Evaluation of Alkali and Acid Pretreatments on Brown Mid Rib Sorghum Varieties for Maximum Cellulose Yield

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Abstract: **Producing bioethanol from lignocellulosic plant material is considered as an alternative energy-production system. Plant biomass comprises cellulose, hemicellulose and lignin as major components among others. It is necessary to convert the cellulose and hemicellulose components of plant biomass into fermentable sugars for the production of bioethanol. The major obstacle in converting the cellulose and hemicellulose to fermentable sugars is lignin which is present as an interrelating agent with cellulose and hemicellulose and hinders the activity of cellulases. In this study we investigated alkali and acid pretreatment efficiency to get maximum cellulose yield for low lignin content sorghum raw materials such as Brown Mid Rib 22, 24 and Non-Brown Mid Rib CSH 22. After removing water and alcohol extractives, TAPPI method was applied to determine the cellulose, hemicellulose and lignin content. Raw material was treated with NaOH and H_2SO_4 concentrations ranging from 2% to 10% and 1 to 5% respectively time of pretreatment ranging from 15 minutes to 60 minutes at temperatures ranging from 60°C to 120°C. For BMR 24 variety 93% highest cellulose yield was observed when treated with 6% NaOH for 30 minutes at 100°C following 89% for BMR 22 variety 76% for non-BMR CSH 22 variety when treated with 4% NaOH for 45 minutes at 60°C and 3% H_2SO_4 for 30 minutes at 80°C respectively. The cellulose yield has substantially increased after alkali and acid pretreatments compared with the cellulose yield without pretreatment.**

Index Terms: **Sorghum, brown mid rib, Pre-treatment, Cellulose, Hemicellulose, Lignin.**

I. INTRODUCTION

In general, among the pressing issues the most three issues that are faced by the society are over reliance on the imported non-renewable fuels, the increasing levels of the greenhouse gases, and the ability of the agricultural systems to maintain the production at the rates that are needed to feed the growing population (Wilhelm W.W et al. 2004). Biofuels are produced from the biomass. The biofuels can be in the solid form (some urban wastes, vegetable wastes and industrial wastes), the liquid form (biodiesel and bio-alcohols) or the gaseous form (biogas and hydrogen) (Demirbaş A, 2008). Biofuels are the sustainable and the renewable source of energy that are derived from the organic matter in the form of the biomass. The biofuels can be derived from the plant as well as the animal biomass. The studies have showed that the plants grown for the biofuel purposes have the potential to reduce net greenhouse gas emissions. Schmer and colleagues have reported that the usage of corn and the switch grass as the source of biofuels has reduced greenhouse gas emissions by −29 to −396 g of the carbon dioxide equivalent per mega joule of the ethanol per year. Presently, about 2.5% of world's transportation, the fuels which are produced are from the crop plants including sugarcane, maize, and vegetable oils (Schmer M.R et al. 2014; Searchinger T et al. 2015). Agriculture and the energy have always been linked very closely. For the energy production, agriculture had always been a source for the fuels such as the feed stock for the draught animals and many more. In the recent times the juices from the stalk became source for the production of the fuels for e.g.; biodiesel and the bioethanol. A major input in the modern mechanised crop production is by the fossil fuel through which energy is being supplied (Belum V.S. Reddy et al. 2009).

One of the most significant crops which are grown around the globe and in India on the dry land is Sorghum. Both in the tropical and in the temperate climatic conditions it has the capacity to adapt and to tolerate the stress conditions. In the view of raising the petroleum prices, it has the potentials and it is the versatile crop which can be utilized as the alternate energy crop. In the early 1960s, the ICAR, initiated research on hybrid sorghum with Rockefeller Foundation assistance. The ICAR then initiated the AICSIP in 1969. Sorghum being the high biomass producer it can be potentially utilized for the production of bioethanol from the lignocellulosic biomass than any other

dry land cereals (Rao S.S et al. 2010). To improve the lignocellulosic biomass digestibility for the cattle brown midrib *(bmr)* enables traits via genetic modifications (Corredor D.Y et al. 2009; Sattler S.E et al. 2010). The first sorghum hybrid, which is CSH1 was bred in India and released officially for commercial cultivation in 1964 (Kumara Charyulu D et al. 2011). In this present study improved varieties with reduced lignin content sorghum *bmr-22*, *bmr-24* and sorghum hybrid *CSH-22* were used.

Lignocellulosic biomass is acomplex structure composed of cellulose, hemicellulose, lignin and extraneous compounds such are waxes, fats, gums, starches, alkaloids, resins, tannins, essential oils, silica, carbonates, oxalates, etc. Cellulose and hemicellulose are useful after conversion of simple sugars only; it can be accessed for production of cellulosic bioethanol through ethanologenic microorganisms. Lignin is composed of phenylpropanoid units binding hemicellulose and cellulose; it is responsible for resistance and recalcitrance to biological and chemical degradation during enzymatic hydrolysis and saccharification. Because of this low availability of cellulose and hemicellulose makes procedure uneconomically feasible hence efficient pretreatment methods require initial breakdown of lignocellulosic biomass (Sluiter A et al. 2012).

The pretreatment process makes cellulose and hemicellulose more available for enzymatic hydrolytic process by disrupting the crystalline structure and reduces the degree of polymerization of cellulosic region (Iroba K.L et al. 2014; Tabil L.G et al. 2014). Therefore, to degrade the complex network of lignocellulosic material cellulose, hemicellulose and lignin, pretreatment is an essential step. To depolymerize the lignocellulosic materials several pretreatment methods have been developed which include steam explosion, acid and alkali hydrolysis and hot water pretreatment (Mosier N et al. 2005), most of pretreatment processes need high pressure, and temperature. Therefore, the present study has been focused on the development of an effective dilute acid and alkali pretreatment process for sorghum hybrid series such as *bmr-22, bmr-24* and *csh-22* to maximize the cellulose hydrolysis to achieve the high yield bioethanol production.

II. METHODOLOGY

A. Raw Material Preparation

Cultivated Low lignin varieties Sorghum raw biomass such as Brown Mid Rib 22, 24 and Non-Brown Mid Rib CSH 22 were collected from ICAR-Indian Institute of Millets Research, Rajendranagar, Hyderabad, Telangana, India. Sorghum raw materials were washed with distilled water thoroughly to remove the soluble sugars, air dried at room temperature (~27˚C). The dried sorghum raw materials were ground and screened to a particle size 1 mm using a sieve shaker. This method was divided into three steps namely, preparation and

Pretreatment of materials, Preparation of extractive, determination of holocellulose, α-cellulose, lignin and ash contents.

B. Pretreatment of Sorghum Raw Materials

Sorghum raw materials were pretreated by two methods dilute alkali (NaOH) and dilute acid (H_2SO_4) and controls with no pretreatment. For Sorghum raw materials H_2SO_4 treatment was used at different concentrations ranging from 1% to 5% (v/v), time of pre-treatment ranging from 15 min to 60 min at different temperatures from 60^0C to 120^0C.

The Sorghum biomass was pretreated with various concentrations of sulphuric acid (1%-5%) and Sodium Hydroxide (2 % -10%) at different time intervals (15 to 60 min), temperatures (60^OC to 120^OC) and mass of Sorghum biomass constant. 10 gm of Sorghum biomass was taken in separate conical flasks and different concentrations of sulphuric acid (1%, 2%, 3%, 4%, and 5%) and different concentrations of NaOH (2%, 4%, 6%, 8%, and 10%) were added and autoclaved at different temperatures 60 ^{O}C, 80 ^{O}C, 100°C, 120°C at 15 min, 30 min, 45 min, and 60 min intervals. Autoclaved samples were filtered and remaining solid fraction was washed till get neutral pH and dried at 60^OC overnight .This solid residue was used for further analysis (Li Y et al. 2004).

C. Composition Analysis

The chemical compositions of the different pretreated Sorghum raw materials were determined modified TAPPI test method (Goering H.K et al. 1970). T2 04 cm-88 and T2 64 cm-88 methods (Anonymous, 1993; Anonymous 1999) were used for the determination of extractives. Hemicellulose was determined following the procedure of Le et al. 1946 and T203 cm-74 (Anonymous, 1999) for cellulose base. The lignin content was carried out following the T222 cm-88 method (Anonymous, 2002). The inorganic constituent ash content was determined by followed the procedure outlined in T 211 cm-93 method (Anonymous, 1999).

1) Solid Recovery

Five grams of three varieties of sorghum biomass was initially weighed. Pretreated biomass was filtered and washed several times with distilled water to recover solids. During analysis porcelain evaporating dish were weighed in hourly intervals, until a constant weight was reached. When constant weight was obtained, porcelain evaporating dish were taken from oven and put into desiccator for 20 min to cool down to room temperature. Then final weight was recorded. Analysis was done in duplicate (Revve D.W, 2002).

