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Alkaline Pretreatment: A Potential Tool to Explore Kallar Grass (*Leptochloa fusca*) as a Substrate for Bio-Fuel Production

[1]Muhammad Nadeem, [2]Umar Ashgar,
[1]Sajjad Abbas, [2]Saif Ullah and [1]Quratulain Syed

[1]Food and Biotechnology Research Center (FBRC),
PCSIR Laboratories Complex, Lahore-54600, Pakistan
[2]Institute of Molecular Biology and Biotechnology (IMBB),
The University of Lahore, Punjab, Pakistan

Abstract: In present study, the substrate kallar grass (Leptochloa fusca) was treated with alkali to explore its best possible use as a cheap substrate for bio-fuel production. In the beginning, physically processed kallar grass was soaked in various concentrations of NaOH (0.5% to 3%) for different soaking times (30min to 48h). The results showed that cellulose contents and delignification increased with the increase in concentration of alkali and soaking time. However, maximum cellulose content (40.17%) was observed with 2.5% NaOH after 24 h of soaking period. In physiochemical pretreatment, the substrate was soaked for 2h in alkaline solution having various concentrations (0.5 % to 3%) of NaOH and thereafter autoclaved at 121°C for 20 to 60min. Maximum cellulose content (58%) was achieved with 2.5% alkali solution at 121°C after 60 min while delignification was found 52%. All these results of the present study indicate that suitable concentration of alkali as well as autoclaving period at specific temperature is very much crucial to expose maximum cellulosic contents prior to employing kallar grass as a substrate for bio-fuel production.

Key words: Kallar grass · Physiochemical pretreatment · Cellulose contents · Delignification

INTRODUCTION

Lignocellulosic material in plant cells gets energy from sun light during photosynthesis and contains different organic molecules likes carbon and hydrogen etc [1]. The production of bio-fuel from lignocellulosic material is the second generation phenomena. In this process the lignocellulosic material that has six carbon sugars is used for the production of bio-ethanol [2]. The lignocellulosic biomass consisted of three components, lignin, hemicellulose and cellulose which are associated with each other and form a compact structure. The lignin is outermost part of biomass consisting of phenolic material that provides resistance from microbial and chemical attacks [3]. The second part present inside the lignin is hemicellulose which contains different types of sugars like pentose, arabinose and glucose. The most important part of biomass is cellulose which consisted of six carbon sugar (glucose). The cellulosic contents of biomass can be converted into glucose by employing saccharifying enzymes [4]. However, the pretreatment of lignocellulosic material to expose maximum cellulose contents is the basic step in bio-fuel production prior to saccharification and fermentation process. Several methods of pretreatments have been discovered but chemical treatment with various acids or bases are most commonly employed [5-8].

The present study was therefore undertaken to produce potential biomass from locally available agriculture waste (Kallar grass) after treated with various concentrations of alkali (NaOH) to explore its application as a cheaper substrate for the production of bio-fuel.

MATERIALS AND METHODS

Substrates Collection: The substrate (Kallar grass) was collected from Tehsil Pirmahal, District Toba Tek Singh, from remote area of the Punjab, Pakistan. The substrate

Corresponding Author: Muhammad Nadeem, Food and Biotechnology Research Center (FBRC),
PCSIR Laboratories Complex, Lahore-54600, Pakistan.

was chopped into small pieces, dried in sunlight and packed in polyethene bag before taken into laboratory.

Processing of Substrate: The substrate (Kallar grass) was then dried at 80°C in hot air oven in the laboratory and processed through physical, chemical and physiochemical treatments.

Physical Pretreatment: The dried material was further pulverized up to 2mm mesh size with hammer beater mill (PX-MFC 90 D) and stored at room temperature in polythene bags. This physical pretreated substrate was further used in chemical and physiochemical pretreatments to expose its maximum cellulosic contents.

Chemical Pretreatment: In this pretreatment, 10 g quantity of substrate (2mm size) was treated with 100 ml alkaline solution having various concentrations of NaOH (0.5-3%) in separate flask. Thereafter, treated materials were soaked for various time intervals (30 min to 48 h) at room temperature. After soaking the sample was filtered and filtrate was saved at 4°C for the analysis of reducing sugar, total sugar and estimation of phenol. The residues were washed with distilled water 5-6 times to get pH 7.0. The washed residues were dried in oven at 105°C and stored at room temperature for estimation of cellulose and lignin contents.

