Research Note

FERMENTATION OF BLACKSTRAP MOLASSES SUPPLEMENTED WITH FOUR DIFFERENT NUTRIENTS[1]

The economics of the alcohol fermentation process is always subject to revision. The costs of the fermentable substrates and the fermentation time are among the most important factors. To reduce the production costs in batch fermentation the use of the less expensive and most abundant substrates is recommended. It is also important to use the best microorganism strains and the optimum nutrient concentrations to accelerate the fermentation time. The development of new fermentation processes for ethanol production has been intensified in the last decade, e.g., cell immobilization, continuous fermentation. These processes are more efficient and less time consuming than batch fermentations. However, the major distilleries still use the batch fermentation process, and it seems that this method will be used for a long time.

The experiments discussed here are a perennial effort to accelerate the fermentation time by adding a nutritional supplement as nitrogen source, and thus reducing cost.

Blackstrap molasses is used in rum production because of its price and availability in the Caribbean region. The distillery yeast *Saccharomyces cerevisiae* has always been used for the fermentation of cane sugar molasses. No other microorganism, until now, had shown superiority in fermenting this substrate.[2,3,4]

The batch fermentation of 23° Brix (18% sugar concentration, total sugar as invert) blackstrap molasses wort lasts 40 hours when the distillers' yeast is used as fermenter. To this wort 1.5 g/L of ammonium sulfate is added as nutritional supplement. To find the best nitrogen source and supplement, we tested three other commercial supplements: Yeastex-61, a mixture of mineral salts and essential and organic nutrients (7 to 8% nitrogen); yeast extract and the less pure yeast product Bi-Tek yeast extract (9 to 12% nitrogen) supplied by DIFCO.

Two different yeast strains were used, RPP-80 and RPP-300, the latter a petite mutant commonly used for wine production.[5] Three different experiments were conducted, two of them with the strain RPP-80. The following minimal medium was prepared as pre-seed: (in g/100 ml) glucose, 4.0; yeast extract, 1.0; peptone, 0.6; KH_2P0_4, 0.2; $MgS0_4$, 0.1. This medium was sterilized at 121° C and 15 lb pressure for 15 min. The blackstrap molasses was diluted to a 23° Brix wort and the pH adjusted to 4.7 with concentrated sulfuric acid (0.6 ml/L). The nutrients were added individually or as indicated, mixed in equivalent amounts. The nutrient concentration never exceeded 2.0 g/L. The molasses wort was pasteurized at 170° F for 30 min. The inoculum was built up on transfers conducted on a daily basis. The fermentation was conducted in 20-L glass fermentors at room temperature, containing 14 L total fermenting mash which included 2 liters of molasses medium as seed. With strain RPP-80, the fermentations were allowed to proceed for

[1]Manuscript submitted to Editorial Board 26 February 1990.

[2]Kampen, W. H., 1985. Technology of the rum industry. *Sugar y Azúcar* 8: 36-43.

[3]Murphy, N. F., 1988. Ethanol production from blackstrap molasses by *Z. mobilis* and *Sacharomyces sp. J. Agric. Univ. P. R.* 72 (3): 483-84.

[4]VanVuuren, H. J. J. and L. Meyer, 1982. Production of ethanol from cane sugar molasses by *Z. mobilis. Biotechnol. Letters* 4 (4): 253-56.

TABLE 1.—*The comparative fermentation of 23° Brix blackstrap molasses wort with four different nutritional supplements*

Yeast Strain	Nutrient[1] concentration	Fermentation time	Final °*Brix*	Residual sugars (g/100ml)	Alcohol (%/vol)
	g/L	*h*			
RPP-80	0.0	42	15.0	10.40	4.39
	YE 1.5	42	11.4	5.40	5.08
	BT 1.5	42	12.1	6.47	6.53
	YX-61 1.5	28	9.2	2.71	8.44
	YX-61 1.5	42	8.1	1.96	8.62
	AS 1.5	28	7.1	1.27	9.20
	AS 1.5	42	7.0	1.24	9.20
	AS 0.75 + YX-61 0.75	28	7.6	1.20	9.01
	AS 1.0 + YX-61 1.0	28	7.5	1.20	9.20
RPP-300	YX-61 1.5	40	7.9	–	–
	AS 0.75 + YX-61 0.75	40	7.2	–	–
	AS 1.0 + YX-61 1.0	40	7.1	–	–
	AS 1.5	40	7.0	–	–

[1]YE, yeast extract; BT, Bi-Teck Yeast Extract; YX-61, Yeastex-61; AS, ammonium sulfate.

28 and 42 hr. With strain RPP-300 the fermentation lasted 40 hours. During this process periodic degree Brix readings were recorded. The residual sugars were analyzed with the Lane-Eynon method, and the ethanol concentration was measured through distillation with an immersion refractometer. These two analyses were not done with strain RPP-300.

Table 1 shows the results. As nutrient for the alcoholic fermentations, ammonium sulfate was the best. With strain RPP-80 the fermentation supplemented with this nutrient yielded a higher ethanol concentration than with any other nutrient. After 28 hours of fermentation, the ethanol concentration was 9.20% higher than the one obtained at 42 hours with other nutrients. This higher ethanol yield is directly related to the lower degree Brix and residual sugar. The ethanol yields were also higher at 28 hours with the mixture of ammonium sulfate and Yeastex-61 than the other nutrients at 42 hours. Yeastex-61 with less nitrogen content than the Bi-Tek yeast extract gave better results as nutrient for blackstrap molasses alcoholic fermentation. It is known that molasses contains between 0.5 to 1.5% nitrogen and the essential nutrients to support yeast growth.[6] The extra vitamin input provided by Yeastex-61 makes the yeast ferment faster than with Bi-Tek yeast extract.

Similar results were obtained with the strain RPP-300. The final degree Brix was lower when ammonium sulfate was used as nutrient.

Ammonium sulfate is 21% nitrogen, more than twice the amount in other nutrients tested. This high concentration of an essential element is probably the explanation for the results. Other nutrients with higher nitrogen content like urea (46%) are commonly used in alcoholic fermentation.

[5]KunKee, R. E., 1979. Production of fortified sweet wine. *Am. J. Enol. Viticult.* 30: 81-7.

[6]Meade, A. P., 1964. Cane Sugar Handbook, 9th ed., John Wiley & Sons, pp. 271-74.

However, urea has been related with the appearence of carbamates (urethane) in alcoholic beverages.[7]

Henry Huertas-Díaz
Research Assistant
Rum Pilot Plant

[7]Conacher, H. B. S., B. D. Page, B. P. -Y. Lau, J. F. Lawrence, R. Bailey, P. Calway, J.-P. Hanchag and B. Mori, 1987. Capillary column gas chromatographic determination of ethyl caramate in alcoholic beverages with confirmation by gas chromatography/mass spectrometry. *J. Assoc. Off. Anal. Chem.* 70 (4): 749-51.