DOI: https://doi.org/10.55302/JAFES18721163n

EFFECTS OF DIFFERENT CHEMICAL PRETREATMENTS ON CELL WALL COMPOSITION AND ASH CONCENTRATION OF SWEET SORGHUM BAGASSE FOR BIOETHANOL PRODUCTION

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Abstract

Pretreatment is one of the key processes in lignocellulosic bioethanol production, which is needed to improve accessibility of enzymes to cellulose. This study was conducted to investigate the effects of different chemical pretreatments on cell wall composition and ash concentration of sweet sorghum bagasse. 9 different pretreatment methods used in the study can be categorized into 3 different methods such as dilute sulphuric acid (1, 1.5 and 2 % H_2SO_4 w/v), dilute sodium hydroxide (1, 1.5 and 2 % NAOH w/v) and sequential dilute sulphuric acid and sodium hydroxide (1 % H_2SO_4 w/v + 0.5 M NAOH, 1.5 % H_2SO_4 w/v + 0.5 M NAOH and 2 % H_2SO_4 w/v + 0.5 M NAOH). According to results, while 2 % H_2SO_4 w/v + 0.5 M NAOH gave the highest cellulose (91.51 %) and lowest lignin (1.7 %) concentrations, the lowest cellulose (65.11 %), hemicellulose (0.4 %), and highest lignin concentrations (23.42 %) were provided by 1.5 % H_2SO_4 w/v among pretreatments. Cellulose, hemicellulose and lignin contents of sweet sorghum bagasse after sodium hydroxide pretreatments ranged from 76.72 to 79.88, 11.75 to 14.62, and 2.05 to 4.11 %, respectively. The most appropriate cell wall composition for enzymatic hydrolysis was derived from sequential dilute sulphuric acid and sodium hydroxide pretreatments due to the fact that they provided the highest cellulose (90.68 – 91.51 %), lowest lignin (1.7 – 3.41 %) and desirable hemicellulose (1.10 – 1.82 %) contents. However, enzymatic hydrolysis must be done to learn which method enables the highest fermentable sugar production.

Keywords: Lignin, cellulose, hemicellulose, biomass.

Introduction

The inevitable depletion of fossil fuel sources and their adverse effects on environment, particularly greenhouse gas emissions has strengthened the interest in renewable energy sources (Hahn-Hagerdal et al. 2006; Chen et al. 2012; Dogaris et al. 2012). Among renewable energy sources, advanced biofuels derived from lignocellulosic biomass such as agricultural residues, forest products, and energy crops are the potential resources for the production of second generation ethanol reducing substantially carbon emissions (Liu et al. 2008; Arora et al. 2010; Aita et al. 2011). The main components of lignocellulosic biomass are two structural carbohydrates (cellulose and hemicellulose) and lignin (Sipos et al. 2009). Cellulose and hemicellulose can be hydrolyzed to fermentable sugars by enzymes prior to microbial fermentation but lignin is highly resistant to deconstruction and restricts enzymatic hydrolysis because of its intricate structure (Aita et al. 2012; Cao et al. 2012). Hemicellulose and lignin form a physical barrier which avoids enzymes to access cellulose (Qing and Wyman et al. 2011). Therefore, lignocellulosic biomass must be pretreated before enzymatic hydrolysis to remove lignin and/or hemicellulose thereby increase enzyme accessibility and cellulose degradation (Hendricks and Zeeman 2009; Zhang et al. 2010). For the sustainable lignocellulosic bioethanol production, pretreatment must be carry out in maximum efficiency because it covers approximately 30 – 40 % of the total processing cost (Eggeman and Elander 2005; Zhang et al. 2009; Alvira et al. 2010). Numerous pretreatment methods have been developed for improving hydrolysis of lignocellulosic biomass and categorized as mechanical (e.g., milling, grinding), thermal (e.g., steam explosion), chemical (e.g., acid, alkaline) and biological (e.g., fungi) processes or combinations of these methods (Aita et al. 2011; Cao et al. 2012; Chen et al. 2012). Among these, chemical pretreatments, usually performed by dilute acids (e.g., sulphuric acid, hydrochloric acid) and alkalines (e.g., sodium hydroxide, lime), have been found to be the most cost effective (Pandey et al. 2000; Barcelos et al. 2013). Dilute sulphuric acid (H_2SO_4) pretreatment enables conversion of hemicellulose to monomeric sugars and thereby disrupt the lignocellulosic composite material linked by covalent bonds, hydrogen bonds and van der Waals forces (Li et al. 2010; Shatalov and Pererira 2012). However, it can result in the formation of polysaccharide degradation products that are often inhibitory to downstream fermentation organisms and lower the overall sugar yields (Fengel and Wegener 1984; Ramos, 2003; Li et al. 2010). Dilute sodium hydroxide (NAOH) pretreatment increases internal surface of cellulose and decreases the degree of polymerization and crystallinity, which provokes lignin disruption (Taherzadeh and Karimi 2008; Gao et al. 2013). In comparison with the dilute acid, it does not cause corrosion and is more effective in solubilizing the lignin but have a limited effect on solubility of hemicellulose (Carvalheiro et al. 2008; Gao et al. 2013; Menezes et al. 2014). Apart from these, a combined process using sequential dilute acid and alkali pretreatment steps have received increasing attention as a promising strategy because it can remove largely of lignin and hemicellulose fractions (Weerasai et al., 2014). In this process, hemicellulose is eliminated by dilute acid pretreatment in the first stage, while second stage is carried out by dilute alkali pretreatment primarily for delignification (Gao et al. 2012). Sweet sorghum is an annual C_4 crop which can be adapted to warm and dry areas thanks to its high drought tolerance. Its juicy stalk has high concentrations of fermentable sugars, mainly sucrose, making it one of the most promising energy crops for first generation bioethanol production (Cao et al. 2012). Besides, sweet sorghum bagasse is a valuable feedstock for lignocellulosic bioethanol production due to its high concentrations of structural carbohydrates, which can be hydrolyzed to fermentable sugars. This study was carried out to investigate the effects of different chemical pretreatments on cell wall composition and ash concentration of sweet sorghum bagasse for bioethanol production.

