PREPARATION OF IMMOBILIZATION MATRIX WITH LOW OXYGEN PERMEABILITY AND ITS APPLICATION TO BIOLOGICAL DENITRIFICATION

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The effects of chemical composition of alginate (i.e., M/G ratio) and gelling agents on the effective diffusivity \(D_e\) of oxygen, nitrate and glucose were investigated for preparation of an immobilization matrix to entrap denitrifier. The ratio of \(D_e\) of oxygen to \(D_w\) in water for Ba-alginate gel was the lowest among Ca-, Sr- and Ba-alginate gels, and the value was only about one half. With increasing M/G ratios above about 0.5, the value of \(D_e\) of nitrate or glucose of alginate gels tended to decrease. Ba-alginate gel with M/G ratios below 0.5 was found to be suitable for immobilization of denitrifier.

The effects of bulk dissolved oxygen (DO) concentration on the denitrification activity of the immobilized denitrifier was investigated. At DO concentrations up to about 2.5 mg/L, the immobilized denitrifier in Ba-alginate gel had almost the same activity to that under anoxic conditions. In addition, denitrification activity under oxic conditions was highest at a cell loading of about 3 g/L, and increased with increasing bead radius.

Introduction

Alginate gel has been widely used for immobilization of biocatalyst because of its extremely mild immobilization conditions (Tanaka and Nakajima, 1990). Immobilization of biocatalyst generally causes loss of the reaction rate compared to the suspended system, because the immobilization matrix can obstruct transportation of the substrate. An immobilization matrix which has less diffusional obstruction is preferred for higher reaction rates. Many researchers have reported on diffusional properties in alginate gel (Kuroswa et al., 1989; Merchant et al., 1987; Ogbonna et al., 1989; Scott et al., 1989; Sun et al., 1989; Tanaka et al., 1984). However, they have focused only on relationships between physical structure of the matrix, such as pore size, and the diffusional properties in the gel. Other physicochemical characteristics of immobilization matrices such as affinity to substrate or selective permeability against inhibitor, have hardly been reported. When an immobilization matrix has high affinity to a substrate, the substrate concentration within the matrix becomes higher than that of the bulk solution, and the reaction rate is expected to be accelerated. When the immobilization matrix has a low permeability against the inhibitor, the reaction rate becomes higher than that of the suspended system, because the inhibitor concentration within the matrix becomes lower than that of the bulk solution. The microenvironment within an immobilization matrix may be controlled to be suitable for biocatalytic reaction by selecting the material and its preparation procedure. However, such an approach has hardly been attempted.

On the other hand, nitrogen removal from sewage and domestic wastewater is becoming a significant environmental protection concern. Biological denitrification is an economically promising method for nitrogen removal. However, denitrifier (a facultative anaerobe), utilizes oxygen for assimilation at dissolved oxygen (DO) concentrations above about 0.2 mg/L. Consequently, denitrification is inhibited under oxic condition (Dawson and Murphy, 1972). Namely, oxygen acts as an inhibitor to denitrification. When denitrifier is immobilized in a matrix, oxygen is consumed near the surface and an anoxic core may be exist. When denitrifier was immobilized within a matrix having lower oxygen permeability, the volume of the anoxic core becomes larger than that of the matrix having higher oxygen permeability because of the decreased oxygen flux. Successful denitrification is expected to be carried out, even if the bulk solution is under oxic conditions, provided that a denitrifier immobilized within the matrix having low oxygen permeability is used.

The objectives of the present study are to prepare an immobilization matrix with low permeability and to evaluate denitrification activity of the immobilized denitrifier within the matrix under oxic conditions. We investigated the effects of chemical composition of
alginate and gelling agents on the effective diffusivity of oxygen, nitrate and glucose in alginate gel. Based on the results obtained, we selected the alginate gel suitable for immobilization of denitrifier. Furthermore, the effects of bulk DO concentration, cell loading and gel radius on denitrification activity of the immobilized denitrifier under oxic conditions were evaluated.

