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1 **Microwave-assisted Organosolv pretreatment of a sawmill mixed feedstock for**  
2 **bioethanol production in a wood biorefinery**

3 A

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9 **Abstract**

10 A mixture of sawmill feedstocks comprising four softwood species (fir, spruce, Scots pine,  
11 and Douglas fir) was subjected to a microwave-assisted Organosolv pretreatment. The  
12 influence of H<sub>2</sub>SO<sub>4</sub> as a catalyst, ethanol-water ratio as the solvent, and temperature on wood  
13 fractionation, cellulose yield and purity, lignin recovery, and inhibitor formation were  
14 investigated. Minute addition of H<sub>2</sub>SO<sub>4</sub> (0.25% w/w) was found to enhance lignin extraction  
15 and hemicellulose hydrolysis, thus providing cellulose yield and purity above 68%±2% and  
16 61%±3%, respectively. The increase in H<sub>2</sub>SO<sub>4</sub> up to 1% and temperature up to 190°C,  
17 impaired cellulose yields a result of intense hydrolysis, thus degrading cellulose and  
18 hemicellulose. Conversely, the increase in ethanol-water ratio enhanced delignification  
19 without cellulose degradation. Similarly, inhibitor formation increased with pretreatment  
20 severity (H<sub>2</sub>SO<sub>4</sub> content and temperature), but was mitigated by higher ethanol-water ratio.  
21 Finally, under best conditions (60:40 ethanol-water, 175°C, 0.25% H<sub>2</sub>SO<sub>4</sub>), cellulose yield  
22 and purity reached 82%±3% and 71%±3%, respectively.

23 **Keywords:** Organosolv pretreatment, mixed sawdust, cellulose, ethanol, lignin

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## 1 **1. Introduction**

2 Shortage of fossil energy and increasing concerns regarding environmental pollutions have  
3 caused a rising interest in renewable energy research in many countries. In France, as in many  
4 other countries, bioethanol is considered as a promising renewable fuel because of its heat and  
5 power potential aspects obtained through a combustion process (Zhao et al., 2009).

6 Bioethanol can be produced from different resources. Therefore, depending on the resources,  
7 it is called either first-generation or second-generation bioethanol. First-generation bioethanol  
8 is produced from edible food crops, such as corn, beef, sugar cane, etc., while second-  
9 generation ethanol derives from lignocellulosic biomass or agricultural residues that do not  
10 compete with agri-food production. The latter is the most interesting because lignocellulosic  
11 biomass represents one of the most abundant renewable resources on Earth and certainly one  
12 of the most affordable. It is estimated a worldwide production of 200 10<sup>9</sup>dry tons a year  
13 (Kuhad and Singh, 1993). A wide variety of substrates may be considered, such as softwood  
14 and hardwood, agricultural byproducts, such as wheat straw and corn stover, or even  
15 lignocellulosic waste generated by pulp, sawmill, and agricultural industries. Their conversion  
16 to ethanol as a biofuel could satisfy a wide part of the global energy demand, still essentially  
17 covered by-products derived from oil, while generating a new opportunity for the forest and  
18 agricultural sector. However, the chemical structure of lignocellulose, mainly composed of  
19 cellulose, hemicellulose, and lignin, makes it recalcitrant to deconstruction, thereby limiting  
20 enzyme ability to convert cellulose and hemicellulose into fermentable sugars (Mupondwa et  
21 al., 2017). This constitutes both a technological and economic issue to the development of  
22 second-generation biofuels. Owing to these structural characteristics, biomass pretreatment is,  
23 thus, an essential step to break down the lignin structure and simultaneously disrupt the  
24 crystalline structure of cellulose for enhancing enzyme accessibility to cellulose during the  
25 hydrolysis step (Alvira et al., 2010).

1 In the last decades, various biomass pretreatment methods were developed. One of these, the  
2 Organosolv process, appears to be an attractive and eco-friendly method to remove lignin  
3 from lignocellulose materials using organic solvents in order to purify cellulose (Akgul and  
4 Kirci, 2009; Zhao et al., 2009; ). This process was originally developed as an alternative  
5 treatment in the pulp and paper industries (Kleinert, 1974). Most of the employed solvents can  
6 be easily removed and recycled due to their low boiling point. Commonly used organic  
7 solvents are ethanol, methanol, glycerol, acetone, organic acids, or a mixture of water/organic  
8 solvent at a high temperature ranging from 100 to 250°C, using a long extraction time between  
9 30 and 120 min, and a solid-liquid ratio from 1:20 to 1:5 (Borand and Karaosmanoğlu, 2018).  
10 In the case of ethanol-water solvent, an acid catalyst (mainly H<sub>2</sub>SO<sub>4</sub> or HCl, 0.5–2.0 % w/w)  
11 has often been added because non-catalyzed conditions typically require more severe  
12 conditions. In the 1990s, several processes had reached industrial production: the  
13 ORGANOCELL process using methanol (Lindner and Wegener, 1990, 1989, 1988), the  
14 ALCELL process using ethanol (Pye and Lora, 1991), and the MILOX process using  
15 peroxyformic acid (Seisto et al., 1997), but for pulp and paper, they often failed to achieve  
16 economic sustainability in comparison to alternative Kraft, sulfite or soda pulp processes.  
17 Organosolv pretreatments constitute, however, an attractive alternative for the production of  
18 second-generation bioethanol because they can provide both cellulose and lignin with high  
19 purity, and minimize at the same time the production of fermentation inhibitors (Mupondwa  
20 et al., 2017). In practice, ethanol Organosolv pretreatment has been the most commonly  
21 applied to a wide range of lignocellulose feedstocks, including hardwood and softwood.  
22 Typical pretreatment conditions were studied for different woody biomass. Bouxin et al.  
23 (2014) pretreated Sitka spruce with an ethanol-water ratio ranging 50–70% v/v while using  
24 0.75–1.25% H<sub>2</sub>SO<sub>4</sub> for a temperature range of 165–205°C for 40 to 94 min. As a result, they  
25 recovered a pulp with 18–24% w/w residual lignin. Mabee et al. (2006) used 50% v/v ethanol

1 to treat Douglas fir feedstock at pH 2 (H<sub>2</sub>SO<sub>4</sub>). They varied temperature from 181 to 202°C  
2 and the reaction time from 14 to 90 min. In this case, they recovered a pulp containing 90%  
3 cellulose.

