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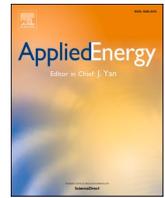
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## Determination of trade-offs between 2G bioethanol production yields and pretreatment costs for industrially steam exploded woody biomass

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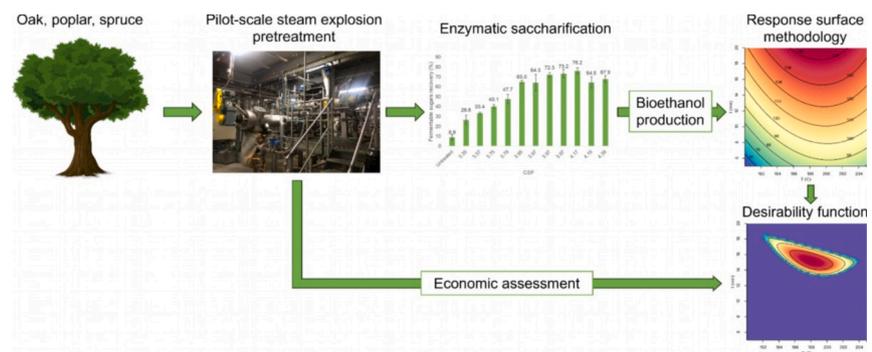
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### HIGHLIGHTS

- Different wood species are pretreated by steam explosion with contrasted severities.
- Bioethanol production yield depends on the wood species.
- Steam explosion improves bioethanol production until a mid-range severity.
- Optimal conditions to maximize bioethanol yield and limit CAPEX are determined.
- High temperatures of pretreatment are preferable to high residence times.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

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### ABSTRACT

Lignocellulosic biomass, including wood, can be transformed into bioethanol using steam explosion as pretreatment to improve saccharification and fermentation steps. Pretreatment is however the most expensive part of the process in terms of CAPEX and OPEX and requires to be optimized. In order to evaluate the link between pretreatment efficiency and cost, three contrasted wood species (oak, poplar and spruce) were pretreated with continuous steam explosion at pilot-scale following full factorial designs. Response surfaces obtained were combined with an economic assessment to determine trade-offs aiming at maximizing both fermentable sugars released during the enzymatic hydrolysis step and bioethanol yield during the fermentation step as well as minimizing costs of pretreatment in an industrial context. Results showed that bioethanol yields were highly dependent on wood species and that high severities of pretreatment were not the most relevant to apply. Optimal conditions of pretreatment corresponding to 70 % and 48 % of bioethanol producible from oak and poplar, respectively, were defined. The desirability function that has been modelled thus helps designing adapted pretreatment conditions regarding bioethanol production and process cost.

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## 1. Introduction

Given the depletion of fossil resources and the urge to reduce greenhouse gas (GHG) emissions, new processes are developed worldwide to produce bioenergy. Bioethanol is leader in the field of biofuel for transportation and seems like a promising alternative to fossil fuel. [1] Nowadays, bioethanol production results mostly from the transformation of food crops such as sugarcane, corn, wheat or sugar beet. [2] Although it is well developed and allows to reduce GHG emissions, the major drawback is the competition with the food industry. Moreover, the Renewable Energy Directive for European Union was revised to raise the target for 2030 to 40 % of renewable sources of energy, with mentions of advanced second-generation (2G) biofuels. [3] Therefore, research has focused for the past decades on the production of 2G bioethanol from lignocellulosic biomass such as agricultural residues, [4] dedicated energy crops [5] and wood. [6]

Woody biomass is constituted of three main biopolymers: cellulose (40–55 %), hemicelluloses (10–30 %) and lignin (15–25 %). [7] Cellulose and hemicelluloses offer a source of fermentable sugars for bioethanol production. However, these two macromolecules form a complex network with lignin in cell walls. This makes wood highly recalcitrant to biological conversion to obtain 2G sugars. [8]

In order to overcome lignocellulosic recalcitrance, a pretreatment step is thus usually required prior to enzymatically catalyzed hydrolysis, where pretreatments can be of physical, chemical or biological nature or a combination of these. [9,10] Steam explosion is a physicochemical pretreatment and belongs to the class of hydrothermal pretreatments. Lignocellulosic feedstock is put in contact with steam at a high pressure (1–3.5 MPa) and high temperature (180–240 °C) for a short residence time (2–20 min), then pressure is promptly reduced to atmospheric pressure causing the explosion of the material. [11] Steam explosion offers several advantages: it has been proven as one of the most cost-effective pretreatment processes and only water can be used as a solvent, so it is also environmentally friendly. [12]

Steam explosion pretreatment leads to chemical and physical modifications that alter wood reactivity. Hemicelluloses are degraded into oligo- or monomeric sugars due to an autohydrolysis reaction taking place because of the solubilization of acetyl groups during pretreatment, [13] while lignin undergoes rearrangement by depolymerization and repolymerization, [14] and morphological changes can also be observed. [15] These modifications contribute greatly to reduce wood recalcitrance by increasing material accessibility and thus to improve bioethanol production. However, cellulosic ethanol costs of production are not currently competitive with oil-based processes mostly because pretreatment steps constitute 30–50 % of the total equipment cost and 20–25 % of operational costs. [16] Pretreatment optimization is thus required to support the creation of cost-effective industrial facilities [17] and pretreatment conditions must be carefully chosen regarding economic trade-offs as stated in a techno-economic assessment on hydrothermal pretreatment that revealed 25 % of reduction in pretreatment equipment cost leads to considerably reduced production cost of succinic acid. [18]

For the past years, experimental design tools have been increasingly used to optimize 2G bioethanol production [19] and applied to different pretreatments such as wet torrefaction [20] or steam explosion combined with alkali pretreatment. [21] Likewise, several studies have demonstrated the benefits of using hydrothermal pretreatments to enhance enzymatic saccharification and bioethanol fermentation. [22,23] Others have focused on the optimization of the steam explosion process to maximize bioethanol yields. The best pretreatment conditions to reach high bioethanol yields from rapeseed straw at lab-scale were determined. [24] The transposition of 2G bioethanol production from agricultural residues to larger-than-pilot scale was demonstrated. [25] Recently, high bioethanol production from wheat straw was achieved by maintaining the amount of inhibitory substances low through optimization of steam explosion parameters [26] and a recent study revealed

that temperature of pretreatment had the strongest impact on saccharification and fermentation of steam exploded eucalyptus. [27]

Regarding the economic viability of the steam explosion process for recovery of fermentable sugars [28] and for bioethanol production, its high potential [29] and economic sustainability [30] were widely showcased and steam explosion was presented as a promising alternative for industrial 2G bioethanol production in Europe, especially in France, through an environmental techno-economic assessment. [31] Recently, studies tend to be closer to reality by confronting prediction and actual data: Priadi et al. [32] carried out a techno-environmental-economic analysis of a process simulation displaying the advantages of hydrothermally pretreated palm tree for 2G bioethanol production; Raina et al. [33] demonstrated that bioethanol production from hydrothermally pretreated sugarcane bagasse is promising regarding the energy efficiency of the process. However, López-Sandin and co-authors [34] found the optimal conditions to maximize bioethanol production from sorghum bagasse after hydrothermal pretreatment were not energetically sustainable as more energy was consumed than produced: this demonstrates that the selected conditions were not economically viable to be implemented in an industrial process.

Based on the literature survey, although the optimization investigations contributed to highlight steam explosion efficiency, only a few investigated its effects on wood digestibility [21,27]. Overall, most of them were restricted to a very limited number of samples using pretreatments carried out in lab scale batch reactors with poor process control and repeatability that make difficult to scale-up to an industrial process. More importantly, these studies focused on determining the optimal conditions to maximize bioethanol production without taking into account economic aspects. Also, most of techno-economic studies are theoretical simulations and are therefore not related with actual bioethanol production. Finally, most of the studies based on experimental data investigated the sustainability of hydrothermal pretreatment but not that of steam explosion pretreatment. Therefore, there is a need to combine and model bioethanol production from steam exploded woody biomass and economic data related to the pretreatment process in order to highlight trade-offs between production yields and pretreatment costs.

In this study, we propose a thorough analysis of contrasted wood samples, with three chemically and structurally different wood species widespread across Europe (oak, poplar and spruce). They were pretreated at pilot-scale by continuous steam explosion at various levels of severity, by varying temperature and residence time, to explore bioethanol production in an industrial context. Economic parameters related to the pretreatment process and calculated from an actual industrial plant were also discussed. Bioethanol production was related to an economic assessment of the process in order to design a desirability function aimed at finding trade-offs between maximizing bioethanol yields and reducing pretreatment cost. This will facilitate the application of 2G bioethanol process using steam explosion to an industrial context of production.

