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BIOTECHNOLOGY & BIOENGINEERING

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Cell Immobilization Using PVA Crosslinked with Boric Acid

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A new cell immobilization technique is described in which polyvinyl alcohol is crosslinked with boric acid, with the addition of a small amount of calcium alginate. The presence of the calcium alginate improves the surface properties of the beads, preventing agglomeration. A pure culture of phenol-degrading *Pseudomonas* was immobilized in the PVA-alginate beads. Phenol was successfully degraded in a fluidized bed of the beads, indicating that cell viability was maintained following the immobilization procedure. The PVA-alginate beads proved to be very strong and durable, with no noticeable degradation of the beads after 2 weeks of continuous operation of the fluidized bed.
Key words: immobilized cell • PVA • polyvinyl alcohol • fluidized bed

INTRODUCTION

Immobilization of living cells has become an established technique for increasing the productivity of biochemical engineering processes. One of the most widely used techniques for cell immobilization is cell entrapment, in which the living cells are enclosed in a polymeric matrix which is porous enough to allow the diffusion of substrates to the cells and of products away from the cells. Materials which have been successfully used for cell entrapment include agar, agarose, kappa-carragennan, collagen, alginates, chitosan, polyacrylamide, polyurethane, and cellulose.[9] However, each of these polymers has drawbacks, such as poor mechanical strength and durability (agar, agarose, kappa-carragennan, collagen, alginates, chitosan), toxicity to microorganisms (polyacrylamide, polyurethane), or high cost.[1,5]

Recently, the use of polyvinyl alcohol (PVA) for cell immobilization has been investigated. Ariga et al.[1] used the technique of iterative freezing and thawing of PVA to form a gel suitable for cell immobilization. They found that this technique produced a low-cost material with a rubber-like elasticity and high strength. Hashimoto and Furukawa[3] used a simpler and less energy-intensive

duced by other cell immobilization techniques such as polyacrylamide and calcium alginate.[1]

The PVA-boric acid technique provides an easy and low-cost method of cell immobilization, producing elastic beads of high strength and durability.[3] There are two potential problems with this technique, however. The saturated boric acid solution used to crosslink the PVA is highly acidic (pH of approximately 4) and thus could cause difficulty in maintaining cell viability. Hashimoto and Furukawa[3] hypothesized that activated sludge could be successfully immobilized without loss of biological activity due to the presence of extracellular polymer in the sludge, which enables the microorganisms to endure changes in cultivation conditions, and cast doubt on whether the technique could be applicable for other types of cells. To date, the use of the PVA-boric acid immobilization technique for other types of microorganisms has not been demonstrated. In addition, PVA is an extremely sticky material; PVA beads, therefore, have a tendency to agglomerate. This is particularly a problem in applying PVA-immobilized cells to fluidized bed reactors. The elasticity and high strength of the PVA beads are ideally suited to the high shear stresses encountered in fluidized beds,[1] but the tendency of PVA beads to agglomerate can make fluidization difficult or impossible.

In this article, a new cell immobilization technique is described which eliminates the agglomeration problem of the PVA-boric acid method by the addition of a small amount of calcium alginate. The technique was demonstrated for a pure culture of *Pseudomonas.* The resulting beads were utilized in a three-phase fluidized bed bioreactor to demonstrate viability of the immobilized cells.

MATERIALS AND METHODS

The microorganisms used in this work was a pure strain

[a] Trace elements include (mg/L): zinc, 0.01; copper, 0.001; selenium, 0.001.

OD (460 nm) of approximately 1.1, a period of approximately 24–30 h, at which time they were centrifuged at 1000 rpm for 10 min.

The cell immobilization process is illustrated in Figure 1. Water was added to 43.7 g of polyvinyl alcohol (100% hydrolyzed, average MW 77,000 to 79,000) to obtain 330 mL of solution. The solution was then carefully heated to a temperature of 60°C to completely dissolve the PVA. A solution of 3.5 mL of 2% sodium alginate (low viscosity, approximately 250 cps) in water was prepared by gently stirring for 30 min and added to the PVA solution. The PVA-alginate solution was then cooled to a temperature of 35°C.

The centrifuged cells (45.6 g wet weight) and 35 mL of distilled water were mixed with 6.3 mL of the growth medium (Table I) to supply nutrients for the microbial

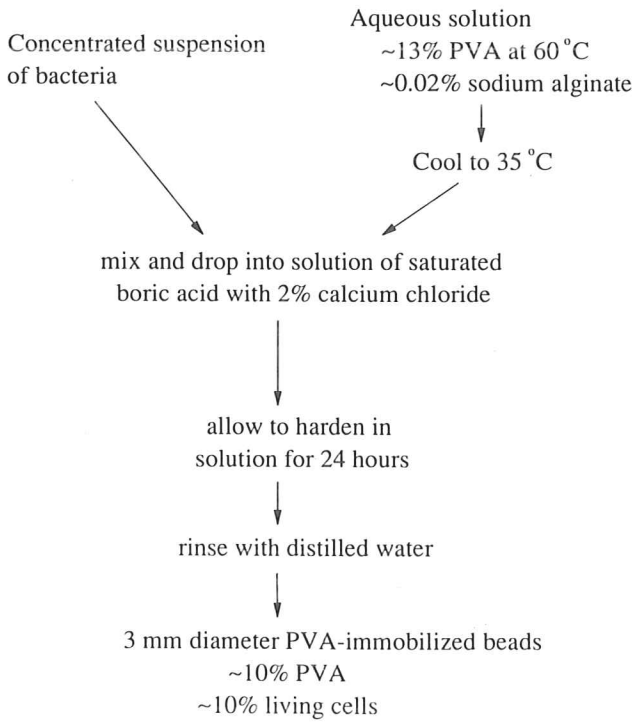

Figure 1. Cell immobilization using PVA-boric acid with calcium alginate.

