

## Article

# A Novel Method to Detoxify Steam-Exploded Biomass and Produce a Substrate for Biorefinery

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**Abstract:** Pre-treatments at relatively high temperatures (range 160 °C–220 °C) are currently used to transform lignocellulosics into biofuels and chemicals. In this step, several molecules with an inhibitory effect in the subsequent fermentation processes are generated. These inhibitors include low-molecular-weight molecules and lignin fragments that can be removed by water washing. However, this procedure also removes valuable soluble carbohydrates which are then difficult to recover from the diluted stream. In this work, a new method to detoxify steam-exploded substrates is reported. The procedure is based on the evaporation of low-weight acids and aldehydes, which leaves all the sugars in the solid matrix, while the cellulose hornification (an irreversible modification of the cellulose fibres that depresses the saccharification yield) is prevented by adding steam to the hot fluidizing flow stream. Two systems were tested: a 0.1 kg/batch oscillating fluidized bed and a continuous fluidized bed dryer operating downstream of a steam explosion plant with a treatment capacity of 150 kg/h. The detoxified substrates were subjected to enzymatic hydrolysis and fermentation to obtain bioethanol, with a yield that was 14% higher than that obtained from substrates detoxified with conventional methods of drying or washing.

**Keywords:** detoxification; fluid bed; steam explosion; inhibitors; biomass; fermentation



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## 1. Introduction

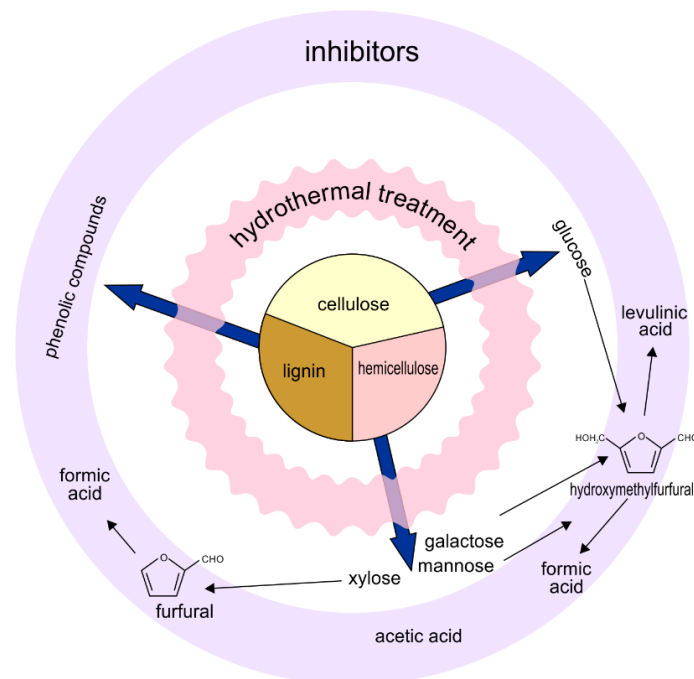
The destructuring of lignocellulosic biomasses, such as wheat straw (*Triticum*) and reeds (*Arundo donax*), is one of the fundamental steps in the production of carbohydrates, from which biofuels and chemicals can be obtained. It is performed using a step of pre-treatments that precedes the enzymatic hydrolysis of the polysaccharides, essential to obtain sugars in monomeric form, which can be fermented in a plethora of products. The pre-treatment modifies the morphology of the feedstock and improves the accessibility of polysaccharides using biological and chemical agents. One of the most common pre-treatments is the steam explosion (SE), which uses steam at high pressure and temperature (15–20 bar and 180–220 °C). An increase in the temperature causes extensive hydrolysis of intra- and inter-molecular bonds, resulting in the chemical destructuring and mechanical weakness of the matrix at a molecular level. An equivalent effect is obtained by increasing the residence times, and the two parameters are used to obtain a semiempirical relationship called the severity parameter, Ro (Equation (1)) [1]:

$$Ro = t \times \exp [(T - 100)/14.75] \quad (1)$$

where  $t$  is expressed in minutes and  $T$  in °C.

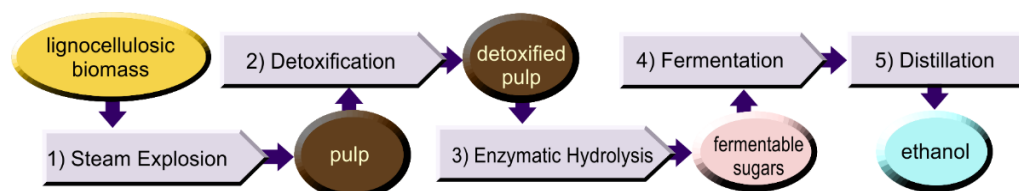
An optimal pre-treatment should satisfy several criteria, including: (1) efficient hydrolysability of the residual solid by enzymes; (2) complete fermentability of the resulting sugars; (3) minimal carbohydrate degradation; (4) economic sustainability; (5) low environmental impact. In practice, none of the pre-treatments developed so far meet all these criteria. The steam explosion has the advantage of requiring only water as a hydrolytic

reactant at high temperatures; moreover, it produces cellulose, which is very digestible by enzymes, and can be performed with relatively simple and cheap machinery. For these reasons, SE is one of the most used pre-treatments at a large scale. Nevertheless, the degradation of hemicellulose has negative consequences on the yield of products and causes the formation of molecules that inhibit the fermentation process (Figure 1). The effect of these inhibitors on microorganisms that carry out the bioconversion into bioethanol has been extensively studied [2].



**Figure 1.** Schematic representation of the hydrolysis and decomposition of biomass during the hydrothermal treatments.

Under the current conditions of pre-treatment, the production of inhibitors accounts for a few percentages in weight of the feedstock. These include volatile molecules such as formic acid, acetic acid, furfural, hydroxymethyl furfural and benzaldehyde, but also larger and non-volatile lignin fragments with an inhibitory effect. The general process of transformation of lignocellulosic biomass into bioethanol used in our approach is shown in Figure 2 and it is constituted of the following steps: (1) pre-treatment (steam explosion); (2) detoxification of the pre-treated material; (3) enzymatic hydrolysis; (4) alcoholic fermentation; (5) distillation.



