Research Note

PILOT PLANT GROWTH OF CANDIDA UTILIS *ON RUM DISTILLERY SLOPS TO LOWER CONTAMINATION POTENTIAL AND GENERATE AN ANIMAL FEED SUPPLEMENT*

The Puerto Rican Industry has devoted significant time and money to find a satisfactory treatment of rum distillery wastes. These efforts are in compliance with the Clean Waters Act, which stipulates that by 1983 a 70-75% BOD reduction should be achieved. Bacardí Corporation has been successfully operating an anaerobic digestor since December 1981,[2] but other rum industries claim that the capital investment necessary is beyond their means. Since 1972, the Rum Pilot Plant (RPP) has been working on the development of a process to prepare rum distillery slops to be utilized as a supplement in animal feeds. Of the process studied, fodder yeast growth in slops seems to be an attractive alternative in terms of reduction of contamination measured as biochemical oxygen demand (BOD). Ramírez and González[3] performed a laboratory scale study which resulted in a 60% BOD reduction and a dried yeast yield of 10 g/L, with a 32-40% protein content, when growing *Candida utilis* on blackstrap molasses distillery slops.

The use of *C. utilis* as a water treatment microorganism is being studied at many locations, especialy where effluents have high BOD levels, high organic acid or high salt levels which are not amenable to other aerobic treatments. Peppler[4] states that *C. utilis* attacks more carbon and nitrogen compounds than other common yeasts, and is favored industrially to modify molasses, wood hydrolysates, spent sulphite liquors, distillery wastes and residual liquors from food and chemical processing.

According to Davy,[5] the use of *C. utilis* for waste treatment has the advantage that a saleable by-product is generated by a process whose cost is similar to that of a conventional biological treatment. He determined that capital cost will be repaid within 4 years. Because wastes are dilute, continuous culture with biomass recycling is recommended by Bu'Lock[6] since theoretically, with a sufficiently high retention factor, the productivity of the reactor is proportionally increased and its required size proportionally reduced.

The objective of the present study was to try at pilot plant scale (1500 L) the procedures that were optimized for laboratory scale for blackstrap molasses (BM) slops[3]. In tune with other investigations currently underway in the Rum Pilot Plant, an exper-

[1]Manuscript submitted to Editorial Board June 30, 1986.

[2]Szendrey, L. M., P. E. Schafer and G. H. Dorion, 1982. Pollution and energy management through the anaerobic approach. Ind. Wastes, 1982 WPCF Issue, September/October, 31–34.

[3]Ramírez, M. and I. M. González, 1980. Potential use of rum distillery slops as animal feed supplement. IV. Fodder yeast growth in slops, J. Agric. Univ. P. R. 64 (2): 148–63.

[4]Peppler, H. J., 1970. Food Yeasts-Chapter 8 of the Yeasts, Vol. 3, Anthony H. Rose, Ed, Academic Press, New York.

[5]Davy, C. A. E., D. Wilson and J. C. M. Lyon, 1980. Commercial production of feed yeasts from carbohydrate waste, Proc. Sixth Int. Fermentation Symp., London, Canada, July 20-25, Pergamon Press, pp 343–50.

[6]Bu'Lock, D., 1980. Reactor design for conversion of dilute sugar wastes into useful biomass. Paper presented at the Sixth Int. Fermentation Symp., London, Canada, July 20-25, unpublished.

iment with slops of high test molasses (HTM) alcoholic fermentation[7,8] was included. These slops are significantly lower in contamination than slops from blackstrap molasses (BM) fermentations[9]. HTM is an alternative to BM, which is now a scarce commodity in Puerto Rico and has to be imported. It could replace BM in the event of a worldwide scarcity of BM. The HTM experiment used the same experimental conditions as the BM runs, conditions that may not be optimum for HTM runs.

The *Candida utilis* strain ATCC 9256 was maintained at 5° C in agar slants of synthetic medium: 0.01% $MgSO_4$, 0.2% KH_2PO_4, 0.4% yeast extract, 0.6% peptone and 4% sucrose. The organisms were activated by an initial transfer to 50 ml of synthetic medium. By daily transfers to slops, increasing volume tenfold each time, a final seed inoculum of 150 liters was prepared.

The distillery wastes that were treated included two blackstrap molasses slops from Bacardí Corporation, Cataño, Puerto Rico, and one high test molasses slops from HTM fermentation studies conducted at the Rum Pilot Plant. Table 1 shows the chemical analysis of the slops, before and after treatment.

All wetted surfaces, including the centrifuge were 316 stainless steel. The tank (1900 L) was equipped with agitator, cooling water coil, aeration through a sparger equipped with a pressure and flow controller, antifoam addition tank and a centrifugal pump leading to a Westfalia centrifuge (Model NA-7-06-076)[10]. The Westfalia is a high-speed centrifuge (8500 RPM) fitted for continuous operation with a self cleaning cycle that is automatically controlled. Yeast slurry from the centrifuge was dried in a Proctor & Schwartz Cabinet Dryer. After drying, the material was ground in a Fitzmill Model-D comminuting machine.