Physiochemical Pretreatment: In physiochemical pretreatment, initially 10 g substrate was treated with 100 ml NaOH solution having concentrations from 0.5% to 3% in separate 250 ml conical flask and then each flask was soaked at room temperature for 2 h. Thereafter each flask was autoclaved at 121°C for various time intervals (20-60 min). The autoclaved samples were filtered and residues were the washed with distilled water 5 to 6 times up to neutrality. The filtered of each treated material were stored at 4°C while residues were dried at 105°C for further analysis.

Analytical Analysis:

Estimation of Reducing Sugars: The reducing sugar was measured according to the DNS method [9]. One ml of filtrate obtained from each treatment was taken in separate test tubes and then added 3 ml of DNS reagent in each test tube. The tubes were boiled in a boiling water bath for 10 min. After that the optical density of each sample was measured by spectrophotometer at 550 nm against blank.

Estimation of Total Sugar: Total sugar contents of the filtrate were measured according to the method of Dubois *et al.* [10]. One ml of each filtrate was taken in separate test tubes and 5 ml of concentrated H_2SO_4 was added. Each sample was left for 20 min at room temperature after proper mixing and then optical density (OD) was measured by spectrophotometer at 470 nm against blank.

Estimation of Cellulose: The cellulose was estimated by method of Gopal and Ranjhan [11]. One gram of sample (W1) was taken in 500 ml round bottom flask and then added 30 ml of 80% acetic acid. After that 2 ml concentrated nitric acid was added and the sample was refluxed for 20 min. The sample was the filtrated and the residues were dried in oven at 105°C for overnight and weighed the sample (W2). The sample was then charred and placed in Muffle furnace at 550°C for 6 h to produce ash and weighed again as W3. The cellulose content (%) was calculated by the following formula.

$$Cellulose\,contenst(\%) = \frac{w_2 - w_3}{w_1} \times 100$$

Estimation of Lignin: The lignin of the pretreated substrate was estimated by the method as described by Milagres [12]. According to the method 1 g sample (W1) was added into 70 ml of 1.25 % H_2SO_4 and then refluxed for 120 min. After that the sample was filtered and residue was put into 30 ml of 72 % H_2SO_4 and stirred up to 2 h by magnetic stirrer. After that excesses water was added to dilute the sample. The sample was filtrated and residues were dried in an oven for overnight at 105° C and weighed again as W2.

$$Lignin\,content(\%) = \frac{w_2}{w_1} \times 100$$

Estimation of Total Phenol: Total phenol in filtrate was estimated by the method of Carralero *et al*. [13]. According to the procedure 1ml of filtrate was mixed with 2.5 ml of diluted folin reagent (1: 10) and then 4 ml of sodium carbonate (Na_2CO_3) solution was added and left for 2 h at room temperature. The optical density was measured by spectrophotometer at 765nm. Vanillin was used as standard for estimation of total phenol.

$$Total\ phenol\,(\%) = \frac{Abs.of\ sample}{Abs\,of\ \ s\tan dard} \times conc.of\ s\tan dard$$

Statistical Analysis: All the experiments in the present study were conducted in triplicates. Mean values and standard deviation (SD) among each triplicate were calculated through Microsoft Office Excel 2007.

RESULT AND DISCUSSION

The results of the present study show that the proper concentration of NaOH and exact time period of soaking play an important role for the maximum exposure of cellulose. In chemical pretreatment, the substrate was treated with various concentrations of NaOH from 0.5% to 3% and thereafter soaked for different time intervals from 30 min to 48 h. Fig.1 1.

Represents the effect of 0.5% of NaOH at different soaking time. Maximum cellulose content (34%) was observed after 24 h soaking while delignification was found 15.92% at this soaking period. Similarly a further increase in concentration of NaOH (1%) resulted in an increase in cellulose contents and delignification as compared to 0.5% NaOH (Fig. 2).

However, increase in cellulose contents and delignifications were found gradually with the increase in the concentration of sodium hydroxide as shown in Fig. 3-4.

Maximum cellulose contents (40.17%) were achieved at 2.5% NaOH after 24 h soaking time while delignification was found 24.60% (Fig. 5).

A further increase in soaking period up to 48 h slightly decreased the cellulose contents of substrate during pretreatment. However, the results of 3% NaOH concentration at similar soaking periods also illustrated an insignificant decline in cellulose contents of pretreated substrate (Fig. 6).

