



Research Article

DEVELOPMENT OF SIMULTANEOUS SACCHARIFICATION AND FERMENTATION SYSTEM FOR BIOETHANOL PRODUCTION FROM LIGNOCELLULOSIC BIOMASS

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Abstract: Bioethanol is a liquid biofuel produced from lignocellulosic biomass. Seven number of lignocellulosic biomass were selected for the biomass characterization such as arecanut shell, arecanut sheath, corn cob, cotton stalk, maize shank, paddy husk and pearl millet stalks. From the physicochemical analysis three biomass (pearl millet stalks, arecanut husk, cotton stalk) were selected for further study. In this study, a pilot scale system of 50 l capacity was designed and developed for fermentation with working volume of 33.33 l. The bioethanol yield, bioethanol concentration, bioethanol production rate was found as 0.023 g g⁻¹, 23.12 g l⁻¹ and 0.24 g l⁻¹h⁻¹ respectively after 96 h of fermentation. The practical yield, theoretical yield and the fermentation efficiency were 6.26, 11.74 g l⁻¹ and 53.30 % respectively at 24 h which increased to 23.12, 27.29 g l⁻¹ and 84.69 % respectively after 96 h of fermentation.

Keywords: Bioethanol, Lignocellulosic biomass, Pretreatment, SSF reactor

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Introduction

Lignocellulosic biomass feedstocks are an ideal raw material suitable for the bioenergy production due to most abundant availability of biomass. In addition, biofuel synthesized from lignocelluloses does not compete with food [1-3]. The bioethanol and petrol can be blend together resulting in gasohol for internal combustion engines that has a higher-octane value and replaces leaded gasoline [4-6]. Using ethanol fuel blended with petrol can significantly reduce greenhouse gas emissions and use of petroleum fuels. Fuel ethanol produced from corn has been used in gasohol or oxygenated fuels since the 1980s. These blended fuels contain up to 10 % ethanol by volume [7]. Bioethanol can be produced from sugar, starch etc. but it will compete for the limited agricultural land needed for food and feed production [8-10]. Hence, the potential raw material adopted can be crop residues, saw dust, solid animal waste, municipal waste, grasses, paper and yard wastes for cost effective ethanol production [11,12].

In order to obtain a high overall ethanol yield, the pretreatment step should improve the accessibility of the cellulose component to hydrolytic enzymes while avoiding degradation of solubilised hemicelluloses and cellulose. Bioethanol production from lignocellulosic materials (second generation bioethanol), including pretreatment processes and enzymes technology for cellulose saccharification, has been investigated with increasing interest for the past few years, due to the growing concerns about climate change, increased energy demand and the forecast depletion of petroleum resources. For the production of ethanol from lignocelluloses 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

Seven lignocellulosic biomass such as arecanut shell, arecanut sheath, corn cob, cotton stalk, maize shank, paddy husk and pearl millet stalks, which are selected based on availability in the local area and low cost. Proximate and physicochemical properties of the seven biomasses were analyzed. 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 [13].

Pretreatment of selected feedstock

The aim of biomass pretreatment for bioethanol production from lignocellulosic feedstocks is to break the lignin barrier and remove the lignin, 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 [14]. Generally, pretreatment of biomass can be physical, chemical and biological or combined methods. However, present pretreatment is examined the physical and chemical method for selected different biomass materials.

i. Physical pretreatment (size reduction)

Reduction of particle size aimed at reducing limitation 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.

ii. Chemical pretreatment

The powdered biomass samples were chemical pretreated with 7.5 % of ortho-phosphoric acid at 121°C with 3 h at 12.5 % of total solid loading [15].

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. Then, the pretreated substrates were dried at 45°C. Hydrolysate obtained from pretreated substrates were further subjected to bioethanol fermentation.

