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Optimization of fermentation parameters of acid hydrolysate of pods of *Samanea saman* for ethanol production

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Abstract
In recent years, the increasingly distressing problems associated with the amount of energy consumption have emphasized the importance of developing alternate renewable sources for energy materials. The polysaccharides in agricultural and forestry products are natural organic resources which are utilized wastes for the production of energy.

Keywords: Polysaccharides, agricultural wastes, forestry products and natural organic

Introduction
In India, there are 295 distilleries producing 1058 million litres of alcohol as against annual consumption of 1266 million litres (Arbatti, 2001). Nearly 90 per cent of alcohol is produced from molasses, a by-product of the sugar industry, which is the cheapest feed stock for ethanol fermentation. However, due to the recent hike in the cost of molasses, it has become necessary to search for an alternate biomass substrate for ethanol production. Ethanol production from corn, sweet sorghum and sugar beet have been well established. The other alternate biomass sources tried successfully are the pineapple, cannery waste (Nigam, 1999) and starch (Verma *et al*. 2000). From the preliminary observations, aqueous extract of deseeded pods of *Samanea saman* showed maximum sugars of 11° brix value indicating its potential for ethanol production (Geeta *et al*. 2000). *Samanea saman* is a tropical tree listed as an economic plant. The trees are cultivated in many parts of India as an ornamental/shade tree particularly on either side of the road.

Review of literature
Fermentation time
Zossi *et al*. (1990) performed ethanol fermentation of sugarcane juice for 7 days at pH 6.0 with supplementation of $S0_2$ (700 ppm) and achieved fermentation efficiency of 96.916+0.83 per cent. Marakis and Marakis (1996) studied the effect of six different fermentation periods viz., 0, 24, 48, 72, 90 and 100 hours on ethanol production from $_aq_{Ueo}$us carob extract and achieved maximum alcohol concentration of 4.75 per cent (v/v) at 100 hours of fermentation period.
Ethanol fermentation from Dunaliella microalgae at 25°C was within (Shirai *et al*, 1998) 5 days and were able to obtain ethanol yield of 11 mg g^{-1} of substrate. An experiment was conducted for conversion of corn starch to fuel ethanol which was 72.2 g L^{-1} ethanol produced in 120 minutes residence time (Krishnan *et al*. 1999). Damaged sorghum and rice grains were utilized by Suresh *et al*. (1999) for ethanol production and obtained ethanol yield of 2.90 per cent v/v at 30°C, after
5 days of fermentation. A maximum alcohol yield in 3 days during fermentation of yam to ethanol by Saccharomyces cerevisiae was observed by Ramanathan (2000).
Sreenath and Jeffries (2000) fermented wood hydrolyzate using yeasts for 3-5 days at temperature and pH of 25-27°C and 5.5 to 7.0 respectively, and obtained average ethanol concentration of 34.8 ± 2.42 g L^{-1}. The effect of four different fermentation periods viz., 24, 48, 72 and 96 hours on ethanol production from starch medium was studied by Verma *et al*. (2000). A maximum ethanol concentration of 24.8 g L"[1] at 48 hours was achieved as compared to 13.7 and 21.6 g L^{-1} at 24 and 96 hours respectively. Ramakrishna *et al*. (2001) [4] obtained maximum ethanol yield of 120 g L^{-1} at 120 h of fermentation from water soluble carob sugars by mutant strain of Zymomonas mobilis KLR 4.

Effect of ph on ethanol yield

Nimbkar *et al*. (1989) obtained maximum alcohol concentration of 12.45 per cent (v/v) at pH 4.5 from fermentation of unsterilized juice of sweet sorghum by S. cerevisiae strain 3319.

A maximum ethanol concentration of 79.1 ml L^1 from cane molasses at operating variables of pH 4.2, temperature 32°C in 30 days of operation was obtained by Garcia and Suarez (1992). Marakis and Marakis (1996) obtained maximum alcohol concentration of 5.8 per cent (v/v) at pH 4.5 from aqueous carob extract after 120 hours of fermentation.

