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

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13 **Abstract**

14 This work addresses a novel microwave-assisted, acid-catalysed, organosolv (EtOH/H_2O)

15 system for the selective fractionation of bamboo, examining the effects of the temperature

16 (110-190 ºC), solvent system (EtOH/H_2O) 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_2O) has on the process. At low temperature (110 ºC) the solvent

24 system does not have much influence, while a synergetic interaction between EtOH and H_2O

25 took place at higher temperatures, which allowed better results to be obtained with

26 EtOH/H_2O mixtures than with the pure solvents alone. The effect of the catalyst was

27 relatively weak, being greatest when using a high temperature (190 ºC) 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

fraction consisted of a mixture of oligomers, sugars, carboxylic acids, ketones and furans; and the solid rich lignin fraction comprised high purity (>95 wt.%) organosolv lignin. The optimisation of the process revealed that by using a temperature of 190 ºC, a solvent system consisting of 45 vol.% EtOH and 55 vol.% H_2O with a concentration of formic acid of 5 vol.% it is possible to fractionate bamboo into a high purity (84 wt.%) cellulose solid fraction, very pure (>95%) organosolv lignin and a rich water-soluble hemicellulose fraction consisting of a mixture of oligomers (27 wt.%), sugars (56 wt.%) and carboxylic acids (14 wt.%); thus converting this process into a very promising method for the selective fractionation of bamboo.

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

Highlights (85 characters including spaces)

- Microwave-assisted bamboo fractionation into cellulose, hemicellulose and lignin

- Analysis and optimisation of the temperature, EtOH/H_2O system and catalyst amount

- Synergetic effect between EtOH & H_2O during bamboo fractionation at high temperature

- Optimum conditions for bamboo fractionation: 190 ºC, 45% EtOH/H_2O and 5 vol.% HCOOH

1. Introduction

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

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

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_2O/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_2O/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   orgnaosolv 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_2O-ethanol organosolv system at 160°C for

115   2h, examining the effect of different homogeneous Lewis acidic catalysts, such as $FeCl_2$,

116     $CuCl_2$, $FeCl_3$, $Ga(OTf)_3$, and $ZrOCl_2$. 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_2O 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_2O 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_2O/γ-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_2SO_4 as a catalyst, achieving high yields for both lignin (75%) and

129　hemicellulose (72%) [23]. H_2O/tetrahydrofuran (THF) assisted by Na_2CO_3 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_2SO_4 catalysed systems: H_2O/tetrahydrofuran (THF) and pure H_2O 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 ºC)

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 assesement 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

2. Experimental

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-thiouronium 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_2O) 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.

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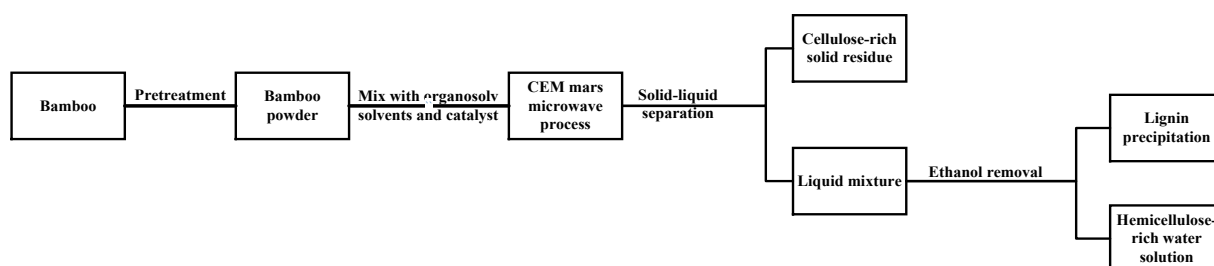
208   Figure 1. Fractionation process schematic diagram

