

# Acetosolv delignification of marabou (*Dichrostachys cinerea*) wood with and without acid prehydrolysis

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**Abstract** The chemical composition of marabou (*Dichrostachys cinerea*) wood and its treatment with acetic acid were investigated. Two different treatment approaches, direct acetosolv and combined acid prehydrolysis/acetosolv, were evaluated. The effects of acetic acid concentration (50%, 70% and 90%) and temperature (normal boiling temperature and 121°C) on yield of solids, solubilization of lignin and hemicelluloses and recovery of cellulose were evaluated for both treatments. High solubilization of marabou components was observed in the direct acetosolv treatment at 121°C, especially at the highest acetic acid concentration, where around 84.8% of lignin and 78% of hemicelluloses were removed. When the material was subjected to acid prehydrolysis prior to acetosolv treatment, lignin solubilization was improved, especially at low acetic acid concentrations. Above 80% of the solubilized lignin was recovered from the liquors in the direct acetosolv treatment, but the recovery was lower in the combined treatment. Cellulose was well preserved in all the treatment schemes.

**Key words** *Dichrostachys cinerea*, delignification, acetosolv, acid hydrolysis

## 1 Introduction

The interest in using lignocellulosic materials from forestry and agriculture as feedstocks for the production of second generation ethanol is continuously growing (Wyman, 2008). The reason for that is the search for alternatives to the raw materials used for producing first generation ethanol. Nowadays, ethanol is produced from bioresources which are also sources of food and feed; their use for energetic purposes is an ethically-controversial issue (Martín and Martín, 2008).

Marabou (*Dichrostachys cinerea*) is an abundant lignocellulosic bioresource that could be of interest for ethanol production in Cuba. It is a fast-growing invasive shrub (Wakeling and Bond, 2007), which was introduced in Cuba as an ornamental plant during the 19th century. But today it has expanded all over the island infesting arable and grazing areas and causing significant losses in agricultural production (Moyroud, 2000). Frequent management is necessary to control

this species; this generates huge amounts of biomass which could be a potential source of sugars for the production of ethanol and other chemicals.

Pretreatment is an important step in the bioconversion of lignocellulosic materials, since it allows the separation of the main components and facilitates enzymatic hydrolysis of cellulose. Different pretreatment methods have been proposed and developed for different lignocellulosic materials (Mosier et al., 2005; Taherzadeh and Karimi, 2008). For a rational utilization of lignocellulosic materials, according to a biorefinery philosophy, the pretreatment should efficiently separate cellulose, hemicelluloses and lignin and preserve their properties to produce various valuable by-products.

The organosolv methods, based on the solubilization of lignin by organic solvents, have been reported to be effective for separation of the main components of lignocellulosic materials and as pretreatment for the enzymatic hydrolysis of cellulose (Zhao et al., 2009). A variety of organic solvents, including alcohols and

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aliphatic acids, can be utilized as pulping agents. Utilization of acetic acid in delignification has been assayed in HCl-catalyzed media (acetosolv process), formic acid-catalyzed media (formacell process) and uncatalyzed media (acetocell process) (Ligero et al., 2008). These three processes have proven to be promising in achieving complete utilization of lignocellulosic materials with minimal environmental impacts. Under mild conditions, these processes cause extensive removal of lignin and hemicelluloses with no significant cellulose degradation (Xu et al., 2006; Ligero et al., 2008).

The organosolv methods based on acetic acid have been applied successfully to hardwood, softwood and non-woody plant materials (Ligero et al., 2005, 2007). However, no previous research on acetic acid-based organosolv processes of marabou wood has been reported. The objective of this study is to evaluate different reaction conditions for acetosolv delignification of marabou wood.

## 2 Materials and methods

### 2.1 Preparation of raw material

Samples of marabou wood were collected in an infested area northeast of Matanzas (23°02'N, 81°30'W), Cuba. The material was air-dried for a week and sawed into 1-cm-thick disks. Then, the disks were air-dried at room temperature for a week followed by drying at 50°C for 48 h. Subsequently, the dry disks were milled, sieved to a particle size of 2 mm and used in the experiments. A 100 g portion of the material was further milled to pass a 1-mm screen and used for compositional analysis.

