

## Assessment of the Influence of the Temperature in the Microwave-Assisted Alkaline Delignification of Vine Shoots

Izaskun Dávila<sup>a,\*</sup>, Patricia Gullón<sup>a</sup>, Jalel Labidi<sup>a</sup>, Vitaliy Budarin<sup>b</sup>

<sup>a</sup>Chemical and Environmental Engineering Department, University of the Basque Country UPV/EHU, Plaza Europa 1, 20018 San Sebastian, Spain.

<sup>b</sup>Green Chemistry Centre of Excellence, Department of Chemistry, University of York, Heslington, York YO10 5DD, United Kingdom  
 izaskun.davila@ehu.eus

The limitation of the fossil-resources has led the research to focus on the production of biomass-based fuels from lignocellulosic agricultural residues, as it is the case of the bio-ethanol. Due to the complex and heterogeneous structure of these residues, a delignification stage is usually necessary prior to a simultaneous saccharification and fermentation treatment (SSF) for the production of bio-ethanol. In a previous work, an alkaline delignification of hydrothermally pretreated vine shoots using conventional heating was carried out in order to obtain a solid residue with low lignin and hemicellulosic content, which could be further subjected to a SSF treatment. However, when this biorefinery scheme was evaluated by a Life Cycle Assessment it was observed that the energy and chemical requirements of the delignification stage needed to be reduced. Therefore, in this work the assistance of the alkaline delignification stage by microwave was studied. The treatments were carried out at different temperatures, in order to study its influence on the lignin removal. It was observed that the microwave assistance could permit the improvement of the delignification treatments not only by increasing the delignification yield but also by reducing the temperature, time and NaOH concentration used in the treatments. The strong influence of the temperature on the chemical composition, structure and crystallinity of the delignified solids was also appreciated during this study. The solid residues obtained under the most severe conditions of the microwave-assisted alkaline delignification treatment (125 °C) could be a promising solid for the employment in a SSF treatment due to its low lignin and hemicellulosic content (16.68 % and 0.01 % respectively) and its high glucan content (50.75 %), although it presented a higher crystallinity index than the hydrothermally pretreated vine shoots.

### 1. Introduction

The production of biomass-based ethanol has increased throughout the years as the limitation of the employment of the fossil-fuel resources has directed the research towards this initiative. In 2014, 927.42 GL of bio-ethanol were produced worldwide, being around 60 % of it produced from sugarcane while the remaining 40 % was obtained from other crops (Achinas and Euverink, 2016). Although the bio-ethanol seems to be an encouraging alternative for the gasoline used in the transportation sector, its production amount continues to be insufficient compared with the annually consumed quantity (Achinas and Euverink, 2016). This fact has driven the attention towards the production of bio-ethanol from unexploited lignocellulosic biomass, such as vine shoots for instance.

The greenest and the most common procedure for the obtaining of bio-ethanol from this kind of residues consist on a simultaneous saccharification and fermentation (SSF). However, prior to the SSF the biomass needs to be pretreated, as it is necessary to disrupt the close inter-component association between the main constituents of the cell walls. It has been reported that the hemicellulosic fraction tend to block the access of the enzyme to the cellulose microfibrils (Zhang et al., 2012) while the lignin fraction apart from acting as a barrier it is also supposed to bind irreversibly by hydrophobic interactions to the cellulase, provoking a decrease of its activity (Hu et al., 2013).

Therefore, the vine shoots, which are the raw material used in this work, were subjected to a hydrothermal treatment followed by a delignification stage. The conditions used during the hydrothermal treatment were 200°C using a LSR of 8 Kg/Kg (oven-dried basis) in a non-isothermal regimen. It has been previously estimated that these were the optimum conditions for the maximum obtaining of oligosaccharides with the minimum formation of monosaccharides and it permitted the removal of 61.5 % of the hemicelluloses present in the vine shoots (Dávila et al., 2016).

