Potential Use of Rum Distillery Slops as Animal Feed Supplement. IV. Fodder Yeast Growth in Slops[1]

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ABSTRACT

Experiments on fodder yeast growth in rum distillery slops showed best results in slops with added nutrients, 0.15% N as $(NH_4)_2SO_4$ and 0.10% P as KH_2PO_4. More than 60% BOD reduction was obtained with a 24-h growth. Average yeast yield obtained was 10 g/L of dried yeast with a 32–40% protein content. The 1:1 slops-water dilution gave the best results.

INTRODUCTION

The revenue from rum sales in Puerto Rico amounts to more than 160 million dollars yearly. This income is presently threatened by the industry's inability to dispose properly of its effluents. A total of 300 million gallons of slops, with a biological oxygen demand (BOD) of 30,000 to 40,000 p/m is dumped yearly on land and into coastal waters. The

TABLE 1.—*Composition of molasses and slops*[1]

%	Molasses	Slops
H_2O	25.4	90.0
N	1.1	0.2
P	0.1	0.003
K	2.7	0.6
Ca	1.0	0.2
Mg	0.5	0.1
Na	0.2	0.02
SO_4	2.4	0.3
Cl	1.1	0.2
Sucrose	32.1	4.0
Reducing Sugar	30.0	0.6
Caramel	—	2.9

[1] Pérez Escolar, R., Reclamation of a saline-soil by use of molasses and distillery slops, J. Agri. Univ. P.R. 50 (3): 209–17, 1966.

Environmental Protection Agency (EPA) has ruled that this industry must find a solution to the problem before 1983.

Realizing the magnitude of this problem and the catastrophic effect it could have upon the economy of Puerto Rico, researchers initiated several

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years ago a development program in this area at the Rum Pilot Plant. One of the research areas included the fermentation of slops with fodder yeasts to enrich the protein content for their potential use as an animal feed supplement.

The nutritive value of slops in terms of nitrogen level is low (table 1), especially if they are to contribute significantly to the animal diet. Aguinaldo (1) stated that in the propagation of yeast the necessary requirements for favorable growth are low concentration of sugar, low pH, high organic nitrogen content, adequate supply of air, and presence of growth-promoting substances. Rum slops fill the majority of these requirements. These microorganisms are important sources of food because their cell matter is rich mostly in B-group vitamins and in protein that contains essential amino acids.

The following tabulation shows the Chang and Yang approximate analysis of dried *Torula* yeast (4).

General composition	%
Moisture	7.73
Crude protein	45.17
Crude fat	2.79
Crude fiber	1.82
Ash	7.68
N free extract	34.81

Minerals	*Ash %*
CaO	3.28
MgO	4.21
P_2O_5	39.17
Fe_2O_2	0.74
K_2O	37.56
Na_2O	14.49
Zinc	2 mg/100 g
Copper	7.8 mg/100 g

Amino acids	*Protein %*
Alanine	6.01
Arginine	5.19
Aspartic acid	8.68
Cystine	0.31
Glutamic acid	16.09
Glycine	4.25
Histidine	1.61
Isoleucine	4.54

Leucine	6.43
Lysine	6.45
Methionine	1.28
Phenylalanine	4.18
Proline	3.04
Serine	4.24
Threonine	4.54
Typtophan	1.11
Tyrosine	3.53
Valine	5.33
Vitamins	*mg/100 g*
Thiamine	0.9
Riboflavin	3.5
Niacin	35.0
Pantothenic acid	5.64
Biotin	0.59
Choline	114.8
Vitamin B_6	4.8

They mentioned three advantages in the production of yeast: 1) a large quantity of protein can be produced in a limited area; 2) the same quantity of biomass is produced in less time with yeasts than with plants or livestock; 3) 90% of the nutrients are absorbed by the yeast in the fermentation tank, whereas when fertilizers are applied to land part of them are leached off through the soil.

Protein production for animal feed has been discussed by López Hernández (7). He suggested that a product rich in protein can be obtained from cane molasses slops fermented with *Candida utilis* and *Torulopsis utilis*. He stated that 15–20 kg of dried yeast can be obtained per m^3 of slops. Seven thousand tons of dried yeast per year would produce 3,000 tons of protein.

Skripnik (15) produced feed concentrates from refuse of the alcohol industry containing protein, antibiotics and vitamins. He found that fermentation of slops with *Candida tropicalis* gave a product that contained biomycin and vitamin B_{12}, suitable for feed. Zabrodskii (17), studying the possibility of obtaining higher yields of nutrient yeast from molasses residues, found that cultivating it in a 2-step process using certain strains of *Saccharomyces* increased the yield of biomass. A thoroughly fermented mash and a spent wash high in inorganic matter, but low in salinity, gave higher yeast yields. He also studied (18) the biosynthesis of fat by *Rhodotorula glacilis* yeast cultured in molasses vinasses, obtaining yeast biomass with 22–25% fat from 1 kg of vinasses.

Brahmer (3) presented the technique and economics of fodder yeast

production from *Torula utilis* and the possibility of its growing in pentose-rich distillery residues. Boruff (2) recovered feed products from stillage by re-fermentation.

