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Comparison of Industrial Scale Ethanol Production from a Palmyrah-Based Carbon Source by Commercial Yeast and a Mixed Culture from Palmyrah Toddy

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ABSTRACT

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Palmyrah (*Borassus flabellifer*) based products were used as an alternative carbon source for industrial scale ethanol production**.** The fermentation medium was enriched with spent wash obtained from a distillation column. The performance of a commercially available baker's yeast in the media was compared with a 'palmyrah toddy mixed culture' where the organisms were obtained from the sedimentation of palmyrah toddy. In a laboratory scale study, the ethanol produced from a palmyrah fruit pulp extract, diluted with distilled water, was 16.5 gL^{-1} (36 h) and 13.0 gL^{-1} (48 h) with 'palmyrah toddy mixed culture' and baker's yeast respectively. The 'palmyrah toddy mixed culture' performed better than the baker's yeast with palmyrah fruit pulp extract, diluted either with distilled water or spent wash. Among the different palmyrah based carbon sources, both cultures preferred molasses diluted with spent wash and both performed best in the medium containing the spent wash supplemented with sucrose. In a 5,000 L industrial scale fermentation of 20° Brix molasses supplemented with 10 gL^{-1} ammonium sulphate, 72 gL^{-1} and 65 gL^{-1} ethanol was produced by the 'palmyrah toddy mixed culture' (72 h) and the baker's yeast (90 h) respectively. As the performance of the 'palmyrah toddy mixed culture' was better than that of the baker's yeast, the former was selected for the industrial scale studies of molasses fermentation media diluted with spent wash. In these studies the temperature reached 42°C by 36 h and resultant cell death was observed. However ethanol production was higher and more rapid in the molasses diluted with spent wash, rather than in the molasses diluted with tap water and supplemented with $(NH_4)_2SO_4$. Cell recycle operation obviated the interruption in fermentation caused by temperature induced cell death and increased rates and efficiency of ethanol production were observed.

Key words: fermentation, molasses, palmyrah, 'palmyrah toddy mixed culture', yeast.

Abbreviations: PFP - palmyrah fruit pulp, PC - palmyrah toddy mixed culture, DPFP - depectinized palmyrah fruit pulp, BY - Baker's yeast.

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INTRODUCTION

Ethanol is produced on an industrial level mainly for the production of alcoholic beverages and in some parts of the world as a substitute for fuel[13]. In Sri Lanka, alcohol is produced for the manufacture of alcoholic beverages such as beer, arrack and brandy. The ethanol produced in the northern part of Sri Lanka, especially in the Jaffna peninsula, is from the naturally fermented palmyrah and coconut sap called 'palmyrah toddy' and 'coconut toddy' respectively. The inflorescence of palmyrah and coconut palm is seasoned and the sap (containing 100–160 gL^{-1} of total sugar) is collected in clay pots (approximately 3L capacity) where it is fermented to mixed alcohols such as ethanol, fusel oils (higher alcohols), acetaldehyde, vicinal diketones and esters[4] by air borne microorganisms. Usually the time given for the sap to ferment is about 10 to 18 h and the toddy is collected in the early morning and evening. When the toddy is collected, the sedimented cells are left out in the pots and are reutilised. The fermented sap, i.e. 'toddy', is taken to the distilleries for the manufacture of potable spirit. Spent wash is the effluent waste from the distillation column.

There are over 10 million palmyrah palms in Sri Lanka, spreading over 60,000 acres. The Jaffna peninsula has 7×10^6 palmyrah palms growing widely[9]. A palmyrah palm gives 200–300 fruits per season. About 125 million fruits are produced by all palmyrah palm in Sri Lanka. The average weight of a fruit is 2.25 kg, of this about 40% is pulp[7] and the total sugar content of the pulp is 100 gL^{-1}. As a growing interest emerges in renewable feed stocks for fermentation, the palmyrah based carbon sources are being considered. Previously attempts were made to mechanically extract palmyrah fruit pulp (PFP)[7] and to ferment the palmyrah fruit pulp on a large scale[1].