In this work, the attention was focused on the delignification stage of the hydrothermally pretreated vine shoots. There are not many examples in the literature of the delignification of this pretreated and untreated residue. However, it has been reported that the alkaline delignifications presented a higher delignification yield than the organosolv treatments (Dávila et al., 2017a). These researchers observed that during the alkali delignification carried out with conventional heating around 60 % of the lignin present in the untreated and pretreated vine shoots was removed, while during the organosolv treatments only 26 % and 12 % of the lignin was removed respectively. However, the alkaline delignification presented a high energy and chemical requirements as it was observed during the Life Cycle Assessment of the biorefinery approach of the vine shoots, which consisted in a hydrothermal treatment, followed by an alkaline delignification and a SSF (Gullón et al., 2018). Therefore, in this work the intensification of the alkaline delignification by the assistance of microwave was studied. The microwave heating is considered as a green technique that could allow the reduction of time, and a more energy efficient process than the conventional heating as the heating transfer is faster (Aguilar-Reynosa et al., 2017). The aim of this work was to observe the benefits that the assistance by microwave of the alkaline delignification treatment of the hydrothermally pretreated vine shoots could exert on the delignification yield compared with the conventional heating. The scheme of the employed experimental procedure is shown in the Figure 1. The influence of an important variable such as the temperature of the delignification treatment on the lignin removal was also studied; therefore, the delignification treatments were carried out at several temperatures (50, 75, 100 and 125 °C). The influence of the microwave-assisted delignification treatment and its temperature on the structure of the hydrothermally pretreated vine shoots was analyzed. Thus, this solid and the delignified solids were subjected to compositional, FTIR and XRD analysis.

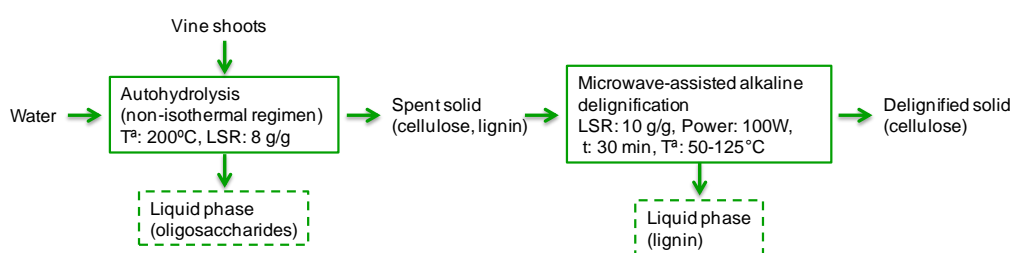


Figure 1: Biorefinery scheme for the revalorization of the vine shoots

## 2. Materials and methods

### 2.1 Raw material

Vine shoots from the grape variety Hondarribi Zuri were obtained from the pruning of the vine after the vintage of 2015 and they were supplied by the local winery Aldakoa S.L. (Basque Country, Spain). Once collected the residues were milled and sieved in order to have a single and homogenized lot with a particle size smaller than 0.4 mm. This lot was stored in a dark and dry place until it was used. The composition of the raw material has been reported in previous works (Dávila et al., 2016).

### 2.2 Hydrothermal treatment of the vine shoots

The vine shoots were subjected to a hydrothermal treatment in a non-isothermal regimen by mixing them with water in a LSR of 8 Kg/Kg (oven-dried basis) in a 1.5 L stainless steel Parr reactor. The treatment was carried out at 200 °C in a non-isothermal regimen being the temperature controlled by a Parr PID control. Once reached the temperature the reactor was cooled down and the solid and liquid fractions were separated by filtration, being the solid phase washed and stored at 4 °C without being dried.

### 2.3 Microwave assisted delignification of pretreated vine shoots

The alkali delignification was carried out in a CEM-Discover SP microwave by mixing the hydrothermally pretreated vine shoots with a solution of 3.1 w/w% NaOH in an LSR of 10 Kg/Kg (oven-dried basis), using 35

mL Teflon PFA liners. The treatments were carried out at different temperatures (50, 75, 100 and 125 °C) for 30 min and using a microwave power of 100 W. After the reaction time, the system was cooled down and the solid and liquid phases were separated by filtration, being the solid phases washed with a solution of 3.1 w/w% NaOH and then they were neutralized with water. The obtained solid phase was air-dried, quantified and subjected to a moisture determination in order to determine the solid yield and to estimate the amount of solubilized substrate.

## 2.4 Analysis of the obtained solid fractions

### Chemical composition

The hydrothermally pretreated vine shoots, together with the delignified solids were subjected to several analysis in order to determine the structural changes that the residue suffers with the temperature of the delignification treatments. The different solids were milled and sieved in order to have a particle size smaller than 0.25 mm, once the hydrothermally pretreated vine shoots were air-dried.