**Figure 2.** Scheme of the conversion of lignocellulosic biomass into bioethanol.

Several methods of removing inhibitors have been tested. Since most of the inhibitors are water-soluble, the most common and simple method is washing the pre-treated biomass with water [3,4]. However, this method has the drawback of also removing soluble oligomers, in particular those obtained from the hydrothermal hydrolysis of hemicellulose that occurs in the pre-treatment [5]. Another simple method is drying the exploded material at a temperature higher than 60 °C [4,6]. Although this method is effective in

removing volatile inhibitors, it generates a rearrangement of the cellulose polymer chains (called hornification) which makes saccharification less effective [7]. Other methods that chemically transform the inhibitors have been proposed, for example, by adding calcium hydroxide or sodium sulfite [8–10]. The selective removal can be achieved by adding activated carbon or organic solvents to the slurry of pre-treated biomass [11–16]. In some cases, enzymatic methods are used to detoxify (laccase) [17,18]; in others, the inhibitory molecules are complexed with Alamine and separated with membranes [19]. One study proposes the use of polymers (PEI) as adsorbents of inhibitors on slurries obtained from acid pre-treatment [20]. Almost all the detoxification methods proposed in the literature are employed on the hydrolysates downstream of enzymatic hydrolysis. Though efficient, these methods have the drawback of adding chemicals to the process, with a consequent increase in the cost and the complexity of the procedures, such as the disposal of the waste stream. In this work, a new approach is tested: the removal of inhibitors on the solid substrate just after the pre-treatment, based on the use of hot and humidified air. The pre-treated biomass is fluidized with this mix that flows through the bed and removes volatile molecules. The technology of fluidized beds is relatively simple and commercially available to also be implemented at a large scale. The method is free of chemicals, does not remove soluble carbohydrates from the substrate, and does not induce hornification. The characteristics of the biomass detoxified using this novel method were compared with those achievable with other well-assessed methods, including water washing and drying, in terms of enzymatic hydrolysis and ethanol yield.

## 2. Materials and Methods

### 2.1. Biomass Pre-Treatment

The pulp was produced with a continuous SE plant with a treatment capacity of 150 kg/h as dry feedstock, which operates at the research centre of ENEA, and is described elsewhere [3]. Common reed (*Arundo donax*) and wheat straw (*Triticum* sp.), supplied by a local farmer in the Basilicata region, were used. The biomasses were shredded by means of a straw chopper to an average size of 2 cm, then humidified to reach a dry matter (DM) of 50% and fed into the SE plant. The biomass was treated at 210 °C for 6 min to obtain a suitable substrate for efficient enzymatic hydrolysis. These pre-treatment conditions resulted from an extensive optimization campaign, as elsewhere reported [5]. The material was produced and collected in a lump, and then carefully homogenized in batches of 50 kg that were stored at 4 °C in closed drums. Samples were taken for chemical analysis.

### 2.2. Detoxification by Water Washing

About 8 kg of pulp was detoxified by water washing at 65 °C at a solid-to-liquid ratio (S/L) of 0.2. The aqueous phase was separated from the solid using a filter press with a filter-grid with holes of 1 mm. The solid phase was rinsed with water and refiltered, and then stored in plastic bags at 4 °C (DM 26%). Samples of the solid and aqueous phases were analysed [3].

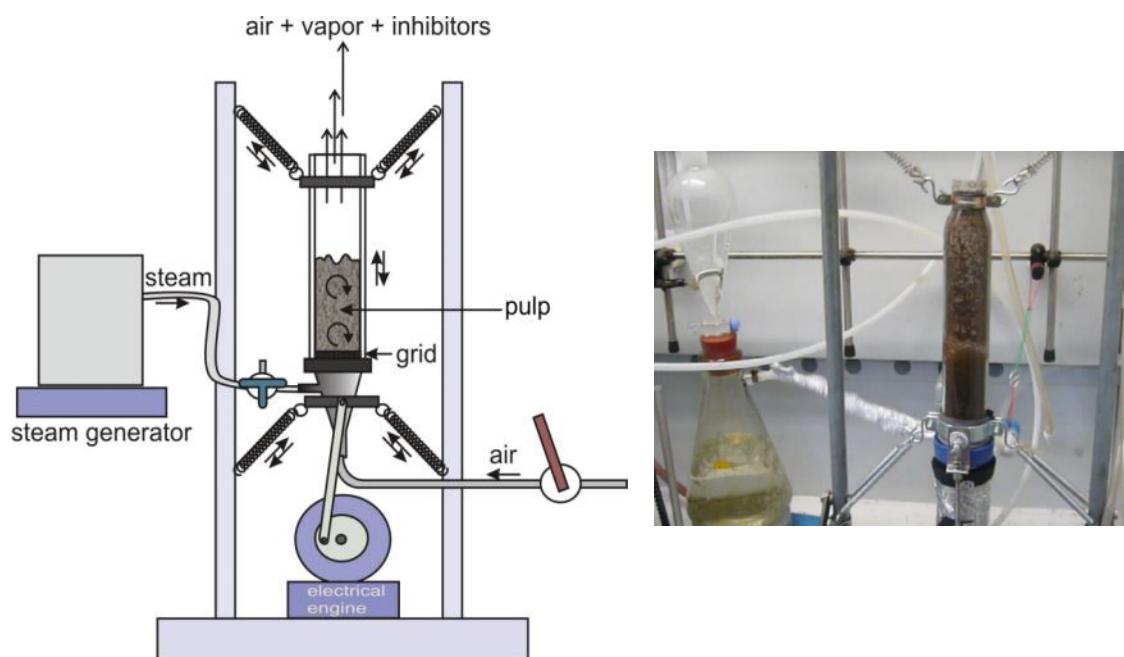
### 2.3. Detoxification by Drying

About 8 kg of pulp was deposited in trays in thick layers of 2 cm and dried in a vented oven at 60 °C for 48 h; it was manually mixed every 6–8 h. The material obtained was closed in bags and analysed. When heated at 105 °C for two hours, it lost less than 1% of the weight [6].

