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Bioethanol Production from Foxtail Straw

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Abstract

Biofuel is a good substitute for fossil fuel as it is economical, renewable and environment friendly emitting about 90 per cent fewer greenhouse gases (GHGs) than gasoline. The long-term viability of bioethanol produced from first-generation feedstock is in question because it will ultimately lead to food insecurity. Therefore, to produce bioethanol, second-generation processes that include lignocellulosic materials are gaining importance. The current study aimed to produce the ethanol from lignocellulosic biomass such as Foxtail straw. The Foxtail straw was pretreated with different combination of acid and alkali to breakdown the cell wall composition of the straw. Then the pretreated samples were subjected to hydrolysis method such as, Simultaneous Saccharification and fermentation (SSF) and Separate hydrolysis and fermentation (SHF) using the fungi *Saccharomyces cerevisiae* with commercial enzyme. With different combination of acid and alkali, the combination of NaOH and H_2O_2 pretreatment was considered as the most suitable pretreatment because highest delignification (39%) was observed. NaOH alone and combination of NaOH and H_2O_2 with Simultaneous Saccharification and fermentation (SSF) method of hydrolysis and fermentation of Foxtail straw yields high ethanol of 11.31 g/L and 11.00 g/L, respectively, which is four times higher than the control. Significant structure and chemical bond changes in the feedstock after pretreatment were found.

Keywords : Bioethanol, Foxtail Straw, Simultaneous Saccharification and fermentation, Separate hydrolysis and fermentation, *Saccharomyces cerevisiae*

Production of biofuels from renewable energy sources is gaining importance in the light of the increase in dependency on non renewable resources due to the advancement of technology, depleting oil reserve, rising fossil fuel prices and increasing greenhouse effect associated with the use of fossil fuels. There are several advantages of biofuels such as environmental friendliness, biodegradability and high potential for local production from various feedstocks. There is a renewed interest in using sugar rich agricultural crops as feedstock for the biofuel production (Shalini *et al.,* 2019) Ethanol accounts for 90 per cent of total biofuels production and is used in different parts of the world. More over, global crude oil production is predicted to decline. Today, about 13 per cent of the total energy consumption is contributed by renewable energy, out of which bioenergy accounts for about 10 per cent. The energy content in solid, liquid and gaseous products derived from biomass is referred to as Bioenergy (IEA, 2010).

Bioethanol is a good substitute for fossil fuel as it is economical, renewable and environment friendly emitting about 90 per cent fewer greenhouse gases (GHGs) than gasoline. Bioethanol can be produced by the process of fermentation using different sugars such as starch, glucose, xylose, etc. The feedstock for bioethanol production is mainly divided into three categories, *i.e.,* starch-based, sugar-based (First generation) and lignocellulosic material (Second generation) (Choudhary *et al*., 2013).

India's National Policy on Biofuels, 2018 sets ambitious biofuel blending targets and aims to source biofuels only from sustainable feedstocks. Feedstocks are primarily defined as non-food feedstocks that do not threaten food security. Specifically, India intends to build upon its previous ethanol mandate by expanding ethanol blending to 20 per cent by 2030; the policy also adds a supplemental biodiesel mandate of 5 per cent.

The long-term viability of bioethanol produced from first-generation feedstock such as sugarcane and starch-rich feedstocks such as potato, corn, etc., is in question because it will ultimately lead to food insecurity by significantly increased dependence of amounts of cultivatable land and a significant hike in food prices. This indicated that first-generation biofuel is not sufficient to meet the global energy demand. Therefore, to produce bioethanol, second-generation processes that include lignocellulosic materials are gaining importance. The production of ethanol from lignocellulosic biomass such as agriculture residues (straw, cobs, wood chips, hull, sugarcane bagasse, etc.) has become one of the best alternatives because of their widespread abundance and the procurement cost is relatively economical (Joshi *et al.*, 2011).

Even though the plenty of lignocellulosic biomass, the commercialization of the bioethanol production process is limited due to insufficient research, related to minimization of the production cost. Bioethanol production from lignocellulosic materials relies on technologies that will efficiently hydrolyze cellulosic biomass to fermentable sugars (Joshi *et al.*, 2011).