### 2.2 Acetosolv treatment

Three delignification media containing 0.2% HCl and different concentrations of acetic acid (50%, 70% and 90%) were prepared. Five grams (DW) of marabou wood were mixed with delignification media to give a 1:10 solid-to-liquid ratio. The experiments were carried out either in glass flasks equipped with reflux condensers at normal boiling temperature (NBT) under atmospheric pressure (heated by an electric mantle) or in sealed glass flasks placed in an autoclave at 121°C. The reaction time was 1 h.

### 2.3 Dilute-acid prehydrolysis (DAPH)

Twenty grams of marabou biomass were mixed with

a solution containing 1% (w/v) sulphuric acid and 1% (w/v) acetic acid in a 500 mL glass flask giving a solid-to-liquid ratio of 1:10. The hydrolysis was performed at 121°C in an autoclave and lasted 1 h. Five batches were run in order to get enough cellulignin for the further acetosolv experiments.

### 2.4 Experimental setup

Acetosolv treatments were applied either directly on raw biomass or on cellulignin obtained by DAPH. For both direct acetosolv and DAPH-acetosolv, experiments were performed at two temperatures (NBT and 121°C). Duplicate experiments were run for each condition.

### 2.5 Analysis of raw material

The contents of moisture, mineral components, extractives, structural carbohydrates and lignin in the raw material were analyzed according to National Renewable Energy Laboratory (NREL) analytical procedures (Sluiter et al., 1998a, b, c, d). Moisture content was determined gravimetrically after drying the material at 105°C (Sluiter et al., 1998a). Mineral components were determined as ash after incineration of an aliquot of the material at 550°C (Sluiter et al., 1998b). Extractives were determined by ethanol extraction in a Soxhlet apparatus (Sluiter et al., 1998d). Cellulose and hemicelluloses were determined by analytical acid hydrolysis of the extractive-free material (Sluiter et al., 1998c).

Sugars and degradation products in the hydrolysate were analyzed by high-performance liquid chromatography (HPLC) with a C-R7A integrator (Shimadzu, Kyoto, Japan). Glucose, cellobiose, xylose, arabinose and acetic acid were separated with an Aminex HPX 87H column (Bio-Rad Laboratories, Hercules, CA, USA) at 45°C using 5 mmol·L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> as a mobile phase at a flow rate of 0.6 mL·min<sup>-1</sup> and detected with a refractive index detector (Shimadzu RID-6A). Lignin was determined by gravimetry of acid-insoluble residue (Sluiter et al., 1998c).

Furfural and hydroxymethylfurfural (HMF) were separated on an RP-18 column (Hewlett-Packard, Avondale, PA, USA) and detected with a UV detector (Shimadzu SPD-10A) at 25°C using a mobile phase consisting of 1% acetic acid and 1:8 acetonitrile-water solution pumped at a flow rate of 0.8 mL·min<sup>-1</sup>. The concentrations of glucose, cellobiose and HMF were used for calculating the cellulose content, whereas the concentrations of xylose, arabinose, acetic acid and furfural were used for calculating the hemicel-

lulose content. Conversion factors for glucose, cellobiose and HMF were 0.9, 0.95 and 1.29, respectively; whereas for xylose, arabinose, acetic acid and furfural, conversion factors were 0.88, 0.88, 0.72 and 1.37, respectively.

## 2.6 Analysis of treated material

The yield of treated solids was determined gravimetrically. Hemicelluloses and cellulose in the treated solids were determined as easy- and difficult-to-hydrolyze polysaccharides, respectively, using a modification of the method described by Bazarnova (2002). The method is based on a two-step acid hydrolysis followed by the spectrophotometric quantification of total reducing sugars using the dinitrosalicylic acid (DNS) method (Miller, 1959). Lignin was determined by gravimetric quantification of the hydrolysis residue.

## 2.7 Precipitation of lignin

For precipitating the solubilized lignin, the acetosolv liquors were vacuum-concentrated to one third of the initial volume using a rotary evaporator (IKA, RV 05 basic, Germany). The concentrated liquors were mixed with ten volumes of water, heated in water bath at 80°C for 2 h and left to stand at room temperature for 24 h. Then, the precipitated lignin was recovered by filtration and dried at 105°C until constant mass.

# 3 Results and discussion

## 3.1 Raw material composition

The compositional analysis revealed that marabou has a carbohydrate content of approximately 61% (w/w), including around 40% of cellulose (Table 1). The high cellulose content indicates the potential of this material for its conversion through the saccharification route to ethanol, lactic acid or other glucose derivatives. The analytical procedure used for cellulose determination, which considered not only the glucose formed upon analytical acid hydrolysis and the HMF resulting from glucose degradation, but also the cellobiose accumulated as a consequence of incomplete cellulose hydrolysis, ensured a reliable and accurate result that could be used as a reference for future marabou studies.

