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Polyaniline nanofiber coated monolith reactor for enzymatic bioconversion

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ABSTRACT

The monolith reactor has many advantages over other reactors including good mass transfer, low-pressure drop and high enzyme stability. In this study, an enzyme-coated monolith with high enzyme stability and activity was developed. The monolith surface was coated with polyaniline nanofibers containing glucose oxidase immobilized on its surface. The developed enzyme-coated monolith was used for the bioconversion of glucose to glucolactone at different residence times. The conversion ratio increased up to 83% at increasing residence time. In addition, the polyaniline-coated monolith showed excellent enzyme activity and long-term stability. The stability of the enzyme-coated monolith containing the polyaniline nanofibers showed almost 100% residual activities after 8 days operation, while rapid enzyme deactivation and detachment was observed when the enzymes were immobilized without the polyaniline nanofibers.

1. Introduction

Monoliths are referred to as honeycomb structures, and are essential for improving interfacial mass transfer and increasing the surface area. In addition, they are easy to scale up. Parallel straight channels separated by thin porous silica walls with a functional surface have been widely used as structured catalysis for chemical reactions, such as hydrogenation, decontamination and proteomic analysis [1–4]. The channels of most common honeycomb monoliths normally have square cross-sections. Monolithic reactors are generally operated after being filled with a porous catalytic material or after a catalytic material has been immobilized on the channel surface [5,6]. In many cases, enzymes that have been immobilized on the channel surface function as a biocatalyst [7–10].

Monolith catalysts or monolith reactors have many advantages over packed bed reactors in several applications, including a lower pressure drop, particularly under high fluid throughput, and a higher specific external catalyst surface area for mass transfer and various reactions. In addition, when this approach is used fouling and plugging are significantly reduced and the catalyst lifetime can be increased [11].

Enzymes have been immobilized on monolithic supports that were modified with (3-aminopropyl) triethoxysilane and sugar, and activated with glutaraldehyde [12]. The kinetics of monolith-supported enzymes have been analyzed in a continuous tubular reactor system. Due to the advantages of this system, there is a low resistance to flow and lower pressure during continuous operation [13]. However, these monoliths, which are typically produced by extrusion, are large and the immobilization efficiency is often quite low [14].

The immobilization of enzymes on several inorganic monolithic supports has been studied and it has been reported that immobilized enzymes can be used to run complex biotechnological processes [1,2,8,15]. A monolith-supported ionic liquid phase for enzyme immobilization has been previously used but the operational catalytic activity was maintained for only 5 h [16]. Although the monolith has advantages in terms of enzyme immobilization, the stability of the immobilized enzyme is currently unsatisfactory. Therefore, for practical application of enzyme-coated monoliths, the enzyme stability needs to be extended.

In this study, a very stable polyaniline-coated monolith reactor with a cross-linked aggregation of enzyme was developed. Polyaniline nanofibers, which consist of conducting polymer nanowires and nanofibers, have attracted increasing interest and hold great promise for use as active components in nanoscale molecular devices and can be used as templates to immobilize enzymes and confine them in a polymer matrix. The stability and performance of the developed monolith enzyme reactors were examined at various residence times.

2. Materials and methods

2.1. Materials

Glucoseoxidase (EC 1.1.3.4) from *Aspergillus niger* (Type VIIS), o-dianisidine dihydrochloride, beta-D-glucose, and peroxidase were purchased from Sigma Chemical Co. (St. Louis, MO). All salts were of analytical-grade and used without further purification. All solvents were of the highest quality commercially available.

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2.2. Preparation of the supporting nanofibers

Polyaniline nanofibers were made from rapidly mixed reactions. 0.1% (weight fraction) of an initiator (ammonium peroxydisulfate) and aniline monomer solutions (15%) in 1 M HCl were mixed rapidly [17]. After the initiator molecules were depleted during the formation of the nanofibers, no further polymerization leading to overgrowth was observed. The samples were washed four times with distilled water to eliminate the remaining HCl and stored in a refrigerator until needed.