### 2.4. Detoxification with a Bench Scale Vibro-Fluidized Bed System

This system was designed and built at the ENEA laboratories and is shown in Figure 3. It consisted of a glass tube ( $d = 5$  cm,  $h = 30$  cm) inside which the exploded material was loaded to be detoxified (approx. 100 g). A flow of air and steam crossed the biomass bed while the tube was subjected to a vertical oscillation using a rod connected to an electric motor. The vibration was guaranteed by a vertical rod connected to the base of a

glass tube, which was anchored with steel springs to a vertical scaffold. The base of the rod was connected to the mid-radius of an engine wheel with variable rpm, to give the reactor vertical oscillatory motions at the desired frequency. The allowed amplitude of the oscillation ranged from 1 to 5 cm. The temperature of the air–vapour flow was measured using a digital thermocouple. The steam was produced by a boiler consisting of a copper coil immersed in an oil bath at 120 °C. A suitable combination of the air–steam flow rate and the oscillation (frequency and amplitude) maintained the biomass in the fluidized state. Table 1 shows the parameters that were used. The tests were conducted for 1.5 h, using steam-exploded pulp from Arundo; during this period, samples were taken and analysed to determine the residual inhibitors.



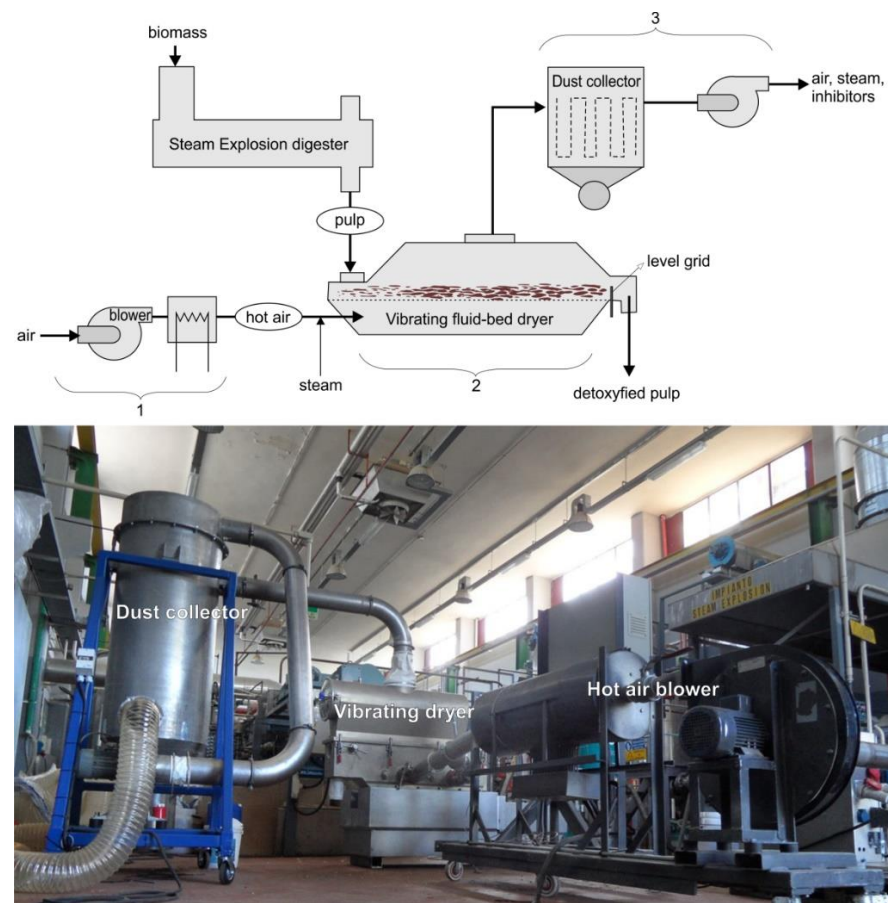
**Figure 3.** Scheme and picture of the bench scale vibro-fluidized bed detoxifier.

**Table 1.** Optimized parameters for the vibro-fluidized bed reactor.

Parameter	Setting
Oscillation amplitude	2 cm
Oscillation frequency	7 Hz
Airflow	9 m <sup>3</sup> /h
Air pressure	0.4 Barg
Steam flow 80 mL/h	80 mL/h
Air–steam flow temperature	60 °C

### 2.5. Detoxification with a Pilot Scale Vibro-Fluidized Bed System

The system was based on a commercial vibrating fluid-bed dryer, which was modified to use humidified hot air. The vibrating fluid-bed dryer was hired by TEMA process B.V. (<http://temaprocess.com/en/> accessed on 1 January 2012). This machine is currently used to dry organic material, such as orange peels, since it allows optimal heat exchange between the material and the air stream. The biomass is dried in mild conditions, since the heating rate is low but the exchange surface is large. Moreover, the material inside the machine is gently mixed through vibro-fluidization from the input to the output. The vibration is achieved using an electric motor. The system used to detoxify the steam-exploded biomass consisted of 3 units: (1) hot air blower; (2) vibrating dryer; (3) and cyclone for powder abatement. These machines were assembled in series downstream of the continuous SE digester, as shown in Figure 4.



**Figure 4.** Scheme of the detoxification by the vibrating fluid-bed pilot-scale system. (1) Hot air blower; (2) Vibrating dryer; (3) Cyclone for powder abatement.

The residence time of the pulp inside the dryer is regulated using a level grid, which can be manually moved vertically. To perform detoxification with a stream of air and steam, the original machinery was modified by adding a steam line. One hygrometer was placed downstream of the treated product, while the steam injector was placed upstream of the airflow line. The treatment temperature, measured in the fluidized bed, was on average 70 °C. This value was not stable, as it was very sensitive to the flow rate and humidity of the hot air stream, and variations of  $\pm 10$  °C were observed. The flow rate was set to guarantee efficient fluidization of the pulp in each test. Below the grid of the fluid bed, a relative pressure of 4–5 mBar was measured. The detoxification tests were optimized using DOE (Design-Expert® version 10.0.8.0 by Stat-Ease Inc., Minneapolis, MN, USA) with the following model: Response Surface, Randomized, Central Composite, Quadratic, 2 factors, 2 centre points, 10 Runs. The temperature of the humidified hot air was adjusted to keep the pulp at 70 °C. Higher temperatures were avoided to limit energy consumption and the decomposition of thermolabile sugars (hemicellulose). The factors examined in the DOE were the pulp residence time, which ranged between 10 and 50 min, and relative humidity ( $U_r$ ) in the hot stream, which ranged between 40 and 80%. Coded parameters and the experimental runs are listed in Table 2.