In areas where the fermentation technology is not refined, any available yeast is used for the fermentation of the pulp. This is likely to be a baker's yeast, and in most cases this gives reasonable results as these yeasts have good fermentative activity. However, with substrates such as palmyrah based carbon sources, they may not perform satisfactorily. Therefore this study was undertaken to compare the performance of baker's yeast (BY) with that of the organisms obtained from the sedimentation of 'palmyrah toddy'. In this paper the utilization of PFP and un-

derutilized palmyrah molasses as feed stocks for ethanol fermentation at a laboratory and an industrial scale is presented. To ferment these palmyrah based products, a commercially available baker's yeast (which is also used by local industries) and the cells from the sediment of palmyrah toddy were used.

MATERIALS AND METHODS

Materials

Palmyrah fruits were obtained from the palmyrah palm (*Borassius flabellifer*) grown in the Jaffna peninsula, in the Northern part of Sri Lanka. Spent wash (collected from the distillation column effluent) and molasses were obtained from Palm Products Distilleries, Thikkam, Jaffna, Sri Lanka. Palmyrah toddy (24 h old) was supplied by Palm Products Cooperative Societies Cluster Ltd., Jaffna Sri Lanka. The baker's yeast was Fermipan (Gist Brocades, The Netherlands). All the other materials were purchased from standard sources, unless otherwise stated.

Analytical methods

Standard methods for the estimation of glucose[8] reducing sugar[8], soluble proteins[6], amino acids[12], alcohol[16] total nitrogen[11], viable cell count[14], total cell count[3] and total acidity[15] were used to analyse the various samples. Estimation of total sugars in the samples was determined after acid hydrolysis[11].

Peptone, yeast extract and nutrient (PYN) medium

The PYN medium described by Odumeru et al.[10] was used with a slight modification. The medium contained (g L^{-1}) peptone, 3.5; yeast extract, 3.0; $MgSO_4$ $7H_2O$, 1.0; KH_2PO_4, 2.0 and $(NH_4)_2SO_4$, 1.0 at pH 5. Under different experimental conditions, different amounts of glucose were added to the medium and the medium was represented as 'glucose (amount in g) – PYN medium'.

Inoculation of laboratory scale fermentations

Palmyrah toddy mixed culture (PC) inoculum. Palmyrah toddy (10 h old) was collected from 'clay pots' and shaken in a reciprocal water bath (150 rpm) at 36°C after the addition of the antibiotic penicillin (1 gL^{-1}). Cells were separated by centrifugation after 24 h of incubation, resuspended in normal saline (6×10^7 cells mL^{-1}) and used for inoculation.

Baker's yeast inoculum. Baker's yeast (0.5 g) was grown in glucose (50 gL^{-1}) – PYN medium at 36°C with shaking (150 rpm) for 24 h.

Inoculum development for industrial scale fermentations

Palmyrah toddy mixed culture inoculum. Inoculum development was carried out by adding toddy sediment collected from 5 bottles of 24 h old toddy to 10 L of diluted molasses (10° Brix) supplemented with 10 gL^{-1} of $(NH_4)_2SO_4$ and the pH was adjusted to 5 with phosphoric acid. After 18 h, this was transferred to 100 L of the same fresh medium and incubated as above. Finally 1,100 L inoculum was developed by inoculating this 100 L inoculum to 1,000 L of the above medium followed by incubation.

Baker's yeast inoculum. Baker's yeast inoculum (BY) (500 g) was added to 10 L of 37°C tap water and left for 30 min. This activated yeast was inoculated into 10 L of diluted molasses (20° Brix) and supplemented with 20 gL^{-1} of $(NH_4)_2SO_4$ and stirred. After 18 h, this was transferred to 100 L of fresh medium and incubated as above. Finally 1,100 L inoculum was developed by inoculating this 100 L inoculum to 1,000 L of the above medium followed by incubation.

