j,j=2}^{3} b_{ij} X_i X_j \quad (1)$$

where Y is a predicted response (xylan conversion or hydrolysis selectivity), b_0 is an interception coefficient (regression coefficient at central point), b_i are the linear coefficients; b_{ii} are the quadratic

coefficients, b_{ij} are the interaction coefficients, X_i and X_j are the independent variables (temperature, time and acid concentration).

The statistical significance of regression coefficients and effects was checked by analysis of variance (ANOVA).

Results and Discussion

Compositional analysis of raw material

Since biomass composition may be variable due to season, growing conditions etc., it is extremely important to be able to relate process performance to actual experimental material. The chemical composition of cardoon whole stalk (with pith) material used in this study is presented in Tables 1 and 2. Similar to other commercially important agro-crops, cardoon has less lignin but more extractives, as compared with wood species [25], what indicates the higher degree of accessibility and therefore reactivity of carbohydrate complex to chemical processing, e.g., in acid hydrolysis, under relatively mild reaction conditions. The content of cellulose (ca. 39%) and hemicelluloses (ca. 30%) in cardoon is close to that of hardwoods (38-50% and 20-28%, respectively) [25], underlining the value of this plant as an important agro-source of sugars for subsequent (bio)-chemical conversion. Generally, the found proportion between principal chemical constituents (i.e., lignin, cellulose, hemicelluloses and extractive) does not differ greatly from the Spanish cardoon reported in [19,20], displaying however some obvious deviations connected first of all with the reduced content of extractives and the elevated portion of carbohydrate polymers.

The hemicellulose fraction of cardoon stalk consists mainly (by ca. 90%) of xylan polysaccharide (Table 2), what comprises about 27% of total crop carbohydrates and gives the value of maximal (theoretical) xylose yield of 21.49% (on oven-dry material) after complete hydrolytic depolymerization of xylan.

Preliminary hydrolysis experiments

To define the current levels (settings) of the independent process variables to be used in statistical experimental design on xylose recovery, a series of experiments on cardoon hydrolysis was performed under variable conditions of reaction temperature (130, 140 and 150°C), reaction time (30 and 60 min) and sulfuric acid concentration (0.5, 1.0 and 1.5%) and the yield of the principal monosaccharides (glucose and xylose) and decomposition side-products (acetic acid, furfural and 5-hydroxymethylfurfural) was determined in hydrolyzate.

As can be seen from Table 3, even moderate reaction conditions applied for xylan hydrolysis inevitably cause the partial hydrolysis of cellulose (as a more accessible amorphous cellulose portion) and decomposition of formed monosaccharides to furans, decreasing thereby selectivity and efficiency of xylan conversion as a whole. Generally, increase in reaction temperature and acid concentration, while accelerating xylan hydrolysis to xylose, intensifies substantially the secondary degradation reactions of monomeric sugars. The process temperature of 130°C provides the most preserving reaction conditions, revealing only traces of furfural and limited cellulose degradation. But, the xylose recovery in solution is low pointing to incomplete xylan conversion. In contrast, the harsh conditions of 150°C cause substantial monosaccharide degradation (up to 4% of furfural and 6% of acetic acid) and cellulose hydrolysis (up to 3.6% of glucose); what also lowers the yield of xylose, particularly under increasing acid concentration.

The most balanced interrelation between hydrolysis efficiency and selectivity can be achieved under reaction temperature about 140°C. Within the tested range of experimental conditions, the maximal xylose recovery of ca. 81% (or 17.48% of oven-dry material) was observed after cardoon hydrolysis at 140°C for 30 min in 0.5% acid solution. The low content of furfural (0.46%) and glucose (1.72%) indicated only limited monosaccharide decomposition and cellulose hydrolytic depolymerization under these reaction conditions.