## 2. Materials & methods

### 2.1. Wood materials and pretreatment

Oak, poplar, and spruce were collected from forests of Marne Département (Great-East Region, France) and were pretreated using continuous steam explosion (SE). We had access to an industrial SE process unit [35], but for the purpose of this study, the experiments were downscaled to pilot-scale unit to allow the preparation of a broad field of study with 30 conditions investigated. Similarities and differences between the two processes are compiled in Fig. 1.

Wood was first chopped into small pieces and 80 % of woodchips were screened through a 16 mm sieve before entering the SE reactor without previous impregnation (initial moisture content between 20 % and 50 %). Pretreatment temperature (190–207.5 °C) and residence

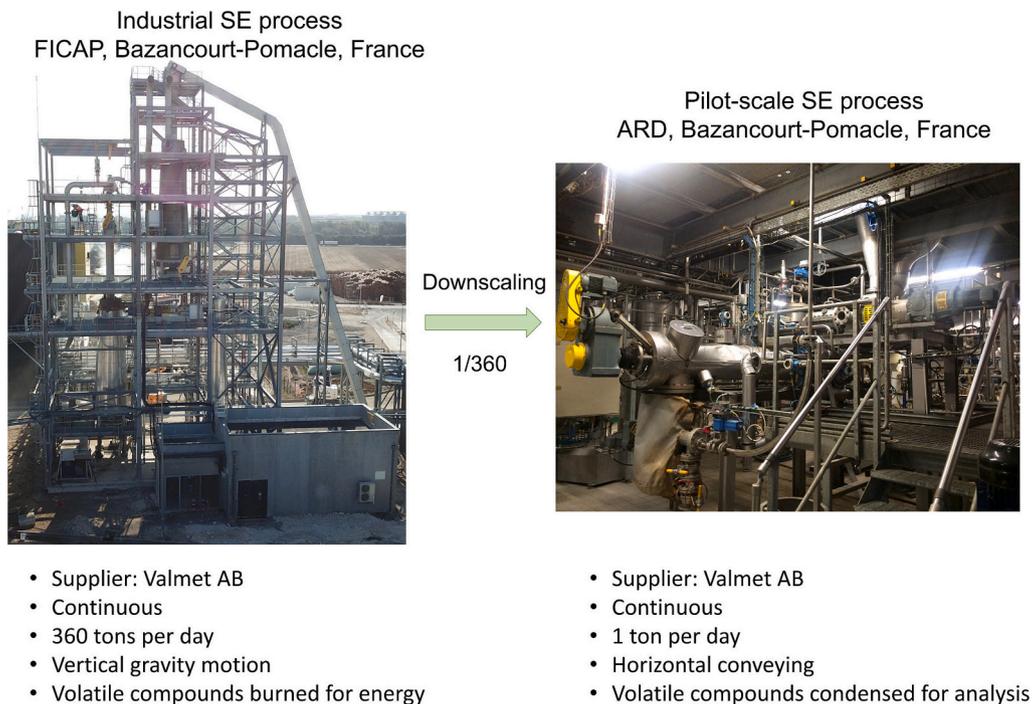


Fig. 1. Comparison of industrial and pilot-scale continuous steam explosion processes. ©Européenne de Biomasse.

time (5–20 min) were defined following a two-factor full factorial experimental design and each couple of operating conditions was associated to a severity value calculated using the combined severity factor (CSF) [36]. Spruce was pretreated at slightly higher severities as softwoods are reported to be more recalcitrant and require drastic conditions of pretreatment to deconstruct lignocellulosic cell wall. [11] Steam exploded wood powder was recovered and 10 g of each were ground at 200  $\mu\text{m}$  for chemical compositional analyses (see below).

## 2.2. Design of experiments

Two two-factor full factorial designs (Fig. 2) were implemented with three replicates for one of the conditions for each biomass. Temperature and residence time were the two variables under study and the variation ranges were selected based on the pilot unit limits. Experimental data were statistically analyzed by response surface methodology using Azurad [37] software. Desirability functions were also obtained from the combination of the model responses for bioethanol production and economic investment to provide zones of pretreatment parameters that fulfill constraints, here maximizing bioethanol production while

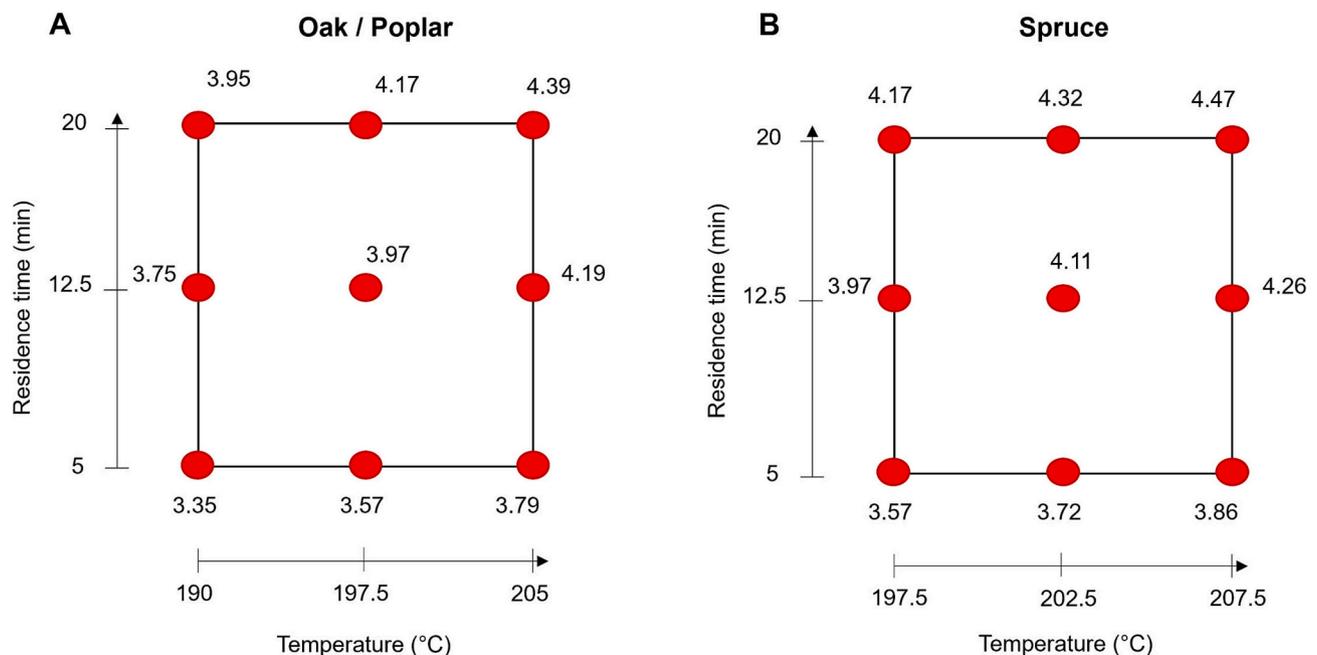


Fig. 2. Two-factor full factorial experimental designs applied to (A) oak and poplar and (B) spruce pretreatment. Each red dot is associated to a severity expressed as the combined severity factor. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

minimizing economic investment.

### 2.3. Mass loss during pretreatment

Mass yields after pretreatment were calculated using the fixed carbon (FC) content (Eq. 1), which is determined by subtracting the moisture, volatile matter and ash contents from the initial mass of the sample.

$$\text{Mass yield} = \frac{\text{FC in untreated sample}}{\text{FC in pretreated sample}} \quad (1)$$

### 2.4. Chemical composition

Pretreated wood samples were milled to quantify cellulose, hemicelluloses and lignin content as described previously. [38] Monomeric sugars were quantified after a two-step H<sub>2</sub>SO<sub>4</sub> hydrolysis using HPAEC-PAD (ThermoScientific DIONEX, USA). Klason lignin was quantified by gravimetry after H<sub>2</sub>SO<sub>4</sub> hydrolysis.