4 In this work, the goal is, therefore, to fractionate an industrial and readily available sawmill  
5 feedstock (a mixed sawdust consisting of four softwood species: fir, spruce, Scots pine, and  
6 Douglas fir) in a perspective of cellulose recovery and purification for bioethanol production  
7 through an “Organosolv pretreatment” based on an original microwave heating system, also  
8 called dielectric heating (Jian, 2015). The novelty and originality of this study, thus, result  
9 from two main points. First, mixed feedstocks have not been investigated up to now, except  
10 by Pan et al. (2005). However, in many countries of the world, dealing with a mixed  
11 feedstock including several wood species is compulsory for economic and environmental  
12 reasons because each species is not abundant enough within a distance lower than 100 km for  
13 supplying a wood biorefinery. Secondly, hybrid microwave-assisted Organosolv processes  
14 have also been disregarded in the literature up to now, as only the works of Gong et al. (2010)  
15 and Liu et al. (2010) can be found, dealing with organic acid on rice straw and aqueous  
16 glycerol solutions on recalcitrant softwood, respectively. In the present study, an original  
17 ethanol microwave-assisted Organosolv pretreatment was applied to the sawmill feedstock.  
18 Organosolv cellulose recovery yield and purity were investigated as a function of the  
19 operating parameters, but also the formation of fermentation inhibitors and lignin recovery  
20 yield, as lignin valorization may also constitute a key point in the economic sustainability of  
21 bioethanol production from wood.

22

## 23 **2. Materials and methods**

### 24 ***2.1. Materials: Feedstocks and reagents***

1 Samples of mixed sawdust from four softwood species were used in this study: fir, spruce  
2 (*Picea abies*), Scots pine (*Pinus sylvestris*), and Douglas fir. The mixture was provided by a  
3 local sawmill company (*Les Scieries du Centre Marcel Esbelin et Cie*), located in Cournon  
4 d’Auvergne (France), which has been extracted from a monthly feedstock. Ethanol, sulfuric  
5 acid, sodium chlorite, and other chemicals (reagent grade) were purchased from Sigma-  
6 Aldrich (France).

7

## 8 **2.2. Feedstock chemical characterization**

9 After oven-drying at 110°C until constant weight has been achieved, and further removal of  
10 any visible bark material, the dried mixed sawmill sawdust was ground using a ball mill  
11 (BM500, *Anton Paar GmbH*, Austria) to a granulometry lower than 1mm and screened to  
12 obtain sawdust with an average size of 0.5mm.

13 Ash content was determined, first, according to NREL standard method (Sluiter et al., 2008a)  
14 by complete incineration of 2 g sawdust in a muffle furnace at 525°C for 6 hours, by weighing  
15 the residues.

16 The extractives were determined by successive Soxhlet extractions, first with  
17 dichloromethane for 6 h, then with ethanol for 6 h, and finally with water for 8 h. After each  
18 extraction, the samples were oven-dried at 110°C and weighed (Sluiter et al., 2008b).

19 The lignin content was determined using the Klason method according to Sluiter et al.  
20 (2008a). In this method, 200 mg of free extractives sawdust was hydrolyzed with 2 ml of  
21 concentrated sulfuric acid (72% w/w) at 30°C in a water bath. After 1 h, the mixture was  
22 diluted to a concentration of 4% w/w by adding 56 ml of pure water, and then autoclaved at  
23 125°C for 1 h. The sample was filtered through a crucible using a vacuum pump. The solid  
24 residue, referred to as Klason lignin, was washed with distilled water; the crucible was placed  
25 in an oven at 110°C overnight and, then, weighed. The hydrolysate was sampled to determine

1 the acid-soluble lignin by measuring its absorbance at 205 nm using a UV/Vis  
2 spectrophotometer.

3 The holocellulose (*i.e.*, cellulose and hemicelluloses) content was determined using sodium  
4 chlorite as a delignification reagent according to Browning method (Browning, 1967; Rowell,  
5 1980). This method consisted in treating 1 g of sample in 32 ml of distilled water, in which  
6 0.2 ml of acetic acid and 0.4 g of sodium chlorite were added in a pressure tube. The mixture  
7 was then heated in a water bath at 70°C equipped with a shaker for 6 h to 8 h; after each hour,  
8 the same amount of acetic acid and sodium chlorite was being added until bleaching was  
9 achieved. After 6 to 8 hours reaction, almost all of the lignin was generally removed. The tube  
10 was then removed from the water bath and allowed to cool down at room temperature. The  
11 mixture was filtered under vacuum using a filter crucible (Porosity 3). The solid residue was  
12 first washed with distilled water, and then with acetone, oven-dried at 110°C and weighed.  
13 Finally, the cellulose (*i.e.*,  $\alpha$ -cellulose) content was determined using an alkali hydrolysis with  
14 NaOH 27% for 1 h. This allowed the release of the hemicellulose fraction from the  
15 holocellulose. The solid phase was recovered by filtration, washed, dried and weighed.  
16 All these experiments were carried out in triplicate.

17

### 18 **2.3. Organosolv pretreatment**

19 The Organosolvpretreatmentexperiments were performed in a microwave reactor system  
20 (Monowave 450, Anton PaarGmbH, Austria)equipped with an autosampler for up to 24-  
21 closed-vessel easily queued and processed automatically. As this work is a preliminary study  
22 on the hybrid microwave-assisted Organosolv pretreatment, the main operating parameters  
23 and their respective range had to be defined from the state of the art on Organosolv  
24 pretreatment, and then assessed from preliminary treatments that are not detailed in this paper.  
25 Finally, the sawdust mixture (same granulometry as in section 2.2) was suspended in an

1 ethanol-water mixture as the solvent with a fixed solid-to-liquid ratio (S/L=1:15 g dry weight  
2 sawdust/15 mL solvent) and a fixed treatment time (60 min). A variable sulfuric acid (H<sub>2</sub>SO<sub>4</sub>)  
3 concentration (0, 0.25, 0.5, 0.7, 1% w/w) was used as a catalyst; several ethanol-water ratios  
4 (40:60, 50:50, 60:40, 70:30, 80:20 v/v) and temperature conditions (175 and 190°C) were  
5 applied. A control program with the appropriate temperature, ramp time, treatment time, and  
6 cooling time was defined. For all the experiments, the reactor was heated to the appropriate  
7 temperature for a constant treatment time of 60 min, with a ramp time of 15 min for reaching  
8 the target temperature (**Figure 1**). A key advantage of microwave-assisted heating was to  
9 achieve a fast temperature ramp: for example, Park et al. (2010) when treating Pitch pine  
10 (50% v/v ethanol, 1% w/w H<sub>2</sub>SO<sub>4</sub> for a range temperature 150-2010) applied a treatment  
11 time lower than 20 min, which may appear short in comparison to this work, but after a 50  
12 min heating ramp to reach the treatment temperature; this highlights that the length of a  
13 treatment cycle was very close to the present work.

