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DETERMINATION OF THE EFFECTS OF AQUEOUS AMMONIA PRETREATMENT ON THE STRUCTURE OF SOLID CAKE VIA STATISTICAL ANALYSES AND CHARACTERIZATION METHODS

**İbrahim Ethem AYHAN [1], Berkan YILMAZ [1], Oğuzhan KARAMAN [1],
Yunus Emre ŞİMŞEK [1], Levent DEĞİRMENCİ [1, *]**

[1] Department of Chemical and Process Engineering, Engineering Faculty, Bilecik Seyh Edebali University, Bilecik, Turkey

ABSTRACT

Pretreatment of biomass prior to use for ethanol production is considered as an important step in increasing efficiency of process. Among various procedures, treatment with NH_3 is an effective and facile method for delignification of biomass with high lignin content. Solid cake, utilized in this study is a lignocellulosic biomass with rich organic content. NH_3 pretreatment was conducted in the course of study to determine its effect on lignin degradation and decreasing cellulose crystallinity. This treatment procedure is a one-step process unlike consecutive dilute acid hydrolysis and alkaline treatment with NaOH which were currently being used in sugar production. Temperature used in experiments were varied between 30-70ºC. These values were much lower than conventional dilute acid hydrolysis which enabled the treatment to be energy efficient.

Alternative use of solid cake as a biomass source in ethanol production would be beneficial in reducing the costs of olive oil production. Efficient use of this biomass depends on degradation of its high lignin content and the decrease in its cellulose crystallinity. Hence determination of optimum conditions utilized in NH_3 pretreatment is crucial to achieve economic production ethanol. The pathway in NH_3 treatment of olive oil cake was presented with this study. Solid cake was treated at varying times, NH_3 amounts and temperatures and the changes in biomass structure were determined in terms of lignin content and cellulose crystallinity. Effect of parameters was statistically validated and interpreted in accordance with FT-IR and TGA analyses.

Chemical treatment of solid cake resulted in lignin degradation which was followed by consecutive hemicellulose decomposition. Cellulose crystallinity decreased at elevated time intervals due to detoriation of its structure. Results indicated the significance of time especially in decreasing cellulose crystallinity. Lignin content was decreased from 47% to 20-22% independent of conditions. In other words, lignin degradation was stable in investigated regions and it was concluded that mildest conditions such as low temperature (30 ºC) and ammonia (NH_3) (5%) would have been sufficient to achieve successful treatment provided that the procedure be maintained for long time (12h) intervals. This was one of the highlights of this study as it was shown that mildest conditions would have been adequate to achieve an effective treatment with energy conservation compared to conventional methods still in use. NH_3 treatment also enabled preservation of glucan in the structure which was the main problem in other treatment procedures. Results obtained from experiments indicated the presence of ß-glucan even in highest treatment times.

Keywords: Biomass, Lignin, Cellulose crystallinity, Solid cake

1. INTRODUCTION

Fossil fuels are still in use despite the efforts to develop their alternatives and replacement of these with their alternatives would not be possible at least in near future. Increasing attention is being payed to developing systems that uses alternative energy sources [1-5]. However, there is also appreciable effort on replacing these fuels with alternatives that could easily be adopted with minor modifications on systems using fossil fuels. The most important feature of fossil fuels is the amount of greenhouse gases (GHG) released to the atmosphere. GHG is merely responsible for global warming and the increase in its amount with increasing use of fossil fuels is the most imminent threat worthy of notice. Reducing the amount of fossil fuel to decrease GHG emission is out of picture considering the elevated increase in

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*Corresponding Author:levent.degirmenci@bilecik.edu.trr

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population. Consequently, utilization of a renewable alternative which could easily be utilized via minor modifications in transport systems is proposed as a rapid solution [6].

Bioethanol is being used as replacement of fuels and/or fuel additives since the invention of internal combustion engine [6]. Renewable biomass such as sugarcane, corn, switchgrass, algae, rice straw, olive oil extraction residues can be used in its production which is the most important advantage of bioethanol over petroleum based fossil fuels [7, 8]. Bioethanol should be recognized as an important alternative based on the amount of production and availability of biomass. Ethanol production when utilized in increasing amounts in fuel had the potential of decreasing CO emissions [6].

Molecular structure of renewable biomass is composed of lignocellulose which is simply a complex carbohydrate containing cellulose, hemicellulose and lignin. The chemical composition of biomass is important as ethanol yield is merely dependent on the ratio of lignin, hemicellulose and cellulose. The process for ethanol production can be divided into three major operations. The first step is delignification which is an optional pretreatment process applied to biomass with high lignin content in the structure. Consecutive hydrolysis and fermentation are then applied respectively to achieve fermentation of reducing sugars [7, 10, 11].

Pretreatment of biomass which usually occurs as delignification is an important step although stated as an optional process. Susceptibility of cellulose to enzymatic hydrolysis is far more enhanced in treated samples and hence the yield of ethanol production is increased [11]. The primary objectives in delignification are removal or reduction of lignin and hemicellulose content. Reducing the amount of cellulose crystallinity and increasing porosity of biomass are also important [10, 11]. Literature survey revealed many studies on various pretreatment technologies [10-17] to achieve all or some of these objectives. Steam explosion can be given as an example to treatment of biomass. Studies indicated that this treatment procedure indeed had decreased the lignin content of the biomass. However, results also showed an increase in cellulose crystallinity which is still one of inhibiting factors in ethanol production [12]. Biological pretreatment applied mainly in the presence of fungus are also gaining importance as they are environmentally friendly procedures [12]. Mechanical pretreatment and thermal treatment procedures are also shown to be effective in decreasing the lignin content of biomass and increasing ethanol efficiency [13].