The Solid recovery of the sample was calculated using the following formula:

% Solid recovery = [(A-B)/B] X 100
where;

A = Weight of initial sample (g) and B = Weight of pre treated sample (g)

2) Extractives

Extractives were determined with the aim to remove all the non-structural material from biomass of sorghum. The non-structural material is referred to inorganic material, non-structural sugars, nitrogenous material, chlorophyll, waxes among other compounds which are soluble in water and ethanol (ASTM, 2005).

a) Determination of Water Extractive

2.5 g of pre treated Sorghum raw materials was loaded in a closed flask and 150 mL of water was used for extraction in the Soxhlet apparatus. Residence times for boiling and rising stages was adjusted to 80^0C and 30 min respectively on the heating mantle for a 4 h run period. After extraction, the samples were air dried at room temperature in a hot air oven at 105^0 C. The extractives % (w/w) content was measured by using electric balance as the difference in weight between the Sorghum raw extractive and extractive-free material (Sluiter A et al. 2002).

Extractives (%) =B1/B2 * 100%

Where B1 is the final biomass dried after extractives extraction (g) and B2 initial dry biomass before extractives extraction (g)

b) Determination of Alcohol Extractive

1 g of dried Sorghum raw water extractive was macerated with 100 ml alcohol in closed flask for 6 h in the Soxhlet extractor set up. It was filtered rapidly taking precaution against loss of alcohol. After filtration, samples were dried at 100^0C. The percentage of residual alcohol extractive was calculated by measuring weight.

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3) Neutral Detergent Fiber (NDF) Estimation (Hemicellulose, Cellulose, Lignin, Minerals)

About 1 g of dried Sorghum raw material (pre-treated extractive) was refluxed in a 250 mL round bottom flask containing neutral detergent solution, 2 mL of decahydronaphthalene and 0.5 g of sodium sulphite.

After 1 h reaction materials were filtered in a crucible and washed thrice with hot distilled water. Finally, the materials were washed with acetone and dried for 8 h at 100^0C and cool the crucible in a desiccator.

To get NDF value crucible was weighed and calculated using the following formula:

$NDF = [Wt_{Crucible+residues}-Wt_{Crucibils}/1(Intial\ Weight)]$

4) Acid Detergent FIber (ADF) estimation

In the NDF obtained Solid fraction was transferred in a 250 mL round bottom flask containing 100 mL acid detergent solution, the contents were refluxed for 60 min from the onset of boiling. The materials were filtered, washed three times with hot distilled water and finally washed with acetone after reaction. The sample was dried overnight at 100° C and weighed.

$ADF = [Wt_{Crucible+residues}-Wt_{Crucibils}/1(Intial\ Weight)\ X\ 100/0.9]$

5) Estimation of Hemicelluloses

Difference between NDF and ADF gave the amount of hemicelluloses present in the pre- treated sorghum raw materials.

Hemicelluloses = NDF-ADF

6) Estimation of Cellulose

The ADF solid residues were transferred in a crucible and half filled with 72% H_2SO_4, material was stirred with glass rod to wet the material completely. After 3 h, H_2SO_4 was removed by suction and the residue was washed thoroughly with hot distilled water. The crucible was dried overnight at 100° C in hot air oven and weighed. Cellulose was estimated as the loss in weight from ADF residues.

Cellulose=ADF-Residues after extracted with 72% H_2SO_4

The % cellulose was determined by using formula:

% cellulose = *ADF-Residues after extracted with 72% H_2SO_4 / Weight of sample taken X 100*

7) Estimation of Lignin and ASH

Solid residues after extracted with 72% H_2SO_4 was ashed in the crucible for 3 h at 550 C and cooled keeping overnight. Loss in weight upon ashing gives the lignin content.

Lignin = Residues after extracted with 72% H_2SO_4 - Ash

III. STATISTICAL ANALYSIS

All the experiments were carried out in duplicate and the average values were reported. The statistical analysis two-way ANOVA was applied to estimate whether it is statistically significant among the groups in analysis. The statistics were performed using simple excel software.

IV. RESULTS AND DISCUSSION

In the present study, acid and alkali pretreatments was done for Sorghum Brown Mid Rib 22, 24 and CSH 22 to achieve maximum cellulose yield and delignification, which results in digestion of biomass sample and production of fermentable sugars and ethanol production. Less lignin biomass will be good substrate for cellulolytic enzymes and application of pretreatment methods is easier as plants are softer (Bruce S et al. 2009). Hence in this study we used low lignin Sorghum varieties such as Brown Mid Rib 22, 24 and compared with wild type Sorghum Non-Brown Mid Rib CSH 22. An ideal chemical pretreatment remove hemicellulose and lignin, reduce the crystallinity of cellulose and increase hydrolysis of cellulose.

A. Composition of Sorghum Biomass (Untreated Sorghum Biomass)

The Untreated composition of Sorghum Biomass used in this study composition analysis values presented in Table 1. Cellulose content in the Sorghum *bmr-22, 24* and *CSH 22* were 54.12 ±6.25%, 69.25 ±7.14% and 36.63 ±5.93% respectively. Hemicellulose content in the Sorghum *bmr-22, 24* and *CSH 22* were 14.72 ± 3.25%, 16.89 ± 4.25% and 28.93 ± 3.78% respectively. Total lignin (including Acid Soluble Lignin and Acid Insoluble Lignin), which is the main non-carbohydrate component, was determined to be 9.4%, 9.2% and 23.1%. Moisture content and Ash content were more in the Sorghum *CSH 22* biomass compared to biomass of Sorghum *BMR-22*, 24 (Table.1). Sorghum mutant varieties *bmr- 22* and *24* would have the advantage of a lower industrial cost, because it has less lignin than other agricultural biomass and wood. In the current study, the composition of sorghum low lignin mutant varieties *bmr-22* and *24* was characterized and compared with Normal Sorghum varieties *csh-22*. But in the research there are gaps in our knowledge on recent developed low lignin sorghum hybrids biomass composition hence we selected these crops for composition analysis and studied acid and alkali pretreatment effects. In our knowledge this is may be first report on the use of Sorghum mutant*bmr-22* and *24*.

A pretreatment process is essential in order to make cellulose, hemicellulose, and lignin availability for bioethanol production by reducing cellulose crystallinity, and increase the porosity of the materials. Dilute-acid and alkali pretreatment has been successfully developed chemical process for lignocellulosic materials. Dependent on the substrate and the conditions used in the process up to 95% of the cellulose, hemicellulosic polysaccharides can be recovered by dilute-acid and alkali hydrolysis from the lignocellulosic feedstock (Chen Y et al. 2013; Zhu Y et al. 2005; Sun Y et al. 2005).

B. Effect of Pretreatment Conditions

Biomass of the Sorghum *bmr-22, 24* and *csh-22*pretreated with different concentrations of acid and alkali agents and their composition analysis studied using the TAPPI method. The effects of pretreatment of acid and alkali concentrations at different temperature and different time intervals on the solid recovery, cellulose, hemicellulose, acid insoluble lignin, acid soluble lignin and ash was studied in three varieties of Sorghum biomass. The pretreatment conditions were selected according to the literature (Chen Y et al. 2013; Zhu Y et al. 2005; Sun Y et al. 2005) and intensity of treatment had varying effects on composition of biomass. Pretreatment is essential for ensuring good ultimate yield of cellulose and to reduce total lignin. According tomany studies enzymatic hydrolysis with pretreatment raises sugar yield over 90% (Hamelinck C. N, 2005).

C. Effect of pretreatment on Solid recovery

After pretreatment of H_2SO_4 various concentrations at different temperature and time combinations solid recovery ranged between 65.12-83.04% for *bmr-22*, 64.12-82.84% for *bmr-24* and 64.58-85.59% for *csh-22*. Maximum solids observed for *csh-22* with 85.59+/-4.98% pretreated with 1% H_2SO_4 at 60 °C, 15 min combination, whereas, it was minimum 65.21 ± 5.22% in the sample pretreated with 5% H_2SO_4 at 120 °C, 60 min for *bmr-22* (Table 2 and 3).

Solid recovery after pretreatment of NaOH different concentrations at different temperature and time combinations ranged between 64.36-84.12% for *bmr-22*, 50.19-83.14% for *bmr-24* and 65.2-84.12% for *csh-22*. Maximum solids observed for *csh-22* with 84.12+/-5.85% pretreated at 120 °C, 60 min combination with 8% NaOH, whereas, it was minimum 64.36 ± 6.58 % in the sample pretreated with 5% H_2SO_4 at 120 °C, 60 min for *bmr- 22*. It was observed that lesser solids recovered as intensity of the H_2SO_4 and NaOH pretreatment increased (Table 2 and 3).

