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# Bioethanol production from common reed (*Phragmites australis*): Biological and chemical pretreatments

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## Abstract

*The present study was aimed at examining the feasibility of using common reed for the production of bioethanol by means of biological and chemical pretreatments. The effectiveness of sodium hydroxide (NaOH), hydrogen peroxide ($H_2O_2$), sodium borohydrate ($NaBH_4$), boron oxide ($B_2O_3$) and sulfuric acid ($H_2SO_4$) for conversion of common reed to ethanol was investigated via chemical pretreatments. Fungal pretreatment (C. subvermispora) degraded the reed structure and 10 weeks of incubation time removed 11.3 and 14.0% of glucan and xylan from the structure, respectively. Statistical analysis showed that fungal pretreatment had a significant effect ($p < 0.001$) on lignin removal. The lignin loss of common reed was 9.16, 10.7 and 16.6% in 2, 4 and 8 weeks, respectively. Chemical pretreatments of NaOH, $H_2O_2$, $NaBH_4$, $B_2O_3$, $H_2SO_4$ dissolved 7.66, 12.2, 4.50, 15.7 and 15.0% of glucan from the structure. The highest xylan dissolution (63.7%) was observed when common reed was pretreated with $H_2SO_4$. In addition, NaOH and $NaBH_4$ pretreatments removed 55.8 and 52.0% of lignin, respectively.*

**Keywords:** Common reed, fungal pretreatment, chemical pretreatment.

## 1. Introduction

Cellulose hydrolysis for the production of cellulosic ethanol is a complicated process employing a number of pretreatment techniques designed to improve the efficiency of enzymatic saccharification and thus, bioethanol production from lignocellulosic materials. These pretreatment methods include physical, physico-chemical, chemical and biological processes (Y. SUN [1]). Chemical methods were first developed and widely used in the paper industry for the production of high-quality paper products via delignification of cellulosic materials (L.T. FAN & al. [2]). The conventional pretreatment chemical utilized in this context is usually NaOH, as well as other chemicals including $H_2O_2$, $H_2SO_4$, $NaBH_4$, etc. (Y. COPUR & al. [3,4,5]). For biological pretreatments, lignin degradation is achieved by inoculation with natural wood-attacking microorganisms (fungi) which are allowed to grow on the biomass. During lignin hydrolysis, the xylan and mannan components of the hemicellulose are lost in significant amounts (L.T. FAN & al. [2]). The presence of a carbohydrate such as cellulose or hemicellulose is a necessary component for the process, because white rot degradation of lignin is oxidative. The most widely used organism for delignification is the white-rot fungus *Phanerochaete chrysosporium* (K.C. WILLIAMS [6]; Y. SUN & J. CHENG [7]). Biological pretreatments require little energy input and are environmentally friendly. Despite these advantages, the economic feasibility of a non-optimized biological pretreatment process is still poor because of the extended cultivation

time of 10 to 14 days. Therefore, biological treatments are sometimes used in conjunction with chemical treatments (C.N. HAMELINCK & al. [8]).

The idea that the selective delignification of fungal pretreatment may improve the process yield and may result in better enzymatic digestibility in bioethanol production was the basis for this study. For this reason, the white-rot fungi *C. subvermispora* FP-90031-sp was utilized in the biological pretreatment step. To compare the obtained results, the chemicals NaOH, $H_2O_2$, $NaBH_4$, $B_2O_3$ and $H_2SO_4$ were also investigated in the chemical pretreatment step.

## 2. Materials and Methods

### *2.1. Materials*

For the present work, common reed (*Phragmites australis*) from Yeniçağa Lake (Bolu Province, Black Sea region of Turkey) was used. A garden chopper was used to cut the reeds to a suitable size (3-5 cm). The chopped material was then air dried at room temperature and the moisture content was determined (Tappi T 412 om-06). The material was sealed in plastic bags and stored at 5 °C. The Center for Forest Mycology Research at the USDA Forest Products Laboratory, Madison, Wisconsin, provided the *C. subvermispora* FP-90031-sp used in the study.