Rachlevicius(13) manufactured dry fodder yeast from distillery residues, and set the following conditions for the fermentation: 34–36° C, pH 5 to 5.5, and foaming induced by sparging with filtered air. Conditions for cultivating fodder yeasts were presented by Semenets (14). He used H_3PO_4, H_2PO_4, H_2SO_4, $(NH_4)_2SO_4$ and H_2O for dilution during the cultivation of *Candida tropicalis*, strains SK-4 and D-3. Best yields were obtained at 30–32° C and at pH 4.7 to 5.2 with strain D-3.

Yeast cultivation in molasses slops supplemented with molasses was studied by Malanowska (8). She obtained yields of 18–20% yeast with respect to the total solids of the slops by cultivation of fodder yeast *Torulopsis utilis* in distillery molasses slops supplemented with 10% beet molasses at 33–35° C and pH 4.5 to 5.0.

Experiments conducted by Matsuo et al. (9) with various yeast strains in modified slops media showed that *Candida utilis*, *C. arborea* and *C. tropicalis* were superior strains for yeast production in slops supplemented with 3% $(NH_4)_2HPO_4$ and 0.03% $MgSO_4 \cdot 7H_2O$. He also found (10) that the yeast *C. utilis* cultured in distillery slops contained more than 50% crude protein, and reduced the BOD of the medium by 50%. Karaki (5) found that the addition of 0.2–0.7% sucrose to slops to be fermented with *Candida utilis* increased the yield of yeast to about 0.15% and that it had no effect on the fermentation process. Clarification of molasses showed no appreciable effect on the yield and quality of the yeast. Kozlowska (6) studied the utilization of some components from vinasse and molasses during yeast production, and found that *Torulopsis utilis* grown in vinasses containing 10% molasses utilized more reducing substances and organic non-carbohydrate substances than that grown in vinasse or molasses alone.

If rum distillery slops prove to be valuable for the growth of fodder yeast, Puerto Rico will have a way of increasing the production of edible protein. In this case, rum slops instead of being an undesirable waste would become a valuable agricultural and industrial by-product resource. This paper reports work initiated in Puerto Rico.

MATERIALS AND METHODS

Fodder yeast growth experiments were performed both on laboratory and small-plant scales. Laboratory scale experiments were carried out in a New Brunswick Scientific Co., Magnaferm Fermenter Model MA-100.[3]

[3] Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

This fermenter consists of a 14-liter glass vessel equipped with a magnetically-driven triple propeller stirrer and a mechanical foam breaker. Temperature and pH were automatically controlled and continuously recorded during the experiments. The temperature of the fermenter was kept betwen 28–30° C and aeration was controlled as specified in tables 2 to 4. Small-plant scale experiments were carried out with continuous aeration and agitation in a pre-seed and a seed tank, 25- and 250-gal capacity, respectively. Sulphonated castor oil plus internal pressure was used to minimize foaming.

Yeast strain PPR-291, *Candida utilis*, obtained from the Northern Regional Research Laboratories was used as growth organism in all

TABLE 2.—*Fodder yeast growth in slops*

	pH				Yeast cell counts (million/ml.)				BOD (p/m)				BOD Reduction (%)			
Hr	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
0	4.9	4.6	4.9	5.0	20	5	9	24	24,600	29,500	25,600	26,400	0	0	0	0
24	5.2	5.4	7.0	7.0	493	242	510	734	20,800	22,300	16,800	13,700	15	25	34	48
48	6.1	7.2	7.0	7.1	557	660	922	748	13,200	14,600	13,100	10,200	46	51	49	61
72	6.4	7.2	7.0	7.2	538	708	548	621	11,000	12,200	10,200	9,500	55	59	60	64
96	6.6	7.6	7.4	—	542	—	590	—	12,700	8,100	10,000	—	48	73	61	—
120	—	7.7	—	—	—	—	—	—	—	9,600	—	—	—	67	—	—
144	—	7.0	—	—	—	535	—	—	—	10,100	—	—	—	66	—	—

Experimental Conditions	I	II	III	IV
Volume (L)	8	56	56	606
Temperature (°C)	30	28–30	28–30	28–30
Aeration (air vol./slop vol. × min.)	0.7	1	1	0.4—0.9
Agitation (r/min)	400	431	431	431
Dilution	1:0	1:0	1:0	1:0

experiments. Slops used in the experiments were obtained from the Bacardí distillery in Cataño, Puerto Rico.

The study considered three aspects: 1) yeast growth in sterile raw slops; 2) effect of slops dilution on yeast growth; and 3) addition of nutrients and their effect on yeast growth. For the preparation of inocula, a purified yeast culture was transferred from agar slants of a synthetic medium (12) to 35 ml liquid synthetic media. Samples were agitated continuously at room temperature in a mechanical shaker; and after 48 hours, each one was transferred to 250 ml sterile distillery slops in 500 ml Erlenmeyer flasks and again shaken continuously for 24 hours. A seed of 5% fermentor volume was used as inoculum.

For dilution experiments, slops were diluted with tap water. When necessary, nutrient salts were added: $(NH_4)_2SO_4$ for N and KH_2PO_4 for P.