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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 Kα 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 2θ 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
Overall results	
$\text{Overall conversion } (\%) = \left(1 - \dfrac{\text{mass of solid residue}}{\text{mass of bamboo}}\right)100$	Mass difference
$\text{Hemicellulose rich fraction yield } (\%) = \left(1 - \dfrac{\text{mass of gas (g)} + \text{mass of solid residue (g)}}{\text{mass of bamboo (g)}}\right)100$	Mass difference
$\text{Cellulose rich fraction yield } (\%) = \dfrac{\text{mass of solid residue (g)}}{\text{mass of bamboo (g)}}100$	Mass difference
$\text{Lignin rich fraction yield } (\%) = \dfrac{\text{mass of lignin (g)}}{\text{mass of bamboo (g)}}100$	
$\text{Gas yield } (\%) = \dfrac{\text{mass of gas (g)}}{\text{mass of bamboo (g)}}100$	Mass difference
Cellulose rich fraction phase properties	
$\text{Composition (wt.\%)} = \dfrac{\text{mass of structural component (g)}}{\text{mass of solid residue(g)}}100$	Chemical titration
Lignin rich fraction phase properties	
Molecular weight (g/mol)	GPC (Gel permeation chromatography)
Hemicellulose rich fraction phase properties	
TOC (Total Organic Carbon)	TC-IC (Total Carbon- Inorganic Carbon) measurement
$\text{Composition (C} - \text{wt.\%)} = \dfrac{\sum \text{mass of C of each compound (g)}}{\text{total mass of C in solution (g)}}100$	HPLC (High performance liquid chromatography)

242   wt.% = weight percentage

243   C-wt.% = percentage in carbon basis

244

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, α: ±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_2O

266   and catalyst amount) were normalised from -1 to 1 (codec 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.

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

281 *Oxygen was calculated by difference

282

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

Table 3. Experimental conditions: temperature (ºC), solvent system (EtOH/H₂O, vol. %) and formic acid (vol. %) and results obtained in the
293

experiments
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Run	1	2	3	4	5	6	7	8	9-12	13	14	15	16	17	18	19
T (ºC)	110	190	110	190	110	190	110	190	150	110	190	150	150	150	150	190
EtOH/EtOH+H₂O (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
Overall results																
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
Cellulose rich fraction fibre analysis																
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
Lignin rich phase																
Mw (g/mol)	600	1528	3177	2758	2039	1053	3133	2540	2142±261	2262	1614	1567	2768	3060	2201	2323
Hemicellulose rich phase composition																
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^2	I.Term	A (T)	B (EtOH%)	C (formic acid)	AB	AC	BC	ABC	A^2	B^2	C^2	A^2B	A^2C	AB^2	AC^2	A^2B^2
Overall results																	
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.	-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	-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	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	-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)	1.24 (18)
Solid residue fractionation composition																	
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
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	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)	-3.65 (10)
Lignin phase																	
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	-537.15 (13)
Water phase properties																	
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
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	-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
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)	-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^2·A^2 + Coefficient B^2·B^2 + Coefficient
310   C^2·C^2 + Coefficient A^2B·A^2B + Coefficient A^2C·A^2C + Coefficient AB^2·AB^2 + Coefficient AC^2·AC^2 + Coefficient A^2B^2·A^2B^2
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 ºC 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 ºC 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 ºC) 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_2O 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_2O mixture than using the pure solvents (EtOH or H_2O 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 (110ºC) the effect of the

399   addition of up to 5 vol.% of formic acid in the process is almost negligible. However, at 190