Extraction of palmyrah fruit pulp

Three ripened palmyrah fruits (4.7 kg) were peeled. The pulp was extracted manually by squeezing the peeled fruits after maceration in 450 mL distilled water. The extraction was repeated with 450 mL of distilled water and the two extracts were pooled and strained through a muslin cloth to remove fibres. Weight and volume of the extract and weight of the seeds after the extraction were measured. Total sugars in the PFP extract were estimated.

Depectinization of palmyrah fruit pulp extract

The pH of the PFP extract (200 mL) was adjusted to 9 using 2N NaOH. Calcium chloride solution (4.8 mL, equivalent to 0.48 g $CaCl_2 \cdot 2H_2O$) was mixed with the PFP extract and gelation was allowed. After 12 min, the gel was cut into small pieces with a stainless steel knife. These pieces were placed in a muslin cloth and gently pressed by hand. Total sugars and volume of the depectinized palmyrah fruit pulp (DPFP) extract were determined.

Ethanol production on a laboratory scale

Fermentation of palmyrah fruit pulp extract. PFP extract (50 mL containing 100 gL^{-1} total sugars) was diluted with distilled water (50 mL) or spent wash (50 mL) and the final pH was adjusted to 5. This made the final concentration of the total sugar to 50 gL^{-1}. The media were inoculated with either a PC or a BY inoculum (10%, v/v) (6×10^7 cells mL^{-1}) and incubated at 36°C with shaking (150 rpm). Residual sugar and ethanol produced were monitored.

Fermentation of palmyrah fruit pulp extract diluted with spent wash and supplemented with sucrose

Sucrose (5 g) was added to PFP (50 mL) and diluted to 100 mL with spent wash. This medium was prepared in duplicate. The PFP extract diluted with spent wash was used as a control and the experiment proceeded as above.

Ethanol from different palmyrah based carbon sources. Media with different palmyrah based carbon sources were prepared (Table I) and the experiment was carried out as described above.

Table I. Composition of media prepared from palmyrah based carbon sources[a].

Type of carbon source	Amount (mL)	Spent wash (mL)	Sucrose (g)	Distilled water (mL)
PFP	50.0	50.0	5.0	-
DPFP	50.0	50.0	4.8	-
Molasses	17.9	50.0	-	32.1
Control	-	50.0	10.0	50.0

[a] The final sugar concentration in palmyrah fruit pulp extract (PFP) and depectinized palmyrah fruit pulp (DPFP) media was made to 100 gL^{-1} with sucrose, while the molasses was diluted with distilled water and spent wash. Control medium was prepared by supplementing spent wash with sucrose. The total sugar content was 100 gL^{-1}.

Table II. Comparison of fermentation of palmyrah fruit pulp extract (PFP) extract diluted with distilled water or spent wash, PFP extract supplemented with sucrose (50 gL^{-1}), PFP extract diluted with spent wash, depectinized palmyrah fruit pulp extract (DPFP) diluted with spent wash and supplemented with sucrose (48 gL^{-1}), molasses diluted with spent wash and spent wash supplemented with sucrose (100 gL^{-1}) by 'palmyrah toddy mixed culture' (PC) and baker's yeast (BY) at pH 5 and 36°C[a].

	Ethanol (gL^{-1})[b]									
	PFP + DW[c]		PFP + SW		PFP + SW + S		DPFP + SW + S		M + SW	
Time (h)	PC	BY	PC	BY	PC	BY	PC	BY	PC	BY
16	5.0 (37.0)	0.20 (44.0)	20.0 (50.0)	23.0 (43.0)	22.0 (48.0)	22.0 (48.0)	23.0 (43.0)	9.0 (76.0)	26.0 (42.0)	18.0 (53.0)
24	10.0 (23.0)	6.0 (32.0)	32.0 (20.0)	35.0 (13.0)	33.0 (23.0)	33.0 (23.0)	35.0 (13.0)	21.0 (43.0)	40.0 (08.0)	33.0 (14.0)
36	16.5 (7.0)	9.0 (24.0)	38.0 (05.0)	39.0 (4.0)	38.0 (10.0)	38.0 (10.0)	39.0 (4.0)	30.0 (19.0)	43.0 (1.0)	38.0 (3.0)
48	16.5 (7.0)	13.0 (13.0)	38.0 (5.0)	39.0 (8.0)	40.0 (4.0)	40.0 (4.0)	39.0 (8.0)	34.0 (8.0)	43.0 (1.0)	38.8 (1.0)