1. Experimental

1.1 Preparation of alginate gels

Seven kinds of sodium alginate were purchased from Kibun Food Chemifa Co. Ltd. The M/G ratio of the algae was reported by the manufacturer to range from 0.21 to 1.05. Sodium alginate from Junsei Chemical Co. Ltd. was also used. The M/G ratio of the alginate was estimated to be 0.38 by colloid titration (Kozawa et al., 1994).

Alginate gel beads were prepared by dropping 2% (w/w) sodium alginate solution into a 0.18 M gelling solution through a glass pipe (3.0 mm in outer diameter). CaCl₂, SrCl₂ and BaCl₂ were used as the gelling agents. The radius of the gel beads was changed from 1.63 to 2.23 mm, depending on the M/G ratio and the gelling agents. Alginate gel membranes were prepared by the cast method with a 0.18 M gelling solutions. The thickness of the gel membranes was changed from about 0.22 to 0.61 mm, depending on the M/G ratio and the gelling agents. The alginate gel beads and membranes were cured in the gelling solution for more than 12 h. The gelling temperature was 303 K and pH was 7.0.

1.2 Effective diffusivity of oxygen in alginate gel membranes

The experimental apparatus was the same as that described by Sun et al. (1989). The reactor was a cylindrical vessel (50 mm in diameter) and consisted of upper and lower chambers. The alginate gel membrane was set between the two chambers. The oxygen concentration in the upper chamber was set at zero by stripping the dissolved oxygen with nitrogen gas. The change in the oxygen concentration in the lower chamber was monitored with a DO meter. The change in DO concentration in the lower chamber is expressed by the following equation.

\[
\ln(C/C_0) = - \frac{AD_EK_F}{LV} t
\]  

(1)

where \( K_F \) is the partition coefficient of oxygen in the membrane. The value of \( K_F \) was reported to be unity for oxygen in Ca-alginate gels (Adlercreutz, 1986). In this work, we assumed that \( K_F \) is unity. The effective diffusivity \( D_E \) of oxygen in the alginate gel was determined by eq. (1). The working volume of the upper chamber was 200 ml and that of the lower chamber was 130 ml. The thickness \( L \) of the gel membrane was determined with a micrometer of which resolution was 1 \( \mu \)m. The effective membrane area \( A \) was 19.0 \( \text{cm}^2 \). All the diffusion experiments were conducted at a temperature of 303 K and a pH of 7.0. Diffusivity of oxygen in water \( D_w \) at 303 K is 2.56 x 10^{-9} \( \text{m}^2/\text{s} \) (Adlercreutz, 1986).

1.3 Effective diffusivity of nitrate and glucose in alginate gel beads

Effective diffusivity of nitrate and glucose in the gel beads was measured by use of the unsteady-state method (Tanaka et al., 1984). The initial concentration of nitrate was 11.1 kg/m³ and that of glucose was 50 kg/m³. The nitrate concentration was determined with an ion chromatography. The glucose concentration was determined by an enzyme-colorimetric method. Diffusivity of glucose in water \( D_w \) at 303 K is 6.80 x 10^{-10} \( \text{m}^2/\text{s} \) (Tanaka et al., 1984). The value of \( D_w \) of nitrate at 298 K is 1.90 x 10^{-9} \( \text{m}^2/\text{s} \) (Arvin and Kristensen, 1982). The value of \( D_w \) of nitrate at 303 K was estimated with the Stokes-Einstein equation.

1.4 Denitrification rate constant

1.4.1 Preparation of immobilized denitrifier

The denitrifier used was accumulated from the activated sludge for more than six months under anoxic conditions in our laboratory. The medium contained glucose, 28.6 g; peptone, 28.6 g; KH₂PO₄, 3.64 g; K₂SO₄, 15.0 g; MgSO₄·7H₂O, 7.84 g; NaCl, 6.40 g; KNO₃, 40.0 g in 1 liter of a distilled water.

A 4% (w/w) Na-alginate solution was added to the same volume of denitrifier suspension to obtain the desired cell loading. The denitrifier in the solution was well dispersed with an ultrasonic disperser. Immobilized denitrifier beads were prepared by dropping the solution into 0.18 M gelling solution through a glass pipe. The immobilized denitrifier gel beads were cured in the gelling solution for 3 h and stored in the solution containing 0.5% (w/w) gelling agent and 1.0% (w/w) glucose at 277 K. CaCl₂ and BaCl₂ were used as gelling agents. The radii of the gel beads was varied by changing the diameter of the glass pipe.

