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Comparison of High Temperature Alkali and Acid Pretreatment for the Enzymatic Saccharification of Poppy Stalks

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In this study, the effect of dilute alkali (NaOH) and dilute acid (H_2SO_4) concentrations on the enzymatic digestibility, and sugar recovery from poppy stalks at the pretreatment temperature of 120 °C, and pretreatment time of 30 min were investigated. While highest lignin removal was at the concentration of 2.25 % of NaOH concentration, acid pretreatment led to highest hemicellulose removal. Glucose recovery gradually increased from 39.50 % to 85.87 % with the increase in the NaOH concentration. Maximum total sugar yield (63.32 %) was achieved at the concentration of 3.0% NaOH, whereas only 38.16 % of total sugar yield was obtained with acid pretreatment. The results obtained in this study showed that alkali pretreatment for fermentable sugars of poppy stalk was more effective on the sugar recovery than acid pretreatment at 120 °C.

1. Introduction

Lignocellulosic materials are natural renewable resource that can be converted into useful materials and energy. Utilization of lignocellulosic materials for these purposes requires effective pretreatment step to disrupt the structure of lignocellulose structure, to increase accessible surface area and porosity (Kumar and Wyman, 2009; Kravanja and Friedl, 2010). A range of chemical, physical and biological processes have been configured to release constituent sugars from lignocellulose. Treatment of lignocellulosic materials with acid and alkali at a high temperature can efficiently improve the enzymatic hydrolysis. High temperature pretreatment operation undergoes generally with low acid or alkali concentrations. Dilute alkali pretreatment is one of the most effective pretreatment method for high lignin contained biomass since the major effect of alkali is to remove lignin and part of hemicellulose (Dagninoa et al., 2013). Dilute-acid pretreatment can be performed in short retention time at high temperature. Although dilute acid pretreatment is not effective on the lignin removal, acid can disrupt lignin and increases the cellulose digestibility (Binod et al., 2012).

Poppy (*Papaver somniferum* L.) has been grown since ancient times for its oil rich seeds and the opium, which is exuded from incised seed capsules. The area under opium poppy cultivation in Turkey is reported as 55,000 hectares for the year of 2011 (Turkish Statistical Institute, 2011). Assuming that average of 50 % of whole plants are fibrous waste residue (poppy stalk) (Laughlin, 1978), total poppy stalk yield from opium poppy cultivation can be estimated more than 100,000 t/y.

The objective of this study was to investigate the effect of dilute NaOH and dilute H2SO4 pretreatment of poppy stalk at high temperatures on the pretreated poppy stalk composition and enzymatic hydrolysis efficiency.

2. Material and Methods

2.1 Material

Papaver somniferum stalks used in this study were kindly provided by Afyon Alkaloids Factory, Bolvadin, Turkey. Raw material was air dried until 10% moisture. The dried materials were grounded by a grinder

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and screened with a sieve shaker to obtain particle sizes between 0.224-0.850 mm. Samples were stored in plastic bags at +4 ^{0}C for future use. Celluclast 1.5 L and Novozyme 188 were purchased from Sigma Aldrich (St. Louis, USA). Aminex HPX 87P column was purchased from Bio-Rad Laboratories (California, USA). All chemicals used were standard analytical grades.

2.2 Pretreatment and enzymatic digestion of poppy stalk

High temperature treatment of poppy stalks was performed in a PARR stainless steel reactor at 120 °C for 30 min using NaOH (1.5-3.0 % (w/v)) and H_2SO_4 (0.5-1.0 % (v/v)). Approximately 7 g of dry poppy stalks were mixed with 70 mL of acid or alkali solution in a Teflon liner. The vessel was heated until desired temperature and pretreatment time was initiated. Solution was kept liquid under N_2 atmosphere. After treatment the reactor vessel was moved from heating jacket. The content of the reactor was cooled down to 80^{0}C. Then the wet material was filtrated for solid recovery and washed with distilled water until the wash water turn to pH 7.0. The pretreated solid was used as the substrate for enzymatic hydrolysis, and stored in plastic bags at +4 ^{0}C.

