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Flexible film-type catalysts encapsulating urease within κ-carrageenan hydrogel network

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HIGHLIGHTS

- We successfully immobilized urease in a thin film of a κ-carrageenan gel.
- The film-type catalyst is flexible enough to be molded into various shapes suitable for a specific reactor configuration.
- Urease is retained within the film during the hydrolysis reaction of urea.
- The immobilized urease exhibited catalytic activity comparable to that of free urease.
- The film-type catalysts can be used multiple times with minimal activity loss.

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ABSTRACT

Flexible film-type catalysts were synthesized by encapsulating urease within a κ-carrageenan gel network supported on a cellulose-acetate membrane filter. Synthesized film-type catalysts are flexible and can be easily formed into a shape that is suitable for a specific process configuration. The filter membrane has macropores about 200 nm, which are formed by interconnected bead-shaped membrane material. Scanning electron microscopy shows that the surface of the membrane material is coated homogeneously with the κ-carrageenan hydrogel. Urease entrapped within the gel network hardly leaches out during urea hydrolysis catalysis conducted at 310 K in a batch reactor and exhibits high catalytic performance. Analysis of the reaction data by the Michaelis–Menten equation shows that the film-type catalyst shows kinetic parameters (K_M and V_{max}) and apparent activation energy (24 kJ mol^{-1}), which are all similar to those found for free urease. Estimation of the mass transfer rate of urea within the κ-carrageenan network and the urea hydrolysis rate show that the diffusion rate of urea within the thin film is five orders of magnitude higher than the reaction rate. The film catalyst can be recycled at least 7 times with <20% decrease in activity. High flexibility as well as high catalytic performance of the film catalyst shows prospective features of this catalyst for future applications.

1. Introduction

Immobilization of enzymes in a support material allows easy separation of enzymes while taking advantage of their high reaction selectivity. Therefore, many investigations have been devoted to find suitable immobilization methods for enzymes [1–8]. One method to obtain an ideal immobilized enzyme catalyst is to encapsulate soluble enzymes within thin films of a solid support material, which prevents leaching of the enzyme from the support while allowing mobility of the enzyme and unrestricted transport of small molecules including reactants and products in and out of the porous network of the support material [9–12]. Thin and porous films allow fast diffusion of reactants and products via short diffusion path lengths so that the enzymes can exhibit their intrinsic catalytic properties [13–16]. Hard metal oxide materials such as silica encapsulating enzyme can be molded into thin films via sol–gel synthesis [17–20]. However, more flexible and deformable films are preferred because they can be structured into a form that is suitable for each specific process configuration. For example, a flexible film-type catalyst can be folded into a corrugated card board form that enables a low fluid resistance while allowing fast mass transfer by convection through straight channels and can process a large amount of reactants like honeycomb-type catalysts [21,22]. From this perspective, paper and membrane filters serve as an appealing scaffold to support enzymes. In addition to catalyst supports, these materials have been exploited in a wide variety

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of fields such as energy-storage devices and organic photodiodes [23,24].

Here, we report synthesis of flexible film-type catalysts by encapsulating urease within a κ-carrageenan gel that is supported on a cellulose-acetate membrane. Urease has been used in a wide variety of applications such as decomposition of urea to ammonia and carbon dioxide in water treatment, artificial dialysis, and biosensors [4,18,25,26]. Immobilization of urease has been investigated using various supports such as polymers, alginate, silica and chitosan [9,27–35]. We chose κ-carrageenan gel (Fig. 1) because it is known to be compatible with urease and allows it to retain its intrinsic catalytic performance [29]. κ-Carrageenan is a sulfated galactan polymer (Fig. 1) that is often used in the food industry and is expected to exhibit good adhesion on the surface of membrane filters via hydrogen bonding as well as electrostatic interactions. Furthermore, the stiffness of the κ-carrageenan gel network can be tailored to some extent by adjusting the potassium ion content within it [36–38]. Thus, urease can be encaged by adding an appropriate amount of potassium ions to κ-carrageenan containing urease. We used a cellulose-acetate membrane filter because it appears to be compatible with urease and it adds mechanical strength to κ-carrageenan gel. A porous membrane filter also induces capillary force and draws the aqueous solution into its pores and enables tight binding of κ-carrageenan to its surface. We demonstrate that leaching hardly occurs from the urease encapsulated within flexible film-type catalyst during urea hydrolysis at 310 K, and yet it exhibits high catalytic activity, which is similar to that of free urease revealed through basic kinetic analysis. We also show that the film catalyst can be recycled at least 7 times with minimal decrease in activity.

2. Experimental methods

2.1. Materials

Urease (Jack Bean, 130 U/mg, Wako Pure Chemical Industries Ltd.), κ-carrageenan, potassium chloride (KCl, 99.5%), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$, 99%), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$, 99%) and urea (NH_2CONH_2, 99%) were purchased from Wako Pure Chemical Industries and used as received.