One such residue is Foxtail straw which is available in plenty, unexplored and it is cheaper for large-scale production of bioethanol. The present investigation was undertaken to evaluate the potential of Foxtail straw in the production of bioethanol through different physicochemical pretreatment and hydrolysis methods.

Material and Methods

Collection and Preparation of Raw Material

The Foxtail straw (FS) was collected from the threshing yard of the zonal agriculture research station (ZARS), UAS, GKVK, Bengaluru. The collected FS cut into small pieces was shade dried and oven-dried (80ºC) for 48 hours. Then they were grounded and sieved using 2 mm sieve. They are stored at room temperature in air-tight bags for further use.

Characterization of Biomass

The composition of biomass was analyzed prior to the different pretreatment of FS. The FS was characterized for physicochemical properties such as cellulose, hemicellulose, lignin, ash content, carbon, nitrogen. The cellulose and hemicellulose were estimated by the procedure outlined by Fruedenburg (1955), Lignin was estimated by the procedure given by Pandey *et al.* (2007), the ash content determined by standardized method. The ADL was determined by the formula given by Raffrenato and Van Amburgh (2011) and the total carbon and nitrogen were estimated using the CN analyzer (LECO Truspec, USA 2009).

Pretreatments

The FS with solid loading 8 per cent (w/v) was pretreated with the following chemicals (Table 1). Samples were autoclaved for 1 hour at 121°C (at 15 psi pressure) and after the pretreatment, samples were filtered, solid part was collected, oven-dried and stored at room temperature in air-tight bags.

Inoculum Preparation

The *Saccharomyces cerevisiae* fungal culture was obtained from the Department of Agricultural Microbiology, UAS, GKVK, Bengaluru and maintained on MGYP medium (Composition: Malt extract 3g, Glucose 10g, Yeast extract 3g, Peptone 5g, Agar 20g, distilled water 1000 mL, pH to 4.4-4.6).

The Mysore Journal of Agricultural Sciences

TABLE 1

Pretreatments of feedstock

Pretreatments	Treatment	Concentration
Hydrogen peroxide	FSO1	5 % H_2O_2
	FSO2	10 % H_2O_2
Dilute alkali and hydrogen peroxide	FSN1O1	2 % NaOH + 5 % H_2O_2
	FSN1O2	2 % NaOH + 10 % H_2O_2
	FSN2O1	4 % NaOH + 5 % H_2O_2
	FSN2O2	4 % NaOH + 10 % H_2O_2
Dilute acid and hydrogen peroxide	FSH1O1	1 % H_2SO_4 + 5 % H_2O_2
	FSH1O2	1 % H_2SO_4 + 10 % H_2O_2
	FSH2O1	2 % H_2SO_4 + 5 % H_2O_2
	FSH2O2	2 % H_2SO_4 + 10 % H_2O_2
Dilute alkaline	FSN1	2 % NaOH
	FSN2	4 % NaOH
Dilute acid	FSH1	1 % H_2SO_4
	FSH2	2 % H_2SO_4
Control	FSC	Soaking in water for 24 hours

Fermentation

100 g of FS sample in 1 L fermenting bottle, 1:10 ratio of solution containing the media (composition : 3.5 gL^{-1} K_2HPO_4; 7.5 gL^{-1} $(NH_4)_2SO_4$; 0.75 gL^{-1} $MgSO_4.7H_2O$; 1 gL^{-1} $CaCl_2.2H_2O$; 5 gL^{-1} yeast extract) and citrate buffer (0.05M) were added, the pH was adjusted to 4.5 and autoclaved for 20 minutes at 121°C, 15 psi and cooled to room temperature. These samples were used for fermentation.

Separate Hydrolysis and Fermentation (SHF) : Before fermentation, the samples were hydrolyzed using 1 per cent v/v of commercial enzyme and maintained at 30°C. The samples were allowed for enzymatic hydrolysis for four days. After that, 10 per cent v/v inoculum was added and these bottles were incubated at 30°C for fermentation for seven days.