Suchi Srivastava *et al*. (1997) showed that the optimum, initial pH of guava pulp medium for the production of ethanol was 5.0 for all three yeast strains employed and obtained maximum ethanol yield of 5.8 per cent (w/v) during 36 hours fermentation of guava pulp by isolate-2 and standard culture of S. cerevisiae. The rate of ethanol production from wood hydrolyzates was enhanced by pH in the range of 5.1-7.0. Ethanol production was maximum (20 g L^{-1}) at pH 6 and hydrolysate having pH 7.7, produced 30 per cent less ethanol (Sreenath and Jeffries, 2000).

Effect of temperature and ethanol yield

Hermann *et al*. (1986) proved that, optimal growth temperature and pH for maximum ethanol production (41-42 g L^{-1}) are 30°C and 5.0 respectively during whey fermentation by Zymomonas mobilis. Nimbkar *et al*. (1989) studied the effect of three different incubation temperatures viz., 25, 30 and 35°C on the ethanol production from unsterilized juice of sweet sorghum with S. cerevisiae and obtained maximum alcohol of 12.45 per cent (v/v) at 30°C and also observed inconsistent alcohol production at 35°C due to a temperature shock.

A maximum ethanol concentration of 88 g L^{-1} from 200 g L^{-1} sucrose medium at optimum temperature of 30°C and pH 6.0 with ethanol yield of 0.48 g g^1 was obtained by Singh and Jain (1994). An ethanol concentration of 91 g L^1 and productivity 2.7 g L^{-1} h^{-1} at the higher temperature of 35°C was achieved by Lother and Oetterer (1995) in a molasses medium containing 22 per cent (w/v) total sugar when thermotolerant strain (KF7) of S. cerevisiae was used. Abate *et al*. (1996) obtained ethanol yield and an average hourly productivity of 0.5 g g^{-1} sugar consumed and 1.5 g L^{-1} respectively in 14 hours after inoculation of S, cerevisiae at temperature of 30°C from 200 g L^{-1} of sucrose.

Both simultaneous saccharification and fermentation (SSF), and separate hydrolysis and fermentation (SHF) in the fluidized bed reactor (FBR) system was studied by Krishnan *et al*. (1999). The hydrolysis and fermentation steps were performed at their optimum temperatures of 55 and 30°C, respectively and were able to obtain volumetric ethanol productivity of 19-25 g L^{-1}. Chandrakant and Bisaria (2000) carried out simultaneous isomerisation and fermentation (SIF) of xylose and simultaneous isomerisation and cofermentation (SICF) of a glucose/xylose mixture by Saccharomyces cerevisiae in the presence of xylose isomerase by addition of borate to the medium which resulted in ethanol concentration and metabolic yield of 29.8 g L^{-1} and 0.42 respectively and temperature modulation from 30 to 35°C during fermentation further enhanced the above parameters to 39 g L^{-1} and 0.45 respectively.

The effect of five different temperatures viz., 25, 30, 35, 37 and 40°C on the ethanol production from starch (100 g L^{-1}) was studied by Verma *et al*. (2000). They observed that maximum ethanol concentration of 21.8 g L^{*1} was at optimum temperature of 30°C in 48 hours of fermentation period. Panesar *et al*. (2001) optimized the fermentation parameters viz., temperature, pH and inoculum level as 30°C, 6,0 and 10 per cent (v/v) respectively for ethanol production from molasses medium.

Effect of inoculum size on ethanol production

Gibbons and Westby (1986) [2] studied the effects of inoculum size on solid phase fermentation of fodder beets for fuel ethanol production and proved that 5 per cent of inoculum of S. cerevisiae (w/w) resulted in rapid ethanol (9% v/v) production. Increase in the inocula indicated no advantages, and lower inocula resulted in increased fermentation time.

An alcohol content of 2.18 per cent (w/v) from whey inoculated with 1.00 per cent of S. cerevisiae at pH 4.0 and temperature of 22°C after 18 hours of fermentation period was obtained by Mathur *et al*. (1986). The highest alcohol recovery of 52 per cent by adding yeast inoculum at the rate of 10 per cent (v/v), during fermentation of sweet sorghum juice was achieved by Swaminathan *et al*. (1987).

