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Design of a Fluidized Bed Reactor for Microencapsulated Urease

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ABSTRACT

A fluidized bed reactor was designed, constructed, and tested for handling microencapsulated urease. The working volume of the reactor was 10 mL, with a minimum fluidization velocity of 7.7×10^{-5} m/s. An even suspension of the microcapsules was obtained at fluid velocities between 1.5×10^{-4} and 6.0×10^{-4} m/s without breakage of the shear-sensitive microcapsules. The mixing behavior in the reactor was evaluated using pulse input tracer experiments and the hydrolysis rates of urea in continuous flow experiments were evaluated under various operating conditions.

Index Entries: Fluidized bed reactor, for microencapsulated urease; reactor, for microencapsulated urease; microencapsulated urease, fluidized bed reactor for; urease, fluidized bed reactor for microencapsulated.

INTRODUCTION

Three main types of immobilized enzyme reactors have been studied for various applications: the continuous stirred tank reactor (CSTR), the packed bed reactor (PBR), and the fluidized bed reactor (FLBR). Most of

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the immobilized enzyme processes use the CSTR or the PBR. However, fluidized bed reactors present many advantages: they are more efficient than PBR from the mass transfer point of view and are more suitable than the CSTR for shear-sensitive enzymatic systems (*1*).

Chang has studied the flexibility of microcapsule membranes by suspending the capsules in silicone fluid and subjecting them to shear stress and collision (*2*). The microcapsules elongated in the line of shear stress, and on collision the membranes flattened at the point of contract.

The fragility of the membranes is a constraint in the design of microencapsulated enzyme reactors using microcapsules with ultrathin (200 Å) membranes. For example, a study of microencapsulated urease in a CSTR showed that for stirring rates higher than 220 rpm, breakage of the microcapsules occurred (*3*).

A fluidized bed reactor is the most suitable system to contain microencapsulated enzymes that are both shear-sensitive and limited by mass transfer.

The enzymic system chosen to evaluate the reactor was microencapsulated urease and the operating conditions (temperature, pH, and urea concentration) were those used in extracorporeal devices (*2*).

MATERIALS AND METHODS

Urease was co-encapsulated with hemoglobin using the method developed by Chang (*2,4–6*) for cellulose nitrate membrane microcapsules.

Because of the wide diameter distribution of the prepared microcapsules, a preliminary size separation was required prior to testing. This was accomplished by sedimentation in 1% Tween 20 solution in a tall column. This technique permitted a rough separation of large microcapsules (diameter>200 μm). After separation, the diameter of the capsules was measured microscopically, and the mean diameter calculated.

The rate and extent of urea hydrolysis was estimated from the increase in pH resulting from the formation of ammonium carbonate (*3*). The urease activity was measured by a method developed by Van Slyke and Archibald (*8*).

The reactor described below was operated under flow conditions of 20–30 mL/min with urea solutions of 6–11 g/L (in pH 7.4 phosphate buffer). The temperature was maintained at 34°C in a temperature-controlled bath. The change in pH resulting from urea degradation was measured using a combination pH microprobe at the exit from the reactor.

Tracer pulse input experiments were performed by injecting 1 mL of 0.01*N* NaOH through the septum port at the base of the reactor at a specific fluid flow rate. The system response to the impulse was continuously recorded by means of a pH recorder.

RESULTS

Reactor Design

A uniform fluidization must be achieved with the shear-sensitive microcapsules to avoid breakage problems. The quality of fluidization is highly dependent on the distributor plate installed in the reactor. The ideal fluid distributor should present a porous structure so that the fluid is introduced through a multitude of injection points, thus avoiding high entrainment of particles at localized points. A smooth fluidization was achieved with a glass-fritted disk (10–15 μm pore size).

The minimum fluidization velocity and the quality of fluidization were evaluated by observation of the microcapsule behavior with increasing values of flow rate. The minimal fluidization velocity was $U_{mf} = 7.7 \times 10^{-5}$ m/s. An evenly suspended bed with gently movement of particles was obtained for velocities between 1.5×10^{-4} and 6×10^{-4} m/s. At higher velocities the particles started to segregate according to their size, and for velocities higher than 10^{-3} m/s, the smallest particles were entrained.