### *2.2. Methods*

#### *2.2.1. Pretreatments*

*Fungal pretreatment*: The inoculum preparation, raw material sterilization, and inoculation were conducted as previously explained by P. BAJPAI & al. [9]. The common reed samples were incubated at 27 °C and 75% relative humidity, and then removed at intervals of 2, 4, 6, 8, and 10 weeks for analyses. In addition, untreated control samples were subjected to identical preparations. The optimum treatment condition was defined regarding the specificity (% lignin loss / % carbohydrate loss) value. The optimum sample was washed with warm water to remove hyphae, air dried, sealed in plastic bags and held at 4 °C.

*Chemical pretreatments*: For this step, 1% (w/v) concentrations of NaOH, $H_2O_2$, $NaBH_4$, $B_2O_3$ and $H_2SO_4$ were used to treat each of the 40 g (o.d.) samples. The treatments were made at 10% (w/v) solid loading at 121 °C (15 psi) for 60 min residence time. After pretreatments, the liquid portions were filtrated and the solid portions were held at 4 °C in sealed plastic bags. Each of the solid samples was assessed for treatment yield, acid soluble/insoluble lignin and sugar contents.

#### *2.2.2. Analytical methods*

The weight loss in the samples due to the fungal and chemical pretreatments was determined by gravimetric measurements (TappiT 210 cm-03). Appropriate analytical tests including hot and cold water (Tappi T 207 om- 88), 1% NaOH solubility (Tappi T 212 om-88) and extractives (Tappi T 204 om-88), ash (Tappi T 211 om-85), α-cellulose (J.S. HAN & J.S. ROWELL [10]) and holocellulose (L.E. WISE [11]) content were used to obtain the chemical composition of the samples. The sugar and lignin contents of the samples were determined using the Laboratory Analytical Procedures (LAP) guidelines from the NREL (A. SLUITER & al. [12]). High performance liquid chromatography (HPLC) (Agilent 1200 system) equipped with a Shodex SH1011 column (mobile phase: 5 mM $H_2SO_4$, flow rate: 0.5 ml/min, column temperature: 60 °C) and a refractive index detector was utilized to determine the sugar contents. The acid-insoluble and soluble lignin was determined by weighing and the adsorption at 320 nm against deionized water blank, respectively. All investigations were carried out in triplicate. The percentage of weight loss and glucan, xylan and lignin

reductions were calculated as explained earlier by Y. COPUR & al. [3,4,5]. In addition, the increase in 1% NaOH solubility was calculated by Y. COPUR & A. TOZLUOGLU [13].

#### *2.2.3. Statistical analysis*

The SPSS 16.0 program was utilized for statistical analysis of the obtained data. The one-way ANOVA was used for identification of significant differences in the effects of pretreatments on the chemical composition of common reed. The Duncan test was employed for detection of significant differences between the groups.

## 3. Results and discussion

### *3.1. Chemical composition of common reed*

Certain chemical properties of common reed are listed in Table 1. The sugar content of common reed was seen to be similar to that reported by other researchers (J.Y. JUNG & al. [14]). The HPLC results revealed that the sugar fraction of the common reed was 51.8% of the dry biomass. Glucan, the major component of the cell wall, made up 33.6%, xylan, the major hemicellulose constituent, 16.5% and arabinan and galactan, only 1.45 and 0.21%, respectively, of the biomass. No mannan was detected in this study. Because of the high sugar content of common reed, it was shown to be a suitable lignocellulosic substrate for the production of ethanol. The lignin (acid insoluble+soluble) content observed in this study (26.2%) was found to be slightly higher compared to that reported in the literature for common reed (J.Y. JUNG & al. [14]).

Table 1. Chemical composition of common reed (*Phragmitesaustralis*), wheat straw (Y. Copur & al. [3]), tobacco stalk (O. AKPINAR & al. [15]), sunflower stalk (P. VAITHANOMSAT & al. [16]) and hard/softwoods (D. FENGEL & G.WEGENER [17]).

