生物工程学报 1(3): 1—7, 1985
Chinese journal of Biotechnology

PARTICLE FORMATION OF IMMOBILIZED GLUCOSE ISOMERASE

Y.C.Jao

(Department of Bioprocess Technology Biotechnology Group Miles Laboratories,Inc.)

INTRODUCTION

The major commercially available immobilized glucose isomerase (IGI) products are represented by Sweetzyme$_R$ (*Bacillus coagulans*) of Novo Industri A/S, Maxazyme$_R$ GI-Immob(*Actinoplanes missouriensis*)of Gist-Brocades N.V.,Take-Sweet$_R$ (*Streptomyces olivaceous*) (no longer available)and Taka-Sweet$_R$ F (*Flavobacterium arborescens*) of Miles Laboratories,Inc., Ketozyme$_R$ (*Actinoplanes missouriensis*) of Universal Oil Products, Inc.and Optisweet 22 (*Streptomyces rubiginosus*) of Kali-Chemie.The methodology used for manufacturing of each product is quite different. The first three products are immobilized using whole cell or cell debris coupled with particle formation technique (Amotz et al., 1976; Outtrup, 1976; Van Velzen,1974; Chen and Jao, 1981), while the others are derived from refined and soluble enzyme covalently bound to chemically modified matrices such as inorganic oxide (Jackson and Yoshiba, 1980; Teague and Huebner,1982). Particle formation of the first three products will be discussed.

PARTICLE FORMATION

Novo Industri A/S Process

The simplified manufacturing scheme of Sweetzyme$_R$ presented by Amotz et al. (1976) indicated that a fermented culture broth was centrifuged to a biomass slurry followed by either flocculation or homogenization, and then cross-linked with glutaraldehyde.The coherent gel was dewatered and shaped into a particulate form with a final moisture of about 12%. Size distribution of commercially available Sweetzyme$_R$ type Q is in the range of 0.25 to 1.10 mm with the majority being from 0.50 to 0.90 mm. The bulk density is about 66 g/100 cc. Morphological observation indicated that the particle extrusion was apparently followed by either downsizing on the die face before drying, or by grinding after drying (Hemmingsen, 1979). It is also possible that the extrudates are downsized before drying by a brief spheronization process (Bucke,1977).

Gist-Brocades N.V.Process

Immobilization and Particle formation of Maxazyme$_R$ GI-Immob have been reported quite extensively (Van Velzen,1974; Hupkes and Van Tilburg, 1976;Hupkes,1978;and Proefschrift and Van Tilburg,1983). The process is as follows.Fermented beer is concentrated to a crude enzyme-containing mycelium and mixed with gelatin-water mixture above 40℃ to a final gelatin concentration of 8% (w/v).The mixture is spray-chilled into a cold,water-immiscible solvent such as n-butanol or butyl acetate for particle formation. Particles so collected are washed repeatedly with water-miscible solvent such as ethanol. The solvent is then removed by filtration or suction. The particles so obtained are then suspended in water and cross-linked with glutaraldehyde at a concentration of 2.5% (v/v) to develop the desirable consistency and hardness. The excess glutaraldehyde and soluble impurities are washed out with tap water. The product is then transferred to a 25% (v/v) aqueous solution of propylene glycol, parahydroxybenzoic acid or formaldehyde, and drained before shipment.The bulk density of the product is about 70 g/100 cc. Particle size falls in a wide range with an average of 1.5 mm.

Miles Laboratories,Inc.Process

Since 1974, Miles has introduced Taka-Sweet$_R$ and Taka-Sweet$_R$ F to the wet milling industry. A process scheme diagram is presented in Figure 1 covering the outlines for both products.

Taka-Sweet$_R$

The bacterial ceell aggregates used as the starting material in making particles can be produced by contacting the fermented broth with the cross-linking reagents mixture of glutaraldehyde and epihalohydrin polyamine copolymer at pH 8 to 9 at room temperature for 0.5 to 1.5 hours.The resulting bacterial cell aggregate slurry is processed through a rotary vacuum filter resulted in a filter cake with a moisture content of 70 to 75%. The moist cake is then extruded through die with opening diameter of 3.18 mm followed by drying, milling and sieving. The physical toughness of the particles is improved by recycling down-sized dry fines to the filter cake prior to extrusion (Chen and Jao, 1981). The final product size ranges from 0.71 to 1.00 mm with a bulk density of 56 g/100 cc.