**Table 2.** Coded parameters in DOE.

Factor	Name	Units	Minimum	Maximum	Coded Values		Mean
A	$U_r$	%	40	80	−1.0 = 40	1.0 = 80	60
B	t	min	10	50	−1.0 = 10	1.0 = 50	30



## 2.6. Analytical Methods

The lignocellulosic materials were characterized according to the NREL procedure. Monomeric sugars were determined by HPLC ion chromatography (DIONEX, model DX 500) using a NaOH solution (concentration gradient 2–200 mM) as an eluent, CarboPack PA1 as a column, and a pulsed electrochemical detector. The inhibitors were analysed by HPLC chromatography (HP 1100 series), with a column Phenomenex 4u synergy RP-80, and using a diode-array detector. The eluent flow was water and acetonitrile with a gradient of acetonitrile (0–15 min; 3–10%; 15–35 min; 10–30%; 35–60 min; 30–50%).

## 2.7. Hydrolysis and Fermentation Tests

The bioconversion of the pulp was carried out by enzymatic hydrolysis for 24 h, followed by simultaneous saccharification and fermentation (SSF) for 72 h [6]. A commercial cocktail of enzymes Celluclast 1.5 L (65 FPU/g and 17  $\beta$ -glucosidase IU/g) and Novozyme 188 (376  $\beta$ -glucosidase IU/g) was used (Novozymes A/S, Bagsværd, Denmark). The hydrolysates were fermented with *Saccharomyces C.* (SIGMA II Type). Enzymatic hydrolysis was carried out with an S/L ratio of 7.2% (*w/v*) using a solution containing 3.5 g/L of Celluclast, 0.8 g/L of Novozyme (protein content: 125 mg per g of enzyme), and 0.05 M of sodium acetate buffer, for a total volume of 40 mL, in 100 mL of Erlenmeyer flasks. The hydrolysis was carried out at 45 °C and pH 4.8, stirring at 150 rpm. After 24 h, the temperature was lowered to 35 °C and the yeast and nutrients were added to obtain a broth with 3 g/L of *Saccharomyces*, 2.5 g/L of yeast extract, 0.25 g/L of  $(\text{NH}_4)_2\text{HPO}_4$ , and 0.025 g/L of  $\text{MgSO}_4 \cdot \text{H}_2\text{O}$ . The tests were done in duplicate. The ethanol produced was analysed by ion chromatography (HPIC DIONEX, Nucleogel OA40 column, 0.1 M  $\text{H}_2\text{SO}_4$  eluent, RI detector). In all experiments, 10 mg of antibiotic (tetracycline) was added to avoid bacterial contamination.

## 3. Results and Discussion

### 3.1. Substrates Characterization

The compositions of the two starting biomasses as well as the obtained exploded and detoxified substrates are reported in Tables 3 and 4. The SE involved a mass loss of 10.2% for the Arundo and 15.7% for wheat, comprising mainly water and volatiles organic compounds that left the process as a separate stream in the explosion step. The exploded biomass was separated into insoluble and soluble by water extraction. More than 20% of the biomass was solubilized, and 7–10% of it resulted in carbohydrates.

**Table 3.** Substrates composition and flow of the constituents after SE and water washing (WI = water-insoluble, WS = water soluble).

Constituent, Wt% <sup>1</sup>	Arundo Donax				Wheat Straw			
	Raw	SE Pulp	WI Pulp	WS Pulp	Raw	SE Pulp	WI Pulp	WS Pulp
Glucan	37.6	35.6	33.2	2.4	38.0	34.2	32.6	1.6
Galactan	0.7	0.4	0	0.4	0.7	0	0	0
Xylan	19.7	12.1	5.7	6.4	19.4	7.1	2.2	4.9
Arabinan	1.5	0.6	0.3	0.3	2.3	0.2	0	0.2
Ashes	4.9	4.9	2.2	2.5	6.9	6.9	2.9	4.0
Lignin	26.6	29.9	25.4	3.09	22.0	30.4	21.6	8.8
Extractives <sup>2</sup>	6.0				8			
Undetermined	3.1	6.3	0.5	5.8	2.7	5.5	1.4	4.1
Water	61.3	144	164	995	13.6	101	250	665
Inhibitors <sup>3</sup>		2.75		2.75		2.41		2.41
DM balance	100.0	89.8	67.2	22.6	100	84.3	60.7	23.6

<sup>1</sup> Value expressed as g/100 g of raw material; <sup>2</sup> Extractives were not determined in the pulps <sup>3</sup> Inhibitors are volatile, so they don't contribute to the DM balance. Error: <5%. SE = Steam-exploded; WI = Water Insoluble fraction of SE pulp; WS = Water Soluble fraction.