The effect of inoculum size on fermentation of whey for fuel ethanol production was studied by Cuesta and Cornejo (1988) and proved that increasing the inoculum level from 1.8 million to 1.9 million cells per ml decreased the time taken to maximum fermentation (about 73% theoretical value) from 360 to 84 h. The cultural conditions was optimised by Kahlon and Chaudhary (1988) for water hyacinth for maximum alcohol production which required 8 per cent yeast inoculum and 36 h fermentation period. Nimbkar *et al*. (1989) studied the effect of different inoculum levels viz., 2, 4, 6, 8 and 10 per cent on the ethanol production from unsterilized juice of sweet sorghum and obtained maximum alcohol concentration of 12.45 and 12.23 per cent (v/v) at 6 and 2 per cent respectively, and also reported that, 2 per cent inoculum level appears to be sufficient for alcohol production.

Mendoza and Raymundo (1990) optimized the fermentation conditions for batch alcohol production from molasses. The parameters selected were, inoculum size, temperature, initial sugars and pH which resulted as 10 per cent (v/v), 30°C, 10 per cent and 5.5 respectively and observed that fermentation efficiency and ethanol yield of 83.3 to 92.6 and 4.6 per cent respectively. A maximum ethanol production of 88 g L'^1 from 200 g $I/^1$ sucrose medium at 10 per cent inoculum size in 16 to 18 hours was obtained by Singh and Jain (1994). Ramanathan (2000) could achieve maximum alcohol recovery of 5.85 per cent at (5%) yeast concentration during fermentation of yam to ethanol.

Effect of nutrient supplementation on ethanol production

Swaminathan *et al*. (1987) achieved highest alcohol recovery of 52 per cent by supplementation of sweet sorghum juice (4.5 pH) with 2 per cent ammonium sulphate after 48-62 hours of fermentation. Cuesta and Cornejo (1988) proved that, addition of equal amounts of H3PO4 and (NH4)2 SO4 upto 0.7 per cent each increased the fermentation rate and level of ethanol production and addition of 0.3 per cent of each of these salts to 0.5 per cent yeast extract had an even greater effect during the whey fermentation by Candida pseudotropicalis IFI 268. Nimbkar *et al*. (1989) supplemented unsterilized sweet sorghum juice with 2 per cent ammonium sulphate during fermentation by S. cerevisiae and able to obtain only 9.05 per cent (v/v) of ethanol concentration as compared to unsupplemented juice (9.08% v/v).

Patil *et al*. (1989) [3] obtained 20-30 per cent more ethanol in the presence of novel supplements viz., skim milk, chitin and

waste mycelium individually or in combination at 30°C with 15 per cent fermentable sugars by S. cerevisiae and also the efficiency of the process was improved from 66 to 87 per cent. Cane molasses (18% total sugars) at pH 4.7 was fermented for 40 hour by S. cerevisiae with ammonium sulphate (1.5 g L"[1]) and other N sources viz., yeast extract and ammonium.

The influence of sulphur dioxide (700 ppm) on fermentation of sugarcane juice by distillery yeast at pH 6.0 was studied by Zossi *et al*. (1990). They obtained fermentation efficiency of 96.916±0.83 per cent. Dubey *et al*. (1991) [1] studied the effect of hormonal growth factor formulations containing oestrogen, progesteron, oxytocin and lecithin complex on the fermentation efficiency of distillery yeast during fermentation of molasses medium at 24, 48, 60 and 72 h. It was reported that all the growth factors had a stimulatory effect on fermentation with fermentation efficiency of 94.72, 93.51, 94.92 and 89.67 per cent at 2 ppm concentration in 60 hour fermentation period compared to that of control (79.15% in 72 h).

The effect of supplementation of ammonium sulphate on ethanol fermentation from molasses medium at concentrations of 0.1 per cent and 1 g L-[1] using Z mobilis strain B-61147 was studied by Iida *et al*. (1993). They obtained ethanol concentration and hourly productivity of 65 to 70 g L[1], 58.4 g L-[1] and 75.1 g 68.8 g Irrespectively, at 0.1 per cent and 1 g L/[1] (NH_4)**2** SO4, compared with 43.9 g L"[1] for S. cereuisiae at 0.5 per cent concentration at pH and temperature 4.8 and 30°C respectively. Amartey and Jeffries (1994) showed that addition of inexpensive supplement such as corn steep liquor (CSL) as a substitute for expensive nitrogen in pentose fermentation mediated by strains P. stipitis and C. shehate had increased the ethanol concentration to 26.5 g Lr[1] in 205 hours of fermentation. Marakis and Marakis (1996) supplemented aqueous carob extract with ammonium sulphate and sodium dihydrogen phosphate at the rate of 0.3 and 0.1 per cent respectively during fermentation by S. cerevisiae and obtained maximum ethanol concentration of 5.8 per cent (v/v) after 120 hours of fermentation