| Composition, %[a] | | Common reed[b] | Common reed[c] | Wheat straw | Tobacco stalk | Sunflower stalks | Hardwoods | Softwoods |
|---|---|---|---|---|---|---|---|---|
| Extractives [d] | | 7.25±0.18[e] | 5.80 | 3.66 | - | 9.03 | 2-6 | 2-8 |
| Cellulose as glucan | | 33.6±0.28 | 37.3 | 36.6 | 33.0 | 27.1 | - | - |
| α-cellulose | | 37.2±1.13 | - | - | - | 45.7 | 38-50 | 29-47 |
| Hemicellulose[f] as | | 18.2 | 16.4 | 23.3 | 21.8 | - | - | - |
| | Xylan | 16.5±1.34 | 14.0 | 19.7 | 21.0 | 7.69 | - | - |
| | Galactan | 0.21±0.08 | 0.5 | - | - | - | - | - |
| | Arabinan | 1.45±0.56 | 1.9 | 3.63 | 0.77 | - | - | - |
| | Mannan | nd[g] | - | - | - | - | - | - |
| Holocellulose | | 58.5±0.26 | - | - | - | - | 70-78 | 63-70 |
| Lignin | | 26.2 | 24.8 | 35.2 | 24.5 | 21.9 | 30-35 | 25-35 |
| | Acid insoluble lignin | 22.9±0.57 | 20.7 | 34.0 | 23.0 | - | - | - |
| | Acid soluble lignin | 3.34±0.31 | 4.10 | 1.22 | 1.50 | - | - | - |
| Ash | | 2.99±0.01 | 3.90 | 10.1 | 6.40 | 10.7 | 0.35 | 0.35 |
| 1% NaOH solubility | | 37.8±0.55 | - | 45.5 | - | - | 14-20 | 9-16 |
| Hot water solubility | | 14.4±0.56 | - | 13.0 | - | - | 2-7 | 3-6 |
| Cold water solubility | | 7.54±0.15 | - | 9.30 | - | - | 4-6 | 2-3 |

[a] Composition percentages are on a dry-weight basis, [b] Chemical composition of common reed (*Phragmitesaustralis*) (current), [c] Chemical composition of common reed (*Phragmitesaustralis*) (J.Y. JUNG & al. [14]), [d] Alcohol-benzene solubility, [e]Mean values of triplicate samples with standard deviations, [f]Hemicellulose: xylose+galactose+arabinose+mannose, [g] nd: Not detected

### *3.2. Pretreatments*

#### *3.2.1. Effect of fungal pretreatment with C. subvermispora on the chemical composition of common reed*

The weight and component losses due to the fungal pretreatment of common reed by *C. subvermispora* for periods of 2-10 weeks are shown in Table 2. There was 0.60% mass loss after two weeks of fungal pretreatment and 5.40% after 10 weeks. The additional mass loss from 2 to 10 weeks was statistically significant based on the confidence intervals of the

data ($p$<0.001). These results were comparable to those observed for loblolly pine pretreated with *C. subvermispora*, i.e., 2 and 9% after 2 and 8 weeks of pretreatment, respectively (M. AKHTAR & al. [18]). The weight loss may be predominantly ascribed to the degrading of the nutrient-rich ray cells (L.L. VILLALBA [19]).

The carbohydrate content (glucan and xylan) as a function of fungal pretreatment time is given in Table 2. Statistical analysis showed that fungal pretreatment had a significant effect ($p$< 0.01) on glucan, but none ($p$> 0.05) on xylan content. The glucan and xylan losses of the common reed at the end of 10 weeks of incubation time were 11.3 and 14.0%, respectively. A comparison of the lignin and carbohydrate data for the degradation of common reed by *C. subvermispora* revealed that at two weeks, there was a 0.22% loss of lignin, and during the same period, a 0.60% loss of glucan also occurred. After 2 weeks of incubation time, the lignin losses surpassed the carbohydrate losses. As a result, it can be surmised that, at the beginning of a fungal attack, some cellulose-degrading enzymes are capable of causing carbohydrate degradation. At longer times (10 weeks), the lignin was degraded to a greater extent than the carbohydrates. In general, the specificity (% lignin loss / % carbohydrate loss) of decay caused by an organism appears to increase to a maximum value of between 20 and 40% weight loss and then decline (E.C. SETLIFF & al. [20]; L.L. VILLALBA [19]). The higher selectivity at lower weight losses suggests a greater accessibility of the initially removed lignin. The specificity value of 0.18 for the common reed incubated for 2 weeks indicated that there was no preferential lignin degradation. At 4 weeks, the specificity was 1.31, showing that at later stages of decay with *C. subvermispora*, lignin was removed preferentially over the carbohydrates. This ratio increased from 0.18 to 1.34 for extended incubation periods, but there was no significant difference between 4 weeks and 10 weeks of incubation time. Thus, the samples pretreated with *C. subvermispora* for a 4-week incubation time were selected as optimum samples for further analysis. These data concur with an earlier study on biodegradation of loblolly pine by L.L. VILLALBA [19].

