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DETERMINATION OF OPTIMUM CONDITIONS FOR THE PROPAGATION OF BAKER'S YEAST ON MOLASSES

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Abstract. A yeast strain, *Saccharomyces cerevisiae* IMI-39916 was grown in a medium based on cane molasses to determine the optimum conditions for its growth. Maximum yield of yeast on the basis of total sugars was obtained when the mash contained 1.0% total sugars, 20 and 30 mg/g of sugar of added phosphorus and nitrogen respectively. Variations in pH of the mash between 4.0 and 5.0 did not affect the yield significantly. The highest yield of yeast up to 46.10% of total sugars was obtained during these studies. The sample of molasses used, contained 17.82% reducing sugars, 49.26% nonreducing sugars, 15.03% ash, 0.75% total nitrogen and 1876 p.p.m. phosphorus on dry weight basis.

Molasses, a byproduct of sugar-cane industry, is being produced in bulk by 22 sugar mills in West Pakistan. Bulk of it is being exported whereas finished products based on molasses are being imported. Baker's yeast is one of these items which is being imported to the tune of Rs. 2½ lacs per annum at a high price. It is anticipated that the same could be produced within the country at a reasonably low cost.

The propagation of yeast is affected by such factors as sugar concentration, pH of the medium and the presence of yeast nutrients such as nitrogen and phosphorus. Harris *et al.*[1] studied the growth of food yeast on wood hydrolysate and observed that at 1% sugar concentration in the mash, the yield was 63.3% and that it decreased to 49.9% at 2% sugar concentration. Further increase in sugar concentration resulted in corresponding decrease in yield. Trubchaninov *et al.*[2] cultivated yeast on sunflower and rice-husk hydrolysates and reported that in the undiluted sample containing 1.98% total sugars, the yield was 51.5% whereas it increased to 70% when hydrolysate was diluted 1:1 with water. Peterson *et al.*[3] had reported similar effect of sugar concentration on the propagation of yeast. Protiva and Negrete[4] studied the propagation of *Rhodotrula gracilis* on molasses for fat production and observed that 4% total fermentable sugars in the mash was the optimum concentration for its growth.

Supplementation of the mash with nitrogen and phosphorus have been reported to be beneficial for the growth of yeast by Frey *et al.*[5] Lorenz[6] reported that the growth of *T. utilis* in the mash prepared from sugar beet molasses or raw cane-sugar decreased when the nitrogen and phosphorus were added to the mash at a lower rate. Harris *et al.*[1] studied the propagation of yeast on wood hydrolysate and concluded that 3.2 lb nitrogen and 1.5 lb phosphorus pentaoxide per 100 lb of reducing sugars in the mash were the minimum requirements for the optimum growth of yeast.

Wide variations in the optimum pH level for the propagation of yeast have been reported by different workers. Maxon and Johnson[7] observed no significant difference in yields of baker's yeast when pH was varied between 4.5 and 5.3. Visuri and Kirsop[8] reported that in a metal-free buffer, the optimum pH for yeast growth was 5.0 whereas the ability of yeast to utilize maltose decreased with increase in pH. Protiva and Negrete[4] reported 5.0–6.0 as the optimum pH range for the propagation of *Rhodotrula gracilis*.

The present studies deal with propagation of *Saccharomyces cerevisiae* IMI-39916 on molasses with and without fortification with a source of nitrogen and phosphorus.

Materials and Methods

Analysis of Molasses. The sample of molasses used in the present studies was obtained from Crescent Sugar Mills and Distillery Ltd., Lyallpur. The sample was analysed for dry matter, reducing sugars, nonreducing sugars, ash and nitrogen contents according to the methods described in A.O.A.C.[9] Phosphorus was determined colorimetrically accroding to the methods described by Richards.[10]

Yeast Strain. A strain of baker's yeast *S. cerevisiae* IMI-39916 was maintained on nutrient agar slants containing 0.3% yeast extract, 0.3% malt extract, 0.1% glucose, 0.05% peptone and 2% agar. The inoculum was prepared by transferring yeast cells from a slant to 300 ml sterilized nutrient broth. The flasks were set on a rotary shaker (100–110 rev/min) and incubated at 30±1°C for 24 hr.

Preparation of Mash. Molasses were diluted with distilled water to 20% total sugars, heated after adding a few drops H_2SO_4 (concd) and cooled. The supernatant was decanted off and used as stock mash.

In order to ascertain the effect of different variables, i.e. sugar concentration, level of added nitrogen and phosphorus and pH of the medium, on the growth of yeast the media were prepared (Table 1).

Mash samples of different treatments were autoclaved at 15 psi for 15 min. The sterilized samples were cooled and inoculated aseptically in fermentation flasks which were set on a shaker and incubated at 30°C. At the end of the desired period of incubation, yeast cells were harvested by centrifuging the mash at 2500 rev/min for 15 min and cells were dried.

Measurement of Yeast Activity. The activity of the yeast was determined by measuring the volume of CO_2 from a piece of dough according to the method described by White.[11]

Results and Discussion

Composition of Molasses

The analysis of the sample of molasses showed that it contained 84.6% dry matter and 17.82% reducing sugars, 49.26% sucrose, 15.03% ash, 0.75% total nitrogen and 1876 p.p.m. phosphorus on dry weight basis. The dry matter contents of the sample were comparatively higher than those reported by most of the early workers[12,13] which could be due to differences in the processing techniques, variety of the sugar-cane, and other agronomical factors.

