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1 **Towards the development of a novel “bamboo-refinery” concept: selective bamboo**  
2 **fractionation by means of a microwave-assisted, acid-catalysed, organosolv process**

3  
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12  
13 **Abstract**

14 This work addresses a novel microwave-assisted, acid-catalysed, organosolv (EtOH/H<sub>2</sub>O)  
15 system for the selective fractionation of bamboo, examining the effects of the temperature  
16 (110-190 °C), solvent system (EtOH/H<sub>2</sub>O) and catalyst amount (0-5 vol.% formic acid) on  
17 the process. The statistical analysis of the results revealed that the operating variables have a  
18 significant influence on bamboo fractionation, allowing the selective production of i) a  
19 cellulose-rich solid fraction, ii) a hemicellulose rich water-soluble fraction and iii) a lignin  
20 rich solid fraction. The yields of each of these fractions varied between 51-94%, 2-23% and  
21 2-32%, respectively. Increasing temperature exerted a positive effect on bamboo  
22 decomposition, increasing the overall bamboo conversion and influencing the effect that the  
23 solvent system (EtOH/H<sub>2</sub>O) has on the process. At low temperature (110 °C) the solvent  
24 system does not have much influence, while a synergetic interaction between EtOH and H<sub>2</sub>O  
25 took place at higher temperatures, which allowed better results to be obtained with  
26 EtOH/H<sub>2</sub>O mixtures than with the pure solvents alone. The effect of the catalyst was  
27 relatively weak, being greatest when using a high temperature (190 °C) and high proportions  
28 of water (>85 vol.%) in the solvent system. With respect to the properties of each fraction,  
29 the cellulose rich solid fraction was made up of un-reacted cellulose (44-83 wt.%),  
30 hemicellulose (0-21 wt.%) and lignin (12-34 wt.%); the water-soluble hemicellulose rich

31 fraction consisted of a mixture of oligomers, sugars, carboxylic acids, ketones and furans;  
32 and the solid rich lignin fraction comprised high purity (>95 wt.%) organosolv lignin. The  
33 optimisation of the process revealed that by using a temperature of 190 °C, a solvent system  
34 consisting of 45 vol.% EtOH and 55 vol.% H<sub>2</sub>O with a concentration of formic acid of 5 vol.%  
35 it is possible to fractionate bamboo into a high purity (84 wt.%) cellulose solid fraction, very  
36 pure (>95%) organosolv lignin and a rich water-soluble hemicellulose fraction consisting of a  
37 mixture of oligomers (27 wt.%), sugars (56 wt.%) and carboxylic acids (14 wt.%); thus  
38 converting this process into a very promising method for the selective fractionation of  
39 bamboo.

40

41 **Keywords:** microwaves, bamboo, organosolv, biomass fractionation, value-added products

42

43 **Highlights (85 characters including spaces)**

44

- 45 - Microwave-assisted bamboo fractionation into cellulose, hemicellulose and lignin
- 46 - Analysis and optimisation of the temperature, EtOH/H<sub>2</sub>O system and catalyst amount
- 47 - Synergetic effect between EtOH & H<sub>2</sub>O during bamboo fractionation at high temperature
- 48 - Optimum conditions for bamboo fractionation: 190 °C, 45% EtOH/H<sub>2</sub>O and 5 vol.%  
49 HCOOH

50

51

52

## 53 **1. Introduction**

54 Throughout the world new policies to mitigate global climate change have led researchers to  
55 seek new processes, alternative renewable materials and more sustainable strategies to  
56 replace the current petroleum-based energy industry with a greener and more  
57 environmentally-friendly energy market. As part of this, the bio-refinery concept is gaining  
58 increasing attention, as biomass is the only renewable source of carbon that can be converted  
59 into gas, liquid and solid products [1]. Unfortunately, the development of a lignocellulosic  
60 biorefinery has received some criticism, since energy and chemical production in this way  
61 could affect the food supply and total forestry area. To overcome this issue, researchers are  
62 looking for fast growing and low “food-conflict” biomasses.

63

64 Among all the potential biomass feedstocks, bamboo is regarded as an excellent candidate.  
65 Bamboo is a big wooden plant large, which is widely distributed in subtropical temperature  
66 zones around the world (latitudes from 46°N to 47°S) [2]. As one of the fastest growing  
67 woody plants, bamboo can grow at a rate of 30-60 cm/day to 15-40 m with a culm diameter  
68 of 30 cm [2, 3]. Due to its fast-growing property, bamboo generally could produce a higher  
69 yield of biomass than other common crops [4]. Typically, bamboo contains 37-47 wt.%  
70 cellulose, 15-30 wt.% of hemicellulose and 18-31 wt.% lignin, depending on the species [4].  
71 In the past, bamboo was widely cultivated in Asian countries and used as a raw material for  
72 food, handicrafts, paper and construction. In recent years, bamboo has shown a huge potential  
73 to be used for the production of biofuel and other value-added chemicals via bioconversion  
74 [4-7].

75

76 Bioconversion is believed to be one of the most attractive methods for lignocellulosic  
77 biomass utilisation [8, 9]; enzymes and bio-catalytically active cells (fermentation) being the  
78 two pathways commonly employed [10]. This biotechnology is mainly used for bioethanol,  
79 biodiesel, biobutanol, methane and fine chemicals production [11]. In the past few years,  
80 bioconversion technology has undergone dramatic growth, which has increased the attention  
81 of researchers and industry. Nevertheless, the intrinsic complexity of biomass hampers the  
82 development of this technology, and more research is needed. In particular, within the  
83 lignocellulosic biomass structure, cellulose and hemicellulose are intimately associated with  
84 lignin in the cell wall, and part of the cellulose is in crystalline status. This hinders enzymes  
85 and microbes from achieving biomass digestion and, therefore, the pre-treatment of biomass  
86 for its selective fractionation into its main constituents (cellulose, hemicellulose and lignin) is  
87 still a challenge.

88

89 In the midst of the different strategies for biomass fractionation, the use of water/organic  
90 solvent (organosolv) processes is gaining increasing attention. Several works have been  
91 conducted with rice straw [12], cotton stalk [13], wheat straw [14-16], hemp hurds [17, 18],  
92 corncob [19, 20], maple wood [21], reed [22], corn stover [23] and bamboo [24, 25]. This  
93 route is well known for its higher efficiency in removing lignin under acidic condition than  
94 other processing methods. Organosolv systems can be classified into two categories: pure  
95 organic (simple) and aqueous-organic solvent (multiple) systems. In general, aqueous-organic  
96 systems perform better than single solvent systems [26, 27]. The multiple systems consist of  
97 a nucleophile agent ( $H_2O$ , in the vast majority of the cases, to react with the activated  
98 linkages in biomass) and an organic solvent to help dissolve the liberated fragments [20].  
99 This water/organic mixture has a significant influence on biomass depolymerization kinetics  
100 and thermodynamics, which affects not only the solubility of biomass but also the dissolution

101 of biomass-derived products. This is of particular interest for the solubilisation of lignin  
102 which has both nonpolar and polar functional groups [28]. In addition, a homogeneous acid  
103 catalyst is used in some cases to increase the kinetics of biomass depolymerization in  
104 organosolv systems and, therefore, help increase the overall efficiency of the process.

105

106 Regarding the organic compounds used in H<sub>2</sub>O/co-solvent systems, alcohols are the most  
107 commonly used as they have a low boiling point and can efficiently fractionate biomass into  
108 relatively high purity lignin and cellulose for further applications [29]. In particular, Hu et al.  
109 [30] regarded the H<sub>2</sub>O/ethanol organosolv system catalysed by oxalic acid as one of the  
110 greenest co-solvent systems for biomass fractionation. They reported that by using this  
111 organosolv system and employing a temperature of 140°C and a pressure of 2MPa for 1h, it  
112 was possible to recover 88 wt.% of the hemicellulose and 89 wt.% of the lignin present in  
113 corn stover, the cellulose remaining in the solid residue. Quignard et al. [16] worked on the  
114 extraction of lignin from wheat straw by using a H<sub>2</sub>O-ethanol organosolv system at 160°C for  
115 2h, examining the effect of different homogeneous Lewis acidic catalysts, such as FeCl<sub>2</sub>,  
116 CuCl<sub>2</sub>, FeCl<sub>3</sub>, Ga(OTf)<sub>3</sub>, and ZrOCl<sub>2</sub>. It was found that lignin extraction was related to the  
117 acidity of the catalyst, with a maximum of 80% lignin conversion. Methanol has also been  
118 used in water-organic systems. In particular, Shimizu and Usami [31] used a catalysed (0.2  
119 wt.% of HCl) methanol/H<sub>2</sub>O mixture at 170 °C for 45 min to fractionate pine wood,  
120 achieving very high hemicellulose (100%) and lignin (90%) recoveries. In addition, high  
121 boiling point alcohols, such as ethylene glycol and glycerol, have also been tested in the  
122 presence of H<sub>2</sub>O for organosolv processes. This allows a low-pressure treatment to be  
123 conducted, but hinders solvent removal; thus decreasing the energy efficiency of the overall  
124 treatment [29].