The solid phases were subjected to a quantitative acid hydrolysis (HAC) in order to determine their composition in glucan, hemicelluloses and acid insoluble lignin (modification of the TAPPI T-249-cm-09). The solids were subjected to an acid hydrolysis with H<sub>2</sub>SO<sub>4</sub> 72 w/w% for 1 h at 30 °C followed by a second acid hydrolysis carried out by diluting the samples to 4 w/w% H<sub>2</sub>SO<sub>4</sub> and keeping them in an autoclave for 1 h at 121 °C. After the hydrolysis, the liquid and solid phases were separated by filtration. The obtained solid phase was oven-dried and it was considered as acid insoluble lignin, while the liquid phase was analysed by High Performance Liquid Chromatography (HPLC) for the determination of monosaccharides (glucose, xylose and arabinose) galacturonic and acetic acid and degradation products (furfural and 5-(hydroxymethyl)furfural). The liquid phases were analysed using a Jasco LC Net II/ADC chromatograph equipped with a refractive index detector and an Aminex HPX-87H column (Bio-Rad Laboratories, USA). The samples were eluted with a solution of 0.005M H<sub>2</sub>SO<sub>4</sub> with a flow rate of 0.6 mL/min at 50°C. All the analysis was carried out in triplicates.

### Fourier transform infrared spectroscopy (FTIR) analysis

The chemical structure of the different obtained solids was determined by FTIR to study the changes that took place depending on the temperature of the treatment. The samples were analysed in a PerkinElmer Spectrum Two FT-IR spectrometer working with a resolution of 4 cm<sup>-1</sup> and accumulating 8 scans in a transmission mode. The spectrums were recorded from 4000 to 600 cm<sup>-1</sup>.

### X-ray diffraction (XRD) analysis

The different solids were subjected to an XRD analysis in order to determine the crystallinity index. The samples were analysed using a Bruker AXD-D8 Advance diffractometer with a Kristalloflex 760 X-ray generator, which generated focused and monochromatized K $\alpha$  X-rays from a Cu source. The recording of the scans was carried out between 5-35 2 $\theta$  over 10 min with a 40 kV and 40 mA current. The phases present in the samples was carried out using the EVA evaluation programma and the Bruker CDS database. The crystallinity index was calculated taking into account the height ratio between the intensity of the crystalline peak ( $I_{002} - I_{AM}$ ) and the total intensity  $I_{002}$  (Park et. al., 2010).

## 3. Results and discussion

### 3.1 Delignification yield and chemical composition

In this study the influence of the temperature on the microwave-assisted alkaline delignification treatment of hydrothermally pretreated vine shoots was analysed. The solid used during the delignification treatments presented very little hemicellulosic content as it can be seen from the results collected in the Table 1. This demonstrates that during the hydrothermal pretreatment the hemicellulosic fraction of the untreated vine shoots was efficiently solubilized as the composition of this solid differs completely from the one of the untreated vine shoots, which presented 27.0 % of hemicelluloses, 33.0 % of glucan, and 26.7 % of lignin (on oven-dry basis) (Dávila et al. 2016).

From the composition of the microwave assisted alkaline delignified solids, shown in the Table 1, it can be seen that the microwave technology permitted the reduction of time, NaOH concentration and temperature of the treatment compared with the delignification carried out with conventional heating. Davila et al. (2017b) were able to remove 67.7 % of the lignin present in hydrothermally pretreated vine shoots when the delignification was carried out at 124 °C for 75 min and using a solution of 12 w/w% NaOH. However, when the delignification treatment was assisted by microwave better delignification yields were obtained working at lower and similar temperatures (100-125°C) but carrying out the treatment for 30 min and using a NaOH solution of 3.1 w/w%.

Table 1: Composition of the solid obtained after the hydrothermal and delignification treatments

	Hydrothermal	Delig. 50°C	Delig. 75°C	Delig. 100°C	Delig. 125°C
Solid yield (%)	-	89.46	72.75	56.48	46.69
Glucan (%)	32.25	33.30	35.82	44.83	50.75
Lignin (%)	52.65	37.08	32.56	14.32	16.68
Hemicelluloses (%)	7.38	4.76	3.5	3.16	0.01
Xylan (%)	6.37	4.49	3.28	2.97	0.01
Acetyl groups (%)	1.01	0.27	0.22	0.19	-
Delignification yield (%)	-	37.00	55.01	84.63	85.20
Glucan loss (%)	-	7.63	19.20	21.50	26.53

The strong influence of the temperature of the delignification treatment on the composition of the delignified solids and on the delignification yield can also be appreciated from the results shown in the Table 1.