According to the relative high content of pentosanes, which were determined as the sum of xylose and arabinose and corrected for sugar degradation during analytical acid hydrolysis, marabou hemicel-

**Table 1** Chemical composition of marabou wood

| Component           | Content, % (w/w) |
|---------------------|------------------|
| Cellulose           | 39.5 ± 0.43      |
| Hemicelluloses      | 21.7 ± 0.35      |
| Pentosanes          | 19.4 ± 0.30      |
| Acetyl groups       | 2.3 ± 0.01       |
| Lignin              | 32.1 ± 0.37      |
| Ethanol extractives | 3.8 ± 0.15       |
| Ash                 | 1.9 ± 0.04       |
| Unknown             | 1.0              |

Note: The data represent mean ± SD. The same below.

luloses are suitable for producing xylose derivatives, such as xylitol or furfural (Table 1). The amount of acetyl groups detected is favorable for the hydrolytic conversion of polysaccharides to simple sugars. The high lignin content (32.1%) is comparable with that of several softwood species (Fengel and Wegener, 1989). The amounts of extractives and mineral components were similar to those of other lignocellulosic materials. To the best of our knowledge, this is the first report on chemical composition of marabou wood. Most of the components of marabou wood were identified in this study and only 1% of the dry matter remained unidentified. This shows the comprehensiveness of the information obtained in this investigation.

## 3.2 Direct acetosolv treatment of marabou biomass

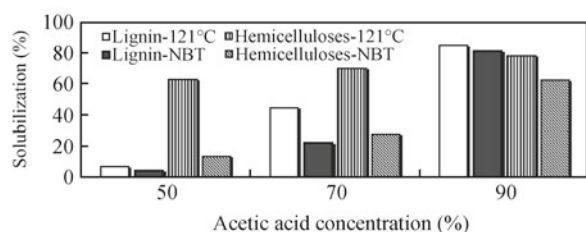
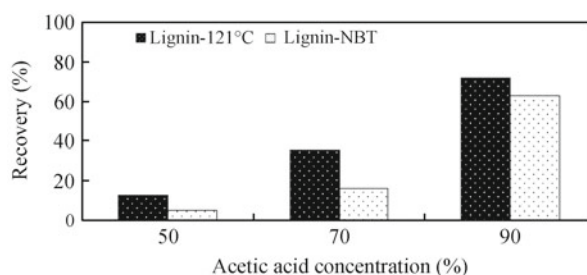
The experimental results of the direct acetosolv treatment of marabou biomass are shown in Table 2. In the experiments at 121°C, a yield of 69.1 g treated solids per 100 g raw material was obtained when using 50% acetic acid. Due to a higher solubilization of hemicelluloses and lignin, the yield of solids decreased to 60.4% and 48.1% when acetic acid concentration increased to 70% and 90%, respectively. At low acetic acid concentration, the solubilization of hemicelluloses was higher than that of lignin (Fig. 1). The solubilization of hemicelluloses can be a consequence of the combined hydrolytic action of acetic and hydrochloric acids. A clear increase of lignin solubilization was observed with increasing acetic acid concentrations. Only 6.8% of the lignin initially contained in the raw materials was removed when 50% acetic acid was used, while the removal reached 44.6% for 70% acetic acid and 84.8% for 90% acetic acid. The solubilization of hemicelluloses (78%) also increased at higher acetic acid concentrations, although the increase was less remarkable than that of lignin.

As a result of the solubilization of lignin and hemicel-

**Table 2** Yield of solids and composition of the solid material obtained by direct acetosolv treatment of marabou wood at normal boiling temperature (NBT) and 121°C with or without dilute-sulfuric acid prehydrolysis.