TABLE 3.—*Fodder yeast growth in diluted slops*

	pH				Yeast cell counts (million/ml.)				BOD (p/m)				BOD reduction (%)			
Hr	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
							Section A									
0	5.0	5.2	4.8	5.0	32	18	10	17	26,000	12,800	14,400	15,000	0	0	0	0
24	6.4	6.5	7.2	7.5	296	254	487	390	8,800	6,800	3,700	6,300	66	47	74	58
48	7.1	6.6	7.2	7.8	256	272	455	322	7,600	7,900	3,500	4,800	71	38	76	68
72	7.3	6.6	7.6	8.2	218	262	430	324	6,000	8,000	5,300	5,600	77	37	63	63
96	—	6.6	7.4	—	—	287	526	—	—	7,800	5,400	—	—	39	63	—

Experimental Conditions	I	II	III	IV
Volume (L)	7	8	56	56
Temperature (°C)	30	30	28–30	28–30
Aeration (air vol./slop vol. × min.)	1	1	1	1
Agitation (r/min)	430	400	431	431
Dilution	1:1	1:1	1:1	1:1

Section B

Hr	V	VI	VII	VII I	V	VI	VII	VII I	V	VI	VII	VIII	V	VI	VII	VIII
0	5.1	5.3	5.0	5.1	20	16	19	24	10,400	8,500	17,800	18,900	0	0	0	0
24	6.5	6.6	7.2	6.8	189	206	550	548	6,000	4,500	9,300	9,100	42	47	48	52
48	7.1	6.8	7.2	7.0	275	163	512	520	5,700	4,900	9,400	9,700	45	42	47	49
72	7.2	6.8	7.1	7.0	199	167	467	556	3,700	4,900	9,600	11,500	64	42	46	39
96	—	6.9	—	—	—	165	—	—	—	4,700	—	—	—	45	—	—

Experimental Conditions	V	VI	VII	VIII
Volume (L)	7	8	8	8
Temperature (°C)	30	30	30	30
Aeration (air vol./slop vol. × min.)	1	1	1	1
Agitation (r/min)	430	400	400	400
Dilution	1:2	1:2	2:1	3:1

The laboratory scale experiments showing best data were later conducted in small-plant scale.

BOD, yeast cell count, and pH were determined in all experiments. Dry weight, ° Brix, K content, total sugars and percentage protein of the yeast were measured in selected experiments. BOD analyses were performed by two different methods (16). Since no significant difference was found between the two, the electrometric method was selected for convenience. Yeast was recovered by centrifuging 10 ml sample of the liquid and drying the residue at 105° C. Protein was determined by the Kjeldahl method and K by atomic absorption spectrophotometry method. Other analyses were performed following official methods (11, 12).

TABLE 4.—*Fodder yeast growth in slops, nutrients added*

	pH				Yeast cell counts (million/ml.)				BOD (p/m)				BOD reduction (%)			
Hr	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
0	4.6	4.6	4.5	4.5	10	11	18	14	33,100	17,900	27,000	26,500	0	0	0	0
24	4.7	5.6	5.3	5.8	273	768	1227	768	19,300	6,400	9,100	9,700	42	64	66	63
48	5.4	5.7	5.6	5.8	1092	822	1278	854	5,100	6,000	8,400	7,900	85	66	69	70
72	5.6	5.7	5.6	6.6	1200	739	1093	826	4,300	4,900	8,000	7,100	87	73	70	73
96	5.6	5.7	5.6	6.6	1274	749	1203	917	7,800	9,700	5,900	3,000	76	46	78	89

Experimental Conditions	I	II	III	IV
Volume (L)	8	8	8	56
Temperature (°C)	30	30	30	28–30
Aeration (air vol./slop vol. × min.)	1	1	1	1
Agitation (r/min)	400	400	400	431
Dilution	1:0	1:0	1:0	1:0
Nutrients % N	0.15	0.05	0.15	0.15
% P	0.10	0.15	0.10	0.10

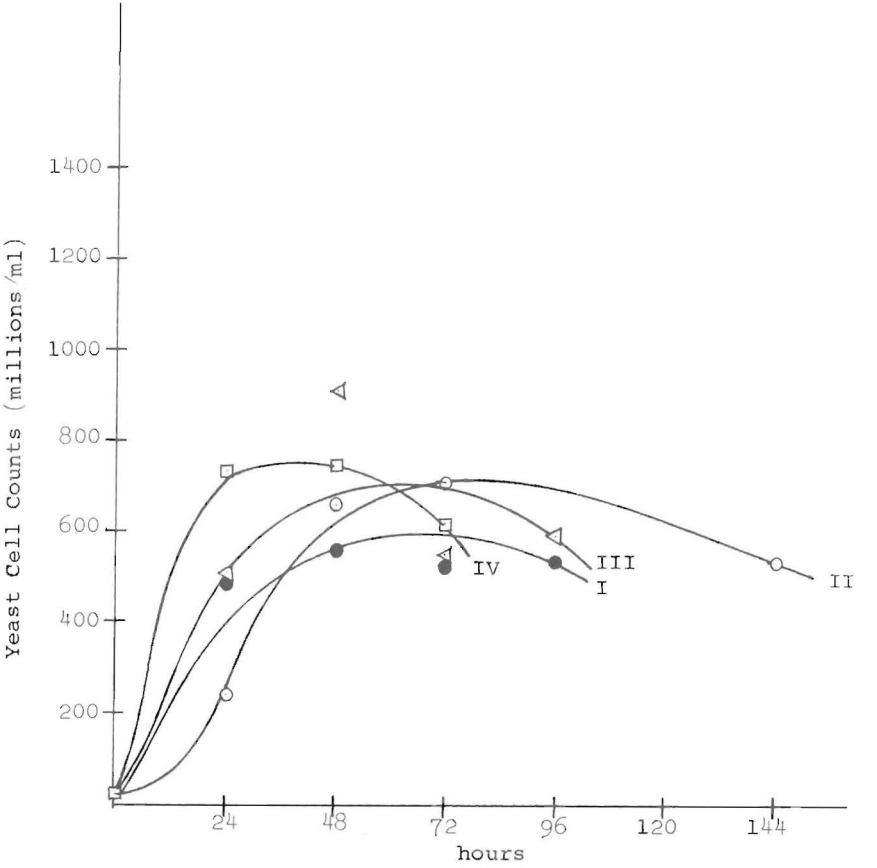

FIG. 1.—Yeast cell counts in slops.