400   ºC 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

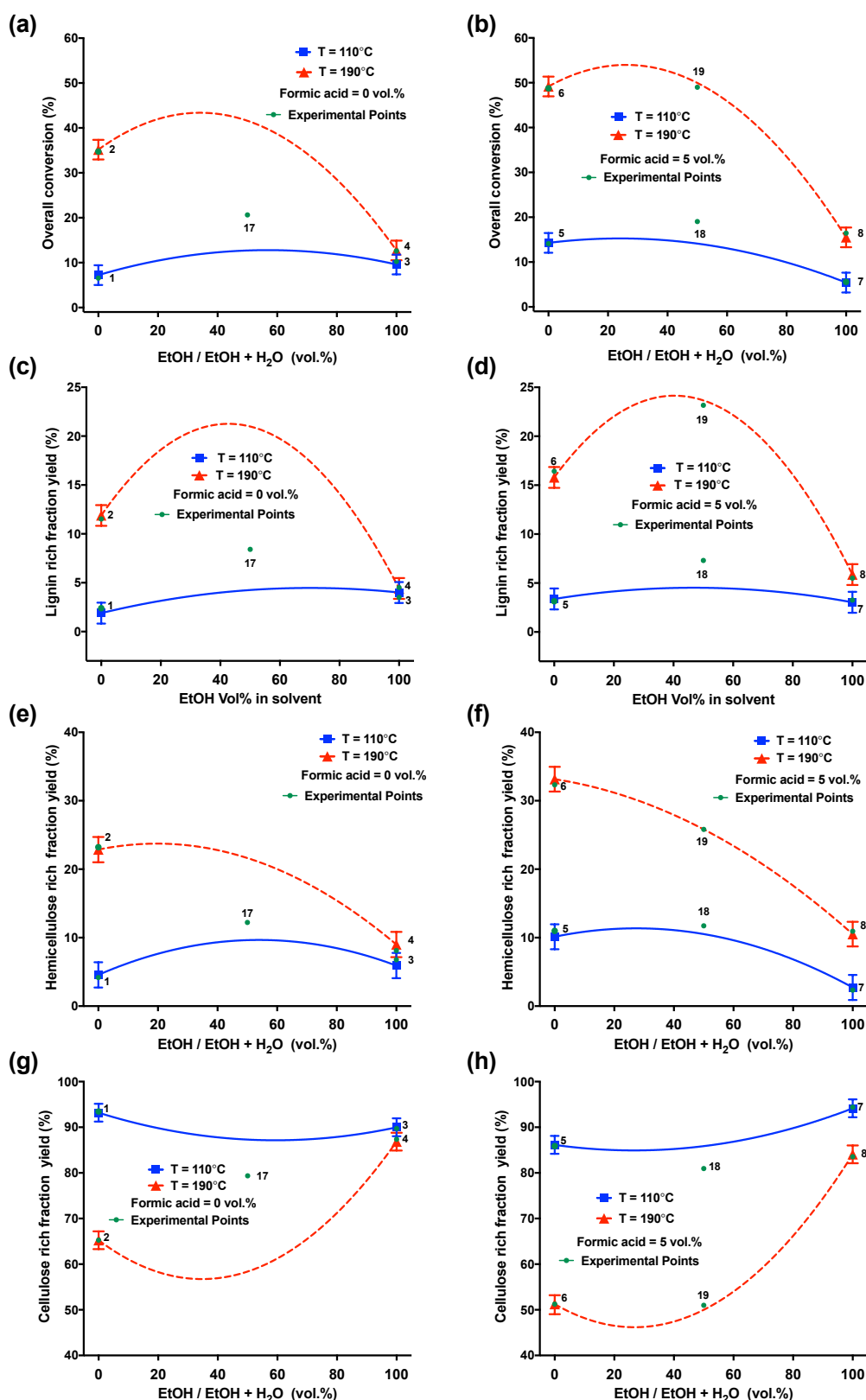406   lower solubilisation of formic acid protons in ethanol than in water [30].

407

Figure 2. Interaction plots between the temperature and the solvent system (EtOH/H₂O) without/with catalyst for the overall bamboo conversion (a/b), lignin rich solid fraction yield (c/d), hemicellulose rich liquid fraction yield (e/f), and cellulose rich solid fraction yield (g/h). Bars are LSD intervals with 95% confidence.