125 Besides alcohols, other organic solvents have also been tested with positive results for  
126 biomass fractionation. For example, the H<sub>2</sub>O/ $\gamma$ -valerolactone (GVL) co-solvent system was  
127 positively used for the simultaneous separation of lignin from corn stover at 150 °C for 30  
128 min, employing H<sub>2</sub>SO<sub>4</sub> as a catalyst, achieving high yields for both lignin (75%) and  
129 hemicellulose (72%) [23]. H<sub>2</sub>O/tetrahydrofuran (THF) assisted by Na<sub>2</sub>CO<sub>3</sub> also showed 94.6%  
130 conversion of lignin from corncob residue, at 2 MPa of pressure at 140 °C for 1h, preventing  
131 the dissolution of cellulose [20]. Wyman et al. [21] compared the difference between two  
132 H<sub>2</sub>SO<sub>4</sub> catalysed systems: H<sub>2</sub>O/tetrahydrofuran (THF) and pure H<sub>2</sub>O for maple wood  
133 degradation. The experiments were conducted at temperatures ranging 170-200°C with  
134 several reaction times (40-120 min). This comparison revealed that THF could significantly  
135 boost the efficiency of organosolv processes due to the good solubility of biomass  
136 decomposition products in THF, with more than 90% lignin recovery [21]. Furthermore,  
137 several organic acids were also tested in organosolv processes. Sun et al. [25] used an acetic  
138 acid-water system catalysed with HCl to extract lignin from bamboo. 95% of lignin and 48%  
139 of cellulose were recovered. The optimal delignification degree was achieved with 4 wt.%  
140 HCl in a 90 wt.% acetic acid-water solution using a batch reactor at 114°C for 2 h.

141

142 All these works published to date provide valuable information on the use of different  
143 organosolv systems for biomass fractionation. However, the work on analysing bamboo  
144 fractionation is very scarce [24, 25, 32-34] and, consequently, more research is needed for the  
145 valorisation of this biomass. In addition, these studies showed that the reaction conditions  
146 (especially the temperature, solvent system and catalyst) exert a significant influence on  
147 biomass fractionation, however parametric studies to thoroughly analyse the specific effect of  
148 the variables have never conducted. Therefore, the large number of factors significantly  
149 influencing the process increases its intrinsic complexity. Moreover, some interactions

150 between some of these factors could occur so that the effects of some variables may depend  
151 on others, resulting in different consequences for the process. These interactions have never  
152 been considered in the works addressing organosolv processes for biomass fractionation. In  
153 addition, optimum conditions for bamboo fractionation and synergetic effects between  
154 solvents have never been analysed in depth. Given this background, this work addresses the  
155 microwave-assisted fractionation of bamboo by means of an ethanol/water organosolv  
156 process catalysed by formic acid. In particular, the effects of the ethanol/water concentration  
157 (0-100 vol.%), catalyst (formic acid) amount (0-5 vol.%) and temperature (110-190 °C)  
158 together with all the possible interactions between these variables on bamboo fractionation  
159 have been analysed in depth. Microwave heating represents a potentially faster, more  
160 efficient and selective process for the thermal treatment of biomass [35, 36]. As water and  
161 ethanol are highly effective in microwave energy absorption, the combination of an  
162 organosolv system together with microwave assisted heating offers an interesting new  
163 technology for the valorisation not only of bamboo but also of many other types of biomass  
164 The fact that the combined effects of the operating variables and their interactions on an  
165 organosolv system has never been reported before demonstrate, together with the results  
166 provided by the in-depth study and the optimisation and energetic assestement conducted,  
167 that this work represents a novel investigation in this field, which can help to develop a novel  
168 biorefinery concept based on bamboo fractionation, “the bamboo-refinery”.

169

## 170 **2. Experimental**

### 171 **2.1 Material**

172 Dried bamboo (*Phyllostachys heterocycle cv. pubescens*) was purchased from Anji County,  
173 Zhejiang Province, China. The raw bamboo was ground to 300 meshes. After grounding, the



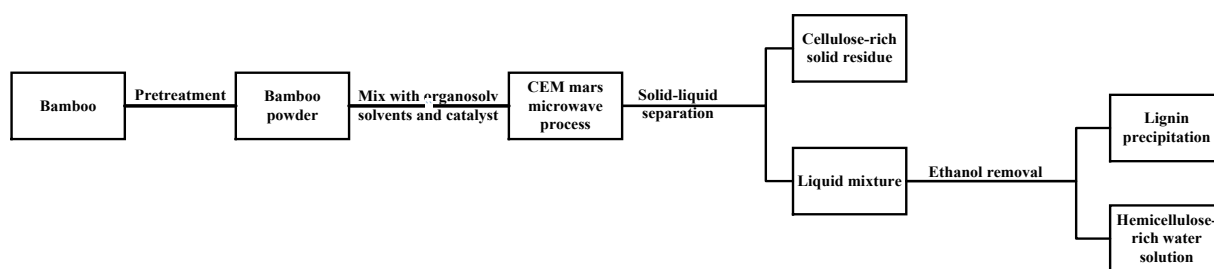
174 bamboo powder was washed by 50°C distilled water and then dried overnight at 105°C. This  
175 bamboo powder was characterised by means of proximate, ultimate and fibre (cellulose,  
176 hemicellulose and lignin) analyses, calorific value and ash content. Proximate analyses were  
177 performed according to standard methods (ISO-589-1981 for moisture, ISO-1171-1976 for  
178 ash and ISO-5623-1974 for volatiles). Elemental analysis, based on carbon, hydrogen and  
179 nitrogen content, was carried out using an Exeter Analytical (Warwick, UK) CE440  
180 Elemental Analyser, calibrated against acetanilide with a S-benzyl-thiuronium chloride  
181 internal standard. Fibre characterisation was performed by using the chemical titration  
182 method described by Hu et al. [24] to determine the amount of cellulose, hemicellulose and  
183 lignin in the material. In addition, Inductively Coupled Plasma Mass Spectrometry (ICP-MS)  
184 was used to identify and quantify the amounts of metals.

185

## 186 **2.2 Microwave experiments**

187 The experiments were carried out in a CEM-Mars microwave system using a 70 mL scale  
188 PrepPlus® reactor copped with a fibre optic temperature sensor. For each experiment, 1.50 g  
189 bamboo powder along with 30 mL solvent (EtOH+H<sub>2</sub>O) and catalyst (formic acid) mixture  
190 were loaded into the reactor. Before placing the reactor inside the microwave unit, the  
191 reaction mixture was pre-stirred at room temperature for 10 min. A ramping time (time to  
192 reach the reaction temperature) and a reaction time of 5 min and 15 min were respectively  
193 used for all the experiments. After reaction, the reactor cooled down to room temperature by  
194 CEM-Mars default programme. Then, the reactor was opened and its content, consisting of a  
195 mixture of liquid and solid, was transferred to a centrifuge tube. After that, the reactor was  
196 carefully rinsed with 10 mL of an ethanol/water (50 vol.%) solution for 3 times and  
197 transferred to the centrifuge tube to recover all the material. Centrifugation was used to

198 separate the solid from the liquid. Then, the solid residue obtained after centrifugation was  
 199 rinsed with the 50 vol.% ethanol-water solution until the solution remained clear, and dried  
 200 overnight at 105°C. The liquid phase obtained was made up of a mixture of ethanol in water  
 201 along with the hemicellulose and lignin fractions solubilised from bamboo during the  
 202 microwave experiments. Finally, ethanol was removed from the liquid phase by means of a  
 203 rotary evaporator, leading to lignin precipitation; thus allowing the separation of  
 204 hemicellulose (water fraction) and lignin (solid fraction) by filtration. After this separation,  
 205 part of the rich lignin solid was re-solubilised in ethanol (ethanol fraction) for further analysis.  
 206 Figure 1 shows a schematic diagram of the fractionation process developed.



207

208 Figure 1. Fractionation process schematic diagram

209

### 210 2.3 Response variables and analytical methods

211 Several response variables were used to analyse the effect of the operating conditions on the  
 212 process. These include the overall bamboo conversion, the gas yields as well as the yields and  
 213 the most important properties of the three fractions produced: i) a cellulose-rich solid fraction,  
 214 ii) a hemicellulose rich water-soluble fraction and iii) a lignin rich solid fraction. Table 1  
 215 summarises the response variables and the analytical methods used for their calculation.

216

217 The solid residue produced after centrifugation was used to determine the conversion of  
218 cellulose, hemicellulose and lignin by using the chemical titration method described by Hu et  
219 al. [24]. This calculation was also used to analyse the relative amounts of cellulose,  
220 hemicellulose and lignin in the cellulose rich fraction. In addition, this solid fraction was also  
221 analysed by X-ray Powder Diffraction (XRD) and Scanning Electron Microscopy (SEM).  
222 The XRD patterns were obtained from a Bruker AXS-D8 Advance diffractometer with a  
223 Kristalloflex 760 X-ray generator, which generates focused and monochromatized  $K\alpha$  X-rays  
224 from a Cu source. The samples were carefully milled into fine powders and placed evenly on  
225 a small round hole bevelled out of an aluminium sample holder. Scans were recorded across  
226 the range of 5-80  $2\theta$  over 10 minutes with a 40kV and 40mA current. Evaluation programme  
227 EVA and the Bruker CDS database were used to identify the phases present in the samples.  
228 SEM pictures were taken with an INSPECT F, at an acceleration voltage of 20KV. Samples  
229 were coated with gold using a vacuum sputter-coater to improve the conductivity of the  
230 samples and the quality of the SEM pictures.

231

232 The water liquid phase was analysed by high-performance liquid chromatography (HPLC)  
233 and Total Organic Carbon (TOC), while the ethanol liquid phase containing ethanol-soluble  
234 lignin was characterised by GPC. HPLC analyses were conducted with an Agilent  
235 Technologies 1200 series HPLC system employing a XDB-C18 column and a UV detector.  
236 A Waters GPC device equipped with a 2414 refractive index detector was used with Waters  
237 Strygel columns HT4 and HT3 in series. Empower III GPC software was used for running  
238 the GPC instrument and for calculations. Both the columns and the RI detector were  
239 maintained at 45°C for analysis. TOC analyses were conducted in a Vario TOC Cube  
240 Analyser.