Taka-Sweet$_R$ F

Biomass from a fermented broth is concentrated and fixed with a cross-

linking system consisting of polyethyleneimine, chitosan and glutaraldehyde. The coherent gel slurry is then filtered to a moisture about 72%, followed by preconditioning and extruding the filter cake onto a spheronizing machine through openings with diameter approximately that of the spherically shaped cell aggregates to be produced. The discharged spheres are dried in a fluidized bed dryer and classified. Compared with the old Taka-Sweet$_R$, the new Taka-Sweet$_R$ shows a better size and morphological uniformity, better physical toughness, and less friability. product particle size ranges from 0.30 mm to 0.85mm. Bulk density of the product is about 70 g/100 ml.

PHYSICAL PROPERTY EVALUATION

Various types of bio-reactors are available for immobilized glucose isomerase to convert dextrose syrup into a high fructose syrup. However, the down flow fixed bed reactor has been overwhelmingly utilized through the industry. Study on mathematical modeling employed to translate laboratory and pilot plant data into a production-scale operation is extensive (Hupkes, 1978; Proefschrift and Van Tilburg, 1983; Verhoff and Furjanic, 1983; Bucholz and Godelmann, 1979). Evaluation of physical parameters on the immobilized enzyme is essential to validate the theoretical model. Following parameters of Taka-Sweet$_R$ were evaluated with the collaboration of the Soil Mechanics department of Ohio State University (Goldstein et al., 1979). Void volume (ε): 42—49%; static friction against stainless steel (μ_s): 0.44; sliding friction (μ'): 0.16—0.17; coefficient of internal friction (K'): 0.5—0.6; and compressibility (α): 0.28—0.30. Some major enzyme particles testing method is presented in the following section.

Mechanical Toughness Test

This testing procedure was described in a great detail by Chen and Jao (1981). Immobilized glucose isomerase is hydrated using 40—45% (w/w) dextrose solution at pH 8.0 and room temperature for one hour before testing. The apparatus depicted in Figure.2 shows an Instron Universal Tester Model 1102 with a stainless steel cylindrical plunger of 4.3 cm diameter and 13.6 cm length attached to a 50 kg load cell for an axial compression. The lower part of the apparatus is a sample cell with an inside diameter of 4.37 cm and a height of 21.75 cm. A spinnerette with 14500 holes of 0.2 mm opening diameter is located on the bottom of the cell to support the sample and to drain the syrup during compression. Hydrated enzyme sample is charged to the container to a height of 11 cm. The plunger is manually moved down

to slightly touch the sample surface, followed by an automatic downward movement for one inch and withdrawal. This movement is repeated for a second compression in one minute interval. The resistant force and plunger travel distance of the second compression is correlated to a quadratic equation which is integrated from O to one inch for the area representing a work value as sample toughness in the unit of kg-in.

A modified system with a plunger of 1.58 cm diameter and a sample cell of 1.75 cm diameter and 3.5 cm height requires only two to three grams sample with only one compression. Table I presents a computer analysis on data obtained from a Taka-Sweet$_R$ F sample. From the small cell testing system, Sweetzyme$_R$ type Q shows a toughness of 2.56 kg-in. A linear relationship between the roughness of Take-Sweet$_R$ obtained from the large cell and the small cell is shown in Figure 3.

Hydraulic Test

Application of fluid dynamics from a compressible packed bed to an immobilized glucose isomerase reactor were extensively studied (Zittan et al., 1979; Verhoff and Furjanic, 1983), A research apparatus in the laboratory was febricateb for measuring pressure drop across the enzyme bed; enzyme bed height; and, syrup flow rate through the bed. The correlations of these parameters were used to predict the potential hydraulic behavior of the enzyme bed. Zitton et al. (1979) presented a device which can be used to examine the pressure effect of both fluid and dead load on the packed enzyme bed. Verhoff and Furjanic (1983) presented a bench device used for testing Taka-Sweet$_R$. A pilot plant 18 inch enzyme column used for studying the hydrodynamic behavior of the enzyme bed is shown in Figure 4 (Goldstein et al., 1979).

Hydraulic behavior of an enzyme bed reactor is expressed as a function of physical parameters previously mentioned as well as substrate viscosity and specific gravity (Furjanic and Verhoff, 1983). Enzyme bed hydrodynamics and enzyme productivity evaluated from pilot scale are criteria used for introducing the product to the market. The relationship of pressure drop and superficial velocity from an 18 inch Taka-Sweet$_R$ F column is shown in Figure 5. The hydraulic test data was collected after the enzyme column produced high-fructose syrup for 28 days. Result from this test revealed that the pressure drop through the bed is 3.4 psi at a syrup superficial velocity of 23 cm/min. The pressure drop profile became repetitive when superficial velocity of syrup through the bed was varied with up and down pattern.