Material and methods

Sweet sorghum was harvested at research and experimental area of Field Crops Department of Cukurova University, Adana, Turkey when grains were at a hard dough stage. Leaves, roots and panicles were removed by hand then stalks were crushed five times to extract the juice through a roller press. 1 kg bagasse sample was washed with distilled water at least three times to remove remaining soluble sugars in the stalk. Finally, it was dried in an oven at 65 °C until a constant weight was achieved then ground to pass through a 1 mm sieve. 9 different pretreatment methods used in the study can be categorized into 3 different groups such as dilute sulphuric acid (1, 1.5 and 2 % H_2SO_4, w/v), dilute sodium hydroxide (1, 1.5 and 2 % NAOH, w/v) and sequential dilute sulphuric acid and sodium hydroxide (1 % H_2SO_4, w/v + 0.5 M NAOH, 1.5 % H_2SO_4, w/v + 0.5 M NAOH and 2 % H_2SO_4, w/v + 0.5 M NAOH). Untreated bagasse was used as a control in the study. The experiment was arranged according to complete randomized plot design with 4 replications. In dilute sulphuric acid and sodium hydroxide pretreatments, 10 gr of dry bagasse samples were slurried with 100 ml 1, 1.5 and 2 % H_2SO_4 (w/v) and NAOH solutions in a 250 ml flasks and heated in an autoclave at 121 °C for 30 min. After treatments, each sample were washed three times with distilled water and dried at 65 °C until a constant weight was achieved. Sequential dilute sulphuric acid and sodium hydroxide pretreatments were carried out as two-stages, differently from the other pretreatments. In the first stage, 10 gr of dry bagasse samples were slurried with 100 ml 1, 1.5 and 2 % H_2SO_4 (w/v) solutions in in a 250 ml flasks, then samples were washed with distilled water and dried at 65 °C until a constant weight was achieved. In the second stage, dried samples were slurried in 0.5 M NAOH solutions with solid: liquid ratio of 1:20 g/ml (Barcelos et al., 2013), then heated in an autoclave at 121 °C for 30 min. After treatments, each sample were washed with distilled water and dried at 65 °C until a constant weight was achieved. Cell wall compositions of samples were determined by Van Soest

(1963) method. In addition, ash concentrations of samples were determined by Kutlu, (2008) method in the study. Variance analysis of experimental results were carried out using JMP 7.0 (SAS Institute, 1994) statistical software and least significant differences (LSD) test was used to test the differences among means.

Results and discussion

As shown in Table 1, DM (Dry matter) loss ranged from 41.99 to 76.54 %. The pretreatments significantly differed in terms of DM loss, with 1.5 % H_2SO_4 (w/v) + NAOH leading the highest DM loss (76.54 %), followed by 2 % H_2SO_4 (w/v) + NAOH (76.28 %) and 1 % H_2SO_4 (w/v) + NAOH (75.74 %). On the other hand, dilute H_2SO_4 pretreatments led to significantly higher DM loss (43.15 – 52.31 %) compared to dilute NAOH (41.99 – 48.73 %) pretreatments. These results were in accordance with findings of Lee et al. (2015) and E Silva et al. (2015). Lee et al. (2015) reported that while dilute H_2SO_4 pretreatments led to DM losses between 42.2 – 58.1 %, DM loss was increased by sequential dilute H_2SO_4 and NAOH pretreatment up to 71.5 % in corn stover. In addition, E Silva et al. (2015) reported that sequential dilute H_2SO_4 and NAOH pretreatment lead to significantly higher DM loss with of 35.3 % than dilute H_2SO_4 pretreatment with of 28.6 %.