3.3 Effects of operating conditions on the properties of each fraction

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

3.3.1 Cellulose-rich solid fraction

This cellulose-rich solid fraction consists of the solid residue obtained by filtration after the microwave treatment of bamboo. The characterisation of this solid by chemical titration revealed that this fraction contains cellulose and, in less proportion, unreacted hemicellulose and lignin with varying compositions depending on the operating conditions. The amounts of cellulose, hemicellulose and lignin in the spent solid vary by: 44-83 wt.%, 0-21 wt.% and 12-34 wt.%. The effect of the operating conditions on the composition of this fraction according to the ANOVA analysis, and the cause-effect Pareto principle is shown in Table 4. The temperature and its interaction with the catalyst amount and in lesser extent with the solvent system are the factors exerting the greatest influence on the proportions of cellulose and lignin. The temperature, the solvent system and the interaction between these two variables primarily influence the proportion of hemicellulose. The effects of the operating conditions and interactions between them on the relative amounts of cellulose, hemicellulose and lignin are plotted in Figure 3. In particular, Figure 3 a, b and c shows the effects of the solvent system and the temperature in the absence of catalyst. Figures 3 d, e and f show these effects when using the highest concentration of catalyst (5 vol.%) employed in this work.
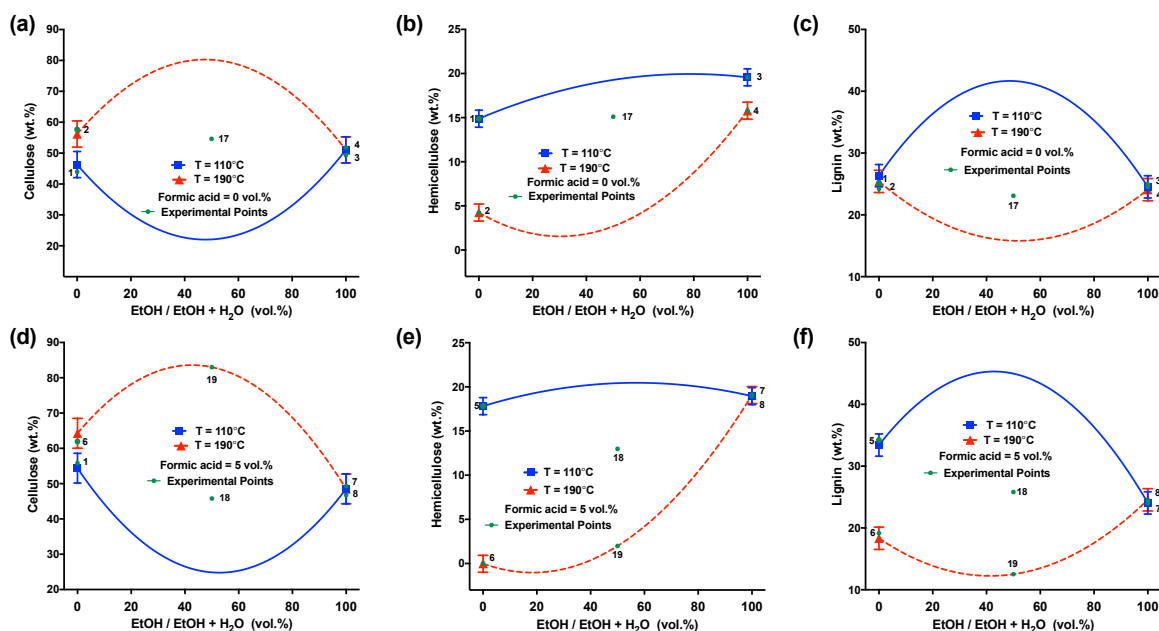
Figure 3. Interaction plots between the temperature and the solvent system (EtOH/H$_2$O) without/with catalyst for the relative amounts of cellulose (a/d), hemicellulose (b/e) and lignin (c/f) of the cellulose rich solid fraction. Bars are LSD intervals with 95% confidence.

With respect to the effects of the temperature on the composition of the cellulose rich fraction, Figure 3 shows how regardless of amount of catalyst employed (0-5 vol.% formic acid), an increase in the temperature between 110 and 190 ºC results in a decrease in the proportions of hemicellulose and lignin, and consequently, the proportion of cellulose increases. An increase in the temperature helps to solubilise the hemicellulose and lignin fractions of bamboo into the solvent system, and therefore a cellulose-rich solid is obtained due to the positive kinetic effect of the temperature in the process. A solid with a high proportion of hemicellulose (80-85%) can be obtained at 190 ºC employing a concentration of EtOH of around 40-50 vol.%. This is in good agreement with other works where the EtOH/H$_2$O solvent system was used for the fractionation of biomass [41-45].