241 Table 1. Response variable and analytical methods used for their determination

Response variables	Analytical method
<b>Overall results</b>	
Overall conversion (%) = $\left(1 - \frac{\text{mass of solid residue}}{\text{mass of bamboo}}\right) 100$	Mass difference
Hemicellulose rich fraction yield (%) = $\left(1 - \frac{\text{mass of gas (g)} + \text{mass of solid residue (g)}}{\text{mass of bamboo (g)}}\right) 100$	Mass difference
Cellulose rich fraction yield (%) = $\frac{\text{mass of solid residue (g)}}{\text{mass of bamboo (g)}} 100$	Mass difference
Lignin rich fraction yield (%) = $\frac{\text{mass of lignin (g)}}{\text{mass of bamboo (g)}} 100$	
Gas yield (%) = $\frac{\text{mass of gas (g)}}{\text{mass of bamboo (g)}} 100$	Mass difference
<b>Cellulose rich fraction phase properties</b>	
Composition (wt. %) = $\frac{\text{mass of structural component (g)}}{\text{mass of solid residue(g)}} 100$	Chemical titration
<b>Lignin rich fraction phase properties</b>	
Molecular weight (g/mol)	GPC (Gel permeation chromatography)
<b>Hemicellulose rich fraction phase properties</b>	
TOC (Total Organic Carbon)	TC-IC (Total Carbon- Inorganic Carbon) measurement
Composition (C – wt. %) = $\frac{\sum \text{mass of C of each compound (g)}}{\text{total mass of C in solution (g)}} 100$	HPLC (High performance liquid chromatography)

242 wt.% = weight percentage

243 C-wt.% = percentage in carbon basis

244

245 **2.4 Experimental design and data analysis**

246 The influence of reaction temperature (110-190°C), ethanol concentration in water (0-100  
247 vol.%) and the amount of formic acid, used as a homogeneous catalyst, (0-5 vol.%) on  
248 bamboo fractionation was experimentally investigated. This ethanol/water system was very  
249 stable under the microwave conditions tested; i.e. the pressure achieved under the  
250 experiments was in good agreement with the saturation pressure of the solvent system at the  
251 temperature of the experiment, and neither runaway reactions nor explosions took place. The  
252 experiments were planned according to a 2 level 3-factor Box-Wilson Central Composite

253 Face Centred (CCF,  $\alpha: \pm 1$ ) design. This corresponds to a  $2^k$  factorial design, where k  
254 indicates the number of factors studied (in this case 3 operating variables) and  $2^k$  represents  
255 the number of runs (in this case 8) for the simple factorial design. 9 axial experiments were  
256 performed to study non-linear effects and interactions according to the CCF design. In  
257 addition, 3 replicates at the centre point (centre of the variation interval of each factor) were  
258 carried out in order to evaluate the experimental error. This experimental design is suitable  
259 not only for studying the influence of each variable (linear and quadratic effects) but also for  
260 understanding possible interactions between variables. The results were analysed with an  
261 analysis of variance (ANOVA) with 95% confidence. The ANOVA analysis helped for the  
262 selection of the operating variables and interactions that significantly influence the response  
263 variables under consideration. In addition, the cause-effect Pareto principle was used to  
264 calculate the relative importance of the operating variables in the response variables. In these  
265 analyses, the lower and upper limits of all the operating variables (temperature, EtOH/H<sub>2</sub>O  
266 and catalyst amount) were normalised from -1 to 1 (coded variables). This codification  
267 permits all operating variables (factors) to vary within the same interval and helps to  
268 investigate their influence in comparable terms. In the interaction Figures, the evolution of  
269 these variables obtained from the ANOVA analysis of all the experiments performed was  
270 represented. In addition, when possible, some experimental points were added.

271

## 272 **3. Results and discussion**

### 273 **3.1 Bamboo characterisation**

274 Table 2 lists the characterisation results of bamboo. These include the proximate, ultimate  
275 and fibre analyses, the ash content and higher heating value (HHV). These results are fairly  
276 similar to those reported in the literature [37-39]. The presence of inorganics in bamboo is

277 accounted for by the presence of powder derived from soil. Ca, Mg, K, Na, P and Si are the  
 278 major inorganics detected. In addition, traces of other inorganic components, such as Fe, Al,  
 279 S and Mn were also found in the feedstock.

280 Table 2. Proximate, fibre, elemental and calorific analyses of the bamboo used in this work.

<b>Proximate analysis (wt.%)</b>	
Moisture	3.46
Ash	8.15
Volatiles	63.52
Fixed carbon	20.31
<b>Fibre analysis (wt.%)</b>	
Cellulose	45.01±1.41
Hemicellulose	25.98±0.66
Lignin	20.80±0.17
Ash	8.15%
<b>Elemental analysis (wt.%)</b>	
C	47.58±0.34
H	5.83±0.03
N	0.48±0.04
O*	37.96±0.33
<b>HHV (MJ/kg)</b>	19.61±0.13
<b>Ash composition (ppm)</b>	
Ca	500
Mg	257.4
K	292
Na	170.41
P	108
Si	107

281 \*Oxygen was calculated by difference

282

### 283 **3.2 Effects of operating conditions on the overall conversion and products distribution**

284 Table 3 summarises the operating conditions for the experiments and the experimental results.

285 The overall bamboo conversion and the yields to gas, lignin-rich solid fraction, hemicellulose

286 rich water fraction and cellulose rich solid fraction vary as follows: 6-49%, 1-9%, 2-23%, 2-

287 32% and 51-94%, respectively. The relative influence of the operating variables on the global

288 bamboo conversion and the yields of the three main fractions produced according to the

289 ANOVA analysis, and the cause-effect Pareto principle is shown in Table 4. The gas yield is

290 low and its not influenced by the operating conditions. This might suggest that formic acid

291 decomposition did not take place due to the relative low temperature employed [40].

292

293 *Table 3. Experimental conditions: temperature (°C), solvent system (EtOH/H<sub>2</sub>O, vol. %) and formic acid (vol. %) and results obtained in the*  
 294 *experiments*

295

Run	1	2	3	4	5	6	7	8	9-12	13	14	15	16	17	18	19
T (°C)	110	190	110	190	110	190	110	190	150	110	190	150	150	150	150	190
EtOH/EtOH+H <sub>2</sub> O (vol.%)	0	0	100	100	0	0	100	100	50	50	50	0	100	50	50	50
Formic acid (vol. %)	0	0	0	0	5	5	5	5	2.5	2.5	2.5	2.5	2.5	0	5	5
pH	4.16±0.39	4.16±0.39	5.42±0.02	5.42±0.02	1.53±0.04	1.53±0.04	1.37±0.02	1.37±0.02	2.12±0.03	2.12±0.03	2.12±0.03	1.83±0.03	1.83±0.03	3.46±0.21	1.45±0.03	1.45±0.03
<b>Overall results</b>																
Overall conversion (%)	6.55	34.74	10.27	12.62	14.12	48.75	5.59	16.45	20.27±1.71	13.43	46.91	29.79	10.80	20.63	19.04	49.00
Hemicellulose fraction yield (%)	4.17	23.22	6.77	8.12	10.98	32.33	2.33	10.94	12.35±1.46	9.04	24.04	23.60	7.44	12.22	11.73	25.82
Cellulose fraction yield (%)	93.45	65.26	89.73	87.38	85.88	51.25	94.41	83.55	79.73±1.71	86.57	53.09	70.21	89.20	79.37	80.96	51.00
Lignin fraction yield (%)	2.39	11.52	3.50	4.51	3.14	16.42	3.26	5.50	7.93±0.60	4.39	22.87	6.19	3.35	8.42	7.31	23.17
Gas yield (%)	1.36	3.23	2.94	2.31	4.85	8.67	3.76	2.39	2.79±0.64	2.45	2.47	2.25	2.58	1.47	2.44	3.74
<b>Cellulose rich fraction fibre analysis</b>																
Cellulose (wt.%)	43.94	57.62	49.33	51.85	55.82	61.92	49.33	46.78	52.86±2.87	49.65	61.55	49.32	56.79	54.61	45.79	83.00
Hemicellulose (wt.%)	14.88	4.22	19.57	15.76	17.82	0.00	18.95	19.10	14.09±0.79	19.85	2.46	7.20	20.86	15.10	12.99	1.99
Lignin (wt.%)	25.15	24.22	24.98	24.53	34.23	19.16	23.99	24.49	23.65±1.34	26.95	30.53	25.29	22.21	23.10	25.82	12.52
<b>Lignin rich phase</b>																
Mw (g/mol)	600	1528	3177	2758	2039	1053	3133	2540	2142±261	2262	1614	1567	2768	3060	2201	2323
<b>Hemicellulose rich phase composition</b>																
TOC (ppm)	0	353.05	174.37	34.31	206.44	157.76	89.69	56.06	148.75±90.27	34.52	172.94	126.11	56.58	177.44	93.28	397.27
Sugars (C- wt.%)	0	2.66	0	1.19	0.53	42.68	0.66	1.66	2.33±1.75	3.86	15.98	32.01	1.95	1.42	1.52	11.52
Acids (C- wt.%)	0	6.43	0	19.56	95.45	53.94	99.34	37.98	81.43±11.07	85.84	27.17	26.59	98.03	32.86	82.99	60.65
Oligomers (C- wt.%)	100.00	90.88	100.00	79.19	0	0	0	60.36	5.50±4.20	0	56.19	41.39	0.00	65.72	0	27.71

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306 Table 4. Relative influence of the operating conditions according to the ANOVA analysis