Labe scale SSF reaction

The laboratory scale SSF experiment was conducted with the hydrolysate alone, hydrolysate with artificial sugar (total sugar concentration of 60 g l⁻¹), hydrolysate with 10 % (w/v) of yeast extract. Two enzymes namely cellulase (40 FPU g⁻¹) and xylanase (25 U ml⁻¹) were used in all the treatments for saccharification. Two yeasts (*S.cerevisiae* and *P.stipitits*) were used for the optimization of fermentation. The practical yield of ethanol was calculated as 32.13, 28.43 and 24.96 g l⁻¹ from pearl millet stalk, arecanut husk and cotton stalk respectively after 96 h of fermentation with *S.cerevisiae* from the hydrolysate with added artificial sugar (total sugar concentration of 60 g l⁻¹ [15]. The sugar consumption was highest in pearl millet (58.48 g l⁻¹) in the condition 2 with *S.cerevisiae* followed by arecanut husk (57.98 g l⁻¹) and the lowest in cotton stalk (55.54 g l⁻¹). Hence, the hydrolysate with added artificial sugar with *S.cerevisiae* was selected for fermentation up to 96 h for all the biomass. The process parameters for SSF i.e., temperature and agitation speed were optimized with the above treatment for all the biomass. 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) followed by arecanut husk (36.95 g l⁻¹) and cotton stalk (32.65 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. According to the ethanol yield and sugar consumption pearl millet stalk was selected for pilot scale fermentation.

Design and development of SSF reactor

SSF reactor was designed and developed for the production of bioethanol from selected lignocellulosic materials. The reactor consists of fermenter, mechanical agitator, heating source and panel for temperature and pH control, heat supply etc. The 50l capacity of fermenter was designed [Fig-1] and fabricated with stainless steel [Fig-2]. In order to avoid corrosion and abrasion of reactor vessel during the process of sterilization, the stainless steel was used. Top and bottom plates are hemispherical to withstand pressures. For an ideal reactor the height to diameter ratio is 1-1.5. Here, the height to diameter ratio taken was 1.5. The volume of the reactor can be given as

$$V = \pi \times R^2 \times H$$

Where,

V = Volume of reactor, m³

R = Radius of the reactor, m

H = Height of the reactor, m

For an ideal reactor height to diameter ratio for fermentation is nearly 1. (Assume,

$$D = H, D = R/2)$$

$$V = \pi/4 \times H^2 \times H$$

$$50 \times 10^{-3} = \pi/4 \times H^3$$

$$H = 0.39 \text{ m}$$

$$D = 0.39 \text{ m}$$

Mechanical Agitator

For growth of the culture and successful fermentation process uniform maintenance of environmental conditions throughout the reactor is necessary, which can be obtained by agitating the complete mixture to facilitate the mixing of the nutrients, substrates, oxygen and microbial cells. An impeller was mounted to a shaft extending through a bearing in the lid of the reactor and driven by a motor.

a. Impeller Diameter: The ideal diameter of the impeller (Di) is 1/3 to 1/2 of vessel diameter of the above the base vessel (D).

$$Di/D = 1/3$$

$$Di = 0.3 \times 0.39$$

$$Di = 0.11 \text{ m}$$

b. Disc Diameter: The disc diameter (Dd) of the impeller is 2/3 of the impeller diameter.

$$Dd/Di = 2/3$$

$$Dd = 0.07 \text{ m}$$

c. Clearance: The clearance between the impeller and bottom of the tank is 1/3 of the tank diameter.

$$C/D = 1/3$$

$$C = 0.13 \text{ m}$$

d. Length of blade: The length of the blade (L) is 1/3 of the impeller diameter.

$$L/Di = 1/3$$

$$L = 0.03 \text{ m}$$

e. Width of blade: The width of the blade (W) is 1/5 of the impeller diameter.

$$W/Di = 1/5$$

$$W = 0.02 \text{ m}$$

Jacketed vessel offers provides advantages over the internal coils, which will become rapidly fouled by microbial growth, decreasing heat transfer and often adversely affecting mixing. The space between the jacket is 50 mm for small vessels and 300 mm for large vessels. Steam generator is used to produce steam which is required to provide sufficient heat during fermentation process. A steam jacket heats the fluids from a low to a high temperature. The rate of heat transfer can be calculated as:

$$Q = m \times cp \times \Delta T/t$$

Where,

Q = mean heat transfer rate, kW

m = mass of the product, kg

cp = specific heat of the product, kJ kg⁻¹ °C

ΔT = change in temperature of the fluid, °C

t = total heating time, s

$$= 33.33 \times 4 \times (30-25) / 10 = 66.6 \text{ kW}$$

The panel consists of the individual controller and display for temperature controller and the agitator speed of SSF reactor controller with digital display. By pressing the temperature set button, the desired temperature can be maintained.

