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Research Article

PROCESS OPTIMIZATION OF SIMULTANEOUS SACCHARIFICATION AND FERMENTATION SYSTEM FOR BIOETHANOL PRODUCTION FROM PEARL MILLET STALK

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Abstract: Bioethanol can be produced from sugar and starch crops, but lignocellulosic biomass can also be utilized for bioethanol production. The physicochemical analysis pearl millet stalk was carried out. Pretreatment process optimization of the selected biomass was done with 7.5, 10 and 12.5 % of total solid loadings for all the biomass with the ortho-phosphoric acid concentration of 5, 7.5 and 10 % at 100˚C and 121˚C for 1, 2 and 3 h of time interval. With 12.5 % of total solid loading and 7.5 % of ortho-phosphoric acid at 121˚C the sugar release and lignin reduction were highest after 3 h of pretreatment. Hence, it was selected as the optimized conditions for ortho-phosphoric acid pretreatment. Pearl millet stalk released about 38.96 g l^{-1} of total sugar while the lignin content was 9 % at the optimized condition. The lab scale Simultaneous Saccharification and Fermentation (SSF) experiment was done with the hydrolysate alone, hydrolysate with artificial sugar (total sugar concentration of 60 g l^{-1}), hydrolysate with 10 % (w/v) of yeast extract. The two types of enzymes cellulase of 40 FPU g^{-1} and xylanase of 25 U ml^{-1} was used in all the treatments for saccharification. The two types of yeasts (*S.cerevisiae* and *P.stipitis*) were used for the optimization of fermentation. The ethanol yield was calculated as 32.13 g l^{-1} from pearl millet stalk after 96 h of fermentation with *S.cerevisiae* from the hydrolysate with added artificial sugar (total sugar concentration of 60 g l^{-1}). The sugar consumption was highest in pearl millet (58.48 g l^{-1}) in the condition 2 with *S.cerevisiae*. Hence, the hydrolysate with added artificial sugar with *S.cerevisiae* was selected for fermentation up to 96 h for all the biomass samples. The process parameters for SSF *viz*., temperature and agitation speed were optimized with the above treatment. The SSF experiment in the above optimized treatment with *S.cerevisiae* was done at 25, 30 and 35˚C. The three different agitation speed were used such as 75, 100 and 125 rpm for optimization. The highest ethanol concentration of was achieved from pearl millet stalk (44.24 g l^{-1}) at 30˚C with 100 rpm at 96 h compared to other temperatures and agitation speed. Hence, the optimized temperature and agitation speed selected were 30˚C and 100 rpm respectively for bioethanol production from pearl millet stalk.

Keywords: *Bioethanol, Pearl millet stalk, S.cerevisiae, Pretreatment, Simultaneous Saccharification and Fermentation*

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Introduction

Lignocellulosic feedstock is considered as an attractive raw material not only for the liquid transportation fuel but also for the production of chemicals and materials. Bioethanol production from lignocellulose biomass depends on two steps *viz*.,pretreatment and hydrolysis [1,2,3]. Cellulose and hemicellulose, when hydrolyzed into their component sugars, can be converted into ethanol through well-established fermentation technologies. However, sugars necessary for fermentation are trapped inside the cross-linking structure of the lignocellulose. Pretreatment refers to a process that converts lignocellulosic biomass from its native form, in which it is recalcitrant to cellulase enzyme systems, into a form for which cellulose hydrolysis is much more effective. Pretreatment methods should improve enzyme accessibility by removing most of the lignin and/or hemicelluloses, increase the porosity of biomass and reduce cellulose crystallinity. A number of pretreatment methods have been developed for improving hydrolysis of lignocellulosic biomaterials. Simultaneous Saccharification and Fermentation (SSF) results in higher yield of ethanol compared to SHF by minimizing product inhibition [4,5,6]. For the production of ethanol from pearl millet stalk a suitable low-cost pretreatment should be selected for the maximal removal of lignin and to increase the glucose concentration. The selection of enzyme and yeast after the pretreatment procedure is also critical. Hence, this study has been selected to carry out suitable process of lignocellulosic material and production of maximum possible ethanol by optimizing the process parameters.

Materials and methods

This study was carried out with pearl millet stalk and this feedstock was selected based on the availability in the local area and having low price. The essential properties of biomass were determined by using the ASTM methods *viz*., moisture content (ASTM, E-871), Ash content (ASTM, E-830). The cellulose, hemicelluloses and lignin were estimated by a standard method [7].