Hydrolysis modeling and optimization

The response surface methodology (RSM) was used for statistical modeling and optimization of cardoon hydrolysis reaction [26]. The 2^3 central composite rotatable design (CCRD) was employed to optimize the effect of the principal independent variables, i.e., reaction time (X_1), temperature (X_2) and acid concentration (X_3), on efficiency (Y_1) and selectivity (Y_2) of xylan conversion to xylose.

Based on results of the preliminary tests, the current settings (range and levels) of independent process variables to be used in RSM were defined (Table 4) and the RSM experimental design matrix for three coded independent variables at five levels each, with six star (axial) points and five replicates at the central point (total 19 runs) was developed according to CCRD (Table 5). The obtained experimental data were used to calculate the significant effects having the greatest impact on reaction outputs. Figure 1 illustrates the Pareto charts of significant effects estimated, respectively, for xylan conversion and hydrolysis selectivity. The graphs show the standardized linear, quadratic and interaction effects of process factors (independent variables), sorted by their absolute magnitude in relation to the statistical significance (p) level of 0.05. Apparently, the linear and quadratic effects of acid concentration, along with quadratic effect of reaction temperature and interaction effect between temperature and concentration, are the most important in determining the resultant degree of xylan conversion to xylose. The hydrolysis selectivity, in its turn, is controlled almost solely by linear effect of reaction temperature.

Component	Content (% on oven-dry matter)
Ash	5.44 ± 0.02
silica SiO_2	0.08 ± 0.01
Extractives	7.36 ± 0.14
dichloromethane	0.41 ± 0.01
ethanol	3.25 ± 0.19
water	3.70 ± 0.21
Lignin	18.19 ± 0.05
Klason (acid-insoluble)	15.46 ± 0.03
acid-soluble	2.73 ± 0.07
Holocellulose	69.87 ± 0.03
α-cellulose	39.28 ± 0.05
hemicelluloses	30.59 ± 0.08
fraction A	25.30 ± 0.06
fraction B	5.29 ± 0.09

Table 1: Chemical composition of cardoon stem-wall material.

	Holocellulose	Hemicellulose	
		Fraction A	Fraction B
Rhamnose	0.96	2.01	1.28
Arabinose	1.48	2.05	2.34
Xylose	27.07	91.98	77.43
Mannose	1.42	0.70	3.22
Galactose	1.74	2.56	4.28
Glucose	67.33	0.70	11.45

Table 2: Carbohydrate composition of holocellulose fraction of cardoon stem-wall material (%).

Temperature (°C)	Time (min)	Acid Concentration (%)	Yield (% on oven-dry matter)				
			Glc[1]	Xyl	AcA	F	HMF
130	30	0.5	0.35	3.72	Traces	Traces	Traces
		1.0	0.63	9.46	2.10	Traces	Traces
		1.5	0.98	12.89	2.60	Traces	0.23
	60	0.5	0.47	8.17	1.23	Traces	0.20
		1.0	0.93	13.35	2.64	0.09	0.27
		1.5	1.38	15.61	3.72	0.33	0.29
140	30	0.5	0.57	9.43	1.24	Traces	0.27
		1.0	1.33	15.78	2.95	0.16	0.28
		1.5	1.72	17.48	3.82	0.46	0.29
	60	0.5	1.06	13.91	2.71	0.20	0.28
		1.0	2.01	15.81	3.05	0.95	0.30
		1.5	2.59	16.12	3.93	1.78	0.33
150	30	0.5	1.20	12.36	2.15	0.20	0.28
		1.0	2.20	16.43	3.67	0.95	0.31
		1.5	2.88	15.97	4.35	2.00	0.36
	60	0.5	2.21	17.09	3.83	1.01	0.31
		1.0	2.82	12.49	4.84	3.38	0.44
		1.5	3.57	10.95	6.30	4.15	0.47

[1]Glc, Xyl, AcA, F,HMF as glucose, xylose, acetic acid, furfural and 5-hydroxymethylfurfural, respectively

Table 3: Experimental data on monosaccharide formation and decomposition under variable conditions of dilute acid hydrolysis of cardoon stalks.