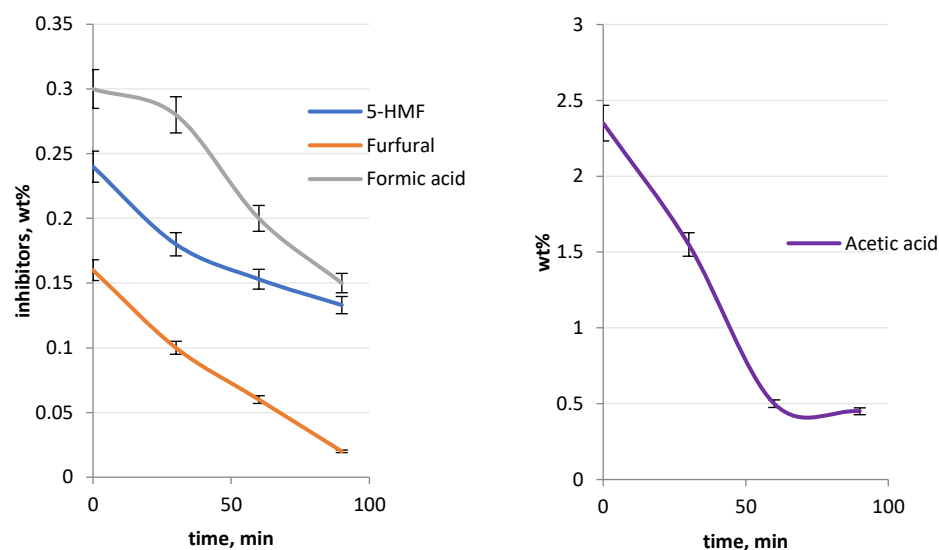
**Table 4.** Inhibitors detected in the steam-exploded biomass.

Compound, Wt% <sup>1</sup>	SE Arundo	SE Wheat Straw
Acetic acid	2.35	2.44
Formic acid	0.3	0.01
Furfural	0.16	0.26
5-HMF	0.24	0.14
Catechol	traces	0.01
4-hydroxybenzaldehyde	0.01	traces
Syringaldehyde	traces	traces

<sup>1</sup> Value expressed as g/100 g of pulp. Error < 5%.

### 3.2. Detoxification with the Bench Scale Vibro-Fluidized Bed System

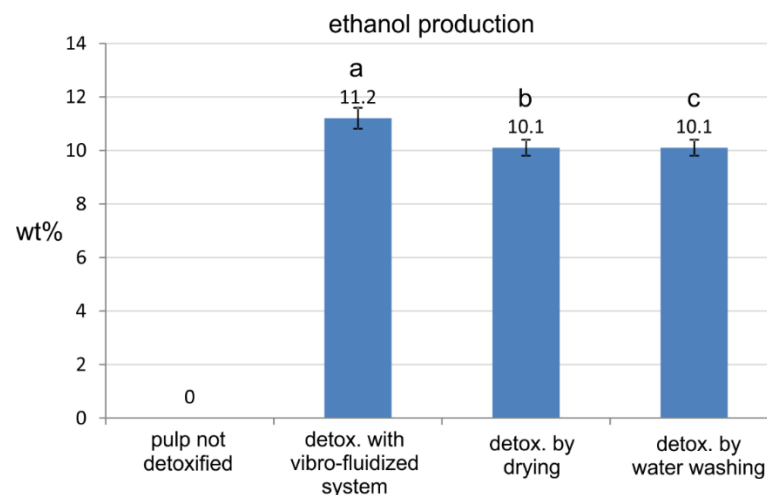
When considering the content of carbohydrates and lignin, this method did not alter the composition of the detoxified product, which remained the same as the starting SE pulp (Table 3), where the glucan was 39.6 wt% of Arundo pulp (DM). Using this system with hot air (60 °C) and without steam, drying 100 g of Arundo pulp (starting DM 33%) was faster than in the oven, i.e., 1 h vs. 30 h. Nevertheless, the process also induced hornification, and to avoid it, a steam flow was added to the air stream. During the detoxification test, 1 g of the sample was taken at 30, 60, and 90 min for the analysis of residual inhibitors. The results are shown in Figure 5. After 90 min of treatment, the detoxified pulp was tested for alcohol production, and the results were compared with those obtained by conventional detoxifying with water washing and drying (Figure 6).



**Figure 5.** The residual content of inhibitors in the pulp achieved with the bench scale vibro-fluidized system, as g/g × 100 of dry pre-treated biomass. Ethanol production after different detoxification methods, as g/g × 100 of dry pre-treated biomass.

The data reported in Figure 6 lead to the following considerations:

- To use steam-exploded biomass as a substrate for the fermentation process, the pulp needs to be detoxified;
- The detoxification with the vibro-fluidized system leads to higher yield of ethanol;
- When the substrate was detoxified by drying, a lower alcoholic production was observed. This could be related to a lower saccharification yield due to hornification;
- When the substrate is detoxified by washing, a lower alcoholic production was observed. This could be due to the loss of soluble carbohydrates.



**Figure 6.** Ethanol production after different detoxification methods, as  $\text{g/g} \times 100$  of dry pre-treated biomass. The Tukey HSD test (significance level 0.05) gives  $p < 0.015$  for a–b and a–c, while no difference results between b and c ( $p = 1$ ).

In addition, the use of the vibro-fluidized system allows the reduction of treatment times, as one can obtain a detoxified product in less than two hours.

### 3.3. Detoxification with the Pilot-Scale Vibro-Fluidized Bed System

Additionally in this case, the content of carbohydrates and lignin was not modified in the detoxified product, which remained the same as the starting SE pulp (Table 3), where the glucan was 40.6% by weight of the straw pulp (DM). The treated biomass was analysed during each run to determine the dry matter and the inhibitor content. The results were analysed by the DOE software, which returned surfaces as a function of the experimental variables, and the criterion to select the polynomial terms in the model analysis was  $p < 0.1$ . The experimental results for each run are reported in Table 5.