1.4.2 Experimental procedure

The reactor was a cylindrical vessel (100 mm in diameter) made of transparent acrylic resin with four baffle plates (13 mm in width). The liquid was agitated by a Teflon bar (35 mm in length) on a magnetic stirrer and the stirrer speed was 15 s⁻¹. The volume fraction of immobilized denitrifier in Ba-alginate gel was 8.1 – 9.4% and that in Ca-alginate gel was 9.8 – 10.1%. All the experiments were conducted at a temperature of 303 K and a pH of 7.0. The bulk DO concentration was controlled with a DO-controller. The initial nitrate concentration was 57.9 mg/l.
pended in 505 ml of a distilled water. The medium contained glucose, 10 g; pepton, 10 g; K₂H₂PO₄, 1.3 g; K₂SO₄, 4.2 g; MgSO₄·7H₂O, 2.2 g; NaCl, 1.8 g; KNO₃, 14.5 g in 1 liter of distilled water. The change in the nitrate concentration with time was measured. The plots of nitrate concentration against time yielded straight lines in all experiments. The denitrification rate constant $k_D$ was calculated from the slope of the line.

2. Results and Discussion

2.1 Preparation of immobilization matrix suitable for immobilization of denitrifier

An immobilization matrix suitable for immobilization of denitrifier requires larger diffusivity of nitrate and glucose and smaller diffusivity of oxygen. To prepare such a matrix, we investigated the effects of M/G ratio of alginate and the gelling agents on the effective diffusivity of oxygen, nitrate and glucose in the alginate gels.

2.1.1 Diffusivity of oxygen  

Figure 1 shows the effects of M/G ratio and gelling agents on effective diffusivity $D_E$ of oxygen in alginate gel. The ordinate shows the ratio of $D_E$ of oxygen to that $D_W$ in water. The relative diffusivity of oxygen was almost constant at M/G ratios ranging from 0.38 to 1.05. The relative diffusivity of oxygen in the Ca-alginate gel was about 0.8 and that in the Sr-alginate gel was about 0.7. The value of $D_E/D_W$ in the Ba-alginate gel was lowest among three kinds of gels and was only about one-half. That is, Ba-alginate gel was found to have low oxygen permeability.

2.1.2 Diffusivity of nitrate  

Figure 2 shows the effects of M/G ratio and gelling agents on $D_E$ of nitrate in the alginate gel beads. No significant effect of gelling agents on $D_E$ was observed. With increasing M/G ratio, the relative diffusivity of nitrate decreased slightly. At M/G ratios up to about 0.5, the relative diffusivity was about 0.9. It was found that nitrate can easily diffuse into the alginate gels at M/G ratios below about 0.5 independent of gelling agents.

2.1.3 Diffusivity of glucose  

Figure 3 shows the effects of M/G ratio and gelling agents on $D_E$ of glucose in the alginate gel beads. The relative diffusivity of glucose in Ca-alginate gels was almost equal to $D_W$ at M/G ratios up to about 0.4. With increasing M/G ratio above 0.4, $D_E$ in Ca-alginate gel decreased rapidly. The value of $D_E$ in Sr-alginate gels was almost constant at 0.9 at M/G ratio of 0.21, and decreased slightly with increasing M/G ratio. The decrease in $D_E$ of glucose in Ca-alginate gels with increasing M/G ratio was explained by a decrease in the network size of the gel structure (Yamagiwa et al., 1995). The results above show that alginate gel structure depended on the gelling cation. The affinity of divalent cation to alginate was reported in the order of Ba$^{2+}$ > Sr$^{2+}$ > Ca$^{2+}$ (Yonese et al., 1988). A difference in $D_E$ of glucose in Ca-, Sr- and Ba-alginate gels may attributed to the difference in the affinity of the cation to alginate.