stirred in this solution for 24 h to complete the solidi
fication, and then rinsed with distilled water to remov
any excess boric acid. Approximately 420 cm^3 of bead
containing the immobilized cells were thus formed. Th
diameter of the beads was approximately 3 mm.

Cell viability and bead integrity were tested in an 8-l
fluidized bed column. The 10.16 cm ID column wa
sparged with air at a rate of 1.4 L/min. Growth mediun
(Table I), containing varying concentrations of phenol
was fed continuously from the top of the column, an
effluent withdrawn at the column bottom, at a rate o
2.5 L/h. The fluidized bed experiments were conducte
at room temperature.

Phenol concentrations of all the samples in thi
work were analyzed by gas chromatography[8] (Hewlett
Packard model 5890A with flame ionization detector).

RESULTS AND DISCUSSION

Initial attempts at cell immobilization were made usin
only PVA crosslinked with boric acid. However, th
beads thus formed had a strong tendency to agglomerat
into a mass of polymer which was very difficult to breal
up. The beads could, therefore, not be utilized in a flu
idized bed. This agglomeration problem appears to b
due to the relatively slow crosslinking of the PVA b
boric acid. Droplets of PVA, which have not been suffi
ciently crosslinked, tended to agglomerate into a mass
This problem persisted even with vigorous stirring o
the boric acid solution to keep the beads suspended.

Attempts were then made to use a combination o
PVA-boric acid and calcium alginate, by hardening a
mixture of PVA and sodium alginate with a mixed solu
tion of boric acid and calcium chloride. In this way, i
was thought that the PVA might contribute durability
and strength to the beads, while calcium alginate migh
improve the surface properties of the beads, reducin
the tendency to agglomerate. The percentage of PVA i
the beads was kept in the range of 10% to 12.5%, a
recommended by Hashimoto and Furukawa,[3] to main
tain maximum bead strength. Varying ratios of dr
PVA to 2% sodium alginate solution were attempted fo
the immobilization procedure. It was found that ag
glomeration of the beads was entirely prevented fo
PVA to alginate solution ratios of 5:1, 8:1, 10:1, an
12.5:1, while agglomeration persisted for a 20:1 ratio
The 12.5:1 ratio (43.7 g PVA to 3.5 mL of 2% sodium

lginate), which corresponds to a total alginate concentration in the beads of approximately 0.02%, was concluded to be approximately the lowest concentration of lginate that would prevent bead agglomeration. It is heorized that calcium alginate is formed nearly instantaneously when the sodium alginate solution contacts he calcium chloride solution, and that the resulting olymeric structure is sufficient to keep the beads from gglomerating during the PVA crosslinking process. The esulting beads were strong and highly elastic, and of early spherical shape.

Fluidized bed experiments were conducted continuusly over a period of 2 weeks. Inlet phenol concentraion was increased in steps from 250 to 1300 mg/L (inlet henol concentrations of 250, 350, 450, 550, 650, 750, 50, 950, 1000, 1100, 1200, and 1300 mg/L were used), /ith the bioreactor allowed to achieve steady state beween each step increase. This range was chosen to be lose to the range of phenol concentrations to which the nicroorganisms had been acclimated. The steady-state utlet phenol concentration, measured by gas chronatography, was found to be zero for each of these inlet henol concentrations.

Phenol removal due to stripping was measured by dierting the off-gas from the column through a NaOH olution (pH 10) and measuring the absorbed phenol. 'he phenol removed by stripping was found to be less han 0.1% of the total phenol degradation. Bioadsorpion has been found by a number of investigators to be mall in comparison with biodegradation.[2,4,7] Therefore, can be concluded that phenol removal in the fluidized ed was due almost entirely to biodegradation, indicatng that cell viability was maintained through the imobilization procedure.

The bioparticles made from the PVA-alginate immoilization technique proved to be very durable, showng no sign of breakage or disintegration after 2 weeks f continuous operation of the fluidized bed. The PVAlginate beads showed no tendency to agglomerate at any time during the fluidized bed experiments. Measurements of bead size, before and after the fluidized bed experiments, showed no discernable difference.

CONCLUSIONS

Immobilization of living cells of a phenol-degrading *Pseudomonas* isolate in a PVA-boric acid gel was demonstrated. Cell viability was indicated by degradation of phenol in a fluidized bed reactor of the beads. The presence of a small amount of calcium alginate in the beads, as small as 0.02%, was shown to prevent agglomeration of the PVA beads. The resulting beads were highly elastic and durable; they were able to withstand high shears in a three-phase fluidized bed for 2 weeks of continuous operation with no noticeable breakage or shrinkage of the beads. The successful immobilization of a *Pseudomonas* isolate by the PVA-boric acid method indicates that the technique might be applicable to a wide variety of microorganisms.

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