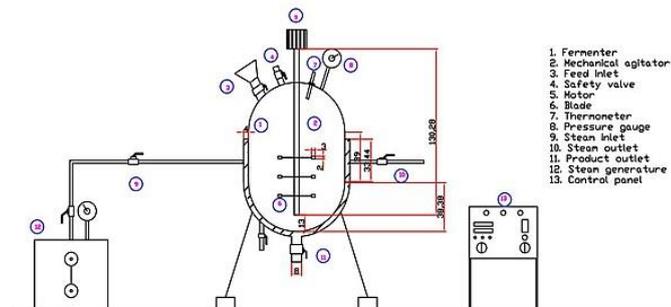


Fig-1 Designed pilot scale SSF system-All dimensions in cm



Fig-2 Set up of fermenter and steam generator

Table-1 Physicochemical properties of cotton stalk biomass

Biomass samples	Moisture Content, %	Ash Content, %	Bulk density, kg m ⁻³	Hemi cellulose, %	Cellulose, %	Lignin, %
Areca nut Sheath	4.05 ± 0.23	5.03 ± 0.35	126.78 ± 9.15	26 ± 1.87	36.85 ± 2.67	18.04 ± 1.29
Areca nut Shell	3.53 ± 0.34	4.98 ± 0.46	113.41 ± 10.43	29.54 ± 2.73	39 ± 3.6	18.34 ± 1.68
Corn Cob	4.36 ± 0.24	2.52 ± 0.15	110.55 ± 6.58	26.52 ± 1.59	37.43 ± 2.22	20.92 ± 1.24
Cotton Stalk	3.89 ± 0.34	5.31 ± 0.47	119.62 ± 10.05	29.92 ± 2.52	38.33 ± 3.21	22.11 ± 1.86
Paddy Husk	4.52 ± 0.24	22.68 ± 1.01	147.17 ± 6.67	19.03 ± 0.84	24.23 ± 1.1	26.02 ± 1.16
Pearl Millet stalk	4.13 ± 0.2	2.73 ± 0.14	100.01 ± 5.16	31.05 ± 1.61	39.98 ± 2.07	17.5 ± 0.89
Maize Shank	8.74 ± 0.61	7.91 ± 0.58	165.62 ± 12.10	24.1 ± 1.77	28.08 ± 2.04	22.16 ± 1.6

Performance evaluation of the system

The methods adopted in the performance evaluation of the SSF reactor for bioethanol production are given below.

Reactor was loaded with 33.33 l of fermentation media contained 12.5 % substrate concentration pretreated with 7.5 % of ortho-phosphoric acid for 3 h at 121°C.

Required quantity of nutrients was added to the reactor after autoclaving of media at 121°C.

The inoculums were introduced into the reactor and the reactants were maintained at 32°C for 96 h at 50 rpm.

The fermented slurry was drained from the reactor and subjected to the distillation to recover ethanol.

Results and Discussions

Physicochemical properties of raw materials

The seven different lignocellulosic biomass were selected for the proximate and physico-chemical analysis and presented in [Table-1]. For the production of ethanol, a biomass with high cellulose and hemicelluloses content will produce higher yield (l/t). Based on the cellulose, hemicelluloses and lignin content, three substrates were selected for further pretreatment from the seven different substrates. Pearl millet stalk contains more hemicelluloses (31.05 %), cellulose content (39.98 %) and less lignin content (17.05 %) followed by areca nut shell and cotton stalk. Hence, from the above seven biomass three substrates (pearl millet stalk, areca nut shell and cotton stalk) were selected for further studies on ethanol production.

Pretreatment for selected feedstock

The selected three biomass were pretreated with 12.5 % of total solid load and 7.5 % of ortho-phosphoric acid at 121°C with duration of 3 h and results are presented in [Table-2]. In the case of pearl millet stalk, higher amount of sugar was obtained 38.96 g l⁻¹ followed by areca nut shell (36.41 g l⁻¹) and least sugar was obtained from cotton stalk (32.81 g l⁻¹). The lignin in pearl millet stalk after pretreatment was found to be the lowest (8.34 %) followed by areca nut husk (10 %) and the highest lignin content was found from cotton stalk (11 %).

