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ENTRAPMENT OF LIVING MICROBIAL CELLS IN COVALENT POLYMERIC NETWORKS: I. PREPARATION AND PROPERTIES OF DIFFERENT NETWORKS

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Different procedures for the formation of polymeric networks to be applied in whole cell entrapment for the preparation of biocatalysts are outlined and compared. These methods include suspension polymerization of water soluble monomers (acrylamide, methacrylamide), water insoluble monomers (methylmethacrylate), and polycondensation of urea and formaldehyde. Special emphasis is given to the detailed description of possible variations in polymer composition and polymerization conditions in relation to the catalytic and mechanical properties of the biocatalyst preparations. The mechanical properties of polymethacrylamide networks can be shown to be significantly improved if compared to conventional polyacrylamide preparations.

INTRODUCTION

Research on immobilization of whole microbial cells has led to an impressive number of contributions to the literature, as has been reviewed recently (1–3). A variety of techniques for immobilization has been developed, from simple flocculation to specific covalent attachment. Entrapment procedures, however, still seem to have the most general applicability, where the term "entrapment" summarizes a variety of different structural and procedural solutions.

In conducting our work, we have been trying to improve the procedure of entrapment in polymer networks with regard to (*a*) particle formation, (*b*) mechanical stability, and (*c*) toxicity. Polyacrylamide (PAAm) preparations so far obtained (4,5) are irregularly shaped and rather weak with regard

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to compression forces. Collagen entrapment (6,7) leads to membrane preparations, which require specific reactor design. Problems in toxicity primarily arise from chemicals like glutardialdehyde used in hardening procedures, or in the choice of organic solvents involved in the processing of polymer/cell mixtures.

Phenol degradation performed by whole cells of *Candida tropicalis* was chosen as a model reaction. The corresponding overall reaction is given as

$$C_6H_5OH + 7O_2 \rightarrow 6CO_2 + 3H_2O$$

while a more detailed description of intermediates and products has been given elsewhere (8). This is an example of a general type of microbial cell catalysis, where a multienzyme system is involved in the reaction pathway; emphasizing the point that such systems provide unique possibilities in biochemical catalysis. It furthermore belongs to the important group of reactions in which oxygen is required as a cosubstrate.

Results of this work have been the subject of two previous short communications by the authors and their coworkers (9,10). It is the purpose of this paper to give a complete report on the procedures of entrapment by polymerization techniques and the properties of the catalyst preparation with regard to the factors (*a*)–(*c*) mentioned above. In a subsequent paper the quantitative evaluation of catalytic performance with regard to diffusional limitations will be outlined.

MATERIALS

Monomers. acrylamide (AAm); methacrylamide (MAAm), from Röhm, Darmstadt; hydroxyethylmethacrylate (HEMA); 2-vinylpyridine (2-VPin), from EGA, Heidenheim; *N*-vinylpyrrolidone (VPon); methylmethacrylate (MMA); *N,N*-2-dimethylaminoethylmethacrylate (DMAEMA); methacrylic acid (MAA); glycidylmethacrylate (GMA), from Röhm, Darmstadt; urea; formaldehyde (37%).

Crosslinkers. *N,N'*methylenebisacrylamide (BisAAm); tetraethyleneglycoldimethacrylate (TEGDMA), from Fluka, Buchs.

Initiator. $(NH_4)_2S_2O_8$.

Coinitiator. tetramethylethylenediamine (TEMED), from Serva, Heidelberg.

Solvents. Dibutylphthalate and water.

Other chemicals. NaCl and phosphate buffer.

Suspension stabilizers. Pluronic L 61 (ethylenoxide-propylenoxide copolymer, Serva, Heidelberg) and Mowiol 18/88 (polyvinylalcohol, Hoechst)

The detergent used is Tween, and the substrate is phenol (99%). If not otherwise mentioned, reagents were purchased from E. Merck, Darmstadt.

Microbial (yeast) cells were *Candida tropicalis*. The centrifuge-wet cell mass with about 80% water was kindly supplied by Prof. F. Wagner, Lehrstuhl für Biotechnologie der Technischen Universität Braunschweig, and was stored in a refrigerator at 4°C for a month.

METHODS FOR POLYMER ENTRAPMENT

This section is intended to outline the general procedures by giving an appropriate example of each type in detail. Variations in compositions and their consequences are given below under Results and Discussion.

