FORMATION OF CALCIUM-ALGINATE GEL COATING ON BIOCATALYST IMMOBILIZATION CARRIER

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Biocatalyst immobilization carrier was coated with calcium alginate gel by a two-step preparation procedure for prevention of leakage of the biocatalyst from the carrier. A uniform gel coating film could be formed around not only calcium alginate gel beads but also chitosan beads. The thickness of calcium alginate gel coating increased with decreasing sodium alginate concentration and stirring rate, and with increasing concentration of calcium ion and diameter of the core bead. The volume of gel coating film was shown to be proportional to the mass of calcium ion initially contained in the core bead. It was also found that the thickness of gel coating film could be controlled by adjusting the calcium concentration in the core bead.

Introduction

Entrapment of biocatalyst with polymeric matrices, such as calcium alginate or κ-carageenan, is preferred by many researchers because of the extremely mild immobilization conditions. During application of immobilized growing cells or immobilized enzyme, however, there is still a general problem of leakage of the biocatalyst from the immobilization carrier into the medium. In the case of calcium alginate gel beads prepared by the conventional immobilization method, cells on and near the surface can easily leak from the beads and subsequently grow rapidly in the medium rather than in the beads. Cell leakage followed by the subsequent rapid growth of the leaked cells may obstruct the evaluation of reaction kinetics inherent in the immobilized biocatalyst. In addition, easy separation of the biocatalyst from the medium, which is considered one of the important merits of immobilization, is not fully realized due to the leakage of biocatalyst.

Several studies have been reported on the prevention of cell leakage from the polymeric matrix. However, complete prevention of cell leakage has been reported only by Tanaka et al., who immobilized yeast cells in calcium alginate gel fiber which was coated with cell-free calcium alginate gel using a double nozzle. Coating of immobilized biocatalyst with catalyst-free polymeric matrix is expected to be effective also for the prevention of cell leakage from spherical gel beads. There are two possible techniques for the preparation of double-layered spherical gel beads. One is single-step gelation of the double-layered droplet using a double nozzle. This procedure is similar to that applied to the production of synthetic salmon roe. The other is two-step formation by which immobilized biocatalyst is coated with catalyst-free gel matrix after preparation of the immobilized biocatalyst. This procedure is slightly complicated but gives higher reproducibility and productivity compared to the single-step preparation. Although the latter procedure was proposed schematically by Vorlop et al., no data are available for the formation kinetics of gel coating nor the controllability of the thickness of coating film.

The objective of the present study was to obtain basic data concerning the formation kinetics of gel coating by the two-step formation procedure. In this study, calcium alginate gel coating was first formed on an immobilization carriers such as calcium alginate gel beads and chitosan beads. The process of gel coating formation was investigated at various sodium alginate concentrations, stirring rates, calcium concentrations in the core bead and bead diameters. The relationship between coating thickness and these operational conditions were investigated in terms of formation kinetics. A method for controlling the thickness of the gel coating was also examined.

1. Experimental

1.1 Two-step coating procedure

Figure 1 shows the schematic procedure of the two-step preparation of coated beads. The procedure included the following steps: (1): Core beads, that is, immobilized biocatalyst, to be encapsulated are...
prepared beforehand. (2): The core beads are immersed in a solution containing calcium ions, which are allowed to diffuse into the water contained in the gel pores. (3): The beads are then collected from the solution, and the excess solution attached to the bead surface is removed by placing the beads on filter paper. (4): The beads are then introduced into a stirred solution containing sodium alginate. The calcium ions contained in the core bead diffuse into the solution. Those reaching the surface of the bead react with sodium alginate to form a calcium alginate gel coating around the bead. (5): After a given time of gelation, the coated beads are collected and rinsed with water to remove excess sodium alginate attached to the surface. (6): The coated beads are stored in a curing solution containing the proper concentration of calcium ions.

The two-step preparation procedure of coated bead has the following potential advantages.
1) Growing immobilized cells as well as resting immobilized cells can be coated.
2) There is wide versatility in the type of core bead used. Any bead that holds a solution containing calcium ion can be coated, irrespective of chemical or structural nature of core material.
3) Immobilized biocatalyst having a shape other than spherical, cubical or cylindrical, for example, also can be coated.