Variable		Range and levels				
		-α	-1	0	+1	+α
Temperature (°C)	X_1	123.2	130	140	150	156.8
Time (min)	X_2	19.8	30	45	60	70.2
Acid concentration (%)	X_3	0.2	0.5	1.0	1.5	1.8

Table 4: Range and levels of independent process variables used in experimental design.

Run Nº	Coded variables				Responses	
	X_1	X_2	X_3		Y_1	Y_2
1	-1	-1	-1		0.18	10.55
2	-1	-1	+1		0.61	13.18
3	-1	+1	-1		0.39	17.28
4	-1	+1	+1		0.74	11.30
5	+1	-1	-1		0.58	10.27
6	+1	-1	+1		0.76	5.55
7	+1	+1	-1		0.82	7.88
8	+1	+1	+1		0.52	3.07
9	-α	0	0		0.53	19.29
10	+α	0	0		0.51	3.28
11	0	-α	0		0.58	16.32
12	0	+α	0		0.86	7.73
13	0	0	-α		0.08	3.43
14	0	0	+α		0.87	6.85
15 (C)	0	0	0		0.81	9.72
16 (C)	0	0	0		0.81	9.42
17 (C)	0	0	0		0.80	9.86
18 (C)	0	0	0		0.81	9.91
19 (C)	0	0	0		0.83	9.37

Table 5: Central composite rotatable design (CCRD) applied for cardoon hydrolysis and the corresponding experimental responses on xylan conversion (Y_1) and process selectivity (Y_2) used for RSM modeling.

The significance of each estimated effect was checked by analysis of variance (ANOVA). The low p values ($p<0.01$) of the main effects influencing the xylan conversion and hydrolysis selectivity, along with results of the Student's (t) and Fisher's (F) tests (Table 6), indicate the high statistical significance of the estimated relation between variables within a 99% confidence interval.

The second-order polynomial model (Eq.1) was fitted to experimental data to determine the optimum levels (conditions) of the independent process variables by multiple regression analysis. The regression coefficients for process modeling were calculated and the statistical significance of each coefficient was estimated by p-values. Two model equations (Eqs. 2,3) describing, respectively, xylan conversion (Y_1) and hydrolysis selectivity (Y_2) were obtained using more statistically significant regression coefficients:

$$Y_1 = - 26.2065 + 0.3218X_1 - 0.001X_1^2 + 0.0644X_2 + 4.8553X_3 - 0.4642X_3^2 - 0.023X_1X_3 - 0.0093X_2X_3 \quad (2)$$

$$Y_2 = 120.6866 - 1.7215X_1 + 0.8381X_2 + 0.0041X_2^2 - 6.0414X_3^2 - 0.0081X_1X_2 - 0.1452X_2X_3 \quad (3)$$

The values of the determination coefficient R^2=0.91 and R^2=0.89, found respectively for Eqs. 2 and 3, pointed to good correlation

between experimental and predicted data (model fit) and indicated the high statistical significance of the models as a whole, which can explain 89-91% of the total variability in responses within examined range of reaction conditions.

The 3D response surfaces and the corresponding contour plots illustrating the modeled effects of independent variables on reaction outputs are shown in (Figures 2-4). As can be seen, the hill-shaped surfaces of xylan conversion during hydrolysis (upper plots of Figures 2-4) reveal some maximum values (stationary points) near the center point of the experimental design, thereby allowing locating and characterizing the optimum responses under variable reaction conditions. Evidently, keeping constant acid concentration at 1% as a center point, the temperature range of 135-145°C and reaction period of 40-60 min are desirable to maximize the yield of xylose (Figure 2). In its turn, at constant temperature of 140°C as a center point, the highest efficiency of xylan hydrolysis can be achieved within the range of acid concentration 1-1.5% and reaction period 40-60 min (Figure 3). The marked desirable ranges of reaction temperature and acid concentration are particularly notable in Figure 4, where the reaction time was kept constant at 45 min as a center point.