Polyacrylamide Preparations

PAAm preparations, required for comparison with the otherwise prepared samples, have been obtained by (*a*) block polymerization, and (*b*) suspension polymerization. Block polymerizations were carried out using variable total monomer concentrations. Highly active gel beads have been obtained starting from an aqueous monomer solution containing 4.5 g acrylamide, 0.5 g bisacrylamide, and 100 mg $(NH_4)_2S_2O_8$ in 35 ml 0.05 M phosphate buffer of pH 7.0. The mixture was deaerated with nitrogen, mixed with a suspension of 1 g wet cells in 9 ml 0.9% NaCl solution and brought to polymerization by adding 0.1 ml TEMED. Gelation occurred 1–3 min after initiation and polymerization was completed in 10–20 min. The resulting gel block was granulated by pressing it through a sieve.

In the case of a suspension polymerization, the polymerizing system was suspended immediately after mixing in 200 ml dibutylphthalate (containing 0.1% Pluronic L 61) under continuous stirring, deaeration and temperature control. Gelation occurred after 2–5 min, and polymerization was complete in 20–30 min. Figure 1 shows a scanning electron microscope picture of a cross-section including immobilized *C. tropicalis* cells.

Polymethacrylamide Preparations

Suspension polymerization of MAAm was carried out in the device shown in Fig. 2. The following mixtures were prepared:

Vessel A: 10 g wet cells of *C. tropicalis* in 5 ml of a 0.9% NaCl solution

Vessel B: 16 g MAAm, 0.5 ml MAAc, 4 g BisAAm, 0.1 ml TEMED, 40 ml of 0.1 M phosphate buffer at pH 9, and 40 ml distilled H_2O

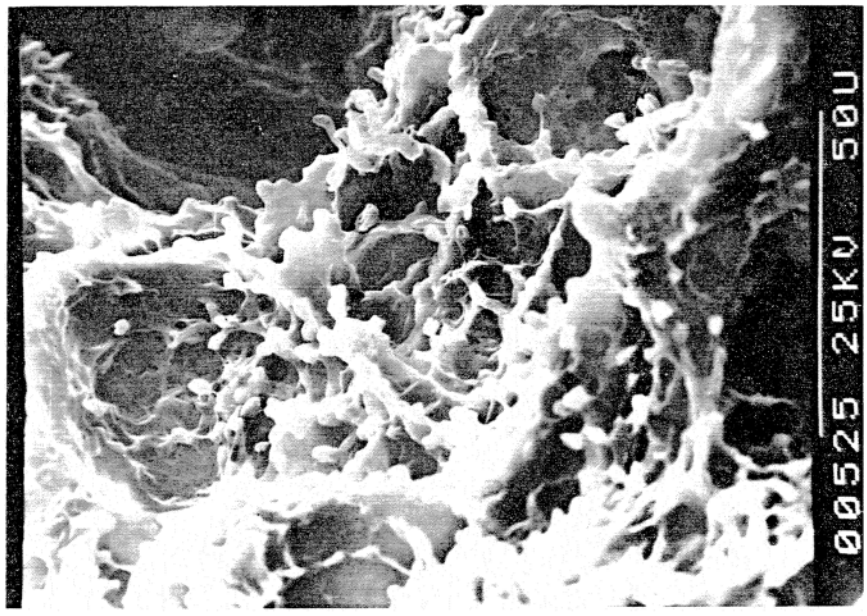

FIG. 1. Scanning electron microscope picture of a cross-section of a PAAm preparation with *C. tropicalis* cells.

Vessel I: 200 mg $(NH_4)_2S_2O_8$ in 2 ml distilled H_2O

Vessel C: 400 ml dibutylphthalate and 0.4 g Pluronic L 61

(Vessels B′ and I′ had same composition as B and I).

After purging the whole system with nitrogen, the content of I′ was added to B′, and gelation due to crosslinking was observed after 10 min. Then I was added to B, and the contents of A added to B after 9 min (thus 1 min before gelation); this mixture was then emptied quickly into vessel C under stirring at 250 rpm. If the temperature in A, B, and C was kept at 0–5°C, about 60–90 min were needed to complete the suspension polymerization. Quicker hardening could be achieved by shutting off the coolant for vessel C after addition of the aqueous phase. Beads from suspension polymerization were separated from dibutylphthalate by filtering off.

Polymer beads of spherical shape could be separated and fractionated with regard to particle size by wet sieving. A typical diameter composition was 0.75–0.5 mm = 51%, 0.5–0.4 mm = 32%, 0.4–0.25 mm = 11%, loss = 6%. All beads, immediately after polymerization, were roughly washed with 0.9% NaCl solution to remove residual solvent and toxic substances. A light microscopic picture of the 0.25–0.4 mm fraction is shown in Fig. 3, while Fig. 4 gives an SEM picture of a cross-section with *C. tropicalis* cells.