Enzymatic hydrolysis was carried out in stoppered conical flasks (50 mL). The pH was adjusted to 4.8 with acetate buffer, and a mixture of cellulase (60 FPU/g dry biomass) and β-Glucosidase (40 CBU/g dry biomass) was added to pretreated substrate in a total working volume of 20 mL. The hydrolysis reactions were carried out at 50 °C in an incubator for 48 h by shaking at 150 rpm. The reactions were stopped in a boiling water bath for 15 minutes and hydrolysates were clarified by centrifuging at 5000 rpm for 5 min. The supernatants were analyzed for glucose and xylose using HPLC.

2.3 Analytical methods

The chemical composition of raw and pretreated poppy stalks were determined according to NREL (Sluiter et al., 2008) methods. 0.3 g solid was hydrolyzed by 3 mL of 72 % (w/w) H_2SO_4 at 30 °C for 60 min then, the reaction mixture was diluted to 4 % (w/w) and autoclaved at 121 °C for 60 min. Lignin was determined by solid residue, cellulose and hemicellulose amount were determined from filtrate by using High Performance Liquid Chromatography (Agilent 1100). The HPLC system was mainly equipped with a Bio-Rad Aminex HPX-87P column (300 mm × 7.8 mm), and a refractive index detector. The analytical column was operated at 80 °C with 0.2 µm filtered HPLC grade water as the mobile phase. Mobile phase flow rate was 0.6 mL/min.

Enzyme activity of Celluclast 1.5L® was determined by NREL protocols and reported as Filter Paper Unit (FPU) (Adney and Baker, 2008). One unit of FPU is defined as the amount of enzyme required to liberate 1µmol of glucose from Whatman no:1 filter paper per minute at 50 °C. One cellobiose unit (CBU) is the amount of enzyme that converts 1 mmoL of cellobiose to 2 mmoL of glucose per min. Cellulose (Eq.1) and hemicellulose digestion (%) (Eq.2), glucose recovery (%) (Eq.3) and total sugar yield (%) (Eq.4) are calculated as follows;

$$Cellulose\ Digestion\ (\%) = \frac{\text{Amount of glucose producedx0.9}}{\text{Amount of cellulose in pretreated poppy}} x100 \tag{1}$$

$$Hemicellulose\ Digestion\ (\%) = \frac{\text{Amount of xylose producedx0.88}}{\text{Amount of hemicellulose in pretreated poppy}} x100 \tag{2}$$

$$Glucose\ Recovery\ \ (\%) = \frac{\text{Amount of glucose producedx0.9}}{\text{Amount of cellulose in unpretreated poppy}} x100 \tag{3}$$

$$Total\ Sugar\ Yield\ (\%) = \frac{\text{Amount of total reduced sugar producedx0.9}}{\text{Amount of cellulose and hemicellulose in unpretreated poppy}} x100 \tag{4}$$

3. Results and discussions

3.1 Composition of poppy stalks

The composition of unpretreated poppy stalks used in this study is presented in Figure 1. The holocellulose fraction was 44.81±2.72 % of the dry biomass being a major component at 24.36±1.29 % cellulose and the remaining 20.45±1.42 % hemicellulose. Lignin content was 19.79±0.95 %.

3.2 Effect of alkali and acid pretreatment on the cellulose recovery (%), and lignin and hemicellulose removal (%)

Effect of alkali and acid concentrations on the cellulose recovery (%), lignin and hemicellulose removal (%) is shown in Figure 2 and Figure 3, respectively. The insoluble solid recovery of acid pretreated poppy stalks was lower than that of alkali pretreatment. Solid recovery ranged from 42.65 % to 56.66 % for alkali pretreatment, it was around 46 % for acid pretreatment. The weight loss was mainly due to the removal of lignin and degradation of hemicellulose (Karunanithy and Muthukumarappan, 2011). The highest lignin removal (58.89 %) at 120 °C for 30 min was obtained when NaOH concentration of 2.25 %. While cellulose recovery (%) ranged from 56.82 % to 94.58 % for alkali pretreatment, it was between 63.66 % and 75.33 % for acid pretreatment. Average hemicellulose removal with acid pretreatment was almost 15 % more than that of alkali pretreatment. Acid concentrations used in this study did not effect on the hemicellulose removal, however, hemicellulose removal was decreased with an increase in NaOH concentration from 2.25 % to 3.0 %. Results obtained in this study was in agreement with those obtained from different crop residues pretreated under similar conditions. The high temperature sodium hydroxide pretreatment of cotton stalk was completely removed lignin and efficiency of the enzymatic hydrolysis improved to 96 % (Binod et al., 2012). Lignin removal, accessibility, and digestibility of cellulose of corn stover were increased by sodium hydroxide pretreatment. Enzymatic hydrolysis efficiency of sodium hydroxide pretreated sample was improved (Chen et al., 2009). Highest cellulose in the solid fraction was obtained from rice hulls with 1.3 % acid and 33 min pretreatment time (Dagninoa et al, 2013). The maximum glucose yield in dilute acid pretreatment of the wheat straw was approximately 50 % where retention time, acid concentration and temperature were 30 min, 0.75 % and 160 °C respectively (Baboukani et al., 2012). Dilute-acid pretreatment with low severity of barley straw is enhanced the enzymatic digestibility and decreased the amount of enzyme loading (Panagiotopoulos et al., 2012).