The efficiency of pretreatment procedure depends on the type and composition of biomass and various procedures were applied to determine the most effective pretreatment procedure. In a study conducted by Eliana et.al. [14] alkaline delignification, dilute acid hydrolysis, steam explosion alkaline peroxide and aqueous ammonia soaking were applied to elephant grass *(Pennisetum purpureum).* Results indicated highest concentrations of reducing sugars in the presence of alkaline pretreatment at 120°C for 1h. This treatment procedure was also shown to achieve highest lignin removal (88%) [14]. Sweet sorghum, another potential biomass source was considered in ethanol production in the work of Zhang et.al. [15]. Biomass was treated with steam explosion, ionic liquid, lime and dilute acid. Results indicated highest cellulose conversion in the presence of biomass treated with steam explosion [15]. Acid and alkali pretreatments are known as the effective pretreatment methods. However, their use are limited due to high energy requirements. Microwave was defined as an alternative pretreatment method with less energy input in the work of Binod et.al. [16]. Microwave treatment combined with acid and alkali treatment procedures were compared for sugarcane bagasse and results showed 90% lignin removal in the presence of combined microwave and alkali treatment [16]. Dilute acid and ionic liquid pretreatment were compared with application on switchgrass. Higher decrease of cellulose crystallinity, higher enhancement in the rate of cellulose hydrolysis was achieved at the expense of higher cost with ionic liquid [17].

Soaking in aqueous ammonia was selected as the treatment procedure in the present study. This method prevents the loss of glucan and xylan which was reported to be minimum for biomass treated with this procedure [18,19]. The aim was to determine the conditions that indicated highest decrease in lignin content and cellulose crystallinity of solid cake obtained during olive oil production. Effect of the parameters, namely NH_3 concentration, time and temperature were altered to give best conditions. An experimental design was initially conducted by using response surface methodology with the aim to decrease the number of applied treatment procedures. Results obtained at the end of treatment procedures were evaluated with statistical analysis.

Olive oil is produced in high amounts especially in Mediterranean countries. The conventional press mill method was recently replaced with two phase extraction in order to enable consumption of lower water during production. The solid cake obtained during production is a valuable residue, rich in organic matter. However, the oil and organic acids present in solid residue are harmful for the environment unless separated from the solid and alternative methods for disposal of this solid cake is currently being investigated. Utilization of solid cake in bioethanol production was considered as a potential alternative and hence it was selected as the biomass source in the present study [20-22].

The use of solid cake as biomass fuel was recently investigated in the work of Zuhal [23]. Results obtained from conventional olive oil fired boilers in Turkey indicated the necessity of suitable combustion systems for efficient energy production. The main problem in utilizing solid cake in energy production is emissions obtained during the process. Carbon monoxide emissions obtained from combustion experiments were reported to vary from 1800-10000 mg/m^3 in the work of Zuhal [23]. Nitrogen and sulphur contents should be monitored continuously in energy production. Polluting gases containing these elements are considered as a serious threat to environment and their amount in the emissions are considered as an indicator for convenience. Nitrogen oxide emissions and Sulphur content obtained from olive oil pellets were stated to vary between 0.69-1.07% and 0.07-0.08%, respectively. Nitrogen oxide emissions for coal was recently reported as 1%, and Sulphur content for coal varied between 2-3%. These results indicated the potential of olive oil pellets to be used as a replacement of coal [24]. However, the need for suitable combustion systems in utilization of solid cake in energy production decreases the popularity of solid cake as combustion fuel [23,24].

Considering the amount of solid cake obtained from olive oil, this by-product should be considered as an important lignocellulosic source for bioethanol production. Hence, sugar production from lignocellulose to lead ethanol production is an important step for its evaluation. Dilute acid pretreatment and consecutive treatments including dilute acid hydrolysis and alkaline treatment indicated successful results in the works of Asli et. al [25] and Fernandes et. al [26]. This study was conducted to offer a significant contribution and a more efficient alternative to these pretreatments with single utilization of NH_3 instead of H_2SO_4 and NaOH [25] and between 30-70˚C which was lower than 160˚C as applied in the work of Asli et.al [25].

2. Experimental

2.1. The Procedure and Experimental Design of Parameters in Soaking Ammonia Treatment

Lignin analyses of bare solid cake and NH_3 treated samples were conducted by 2.5g samples which were grinded to 0.5mm. A consecutive extraction procedure initially by hot water at 95°C for 3 hours and then by acetone was applied to samples prior to analyzes. Samples obtained after drying and homogenization were analyzed to determine their lignin contents according to a modified version of TAPPI T222 method [27]. Samples were hydrolyzed with 0.313N NaOH for cellulose analyses. Samples (3g) collected after washing step with deionized water and H_2SO_4 acid (0.255N) were burned in an oven at 550°C and weighed to detect the difference for cellulose determination (TS324) [28]. Extractive content of the samples were determined by extraction with ethanol-toluene mixture. Samples, filtered after extraction and washed with ethanol, were further extracted with ethanol. Extractive content were calculated by difference of the samples obtained after consecutive washing and drying steps (ASTM D-1105-96). Hemicellulose content was calculated from difference. Cellulose, hemicellulose, lignin and extractives of solid cake utilized in experiments was determined as 3.6, 11.5, 47, and 38%, respectively. Solid cake were dried prior to treatment in aqueous ammonia which was conducted in a shaken batch system. The system was operated in lowest value of mixing and the volume/weight ratio of ammonia to solid cake was determined as 10/1 in the course of experiments. The samples were immersed in ice at the end of treatment to terminate ongoing reaction.

Response surface methodology is a versatile statistical approach utilized in the modelling and optimization of biological and chemical processes. The aim of the present study was to investigate thoroughly cellulose crystallinity and lignin content by applying NH_3 pretreatment to find the optimum conditions i.e. temperature, soaking time and concentration. A similar study was recently carried out by Kang et.al. [29]. In that study, the authors employed the central composite design of response surface methodology and optimized pretreatment conditions for rapeseed straw which was treated by soaking in aqueous ammonia. Response surface methodology was applied in a similar fashion to determine the effects of experimental parameters on the response (crystallinity Y_C and lignin content Y_L).

Response surface methodology was applied to determine the order and the number of runs that would be conducted on pretreatment procedure. Three intervals for three parameters were initially selected for application and in the case of applying a conventional experimental design, a total of 27 experiments were required to obtain results. The number of experiments were decreased to 20 with this methodology and repeated runs in identical conditions were also included to this number. The parameters applied in treatment were illustrated in Table 1 and the order of the experiments were given in Table 2.