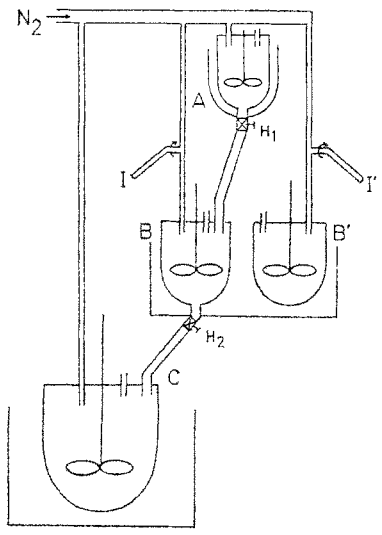

FIG. 2. Experimental device for a step controlled suspension polymerization of methacrylamide/bisacrylamide (see text for detailed description).

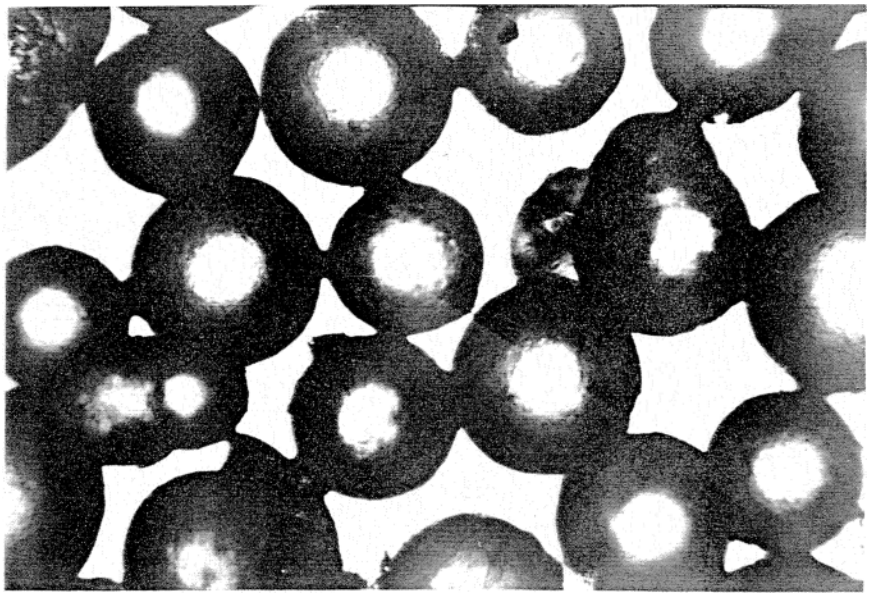

FIG. 3. Light microscopic picture of PMAAm beads obtained by suspension polymerization.

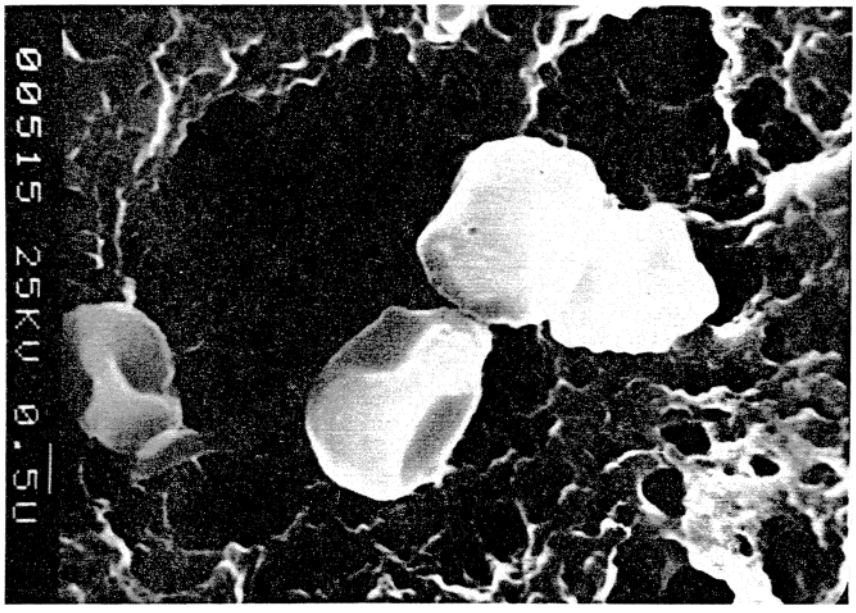

FIG. 4. SEM picture of a cross-section of a PMAAm preparation, showing entrapped *C. tropicalis* cells.