Table 1. Effects of different pretreatment methods on DM loss, cell wall composition and ash concentration of sweet sorghum bagasse

Pretreatments	DM Loss (%)	Cellulose (%)	Hemi-cellulose (%)	Lignin (%)	Ash (%)
Untreated	-	44.98	24.81	12.98	1.94
1 % H_2SO_4 (w/v)	43.15 h	65.21 g	0.90 f	20.96 c	1.19 c
1.5 % H_2SO_4 (w/v)	49.82 h	65.11 h	0.40 g	23.42 a	1.63 b
2 % H_2SO_4 (w/v)	52.31 d	65.13 h	0.44 g	22.91 b	1.74 a
1 % NAOH (w/v)	41.99 ı	76.72 f	11.75 c	4.10 d	0.83 d
1.5 % NAOH (w/v)	46.61 g	77.89 e	14.63 a	2.07 g	0.79 d
2 % NAOH (w/v)	48.73 f	79.88 d	13.28 b	2.05 g	0.59 g
1 % H_2SO_4 + 0.5M NAOH (w/v)	75.74 c	91.21 b	1.82 d	2.49 e	0.70 e
1.5 % H_2SO_4 + 0.5M NAOH (w/v)	76.54 a	90.68 c	1.72 e	2.21 f	0.65 f
2 % H_2SO_4 + 0.5M NAOH (w/v)	76.28 b	91.51 a	1.80 d	1.70 h	0.67 ef
Mean	56.80	78.15	5.19	9.10	0.98