1.2 Methods
Calcium alginate gel beads were used as core material. They were prepared by dropping 20 kg/m³ sodium alginate solution into 20 kg/m³ calcium chloride solution. To clarify the boundary between the core bead and the coating film, 0.10 kg/m³ titanium oxide was added to the sodium alginate solution in the preparation of the core beads. After a given time of gel coating formation as described above, the coated beads were cured for more than 12 hours.

Concentrations of calcium chloride solution $C_{Ca}$ and sodium alginate solution $C_{Alg}$ for the formation of gel coating were varied in the range of 5.0–60.0 kg/m³ and 2.0–8.0 kg/m³ respectively. Stirring rate $N$ was varied from 6.67 to 13.3 s⁻¹. The mean diameter $D_n$ of core beads was varied from 2.20 to 3.87 mm by changing the diameter of the nozzle through which sodium alginate was dropped. All the experiments were carried out at 303 K. Coating film thickness was measured by taking microphotographs of the coated beads.

2. Results and Discussion
2.1 Formation of gel coating around immobilization carrier
A potential merit of the two-step coating procedure is that not only calcium alginate gel beads but also beads made of other materials can be coated. This allows a wide selection of core and coating materials. To verify this potential merit, calcium alginate gel beads and chitosan beads were used as core materials to be coated. Figure 2a shows a photograph of coated calcium alginate gel beads. Bead diameter and thickness of the coating film in the figure were about 3.7 mm and about 290 μm respectively. A gel coating of uniform thickness could be formed around each bead, as shown in the figure. Figure 2b shows a photograph of chitosan beads coated with calcium alginate gel. Bead diameter and thickness of the coating gel were about 2.0 mm and about 160 μm respectively. In this case also, the thickness of the gel coating was uniform. The chitosan beads (Chitoparl HP-5020, Fuji Spinning Co. Ltd.) were reported to have a mean pore size of 50 μm by the manufacturer. The calcium alginate gel was reported to have a maximum pore size of nanometer order. Therefore, the coated chitosan beads are considered to have two different structures: macroporous core bead and microporous coating film.

2.2 Process of coating film formation
2.2.1 Effects of sodium alginate concentration and stirring rate
1) Effect of sodium alginate concentration
Figure 3 shows the relationship between coating film thickness $L$ and gelation time $t$ at various sodium
alginate concentrations $C_{\text{Alg}}$. The thickness of the gel coating increased rapidly within the first ten minutes and then leveled off. The thickness of the coating film arrived faster at its maximum value $L_{\text{max}}$ with increasing $C_{\text{Alg}}$. The value of $L$ decreased with increasing $C_{\text{Alg}}$. With increasing $C_{\text{Alg}}$ the number of alginate molecules in the vicinity of the surface of the core bead increased. Calcium ions diffused from the core bead react with homopolymeric blocks of l-guluronic acid in the alginate molecules to form an ionotropic gel. The number of l-guluronic acid residues in the alginate molecules are in excess at the reaction front compared to that of calcium ions diffused. The gelation reaction will take place closer to the bead surface when more alginate molecules exist in the vicinity of the bead surface. Therefore, the effect of sodium alginate concentration on coating film thickness can be explained by the dependency of the position of the reaction front on sodium alginate concentration in the vicinity of the bead surface.

2) Effect of stirring rate

Figure 4 shows the process of gel coating formation at various stirring rates $N$. With increasing $N$, the thickness $L$ of coating film decreased and reached its maximum value more quickly. Figure 5 shows a typical result for the effect of rate $N$ on $L$ with sodium alginate concentration $C_{\text{Alg}}$ as a parameter. The value of $L$ decreased with increasing $N$. With increasing stirring rate, the thickness of the concentration boundary layer around the core bead decreased because of the enhanced turbulence. The local concentration of sodium alginate in the vicinity of the bead surface will increase with decreasing thickness of the concentration boundary layer. The increase in local concentration of alginate in the vicinity of the bead surface will result in the increase in thickness of the calcium alginate gel coating, as described above. An increase in stirring rate $N$ is considered to have a similar effect of increase to that of sodium alginate concentration $C_{\text{Alg}}$ on the coating film thickness $L$.