### 2.5. Enzymatic saccharification

Enzymatic hydrolyses were carried out using the Cellic® CTec2 cocktail (Novozymes, Denmark) with a global cellulase activity of 130 FPU/mL quantified following the NREL procedure. [39] Pretreated wood samples (10 % w/v) were hydrolyzed in 50 mL citrate-phosphate buffer (50 mM, pH 5) for 72 h at 50 °C and 150 rpm stirring with an enzyme loading of 10 FPU/g biomass. Mixtures were preincubated overnight and saccharification was initiated by addition of enzymes. Released sugars were then quantified by HPAEC-PAD (ThermoScientific DIONEX, USA) with a PA1 column as described previously. [40] Enzymatic saccharification yields were calculated as the ratio of C6 sugars (glucose and mannose) released after enzymatic action and the total amount of C6 sugars in wood samples. All assays were performed in triplicate.

### 2.6. Fermentation

After centrifugation and filtration (0.22 µm), hydrolysates recovered after enzymatic saccharification were fermented into bioethanol using *Saccharomyces cerevisiae* (Ethanol Red, Lesaffre, France). 17 mL of hydrolysates were incubated with 2 mL of culture medium and 1 mL of yeast suspension (30 g/L) for 96 h at 30 °C and 170 rpm. Culture medium was composed of yeast extract (50 g), ammonium sulfate (4 g) and chloramphenicol (0.5 g) in 1 L citrate-phosphate buffer (500 mM, pH 5). Quantity of ethanol produced was determined by high-performance liquid chromatography (Shimadzu, Japan) with an Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad) and equipped with infrared detection. All assays were performed in triplicate.

### 2.7. Inhibitors quantification

5-hydroxymethylfurfural (HMF), furfural, acetic acid and formic acid were quantified from the pretreated wood powders using HPLC with UV detection. Phenolics compounds were quantified from the pretreated powders and from the hydrolysates after enzymatic saccharification with Folin-Ciocalteu reagent and measurement by spectrophotometry.

### 2.8. Economic assessment

For each condition of pretreatment, a relative investment (RI) was calculated with the lowest severity condition as basis and using the following formula (Eq. 2), with the base cost set at 1 and 0.7 the scaling change factor. [41]

$$RI = \text{base cost} \times \left( \frac{\text{new size}}{\text{base size}} \right)^{0.7} \quad (2)$$

For the industrial process, the total investment is composed of 16 % for steam consumption in the reactor, 27 % for steam production and 57 % for other consumptions which is constant for all conditions of pretreatment. Changing residence time has an impact on the quantity of steam in the reactor and changing pretreatment temperature has an impact on the steam production. These data were obtained from industrial measurements at the FICAP plant (Bazancourt-Pomacle) and were used to calculate a total investment for each couple of conditions as follows (Eq. 3):

$$\text{Investment} = 0.16 \times \text{time RI} + 0.27 \times \text{temperature RI} + 0.57 \quad (3)$$

Finally, the total investments were brought to a percentage of additional charge in comparison to the base investment at lowest severity of pretreatment.

## 2.9. Statistical analyses

R software [42] was used to perform one-way analysis of variance (ANOVA) and pairwise *t*-test (Holm) to determine the significance of the results.

## 3. Results & discussion

### 3.1. Chemical composition

Wood composition was greatly affected by SE pretreatment (Table 1). Mass loss was directly correlated to pretreatment severity and increased up to 20 % for oak, 18 % for poplar and 30 % for spruce with R<sup>2</sup> = 0.89, R<sup>2</sup> = 0.85 and R<sup>2</sup> = 0.85, respectively. Mass loss was due to partial degradation of lignocellulosic constituents into volatile compounds during pretreatment (Supplementary Table 1), such as HMF from C6 sugars, furfural from C5 sugars, acetic acid from hemicellulose acetyl groups and formic acid from the further degradation of HMF and furfural. [43] This led to chemical modifications of the solid fraction of wood after pretreatment. As expected regarding the effect of SE, hemicellulose content was severely decreased as this polymer was almost totally degraded into HMF and furfural. Losses of 81 %, 74 % and 86 % were monitored for oak, poplar and spruce, respectively. Hemicelluloses are the most labile components of lignocellulosic matrix under acidic conditions, therefore they are first to degrade because of the autohydrolysis taking place during pretreatment. [44] Hemicelluloses degradation led consequently to cellulose and lignin enrichment in pretreated wood samples.

### 3.2. Bioethanol production

#### 3.2.1. Enzymatic saccharification

Untreated and pretreated wood samples underwent enzymatic saccharification using a commercial enzymatic cocktail containing cellulases. Fig. 3 (A-C) depicts the enzymatic saccharification efficiency as the recovery of fermentable C6 sugars (glucose and mannose) after 72 h of reaction. For each biomass species, SE improved enzymatic saccharification compared to untreated wood: fermentable sugars release was increased at least 3 times for oak, 4 times for poplar and 5 times for spruce, and at most 9 times for oak and 6 times for poplar and spruce.

But increase in pretreatment severity increased the saccharification efficiency of oak and poplar until a mid-range severity: after CSF = 3.95 and CSF = 3.79, respectively, a plateau with no significant differences (*p* > 0.05) appeared. While for spruce, increasing pretreatment severity did not improve enzymatic saccharification. This could be due to lignin structure as softwood lignin is mainly constituted of G units in opposition to hardwood lignin composed of both G and S units. Since G units

**Table 1**  
Mass yields after pretreatment and chemical composition of solid fraction.

Biomass	CSF	Mass yield after SE	Cellulose (% dry matter)	Hemicelluloses (% dry matter)	Lignin (% dry matter)	
Oak	Untreated	–	34.55 ± 1.40	20.24 ± 1.09	29.05 ± 0.24	
	3.35	100 %	41.38 ± 1.07	19.29 ± 0.66	28.89 ± 0.09	
	3.57	97.9 %	39.89 ± 0.53	15.73 ± 0.37	30.80 ± 0.11	
	3.75	94.4 %	39.86 ± 0.78	16.48 ± 0.04	30.34 ± 0.06	
	3.79	89.9 %	42.58 ± 0.63	10.77 ± 0.15	34.12 ± 0.42	
	3.95	91.6 %	41.03 ± 1.90	13.99 ± 0.14	32.28 ± 0.88	
	3.97	90.29 %	44.09 ± 0.65	11.49 ± 0.20	31.47 ± 0.31	
	3.97	91.63 %	42.30 ± 1.84	12.94 ± 0.26	33.68 ± 1.09	
	3.97	91.63 %	43.75 ± 1.66	12.80 ± 0.52	34.14 ± 0.33	
	4.17	87.74 %	46.29 ± 0.80	8.21 ± 0.11	35.24 ± 0.40	
	4.19	83.0 %	46.62 ± 1.40	6.28 ± 0.10	37.92 ± 1.52	
	4.39	80.5 %	48.57 ± 1.23	3.81 ± 0.07	37.27 ± 0.43	
	Poplar	Untreated	–	48.56 ± 0.28	20.12 ± 0.20	25.23 ± 1.19
		3.35	100 %	49.22 ± 0.46	20.64 ± 0.98	25.02 ± 0.52
		3.57	98.2 %	49.14 ± 0.40	17.87 ± 0.15	25.41 ± 0.43
3.75		92.5 %	51.32 ± 0.14	17.90 ± 0.17	25.61 ± 0.17	
3.79		95.3 %	51.64 ± 2.01	15.71 ± 0.52	27.51 ± 0.93	
3.95		96.4 %	53.09 ± 0.81	14.78 ± 0.08	27.86 ± 0.97	
3.97		90.96 %	50.66 ± 0.58	15.66 ± 0.16	27.58 ± 0.19	
3.97		90.96 %	51.32 ± 0.62	14.25 ± 0.32	28.64 ± 0.42	
3.97		90.96 %	54.22 ± 0.77	15.06 ± 0.02	28.72 ± 0.93	
4.17		86.56 %	50.76 ± 0.90	13.55 ± 0.15	29.98 ± 1.88	
4.19		89.0 %	54.71 ± 0.85	9.34 ± 0.30	30.49 ± 0.53	
4.39		81.7 %	58.50 ± 0.28	5.27 ± 0.07	32.24 ± 0.24	
Spruce		Untreated	–	48.81 ± 0.79	24.08 ± 0.38	27.27 ± 0.29
		3.57	92.3 %	48.71 ± 0.47	21.76 ± 0.42	29.68 ± 0.06
		3.72	94.0 %	49.06 ± 2.39	18.73 ± 0.41	30.99 ± 0.35
	3.72	89.08 %	47.96 ± 0.08	18.88 ± 0.35	31.75 ± 0.49	
	3.72	91.18 %	47.06 ± 1.09	19.35 ± 0.20	30.09 ± 0.50	
	3.86	78.6 %	50.09 ± 0.44	14.17 ± 0.22	33.77 ± 0.29	
	3.97	86.5 %	50.12 ± 2.69	16.00 ± 0.46	32.92 ± 0.05	
	4.11	78.0 %	49.22 ± 0.61	12.41 ± 0.13	34.04 ± 0.55	
	4.17	79.0 %	51.07 ± 0.75	11.57 ± 0.11	35.20 ± 2.26	
	4.26	73.0 %	49.28 ± 2.71	5.68 ± 0.06	36.40 ± 0.80	
	4.32	76.29 %	49.79 ± 2.88	6.78 ± 0.26	40.64 ± 0.68	
	4.47	69.7 %	51.08 ± 0.93	3.44 ± 0.05	40.82 ± 0.62	

were reported to interact more strongly through hydrophobic and hydrogen bonding with enzymes than S units, leading to a stronger inefficiency of the enzymes in case of spruce. [45–47] Another difference between hardwood or softwood species is hemicellulose composition. Xyloglucans are found in oak and poplar, while in spruce glucomannans and galactoglucomannans are more abundant. Mannans are known to cause competitive inhibition of cellobiohydrolases and irreversible inhibition of endoglucanases. [48] Both enzymes being glycoside hydrolases degrading cellulose, this likely explains spruce recalcitrant behavior to saccharification.

