IMMOBILIZATION OF MICROBIAL CELLS IN EPOXY CARRIER SYSTEMS

J. Klein*, H. Eng
Institute of Chemical Technology
Braunschweig, Federal Republic Germany

Epoxy polymer networks are obtained by well defined polycondensation reactions, and the characteristics of such materials are their high mechanical strength and chemical stability under very variable conditions. These properties are advantageous for their technological application as carriers for biocatalysts in many different systems.

In screening for a suitable epoxy/curing system we developed some different types of biocatalysts.

A very important advantage of the epoxy/curing reactions we describe is formation of the polymer network at room temperature without formation of any by-products.

Epoxy resins, obtained by polycondensation of a water soluble epoxy precursor and a polyfunctional amine in the presence of a water soluble filler, gave a micro- and macro-porous epoxy membrane (Klein, Hackel, Schara and Eng, H. 1970). The microbial cells were entrapped directly into the epoxy matrix; however, their catalytic activity may be limited by the initial pH, between 9 and 11, used in the polycondensation reaction. In another application this "epoxy disc" was used as support for a surface coating with microbial cells stabilized by entrapment in a polymer film (Eng, H. 1977).

Epoxy resins obtained by polycondensation of water-emulsifiable epoxy- and water-soluble polyaminoamide precursors can usefully be applied under more physiological conditions (Klein, Wagner, Washausen and Eng, 1978; Klein and Wagner, 1979). This epoxy/curing system allows the preparation of epoxy particles and epoxy beads.

Figure 1 Schematic Diagram for the Preparation of Epoxy Particles

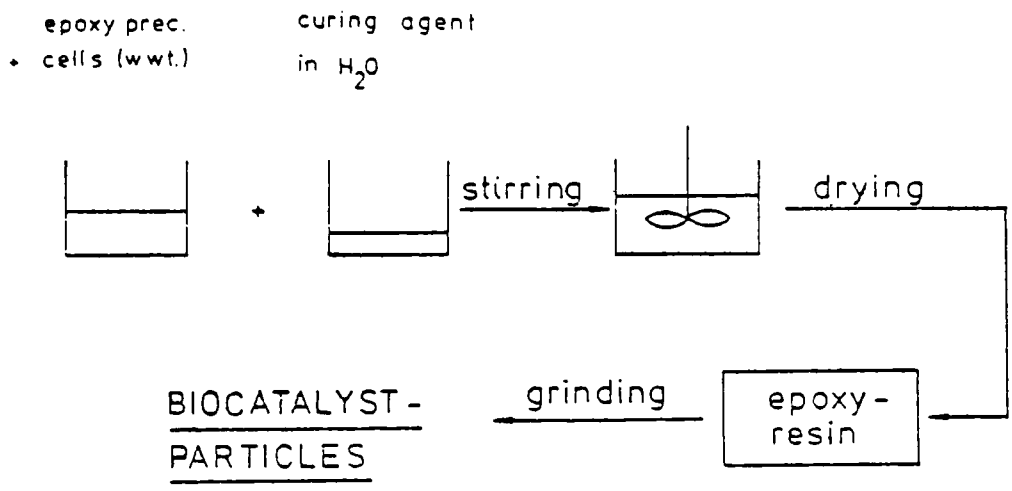


Fig. 1 shows a schematic diagram for preparation of epoxy particles. An example is given as follows:

Mixing 10 g of the epoxy precursor "Epikote DX 255" (Shell) with the wet cells and adding 10 g of the curing agent "Casamide" (Akzo) in 10 ml H_2O under stirring, the bulk condensation is completed after 36 hours. A grinding process then gives irregularly shaped particles of an average diameter of 100 μ. The microporosity of the prepared epoxy polymer network is determined by using a high proportion of cellmass compared to the amount of epoxy/curing agent. The volume concentration of the carrier system during the bulk condensation then causes particles with high cell loading and high enzymatic activity, which is a clear proof of a rather mild immobilization method (see Table 1). The coating of the microbial cells with the epoxy precursor prior to adding the curing agent may be a favourable factor.

For a technical application requiring a fluidized or packed bed reactor, particles with a diameter of 2 - 4 mm are advantageous. As a result of severe diffusion limitation, particles of this size, prepared as above, show 20% the original enzymatic activity of the 100 u particles.