Pretreatment of selected feedstock

The purpose of pretreatment of lignocellulosic biomass for bioethanol production are break the ligno-hemicellulose-pectin complex, disrupt/loosen-up the crystalline structure of cellulose and increase the porosity of biomass. These changes in lignocellulosic materials make it easier for enzymatic saccharification (hydrolysis), results in higher fermentable sugars levels and will have a significant impact on the overall process [8]. Pretreatment of biomass can be physical, chemical and biological etc. For this study physical and chemical pretreatment were adopted for pretreating selected different biomass materials.

Physical pretreatment (size reduction)

Reduction of particle size aimed at reducing limitations of mass and heat transfer during the pretreatment and fermentation process. The selected substrates were dried at 45˚C for moisture removal and powdered in a milling machine. The powdered samples were sieved to obtain uniform particle size of 500 µm.

Chemical pretreatment

Different total solid contents of selected biomass (7.5, 10, and 12.5 %) were taken and transferred to Erlenmeyer flask of 250 ml capacity. The substrates were treated with 5, 7.5, and 10 % of ortho-phosphoric acid. These chemically pretreated substrates were autoclaved at 121 and 100˚C for different time periods of 1, 2 and 3 h respectively. After cooling, samples were taken in each interval and the hydrolysates were collected. Reducing sugars were estimated for hydrolysates using DNSA methods. The substrates were neutralized with sodium hydroxide. The pretreated substrates were dried at 45˚C. Hydrolysate obtained from pretreated substrates were further subjected to bioethanol fermentation.

Estimation of reducing sugar and lignin in pretreated biomass

The amount of reducing sugar was estimated by dinitrosalicylic acid (DNSA) method [9]. A quantity of 0.3 g of dried pretreated sample was taken and 3 ml of % of H_2SO_4 was added. It was incubated in room temperature for 1 h. After 1 h it was diluted with 84 ml of distilled water to make it to 4 % of H_2SO_4. It was autoclaved for 1 h at 121˚C after which the sample was cooled and filtered in a pre-weighed filter paper. The filter paper was dried and the weight of the dried residue was taken. The acid insoluble lignin can be estimated by using the following formula.

Lignin, % = ((Final dry weight-Initial dry weight))/ODW x 100

Laboratory scale experiment on Simultaneous Saccharification and Fermentation (SSF) process

After the pretreatment, the glucose molecules are still imprisoned in long chains of cellulose and hemicellulose and therefore not readily available for fermentation. SSF is a method for producing bioethanol that utilizes enzymatic bond breaking and parallel to the enzymatic activity and simultaneously, sugar fermented into bioethanol by yeast. The SSF process offers benefits such as improved bioethanol yields by reducing the product inhibition exerted by saccharification and fermentation, which results in cost reductions [10].

Measurement of cellulase enzyme activity (FPase assay)

The filter paper assay for commercial cellulase enzyme was performed. One ml of 0.05 M sodium citrate having pH 4.8 was added with 0.5 ml of enzyme in a test tube. One strip of Whatman No. 1 filter paper (weighing 50 mg) was placed into a test tube. The tube along with blank was kept in water bath at 50˚C for 60 min. DNSA method was followed further to assess for the amount of sugars released by the cellulase. One unit of enzyme activity is the one mole of reducing sugar in terms of glucose released per minute.

Measurement of xylanase enzyme activity

The endoxylanase activity xylanase enzyme was performed. 0.5 ml of 0.05 M sodium acetate trihydrate buffer diluted with 0.01 M of glacial acetic acid to maintain the pH 4.8 was added with 0.5 ml of enzyme in a test tube. The 2 % xylan (2 g in 100 ml of 0.05 M acetate buffer) was added in the test tube. The test tube along with blank was kept in the water bath at 50˚C for 60 min. At least two dilutions must be made of each enzyme sample. One dilution should release slightly more and one slightly less than 0.5 mg of xylose in the reaction conditions. DNSA method was followed further to assess for the amount of sugars released by the xylanase. One unit of enzyme activity is the one mole of reducing sugar in terms of xylose released per minute.