Although considered as an important step, pretreatment procedures increase the economic burden when utilized in accordance with hydrolysis and fermentation steps in bioethanol production. Hence in order to maintain an increase in efficiency without increasing operational costs, the procedure itself should be conducted in the mildest conditions possible. The parameters stated in Table 1., represents these conditions with a maximum temperature of 70ºC and NH_3 concentration of 25%. Based on the success of this applied procedure, a more efficient alternative will be introduced to researchers which was the fundamental aim of this study.

Table 1. Parameters utilized in treatment of solid cake with aqueous ammonia

Codes	**Temperature (°C)**	**Time (h)**	**NH_3 (%) w/w**
-1	30	6	5
0	50	9	10
1	70	12	25

2.2. Determination of Lignin Content and Cellulose Crystallinity

Lignin content was determined according to [27]. X- ray diffraction (XRD) patterns were obtained by a Panalytical Empyrean instrument at 200 kV, 50 mA and in the range of 2ϴ value between 5° and 80° (10° min-1). Results were interpreted to obtain cellulose crystallinity according to literature [28].
Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FT-IR) studies were performed in a Cary 630 Fourier transform infrared spectrometer equipped with a single reflection diamond attenuated total reflectance (ATR) accessory. TGA analyses of selected samples were conducted in Hitachi Exstar SII TG/DTA 7300 device under argon atmosphere and 10°C/min heating rate.

Table 2. Experimental design of samples utilized in treatment

Code Name	**Temperature (°C)**	**Time (h)**	**NH_3 (%) w/w**
A	1	-1	1
B	-1	-1	1
C	0	0	-1
D	0	0	0
E	1	0	0
F	1	-1	-1
G	0	-1	0
H	-1	1	1
I	0	1	0
J	0	0	1
K	0	0	0
L	-1	-1	-1
M	-1	0	0
N	-1	1	-1
O	0	0	0
P	0	0	0
R	1	1	-1
S	0	0	0
T	1	1	1
U	0	0	0

2.3. Statistical Analysis

Samples were soaked in aqueous ammonia and treatments were conducted in conditions stated above (Table 2). Samples were then recovered, dried and stored in refrigerator at +4°C prior to FT-IR and XRD analyses. Results were obtained in terms of lignin content and cellulose crystallinity index and they were separately analyzed by utilization of multivariable non-linear regression models. These models were tested to determine dependency of lignin % and cellulose crystallinity on NH_3 concentration, time and temperature. Statistical analyses were conducted by one-way variance analysis (ANOVA) via MATLAB 2013R software. $p<0.05$ were determined as the threshold of parameter significance.

Second order interaction coefficients were produced by multiple regression analysis. The evaluation of the fit of the model proposed in this study was assessed by determination of the model coefficients and analysis of variance. The second order quadratic response surface model was fitted to the below equation.

$$Y_C \; and \; Y_L = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ij} X_i^2 + \sum_{i,i>j}^{k} \sum_{j}^{k} \beta_{ij}\, \beta_{ij} X_i X_j$$

In which Y is the responses (crystallinity Y_C and lignin content Y_L, respectively) i and j are the linear and quadratic coefficients, β is the regression coefficient and k is the number of factors investigated and optimized in the aqueous ammonia pretreatment experiments [30].

3. RESULTS AND DISCUSSION

Lignin content and cellulose crystallinity of the samples, determined according to literature [27,28], were given in Supplementary file. Initially, variance analysis applied to lignin content of the samples will be evaluated. The response equation obtained with variance analysis for lignin content was:

$$\begin{aligned} Y = 21.2969 - 1.06709A + 0.726674B - 1.33959C - 0.499539A^2 - 0.493423B^2 \\ - 0.727507C^2 + 0.505672AB - 1.78111AC + 0.725511BC \end{aligned}$$

The terms A, B, C corresponded to temperature, time and NH_3 concentration. The R square and adjusted square values for the equation was obtained as 0.9131 and 0.8349, respectively. The *p* values of A, B, C, AC and BC were smaller than 0.05 showing that lignin content was dependent on these parameters (Table 3).

Table 3. Analysis of variance for lignin content determined according to [27]

Source	DF	Sum of squares	Mean square	F	*p value*
Model	9	77.172	8.5747	11.67	0.000
A	1	11.387	11.3869	15.50	0.003
B	1	5.281	5.2806	7.19	0.023
C	1	17.945	17.945	24.43	0.001
A*A	1	7.590	0.6862	0.93	0.357
B*B	1	1.879	0.6695	0.91	0.362
C*C	1	1.455	1.4555	1.98	0.190
A*B	1	2.046	2.0456	2.78	0.126
A*C	1	25.379	25.3787	34.55	0.000
B*C	1	4.211	4.2109	5.73	0.038
Residual	10	7.346	0.7346		
Lack of fit	5	6.150	1.2301	5.14	0.048
Pure error	5	1.195	0.2391		
Total	19	84.518			

Contour plots indicating the effect of parameters on lignin content and three-dimensional response surface plot indicating the correlation between the parameters and lignin content were given in Figure 1 and 2. Lignin content of solid cake prior to experiments was determined as 47%. Results obtained after treatment procedures showed that lignin content of biomass due to applied experimental conditions remained mostly in the range of 20-22% implying a certain independence from treatment conditions (Figure 1). As previously stated, delignification of biomass is required in bioethanol production to enhance enzyme digestibility. The interval of determined lignin amounts was close implying that lowest values of temperature, time and NH_3 concentration would be sufficient for effective delignification. Similar observation could be deducted from response plots (Figure 2.).

Although the pretreatment conditions applied in this study was stated to be independent of applied parameters, they should still be considered as effective in terms of lignin removal. Lignin removal values close to 50% was achieved by NH_3 pretreatment. The decrease value obtained with this procedure was in the similar range obtained for alkaline pretreatment (60 %) and wet oxidation treatment procedures with delignifation values of 50-70% depending on the type of biomass [31]. The main advantage of this procedure was its economic feasibility compared to other techniques. Economic evaluation of this procedure with dilute acid and lime pretreatment techniques indicated lower costs in terms of chemical expenses [32].

In the case of solid cake obtained during olive oil production delignification is relatively easy and mildest conditions can be applied as seen from figures. However, lignin content is not the only factor effecting bioethanol efficiency. Other factors such as crystallinity of cellulose must be taken into consideration as the lower values of cellulose crystallinity obtained at the end of pretreatment enable production of higher bioethanol amount.