### 3.2.2. Fermentation

Hydrolysates containing the released sugars from the enzymatic saccharification were fermented using *Saccharomyces cerevisiae* and fermentation efficiency was calculated as the quantity of bioethanol produced with respect to the quantity of fermentable sugars available in hydrolysates (Fig. 3, D–F). No significant differences were found between samples from a same wood species ( $p > 0.05$ ), meaning that no fermentation inhibitors were generated from the pretreatment in sufficient quantity to impact bioethanol production. However, fermentation efficiency was dependent on the wood species with an average conversion percentage of 80 % for oak, 62 % for poplar and 58 % for spruce.

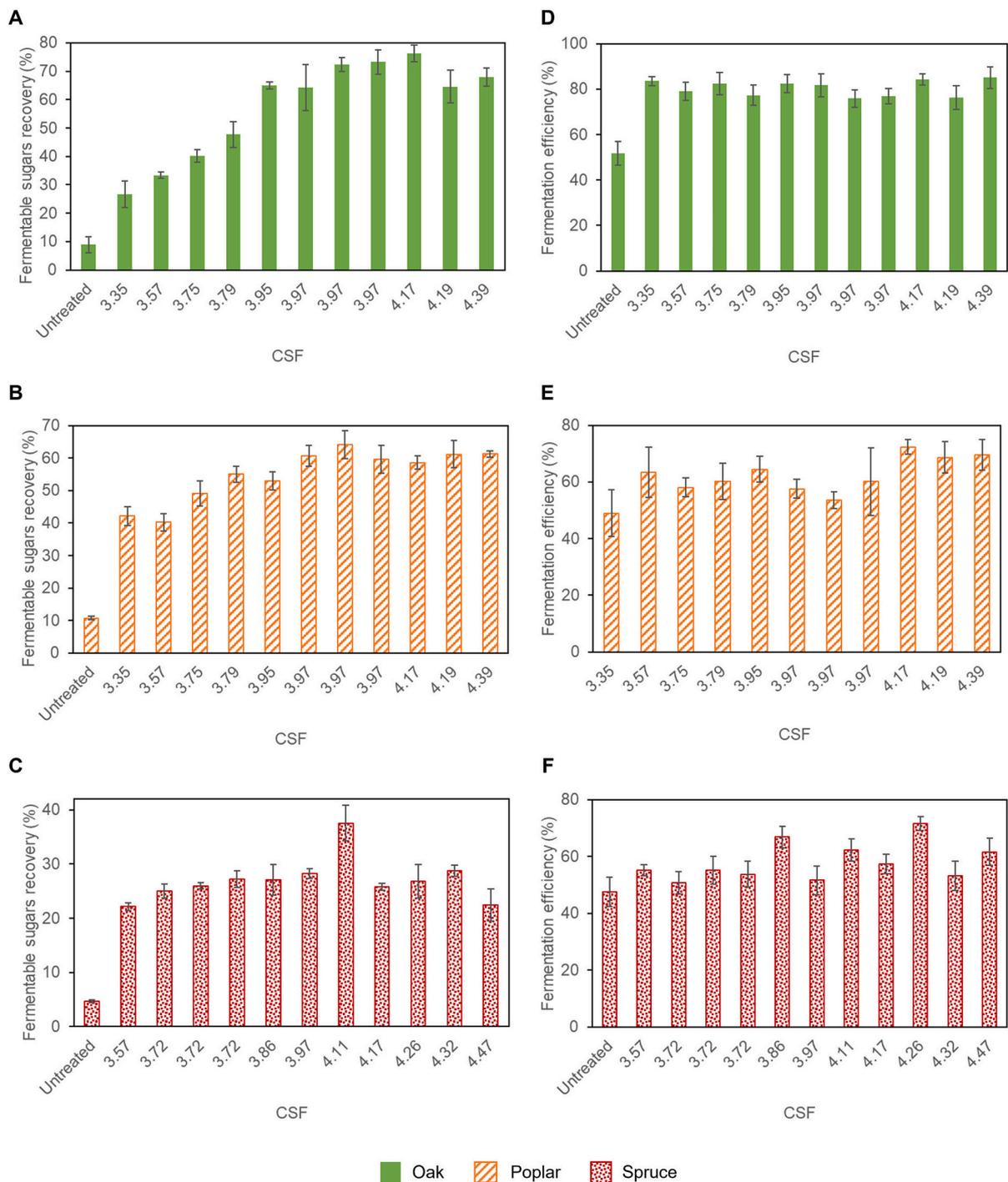
Bioethanol yields per gram of initial dry matter were calculated in order to take into account the mass loss during pretreatment (Fig. 4). Bioethanol production strongly and positively correlated to enzymatic saccharification with correlation coefficients equal to 0.96, 0.80 and 0.78 for oak, poplar and spruce, respectively, meaning bioethanol production mainly depends on enzymatic saccharification efficiency. Evolution of bioethanol yields was identical to that of hydrolysis yields since it also reached a plateau from mid-range severities for oak and poplar samples, while bioethanol yield from spruce remained constant. In average, 124.4 mg of bioethanol per gram (dry basis) of oak were recovered, 106.9 mg/g for poplar and 44.1 mg/g for spruce. Yields for oak and poplar were almost equivalent and this can be explained by the fact that untreated poplar contains more sugars and was less degraded during pretreatment, but saccharification and fermentation steps were less efficient for poplar than for oak.

### 3.2.3. Inhibitors quantification

Saccharification and fermentation inhibitors were quantified to investigate their potential role to explain the differences between the contrasted yields measured (Fig. 5). Main saccharification inhibitors are the phenolic compounds resulting from lignin degradation during steam explosion that can cause deactivation of cellulases. [49] Fermentation inhibitors are also phenolic compounds trapped in lignocellulosic structure that can be released after enzymatic hydrolysis which disrupt membrane integrity of microorganisms. Other molecules are detrimental to fermentation: the organic acids (formic acid and acetic acid) generated during pretreatment which acidify yeast cells cytoplasm leading to their death; and furfural and HMF resulting from sugar degradation which prevent yeast cell growth. [50]

Before saccharification, amounts of phenolics were equivalent in oak and poplar, and much lower in spruce (Fig. 5A). They increased with pretreatment severity, therefore it could impact the saccharification. But this should not be the main factor leading to the appearance of the saccharification plateau. Indeed, saccharification yields for poplar were inferior compared to oak, while the same contents of phenolics were measured in both woods, meaning this does not affect the saccharification process.

The same observations were made for fermentation inhibitors where the same contents were found for phenolics in hydrolysates (Fig. 5B), acetic acid (Fig. 5D) and HMF (Fig. 5E). Percentages in formic acid (Fig. 5C) and furfural (Fig. 5F) were even greater in oak, while its fermentation efficiency was the best. Moreover, even if inhibitor concentrations increased with severity, fermentation efficiency did not decrease, which means inhibitors generated by steam explosion did not



**Fig. 3.** Fermentable sugars recovery (A–C) and fermentation efficiency (D–F) depending on pretreatment severity and wood species.