Table 2. Chemical composition of common reed treated with *C. subvermispora*.

| Experiments | Untreated | Biotreated | | | | | p[y] |
|---|---|---|---|---|---|---|---|
| | | 2 weeks | 4 weeks | 6 weeks | 8 weeks | 10 weeks | |
| Weight, g | 100±0.00[a] | 99.4±0.02[a] | 95.6±0.08[b] | 95.1±0.01[cb] | 94.7±0.03[cb] | 94.6±0.01[c] | * |
| Glucan, % | 33.6±0.28[a] | 33.6±0.45[a] | 31.9±0.17[b] | 31.6±0.53[b] | 31.6±0.19[b] | 31.5±0.40[b] | ** |
| Xylan, % | 16.5±1.34[a] | 16.5±0.40[a] | 15.3±0.14[a] | 15.3±0.45[a] | 15.2±0.04[a] | 15.0±0.06[a] | NS |
| Lignin, % | 26.2±0.88[a] | 26.3±0.40[a] | 20.0±0.77[b] | 19.3±0.31[cb] | 18.9±0.10[cb] | 18.3±0.23[c] | * |
| Extractives, % | 7.25±0.18[a] | 5.27±0.69[b] | 4.73±0.37[cb] | 4.28±0.07[c] | 4.28±0.23[c] | 4.02±0.04[c] | ** |
| α-cellulose, % | 37.2±1.13[a] | 37.2±0.60[a] | 37.1±0.64[a] | 37.1±0.07[a] | 37.0±0.16[a] | 36.9±0.11[a] | NS |
| 1% NaOH solubility, % | 37.8±0.55[a] | 46.2±0.64[b] | 52.3±0.80[c] | 53.3±0.67[cd] | 54.6±0.62[de] | 55.8±0.40[e] | * |
| Weight loss, % | - | 0.60 | 4.40 | 4.90 | 5.30 | 5.40 | - |
| Glucan loss, % | - | 0.60 | 9.24 | 10.6 | 10.9 | 11.3 | - |
| Xylan loss, % | - | 0.60 | 11.4 | 11.8 | 12.8 | 14.0 | - |
| Lignin loss, % | - | 0.22 | 27.0 | 29.9 | 31.7 | 33.9 | - |
| Extractives loss, % | - | 27.7 | 38.0 | 43.6 | 43.8 | 47.8 | - |
| α-cellulose loss, % | - | 0.60 | 4.66 | 5.16 | 5.81 | 6.16 | - |
| Increase in 1% NaOH solubility, % | - | 14.9 | 32.3 | 34.1 | 36.8 | 39.6 | - |
| Specificity(% lignin loss/% glucan+xylan loss) | - | 0.18 | **1.31** | 1.34 | 1.34 | 1.34 | - |

[y] Significance level, * Significant at 0.001 for ANOVA, ** Significant at 0.01 for ANOVA, NS: non-significant for ANOVA, [a,b,c,d,e] Values having the same letter are not significantly different (Duncan test).

The lignin content of common reed was very susceptible to the pretreatment, except in the first two weeks, when no statistical difference was observed with the untreated sample.