[a] The pH of the medium was adjusted to 5 and the fermentation temperature was 36°C.
[b] The value given in the parenthesis is the residual sugar content (gL^{-1}).
[c] DW: Distilled water; SW: spent wash; S: sucrose; M: molasses.

Industrial scale fermentation of palmyrah molasses

Comparison of the fermentation of palmyrah molasses supplemented with $(NH_4)_2SO_4$ by 'palmyrah toddy mixed culture' or baker's yeast. The molasses substrate (60° Brix) was diluted to 20° Brix, with tap water to a volume of 10,000 L and supplemented with 10 gL^{-1} of $(NH_4)_2SO_4$. The pH of the medium was adjusted to 5 with phosphoric acid. The medium was divided into 5,000 L portions and the first portion was inoculated with a 10% (v/v) PC inoculum (labelled test) and mixed thoroughly by recirculating the same with the aid of a pump. Temperature at the centre of the fermenter, initial cell number, ethanol and residual sugar contents were monitored. As a control, the second portion (5,000 L) was inoculated with a 10% (v/v) yeast inoculum.

Fermentation of palmyrah molasses supplemented with either $(NH_4)_2SO_4$ or spent wash by 'palmyrah toddy mixed culture'. A molasses substrate (60° Brix) was diluted to 20° Brix, either with tap water (labelled control) or spent wash (labelled test) to a volume of 5,000 L. The molasses diluted with tap water (control) was supplemented with 10 gL^{-1} of $(NH_4)_2SO_4$. The pH of both media was adjusted to 5 with phosphoric acid. The experiment was carried out as described above.

'Palmyrah toddy mixed culture' cell recycle operation in an industrial scale fermentation of palmyrah molasses diluted with spent wash. The molasses substrate (60° Brix) was diluted to 20° Brix with spent wash to a volume of 5,000 L. The pH of the medium was adjusted to 5. The medium was inoculated with a 10% (v/v) PC inoculum. Mixing was facilitated and proceeded as described above. At the end of the fermentation the cells were allowed to settle. The fermented medium from the fermentation tank was removed by tapping the spent wash above the level of the cell deposit. To the deposited cells in the fermentation tank, fresh molasses diluted with spent wash (5,000 L) was introduced and the process was repeated.

RESULTS AND DISCUSSIONS

Extraction and depectinization of palmyrah fruit pulp

The pulp was extracted by manually squeezing the pulp with water. The total volume and weight of the PFP extract obtained from three palmyrah fruits were 2,500 mL and 2.38 kg respectively. After filtration through muslin cloth (to remove fibres), 1.65 kg PFP extract was obtained. (The weight of the seeds and the fibres was 2.65 kg). Hence the net weight of the PFP extract from 4.7 kg palmyrah fruit was 1.65 kg and the pulp content of the fruit was 35% (w/w). The average amount of the PFP extracted from a fruit was 0.55 kg. Mahendran et al.[7] obtained 0.630 kg of pulp per fruit. The pH and the total sugar content were 4.1 gL^{-1} and 100 gL^{-1} respectively. The liquid entrapped in the interstitial spaces within the gel of DPFP extract was a pale yellow colour extract (160 mL) and contained 104 gL^{-1} total sugars. The recovery of the sugar was 83.2%. The Brix value of the extracted pulp was measured with a hand refractometer and was 12° Brix and similar to previous observations[7].