Variable	R <sup>2</sup>	I.Term	A (T)	B (EtOH%)	C (formic acid)	AB	AC	BC	ABC	A <sup>2</sup>	B <sup>2</sup>	C <sup>2</sup>	A <sup>2</sup> B	A <sup>2</sup> C	AB <sup>2</sup>	AC <sup>2</sup>	A <sup>2</sup> B <sup>2</sup>	
<b>Overall results</b>																		
Overall conversion (%)	0.99	20.17	16.21 (28)	-7.82 (17)	n.s.	-6.2 (12)	1.74 (7)	-2.8 (5)	n.s.	9.48 (3)	n.s.	n.s.	n.s.	2.46 (8)	-6.71 (8)	n.s.	n.s.	-11.01 (11)
Hemicellulose fraction yield (%)	0.99	12.22	6.52 (28)	-8.08 (22)	n.s.	-3.8 (13)	1.18 (6)	-2.19 (7)	n.s.	4.25 (1)	3.3 (3)	n.s.	2.76 (4)	1.77 (7)	n.s.	n.s.	n.s.	-7.41 (8)
Cellulose fraction yield (%)	0.99	79.83	-16.21 (28)	9.5 (17)	n.s.	6.2 (12)	-1.74 (6)	2.8 (5)	n.s.	-9.47 (3)	n.s.	n.s.	-2.09 (2)	-2.46 (8)	6.71 (8)	n.s.	n.s.	11.01 (11)
Lignin fraction yield (%)	0.99	7.91	8.98 (26)	-1.95 (9)	n.s.	-2.39 (10)	0.61 (7)	-0.61 (3)	n.s.	5.46 (4)	-3.14 (18)	n.s.	n.s.	0.73 (5)	-5.77 (13)	n.s.	n.s.	-3.95 (5)
Gas yield (%)	0.92	2.45	n.s.	n.s.	n.s.	-0.96 (17)	n.s.	-1 (18)	n.s.	n.s.	n.s.	n.s.	-0.84 (18)	1.19 (23)	n.s.	0.42 (7)	n.s.	1.24 (18)
<b>Solid residue fractionation composition</b>																		
Cellulose (wt.%)	0.92	52.54	5.95 (20)	n.s.	-4.41 (9)	-2.48 (7)	n.s.	-2.66 (8)	n.s.	n.s.	n.s.	n.s.	-2.75 (9)	5.8 (10)	-26.6 (17)	23.12 (20)	n.s.	n.s.
Hemicellulose (wt.%)	0.99	14.06	-8.75 (26)	6.83 (22)	-1.05 (3)	3.1 (12)	-0.41 (4)	0.5 (2)	1.39 (6)	-2.96 (4)	n.s.	n.s.	-2.27 (4)	1.22 (1)	4.73 (10)	n.s.	n.s.	2.69 (6)
Lignin (wt.%)	0.95	23.88	1.79 (16)	-0.79 (5)	n.s.	2.01 (12)	-1.65 (17)	n.s.	1.89 (12)	4.86 (5)	n.s.	n.s.	n.s.	n.s.	12.58 (0)	-16.36 (22)	n.s.	-3.65 (10)
<b>Lignin phase</b>																		
Mw (g/mol)	0.92	2097.5	-169.77 (8)	758.9 (40)	-429.5 (1)	n.s.	-258.46 (10)	n.s.	217.5 (11)	n.s.	n.s.	543.15 (5)	n.s.	519.79 (13)	n.s.	n.s.	n.s.	-537.15 (13)
<b>Water phase properties</b>																		
TOC (ppm)	0.92	125.36	69.21 (15)	-43.23 (13)	n.s.	-59.76 (16)	-36.91 (3)	n.s.	63.52 (18)	n.s.	n.s.	n.s.	n.s.	n.s.	-292.49 (17)	239.61 (17)	n.s.	n.s.
Sugars (C- wt. %)	0.93	13.5	n.s.	27.83 (5)	n.s.	-22.44 (18)	n.s.	n.s.	22.15 (18)	n.s.	57.79 (12)	n.s.	-28.18 (10)	24.18 (21)	n.s.	n.s.	n.s.	-44.9 (15)
Acids (C- wt.%)	0.96	76.79	-29.33 (10)	35.72 (6)	31.54 (26)	n.s.	-15.54 (12)	n.s.	n.s.	-14.52 (15)	-10.97 (7)	-13.09 (6)	-35.59 (11)	n.s.	n.s.	20.29 (7)	n.s.	n.s.
Oligomers (C- wt.%)	0.99	6.01	28.1 (5)	-20.7 (1)	-37.75 (26)	6.08 (4)	11.03 (8)	9.01 (6)	9.01 (6)	21.07 (15)	14.69 (6)	25.83 (8)	26.78 (7)	n.s.	n.s.	-24.55 (7)	n.s.	-13.8 (2)

307

308 n.s: Non significant with 95% confidence

309 Response = Indep. + Coefficient A · A + Coefficient B · B + Coefficient C · C + Coefficient AB · AB + Coefficient AC · AC Coefficient BC · BC Coefficient ABC · ABC + Coefficient A<sup>2</sup> · A<sup>2</sup> + Coefficient B<sup>2</sup> · B<sup>2</sup> + Coefficient

310 C<sup>2</sup> · C<sup>2</sup> + Coefficient A<sup>2</sup>B · A<sup>2</sup>B + Coefficient A<sup>2</sup>C · A<sup>2</sup>C + Coefficient AB<sup>2</sup> · AB<sup>2</sup> + Coefficient AC<sup>2</sup> · AC<sup>2</sup> + Coefficient A<sup>2</sup>B<sup>2</sup> · A<sup>2</sup>B<sup>2</sup>

311 Numbers in brackets indicate the percentage Pareto influence of each factor on the response variable. Pareto values represent the percentage of the orthogonal estimated total value.

312



313 According to the cause-effect Pareto analysis, the temperature (with more than 26% of  
314 influence) is the operating variable exerting the greatest influence on the overall bamboo  
315 conversion and the yields to lignin, hemicellulose and cellulose rich solid fractions. In  
316 addition, the concentration of ethanol and the interactions (both linear and quadratic) between  
317 the temperature and the concentration of ethanol also have a significant influence on these  
318 response variables with more than 12% influence in all cases. The concentration of formic  
319 acid (catalyst) alone does not have a significant influence on these response variables.  
320 However, its interaction with the temperature is significant, thus suggesting a synergistic  
321 effect between these two variables; i.e. a certain temperature is needed for the acid to catalyse  
322 the process significantly. The gas yield is greatly influenced by the interaction between the  
323 temperature and the concentration of ethanol in water.

324

325 As regards the influence (positive or negative) of the operating variables on the process, the  
326 coefficients in the models indicate that an increase in the temperature leads to an increase in  
327 the overall conversion, the hemicellulose rich fraction and lignin rich fraction yields (positive  
328 terms), and a decrease in the cellulose rich solid fraction yield (negative term). The  
329 concentration of ethanol in water has the opposite effect; i.e. an increase in the proportion of  
330 ethanol results in a decrease in the overall bamboo conversion and the hemicellulose rich  
331 fraction and lignin rich fraction yields (negative terms) along with an increase in the cellulose  
332 rich solid fraction (positive terms). Moreover, as commented above, significant interactions  
333 between variables also take place, demonstrating that the other operating variables influence  
334 the effect that each variable has on the process. To gain a better understanding of the process,  
335 Figure 2 displays the effect of the operating variables and the most important interactions  
336 detected with the ANOVA analysis. Specifically, Figure 2 a and b illustrates the effect of the  
337 ethanol concentration and the temperature on the overall bamboo conversion in the absence

338 of catalyst (formic acid = 0 vol.%) and for the highest amount of catalyst used in this work  
339 (formic acid = 5 vol.%), respectively. These effects are also shown for the lignin rich fraction,  
340 hemicellulose rich fraction and cellulose rich fraction yields in Figures 3 c-d, e-f and g-h,  
341 respectively.

342

343 As regard the effects of the temperature, Figure 2 shows that regardless of the other operating  
344 conditions, the temperature positively potentiates bamboo decomposition. Specifically, an  
345 increase in temperature from 110 to 190 °C substantially increases the overall bamboo  
346 conversion as well as the yields of the lignin and hemicellulose fractions; which consequently  
347 results in a decrease in the cellulose fraction yield. The temperature positively enhances the  
348 kinetics of the process and greater microwave power is also needed to achieve higher  
349 temperatures, thus promoting the break of the intramolecular bonds between cellulose,  
350 hemicellulose and lignin in bamboo. Kassaye et al. [37] reported a similar trend during the  
351 hydrolysis of bamboo between 90 and 150 °C with NaOH for 30 min.

352

353 The effect of the concentration of ethanol in water (solvent system) depends on the  
354 temperature due to the significant interaction detected between both variables, with two  
355 different developments occurring. At low temperature (110 °C) the solvent system does not  
356 have a very important influence on the process, and similar conversions and yields to  
357 products are obtained regardless of the solvent composition, either using the pure solvents  
358 (ethanol and/or water) or any binary mixture. This suggests that a certain temperature is  
359 needed to both break the intermolecular bonds between the bamboo structural components  
360 and solubilise the released fractions into the solvent system. This is a consequence of the  
361 kinetics of the process and is in good agreement with the cause-effect Pareto results, which  
362 indicated that the temperature was the operating variable exerting the greatest influence on

363 the overall conversion and the yields to different fractions. High temperatures are needed to  
364 achieve good conversion during the very short reaction (15 min) time employed in this work.

365

366 Conversely, as the temperature increases, the solvent system has a more important influence  
367 on the process, and an interaction between ethanol and water takes place. In particular, a  
368 synergetic interaction between EtOH and H<sub>2</sub>O occurs for the overall bamboo conversion and  
369 the yields to lignin and cellulose fractions. Higher values are obtained for the bamboo  
370 conversion and the yield of lignin fraction and lower values for the yield of cellulose fraction  
371 with an EtOH/H<sub>2</sub>O mixture than using the pure solvents (EtOH or H<sub>2</sub>O alone), with a  
372 maximum occurring with a concentration of EtOH of around 40-50 vol.%. This maximum is  
373 in good accordance with other works reported in the literature [41-45] where maxima for  
374 lignin conversion occurred employing a concentration of 40-60 vol.% of EtOH in water. This  
375 solvent system-temperature interaction has been never detected in other works dealing with  
376 organosolv systems and can help to gain a deeper insight into this complex process.