However, as can be seen, there was a very significant difference in the lignin content after extended incubation periods of pretreatment. Statistical analysis showed that fungal pretreatment had a significant ($p$<0.001) effect on lignin content. The lignin loss of common reed at the end of 10 weeks of incubation time was 33.9%. R. MENDONCA & al. [21] reported that *C. subvermispora* lowered the lignin content of loblolly pine by 9.16, 10.7 and 16.6% in 2, 4 and 8 weeks, respectively. The higher lignin reduction in this study could be explained by the lower lignin content of common reed,which resulted in more open structure available to fungal activity. The reduction in the lignin content may be attributed to the degradation of the lignin β-O-aryl ether linkages by the fungus (A. GUERRA & al. [22]). The α-cellulose content of common reed was decreased with fungal pretreatment. The highest α-cellulose loss was found to be 6.16% after an incubation time of 10 weeks. S.K. GULSOY and H. EROGLU [23] reported 5.45% α-cellulose loss after 100 days of incubation time with *C. subvermispora*-pretreated European black pine. Statistical analysis showed that fungal pretreatment had no significant ($p$>0.05) effect on α-cellulose content. Recent studies on *Eucalyptus grandis* (A. FERRAZ & al. [24]) and *Pinus taeda* (A. GUERRA & al. [25]) biodegradation by *C. subvermispora* have noted that the decrease in the α-cellulose content due to cellulose depolymerization was caused by extended biotreatment periods.The increase in alkali solubility of materials in the common reed as a function of the pretreatment time is given in Table 2. Statistical analysis showed that fungal pretreatment had a significant ($p$<0.001) effect on alkali solubility. There was clearly more soluble material and a steady degradation was observed as the incubation time progressed up to 10 weeks, at which the solubility had increased by 39.6%. The increase in solubility could be due to the hot alkali solution extraction of the low-molecular weight carbohydrates in the structure (mainly hemicellulose and degraded cellulose) (R.M. SHRESTHA & N. BUDHATHOKI [26]). It could also signifythat the cell wall structure had been opened up by the fungus, thus allowing greater access to the 1% NaOH. L.L. VILLALBA [19] reported that *C. subvermispora* increased the alkali solubility of loblolly pine by almost 29 and 63% in 2 and 4 weeks, respectively. The lower alkali solubility in this study could be explained by the lower carbohydrate degradation of common reed.The findings of the chemical composition analysis definitely indicate that fungal pretreatment of common reed offers benefits for bioethanol production which include the depolymerization of lignin and extractives removal. However, cellulose depolymerization emerges as a disadvantage for bioethanol production.

### *3.2.2. Effect of chemical pretreatments on the chemical composition of common reed*

In Table 3, details of the solids recovered after chemical pretreatments are given. The lower yields after pretreatment can be mainly attributed to the removal of lignin, hemicellulose and other solubles from the structure. The $B_2O_3$-pretreated samples exhibited the highest solid recovery (83.1%), while the NaOH-pretreated samples showed the lowest (62.3%). Statistical analysis revealed that chemical pretreatments had a significant effect on glucan ($p$<0.001), xylan ($p$<0.001) and lignin ($p$<0.01).

#### *Effects of chemical pretreatment on glucan content*

Using NaOH as the pretreatment agent degrades the cellulose and hemicellulose H-bonds and is able to break the ester linkages between lignin and xylan (X.F. SUN & al. [27]). Consequently, this results in swelling that causes hemicelluloses and lignin dissolution (Y. CHEN & R.R. SHARMA-SHIVAPPA [28]). The glucan content of the NaOH pretreated common reed was 49.8%. On the other hand, dissolved glucan was found to be 7.66%. Z. WANG & al. [29] observed 2.03-9.77% of solubilization for Bermuda grass pretreated with

0.5-3% NaOH (121 ºC/15-90 min). F. MONLAU & al. [30] found a much lower (<8%) glucan solubilization when sunflower stalks were pretreated with 4% NaOH (55 °C/ 24 h or 170 °C/ 1 h). In contrast, higher glucan solubility was reported by R.A. SILVERSTEIN & al. [31], who observed nearly 21.0% glucan solubilization for cotton stalks treated with 2% NaOH (121 °C/ 60 min). This indicates that glucan solubility is affected by both the raw material and the pretreatment conditions. Alkaline $H_2O_2$ is a well-known bleaching agent used in the paper industry. Its main advantage is that it does not leave any residue (secondary products) in the material after treatment, as it is decomposed into oxygen and water (S.C. RABELO & al. [32]). The percentage of glucan in the pretreated solids remaining as a result of $H_2O_2$ pretreatment was 36.7%. Glucan solubilization on average was 12.2%. R.A. SILVERSTEIN & al. [31] found 29.1% glucan solubilization for cotton stalks when samples were treated with 2% $H_2O_2$ at 121 ºC for 30 min.