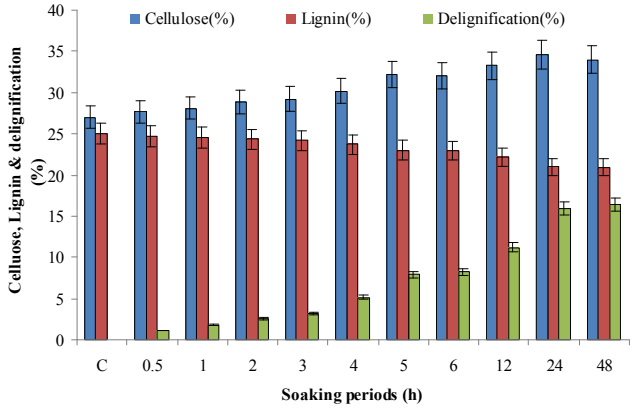

Fig. 1: Effect of 0.5% Concentration of NaOH on Cellulose and Lignin Residues of Substrate at Various Soaking Periods. C: Control (Without Treatment. Bars Represented SD among Triplicate Values.

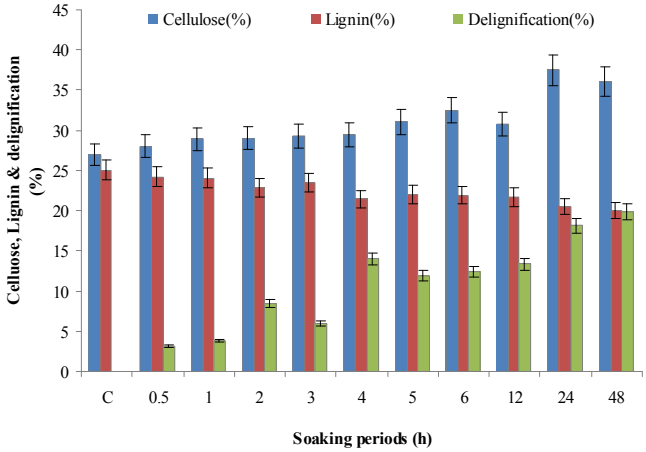

Fig. 2: Effect of 1.0% Concentration of NaOH on Cellulose and Lignin Residues of Substrate at Various Soaking Periods. C: Control (Without Treatment. Bars Represented SD among Triplicate Values.

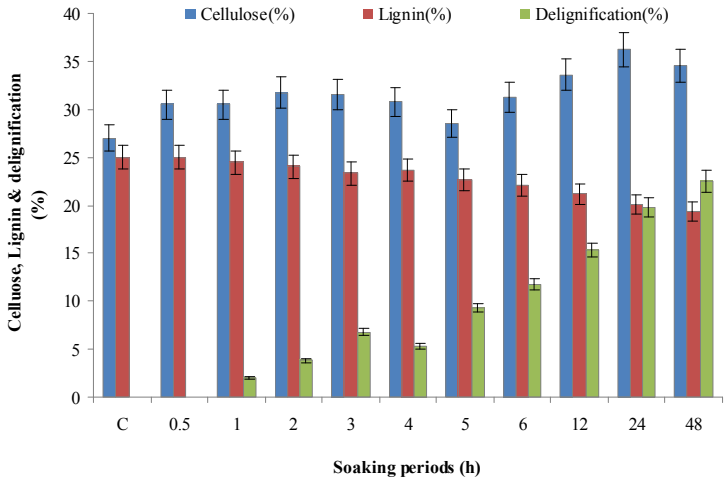

Fig. 3: Effect of 1.5% Concentration of NaOH on Cellulose and Lignin Residues of Substrate at Various Soaking Periods. C: Control (Without Treatment. Bars Represented SD among Triplicate Values.

