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EFFECT OF FERROCYANIDE ON CITRIC ACID PRODUCTION FROM BEET MOLASSES BY ASPERGILLUS NIGER

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Effect of ferrocyanide (0–200 ppm) was studied on the growth of *Aspergillus niger* and citric acid formation in the molasses medium. The growth of *Aspergillus niger* in the molasses medium was sensitive to ferrocyanide when added during the exponential growth phase. The mould growth was modified to the form of small, separate and round pellets with the result that both the agitation and aeration or oxygen supply to the cultures was increased. Ferrocyanide concentration of 30 ppm stimulated maximum citric acid formation.

Introduction

In an earlier communication[1] citric acid production from cane molasses by *Aspergillus niger* has been reported. Ferrocyanide was added to the molasses medium for reducing trace metals and the factors studied were (i) the determination of optimum amount of ferrocyanide, (ii) time of its addition, (iii) initial pH of the medium and (iv) changes in the ferrocyanide concentration during the fermentation. Beet molasses is also being produced by sugar factories, such as the Premier Sugar Mills & Distillery Mardan, Frontier Sugar Mills & Distillery Takht-i-Bahi and Charsadda Sugar Mills Charsadda, amounting to five to seven thousand tons per annum. The use of beet molasses as substrate for citric acid fermentation is preferred for its low content of heavy metals as compared with cane molasses.[2–3] The present paper, therefore, describes the citric acid production in shake flask cultures from beet molasses and optimum concentration of ferrocyanide and time of its addition have been determined for its exploitation on pilot scale.

Material and Methods

The fermentation procedure and the methods of analysis of citric acid, sugar and ferrocyanide have been reported.[1] Briefly, fermentation medium was prepared from beet molasses by dissolving with tap water to 15% sugar concentration. One litre of molasses solution was clarified by boiling for ½ hr with 35 ml 1N H_2SO_4, neutralized after cooling with lime water and left to stand overnight. The clear supernatant was used for experiments. All fermentations were carried out at 30°C in 300 ml shake flasks containing 25 ml molasses medium. The rotary shaker was rotated at 125 rev/min with 1½ in amplitude throw. The spores of *A. niger* WIS 72-4 were used in the present study. All cultures were analysed 7 days after spore inoculation.

Results and Discussion

The citric acid production and sugar utilization by *A. niger* in control cultures were about 10–20 g/l and 70–80 g/l respectively. The mould growth in control cultures was in the form of large filamentous and gelatinuous pellets and thus both the agitation and aeration of the cultures were greatly affected. The addition of 5–200 ppm of the ferrocyanide at the time of inoculation to shake flask cultures modified the mould growth to the form of small separate and round pellets and both agitation and aeration or oxygen supply of the cultures were greatly improved. The addition of ferrocyanide stimulated citric acid formation and maximum stimulation was reached with 10–30 ppm of ferrocyanide (Fig. 1). This is in agreement with the findings of Clark.[4] Further increase in the concentration of ferrocyanide, however, reduced the sugar utilization, mycelial formation and citric acid synthesis. The size of the pellets produced was related to ferrocyanide concentration. Pellets capable of producing optimum yields of citric acid were of the size of 1–2 mm diameter in the presence of 30 ppm ferrocyanide and their size was decreased by increasing the ferrocyanide level showing its inhibitory effect on the growth process of the mould (Fig. 2). The amount of citric acid produced was 63 g/l (70% on the basis of sugar used). Effect of time of addition of ferrocyanide (30 ppm) was also investigated on both the growth process and citric acid formation (Fig. 2). The purpose of the experiment was to determine whether stimulatory effect of ferrocyanide is due to its action on the growth process during exponential growth or on enzymic system in the stationary phase. The mould growth was modified to the form of small, separate and round pellets by adding ferrocyanide at 0–24 hr after spore inoculation. The pellets produced were, however, large filamentous and gelatinuous when ferrocyanide addition was made at 48–72 hr after inoculation.