The response equation obtained with the variance analysis of cellulose crystallinity was given below:

$$Y = 49.0234 - 1.16500A - 1.63700B - 1.17600 + 0.159091A^2 - 7.86091B^2 + 1.16409C^2 + 0.161250AB - 0.473750AC + 0.0912500BC$$

With A, B, C corresponded to temperature, time and NH_3 concentration. R square and adjusted square values for the equation was obtained as 0.9551 and 0.9147. The $p<0.05$ values obtained for A, B, C, AA and BB indicated the dependency of cellulose crystallinity on these parameters (Table 4).

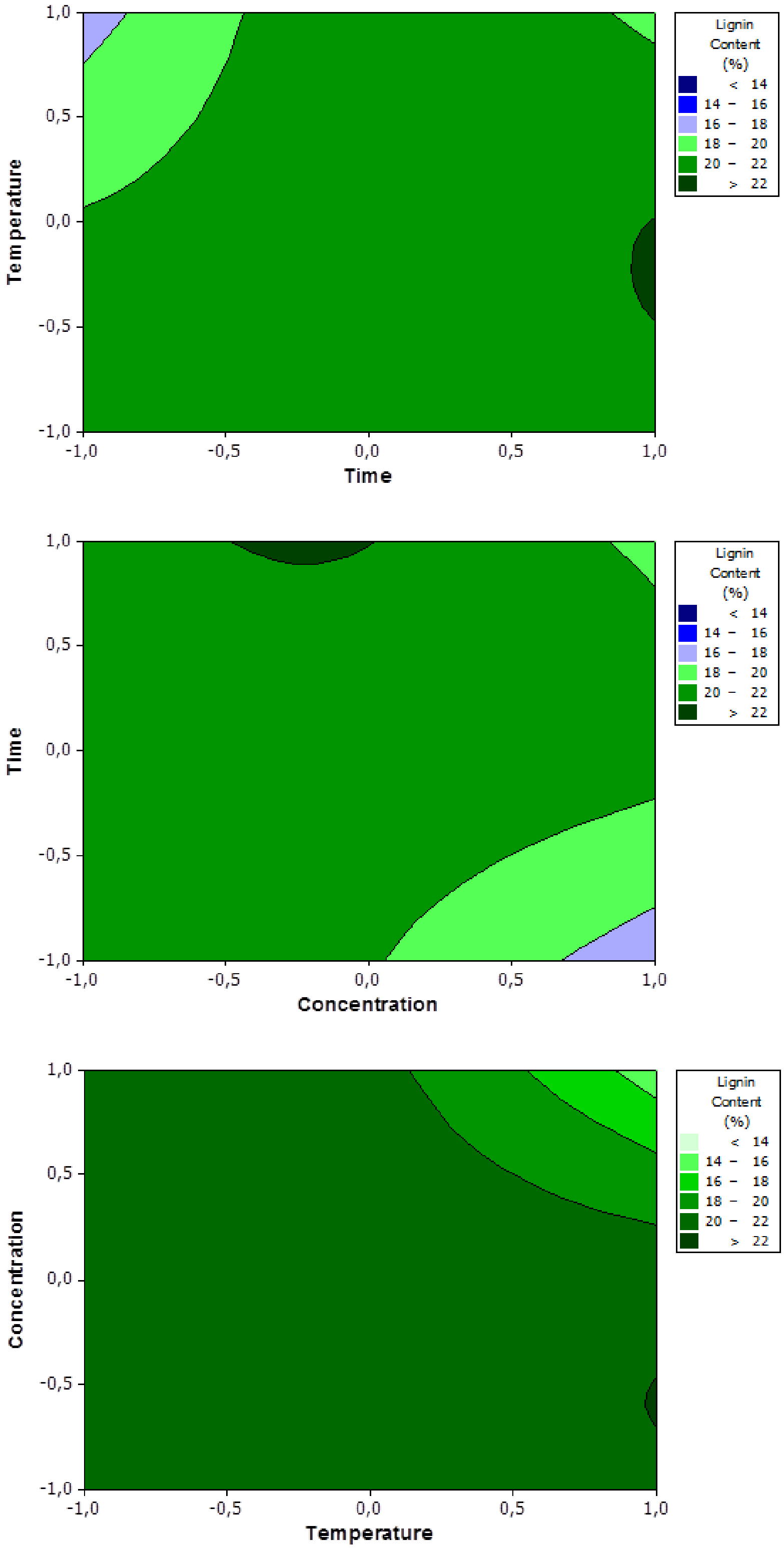
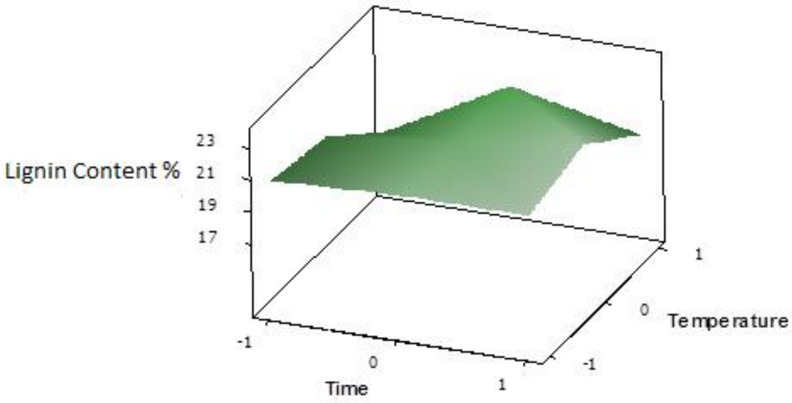
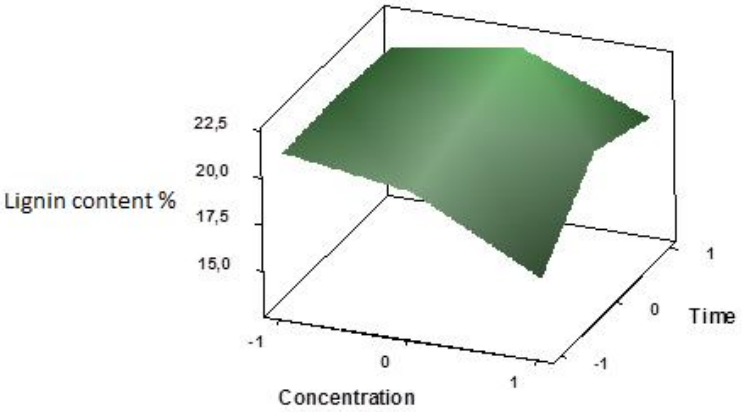