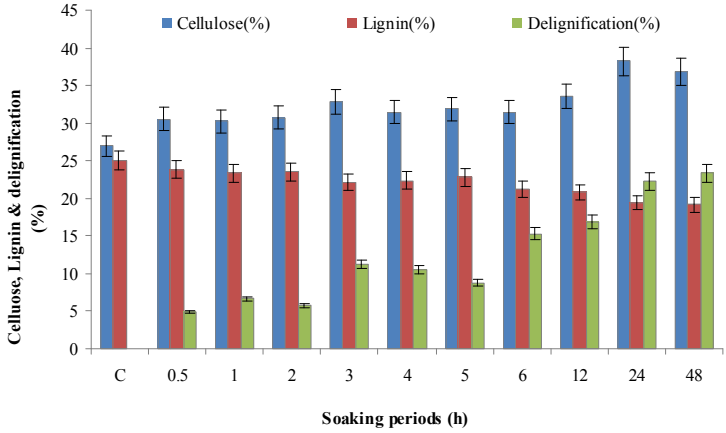

Fig. 4: Effect of 2% Concentration of NaOH on Cellulose and Lignin Residues of Substrate at Various Soaking Periods. C: Control (Without Treatment. Bars Represented SD among Triplicate Values.

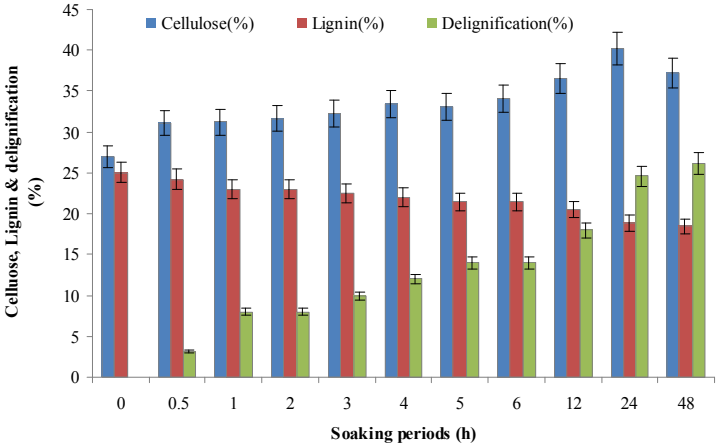

Fig. 5: Effect of 2.5% Concentration of NaOH on Cellulose and Lignin Residues of Substrate at Various Soaking Periods. C: Control (Without Treatment. Bars Represented SD among Triplicate Values.

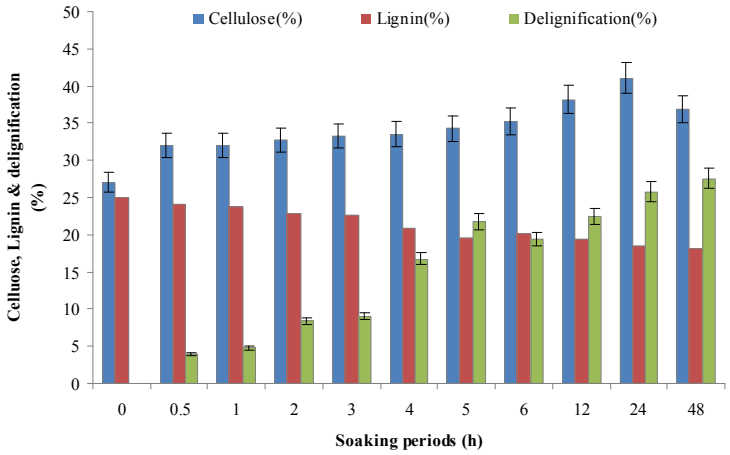

Fig. 6: Effect of 3% Concentration of NaOH on Cellulose and Lignin Residues of Substrate at Various Soaking Periods. C: Control (Without Treatment. Bars Represented SD among Triplicate Values.

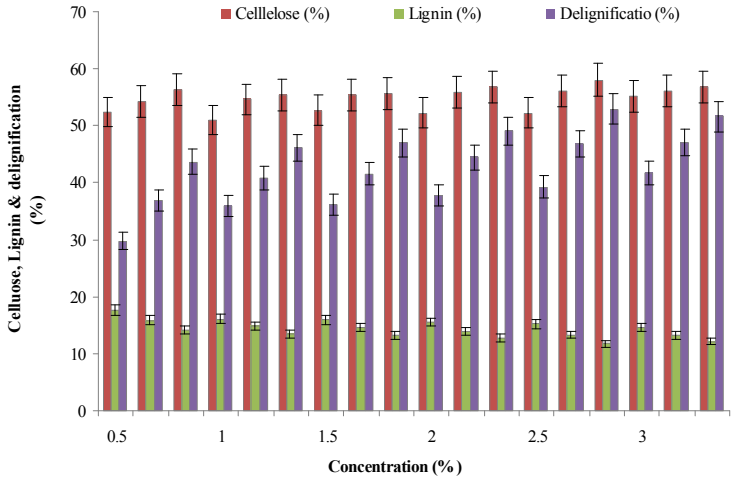

Fig. 7: Effect of Various Concentrations (0.5-3%) of NaOH on Cellulose, Lignin and Delignification after Autoclaving at 121°C for Different Intervals (20-60 Min). Bars Represented SD among Parallel Triplicate Values.