The effect of process inputs on hydrolysis selectivity is more complex and depicted by saddle-shaped response surfaces with maximum and minimum values encountered at various combinations of independent variables (bottom plots of (Figures 2-4). As mentioned above, the reaction temperature has a vital importance for hydrolysis selectivity. Under fixed acid concentration of 1% as a center point, the

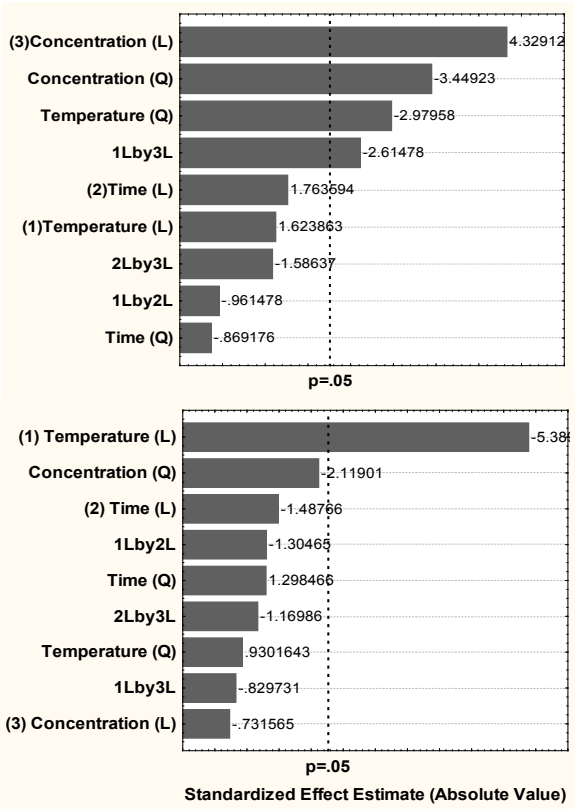

Figure 1: Pareto charts of standardized effects for xylan conversion to xylose (top) and hydrolysis selectivity (bottom).

Factor	Y_1					Y_2			
	Estimated effect	*t*-test	*F*-test	*p*		Estimated effect	*t*-test	*F*-test	*p*
(1) Temperature (L)	0.1092	1.6239	2.6369	0.1389		-7.6815	-5.3898	29.0504	0.0004
Temperature (Q)	-0.2005	-2.9796	8.8779	0.0155		1.3260	0.9302	0.8652	0.3766
(2) Time (L)	0.1186	1.7636	3.1103	0.1116		-2.1202	-1.4877	2.2131	0.1710
Time (Q)	-0.0585	-0.8692	0.7555	0.4073		1.8510	1.2985	1.6860	0.2264
(3) Concentration (L)	0.2912	4.3291	18.7413	0.0019		-1.0426	-0.7316	0.5352	0.4830
Concentration (Q)	-0.2321	-3.4492	11.8972	0.0073		-3.0207	-2.1190	4.4902	0.0631
1L by 2L	-0.0845	-0.9615	0.9244	0.3614		-2.4294	-1.3047	1.7021	0.2244
1L by 3L	-0.2298	-2.6148	6.8371	0.0281		-1.5450	-0.8297	0.6885	0.4282
2L by 3L	-0.1394	-1.5864	2.5165	0.1471		-2.1784	-1.1699	1.3686	0.2721

Table 6: ANOVA of estimated linear (L), quadratic (Q) and interaction effects for xylan conversion (Y_1) and process selectivity (Y_2).