A maximum ethanol yield of 30.8 g L~] was obtained when the grape skin pulp extract was supplemented with NH4NO3 (0.1%) and K2HPO4 (0.1%) compared to ethanol yield (23.3 g L"[1]) without supplementation (Israilides et ah, 1998). Anantha and Gunasekaran (2000) obtained higher ethanol yiled of 44.2 and 54.9 g L[1], respectively by supplementation of liquefied cassava starch with ammonium sulphate (1 g L-[1]) and yeast extract (10 g L"[1]) which was higher than that of without supplementation (36.5 g L-[1]) in a mixed culture of Z. mobilis and S. diastaticus.

Each tonne of cassava requires 200 g of triple superphosphate and ammonium sulphate each and 20 g of Magnesium sulphate also for fermentation of cassava to ethanol (25-30%) (Ramanathan, 2000). An addition of zinc sulfate (ZnSCU) @ 10 mg I/[1] in the fermentation medium of wood hydrolysate increased the rate of sugar utilization and ethanol yield due to high activity of zinc dependent alcohol dehydrogenase and, obtained higher ethanol yield of 26-30 g L–[1] (0.33 to 0.49 g g"[1]) (Sreenath and Jeffries, 2000). Geeta *et al*. (2001) studied the effect of zinc sulphate (ZnS04) at various concentrations viz., 0, 10 and 15 mg L[1] on ethanol production from the pods of *Samanea saman* and obtained maximum ethanol concentration at 10 mg L"[1] ZnS04.

Shiva *et al*. (2001) conducted studies by supplementing cholesterol, soya lecithin, oleic acid and tween 80, individually and their combinations, in the fermentation medium used for ethanol production and found that, of all the supplements tested, combination of cholesterol and oleic acid could produce 19.74 per cent enhanced yields as compared to unsupplemented control. Ramakrishna *et al*. (2002) [4] carried out investigations to determine alcohol and fructose syrup at different temperature levels employing Z. mobilis (KLR5) and Arthrobacter sps. on carob pod syrup. Maximum alcohol and fructose syrup of 4.2 per cent and 126 g kg^{-1} respectively was observed in Z. mobilis followed by Arthrobacter (4.1% and 122 g kg^{-1}) at 35°C.

Sridhar and Rao (2002) studied the influence of nitrogen sources (ammonium sulphate, ammonium chloride, urea, casein hydrolysate, yeast extract and peptone) and organic ions (magnesium, calcium, manganese, zinc and potassium) at different concentrations on thermotolerance, osmotolerance and ethanotolerance of Saccharomyces cerevisiae VS3 at 30 and 42°C and obtained enhanced ethanol yield of 12 and 8 g L"[1] at 30°C when medium was supplemented with yeast extract and ammonium sulphate respectively.

Material and Methods

Fermentation

The acid hydrolysate of the pods of S. saman was adjusted to a pH of 4.5 using pellets of potassium hydroxide and inoculated with different yeast cultures and incubated under aerobic conditions for 24 hours. Anaerobic condition was then created by plugging with cork, making a provision for trapping carbon dioxide and incubated at room temperature for 7 days. The amount of ethanol produced and residual sugars left unfermented were estimated. The best performed strains (BCY-108, C. wickerhami, NCIM 3463 and K. marxianus NCIM 3551) were selected for further standardization.

Effect of fermentation time on bioethanol production

The efficient yeast cultures were tested to standardise the optimum period of fermentation for maximum ethanol production. The acid hydrolysate was inoculated @ one per cent yeast inoculum and was allowed for fermentation for 4, 5, 7 and 9 days. The residual sugar content and ethanol produced were analysed.