The conditions in flow rate to be evaluated were set between 20 and 30 mL/min. Given the fluid velocity constraints noted above to achieve suspension of the bed, a range of possible reactor diameters was calculated to be between 32 and 53 mm. The working volume of the test reactor was limited to 10 mL. In order to have feasible dimensions of diameter and height, the following values were chosen: $D = 35$ mm and $H = 10$ mm. These dimensions represented the section containing the microcapsules since a distribution section was also provided, as illustrated in Fig. 1.

The reactor body was constructed of plexiglass with copper inlet and outlet tubings (id, 5 mm). The distribution section consisted of a cone packed with 4-mm-diameter glass beads, on top of which was placed the glass-fritted distributor. The combination of the glass beads and the fritted distributor provided for a very smooth fluidization. On the upper part of the reaction section, a 74-μm mesh grid was installed to prevent the capsules from escaping the reactor.

In order to characterize the mixing behavior of the reactor, residence time distribution experiments were performed. Mixing characteristics in a fluidized bed reactor are intermediate to the extremes as represented by the ideal plug flow reactor and the ideal stirred tank reactor. The reactor dispersion number D/UL is a dimensionless parameter that measures the extent of axial dispersion (*8*), where D is the axial dispersion coefficient, U the fluid velocity, and L the distance from inlet to outlet in the reactor. The dispersion number may be obtained from tracer experiments by applying the following equation,

$$\frac{\sigma^2}{\bar{t}^2} = 2\frac{D}{UL} - 2\left(\frac{D}{UL}\right)^2\left(1 - \exp - \frac{UL}{D}\right) \quad (1)$$

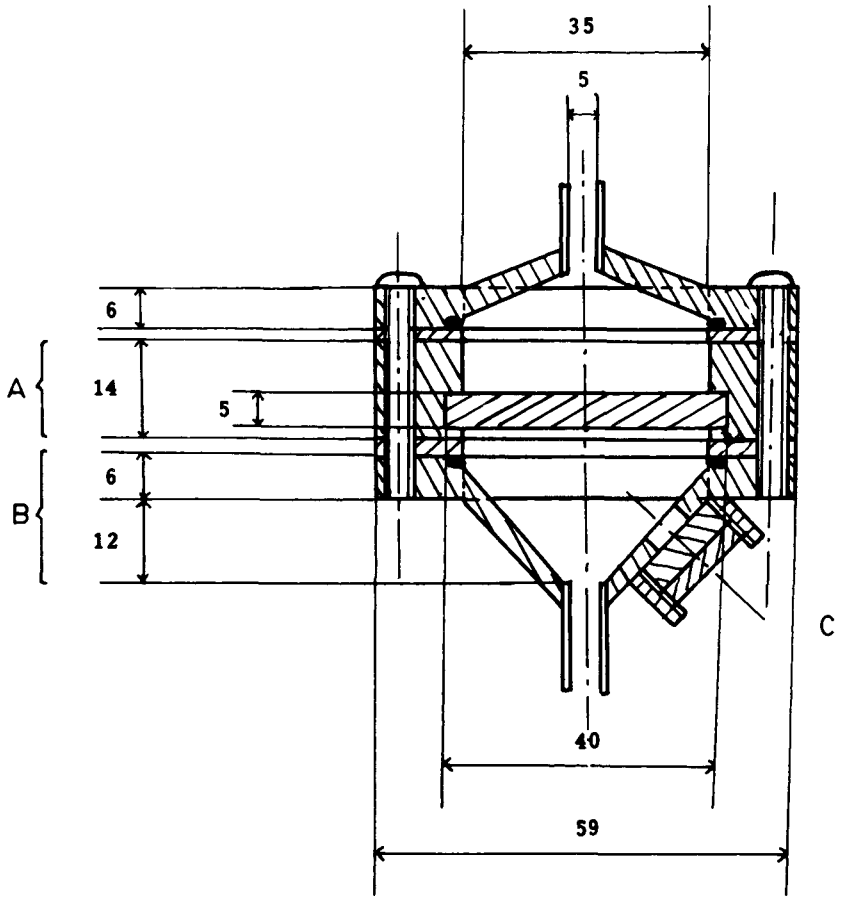

Fig. 1. Fluidized bed reactor composed of a reaction section (A) with 5-mm fritted disk at base, a distribution section (B) containing glass beads, and a port at the base (C) for tracer injections. Inlet and outlet ports are at the extreme bottom and top, respectively. All dimensions are in millimeters.

where $\bar{t}$ is the experimentally determined mean residence time, and σ^2 is the variance or spread of the residence time distribution about the mean.