22

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 ºC, 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 α-hydroxyl groups in bamboo to increase the solubility of lignin, preventing

464   its condensation; thus decreasing the lignin content of this fraction at 110 ºC [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 a low temperature. The opposite effect takes places 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 (190ºC). 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

23

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 ºC); 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 ºC)

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
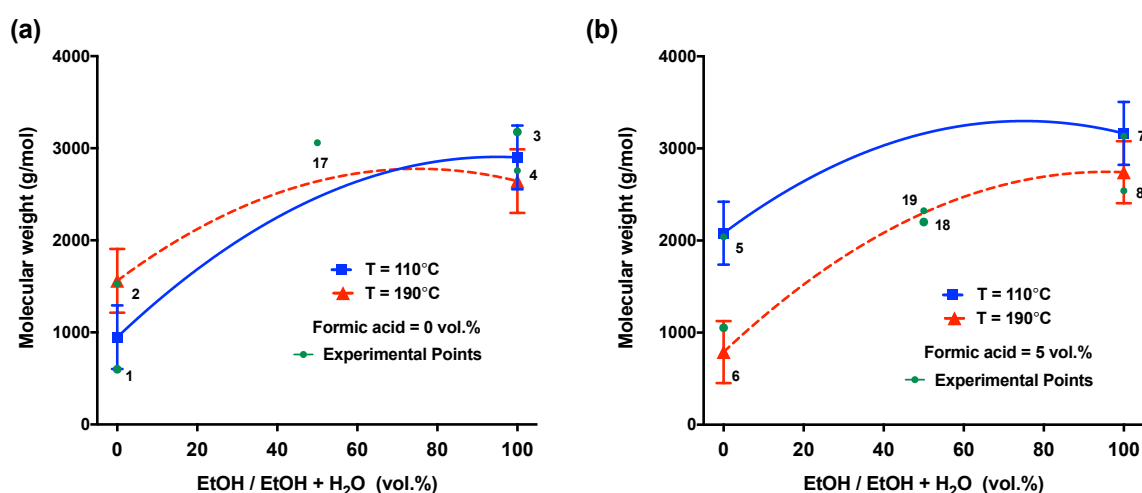
509   lignin produced [20].

510

Figure 4. Interaction plots between the temperature and the solvent system (EtOH/H_2O)
without/with catalyst (a/b) for the molecular weight of the lignin produced. Bars are LSD
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

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 (γ-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
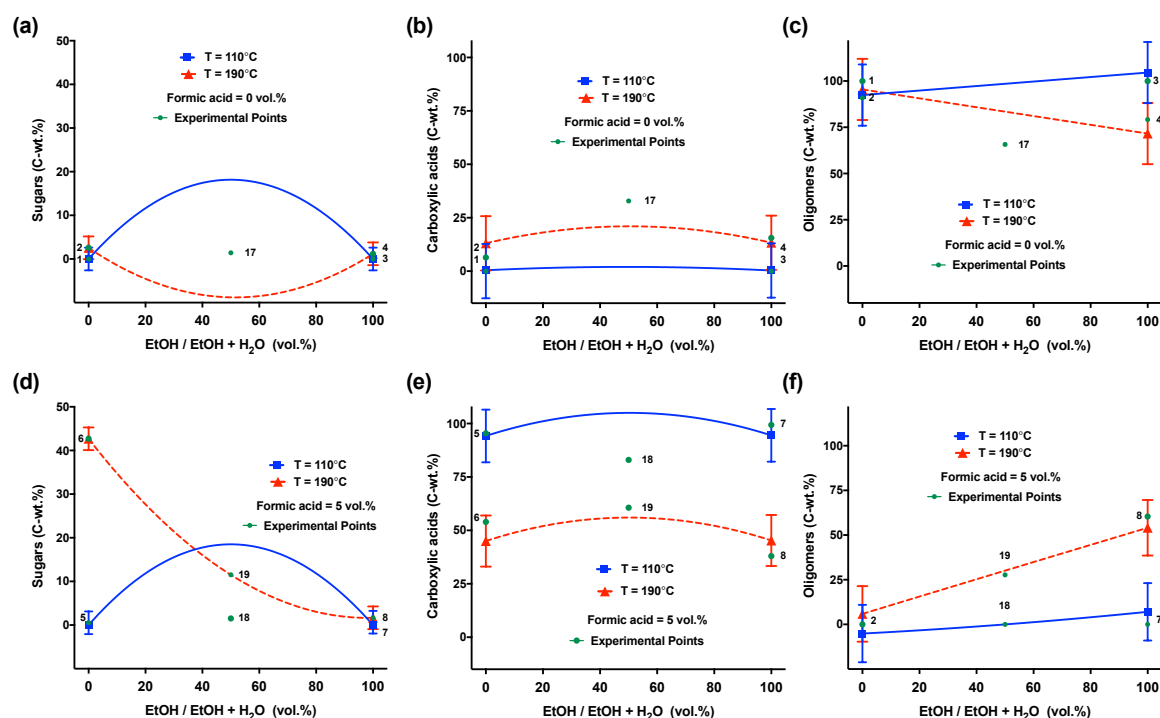
542   between the operating variables also take place.