Effect of ph on bioethanol production

The pH of the acid hydrolysate was adjusted to 3.5, 4.5, 5.5 and 6.5 with potassium hydroxide pellets. The efficient yeast cultures viz., C. wickerhami NCIM 3463, K. marxianus NCIM 3551 and local isolate BCY-108 were inoculated and fermentation was continued upto 7 days as described earlier. The samples were analysed for ethanol and residual sugar content after fermentation.

Effect of temperature on bioethanol production

After determination of optimum pH which was 4.5, hydrolysate was inoculated with efficient yeast cultures @ 1.00 per cent and incubated at different temperatures viz., 25, 30 and 37°C for 7 days. After complete fermentation, the samples were analysed for ethanol and residual sugar content.

Standardisation of inoculum size for bioethanol Production

The pod extractant was further studied to know the effect of various levels of inoculum on ethanol production.

The pod extract (100 ml of pH 4.5) was inoculated with 1.0, 1.5 and 2.0 per cent of inoculum levels and kept for

fermentation at 30°C for 7 days and samples were analysed for residual sugar and ethanol yield.

Effect of nutrient supplementation on bioethanol Production

The pod extractant was further studied to know the effect of supplementation of various nutrients and optimisation of their concentrations on ethanol yield. Mainly nitrogen, phosphorus and zinc sulphate were included in the study.

Ammonium sulphate was used to supply additional nitrogen, as it was proved elsewhere to be best source compared to other source. Phosphate was supplied in the form of orthophosphoric acid and sodium dihydrogen phosphate.

Zinc sulphate was supplemented as stimulant, since it is known to stimulate the alcohol dehydrogenase (ADH) enzyme which is responsible for alcohol production.

The concentrations of all the nutrients were used individually which are listed below. A control was also maintained without supplementation of any nutrients.

1. Ammonium sulphate - 0.1, 0.3 and 0.5 per cent (Anantha and Gunasekaran, 2000).
2. Orthophosphoric acid - 0.002, 0.004 and 0.006 per cent (Cuesta and Cornejo, 1988).
3. Sodium dihydrogen phosphate -0.1, 0.3 and 0.5 per cent (Marakis and Marakis, 1996).
4. Zinc (as zinc sulphate) - 5, 10 and 15 mg L^{-1} (Sreenath and Jeffries, 2000).

The substrates supplemented with the above mentioned nutrients were inoculated individually with efficient yeast cultures @ 1.5 per cent inoculum and kept for fermentation for 5 days at 30°C. The samples were analysed for ethanol and residual sugar content.

Pilot scale production of ethanol

From the previous experiments, BCY-108 yeast strain was found to be efficient in conversion of the hydrolysate to ethanol. Hence, this strain was used for pilot scale fermentation of ethanol. The optimum parameters obtained from the previous studies were adopted in this trial. A fermentor of 3L was designed for the pilot scale study in which 1.5 L of the hydrolysate was fermented by following the procedure described before. The performance was evaluated in terms of ethanol yield and residual sugars. The population density was also observed at intervals.

Optimum fermentation parameters followed for pilot scale production of ethanol

Parameters

Fermentation time : 5 days
pH : 4.5
Temperature : 30°C
Inoculum size : 1.5 per cent (30 x 10^6 cells ml^{-1})
Nutrient supplementation : ZnS04 @ 10 mg L^{-1}

Statistical analysis

The results obtained were analysed statistically using completely randomised design as described by Panse and Sukhatme (1985).

Experimental results

Effect of different fermentation periods on ethanol yield and residual sugar content in deseeded pods of S. Saman

The ethanol yield in deseeded pods of S. saman was found to increase due to inoculation of the selected efficient yeast cultures at different periods of fermentation. Accordingly the residual sugar content also decreased, as observed in Table 9.

The ethanol yield increased significantly in the acid hydrolysate of deseeded pods of S. saman, when inoculated with yeast cultures viz., BCY-108, C. wickerhami NCIM 3463 and K. marxianus NCIM 3551 (9.582, 9.512 and 9.377 g L^{-1} respectively) over respective control (Table 9). However, the three strains were on par with each other. The uninoculated control exhibited 4.267 g L^{-1} of ethanol during 9 days of fermentation.