14 Each pretreatment was carried out in 10 borosilicate glass vessels of 30 mL under our  
15 investigation conditions including substrate concentration, temperature, time, and acid  
16 concentration, as defined above. According to the S/L ratio, ground mixed sawdust samples  
17 were accurately weighed and transferred to the borosilicate glass extraction vessels. The  
18 sulfuric acid solutions at different concentrations were added to each sample (1 mL) to reach a  
19 volume about 20 mL suspension for all treatments, *i.e.* which occupies nearly 66% of the  
20 borosilicate vessel volume. The extraction vessels were sealed, and the mixtures were  
21 homogenized with a magnetic stirrer for all the pretreatment period. Then, the microwave  
22 extraction process was applied according to the experimental conditions. Due to heating,  
23 pressure increased in the sealed vessel (**Figure 1**), but was an uncontrolled variable that could  
24 only be measured over time and resulted from temperature and solvent composition; in  
25 practice, pressure reached a nearly constant value within the 60 min when treatment



1 temperature was constant, as illustrated in **Figure 1**. Power consumption was also recorded  
2 over time, but remained very low except in the first minute of the heating ramp, as highlighted  
3 by **Figure 1**. Finally, the extraction vessels were allowed to cool down to room temperature  
4 (air cooling) before removal from the system and the mixture was filtered using a crucible  
5 (Porosity 3). The solid fraction called "pulp" recovered after the thermomechanical  
6 Organosolv treatment was washed with hot water to remove residual lignin, dried and  
7 weighed so as to measure the mass of remaining solid. This solid phase was then submitted to  
8 cellulose, hemicellulose, and residual lignin chemical analyses already described in section  
9 2.2. The performance of the Organosolv pretreatment was assessed by estimating cellulose  
10 recovery yield and cellulose purity in the remaining solid fraction, defined as follows:

$$11 \text{ Cellulose yield (\% w/w)} = 100 \frac{\text{Cellulose content in the remaining solid (g)}}{\text{Initial cellulose amount in the raw sawdust(g)}} \quad (1)$$

$$12 \text{ Cellulose purity (\% w/w)} = 100 \frac{\text{Cellulose content in the remaining solid (g)}}{\text{Mass of the remaining solid (g)}} \quad (2)$$

13 The liquid phase(black liquor)was sampled to investigate the formation of fermentation  
14 inhibitors, such as furfural and 5-hydroxymethyl-2-furaldehyde (HMF) using an Agilent  
15 HPLC system (1100 Series) fitted with a Luna® C18 column (*Phenomenex*, USA) operated at  
16 80°C and a UV detector used at 280 nm. Distilled water, methanol and acetic acid (80:10:3:  
17 v/v/v) were used as the eluent at a flow rate of 0.5 mL/min. Appropriately diluted aliquots (20  
18 µl) were injected after passing through a 0.45 µm syringe filter. The remaining liquor was  
19 added to three volumes of acidic water (pH 2) to promote the precipitation of the lignin. The  
20 precipitated lignin was then recovered, dried (105°C), and weighed after centrifugation,  
21 filtration and washing processes. Lignin recovery yield was expressed as follows:

$$22 \text{ Lignin yield (\% w/w)} = 100 \frac{\text{Precipitated lignin from black liquor (g)}}{\text{Initial lignin amount in the raw sawdust(g)}} \quad (3)$$

23 All the experiments were carried out in triplicate; confidence intervals could then be deduced  
24 based on standard deviation.

1

## 2 **3. Results and discussion**

### 3 *3.1. Chemical composition of the sawmill mixed feedstocks*

4 **Table 1** displays the mixed sawmill feedstock composition prior to pretreatment deduced  
5 from chemical analyses. Due to the lack of studies involving such a mixture in the literature,  
6 the chemical composition of the mixed sawdust was compared to the composition reported in  
7 the literature of the different softwood species that composed the mixture in order to validate  
8 the chemical characterization analyses. Roughly, the composition of the mixed sawdust  
9 appears to lie between the values found in the literature and reported in **Table 1** for three  
10 softwood species of the mixture for cellulose and lignin, while experimental data are just  
11 above the maximum value from the literature for hemicelluloses. This highlights that  
12 chemical analyses are consistent with literature data.S

13

### 14 *3.2. Effect of sulfuric acid and temperature on the Organosolv pretreatment*

15 The Organosolv pretreatment assays were conducted, first, at constant ethanol-water ratio,  
16 60:40 v/v, under the preselected conditions of section 2 in terms of sulfuric acid concentration  
17 (0%, 0.25, 0.50%, 0.75% and 1% w/w) and temperature (175°C and 190°C) using the  
18 assisted-microwave reactor for heating.

#### 19 *3.2.1. Effect on cellulose yield and purity*

20 Experimental results are summarized in **Table 2**. First, this table shows that without a  
21 catalyst, the mass of remaining solid recovered decreased moderately from 80%±2% to  
22 73%±4% when the temperature was increased from 175 to 190°C, which highlights that  
23 temperature favors slightly wood fractionation. From chemical analyses, it arises, however,  
24 that cellulose yield decreased at the same time from 98%±2% to 89%±3%. So, without  
25 catalyst, higher temperature reduced cellulose recovery yield, while purity in the solid phase

1 was not improved, about  $54\% \pm 2\%$  for both temperatures. As an increase in temperature also  
2 induced an increase in pressure (**Table 2**), both effects could not be distinguished.

3 Conversely, the addition of the catalyst, even at the smallest quantity of 0.25% w/w, induced  
4 a steep decrease of the mass of remaining solid after pretreatment. The same trend could be  
5 observed for cellulose yield between 0% and 0.25% catalyst content, but not for purity that  
6 increased with acid concentration for both temperatures. As the addition of sulfuric acid  
7 concentration increased (0.25, 0.5, 0.75 and 1%), the amount of remaining solid always  
8 decreased, but the decrease remained moderate at 175°C (from  $52\% \pm 4\%$  to  $46\% \pm 4\%$ ), while a  
9 steep fall was observed at 190°C (from  $49.8\% \pm 0.2\%$  to  $19\% \pm 4\%$ ) in **Table 2**. A careful  
10 analysis of the chemical composition of the remaining solid is described in **Figure 2**.