Polymeric Fillers

For filler no. 1 (F1), 20 g AAm, 25 ml AN, and 15 g BisAAm were dissolved in 75 ml H_2O and polymerized under N_2 at 35°C with the addition of 200 mg $(NH_4)_2S_2O_8$ as initiator. The resulting gel block was mechanically disrupted, dried at 95°C, and finally pulverized in a ball mill to obtain sizes of 0.1–10 μm. For filler no. 2 (F2), 10 g BisAAm, dispersed in 90 ml H_2O, was polymerized, using 200 mg $(NH_4)_2S_2O_8$ and 0.5 ml TEMED as initiator.

Polymethylmethacrylate Preparations

Application of water insoluble monomers like MMA requires a double emulsion technique involving two aqueous mixtures (*1*) and (*3*), and one nonaqueous (*2*) mixture, as given below:

1. 2 g wet whole cells of *C. tropicalis* in 10 ml of a 0.9% NaCl solution; gelatine up to 15% may be added to improve cell stability in contact with the organic media
2. 5 ml MMA, 2 ml glycidylmethacrylate, 3 ml TEGDMA, 0.2 g benzoylperoxide, 0.2 ml DMPT, 10 ml dibutylphthalate, 0.2 ml Pluronic L 61, and 0.1 ml Span 80

3. 100 ml of a 0.9% NaCl solution, 0.1 g Mowiol 18/88, and 0.02 g Tween 80

Mixture 1 (above) was suspended into mixture 2 and this suspension was carefully transferred into (3) to maintain the "1 in 2" suspension. Under gentle stirring the polymerization in the organic phase proceeded, transforming the liquid wall 2 around 1 into a solid wall. Due to agglomeration, not only "monocapsule" but also "multicapsule" particles were finally obtained. Particle size varied from 1 to 3 mm. Corresponding light microscopic pictures are shown in Figs. 5 and 6.

Urea-Formaldehyde Preparations

Urea, 41 g (0.68 M), was dissolved in 110 ml (1.36 M) of a 37% formaldehyde solution, and the pH was adjusted to 8.5 with 10% NaOH solution. The mixture was heated for 90 min to 70°C to give the additional product dimethylolurea. This clear solution could be stored for some days at 4°C.

Bulk condensation occurred when the pH was adjusted to 3.5, with a gelation point after 30 min; the wet cell suspension was added to the

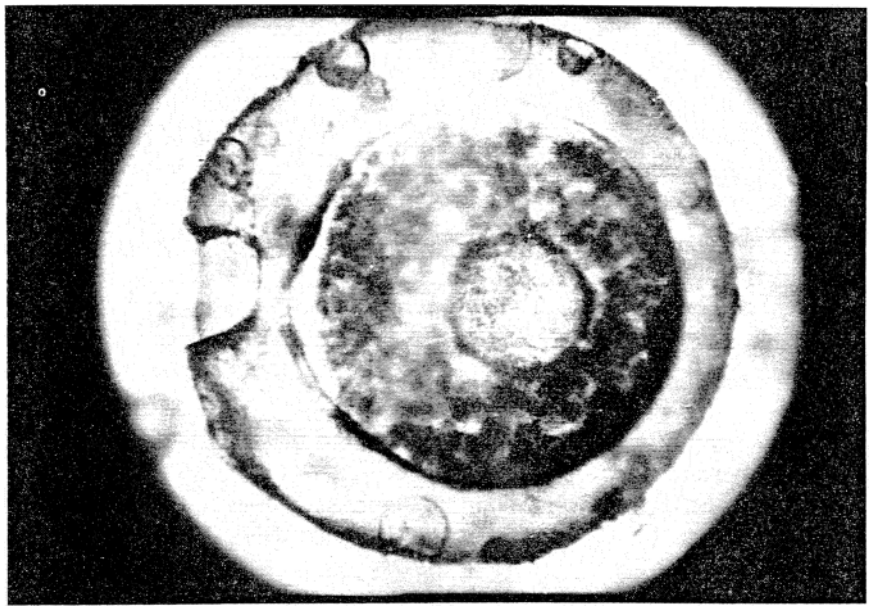

FIG. 5. Light microscopic picture of a PMMA capsule, obtained by reversed suspension polymerization.