RESULTS AND DISCUSSION

Table 2 and figures 1 and 2 show the results on yeast growth in raw slops. Fodder yeast increased pH from acidic to neutrality levels during the first 48-hour growth period. High yeast yields in terms of total cell counts were obtained during the first 48 hours in sterile raw slops: an average of 10 g dried yeast of 32–40% protein content per liter of slops. BOD reductions of 55–60% were observed in 72 hours. Reductions in BOD were found directly proportional to yeast cell counts.

Table 3 and figures 3–6 summarize the data of the dilution experiments with fodder yeast. As in raw slops, the pH turned from acid to about

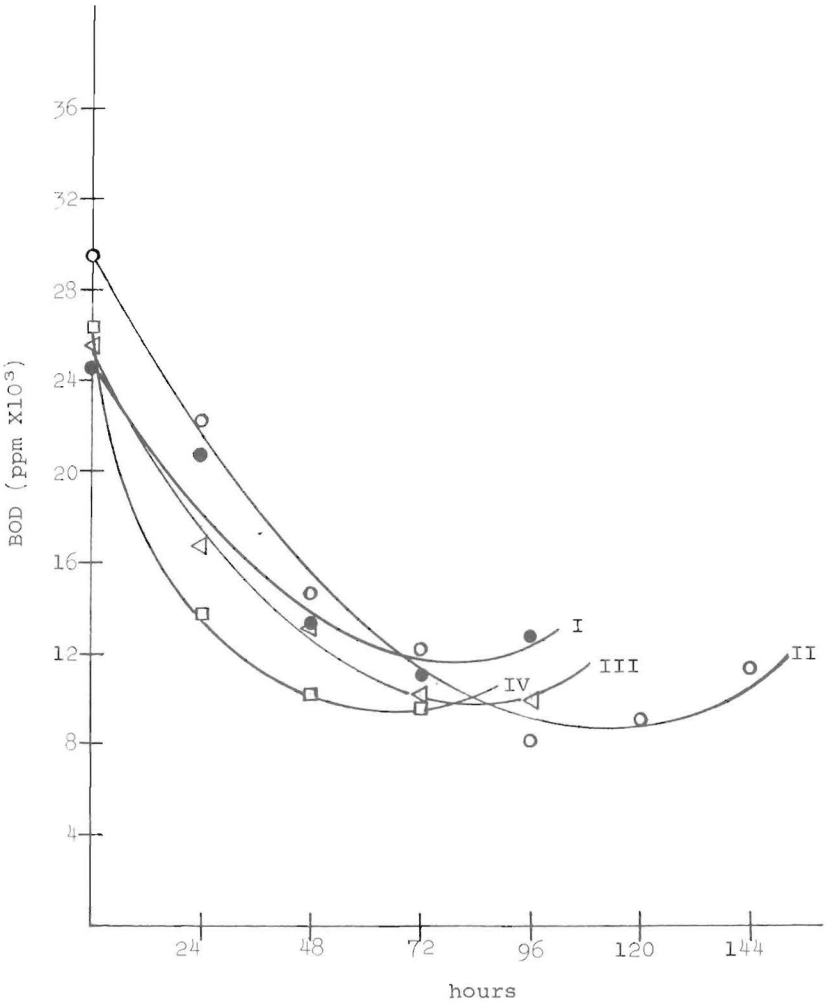

FIG. 2.—Effect of yeast growth on BOD of slops.