| No. | Prehydrolysis | Temperature (°C) | Acetic acid concentration (%) | Yield (%)   | Hemicelluloses* (%) | Cellulose** (%) | Lignin (%)  |
|-----|---------------|------------------|-------------------------------|-------------|---------------------|-----------------|-------------|
| 1   | No            | 121              | 50                            | 69.1 ± 0.64 | 15.9 ± 0.26         | 39.5 ± 0.32     | 31.6 ± 0.78 |
| 2   | No            | 121              | 70                            | 60.4 ± 1.20 | 14.9 ± 0.18         | 55.4 ± 1.05     | 21.5 ± 0.64 |
| 3   | No            | 121              | 90                            | 48.1 ± 0.42 | 13.5 ± 0.30         | 77.6 ± 0.27     | 7.4 ± 0.23  |
| 4   | No            | NBT              | 50                            | 86.9 ± 0.64 | 29.0 ± 0.07         | 38.3 ± 0.31     | 25.8 ± 0.00 |
| 5   | No            | NBT              | 70                            | 82.7 ± 0.57 | 26.4 ± 0.14         | 44.1 ± 0.28     | 22.1 ± 0.23 |
| 6   | No            | NBT              | 90                            | 58.2 ± 0.35 | 19.9 ± 0.09         | 64.9 ± 0.53     | 7.6 ± 0.07  |
| 7   | Yes           | 121              | 50                            | 74.3 ± 0.71 | 15.8 ± 0.17         | 46.7 ± 0.21     | 23.9 ± 0.02 |
| 8   | Yes           | 121              | 70                            | 64.7 ± 0.71 | 13.7 ± 0.07         | 59.9 ± 0.43     | 17.6 ± 0.00 |
| 9   | Yes           | 121              | 90                            | 52.4 ± 0.14 | 13.1 ± 0.42         | 73.4 ± 0.95     | 5.8 ± 0.23  |
| 10  | Yes           | NBT              | 50                            | 90.6 ± 0.28 | 20.6 ± 0.21         | 44.7 ± 0.17     | 26.5 ± 0.14 |
| 11  | Yes           | NBT              | 70                            | 83.2 ± 0.78 | 19.8 ± 0.07         | 47.1 ± 0.19     | 23.4 ± 0.07 |
| 12  | Yes           | NBT              | 90                            | 60.3 ± 0.28 | 13.2 ± 0.14         | 70.5 ± 0.34     | 10.2 ± 0.07 |

Note: The data are the average of two replicates. \* was determined as easy-to-hydrolyze polysaccharides, \*\* was determined as difficult-to-hydrolyze polysaccharides.

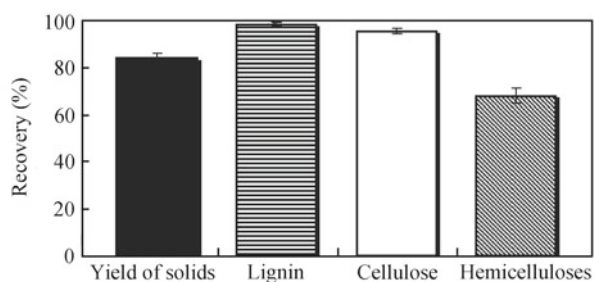
**Fig. 1** Solubilization of lignin and hemicelluloses of marabou wood during direct acetosolv treatment at 121°C and normal boiling temperature (NBT)**Fig. 2** Recovery of lignin from the liquors obtained by direct acetosolv treatment at 121°C and NBT

celluloses, an increase of the content of cellulose was observed in the treated material (Table 2). The content (77.6%) of cellulose in the material treated with the highest acetic acid concentration was almost two-fold higher than that in the raw marabou. This means that acetosolv at 121°C using 90% acetic acid is effective for producing cellulose-rich marabou pulps that could be of high relevance for obtaining not only cellulosic

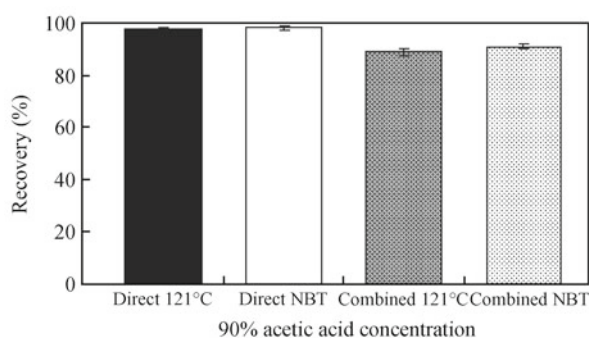
ethanol, but also various cellulose derivatives.