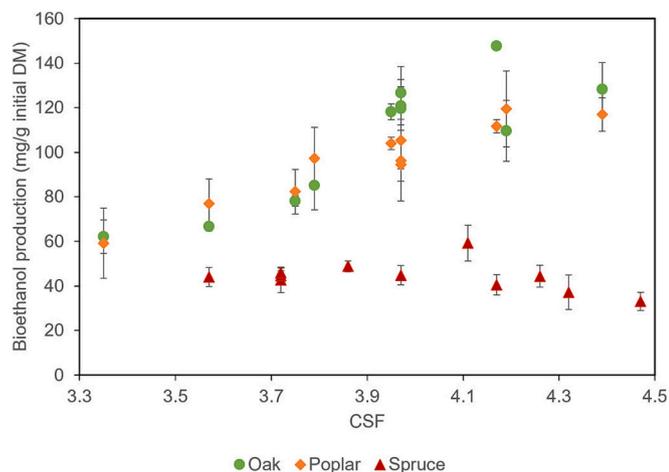
affect bioethanol production by the yeast used in our study. Presence of inhibitors does not explain differences between yields and thus other chemical and physical features such as interactions between polymers [51], accessible surface area [52] or particle size [53] known to play a key role in accessibility and digestibility of the substrate during enzymatic hydrolysis will be further investigated in future studies.

### 3.3. Modelling optimal conditions for bioethanol production

Before moving on to data processing, spruce sample pretreated at CSF 4.11 was removed from further data analysis as it clearly stood out from others and was considered as an outlier, especially as this sample

was pretreated apart from others. This prevented this sample to strongly influence the prediction and desirability models.

Bioethanol production response surfaces modelled from the experimental design data (Fig. 6) using eqs. 4 to 6 revealed that the optimal conditions of pretreatment needed for each wood species to maximize the bioethanol yields were slightly different: 199.1 °C / 20 min for oak (CSF 4.22), 205 °C / 19.4 min for poplar (CSF 4.38) and 207.5 °C / 5 min for spruce (CSF 3.86). The high adjusted  $R^2$  values (0.83 for oak, 0.93 for poplar and 0.90 for spruce) testify a good fit between experimental data and data predicted by the model.



**Fig. 4.** Bioethanol production from pretreated wood species. Bioethanol yields from oak (green circles), poplar (orange diamonds) and spruce (red triangles) are expressed taking into account the mass loss during pretreatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$$Bioethanol_{oak} = -18.074T^2 - 4.824t^2 + 10.8T + 30.05t - 3.2Tt + 118.189 \quad (4)$$

$$Bioethanol_{poplar} = 1.066T^2 - 5.584t^2 + 14.717T + 16.533t - 6.325Tt + 99.174 \quad (5)$$

$$Bioethanol_{spruce} = 1.125T^2 - 3.025t^2 - 0.517T - 4.3t - 3.075Tt + 43.475 \quad (6)$$

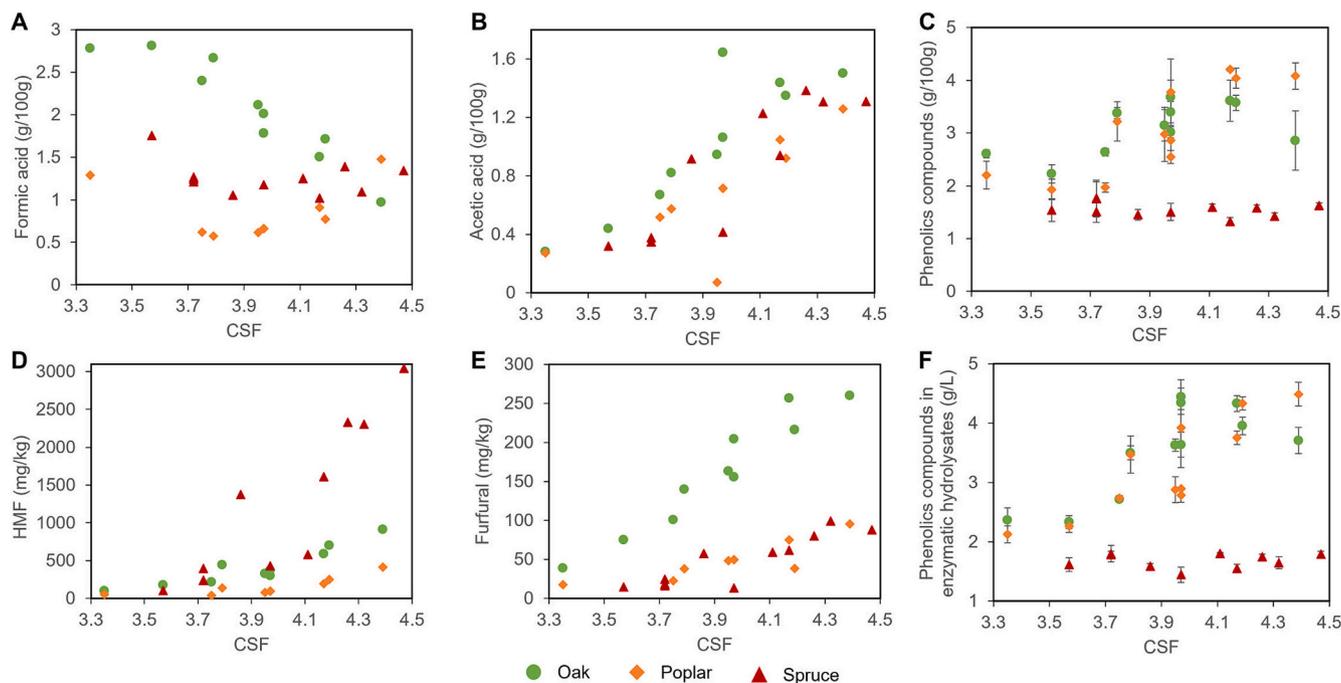
It is brought out from the response surfaces that too high temperatures of pretreatment were not efficient for oak (Fig. 6A). For poplar, optimal conditions being at the extremity of the domain under study suggests that yields could be further improved with higher conditions of

temperature and residence time (Fig. 6B). But this would require using another equipment with higher temperature limits. For spruce, long residence times were not efficient but increasing further the temperature could also improve bioethanol production (Fig. 6C). With these optimal conditions, maximum yields would be 144.2 mg/g dry basis, 119.6 mg/g dry basis and 48.4 mg/g dry basis which are equal to 79 %, 52 % and 20 % of bioethanol producible from oak, poplar and spruce, respectively.

Coefficients of eqs. 4 to 6 show that residence time has a stronger impact on bioethanol production for oak, while for poplar temperature and time have equivalent effects. For spruce, residence time also has a more important impact, but it is noteworthy that yields evolution with severity is moderate, with an increase of only 14 mg/g at most which is not significant given the standard deviations.

Yields for oak and poplar increase quicker for low severities: the first isocurves are closer to one another and are increasingly remote. The rapid increase can also be seen on 3D representations of the response surfaces (Supplementary Fig. 1). This highlights that SE helped to enhance bioethanol production from wood, but from a mid-range severity its efficiency was limited, maybe due to physicochemical features modified by pretreatment that could have a negative impact on saccharification or fermentation, as explained previously.

It is interesting to note here that the highest severity of pretreatment is not necessary the most efficient. Moreover, for oak and poplar, there is a whole zone of pretreatment conditions around the optimal point described above for which yields are almost equal. For instance, decreasing residence time to 15 min with temperature remaining at 199.1 °C results in a predicted bioethanol yield equal to 128.9 mg/g, *i.e.* a 15.3 mg/g decrease compared to optimum. Similarly, a decrease in temperature to 200 °C and residence time kept at 19.4 min for poplar results in a predicted bioethanol yield of 112.7 mg/g, *i.e.* a 6.9 mg/g decrease. These differences are not significant regarding the  $I_{95}$  (95 % confidence interval) for predicted optima which are equal to 36.7 mg/g and 16.1 mg/g for oak and poplar, respectively. But this emphasizes that multiple conditions of pretreatment can be used to obtain equivalent bioethanol quantities. The same observation was made in a study by Troncoso-Ortega et al. [54] for steam-exploded eucalyptus for which authors found the yield obtained for optimized conditions was not



**Fig. 5.** Saccharification and fermentation inhibitors in pretreated wood. Saccharification inhibitors: phenolics compounds (A), fermentation inhibitors: phenolics compounds after enzymatic hydrolysis (B), formic (C) and acetic (D) acids, HMF (E) and furfural (F).