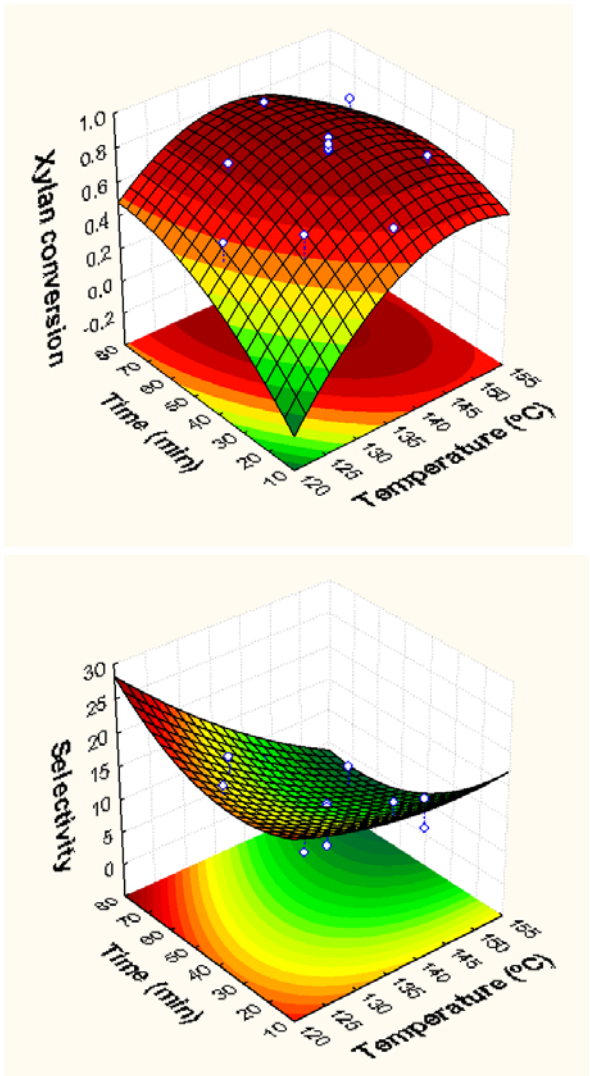

Figure 2: Response surfaces and contour plots of modeled xylan conversion to xylose (top) and hydrolysis selectivity (bottom) as a function of reaction time (*t*, min) and reaction temperature (*T*,°C) at fixed acid concentration of 1% set as a central point.

maximum estimated selectivity (ca. 20) of acid hydrolysis of cardoon hemicelluloses, i.e., the minimal hydrolytic cellulose depolymerization, can be obtained under temperatures below 120°C (Figure 2), what is however far removed from the desirable temperature range needed for maximal xylan conversion to xylose. The effect of acid concentration on selectivity is shown in Figure 3. It can be seen that at constant temperature of 140°C as a center point, the highest selectivity can be provided only by short (less than 20 min) reaction under high acid concentrations or by prolonged (over 70 min) reaction under low acid concentration, what also falls outside the desirable range of reaction conditions required for maximal xylan conversion. Hence, in support of experimental data of the preliminary tests, the statistical modeling showed the practical unfeasibility to achieve maximum xylan conversion to xylose without partial degradation of cellulose, i.e., without some loss in hydrolysis selectivity. This is obviously connected with close reactivity/accessibility of hemicelluloses and the less-ordered (amorphous) cellulose portion in the cell-wall material of plants [27]. In fact, there is a good agreement between amount of glucose released into solution during cardoon hydrolysis and the content of alkali-extractable (amorphous) cellulose portion quantified during hemicellulose analysis of cardoon stalks (Fraction B, Table 2). Based on Fig. 4, the estimated selectivity level of only ca. 6-10 (1.5-3% glucose in solution) can be expected when hydrolysis reaction is performed under conditions designed to maximize the xylose yield in solution. It is significantly better of data reported for high temperature (180-200°C) dilute sulfuric acid (0.1-0.2%) hydrolysis of cardoon [19,20], where release of 10-20% glucose was observed lowering substantially hydrolysis selectivity. The higher cellulose degradation (4.4-11.2% glucose released) was also reported for dilute acid hydrolysis of other herbaceous species [28,29].

