Pakistan J. Sci. Ind. Res., Vol. 30, No. 12, December 1987

# CALCIUM GLUCONATE FERMENTATION OF MAIZE GUR (HYDROL) IN STIRRED 50 L. FERMENTER

M. Afzal Baig

*PCSIR Laboratories, Lahore-16*

(Received October 24, 1985; revised November 26, 1987)

Various raw products such as maize gur and liquid glucose were tried as substrate for the production of calcium gluconate by *Aspergillus niger* WRL 51. The effect of temperature, different concentrations of substrates and rate of aeration was also investigated.

The maximum conversion of glucose present in the substrates into calcium gluconate was achieved at 16-24 hr. after inoculation. Optimum temperature and the rate of aeration was 30 ± 2° and 500 cc/l/m respectively. The production of calcium gluconate was higher in medium containing maize gur than that of liquid glucose i.e. 132 g/l and 118 g/l respectively.

*Key words:* Maize gur (hydrol), Calcium gluconate, Liquid and commercial glucose.

## INTRODUCTION

The production of gluconic acid and its salts such as calcium gluconate has been carried out by microbiol oxidation of glucose. The species of bacteria such as Acetobacter [10], Pseudomonas [11] and of molds i.e. *Aspergillus* [1-4] and Penicillium [5, 6] have been studied mostly for the biosynthesis of gluconic acid and its salts on commercial scale. The present work describes the production of calcium gluconate by a locally isolated strain of *A. niger* WRL-51 in 50 1 glass stainless steel fermenter using maize gur (hydrol), a byproduct of glucose manufacturing plant (Glaxo Laboratories Lahore).

Raw materials abundantly available here and their use in poultry feed could be obtained. The effect of different levels of substrates such as maize gur, liquid glucose, temperature and rate of aeration on glucose oxidation by growing *Aspergillus niger* in the fermenter has been studied in the present paper prior to pilot plant investigation.

## MATERIALS AND METHODS

The strain of *Aspergillus niger* locally isolated WRL-51 was used throughout the present studies. The culture was maintained on the agar medium consisting of (g/l) glucose 30.0, $MgSO_4$, $7H_2O$, 0.1, $K_2HPO_4$ 0.12, $NH_4NO_3$ 0.25, potatoes 44.00, $CaCO_3$ 4.00. The culture was incubated at 30 ± 2° for 5-7 days for maximum sporulation and then slants were kept in the refrigerator.

*Inoculum preparation.* The spores of 5-7 days old cultures were washed with 5 ml 0.5% Monoxol O.T. The agar surface was washed twice with sterile distilled water. The combined washing were made upto 25 ml and shaken with glass beads to break the clumps of spores. A vegetative inoculum was prepared by incubating 200 ml of fermentation medium in one litre conical flasks combined with spore suspension. The shake flasks cultures were placed on a rotary shaker for growth at 30 ± 2° for 48 hr. before asceptic transfer to a fermenter.

*Fermentation medium.* The fermentation medium consisting of (g/l) glucose 150.0 $(NH_4)_2HPO_4$ 0.8 $KH_2PO_4$ 0.3 $MgSO_4$ 0.25 and calcium carbonate 26.0. The medium was divided into two parts for sterilization: (a) glucose + salt solution and (b) $CaCo_3$ suspension. The sterilization was carried out by boiling the both solutions in a open steam pan continuously for 30-50 min. and then asceptically transferred to a fermenter which was sterilized by steaming for 1 hr. The fermentation medium was cooled by passing water through the coils and then inoculated with a vegetative inoculum.

*Culture vessel.* The glass stainless steel fermenter of 50 l capacity was fabricated in the PCSIR Laboratories Workshop, Lahore. The glass pipe (Ps 24/1000) was obtained from QUF, UK. The vessel was equipped with an agitater, cooling coils, baffle, air inlet, outlet, medium transfer lines and a sampling device. The agitator was rotated at 250 rpm by variable speed motor. The temperature of the culture medium was kept at 30 ± 2° by passing tap water through the coils. The rate of air

flow was adjusted using flow meter. Working volume of 20 l in the fermenter was used throughout the present investigation for gluconate production.

*Analytical method*

Glucose was estimated by the ferricyanide reduction method a modification of Fugita and Iwatake [7]. For calcium gluconate assay a determination of soluble calcium was made by the EDTA titration methods [8].