These findings indicated that the higher concentration of alkali and longer soaking period might be degraded the cellulose contents during pretreatment. Furthermore the effects of physiochemical pretreatment on kallar grass (*Leptochloa fusca*) have also been investigated to explore its exploitation as a substrate of choice for biofuel production. During physiochemical the substrate was soaked for 2 h in alkaline solution having various concentrations of NaOH solution ranging from 0.5% to 3.0% and then autoclaved each treatment at 121°C for different intervals. The results showed that exposure of cellulose contents increase gradually with increase in alkaline concentration from 0.5% to 2.5% along with increase in autoclaving time i.e. 20 to 60 min (Fig. 7).

Maximum cellulose contents (58%) and delignification (52%) was observed after 60 min when the substrate was treated with 2.5% concentration of NaOH at 121°C for 60 min. About 70 % delignification of sugarcane bagasse with 2.5% conc. of KOH was reported in an earlier investigation under steaming of 121°C [14]. These variations in the results of delignification might be existed due the difference in nature of substrates as well as alkaline materials used in the pretreatment process. However, a further increase in concentration of NaOH up to 3.0% (w/v) slightly decreased the cellulose contents and delignification as shown in Fig. 7. Similarly, total sugars, reducing sugars and total phenols in filtrate which representing the degradation of hemicelluloses and lignin contents during physiochemical treatment were also

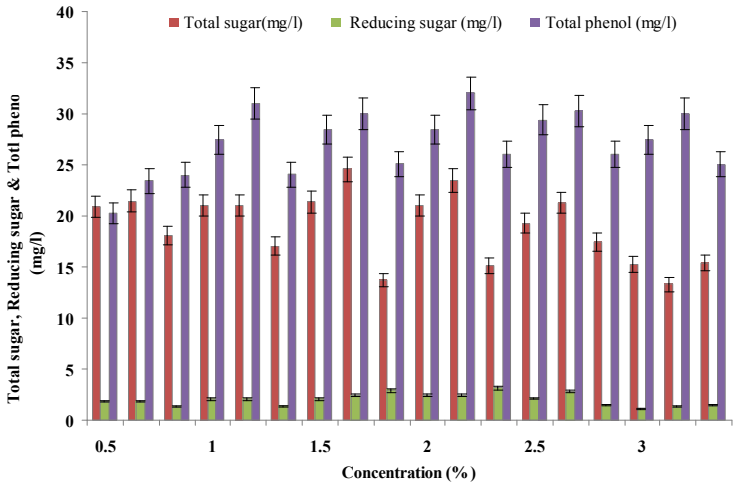

Fig. 8: Effect of Various Concentrations (0.5-3%) of NaOH on Cellulose, Lignin and Delignification after Autoclaving at 121°C for Different Intervals (20-60 Min). Bars Represented SD among Parallel Triplicate Values.

analyzed. Maximum values of total sugar (17.4 mg/ml), reducing sugar (1.5 mg/ml) and total phenol (26 mg/ml) were estimated during the treatment of substrate with 2.5% NaOH solution at 121°C for 60 min (Fig. 8).

Total sugar and reducing sugars having values of 20.37 mg/ml and 7.56 mg/ml, respectively in filtrate of pretreated sugarcane bagasse had been reported in another investigation [15].

CONCLUSION

Alkaline pretreatment of lignocellulosic biomass was conducted for the maximum exposure of cellulose and degradation of lignin which provides accessible area for enzymatic activity during the saccharification of pretreated biomasses. The substrate kallar grass (*L. fusca*) was treated with various concentrations of alkali (NaOH) for chemical and physiochemical pretreatments. During physiochemical treatment, maximum cellulose and delignification achieved was 58 % and 52 % respectively. These findings clearly indicate that suitable pretreatment method is very much essential to expose maximum cellulose contents and delignification of a substrate for its proper exploitation in saccharification process.

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