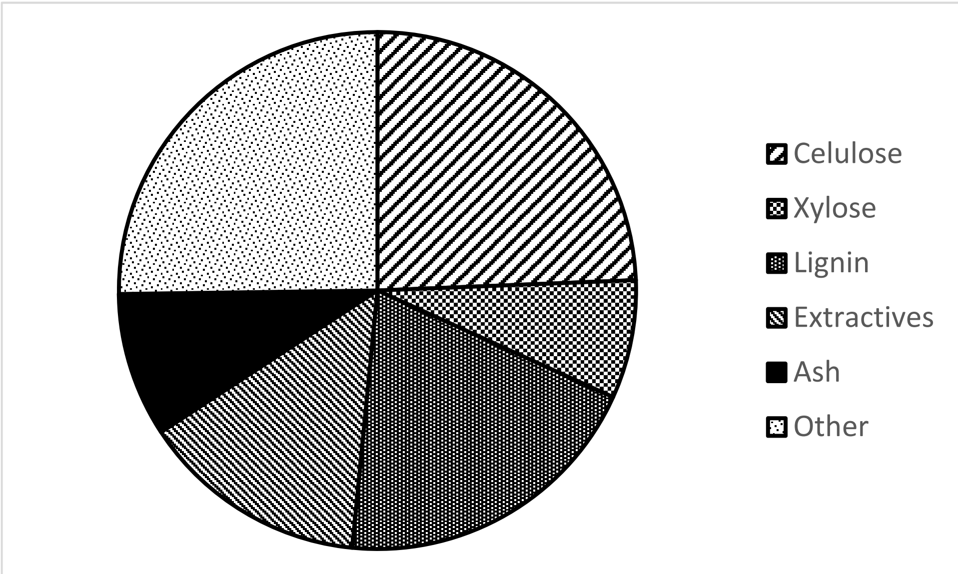

Figure 1. Composition of unpretreated pappy stalk

3.3 Effect of alkali and acid pretreatment on the enzymatic hydrolysis

Table 1 shows the effect of NaOH and H_2SO_4 concentrations on cellulose and hemicellulose digestibility. With the enzyme combination used in this study (a mixture of 60 FPU cellulase/g dry biomass and 40 CBU β-Glucosidase /g dry biomass), cellulose and hemicellulose digestibility increased with increased concentration of alkali, and reached to 90.68 % and 84.70 %, respectively, at 3.0 % of NaOH. At this concentration released glucose and xylose amounts after enzymatic hydrolysis were 409 and 122 mg/g, respectively. In contrast to NaOH, glucose content slightly increased from 335.15 mg/g to 366.96 mg/g when acid concentration increased from 0.5 % to 1.0 %, whereas xylose content decreased to 3.33 mg/g with acid concentration due to solubility of xylose in the acid solution.

Increasing NaOH concentration from 1.5 % to 3.0 % (w/v), increased the glucose recovery (%) and total sugar yield (%) almost 2-fold (Figure 4). The highest recoveries of glucose and total sugar were 85.87 % and 63.32 % respectively, and reached at the concentration of 3.00 % NaOH.