As it can be seen the increase of the temperature, which makes the treatment more severe, provoked a reduction of the solid yield due to the higher lignin and glucan removal. During the alkaline delignification treatment, mainly the degradation and solubilization of the lignin fraction takes place by the breaking of the ether linkages that maintain the inter-units of the lignin together (Xu et al., 2016). However, this treatment is not as selective as it ideally could be as the degradation of carbohydrates is unavoidable (Xu et al., 2016). As it can be seen in the Table 1, the lignin removal increases with the temperature until it reached 100 and 125 °C, when the delignification yield remained almost constant. Stoklosa and Hodge (2015) also observed that the degradation of the lignin increased with the increase of the severity of the treatment when they studied the alkaline delignification of hardwoods such as sugar maple, silver birch and hybrid poplar. They also observed a decrease of the delignification rate with the increase of the severity of the treatment together with additional polysaccharide losses. It can also be seen that the increase of the severity of the delignification treatment produced an increase of the glucan loss. Xu et al. (2016) suggested that the glucan loss could be attributed to peeling and hydrolysis reactions of cellulose, which could be favored at high temperatures and strong alkaline solutions.

### 3.2 FTIR analysis of the solids obtained after the hydrothermal and the delignification treatments

The solids obtained after the hydrothermal and microwave assisted alkaline delignification treatments were analysed by FTIR in order to study how the delignification treatment and its temperature influence the chemical structure of the hydrothermally pretreated vine shoots. The Figure 2 collects the FTIR spectra obtained from each analysed solid and as it can be seen the temperature used during the delignification treatment affected the intensity and the presence of the bands. The band assignment was carried out taking into account what it has been described in the literature (Pereira et al., 2016).

The bands observed at 1,604 and 1,508  $\text{cm}^{-1}$  were associated to the lignin structure as they correspond to the typical skeletal vibration of aromatic rings and to the presence of guaiacyl units, respectively. The band observed at 1,234  $\text{cm}^{-1}$  corresponds to the characteristic C-O stretching of hemicelluloses and lignin. On the other hand, the bands observed at 1,425, 1,375 and 1,325  $\text{cm}^{-1}$  were attributed to the crystalline structure of the cellulose. Another two bands associated to the cellulose structure were observed at 1,164  $\text{cm}^{-1}$  and 1,035  $\text{cm}^{-1}$  as they correspond to the C-O-C asymmetric stretching and to the C-O stretching of cellulose. The bands that appeared at 1,114 and 1,051  $\text{cm}^{-1}$  were also associated to the cellulose as they correspond to the  $\gamma$  ring in plane vibration (Oh et al., 2005) and to the linkages present in the cellulose. Another characteristic band of the cellulose was observed at 872  $\text{cm}^{-1}$  which corresponds to the  $\beta$ -glycosidic linkages.

As it can be appreciated from the Figure 2, the FTIR spectra of the solid residues obtained after the delignification treatments carried out at 50 and 75 °C resembled more to the hydrothermally pretreated solid. While the FTIR of the solids obtained under more severe delignification conditions (100 °C and 125 °C) presented the greatest differences compared with the hydrothermally pretreated solid, being its chemical structure more affected during the treatment. The main bands affected during the treatments were those associated with the lignin and cellulose structure.

It can be observed that the bands associated to the lignin structure as can be the ones observed at 1604 and 1508  $\text{cm}^{-1}$  presented lower intensity with the increase of the severity of the treatment, having them almost disappeared in the spectra of the solids obtained after the delignification at 125 °C. Although all the FTIR spectra, presented the bands associated with the cellulose it can be seen that the intensity of those associated to the crystalline of the cellulose, as it is the case of the bands observed at 1,425, 1,375 and 1,325  $\text{cm}^{-1}$ , presented an increase of their intensity with the increase of the severity of the delignification conditions. It can also appreciate the increase of the intensity of the band observed at 872  $\text{cm}^{-1}$  with the increase of the temperature of the delignification treatment. The tendencies of the reduction of the intensity of the bands associated with the lignin

and the increase of the intensity of the bands associated with the cellulose with the increase of the severity of the delignification treatment is in agreement with the trend observed for the chemical composition of the delignified solids.