Simultaneous Saccharification and Fermentation (SSF) : 10 per cent v/v inoculum and 1 per cent v/v of the commercial enzyme, was added at a time and these bottles were incubated at 30°C for 12 days for fermentation.

Estimation of Ethanol

One ml of SHF and SSF samples drawn from each bottle and diluted with 35 ml of distilled water. Each sample was distilled at 70°C and the distillate containing alcohol was collected in 25 ml of 0.23 N $K_2Cr_2O_7$ solution, till a total volume of 45 ml was obtained. Similarly, ethanol standards (10-200 mg ethanol) were prepared separately using ethyl alcohol. These samples and standards were kept in water bath at 60°C for 30 min and were cooled, volume was made up to 50 ml with distilled water and optical density was measured at 600 nm using a spectrophotometer (Multiskan Sky, Thermoscientific). The standard curve was plotted considering the concentration against absorbance. From the standard graph, the amount of ethanol in the sample was calculated (Caputi *et al.,* 1968).

Statistical Analysis

The data were analyzed statistically for ethanol yield by factorial design using R software (version 4.1.0). Means for pretreatment and hydrolysis and fermenting methods were considered to be statistically significant at ($P<0.05$) level of significance.

RESULTS AND DISCUSSION

Raw Material Characterization

The Foxtail straw was characterized to determine the components of feedstock were presented in Table 2. Cellulose accounts for 35.9 ± 0.06

TABLE 2

Feedstock composition of Foxtail straw

Parameters	Composition (%)
Cellulose	35.9 ± 0.06
Hemicellulose	24.6 ± 0.95
Lignin	26.3 ± 0.20
Ash	17.7 ± 0.05
Total carbon	37.3 ± 0.07
Nitrogen	1.3 ± 0.01
C/N ratio	29.9 ± 0.32

per cent to the dry weight of raw material and hemicellulose content was found to be 24.6 ± 0.95 per cent of dry biomass. The presence of high holocellulose (Hemicellulose + Cellulose) content (60.5±1.01%) in the cell wall of Foxtail straw, provides a potential feedstock for bioethanol production. Foxtail straw contains 26.3±0.20 per cent lignin, 17.7±0.05 per cent ash content, 37.3±0.07 per cent of total carbon and 1.3±0.01 per cent of nitrogen content. The similar results were reported by Zhang *et al.* (2019) in millet straw *i.e.,* cellulose 36.68 per cent and lignin 19.38 per cent, hemicellulose content 17.3 per cent and ash content 4.5 per cent. The difference in the cell wall composition was due to heterogeneity in raw material, geographical location, season, processing methods and analytical methods used for chemical composition (Silverstein *et al.,* 2007 and Binod *et al.,* 2012).

Effect of Pretreatments on the Feedstock Composition of Foxtail Straw

The pretreatments were imposed on the Foxtail straw for the removal or to breakdown the lignin and hemicellulose to reduce the crystallinity of cellulose (Tan *et al.,* 2021). The suitable pretreatment and condition usually depend on the type of the lignocellulosic content present in the raw material (Taherzadeh and Karimi, 2008).

The composition of Foxtail straw after pretreatment was presented in the Table 3. The combination of pretreatment showed the highest content of cellulose (65.90%), ADL (24.8%), lignin content (10.80%) and low content of Hemicellulose were reported in pretreatment FSN2O1 (4% NaOH and 5% H_2O_2). The same trend was observed in all the pretreatment with NaOH and H_2O_2 combination. Fig. 1, shows the variation in the composition of Foxtail straw after pretreatment, that lower the lignin higher the cellulose content in all the pretreatment methods. Dilute NaOH pretreatment of the lignocellulosic material was found to cause swelling, leading to an increase in internal surface area of the Foxtail straw and rupture of lignin structure. Silverstein *et al*. (2007) reported that 2 per cent of NaOH in 90 min at 121°C was the best condition, resulting in 65 per cent of lignin removal.