11 According to **Figure 2a**, the cellulose, hemicellulose and lignin contents were almost stable  
12 as the sulfuric acid concentration increased at 175°C; only the amount of impurities clearly  
13 decreased when catalyst concentration was increased. Conversely, it can be noticed in **Figure**  
14 **2b** a sharper evolution of the chemical composition at 190°C compared to that at 175°C. In  
15 practice, the increase of H<sub>2</sub>SO<sub>4</sub> content at 190°C did not improve purity and finally lead to a  
16 remarkable decrease in cellulose and hemicellulose content when 1% w/w acid was used.

17 Similar results were also observed by Pan et al. (2007) when using sulfuric acid as the catalyst  
18 and by Park et al. (2018) using peroxide as a catalyst. This important decrease may be due to  
19 the combined increase of two major Organosolv pretreatment parameters (temperature and  
20 sulfuric acid concentration), which rather than allowing just the lignin degradation, appear to  
21 degrade both lignin and cellulose at the same time. At 190°C, the lignin content in the  
22 pretreated substrate at maximum sulfuric acid (1% w/w) was found to be higher than cellulose  
23 content. According to Pan et al. (2006, 2007), the explanation of this behavior is that  
24 carbohydrates are hydrolyzed faster than lignin at high temperature, which results in a relative  
25 increase in residual lignin from the solid fraction. Conversely, as pressure was insignificantly

1 affected by the acid content, pressure could not explain the variability of cellulose yield and  
2 purity in section 3.2; the same conclusion stands for lignin recovery and inhibitor formation.  
3 As a result, it emerges from these results that cellulose recovery and yield are both favored by  
4 a small addition of catalyst in the microwave-assisted Organosolv treatment, but that working  
5 at 190°C is useless.

6

### 7 3.2.2. Effect on the Organosolv lignin recovery

8 Organosolv pretreatments are also known to provide high-quality lignin with a good purity.  
9 This may be an advantage for the economic sustainability of the process. For this reason,  
10 lignin recovery has also been investigated. **Table 3** presents the evolution of Organosolv  
11 lignin recovery yield as a function of sulfuric acid for both temperatures. Regarding the  
12 results, it appears that without sulfuric acid, Organosolv lignin yield is very low, but increases  
13 with temperature from 13.1%±0.9% to 26%±1% w/w on the basis of the total lignin from the  
14 raw sawdust feedstock. However, the yield more rapidly increased with increasing catalyst  
15 concentration because acid is known to promote cleavage and lignin dissolution. Furthermore,  
16 an increase in lignin yield was also observed as the temperature rose in the presence of  
17 catalyst. Consequently, the maximum Organosolv lignin recovered was usually obtained  
18 between 0.75–1% H<sub>2</sub>SO<sub>4</sub> for both temperatures, with an overall maximum of about  
19 57.5%±0.4% at 190°C (**Table 3**). A comparison with literature data shows that this value is  
20 higher than in Organosolv pretreatments aimed at bioethanol production (see, *e.g.*, Monrroy  
21 Almengor et al., 2012), but lower than when lignin production is targeted (*e.g.*, in the data  
22 reviewed by Borand and Karaosmanoğlu, 2018), as expected.

23 According to these results, it appears that sulfuric acid used as a catalyst for the Organosolv  
24 fractionation at different concentrations (0, 0.25, 0.5, 0.75, and 1% w/w) is an influential

1 parameter for the delignification of the sawdust feedstock and for an efficient recovery of  
2 purified lignin. This was confirmed by Sun and Cheng (2002): the addition of an acid catalyst  
3 to pulping media causes the cleavage of more ether-linkages, but also favors the occurrence of  
4 intramolecular condensation reactions, giving rise to an enhanced cellulose-lignin separation  
5 and a more complex lignin structure. To assess the quality of the Organosolv lignin recovered  
6 in this work, infrared spectra measurement using an FTIR-ATR (Attenuated Total Reflection)  
7 equipment (Vertex 70 from *Bruker*, Germany) were confronted to those of a commercial  
8 lignosulfonic acid sodium salt (*Sigma-Aldrich*, France). The current publication provides a  
9 supplementary figure (a typical example of comparison IR-spectra between commercial  
10 lignosulfonic acid sodium salt from *Sigma-Aldrich* ( France) and our Organosolv lignin  
11 obtained during pretreatment) that shows that the IR spectra are very similar, highlighting the  
12 quality of the lignin recovered by the Organosolv treatment.

### 13 *3.2.3. Effect on inhibitor formation*

14 Under severe pretreatment conditions such as long reaction time, high temperature and acid  
15 concentration, typical for Organosolv pretreatment, the pentoses resulting from hydrolysis of  
16 the hemicelluloses undergo dehydration with formation of 2-furaldehyde, hereafter referred to  
17 as furfural, while the hexoses are dehydrated to 5-hydroxymethyl-2-furaldehyde, hereafter  
18 referred to as HMF (Pan et al., 2007; Jönsson and Martín, 2016). These compounds may have  
19 a negative impact on the downstream hydrolysis and fermentation processes. **Table 3**  
20 highlights the evolution of furfural and HMF concentration in the solvent after pretreatment at  
21 175°C and 190°C as the sulfuric acid concentration augments. According to experimental  
22 data, very low concentrations of furfural and HMF were produced at 175°C without catalyst.  
23 However, these concentrations increased as the sulfuric acid concentration increased.  
24 Moreover, the same behavior was observed at 190°C with almost twice the amount of each  
25 component compared to the quantity measured at 175°C. These results were confirmed by

1 Harmsen et al. (2010) who reported that furfural and HMF formation were enhanced at higher  
2 temperature and treatment time, and by Agbor et al. (2011) concerning the increase in acidic  
3 concentration. Kim et al. (2018) when treating *Miscanthus x giganteus* explained this result  
4 by the increase in hydronium ion concentration in water at high temperature. Finally, these  
5 results confirm the conclusion of section 3.2.1 which claims that high temperature must be  
6 avoided.