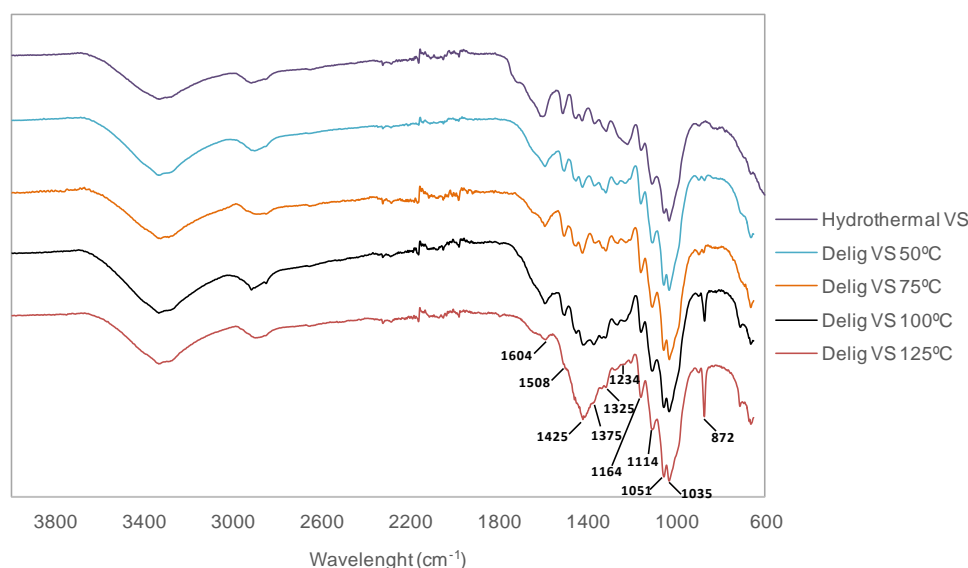


Figure 2: FTIR spectra of the solids obtained in the hydrothermal and delignification treatments

### 3.3 XRD analysis of the hydrothermally pretreated and delignified solids

The alkaline treatments apart from provoking the disruption of the lignin structure, as it has been seen for the chemical composition of the delignified solids, they could alter the morphology and conformation of the cellulose decreasing its degree of polymerization and crystallinity (Taherzadeh et al., 2007). The crystallinity of the solid phases is an important parameter to take into account for the employment of the delignified solids in a subsequent enzymatic saccharification, which is part of the SSF treatment (Binod et al., 2012). In order to study the crystallinity index of the hydrothermally pretreated vine shoots and of the obtained delignified solids they were subjected to an XRD analysis, being the crystallinity index collected in the Table 2.

Table 2: Crystallinity index of the solid obtained after the hydrothermal and delignification treatments

	Hydrothermal	Delig. 50 °C	Delig. 75 °C	Delig. 100 °C	Delig. 125 °C
Crystallinity index	43.60	40.21	37.91	44.42	49.29

As it can be seen in this table, compared with the hydrothermally pretreated vine shoots the solid residues obtained in the microwave-assisted alkaline delignification treatments carried out below 100 °C presented a lower crystallinity index, as it was expected. However, in those delignification treatments carried out above 100°C the crystallinity index of the obtained solids was higher than the one estimated for the hydrothermally pretreated vine shoots. Binod et al. (2012) also observed this tendency when they carried out a microwave-assisted alkaline delignification of sugarcane bagasse at high temperatures. They attributed the increase of the crystallinity to the removal of the lignin fractions during the treatment, which could be a possible explanation for what it has been observed during the delignification treatments carried out above 100 °C. The increase of the crystallinity of the solids obtained with the severest conditions could also be associated with the higher glucan loss observed in these conditions, which could be part of the amorphous cellulose present in the solid.

It could be seen that the crystallinity index of the solids obtained by the microwave-assisted alkaline delignification of hydrothermally pretreated vine shoots didn't follow a simple trend, as it could be influenced by the decrease of the crystallinity of the cellulose, the removal of cellulose and the removal of lignin during the delignification treatments. Karp et al. (2014) also found it difficult to correlate the crystallinity results of alkaline pretreated corn stovers, as according to them the crystallinity measurements was convoluted by all the effects that takes place during the alkaline delignification treatments.