The partial differentiation of the multivariate function described by Eq. 2 was used to find an optimal combination of the independent process variables in the stationary point of the fitted polynomial model corresponding to maximum xylan conversion to xylose during hydrolysis. The partial derivatives of Y_1 with respect to X_1, X_2 and X_3 gave the following equations:

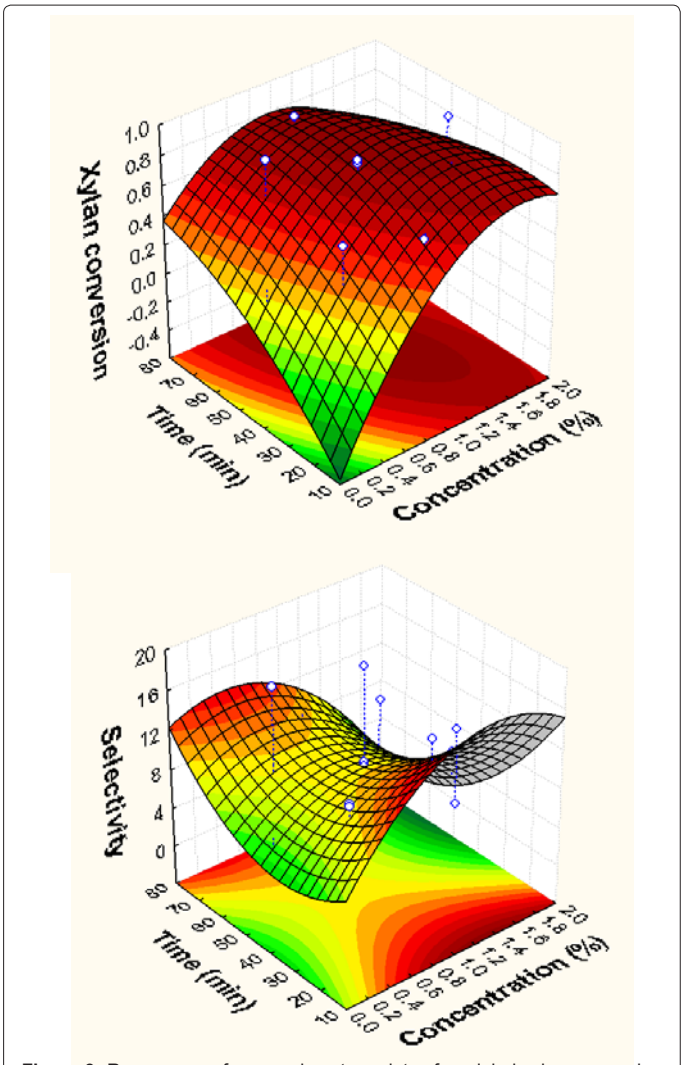

Figure 3: Response surfaces and contour plots of modeled xylan conversion to xylose (top) and hydrolysis selectivity (bottom) as a function of reaction time (*t*, min) and acid concentration (*C*, %) at fixed reaction temperature of 140°C set as a central point.

$$\partial Y_1/\partial X_1 = 0.3218 - 0.002X_1 - 0.023X_3 = 0 \quad (4)$$

$$\partial Y_1/\partial X_2 = 0.0644 - 0.0093X_3 = 0 \quad (5)$$

$$\partial Y_1/\partial X_3 = 4.8553 - 0.9284X_3 - 0.023X_1 - 0.0093X_2 = 0 \quad (6)$$

Resolution of Eqs 4-6 revealed the optimum condition set required for maximal predicted response of xylan conversion to xylose: reaction time of 51.7 min, reaction temperature of 138.5°C and sulfuric acid concentration of 1.28% with a maximum expected xylan conversion of 0.87 (87% of xylose recovery in solution).