The ethanol yield increased significantly with increase in fermentation period from 4 to 7 days. Further increase in ethanol yield did not differ significantly between 7^{th} and 9^{th} days (20.002 and 20.010 g L^{-1} respectively). The ethanol yield recorded on 7^{th} and 9^{th} day was significantly superior as compared to 4^{th} and 5^{th} day of fermentation (Table 9).

Similarly, the residual sugar content also decreased significantly in the acid hydrolysate of pods of S. saman due to inoculation of efficient yeast cultures viz., BCY-108, C. wickerhami NCIM 3463 and K. marxianus NCIM 3551, which recorded 6.921, 6.972 and 7.060 g L^{-1} respectively (Table 1).

Table 1: Effect of fermentation periods on ethanol yield and residual sugar content in the extract of deseeded pods of S. saman

S. No.	Fermentation period (days)	Ethanol yield (gL^{-1})				Residual sugar (gL^{-1})			
		BCY-108	C. wickerhami NCIM 3463	K. marxianus NCIM 3551	Mean	BCY-108	C. wickerhami NCIM 3463	K. marxianus NCIM 3551	Mean
1.	Control	1.633	1.633	1.633	1.633	9.676	9.767	9.767	9.767
2.	4	10.537	10.143	10.198	10.198	6.723	6.847	6.983	6.851
3.	Control	1.833	1.833	1.833	1.833	9.200	9.200	9.220	9.200
4.	5	15.200	14.743	14.99	14.999	5.247	5.377	5.530	5.384
5.	Control	2.983	2.983	2.983	2.983	8.880	8.880	8.880	8.880
6.	7	20.260	19.733	20.002	3.633	3.663	3.703	3.943	3.770
7.	Control	3.933	4.433	4.267	8.233	8.233	8.233	8.233	8.233
8.	9	20.277	19.747	20.010	3.653	3.653	3.770	3.937	3.787
	Mean	9.582	9.512	9.377		6.921	6.972	7.06	
	Source	SEm±	CD at 1%			Sem±	CD at 1%		
	Fermentation period (A)	0.0763	0.2889			0.2924	1.1072		
	Cultures (B)	0.0467	0.1768			0.1790	0.6778		
	A X B	0.1322	0.5006			0.5064	1.9175		

The residual sugar content also decreased due to extended fermentation period from 4^{th} to 9^{th} day which ranged from 6.851 to 3.787 g L^{-1}. However, it decreased significantly upto 7^{th} day of fermentation (3.770 g L^{-1}) beyond which decrease

in residual sugar content did not differ significantly. In control, the residual sugars remained almost constant (8.233 g L^{-1}) during 9 days of fermentation. The interaction effect was non-significant between fermentation time and yeast cultures on ethanol yield and residual sugar content in the acid hydrolysate of deseeded pods of S. saman. The ethanol yield was significant at 7 days of fermentation with BCY-108 strain (20.260 g L^{-1}), but it did not differ significantly on 9th day (20.277 g L^{1}) of fermentation. The residual sugar content also decreased significantly.

Effect of different ph levels on ethanol yield and residual sugar in the acid hydrolysate of deseeded pods of S. saman

In general the ethanol yield was affected by pH levels as indicated in Table 10.

The ethanol yield was 13.591 and 19.953 g L^{-1} at pH levels of 3.5 and 4.5, respectively which was significant. Beyond pH 4.5 ethanol yield decreased significantly. The highest ethanol yield was recorded in acid hydrolysate at pH 4.5 inoculated with BCY-108 (20.257 g L^{-1}) which was significant as compared to C. wickerhami NCIM 3463 and K. marxianus NCIM 3551. In control also, higher pH inhibited ethanol production with a corresponding increase in residual sugars.

Accordingly, the residual sugar content also decreased significantly at pH 4.5 which was optimum. As the pH increased, there was increase in the residual sugar which was 4.734 g L^{-1} at pH 5.5 and 5.217 g L^{-1} at pH 6.5, indicating their inhibitory effect on fermentation process (Table 2).

Table 2: Effect of different levels of pH on ethanol yield and residual sugar content in the extract of deseeded pods of S. saman.