Figure. 1. Contour plots indicating the effect of investigated parameters on lignin content





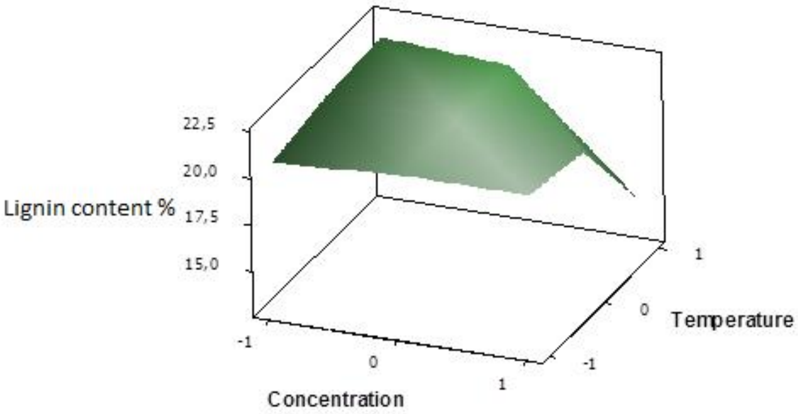


Figure 2. Response surface plots indicating the effect of parameters on lignin content

Table 4. Analysis of variance for cellulose crystallinity calculated by interpretation of XRD analyses [28].

Source	**DF**	**Sum of squares**	**Mean square**	**F**	***p value***
Model	9	310.844	34.538	23.65	0.000
A	1	13.572	13.572	9.29	0.012
B	1	26.798	26.798	18.35	0.002
C	1	13.380	13.830	9.47	0.012
A*A	1	74.459	0.070	0.05	0.832
B*B	1	176.388	169.933	116.34	0.000
C*C	1	3.727	3.727	2.55	0.141
A*B	1	0.208	0.208	0.14	0.714
A*C	1	1.796	1.796	1.23	0.294
B*C	1	0.067	0.067	0.05	0.835
Residual	10	14.606	1.461		
Lack of fit	5	3.874	0.775	0.36	0.856
Pure error	5	10.733	2.147		
Total	19	325.450			

The changes in crystalline content due to applied experimental conditions were given in Figure 3. Initial interpretation of the figure indicated that crystalline cellulose content of the biomass had mostly remained between 48-50% independent of applied procedure. At first sight the results implied that any treatment parameter utilized for decreasing cellulose crystallinity would result in similar % ranges. However, two separate regions in which crystallinity had varied between 46-48% were also determined from Figure 3 a and b. These regions illustrated dependence of crystallinity on time and a decrease was observed independent of temperature and concentration at higher time values. In other words, obtaining cellulose crystallinity at similar % ranges was not the case and the distinction could also be seen from the response surface plots in Figure 4. Response surface plots given for concentration-time and temperature-time vs cellulose crystallinity indicated two separate regions with lowest crystallinity. Results implied the presence of different contents of the biomass in these regions. In the first region, with lower time values (between 6 and 9h) crystallinity was low in any concentration and temperature. It was thought that decomposition of hemicellulose had occurred simultaneously with delignification in this region. Early stages of hemicellulose degradation were previously reported to influence the content of biomass [33, 34]. Moreover, hemicellulose is known as the least stable constituent of biomass which is likely to decompose in relatively shorter times during soaking on aqueous ammonia [35].