The validity of the developed statistical model was checked by replicated (four times) control experiments performed under found optimal conditions. The obtained experimental values on xylan conversion (0.86 ± 0.01) and hydrolysis selectivity (7.85 ± 0.11; with 2.03% of glucose) showed very close agreement with calculated (predicted) data thereby confirming the high model validity and suitability as a whole. As it shown on the mass balance flow diagram (Figure 5), only limited monosaccharide degradation (furfural 1.04 g, 5-hydroxymethylfurfural 0.33 g and acetic acid 3.61 g per 100 g of dry biomass) was observed under optimized reaction conditions providing the desired quality of sugar substrate (hydrolyzate) for subsequent (bio)chemical processing. The similar results on xylose recovery after dilute sulfuric acid hydrolysis were also reported for other perennial herbs, such as silvergrass (xylose yield of ca. 75%) [29], switchgrass, reed canarygrass and alfalfa (xylose yield of 73-86%) [28], correlating well with the data of the present study.

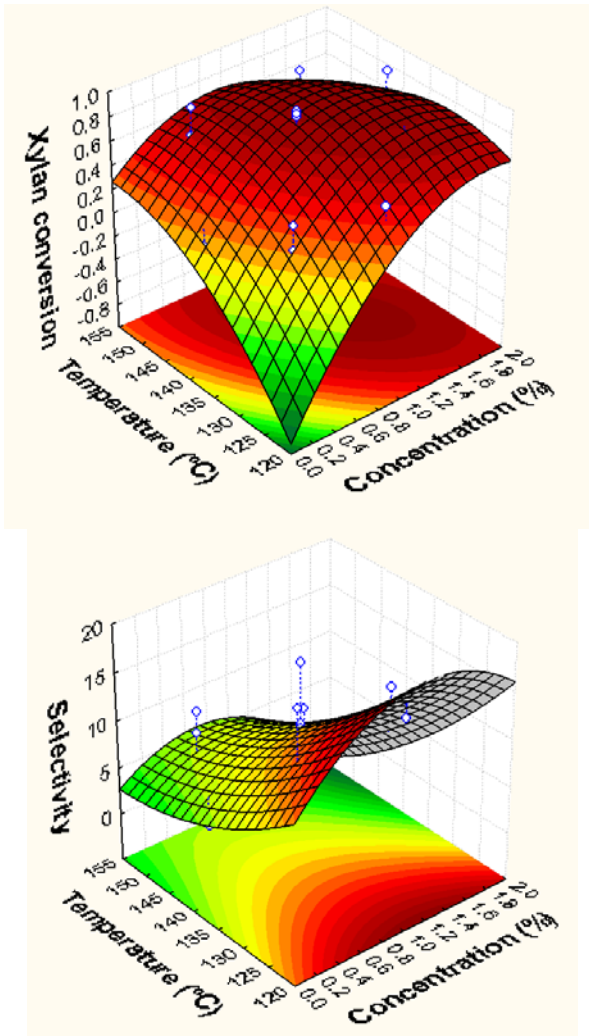

Figure 4: Response surfaces and contour plots of modeled xylan conversion to xylose (top) and hydrolysis selectivity (bottom) as a function of reaction temperature (*T*,°C) and acid concentration (*C*, %) at fixed reaction time of 45 min set as a central point.

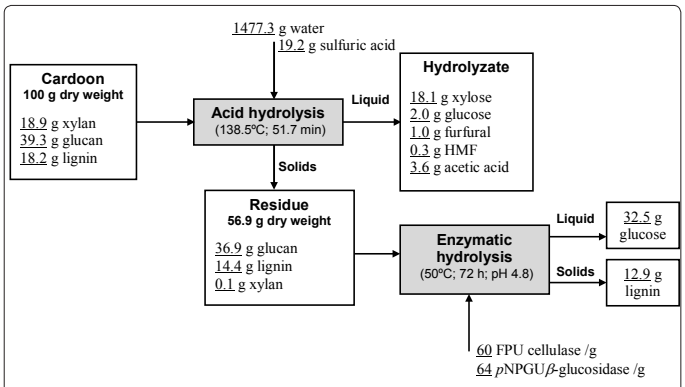

Figure 5: Mass balance flow diagram for acid hydrolysis of cardoon (performed under optimized conditions designed for total xylan conversion to monomeric xylose) and enzymatic digestibility of unhydrolyzed solid residue.