*Identification of gluconic acid.* The formation of gluconic acid in the culture broth was also confirmed by paper chromatography [9]. A solvent system of butanol, acetic acid, water (4.1.5 v/v) was used and chromatograms were developed by a slightly alkaline solution of 0.04% alcoholic solution of bromophenol blue.

## RESULTS AND DISCUSSION

Fig. 1 and the data in Table 1 show the effect of glucose concentration level in the different substrates on the production of calcium gluconate in the fermenter by *A. niger*. The rate of aeration and size of inoculum was 4% and 500 cc/l/m respectively. The utilization of glucose by mould strain was almost complete at 24 hr. after inoculation when the level of glucose was 10% and 15%. Furthermore liquid glucose was slightly better than maize gur in the conversion of glucose to gluconic acid, because liquid glucose has a lower content of impurities as compared to maize gur. The consumption of glucose was quite slow when 20% glucose level was kept both in liquid glucose and maize gur. The maximum yield of calcium gluconate in maize gur and liquid glucose was 132/l and 188 g/l respectively. Maize gur resulted in a better yield of calcium gluconate than liquid glucose due to the presence of amino acids, minerals and vitamins.

The data in Table 2 indicate the effect of temperature such as 22 ± 2° and 30 ± 2° on the production of calcium gluconate in a stainless steel fermenter using different substrates. The glucose level, size of inoculum and rate of aeration were kept constant. It was observed that mould growth and morphology were greatly affected by the temperature. There was a correlation between the fermentation time and yield. Regular increase in the formation of calcium gluconate was found at both temperatures. However, the mould resulted in a heavy cell mass at 22 ± 2° and decreased the formation of gluconic acid. The maximum production of calcium gluconate at 30 ± 2° in maize gur, liquid

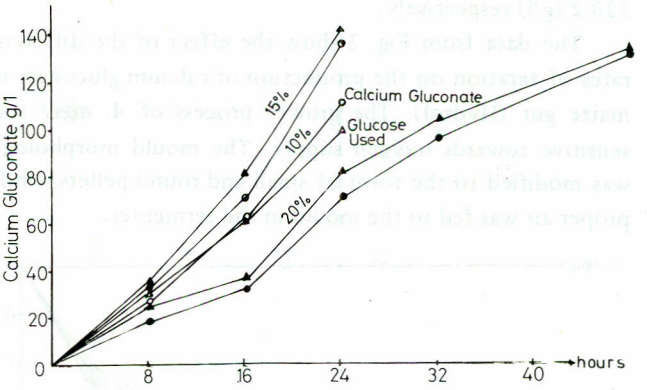


Fig. 1. Effect of different concentration of maize gur on the production of calcium gluconate in 50 L fermenter by *Aspergillus niger* WRL-51.

Table 1. Effect of different concentration of liquid glucose on the production of calcium gluconate in 50l fermenter.

| Hours | 10% Liquid glucose | | | 15% Liquid glucose | | | 20% Liquid glucose | | |
|---|---|---|---|---|---|---|---|---|---|
| | used g/l | unused g/l | Calcium gluconate g/l | used g/l | unused g/l | Calcium gluconate g/l | used g/l | unused g/l | Calcium gluconate g/l |
| 8 | 39 | 61 | 30.8 | 41 | 109 | 42.9 | 30.0 | 170 | 23.2 |
| 16 | 62 | 38 | 63.8 | 80 | 70 | 75.9 | 42 | 158 | 34.1 |
| 24 | 87 | 13 | 94.6 | 128 | 22 | 130.8 | 75 | 125 | 61.8 |
| 32 | | | | | | | 105 | 95 | 92.4 |
| 48 | | | | | | | 115 | 85 | 114.4 |

Table 2. Effect of different temperature on the production of calcium gluconate in 50l fermenter using different substrates

| Hours | 15% Maize gur | | 15% Liquid glucose | | 15% Comm. glucose | |
|---|---|---|---|---|---|---|
| | Temp. 22 ± 2°C | Temp. 30 ± 2°C | Temp. 22 ± 2°C | Temp. 30 ± 2°C | Temp. 22 ± 2°C | Temp. 30 ± 2°C |
| | Calcium gluconate | | Calcium gluconate | | Calcium gluconate | |
| 8 | 28 | 36 | 24.2 | 35.2 | 22.0 | 45.1 |
| 16 | 40 | 60.5 | 37.4 | 67.1 | 36.3 | 68.2 |
| 24 | 56.1 | 130.1 | 50.8 | 118.8 | 56.1 | 128.2 |
| 32 | 72.6 | | 61.6 | | 68.2 | |
| 48 | 80.8 | | 72.6 | | 78.1 | |

glucose and commercial glucose was 130, 118.8 and 128.2 (g/l) respectively.