Table-2 Pretreatment of biomass with 12.5 % total solid loading 7.5 acid, 3 h reaction time at 121°C

Biomass	Sugar released, g l ⁻¹	Lignin content, %
Pearl millet stalk	38.96	8.34
Areca nut husk	36.41	10.00
Cotton stalks	32.81	11.00

Lab scale SSF study

Lab scale SSF study was conducted for the three lignocellulosic biomass viz., pearl millet stalk, areca nut husk and cotton stalk. The ethanol yield was highest from pearl millet (32.13 g l⁻¹) followed by areca nut husk (28.43 g l⁻¹) and cotton stalk (24.96 g l⁻¹). The sugar consumption was also highest from pearl millet stalks (58.48 g l⁻¹) followed by areca nut husk (57.98 g l⁻¹) and the lowest sugar consumption was from cotton stalk (55.54 g l⁻¹). It can be concluded that among the three biomass ethanol concentration was highest from pearl millet stalk hydrolysate with artificial glucose with *S.cerevisiae* compared to the other biomass feedstocks after 96 h of fermentation.

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

The sugar reduction and ethanol yield of acid pretreated hydrolysate using the

S.cerevisiae NCIM 3204 and commercial cellulase and xylanase enzymes at 25, 30 and 35°C and agitator speed of 75, 100 and 125 rpm were presented in [Table-3]. The sugar concentration of the fermentation broth was in the range of from 34.05 to 40.56 g l⁻¹ after 24 h which was reduced to 0.30 to 1.82 g l⁻¹ at the end of fermentation 96 h [Table-3]. The process temperature affected the reduction of sugar concentration significantly. The concentration decreased from 60 g l⁻¹ to 0.61, 0.83 and 0.94 g l⁻¹ for pearl millet stalk, areca nut husk and cotton stalk respectively when the temperature was 25°C after 96 h which further reduced to 0.14, 0.54 and 0.75 g l⁻¹ respectively, when the temperature was increased from 25 to 30°C. Pearl millet stalk had relatively higher reduction in sugar concentration compared to areca nut husk and cotton stalk. Among all the three-feedstock pearl millet stalk has the higher sugar consumption at 30°C with 100 rpm at 96 h followed by areca nut husk and the lowest consumption in cotton stalk. The sugar consumption increased as the temperature increased from 25°C to 30°C. The sugar consumption was decreased in all the feedstock as the temperature increased from 30 to 35°C.

The ethanol was recovered from the fermentation broth by simple distillation method. In the present study, 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 bioethanol production. The ethanol production was varied from 6.02 to 44.24 g l⁻¹. With increase in time of fermentation, ethanol production was increased up to 96 h. Pearl millet stalk had higher ethanol production compared to the other feedstock [Table-3]. Pearl millet stalk at 30°C with 100 rpm produced highest ethanol 44.24 g l⁻¹ followed by areca nut husk of 36.95 g l⁻¹ ethanol yield and the lowest ethanol from cotton stalk of 32.65 g l⁻¹. This was because the cellulose and hemicelluloses content in pearl millet stalk was more and the lignin content was low compared to the other feedstock. In cotton stalk the lignin content was more while the cellulose, hemicelluloses content was comparatively less. The ethanol production was less with 75 rpm at 25°C (6.02 to 34.29 g l⁻¹) for all the biomass, which increased gradually with the increase of temperature up to 30°C (8.09 to 39.97 g l⁻¹) but decreased (6.97 to 35.9 g l⁻¹) with further increase in temperature to 35°C. With the increase in agitation speed to 100 rpm the ethanol production was increased. The highest ethanol was achieved with 100 rpm at 30°C from all the biomass which gradually decreased and further increased to 35°C. The ethanol yield was increasing with the increase in agitation speed and temperature up to certain limit but with further increase in agitation speed and temperature the ethanol production reduced [Table-3]. With the increase in agitation speed from 75 rpm to 100 rpm the ethanol production increased but with further increase in speed to 125 rpm affected reversely on the ethanol production. Hence, the optimized parameters were agitation speed with 100 rpm and 30°C up to 96 h of fermentation.