7

### 8 ***3.3. Effect of ethanol-water ratio on the Organosolv pretreatment***

9 For bioethanol production, the downstream pretreatment processes require, as much as  
10 possible, to recover an insoluble fraction which consists mostly of cellulose, together with  
11 some minor amounts of unhydrolyzed hemicelluloses and some residual lignin (Arato et al.,  
12 2005). In the previous section, our findings revealed that the increase of both acid  
13 concentration and temperature improved the recovery of Organosolv lignin, *i.e.* the  
14 delignification yield, but hampered the recovery of the solid fraction, particularly cellulose  
15 which was being degraded in the process when severe conditions of temperature and acid  
16 content were applied simultaneously. So, a trade-off is necessary between cellulose purity and  
17 cellulose recovery in the solid fraction for subsequent enzymatic hydrolysis and fermentation  
18 steps. From **Table 2** and **Table 3**, the treatment with 0.25% w/w sulfuric acid at 175°C  
19 emerges as a good trade-off between cellulose purity (about 63%) and yield (about 74%),  
20 while inhibitor formation remained rather limited. So, further experiments investigating the  
21 ethanol-water ratio between 40:60 and 80:20 v/v were carried out at a fixed temperature  
22 (175°C) and catalyst content (0.25% w/w).

23

#### 24 ***3.3.1. Effect on cellulose yield and purity***

1 The effect of ethanol-water ratio on the amount of remaining solid and on its chemical  
2 composition is summarized in **Table 2** and in **Figure 3**, respectively. Contrary to the  
3 observations made in section 3.2.1, the mass of solid recovered varied less significantly as a  
4 function of the ethanol-water ratio than as a function of catalyst content, between  $55\% \pm 2\%$   
5 and  $67\% \pm 3\%$ . It emerges that cellulose dissolution was not enhanced by the increase in  
6 ethanol content and remained nearly constant, about 37–39g cellulose per 100 g sawdust  
7 feedstock (**Table 2**). The amount of impurities also decreased when ethanol concentration  
8 was increased (**Figure 3**). Cellulose purity lay between  $59\% \pm 2\%$  and  $71\% \pm 3\%$ , with a  
9 maximum achieved for 60:40 v/v ethanol-water ratio. Conversely, lignin content in the solid  
10 phase was almost halved in comparison to the raw sawdust, highlighting a very good  
11 delignification yield under this condition. These results are in agreement with the review  
12 paper from Borand and Karaosmanoğlu (2018) in which the optimum ethanol-water ratio is  
13 always between 40:60 and 60:40 for woody biomass. According to Pan et al. (2007), with the  
14 increase of ethanol-water ratio, the lignin binding the cellulose fibers together is removed in a  
15 manner that substrates were defibered, thus resulting in more cellulose than when catalyst  
16 content and temperature were increased. **Table 2** reveals, however, that when ethanol content  
17 was higher than 60% v/v, the effect of ethanol remained weak for the sawdust mixture studied  
18 in this work, so that the best condition can be defined with a 60% v/v concentration of ethanol  
19 in the solvent, which makes solvent regeneration easier in the process.

20

### 21 *3.3.2. Effect on the Organosolv lignin recovery*

22 For the liquid phase, **Table 3** summarizes the amount of lignin that can be recovered by  
23 precipitation as Organosolv lignin for 0.25% w/w sulfuric acid at 175°C as a function of  
24 ethanol-water ratio. According to these data, it can be observed that any increase in ethanol-  
25 water ratio improved the recovery of the Organosolv lignin recovery yield. Obviously, it can

1 be pointed out that lignin yield was above 44% when ethanol content was 60% v/v or higher,  
2 and the maximum lignin yield,  $49.2\% \pm 0.6\%$ , was achieved at the highest ethanol-water ratio  
3 80:20 v/v. But an efficient lignin recovery, about  $45.2\% \pm 0.3\%$  yield, could also be achieved  
4 using lower ethanol concentration in an economic perspective. Nevertheless, the ethanol-  
5 water ratio increase was found to be an important parameter for promoting lignin  
6 solubilization of sawdust feedstock, while preventing cellulose dissolution, because it allowed  
7 increasing the lignin solubility without an intense dissolution of cellulose from the raw  
8 sawdust, contrary to high-temperature condition or high catalyst content in **Table 2**. These  
9 results are in accordance with those reported by Domínguez-Robles et al. (2018) in a solvent  
10 fractionation process using acetone-water, and those reported by Ni and Hu (1995) using  
11 ethanol-water as the solvent. According to both studies, the solubility of the lignin was  
12 usually improved with increasing the concentration of the organic solvent. However,  
13 Gonçalves and Ruzene (2003) claimed that the reason for enhanced lignin dissolution was  
14 mainly the increase in pressure in the Organosolv pretreatment reaction vessel which was  
15 observed when increasing ethanol-water ratio at constant treatment temperature. This increase  
16 in pressure, from 15.6 to 18.8 bars, was also observed in this work (**Table 2**), but process  
17 condition made it not possible to distinguish the chemical effect of the solvent from the  
18 physical effect of pressure on enhanced lignin recovery. From a mass balance on lignin in the  
19 solid and the liquid phase in **Table 2** and **Table 3**, it emerged, however, that more lignin was  
20 recovered in both phases when the ethanol content was increased in the solvent, which means  
21 that ethanol probably prevented lignin degradation in the Organosolv pretreatment. However,  
22 accounting for cellulose and lignin recovery with cellulose purity, it arises that 60:40 ethanol-  
23 water ratio constitutes a good trade-off, in agreement with conventional Organosolv  
24 pretreatments from the literature.



### 1 3.2.3. *Effect on inhibitor formation*

2 Finally, **Table 3** describes the inhibitor formation due to Organosolv pretreatment using  
3 0.25% w/w sulfuric acid at 175°C, with ethanol-water ratio as the variable parameter. The  
4 main outcome is that furfural and HMF production remained low and even decreased with  
5 increasing ethanol concentration, especially above 60% ethanol, which is a beneficial effect  
6 because furfural and HMF at high concentration are toxic for hydrolysis and fermentation  
7 processes. This may be due to the reduced degradation of carbohydrates, which agrees with  
8 the lower degradation of lignin discussed in section 3.3.2 at increasing ethanol content. Kim  
9 et al. (2018) also attributed the decrease in inhibitor formation at increasing ethanol content to  
10 the reduced formation of hydronium cations when water content decreases.

11 Thus, experimental data confirm that increasing ethanol content enhances lignin solubilization  
12 and cellulose purity in the solid phase (**Table 2**), while preventing inhibitor formation at the  
13 same time (**Table 3**), contrary to temperature and high acid concentrations that increase  
14 simultaneously lignin recovery and inhibitor formation.