To prepare epoxy beads with sufficient porosity and the required size of 2 - 4 mm, the polycondensation process was combined with the ionotropic gelformation technique (Klein and Vorlop, 1979).

Figure 2 Schematic Diagram for the Preparation of Epoxy Beads

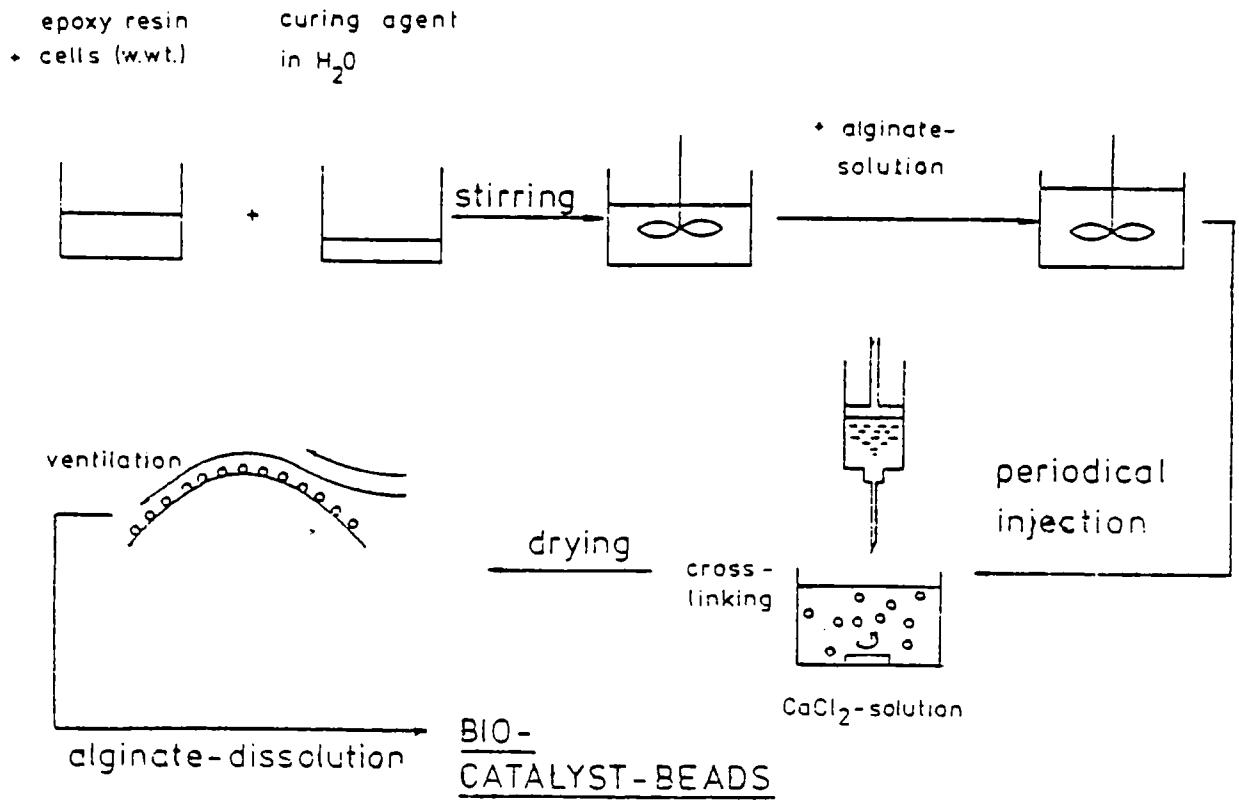


Fig. 2 is a schematic diagram for the preparation of these porous epoxy beads. An example is given as follows:

The first step is the same as in the preparation of epoxy particles. In the next step the epoxy/curing/cell mixture is mixed with a polyelectrolyte e.g. a 30 ml of 8% Na-alginate solution. Periodical injection of this into aqueous 2 wt % $CaCl_2$-solution gives a stabilized drop by crosslinking of the polyelectrolyte at the drop surface. Inside the drop the polycondensation of the epoxy/curing system will take place. The particles are isolated after 40 min. and dried using mild ventilation. After the drying process the polyelectrolyte is redissolved by washing with 0,1 M phosphate buffer. This leaching process leads to the formation of capillary channels throughout the matrix and this additional porosity is very favourable for transport processes. This interpretation is supported by the observation that during the leaching operation the particle diameter increases by about 30% compared to the dry beads. These biocatalysts have good enzymatic activity, as shown by the comparative data of Table 1.