The pulping additive $NaBH_4$ is conventionally used in paper productionfor the improvement of the pulping selectivity in the kraft method. The chemical acts as a catalyst and results in selective delignification (Y. COPUR & A. TOZLUOGLU [13]). By stopping carbohydrate degradation, it increases the pulp yield (Y. COPUR & al. [3]). However, little has been written on utilizing $NaBH_4$ as a pretreatment chemical for the production of bioethanol. The effects of $NaBH_4$ used as a pretreatment chemical for wheat straw, corn stalks and hazelnut husks have been studied (Y. COPUR & al. [3,4,5]), and it has been shown to be as efficient as NaOH for chemical pretreatment. The glucan content of the $NaBH_4$-pretreated samples was 42.9% and the glucan solubilization was 4.50%. Y. COPUR & al. [3,5] reported 7.27% and 31.6% glucan solubilization, respectively, when wheat straw and hazelnut husks were pretreated with 2% and 4% $NaBH_4$ at 121 °C for 30 min. One of the oxides of boron, $B_2O_3$,has recently begun to attract the attention of researchers as a pretreatment chemical in the production of bioethanol. In the present study,glucan content of the $B_2O_3$-pretreated samples was 34.1% and the glucan solubilization was 15.7%. However, glucan solubilization of 28.5% for sunflower stalks pretreated with 2% $B_2O_3$ (121 °C/ 90 min) was obtained by A. TOZLUOGLU & Y. COPUR [33]. Pretreatment with dilute acids at high temperatures hydrolyzes the hemicelluloses. This is accomplished whenthe monomeric sugars and soluble oligomers are released from the cell wall matrix into the hydrolyzate. Thus, one of the most effective methods employed for lignocellulosic biomass is acid pretreatment. The porosity of the structure is increased when the hemicelluloses are removed, thereby enhancing enzymatic digestibility. Studies in the literature (J.D. MCMILLAN [34]) have indicated that removal of all hemicellulose from the structure enables maximum enzymatic digestibility to be achieved. The glucan content of the $H_2SO_4$-pretreated common reed was 35.9%. The percentage of glucansolubilizationwith $H_2SO_4$ pretreatment was 15.0%. It is important that the cellulose in the biomass be essentially unaffected during pretreatment. A slightly higher glucan reduction was reported during the acid pretreatment of cotton stalks (S.B. KIM & al. [35]) because the cellulose-rich, loose cotton fiber in the stalks is not imbedded in lignin and hemicellulose, and as a result, the acid has direct access to the cellulose during pretreatment and can initiate more degradation of glucan than is usually seen with other feedstock.

***Effects of chemical pretreatment on xylan content***

The xylan content of NaOH-pretreated solid was 19.5%, whereas the dissolved xylan was 26.4%. The xylan solubility found in this study was similar to that of Y. CHEN & R.R. SHARMA-SHIVAPPA [28], who reported 40, 35 and 35% xylan solubilization in barley straw, triticale straw and wheat straw, respectively, using 2% NaOH (121 ºC/ 60 min). In

contrast, a higher xylan solubility value was reported by Z. WANG & al. [29], who achieved 60.5% xylan solubilization in Bermuda grass (3% NaOH/121 ºC/90 min). Results showed that the NaOH pretreatment removed more xylan than glucan; this could be due to the vulnerability of hemicelluloses to the chemicals (A.S. SCHMIDT & A.B. THOMSEN [36]). Although the xylan solubilization due to the NaOH pretreatment was lower than that from the $H_2SO_4$ pretreatment (Table 3), the solubilization of xylan together with substantial lignin reduction was expected to improve enzymatic hydrolysis.