Selection of yeast for bioethanol production

The ethanogenic yeast *Saccharomyces cerevisiae* NCIM 3204 and *Pichia stipitis* NCIM 3298 has been selected for simultaneous saccharification and fermentation process. *Saccharomyces cerevisiae* is the yeast that most commonly used in bioethanol production, which converts the glucose into bioethanol. Nevertheless. It is not capable of converting xylose to ethanol. For fermentation of both of glucose and xylose sugars, various xylose fermentable microorganisms including *Candida shehatea, Candida guilliermondi, Pichia stipitis, Zymomonas mobilis, Pachysolen tannophilus, Kluyveromyces marxianus, Mucor indicus* and *Rhizopus oryzae* have been used. Among these yeasts, *P. stipitis* is one of the most promising species to ferment xylose to ethanol owing to its low by-product formation [11]. Hence, two types of yeasts were selected for optimization of ethanol production from hydrolysate.

Maintenance of yeast cultures

Saccharomyces cerevisiae NCIM 3204 and *Pichia stipitis* NCIM 3498 procured from National Collection of Industrial Microorganisms (NCIM). The yeast cultures used for simultaneous saccharification and fermentation was maintained on YEPD slant, which contained yeast 20 g l^{-1}, yeast extract 10 g l^{-1}, Peptone 20 g l^{-1} and agar 20 g l^{-1} [12].

Preparation of yeast inocula

The yeast inoculum was prepared by transferring the organisms maintained on YEPD slant into 100 ml of sterile medium having glucose 10 g l^{-1}, malt extract 5 g l^{-1}, yeast extract 3 g l^{-1} and peptone 5 g l^{-1} [12]. Seed inocules was grown for 24 h at 30˚C on a rotary shaker speed of 100 rpm.

Simultaneous saccharification and fermentation process

The lab scale SSF experiments were started with a working volume of 100 ml in 250 ml flask. SSF was performed with the hydrolysate obtained after pretreatment with and without addition of glucose (60 g l^{-1}) and yeast extract (10 %) as supplementary substrates. The other media was placed in the flask along with the nutrient solution with the yeast extract 10 g l^{-1}, urea 6.4 g l^{-1}, KH_2PO_4 2 g l^{-1} and $MgSO_4.7H_2O$ 1 g l^{-1} [6]. The flasks were autoclaved and added with enzyme (cellulase and xylanase) and inoculated with yeast *Saccharomyces cerevisiae* and *Pichia stipitis*. The dosages of enzymes and yeast were 40 FPU g^{-1} of cellulase, 25 U ml^{-1} of xylanase and 10 % (v/v) respectively. The inoculated flasks were incubated under anaerobic condition. The flasks were incubated for 96 h. The ethanol was estimated by gas chromatography method and the residual reducing sugar was estimated by following DNSA method.

Results and Discussions

Physiochemical properties of raw materials

The physiochemical properties of selected biomass were determined as per the standard procedure and the water extractives, ethanol extractives, cellulose, hemicelluloses and lignin were calculated and given in the [Table-1]. For bioethanol production, a biomass with high cellulose and hemicelluloses content will produce higher yield (l/t). The effect of cellulose content of switchgrass and reported that ethanol yield 280 l t^{-1} compared to wood (205 l t^{-1}) due to increased proportion of lignin in wood [13].

Table-1 *Physicochemical properties of pearl millet stalk*

Pearl Millet stalk	Moisture Content, %
Moisture Content, %	4.13 ± 0.2
Ash Content, %	2.73 ± 0.14
Bulk density, kg m^{-3}	100.01 ± 5.16
Water Extractives, %	5.83 ± 0.28
Ethanol Extractives, %	6.61 ± 0.34
Hemi cellulose, %	31.05 ± 1.61
Cellulose, %	39.98 ± 2.07
Lignin, %	17.5 ± 0.89

Pretreatment for selected feedstock

The different total solid concentration of the raw biomass was taken i.e., 7.5, 10 and 12.5 %. The raw biomass was pretreated with 5, 7.5 and 10 % of ortho-phosphoric acid at 100 and 121˚C at different time intervals of 1, 2 and 3 h. The reducing sugar and lignin were estimated with different intervals. From the results, highest reducing sugar and lowest lignin content was observed at 12.5 % total solid content, 7.5 % acid concentration with 121˚C at 3h. Hence, optimal pretreatment conditions were fixed as 7.5 % ortho-phosphoric acid with 3 h at 121˚C. The acid pretreatment on lignocellulosic biomass was capable of solubilising the lignin content and release some cellulose and hemicelluloses. During initial stages of pretreatment, 10 % acid pretreatment released higher quantities of total sugars than lower acid levels. However, over the time of treatment an increasing sugar release was evident from experimental results.