Ethanol production in laboratory scale

Fermentation of palmyrah fruit pulp extract. The PFP extract (initial sugar 100 gL^{-1}) was diluted either with distilled water or spent wash and the fermentation was carried out with either PC or BY. The ethanol produced from PFP extract diluted with distilled water was 16.5 gL^{-1} (36 h) and 13 gL^{-1} (48 h) with PC and BY respectively (Table II). This suggested that in addition to fermentable sugars, PFP extract contained the nutrients necessary to

Table III. Kinetics of ethanol production rate by palmyrah toddy mixed culture (PC) and baker's yeast (BY) in different combinations of palmyrah fruit pulp (PFP) extract medium[a].

Parameter	PFP + DW[b]		PFP + SW	
	PC	BY	PC	BY
Ethanol (gL^{-1})	16.5 (36 h)	13.5 (48 h)	20.0 (36 h)	17.0 (48 h)
Efficiency (%)[c]	64.5	53.0	78.2	66.5
Ethanol yield[d]	0.33	0.27	0.40	0.34
Ethanol production rate ($gL^{-1}h^{-1}$)[e]	0.46	0.28	0.55	0.35

[a] The time at which maximum ethanol produced is given in parenthesis.
[b] DW: Distilled water; SW: spent wash.
[c] Ethanol produced / theoretical maximum × 100.
[d] g ethanol / g of sugar used.
[e] Ethanol produced / time taken to produce the ethanol.

support fermentation. Ethanol produced with PC and BY was 20 gL^{-1} (36 h) and 17 gL^{-1} (48 h) respectively in PFP extract diluted with spent wash medium. The PC showed better performance than BY and the time taken to complete the fermentation was shorter. Utilization of spent wash instead of distilled water improved the ethanol production by both the PC and BY cultures (Table II). The spent wash contained reducing sugar (0.5 gL^{-1}), total sugar (0.8 gL^{-1}), total acids (3.34 gL^{-1}), free amino acids (3.18 gL^{-1}) and total nitrogen (11 gL^{-1}). The pH was 3.5. Some nutrients would have leaked from the yeast cells at the end of the fermentation[5], leading to a biologic oxygen demand of 20,000 to 25,000. Such nutrients present in the spent wash may be giving beneficial effects to newly inoculated PC and BY cells. Therefore the improved performance of both of the cultures in the PFP extract diluted with spent wash medium could perhaps be attributed to the presence of nutrients from yeast, in the spent wash.

Table III depicts the amount and rate of ethanol production, efficiency and yield. The spent wash improved the performance of both PC and BY and alcohol produced was 17 gL^{-1} and 20 gL^{-1} respectively. These low levels of alcohol are economically not suitable for distillation on an industrial scale. Therefore in subsequent experiments, the PFP extract was diluted with spent wash that was supplemented with sucrose.

Ethanol production from palmyrah fruit pulp extract diluted with spent wash and supplemented with sucrose. PC produced 38 gL^{-1} ethanol at 36 h while BY produced 30 gL^{-1} and 34 gL^{-1} ethanol at 36 and 48 h respectively in a medium comprising PFP extract diluted with spent wash and supplemented sucrose (Table II). Therefore the increase in total sugar concentration from 50 - 100 gL^{-1} increased ethanol production with both PC and BY. The efficiency and yield of ethanol production by PC were 74.3% and 0.38 g ethanol/g sugar respectively and those by BY were 66.5% and 0.34 g ethanol/g sugar respectively. Therefore, in the subsequent fermentation studies, tap water was replaced with spent wash for dilution. Although PC performed superior to BY, the latter is not affected by seasonal and locational variations. Hence further experiments used the BY culture.