Table 3. Chemical composition of chemically pretreated common reed.

| Experiments | Untreated | Fungal pretreatment | Chemical pretreatments | | | | | p[y] |
|---|---|---|---|---|---|---|---|---|
| | | | NaOH | $H_2O_2$ | $NaBH_4$ | $B_2O_3$ | $H_2SO_4$ | |
| Weight, g | 100±0.00[a] | 95.6±0.08[b] | 62.3±0.42[c] | 80.4±0.99[d] | 74.8±1.41[e] | 83.1±0.57[f] | 79.6±0.28[d] | * |
| Glucan, % | 33.6±0.28[ab] | 31.9±0.17[a] | 49.8±1.77[c] | 36.7±1.53[b] | 42.9±1.60[d] | 34.1±1.12[ab] | 35.9±2.08[b] | * |
| Xylan, % | 16.5±1.34[ab] | 15.3±0.14[a] | 19.5±1.43[c] | 16.8±1.34[ab] | 18.1±0.93[bc] | 15.4±1.10[ab] | 7.53±0.48[d] | * |
| Lignin, % | 26.2±0.88[ab] | 20.0±0.77[cd] | 18.6±1.61[c] | 24.7±1.81[ab] | 16.8±1.13[c] | 23.1±0.91[ad] | 28.1±2.66[b] | ** |
| Weight loss, % | - | 4.40 | 37.7 | 19.6 | 25.2 | 16.9 | 20.4 | - |
| Glucan loss, % | - | 9.24 | 7.66 | 12.2 | 4.50 | 15.7 | 15.0 | - |
| Xylan loss, % | - | 11.4 | 26.4 | 18.1 | 17.9 | 22.4 | 63.7 | - |
| Lignin loss, % | - | 27.0 | 55.8 | 24.2 | 52.0 | 26.7 | 14.6 | - |
| Specificity(% lignin loss/% glucan+xylan loss) | - | 1.31 | 1.64 | 0.80 | 2.32 | 0.70 | 0.19 | - |

[y] Significance level, [*] Significant at 0.001 for ANOVA, [**] Significant at 0.01 for ANOVA, [a,b,c,d] Values having the same letter are not significantly different (Duncan's test).

The solubilization of xylan due to the $H_2O_2$ pretreatment was 18.1%, while the xylan content in the pretreated solid was 16.8%. R.A. SILVERSTEIN & al. [31] observed that cotton stalk samples treated with 2% $H_2O_2$ (121 ºC /30 min) resulted in 30.6% xylan solubilization. When the $NaBH_4$ pretreatment is examined, the xylan content of the pretreated common reed was 18.1%, whereas xylan removal was 17.9%. Y. COPUR & al. [3,5] reported almost 58.7% and 66.2% xylan solubilization for wheat straw and hazelnut husks when the materials were pretreated with 4% $NaBH_4$ (121 °C/ 90 min and 30 min, respectively). The lower xylan solubility in the present study could be explained by differing characteristics of the raw material. The xylan content of the $B_2O_3$-pretreated samples was 15.4% and xylan solubilization was 22.4%, whereas A. TOZLUOGLU & Y. COPUR [33] observed 16.8% xylan solubilization when sunflower stalks were pretreated with 2% $B_2O_3$ at 121 °C for 90 min. The xylan content of the $H_2SO_4$-pretreated samples was 7.53%. The findings revealed that $H_2SO_4$ had a significant effect on the reduction of xylan. With the $H_2SO_4$ pretreatment, 63.7% of the xylan was dissolved from the common reed structure. Because of its heterogeneous, non-crystalline structure, xylan is more labile (J.D. MCMILLAN [34]). This fact could be the reason for the higher xylan dissolution compared to glucan. Similar to the findings of the present study, D.J. SCHELL & al. [37] reported 77% xylan reduction in corn stover treated with 1.35% acid (190 ºC/ 60 min), and K. GROHMANN & al. [38] found more than 80% reduction of xylan in wheat straw treated with dilute $H_2SO_4$ (140 ºC/ 1 h).

