Biological Sciences Section

Pakistan J. Sci. Ind. Res., Vol. 18, No. 5, October 1975

PRODUCTION OF CALCIUM GLUCONATE BY ASPERGILLUS NIGER IN 50-L FERMENTER

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(Received December 24, 1974 ; revised May 22, 1975)

Abstract. The production of calcium gluconate by *Aspergillus niger* in 50-1 glass–stainless steel fermenter was studied. The optimum levels of glucose concentration and size of inoculum were determined for maximum conversion of glucose to gluconic acid as calcium salt. Rates of aeration (500 ml 1/m) and agitation (200 rev/min), however, remained unchanged.

The production of calcium gluconate by *Aspergillus*[1] and *Penicillium*[2] strains, was carried out in shake flasks to select the mould strains and their optimum cultural conditions. The present study describes the production of calcium gluconate by *A. niger* in a 50-1 glass–stainless steel fermenter. Effect of the rate of aeration and inoculum size on the glucose oxidation by growing mould in the fermenter has been studied in the present report before pilot-plant investigations.

Materials and Methods

Organism. The strain of *Aspergillus niger* WRL-51 was used in the present work. The culture was maintained on the agar medium consisting of (g/l): glucose, 150; agar, 20; NH_4NO_3, 2.5, KH_2PO_4, 1.0, $MgSO_4.7H_2O$, 0.25; and trace metals (mg/l): Fe^{+++}, 2.2; Cu^{++}, 0.48, and Zn^{++}, 3.80. The mould was grown on the agar medium (15 ml) contained in 6-oz flat bottles plugged with cotton wool. The cultures were incubated at 30±2°C for 5–7 days and then kept in the refrigerator before use.

Inoculum Preparation. Vegetative inoculum was used to inoculate the fermentation medium. The spore suspension was prepared by wetting the spore, with 5 ml 0.005% aqueous solution of Manoxol O.T. (dioctyl ester of sodium sulphosuccinic acid) and then washed with sterile distilled water. The washings were combined and made up to 50 ml. Vegetative inoculum was prepared by inoculating 200 ml of frementation medium contained in one litre conical flask, with spore suspension. The shake flask cultures were incubated at 30±2°C for 36 hr before asceptic transfer to the fermenter.

Fermentation Medium. The fermentation medium consisting of (g/l) : glucose, 150.0; $(NH_4)_2HPO_4$, 0.4; KH_2PO_4, 0.2; $MgSO_4.7H_2O$, 0.156; and $CaCO_3$, 26.0 was divided into two parts for sterilization (a) glucose + salt solution and (b) $CaCO_3$ suspension. The sterilization was carried out by boiling both solutions in a steam pan for 30–45 min and then they were asceptically transferred to the fermenter which was previously sterilized by steaming for 1 hr. The fermentation medium was cooled by passing water through the coils and inoculated with the vegetative inoculum.

Culture Vessel. The stainless steel–glass fermenter of 50-1 capacity was fabricated in the laboratory workshop. The glass pipe (PS24/1000) was obtained from QVF U.K. The fermenter was equipped with agitator, cooling coils, baffles, air, inlet and outlet, medium transfer lines and sampling devices. The agitator was rotated at 250 rev/min by a variable speed motor. The temperature of the culture medium was kept at 30°±2 by passing tap water through the coils. The air at 10 lb pressure was used for aeration. The rate of air flow was adjusted using flowmeter. Working volume of 25 1 in the fermenter was used throughout the present work (Fig. 1).

Analytical Methods. Glucose was estimated by ferricyanide reduction method— modification of Fungita and Iwatake.[3] For calcium gluconate

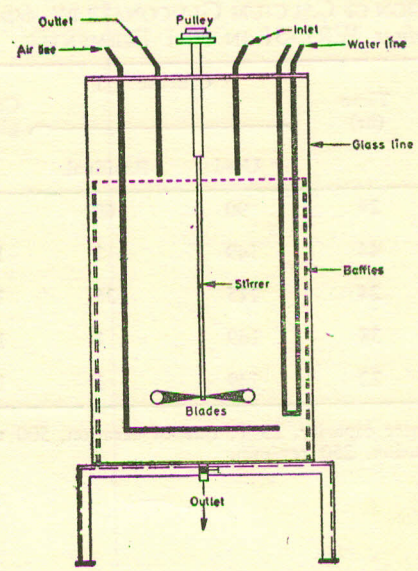

Fig. 1. Glass–stainless steel fermenter.

assay, a determination of soluble calcium was made by EDTA titration methods.[4]

Identification of Gluconic Acid. The biosynthesis of gluconic acid in the culture growth was also confirmed by paper chromatography.[5] 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 Discussions

The data of Table 1 shows the effect of golucse concentrations on the production of calcium gluconate in the fermenter by *Aspergillus niger*. The size of the inoculum was 4% of the culture medium.

TABLE 1. EFFECT OF GLUCOSE CONCENTRATION ON THE PRODUCTION OF CALCIUM GLUCONATE BY (*Aspergillus niger*) WRL5-1 in 50-1 FERMENTER.

Glucose (%)	Time (hr)	Glucose (g/1)		Calcium gluconate (g/1)
		Used	Residual	
10	24	80	20	70·5
	42	99	1·0	90·8
15	24	90	60	79·6
	42	148·5	1·5	133·2
30	24	110	190	91·7
	42	175	125	155·8

Fermenter capacity, 25 1; rate of aeration, 500 ml l/m; rate of rotation, 250 rev/min.

TABLE 2. EFFECT OF SIZE OF INOCULUM ON THE PRODUCTION OF CALCIUM GLUCONATE BY *Aspergillus niger* WRL-51 IN 50-1 FERMENTER.

Glucose (%)	Time (hr)	Glucose g/1		Calcium gluconate (g/1)
		Used	Residual	
4	24	90	60	78·8
	44	149	1	134·2
10	24	115	35	103·5
	38	149	1	134·4
20	22	149	1	135·2

Fermenter capacity, 25 1; rate of aeration, 500 ml l/m; rate of rotation, 250 rev/min.

The consumption of glucose by the mould strain was almost complete after 42 hr inoculation when glucose level was kept at 10 or 15% in the cultuer medium. The residual glucose was about 1.0–1.5%. Further increase in glucose concentration, e.g. 30% resulted in lowering glucose consumption. The amount of residual glucose after 42 hr spore inoculation was 14.5%. Thus the use of higher concentration of glucose in the culture medium was not economical. The adequate level of glucose in the fermentation mash was 15%. This is in accordance with the findings reported by other workers.[6–10] Glucose concentration higher than 15% in the culture medium can be used by adding boron salt, it increases the solubility of calcium gluconate but its use creates problems in purification process for the production of the salt of pharmaceutical grade.

The size of inoculum has a great influence on the rate of fermentation. Table 2 shows the effect of the size of vegetative inoculum (24-hr old) on glucose consumption and calcium gluconate formation. The rate of glucose utilization hence calcium gluconate formation were increased with the increase in the size of inoculum. Glucose consumption was almost complete after 44, 38 and 22 hr inoculation with the inoculum size of 4, 10 and 20% respectively. Thus the use of 20% inoculum size is of great commercial importance in making the process more economic.

Acknowledgement. The authors are grateful to Messrs. Hamid, Mahboob Ali Qureshi, Noor Mohammad and Mohammad Saleem, for their technical help during the course of the studies.

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