Performance evaluation of the reactor and efficiency of fermenter

As pearl millet stalk yielded higher quantity of sugar compared to areca nut husk and cotton stalk, pearl millet stalk was selected as the feedstock for pilot scale studies. From lab scale SSF studies the hydrolysate with artificial glucose (total sugar concentration of 60 g l⁻¹) was optimized with *S.cerevisiae* at 30°C, 100 rpm for 96 h. Hence, optimized conditions were used for pilot scale experiment. After 24 h, the sugar consumed from the fermentation broth was 20.98 g l⁻¹. Sugar was estimated after every 24 h in the fermentation broth. After 96 h, the amount of sugar reduced in pearl millet stalk was up to 6.58 g l⁻¹ and the sugar consumption was about 53.42 g l⁻¹. Similarly, the ethanol yield was increasing with the increase of time up to 96 h.

Table-3 Effect of temperature and agitation speed on ethanol production and sugar

Temp (°C)	Speed, rpm	Fermentation time, h	Sugar reduction, g l ⁻¹			Ethanol Production, g l ⁻¹		
			Pearl millet stalk	Areca nut husk	Cotton stalk	Pearl millet stalk	Areca nut husk	Cotton stalk
25	75	24	38.78	39.57	40.56	10.34	7.18	6.02
25	75	48	24.59	28.22	25.50	14.91	10.04	8.56
25	75	72	8.63	9.59	11.88	29.26	22.27	13.55
25	75	96	1.16	1.82	1.71	34.29	29.57	27.85
30	75	24	38.10	38.2	38.54	14.59	8.32	8.09
30	75	48	22.49	25.34	24.04	19.82	11.05	10.67
30	75	72	6.27	8.92	9.38	34.53	25.20	15.92
30	75	96	0.84	0.94	1.16	39.97	30.96	29.37
35	75	24	38.41	39.89	39.66	11.38	7.63	6.97
35	75	48	23.58	26.19	25.37	16.96	10.87	9.56
35	75	72	7.52	9.15	10.12	31.53	23.41	14.54
35	75	96	0.94	1.59	1.63	35.90	30.12	28.76
25	100	24	37.26	38.10	39.39	12.83	9.55	9.69
25	100	48	20.45	24.37	23.91	16.98	11.02	11.28
25	100	72	5.42	6.49	9.48	33.36	23.88	16.03
25	100	96	0.61	0.83	0.94	35.39	31.02	29.24
30	100	24	34.05	36.40	37.29	16.14	12.67	11.01
30	100	48	20.17	21.57	22.57	21.71	15.56	13.61
30	100	72	3.28	4.26	5.64	39.43	29.55	18.94
30	100	96	0.14	0.54	0.75	44.24	36.95	32.65
35	100	24	34.97	37.35	38.28	13.98	11.16	10.52
35	100	48	20.27	22.34	22.94	18.38	12.70	12.42
35	100	72	4.45	5.66	6.98	35.16	24.90	17.80
35	100	96	0.41	0.64	0.83	37.72	32.14	30.86
25	125	24	37.98	39.27	39.53	11.47	8.21	7.29
25	125	48	22.84	26.68	24.54	15.60	10.63	8.96
25	125	72	7.10	8.61	10.50	30.23	22.73	14.77
25	125	96	0.94	1.19	1.48	34.71	30.50	28.51
30	125	24	36.17	37.38	37.77	15.85	9.45	8.85
30	125	48	21.95	23.43	23.64	20.98	12.84	11.42
30	125	72	5.07	7.19	8.26	35.43	26.92	16.56
30	125	96	0.74	0.86	0.95	40.90	31.96	30.71
35	125	24	36.37	37.46	38.50	12.67	9.34	7.58
35	125	48	22.11	24.60	23.80	17.67	11.22	10.49
35	125	72	6.01	7.38	9.39	32.53	24.32	15.67
35	125	96	0.81	0.91	1.22	36.95	31.10	29.45

Table-4 Sugar consumption, ethanol yield, theoretical yield during fermentation in pilot scale

Time, h	Sugar consumption, g l ⁻¹	Ethanol yield, g l ⁻¹	Theoretical yield, g l ⁻¹	Efficiency, %
24	20.98	6.26	11.74	53.30
48	33.56	9.37	17.14	54.63
72	47.02	19.77	24.02	82.28
96	53.42	23.12	27.29	84.69

After 24 h, the ethanol yield was 6.26 g l⁻¹ which gradually increased to 23.12 g l⁻¹ after 96 h of fermentation. The ethanol yield, sugar consumption, theoretical yield and fermentation efficiency were calculated and furnished in the [Table-4].