D. Extractives (%)

Extractives are unbounded chemical components include inorganic material, carbohydrates, aromatics hydrocarbons, phenols, lipids, fats, and waxes (Sluiter A et al. 2002). It can be extracted from lignocellulosic biomass using various solvents like water, ethanol, acetone, benzene, ether, toluene, and their mixtures (Sluiter et al. 2010; Chen S. F et al. 2010; Vassilev S. V et al. 2012).

According literatures high extractives content might affect Cellulose availability (Li, Z et al. 2016). In this study, H_2SO_4 and NaOH pretreated sorghum biomass extractives were removed by using water and alcohol. Water Extractives were more in untreated sorghum biomass compared to H_2SO_4 and NaOH pretreated biomass, 5.2 ± 0.35 % Water extractives were observed in untreated Sorghum *bmr*-24 and 3.5 ± 0.47% extractives were observed in same variety after pretreatment of both H_2SO_4 and NaOH (Table 1, 2 and 3). But alcohol extractives were nearly same percentage for untreated and pre-treated sorghum biomass (Tyskiewicz et al. 2019) of three varieties. Generally biomass have high phenolic compounds, alcohol significantly dissolve higher amounts of phenolic compounds compared to water may be because of this alcohol extractives percentage were more.

E. Effect of Pretreatment on cellulose yield

In this investigation we studied improved cellulose Yield by H_2SO_4 and NaOH pretreatment for low lignin Sorghum mutant varieties *bmr-22, 24* and wild type CSH-22. The amounts of cellulose yield in untreated, acid pretreated and alkaline pretreated values were given in Tables 1,5 and 6. The maximum cellulose yield were observed 93 ± 8.65% for *bmr-*24 at 6% NaOH pretreatment 100^0C temperature and 30min

reaction time follows 89 ± 6.97% observed for *bmr*-22 at 60^{0}C, 45 min reaction time min (Fig. 1 and 2) Using H_2SO_4 pretreatment 89 ± 6.58% maximum cellulose yield was observed at 5% 60°C 15 min for b*mr-22* follows 85% maximum for *bmr-24* at 4% 80^{0}c 30. From these results clearly understanding application of NaOH pretreatment is a promising method comparative H_2SO_4 pretreatment for *bmr-22, bmr-24* mutant Sorghum varieties.

NaOH pretreated cellulose yield ranged from 60%-89%, 67-93% for bmr-22 and bmr-24 respectively, which is high comparative to wild type csh-22 sorghum raw material 59-72% (Fig. 1a, 2a and 3a). Ammonium hydroxide pretreatment has been shown to be effective for processing Sorghum *bmr-6* and *12*. Bali G el al. 2015 observed highest increase in the cellulose accessibility in dilute sodium hydroxide in Populus. H_2SO_4 pretreated cellulose yield ranged from 61%-85%, 65-89% for *bmr-22* and *bmr-24* respectively, which is high comparative to wild type csh-22 sorghum raw material 61-76% (Fig 1b, 2b and 3b). Rocha et al. 2011 studied dilute mixed-acid pretreatment of sugarcane bagasse for ethanol production and observed 85.4% cellulose yield at 190^{0}C for 10 min.

Both pretreatment cellulose yields were high when compared to untreated 54.12 ±6.25%, 69.25 ±7.14%, 36.63 ±5.93% for bmr-22, bmr-24 and csh-22 respectively. *Kim* et al. 2006 acknowledged that the effectiveness of the pretreatment depends on the biomass source. For instance, due to the presence of guaiacyl lignin in softwood, a major effect of the pretreatment was observed for hardwood than for softwood (Ramesh et al. 1993; Fan, L.T et al. 2006a; Fan, L.T et al. 2006b; Dekui et al. 2013).

Increase of cellulose yields at high concentration low temperature, less reaction time observed in both pretreatments and also at low concentration high temperature and long-time reaction also increasing cellulose yields. So it is clearly indicating in pretreatment along with H_2SO_4 and NaOH concentrations pretreatment temperature and time also represent one of the most important issues to be considered.

To study the effect of temperature and time on cellulose yield, experiments were carried out at different temperatures ranging from 45 to 120^0 C and time 15-60min remaining parameters were maintained at their optimum values (Tables 5 and 6). There was an increasing trend in cellulose recovery and with respect to temperature and time, but after 100^{0}C and 45 min it was inverse. This may be due to the formation of more amount of free radical at higher temperature, which initiated the breakdown of covalent bonds leading to higher reaction rates after reaching 100^0 C and 45 min may be cellulose decompose into various low molecular weight compounds(C_{2-4} compounds or light gases) (Dekui et al. 2013). Very Short pretreatment

times and temperatures (15min and 45^{0}C) could be not enough to penetrate and transform the matrix.

The major obstacle for efficient enzymatic saccharification/hydrolysis is the crystallinity nature of cellulose fibers. Also, the largely lignified cell wall that surrounds the cellulose fiber reveals the cementing contribution of lignin and hinders the hydrolysis of cellulose (Fan et al. 2006a). Fan et al. (2006a), Fan et al. (2006b) reported that the hydrolytic enzyme attack on cellulose depends on its structural features, which includes: the crystallinity of the cellulose, the surface area, degree of polymerization, and the lignin seal surrounding the cellulose fibers, which leads to the structural resistance of cellulose (Jeoh, T et al. 2007).

F. Effect of Pretreatment on Hemicellulose

Hemicellulose content was measured after pretreatment of H_2SO_4 and NaOH at different concentrations, temperature and time combinations. After H_2SO_4 pretreatment, hemicelluloses content ranged between (Table 3) 6.5-9.8% for *bmr-22*, 6.4-10.1 for *bmr-24* and 16.5-21.9 for *csh 22* (Fig. 3a). The minimum hemicellulose content of 6.5 ± 4.12% and 6.4 ± 4.78% was observed when pretreated with 4% H_2SO_4, 60^{0}C 30 min for *bmr*-22for *bmr-24* respectively whereas, it was minimum 16.7 ± 3.78% for *CSH-22* in the sample pretreated with 5% H_2SO_4 at 80^{0}C, 45min.

After NaOH pretreatment, hemicelluloses content ranged between (Table 2) 9.4-10.4% for *bmr-22*, 9.3-10.9 for *bmr-* 24 and 19.4-23.28 for *csh 22* (Fig. 3b). The minimum hemicellulose content 9.4 ± 4.15% for *bmr-22* and 9.3 ± 3.93% *bmr-24* observed when pretreated with 8% NaOH 60^{0}C 30 min and 6% NaOH 80^{0}C 30 min respectively whereas, it was minimum 19.4 ± 3.62% for *csh 22* in the sample pretreated with 8% NaOH at 60 °C, 30min. NaOH solution may be hydrolyzes the acetyl units of the hemicellulose portions to form organic acids such as acetic and uronic acids. These acids act as catalysts in the depolymerization of hemicellulose, yielding xylan and restricted quantity of glucan (Chornet E et al. 1991).

In comparison with the 120^{0}C reactions, the 80^{0}C and 100^{0}C min reactions resulted in higher hemicelluose content for both acid and alkali concentrations values were presented in Table2 and 3. During the pretreatment process, increasing the temperature up to a certain amount (80-100^{0}C) can adequately release hemicellulosic sugars by hydrolysis of glycosidic bonds/linkages of hemicellulose. H_2SO_4 pretreatment processes result to lesser hemicellulose content unlike the NaOH pretreatment, as a result acid pretreatment is efficient method for hemicellulose degradation.

The aim of the pretreatment is to improve the yield of hemicellulose also not only cellulose yield. Hemicellulose is chemically bonded to lignin and it serves as an interface between the lignin and the cellulose (Ramesh et al. 1993; Jeoh, T et al. 2007) . Compared to cellulose, hemicellulose is a highly branched heteropolymer, consisting primarily of five- and six-carbon sugars. The main hemicellulose is xylose, which consists of a xylan as the main 19 chain (backbone) made up of β-1, 4-linked D-xylose units with the other groups mentioned above as branches, which could be utilized in the production of fuels and chemicals such as bioethanol[36].

Bruce S. Dien (2009) also worked on mutant sorghum varieties *bmr*-6 and *bmr*– 12, they also recovered 98.6±4.0% of the xylan at low-severity dilute-acid pretreatment (1.75% H_2SO_4, 121°C for 1 h). Zhu et al. (2005) studied Optimization of dilute-acid (H_2SO_4) pretreatment of corn stover and they reported that the digestibility and accessibility was related to the degree of xylan removal. The effect of increasing the concentration of sulphuric acid and residence time of the pretreatment process is significantly higher for Bermuda grass than rye straw for ethanol production (Sun Y et al. 2005). The effect of alkaline pretreatment is dependent on the lignin content of the biomass materials (Kim, S et al. 2006). Kumar et al. (2009) observed efficient alkaline pretreatment at low temperatures and relatively long period of time with high concentration.