The diffusion resistance of glucose in the alginate gel beads with M/G ratios up to about 0.5 was relatively small because the values of $D_E/D_W$ of glucose in these alginate gel beads was higher than 0.85.

2.1.4 Selection of matrix suitable for immobilization of denitrifier  

The requirements for immobiliza-
tion matrix of denitrifier are larger $D_E$ of nitrate and glucose and smaller $D_E$ of oxygen. The value of $D_E$ of oxygen in Ca-, Sr- and Ba-alginate gels was almost independent of M/G ratio. The value of $D_E$ of nitrate and glucose at M/G ratios below about 0.5 was larger than that at M/G ratios above about 0.5.

Figure 4 summarizes the effect of gelling agents on relative diffusivity of oxygen, nitrate, and glucose in alginate gels with an M/G ratio of 0.47. The value of $D_E/D_W$ of nitrate and glucose was higher than 0.85, regardless of the gelling cation. Nitrate and glucose can diffuse easily into three kinds of alginate gels. On the contrary, $D_E/D_W$ of oxygen in the Ba-alginate gel was only about one half, and the lowest among them. It was found from these results that Ba-alginate gel with M/G ratios below 0.5 meets the requirements.

Nitrate is ionic and glucose is a hydrophilic solute. Oxygen, however, is hydrophobic and hydrates hydrophobically with water. Diffusivity in the alginate gel may also depend on hydrophilicity or hydrophobicity of the diffusant.

2.2 Denitrification rate of immobilized denitrifier

2.2.1 Effect of bulk DO concentration on denitrification rate

The denitrification rate constant $k_{D0}$ by suspended denitrifier under anoxic conditions was 15.4 mg/g·h. This value was slightly larger than the reported value of 11.6 mg/g·h at 303 K and pH 7.0 (Ciziniska, 1992). The value of $k_{D0}$ of the immobilized denitrifier in Ba-alginate gel was 15.2 mg/g·h and that in Ca-alginate gel was 16.0 mg/g·h. That is, $k_D$ of the immobilized denitrifier was almost equal to that in the suspended cells. This result clearly shows that the diffusional resistance of nitrate and glucose in Ca- or Ba-alginate gel was negligible.

Figure 5 shows the effect of bulk DO concentration on $k_D$ of the immobilized denitrifier. The ordinate represents the relative denitrification rate constant, namely the ratio of $k_D$ to that under anoxic conditions $k_{D0}$. It should be noted that denitrification in suspended systems is completely inhibited at DO concentrations above about 0.2 mg/l (Dawson and Murphy, 1972). In the case of immobilizing denitrifier in Ba- or Ca-alginate, sufficient denitrification activity was obtained even if the bulk DO concentration was almost saturated. The Ba-alginate immobilized denitrifier had relative denitrification activities above 0.9 with DO concentrations up to about 2.5 mg/l. On the other hand, the relative denitrification activity of the Ca-alginate immobilized denitrifier decreased rapidly with increasing bulk DO concentration, and was only about 0.6 at DO concentration of 2.5 mg/l. The oxygen flux through Ba-alginate gel was smaller than that through Ca-alginate gel due to the smaller oxygen diffusivity. The volume of the anoxic core within the Ba-alginate beads was considered to be larger than that within Ca-alginate beads. The microenvironment within the Ba-alginate gel beads was found to be more suitable for denitrification than that within Ca-alginate gel beads. Therefore, successful denitrification is expected to proceed by immobilized denitrifier in the Ba-alginate gel irrespective of the oxygen level of the bulk solution.

2.2.2 Effect of cell loading on denitrification rate constant

Figure 6 shows the effect of cell loading on the relative denitrification activity of Ba-alginate immobilized denitrifier. The value of $k_D/k_{D0}$ increased with increasing cell loading up to about 3 g/l. The value of $k_D/k_{D0}$ at DO concentration of 2.2 mg/l was almost constant at cell loadings above
Fig. 7  Effects of beads radius and bulk DO concentration on relative denitrification activity

3 g/l. With increasing cell loading, the oxygen consumption rate increased and hence the thickness of the oxic shell decreased. At the cell loading of 6.92 g/l, \( k_D/k_{D,0} \) was almost unity. This result means that the presence of denitrifier hardly obstructed diffusion of nitrate and glucose.