2.2. Synthesis of flexible film-type catalysts

Synthesis of a flexible film-type catalyst was conducted as schematically shown in Scheme 1. Urease (0.05 mg) and κ-carrageenan (0.89 mg) were mixed in 50 mL deionized water at 323 K to prepare a sol (5.3 wt% urease with respect to the sum of κ-carrageenan and urease). A membrane filter (Advantec Toyo A020A047A, mixed cellulose ester, pore diameter = 200 nm, filter thickness = 0.133 mm, porosity = 80%) was immersed into this sol for 10 s. After the film was taken out of the solution, it was left in air at ambient temperature for 1 h. Then, after the excess gel was scraped off the membrane surface using a spatula, the film was soaked in an aqueous KCl solution for 3 h to strengthen the κ-carrageenan gel network.

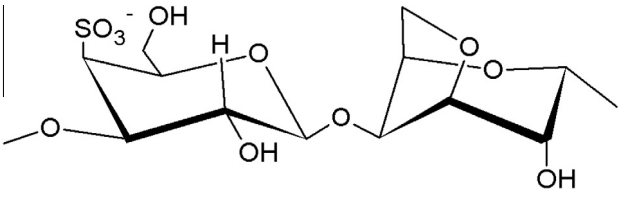

Fig. 1. The chemical structure illustrating the monomer unit of κ-carrageenan.

2.3. Synthesis of a cube-type catalyst

A cube-type catalyst was prepared from the same urease-containing the same κ-carrageenan gel to prepare the film-type catalysts. A portion of the gel was poured into a plastic petri dish and dried in air at ambient temperature for 1 h. The dried gel was immersed into a solution containing 0.5 mol L^{-1} KCl aq. for 3 h. After washing the gel with distilled and deionized water, it was cut into a 1-cm cube.

2.4. Characterization

The morphology of the synthesized catalysts was examined using a field emission scanning electron microscope (FE-SEM) (JEOL JSM-6500F) operating at an acceleration voltage of 10 kV. Before analysis, the samples were mounted on carbon tape. The samples were then coated with a thin layer of gold in a JEOL JFC-1200 sputtering unit to prevent sample charging during SEM analysis. The pore structure of the synthesized materials was characterized through nitrogen gas adsorption experiments using a gas adsorption analyzer (BELSORP-max, BEL Japan). For all measurements, approximately 80 mg of the sample was transferred to a pre-weighed glass sample tube and capped. Samples were then transferred to the analyzer and heated at 323 K under dynamic vacuum for 24 h. Evacuated tubes were refilled with nitrogen and then transferred to a balance and weighed to determine the mass of the sample. Then, the tube was transferred to the analyzer again. Nitrogen gas adsorption/desorption isotherms were measured at 77 K. Surface areas were calculated from BET plots and expressed per geometrical surface area of the membrane filter. Mesopore size distributions were determined by applying the DH method to the adsorption branches.

2.5. Urease hydrolysis reaction

Urease hydrolysis reactions were performed using a batch reactor at 310 K. A stock urea solution (200 mmol-urea L^{-1}) was prepared by dissolving 600.6 mg of urea in a pH 7.2 buffer solution, which had been prepared by dissolving 1.092 g of $NaH_2PO_4{\cdot}2H_2O$ and 6.45 g of $Na_2HPO_4{\cdot}12H_2O$ in 50 mL of distilled and deionized water. A 100 mL glass vessel was charged with 50 mL of the stock solution and its temperature was maintained at 310 K using a thermostat. A weighed film-type catalyst (255.6 mg, urease content of 8.605 μg) was added to the solution (corresponding urease concentration was 3.58×10^{-10} mol of urease per L of the solution) and stirred by using a straight-shaped Teflon-coated stirring bar (30 mm) and the transient change of the ammonia concentration in the solution was measured using an ammonia meter (Toko Chemical Laboratories, TiN-9001). As a control, the same reaction experiment was conducted using unsupported free urease under otherwise identical conditions. Hydrolysis of urea was also performed at various pH or temperature values ranging from 5.8 to 8.0, 305 K to 338 K, respectively.

Kinetic parameters for the film-type catalyst and free urease were determined using the Michaelis–Menten equation shown in Eq. (1) where r [mM (mg min)$^{-1}$], S [mM], V_{max} [mM (mg min)$^{-1}$], and K_m [mM] represent rate of hydrolysis, substrate (urea) concentration, maximum rate, and substrate concentration when r is 0.5 V_{max} (K_m is often denoted as Michaelis–Menten constant), respectively. To determine K_m and V_{max}, the inverse rate ($1/r$) was plotted against the inverse substrate concentration ($1/S$) and the resultant data were fitted with Eq. (2) [Lineweaver–Burk plot]:

$$r = \frac{V_{max} S}{K_m + S} \quad (1)$$