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# SOLID STATE FERMENTATION FOR L-GLUTAMIC ACID PRODUCTION USING *BREVIBACTERIUM* SP.

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**SUMMARY** : Solid state fermentation system was used to cultivate *Brevibacterium* sp. on sugar cane bagasse impregnated with a medium containing glucose, urea, mineral salts and vitamins for producing L-glutamic acid. Maximum yields (~80 mg glutamic acid per g dry bagasse with biomass and substrate - mg/gds) were obtained when bagasse of mixed particle size was moistened at 85-90% mositure level with the medium containing 10% glucose. This is the first report on the cultivation of *Brevibacterium* sp. in solid cultures for production of glutamic acid.

## INTRODUCTION

Solid state fermentation (SSF) carried out on inert support materials, which differs from the process of microbial growth on or in solid particles floating in a liquid medium, has been regarded as one of the future development of the SSF systems (Aidoo *et al.* 1982, Pandey 1992). The use of solid inert material impregnated with suitable liquid media would provide homogenous aerobic conditions throughout the fermenter and the purity of the product would also be comparatively high. Literature survey revealed that a few attempts have been made on SSF processes employing inert supports. Meyrath (1965, 1966) reported high yields of dextrinogenic amylases with *Aspergillus oryzae* on vermiculite impregnated with starch solution. Based on Cahn's method, successful production of citric acid on solid inert materials by *A. niger* were reported by Terui *et al.* (1957) and Lakshminarayana *et al.* (1975). They used saw dust and sugar free cane bagasse, respectively. Auria *et al.* (1990) developed a process using ion exchange resin, Amberlite IRA 900, as inert carrier for *A. niger*. Recently, Kobayashi *et al.* (1991) used polyurethene foam (PUF) as inert support for glucoamylase production by *A. oryzae*. Since the discovery of *Micrococcus glutamicus* by Kinoshita *et al.* (1957), many bacteria have been reported to produce L-glutamic acid directly from sugar in high yields in liquid fermentaion. But to the best of our knowledge, no attempts have so far been made to use amino acid producing bacteria in SSF systems.

In the present paper, we describe our findings on cultivation of *Brevibacterium* sp., grown on cane bagasse as inert support for production of L-glutamic acid which is such first report on the subject.

## MATERIALS and METHODS

*Microorganism and culture media* : A strain of *Brevibacterium* sp. (DSM 20411) was used in the present study. Culture preservation conditions and growth medium were similar as described elsewhere (Nampoothiri and Pandey 1995a,b).

*Inert carrier* : Milled sugar cane bagasse, obtained from a local sugar mill, was sieved to obtained the particles of 0.45-3.00 mm size. After washing it twice with deionized water (using ten times water volume each time) to remove soluble sugars and other soluble constituents adhering to the particles, it was dried in an hot air oven at 60°C for 30 h and stored at room temperature in sealed polythene bags. For each set of the experiment, an adequate amont of the bagasse was autoclaved at 121°C for 30 min.

*Production medium* : The production medium used for impregnating the bagasse contained : glucose 2 g, urea 1 g, $KH_2PO_4$ 0.12, mineral solution 1 ml (prepared by dissolving 1 mg of each of $FeSO_4.7H_2O$, $MnSO_4.4H_2O$, $MgSO_4.7H_2O$, $ZnSO_4.6H_2O$ and NaCl in 100 ml distilled water), thiamine hydrochloride 100 µl, corn steep liquor 0.25 g and two drops of Tween-80 in 100 ml distilled water (pH 7.2). It was sterilized at 115°C for 15 min. In the studies on the effect of glucose concentration in the medium, however, instead of 2%, varying concentrations of glucose, as shown in the relevent text, were used.

*Fermentation* : Fermentation was carried out by taking 2 g of dried bagasse in 250 ml conical flask. This was impregnated with 18 ml of pre-inoculated (20 h old culture of *Brevibacterium* sp.) production medium and mixed well. The flasks were incubated at 30°C for 120 h. Samples, as whole flask in duplicate, were withdrawn after each 24 h. The results shown are the average of four sets of the experiments.

*Extraction of the fermented matter* : Fermented matter (5 g, wet wt) was mixed thoroughly on a magnetic stirrer with 25 ml of distilled water for 30 min and the contents were filtered through ordinary filter paper. The residue was again treated similarly and both the filtrates were combined. The filtrate, so obtained, after centrifugation at 5000 rpm for 10 min was used for various assays as below.