2.3. Development of monolith enzyme reactor

Monolith, which was kindly donated by Heesung Engelhard Co. (Ansan, Korea), was used for enzyme immobilization. Monolith containing nine cells (each cells: 0.8 mm × 0.8 mm × 57.6 mm) was used to immobilize glucoseoxidase (GOx). Two different immobilization methods were used. One of the methods was to directly cross-link the enzyme to the monolith surface and the other was to immobilize the enzyme to a polyaniline nanofiber packed monolith reactor. For the development of the polyaniline nanofiber packed monolith reactor, monolith was coated with polyaniline nanofibers (Fig. 1(a)). 30 ml of the nanofiber mixture solution (nanofiber was 60 mg) was circulated to form a PANI film on the surface of the monolith.

A plug flow process was developed for enzyme coating. The system was connected to a low speed peristaltic pump (10 ml/h, Watson-Marlow, UK). Two types of monoliths were incubated in 20 ml of 100 mM sodium phosphate buffer (pH 7.0) containing 200 mg of GOx. The enzyme solution was circulated (10 ml/h) using a peristaltic pump at room temperature for 30 min, and placed in a refrigerator for 2 h. A glutaraldehyde (GA) solution was then added (final GA concentration was 0.2%, w/v), and the mixture was circulated (10 ml/h) with a peristaltic pump overnight at 4 °C. The enzyme-immobilized monoliths were washed with 10 mM phosphate buffer (pH 7.0) and 100 mM Tris–HCl (pH 7.9). The unreacted aldehyde groups were capped by incubating the monolith in 100 mM Tris–HCl (pH 7.9) buffer for 30 min. After capping, the monolith was washed extensively with 100 mM sodium phosphate buffer (pH 7.0) until no enzyme was present in the washing solution. The enzyme-coated monolith was stored in 100 mM sodium phosphate (pH 7.0) at 4 °C for further use.

2.4. Scanning electron microscopy (SEM)

The surface morphology was examined by scanning electron microscopy (SEM Hitachi S-4800 Hitachi, Tokyo, Japan). Images were obtained before and after GOx immobilization. The SEM images were digitized under the following conditions: files, 1280 × 960 pixel; voltage, 15 kV; probe size, 20 nm; and magnification, 10,000×. The photographs represent a magnification of 10,000×.

2.5. Protein assay

The protein concentration was determined using the Lowry method with the Folin reagent, and the absorbance was read at 590 nm using a spectrophotometer [18]. The protein concentration was determined with a Bio-Rad protein assay kit (Hercules, CA) using bovine serum albumin (BSA) as the standard.

2.6. Enzyme assay

2.6.1. Glucose oxidase

GOx activity was determined using a colorimetric assay, which is based on the oxidation of o-dianisidine in a peroxidase-coupled system. GOx catalyzes the oxidation of glucose to gluconic acid producing hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide participates in a reaction involving *p*-hydroxybenzoic acid and 4-aminoantipyrine and a quinone imine dye complex is formed, which can be measured at 500 nm. The reaction substrate contained 0.98 ml of a reaction cocktail (0.17 mM of o-dianisidine and 95.7 mM of glucose solution) and 0.01 ml of peroxidase (600 units/ml) were used to measure GOx activity. After addition of 0.01 ml of free or immobilized GOx (2 mg/ml), the absorbance at 500 nm was monitored as a function of time. The GOx activity and the stability of the immobilized monolith were assessed from the changes in glucose concentration in the product solution. The experiments were performed in triplicate, and the data were normalized.