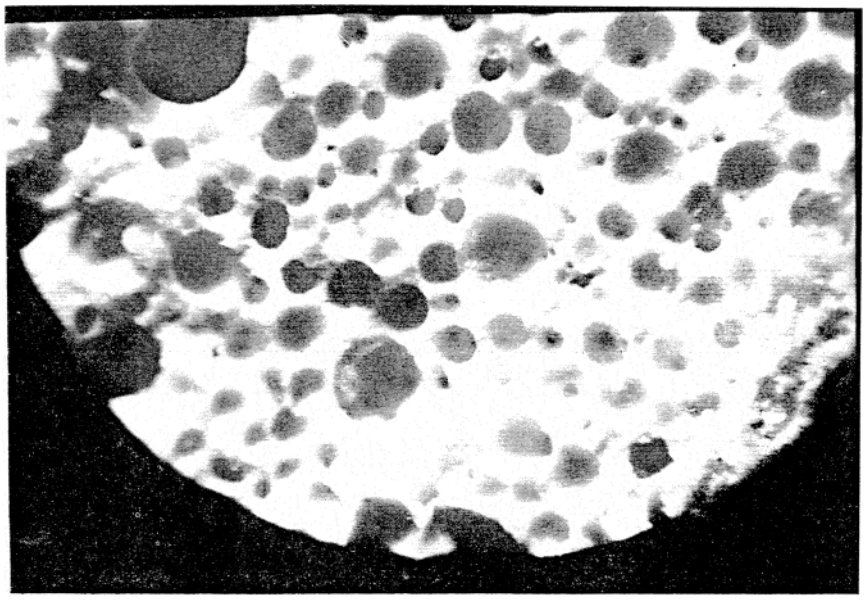

FIG. 6. Light microscopic picture of a multicore PMMA capsule obtained by reversed suspension polymerization.

prepolymer solution shortly before gelation. Irregularly shaped and breakable particles were obtained. A membrane type catalyst was obtained if the dimethylolurea solution of pH 3.5, including suspended cells, was soaked into dry paper with subsequent drying under air for 12 h.

ANALYTICAL PROCEDURES

Mechanical Strength

Critical compression forces (force required to break the gel) of cross-linked polymer gels were obtained, using two different experimental devices: (*a*) Pfizer hardness tester, requiring test cubes of 0.5 cm^3; (*b*) the Stokes Monsanto Hardness Tester, requiring test cubes of 1 cm^3. The area of the piston of both instruments is 0.3 cm^2, so that the pressure can be calculated from an instrumental reading given in kg. Both instruments have been described elsewhere (13).

Pictures

Scanning electron microscope pictures have been obtained, using a Hitachi S-550 instrument. Samples were prepared by freeze-drying at −60°C.

Reaction Rate

Determination of activity data of the immobilized cells was based on the comparison of the phenol consumption rate of free suspended and immobilized cells. Phenol consumption of free cells was independent of phenol concentration in the range 5×10^{-3} to 1×10^{-4} M and of oxygen concentration in the range 5×10^{-4} to 5×10^{-5} M. Phenol consumption of entrapped cells was measured with low cell loaded catalyst particles under controlled oxygen saturation with air to avoid a reaction rate limitation by the oxygen diffusion rate. Phenol concentration was determined by UV photometry at $\lambda = 270$ nm.

RESULTS AND DISCUSSION

It was one intention of this work to explore in a screening type procedure the feasibility of other polyreaction systems besides PAAm for a whole cell entrapment process. Thus information about the catalytic activity of the immobilized cells will be the first criterion for evaluation of the procedures. Second, a more detailed analysis of mechanical properties of PMAAm preparations in comparison with PAAm will be given.

Catalytic Activity

The results of activity tests for phenol degradation with the various polymer-cell preparations are summarized in Table 1. If care is observed with regard to comparability of particle size, cell concentration, and oxygen saturation, the activity yield may, in a first approximation, be a measure of toxicity during immobilization. It becomes obvious that the activities of urea/formaldehyde and PMMA systems are much too low to justify further development.

The urea/formaldehyde system is an example of a *polycondensation* type reaction. Due to the kinetics of chain growth in such a system, much more monomer has been transformed into oligomers and polymers at the point of gelation than in a free radical polymerization process. Thus addition of cells shortly before gelation should lead to less toxicity damage, if the

TABLE 1. **Activity Yields for Phenol Degradation with Immobilized *C. tropicalis* in Different Polymer Structures**

Polymer	Polymer concentration (%w/v)	Cell concentration (g wet w/ml)	Polyreaction temperature (°C)	Activity yield (%)
Free suspended cells				100
PAAm				
(block)	30	0.04	20–25	17–26
(block)	10	0.02	20–25	30–70
(susp.)	10	0.02	0–5	90–100
PMAAm				
(susp.)	20	0.02	20–25	20–30
(susp.)	20	0.02	0–5	40–45
PMMA (microcapsules)	40 (wall)		20–25	2–3
Urea/formaldehyde (paper)			20–25	3

dimethylolurea concentration is the critical parameter. This favorable effect was presumably overcompensated by low amounts of high toxic formaldehyde originating from acidification and the low pH value required to obtain an appropriate reaction rate for condensation.