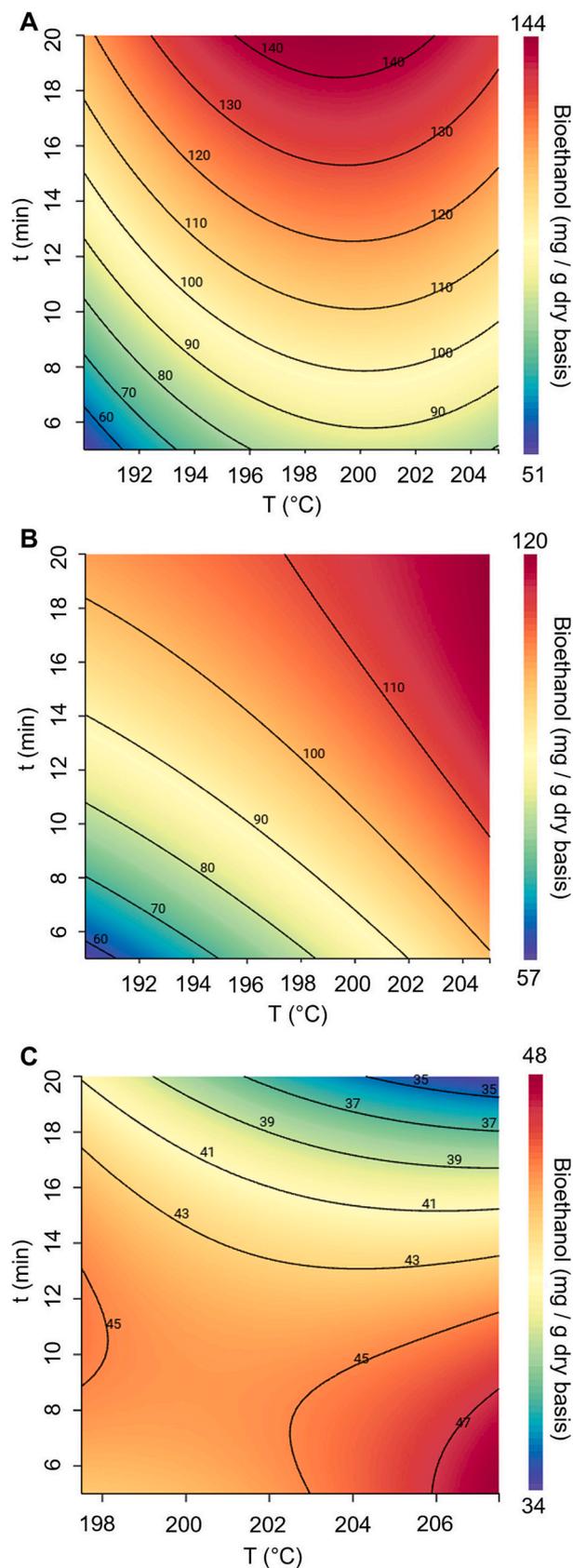


Fig. 6. Response surfaces of bioethanol production from steam exploded oak (A), poplar (B) and spruce (C).

significantly different from yields obtained with other pretreatment conditions. This result is key for industrial applications: if it is possible to lower temperature and residence time of pretreatment for an equivalent bioethanol yield, this enables to make 2G bioethanol production more cost-effective.

### 3.4. Economic desirability

As increasing SE severity did not significantly improve bioethanol production from spruce, the economic assessment of the process was done only for the conditions investigated for oak and poplar pretreatment.

The economic assessment (Table 2) revealed that it is more profitable to pretreat at high temperatures rather than for a long time. At equivalent severities of 3.75 and 3.79, additional cost is lower with pretreatment parameters set at 205 °C and 5 min (+6.4 %) instead of 190 °C and 12.5 min (+14.3 %). Increasing time of pretreatment thus results in a superior additional cost. Here, only the capital expenditure (CAPEX) was investigated in terms of reactor and steam generator sizing. Operational expenditure (OPEX) such as overconsumption of steam when increasing the residence time was not considered. Although if it were, it would amplify the economic impact of increasing residence time and would therefore lead to the same conclusion.

Economic assessment values were combined with bioethanol yields to calculate desirability functions to find trade-offs between maximizing bioethanol production while limiting the operating cost of SE process. Different scenarios with several maximum for bioethanol yield (85 %, 90 % or 95 % of optimum) and investment (+25 % or +32.5 %) were tested (Supplementary Fig. 2 and Supplementary Table 2). In each scenario, the optimal pretreatment temperature remained unchanged and only the optimal residence time increased when the desirability domain was reduced, which is concomitant with a higher CAPEX. Moreover, there was no real benefit to set a higher residence time according to the  $I_{95}$ , equal to 33.5 mg of bioethanol / g at least and 36.6 mg/g at most for oak, and 15.2 mg/g for poplar. For instance, the gain between an optimal bioethanol yield of 143 mg/g and 129 mg/g from oak wood is not significant regarding the  $I_{95}$ , and the same observations are done for poplar. Therefore, desirability parameters were set as follows: lower limit for bioethanol yield at 85 % of optimum value and higher limit for investment at +25 %.

The desirability functions were dependent on the wood species. Optimal bioethanol yield for oak was found in the center of the domain under study (Fig. 7A) while it was at the right of the temperature axis for poplar (Fig. 7B), likely meaning it could be improved by enlarging the design experiment area. The best trade-off for oak was found at 198.1 °C / 15.2 min corresponding to a bioethanol yield of 128.9 mg/g dry basis, while it was 205 °C / 9.9 min with a yield of 110.8 mg/g dry basis for poplar, corresponding to 70 % and 48 % of bioethanol producible, respectively. Compared to optimal yields calculated without desirability, this corresponds to a very moderate loss of 9 % and 4 % for oak and poplar, respectively. More importantly, CAPEX showed dramatic

Table 2

Economic assessment of the steam explosion process. Additional cost is determined regarding the investment needed in comparison to the lowest SE pretreatment severity.

CSF	Temperature (°C)	Residence time (min)	Additional cost
3.35	190	5	0 %
3.57	197.5	5	+3.6 %
3.75	190	12.5	+14.3 %
3.79	205	5	+6.4 %
3.95	190	20	+26.1 %
3.97	197.5	12.5	+17.4 %
4.17	197.5	20	+26.1 %
4.19	205	12.5	+20.7 %
4.39	205	20	+32.5 %

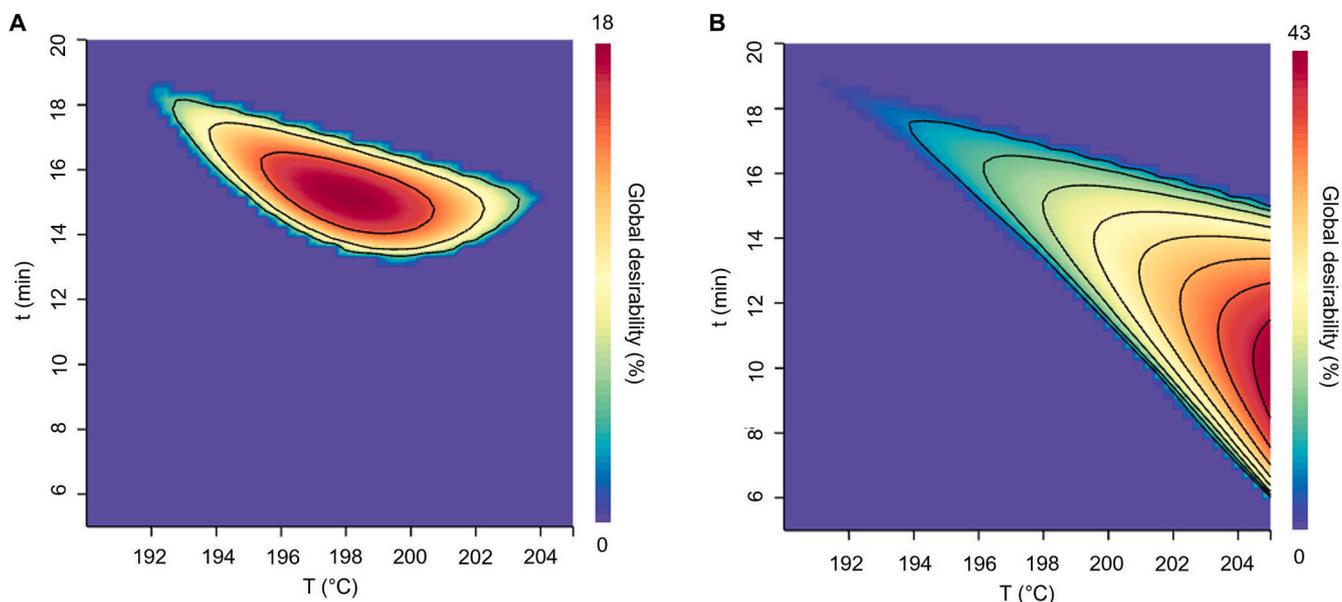


Fig. 7. Desirability function maximizing bioethanol yield and minimizing investment. A: desirability for oak, B: desirability for poplar.

decreases after desirability optimization: from +30 % to +22.5 % for oak and from +31.8 % to +16 % for poplar, corresponding nearly to a two-fold decrease for the latter. This demonstrates the interest of using such a desirability function to define optima more adequate with industrial applications.