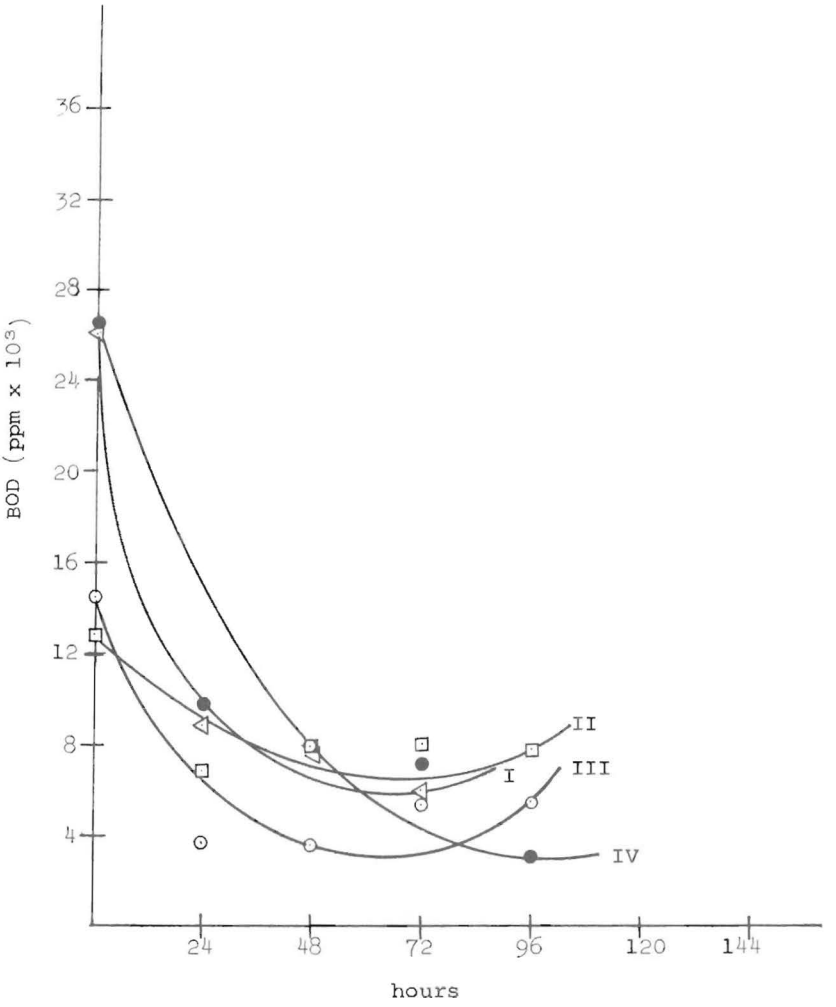

FIG. 3.—Effect of yeast growth on BOD of diluted slops (1:1)

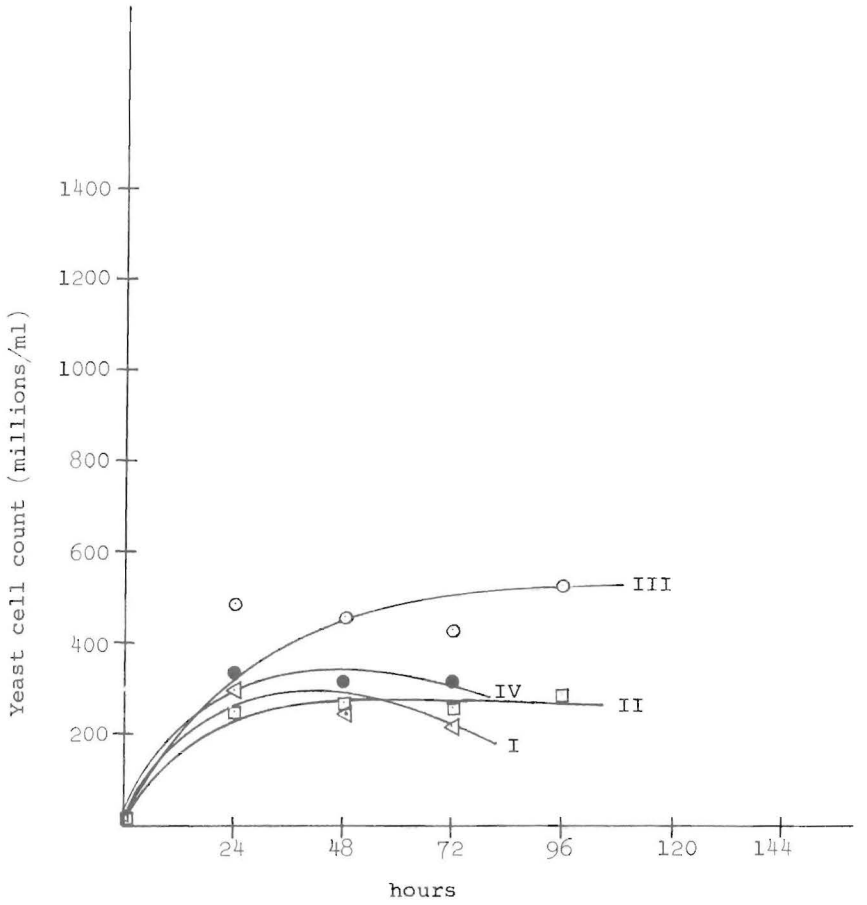

FIG. 4.—Yeast cell counts in diluted slops.