The value of \( k_D/k_{D,0} \) at DO concentrations above 4.0 mg/l also increased with increasing cell loading up to about 3.0 g/l, and then leveled off.

These results show that a cell loading of about 3 g/l was optimal.

2.2.3 Effect of radius of gel beads on denitrification rate constant  

Figure 7 shows the effect of radius of gel beads on the denitrification activity. The value of relative denitrification activity of the beads of radius 1.35 mm rapidly decreased with increasing DO concentration while that of the beads radius 2.31 mm was almost equal to \( k_{D,0} \) at DO concentrations up to about 3 mg/l. At DO concentration of 3 mg/l, \( k_D/k_{D,0} \) of the immobilized denitrifier with radius of 2.31 mm was almost unity while that with radius of 1.35 mm was about 0.7. This is attributed to the increased volume ratio of the anoxic core to the oxic shell.

The denitrifier immobilized within Ba-alginate gel, with a cell loading of about 3 g/l and a radius of 2.31 mm showed relative denitrification rates more than 0.9 at bulk DO concentrations up to about 3 mg/l. The typical DO concentration in the conventional aerobic wastewater treatment is about 2 mg/l. From the results above, it is expected that denitrification will successfully proceed in the conventional aerobic wastewater treatment system by use of the Ba-alginate immobilized denitrifier. It is necessary to further investigate the effect of amount of organic carbon, bulk temperature and pH on denitrification of Ba-alginate immobilized denitrifier prior to the practical application of Ba-alginate immobilized denitrifier to aerobic wastewater treatment.

Conclusion

Preparation of an alginate gel matrix with low oxygen permeability was examined. The effects of M/G ratio and gelling agents on diffusivity of oxygen, nitrate and glucose were investigated. Based on the results obtained, we selected the matrix suitable for immobilization of denitrifier. Furthermore, the effects of bulk DO concentration, cell loading and bead radius on denitrification activity of the immobilized denitrifier was investigated. The results obtained are summarized as follows.

1) Effective diffusivity \( D_E \) of oxygen in Ba-alginate gel was only about one half compared to that \( D_w \) in water, and almost independent of M/G ratio. Ba-alginate gel was of low oxygen permeability.

2) Alginates gel with M/G ratios below 0.5 were preferable for immobilization of denitrifier because of larger diffusivity of glucose and nitrate.

3) Ba-alginate gel with M/G ratios below 0.5 was found to be suitable for immobilization of denitrifier.

4) Under anoxic conditions, immobilization of denitrifier within Ba- and Ca-alginate gel caused no activity loss.

5) At DO concentrations below 2.5 mg/l, the relative denitrification activity of Ba-alginate immobilized denitrifier was above 0.9.

6) The denitrification activity of Ba-alginate immobilized denitrifier was highest at cell loading of about 3 g/l and increased with increasing radius of beads.

Nomenclature

\[ A \quad \text{effective membrane area for oxygen penetration} \quad \text{[m}^2\text{]} \]

\[ C \quad \text{oxygen concentration in lower chamber} \quad \text{[kmol/m}^3\text{]} \]

\[ C_0 \quad \text{initial oxygen concentration in lower chamber} \quad \text{[kmol/m}^3\text{]} \]

\[ D_E \quad \text{effective diffusivity} \quad \text{[m}^2\text{/s]} \]

\[ D_w \quad \text{molecular diffusivity in water} \quad \text{[m}^2\text{/s]} \]

\[ k_D \quad \text{denitrification rate constant under anoxic condition} \quad \text{[mg/g-h]} \]

\[ k_{D,0} \quad \text{denitrification rate constant under anoxic condition} \quad \text{[mg/g-h]} \]

\[ K_P \quad \text{partition coefficient} \quad [-] \]

\[ L \quad \text{membrane thickness} \quad \text{[m]} \]

\[ r \quad \text{bead radius} \quad \text{[mm]} \]

\[ t \quad \text{time} \quad \text{[s]} \]

\[ V \quad \text{liquid volume of lower chamber} \quad \text{[m}^3\text{]} \]

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