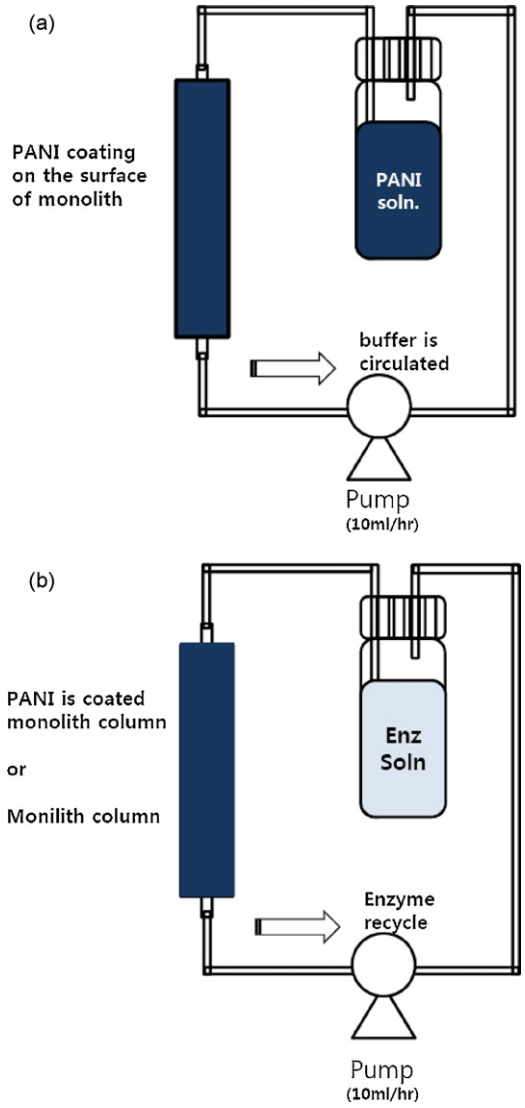

Fig. 1. Schematic diagram of (a) PANI coating on the surface of monolith and (b) enzyme immobilization using cross-linked enzyme aggregation.

3. Results and discussion

3.1. SEM image analysis

In this study, enzymes were immobilized on one of the most commonly used structured reactors, the monolith reactor. The monolith reactor has several advantages over other structured