Table-2 *Total sugar released and lignin content of pearl millet stalk with different of solid loading*

Treatments	Sugar released, g l^{-1}		Lignin content, %		Treatments	Sugar released, g l^{-1}		Lignin content, %	
	121˚C	100˚C	121˚C	100˚C		121˚C	100˚C	121˚C	100˚C
7.5 % of total solid loading									
Water + 1 h	11.54	8.63	14.67	15.66	7.5 % + 2 h	28.11	18.24	10.67	12
5 % + 1 h	22.49	10.51	11	12.67	10 % + 2 h	30.55	23.34	9.67	11
7.5 % + 1 h	27.24	16.84	10.67	12.33	Water + 3 h	14.38	10.77	13.67	14.67
10 % + 1 h	29.74	19.19	10.34	12.34	5 % + 3 h	27.26	17.91	10.34	11.33
Water + 2 h	12.59	10.40	14.33	15.33	7.5 % + 3 h	29.02	21.36	10	11
5 % + 2 h	24.11	14.24	10.67	12	10 % + 3 h	34.05	27.67	10	10.67
10 % of total solid loading									
Water + 1 h	13.26	10.78	14.33	15	7.5 % + 2 h	31.31	23.64	9.67	11.33
5 % + 1 h	25.33	16.95	10.67	12.33	10 % + 2 h	34.27	27.30	10	11
7.5 % + 1 h	29.78	19.69	10.34	12	Water + 3 h	15.15	12.90	13.33	14
10 % + 1 h	32.47	21.33	10	12	5 % + 3 h	30.55	23.34	9.67	11
Water + 2 h	14.08	12.31	14	14.67	7.5 % + 3 h	33.87	28.81	9.34	10.67
5 % + 2 h	27.92	19.83	10	11.67	10 % + 3 h	37.43	32.12	9	10.34
12.5 % of total solid loading									
Water + 1 h	13.85	12.07	13.67	14.67	7.5 % + 2 h	37.96	28.91	9	11
5 % + 1 h	35.51	20.23	10	11.67	10 % + 2 h	38.23	30.08	9	10.34
7.5 % + 1 h	37.38	26.28	9.34	11.34	Water + 3 h	15.34	13.97	12.33	13.33
10 % + 1 h	38.29	27.62	9.34	11	5 % + 3 h	37.01	28.81	9	10.67
Water + 2 h	14.55	13.14	13	14.34	7.5 % + 3 h	38.96	31.84	8.34	10
5 % + 2 h	36.17	23.24	9.34	11.33	10 % + 3 h	38.34	33.84	8.67	10

Table-3 *Effect of yeast with different treatments and time on Ethanol yield and Sugar consumption*

Yeast	Ethanol yield (g l^{-1})				Sugar consumption (g l^{-1})			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Hydrolysate alone								
S.cerevisiae	1.31	4.56	10.39	16.78	13.53	22.4	34.31	36.75
P.stipitis	1.03	3.07	7.21	15.64	9.63	17.98	23.87	29.89
Hydrolysate + glucose (60 g/l)								
S.cerevisiae	8.23	10.35	25.81	32.13	25.43	40.1	49.83	58.48
P.stipitis	6.69	9.1	18.11	25.9	13.58	29.75	41.07	50.80
Hydrolysate alone + yeast extract (10 %)								
S.cerevisiae	2.03	6.06	11.53	17.02	14.46	24.98	35.99	37.56
P.stipitis	1.99	4.23	8.31	16.71	10.31	19.52	24.34	30.64

Table-4 *Ethanol yield from pearl millet stalk*

Time, h	*Saccharomyces cerevisiae*			*Pichia stipitis*		
	Hydrolysate alone	Hydrolysate with artificial glucose, 60 g l^{-1}	Hydrolysate with 10 % of yeast extract (w/v)	Hydrolysate alone	Hydrolysate with artificial glucose, 60 g l^{-1}	Hydrolysate with 10 % of yeast extract (w/v)
24	1.31	8.23	2.03	1.03	6.69	1.99
48	4.56	10.35	6.06	3.07	9.1	4.23
72	10.39	25.81	11.53	7.21	18.11	8.31
96	16.78	32.13	17.02	15.64	25.9	16.71

Hence, highest amount of sugar release (38.96 g l^{-1}) with higher lignin loss was observed at 3 h of treatment with 7.5 % of acid concentration.

