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The use of tamarind waste to improve ethanol production from cane molasses

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Tamarind wastes such as tamarind husk, pulp, seeds, fruit and the effluent generated during tartaric acid extraction were used as supplements to evaluate their effects on alcohol production from cane molasses using yeast cultures. Small amounts of these additives enhanced the rate of ethanol production in batch fermentations. Tamarind fruit increased ethanol production (9.7%, w/v) from 22.5% reducing sugars of molasses as compared to 6.5% (w/v) in control experiments lacking supplements after 72 h of fermentation. In general, the addition of tamarind supplements to the fermentation medium showed more than 40% improvement in ethanol production using higher cane molasses sugar concentrations. The direct fermentation of aqueous tamarind effluent also yielded 3.25% (w/v) ethanol, suggesting its possible use as a diluent in molasses fermentations. This is the first report, to our knowledge, in which tamarind-based waste products were used in ethanol production.

Keywords: yeast; cane molasses; ethanol; tamarind

Introduction

Continuous depletion of petroleum reserves has created an immediate need to search for alternate fuel energy sources. The bioconversion of renewable substrates to ethanol is still the focus of much research. The production of ethanol from sugar cane molasses has been the subject of many studies. In the present context of ethanol production using batch fermentation processes, two areas of investigation are the improvement of the rate of ethanol production and final ethanol concentration by supplementation of cane molasses [4,6,8,9] and the development of strains to be used in cane molasses fermentation [5,7]. In India, ethanol is produced by conventional batch fermentation of cane molasses in which 7–8% (v/v) ethanol is produced from diluted molasses sugar (15–16%) with 80–85% efficiency. Supplementation with ergosterol [1], chitin [9], fungal mycelium [11], skim milk powder [8] or proteolipid from *Aspergillus oryzae* [6] have improved the rate and the yield of ethanol production in cane molasses fermentation. New production techniques such as the use of vacuum fermentation [13], cell recycling [15] and top and bottom yeast [10] have been introduced for better production of industrial alcohol.

In our laboratory, studies were initiated to increase the rate and yield of alcohol production by supplementation technology in which waste materials generated during tartaric acid extraction from tamarind fruit were added to the fermentation medium [12]. The production of tamarind fruit in India is about 300 000 tons per year and only 75 000 tons are properly consumed. Tamarind fruit contains major components like carbohydrates (35–40%), tartaric acid (12–18%) and edible protein and vitamins. A process has been developed in our laboratory for the extraction of tartaric acid from tamarind fruit. This process releases waste material such as tamarind pulp, aqueous effluent in addition to tamarind husk and seeds separated before extraction.

We report here the use of such waste material in cane molasses fermentation to enhance the rate of ethanol production from high molasses sugar concentrations.

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Materials and methods

Organisms and culture media

The yeast cultures *Saccharomyces cerevisiae* NCIM 3526 and 3300 were obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune 411 008, India. These cultures were routinely maintained on MGYP slants and subcultured every 2 months. MGYP medium contained malt extract, 0.3%; yeast extract, 0.3%; peptone, 0.5%; glucose, 2.0% and agar, 2.0%. MUMY medium consisted of molasses sugar, 5%; urea, 0.2%; $MgSO_4$, 0.05% and yeast extract 0.1%. Molasses fermentation medium consisted of variable amounts of molasses sugar supplemented with 0.2% urea. Glucose fermentation medium consisted of glucose, 20%; $(NH_4)_2SO_4$, 0.1%; urea, 0.2%; KH_2PO_4, 0.1%; $MgSO_4$, 0.05% and yeast extract, 0.1%. The pH of both fermentation media was adjusted to 5.0 before sterilization at 121°C for 20 min. Fermentation media were supplemented with additives like tamarind husk, tamarind fruit etc at concentrations varying between 0.1–1.0%.

Yeast extract, malt extract and peptone were obtained from Hi-Media, Bombay, India. Sugar cane molasses containing 53% reducing sugar was obtained from a local sugar factory. All other chemicals were of analytical grade and obtained locally. Tamarind fruit was obtained from local markets. All other waste material generated during tartaric acid extraction was procured from the Organic Chemistry Technology (OCT) Division of this laboratory.

Preparation of hot and cold water extracts

The tamarind supplement (10 g) was suspended in 100 ml of cold water and shaken at 180 rpm and 30°C overnight. The solid material was separated by filtration through muslin cloth and the filtrate was used as a supplement to the fermentation medium. The hot water extract was prepared by boiling the supplements (10 g) in 100 ml of distilled water for 30 min followed by filtration. Five millilitres of each extract contained equivalent amounts of soluble ingredients present in 0.5 g of supplement.

Batch fermentation of glucose or cane molasses

Cultures were grown either in MGYP or MUMY medium for 24 h at 30°C with constant shaking at 180 rpm and used as inocula. The inoculum (10 ml) was transferred to 90 ml of fermentation medium in a 150-ml Pyrex conical flask with added supplements and incubated at 30°C without shaking. Samples (5 ml) of the fermented broth were withdrawn after 24, 48 and 72 h for the determination of ethanol content. The fermentation efficiency was calculated on the basis of total fermentable sugars in the fermentation medium. Results are the average of three independent experiments. The standard deviation of values ranged from ±2–±3%.

