Research Note

ETHANOL PRODUCTION FROM BLACKSTRAP MOLASSES BY ZYMOMONAS MOBILIS *AND* SACCHAROMYCES *SP.*[1]

The gram negative bacterium *Zymomonas mobilis* has been the focus of considerable attention during this decade for its remarkable alcohol production capacity. Researchers[2, 3, 4] working in chemically defined media attribute to this microorganism advantages over yeasts. However, for the development of an industrial process, *Zymomonas* must show its alcohol production potential with inexpensive substrates. We then initiated research in sugarcane molasses, which is the substrate for rum fermentations. Our previous results have indicated that fermentation rate is limited by substrate inhibition.[5] We found that with HTM the fed-batch fermentation process partially lessened this inhibition.[6]

Whereas *Zymomonas mobilis* has been widely investigated as a potential microorganism for producing ethanol, yeasts are still the dominant strains used in industrial processes. The study reported herein compares the fermentation behavior of both *Zymomonas* and *Saccharomyces* in blackstrap molasses (BM) substrate to determine the potential use of *Zymomonas* as an alternative for yeasts in the cane molasses ethanol industry.

Zymomonas mobilis strain ATCC 31822 (B-5) is a nitrosoquanidine mutant which exhibited improved growth and fermentation in molasses when compared to the parent strain ATCC 31921.[7] *Saccharomyces* sp. PPR 80 is a highly productive distillery yeast which has been our best alcohol producer for years.

The seed inoculum for both microorganisms (B-5 and PPR 80) was prepared by daily transfers from stock cultures to 10 ml sucrose defined media, to 100 ml of the same sucrose defined media, to 900 ml BM at approximately 8° Brix (6% sugar). Composition of sucrose-defined media included: 0.1% KH_2PO_4, 0.1% $(NH_4)_2SO_4$, 0.5% $MgSO_4$, 0.2% yeast extract, 4% sucrose. The molasses media consisted of BM diluted to 8° Bx (6% sugars) for seed preparation and to 17° Bx (12% sugars) for the fermentation mash. Ammonium sulfate was added at a concentration of 1.5 g/L. All media were sterilized at 121° C and 15 psi.

Experiments were carried out at 30° C

[1]Manuscript submitted to Editorial Board 3 November 1987.

[2]Swings, J. and J. Deley, 1977. The biology of *Zymomonas*. *Bacteriol. Rev.* 41, pp. 1–46.

[3]Rogers, P. L., D. Phil, K. J. Lee and D. E. Tribe, 1980. High productivity ethanol fermentations with *Zymomonas mobilis*. *Process Biochem.*, Aug.-Sept. 7–11.

[4]Lyness, E. and H. W. Doelle, 1981. Fermentation pattern of sucrose to ethanol conversions by *Zymomonas mobilis*. *Biotechnol. Bioeng.* 23: 1449–460.

[5]Murphy, N. F., 1988. Batch fermentation patterns for different strains of *Zymomonas* in high test molasses and blackstrap molasses. *J. Agric. Univ. P. R.* 72 (3): 000000000.

[6]Murphy, N. F., 1988. Comparative batch and fed-batch fermentations of high test molasses by *Zymomonas mobilis*. Comparative growth in different media. *J. Agric. Univ. P. R.* 72 (3): 000000000.

[7]Rhee, S. K., R. J. Pagán, M. F. Lefebvre, L. Wong and P. L. Rogers, 1984. Ethanol production from desalted molasses using *Saccharomyces uvarum* and *Zymomonas mobilis*. *J. Ferment. Technol.* 62 (3): 297–300.

TABLE 1.—*Comparative alcohol productivity on blackstrap molasses fermentation by* Saccharomyces *(PPR-80) versus* Zymomonas *(B-5)*

Hours of fermentation	Experiment 1		Experiment 2		Experiment 3	
	PPR 80	B-5	PPR 80	B-5	PPR 80	B-5
			Degree Brix			
17	—	—	—	—	6.9	12.7
23	6.1	—	5.6	6.8	5.3	7.5
25	—	—	—	—	5.3	6.3
31	5.9	8.7	5.3	6.5	—	—
41	—	—	—	—	5.2	5.6
48	—	—	5.5	5.6	—	—
96	5.3	5.1	—	—	—	—
Total sugars g/100 ml	12.4	12.4	12.3	12.3	12.0	12.0
Residual sugars g/100 ml	0.71	0.97	0.75	1.15	0.58	1.46
% alcohol v/v	5.9	6.1	5.8	5.8	6.0	5.6
% yield	78	80	76	77	81	76

in New Brunswick Magna Ferm fermentors.[8] Gentle stirring was provided at 150 r/m. Yeast fermentations were conducted according to the traditional batchwise procedure for yeasts, where the inoculum was added initially to the total volume (7L) of fermentation mash. *Zymomonas* fermentations, as recommended by Murphy,[9] were conducted with fed-batch techniques. Feeding 7L of the substrate was accomplished during 16 h with a metering pump at a rate not to exceed a 12° Bx reading inside the fermentor to avoid the deleterious effect of high substrate levels, very common with *Zymomonas* sp. Brix hydrometers were used periodically to follow the course of the fermentation. Our goal was to obtain 6% alcohol from 12 to 13% total sugars. With the methods of the Rum Pilot Plant, total initial and residual sugars as invert and alcohol percentage were determined.

Table 1 shows yeast PPR 80 and *Zymomonas* B-5 were best alcohol producers. A greater amount of non-used sugars in the case of *Zymomonas* points to the possibility of higher alcohol yields, should this residual sugar be further used. Considering that PPR 80 is a highly productive distillery yeast, the performance of *Zymomonas* in cane molasses is encouraging for the possibility of its use in the cane molasses ethanol industry. However, yeasts still behaved faster as indicated by °Brix readings; thus it is evident that the *Zymomonas* process needs improvements.

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[8]Trade names are used solely for the purpose of providing specific information. Mention of trade names does not constitute a guarantee or warranty by the Agricultural Experiment Station of the University of Puerto Rico or an endorsement over other preparations not mentioned.

[9]Manual de Métodos Analíticos de la Planta Piloto del Ron, Estación Experimental Agrícola, Recinto de Mayagüez, Univ. P. R., Río Piedras, P. R.