### 15 3.4. *Comparison with conventional Organosolv pretreatment*

16 Based on the experimental results of previous sections reported in **Table 2** and **Table 3**, the  
17 microwave-assisted Organosolv pretreatment applied to the sawdust mixture was able to  
18 achieve up to 82%±3% and 71%±3% cellulose recovery and purity in the solid phase,  
19 respectively, with very low quantities of inhibitors in the liquid phase (below 0.3 g/100 g of  
20 dry sawdust for 5-HMF and furfural). As highlighted by many studies (Bouxin et al., 2014 ;  
21 Mabee et al., 2006) and reviews from the literature (*e.g.*, Zhao et al., 2009; Borand and  
22 Karaosmanoğlu, 2018), a comparison with data on conventional Organosolv pretreatment is  
23 not straightforward because raw materials and solid-to-liquid ratio strongly vary, even though  
24 ethanol-water content and temperature remain in the same range. The maximum values of  
25 cellulose recovery and purity achieved in this preliminary work appear, however, to be among

1 the highest in the field of woody biomass, and could probably be improved after process  
2 optimization. The same difficulty emerges when a comparison with alternative processes  
3 (steam explosion...) and even with other microwave ethanol-based Organosolv treatment is  
4 considered: literature on woody biomass is rather limited and does not involve ethanol-water  
5 solvent (Gong et al., 2010, Liu et al., 2010), whereas microwave-assisted ethanol-Organosolv  
6 treatment has been applied recently to bagasse of *Agave angustifolia*, which differs  
7 significantly from softwood (Hernández et al., 2018). Thus, an alternative method for  
8 comparison can be based on energy consumption. For example, the model developed by Mafe  
9 et al. (2015) for dilute acid pretreatments in water can be applied to estimate energy  
10 consumption of the microwave-assisted Organosolv treatment. This model accounts for  
11 heating energy, heat loss, heat recovery, treatment temperature, and solid-to-liquid ratio. In  
12 the conditions of this work (temperature, S/L ratio...), energy requirements of dilute acid  
13 treatment with 0.25% sulfuric acid w/w was estimated about  $19 \cdot 10^3$  kJ/kg dry biomass. From  
14 power measurements, heating energy could also be deduced for the microwave-assisted  
15 Organosolv treatment of this work. However, power input for counterbalancing heat loss was  
16 very low and below the accuracy of the power measurement facility, so that the same  
17 assumptions on heat recovery and heat loss as in Mafe et al. (2015) were applied. The results  
18 showed that energy consumption ranged between  $11.1 \cdot 10^3$  and  $13.4 \cdot 10^3$  kJ/kg dry wood,  
19 which shows a significant decrease in comparison to dilute acid treatment, with a value about  
20  $12.5 \cdot 10^3$  kJ/kg dry wood for the highest cellulose recovery yield of this work. As a result,  
21 microwave-assisted Organosolv pretreatment appears as a potential way to achieve at the  
22 same time high performance and reduced energy consumption. It must however be pointed  
23 out that the values estimated in this preliminary work,  $12.5 \cdot 10^3$  kJ/kg and  $19 \cdot 10^3$  kJ/kg for  
24 microwave-assisted Organosolv and dilute acid treatment remain high in comparison to the  
25 optimized conditions of Mafe et al. (2015) in which values close to  $3 \cdot 10^3$  kJ/kg could be

1 achieved when treatment time was reduced to 15 min and solid loading reached 30% w/w in  
2 dilute acid aqueous solutions. This highlights that further optimization of the Organosolv  
3 process must be conducted, increasing first solid-to-liquid ratio, and then, minimizing  
4 treatment time, to achieve process sustainability, in parallel to cellulose enzymatic hydrolysis  
5 into convertible sugars to assess the feasibility of alcoholic fermentation for bioethanol  
6 production.

7

#### 8 **4. Conclusion**

9 Microwave-assisted heating appears as a versatile tool for Organosolv pretreatment of  
10 sawdust feedstock. Under the best conditions (60:40 ethanol-water, 175°C, 0.25% H<sub>2</sub>SO<sub>4</sub>),  
11 cellulose yield and purity reached 82%±3% and 71%±3%, respectively. The use of acid  
12 catalyst appeared to be essential to enhance delignification at lower temperature, but high  
13 H<sub>2</sub>SO<sub>4</sub> concentration promoted delignification, cellulose dissolution, and inhibitor formation.  
14 Increasing ethanol-water ratio could help reduce processing temperature because this  
15 promoted delignification without cellulose dissolution. Now, further studies focusing on  
16 pretreatment energy savings, cellulose enzymatic hydrolysis into convertible sugars, alcoholic  
17 fermentation for bioethanol production, are compulsory to assess process efficiency and  
18 sustainability.

#### 19 **Acknowledgments**

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22 (wood-wastes) on a regional scale.

23

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13

14



1 **Figure captions**

2 **Figure 1.**Temperature, pressure and power profile recorded during the Organosolv  
3 microwave assisted pretreatment

4 **Figure 2.** Effect of sulfuric acid and temperature on the chemical composition of the substrate  
5 after the Organosolv pretreatment, (a): 175°C and (b):190°C

6 **Figure 3.** Effect of ethanol-water ratio on the chemical composition of the substrate after the  
7 Organosolv pretreatment at 0.25% w/w sulfuric acid and 175°C

8

1 **Table captions**

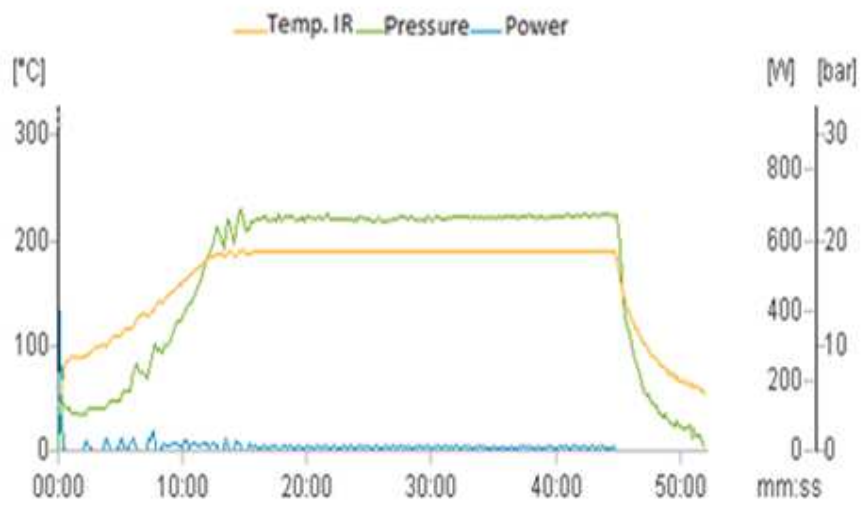
2 **Table 1.** Chemical composition of the mixed sawmill feedstocks (% w/w of the initial dry  
3 sawdust feedstock)