G. Effect of Pretreatment on Total lignin

After pretreatment recovered Acid insoluble lignin using various concentrations of NaOH and H_2SO_4 at different temperature-time combinations ranged between 3.5-4.5%, 2.5-3.8% and 7.6-8.3%, 3.3-4.9%, 2.9-3.8% and 7.2- 8.9% for *bmr-22*, *24* and *CSH-22* respectively. The corresponding maximum Acid insoluble lignin reduction after NaOH pretreatment 7.9-9.2%, 6.7-8.9% and 16.7-18.8% after H_2SO_4 pretreatment 5.8-6.9%, 5.8-6.8%, 15.5-16.8% for *bmr-22*, *24* and *csh 22* respectively.

The maximum lignin reduction observed in NaOH pretreatment at 4% concentration 45 min, 60°C temperature duration in mutant sorghum *bmr*-22 and *24* but wild type maximum we observed at 8% 30min duration, 60°C temperature. The maximum lignin reduction observed in H_2SO_4 pretreatment at 2% concentration 45 min duration, 100°C temperature for mutant sorghum *bmr*-22 and 24 but wild type maximum we observed at 3 % 45 min duration, 80°C temperature. From this results understanding based on the lignin content in the biomass pretreatment conditions were changing. While increasing acid and alkali concentrations and reaction temperature, time of the process it is breaking alkyl-aryl linkages of lignin and it producing low molecular weight

fragments instead of producing sugars (Chornet E et al. 1999; Tanahashi, M et al. 1982). When compared to untreated both pretreatment conditions (acid and alkali) showing maximum lignin reduction so we can consider both pretreatment techniques were efficient.

Alkaline pretreatment indicated that it is more effective on agricultural biomass residues than on wood materials (Xu, F et al. 2007; Zhang, Y.H.P et al. 2008). According Zhang et al. (2008) rice straw pretreatment using 2% NaOH increased cellulose by 54.83% and decreased lignin by 36.24%. The digestibility of different structural polysaccharides was higher for NaOH pretreated straw than the native straw. However, at lower concentrations of alkaline solution, cellulose showed resistance to solubilization, but not at higher levels (above 7% w/w) (Zhang et al. 2008), we also observed at 6% NaOH maximum reduction of lignin with 3.5 ± 0.72, 2.9 ± 0.74% recovery of acid soluble lignin, 7.9 ± 2.45%, 7.2 ± 1.78% recovery of acid insoluble lignin for Sorghum *bmr*-22 and 24 respectively.

Studies have revealed that sulfuric acid below 4% (w/w) concentrations is usually the most interesting condition to effectively pretreat at low cost (Brecc, K.A et al. 1988), in this study also maximum reduction of lignin observed at 2 % H_2SO_4 with 3.3 %, 5.7% recovery of acid soluble lignin, 3.5%, 6.3% recovery of acid insoluble lignin for Sorghum *bmr*-22 and 24 respectively. Bruce S. et al. (2009) studied dilute-acid pretreatment effect on Sorghum *BMR*-6 and 12 and observed maximum reduction of lignin.

By advantage of low lignin in the *bmr* plants and the cost, duration of pretreatments can be reduced thereby decreasing the energy requirement for processing. Therefore Low lignin content sorgum brown mid rib 22, 24 biomass, and altered lignin composition by pretreatment, may increase conversion efficiency over their wild-type counterparts (Pedersen JF et al. 2008).

H. Effect of Pretreatment on ASH

Ash content also needs to be considered when optimizing the pretreatment conditions, in our results observed alkaline and acid pretreatment leads to increase in ash content when compared to untreated (Table 1, 2, and 3). Ash content were increased while increasing pretreatment concentrations of H_2SO_4 and also increasing temperature values were ranged between 3.8-4.9, 5.1-6.5, 5.1-6.5% for *bmr*-22, 24 and csh 22 respectively. Increasing acid concentration break major inter unit linkages β-O-4′, β-5′, and β- β linkages disappeared after pretreatment may these contents we observe in ash. Only insoluble inorganic matter is revealed by chemical analysis, whereas the acidic liquid medium during pretreatment dissolves

acid-soluble ash components (or in the aqueous sulphuric acid medium later during compositional analysis). According to Clementine et al. study (Chambon CL et al. 2019) presence of ash in the pulp did not show negative impacted on its enzymatic digestibility.

The ash content increased with the decreasing ratio of biomass to NaOH solution. This is primarily due to the high concentration of mineral content of sodium in the NaOH solution at lower ratio. This problem of increased ash content can be addressed by washing the pretreated samples until the pH reached around 7. Rai and Mudgal (1987) studied (Rai S.N et al. 1987) the effect of NaOH at six concentrations of 0, 3, 5, 7, 9, and 12% (w/w) on wheat straw. Similar ash content results were observed on barley and wheat straw in pretreatment of 1% and 2% NaOH in microwave (Kashaninejad M et al. 2011). Increased ash content with the lower the ratio of biomass to the NaOH solution may be due to the high concentration of sodium in the NaOH solution. The ratio of biomass to NaOH solution and temperature has significant effects on the ash content, even H_2SO_4 increasing concentration and temperature also showing significant effect on ash (Table2 and 3).

On a relative basis, the effect of reducing lignin content was less than that observed with the dilute-acid pretreatment. This reduced effect is likely due to the overall greater effectiveness of the alkali pretreatment.

CONCLUSION

To use the raw material efficiently optimization should be carried out and also it should be potential economic pretreatment process hence evaluation of low lignin content sorghum mutant varieties *bmr-22* and *bmr-24* highly beneficial for improving biomass conversion yields. Easy to set-up and potentially affordable route for the bio-fuel industry is represented by dilute acid and alkaline pretreatment techniques. According to this study we conclude that NaOH pretreatment played important role in decreasing the degree of polymerization of cellulose, H_2SO_4 pretreatment efficient to make available of hemicellulose and both pretreatment methods are good in removal of lignin and make available for enzymatic hydrolysis. But this requires further analysis and economic validation on ethanol yield using a promising alkaline and acid pretreatment.

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Table 1: Composition of Untreated Sorghum *bmr-22, bmr-24* and *csh-22*

Sorghum Variety	Solid Recovery (%)	Water Extractives (%)	Alcohol Extractives (%)	Cellulose (%)	Hemi Cellulose (%)	Acid Soluble Lignin (%)	Acid Insoluble Lignin (%)	Ash Content (%)
BMR 22	50 ±5.61	4.2 ± 0.42	6.8 ± 1.05	54.12 ±6.25	14.72 ± 3.25	8.22 ± 0.98	9.9 ± 1.82	3.5 ± 0.75
BMR 24	53+/-.8.34	5.2 ± 0.35	8.9 ± 1.82	69.25 ±7.14	16.89 ± 4.25	9. 09 ± 0.87	10.8 ± 2.15	2.9 ± 0.65
CSH 22	48+/-6.82	4.8 ± 0.49	7.7 ± 1.73	36.63 ±5.93	28.93 ± 3.78	10.16 ± 2.74	18.3 ± 2.63	5.8 ± 0.77

Table 2: Effect of NaOH and H$_2$SO$_4$ pretreatment on Cellulose yield for mutant Sorghum bmr-22

Temp/Time		Cellulose yield (%)									
		NaOH					H$_2$SO$_4$				
		2%	4%	6%	8%	10%	1%	2%	3%	4%	5%
60°C	15 M	68 ± 7.25	77 ± 7.98	69 ± 8.25	74 ± 6.35	69 ± 9.12	79 ± 7.44	69 ± 7.25	71 ± 7.55	79 ± 8.25	69 ±6.85
	30 M	72 ± 7.98	85 ± 8.25	72 ± 7.96	79 ± 6.89	68 ± 8.58	77 ± 6.98	74 ± 6.99	72 ± 8.25	77 ± 8.62	70 ±7.25
	45 M	66 ±6.28	89 ± 6.58	74 ± 6.58	70 ± 7.25	70 ± 7.44	69 ± 8.65	77 ± 7.25	69 ± 6.98	69 ± 7.88	73 ±8.66
	60 M	69 ±6.76	83 ± 7.25	80 ± 7.12	69 ± 7.88	66 ± 8.25	75 ± 7.25	73 ± 8.65	70 ± 7.45	75 ± 7.89	71 ±6.35
80°C	15 M	71 ± 8.12	81 ± 8.36	83 ± 7.58	74 ± 8.12	74 ± 7.96	78 ± 9.26	69 ± 7.84	68 ± 8.25	78 ± 6.98	74 ±9.12
	30 M	65 ±8.25	78 ± 7.52	87 ± 6.52	71 ± 6.99	73 ± 7.99	68 ± 9.12	75 ± 7.95	64 ± 8.62	85 ± 6.74	69 ±8.74
	45 M	72 ±6.58	82 ± 6.58	84 ± 7.64	69 ± 7.98	77 ± 6.98	63 ± 8.52	72 ± 6.99	65 ± 6.88	77 ± 9.35	66 ±7.95
	60 M	62 ±7.15	79 ± 7.44	79 ± 6.66	70 ± 7.85	70 ± 8.65	71 ± 7.98	74 ± 7.85	69 ± 9.12	79 ± 7.58	70 ±8.25
100°C	15 M	71 ±7.58	81 ± 8.69	77 ± 6.87	73 ± 6.85	73 ± 7.26	69 ± 6.99	69 ± 8.15	71 ± 7.84	75 ± 7.66	72 ±7.95
	30 M	78 ±6.59	77 ± 8.78	69 ± 7.45	71 ± 7.33	68 ± 7.98	78 ± 7.65	66 ± 8.65	74 ± 7.99	78 ± 8.25	68 ±7.64
	45 M	73 ±8.02	79 ± 6.98	75 ± 8.26	74 ± 8.12	66 ± 8.12	68 ± 8.15	70 ± 7.85	70 ± 8.65	68 ± 8.14	63 ±8.25
	60 M	69 ±7.49	75 ± 7.12	78 ± 8.69	69 ± 8.66	65 ± 8.65	63 ± 8.65	72 ± 7.99	73 ± 9.25	64 ± 9.21	71 ±8.64