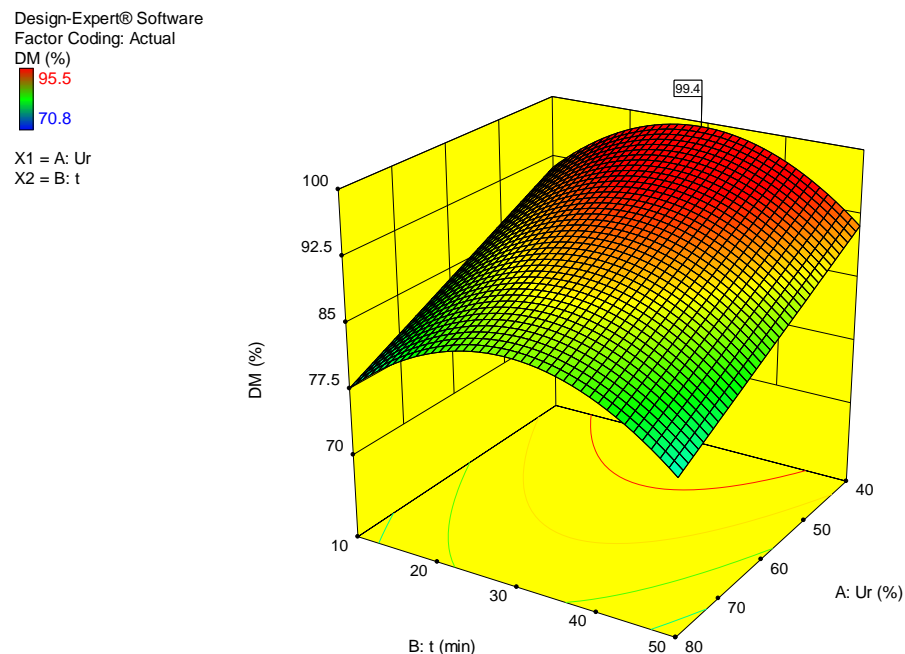
**Table 5.** Experimental <sup>a</sup> responses at different conditions.

Exp.	Ur, %	t, min	DM, %	Inhibitors, %	Enz. Hydrolysis Yield, %
1	80	50	83.5	0.136	80.6
2	60	10	81.5	0.428	88.2
3	60	50	84.1	0.206	83.6
4	40	30	94.7	0.198	81.1
5	80	30	87.5	0.096	85.7
6	80	10	70.8	0.381	85.7
7	40	10	92.6	0.416	85.6
8	60	30	93.2	0.171	88.8
9	40	50	94.9	0.375	86.2
10	60	30	95.5	0.172	84.3

<sup>a</sup> Error < 5%.

Figure 7 shows the variation of the dry matter (DM) as a function of time and relative humidity. The interpolating surface is a 2nd degree polynomial and the correlation with experimental data is good ( $R^2 = 0.895$ ). The final equations in terms of coded and actual factors are, respectively, Equations (2) and (3). More details, including statistical data, are provided in the supplementary section (Tables S2, S3 and S14).





**Figure 7.** Dry matter (DM) of pulp detoxified with the pilot vibro-fluidized bed under different treatment conditions.

Equation in Terms of Coded Factors:

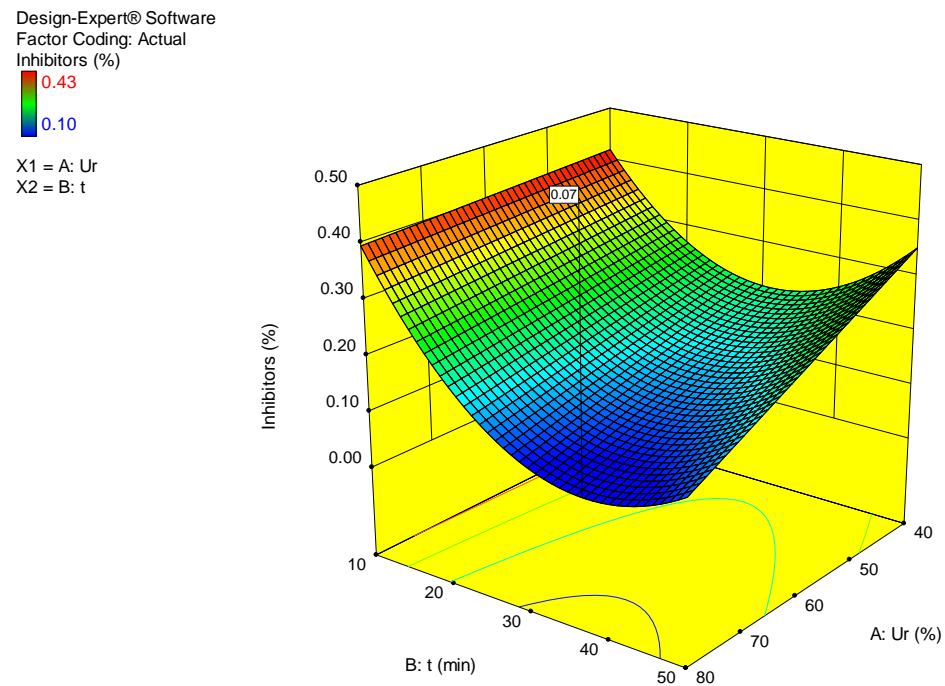
$$DM (\%) = 92.72 - 6.73 \times A - 8.16 \times B^2 \quad (2)$$

Equation in Terms of Actual Factors:

$$DM (\%) = 106.35 - 0.336 \times Ur + 1.47 \times 10^{-3} \times t^2 \quad (3)$$

The treatment times and the relative humidity significantly influenced the final dry matter of the pulp. After 30 min of treatment with 40% Ur, the pulp became almost completely dry but hornification could occur.

On the other hand, the trend of the residual inhibitors found in the pulp at the end of the treatment reported in Figure 8 as the sum of the molecules indicated in Table 4 was more interesting. The surface interpolated very well in the experimental data ( $R^2 = 0.95$ ). The obtained equations are, respectively, Equations (4) and (5). More details, including the analysis of some single inhibitors and statistical outputs, are provided in the supplementary section (Tables S4–S14, Figures S1–S3). The lowest value of inhibitors (0.07%) was obtained at Ur 80% and 38 min of treatment; acetic acid (0.03%) and 5-HMF (0.04%) were identified as the most abundant. Under these conditions, the concentration of the volatile inhibitors in the substrate was reduced by 40 times from the initial content. For 10-min treatments, the residual inhibitor content remained around 0.4%, corresponding to a reduction of approximately one seventh of the initial content. This reduction was not sufficient to detoxify the substrate, since the pulp treated for 10 min did not produce ethanol. Generally, the inhibitor content decreased with Ur and this can be explained by the affinity of the inhibitors with the water. Increasing the treatment time up to about 35 min resulted in a decrease in the inhibitors, but, with longer times, the yield of fermentation decreased. There are some possible explanations for this trend: (1) Prolonged treatment times lead to the formation of new inhibitors. (2) Prolonged times cause a larger removal of fine particles dragged from the bed, as they are more efficiently saccharified because of their dimension. Indeed, after 50 min, 30% of the initial pulp was found to have left the bed as fine, while it was about 15% after 30 min.