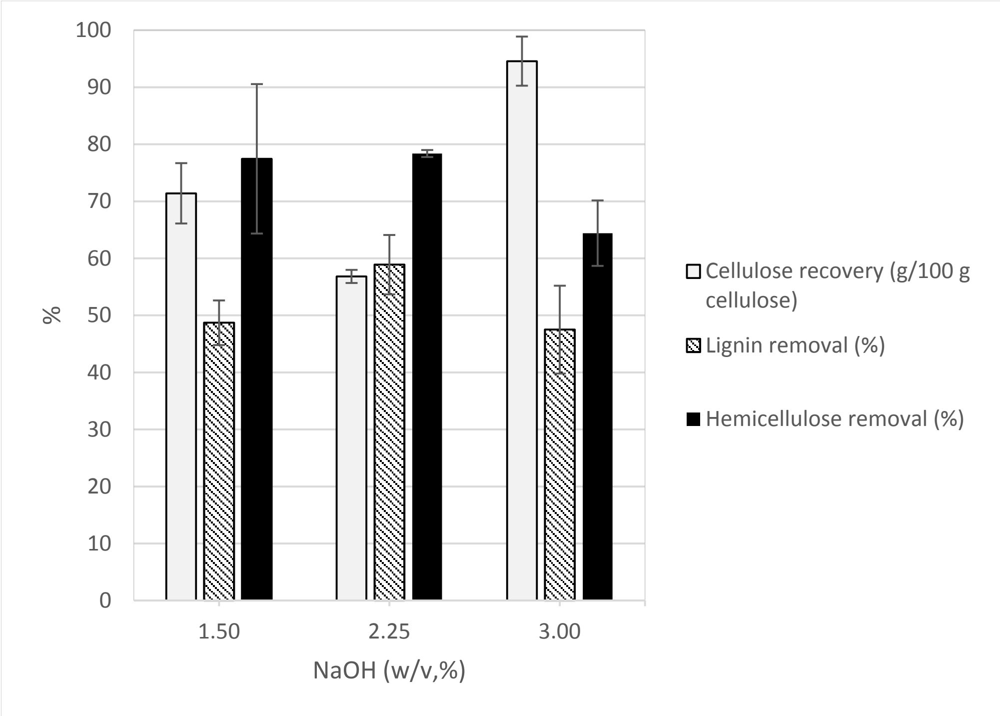

Figure 2. Effect of NaOH concentration on the cellulose recovery, lignin and hemicellulose removal of poppy stalks at pretreated 120^{o}C for 30 min

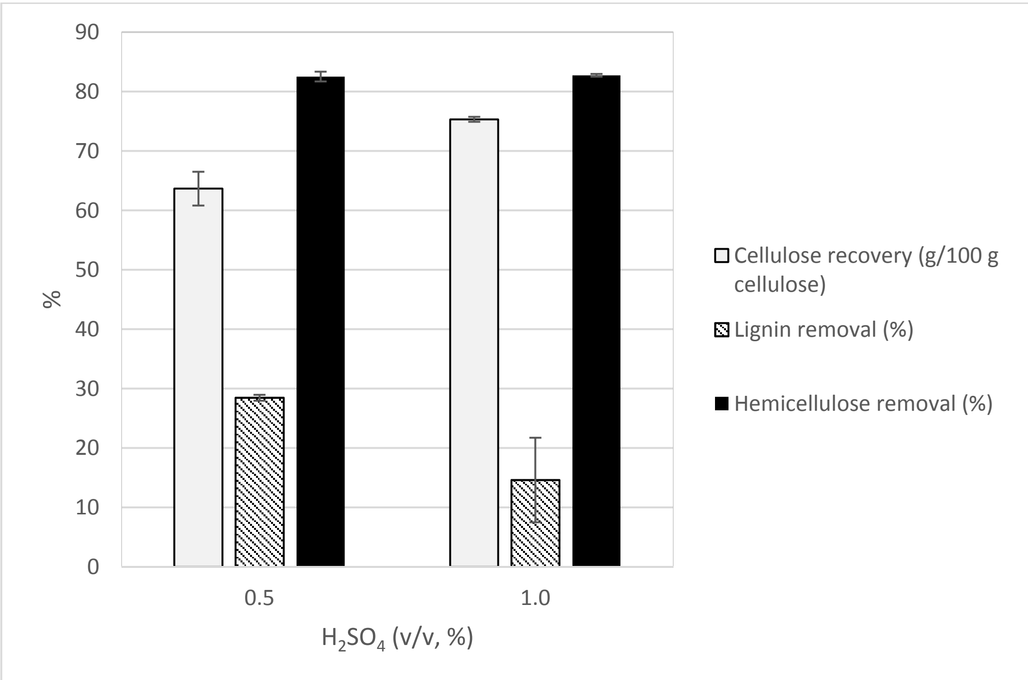

Figure 3. Effect of H_2SO_4 concentration on the cellulose recovery, lignin and hemicellulose removal of poppy stalks pretreated at 120 °C for 30 min