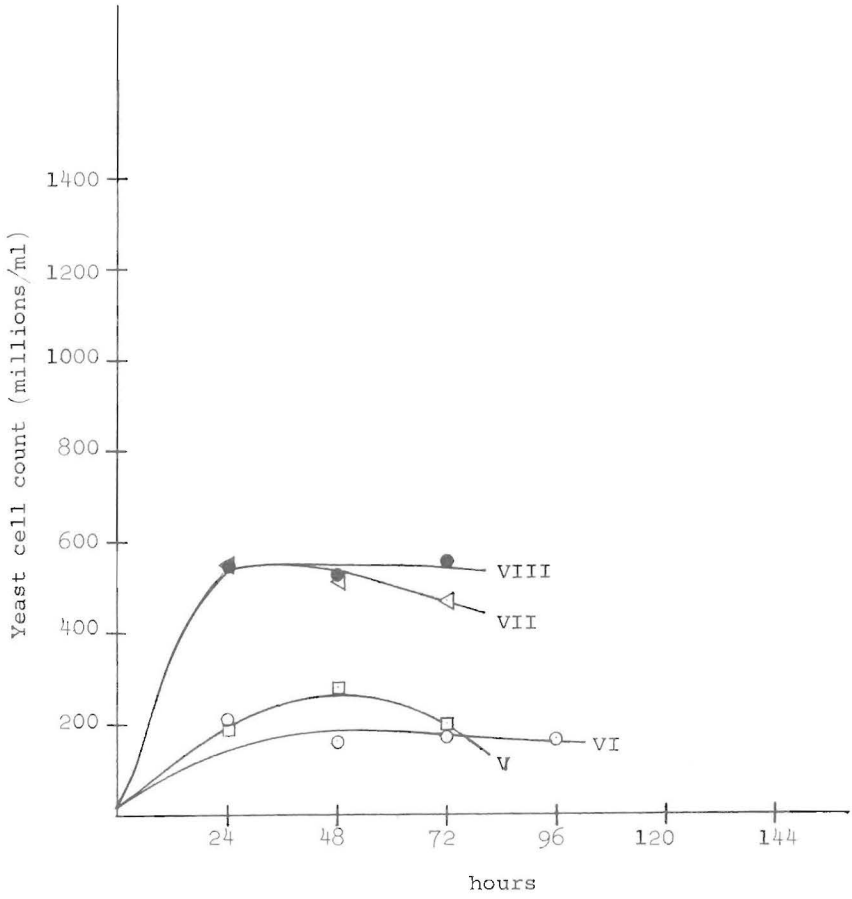

FIG. 5.—Yeast cell counts in diluted slops.

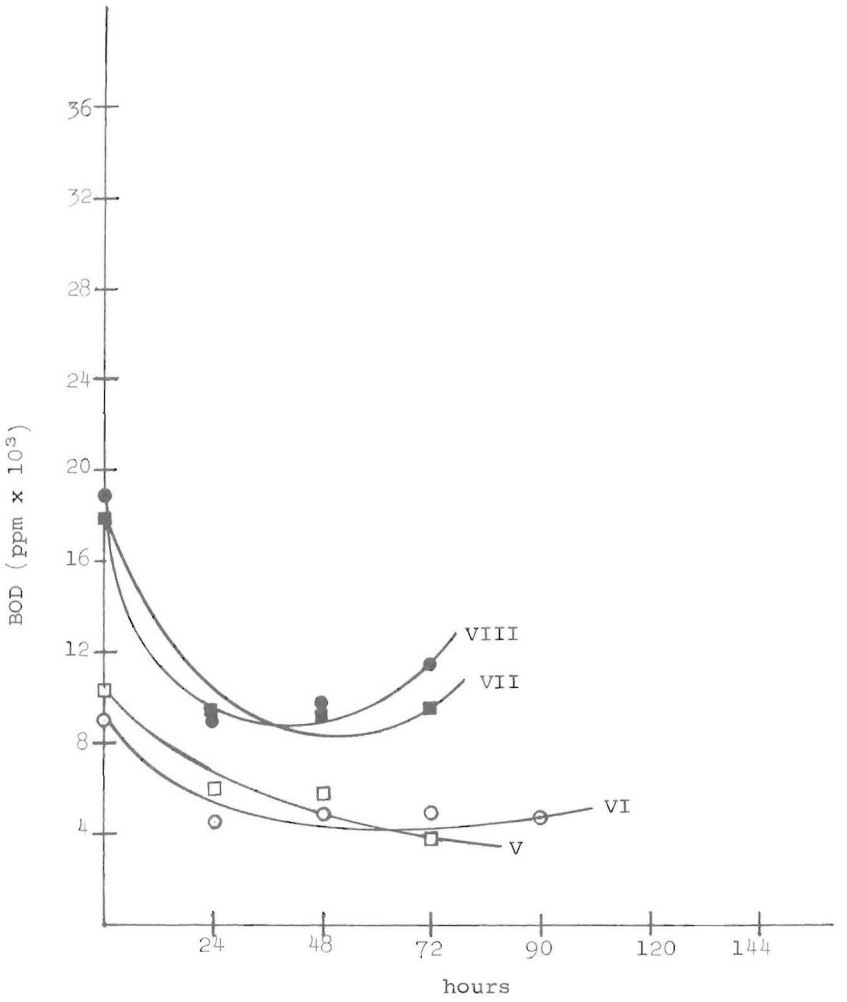

FIG. 6.—Effect of yeast growth on BOD of diluted slops.

neutral in a short time. Maximum yeast cell counts were attained at 24–48 hours with 5 g/L yeast yield for a 1:1 dilution. Best results in terms of BOD reductions were obtained with 1:1 slops-water dilution.

Table 4 and figures 7 and 8 show the effect of added nutrients on yeast growth in slops. A lower increase in pH was observed during yeast growth experiments with added nutrients as a consequence of higher utilization of NH_4^+ ions from the media. Best results were obtained with 0.15% N and 0.10% P. After 24 hours' growth, more than 60% BOD reduction was observed. High yeast cell counts were obtained at 48–72 hours.