544   Figure 5. Interaction plots between the temperature and the solvent system (EtOH/H_2O)
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.

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 ºC 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 assestment**

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
$$D(X) = (d_1^{r1} \cdot d_1^{r2} \cdot ... \cdot d_1^{rn})^{\frac{1}{\Sigma rj}} = (\textstyle\prod_{j=1}^{n} d_j^{rn})^{\frac{1}{\Sigma rj}} \qquad \text{(Eq. 1)}$$

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 (ºC)	none	110-190		190	
EtOH/EtOH+H$_2$O (vol.%)	none	0 - 100		45	
HCOOH (vol.%)	none	0 - 5		5	
Overall Conversion (%)	maximise	0-100	3	52	51.23
Cellulose rich faction					
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
Lignin rich fraction					
Yield (%)	maximise	0-100	3	24	23.87
Molecular weight (DA)	none			2203	2201
Hemicellulose rich fraction					
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 ºC using a

590 solvent system consisting of 45% EtOH/50% H$_2$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
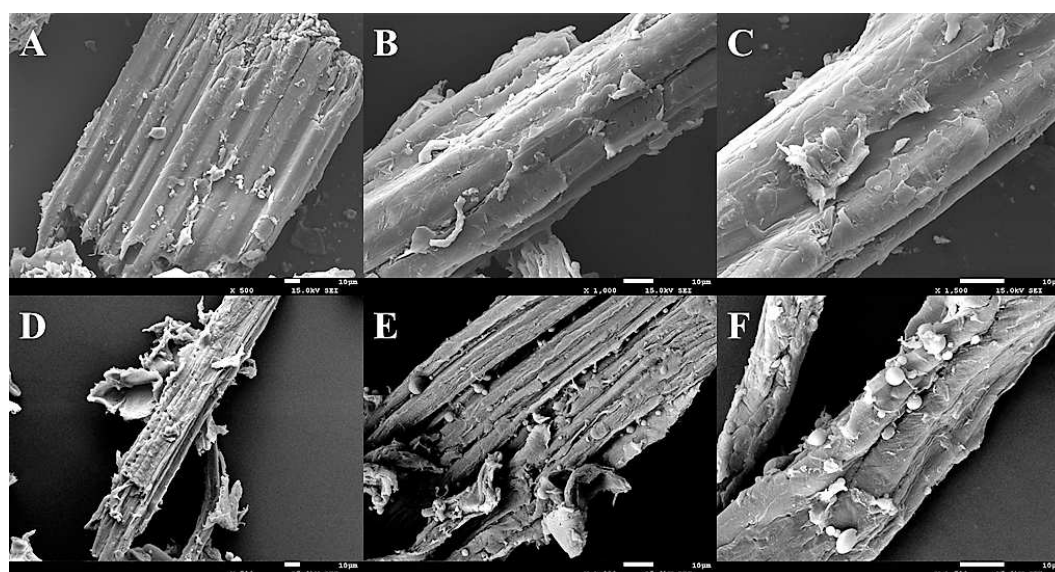
612   results listed in Table 5.