The results obtained from pulse input experiments are presented in Table 1. Values of *D/UL* were calculated using Eq. 1. The reactor dispersion number ranged from 0.185 to 0.39, increasing with decreasing flow rates through the reactor. The mean residence time of fluid in the reactor ranged from 0.62 min at the highest flow rate (31.1 mL/min) to 1.05 min, at the lowest flow rate studied (22.3 mL/min).

Urea Degradation Under Dynamic Flow Conditions

Once the conditions were established to maintain a uniform suspension of the fluidized microcapsules, a series of experiments were performed to examine the extent of urea degradation as a function of resi-

TABLE 1
Mean Residence Time ($\bar{t}$) and Dispersion Number (D/UL) Measured by Tracer Injection Experiments at Different Flow Rates Through the Reactor

Q, mL/min	$\bar{t}$, min	D/UL
22.3	1.05	0.390
24.4	0.81	0.227
27.0	0.77	0.206
28.1	0.76	0.188
31.1	0.62	0.185

dence time. The variables examined were S_o, the urea concentration in the feed; V_c, the volume of microcapsules; d_p, the microcapsule mean diameter; and E_o, the enzyme content in the reactor. All measurements represent steady state values under the specified operating conditions in a continuous feed experiment.

Figure 2 illustrates the effect of varying the feed urea concentration to the reactor. In all the experiments, the percentage degradation increased with the residence time in the reactor, eventually reaching a maximum conversion. In Fig. 2, higher percentage conversions were achieved at the lower feed urea concentrations. Percentage degradation values as given by $(S_o - S)/S_o$ were low, usually less than 10% for a single-pass flow experiment under the flow rates evaluated.

The effect of the total volume of microcapsules in the reactor was examined (*see* Fig. 3) at V_c values of 1.26 and 1.50 mL. The enzyme activity (0.4 Sumner units) was identical in both cases. The maximum percentage conversion achieved (12%) at a capsule volume of 1.5 mL and residence time of 0.5 min represents the highest conversion observed under all of the operating conditions tested. The higher value of V_c also resulted in higher conversions over those achieved at the lower value of V_c.

Two different microcapsule diameters (d_p) are compared in Fig. 4. Since it was difficult to maintain V_c and E_o constant while testing the effects of d_p, the plots presented represent mean microcapsule diameters of 175 and 205 μm and capsule volumes of 1.21 and 1.00 mL, respectively. It may be seen in Fig. 4 that higher conversions were obtained with the 175 μm microcapsules. A maximum urea conversion was achieved (10.2%) at a residence time of 0.5 min.

The effects of enzymatic activity of the microcapsules are compared in Fig. 5. Four levels of activity (0.15, 0.2, 0.3, 0.4 Sumner units) were examined under the operating conditions noted in the legend to the figure. It appears that for E_o values higher than 0.2 units, the urea degradation is independant of the enzyme concentration in the reactor. A substantial difference in conversion was observed with an activity of 0.15 Sumner units when compared to the higher conversion at 0.2 units.