Dilute acid pretreatment is reported to increase surface area, removes hemicelluloses and alters lignin structure (Kumar et al, 2009). Cellulose digestibility of acid pretreated poppy stalks was between 84.39 and 89.04 %, which were similar to those obtain from alkali pretreatment (Table 1). However, hemicellulose digestibility was very low (3.89 % and 27.13 %) when compared to those obtained from

NaOH pretreatment, due to hemicellulose dissolved in acid solution. Glucose recovery only 12 % increased with an increased acid concentration from 0.5 % to 1.0 %. Acid concentration did not alter to total sugar yield, the average sugar yield was only 38.0 %.

Similar results reported in previous studies. For example, Ioelovich and Morag (2012) who applied both dilute H_2SO_4 and NaOH to four materials and found the alkali to be more efficient in terms of sugar yields. Sodium hydroxide pretreatment of cotton stalk waste was reported to effective for producing fermentable sugars. The process efficiency, based on glucose recovery was given as 53 % (Parameswaran Binod 2012)

Table 1. Effect of alkali and acid concentration on the cellulose and hemicellulose digestibility and sugar recovery of pretreated poppy stalks at 120 °C for 30 min

Concentration	Glucose (mg/g pretreated poppy stalks)	Xylose (mg/g pretreated poppy stalks)	Cellulose digestion (%)	Hemicellulose digestion %
NaOH (w/v, %)				
1.50	171.72±1.64	34.43±0.97	45.12±2.68	53.24±0.1
2.25	323.16±8.84	65.34±0.65	89.61±2.43	55.47±2.54
3.00	409.82±10.19	122.80±0.79	90.68±3.78	84.70±6.68
H_2SO_4 (v/v%)				
0.50	335.15±1.50	24.67±0.02	89.04±2.01	27.13±0.40
1.00	366.76±8.67	3.33±0.01	84.39±0.79	3.89±0.02

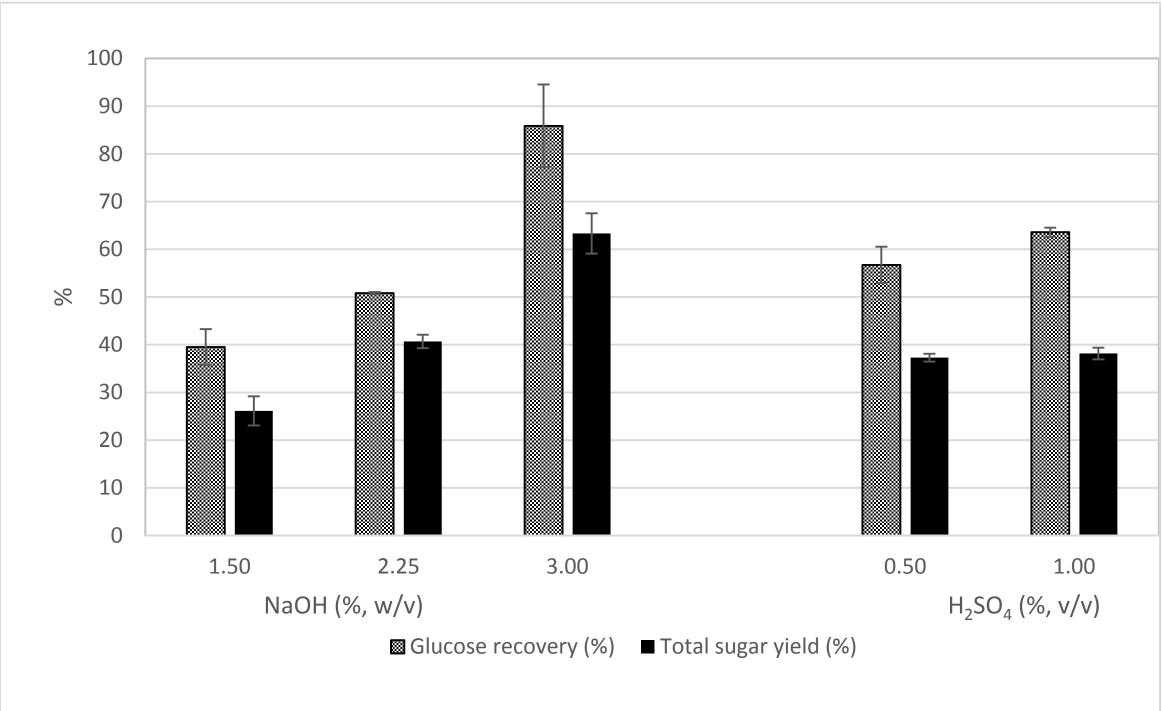

Figure 4. Glucose and total sugar recovery at different alkali and acid concentration at 120 °C, 30 min