This hydraulic phenomenon indicates the enzyme bed was compressed initially by the hydraulic pressure and then no longer yielded further to the pressure impact from syrup flow change. Result from data analysis using Verhoff and Furjanic's (1983) model predicts that the pressure drop at a syrup (45% w/w) flow rate of 60 gallons per minute through a 5 feet diameter column with a 7 feet enzyme bed height is 10 psi. Detail of the analysis will be published elsewhere.

Table 1

Instron Testing of GI Mechanical Strength

Instron Crosshead Speed 0.5 inch/min

Crosshead Travel Distance(inch) X	Resistant Force(kg) Y
0.10	0.78
0.20	2.87
0.30	6.60
0.40	12.55
0.50	21.50

Equation Fit for Quadratic

Example: $Y = ax^2 + bx + c$

$a = 114, \quad b = -17.2$

$c = 1.49, \quad Y^2 = 0.9993$

$\text{Work} = \int_0^{0.5} Y dx, \quad \text{Work} = 3.342 \text{ kg-in}$

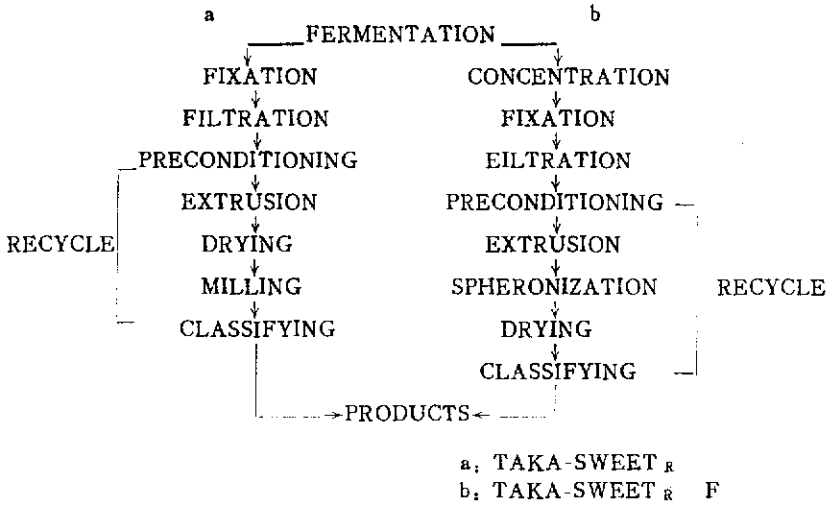

Figure 1. Two processes for particle formation in glucose isomerase

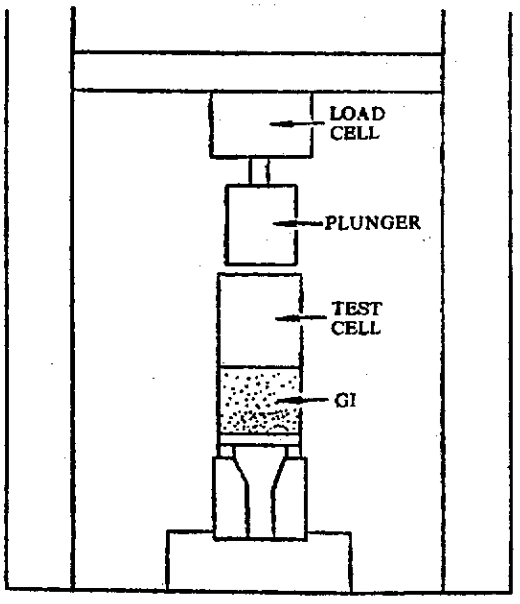

Figure 2. Apparatus for compression testing of immobilized glucose isomerase particles

