

## Continuous synthesis of alpha amylase by immobilized *Bacillus polymyxa*

C M Mohandass & M Chandrasekaran

Cochin University of Science and Technology, Cochin, India

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Industrially important alpha amylase producing *B. polymyxa* was immobilized in sodium alginate and used in a fluid bed reactor for continuous production of alpha amylase. On continuous operation of the fluidized bed reactor packed with immobilized cell beads at a flow rate of 20 ml/hr with the residence time of 120 min, a maximal enzyme production was recorded (41 units/g of beads; 1 unit = quantity of enzyme required to convert 1 mg of starch at 40°C in 10 min). The half life of immobilized *B. polymyxa* cell beads was estimated to be 24 days, and in continuous production, using minimal substrate solution (1%; 100 ml) maximal enzyme could be produced (204.5 units/g of bead in a total time of 10 hr).

Ability of alpha amylase to cause mild changes in random degradation of starch is of vital importance in baking, brewing, textile and paper manufacturing and starch liquefaction. Amylolytic enzymes are widely distributed in plants, animals and microorganisms<sup>1</sup>. Among them the microbial amylases have drawn the attention of many investigators<sup>2-4</sup>. Among the bacteria, bacillus is acknowledged as producer of amylase for various applications<sup>5-8</sup>. *B. polymyxa* produces alpha amylase besides beta amylase<sup>9</sup>. Alpha amylase production can be increased in many ways, such as batch process, solid state fermentation, etc and one of the modern techniques is immobilization. It has a advantage of continuous reuse. Therefore in the present study an attempt has been made to synthesize alpha amylase continuously using immobilization technique.

*B. polymyxa* (CBTB-25) was isolated from soil environments of Cochin University campus during an earlier investigation (1987-89). It is identified and available as a stock culture in the culture collection of the Center for Biotechnology. The bacterium was cultured in the mineral salt starch medium (MSSM)<sup>10</sup>. Growth of the bacterium was carried out in the Eyela mini fermenter 1.51 capacity at 30°C, pH 7 at 200 rpm for 18 hr. The cells were

harvested by centrifugation at 10,000 rpm for 30 min at 5°C in refrigerated centrifuge (Kubota; 6700 model). Cells were suspended in 20 ml sterile saline. This cell slurry (10<sup>8</sup> cells/ml) was mixed with 40 ml sodium alginate solution (4%.w/v). This was then extruded drop wise through 10 ml syringe from a height of about 10 cm into an excess of 0.2 M CaCl<sub>2</sub> solution, beads of calcium alginate entrapped (3 mm) were maintained in CaCl<sub>2</sub> solution for 30 min. After washing with physiological saline thrice, the beads were used for further studies.

A cylindrical glass column (80 mm diam. 150 mm long) was used as a reactor. The bottom of the column which was hemispherical in shape with 5 mm diam. inlet, was packed with small amount of glass wool and glass beads of 4 mm diam. up to a height of 20 mm. A sieve plate with 1 mm perforations was placed over the glass beads and immobilized beads were packed up to 100 mm height. The column was provided with a side tube of 5 mm diam. at the top section, through which the sample broth was withdrawn. The reactor was fed with 1% soluble starch from the bottom by peristaltic pump (Maclins India Ltd.) and the effluent was removed from the top.

Impact of residence time on the performance of the immobilized cell in a fluid bed reactor on continuous operation for the production of alpha amylase and starch conversion was determined<sup>11</sup> by allowing the substrate solution (1% soluble starch) to pass through the fluid bed reactor at different flow rates. The residence time tested included 16, 20, 40, 53.3, 80 and 120 min during the continuous operation.

Percentage of starch conversion and amylase production increased along with increase in residence time (Table 1). A maximum of 97.7% starch conversion and 40.9 units/g of beads (1 unit = enzyme required to convert 1 mg of starch at 40°C in 10 min) of enzyme production at 120 min of residence time could be obtained at a flow rate of 20 ml/hr. Synthesis of alpha amylase was rapid at flow rates varying from 20-45 ml/hr compared to higher flow rates. Moreover rates of enzyme synthesis declined drastically along with increase in flow rates.

