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Effect of Acid Steam Explosion on Enzymatic Hydrolysis of *O. nervosum* and *C. cardunculus*

Scientific Note

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INTRODUCTION

During the last several years, increasing attention has been focused on the transformation of lignocellulosic substrates obtained as residues of agricultural and forestry activities, or from plantations specially cultivated for this purpose, into high added value chemicals or alternative fuels. In order to identify new biomass sources for transformation purposes, several studies have been undertaken in Spain with lignocellulosic and amylaceous biomasses (*1,2*) from different herbaceous plants. Results obtained to date have led to consideration of some species, such as *Onopordum nervosum* and *Cynara cardunculus,* as promising lignocellulosic substrates to be converted into ethanol owing to their composition, growth requirements, and high productivity.

The two species mentioned above (Figs. 1 and 2) have been previously studied as substrates for acid and enzymatic hydrolysis (*3–5*). In the last case, different pretreatments have been tested (*4,6,7*) that show the effectiveness of steam explosion to increase the yield of the subsequent enzymatic hydrolysis of these substrates. In this work, we report some preliminary results obtained in the hydrolysis with enzymes of lignocellulosic

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Fig. 1. *O. nervosum.*

biomass from *O. nervosum* and *C. cardunculus* when they are pretreated by steam explosion in acid medium and different reaction conditions.

MATERIALS AND METHODS

Substrates and Pretreatments

Biomass from *O. nervosum* Boiss. and *C. cardunculus* L. was employed as lignocellulosic substrate. Dried biomass was ground to 4 mesh size and used as such, or pretreated by steam explosion. The pretreatment was carried out in a pilot unit equipped with a reaction vessel of 2 L working volume and designed to reach a maximum operating pressure of 42 kg/cm^2. Dry, wet (5 L of water/kg substrate) or acid impregnated substrates were loaded into the explosion vessel. In the last case, biomass was soaked at a solid/liquid ratio of 1:4 in 0.4 or 1% sulfuric acid solutions and maintained for 24 h at 25°C. Treatment was performed at temperatures ranging from 170 to 230°C and reaction times of 1,2,4, and 8 min. Compositions of substrates and residues in potential glucose and lignin have been determined by total hydrolysis with H_2SO_4 (*8*).

Fig. 2. *C. cardunculus.*

Enzymatic Hydrolysis Tests

The cellulolytic complex employed in this study was obtained from a *Trichoderma reesei* QM9414 culture, as previously described (*5*). Enzyme was dissolved in 0.1*M* acetate buffer, pH 4.8, and used in hydrolysis experiments at a final filter paper activity of 20 IU/g substrate. Substrate concentration in the reaction medium was 5% (w/v). Total reducing sugars and glucose liberated after 48 h hydrolysis at 50°C were quantified by the Nelson-Somogyi method (*9*) and a comercial enzymatic assay (Gluco-quant, Boehringer-Mannheim S.A., Barcelona, Spain, Ref.263826), respectively.

RESULTS

The steam explosion unit employed in this study is shown in Fig. 3. The scheme of the flow diagram of the system is represented in Fig. 4. The content of cellulose, hemicellulose, lignin, and ash of biomass from the two species employed as raw materials is summarized in Table 1.