#### 4. Conclusions

The microwave-assisted alkaline delignification at different temperatures of hydrothermally pretreated vine shoots at 200 °C using a non-isothermal regimen was carried out, concluding that the microwave is a promising technology that could permit the softening of the treatment conditions. The temperature of the treatment influenced strongly on the chemical composition, structure and crystallinity of the delignified solids. The treatment carried out at most severe conditions (125 °C) permitted the obtaining of delignified solid with 16.68 % of lignin and 50.75 % of glucan and a higher crystallinity index than the hydrothermally pretreated solid, making it a promising solid for a subsequent SSF treatment.

#### Acknowledgments

Authors would like to thank to the Basque Government for the scholarship of young researchers training and to the Spanish Ministry of Economy and Competitiveness (FPDI-2013-18748 and CTQ2016-78689-R).

#### References

- Achinas S., Euserink G. J.W., 2016, Consolidated briefing of biochemical ethanol production from lignocellulosic biomass, *Electronic Journal of Biotechnology*, 23, 44-53.
- Aguilar-Reynosa A., Romani A., Rodríguez-Jasso R.M., Aguilar C.N., Garrote G., Ruiz H.A., 2017, Microwave heating processing as alternative of pretreatment in second generation biorefinery: an overview, *Energy Conversion and Management*, 135, 50–65.
- Binod P., Satyanagalakshmi K., Sindhu R., Janu K.U., Sukumaran R.K., Pandey A., 2012, Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse, *Renewable Energy*, 37, 109-116.
- Dávila I., Gordobil O., Labidi J., Gullón P., 2016, Assessment of suitability of vine shoots for hemicellulosic oligosaccharides production through aqueous processing, *Bioresource Technology*, 211, 636-644.
- Dávila I., Gullón P., Andrés M., Labidi J., 2017b, Coproduction of lignin and glucose from vine shoots by eco-friendly strategies: Toward the development of an integrated biorefinery, *Bioresource Technology*, 244, 328-337.
- Dávila I., Robles E., Andrés M.A., Gullón P., 2017a, Delignification alternatives of spent solid from autohydrolysis of vine shoots, *Chemical Engineering Transactions*, 57, 85-90.
- Gullón P., Gullón B., Dávila I., Labidi, J. Gonzalez-Garcia, S., 2018, Comparative environmental Life Cycle Assessment of integral revalorization of vine shoots from a biorefinery perspective, *Science of the Total Environment*, 624, 225-240.
- Hu F., Jung S., Ragauskas A., 2013, Impact of pseudolignin versus dilute acid-pretreated lignin on enzymatic hydrolysis of cellulose. *ACS Sustainable Chemistry and Engineering*, 1, 62-65.
- Karp E.M., Donohoe B.S., O'Brien M.H., Ciesielski P.N., Mittal A., Biddy M.J., Beckham G.T., 2014, Alkaline pretreatment of corn stover: Bench-scale fractionation and stream characterization, *ACS Sustainable Chemistry and Engineering*, 2, 1481-1491.
- Oh S.Y., Yoo D.I., Shin Y., Kim H. C., Kim H.Y., Chung Y.S., Park W.H., Youk J.H., 2005, Crystalline structure analysis of cellulose treated with sodium hydroxide and carbon dioxide by means of X-ray diffraction and FTIR spectroscopy, *Carbohydrate Research*, 340, 2376-2391.
- Park S., Baker J.O., Himmel M.E., Parilla P.A., Johnson D.K., 2010, Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulose performance, *Biotechnology and Biofuels*, 3, 1-10.
- Pereira S.C., Maehara L., Machado C.M.M., Farinas C.S., 2016, Physical-chemical-morphological characterization of the whole sugarcane lignocellulosic biomass used for 2G ethanol production by spectroscopy and microscopy techniques, *Renewable Energy*, 87, 607-617.
- Stoklosa R.J., Hodge D.B., 2015, Fractionation and improved enzymatic deconstruction of hardwoods with alkaline delignification, *Bioenergy Research*, 8, 1224-1234.
- Taherzadeh M. J., Karimi K., 2007, Enzyme-based hydrolysis processes for ethanol from lignocellulosic materials: a review, *BioResources*, 2, 707-738.
- Xu H., Li B., Mu X., 2016, Review of alkali-based pretreatment to enhance enzymatic saccharification for lignocellulosic biomass conversion, *Industrial and Engineering Chemistry Research*, 55, 8691-8705.
- Zhang J., Tang M., Viikari L., 2012, Xylans inhibit enzymatic hydrolysis of lignocellulosic material by cellulases, *Bioresource Technology*, 121, 8-12.