It can be concluded that in all the experiments the best results were obtained in raw slops with added nutrients. Total yeast cell counts at 48

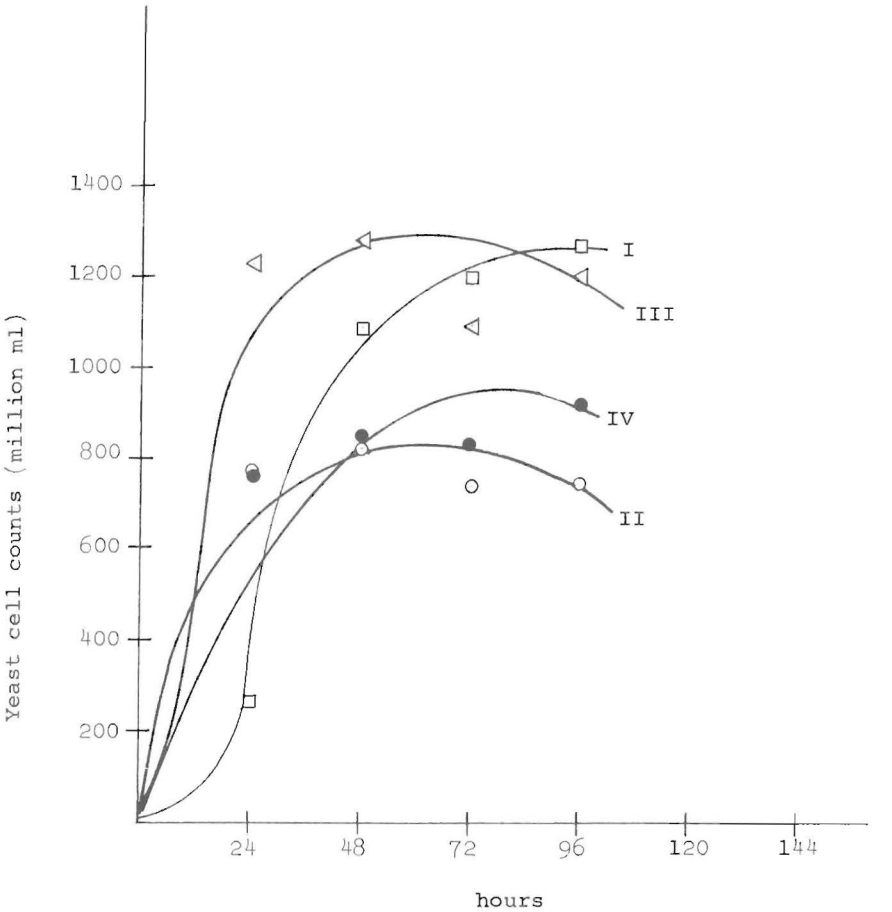

FIG. 7.—Yeast cell counts in slops, nutrients added.

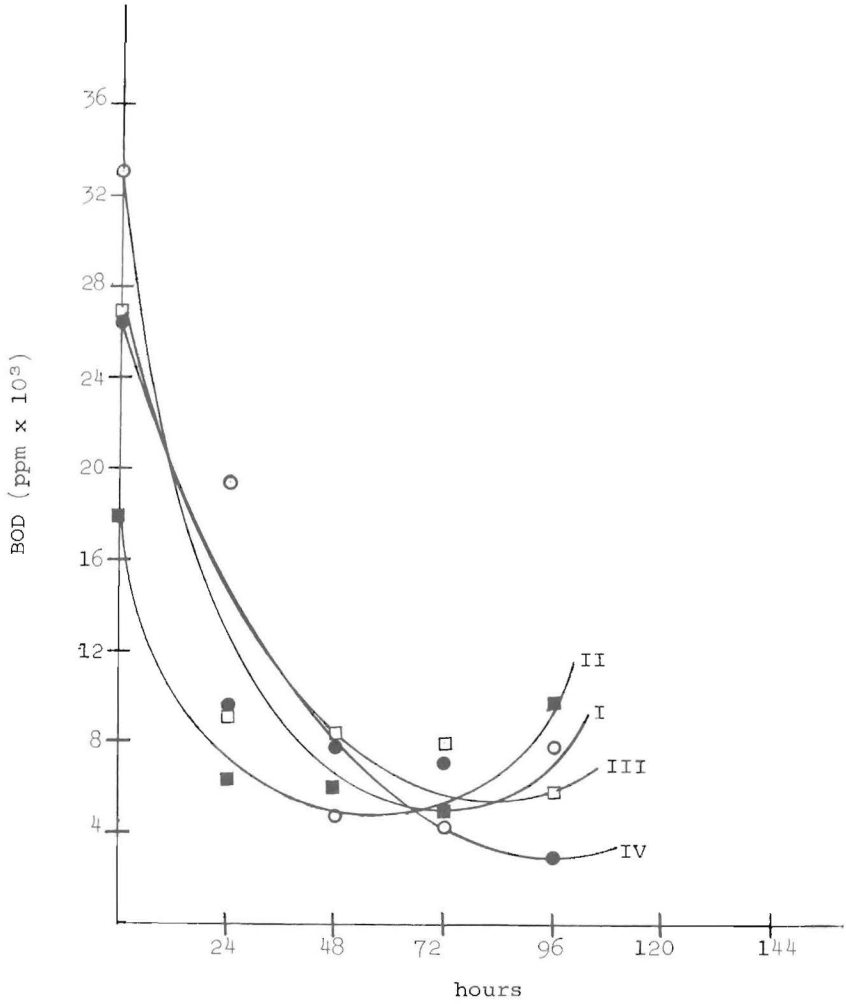

FIG. 8.—Effect of yeast growth on BOD of slops, nutrients added.

hours were higher in slops with nutrients than in raw or diluted slops with an average BOD reduction of 72%.