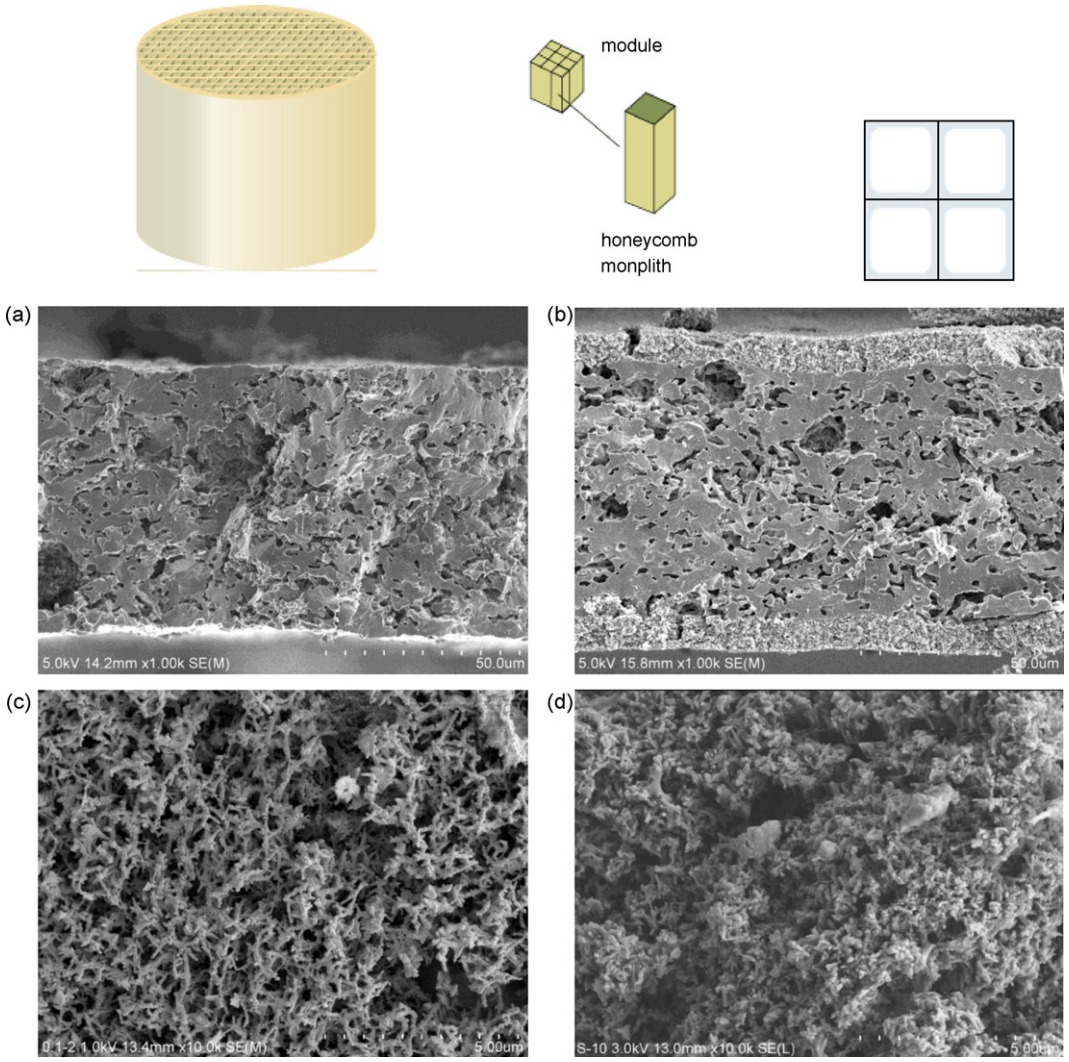

Fig. 2. SEM images of the cross-sectional area of the (a) monolith, (b) monolith coated with PANI, (c) surface of the PANI and (d) surface of the GOx immobilized on PANI.

reactors including a low-pressure drop and high surface area. The monolith surface was coated with polyaniline nanofibers to increase the surface area and enzyme immobilization capability. The prepared polyaniline-coated monolith contained a larger number of nanopores than the monolith without polyaniline. As shown in Fig. 2, the monolith was coated with 10 μm of the polyaniline nanofiber, which resulted in aggregated fibrous nanofibers containing nanopores. The monolith supports allowed PANI to be deposited. The higher enzyme loading on the PANI resulted in a greater activity per unit monolith volume, which corresponds to the amount of adsorbed enzyme. The enzymes were tightly aggregated on the surface of the polyaniline nanofiber and showed high stability for enzyme bioprocessing.

3.2. Evaluation of bioconversion using GOx immobilized monolith reactor

3.2.1. GOx immobilization efficiency after PANI coating

The enzyme loading capacity of the monolith was determined by measuring the protein content of the effluent stream. 83% of GOx was immobilized on the polyaniline nanofiber coated monolith and only 8% of GOx was immobilized on the monolith that was not coated with polyaniline (Fig. 3). Since the polyaniline nanofibers contained many nanopores and had a large surface area for GOx immobilization, the binding of GOx to the monolith

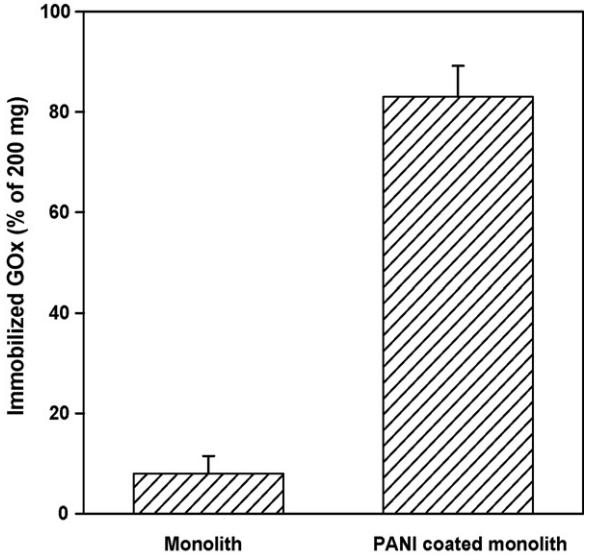

Fig. 3. Effects of the polyaniline nanofiber coating on GOx loading. GOx was immobilized on the surface of the monolith and PANI coated monolith through cross-linked aggregation.