120°C	15 M	68 ±6.25	78 ± 7.69	68 ± 7.25	66 ± 7.95	68 ± 7.98	71 ± 7.98	65 ± 6.98	68 ± 7.45	65 ± 7.63	78 ±7.93
	30 M	66 ±7.62	68 ± 7.58	63 ±7.85	70 ± 8.52	63 ± 8.45	86 ± 7.85	68 ± 6.85	66 ± 8.65	71 ± 8.25	74 ±7.15
	45 M	67 ±7.96	64 ± 6.85	71 ± 6.58	72 ± 7.65	61 ± 9.25	79 ± 6.85	63 ± 8.12	65 ± 7.88	69 ± 8.65	69 ±8.84
	60 M	65 ±6.98	65 ± 7.89	69 ± 6.98	65 ± 7.88	60 ± 8.98	69 ± 9.44	61 ± 8.25	68 ± 7.99	73 ± 7.63	66 ±8.99

Table 3: Effect of NaOH and H_2SO_4 pretreatment on Cellulose yield for mutant Sorghum bmr-24

Temp/Time		Cellulose yield (%)									
		NaOH					H_2SO_4				
		2%	4%	6%	8%	10%	1%	2%	3%	4%	5%
60°C	15 M	73 ±7.11	78 ±8.45	87 ± 7.52	85 ±7.58	79 ± 6.88	73 ± 7.55	81 ±8.25	72 ± 8.11	85 ±8.25	89 ± 7.15
	30 M	76 ± 8.65	74 ±7.15	83 ± 7.66	86 ±8.25	81 ± 9.22	70 ± 8.45	82 ±7.84	77 ± 7.52	86 ±7.82	78 ± 8.26
	45 M	81 ±9.25	78 ±7.25	74 ± 8.65	79 ±7.56	85 ± 7.58	75 ± 7.15	85 ±7.95	71 ± 7.82	78 ±7.85	83 ± 8.22
	60 M	83 ±7.48	78 ±8.65	79 ± 7.98	84 ±7.65	83 ± 7.65	69 ± 7.18	83 ±8.62	69 ± 8.62	80 ±8.25	79 ± 8.25
80°C	15 M	79 ±8.47	74 ±9.12	78 ± 8.25	87 ±8.25	79 ± 8.95	74 ± 7.99	80 ±8.25	78 ± 7.84	75 ±9.12	80 ± 8.62
	30 M	82 ±8.77	75 ±7.48	89 ± 8.66	85 ±9.22	80 ± 8.55	70 ± 8.95	75 ±7.15	75 ± 9.24	78 ±7.25	79 ± 7.94
	45 M	79 ±7.25	88 ±6.98	90 ± 7.91	79 ±7.78	82 ± 8.74	69 ± 9.52	78 ±7.92	79 ± 9.62	68 ±7.95	78 ± 7.82
	60 M	81 ±7.63	87 ±7.45	86 ± 7.28	74 ±7.98	79 ± 7.49	70 ± 7.83	78 ±7.92	73 ± 7.85	64 ±7.15	74 ± 7.15
100°C	15 M	77 ±8.54	79 ±8.62	88 ± 9.21	82 ±8.95	75 ± 7.96	73 ± 7.49	74 ±8.15	75 ± 7.84	65 ±8.33	79 ± 7.94
	30 M	79 ±7.12	85 ±8.52	93 ± 8.65	78 ±8.25	76 ± 8.66	73 ± 8.92	75 ±8.52	76 ± 7.26	71 ±8.48	68 ± 8.26
	45 M	75 ±7.88	81 ±7.88	89 ± 7.15	74 ±9.65	78 ± 7.55	71 ± 7.82	71 ±7.91	72 ± 7.92	69 ±7.95	66 ± 8.74
	60 M	78 ±7.98	78 ±8.65	78 ± 7.84	75 ±8.47	76 ± 7.95	69 ± 7.39	79 ±7.99	74 ± 8.25	83 ±8.25	65 ± 9.15
120°C	15 M	78 ±8.65	74 ±8.77	79 ± 8.48	72 ±7.99	75 ± 8.59	68 ± 8.91	73 ±8.61	78 ± 8.96	80 ±7.16	78 ± 8.15
	30 M	83 ±7.99	77 ±7.88	78 ± 8.94	76 ±8.15	69 ± 8.52	78 ± 8.47	72 ±8.22	76 ± 7.48	75 ±7.93	74 ± 8.95
	45 M	89 ±6.97	75 ±7.92	88 ± 7.55	73 ±8.55	68 ± 7.84	77 ± 8.22	69 ±7.88	70 ± 7.92	78 ±9.15	77 ± 9.15
	60 M	79 ±7.98	74 ±7.15	77 ± 7.98	71 ±6.98	67 ± 7.15	69 ± 7.95	70 ±6.92	69 ± 7.82	70 ±8.25	79 ± 7.98

Table 4: Effect of NaOH and H_2SO_4 pretreatment on Cellulose yield for wild type Sorghum csh-22

Temp/Time		Cellulose yield (%)									
		NaOH					H_2SO_4				
		2%	4%	6%	8%	10%	1%	2%	3%	4%	5%
60°C	15 M	68 ± 7.55	67 ± 7.44	69 ± 7.95	64 ± 8.15	69 ± 8.26	69 ± 8.26	68 ± 7.26	71 ± 8.62	69 ± 7.85	68 ± 9.26
	30 M	62 ± 7.29	65 ± 8.44	70 ± 8.25	59 ± 8.62	67 ± 8.25	67 ± 8.32	64 ± 7.28	73 ± 8.25	71 ± 7.95	67 ± 8.25

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	45 M	66 ± 7.84	63 ± 7.52	64 ± 8.75	60 ± 9.25	72 ± 7.15	69 ± 7.92	69 ± 8.62	69 ± 7.16	70 ± 8.15	66 ± 7.15
	60 M	68 ± 8.95	62 ± 7.85	70 ± 7.58	59 ± 7.15	66 ± 8.62	74 ± 7.93	63 ± 8.49	70 ± 8.26	65 ± 8.52	61 ± 8.25
80°C	15 M	60 ± 7.59	59 ± 8.15	63 ± 7.62	64 ± 7.92	64 ± 7.15	68 ± 8.61	68 ± 8.96	68 ± 9.12	68 ± 8.16	64 ± 8.26
	30 M	65 ± 8.62	67 ± 9.66	71 ± 7.19	61 ± 8.33	63 ± 7.92	69 ± 7.89	65 ± 8.66	76 ± 7.15	69 ± 8.14	69 ± 8.96
	45 M	62 ± 8.16	66 ± 8.25	64 ± 8.92	59 ± 8.25	67 ± 8.19	73 ± 8.95	62 ± 7.91	75 ± 7.18	67 ± 7.16	66 ± 8.11
	60 M	62 ± 6.95	65 ± 7.15	69 ± 7.49	60 ± 8.18	70 ± 7.28	71 ± 7.18	60 ± 7.29	69 ± 7.39	69 ± 7.85	68 ± 7.82
100°C	15 M	59 ± 7.11	68 ± 7.85	67 ± 7.29	67 ± 8.72	71 ± 7.16	69 ± 9.24	59 ± 8.28	72 ± 7.82	65 ± 7.96	67 ± 8.93
	30 M	66 ± 7.62	67 ± 9.52	69 ± 8.92	61 ± 8.92	68 ± 9.16	68 ± 7.88	66 ± 8.92	73 ± 7.19	68 ± 7.88	68 ± 9.16
	45 M	63 ± 8.62	63 ± 8.65	70 ± 8.35	64 ± 8.19	69 ± 8.18	67 ± 8.25	60 ± 9.72	70 ± 8.63	69 ± 7.82	63 ± 8.18
	60 M	68 ± 8.85	65 ± 8.15	68 ± 7.58	59 ± 7.95	66 ±. 7.92	63 ± 7.85	62 ± 7.19	71 ± 8.24	65 ± 8.63	62 ± 7.19
120°C	15 M	67 ± 6.98	60 ± 7.81	67 ± 8.62	66 ± 7.82	65 ±.36	71 ± 8.16	65 ± 7.29	68 ± 7.29	64 ± 8.16	70 ± 8.36
	30 M	69 ± 7.98	58 ± 7.93	63 ± 9.25	60 ± 8.62	63 ±9.17	75 ± 8.98	68 ± 7.82	67 ± 9.36	70 ± 8.17	64 ± 8.72
	45 M	67 ± 6.88	59 ± 7.92	65 ± 7.19	62 ± 8.25	61 ±8.28	68 ± 8.24	63 ± 8.16	65 ± 7.21	69 ± 8.39	69 ± 9.15
	60 M	65 ± 7.95	55 ± 7.16	69 ± 7.95	65 ± 8.18	60 ±8.16	69 ± 7.18	61 ± 8.92	67 ± 7.88	63 ± 7.99	66 ± 8.25