RESUMEN

Experimentos sobre el crecimiento de levadura forrajera en mostos mostraron un aumento en el pH de los mismos durante las primeras 48 horas de crecimiento. Se alcanzaron rendimientos altos de levadura, obteniendo 10 gramos de levadura seca por litro de mostos, con un contenido en proteína de 32–40%. Las reducciones de BOD más altas

se obtuvieron a las 72 horas, 55–64%. Los conteos de levadura se pudieron relacionar directamente con las reducciones en BOD; a más elevado el conteo, mayor la reducción de BOD obtenida. Se estudió además el efecto de la dilución, obteniendo conteos máximos a las 24–48 horas y produciendo 5g/l de levadura seca en la dilución 1:1. Entre las diluciones estudiadas las reducciones de BOD más altas se obtuvieron con esta dilución. El efecto de sales nutritivas se exploró obteniendo los mejores resultados usando 0.15% N y 0.10% P. Un promedio de 72% en la reducción del BOD se obtuvo a las 48 horas de crecimiento. De todas las variables estudiadas, los mejores resultados se obtuvieron en mostos con nutrimentos, ya que la reducción del BOD se obtuvo en un tiempo de retención más corto.

LITERATURE CITED

1. Aguinaldo, J. T., 1975. Utilization of the distillery slops for continuous propagation of Torula yeast, Sugar News 354-6, September.
2. Boruff, C. S., Stone L., Bauernfeind, J. C., and Garey, J. C., Recovery of feed products from stillage by refermentation (to Hiram Walker & Sons, Inc.), U.S. 2, 595, 827, May 6, 1952; C.A. 47, 6090h, 1953.
3. Brahmer, H., Production of fodder yeast, Kgl. Lantbruksakad. Tid. 81, 301-16, 1942; C.A. 39, 2161, 1945.
4. Chang, C. T. and Yang, W. L., 1973 (Yeast & Feed Factory, T.S.C.), Torula yeast production from blackstrap molasses, Taiwan Sugar 20(5), 193-9, September-October.
5. Karaki, I., Nishioka, S., and Konishi, K., Yeast production from alcohol distillation slops of cane molasses, II, Hakko Kyokaishi 26(4), 173-86, 1968 (Japan); C.A. 69, 34675p, 1968.
6. Kozlowska, E. and Malanowska, J., Utilization of some components from vinasse and molasses and molasses and vinasse containing worts during yeast production, Przemysl Ferment. 8(11), 388-9, 1965 (Pol); C.A. 64, 11823a, 1966.
7. López Hernández, J. and Paz, H. A., 1972. Potential of Tucumán province in the protein production, La Industrial Azucarera 113-6. Universidad de Tucumán, Facultad de Agronomía y Zootecnia, Misceláneas No. 48, Tucumán, Argentina.
8. Malanowska, J., Skiba, M., Lebendzinski, S., and Wojcieszak, P. (Inst. Przem. Ferment., Warsaw, Poland), Yeast cultivation on the molasses slops, supplemented with molasses, Pr. Inst. Lab. Badaw, Przem. Spozyw. 17(3). 27-48, 1967 (Pol.); C.A. 68, 10387t, 1968.
9. Matsuo, T., Ishikawa, F., Yamanaka, M., and Konishi, K., Yeast production from alcohol distillation slops of cane molasses I, Hakko Kyokaishi 23(7), 320-4, 1965 (Japan); C.A. 63, 17089h, 1965.
10. ——,——,——, Nishioka, S., and Konishi, K., Yeast production from alcohol distillation slops of cane molasses II, Hakko Kyokaishi 24(10), 457-71, 1966 (Japan); C.A. 64, 20591f, 1966.
11. Official Analytical Methods of the Rum Pilot Plant, 1969. Agri. Exp. Stn, Mayagüez Campus, Univ. P.R.
12. Official Bacteriological Methods of the Rum Pilot Plant, 1969. Agri. Exp. Stn, Mayagüez Campus, Univ. P.R.
13. Rachlevicius, M., Manufacture of dry fodder yeast from slops, Mokslas ir Tech. No. 11, 20-1, 1961; C.A. 57, 3875h, 1962.

14. Semenets, P.A. and Dokienko, O.I., (Alc. Plant, Andrushevsk, Ukraine), Cultivating fodder yeasts on molasses slops, Spirt. Prom. 29(8), 22–4, 1963; C.A. 60, 9871g, 1964.
15. Skripnik, Y. P., Lerner, I. M., Kongin, A. V., and Baramidze, G. A., Production of feed concentrates which contain protein, antibiotics, and vitamins from refuse from the alcohol industry, Spirtovaya Prom. 27(4), 21–5, 1961; C.A. 55, 20259e, 1961.
16. Standard Methods for Examination of Waste Water. 13th ed, American Public Health Association. New York, 1971.
17. Zabrodskii, A. G., Osovik, A. N., Polyanskaya, E. A., and Ztrizhenyuk, E. V., (Ukr. Nauchao-Issled. Inst. Spirt. Prom. USSR) Possibility of obtaining higher yields of nutrient yeast from molasses residues, Ferment. Spirt. Prom. (1), 40–3, 1973 (Russ); C.A. 78, 96056g, 1973.
18. ——, Pogrebnaya, V. F., Kravets, E. N., Osovik, A. N., and Bozhik, M. V., Tr. Nauch.-Issled Inst. Spirt. Likero-Vodoch, Prom. No. 11, 133–7, 1967 (Russ); C.A. 68, 38156x, 1968.