3) Description of process of gel coating formation

Solid lines in the Figs. 3 and 4 represent the process of forming the coating film, which is represented as film thickness $L$, calculated by the following equation. Equation (1) is a relation similar to that derived by Chrastil for a diffusion-limited reaction system.

$$\frac{V}{V_{\text{max}}} = [1 - \exp(-kt)]^n$$

where $V$ and $V_{\text{max}}$ are the volume of coating film formed at time $t$ and $t \to \infty$ (i.e., maximum volume of gel coating) respectively, and $k$ is the gelation rate constant. The values of $V$ and $V_{\text{max}}$ were calculated with $L$ and $L_{\text{max}}$ respectively. The parameter $n$ represents the heterogeneous structural resistance constant, which is indirectly proportional to the diffusional resistance. Chrastil investigated the gelation kinetics of calcium alginate with rice starch and/or rice flour and reported that the value of structural diffusion resistance $n$ decreased significantly in gels made from mixtures of alginate with rice starch or flour. The maximum volume $V_{\text{max}}$ of the coating film was determined experimentally. The value of parameters $n$ and $k$ was determined by the least squares method. The process of forming the gel coating film was found to be expressed quantitatively by Eq. (1),...
as shown in Figs. 3 and 4.

Figures 6 and 7 show the dependencies of parameters \( n \) and \( k \) in Eq. (1) on sodium alginate concentration and stirring rate respectively. The structural resistance constant \( n \) was almost unity within the range of alginate concentration and stirring rate tested. That is, the gelation reaction was almost a first-order reaction. This suggests that the structure of the gel coating is hardly affected by the alginate concentration within the range tested. Chrustal reported that the value of \( n \) was almost unity for calcium alginate gel made from 2% alginate solution.\(^4\) The gelation rate constant \( k \) increased with increasing alginate concentration or stirring rate. The calcium ions reached at the bead surface must diffuse through the coated gel film to react with sodium alginate during the formation of the gel coating. Therefore, higher alginate concentration or stirring rate will result in thinner maximum coating film. The dependency of \( k \) on alginate concentration or stirring rate may include the effect of additional diffusion path which increases successively in the gelation kinetics.

### 2.2.2 Effect of calcium chloride concentration

Figure 8 shows the effect of calcium chloride concentration \( C_{Ca} \) on the thickness of coating film. The maximum film thickness was about 100 \( \mu \)m when the core bead was immersed in \( \text{CaCl}_2 \) solution of 7.0 kg/m\(^3\). The value of \( L_{\text{max}} \) was more than 1000 \( \mu \)m when the \( \text{CaCl}_2 \) concentration was 60 kg/m\(^3\). The thickness of coating film increased remarkably with increasing \( C_{Ca} \). Lower \( C_{Ca} \) gave shorter reaction time required to obtain maximum film thickness. The thickness of the coating film increased rapidly within the first few minutes when the \( \text{CaCl}_2 \) concentration was higher than 7.0 kg/m\(^3\). A thinner coating film with sufficient strength is required for practical application of coated immobilized biocatalyst. Vorlop et al.\(^{13}\) suggested that the thickness of coating film can be controlled by the reaction time. Although this method is possible theoretically, control of film thickness by reaction time alone to produce a thin coating film seems to be very difficult because of the rapid increase in film thickness in the initial gelation process of the coating film, as shown in the figure. For preparation of immobilized biocatalyst coated with thinner calcium alginate gel film, the method of controlling film thickness by the concentration of \( \text{CaCl}_2 \) may be recommended.