TABLE 3

Feedstock composition of Foxtail straw after pretreatment

Pretreatments	Cellulose (%)	Hemicellulose (%)	ADL (%)	Lignin (%)	Ash (%)
FS_CONTROL	34.60 #	20.90 *	43.80 *	27.80 *	16.00 *
FSH1	50.70	4.00	39.40	25.40	14.00
FSH1O1	59.60	7.80	30.80	18.80	12.00
FSH1O2	46.50	6.40	35.60	23.60	12.00
FSH2	52.50	3.00 #	33.60	21.60	12.00
FSH2O1	48.10	4.90	35.00	25.00	10.00
FSH2O2	51.50	4.40	30.20	20.20	10.00
FSN1	64.80	6.70	28.40	18.40	10.00
FSN1O1	60.60	10.10	29.00	19.00	10.00
FSN1O2	53.10	5.60	30.80	16.80	14.00
FSN2	61.60	4.80	22.80 #	12.80	10.00
FSN2O1	65.90 *	5.30	24.80	10.80 #	14.00
FSN2O2	65.20	6.30	26.40	12.40	14.00
FSO1	52.80	14.20	25.40	23.40	2.00 #
FSO2	51.00	12.60	28.00	22.00	6.00

Note : * – Highest, # – Lowest

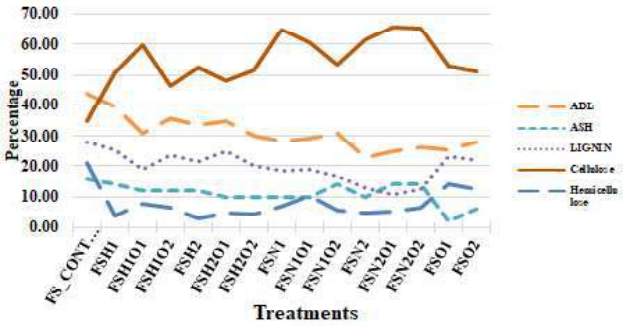

Fig. 1 : Variation in composition of Foxtail straw after pretreatment

In this study, all the pretreatments were conducted at 121°C, 15 psi for 60 min, which resulted in the removal of 52 per cent and 39 per cent, ADL and lignin, respectively in the pretreatment combination, of NaOH and H_2O_2. But the dilute NaOH pretreatment was beneficial for the enzymatic hydrolysis of crop residues, which was supported by the results of Keshav *et al.* (2016); Prabu & Murugesan (2011) and McIntosh & Vancov (2011). Thus, delignification increases the effectiveness of enzymes access to cellulose and hemicellulose for further saccharification process (Bensah and Mensah, 2013).

TABLE 4

Average ethanol yield from different pretreatments and fermentation methods of Foxtail straw

Pre-treatments	Average Ethanol Yield (g/L)		
	SSF	SHF	Average
FSN1	11.31 a	6.89 h	9.10 a
FSN1O1	11.00 b	5.04 s	8.02 b
FSN2O1	8.46 e	6.64 j	7.55 c
FSN1O2	9.86 c	4.71 v	7.29 d
FSN2O2	8.60 d	5.53 o	7.06 e
FSH2O1	6.04 m	7.80 f	6.92 f
FSN2	7.47 g	4.73 u	6.10 g
FSH1O1	5.25 q	6.75 i	6.00 h
FSH1	5.10 r	6.28 k	5.69 i
FSH2O2	6.24 l	4.86 t	5.55 j
FSH2	4.71 v	5.72 n	5.21 k
FSH1O2	5.43 p	2.21 z	3.82 l
FS_CONTROL	3.15 w	2.34 y	2.74 m
FSO2	1.91 A	3.15 w	2.53 n
FSO1	1.59 B	2.51 x	2.05 o
Average	6.41	5.01	5.71
STDEV	3.03	1.78	2.11
SE ±	0.78	0.46	0.54
CD @0.05%	0.01		0.01
Average	5.71		
STDEV	2.54		
SE ±	0.46		

