A Solid State Fermentation Method for Citric Acid Production Using Sugar Cane Bagasse

Since the first report by Cahn[1] in 1936 on the use of beet and sugar cane pulp for citric acid production by *Aspergillus niger*, there has been no other report, to date, on the use of solid inert materials in this type of fermentation. In recent years, the emphasis has been mainly to develop either surface or submerged fermentations to produce this organic acid. In our laboratory Chakrovorty and Vyas[2] isolated a strain of *A. niger* (3/1) which produced low amounts of citric acid under submerged conditions. During our subsequent studies to improve and optimize conditions for maximal citric acid production by this strain of *A. niger*, we found that the yields of citric acid were higher under surface culture conditions than under submerged conditions. Since the production of citric acid is dependent on an appropriate supply of oxygen and also on the ratio of surface area to volume, an alternative approach of providing these conditions was devised by the introduction of sugar cane bagasse in the fermentation medium. Under these conditions, the medium is adsorbed by the sugar cane bagasse and additional surface area is provided for the growth of the organism, thus resulting in high yields of citric acid.

A. niger 3/1 used in these experiments was isolated in our laboratory and was maintained as a soil culture. It was subcultured on potato dextrose agar slants twice before use. Spores from a 7 day old slant were always used as inoculum in fermentation studies.

Two types of liquid media were employed. The sucrose medium, pH 3.0, had the composition described by Millis et al.[3] and contained 14% commercial sucrose. The molasses medium, pH 5.8, contained 28% Indian Cane molasses (14% reducing sugars), 0.25% KNO_3, and 0.1% KH_2PO_4. The sugar cane bagasse employed was finely chopped sugar free material from a local sugar factory.

For surface and submerged culture experiments, 75 ml of the above media were distributed in 500 ml Erlenmeyer flasks, autoclaved at 15 lb/15 min, cooled, and inoculated with 1 ml of the spore suspension (about 10^5–10^6 spores). For solid state fermentation, 10 g of the chopped sugar cane bagasse was introduced into 500 ml Erlenmeyer flasks containing 75 ml of liquid medium, sterilized, and inoculated as above. The media, after inoculation, was mixed thoroughly to give a uniform distribution of spores. In experiments where the effect of methanol was tested, 3% methanol[4] was added to the flasks before incubation. For submerged conditions, the flasks were incubated at 30°C on a rotary shaker operating at 210 rpm with a 2 in. amplitude throw. The flasks were withdrawn after 6 days of incubation. The contents of the flasks from surface and submerged culture were filtered, the mycelia were washed with distilled water, and the volume was made up to 100 ml. In the case of solid state fermentation, the materials were extracted thrice by the addition of 100 ml of distilled water and by shaking on a rotary shaker for 30 min. The extracts were pooled and the volume was made to 400 ml.

In tray experiments, 150 g of bagasse was mixed with 1 liter of sucrose medium, sterilized in a stainless steel container, cooled, and inoculated with 10 ml of the spore suspension. Thirty milliliters of methanol was then added and mixed, then the material was spread in an enamel tray (12 in. × 15 in. × 2 in.) presterilized with ethanol. The trays were covered with polyethylene sheets and incubated at 30°C for 6 days. The material was extracted thrice with 1.5 liters of distilled water and the volume was made up to 5 liters. The culture broths were analyzed by both electrometric titration and paper chromatography for citric acid.[5] Residual sugar was estimated by Cole's method.[6]

Table I summarizes the results of our experiments. The average yield of citric acid under surface culture conditions is more than that under submerged culture conditions and the yield of citric acid is increased only in the presence of methanol. The presence of methanol in submerged culture conditions however, is not conducive to the production of citric acid.

The average yield of citric acid under solid state conditions in the presence of methanol in sucrose medium was as high as 62.8% while in molasses medium it was 56.4%. The yield of citric acid by this method is much higher than the yield reported by Cahn.

To test whether the yields obtained in flask-scale experiments could be successfully reproduced on a larger scale, tray experiments using only sucrose as carbon source in the presence of methanol, were carried out (Table I). The yield of citric acid, based on the amount of initial sugar present in the fermentation

TABLE I

Citric Acid Production by *A. niger* 3/1 under different Cultural Conditions[a]

Medium	Cultural condition	Methanol (3%)	% Residual sugar	% Citric acid (g)[b]	% Citric acid (g)[c]
Sucrose					
	Submerged	−	10.0	4.5	5.0
		+	13.1	3.8	4.3
	Surface	−	20.7	5.2	6.6
		+	24.3	22.9	30.2
	Solid state	−	12.5	14.1	16.1
		+	12.5	62.8	71.8
Molasses					
	Submerged	−	27.1	4.9	6.7
		+		poor growth	
	Surface	−	20.7	9.4	11.8
		+	10.0	30.8	34.3
	Solid state	−	2.7	18.4	18.9
		+	3.9	56.4	58.7
Tray experiments (Sucrose medium)		+	2.1	78.6	80.2

[a] The values for citric acid represent averages of 10 flasks for each treatment and averages of 3 tray experiments.

[b] Based on the initial sugar concentration in the fermentation medium.

[c] Based on the actual sugar consumed.

medium, was 78.6%. Based on the amount of sugar actually consumed, the yield was as high as 80%. Chromatographic analysis of the culture broths both from flask and tray scale experiments have revealed that the only acid present is citric acid with no traces of any other organic acid. The tray scale experiments have therefore demonstrated the feasibility of using this technique effectively on a commercial scale to produce citric acid using either sucrose or molasses as carbon source. However, for the method to be successful, the presence of a 3% methanol concentration is necessary.

References

1. J. Cahn, *Ind. Eng. Chem.*, **27,** 201 (1935).
2. S. C. Chakrovorty and S. R. Vyas, *J. Research (PAU).*, **52,** 266 (1968).
3. N. F. Millis, B. H. Trumphy, and B. M. Palmer, *J. Gen. Microbiol.*, **30,** 365 (1963).
4. A. J. Moyer, *Appl. Microbiol.*, **1,** 1 (1953).
5. K. Chaudhary, K. Lakshminarayana, I. K. Dev, and S. R. Vyas, *Ind. J. Microbiol.*, **14,** 42 (1974).
6. S. W. Cole, *Practical Physiological Chemistry,* W. Heffner and Sons, Cambridge, England, 1949, p. 167.

K. Lakshminarayana
K. Chaudhary
S. Ethiraj
P. Tauro

Dept. of Microbiology
Haryana Agricultural University
Hissar, India

Accepted for Publication November 12, 1974