**Analytical methods** : Soluble sugars and proteins were analysed in the filtrate by the methods of Miller (1959) and Lowry *et al.* (1951), respectively. Thin layer chromatography (Silica gel G, solvent mixture - n-butanol:acetic acid:water, 4:1:1, v/v) was used for the qualitative detection of L-glutamic acid (Brenner and Neiderwieser 1967). The filtrate tested only one spot which was identical with authentic sample of L-glutamic acid (similar to our findings in liquid fermentation, Nampoothiri and Pandey 1995a,b,c), hence ninhydrin colour reaction method (Spies 1957) was used for quantitative estimation. The yields of glutamic acid are reported as mg obtained from one g dry fermented substrate (mg/gds). pH measurements were made by a standard pH meter (Systronics, India). Solid pH measurements were made by the method of Moore and Johnson (1967).

## RESULTS and DISCUSSION

Fig 1 shows the glucose consumption pattern by *Brevibacterium* sp. in SSF system. With course of time, there was gradual decrease in the concentration of sugar which was used by the bacterial strain and this indirectly showed the grwoth of the culture. The amount of soluble proteins well correlated with this. We studied some of the important parameters as under.

*Influence of intitial moisture level* : Five different initial moisture levels, as shown in Table 1, were set in the substrate. The total substrate in each flask was always 20 g (wet wt). Although, bacterial grwoth, as observed by glutamic acid production, occurred even at lowest experimental moisture content, i.e. 65-70%, maximum production was observed

Table 1. Influence of initial moisture on glutamic acid production in Solid State Fermentation by *Brevibacterium sp.*

| Initial moisture level (%) | Ratio between moistening agent (ml)& cane bagasse (gm) | Glucose consumption (%) | Glutamic acid (mg/gds) | Protein mg/gds | pH |
|---|---|---|---|---|---|
| 65-70 | 14:6 | 65.70 | 33.30 | 133.21 | 7.88 |
| 70-75 | 15:5 | 66.65 | 42.14 | 200.87 | 8.05 |
| 75-80 | 16:4 | 69.72 | 48.30 | 242.73 | 8.15 |
| 80-85 | 17:3 | 77.20 | 62.69 | 306.82 | 8.21 |
| 85-90 | 18:2 | 78.32 | 75.39 | 354.32 | 8.60 |

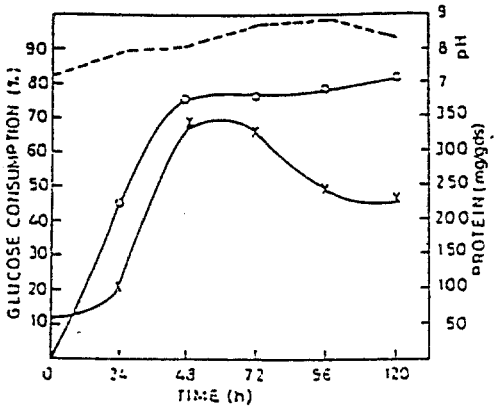


Fig 1 Glucose consumption pattern and pH during glutamic acid prodction in SSF. ---pH, x protein o glucose consumption, %

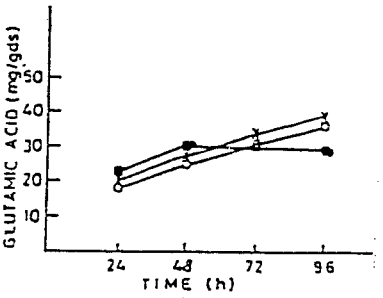


Fig 2 Effect of inoculum size on glutamic acid production in SSF o 0.5%, x 10%, • 20% (all v/w)

Table 2. Effect of particle size of sugar cane bagasse on glutamic acid production in Solid State Fermentation *Brevibacterium sp.*(72h)

| Particle size (mm) | Distribution (%) | Glucose consumption (%) | Glutamic acid (mg/gds) | Proteins mg/gds | pH |
|---|---|---|---|---|---|
| 0.450 | 100 | 69.20 | 29.49 | 72.00 | 7.68 |
| 1.000 | 100 | 73.50 | 43.67 | 114.12 | 8.24 |
| 2.000 | 100 | 70.58 | 30.08 | 72.82 | 8.19 |
| 3.000 | 100 | 65.32 | 27.36 | 81.40 | 8.12 |
| 1 and 0.450 | 50 each | 72.00 | 46.42 | 241.13 | 8.18 |
| 1 and 2.00 | 50 each | 68.73 | 50.10 | 310.58 | 8.20 |
| 0.45, 1.00 2.00 and 3.00 | 25 each | 70.10 | 82.22 | 337.38 | 8.22 |

with 85-90% moisutre substrate in 96 h. By this time, 78% of the glucose was consumed by the bacterial culture and the conversion rate was 29.88% (on the basis of glucose consumed and that 81.74% is the theorical conversion of glucose to L-glutamic acid). There are reports which describe SSF with inert support impregnated with relatively a large quantities of liquid medium of low sugar concentration (Zhu *et al.* 1994). For the homogenous distribution of the nutrients, the moistening agent (production medium) was required in high amounts and so the intitial moisture level too. The amount of moistening agent was shown to influence the physio-chemical properties of the solids (Feniksova *et al.* 1960). At higher moisture contents (90% or more), the support may form clumps which affect bacterial activity adversely.