MMA was selected as an example of a *water nonsoluble monomer* in a free radical polymerization, a system that has not been used so far for immobilization purposes. Since the cell suspension cannot be directly mixed with the polymerizing system, the procedure of encapsulating the aqueous cell suspension in the organic phase was developed. As far as shape and stability of the PMMA beads are concerned, the system, though not easy to handle, obviously works. The drastic loss in catalytic activity can be attributed to three effects:

1. Contact of the cell suspension with the organic phase, and especially here the monomer is highly toxic.
2. Coating of the cell suspension phase with the organic phase may not at all be complete, so part of the cells may leak out into the excess aqueous phase in the second suspension step.
3. To obtain the first emulsion, the volume of the organic phase has to be larger than that of the aqueous cell suspension. This ratio is maintained in the finally obtained capsules, which have a rather small catalytic active volume surrounded by a rather thick polymer wall. Therefore an especially high diffusion barrier has to be considered.

Furthermore, not only singular capsule but also multicapsular conglomerates are obtained as catalyst beads. The latter effect is enhanced by the decreasing ratio of the stirring velocity of the first and second

emulsion steps, and by an increasing concentration of the emulsifying agents in the organic phase. Multicore capsules have thinner walls with the advantage of low diffusion barrier, but with a higher risk of defective capsule production.

Compared to PAAm samples, the PMAAm preparations had a lower activity yield throughout. One reason may be the somewhat higher toxicity of MAAm monomers to *C. tropicalis* cells than observed with AAm monomer. The main reason, however, seems to be the lower polymerization velocity, which results in much longer gelation and polymerization times. Exposure of cells to unreacted monomer is therefore much longer, an effect that is definitely known to be hazardous for remaining enzymatic activity.

Some effort has been made to minimize this cell–monomer contact time (*a*) either by finding better initiation conditions to accelerate the network formation process, or (*b*) by the addition of chain transfer agents to achieve a higher degree of monomer conversion at the point of gelation. Typical examples are summarized in Table 2. It can be seen that DMAEMA is a less effective coinitiator and that a mixture of 1.0% $(NH_4)_2S_2O_8$ and 0.2% TEMED is optimal. Too high a TEMED concentration should be avoided with regard to toxicity, while a higher amount of $(NH_4)_2S_2O_8$ is not critical.

Thioethanol as transfer agent at the acceptable concentration levels has only a negligible influence on the macroscopically observed gelation time. Reports in the literature indicate that the typically observed activity for PAAm entrapped cells is in the order of 60–70%, even for single enzyme activities like aspartase activity in *E. coli* cells (1). The fact that the yield observed by us can be much higher and exceptionally may be even close to 100% has two explanations.

First, temperature obviously plays a very important role with regard to activity yield. In a block polymerization process with AAm, the temperature rise is appreciable and easily exceeds 30°C, a value that is critical for phenol degradation activity in *C. tropicalis*. Thus early attempts at *C. tropicalis* immobilization using the typical block polymerization techniques led to zero activity. *Suspension polymerization* therefore is a convenient step to achieve the necessary temperature control with regard to isothermal conditions and to the desired temperature value itself. As can be seen from Table 1, much higher activities are generally observed if temperature is kept at 0–5°C, compared to 20–25°C. Longer polymerization times, resulting in a prolonged exposure of cells to nonreacted monomer at this temperature is obviously less important than maintenance of enzymatic activity of the cells with regard to temperature alone.

Second, when applying suspension polymerization techniques, the choice of system components becomes critical. Attempts at preparing polyacrylamide pearl catalysts have been undertaken using a mixture of

TABLE 2. **Influence of Initiator, Coinitiator, and Chain Transfer Agent on Gelation Time**