#### 4. Conclusions

Response surface methodology and determination of desirability functions from experimental designs conducted on a large set of 33 contrasted samples highlighted the suitable conditions of SE pretreatment to maximize bioethanol production while limiting the cost of pretreatment in an industrial context. Results revealed that the efficiency of saccharification and fermentation steps was highly dependent on wood species. Increasing severity of pretreatment also played a positive role in the increase of yields until a mid-range severity after which a plateau was reached, demonstrating that the highest severity conditions of pretreatment were not the most appropriate. Inhibitors quantification did not explain the inter- and intra-species yield differences. Other physicochemical features likely have a positive or negative effect on saccharification and fermentation and should be further investigated. The sound scenarios proposed when combining data from the SE pretreatment severity and cost highlight the importance and the possibility of defining relevant trade-offs for bioethanol production in an industrial context.

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#### CRedit authorship contribution statement

**Edwige Audibert:** Writing – original draft, Investigation, Formal analysis, Conceptualization. **Juliette Floret:** Investigation. **Adriana Quintero:** Writing – review & editing, Resources. **Frédéric Martel:** Writing – review & editing, Supervision, Resources. **Caroline Rémond:** Writing – review & editing, Supervision, Conceptualization. **Gabriel Paës:** Writing – review & editing, Supervision, Conceptualization.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apenergy.2024.125028>.

#### References

- [1] Cantarella H, Mendes Souza G, Horta Nogueira L, Maciel Filho R, Costa de Paiva G, Islongo Canabarro N, et al. Assessment of successes and lessons learned for biofuels deployment – Report Work package 2 | Meta-analysis of existing studies. 2023.
- [2] Devi A, Bajar S, Sihag P, Sheikh ZUD, Singh A, Kaur J, et al. A panoramic view of technological landscape for bioethanol production from various generations of feedstocks. *Bioengineered* 2023;14:81–112. <https://doi.org/10.1080/21655979.2022.2095702>.
- [3] Directive (EU). 2018/2001 of the European Parliament and of the Council of 11 December 2018 on the promotion of the use of energy from renewable sources. 2024.
- [4] Guo Y, Liu G, Ning Y, Li X, Hu S, Zhao J, et al. Production of cellulosic ethanol and value-added products from corn fiber. *Bioresour Bioprocess* 2022;9:81. <https://doi.org/10.1186/s40643-022-00573-9>.
- [5] Mironova GF, Budaeva VV, Skiba EA, Gismatulina YA, Kashcheyeva EI, Sakovich GV. Recent advances in *Miscanthus* macromolecule conversion: a brief overview. *IJMS* 2023;24:13001. <https://doi.org/10.3390/ijms241613001>.
- [6] El Hage M, Louka N, Rezzoug S-A, Maugard T, Sablé S, Koubaa M, et al. Bioethanol production from Woody Biomass: recent advances on the effect of pretreatments on the bioconversion process and energy yield aspects. *Energies* 2023;16:5052. <https://doi.org/10.3390/en16135052>.
- [7] Isikgor FH, Becer CR. Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. *Polym Chem* 2015;6:4497–559. <https://doi.org/10.1039/C5PY00263J>.