Table 1 Catalytic Acylase Activity of Immobilized E. coli for Epoxy Carrier Systems

GEOMETRY	LOADING G CELLS W.WT/G CATALYST	LOADING VOL %	REL. ACTIVITY %	ABSOL. ACTIVITY U/L CATAL.
RESIN-PARTICLES				
(1) Ø 100 µ	1.24	70	40	4800
(2) Ø 3-4 mm	1.24	70	11	980
(3) POROUS BEADS Ø 3mm	1.13	67	28	2300

In the work shown in Table 1, E. coli cells (acylase activity = 15 U/g wwt. cells) have been immobilized and tested with respect to their acylase activity for cleavage of Penicillin G to form 6 APA (pH 7.8, temp. 37°C). Comparison of (1) and (2) shows the increasing transport limitation with increasing particle diameter, while the comparison of (2) and (3) shows the effect of the improved porosity as discussed above.

The structural modification of the epoxy polymer network by polycondensation of the epoxy/curing system in a polyelectrolyte matrix gives a change in macroscopic material behaviour. The bulk condensation epoxy particles are friable, whereas the epoxy beads are elastic.

Figs. 3 and 4 show some characteristic data for the mechanical strength of the developed porous epoxy beads. The mechanical strength of the catalyst beads was tested in a special testing apparature (Klein and Washausen, 1979). As shown, Fig. 3 demonstrates that the stability of the beads decreases with increasing cell concentration; however even at 75% high stability is still maintained. Fig. 4 shows the relation between curing time and curing temperature required to optimise precursor conversion in formation of the epoxy resin.

Fig. 3 Mechanical Stability of Epoxy Beads

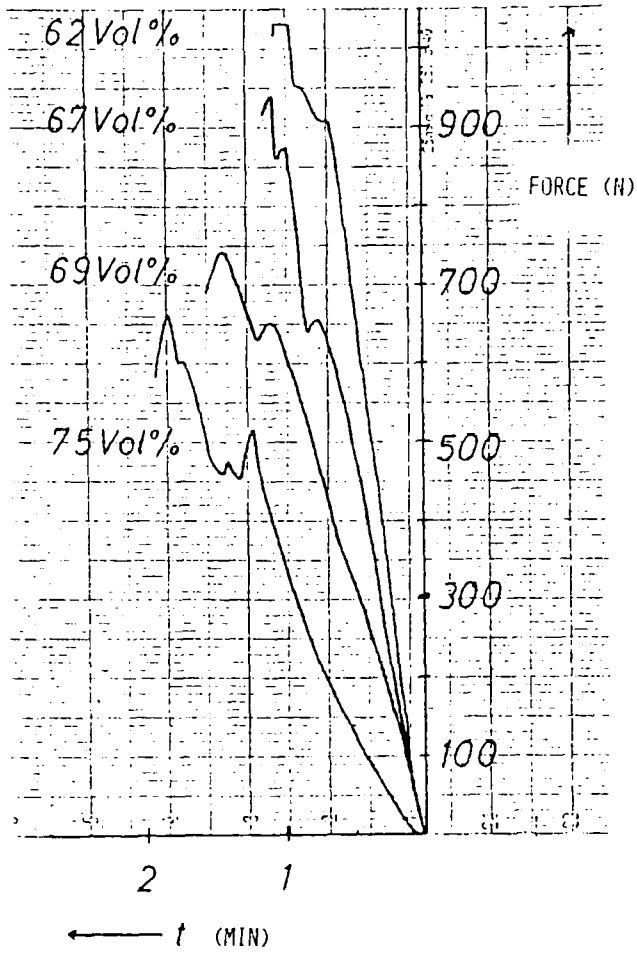


Fig.4 Curing-Time of Epoxy Beads (Loading: 67 Vol %, ø 3mm)