No.	Monomer (1%)	Monomer (2%)	Monomer (3%)	C_2H_5SH (%)	Initiator $(NH_4)_2S_2O_8$	Coinitiator (%)	pH	T (°C)	Gelation (min)
1	MAAm, 15.6	Bis, 4.0	MAA, 0.4		0.4%	TEMED, 0.2	8	20	10
2	,,	,,	,,		1.0	TEMED, 0.2	,,	20	8
3	,,	,,	,,		1.0	DMAEMA, 0.2	,,	,,	30
4	,,	,,	,,		,,	,, 0.4	,,	,,	17
5	,,	,,	,,		,,	,, 1.0	,,	,,	9
6	,,	,,	,,	0.02	,,	TEMED, 0.2	,,	,,	8
7	,,	,,	,,	0.04	,,	,, ,,	,,	,,	10
8	,,	,,	,,	0.08	,,	,, ,,	,,	,,	10
9	,,	,,	,,	0.20	,,	,, ,,	,,	,,	10
10	,,	,,	,,		0.2	,, 0.1	,,	0–5	45–60
11	,,	,,	,,	0.10	,,	,, ,,	,,	0–5	60–80

toluene/chloroform in an enzyme fixation procedure (11) and with mineral oil in the entrapment of microorganisms (12). Low molecular weight organic solvent mixtures like toluene-chloroform were disregarded because of the toxicity of these compounds. Mineral oil seemed to be the better choice; however, only irregularly shaped particles with a high degree of abrasion could be obtained with AAm and MAAm monomers. Addition of different suspension stabilizing agents (butanol, Span 80, Pluronic L 61) did not improve this system. Finally, dibutylphthalate was found to be advantageous: it is practically nontoxic; its density is close to the density of the monomer solution, thus avoiding any flotation or sedimentation problem during suspension polymerization, and addition of 0.1% Pluronic L 61 (ethylene-oxide–propylene-oxide copolymer) leads to the formation of abrasion resistant spherical beads.

Mechanical Network Properties

Only those preparations remain to be discussed that are obtained starting from water soluble monomers. A comparison of a larger variety of systems is given in Table 3, which provides information about monomer composition, polymerization conditions, and times for crosslinking, as well as some qualitative description of the network structure.

The overall monomer concentration (monomer, comonomer, plus crosslinking agent) was kept constant in these experiments at 20% (w/v), as was the initiator system [0.2% (w/v) $(NH_4)_2S_2O_8$ plus 0.2% (w/v) TEMED] and the polymerization temperature of 25°C. The visual classification "white" and "transparent" distinguishes between networks of inhomogeneous (macroporous) and homogeneous (microporous, gel type) structure, respectively.

It can be seen from Table 3 that network formation from HEMA, HPMA, and 2-V-Pin is rather slow and precipitation of polymer occurs before the gelation point is reached. Therefore only inhomogeneous networks of poor rigidity are obtained. VPone and DMAEMA are fast polymerizing monomers and in this respect comparable to AAm. The rigidity of these networks was again very low, the beads very easily deforming and breaking apart.

MAAm appeared to be the only monomer that definitely gave improved network properties. This effect can be observed to some extent if MAAm is used as a comonomer for AAm; however, systems with MAAm as the main component are superior. Addition of ionic comonomers like MAA, which it was thought would be useful to achieve a variation in partition or transport properties of the network, had only a minor effect, resulting in less compressible networks with slightly reduced pressure

TABLE 3. **Network Properties and Preparation from Water Soluble Monomers**[a]

No.	Monomers (%, w/w)		Bisacrylamide (Bis)	Monomer conc./ crosslinker fraction (%)	pH	Gel time (min)	Facing of network	Hardness	Remarks
1	HEMA, 18		2	20/10	7	60	White	Very low	Precipitation of Polymer
2	HPMA,[b] 18		2	,,	7	60	White	Very low	,,
3	DMAEMA, 18		2	,,	9	5	White	Very low	
4	AAm, 18		2	,,	7	2	White	Low	Deformable elastic gel
5	AAm, 14	MAA, 4	2	,,	4[c]	–[c]	Transparent	Medium	Nonelastic, rigid but very pressure sensitive
6	AAm, 14	DMAEMA, 4	2	,,	9	5	Opaque	Medium	,,
7	AAm, 14	AN,[b] 4	2	,,	7	2	White	Low	Very elastic + deformable
8	MAAm, 18		2	,,	7	20	White	Very strong	Rigid hard network
9	VPon, 16		4	20/20	7	5	White	Very low	Deformable weak gel
10	2-VPin, 16		4	,,	9		White	Very low	Very deformable, very heterogeneous gel

11	AAm, 16		4	,,	7	4	White	Low	Deformable, heterogeneous gel
12	AAm, 12	VPon, 4	4	,,	7	5	White	Medium	
13	AAm, 8	MAAm, 6	4	,,	7	13		Strong	Not very deformable
14	AAm, 12	MAA, 4	4	,,	6[c]	30[c]		Strong	
15	AAm, 12	DMAEMA, 4	4	,,	9	1		Medium	Rigid, but pressure sensitive gel
16	MAAm, 16		4	20/20	7	30	White	Very strong	Heterogeneous, rigid network
17	MAAm, 12	DMAEMA, 4	4	,,	5[c]	60[c]	Opaque	Low	
18	MAAm, 12	MAA, 4	4	,,	7	30	Clear	Very strong	Very rigid, but pressure sensitive
19	MAAm, 10	MAA, 2	8	20/40	4[c]	90[c]	White	Very strong	Very rigid
20	AAm, 10	MAA, 2	8	20/40	6	14	Opaque	Low	