Significant differences were observed in cellulose concentration among pretreatments, ranging from 65.11 to 91.51 %. All pretreatments tested in the study increased cellulose concentration of sweet sorghum bagasse. The highest value was observed in 2 % H_2SO_4 (w/v) + NAOH, followed by other dilute H_2SO_4 and NAOH pretreatments. Differently from the DM loss, dilute NAOH pretreatments provided significantly higher cellulose concentrations than dilute H_2SO_4 pretreatments. Similar results also observed in previous comparative studies (Lee et al. 2015; E Silva et al. 2015). Lee et al. (2015) reported that cellulose concentration of corn stover achieved by sequential dilute H_2SO_4 and NAOH pretreatments was found between 80.4 – 81.5 % whereas H_2SO_4 pretreatments led the cellulose concentration between 43.1 – 53.0 %. In addition, E Silva et al. (2015) stated that sequential dilute 1.1% H_2SO_4 (w/v) and 0.5 M NAOH pretreatments increased the cellulose concentration of giant reed from 30.7 to up to 81.5 % whereas highest cellulose concentration derived by dilute H_2SO_4 pretreatments was found as 53.0 %. The hemicellulose concentrations after pretreatments ranged from 0.40 to 14.63 % in the present study. The highest value was achieved by 1.5 % NAOH (w/v) whereas the lowest was in 1.5 % H_2SO_4 (w/v). Dilute H_2SO_4 pretreatments decreased hemicellulose content of sweet sorghum bagasse between 96 – 98 %. Higher efficiency of dilute H_2SO_4 in removal of hemicellulose was also reported by previous authors in sugarcane (Barcelos et al. 2013; Jiang et al. 2013), sweet sorghum (Zhang et al. 2011) and bulbous canary grass (Pappas et al. 2014). Dilute H_2SO_4 pretreatments caused the significantly lower hemicellulose concentrations, when compared to the other pretreatments, indicating that they are more effective in hemicellulose solubilisation than the other pretreatments. This result is also supported by different authors (Weerasai et al. 2014; Lee et al. 2015; E Silva et al. 2015). Lee et al. (2015) reported that Dilute 1 % H_2SO_4 (w/v) pretreatment reduced hemicellulose concentration of switchgrass by 1.2 %, whereas sequential dilute 1 % H_2SO_4 (w/v) + 2 % NAOH (w/v) pretreatment reduced the hemicellulose concentration up to 5.5 %. NAOH pretreatments tested in the study provided the hemicellulose removal between approximately 41 – 53 %, which is comparable to reported by Cao et al. (2012) (45 %) in sweet sorghum and Wang et al. (2010) (41 %) coastal bermuda grass. The pretreatments were significantly differed in terms of lignin concentration, ranging from 1.70 - 23.42 %. While the highest lignin concentration was achieved by 1.5 % H_2SO_4 (w/v), the lowest was in 2 % H_2SO_4 (w/v) + NAOH. All dilute H_2SO_4 pretreatments significantly increased the lignin concentrations, differently from the dilute NAOH and sequential dilute H_2SO_4 and NAOH pretreatments. In spite of the fact that dilute acid pretreatments are generally more effective in extracting the cellulose and hemicellulose fractions than lignin, but only limited amount of lignin could be hydrolyzed compared to cellulose and hemicellulose because the lignin concentration was stabilized by a condensation reaction under acidic conditions (Ramos, 2003; Kim and Kim 2013; Lee et al. 2015). Similar to our results, previous authors also indicated that dilute H_2SO_4 pretreatment remarkably increased the lignin concentration of sugarcane (Barcelos et al. 2013), switchgrass (Li et al. 2010), corn stover (Lee et al. 2015) and sorghum (Zhang et al.2011; Wang et al. 2013). On the other hand, dilute NAOH and sequential H_2SO_4 and NAOH pretreatments led to considerable lignin removal in the study. Our results are associated with those of Xu et al. (2010), Cao et al. (2012), Kim and Kim (2013), Wang et al. (2013), Weerasai et al. (2014), Lee et al. (2015) and E Silva et al. (2015). Xu et al. (2010) reported that 0.5, 1 and 2 % (w/v) dilute NAOH pretreatments provided lignin reduction between 62.9 – 85.8 % in switchgrass, Cao et al. (2012) reported that 2 % dilute NAOH (w/v) pretreatments reduced the lignin from 10.8 to 1.68 % in sweet sorghum, Kim and Kim (2013) declared that 4 % H_2SO_4 (w/v) + 10 N NAOH pretreatment enabled the lignin reduction with the ratio of 70 % in empty palm fruit bunch fiber, Wang et al. (2010) stated that 3 % dilute NAOH (w/v) pretreatment decreased the lignin concentration of coastal bermuda grass from 19.33 to 2.82 %, Weerasai et al. (2014) reported that lignin concentration of rice straw was eliminated between 72 – 93 % by sequential dilute H_2SO_4 and NAOH pretreatments. Lee et al. (2015) reported that 12 different dilute H_2SO_4 pretreatments led to increase in lignin concentration of switchgrass from 14.2 to between 21.6 and 32.1 % whereas 2 % dilute NAOH (w/v) pretreatment after dilute H_2SO_4 pretreatment led to decrease lignin concentration up to 4 %. E Silva et al. (2015) reported that sequential dilute H_2SO_4 and NAOH pretreatment (1.1 % H_2SO_4 w/v + 0.5 M NAOH) reduced lignin concentration of giant reed from 18.49 to 10.05 % whereas 1.1 % H_2SO_4 (w/v) pretreatment increased lignin concentration up to 24.75 %. Lower ash concentration may be considered as an advantage, because biomass containing salts solubilize in the hemicellulose and cellulose hydrolysates during pretreatment. This increase in the concentration of ions leads to an increase in the osmotic pressure in the medium, hindering the fermentability of the generated hydrolysates (E Silva et al. 2015). Ash content of sweet sorghum bagasse ranged from 0.59 to 1.74 %. The pretreatments were significantly differed in terms of ash concentration, with 2 % H_2SO_4 (w/v) pretreatment producing the highest lignin concentration whereas the lowest was in 2 % NAOH (w/v). All pretreatments tested in the study decreased the lignin concentration of sweet sorghum bagasse. Our findings are in accordance with those of Jiang et al. (2013) in which dilute H_2SO_4 pretreatment reduced the ash concentration of sugarcane from 5.7 to 5.3 % and Weerasai et al. (2014) in which sequential dilute H_2SO_4 and NAOH pretreatment considerably decreased the lignin concentration of rice straw. Furthermore, our findings coincide with those of Wang et al. (2013) in which 0.5 % H_2SO_4 (w/v) pretreatment increased the ash concentration of sorghum from 2 to 4.6 %.

Conclusions

Sequential dilute H_2SO_4 and NAOH pretreatments provided the most appropriate cell wall composition for enzymatic hydrolysis among all pretreatments tested in the study, due to the substantially increased cellulose, and reduced lignin and hemicellulose concentrations. However, considerably higher DM loss (90.68 – 91.51 %) in these pretreatments may be a challenge for satisfactory fermentable sugar production from sweet sorghum bagasse during enzymatic hydrolysis. Therefore, enzymatic hydrolysis must be done to learn which method enables to the highest fermentable sugar production.

Acknowledgments

This study was funded by the Scientific Research Project Unit (BAP) of Cukurova University.

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