Ethanol from palmyrah based carbon sources. In this set of experiments PFP extract was diluted with spent wash and supplemented with sucrose. DPFP extract (50 mL, 104 gL^{-1} total sugar) diluted with spent wash (50 mL) and supplemented with sucrose (48 gL^{-1}) and molasses (17.85 mL, 560 gL^{-1} total sugar) supplemented with sucrose was used as the control (Table I). In all the media, the total sugar content was 100 gL^{-1} and the amount of ethanol produced is given in Table II.

Table IV. Glucose utilization, over all utilization, overall ethanol yield and ethanol production efficiency of 'palmyrah toddy mixed culture (PC) and baker's yeast (BY) in different palmyrah based carbon sources at pH 5 and 36°C.

Medium	Sugar utilization (%)[a]		Ethanol yield[b]		Ethanol production efficiency (%)[c]	
	PC	BY	PC	BY	PC	BY
PFP[d] + SW + S	95	91.8	0.4	0.37	74.4	66.5
DPFP + SW + S	97.5	92.4	0.40	0.37	75.3	66.9
M + SW	96.0	92.7	0.41	0.37	75.3	68.5
SW + S	98.9	98.8	0.43	0.38	84.1	74.4

[a] Sugar utilized / initial total sugar × 100.
[b] Ethanol produced / sugar utilized × 100.
[c] Ethanol produced / theoretical amount of ethanol that could be produced from total sugar × 100.
[d] PFP: Palmyrah fruit pulp extract; SW: spent wash; S: sucrose; DPFP: depectinized palmyrah fruit pulp extract; M: molasses.

The PC did not show significant variation in the fermentation of PFP extract and DPFP extract diluted with spent wash and supplemented with sucrose. However the initial rate of fermentation was high in the DPFP extract diluted with spent wash and supplemented with 48 gL^{-1} sucrose when compared to the PFP extract diluted with spent wash and supplemented with 50 gL^{-1} sucrose. In industrial practice, the DPFP medium is preferred to PFP as the pectin found in PFP could be demethylated[2] and result in the production of methanol. The methanol in the fermentation medium could contaminate the spirit produced by distillation and its presence disqualifies the distilled product for human consumption. Further, the sticky nature of pectic substances cause problems in handling the medium, feeding to the plant (blocking the valves and pumps) and may also cause incrustation in the distillation plant. Moreover, the removed pectin can find a place in the food industry.

The rate of sugar utilization (% of initial sugar used), yield (g ethanol/g sugar used) and efficiency of ethanol production efficiency are shown in Table IV. The results showed that the PC performed better than the BY. Among the media, maximum performance by PC was observed in spent wash supplemented with sucrose. The rate of sugar utilization, overall ethanol yield and ethanol production efficiency were highest in spent wash supplemented with sucrose medium (Table IV). The performance of PC was better than BY in all of the media tested (Table II). Therefore with the PC, a wild microbial population can be used as a mixed culture or suitable isolates can be selected from the mixed population.

Industrial scale fermentation of palmyrah molasses

Comparison of the fermentation of palmyrah molasses supplemented with $(NH_4)_2SO_4$ by 'palmyrah toddy mixed culture' and baker's yeast. In the control, where BY was used as the inoculum, the amount of ethanol produced (at 90 h) and residual sugar content was 65

Table V. Industrial scale ethanol production from palmyrah molasses[a].

Time (h)	Ethanol (gL^{-1})	Medium temperature (°C)
24	40	38
36	48	42
48	52	38
72	70	40

[a]The molasses (60° Brix) diluted to 20° Brix with tap water was supplemented with $(NH_4)_2SO_4$ (10 gL^{-1}) and inoculated with the 'palmyrah toddy mixed culture' inoculum. Ethanol and temperature were monitored during the course of fermentation.

gL^{-1} and 0 gL^{-1} respectively. Therefore in the large scale fermentation of molasses (20° Brix) supplemented with 10 gL^{-1} of $(NH_4)_2SO_4$, the ethanol yield was 0.33 and the efficiency was 64%.