Lowering the treatment temperature from 121°C to the boiling point of each reaction mixture led to a decreased solubilization of marabou components. As shown in Table 2, the higher yields of treated solids, 58.2–86.9 g per 100 g raw material, were obtained in the direct acetosolv treatment at normal boiling temperature (NBT). This might be due to the fact that at temperatures below 121°C, only the easy-to-hydrolyze fraction of hemicelluloses (Lavarack et al., 2002) and the fast-reacting fraction of lignin (Parajó et al., 1993) were solubilized. The solubilization of hemicelluloses and lignin at low acetic acid concentration was rather low for the direct acetosolv treatment at NBT (Fig. 1). Just like in the experiments performed at 121°C, a significant increase of lignin solubilization occurred with the increasing concentration of acetic acid in the pulping liquor. At 90% acetic acid concentration, lignin solubilization was comparable for both treatment temperatures. Anyway, due to the lower hemicellulose solubilization, cellulose enrichment of the material was not as remarkable as that at 121°C. The acetosolv pulp contained 64.9% of cellulose at NBT, which is 12.7% lower than the amount detected in the pulp obtained at 121°C.

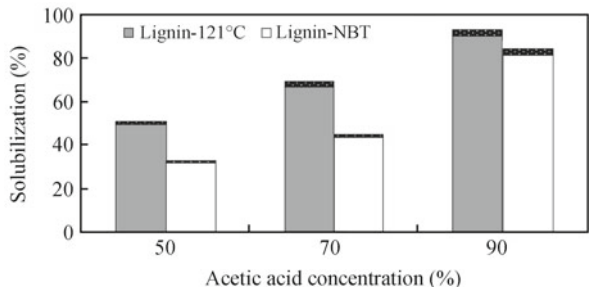
For all the direct acetosolv experiments, the solubilized lignin was easily recoverable by water addition to previously concentrated acetosolv liquors. At the highest acetic acid concentration, approximately 72% of the lignin contained in the raw material was recovered from the liquors at 121°C and 63% at NBT (Fig. 2). Those figures are equivalent to recoveries of 84.7% and 80.8% of the solubilized lignin. Independently of



**Fig. 3** Yield of solids and recovery of the main components after dilute-acid hydrolysis of marabou biomass. The values were represented by the mean of two replicates. The error bars show the standard deviations.



**Fig. 4** Recovery of cellulose in direct and combined acetosolv processes using 90% acetic acid at 121°C and NBT



**Fig. 5** Solubilization of lignin by combined dilute-acid/acetosolv treatment at 121°C and NBT. The top dark rectangles indicate the lignin solubilized in the hydrolysis stage while the rest of the bars show the values obtained in the acetosolv stage.

the treatment conditions, above 80% of the solubilized lignin was precipitated from all the liquors. This high degree of lignin recovery is in agreement with the results reported for acetosolv processing of wood species (Vila et al., 2003).

### 3.3 Combined dilute-acid/acetosolv treatment

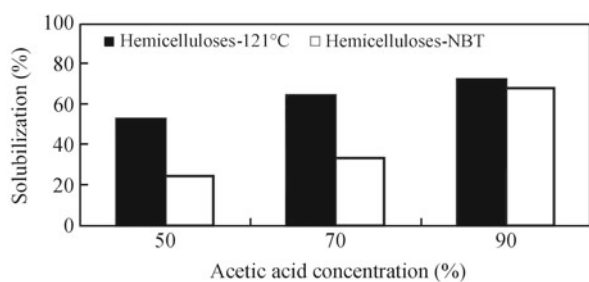
The combined treatment was aimed to potentiate acetosolv delignification by a preliminary solubilization of hemicelluloses. As a first step, dilute-acid

prehydrolysis was performed in order to solubilize as much hemicelluloses as possible without significantly affecting cellulose and lignin. Our former research demonstrated the effectiveness of using a mixture of dilute sulfuric and acetic acids for hydrolyzing the hemicelluloses from sugarcane bagasse (Rocha et al., 2011). In the current work, the application of dilute-acid prehydrolysis approach to marabou wood led to a minor solubilization of the raw material as indicated by the high yield (84%) of treated solids (Fig. 3). The hemicelluloses were the main solubilized component, according to their lower recovery (68%) in the solids. In contrast, the solubilization of lignin and cellulose was only marginal. A higher solubilization of hemicelluloses could be achieved if higher temperature and/or acid concentration would be applied. However, no attempt in this direction was performed in order to avoid possible damage on cellulose, which under the conditions tested was well preserved.