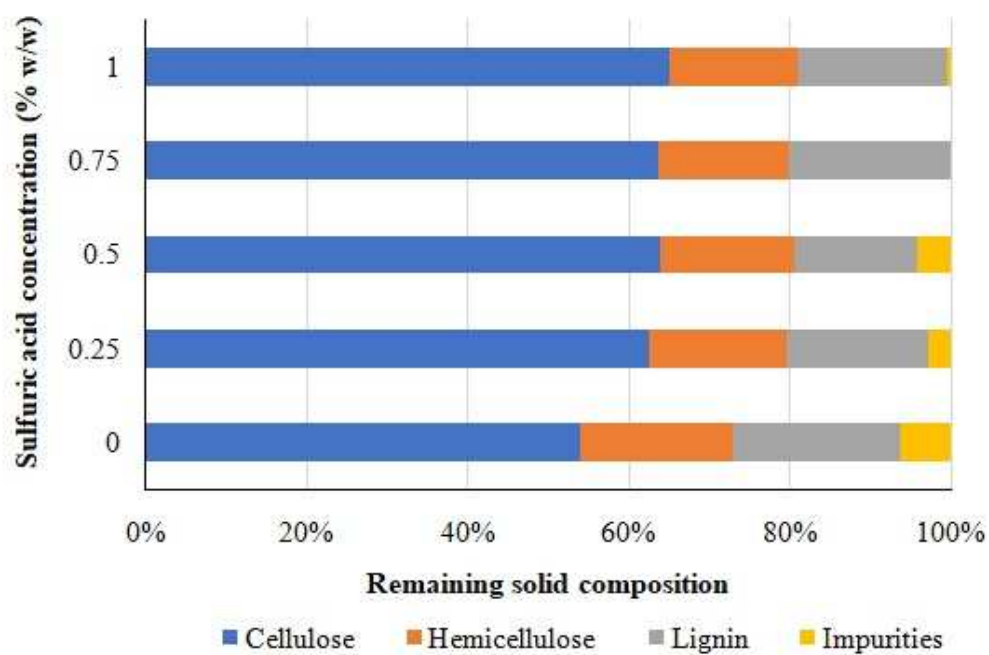
4 **Table 2.** Experimental design and results on the solid phase recovered after microwave-  
5 assisted Organosolv pretreatment of mixed sawdust

6 **Table 3.** Experimental design and results on the liquid phase recovered after microwave-  
7 assisted Organosolv pretreatment of mixed sawdust

