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# IMPROVED PRODUCTION OF GLUCONIC ACID BY GLUCONOBACTER OXYDANS GPM60 ENTRAPPED IN CALCIUM ALGINATE BEADS

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**Keywords:**

Experimental, immobilization, *Gluconobacter oxydans*, calcium alginate

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## ABSTRACT

An experimental study was carried out to evaluate some advantages of immobilization of *Gluconobacter oxydans* GPM60 entrapped in calcium alginate beads. For this purpose, several parameters were examined regarding immobilization to maximize the production.production was increased significantly ($p<0.01$)with immobilized *Gluconobacter oxydans* GPM60 (entrapped in calcium alginate) compared to the production by free cells. Maximum production (19.6 mg/ml) was obtained with CaCl2,0.2 (M);sodium alginate,2.5%;bead diameter,6mm and 24h storage period.

## INTRODUCTION

Immobilization of bio-catalysts (like enzymes) , organelles microbial cells and other living objects in attracting worldwide attention. Immobilized biocatalysts catalyse biochemical reactions under more stabilized conditions than their free counterparts; moreover, they can be reused economically1. Microbial cells and organelles contain metabolic systems that catalyse different complicated metabolic reactions. Furthermore, with immobilization of microbial cells, the procedure of extracting enzymes from cells is no longer is required. This avoid inactivation of enzymes during tedious and time consuming purification procedures and it increases stability of many membrane-associated enzymes unstable in a stabilized state4, 5.These examples illustrate the advantages of immobilized enzymes in various field.

A variety of reactions can also be achieved by immobilized living cells, whether they are resting or growing in gel matrices. Definition of immobilized resting cells is often difficult because the state of immobilized cells was not mentioned in the literature in many cases. Therefore immobilized resting cells sometimes mean non-treated cells6-8.Immobilized cell systems have been reviewed by Chibata and Tosa9, Abbott1, Dunnill10 and very recently Ganguly and Banik11.

Considering the previous literature, our present study was intended to examine the advantage(s) of immobilization (if any)by entrapping *Gluconobacter oxydans* GPM60 in calcium alginate gel beads for gluconic 2 acid production which is comparatively cheap among other immobilization methods and also easier to form and maintain.

## MATERIALS AND METHODS

**Microorganism:** *Gluconobacter oxydans* was isolated from the soil of Sankrail,Howrah,West Bengal,India .the parent strain produced only 0.9% gm/L gluconic acid.To develop a high yielding strain of *Gluconobacter oxydans* ,the parent strain was treated subsequently using Ethyl MethaneSulphonate(EMS) as stated in our previous publication12.

**Composition of production medium:** A synthetic medium for the production of gluconic acid with the following composition was used throughout the study:glucose,8%;(NH4)2SO4 ,0.6%;K2HPO4 , 0.3% ; KH2PO4 , 0.2%; MgSO4 . 7H2O, 0.6%; CaCl2, 0.3%.pHwas adjusted to 6.0.

**Cultural conditions:** Production was carried out using shake-flask method on a temperature controlled shaker (BOD incubator with shaker)shaking at 300 rpm for 96h at 300C with a cell density of 10x108 cells/ml using 48h aged inoculum.

**Estimation of gluconic acid:** Gluconic acid was estimated by isotachophoretic method13.

**Estimation of residual sugar:**Residual sugar was estimated by DNS method14.

**Preparation of calcium alginate:** The cells were suspended was slowly added to the sterile solution of sodium alginate (1%) and mixed thoroughly with the sterile glass rod. The mixture was continuously extruded into 25ml Erlenmeyer flask containing 25 ml synthetic medium. Then the beads were filtered aseptically and washed successively with sterile buffer solution (pH6.5) and with sterile distilled water15.

**Statistical analysis:** All data were expressed as mean± SEM, where n=6.Data were analysed by One Way ANOVA followed by Dennett's post hoc multiple comparison test using Prism4.0 soft-ware (Graph pad inc., USA)considering $p<0.5$ as significant and 0.01 as highly significant.

**RESULTS AND DISCUSSION**

Different parameters for Calcium alginate bead formation and its stability were studied one after another to maximize gluconic acid production as shown in Table 1-5.

**Table 1: Optimization of CaCl2 for the bead formation (values were expressed as mean±SEM,where n=6.*p0.05 when compare to control)**

| CaCl2(M) | Gluconic acid(mg/ml) | Residual sugar (%) |
|---|---|---|
| 0.1 | 16.3±0.871 | 8.6±0.468 |
| 0.2(control) | 16.8±0.663 | 8.3±0.432 |
| 0.3 | 17.1±0.627 | 8.0±0.461 |
| 0.4 | 17.6±0.861 | 7.7±0.663 |

**Table 2: Optimization of sodium alginate for Calcium alginate bead formation (values were expressed as mean±SEM,where n=6.Changesin the data were statistically non-significant compared to control considering p<0.05 and p<0.01)**

| Sodium alginate(%) | Gluconic acid(mg/ml) | Residual sugar (%) |
|---|---|---|
| 1.0 | 16.8±0.682 | 8.5±0.613 |
| 1.5 | 16.9±0.771 | 8.2±0.462 |
| 2.0 | 17.1±0.671 | 8.0±0.613 |
| 2.5(control) | 17.6±0.663 | 7.6±0.631 |
| 3.0 | 17.2±0.614 | 8.1±0.662 |

| **Table 3: Optimization of bead diameter(values were expressed as mean±SEM, where n=6.*p0.05 when compare to control)** | | |
|---|---|---|
| Bead diameter(mm) | Gluconic acid(mg/ml) | Residual sugar (%) |
| 2.0(control) | 17.6±0.631 | 7.6±0.613 |
| 4.0 | 18.1±0.723 | 7.2±0.663 |
| 6.0 | *18.8±0.667 | 7.0±0.631 |
| 8.0 | 18.3±0.613 | 7.3±0.668 |

| **Table 4: Optimization of storage period of bead (values were expressed as mean±SEM,where n=6.Changesin the data were statistically non-significant compared to control considering p<0.05 and p<0.01)** | | |
|---|---|---|
| Storage period(h) | Gluconic acid(mg/ml) | Residual sugar (%) |
| 0.0(control) | 18.8±0.881 | 7.1±0.448 |
| 24 | 19.6±0.673 | 6.6±0.882 |

| **Table 5: Comparison of gluconic acid production between free and immobilized cells of *Gluconobacter oxydans* GPM60 (values were expressed as mean ±SEM,where n=6.**p<0.01 when compare to control)** | |
|---|---|
| Condition of cells | Gluconic acid(mg/ml) |
| Free | 15.2±o.613 |
| Immobilized in Calcium alginate bead | **19.6±0.732 |

Maximum production of gluconic acid was obtained by the mutant entrapped in calcium alginate beads formed using CaCl2,0.2(M);sodium alginate,2.5%;bead diameter,6mm and 24h storage period. Production was increased significantly (p<0.01) compared to the production obtained by the free cells under the same Physico-Chemical conditions. Thus we can tentatively recommended that, the production of gluconic acid by the mutant can be increased significantly by using calcium alginate immobilization technique.

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