In the test with PC, the fermentation was very brisk when compared to the control. Table V depicts the changes in temperature and ethanol production with fermentation time. At 36 h, the fermentation became vigorous and the temperature rose to 42°C. Subsequently the fermentation ceased abruptly. At this juncture the medium was left as such for observation and the fermentation was restarted at 48 h and after 72 h. From the time of addition of the inoculum, 70 gL^{-1} ethanol was produced. The increase in temperature during the vigorous phase of fermentation could be due to the exothermal metabolic activity of microbial populations in the medium. Ingledew[5] reviewed that the heat generated by yeast fermenting 9 g of glucose in a 10% (w/w) solution is about 1.7 Kcal, and this energy is enough to raise the temperature of the medium by 17.9°C. Practically this is not experienced because heat is lost both by evaporation and dissipation. In tropical regions such as Jaffna, where the environmental temperature is high, heat dissipation becomes insufficient to control the temperature of the medium and it often rises to lethal levels. As this stage, the viability of the PC cells was 10% and the reduced viability could be attributed to the thermal death of the cells. The ethanol yield and efficiency with PC was 0.36 kg ethanol/g sugar used and 70.5%, respectively. In addition to higher ethanol yield and efficiency, the PC completed the fermentation in 72 h versus 90 h by the BY.

In the industrial scale fermentation of molasses, the use of PC would result in improved economic gains. PC gave a higher production of ethanol along with reduced fermentation times, saving steam required for distillation due to the high percentage of alcohol in the feed and savings in fermentation house capacity. The final alcohol strength produced from 20° Brix molasses by PC and BY was 70 gL^{-1} and 65 gL^{-1} respectively. Thus the alcohol production by PC was 5 gL^{-1} greater than that of BY. In the 5,000 L industrial scale fermentation, 25,000 g more ethanol was produced by the PC. The 25,000 g additional ethanol was fed to the distillation column without a change in the feed volume. The percentage ethanol recovery from feed by the column distillation in the distillery was 90%. By the additional feed of ethanol, 22,500 g ethanol could be recovered leading to a net gain in ethanol production in the distillery of 22,500 g. The cost of production of 1,000 g ethanol from molasses is USD 9.1. Here a USD 204.6 profit was achieved by the distillation of 5,000 L feed fermented by PC. Further, savings in fermenter capacity were achieved due to a reduced time cycle of fermentation from 70 h to 65 h. The reduced fermentation time gives a 7.1% savings in fermenter capacity.

Savings in steam due to the higher percentage of ethanol in the feed were observed. When the feed ethanol content was increased from 65 to 70 gL^{-1}, the same column distillation plant showed double the amount of steam saving per 4.5 L of distilled spirit (the average of data from 50 operations in the distillery). Therefore a significant amount of steam savings and reduction in spent wash volume was achieved by increasing the feed concentration from 65 gL^{-1} (BY fermented) to 70 gL^{-1} (PC fermented).

In spite of all these advantages, fermentation by PC faces the problem of a sudden decrease in fermentation rate after 36 h, with the depletion of viable cells to 10%. Hence to maintain a steady rate of fermentation, either the fermenter must be cooled to minimize the temperature rise, or a thermotolerant yeast must be used as the inoculum. In the present industrial scale fermentation study, the fermentation was initiated by a mixed culture, which was obtained by the sedimentation of toddy. The progress in the fermentation led to the rise in temperature and a selection pressure was exerted on the microbial population. Thermotolerant strains could possibly have survived in this situation and subsequently those populations could have increased to adequate cell numbers, enough to restart the fermentation.