In the case of pearl millet stalk, higher amount of sugar was obtained with 12.5 % total solids, 7.5 % acid (38.96 g l^{-1}). The lignin in pearl millet stalk after pretreatment was found as lowest (9 %) with 12.5 % of total solid and 7.5 % of acid concentration. The effect of pretreatment on lignocellulosic biomass improved its digestibility, alteration of lignin structure and a small percentage of the hemicelluloses and providing an improved accessibility of the cellulose for hydrolytic enzymes [8]. The results of sugar release and lignin reduction in acid pretreatment from all the substrates are presented in [Table-2]. The sugar concentration at 5, 7.5 and 10 % of ortho-phosphoric acid with 7.5, 10 and 12.5 % of total solids for 1, 2 and 3 h at 100˚C and 121˚C. Maximum sugar concentration of the selected feedstock varied from 38.96 to 8.34 g l^{-1}, while the lignin content varied from 9 to 15.66 %. Before acid pretreatment, the lignin content of the pearl millet stalk sample was found as 17.5 + 0.89 %.

Laboratory scale simultaneous saccharification and fer mentation process

The lab scale SSF experiment was done with three different conditions; pretreated hydrolysate without addition of artificial sugar, hydrolysate with addition of artificial sugar (sugar concentration up to 60 g l^{-1}), pretreated hydrolysate with 10 % of yeast extractives (w/v). 100 ml of media was taken in 250 ml of conical flask. The flasks were autoclaved at 121˚C and the required amount of nutrients was added. After autoclaving, 40 FPU g^{-1} of cellulase enzyme, 25 U ml^{-1} of xylanase was added in all the flasks. Two types of yeasts (10 % of *S.cerevisiae* and 10 % of *P.stipitis*) were used for optimization of the fermentation. The samples were taken at different time intervals of 24, 48, 72 and 96 h. Ethanol were recovered by distillation method of fermented slurry. The effect of different types of yeasts, hydrolysates alone, hydrolysates with different treatments, time on the bioethanol yield from pearl millet stalks was estimated and furnished in [Table-3]. It can be seen from the table value that the interaction effects were found to be significant. *S.cerevisiae* was on par with *P.stipitis* and significantly different from *P.stipitis*. The hydrolysate with artificial glucose had the best yield than other treatments at different time intervals while *P.stipitis* with hydrolysate alone yielded the lowest amount of ethanol at different time intervals. Hydrolysate with artificial sugar gave more ethanol than hydrolysate alone and hydrolysate with 10 % yeast extract. This may be due to presence of more sugar concentration than other two treatments.

The maximum ethanol was yielded by *S.cerevisiae* with hydrolysate and artificial sugar (19.13 g l^{-1}) than other treatments. Both the yeasts produced significantly lowest amount of ethanol with the hydrolysate alone. The ethanol production was significantly increasing with the increase in time; 96 h was proved to be the optimum time period for the fermentation. Hydrolysate with artificial sugar gave highest amount of ethanol at 96 h (29.01 g l^{-1}) which was on par with all other treatments.

Table-5 *Temperature and agitation speed effect on sugar reduction and ethanol production*

Temperature (˚C)	Fermentation time, h	Sugar reduction, g l^{-1}			Ethanol Production, g l^{-1}		
		75 rpm	100 rpm	125 rpm	75 rpm	100 rpm	125 rpm
25	24	38.78	37.26	37.98	10.34	12.83	11.47
	48	24.59	20.45	22.84	14.91	16.98	15.60
	72	8.63	5.42	7.10	29.26	33.36	30.23
	96	1.16	0.61	0.94	34.29	35.39	34.71
30	24	38.10	34.05	36.17	14.59	16.14	15.85
	48	22.49	20.17	21.95	19.82	21.71	20.98
	72	6.27	3.28	5.07	34.53	39.43	35.43
	96	0.84	0.14	0.74	39.97	44.24	40.90
35	24	38.41	34.97	36.37	11.38	13.98	12.67
	48	23.58	20.27	22.11	16.96	18.38	17.67
	72	7.52	4.45	6.01	31.53	35.16	32.53
	96	0.94	0.41	0.81	35.90	37.72	36.95