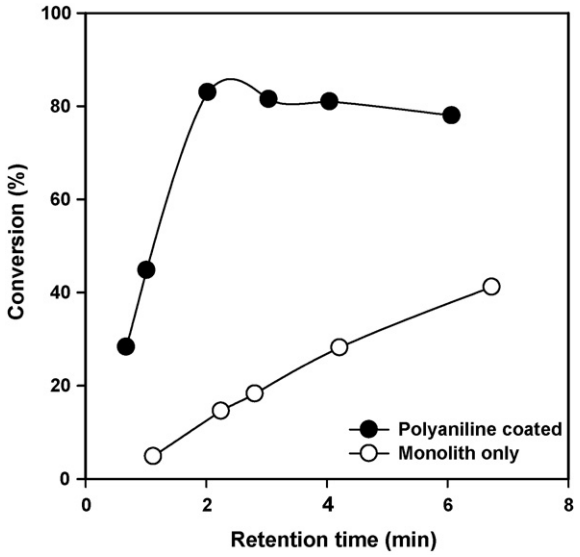

Fig. 4. Effect of the residence time on glucose conversion. The glucose solution was fed into the monolith reactor and the product concentration was measured.

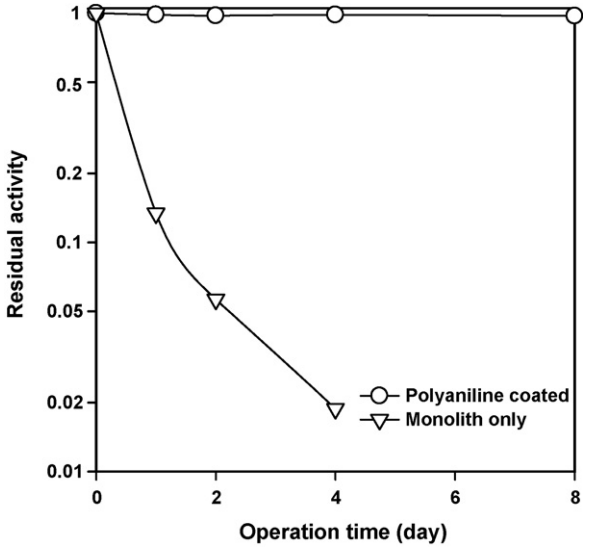

Fig. 5. Operational stability of GOx immobilized on the monolith and PANI coated monolith. The substrate glucose solution was fed continuously into the monolith reactor and the relative activity was calculated from the ratio of residual activity at each time point to the initial activity.

appeared to occur through surface adsorption and covalent attachment. The monolith surface consisted of silica, and its chemical bond was barely altered by immobilization, resulting in a very weak adsorption comparable to covalent attachment to the polyaniline nanofiber. The specific activity of GOx on the polyaniline-coated monolith was found to be significantly higher than on the monolith only. de Lathouder et al. [11] previously proposed using advanced ceramic material l(ACM) for enzyme immobilization. Although this approached affords good accessibility and higher mass transfer, the enzyme loading was low relative to that of the PANI coated monolith.

3.2.2. Effect of residence time on glucose conversion

The effects of residence time (τ) on glucose conversion with GOx were examined by changing the feed rate from 8 ml/h (τ = 6.06 min) to 72 ml/h (τ = 0.67 min). The glucose conversion was presented in Fig. 4 and was shown to increase with retention time.

