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NEW EQUIPMENT FOR THE SCALED UP PRODUCTION OF SMALL SPHERICAL BIOCATALYSTS

U. Matulovic*, D. Rasch and F. Wagner

Institute of Biochemistry and Biotechnology, Technical University Braunschweig, Bültenweg 17, D-3300 Braunschweig, F.R.G.

SUMMARY

A new apparatus with a capacity up to 24 kg per hour for physical entrapment of cell fragments or whole cells in polymeric networks was constructed, based on a high speed rotating nozzle-ring. Immobilized biocatalysts are prepared at laboratory scale with bead size under 1 mm diameter,with a deviation of 10 % or less in size distribution.

INTRODUCTION

The potential for using immobilized cell systems in biocatalysis is well recognized. Physical entrapment of cells or cell fragments in porous polymeric networks is the most widely used immobilization technique (Klein and Wagner, 1983). Favoured are ionotropic networks like alginate or chitosan and gels like carrageenan or agar, prepared as spherical beads because of the simple and mild immobilization procedure (Bucke, 1983). For industrial application an immobilization device should deliver small spherical beads with a high throughput. A disadvantage of large beads (diameter > 1 mm) is their significant mass transport resistance, while a low throughput can lead to a high residence time within the cell-polymer reservoir favouring inactivation of the biocatalyst.

In the present literature the preparation of beads is described by dropping a cell-polymer suspension through a capillary tube into a cross-linking solution. The drop size can be adjusted by a controlled airstream concentric to the nozzle-outlet. Vorlop and Klein (1981) described such an apparatus with a system of 42 outlets and a capacity of 3 - 5 kg per hour biocatalyst with a diameter of 3 mm. In producing smaller beads there is a significant decrease of bead formation capacity . Hulst et al. (1985) developed a mechanical vibration system to form gel beads with 1.1 mm in diameter and a capacity of 24 l biocatalyst per hour. However, this system seems to be practicable only with cell-polymer suspensions of low viscosity, so that in many cases the resulting gel beads show a low mechanical stability. Another method for preparation of alginate beads smaller than 1 mm in diameter (Rehg et al., 1986) uses a two fluid atomizer, but again only polymers of low viscosity can be used. In their experiments the nozzles had a strong tendency to plug up with alginate suspension concentrated higher than 1 % Na-alginate. Efforts to overcome these problems are being made by construction of an apparatus with a high speed rotating nozzle ring for the production of immobilized spherical cell biocatalysts.

MATERIAL AND METHODS

Microorganisms

For all experiments wet cell mass of the strain Pseudomonas sp. DSM 2874 was used, prepared as described previously (Syldatk et al., 1985).

Polymers

Alginate (Manugel DJX) was obtained from Kelco/AIL, Hamburg, F.R.G., Chitosan was purchased from Chugai Boyecki Europe Office, Duesseldorf, F.R.G. and Kappa-Carrageenan (Genugel X 0828) was received from Copenhagen Pectin Factory, Denmark.

Preparation of the cell-polymer suspension

The wet cell mass of was mixed with a 0.9 % NaCl solution (1:1, w:w) and stirred at 4°C to give a homogeneous suspension. This cell suspension was added to different polymer solutions (1:5, w:w) at room temperature, stirred to a homogeneous suspension and filled into the cell-polymer reservoir of the immobilization apparatus.