- [8] Zhao X, Zhang L, Liu D, Biomass recalcitrance.. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. *Biofuels Bioprod Biorefin* 2012;6:465–82. <https://doi.org/10.1002/bbb.1331>.
- [9] Periyasamy S, Karthik V, Senthil Kumar P, Isabel JB, Temesgen T, Hunegnaw BM, et al. Chemical, physical and biological methods to convert lignocellulosic waste into value-added products. A review *Environ Chem Lett* 2022;20:1129–52. <https://doi.org/10.1007/s10311-021-01374-w>.
- [10] Galbe M, Wallberg O. Pretreatment for biorefineries: a review of common methods for efficient utilisation of lignocellulosic materials. *Biotechnol Biofuels* 2019;12:294. <https://doi.org/10.1186/s13068-019-1634-1>.
- [11] Yu Y, Wu J, Ren X, Lau A, Rezaei H, Takada M, et al. Steam explosion of lignocellulosic biomass for multiple advanced bioenergy processes: a review. *Renew Sust Energ Rev* 2022;154:111871. <https://doi.org/10.1016/j.rser.2021.111871>.
- [12] Ruiz HA, Sganzerla WG, Larnaude V, Veersma RJ, Van Erven G, Shiva, et al. Advances in process design, techno-economic assessment and environmental aspects for hydrothermal pretreatment in the fractionation of biomass under biorefinery concept. *Bioresour Technol* 2023;369:128469. <https://doi.org/10.1016/j.biortech.2022.128469>.
- [13] Ziegler-Devin I, Chrusciel L, Brosse N. Steam explosion pretreatment of lignocellulosic Biomass: a Mini-review of Theoretical and experimental approaches. *Front Chem* 2021;9:705358. <https://doi.org/10.3389/fchem.2021.705358>.
- [14] Ruiz HA, Conrad M, Sun S-N, Sanchez A, Rocha GJM, Romani A, et al. Engineering aspects of hydrothermal pretreatment: from batch to continuous operation, scale-up and pilot reactor under biorefinery concept. *Bioresour Technol* 2020;299:122685. <https://doi.org/10.1016/j.biortech.2019.122685>.
- [15] Zhao W, Zhang J, Zhang W, Yuan S, Chen H, Wu A. Effect of bamboo unit morphology on the preparation of bamboo fibers by steam explosion. *Ind Crop Prod* 2023;202:117066. <https://doi.org/10.1016/j.indcrop.2023.117066>.
- [16] Valdivia M, Galan JL, Laffarga J, Ramos J. Biofuels 2020: biorefineries based on lignocellulosic materials. *Microb Biotechnol* 2016;9:585–94. <https://doi.org/10.1111/1751-7915.12387>.
- [17] Lynd LR, Liang X, Biddy MJ, Allee A, Cai H, Foust T, et al. Cellulosic ethanol: status and innovation. *Curr Opin Biotechnol* 2017;45:202–11. <https://doi.org/10.1016/j.copbio.2017.03.008>.
- [18] Shaji A, Shastry Y, Kumar V, Ranade VV, Hindle N. Economic and environmental assessment of succinic acid production from sugarcane bagasse. *ACS Sustain Chem Eng* 2021;9:12738–46. <https://doi.org/10.1021/acssuschemeng.1c02483>.
- [19] Bernardi AV, Gerolamo LE, Dinamarco TM, Tapia-Blácido DR. Optimization of biomass saccharification processes with experimental design tools for 2G ethanol production: a review. *Biofuels Bioprod Biorefin* 2023;17:1789–806. <https://doi.org/10.1002/bbb.2534>.
- [20] Chen W-H, Lo H-J, Aniza R, Lin B-J, Park Y-K, Kwon EE, et al. Forecast of glucose production from biomass wet torrefaction using statistical approach along with multivariate adaptive regression splines, neural network and decision tree. *Appl Energy* 2022;324:119775. <https://doi.org/10.1016/j.apenergy.2022.119775>.
- [21] LMA Chiarello, Ramos CE, LFF Dos Santos, MHL Silveira, Zaccaron S, Schiehsler S, et al. Characterization of pretreated fractions and cellulose ethanol production from steam-exploded *Eucalyptus urograndis*. *Energy Fuel* 2020;34:535–45. <https://doi.org/10.1021/acs.energyfuels.9b03405>.
- [22] Nitsos CK, Choli-Papadopoulou T, Matis KA, Triantafyllidis KS. Optimization of hydrothermal pretreatment of hardwood and softwood lignocellulosic residues for selective hemicellulose recovery and improved cellulose enzymatic hydrolysis. *ACS Sustain Chem Eng* 2016;4:4529–44. <https://doi.org/10.1021/acssuschemeng.6b00535>.
- [23] Yuan S, Ji X, Ji H, Tian Z, Chen J. An optimum combined severity factor improves both the enzymatic saccharification yield and the functional lignin structure. *Cellulose* 2019;26:4731–42. <https://doi.org/10.1007/s10570-019-02442-9>.
- [24] López-Linares JC, Ballesteros I, Tourán J, Cara C, Castro E, Ballesteros M, et al. Optimization of uncatalyzed steam explosion pretreatment of rapeseed straw for biofuel production. *Bioresour Technol* 2015;190:97–105. <https://doi.org/10.1016/j.biortech.2015.04.066>.
- [25] Pérez-Pimentá JA, Papa G, Gladden JM, Simmons BA, Sanchez A. The effect of continuous tubular reactor technologies on the pretreatment of lignocellulosic biomass at pilot-scale for bioethanol production. *RSC Adv* 2020;10:18147–59. <https://doi.org/10.1039/D0RA04031B>.
- [26] Sulzenbacher D, Atzmüller D, Hawe F, Richter M, Cristobal-Sarramian A, Zwirzitz A. Optimization of steam explosion parameters for improved biotechnological use of wheat straw. *Biomass Convers Biorefinery* 2023;13:1035–46. <https://doi.org/10.1007/s13399-020-01266-z>.
- [27] Guignon M, Guarino J, Chiarello LM, Cabrera MN, Vique M, Lareo C, et al. Steam explosion of *Eucalyptus grandis* sawdust for ethanol production within a biorefinery approach. *Processes* 2023;11:2277. <https://doi.org/10.3390/pr11082277>.
- [28] Ding K, Liu D, Chen X, Zhang H, Shi S, Guo X, et al. Scalable lignocellulosic biorefineries: Technoeconomic review for efficient fermentable sugars production. *Renew Sust Energ Rev* 2024;202:114692. <https://doi.org/10.1016/j.rser.2024.114692>.
- [29] Vasilakou K, Nimmegeers P, Thomassen G, Billen P, Van Passel S. Assessing the future of second-generation bioethanol by 2030 – a techno-economic assessment integrating technology learning curves. *Appl Energy* 2023;344:121263. <https://doi.org/10.1016/j.apenergy.2023.121263>.
- [30] Shibukawa VP, Ramos L, Cruz-Santos MM, Prado CA, Jofre FM, De Arruda GL, et al. Impact of product diversification on the economic sustainability of second-generation ethanol biorefineries: a critical review. *Energies* 2023;16:6384. <https://doi.org/10.3390/en16176384>.
- [31] Vasilakou K, Nimmegeers P, Billen P, Van Passel S. Geospatial environmental techno-economic assessment of pretreatment technologies for bioethanol production. *Renew Sust Energ Rev* 2023;187:113743. <https://doi.org/10.1016/j.rser.2023.113743>.
- [32] Priadi H, Awad S, Villot A, Andres Y, Purwanto WW. Techno-enviro-economic analysis of second-generation bioethanol at plant-scale by different pre-treatments of biomass from palm oil waste. *Energy Convers Manage*; X 2024;21:100522. <https://doi.org/10.1016/j.ecmx.2023.100522>.
- [33] Raina N, Boonmee R, Kirdponpattara S, Narasingha M, Sriariyanun M, Phitsuwon P, et al. Process performance evaluation of different chemical pretreatments of lignocellulosic biomass for bioethanol production. *Ind Crop Prod* 2024;211:118207. <https://doi.org/10.1016/j.indcrop.2024.118207>.
- [34] López-Sandin I, Rodríguez-Jasso RM, Gutiérrez-Soto G, Rosero-Chasoy G, Shiva González-Gloria KD, et al. Energy assessment of second-generation (2G) bioethanol production from sweet Sorghum (*Sorghum bicolor* (L.) Moench) bagasse. *Agronomy* 2022;12:3106. <https://doi.org/10.3390/agronomy12123106>.
- [35] Despres JL, Quintero Marquez A, Martel F. Procédé de fabrication en continu d'une matière combustible pour chaudière industrielle, matière et installation correspondantes. FR3087789-B1. 2020.
- [36] Overend RP, Chornet E. Fractionation of lignocellulosics by steam-aqueous pretreatments. *Phil Trans R Soc Lond A* 1987;321:523–36. <https://doi.org/10.1098/rsta.1987.0029>.
- [37] Azurad. 2019.
- [38] Auxenfans T, Crônier D, Chabbert B, Paës G. Understanding the structural and chemical changes of plant biomass following steam explosion pretreatment. *Biotechnol Biofuels* 2017;10:36. <https://doi.org/10.1186/s13068-017-0718-z>.
- [39] Adney B, Baker J. *Measurement of Cellulase activities*. 2008.
- [40] Herbaut M, Zoghli A, Habrant A, Falourd X, Foucat L, Chabbert B, et al. Multimodal analysis of pretreated biomass species highlights generic markers of lignocellulose recalcitrance. *Biotechnol Biofuels* 2018;11:52. <https://doi.org/10.1186/s13068-018-1053-8>.
- [41] Humbird D, Davis R, Tao L, Kinchin C, Hsu D, Aden A. *Process design and economics for biochemical conversion of lignocellulosic Biomass to ethanol*. 2011.
- [42] R Core Team. 2022.
- [43] Kim D. Physico-chemical conversion of lignocellulose: inhibitor effects and detoxification strategies: a Mini review. *Molecules* 2018;23:309. <https://doi.org/10.3390/molecules23020309>.
- [44] Ibbett R, Gaddipati S, Davies S, Hill S, Tucker G. The mechanisms of hydrothermal deconstruction of lignocellulose: new insights from thermal-analytical and complementary studies. *Bioresour Technol* 2011;102:9272–8. <https://doi.org/10.1016/j.biortech.2011.06.044>.
- [45] Li X, Zheng Y. Lignin-enzyme interaction: mechanism, mitigation approach, modeling, and research prospects. *Biotechnol Adv* 2017;35:466–89. <https://doi.org/10.1016/j.biotechadv.2017.03.010>.
- [46] Qin L, Li W-C, Liu L, Zhu J-Q, Li X, Li B-Z, et al. Inhibition of lignin-derived phenolic compounds to cellulase. *Biotechnol Biofuels* 2016;9:70. <https://doi.org/10.1186/s13068-016-0485-2>.
- [47] Sun S, Huang Y, Sun R, Tu M. The strong association of condensed phenolic moieties in isolated lignins with their inhibition of enzymatic hydrolysis. *Green Chem* 2016;18:4276–86. <https://doi.org/10.1039/C6GC00685J>.
- [48] Xin D, Yang M, Chen X, Ma L, Zhang J. Recovering activities of inactivated Cellulases by the use of Mannanase in spruce hydrolysis. *ACS Sustain Chem Eng* 2017;5:5265–72. <https://doi.org/10.1021/acssuschemeng.7b00605>.
- [49] Dos Santos AC, Ximenes E, Kim Y, Ladisch MR. Lignin-Enzyme Interactions in the Hydrolysis of Lignocellulosic Biomass. *Trends Biotechnol* 2019;37:518–31. <https://doi.org/10.1016/j.tibtech.2018.10.010>.
- [50] Ko JK, Um Y, Park Y-C, Seo J-H, Kim KH. Compounds inhibiting the bioconversion of hydrothermally pretreated lignocellulose. *Appl Microbiol Biotechnol* 2015;99:4201–12. <https://doi.org/10.1007/s00253-015-6595-0>.
- [51] Chen Y, Yang D, Tang W, Ma C, He Y-C. Improved enzymatic saccharification of bulrush via an efficient combination pretreatment. *Bioresour Technol* 2023;385:129369. <https://doi.org/10.1016/j.biortech.2023.129369>.
- [52] Torr KM, Love KT, Simmons BA, Hill SJ. Structural features affecting the enzymatic digestibility of pine wood pretreated with ionic liquids. *Biotechnol Bioeng* 2016;113:540–9. <https://doi.org/10.1002/bit.25831>.
- [53] Vaidya AA, Donaldson LA, Newman RH, Suckling ID, Campion SH, Lloyd JA, et al. Micromorphological changes and mechanism associated with wet ball milling of *Pinus radiata* substrate and consequences for saccharification at low enzyme loading. *Bioresour Technol* 2016;214:132–7. <https://doi.org/10.1016/j.biortech.2016.04.084>.
- [54] Troncoso-Ortega E, Valenzuela R, Reyes-Contreras P, Castaño-Rivera P, Schiappacasse L-N, Parra C. Maximizing bioethanol production from *Eucalyptus globulus* using steam explosion pretreatment: a multifactorial design and fermenter development for high solid loads. *Fermentation* 2023;9:965. <https://doi.org/10.3390/fermentation9110965>.