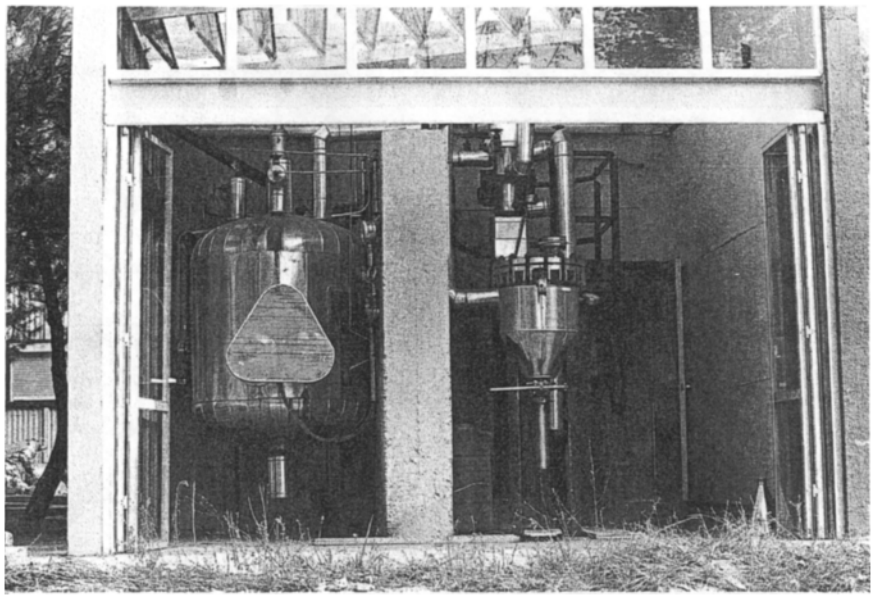

Fig. 3. Pilot plant for steam explosion of lignocellulosic biomass.

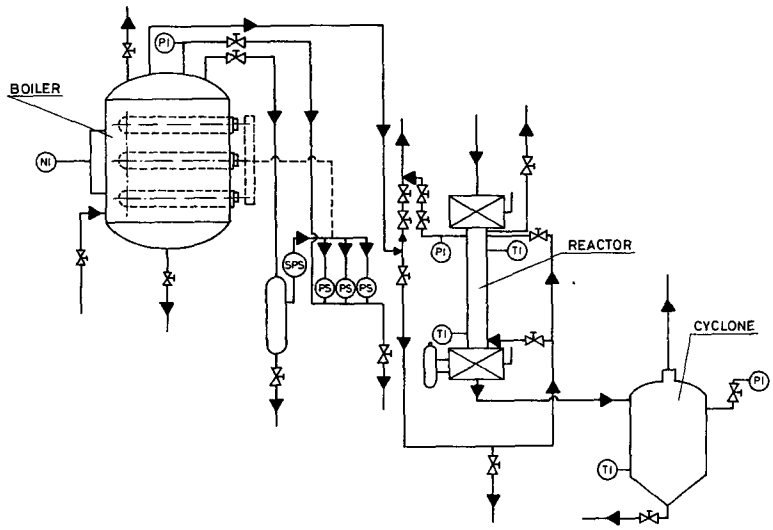

Fig. 4. Flow diagram of the steam explosion system.

Table 1
Composition[a] and Enzymatic Hydrolysis Yield (SE)
of the Untreated Biomass from *O. nervosum* and *C. cardunculus*

	O. nervosum	*C. cardunculus*
Cellulose	30	36
Hemicellulose	18	21
Lignin	21	16
Ash	15	9
SE, %	23	20

[a]Percentages on dry weight.

Table 2
Effect of Steam Explosion Conditions on Solid Recovery and Enzymatic Hydrolysis Yield of Dry Biomass from *O. nervosum* and *C. cardunculus*[a]

Temperature	Residence time	*O. nervosum*			*C. cardunculus*		
		Gy	SE	CC	Gy	SE	CC
210°C	1 min	63	77	63	72	84	84
	2 min	61	72	59	71	97	88
	4 min	62	91	70	63	97	77
	8 min	58	84	77	57	84	65
230°C	1 min	58	97	70	60	88	77
	2 min	60	97	68	50	57	43
	4 min	57	89	69	57	75	64
	8 min	56	81	63	53	59	45

[a]Gy, solid residue recovered (%); SE, saccharification efficiency; and CC, cellulose conversion.

The effect of different pretreatment conditions on the enzymatic hydrolysis yield of substrates has been studied by comparing the SE (Saccharification efficiency) and the CC (Cellulose conversion) values, obtained and calculated as follows

$$SE = Gp/Gr$$
$$CC = Gp \cdot Gy/Gi$$

where *Gp* is the glucose released in the 48 h enzymatic hydrolysis of a substrate, *Gr* is the theoretically maximum glucose yield in that substrate, *Gy* is the percentage of solid residue recovered after pretreatment, and *Gi* is the theoretically maximum glucose yield in the initial untreated substrate.