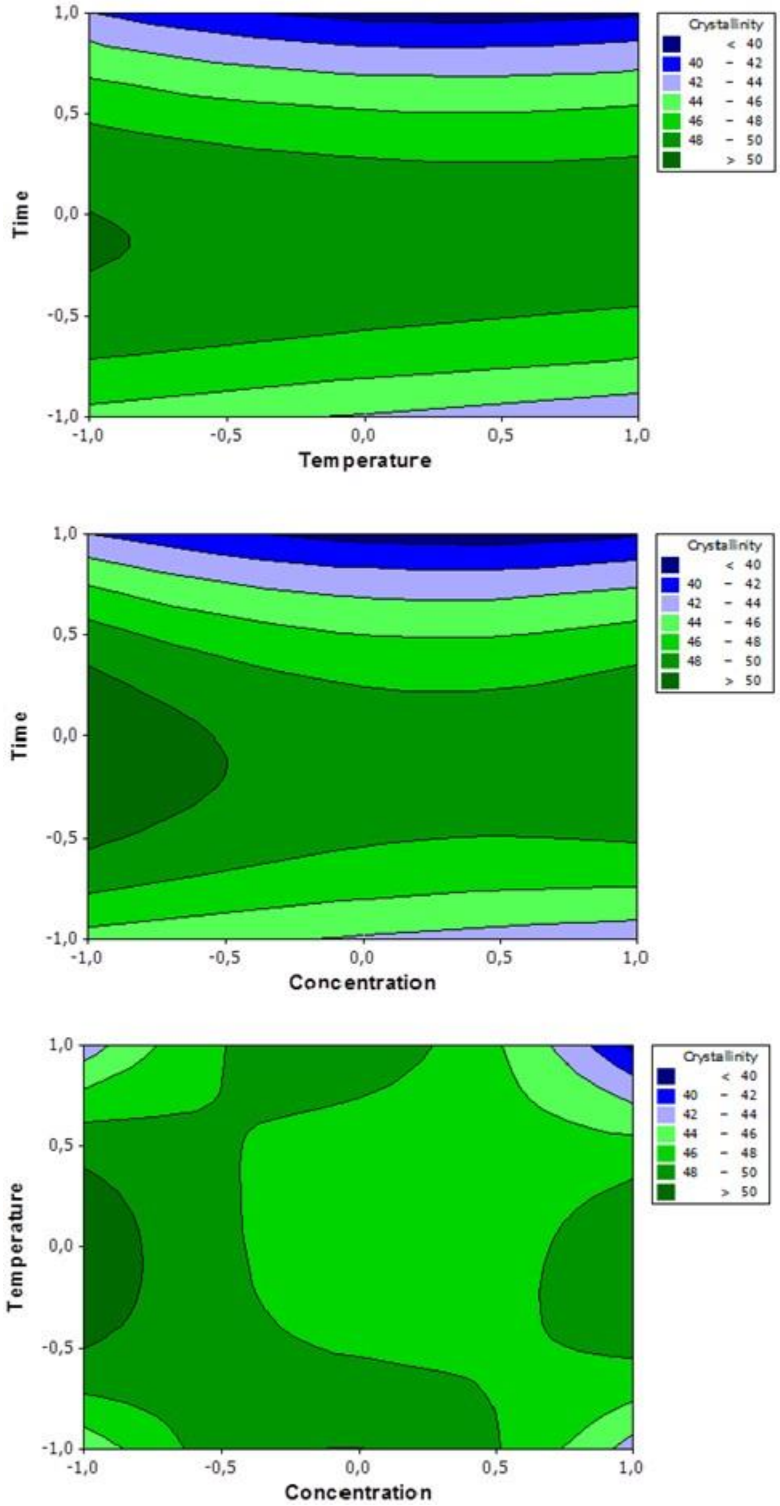


Figure 3. Contour plots indicating the effect of investigated parameters on cellulose crystallinity.

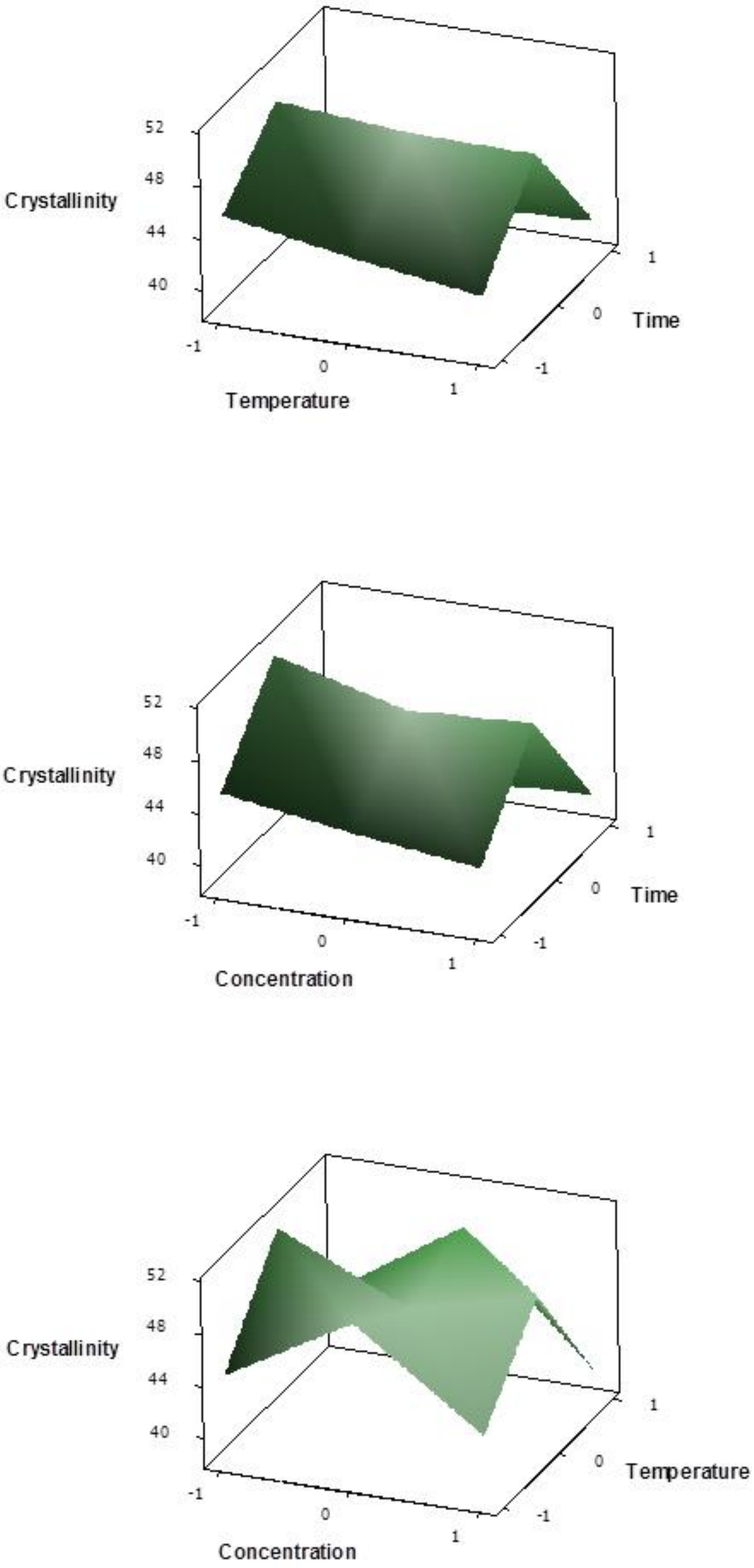


Figure 4. Response surface plots indicating the effect of parameters on cellulose crystallinity

Regression coefficient (R^2) and P-value of lack of fit are two standards of measurements to determine how fit a regression model is. The higher R^2 and P-value of lack of fit > 0.05 indicated that the model was adequate for use. In the present study the high R^2 values of 0.9131 and 0.9551 for lignin and crystallinity, along with P-values of lack of fit for crystallinity (0.048) close to 0.05 and lignin content (0.856) greater than 0.05 implied unnecessity of higher models such as cubic spline regression.

The simplest way to illustrate the changes in the structure of biomass is to interpret FT-IR results (Figure 5). Hence, FT-IR results of samples treated at 6, 9 and 12h at same temperature and concentration were compared in order to show the effect of treatment time on hemicellulose decomposition. Spectra were given between 4000-2500 and 2500-500 cm^{-1} wavenumbers for better observation of the changes in peak intensities. An increase in the intensity of peak values at 1002, 1315 and 1635 cm^{-1} was observed as seen from the figure. These peak values corresponded to C-C, C-OH vibrations for 1002 cm^{-1} , C-H and C-OH stretching for 1315 cm^{-1} and structural vibrations of aromatic C=C and/or C=O stretching of COO^- for 1635 cm^{-1} [35-37]. The increase in the intensity of these peaks, in our opinion, indicated an ongoing hemicellulose decomposition during time.