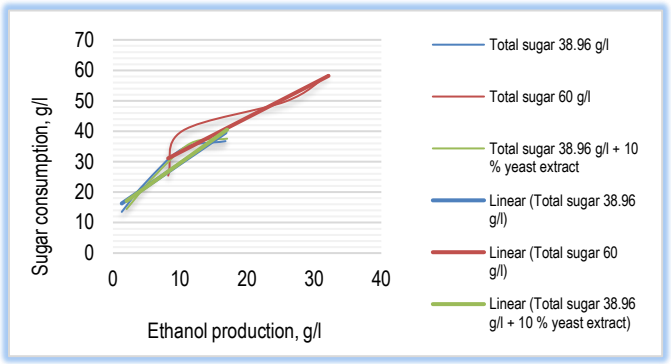

Fig-1 Ethanol production with sugar consumption from pearl millet stalk with different treatments with *S.cerevisiae*

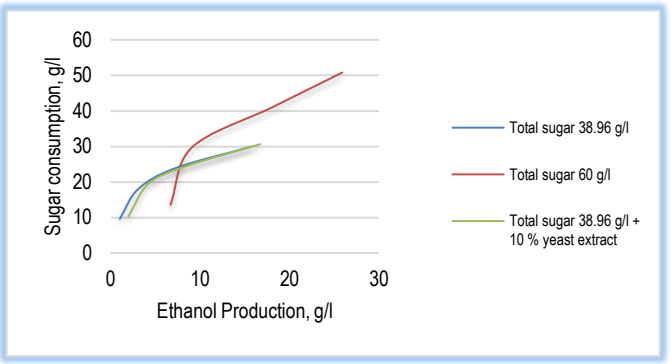

Fig-2 Ethanol production with sugar consumption from pearl millet stalk with different treatments with *P.stipitis*

The best treatment was found to be *Saccharomyces cerevisiae*; hydrolysate with artificial glucose (total sugar 60 g l^{-1}) at 96 h (32.13 g l^{-1}) while the poorest performing treatment was found at 24 h (Y1+H1, Y1+H3, Y2+H1, Y2+H3). After 96 h of fermentation, the bermudagrass and reed flasks produced a maximum ethanol concentration of 56.1 g l^{-1} and 55.0 g l^{-1}, though a large amount of glucose remained in the broth when the fermentation was done by *S.cerevisiae* [14]. Because the SSF experiment was conducted at 38 ˚C, the yeast strain at this temperature produced a maximum ethanol concentration of 56 g l^{-1} from glucose because of ethanol tolerance. This result is consistent with the results of previous SSF studies [15]. The effect of sugar consumption from pearl millet stalk by using different yeasts, with different treatments of hydrolysate at different interval of time [Table-3]. *S.cerevisiae* was significantly different than *P.stipitis* for the consumption of sugar in pearl millet stalk. The hydrolysate with artificial sugar (total sugar concentration 60 g l^{-1}) was proved to be the best treatment than the hydrolysate alone and the hydrolysate with 10 % of yeast extract. The best time for the fermentation was proved to be 96 h. The *S.cerevisiae* with hydrolysate and artificial sugar consumed more sugar (43.46 g l^{-1}) than *P.stipitis* with the same treatment (33.8 g l^{-1}). The hydrolysate with artificial sugar was the best performing treatment at 96 h than other time intervals. The highest amount of sugar was consumed by *S.cerevisiae*; with hydrolysate and artificial sugar at 96 h (58.48 g l^{-1}) than other combination of treatments. Hence, pearl millet stalk with above reaction conditions was found as best treatment.

The ethanol production with the sugar consumption from pearl millet stalks with different hydrolysate treatments by using *S.cerevisiae* and *P.stipitis* respectively. The ethanol production was increasing with the increase in sugar consumption [Fig-1]. The ethanol production and sugar consumption were nearly same from the hydrolysate alone and the hydrolysate with 10 % of yeast extract, while the ethanol production and sugar consumption were highest from hydrolysate with artificial sugar. The ethanol production and sugar consumption was highest by using the *S.cerevisiae* compared to *P.stipitis*. From [Table-3], it can be seen that with the hydrolysate alone the ethanol production was 8.26 g l^{-1} while the sugar consumption was 26.74 g l^{-1} by using *S.cerevisiae*. With the hydrolysate and artificial sugar, the ethanol production was 19.13 g l^{-1} and the sugar consumption was 43.46 g l^{-1}. With the hydrolysate and 10 % of yeast extract the ethanol yield was 9.16 g l^{-1} while the sugar consumption was 28.24 g l^{-1}.