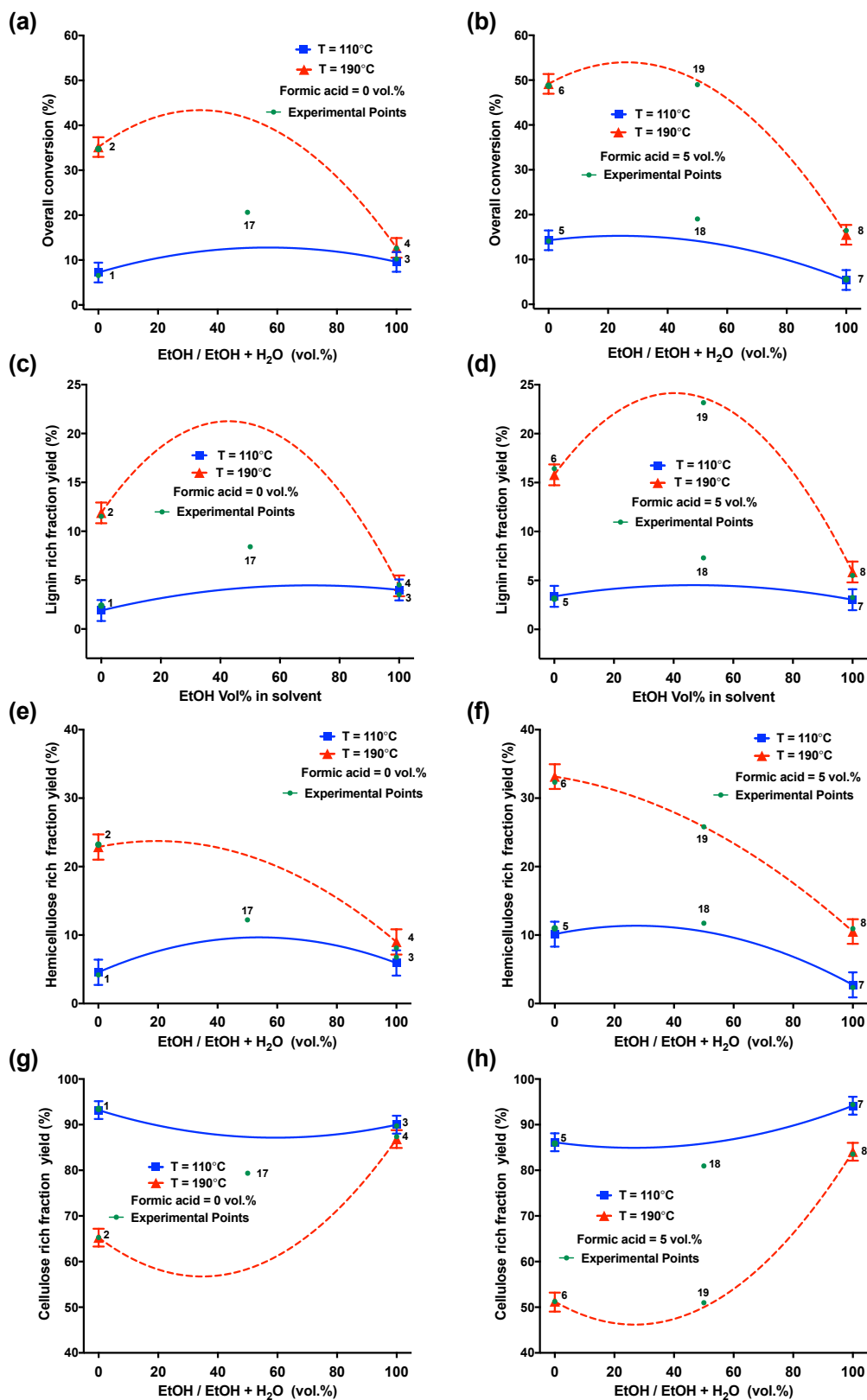
377

378 This interaction is more obvious for the lignin yield than for the other variables, and it does  
379 not take place for the hemicellulose fraction yield, thus suggesting that the solvent system  
380 exerts the greatest influence on lignin fractionation. For lignin extraction, a nucleophilic  
381 solvent such as water is needed for extracting lignin from the biomass structure along with  
382 another solvent to dissolve the extracted lignin [28]. Water alone can extract lignin, however,  
383 the solubilisation of this lignin by water is the limiting step. Conversely, ethanol is not  
384 appropriate for lignin extraction, but it promotes lignin solubilisation. Therefore, the presence  
385 of both solvents in the system at the right temperature promotes both lignin extraction and  
386 solubilisation; which, therefore, significantly increases the lignin yield. This synergetic  
387 interaction does not take place for the hemicellulose fraction yield, for which the highest

388 yield is produced with pure water; the addition of ethanol into the system progressively  
389 decreases the yield of this fraction. In addition, this effect is too weak for the rich cellulose  
390 solid fraction. These developments are a consequence of the different polarities of these two  
391 fractions compared to lignin as well as due to the lesser spread of hydrolysis reactions to  
392 extract hemicellulose in ethanol than in water [30].

393

394 The effect of the catalyst concentration on the overall bamboo conversion and the yields to  
395 the lignin, hemicellulose and cellulose rich fractions can be observed comparing Figure 2  
396 a,c,e, and g with b, d,f and h, respectively. This comparison reveals that the effect of the  
397 catalyst for these response variables is relatively weak as predicted by the Pareto Analysis. In  
398 addition, the effect depends on the temperature. At low temperature (110°C) the effect of the  
399 addition of up to 5 vol.% of formic acid in the process is almost negligible. However, at 190  
400 °C the catalyst has a more pronounced effect on bamboo decomposition as the kinetics of the  
401 process is enhanced. In addition, at this temperature, the specific effect of the catalyst  
402 depends on the solvent system. While an increase in the amount of catalyst promotes bamboo  
403 decomposition (increases bamboo conversion/decreases the cellulose solid yield and  
404 increases the yields to lignin and hemicellulose rich fractions) with pure water, the addition  
405 of ethanol progressively decreases the catalytic effect of formic acid in the process due to the  
406 lower solubilisation of formic acid protons in ethanol than in water [30].



407

408 Figure 2. Interaction plots between the temperature and the solvent system (EtOH/H<sub>2</sub>O)  
 409 without/with catalyst for the overall bamboo conversion (a/b), lignin rich solid fraction yield  
 410 (c/d), hemicellulose rich liquid fraction yield (e/f), and cellulose rich solid fraction yield (g/h).  
 411 Bars are LSD intervals with 95% confidence.

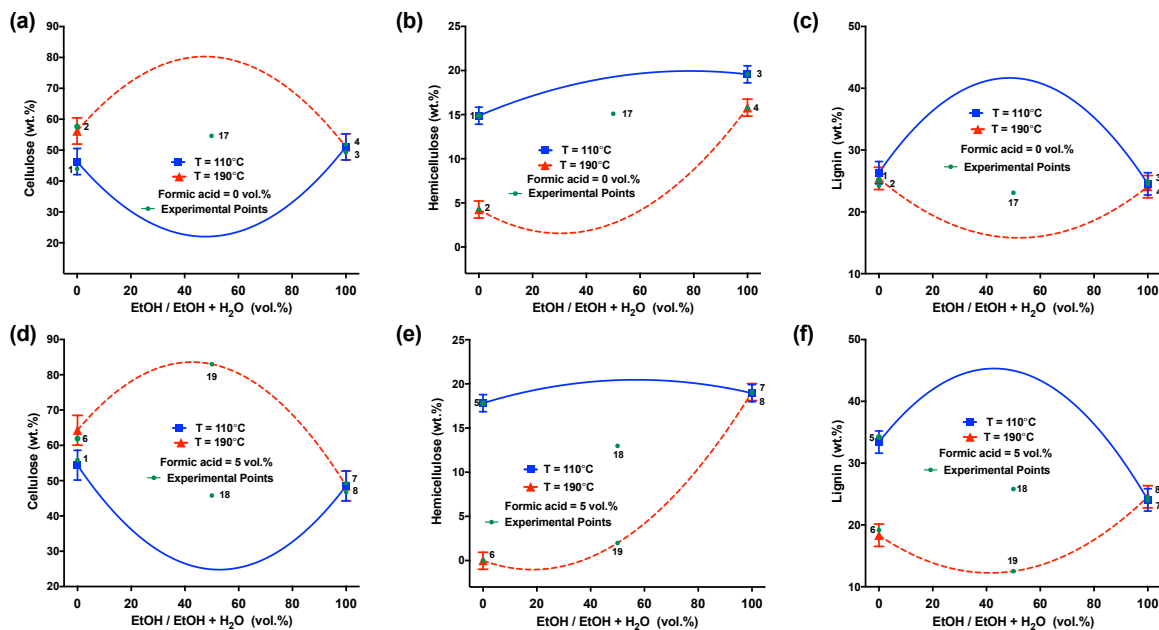
### 412 **3.3 Effects of operating conditions on the properties of each fraction**

413 This section examines the effects of the operating conditions on several of the most important  
414 properties of the three products produced during the fractionation of bamboo: i) the cellulose-  
415 rich spent solid fraction, ii) the hemicellulose rich water sample fraction and iii) the lignin  
416 rich fraction.

417

#### 418 **3.3.1 Cellulose-rich solid fraction**

419 This cellulose-rich solid fraction consists of the solid residue obtained by filtration after the  
420 microwave treatment of bamboo. The characterisation of this solid by chemical titration  
421 revealed that this fraction contains cellulose and, in less proportion, unreacted hemicellulose  
422 and lignin with varying compositions depending on the operating conditions. The amounts of  
423 cellulose, hemicellulose and lignin in the spent solid vary by: 44-83 wt.%, 0-21 wt.% and 12-  
424 34 wt.%. The effect of the operating conditions on the composition of this fraction according  
425 to the ANOVA analysis, and the cause-effect Pareto principle is shown in Table 4. The  
426 temperature and its interaction with the catalyst amount and in lesser extent with the solvent  
427 system are the factors exerting the greatest influence on the proportions of cellulose and  
428 lignin. The temperature, the solvent system and the interaction between these two variables  
429 primarily influence the proportion of hemicellulose. The effects of the operating conditions  
430 and interactions between them on the relative amounts of cellulose, hemicellulose and lignin  
431 are plotted in Figure 3. In particular, Figure 3 a, b and c shows the effects of the solvent  
432 system and the temperature in the absence of catalyst. Figures 3 d, e and f show these effects  
433 when using the highest concentration of catalyst (5 vol.%) employed in this work.