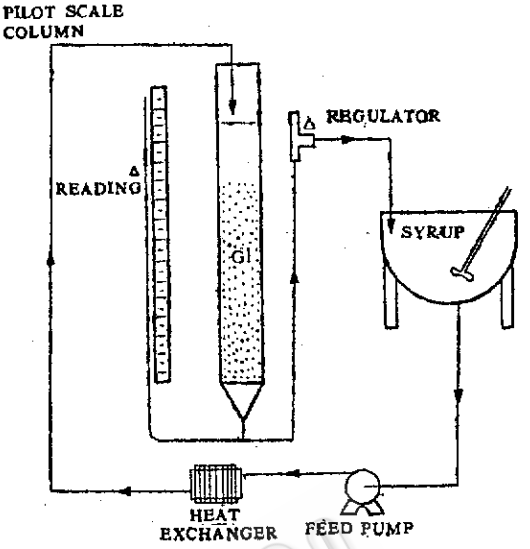

Figure 4. Pilot scale flow test apparatus

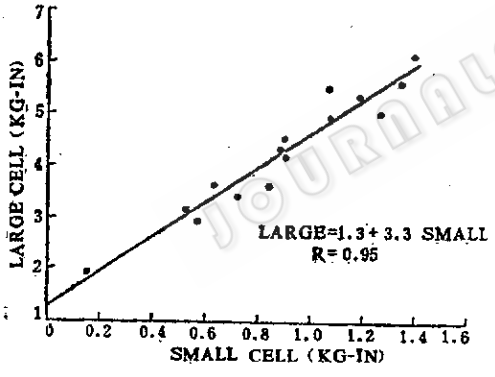

Figure 3. Linear relationship between small and large scale compression tests

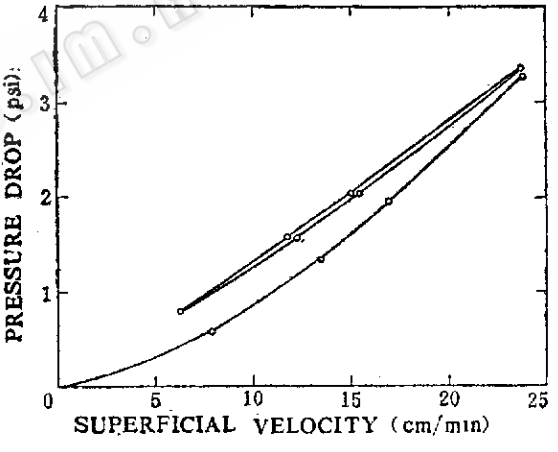

Figure 5. Pressure drop in glucose isomerase bed as a function of flow rate

LITERARY REFERENCES

[1] Amotz,S.,T.K.Nielsen and N.O. Thiesen:Immobilization of Glucose Isomerase, United States Patent 3,980,521,Sept.14,1976.

[2] Bucholz,K.and B.Godelmann: Characterization of Immobilized Biocatalysts, Dechema,84(1979) pp.127—135.

[3] Bucke, C.(1977): Industrial Glucose Isomerase, In A. Wiseman(ed.), Enzyme and Fermentation Biotechnology,Chap. 7 , pp.147—171,Halsted Press, London.

[4] Chen, A.H.and Y.C. Jao: Preparation of a Bacterial Cell Aggregate, United States Patent 4, 251,632,Feb.17,1981.

[5] Goldstein, W. E., A. H. Chen, Y. C. Jao, O. J. Lantero, J.Furjanic, and J.J.Fish (1979): Process Considerations, Kinetic and Stability,Hydraulic Property Testing and Scale-up Studies

for Taka-Sweet$_R$, Research & Development, Biotechnology Group, Miles Laboratories, Inc.
[6] Hemmingsen, S.H. (1979): Development of an Immobilized Glucose Isomerase for Industrial Application. In L.B. Wingard, Jr., E. Hatchalski-Katzir and L. Goldstein (eds.), Applied Biochemistry and Bioengineering, vol. 2, Academic Press, New York, pp.157—183.
[7] Hupkes, J.V.: Practical Process Conditions for the Use of Immobilized Glucose Isomerase, Starch/Starke, 30(1978), pp.24—28.
[8] Hupkes, J.V.and R. Van Tilburg (1976):Production and Properties of an Immobilized Glucose Isomerase, Starke, 28, pp.356—360.
[9] Jackson, D.M., and T. Yoshiba: Method for Purifying Glucose Isomerase and A Composition for Storing Same, U.S. Patent 4,237,231, Dec 2, 1980.
[10] Outtrup, H.: Production of Glucose Isomerase by *Bacillus coagulans*, United States Patent 3,979,261, September 7, 1976, Novo Industri A/S, Bagsvaerd, Denmark.
[11] Proefschrift and R.Van Tilburg, Engineering Aspects of Biocatalysts in Industrial Starch Conversion Technology, Delftse Universitaire Pers, 1983.
[12] Teague, J.R. and Huebner, A.L.: Enhanced Immobilization of a Glucose Isomerase, U.S. Patent 4,337,172, June 27, 1982.
[13] Van Velzen, A. G.: Water-Insoluble Enzyme Composition, United States Patent 3,838,007, Sept. 24, 1974.
[14] Verhoff, F.H., and Furjanic, J.J.:Compressible Packed Bed Fluid Dynamics and Application on a Glucose Isomerase Reactor,I & EC Process Design & Development, 22(1983),pp.192—198.
[15] Zittan, L., Norsker, K.,and Gibson, K.: Starch/Starke, 31(1979), pp.13—16.