Fermentation of palmyrah molasses supplemented with either $(NH_4)_2SO_4$ or spent wash by 'palmyrah toddy mixed culture'. In general, a large volume of spent wash is produced after distillation. To prevent the usage of chemicals and to reduce the need for makeup water and to minimize the volume of the spent wash drained, a trial run was performed using spent wash as a diluent for high gravity molasses. The high gravity molasses substrate (60° Brix) was diluted to 20° Brix with either tap water or spent wash. The pH of the spent wash diluted molasses was 5 and the pH of the tap water diluted molasses was adjusted to 5 with phosphoric acid. Both of the media were inoculated with PC. The initial cell density was 2×10^7 cells/mL^{-1}. Table VI shows the progress of fermentation over time. At 24 h, the fermentation medium became very active (vigorous) and the observations were the same as in the previous experiment. When the temperature of the medium rose to 42°C at 36 h, the fermentation ceased and 48 gL^{-1} and 49 gL^{-1} ethanol was produced in the control and test media respectively. The residual sugar in the control and test media was 0.5 gL^{-1} and 0.3 gL^{-1} respectively. The efficiency of ethanol production in the test medium was 71% versus 68.5% in the control medium. The cost analysis indicates a profit of USD 81.8 per single batch operation of 5,000 L capacity. A cost effective industrial scale fermentation process was developed by replacing the BY with PC. The volume of the makeup water was reduced by spent wash recycling, utilization of chemicals was avoided, 10,000 g ethanol was produced in excess and the spent wash disposal problem (pollution) was minimized.

Table VI. Production of ethanol and changes in temperature in the fermenter, during the course of the fermentation when the palmyrah molasses substrate was fermented at an industrial scale[a].

Time (h)	Ethanol (gL^{-1})		Media Temperature (°C)		Environment temperature (°C)[b]
	Control	Test	Control	Test	
24	40	36	38	38	32
36	48	49	42	42	34
48	52	51	38	38	33
72	70	72	38	38	33

[a] The molasses (60° Brix) was diluted to 20° Brix either with tap water and supplemented with $(NH_4)_2SO_4$ (labeled control) or diluted with spent wash (labeled test) and inoculated with inoculums developed from the palmyrah toddy mixed culture.
[b] Environment temperature was measured at a distance of 30 cm from the outer surface of the fermenter.

Table VII. Production of ethanol and changes in temperature during the cell recycle operation on an industrial scale ethanol production process from palmyrah molasses[a].

Time (h)	Ethanol (gL^{-1})	Medium Temperature (°C)
24	42	39
36	52	41
48	68	42
65	80	39

[a] The sedimented cells obtained from the first batch (spent wash medium) were reused for the second batch industrial scale fermentation. The molasses (60° Brix) was diluted to 20° Brix with spent wash and inoculated with the 'palmyrah toddy mixed culture'.

'Palmyrah toddy mixed culture' cell recycle operation in an industrial scale fermentation of palmyrah molasses diluted with spent wash. In addition to the use of PC for the molasses fermentation, another modification in the normal distillery practice was introduced by implementing a cell recycle operation. The ceased fermentation with the 'palmyrah toddy mixed culture' was restarted with the multiplication of the 10% viable cells which had survived when the temperature rose to 42°C. Therefore, the utilization of the survived cells was investigated for cell recycling.

When compared with the first batch of fermentation, the cell-recycle operation reduced the fermentation time from 72 h to 65 h and also increased ethanol production from 72 gL^{-1} to 80 gL^{-1} (Table VII). The ethanol yield and ethanol production efficiency were 0.4 g ethanol/g sugar used and 78.9%, respectively. The ethanol produced was increased by 11% by implementing the cell recycle process. In the previous experiment, an economic analysis was made and a similar analysis to this process points out the merits of a cell recycle operation. The increase in ethanol produced by 8 gL^{-1} would result in a 40,000 g ethanol increase in the feed to the distillation plant. As the efficiency of ethanol recovery by distillation was 90%, 36,000 g ethanol was obtained in excess when compared to the first batch operation. This resulted in the additional production of ethanol worth USD 327.3.

During the cell-recycle operation, the temperature of the medium increased to 42°C at 36 h and the fermentation continued without slowing. This could be due to the presence of the selected yeast population in the medium. Settled yeast strains were used and the cells could have adapted to spent wash diluted molasses medium during the first cycle of the fermentation. This is an important advantage in cell recycle operations.

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