***Effects of chemical pretreatment on lignin content***

Lignin, a three-dimensional, complex aromatic polymer, surrounds the carbohydrates (cellulose and hemicellulose) in woody biomass. However, lignin limits the accessibility of hydrolytic enzymes to the carbohydrates and thus, its removal is crucial in order to improve the digestibility. The main effect of NaOH pretreatment on lignocellulosic biomass is the increase in porosity of the biomass as a result of delignification, which breaks the ester bonds cross-linking lignin and xylan (L.T. FAN & al. [39]). The amount of lignin in the solids after

NaOH pretreatment was 18.6% and 55.8% of the lignin was removed from the structure. Results showed that delignification and xylan degradation were the cause of the sizeable solid loss during the NaOH pretreatment of common reed. Comparable lignin removal (20.0%) was observed by G. ANTONOPOULOU & al. [40] while pretreating sunflower stalks with 2% NaOH( 80 °C/24 h). In addition, F. MONLAU & al. [30] reported nearly the same lignin removal rate (22.0 and 36.0%) by pretreating sunflower stalks with 4% NaOH (55 and 170 °C/ 24h, respectively). M. GASPAR & al. [41] and Z. WANG & al. [29] recorded 95% lignin removal for corn fiber (2.5% conc./121 °C/ 60 min) and 86% removal for Bermuda grass (3% conc./ 121 °C/ 90 min), respectively. The higher lignin removal (95%) for corn stover reported by E. VARGA & al. [42] might have been because of the higher NaOH concentration (10%). In the present study, the NaOH concentration was only1%. The results indicated that lignin removal depends on pretreatment conditions and the characteristics of the raw material. In the $H_2O_2$ pretreatment, oxidative delignification is utilized to separateand dissolve the lignin and loosen the lignocellulosic matrix, resulting in enzyme digestibility (P. MARTEL & J.M. GOULD [43]). The $H_2O_2$-pretreated samples had 24.7% lignin content and the lignin reduction was 24.2%. A.M. AZZAM [44] and X.F. SUN & al. [27] reported 50% lignin reduction in sugarcane bagasse with 2% alkaline $H_2O_2$ (30 ºC /8 h), and more than 80% lignin reduction in wheat straw treated with 2% $H_2O_2$ (50 ºC/ 5 h). The relatively lower degree of lignin reduction seen in this study could be a result of the shorter treatment time, which may have limited the oxidative delignification.

The lignin content of the $NaBH_4$-pretreated samples was 16.8% and lignin removal was 52.0%. Y. COPUR & al. [3,5] reported almost 58.4 and 49.1% delignification, respectively, for wheat straw and hazelnut husks (4% $NaBH_4$/ 121 °C/ 90 min). More lignin as compared to xylan was removed from the common reed pretreated with $NaBH_4$. It is known that the removal of lignin is extremely crucial for improving enzymatic digestibility. The lignin content of the $B_2O_3$-pretreated samples was 23.1% and lignin reduction was 26.7%, while A. TOZLUOGLU & Y. COPUR [33] observed 19.8% lignin reduction when sunflower stalks were pretreated with 2% $B_2O_3$ (121 °C/ 90 min).One of the most effective pretreatments of lignocellulosic biomass is the dilute acid pretreatment because it predominantly affects hemicellulose and has little impact on lignin degradation. The $H_2SO_4$-pretreated samples, compared with the untreated samples,exhibited lignin content increasein the material due to the removal of carbohydrates from the structure. The lignin content of the samples was 28.1%, whereas the dissolved lignin was 14.6%. These findings are on a level withthose of R.A. SILVERSTEIN & al. [31], who reported 24.2% lignin reduction for cotton stalks treated with 2% $H_2SO_4$ (121 ºC/90 min). In addition, Y. CHEN & R.R. SHARMA-SHIVAPPA [28] found as much as 20% lignin reduction for barley straw, triticale straw and wheat straw treated with 2% $H_2SO_4$ (121 ºC/ 60 min).

## 4. Conclusion

Common reed was pretreated with white-rot fungi (*S. cerevisiae*) as a biological pretreatment. In order to compare the outcomes, the chemicals NaOH, $H_2O_2$, $NaBH_4$, $B_2O_3$ and $H_2SO_4$ were also investigated in a chemical pretreatment step.Fungal pretreatment (incubation time of 10 weeks) dissolved 11.3 and 14.0% glucan and xylan from the structure, respectively. The lignin loss was 9.16, 10.7 and 16.6% in 2, 4 and 8 weeks, respectively. The $H_2SO_4$ pretreatment substantially solubilized xylan in the structure, whereas fungal, NaOH, $H_2O_2$, $NaBH_4$ and $B_2O_3$ pretreatments resulted in greater lignin reduction.

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