[a] Initiator: 0.2% (w/w)$(NH_4)_2S_2O_8$; 0.2% (w/w) TEMED.
[b] Abbreviation not defined in text: HPMA, hydroxypropylmethacrylate; AN, acrylonitrile. For remaining definition, see text under Materials.
[c] Long gelation time because of low pH (inactivation of TEMED by protonation).

TABLE 4. **Critical Pressure Stability of Hydrophilic Networks**[a]

No.	Monomers (%, w/w)		Bis	M/C	Filler	(%)	Vp	Rigidity	Pfizer Hardness Tester			Monsanto Hardness Tester		
									n	$\bar{p}$ (kg)	SD	n	$\bar{p}$ (kg)	SD
1	AAm, 14.4		0.8	15/7			15	Poor	3	5.37	0.15	4	2.03	0.13
2	,, 14.4		0.8	15/7	F2	10	25	Good	6	7.80	0.57	6	4.75	0.94
3	,, 18.8		1.2	20/6			20	Medium	2	5.30	0.14	6	2.13	0.29
4	,, 18.0		2.0	20/10			20	Poor	4	6.72	0.61			
5	,, 16.0		4.0	20/20			20	Poor	8	6.26	0.42			
6	,, 24.0		6.0	30/20			30	Good				2	4.00	0.71
7	MAAm, 12.8		2.3	15/15			15	Poor	3	6.53	0.23	4	3.0	0.0
8	,, 17.0		3.0	20/15			20	Good	4	8.45	0.25	5	5.14	0.34
9	,, 16.0		4.0	20/20			20	Good	14	10.64	1.20	2	7.50	0.71
10	,, 15.6	MAA, 0.4	4.0	20/20			20	Very good	13	10.28	0.79	2	6.00	0
11	,, 15.2	MAA, 0.8	4.0	20/20			20	Very good	10	9.75	1.18	2	4.90	0.14
12	,, 14.0	MAA, 2.0	4.0	20/20			20	Very good	6	7.07	0.48			
13	,, 24.0		4.0	28/14			28	Very good	10	10.90	1.39	2	8.5	0
14	,, 15.6	MAA, 0.4	4.0	20/20	F1	24	44	Very good	8	14.18	0.77	3	15.4	0.79

[a] Initiator concentration: 0.4% (w/w) $(NH_4)_2S_2O_8$; 0.4% TEMED. n = number of measurements; SD = standard deviation; $\bar{p}$ = average pressure.

stability. The higher values in pressure stability of the PMAAm networks can be related to the more hydrophobic nature of the α-methyl-substituted monomer when compared to AAm. Linear chain molecules of PMAAm become insoluble in water at higher molecular weights, and correspondingly, an increasing number of polymer segment interaction points in the network polymer will definitely improve its mechanical properties. A quantitative comparison of the mechanical properties with regard to stability against compression forces is given in Table 4.

A series of block polymerizations had to be carried out to obtain cubic samples ($0.5 \times 0.5 \times 0.5$ cm in the case of the Pfizer Hardness Tester; $1 \times 1 \times 1$ cm in the case of the Monsanto Hardness Tester) and the average forces $\bar{p}$ result from a number n of pressure data, which can be read from the instrument at the point of gel disruption. Since both instruments have different structures, no direct cross-correlation is possible. However, the relative magnitude for different preparations in each line has a quantitative meaning.

All PAAm samples have smaller numbers than the PMAAm samples: This is exemplified by comparing samples 5 and 9 in Table 4. Addition of polymeric solids as "filler" (see samples 2 and 14) obviously improves the network stability; however, the sample 2 composite is still less stable than samples 8 or 9, which consist of PMAAm alone. Comparing the PMAAm samples 7–9 and 13, the 20% monomer with an 80/20 ratio of MAAm to BisAAm is optimal: decrease of monomer concentration gives lower stability, while increase of monomer concentration does not improve the stability to such an extent that increasing transport resistance for substrate diffusion would be justified. Addition of comonomers like MAA shows lower stability values in the PMAAm series, although still higher than the PAAm samples. Thus a limited amount may be added without lowering the superiority of the PMAAm based systems.

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