434

435 Figure 3. Interaction plots between the temperature and the solvent system (EtOH/H<sub>2</sub>O)  
 436 without/with catalyst for the relative amounts of cellulose (a/d), hemicellulose (b/e) and  
 437 lignin (c/f) of the cellulose rich solid fraction. Bars are LSD intervals with 95% confidence.

438

439 With respect to the effects of the temperature on the composition of the cellulose rich fraction,  
 440 Figure 3 shows how regardless of amount of catalyst employed (0-5 vol.% formic acid), an  
 441 increase in the temperature between 110 and 190 °C results in a decrease in the proportions of  
 442 hemicellulose and lignin, and consequently, the proportion of cellulose increases. An increase  
 443 in the temperature helps to solubilise the hemicellulose and lignin fractions of bamboo into  
 444 the solvent system, and therefore a cellulose-rich solid is obtained due to the positive kinetic  
 445 effect of the temperature in the process. A solid with a high proportion of hemicellulose (80-  
 446 85%) can be obtained at 190 °C employing a concentration of EtOH of around 40-50 vol.%.  
 447 This is in good agreement with other works where the EtOH/H<sub>2</sub>O solvent system was used  
 448 for the fractionation of biomass [41-45].

449

450 In addition, an interaction between the temperature and the solvent system was detected in  
451 the ANOVA analysis and can be observed from the results plotted in Figure 3. This indicates  
452 that the effect of the temperature depends on the solvent system and vice versa. The  
453 temperature has a significant influence on the process for the majority of the binary mixtures  
454 considered (between 15 and 85 wt.% of ethanol in water). However, for the pure solvents or  
455 high concentrated in one compound (>85 wt.%) binary mixtures, the effect of the temperature  
456 decreases dramatically, being insignificant in some cases. This development might be a  
457 consequence of the positive influence of the temperature on the solvent system in this work.

458

459 At 190 °C, the positive kinetic effect of the temperature can mask the effect on the solvent  
460 system on lignin solubilisation, resulting in a cellulose solid fraction with similar lignin  
461 content regardless of the solvent system. Conversely, the effect of the solvent can be  
462 observed at low temperature. Pure water helps lignin depolymerisation [28], while ethanol  
463 can react with  $\alpha$ -hydroxyl groups in bamboo to increase the solubility of lignin, preventing  
464 its condensation; thus decreasing the lignin content of this fraction at 110 °C [28]. For these  
465 reactions to take place at low temperature, high biomass/ethanol and biomass/water ratios  
466 might be needed, therefore pure solvents are required, and the synergetic effect between both  
467 solvents does not take place at a low temperature. The opposite effect takes place for the  
468 relative amount of hemicellulose in the solvent system; i.e. the proportion of hemicellulose is  
469 not strongly influenced by the solvent system at low temperature, and this fraction has a high  
470 proportion of hemicellulose due to the low solubilisation of this structural component at low  
471 temperature. Conversely, the solvent system significantly influences the relative amount of  
472 hemicellulose at high temperature (190°C). Using pure water the proportion of hemicellulose  
473 in the solid is very low, and the addition up to a 40 vol.% of ethanol in the system does not  
474 influence the relative amount of hemicellulose in the solid. However, a further increase in the



475 proportion of ethanol from 40 to 100 vol.% results in a sharp increase in the relative amount  
476 of hemicellulose in the spent solid due to the lesser spread of hydrolysis reactions to extract  
477 hemicellulose in ethanol than in water [30]. The variations observed for the relative amount  
478 of cellulose in this fraction are related to the variations observed for hemicellulose and lignin,  
479 as this fraction is not very reactive under the operating conditions tested in this work.

480

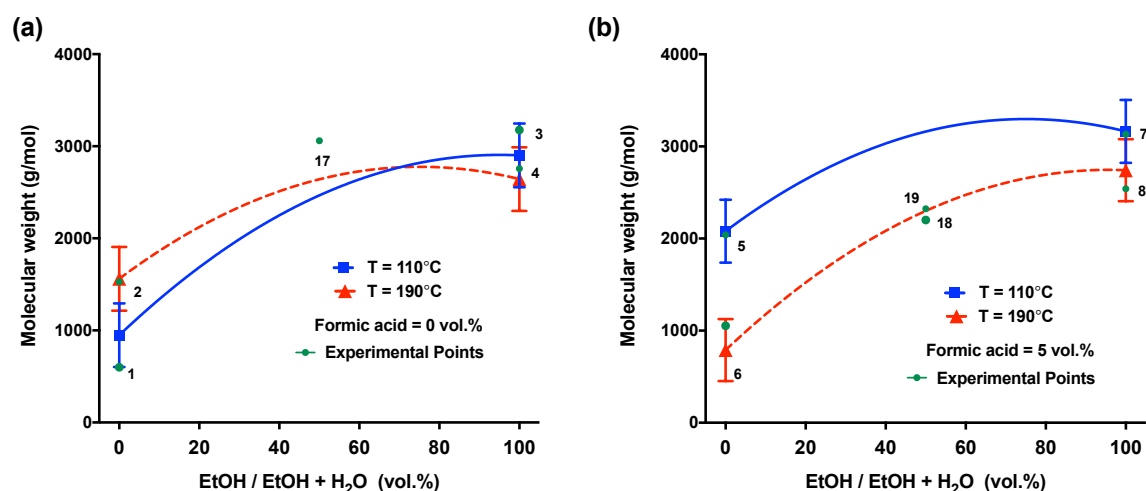
481 The catalyst has a weak effect on the proportions of cellulose, hemicellulose and lignin of  
482 this fraction, with two different effects occurring depending on the temperature. At low  
483 temperature an increase in the amount of formic acid between 0 and 5 vol.% leads to a  
484 decrease in the proportion of cellulose along with an increase in the relative amount of  
485 hemicellulose and lignin, the opposite is observed at high temperature (190 °C); i.e. the  
486 proportion cellulose increases and the relative amounts of hemicellulose and lignin decrease.

487

### 488 **3.3.2 Lignin-rich solid fraction**

489 This rich lignin fraction consists of the solid residue produced after removing the ethanol  
490 from the liquid collected after the microwave experiments. The lignin content of this solid  
491 fraction was greater than 95 wt.%, which is in good agreement with other works reported in  
492 the literature that regard this solid as high purity organosolv lignin and suggests that the  
493 formation of humins does not substantially take place under the operating conditions tested in  
494 this work. This highlights the good controllability and selectivity of this microwave-assisted  
495 organosolv process for the fractionation of bamboo. The molecular weigh of this fraction,  
496 determined by GPC, shifts between 600 and 3177 g/mol. The effects of the operating  
497 conditions on the molecular weight of the organosolv lignin produced according to the  
498 ANOVA analysis, and the cause-effect Pareto principle is shown in Table 4. This analysis

499 shows that the solvent system (ethanol concentration in water) is the factor exerting the  
 500 greatest importance on the molecular weigh of this fraction, the temperature and the catalyst  
 501 amount having a lower influence. Figure 4 a and b show the effect of the solvent system on  
 502 the molecular weight of the organosolv lignin produced at low and high (110 and 190 °C)  
 503 temperatures for two catalyst loadings: 0 vol.% and 5 vol.%, respectively. Regardless of the  
 504 temperature and the catalyst loading, this fraction has the lower molecular weight when only  
 505 water is used in the process. The addition of up to 45-50 vol.% of ethanol in the solvent  
 506 mixture increases the molecular weight of this fraction, while a further increase of up to 100  
 507 vol.% does not modify the molecular of the lignin produced. The presence of water in the  
 508 solvent system helps to depolymerise lignin, thus decreasing the molecular weight of the  
 509 lignin produced [20].



510

511 Figure 4. Interaction plots between the temperature and the solvent system (EtOH/H<sub>2</sub>O)  
 512 without/with catalyst (a/b) for the molecular weight of the lignin produced. Bars are LSD  
 513 intervals with 95% confidence.