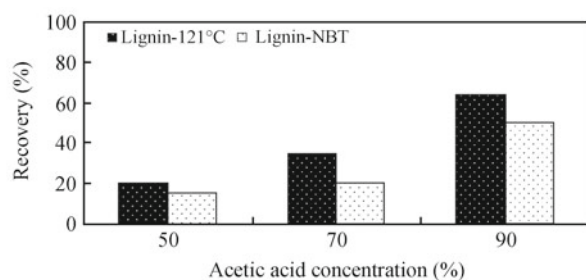
The effect of dilute-acid prehydrolysis was noticeable in the composition of acetosolv treated solids (Table 2). It was apparent that after removal of part of the hemicelluloses in the acid prehydrolysis step, the acetic acid can act rather selectively on lignin during the acetosolv step of the combined process, whereas in the direct acetosolv treatment it acts simultaneously on both lignin and hemicelluloses. All the pulps obtained in the treatments performed at 121°C contained less lignin than those produced by direct acetosolv under the same conditions. This was more remarkable for the process carried out with 50% acetic acid, approximately 8% less lignin and 7% more cellulose than that obtained by direct acetosolv. The pulps produced by the combined treatment at NBT were enriched in cellulose (ranging between 44.7% and 70.5%) compared with those obtained by direct acetosolv at the same temperature (only 38.3%–64.9%) (Table 2).

The pulps produced in the processes using the highest acetic acid concentration contained the maximum cellulose content. The high cellulose recovery detected in the treated solids obtained in direct acetosolv as in combined treatments indicates that cellulose loses in all the processes were low (Fig. 4). An investigation using higher acetic acid concentrations is now underway by our group.

The solubilization of lignin during acetosolv treatment was considerably improved by the dilute-acid prehydrolysis, especially when applying low acetic acid concentrations. Approximately one half of the lignin in the cellulignin was removed in the acetosolv stage when 50% acetic acid was used at 121°C (Fig. 5). That is roughly seven times higher than the 6.8% solubilization achieved by direct acetosolv under the same conditions (Fig. 1). When the acetosolv was performed with 70% and 90% acetic acid, the delignifica-



**Fig. 6** Solubilization of hemicelluloses during acetosolv treatment of the combined treatment at 121°C and NBT



**Fig. 7** Recovery of lignin from the liquors during acetosolv treatment of the combined treatment at 121°C and NBT

tion degree increased to 68% and 91.4%, respectively. These were also higher than the values achieved by direct acetosolv, although the difference was not as spectacular as that in the 50% acetic acid process. However, if taking into account the solubilization of lignin occurred during the prehydrolysis, higher overall delignification has to be reported. When applying 90% acetic acid at 121°C, the overall delignification reached 93.3% in the combined treatment (Fig. 5), almost 9% higher than that in the direct acetosolv under the same condition.

The lignin solubilization was also improved by the prehydrolysis at NBT, but the effect was restricted to low and medium acetic acid concentrations (Fig. 5). When 50% acetic acid was used, the delignification degree increased eight-fold (from 4.2% in the direct acetosolv to 32.7% in the combined process), while a two-fold increase (from 21.9% to 44.8%) occurred for 70% acetic acid. However, only a minor improvement of lignin solubilization was detected when using highest acetic acid concentration; the delignification degree (85.6%) was only slightly higher than the one obtained by direct acetosolv (84.4%).

A considerable fraction of the hemicelluloses which were not removed during the prehydrolysis step was solubilized during the acetosolv treatment (Fig. 6). When using low and medium acetic acid concentrations, the solubilization of the remaining hemicelluloses at 121°C doubled that at NBT. On the other hand, when 90% acetic acid was used comparable

hemicellulose solubilization was achieved for both temperatures (Fig. 6).

The amount of lignin recovered from the liquors was higher for the treatments performed at 121°C and increased steadily with increasing acetic acid concentration (Fig. 7). Compared with the direct acetosolv treatments, lignin recovery was higher for low acetic acid concentration and lower for high acetic acid concentration. The precipitated lignin accounted for 30.3%–68.6% of the lignin solubilized in the acetosolv stage of the combined process.

## 4 Conclusions

Marabou wood has a high carbohydrate content, including 39.5% of cellulose, which is its main chemical component. Compared with lower acetic acid concentrations, acetosolv treatment of marabou using 90% acetic acid was very effective for the removal of lignin and for the enrichment of the pulp in cellulose. A dilute-acid hydrolysis step prior to acetosolv treatment improved the delignification process without causing significant losses of cellulose. Lignin removal was higher at 121°C than at NBT. Lignin recovery from the liquors was better in direct acetosolv than in the combined treatments.