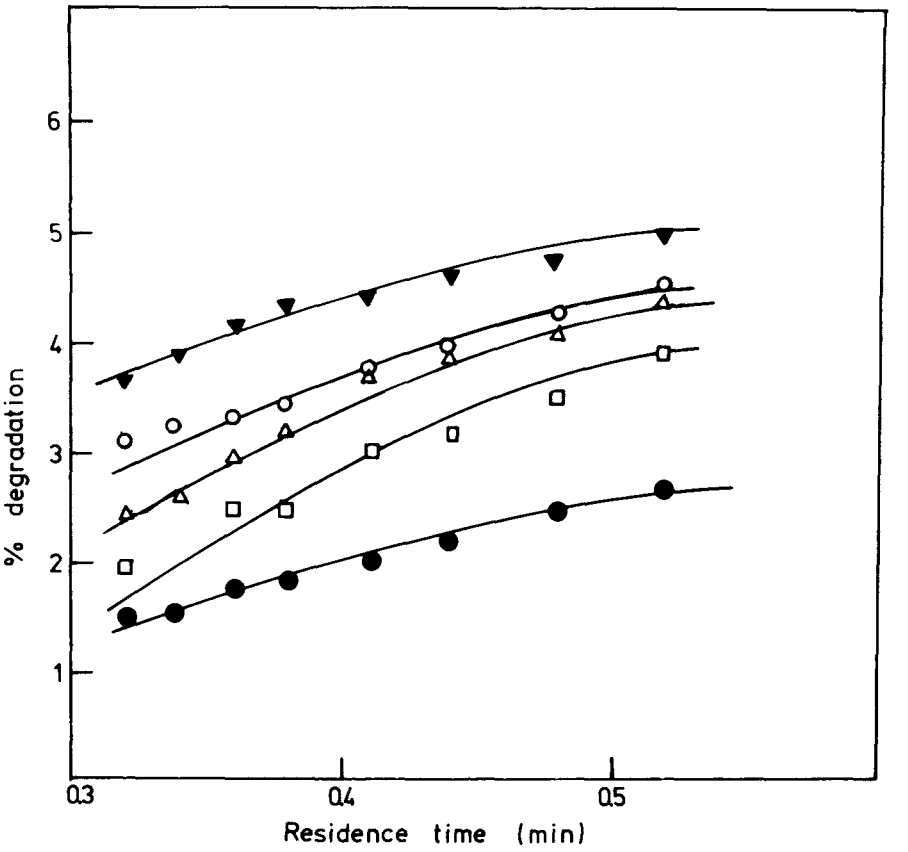

Fig. 2. Percentage degradation of urea at various feed concentrations: E_o, 0.15 Sumner units; d_p, 175 μm; V_c, 0.85 mL; S_o, (▼) 6 g/L; (○) 8 g/L; (△) 9 g/L; (□) 10 g/L; (●) 11 g/L.

Ammonium carbonate, an end product of urea hydrolysis, was examined for its possible effects on hydrolysis. A step change in the feed solution to the reactor was introduced at each flow rate tested. The feed alternated between a solution containing only urea to a solution that consisted of urea plus 1.3 g/L ammonium carbonate. All of the other parameters; d_p, E_o, V_c, and S_o were maintained constant. The results presented in Fig. 6 indicated higher conversions at steady state, in the presence of the ammonium ion, than in the case of urea alone. Maximum degradation was 7.7% at a reactor residence time ranging from 0.45 to 0.5 min.

DISCUSSION

The feasibility of utilizing fluidization technology to handle microencapsulated enzyme systems has been demonstrated using the reactor designed and described in this paper. An even fluidization was achieved