*Effect of particle size of the substrate* : The particle size, and therefore, specific surface area, of the substrate is of great importance in SSF (Pandey 1991). As shown in Table 2, we used particles of four different individual sizes and three of mixed sizes (see table for composition of substrates with mixed mesh). Maximum glutamic acid production (82.22 mg/gds) was obtained with the substrate containing particles of four different sizes in equal amounts. Among the individual particle size substrate, the best yields (43.67 mg/gds) were resulted by 1 mm particles substrate. Thus, evidently, substrates with mixed particle size are the better choice. With smaller particles, the available surface area for microbial growth is larger but the inter-particle space and hence porosity becomes less. With bigger particles substrate, the situation is reverse. These two opposing factors probably interact together and thus determine the growth and activity of microorganisms (Muniswaran and Charayulu 1994). Our results are also in similar lines. The role of oxygen transfer into void space affects the microbial growth and a compromise has always to be made on composition of substrates with mixed particle sizes for optimal activty and mass transfer effects (Pandey 1991).

*Effect of inoculum size* : Three different inoculum size, viz. 0.5, 2.0 and 4.0 ml (containing $7x10^7$ cells/ml), were used to inoculate 20 g wet substrate. The results are shown in Fig 2. An increase in inoculum size from 2.5% (v/wet wt of substrate) to 10% does not have much impact on the production level, although a slight increase was noted. Increasing the inoculum size to 20%, resulted faster glutamic acid formation during the early phases of fermentation (upto 48 h), but after 96 the yields were less when compared with the two others. The marginal decrease seen with larger inoculum doses could be probably due to shortage of nutrients available for the large number of cells.

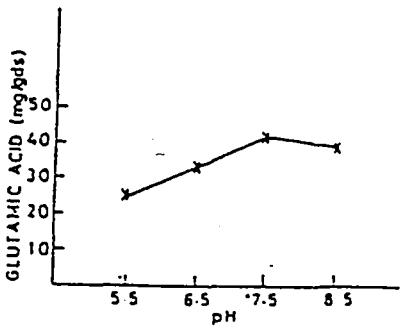


Fig 3 Effect of initial pH on glutamic acid production in SSF (120 h)

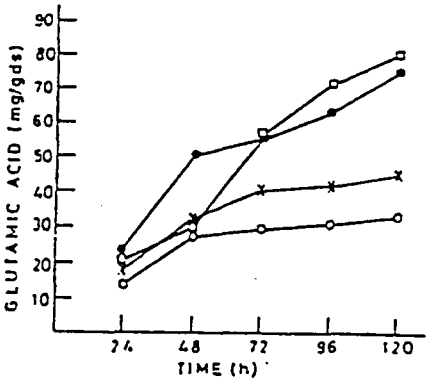


Fig 4 Effect of initial concentration of glucose on glutamic acid productior in SSF. o 1%, x 2%, • 5%, □ 10%

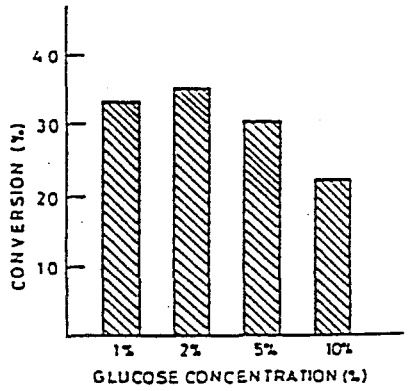


Fig 5 Effect of initial glucose concentration on its conversion to glutamic acid in SSF (120 h) using *Brevibacterium* sp.

*Effect of initial pH* : The pH of the production medium was adjusted to different values (5.5, 6.5, 7.5 and 8.5) before mixing with the bagasse. The results obtained are shown in Fig 3. The maximum production was noted with the substrate having 7.5 initial pH.

*Effect of initial glucose concentration* : It is known that an ideal substrate concentration in any fermentation process results higher conversion efficiencies and optimum substrate utilization (Anderson 1968). Results of our study on the effect of using 1, 2, 5 and 10% glucose in production medium showed maximum glutamic acid with 10% glucose (Fig 4). In this case, glucose consumption was 80% but the conversion efficiency was 22.03%. Maximum conversion (34.75%) was obtained with 2% glucose but this was marked with poor substrate utilization (68.5%). Considering both the factors as crucial, 5% glucose concentration appeared to be the best in which case the bacterial culture consumed 81% substrate with 30% conversion efficiency. In SSF systems utilizing inert supports, adequate availability of energy source (carbon) and nutrients is very crucial. If the carbon source is too less, it affects nutrients uptake due to improper availability resulting from the inadequate distribution and if it is too high, it affects the growth and conversion efficiencies probably by a feedback mechanism.

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