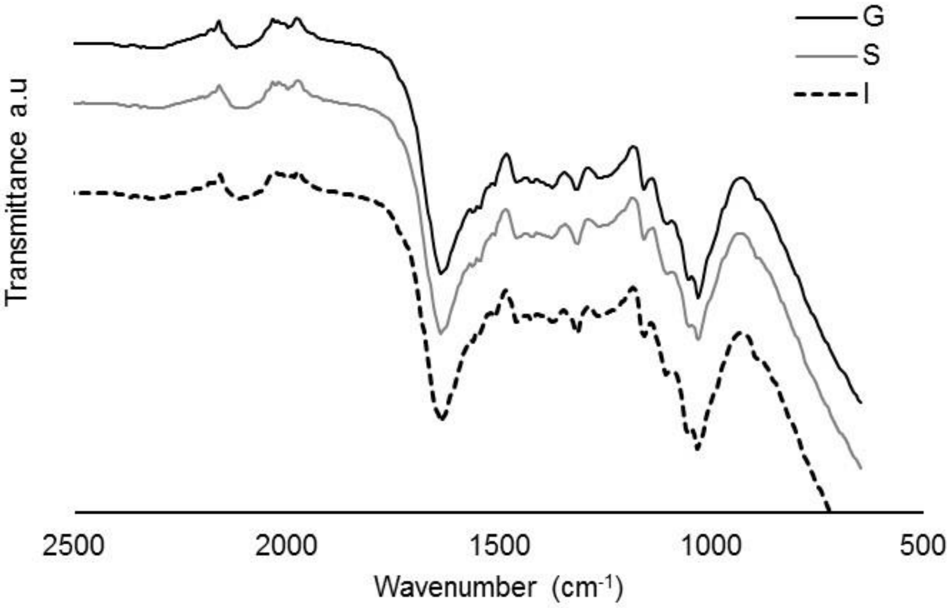


Figure 5. FT-IR spectra of samples between 2500-500 cm-1 wavenumber interval

The decrease in cellulose crystallinity during lower times was due to low % of cellulose in biomass structure. The high value of crystallinity at 9h implied that most of the hemicellulose in the structure be decomposed and cellulose % in biomass was highest at this time. As the time increased cellulose crystallinity had decreased with time (Figure 4). The reason of the decrease in cellulose crystallinity might be due to accelerated cellulose decomposition in biomass structure or simply deterioration of crystal structure. The respective increasing and decreasing trend of cellulose crystallinity could also be observed from XRD data of G, O and I samples in Figure 6. These samples were especially selected to demonstrate the effect of time on crystallinity. The 101, 002, 021 and 040 planes indicated the presence of Cellulose I structure. The increase in the intensity of 002 and 021 planes observed for O samples indicated the increase of crystallinity due to hemicellulose decomposition. Further increase in time of treatment resulted in the decrease of intensities showing a decrease of cellulose crystallinity (I sample) (Figure 6) [38].