Percentages of solids recovered and cellulose conversion values are given in Table 2 for dry biomass from *O. nervosum* and *C. cardunculus* pretreated by steam explosion at different temperatures and residence times in the reaction vessel. The effect of previous acid impregnation on steam

Table 3
Effect of Steam Explosion Conditions
on Solid Recovery and Enzymatic Hydrolysis Yield
of Acid Impregnated Biomass from *O. nervosum* and *C. cardunculus*[a]

		O. nervosum						*C. cardunculus*		
		0.4%			1%			1%		
Temperature	Residence time	Gy	SE	CC	Gy	SE	CC	Gy	SE	CC
170°C	2 min	79	35	34	65	48	45	41	57	41
	4 min	70	37	36	62	42	37	43	53	31
	8 min	70	51	45	64	52	39	43	71	44
190°C	2 min	69	51	43	68	37	34	43	57	34
	4 min	71	40	38	65	40	34	40	58	37

[a]Gy, solid residue recovered (%); SE, saccharification efficiency; and CC, cellulose conversion.

explosion efficiency has been studied, as described in the section of Material and Methods, for two different acid concentrations, 0.4 and 1%. Results obtained for both studied substrates are presented in Table 3.

DISCUSSION

The enzymatic hydrolysis yield undergoes a remarkable increase when substrates are pretreated by different steam explosion conditions assayed (Table 2). The saccharification efficiency reached in the most favorable conditions of pretreatment (230°C, 1–2 min for *O. nervosum* and 210°C, 2–4 min for *C. cardunculus*) values higher than 90%, compared to 23 and 20% obtained for both untreated substrates, respectively (Tables 1 and 2). The cellulose conversion percentages, which relate the initial glucose content of the untreated substrate with the final glucose yield obtained by enzymatic hydrolysis of the pretreated biomass, are in all cases lower than saccharification efficiency values, owing to the loss of glucose that occurred during pretreatment. No significant differences have been found between dry or wet substrates (data not shown). Since it is more difficult to control the reaction times in the last case, it seems advantageous to carry out the pretreatment using dry biomass.

Although the acid impregnation was expected to increase the favorable effect of steam explosion on enzymatic hydrolysis yield, the results show, as can be observed in Table 3, a considerable decrease of saccharification efficiency and cellulose conversion values when substrates were previously impregnated with sulfuric acid. The study of the composition of the solid residues obtained after repeated water washings of the steam exploded biomass (Table 4) revealed that no additional losses of glucose

Table 4
Composition of Residues Obtained After Steam Explosion of Acid Impregnated Biomass from *O. nervosum* and *C. cardunculus*[a]

Temperature	Residence time	*O. nervosum* 0.4% G	RS	L	*O. nervosum* 1% G	RS	L	*C. cardunculus* 1% G	RS	L
170°C	2 min	37	54	27	43	59	32	63	66	31
	4 min	41	55	28	42	54	32	49	59	32
	8 min	38	58	29	36	60	31	51	63	34
190°C	2 min	37	57	30	40	53	29	50	59	32
	4 min	38	54	30	39	53	28	58	65	32

[a]Percentage on dry weight. G, glucose; RS, reducing sugars; and L, lignin.

occurred during steam explosion owing to acid impregnation. This fact suggests the possibility of a certain extent of enzymatic hydrolysis inhibition by some water insoluble degradation products formed during the pretreatment in acid conditions.

From the results discussed above for *O. nervosum* and *C. cardunculus*, it can be deduced the high efficiency of pretreatment by steam explosion to enhance or to increase the enzymatic hydrolysis rate of both substrates. The acid impregnation of the lignocellulosic biomass before the steam treatment has produced a negative effect on the hydrolysis yield. Because of the solubilization of total solids and glucose observed after steam explosion, additional tests will be carried out in order to determine more favorable conditions for an efficient solid recovery from the exploded substrates.

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