## References

- Bazarnova N G. 2002. Khimija Drevesiny y ee osnovnykh komponentov. Utchebnoe posobie. Barnaul, Russia: Altayskii Gosudarstvennyi Universitet
- Fengel D, Wegener G. 1989. Wood: Chemistry, Ultrastructure, Reactions. Berlin: Walter de Gruyter, 274–276
- Lavarack B P, Griffin G J, Rodman D. 2002. The acid hydrolysis of sugarcane bagasse hemicellulose to produce xylose, arabinose, glucose and other products. *Biomass Bioenerg*, 23: 367–380
- Ligero P, Vega A, Bao M. 2005. Acetosolv delignification of *Miscanthus sinensis* bark: Influence of process variables. *Ind Crops Prod*, 21: 235–240
- Ligero P, Villaverde J J, de Vega A, Bao M. 2008. Delignification of *Eucalyptus globulus* saplings in two organosolv systems (formic and acetic acid): Preliminary analysis of dissolved lignins. *Ind Crops Prod*, 27: 110–117
- Ligero P, Villaverde J J, Vega A, Bao M. 2007. Acetosolv delignification of depithed cardoon (*Cynara cardunculus*) stalks. *Ind Crops Prod*, 25: 294–300
- Martín A, Martín C. 2008. An ethical approach to the food vs. fuels debate. Proceedings of the 10th International Congress on Sugar and Sugarcane Derivatives. Havana, Cuba, 176–181
- Miller G L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem*, 31: 420–428

- Mosier N, Wyman C, Dale B, Elander R, Lee Y, Holtzapple M, Ladisch M. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Biores Technol*, 96: 673–686
- Moyroud R. 2000. Exotic weeds that threaten the Caribbean: a brief overview and early alarm call. *Wildland Weeds*, 4–8
- Parajó J C, Alonso J L, Vázquez D. 1993. On the behaviour of lignin and hemicelluloses during the acetosolv pulping of wood. *Biores Technol*, 46: 233–240
- Rocha G J M, Martín C, Soares I B, Souto-Maior A M, Baudel H M, de Abreu C A M. 2011. Dilute mixed-acid pretreatment of sugarcane bagasse for ethanol production. *Biomass Bioenerg*, 35: 663–670
- Sluiter A, Hames B, Hyman D, Payne C, Ruiz R, Scarlata C, Sluiter J, Templeton D, Wolfe J. 1998a. Determination of total solids in biomass and total dissolved solids in liquid process samples. Laboratory Analytical Procedure (LAP). Technical Report NREL/TP-510-42621. Golden, Colorado: National Renewable Energy Laboratory
- Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D. 1998b. Determination of ash in biomass. Laboratory Analytical Procedure (LAP). Technical Report NREL/TP-510-42622. Golden, Colorado: National Renewable Energy Laboratory
- Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D. 1998c. Determination of structural carbohydrates and lignin in biomass. Laboratory Analytical Procedure (LAP). Technical Report NREL/TP-510-42618. Golden, Colorado: National Renewable Energy Laboratory
- Sluiter A, Ruiz R, Scarlata C, Sluiter J, Templeton D. 1998d. Determination of extractives in biomass. Laboratory Analytical Procedure (LAP). Technical Report NREL/TP-510-42619. Golden, Colorado: National Renewable Energy Laboratory
- Taherzadeh M J, Karimi K. 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *Int J Mol Sci*, 9: 1621–1651
- Vila C, Santos V, Parajó J C. 2003. Recovery of lignin and furfural from acetic acid-water-HCl pulping liquors. *Biores Technol*, 90: 339–344
- Wakeling J L, Bond W J. 2007. Disturbance and the frequency of root suckering in an invasive savanna shrub, *Dichrostachys cinerea*. *Afr J Range Forage Sci*, 24: 73–76
- Wyman C E. 2008. Cellulosic ethanol: a unique sustainable liquid transportation fuel. *MRS Bull*, 33: 381–383
- Xu F, Sun J X, Sun R C, Fowler P, Baird M S. 2006. Comparative study of organosolv lignins from wheat straw. *Ind Crops Prod*, 23: 180–193
- Zhao X B, Cheng K K, Liu D H. 2009. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. *Appl Microbiol Biotechnol*, 85: 815–827

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