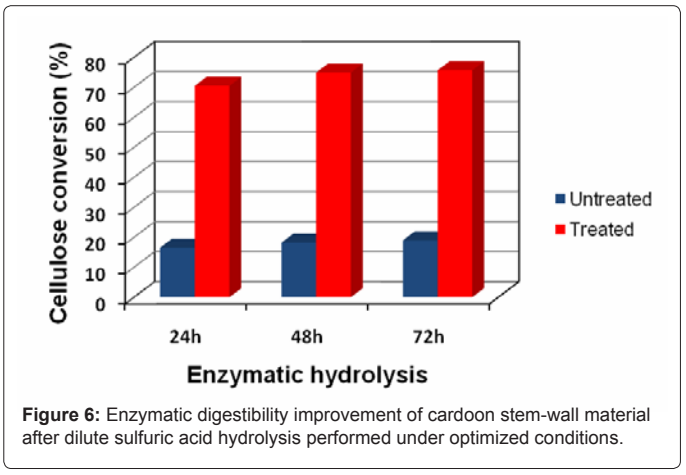

Figure 6: Enzymatic digestibility improvement of cardoon stem-wall material after dilute sulfuric acid hydrolysis performed under optimized conditions.

Enzymatic digestibility/saccharification of insoluble residue

Since hemicellulose removal is presumed to destroy the lignin-carbohydrate matrix shielding cellulose microfibrils in the cell wall material [5,11], the dilute acid hydrolysis of cardoon should therefore substantially improve enzymatic digestibility (saccharification) of cellulose portion in insoluble (unhydrolyzed) residue, providing a valuable source of fermentable sugars (glucose) for biofuel (ethanol) production.

As can be seen from Figure 6, the enzymatic digestibility of cellulose in pre-hydrolyzed biomass was substantially improved over the control (unhydrolyzed cardoon). After 72 h enzymatic hydrolysis performed under standard NREL conditions [21], the cellulose conversion to monomeric glucose of ca. 76% (32.5 g Glc / 100 g dry biomass, Figure 5) was achieved for pre-hydrolyzed biomass, in contrast to ca. 19% conversion (8.2 g Glc / 100 g dry biomass) for unhydrolyzed biomass.

Evidently, the degree of cellulose digestibility can be further improved by optimization study of enzymatic saccharification.

Conclusion

The low temperature one-step dilute sulfuric acid hydrolysis was very effective to convert the cardoon (*Cynara cardunculus* L.) heteroxylan to monomeric sugars providing a quality xylose-rich substrate for subsequent (bio)chemical processing and reactive hemicellulose-free solid residue ready for further fractionation within complex biorefinery scheme. The statistical modeling using Response Surface Methodology made it possible to identify the main factors of the multi-variable hydrolysis process affecting efficiency and selectivity of xylan conversion to xylose and to define the optimum set of reaction conditions for maximal xylose recovery in solution. Under optimum reaction conditions (138.5ºC, 51.7 min and 1.28% acid concentration) the xylan conversion of 0.86 (86% of xylose recovery or 18.08 g /100 g cardoon) was achieved vs. 0.87 predicted by model, with limited cellulose degradation and furans formation (glucose 2.03 g and furfural 1.04 g per 100 g of cardoon). The insoluble residue after xylan hydrolysis can be easily digested to fermentable sugars for ethanol production, providing cellulose-to-glucose conversion of 76% vs. 19% for untreated cardoon.

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