Table 5: Effect of NaOH pretreatment on Composition of Sorghum *bmr-22*, *bmr-24* and *csh-22*

|	BMR 22					BMR 24					CSH 22				
NaOH	2%	4%	6%	8%	10%	2%	4%	6%	8%	10%	2%	4%	6%	8%	10%
	100°C /30min	60°C /45min	80°C /30min	60°C /30in	80°C /45min	120°C/45 min	80°C /45min	100°C /30min	80°C /15min	60°C /45min	120°C/30 min	100°C /15min	80°C /30min	100°C /15min	60°C / 45 min
Solid Recovery (%)	79.18 ± 5.24	84.12 ± 4.52	68.25 ± 5.96	65.25 ± 4.85	64.36 ± 6.58	81.59 ± 6.41	83.14 ± 5.77	75.85 ± 5.79	71.45 ± 4.85	65.28 ± 6.25	84.12 ± 5.85	79.58 ± 5.47	75.52 ± 4.75	69.58 ± 4.95	65.52 ± 5.85
Water Extractives (%)	1.5 ± 0.45	2.9 ± 0.52	1.8 ± 0.54	2.5 ± 0.36	2.6 ± 0.42	2.8 ± 0.38	2.7 ± 0.37	3.5 ± 0.47	2.9 ± 0.41	3.4 ± 0.38	1.5 ± 0.58	2.3 ± 0.57	1.8 ± 0.32	2.2 ± 0.35	1.9 ± 0.47
Alcohol Extractives (%)	8.6 ± 1.02	9.5 ± 1.22	7.9 ± 1.42	6.8 ± 0.98	7.7 ± 2.25	7.7 ± 1.87	7.9 ± 1.82	8.2 ± 2.03	6.8 ± 2.31	7.6 ± 1.98	7.8 ± 1.72	8.2 ± 1.83	7.7 ± 1.77	6.9 ± 1.37	7.9 ± 2.03
Hemi-Cellulose (%)	10.4 ± 3.25	9.6 ± 3.55	10.5 ± 4.25	9.4 ± 4.15	11.53 ± 4.25	10.9 ± 4.72	9.9 ± 3.78	9.3 ± 3.93	9.9 ± 4.35	10.5 ± 3.75	23.28 ± 3.99	22.9 ± 3.74	20.6 ± 3.37	19.4 ± 3.62	21.9 ± 3.18
Acid Soluble Lignin (%)	4.3 ± 0.53	3.9 ± 0.68	4.5 ± 0.57	3.5 ± 0.72	3.9 ± 0.63	2.5 ± 0.72	3.8 ± 0.66	2.8 ± 0.85	2.9 ± 0.74	3.7 ± 0.69	8.3 ± 2.12	7.8 ± 2.54	8.1 ± 3.16	7.9 ± 2.85	7.6 ± 2.79

Acid Insoluble Lignin (%)	9.2 ± 1.58	8.8 ± 1.85	8.5 ± 2.02	7.9 ± 2.45	9.1 ± 1.85	8.6 ± 2.75	8.9 ± 2.85	7.2 ± 1.78	7.3 ± 2.42	6.7 ± 1.99	16.8 ± 1.76	18.8 ± 1.85	17.2 ± 2.14	18.5 ± 2.45	16.7 ± 2.45
Ash Content (%)	5.1 ± 0.58	4.8 ± 0.52	4.3 ± 0.72	4.9 ± 0.68	3.5 ± 0.99	3.4 ± 0.98	3.6 ± 1.11	3.9 ± 1.14	3.4 ± 1.02	3.6 ± 1.15	7.2 ± 0.99	6.5 ± 0.75	6.6 ± 0.85	6.9 ± 0.67	6.8 ± 0.79

Table. 6: Effect of H_2SO_4 pretreatment on Composition of Sorghum *bmr-22, bmr-24* and *csh-22*