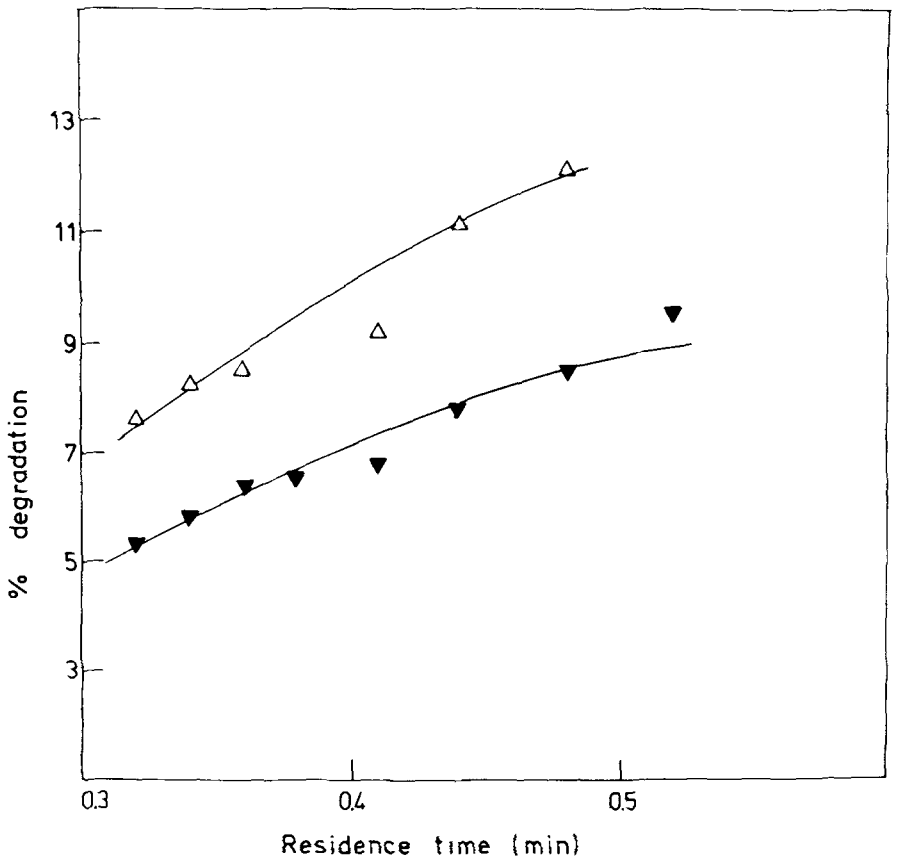

Fig. 3. Percentage degradation of urea as a function of residence time: E_o, 0.4 Sumner units; d_p, 124 μm; S_o, 6 g/L; V_c, (△) 1.5 mL; (▼) 1.26 mL.

without any breakage of the shear-sensitive microcapsules. (Urease activity was determined in the reactor exit stream to test for capsule breakage.) Furthermore, plugging problems, associated with packed bed reactors were eliminated.

Based on the results obtained with pulse input tracer experiments, values of *D/UL* obtained for the fluidized reactor are in the range of large dispersion according to Levenspiel (*8*). It was noted that the dispersion number actually decreased as the flow rate through the reactor increased, indicating better mixing at the lower flow rates. This would have an effect on the rates of reaction in the reactor.

The overall enzymatic reaction comprises three steps: substrate diffusion into the capsule, enzymic reaction, and product diffusion out of the capsule. If the time needed for the whole process to occur is in the same order of magnitude as the residence time (less than 1 min), there will be a time limitation. The percentage degradation will then be dependent on the residence time. This was confirmed by the results obtained.