**Figure 8.** Inhibitors in the pulp after treatment with the pilot vibro-fluidized bed at different treatment conditions.

Equation in Terms of Coded Factors:

$$\text{Inhibitors (\%)} = 0.16 - 0.063 \times A - 0.085 \times B - 0.051 \times A \times B + 0.16 \times B^2 \quad (4)$$

Equation in Terms of Actual Factors:

$$\text{Inhibitors (\%)} = 0.61 + 6.92 \times 10^{-4} \times \text{Ur} - 0.02 \times t - 1.27 \times 10^{-4} \times \text{Ur} \times t + 4.11 \times 10^{-4} \times t^2 \quad (5)$$

The enzymatic hydrolysis carried out on the detoxified pulp produced glucose with a yield between 80 and 90% and was generally not dependent on the investigated parameters (Table 4 and Figure S4). Based on these results, the optimal conditions to detoxify the steam-exploded biomass resulted in 38 min of residence time and Ur 80% of the airflow. The final pulp, including 19% of fines recovered from the cyclone, had a DM of 89% and an inhibitor content of 0.08%, values close to the predicted values (0.07%). Bioconversion tests were performed, obtaining an enzymatic hydrolysis yield of 85% and an alcoholic fermentation yield of 94%. The high yield of alcoholic fermentation confirms the effectiveness of the detoxification method proposed in this work. Although most of the other methods proposed in the literature are equally effective, it is necessary to highlight some key features of the proposed method. First of all, it detoxifies the solid pulp just produced without changing its composition in terms of carbohydrates. Secondly, other methods, which use external agents (chemicals or resins) or act on the liquid phases (slurry or hydrolysates), need additional operations for the neutralization, removal, or recovery of these detoxifying agents. Another advantage in detoxifying the solid pulp is represented by the use of the substrate in fed-batch processes, which lead to a slurry with a high solid/liquid ratio [21]. In fact, starting from a detoxified substrate, the concentration of inhibitors in slurries with high solids load is avoided.

As regards the energetic cost, this was calculated at the optimized conditions reported above by summing up the mechanical work for vibro-fluidization, which was 0.216 MJ per kg of dry feedstock, and the heat required to produce the stream of hot air-steam, which ranged from 0.30 MJ/kg to 1.6 MJ/kg, respectively, depending on whether recycled low enthalpy steam at 100 °C was considered or fresh water. The calculation sheet is

reported in the supplementary data, Table S7. In the optimised process designed to use, for example, the steam downstream the digester after the steam explosion is executed, the energy consumption resulted in 0.30 MJ/kg, which is comparable to other biomass pre-treatments, such as briquetting lignocellulosic biomasses, including wood and forest residues, whose energy requirement is estimated to be as high as 0.35 MJ/kg by summing up the biomass drying, preheating and the mechanical processes [22–24].

#### 4. Conclusions

A new detoxification method to detoxify steam-exploded biomass based on the use of humidified hot air flowing through the fluidized bed of biomass was tested at bench and pilot scales. This method was proven effective for the selective removal of inhibitors and, compared to other similar detoxification methods of water washing and drying, has the advantage of preserving both the content of soluble sugar and the digestibility of the cellulosic fibres by enzymes. An experimental design was carried out on the pilot system to optimize the most important process parameters, including the treatment time and the degree of humidification of the air. The optimal conditions to detoxify the steam-exploded biomass resulted in 38 min of residence time and relative humidity of 80% of the airflow at 70 °C. At these conditions, the energy consumption is estimated to be 0.30 MJ/kg in a process designed to recycle low enthalpy steam to be used as stripper with hot air. The detoxified materials were used as a substrate to produce ethanol, and the conversion was 14% higher than that obtained from substrates detoxified with conventional methods (drying or washing), while the raw steam-exploded biomass was not at all effective for that purpose.

**Supplementary Materials:** The Tables S1–S14 and Figures S1–S4 can be downloaded at: <https://www.mdpi.com/article/10.3390/pr10122611/s1>.

**Author Contributions:** Conceptualization, F.Z.; methodology, G.A. and E.V.; formal analysis, E.V.; supervision and validation, F.Z.; investigation, G.A., V.V. and M.C.; resources, G.A. and V.V.; data curation, E.V.; writing—original draft preparation and visualization, E.V.; Writing—review and editing, F.Z. and E.V.. All authors have read and agreed to the published version of the manuscript.

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#### References

1. Chornet, E.; Overend, R.P. Phenomenological kinetics and reaction engineering aspects of steam/aqueous treatments. In Proceedings of the International Workshop on Steam Explosion Techniques, Milan, Italy, 20–21 October 1988; Focher, B., Marzetti, A., Crescenzi, V., Eds.; Gordon and Breach: Philadelphia, PA, USA, 1998.
2. Olsson, L.; Hahn-Hägerdal, B. Fermentation of lignocellulosic hydrolysates for ethanol production. *Enzym. Microb. Technol.* **1996**, *18*, 312–331. [\[CrossRef\]](#)
3. De Bari, I.; Viola, E.; Barisano, D.; Cardinale, M.; Nanna, F.; Zimbardi, F.; Cardinale, G.; Braccio, G. Ethanol production at flask and pilot scale from concentrated slurries of steam exploded aspen. *Ind. Eng. Chem. Res.* **2002**, *41*, 1745–1753. [\[CrossRef\]](#)
4. Hongqiang, L.; Hongzhang, C. Detoxification of steam-exploded corn straw produced by an industrial-scale reactor. *Process Biochem.* **2008**, *43*, 1447–1451.
5. Zimbardi, F.; Viggiano, D.; Demichele, M.; Cuna, D.; Cardinale, G. Steam explosion of straw in batch and continuous systems. *Appl. Biochem. Biotechnol.* **1999**, *77–79*, 117–132. [\[CrossRef\]](#)
6. Viola, E.; Arcieri, G.; Zimbardi, F.; Valerio, V.; Cerone, N.; De Corato, U. Evaluation of a pilot-scaled paddle dryer for the production of ethanol from lignocellulose including inhibitor removal and high-solids enzymatic hydrolysis. *Biotechnol. Rep.* **2016**, *9*, 38–45. [\[CrossRef\]](#)
7. Luo, X.; Zhu, J.Y. Effects of dryin-induced fiber hornification on enzymatic saccharification of lignocellulose. *Enzym. Microb. Technol.* **2011**, *48*, 92–99. [\[CrossRef\]](#)
8. Bura, R.; Mansfield, S.D.; Saddler, J.N.; Bothast, R.J. SO<sub>2</sub>-catalyzed steam explosion of corn fiber for ethanol production. *Appl. Biochem. Biotechnol.* **2002**, *98*, 59–72. [\[CrossRef\]](#)