Effect of Different Variables on the Propagation of Yeast

(1) *Sugar Concentration.* Data showing the effect of sugar concentration in the mash on the yield of yeast are presented in Table 2. It has been observed that sugar concentration in the mash had a highly significant effect on the propagation of yeast. The maximum cell yield was obtained when the sugar concentration in the mash was 1.0% and it decreased as the concentration of sugar increased. There was no increase in cell mass in the samples containing 1% sugars when incubation period was extended to 48 hr. This is most likely due to the fact that all fermentable sugars had been consumed during the first 24 hr. An increase in cell mass was, however, observed when samples containing more than 1.0% sugar were incubated up to 48 hr, yet the yield was less than that of samples containing 1% sugar.

On the basis of these observations 1% sugar concentration in the mash and 24 hr incubation period were considered to be optimum. Similar results have been reported by Peterson *et al.*,[3] Harris *et al.*[1] and Trubchaninov *et al.*[2] It was further observed that during incubation there was a pronounced alcoholic smell in the samples containing higher sugar concentrations. Low yield on the basis of sugar utilized and alcoholic smell in these samples indicated that high sugar concentration favoured production of alcohol rather than cell multiplications.

(2) *Effect of Nitrogen and Phosphorus Addition.* Data showing the effect of added nitrogen and phosphorus on the yield of yeast cells are presented in Table 3.

Supplementation of the mash with nitrogen and phosphorus increased the cell yield significantly. The optimum levels of nitrogen and phosphorus for supplementing the molasses mash were observed to be 30 and 20 mg/g sugars respectively. Further increase in the level of added nitrogen did not prove beneficial but on the other hand caused a decrease in the yield. This might be due to the inhibitory effect of SO_4- - as the nitrogen was added in the form of $(NH_4)_2SO_4$. Effect of added nitrogen and phosphorus were significant at 1% level. The doses of nitrogen and phosphorus observed to be optimum in these studies were similar to those reported by Harris *et al.*[1]

(3) *pH of the Medium.* The average yield of cell mass was observed to be 44.40 and 45.15% of total sugars in the mash when pH was adjusted to 4.0, 4.5 and 5.0 respectively. The differences in the yield of cell mass were, however, nonsignificant which indicated that the growth of yeast was not affected appreciably by the change in pH of the medium with a range of 4–5. Tolerance of the yeast to slight pH changes has also been reported by Maxon and Johnson,[7] Visuri and Kirsop[8] and Protiva and

TABLE 1

Factor studied	Sugar concn (%)	Added nitrogen (mg/g sugar)	Added phosphorus (mg/g sugar)	pH
Effect of sugar concn.	1·0	40	30	4·5
	1·5	40	30	4·5
	3·0	40	30	4·5
	6·0	40	30	4·5
Effect of added nitrogen	1·0	—	30	4·5
	1·0	20	30	4·5
	1·0	30	30	4·5
	1·0	40	30	4·5
Effect of added phosphorus	1·0	30	—	4·5
	1·0	30	10	4·5
	1·0	30	20	4·5
	1·0	30	30	4·5
Effect of pH	1·0	30	20	4·0
	1·0	30	20	4·5
	1·0	30	20	5·0

TABLE 2. EFFECT OF SUGAR CONCENTRATION ON SUGAR UTILIZATION AND THE YIELD OF YEAST.

Incubation period (hr)	Initial sugar concn(%)	Residual sugar concn(%)	Yield of yeast (%)	
			Total	Utilized
24	1·00	0·05	45·15	47·52
	1·50	0·65	24·53	41·11
	3·00	2·13	11·15	38·67
	6·00	5·17	4·14	30·12
48	1·00	0·05	45·10	47·47
	1·50	0·09	35·96	38·40
	3·00	0·45	31·15	36·71
	6·00	3·55	12·21	29·95

TABLE 3. EFFECT OF NITROGEN AND PHOSPHORUS LEVELS ON THE YIELD OF YEAST.

Nitrogen (mg/g sugar)	Yield on the basis of total	Phosphorus (mg/g sugar) sugars(%)	Yield on the basis of total sugars(%)
0	37·15	0	34·80
20	43·20	10	37·25
30	46·10	20	44·20
40	43·65	30	44·05

Negrete,[4] who have reported optimum pH values for yeast growth ranging from 4 to 6.

(4) *Activity of the Yeast.* Data showing the volume of CO_2 produced in a dough by the yeast prepared during these studies (local) as compared to that of an imported sample (from France) of dried yeast are presented in Table 4.

The imported yeast had comparatively higher activity than the yeast prepared in these studies as evidenced by the volume of CO_2 produced. Imported sample produced 1423 ml CO_2 in 4 hr whereas the local yeast produced only 1190 ml of the gas in the same period.

TABLE 4. CARBON DIOXIDE PRODUCED BY LOCAL AND IMPORTED YEAST.

Time (hr)	CO_2 (ml)	
	Local yeast	Imported yeast
0	0	0
1	272	290
2	295	345
3	308	388
4	315	400
Total	1190	1423

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