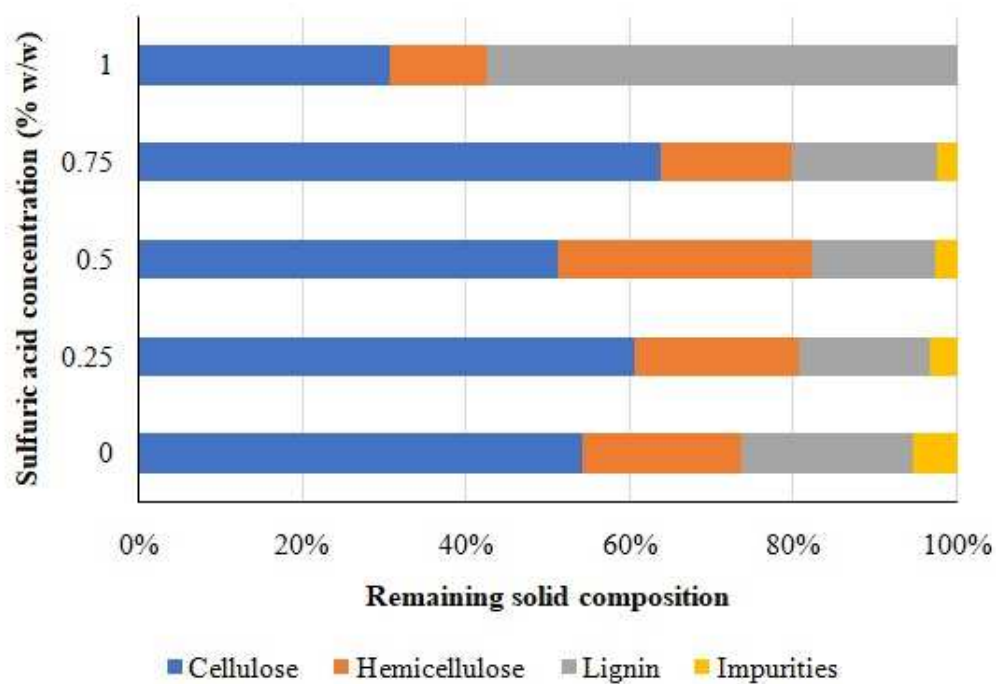
8

9

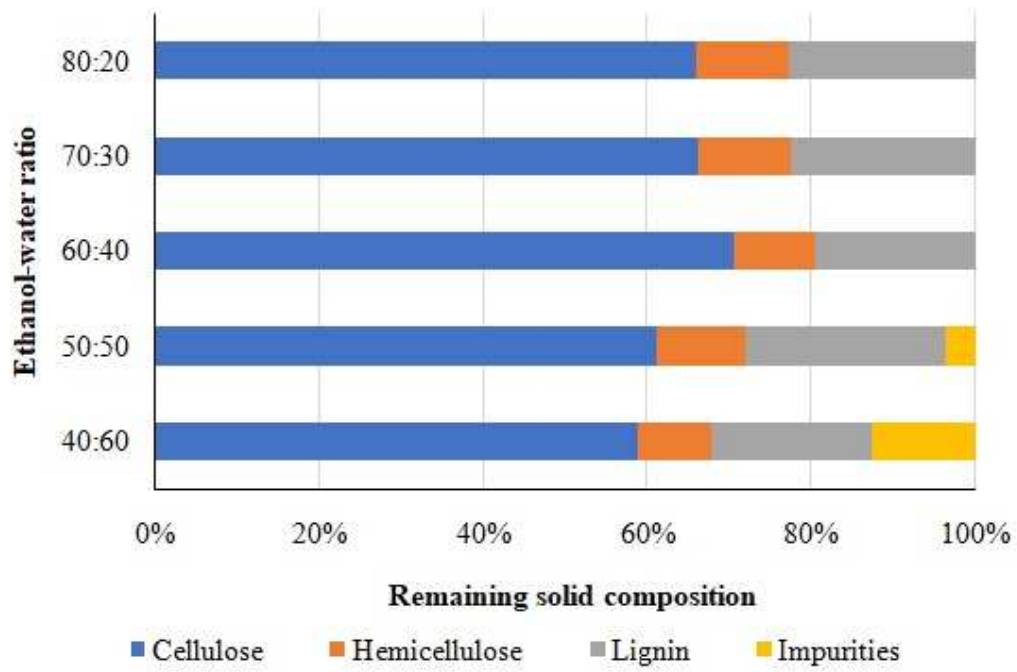




(a)



(b)



Composition % w/w	Mixed sawdust of this work <sup>a</sup>	Spruce(Butler et al., 2013)	<i>Pinus sylvestris</i> (Pettersen, 1984)	Douglas fir(Mabee et al., 2006)
Extractible (dichloromethane, ethanol, water)	3.2 ± 0.2	3.45	2.6	
Cellulose	44.3± 0.5	49.42	47	43
Hemicelluloses	25.6± 0.2	23.68	22.2	23
Insoluble lignin	26 ± 3	27.33	28	28
Ash content	0.3 ± 0.2		0.2	

<sup>a</sup>Standard deviation is based on experiments carried out in triplicate.

Ethanol-water ratio (v/v)	T (°C)	H <sub>2</sub> SO <sub>4</sub> (%w/w)	Pression (Bar)	Solid fraction(g/100g of raw sawdust)				Cellulose recovery yield (%)	Cellulose purity in the solid fraction (%)
				Remaining solid	Cellulose	Hemicellulose	KlasonLignin		
60:40	175	0	18.00 ±0.01	80 ± 2	43.2 ± 0.8	15 ± 1	16.7 ± 0.1	98 ± 2	54 ± 1
		0.25	17.55 ± 0.01	52 ± 4	33 ± 4	8.9 ±0.8	9.3 ± 0.6	74 ± 3	63 ±2
		0.5	17.94 ± 0.01	51 ±3	32.7 ± 0.3	9 ±2	7.9 ± 0.8	74 ±2	64 ± 3
		0.75	18.19 ± 0.01	49 ±2	31.2 ± 0.1	7.9 ±0.3	10 ± 2	71 ± 2	65 ± 3
		1	17.87 ± 0.01	46 ±4	30 ± 4	7 ±1	8.5 ± 0.8	67 ±3	65 ± 3
	190	0	23.72 ±0.01	73 ±4	40 ± 2	14 ± 2	15.4 ± 0.8	89 ± 3	54 ± 2
		0.25	23.80 ± 0.01	49.8 ± 0.2	30.1 ± 0.3	10.1 ± 0.1	7.9 ± 0.2	68 ±2	61 ± 3
		0.5	24.50 ± 0.01	43 ±3	22 ± 2	13 ± 2	6.5 ± 0.7	50 ±4	51 ± 4
		0.75	24.59 ± 0.01	39.3 ± 0.4	25.1± 0.7	6.3 ±0.3	7.0 ± 0.4	57 ±3	64 ± 4
		1	24.25 ± 0.01	19±4	6 ± 4	2 ±2	11 ± 1	14 ± 4	31 ± 5
40:60			15.63± 0.01	67 ±3	39 ±4	6 ±2	23 ± 1	89 ±4	59 ± 2
50:50			16.82 ± 0.01	60.9 ± 0.8	37 ±3	6.5 ± 0.5	14.9 ± 0.1	84 ± 3	61 ± 2
60:40	175	0.25	17.35 ± 0.01	52 ± 1	37 ±3	5 ± 1	10.2 ± 0.6	82 ± 3	71 ± 3
70:30			18.36 ± 0.01	67 ± 1	38.6 ± 0.7	7 ±2	13±1	87 ± 3	66 ± 2
80:20			18.75 ± 0.01	55 ± 2	39 ±4	7 ±3	13.2 ± 0.9	87 ± 3	66 ± 2

Ethanol-water ratio (v/v)	Temperature (°C)	H <sub>2</sub> SO <sub>4</sub> (% w/w)	Liquid fraction “black liquor” (g/100g of raw sawdust)			Lignin recovery yield (%)
			Organosolv lignin	Furfural	5-HMF	
60:40	175	0	3.5 ± 0.4	0.03 ± 0.01	0.00 ± 0.06	13.1 ± 0.9
		0.25	11.8 ± 0.3	0.25 ± 0.02	0.28 ± 0.05	44.5 ± 0.5
		0.5	11.9 ± 0.4	0.40 ± 0.07	0.58 ± 1.41	44.9 ± 0.5
		0.75	12.3 ± 0.8	0.46 ± 0.14	0.9 ± 0.1	46.3 ± 0.5
		1	13.2 ± 0.4	0.55 ± 0.12	1.00 ± 0.04	49.6 ± 0.4
	190	0	6.9 ± 0.4	0.08 ± 0.02	0.08 ± 0.1	26 ± 1
		0.25	11.8 ± 0.1	0.45 ± 0.04	0.92 ± 0.02	44.4 ± 0.5
		0.5	14.1 ± 0.4	0.61 ± 0.06	1.3 ± 0.3	53.2 ± 0.1
		0.75	15.3 ± 0.2	0.70 ± 0.02	1.41 ± 0.06	57.5 ± 0.4
		1	15.0 ± 0.6	0.78 ± 0.01	1.55 ± 0.01	56.4 ± 0.2
40:60	175°C	0.25	6.8 ± 0.6	0.17 ± 0.01	0.26 ± 0.01	25.7 ± 0.6
50:50			7.3 ± 0.0	0.15 ± 0.01	0.24 ± 0.01	27.4 ± 0.5
60:40			11.9 ± 0.3	0.23 ± 0.02	0.30 ± 0.03	44.7 ± 0.5
70:30			12.0 ± 0.7	0.11 ± 0.01	0.16 ± 0.01	45.2 ± 0.3
80:20			13.1 ± 0.4	0.09 ± 0.01	0.08 ± 0.01	49.2 ± 0.6