Laboratory scale experiments had determined that for BM the optimum conditions for pilot plant scale were the following:

Nutrients (to seed and to fermentor)	
%P (as superphosphate – fertilizer grade) – 0.1	
%N (as ammonium sulfate – fertilizer grade) – 0.1	
Inoculum	10% (v/v)
Silicon antifoam	the least needed
Internal pressure	5 psig (to minimize foaming)
Volume	1500 liters
Aeration	10 ft^3/min at 20 lb/in^2
Agitation	190 r/min
Temperature	28–30° C

All lines and tanks were treated with live steam before procedures. Slops, with nutrients added, were cooled to 30° C; aeration was started and inoculum was added. *Conditions as specified above were maintained* until optimum growth of *Candida utilis*, determined by Neubauer Hemacytometer cell count, was attained; then the liquor was pumped to the centrifuge where the yeast was separated from the liquor (fed 22.7 L./min). A total volume of about 75 liters of yeast slurry was collected. This slurry was then dried in the cabinet dryer. The drying process was performed with an air temperature of 70° C and cross air circulation with the air draft adjusted to 0.1 in. The drying time per batch was approximately 8 hr when the thickness of the layer was approximately 3/8" in trays

[7]Martínez, G. and N. F. Murphy, 1984. Congeners from high test molasses alcoholic fermentation, J. Agric. Univ. P. R. 68 (1):59–66.

[8]Murphy, N. F., 1984. Fermentation of high test molasses, J. Agric. Univ. P. R. 68 (1): 33–44.

[9]Ramírez, M., 1982. Characterization of slops of high test molasses alcoholic fermentation, Res. Note, J. Agric. Univ. P. R. 66 (3): 235–37.

[10]Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment of 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.

TABLE 1.—*Characterization of slops before treatment and after treatment with* Candida utilis *and centrifugation*

Yeast cell count million/ml			Total solids %			Ash %			COD p/m			BOD p/m		
BM_1	BM_2	HTM	BM_1	BM_2	HTM	BM_1	BM_2	HTM	BM_1	BM_2	HTM	BM_1	BM_2	HTM
						Before treatment								
			7.6	7.4	2.5	2.0	2.0	0.6	85,800	77,000	36,700	21,900	27,800	11,900
						After treatment								
907[1]	655[1]	262[1]	3.9	6.1	2.6	1.4	2.34	0.8	29,800	57,400	21,300	5,300	12,100	6,000
						% change								
			–48.7	–17.6	–14.0	–30.0	+15.0	+33.3	–65.3	–26.2	–42.0	–75.8	–56.5	–44.5

[1] Final but before centrifugation.

Experimental Conditions	BM_1	BM_2	HTM
Alcoholic fermentation substrate	BM	BM	HTM
Length of treatment (hr)	21.5	27.0	17.0

TABLE 2.—*Dried fodder yeast characterization*

	Yeast grown in Blackstrap Molasses Slops			Yeast grown in High Test Molasses Slops
	P-6	*P-7*	*Average*	*P-8*
Humidity (%)	5.8	9.80	7.8	6.2
Total solids (%)	94.2	90.20	92.2	93.8
Ashes (%)	12.2	18.40	15.3	17.0
*Proteins (%)	45.48	38.21	41.85	42.23
*Fat (%)	1.14	0.46	0.80	0.64
Yield (kg)	15.88	14.06	14.98	4.54

*Dry weight basis.

20"x30"x2". The resultant solid was ground in the comminuting machine. The finished product was forwarded to the Lajas Agricultural Research and Development Center, where it proved to be a useful ingredient when included at 10% in a ration for layer hens[11].

As Ramírez[8] reported, slops from HTM alcoholic fermentation show significantly lower levels of total solids, ash, COD and BOD than those of BM slops. In general, the yeast action was more effective in lowering BOD than COD levels. On average, BOD was reduced 66% for BM runs and 44.5% for the HTM run. The yields of dried yeast were 14.98 kg for BM and 4.54 kg for HTM; equivalent to 9.9 g/L of slops treated for BM and 3.0 g/L for HTM. Table 2 gives the yeast analysis. After-treatment levels of the two runs of BM slops show big differences in all parameters studied, but the original samples did not show these differences. This finding needs further study.

Since the current regulations require a 70-75% BOD reduction for BM alcoholic fermentations, further studied with *Candida utilis* to achieve this goal are recommended. Biomass recycling, which will probably make the system more efficient, should be studied. Another method is the one tested by Bottaro Castlla et al.[12] This method includes a second step whereby the supernatant after treatment with *Candida utilis* is treated with *Paecilomyces variotti* without additives. The reduction of COD reported by Bottaro Castlla for the *C. utilis* treatment was 46%, a figure very similar to ours, after treatment with *P. variotti* COD reduction amounted to 92%.

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[11]Sosa, M. and P. F. Randel, 1985. Fodder yeast grown on rum distillery stillage as protein supplement for layer hens, J. Agric. Univ. P. R. 69 (3): 435.

[12]Bottaro Castlla, R., R. S. Waehner and A. M. Guilretti, 1981. Aerobic microbial treatment of sugar cane stillage by *Candida utilis* and *Paecilomyces variotti* in two step continuous cultures, Biotechnol. Letters 6 (3): 195–98.