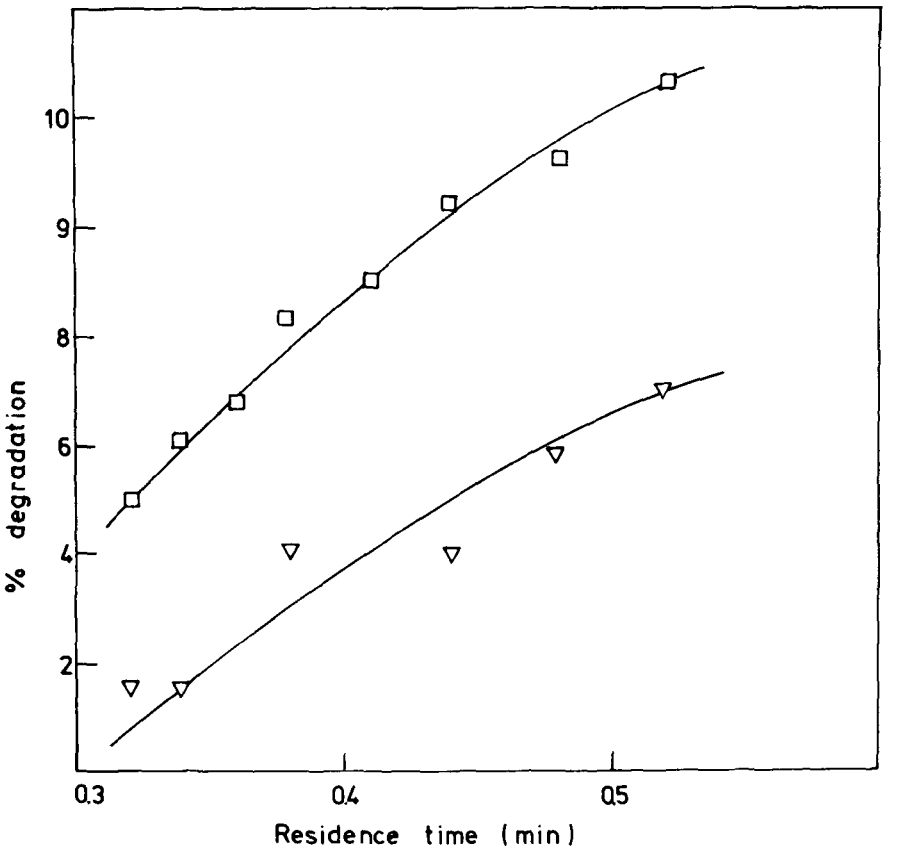

Fig. 4. Percentage degradation of urea as a function of residence time: E_o, 0.2 Sumner units; S_o, 7 g/L; □, d_p = 175 μm; V_c = 1.21 mL; ▽, d_p = 205 μm; V_c = 1.00 mL.

As a consequence, the degradation increases with the enzyme concentration until E_o reaches a limiting value. The residence time limits the amount of substrate to be degraded at this enzyme concentration. Any increase of enzyme load does not affect the reaction if the residence time remains the same.

Percentage urea degradation as given by $(S_o - S)/S_o$ decreased as the urea feed concentration increased. Because of the concentration effect on the diffusional driving force, the rate of diffusion and reaction would increase with the substrate concentration S_o. The total conversion $(S_o - S)$ resulting will be offset by S_o, which appears in the denominator, the net result being a decrease in the percentage degradation.

The percentage degradation of urea was inversely proportional to the mean diameter of capsules and proportional to the volume of capsules. This result may be explained by mass transfer limitations affecting the rate of reaction. For microencapsulated enzymes, in addition to partitioning effects caused by membrane charges, the mass transfer of substrate through the membrane is essentially diffusional. The diffusion rate

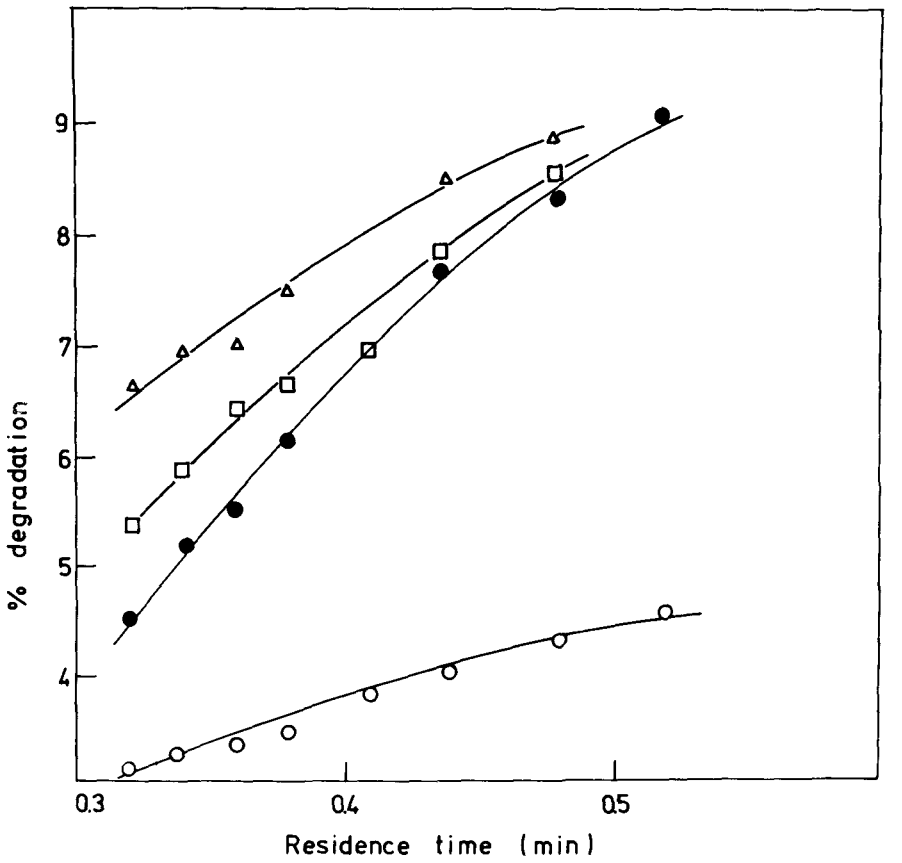

Fig. 5. The effect of enzyme activity in the reactor on the degradation of urea: S_o, 7 g/L; d_p, 150 μm; V_c, 1.20 mL; E_o,(○) 0.15 units; (●) 0.2 units; (△) 0.3 units; (□) 0.4 Sumner units.

is proportional to the surface available for the transfer. Smaller diameter capsules will have a higher surface area per capsule volume than will larger diameter capsules.