S. No.	pH	Ethanol yield (gL^{-1})				Residual sugar (gL^{-1})			
		BCY-108	C. wickerhami NCIM 3463	K. marxianus NCIM 3551	Mean	BCY-108	C. wickerhami NCIM 3463	K. marxianus NCIM 3551	Mean
1.	Control	1.053	1.053	1.053	1.053	10.367	10.367	10.367	10.367
2.	3.5	13.800	13.650	13.323	13.591	5.810	5.927	5.983	5.907
3.	Control	3.597	3.597	3.597	3.597	7.883	7.883	7.883	7.883
4.	4.5	20.257	19.857	19.747	19.747	3.703	3.893	4.010	3.869
5.	Control	2.950	2.950	2.950	2.950	8.943	8.943	8.943	8.943
6.	5.5	17.340	17.153	17.013	17.013	4.613	4.753	4.837	4.734
7.	Control	2.047	2.047	2.047	2.047	9.310	9.310	9.310	9.310
8.	6.5	16.203	15.957	14.517	14.517	4.920	5.200	5.530	5.217
	Mean	9.656	9.533	9.281		6.944	7.035	7.108	
	Source	SEm±	CD at 1%			Sem±	CD at 1%		
	pH (A)	0.0171	0.0647			0.0283	0.1071		
	Cultures (B)	0.0105	0.03976			0.0173	0.0655		
	A X B	0.0296	0.1120			0.0490	0.1855		

Summary

Ethanol yield increased significantly due to different fermentation periods upto 7 days beyond which it did not differ significantly between 7th and 9th day (20.002 and 20.010 g L^{-1}). Accordingly, the residual sugar content was also reduced. But, it was non-significant among the cultures.

The pH had significant influence on ethanol yield in the acid hydrolysate of deseeded pods of S. saman. The highest ethanol yield was recorded at pH 4.5 (19.953 g L^{-1}) beyond which it decreased significantly at pH 6.5. Among yeast cultures, BCY-108 proved its efficiency in producing ethanol yield of 9.656 g L^{-1} and was significantly superior to C. wickerhami NCIM 3463 and K. marxianus NCIM 3551. The residual sugar content also was affected due to different pH levels, which ranges from 5.907 to 3.869 g L^{-1} at pH 3.5 and 4.5 respectively.

Ethanol yield had positive influence due to the variation in temperature and inoculation with efficient yeast cultures. The ethanol yield increased significantly upto 30°C beyond which it decreased significantly. The maximum ethanol yield of 20.04 g L^{-1} was recorded at 30°C. Among yeast cultures, BCY-108 recorded highest ethanol yield of 16.856 g $L^{"1}$ as compared to C. wickerhami NCIM 3463 (15.888 g L^{-1}) and K. marxianus NCIM 3551 (15.311 g L^{-1}). While the residual sugar content also decreased significantly at 30°C with inoculation of BCY-108 (3.952 g L^{-1}) and K marxianus NCIM 3551 (22.163 g L^{-1}).

The inoculum size had significant influence on ethanol yield which increased with increase in inoculum level upto 1.5 per cent. Further increase in ethanol yield did not change significantly, but highest was recorded at 1.5 and 2 per cent inoculum level which accounts to 24.131 and 24.154 g L^{-1} respectively. While residual sugar content also decreased significantly at 1.5 and 2 per cent inoculum level, it did not differ significantly (2.573 and 2.549 g L^{-1} respectively). Among yeast cultures, BCY-108 recorded highest ethanol yield (23.407 g L^{-1}) as compared to C. wickerhami NCIM-3463 (22.77 g L^{-1}).

The acid hydrolysate of deseeded pods of S. saman inoculated with efficient yeast cultures along with supplementation of nutrients had positive influence on ethanol yield and reducing sugar content. Among yeast cultures, BCY-108 was found to be efficient in producing ethanol yield as compared to C. wickerhami NCIM 3463 and K. marxianus NCIM 3551. Among nutrients, when acid hydrolysate was supplemented with zinc sulphate @ 10 mg $I/^{1}$ had significantly higher ethanol yield (29.849 g L^{-1}) followed by orthophosphoric acid @ 0.004 per cent (27.228 g L^{-1}), ammonium sulphate @ 0.3 per cent (26.966 g L^{-1}) and sodium dihydrogen orthophosphate @ 0.1 per cent (19.809 g L^{-1}). Accordingly, the residual sugar content also decreased significantly similar to the response of ethanol production for nutrients.

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