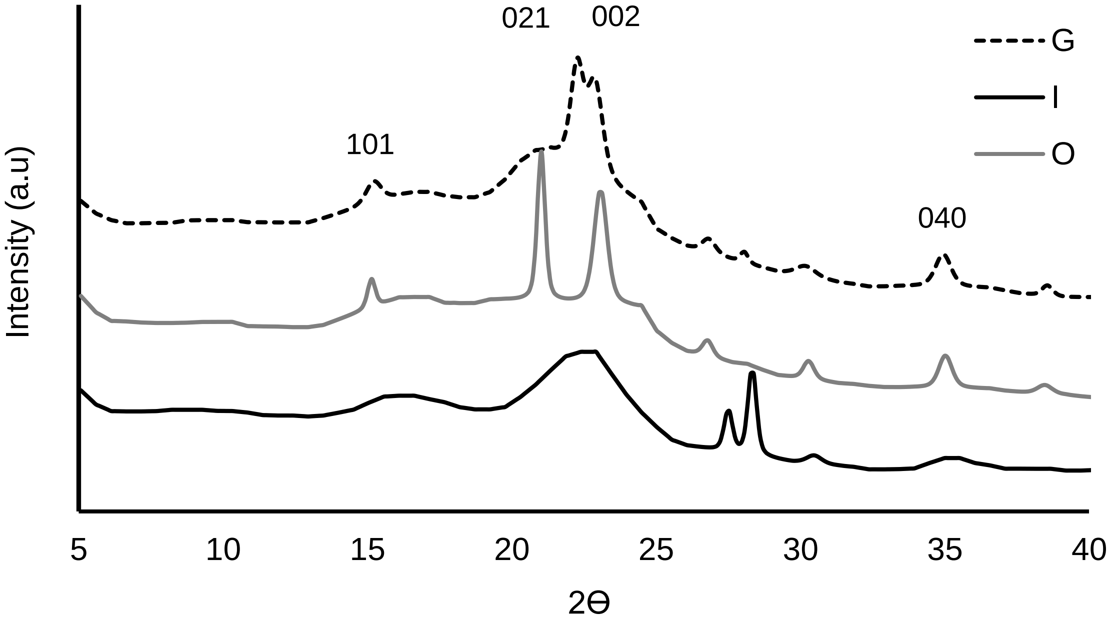


Figure 6. XRD analyses of selected samples

Consequently, results showed that time had been the most important parameter in controlling the ingredients of organic structure. Low treatment time resulted in higher amount of hemicellulose and as time increases the amount of cellulose would increase due to completion of hemicellulose degradation. However, further increase in time would eventually result in cellulose degradation or change in its crystal structure. Interpretation of statistical analysis and FT-IR results was not adequate to precisely determine whether a change in crystal structure or decomposition occurred during treatment. Hence some further validation of the results would be preferable to ensure our conclusion. This was simply achieved by conducting TGA analysis on the samples obtained at same temperature and NH_3 concentration with varying times (samples G, S and I). Besides the region of water removal (30-130°C), a sharp decay was observed between 230-370°C and smoother loss of mass was detected starting from 370°C until completion (700°C). These regions indicated consecutive decomposition of hemicellulose and cellulose in the structure [35] (Figure 7). Comparison of samples revealed higher amounts of decomposition in hemicellulose structure for “I” coded sample which was obtained at the end of highest treatment time. The amount of cellulose loss was similar for S and I coded samples and lower for G coded sample obtained at lowest treatment time. In other words increasing time in treatment resulted in the increase of both hemicellulose and cellulose in the structure and it was our belief that treatment for 12 hour would only change crystalline structure of cellulose and cellulose decomposition, if any, would be negligible. We can support our conclusion with similar results obtained in literature. In the work of Kljun et. al [37], effect of alkaline treatment in crystalline structure of cellulose was investigated and changes in its crystal structure was stated to be due to cleavage of inter molecular hydrogen bonds which might also be valid for NH_3 treatment [39].

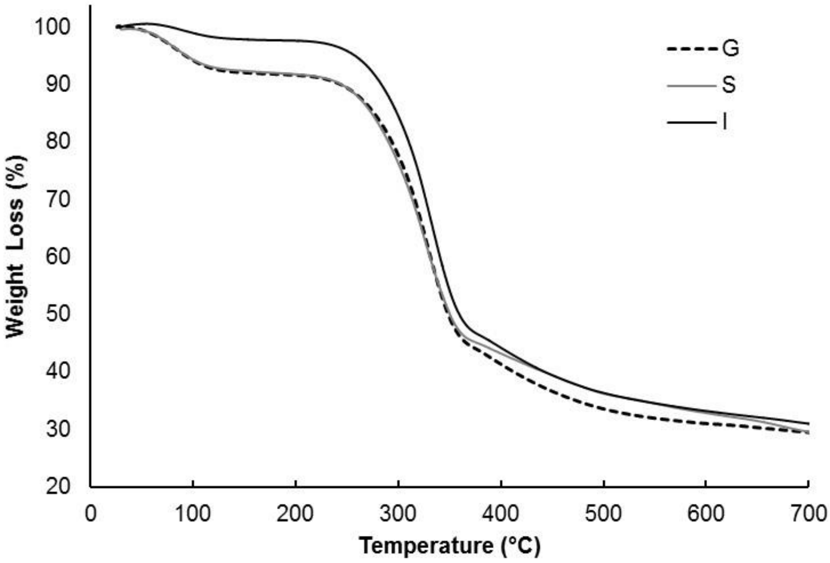


Figure 7. TGA analyses of G, S and I samples

Based on the results, it is our understanding that lowest degree of cellulose crystallinity and lignin content could only be obtained with treatment at higher times. Treatment for higher times would enable consecutive decomposition of lignin, hemicellulose and cellulose. Concomitant decomposition of these structures also appeared which was shown to be due to applied treatment time and employing higher times in treatment was crucial to ensure hemicellulose decomposition and following deterioration of cellulose crystals. The temperature and NH3 concentration utilized in treatment was not as effective as treatment time. As previously stated, TGA analyses indicated a change in crystal structure of cellulose rather than decomposition. This result was also validated by simply determining the loss of glucan from biomass structure which would decrease bioethanol efficiency in fermentation. In order to determine whether applied treatment time had negative impact on glucan content, FT-IR spectra of samples obtained at highest time (samples H, N, R) were compared with the sample obtained after treatment at lowest time (sample L) (Figure 8).

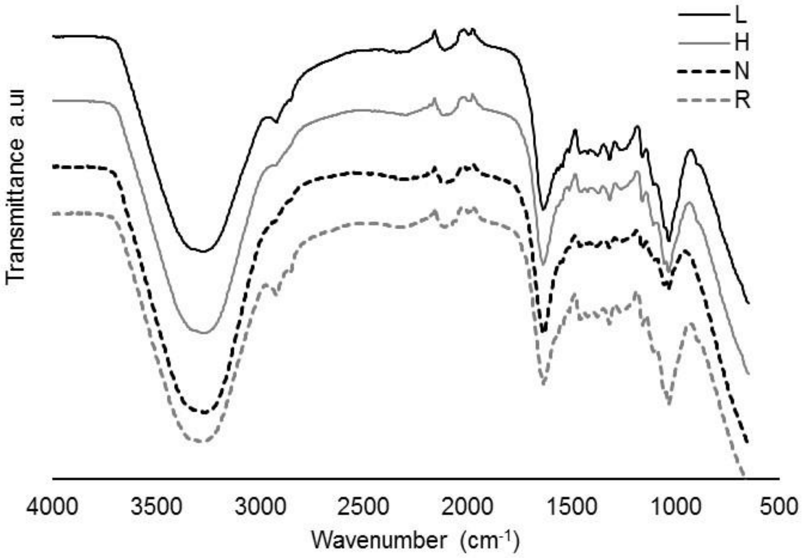


Figure 8. FT-IR spectra of samples obtained at highest (H,N,R) and lowest (L) times of treatment

Besides O-H stretch obtained between 3000-3500 cm^{-1}, peak values at 1636, 1316,1159 and 1010 cm^{-1} corresponding to intra molecular hydrogen bonds, C-H stretch, bridge O stretch and C-O stretch still existed indicating the existence of β-glucan despite high treatment times [40, 41]. This result interpreted with TGA analyses showed that higher treatment times had resulted mostly in deterioration of cellulose crystallinity.

4. CONCLUSION

Solid cake obtained during olive oil production was treated in aqueous ammonia to enhance the efficiency of enzymatic hydrolysis in bioethanol production. Effect of treatment conditions on lignin content and cellulose crystallinity were investigated with varying temperature, time and NH3 concentration. Lignin content and cellulose crystallinity were determined according to literature and variance analysis were conducted on results to determine the effect of treatment conditions on obtained values. Results indicated that mildest conditions would have been sufficient for lignin degradation. However, longer time periods was required to achieve complete decomposition of hemicellulose and reduce cellulose crystallinity. It was concluded that the effect of temperature and NH3 concentration on cellulose crystallinity could have been neglected.

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