The data from Fig. 2 show the effect of the different rates of aeration on the production of calcium gluconate in maize gur (Hydrol). The growth process of *A. niger* was sensitive towards oxygen supply. The mould morphology was modified to the form of small and round pellets, while proper air was fed to the mould in the fermenter.

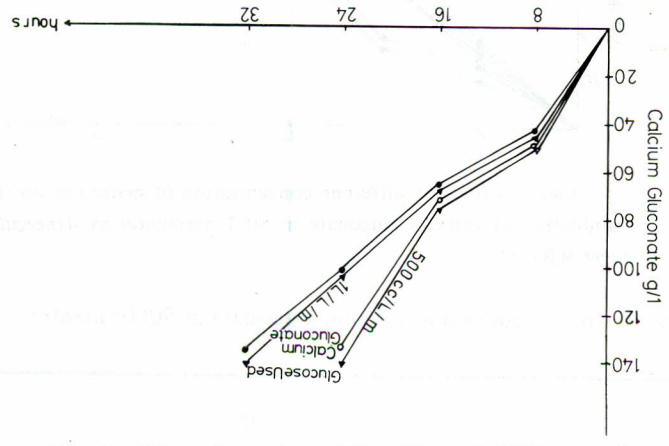


Fig. 2. Effect of aeration (500 cc/I/m and II/I/m) on the production of calcium gluconate in 50 l fermenter using maize gur as substrate by *Aspergillus niger* WRL-51.

Agitation also exercised a sharp effect on the formation of calcium gluconate in a culture with a pellet type growth. Gluconic acid fermentation depends on the regular and proper supply of oxygen. Calcium gluconate was maximum (i.e. 130 g/l) at 24 hr. after inoculation when the rate of aeration was 1 l/l/m. Optimum conversion of maize gur at 500 cc 1/l/m gluconic acid was (133.2 g/l) after 24 hr. inoculation. The formation of small pellets of the mould and large capacity of absorption of oxygen produced the maximum quantity of gluconic acid. Thus the use of 500 cc/l/m aeration is of great commercial importance in making the process more economical.

*Acknowledgement.* The author greatfully acknowledges the technical help of Messrs. Muhammad Salim and Muhammad Hamid throughout the investigation.

## REFERENCES

1. M. Yasin, A.H.K. Niazi, and M.A. Qadeer, Pakistan J. Sci. Ind. Res., **12**, 37 (1969).
2. A.H.K. Niazi, M. Yasin and M.A. Qadeer, Pakistan J. Biochem., **11**, (1) (1969).
3. E.A. Cas Trock, N. Progres, P.A. Wells and A.J. Moyer, Industr. Engng. Chem. (Intern. ed.), **30**, 782 (1938).
4. N. Progres, T.F. Clark and S.I. Aronovasky, Inst. Engng. Chem., **33**, 1065 (194 ).
5. D.E. May, H.T. Herrick, E. Thom and M. Bchurchi, J. Biol. Chem., **75**, 417 (1965).
6. G.R. Ambeaker, S.B. Thadane and V.M. Doctor, Appl.Microbiol.,**13**, 713 (1965).
7. A. Fujita and D. Iwatake DE Biochem., **2**, 242 (1931).
8. H.A. Flaschka, *EDTA Titration – An Introduction to Theory and Practice* (Pergamon Press, London, 1959).
9. S.M. Patridge, Biochem. J, **42**, 238 (1948).
10. L.B. Lockwood, B. Tabenkin and G.E. Ward, J. Bacteriol, **42**, 51 (1941).
11. G.F. Pench and L.L. Kempe, Biotech. & Bioengng., **61**, 347 (1964).