514

515 The effect of the temperature depends on the catalyst amount. On the one hand, in the  
 516 absence of a catalyst, the temperature does not exert a significant influence regardless of the  
 517 solvent system. On the other, when the highest amount of catalyst is used, the effect of the

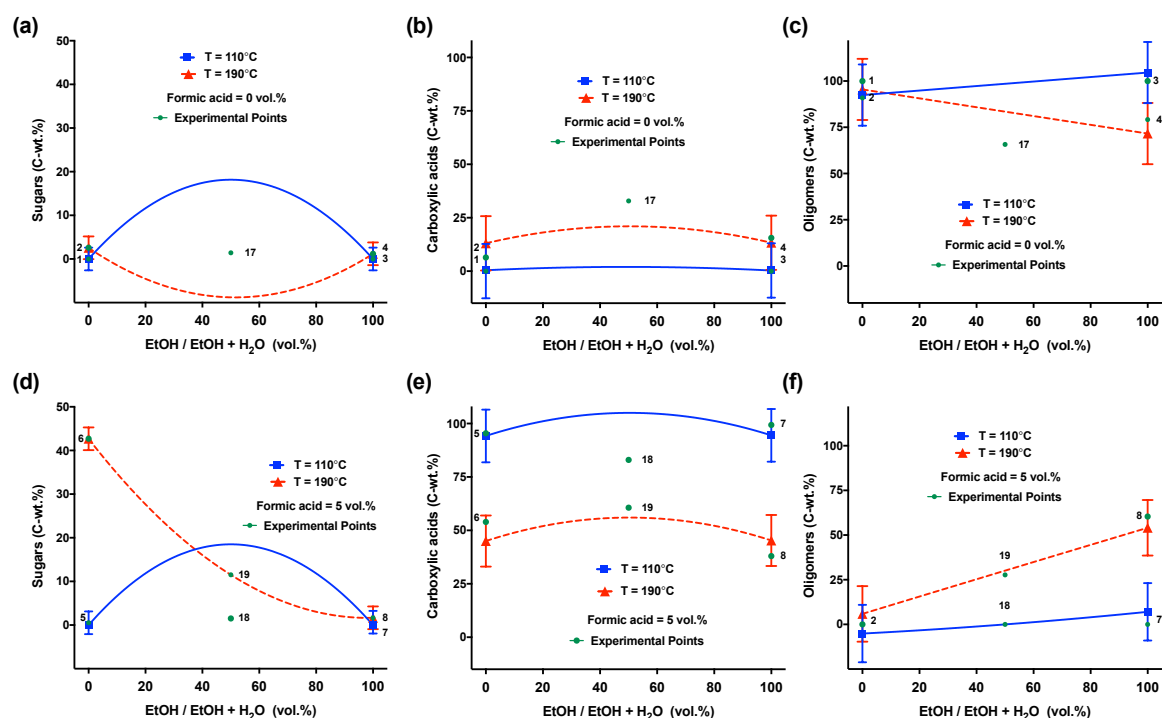
518 temperature depends on the solvent system with two developments observed. In particular, an  
519 increase in the temperature decreases the molecular weight of lignin when pure water is used.  
520 The addition of ethanol progressively reduces the effect of the temperature and as a  
521 consequence, the effect of this variable stops having a significant influence on the molecular  
522 weight of the organosolv lignin produced for concentrations of ethanol higher than 75 vol.%  
523 due to the lower effectiveness of ethanol than water in lignin depolymerisation [20].

524

### 525 **3.3.3 Hemicellulose-rich liquid fraction**

526 This fraction comprises water-soluble hemicellulose decomposition products. It is made up of  
527 a mixture of oligomers, sugars (cellobiose, glucose and xylose), carboxylic acids (acetic acid),  
528 ketones ( $\gamma$ -valerolactone, GVL) and furans (furfural and 5-hydroxymethylfurfural, HMF).  
529 The relative amounts (in C basis) of these families of compounds vary by 0-100 wt.%, 0-100  
530 wt.%, 0-100 wt.%, 0-13 wt.% and 0-4 wt.%, respectively. Table 4 lists the effects of the  
531 operating conditions on the composition of this fraction according to the ANOVA analysis  
532 and the cause-effect Pareto principle. This analysis indicates that the operating conditions do  
533 not significantly influence on the relative amounts of ketones and furans of this fraction,  
534 probably due to their low concentration in the liquid phase under the operating conditions  
535 used in this work. In addition, methyl acetate was not found in the chemical analysis of the  
536 water-soluble hemicellulose rich fraction; thus suggesting that the possible esterification  
537 reaction between ethanol and formic acid did not significantly take place under the operating  
538 conditions tested in this work. The statistical analysis shows that the interaction between the  
539 temperature and the solvent system is the factor with the highest influence on the proportion  
540 of sugars, while the amount of catalyst is the operating variable with the highest influence on

541 the relative amounts of carboxylic acids and oligomers. In addition, significant interactions  
 542 between the operating variables also take place.



543  
 544 Figure 5. Interaction plots between the temperature and the solvent system (EtOH/H<sub>2</sub>O)  
 545 without/with catalyst for the relative amounts of sugars (a/d), carboxylic acids (b/e) and  
 546 oligomers (c/f) present in the water-soluble hemicellulose rich fraction. Bars are LSD  
 547 intervals with 95% confidence.

548  
 549 The effect of the operating conditions and the most important interactions for the proportions  
 550 of sugars, carboxylic acids and oligomers are shown in Figure 5. In the absence of a catalyst,  
 551 this hemicellulose rich water fraction is primarily made up of oligomers, the proportions of  
 552 sugars and carboxylic acids being quite low regardless of the solvent system. Conversely, the  
 553 addition of a catalyst (formic acid) decreases the proportion of oligomers and increases the  
 554 concentration of sugars, and carboxylic acids in this fraction. At low temperature (110 °C)  
 555 carboxylic acids are the most abundant compounds of this fraction, while an increase in the  
 556 temperature increases the relative amounts of sugars and oligomers and decreases the  
 557 proportion of carboxylic acids in the liquid due to greater conversion of hemicellulose and

558 lignin. At 190 °C the proportion of carboxylic acids is not influenced by the solvent system,  
559 while this operating variable exerts a significant influence on the relative amounts of sugars  
560 and oligomers. With pure water, this fraction has a high concentration of sugars, and a  
561 negligible quantity of oligomers is observed. The increase of the proportion of ethanol in the  
562 solvent system decreases the proportion of sugars and increases the proportion of oligomers.  
563 The presence of water in the solvent system promotes hydrolysis reactions, thus decreasing  
564 the proportion of oligomers and, therefore, increasing the amount of sugars [28].

565

### 566 **3.4 Optimisation of the fractionation process and energetic assessment**

567 Optimum conditions were sought for the selective fractionation of bamboo making use of the  
568 experimental models developed (Table 4). The predicted  $R^2$  of all the models are greater than  
569 0.90, allowing their use for prediction purposes within the range of study. The optimisation  
570 process comprises the minimisation of the cellulose conversion (maximisation of the  
571 cellulose solid rich fraction and proportion of cellulose of this fraction) and the maximisation  
572 of the yields of hemicellulose rich and lignin rich fractions. To meet this objective, a solution  
573 that strikes a compromise between the optimum values for all the response variables was  
574 sought. To do so, a relative importance (from 1 to 5) was given to each of the objectives in  
575 order to come up with a solution that satisfies all the criteria. Table 5 lists the relative  
576 importance assigned to each variable as well as the criteria used in the whole optimisation. A  
577 relative importance of 3 was given to the overall conversion and the cellulose, hemicellulose  
578 and lignin yields, while a relative importance of 5 was given to proportions of cellulose,  
579 hemicellulose and lignin in the cellulose rich fraction to increase the purity of this fraction  
580 and therefore the purity of the lignin and hemicellulose fractions produced. This strategy

581 allows striking a compromise between yield and purity to maximise the process by using the  
 582 desirability objective function  $D(X)$  shown in Eq.1.

$$583 \quad D(X) = (d_1^{r_1} \cdot d_1^{r_2} \cdot \dots \cdot d_1^{r_n})^{\frac{1}{\sum r_j}} = (\prod_{j=1}^n d_j^{r_j})^{\frac{1}{\sum r_j}} \quad (\text{Eq. 1})$$

584 Where  $n$  is the number of responses considered in the optimisation and  $r_j$  is the relative  
 585 importance of each response (1-5)

586 Table 5. Theoretical optimisation: operating conditions and response variables: objectives,  
 587 the interval of variations, relative importance, theoretical and experimental optimum values.

Variables	Objective	Interval of variation	Relative importance (1-5)	Optimum Theoretical	Optimum Experimental
Temperature (°C)	none	110-190		190	
EtOH/EtOH+H <sub>2</sub> O (vol.%)	none	0 - 100		45	
HCOOH (vol.%)	none	0 - 5		5	
<b>Overall Conversion (%)</b>	maximise	0-100	3	52	51.23
<b>Cellulose rich fraction</b>					
Yield (%)	maximise	0-100	3	49	50.46
Cellulose content (wt.%)	maximise	0-100	5	84	84.87
Hemicellulose content (%)	minimise	0-100	5	1	1.85
Lignin Content (%)	minimise	0-100	5	12	11.55
<b>Lignin rich fraction</b>					
Yield (%)	maximise	0-100	3	24	23.87
Molecular weight (DA)	none			2203	2201
<b>Hemicellulose rich fraction</b>					
Yield (%)	maximise	0-100	3	27	26.82
Oligomers (C-wt.%)	none	0-100		27	27.80
Sugars (C-wt.%)	none	0-100		56	60.11
Carboxylic acids (C-wt.%)	none	0-100		14	12.65

588

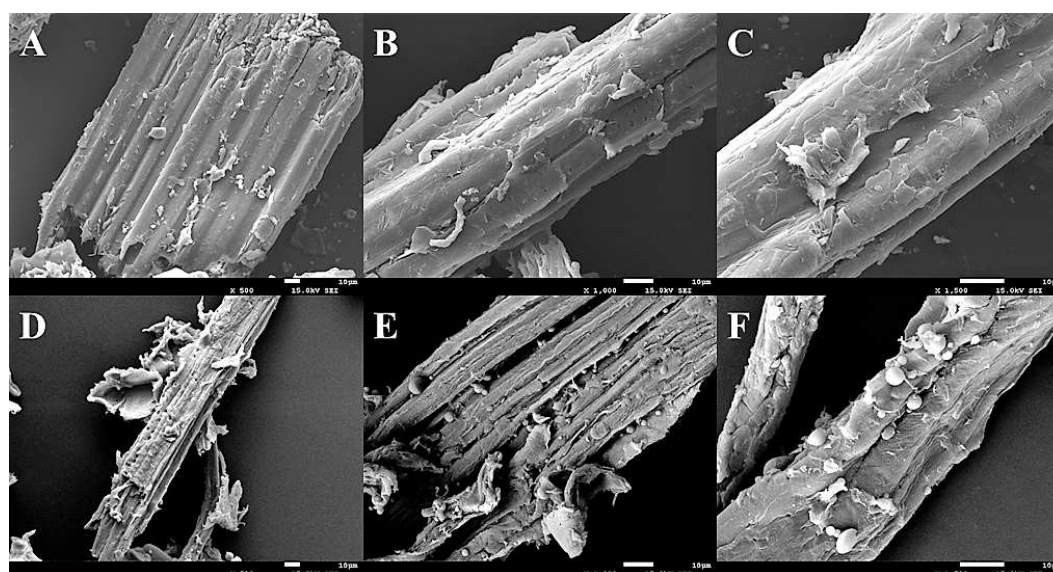
589 Taking these conditions into account, the optimisation predicts an optimum at 190 °C using a  
 590 solvent system consisting of 45% EtOH/50% H<sub>2</sub>O (vol/vol) with a concentration of formic  
 591 acid of 5 vol.%. Under these conditions, it is possible to selectively fractionate bamboo into  
 592 cellulose, hemicellulose and lignin. The cellulose rich fraction contains 84 wt.% of cellulose  
 593 and 12 wt.% of lignin. The yield to organosolv lignin is very high (24%), which corresponds  
 594 to a recovery of 90 wt.% of the lignin present in the original bamboo. In addition,  
 595 hemicellulose is completely removed from the original feedstock, and a water solution  
 596 consisting of a mixture of oligomers, sugars and carboxylic acids can be produced. This  
 597 optimum was checked experimentally (Table 5) and non-statistically significant differences

598 were found between the theoretical prediction and the experimental results with 95%  
599 confidence.