|	BMR 22					BMR 24					CSH 22				
H_2SO_4	1%	2%	3%	4%	5%	1%	2%	3%	4%	5%	1%	2%	3%	4%	5%
Temp/Time	120°C /30 min	60°C /45min	100°C /30min	80°C /30min	120°C /15min	120°C /30min	60°C /45min	80°C /45min	60°C /30min	60°C /15min	120°C /30min	60°C /45min	80°C /30min	60°C /30min	120°C /15min
Solid Recovery(%)	81.89 ± 5.66	83.04 ± 4.85	78.52 ± 4.85	71.68 ± 6.58	65.21 ± 5.22	82.84 ± 5.74	81.74 ± 4.77	77.58 ± 5.82	72.59 ± 4.92	64.12 ± 4.77	85.59 ± 4.98	82.25 ± 5.66	75.87 ± 6.58	71.58 ± 5.98	64.58 ± 4.85
Water Extractives (%)	2.4 ± 0.42	1.7 ± 0.39	2.2 ± 0.49	2.9 ± 0.39	1.9 ± 0.52	1.7 ± 0.48	2.6 ± 0.58	3.5 ± 0.68	2.9 ± 0.57	1.8 ± 0.43	2.7 ± 0.39	1.9 ± 0.47	2.5 ± 0.52	2.9 ± 0.48	1.8 ± 0.38
Alcohol Extractives (%)	6.5 ± 1.25	7.8 ± 1.72	6.4 ± 1.93	8.7 ± 1.27	8.8 ± 1.83	6.6 ± 1.38	8.2 ± 2.35	7.7 ± 0.99	6.9 ± 1.73	7.1 ± 1.79	8.3 ± 1.74	7.7 ± 1.84	6.9 ± 1.73	8.2 ± 1.88	7.8 ± 1.83
Hemi cellulose(%)	9.8 ± 3.77	8.9 ± 3.25	6.9 ± 4.25	6.5 ± 4.12	7.7 ± 3.72	10.1 ± 3.25	9.8 ± 4.52	7.2 ± 3.85	6.4 ± 4.78	7.9 ± 4.36	21.9 ± 3.78	19.6 ± 3.95	18.8 ± 4.25	17.1 ± 4.78	16.7 ± 3.78
Acid Soluble Lignin (%)	4.2 ± 0.69	3.3 ± 0.72	4.3 ± 0.57	3.9 ± 0.82	4.9 ± 0.74	3.8 ± 0.82	3.5 ± 0.72	3.3 ± 0.69	2.9 ± 0.59	2.8 ± 0.85	8.5 ± 2.12	8.9 ± 3.25	7.2 ± 2.78	7.9 ± 2.72	8.3 ± 3.12
Acid Insoluble Lignin (%)	5.9 ± 1.25	5.7 ± 1.63	6.2 ± 1.85	5.8 ± 2.36	6.9 ± 2.14	6.4 ± 2.45	6.3 ± 2.63	5.8 ± 1.24	6.8 ± 1.85	5.9 ± 2.15	16.5 ± 2.02	15.5 ± 2.48	15.8 ± 2.44	16.8 ± 2.08	16.7 ± 1.85
Ash Content(%)	3.8 ± 0.59	4.2 ± 0.86	4.1 ± 0.75	4.4 ± 0.99	4.9 ± 1.14	5.1 ± 1.10	5.4 ± 0.89	6.5 ± 0.92	5.2 ± 0.88	6.8 ± 1.14	7.1 ± 0.93	5.5 ± 1.14	6.9 ± 0.98	5.6 ± 0.87	6.8 ± 0.83
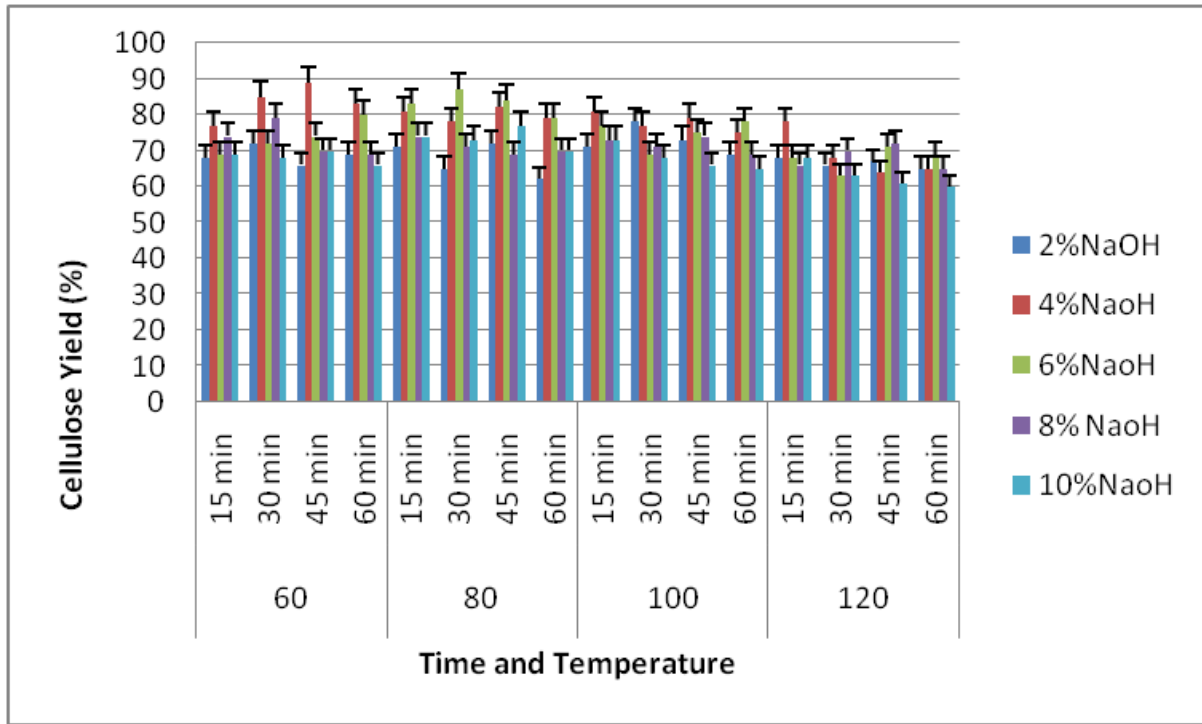
(a)
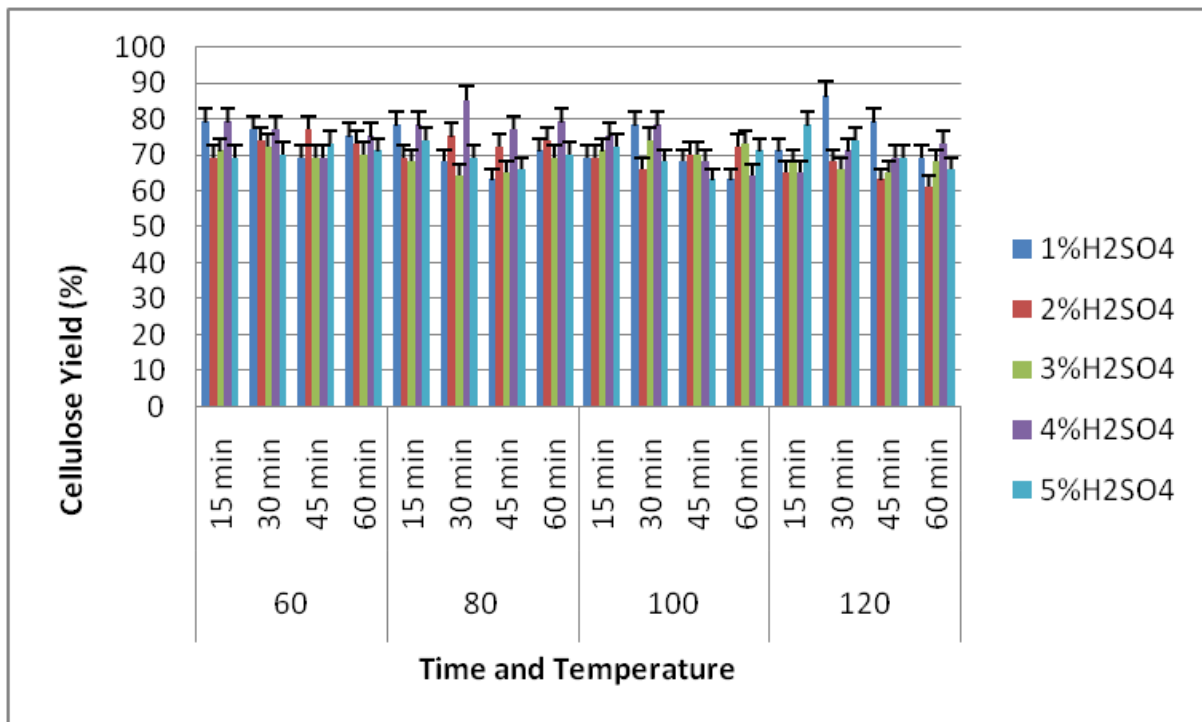
(b)