9. Converti, A.; Domínguez, J.M.; Perego, P.; da Silva, S.S.; Zilli, M. Wood Hydrolysis and Hydrolyzate Detoxification for Subsequent Xylitol Production. *Chem. Eng. Technol.* **2000**, *23*, 1013–1020. [[CrossRef](#)]
10. Marchal, R.; Ropars, M.; Vandecasteele, J.P. Conversion into acetone and butanol of lignocellulosic substrates pretreated by steam explosion. *Biotechnol. Lett.* **1986**, *8*, 365–370. [[CrossRef](#)]
11. Cantarella, M.; Cantarella, L.; Gallifuoco, A.; Spera, A.; Alfani, F. Comparison of different detoxification methods for steam-exploded poplar wood as a substrate for the bioproduction of ethanol in SHF and SSF. *Process Biochem.* **2004**, *39*, 1533–1542. [[CrossRef](#)]
12. Ge, J.-P.; Cai, B.-Y.; Liu, G.-M.; Ling, H.-Z.; Fang, B.-Z.; Song, G.; Yang, X.-F.; Ping, W.-X. Comparison of different detoxification methods for corn cob hemicellulose hydrolysate to improve ethanol production by *Candida shehatae* ACCC 20335. *Afr. J. Microbiol. Res.* **2011**, *5*, 1163–1168. [[CrossRef](#)]
13. Palmqvist, E.; Hahn-Hägerdal, B. Fermentation of lignocellulosic hydrolysates. I: Inhibition and detoxification. *Bioresour. Technol.* **2000**, *74*, 17–24. [[CrossRef](#)]
14. Canilha, L.; Chandel, A.K.; Suzanne dos Santos Milessi, T.; Antunes, F.A.F.; Luiz da Costa Freitas, W.; das Graças Almeida Felipe, M.; da Silva, S.S. Bioconversion of Sugarcane Biomass into Ethanol: An Overview about Composition, Pretreatment Methods, Detoxification of Hydrolysates, Enzymatic Saccharification, and Ethanol Fermentation. *J. Biomed. Res.* **2012**, *2012*, 989572. [[CrossRef](#)] [[PubMed](#)]
15. Devia, A.; Singha, A.; Bajarb, S.; Pantc, D.; Ud Dina, Z. Ethanol from lignocellulosic biomass: An in-depth analysis of pre-treatment methods, fermentation approaches and detoxification processes. *J. Environ. Chem. Eng.* **2021**, *9*, 105798. [[CrossRef](#)]
16. Jönsson, L.J.; Alriksson, B.; Nilvebrant, N.-O. Bioconversion of lignocellulose: Inhibitors and detoxification. *Biotechnol. Biofuels* **2013**, *6*, 16. [[CrossRef](#)] [[PubMed](#)]
17. Moreno, A.D.; Ibarra, D.; Fernández, J.L.; Ballesteros, M. Different laccase detoxification strategies for ethanol production from lignocellulosic biomass by the thermotolerant yeast *Kluyveromyces marxianus* CECT 10875. *Bioresour. Technol.* **2012**, *106*, 101–109. [[CrossRef](#)] [[PubMed](#)]
18. Jurado, M.; Prieto, A.; Martínez-Alcalá, Á.; Martínez, Á.T.; Martínez, M.J. Laccase detoxification of steam-exploded wheat straw for second generation bioethanol. *Bioresour. Technol.* **2009**, *100*, 6378–6384. [[CrossRef](#)]
19. Grzenia, D.L.; Schell, D.J.; Wickramsinghe, S.R. Detoxification of biomass hydrolysates by reactive membrane extraction. *J. Membr. Sci.* **2010**, *348*, 6–12. [[CrossRef](#)]
20. Carter, B.; Squillace, P.; Gilcrease, P.C.; Menkhaus, T.J. Detoxification of a lignocellulosic biomass slurry by soluble polyelectrolyte adsorption for improved fermentation efficiency. *Biotechnol. Bioeng.* **2011**, *108*, 2053–2060. [[CrossRef](#)]
21. de Albuquerque Wanderley, M.C.; Martín, C.; de Moraes Rocha, G.J.; Gouveia, E.R. Increase in ethanol production from sugarcane bagasse based on combined pretreatments and fed-batch enzymatic hydrolysis. *Bioresour. Technol.* **2013**, *128*, 448–453. [[CrossRef](#)]
22. Roman, K.; Barwicki, J.; Rządziejewicz, W.; Dawidowski, M. Evaluation of mechanical and energetic properties of the forest residues shredded chips during briquetting process. *Energies* **2021**, *14*, 3270. [[CrossRef](#)]
23. Muntean, A.; Ivanova, T.; Hutla, P.; Havrland, B. Influence of raw material properties on the quality of solid biofuel and energy consumption in briquetting process. *Agron. Res.* **2017**, *15*, 1708–1715. [[CrossRef](#)]
24. Arrieche, R.; Saloni, D.; van Dyk, H.; Lemaster, R.L. Evaluation of the energy balance for the production of briquettes from biomass. *For. Prod. J.* **2011**, *61*, 302–309. [[CrossRef](#)]