Since free urease has been reported to be inhibited by the presence of NH_4^+ (*9*), the degradation enhancement observed in this study may be explained by a pH effect resulting from the immobilization technique. The optimum pH of the free urease was 6. The collodion membrane that encloses the encapsulated urease is negatively charged (*2*). When the particles are in solution, there is a partitioning of ions between the bulk phase and the inside of the capsules. The protons being positively charged are trapped inside the capsules, thus leading to a pH gradient between the capsules and the bulk phase. The pH inside the capsule may be less than the measured pH in the bulk phase. Similar cases have been reported. As an example (*10*), the comparison of pH activity profiles of chymotrypsin in free solution and of the enzyme immobilized on negatively charged kaolinite particles, showed a shift of two units toward higher values of the optimum pH.

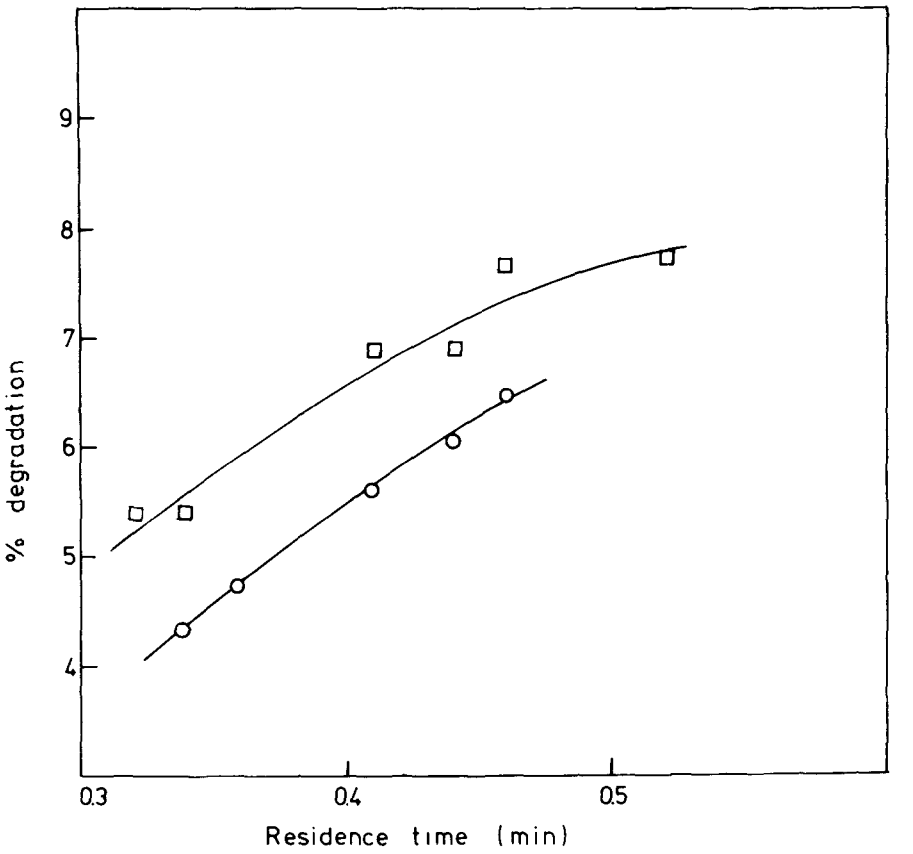

Fig. 6. Degradation of urea with (□) and without (○) the addition of 1.3 g/L ammonium carbonate to the feed stream: E_o, 0.36 Sumner units; S_o, 6 g/L.

At a pH value of 7.4 in the urea solution, the intracapsular pH may be less than 6, which is the optimum pH. The addition of ammonium carbonate in the feed increases the pH outside and inside of the capsules. The pH inside the capsules approaches the optimum value and the degradation increases.

Work to be reported in a future paper will include a mathematical modeling of the reactor behavior under steady- and transient-state flow conditions.

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