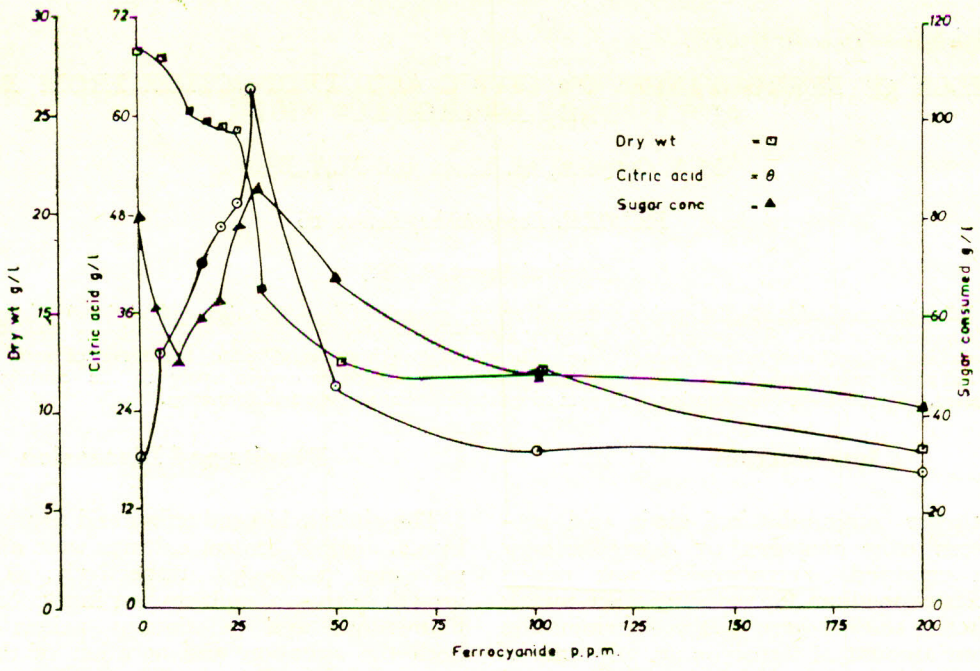

Fig. 1.—Effect of ferrocyanide when added at the time of inoculation on citric acid production, mycelial dry wt and sugar consumption by *A. niger*.

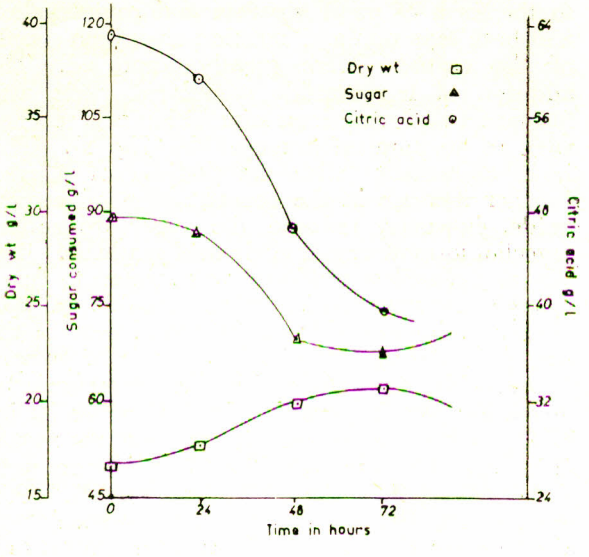

Fig. 2.—Effect of ferrocyanide (30 ppm) when added at different intervals after inoculation on citric acid production, mycelial dry wt and sugar consumption by *A. niger*.

The citric acid formation was maximum when ferrocyanide was added during the early growth stage e.g. 0–24 hr after inoculation. It follows that stimulation of citric acid production by ferrocyanide was because of its action during growth process. This evidence of ferrocyanide effect is against the suggestion of Martin[2] that ferrocyanide has a direct action on the mould in increasing citric acid formation.

The sensitivity of growth process to ferrocyanide was in agreement with the findings of Choudhary and Pirt[5–7] and Akbar *et al.*[1] Moreover, the pH of the medium has great influence on the ferrocyanide action. At pH near neutrality, as in the present work, ferrocyanide has no toxic effect on the growth process but at low pH that is at 3.0 as reported by Choudhary and Pirt[5–7] mould growth is inhibited. It may be concluded from the above results that beet molasses is quite suitable for its exploitation as carbohydrate source for the production of citric acid on commercial scale.

References

1. M. Akbar, F. Ahmad and M.A.Q. Choudhary, Pakistan J. Sci. Ind. Res., **10,** 184 (1967).
2. P.P. Gerhardt, W.W. Dorral and I.L. Baldivin, J. Bacteriol., **52,** 555 (1946).
3. S.M. Martin, Can. J. Microbiol., **1,** 644 (1952).
4. D.S. Clark, Ind. Engg. Chem., Product Research and Development, **1,** 59 (1962).
5. A. Q. Choudhary and S. J. Pirt., J. Gen. Microbiol., **41,** 99 (1965).
6. A. Q. Choudhary and S. J. Pirt, J. Gen. Microbiol., **41,** iii (1965).
7. A. Q. Choudhary and S. J. Pirt, J. Gen. Microbiol., **43,** 71 (1966).