600

601 To gain a better insight into this fractionation process, the original bamboo and the spent  
602 solid produced at the optimum condition were characterised by Scanning Electron  
603 Microscopy (SEM) and X-ray Diffraction (XRD), and a comparison was established between  
604 both materials. The SEM images (Figure 6) show how after the microwave-assisted  
605 organosolv treatment the original micro-fibrous structure of bamboo disappears and the solid  
606 residue surface becomes significantly rougher. In addition, some small spheroidal particles  
607 appeared on the cellulose fibrillar structure after the treatment (under 1500 magnification),  
608 which was possibly caused by lignin precipitation onto the fibres [46, 47]. These differences  
609 suggest that most of the lignin and hemicellulose are removed during the organosolv process.  
610 A small quantity of lignin still remains on the surface of the cellulose rich fraction due to the  
611 lignin condensation. These developments are in a good agreement with the experimental  
612 results listed in Table 5.

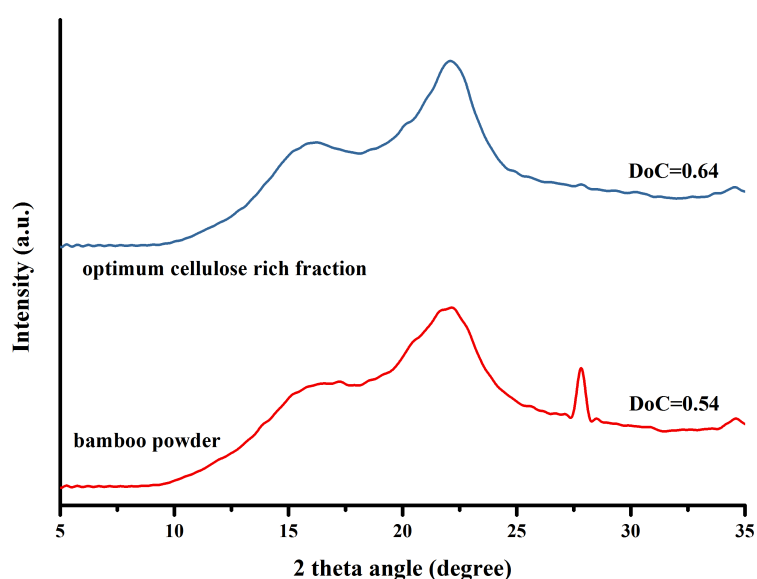
613



614

615 Figure 6. SEM images of bamboo powder (A:  $\times 500$ , B:  $\times 1000$ , C:  $\times 1500$ ) and optimum  
616 experimental run solid residue (D:  $\times 500$ , E:  $\times 1000$ , F:  $\times 1500$ ).

617 XRD was used to evaluate the effectiveness of the optimised microwave assisted organosolv  
618 process by comparing the degree of crystallinity (DoC) of both materials (before and after the  
619 treatment) [48, 49]. Figure 7 shows the XRD patterns for the original material and the spent  
620 solid (high purity cellulose fraction) produced after the microwave treatment. The  
621 comparison of both spectra does not show great differences between both solids, which is in  
622 good agreement with the experimental results, as only crystal cellulose can be detected by  
623 XRD. In addition, the crystallinity degree increases from 0.54 (original bamboo) to 0.64  
624 (cellulose rich fraction). This is in good agreement with the experimental results described  
625 above, as hemicellulose and lignin (amorphous) are removed during the treatment and,  
626 therefore, the proportion of cellulose in this fraction increases; thus augmenting the degree of  
627 crystallinity of this fraction.



628  
629 Figure. 7 XRD patterns for the original bamboo powder and spent solid produced the  
630 optimum conditions.  
631

632 Finally, a basic energetic assessment was conducted to analyse the energy feasibility of this  
633 organosolv process. This process relies on thermal energy, i.e., for heating of the feedstock to



634 reaction conditions, for product separation by drying and for thermal solvent recovery. The  
635 physicochemical properties used for this calculation are listed in Table 6. At the optimum  
636 operating conditions (reaction temperature and solvent system), the energy required to heat  
637 up the solvent system and bamboo from 25 to 190 °C is 10.89 MJ/kg bamboo and 0.25 MJ/kg  
638 bamboo, respectively. In addition, the energy required for ethanol evaporation is 9.4 MJ/kg  
639 bamboo. Therefore, the input energy, calculated as the sum of energy needed to heat the feed  
640 up to the reaction temperature and the energy needed for solvent evaporation is 20.5 MJ/kg  
641 bamboo. In a real case, it would be necessary to include heat losses as well as the energy  
642 demand for all the operation units required in the process. Viell et al. [50] and Garcia et al.  
643 [51] reported energy requirements of 28.8 MJ/kg biomass and 11.84 MJ/kg biomass for wood  
644 biomass, respectively. Very interestingly, it was also reported that the energy requirement of  
645 organosolv processes could be reduced up to around 6 MJ/kg biomass using process unit heat  
646 integration [50], which makes this organosolv process more energetically feasible.

647

648 Table 6. Physicochemical properties of biomass and solvents used for energy calculations

Chemical property	Energy
Water heat capacity	0.00418 MJ·Kg <sup>-1</sup> ·K <sup>-1</sup>
Water enthalpy vaporisation at normal boiling point	2.26 MJ·Kg <sup>-1</sup>
Ethanol heat capacity	0.00242 MJ·Kg <sup>-1</sup> ·K <sup>-1</sup>
Ethanol enthalpy vaporisation at normal boiling point	0.841 MJ·Kg <sup>-1</sup>
Biomass heat capacity (estimated) [52]	0.0015 MJ·Kg <sup>-1</sup> ·K <sup>-1</sup>

649

650

651

#### 652 **4. Conclusions**

653 This work addresses a novel microwave-assisted, acid catalysed, organosolv process for the  
654 selective fractionation of bamboo analysing how and to what extent the reaction temperature  
655 (110-190 °C), solvent system (EtOH/H<sub>2</sub>O) and catalyst amount (0-5 vol.% formic acid) affect  
656 the yields and the most important properties of each fraction. The most important conclusions  
657 obtained from this work are summarised as follows.

658 1. The operating variables have a significant influence on bamboo fractionation, with three  
659 main fractions being obtained: i) a cellulose-rich solid fraction, ii) a hemicellulose rich water-  
660 soluble fraction and iii) a lignin rich fraction. The yields to each one of these fractions varied  
661 by 51-94%, 2-23% and 2-32%, respectively.

662 2. Increasing temperature positively enhances bamboo decomposition, increasing the overall  
663 bamboo conversion. The effect of the concentration of ethanol in water (solvent system)  
664 depends on the temperature. At low temperature (110 °C) the solvent system does not exert a  
665 very important influence, while a synergetic interaction between EtOH and H<sub>2</sub>O takes place  
666 at high temperature, and better results are obtained with EtOH/H<sub>2</sub>O mixtures than with pure  
667 solvents alone. The effect of the catalyst is relatively weak, however it does demonstrate a  
668 noticeable effect when using a high temperature and high proportions of water in the solvent  
669 system.

670 3. The cellulose rich solid fraction consists of the solid residue obtained after the microwave  
671 treatment of bamboo, and it is made up of un-reacted cellulose (44-83 wt.%), hemicellulose  
672 (0-21 wt.%) and lignin (12-34 wt.%). The water-soluble hemicellulose rich fraction consists  
673 of a mixture of oligomers, sugars, carboxylic acids, ketones and furans. The relative amounts  
674 (in C basis) of these families of compounds vary by 0-100 wt.%, 0-100 wt.%, 0-100 wt.%, 0-

675 13 wt.% and 0-4 wt.%, respectively. The solid rich lignin fraction comprises high pure  
676 (>95%) organosolv lignin, with a molecular weight range of 600 to 3177 g/mol.

677 4. An optimum for this bamboo fractionation process was found at 190 °C using a solvent  
678 system consisting of 45% EtOH/50% H<sub>2</sub>O (vol/vol) with a concentration of formic acid of 5  
679 vol.%, employing a reaction time as short as 15 min; thus converting this process in a very  
680 selective and efficient process for the valorisation not only of bamboo but also of many other  
681 types of biomass. These conditions maximise the solubilisation of lignin and hemicellulose in  
682 the organosolv system and minimise cellulose conversion; thus allowing the selective  
683 fractionation of bamboo into a high purity (84 wt.%) cellulose solid fraction, pure (>95%)  
684 organosolv lignin and a rich hemicellulose fraction consisting of a mixture of oligomers (27  
685 C-wt.%), sugars (56 C-wt.%) and carboxylic acids (14 C-wt.%) in water.

686

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