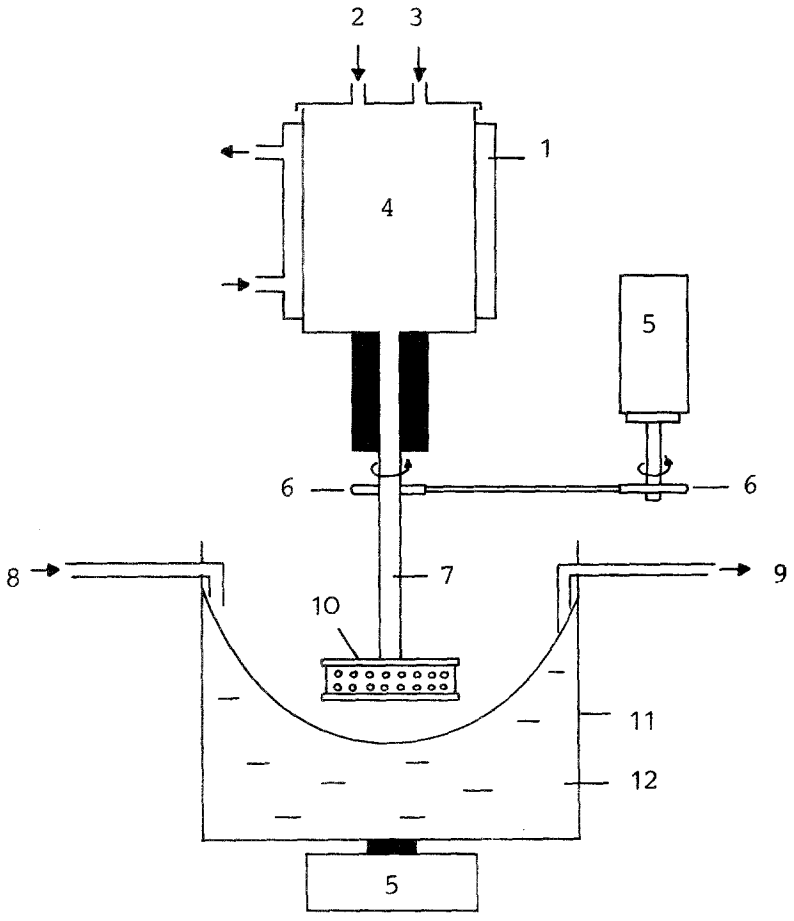

Fig. 1 Schematic diagram of the immobilization equipment: 1 - temperature adjustment; 2 - air pressure; 3 - cell -polymer feeding; 4 - cell-polymer reservoir; 5 - motor with speed governor; 6 - drive pulleys ; 7 - hollow shaft 8 - feed of crosslinking solution; 9 - bead harvesting; 10 - rotating nozzle ring; 11 - rotating vessel; 12 - crosslinking solution.

The whole immobilization equipment (Wagner, 1985) is schematically presented in Fig. 1. The cell polymer suspension in the thermostated storage tank (4) is moved by gravity or compressed air (2) through a revolving vertical hollow shaft

(7) at the nozzle head (10). The rotating nozzle ring calibrates the passing drops to the diameter of its perforations and the drops are centrifuged against a cross linking solution (12) in the rotating vessel (11) which revolves with a speed in the range between 100 to 500 rpm. The immediately formed beads can be harvested continuously (9) while new crosslinking solution will be pumped into the rotating vessel to maintain a constant volume (8) and a residue time in the range of 20 to 120 min.

RESULTS

The results of some experiments for the manufacture of immobilized living cells in Ca-alginate are summarized in table 1. The flexibility of the immobilization equipment is illustrated by the variability of the following parameters:

- Total number of nozzles placed at the nozzle ring
- Diameter of the nozzle
- Revolving speed of the nozzle head
- Controlled overpressure in the cell-polymer reservoir

Table 1: A cell-polymer suspension (viscosity at shear rate of 30 s^{-1} at 25°C of 66 mPas) consisting of cell-suspension and 3 % Na-alginate (1:5, w:w) is filled into the reservoir and projected against a crosslinker container containing a 2 % $CaCl_2$-solution.

number of nozzles	nozzle diameter (mm)	nozzle head (rpm)	pressure (bar)	bead diameter (mm)	capacity (kg/h)
70	1	1400		1	10.0
70	1	1800	-	1	12.0
140	1	1800	-	1	24.0
120	0.5	1400	-	0.5	3.5
120	0.5	1800	-	0.5	4.0
120	0.5	1800	0.5	0.5	18.0

The results of another set of experiments in which Pseudomonas sp. is immobilized in chitosan or kappa-carrageenan are given in table 2 and 3. This process generates cell-biocatalysts with a fairly narrow size distribution. An important attribute of this immobilization method is that it is working also at higher viscosities. Because of the short length of the nozzles (determined by the thickness of the nozzle ring, e.g. in stainless steel , 1 mm) clogging occurs very rarely in comparison to the common apparatus.

Table 2: A cell-polymer suspension(viscosity at a shear rate of 30 s^{-1} at 25°C of 111 mPas) consisting of cell -suspension and 2.7 % chitosan-acetate solution (1:5, w:w) is filled into the reservoir and projected against a crosslinker container containing a 2 % $Na_5P_3O_{10}$ solution.

number of nozzles	nozzle diameter (mm)	nozzle head (rpm)	pressure (bar)	bead diameter (mm)	capacity (kg/h)
70	1	1800	-	1	6.0
140	1	1800	-	1	12.0
70	1	1800	0.5	1	18.0
120	0.5	1800	-	0.5	2.0
120	0.5	1800	0.5	0.5	9.0

The number of nozzles, the revolving speed of the nozzle head and the pressure applied to the cell-polymer suspension in the reservoir can be scaled up, so that an enormous increase of the bead formation capacity can be obtained. Not only the handling of the apparatus but also its scaling-up for production of larger quantities of cell-biocatalysts is very simple.

Table 3: A cell-polymer suspension (viscosity at a shear rate of 30 s^{-1} at 25°C of 65 mPas) consisting of cell -suspension and 4 % kappa-carrageenan solution (1:5, w:w) is filled into the reservoir and projected against a crosslinker container containing a 2 % KCl solution at 4°C.

number of nozzles	nozzle diameter (mm)	nozzle head (rpm)	pressure (bar)	bead diameter (mm)	capacity (kg/h)
70	1	1800	-	1	12.0
140	1	1800	-	1	24.0
120	0.5	1400	-	0.5	3.3
120	0.5	1800	0.5	0.5	17.5

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