Figure 9 shows the dependencies of parameters \( n \) and \( k \) on \( \text{CaCl}_2 \) concentration. The value of \( n \) was almost unity with \( C_{Ca} \) up to 40 kg/m\(^3\) but decreased with increasing \( C_{Ca} \) above 40 kg/m\(^3\). The coating film thickness increased considerably and the time required to obtain maximum film thickness was also considerably prolonged when the \( \text{CaCl}_2 \) concentration was above 40 kg/m\(^3\). The decrease in \( n \) with \( \text{CaCl}_2 \) concentration above 40 kg/m\(^3\) may be due to the enlarged diffusion path caused by the formation of thicker coating film. The gelation rate constant \( k \) decreased rapidly at first and then leveled off with increasing \( \text{CaCl}_2 \) concentration. This is considered to be due to the concentration dependency of the diffusivity of calcium ion\(^5\), in addition to the enlarged diffusion path mentioned above.
2.2.3 Effect of bead diameter

Figure 10 shows the effect of bead diameter $D_B$ on the process of forming the coating film. Maximum coating film thickness increased with increasing $D_B$. This can be explained by the fact that the mass of the calcium ions initially contained in the core bead increases with increasing $D_B$. The time required to obtain maximum film thickness was also prolonged with increasing $D_B$.

Figure 11 shows the dependencies of parameters $n$ and $k$ on core bead diameter $D_B$. The value of the structural resistance constant $n$ was almost unity when core beads having a diameter below 3.72 mm were used. The value of $n$ tended to decrease when core beads having diameter above 3.72 mm were used. The decrease in $n$ with increasing bead diameter above 3.72 mm is considered to be due to the enlarged diffusion path, that is, the increased thickness of coating film, as described above. The gelation rate constant $k$ decreased almost linearly with increasing core bead diameter within the range tested.

2.3. Maximum volume of gel coating film

The maximum volume $V_{\text{max}}$ of coating film is plotted against the amount of calcium ion $m_{\text{Ca}}$ initially contained in the core bead in Fig. 12. The volume fraction of alginate molecules in the core bead was neglected in calculating the mass of calcium ions. The partition coefficient of calcium ion between the core bead and bulk solution was also assumed to be unity. The maximum volume of coating gel was proportional to the mass of calcium ion contained initially in the core bead. This result explains the dependency of coating film thickness on calcium concentration and bead diameter. The maximum volume of coating gel formed per unit mass of calcium ion diffused was estimated to be $5.72 \times 10^{-3}$ m$^3$/mol from the slope of the line.

A bead coated with calcium alginate gel film of desired thickness can be formed by controlling the mass of calcium ions initially contained in the core bead. This mass can be easily adjusted by changing the concentration of calcium ions in the bath in which the core beads are immersed. Therefore, the thickness of calcium alginate gel coating film around the core bead can be easily controlled with the two-step coating procedure.
3. Conclusion

Immobilization carrier was coated with calcium alginate gel by the two-step coating procedure. The relationship between coating film thickness and operational conditions was investigated in terms of formation kinetics. The results obtained are summarized as follows.

1) Uniform calcium alginate gel coating film could be formed around not only calcium alginate gel bead but also chitosan bead.

2) The process of forming gel coating around the core bead was expressed well by the relationship of Eq. (1).

3) The maximum thickness of coating film decreased with increasing sodium alginate concentration and stirring rate. Maximum coating film thickness increased with increasing concentration of calcium ion and core bead diameter.

4) Maximum film volume was proportional to the mass of calcium ions initially contained in the bead. The film thickness can be controlled easily by setting the initial calcium concentration of the bath.

Nomenclature

\( C_{\text{Alg}} \) = concentration of sodium alginate [kg/m³]
\( C_{\text{Ca}} \) = concentration of calcium chloride [kg/m³]
\( D_{\text{B}} \) = bead diameter [m]
\( k \) = gelation rate constant [s⁻¹]
\( L \) = thickness of gel coating film [m]
\( L_{\text{max}} \) = maximum film thickness [m]
\( m_{\text{ca}} \) = mass of calcium ion initially contained in core bead [mol]
\( n \) = heterogeneous structural resistance constant [—]
\( N \) = stirring rate [s⁻¹]
\( t \) = time [s]
\( V \) = volume of coating film [m³]
\( V_{\text{max}} \) = maximum volume of coating film [m³]

Literature Cited