Analytical methods

Total reducing sugars (TRS) in molasses and samples were estimated as glucose equivalents by the dinitrosalicylic acid method after inversion with HCl [3]. Ethanol concentrations were determined by the cerric ammonium nitrate method [14] which was confirmed by gas chromatography using a Shimadzu GC RIA model instrument with a chromosorb 101 column (80–100 mesh, 2 m). The column temperature was maintained at 150°C and the injection temperature was 180°C. The flow rate of the carrier gas (nitrogen) was 40 ml min^{-1} and a flame ionization detector was used at 180°C.

Results

Table 1 summarizes the effect of different tamarind-based supplements on ethanol production using glucose fermentation medium with 20% glucose. In general, all the supplements used showed increased levels of ethanol production after 72 h. Tamarind effluent was the least effective, showing only 16% improvement with respect to controls. The addition of these supplements also resulted in increased fermentation efficiency (>90%) as compared to the unsupplemented control (62.5%). Among all the additives used, tamarind fruit gave maximum production with 98% fermentation efficiency. Calcium pectate and calcium tartarate showed marginal improvement in ethanol production.

Table 2 shows that supplementation of molasses fermentation medium (15% TRS) caused a significant increase in both the rate and efficiency of ethanol production over unsupplemented medium. The improvement varied between 11–50% at 48 h. Fermentation was complete at 48 h in molasses medium supplemented with tamarind fruit (TF), tamarind pulp (TP), tamarind husk (TH) and tamarind seed powder (TSP), producing 6.7% (w/v) ethanol with maximum efficiency (>95%). Extracts of tamarind-based waste materials in hot and cold water also produced improvements in ethanol production. Solids of tamarind-based materials appeared to be more effective.

Fermentations were carried out using supplemented molasses medium containing 20% TRS and the results are shown in Table 3. It is noteworthy that all the tamarind-based waste products used as supplements, gave improved ethanol production with maximum fermentation efficiency (>95%). Significant improvement (>100%) in ethanol production was observed at 48 h. There was a decline in improvement in ethanol production after 72 h indicating a rapid increase in ethanol productivity during the initial phase of fermentation (Table 3). Calcium pectate, calcium tartarate and tamarind effluent did not produce a significant improvement in ethanol production (data not shown). Further experiments were performed using all the effective supplements (TF, TH, TP, TSP) individually at different concentrations. The data given in Table 4 indicate that 0.5% of each supplement was enough to give improved ethanol production with maximum efficiency.

Ethanol production using different concentrations of

Table 1 Effect of tamarind-based supplements on ethanol production in fermentation medium containing 20% glucose

Supplement	Ethanol (%, w/v) at				
	48 h	% IMP	72 h	% IMP	% EFF at 72 h
None	4.1	–	6.2	–	62
Tamarind fruit	6.4	56	9.8	58	98
Tamarind husk	6.3	53	9.6	54	96
Tamarind pulp	6.5	58	9.1	46	91
Tamarind effluent (5 ml)	5.0	22	7.2	16	72
Tamarind seed powder	6.4	56	9.6	54	96
Ca-pectate	5.1	24	7.1	14	71
Ca-tartarate	4.2	–	6.3	–	63

IMP, improvement; EFF, fermentation efficiency.

$$\%\ \text{IMP} = \left[\frac{\text{Ethanol formed in supplemented medium}}{\text{Ethanol formed in unsupplemented (control) medium}} \times 100 \right] - 100$$

$$\%\ \text{EFF} = \frac{\text{Experimental ethanol yield}}{\text{Theoretical ethanol yield}} \times 100$$

Table 2 Effect of tamarind-based products and their extracts on ethanol production from molasses fermentation medium containing 15% TRS

Supplement	Ethanol (%, w/v) at			
	24 h	48 h	% IMP at 48 h	% EFF at 48 h
None	2.4	4.5	–	65
Tamarind fruit	5.5	6.8	51	98
Tamarind pulp	5.4	6.7	48	97
Tamarind husk	5.3	6.7	48	97
Tamarind seed	5.2	6.7	49	97
Ca-pectate	3.6	6.3	40	93
TFEC (5 ml)	4.6	6.0	33	87
TFEH (5 ml)	4.8	6.1	35	88
TPEC (5 ml)	4.2	5.4	20	78
TPEH (5 ml)	4.3	5.5	22	80
THEC (5 ml)	4.1	5.0	11	72
THEH (5 ml)	4.2	5.1	13	74

% IMP and % EFF as per Table 1.
TRS, total reducing sugar; TFEC, tamarind fruit extract in cold water; TFEH, tamarind fruit extract in hot water; TPEC, tamarind paste extract in cold water; TPEH, tamarind paste extract in hot water; THEC, tamarind husk extract in cold water; THEH, tamarind husk extract in hot water.