613

614

615 Figure 6. SEM images of bamboo powder (A: ×500, B: ×1000, C: ×1500) and optimum
616 experimental run solid residue (D: ×500, E: ×1000, F: ×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
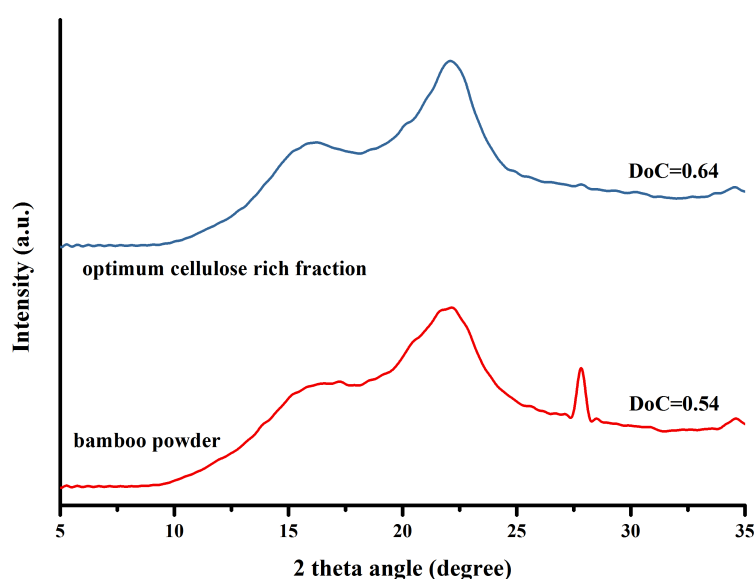
627   crystallinity of this fraction.

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

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

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

Chemical property	Energy
Water heat capacity	$0.00418 \text{ MJ·Kg}^{-1}\text{·K}^{-1}$
Water enthalpy vaporisation at normal boiling point	2.26 MJ·Kg^{-1}
Ethanol heat capacity	$0.00242 \text{ MJ·Kg}^{-1}\text{·K}^{-1}$
Ethanol enthalpy vaporisation at normal boiling point	0.841 MJ·Kg^{-1}
Biomass heat capacity (estimated) [52]	$0.0015 \text{ MJ·Kg}^{-1}\text{·K}^{-1}$

4. Conclusions

This work addresses a novel microwave-assisted, acid catalysed, organosolv process for the selective fractionation of bamboo analysing how and to what extent the reaction temperature (110-190 ºC), solvent system (EtOH/H_2O) and catalyst amount (0-5 vol.% formic acid) affect the yields and the most important properties of each fraction. The most important conclusions obtained from this work are summarised as follows.

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

2. Increasing temperature positively enhances bamboo decomposition, increasing the overall bamboo conversion. The effect of the concentration of ethanol in water (solvent system) depends on the temperature. At low temperature (110 ºC) the solvent system does not exert a very important influence, while a synergetic interaction between EtOH and H_2O takes place at high temperature, and better results are obtained with EtOH/H_2O mixtures than with pure solvents alone. The effect of the catalyst is relatively weak, however it does demonstrate a noticeable effect when using a high temperature and high proportions of water in the solvent system.

3. The cellulose rich solid fraction consists of the solid residue obtained after the microwave treatment of bamboo, and it is made up of un-reacted cellulose (44-83 wt.%), hemicellulose (0-21 wt.%) and lignin (12-34 wt.%). The water-soluble hemicellulose rich fraction consists of a mixture of oligomers, sugars, carboxylic acids, ketones and furans. The relative amounts (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 ºC using a solvent

678 system consisting of 45% EtOH/50% H_2O (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.

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