Flow rate of 30 ml/hr showed percentage of starch conversion 59.47, enzyme synthesis (31.46 units/ml) and reducing sugar (2.170 mg/ml). Maximal amylase (204.5 units) could be produced using minimal starch substrates (100 of 1% soluble starch) in 10 hr, at a flow rate of 20 ml/hr (Fig. 1). Total quantity of substrate

\*Present address: Biological Oceanography Division, National Institute of Oceanography, Dona-Paula 403 004, India

Table 1—Continuous synthesis of alpha amylase by immobilized *B. polymyxa*

Flow rate (ml/hr)	Residence time (min)	Starch* converted (in %)	Enzyme* synthesis (in units)	Reduced sugar (mg/g of bead)
150	16	23.30	9.74	0.663
120	20	28.50	11.92	0.810
60	40	35.50	14.92	0.839
45	53.3	52.10	21.78	1.482
30	80	59.47	31.46	2.170
20	120	97.76	40.90	2.783

\*Average of 5 values

1 unit = enzyme required to convert 1 mg of starch at 40°C in 10 min.

$$\text{Starch \%} = \frac{\text{Initial starch} - \text{Final starch concentration}}{\text{Initial starch concentration}} \times 100$$

SE = 0.05%

solution used within the total period of 5 hr of analysis was 157.3 units/g of bead; 108.88, 74.6, 59.59 and 49.7 units of amylase/g bead was obtained using 150, 225, 300, 600 and 750 ml of starch solution in 5 hr at 30, 45, 60 and 150 ml/hr flow rates, respectively.

Half life period of the immobilized beads were computed based on the performance of beads in a fluid bed reactor immobilized cells over a period of 50 days. The initial concentration of starch (81.9%) was reduced to half of its life (40.8%) during 24 days (Fig. 2). Although a sharp decline in the activity was shown by the beads during the initial 10 days (81.9-55%) in the later period of study the performance of the cells declined in a regular fashion.

Compared to free cells, immobilized cell process can also be operated in a batch mode; though it is commercial to use them in continuous process<sup>12</sup>. Recently Jamuna and Ramakrishna<sup>13</sup> discussed continuous synthesis of amylase with reference to dilution rate, maximum amylase activity at low dilution rate gradually decreased with increasing dilution rate. Whereas in the present study maximal enzyme synthesis (41.2 units/g) along with maximal starch conversion (98.1%) could be recorded at a flow rate of 20 ml/hr i.e., at low rate (0.5) of dilution. The productivity of amylase declined with the increase in flow rate and dilution rate. In the present study *B. polymyxa* showed a progressive decline in the productivity along with increase in dilution rate.

An interesting observation made in the study was that using minimal starch solution (100 ml) maximal enzyme (204.5) could be produced under immobilized state on continuous operation.

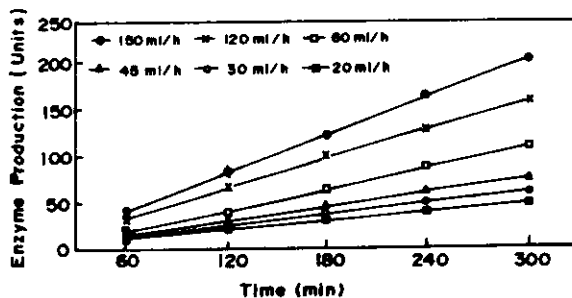


Fig. 1—Continuous synthesis of alpha amylase cumulative (total) production

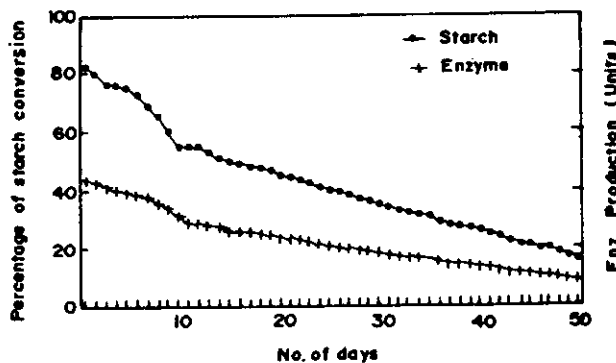


Fig. 2—Half life period of immobilized cells

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