Table 3 Effect of tamarind-based supplements on ethanol production from 20% molasses sugar

Supplement	Estimated alcohol (w/v) at				
	48 h	% IMP	72 h	% IMP	% EFF at 72 h
None	3.4	–	6.1	–	61
Tamarind fruit	7.6	123	8.8	44	97
Tamarind husk	7.4	117	8.8	44	97
Tamarind pulp	7.4	117	8.7	42	95
Tamarind seed	7.3	114	8.7	42	95

% IMP and % EFF as per Table 1.

Table 4 Effect of different concentrations of supplements on ethanol production from 15% molasses sugar

Supplement (%)	Ethanol (%, w/v) at				
	24 h	% IMP	48 h	% IMP	% EFF
None	2.5	–	4.4	–	66
Tamarind fruit					
(0.1)	3.9	56	5.6	27	83
(0.2)	5.2	108	6.5	48	97
(0.5)	5.6	124	6.6	50	95
Tamarind pulp					
(0.1)	5.0	100	6.0	36	89
(0.2)	5.4	116	6.1	38	89
(0.5)	5.5	120	6.2	40	91
Tamarind husk					
(0.1)	4.1	64	6.1	38	89
(0.2)	5.3	112	6.1	38	89
(0.5)	5.2	108	6.5	47	95
Tamarind seed					
(0.1)	3.8	52	6.2	40	90
(0.2)	4.4	76	6.4	45	93
(0.5)	5.1	104	6.7	52	98

% IMP and % EFF as per Table 1.

molasses sugars showed that the optimum concentration for maximum ethanol production was 22.5% (Table 5) in supplemented media. At this concentration 9.7% (w/v) ethanol was produced in the presence of supplements like TF and TH, representing more than 95% of the theoretical yield. Tamarind effluent itself, containing 7.5% reducing sugar, could be fermented directly yielding 3.25% (w/v) ethanol (data not shown).

Discussion

Under the fermentation conditions described, the addition of any of the tamarind-based waste materials substantially enhanced both the rate and yield of ethanol production irrespective of the molasses sugar concentration used in fermentation media. All supplements except tamarind fruit, were waste materials generated during tartaric acid extraction from tamarind fruit. The addition of such supplements (>0.5%) in fermentation medium with 20% glucose produced 9.8% (w/v) alcohol at 72 h of fermentation compared to 6.3% (w/v) in the unsupplemented control. Similar

Table 5 Effect of different molasses sugar concentrations on ethanol production

Supplement (0.5%)	Molasses sugar (%)	Ethanol (%, w/v) at			
		24 h	48 h	72 h	% EFF at 72 h
None	18.0	3.30	4.35	6.60	82.0
Tamarind fruit	18.0	5.75	7.35	8.00	97.5
Tamarind pulp	18.0	5.80	7.30	8.00	97.5
None	22.5	3.10	4.65	6.45	63.0
Tamarind fruit	22.5	6.20	7.85	9.70	95.0
Tamarind pulp	22.5	6.35	7.80	9.70	95.0
None	25.0	2.20	2.85	4.10	36.0
Tamarind fruit	25.0	4.10	5.0	6.35	56.0
Tamarind pulp	25.0	4.00	4.95	5.90	52.0

% EFF as per Table 1.

enhancement was observed when molasses medium containing 20% reducing sugar was supplemented with tamarind-based waste materials. The improvement was more pronounced at higher molasses sugar concentrations. The addition of cold or hot water extracts of tamarind materials resulted in partial improvement in both the rate and yield of alcohol production, which suggests that the soluble fraction could play an important role in improving ethanol production. The tamarind aqueous effluent released after tartaric acid extraction contained 7.5% total reducing sugar which could be directly fermented to yield 3.25% ethanol. This confirmed the presence of fermentable sugars in the tamarind effluent which can also be used as a diluent in place of water in molasses fermentations. The lack of balanced nutrients and the presence of inhibitory substances in cane molasses are probably responsible for the lowered rate of ethanol production in the control experiments. Tamarind fruit contains carbohydrates, proteins and vitamins which can act as a good source of nutrition for yeast.

The supplementation of molasses medium with skim milk powder [8], chitin [9] and fungal mycelium [11] has been shown to improve ethanol production. Ezeogu and Okolo [2] reported that supplementation of molasses medium with protein and lipid rich soybean, groundnut or castor oil seed meal markedly enhanced ethanol productivity. None of these supplements are cost-effective at an industrial scale. The use of cheaper tamarind-based waste products, could provide a cost-effective means for improving ethanol productivity. Though not fully understood, this improvement could be attributed to either increased biomass due to the presence of nutritive factors present in tamarind-based waste products or to the increased ethanol tolerance of the yeast cells. In addition, the supplements may help in increasing membrane permeability, thus enhancing glucose uptake resulting in improved ethanol production. The introduction of this supplementation step will not require additional capital cost for equipment. Efforts are being made for basic studies to isolate and identify the compounds or fractions from the supplements which are responsible for improving ethanol production. Additional studies on the effect of these supplements on ethanol tolerance and on substrate uptake rates will be carried out.

In conclusion, medium supplementation with tamarind-based waste products enhanced ethanol productivity. Thereby the cost of ethanol production might be substantially reduced.

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