Figure 1: a) Cellulose yield after NaOH pretreatmentfor Sorghum *bmr-22*
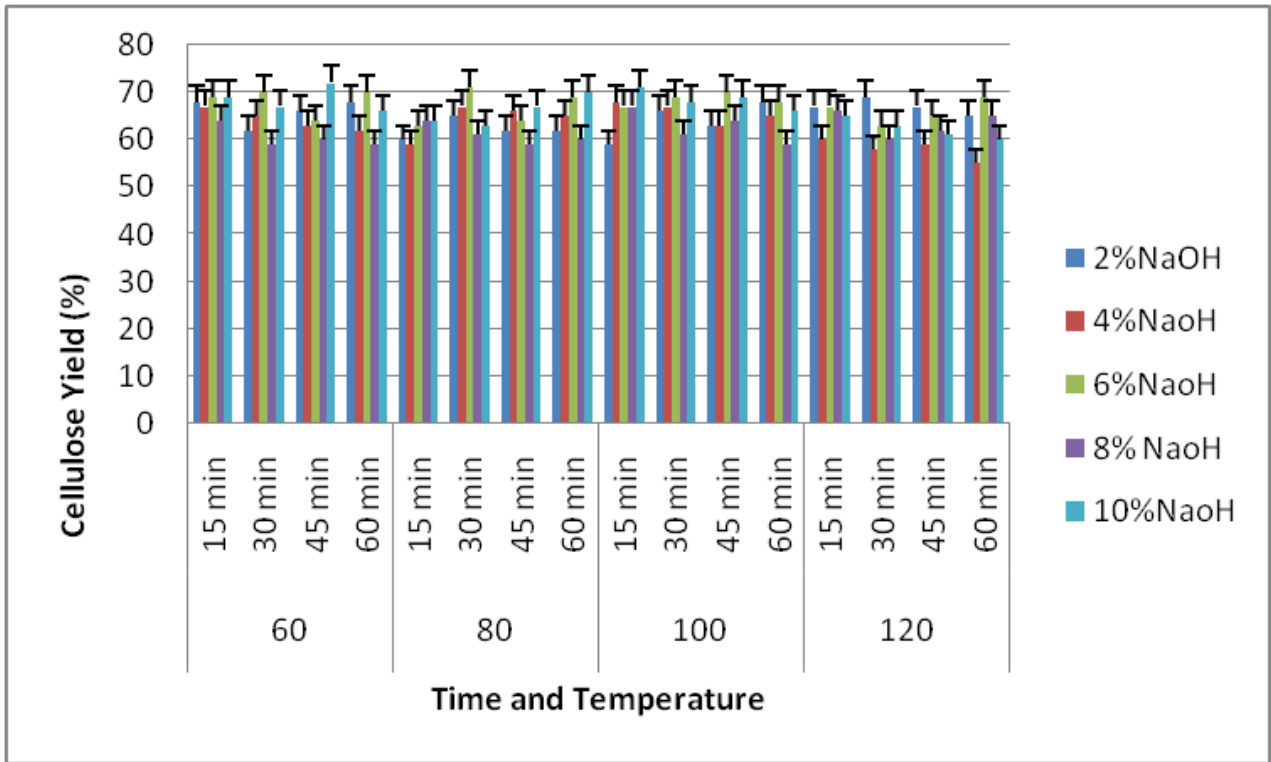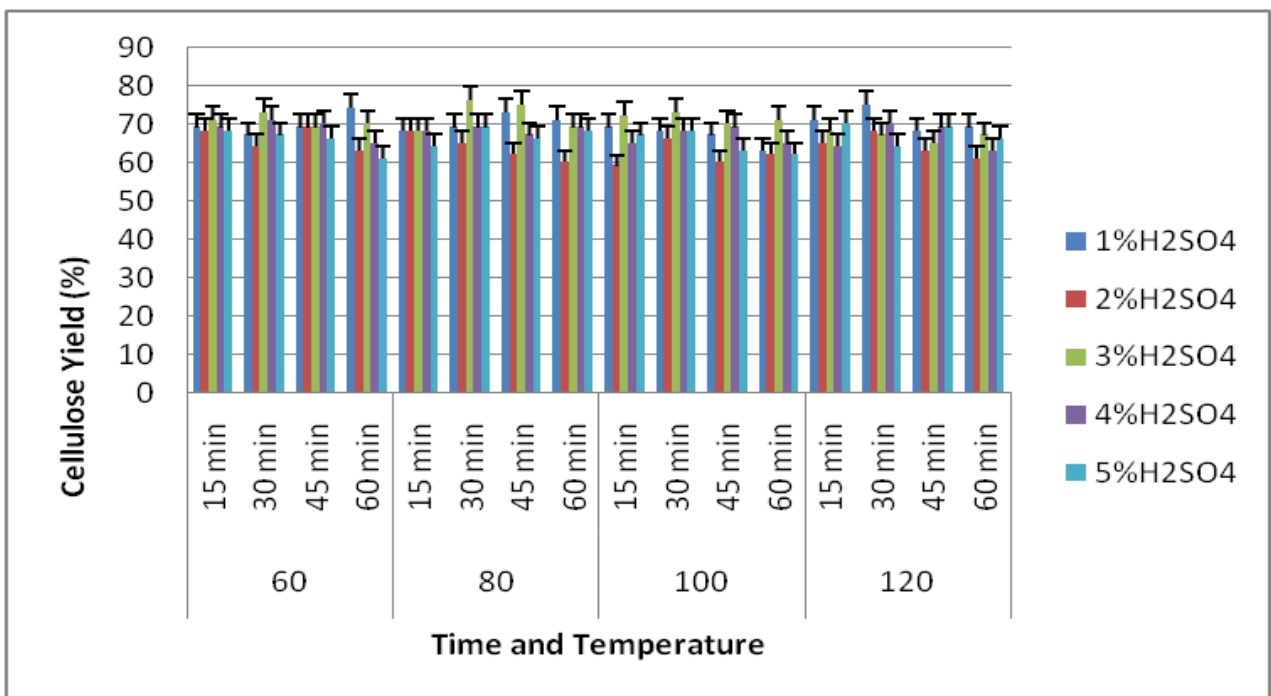b) Cellulose yield after H_2SO_4pretreatment for Sorghum *bmr-22*
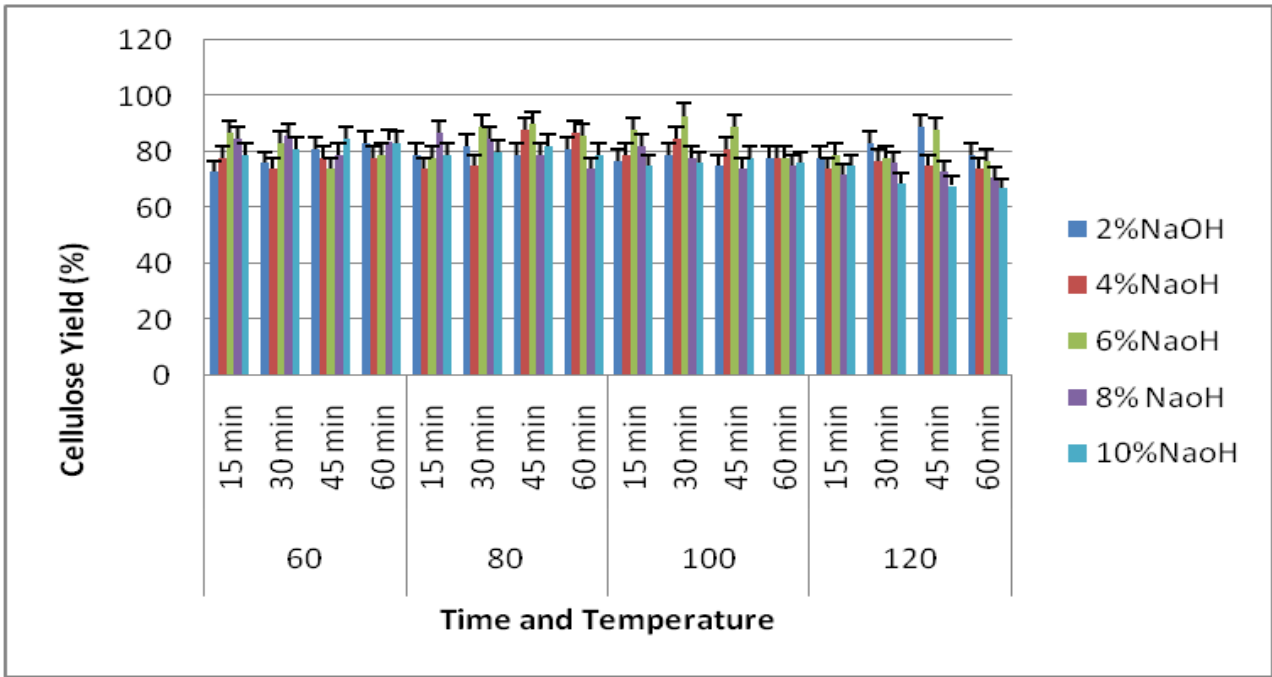
(a)
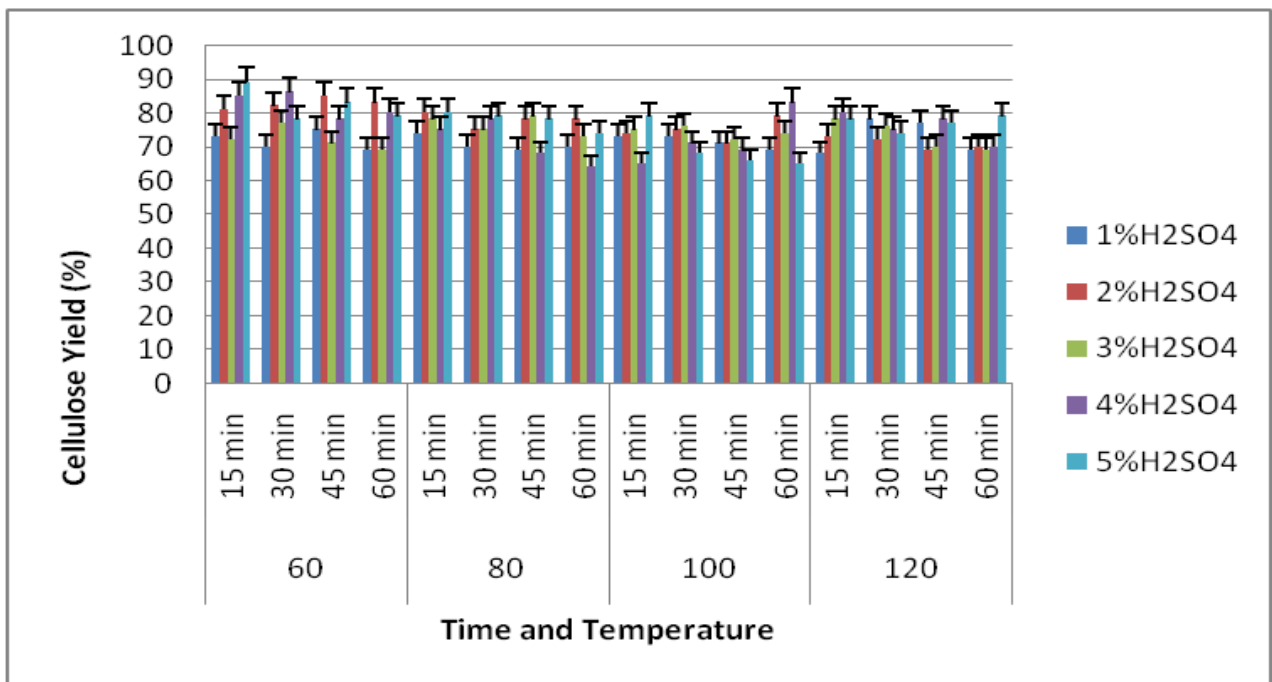
(b)

Figure 2: a) Cellulose yield after NaOH pretreatment for Sorghum *bmr-24*
b) Cellulose yield after H_2SO_4 pretreatment for Sorghum *bmr-24*

(a)

(b)

Figure 3: a) Cellulose yield after NaOH pretreatment for Sorghum *csh-22*
b) Cellulose yield after H_2SO_4 pretreatment for Sorghum *csh-22*

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