4. Conclusion

In response to dilute alkaline (NaOH) and acid (H_2SO_4) pretreatments of poppy stalks at 120^{o}C for 30min, the maximum glucose and total sugar recoveries of 85.87% and 63.32%, respectively were obtained when the poppy stalk was pretreated with 3.0% NaOH concentration. While total sugar recovery increased gradually in increased concentration of NaOH, H_2SO_4 concentration did not effect on the total sugar yield of poppy stalks, and slightly effected on the glucose recovery. Compared to acid hydrolysis, NaOH pretreatment appeared to improve enzymatic biodegradability due to the higher delignification ability of alkali. Further investigations should be done to optimize alkali pretreatment process.

Acknowledgments

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References

Adney B., Baker J., 2008, Measurement of cellulase activities, Technical Report, NREL/TP-510-42628, National laboratory of the U.S. Department of Energy, Colorado, USA.

Baboukani B.S., Vossoughi M., Alemzadeh I., 2012, Optimisation of dilute-acid pretreatment conditions for enhancement sugar recovery and enzymatic hydrolysis of wheat straw, Biosys. Eng. 111, 166-174.

Binod P., Kuttiraja M., Archana M., Usha Janu K., Sindhu R., Sukumaran R.K., Pandey A., 2012, High temperature pretreatment and hydrolysis of cotton stalk for producing sugars for bioethanol production, Fuel 92, 340-345

Chen M. Zhao J., Xia L., 2009, Comparison of four different chemical pretreatments of corn stover for enhancing enzymatic digestibility, Biomass Bioenerg. 33, 1381-1385.

Dagninoa E.P., Chamorro E.R., Romano, S.D., Felissia, F.E., Area M.C., 2013, Optimization of the acid pretreatment of rice hulls to obtain fermentable sugars for bioethanol production, Ind. Crop Prod. 42, 363-368.

Ioelovich M., Morag E., 2012, Study of enzymatic hydrolysis of mild pretreated lignocellulosic biomasses, BioResources, 7(1), 1040-1052.

Karunanithy C., Muthukumarappan K., 2011, Optimization of alkali soaking and extrusion pretreatment of prairie cord grass, Biochem. Eng. J. 54, 71-82.

Kravanja P. and Friedl, A., 2010, Evaluation of Ethanol from Lignocellulosic Biomass-Process Scenarios for Austria, Chemical Engineering Transactions, 21, 1141-1146.

Kumar R., Wyman C. E., 2009, Access of cellulase to cellulose and lignin for poplar solids produced by leading pretreatment technologies, Biotechnol. Prog. 25, 807–819.

Kumar P., Barrett D., Delwiche M., Stroeve P., 2009, Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production, Ind. Eng. Chem. Res. 48, 3713-3729.

Laughlin J.C., 1978, The effect of band placed nitrogen and phosphorus fertilizer on the yield of poppies (*Papaver somniferum* L.) grown in Krasnozem soil, Acta Hort. 73,165-169.

Panagiotopoulos I.A., Lignos G.D., Bakker R.R:, Koukios, E.G., 2012, Effect of low severity dilute-acid pretreatment of barley straw and decreased enzyme loading hydrolysis on the production of fermentable substrates and the release of inhibitory compounds, J. Clean. Prod. 32, 45-51.

Sluiter A., Hames B., Ruiz R., Scarlata C., Sluiter J., Templeton D., Crocker, D., 2008a, Determination of structural carbohydrates and lignin in biomass, Technical Report NREL/TP-510-42618 Revised April 2008, National laboratory of the U.S. Department of Energy, Colorado, USA.

Turkish Statistical Institute (TUIK), 2011. The Summary of Agricultural Statistics, http://www.turkstat.gov.tr, Ankara, Turkey