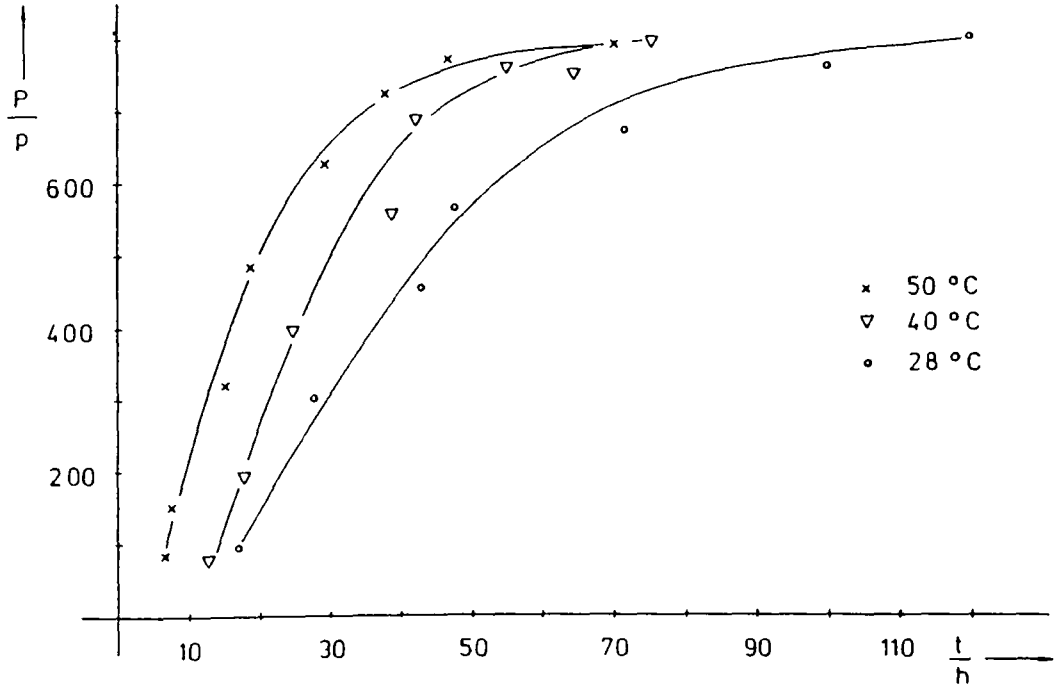
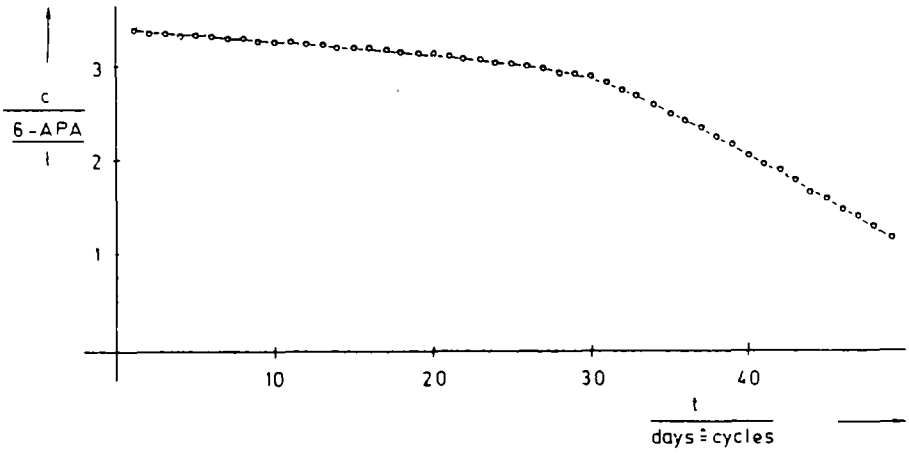


Fig.5 Long-Time-Run with Epoxy Beads



The catalytic properties of porous epoxy beads were also tested in a long-term experiment. A series of repeated batch reactions was performed in a loop configuration using a fluidized-bed reactor (vol. 50 ml), contained 6 ml catalyst; 1 l of 1% Pen-G solution was circulated continuously through the reactor (37°, pH 7,8). The reaction solution was exchanged every 24 hours and the conversion to 6-APA measured. The activity time profile is shown in Fig. 5; the catalytic acylase activity was nearly stable for 30 days.

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