Ethanol Yield from different Pretreatments and Fermentation Methods

The methods used for hydrolysis and fermentation of Foxtail straw after pretreatments were i) Simultaneous Saccharification and Fermentation (SSF), ii) Separate Hydrolysis and Fermentation methods (SHF). The ethanol yield was found to be significant in fermentation methods for all the pretreatments, but highest ethanol yield (11.31 g/L) was recorded in the SSF fermentation method of raw material which is pretreated with 2 per cent NaOH (FSN1) (Table 4) and average ethanol yield (9.10%), followed by the combination of NaOH and the H_2O_2, lowest ethanol yield was observed in the H_2O_2 alone, which is lesser than that of control (Table 4). Whereas in the SHF fermentation method highest ethanol yield (7.80 g/L) was recoded in raw material pretreated with 2 per cent H_2SO_4 and 5 per cent H_2O_2 (FSH2O1) and lowest was observed in the H_2O_2 alone pretreatment (Table 4). Similar results were observed in the sweet sorghum bagasse (6 .12 g/L) (Cao *et al.,* 2012) and higher ethanol production observed in the cotton stalk (23.17 g/L) (Keshav *et al.,* 2016 and Govumoni *et al.,* 2013). From the Table 5, it was clear that SSF method has highest

TABLE 5

Average ethanol yield from different fermentation methods of Foxtail straw

Fermentation methods	Ethanol (g/L)
SSF	6.41 a
SHF	5.01 b
Average	5.71
STDEV	0.99
SE ±	0.70
CD @0.05%	0.004

ethanol yield than the SHF method. Similar results were recorded by Zhu *et al.,* (2012); Alejo *et al.,* (2020).

Correlation of Compositional Parameters of Pretreated Samples to the Ethanol Yield from the different Fermentation Methods

The correlation of compositional parameters and the ethanol produced from the SSF and SHF is shown in Fig. 2. ADL and Lignin are positively correlated with each other (0.79), ADL and cellulose are negatively correlated (-0.81), Lignin and cellulose are highly negatively correlated (-0.859), Lignin and ethanol production in SSF are negatively correlated (-0.62) and Cellulose is positively correlated to both SSF and SHF ethanol yield (0.64 and 0.54, respectively). Higher the delignification more the cellulose and hemicellulose availability for the hydrolysis process sequentially higher the production of ethanol.

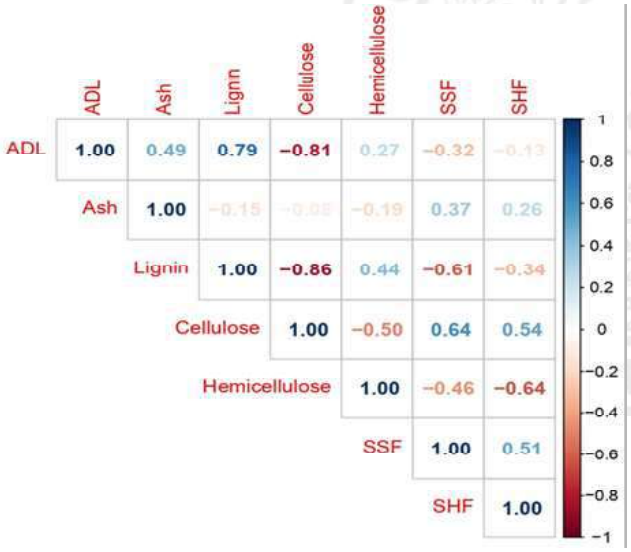

Fig. 2 : Correlation of compositional parameters and the ethanol yield from the different fermentation methods of Foxtail straw

In this study, the combination of NaOH and H_2O_2 pretreatment was considered as the most suitable pretreatment because highest delignification (39%) was observed. NaOH alone and combination of NaOH and H_2O_2 with simultaneous Saccharification and Fermentation (SSF) method of hydrolysis and fermentation of Foxtail straw yields high ethanol 11.31 g/L and 11.00 g/L, respectively, which is four times higher than the control. Significant structure and chemical bond changes in the feedstock after pretreatment were found. Further research is needed on improving the delignification and ethanol yield, as this work provides the foundation for future work.

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