The hydrolysate alone the ethanol production was 6.73 g l^{-1} while the sugar consumption was 20.34 g l^{-1} by using *P.stipitis* [Fig-2]. With the hydrolysate and artificial sugar, the ethanol production was 14.95 g l^{-1} and the sugar consumption was 33.8 g l^{-1}. With the hydrolysate and 10 % of yeast extract the ethanol production was 7.81 g l^{-1} while the sugar consumption was 21.2 g l^{-1}. The yield of ethanol was estimated for all the treatments for the selected feedstocks and presented in [Table-4] and the yield of ethanol was calculated as 32.13 g l^{-1} from pearl millet stalk after 96 h of fermentation.

Optimization of process parameters for ethanol production

The factors affecting bioethanol production are temperature, agitation speed etc. Hence, these parameters were optimized in the optimized treatments with *S.cerevisiae*.

Effect of temperature and agitation speed on sugar reduction and ethanol production

The sugar reduction of acid pretreated hydrolysate using the *S.cerevisiae* NCIM 3204 and commercial cellulase and xylanase enzymes at the temperatures of 25, 30 and 35˚C and mechanical agitator speed of 75, 100 and 125 rpm during SSF. The sugar concentration of the fermentation broth was in the range of from 34.05 to 38.78 g l^{-1} after 24 h which was reduced to 0.41 to 1.16 g l^{-1} at the end of fermentation 96 h [Table-5]. The process temperature affected the reduction of sugar concentration significantly. The concentration decreased from 38.78 g l^{-1} to 1.16 g l^{-1} for pearl millet stalk when the temperature was 25˚C after 96 h which further reduced to 0.84 g l^{-1} respectively when the temperature was increased from 25 to 30˚C. The ethanol production was increased as the temperature increased from 25 to 30˚C while with further increase of temperature from 30 to 35˚C the ethanol production was reduced. The ethanol was recovered from the fermentation broth by simple distillation method. The ethanol production was estimated at different temperatures with different speed and presented in [Table-5]. The ethanol production from pearl millet stalk was maximum at 30˚C when incubated with *S.cerevisiae* for 96 h (44.24 g l^{-1}). The agitation speed affected the ethanol production which was the most important factor for the growth of yeast cells. The mechanical agitator speed of 100 rpm was found out to be the optimum speed for higher ethanol production.

Conclusion

The present study is focused on biomass pretreatment and bioethanol production from pearl millet stalks. The optimized conditions for biomass pretreatment was found as 12.5 % of total solid, 7.5 % of acid at 121˚C for 3 h for the pearl millet stalks. Under the optimized conditions, pearl millet stalk released about 38.96 g l^{-1} of total sugar while the lignin content was reduced from 17 to 9 %. The lab scale SSF experiment was tried with three different hydrolysate treatments; without addition of any artificial sugar in hydrolysate, with addition of artificial sugar in the hydrolysate (total sugar concentration up to 60 g l^{-1}), hydrolysate with addition of 10 % (w/v) of yeast extractives with two types of yeasts, *Saccharomyces cerevisiae* and *Pichia stipitis* for optimization of SSF process up to 96 h. The two types of enzymes cellulase of 40 FPU g^{-1} and xylanase of 25 U ml^{-1} was used in all the treatments for saccharification. The treatment *i.e*., hydrolysate with addition of artificial sugar with *S.cerevisiae* was optimized as the best condition for ethanol production from all the biomass. The bioethanol yield was calculated as 32.13 g l^{-1} from pearl millet stalk after 96 h of fermentation. For further improvement, SSF experiment was focused on optimization of *S.cerevisiae* with three reaction temperatures (25, 30 and 35˚C) and three different agitation speeds (75, 100 and 125 rpm) for optimization. The highest ethanol concentration of was achieved from pearl millet stalk (44.24 g l^{-1}) under optimized conditions such as 30˚C with 100 rpm at 96 h.

Application of research: Renewable fuel for replacing conventional fuel

Research Category: Renewable Energy Engineering

Abbreviations: SSF- Simultaneous Saccharification and Fermentation,
ASTM - American Society for Testing and Materials,

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Study area / Sample Collection: TNAU farms, Coimbatore

Breed name: Pearl Millet stalk

Conflict of Interest: None declared

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