The theoretical yield of ethanol was 11.74 g l⁻¹ after 24 h of fermentation, however, it increased to 27.29 g l⁻¹. The actual yield of ethanol after 96 h of fermentation was 23.12 g l⁻¹ while the theoretical yield was 27.29 g l⁻¹ [Table-4]. The fermentation efficiency was 53.30 % which gradually increased with the increase in the fermentation time. The fermentation efficiency increased to 84.89 % after 96 h of fermentation. The ethanol yield efficiency was 49.69 % in soyabean molasses without addition of any nutrients whereas the efficiency reduced to 46.64, 41.93 and 44.09 % with addition of magnesium source (MgSO₄, 0.1 g l⁻¹), nitrogen source (NH₄NO₃, 3.5 g l⁻¹) and combination of magnesium and nitrogen sources when fermented with *S. cerevisiae*. With the brix of 20° and at 20 h the ethanol yield was 38 g l⁻¹ from the molasses with the sugar reduced from 169.5 to 93.4 g l⁻¹. The ethanol production rate was 1.82 g l⁻¹ h⁻¹ and the average yield efficiency was 42.8 %.

Furthermore, the brix content of 35° after 40 h of fermentation the sugar reduced from 311.6 to 151.3 g l⁻¹ and the ethanol yield was 63.5 g l⁻¹. The ethanol production rate and yield efficiency were 1.53 g l⁻¹ h⁻¹ and 38.53 % respectively (Siqueira *et al.*, 2008). The ethanol concentration, yield and production rate for the selected lignocellulosic biomass was 23.12 g l⁻¹, 0.023 g g⁻¹ and 0.24 g l⁻¹ h⁻¹, respectively at the end of pilot scale fermentation.

Conclusion

A pilot scale fermenter of 50 l capacity was designed and developed for fermentation with working volume of 33.33 l. The system was made up of stainless steel to withstand high pressure and corrosion. The diameter and height of the reactor was 0.39 m, impeller diameter was 0.11 m, disc diameter, length and width of the blade was 0.07, 0.03 and 0.02 m respectively. Furthermore, 66.6 kW heat supply system was used. The pilot scale SSF experiment was done from pearl millet stalk for bioethanol production. The fermentation was done up to 96 h and the ethanol concentration and sugar reduction was estimated at the interval of every 24 h.

The optimized conditions with optimized process parameters were maintained during pilot scale SSF experiment. The sugar reduction was highest from pearl millet stalk of 20.98 g l⁻¹ during 24 h of fermentation while it increased to 53.42 g l⁻¹ after 96 h of fermentation.

The ethanol concentration was increasing with increase in time from 24 h to 96 h. The ethanol concentration was 6.26 g l⁻¹ after 24 h of fermentation which gradually increased to 23.12 g l⁻¹ after 96 h of fermentation. The ethanol yield, ethanol concentration, ethanol production rate was 0.023 g g⁻¹, 23.12 g l⁻¹ and 0.24 g l⁻¹ h⁻¹ respectively after 96 h of fermentation. The practical yield, theoretical yield and the fermentation efficiency were 6.26, 11.74 g l⁻¹ and 53.30 % respectively at 24 h which increased to 23.12, 27.29 g l⁻¹ and 84.69 % respectively after 96 h of fermentation.

Application of research: Renewable fuel for replacing conventional fuel

[15] Sriramajayam S., Tanmayee P., Ramesh D., Gitanjali J. and Palaniselvam V. (2022) *International Journal of Agriculture Sciences*, 14(6), 11381-385.

Research Category: Renewable Energy Engineering

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

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Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: TNAU farms, Coimbatore

Cultivar / Variety / Breed name: Pearl Millet stalk, Arecanut husk, Cotton satlk

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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