When the polyaniline-coated monolith was used for glucose conversion, the conversion increased linearly up to 83% (at a feed flow rate of 24 ml/h and τ = 2.02 min). However, there was no further increase in conversion despite a 3-fold increase in residence time (Fig. 4). This result showed that the glucose diffusion rate into the PANI was the rate-limiting step in this case. The glucose conversion and mass transfer occur simultaneously around the contact plane. Leeuwen et al. [19] previously measured the glucose transfer rate in a microchannel. The glucose mass transfer rates were high at the interface but its diffusion rates were very low in the nanopores [20] as was the conversion ratio.

When the enzyme-immobilized monolith was used for glucose conversion, the conversion ratio increased with increasing residence time. Mass transfer resistance was low for the monolith reactor, and the conversion increased with increasing residence time.

3.2.3. Stability of GOx immobilized monolith reactor

The catalytic stability of the enzyme-immobilized monolith and polyaniline nanofiber coated monolith containing immobilized enzymes were investigated by continuously feeding the reactor a buffer solution (residence time 2.0 min) for 4 days at room temperature. At predetermined times, the remaining activity was measured by comparing the exit glucose concentration with the initial glucose concentration. The leaching of GOx was also monitored by measuring the protein content in the aqueous buffer solution at each time point.

Fig. 5 depicts the results of these operational stability measurements on the enzyme-immobilized monolith reactor. The activity of the enzyme-immobilized monolith rapidly decreased due to the detachment of enzymes, while the adsorbed and covalently attached GOx on the polyaniline nanofiber coated monolith showed significantly improved enzyme stability. When the enzyme-immobilized monolith was used, a small amount of protein was observed in the effluent stream due to the weak attachment of the enzymes on the monolith surface. However, no proteins were detected in the effluent stream when the polyaniline-coated monolith reactor was used. Polyaniline nanofiber coated monolith showed enhanced catalytic stability with no decrease in activity after 8 days. In contrast, the GOx immobilized monolith became rapidly inactivated as a result of enzyme detachment.

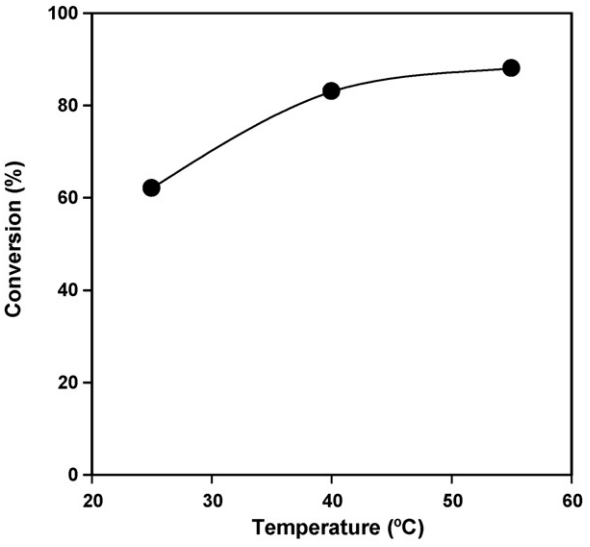

Fig. 6. Effect of temperature on glucose conversion. The glucose retention time was controlled at 2.0 min and the temperature of the reactant and monolith reactor was controlled using a constant temperature bath.

3.2.4. Effect of temperature on glucose conversion

The effect of temperature on conversion was measured at a constant residence time (τ = 2.0 min). At increasing temperature, the enzyme conversion increased to more than 88% through increased molecular diffusion rather than through an increased reaction rate (Fig. 6). The enzyme reaction rate with the free GOx increased with increasing temperature. However, the increase in glucose conversion at high temperature was small relative to that of free enzyme [21]. This shows that the enzyme reaction rate was limited by glucose diffusion rates in the nanopores of PANI.

4. Conclusion

The polyaniline nanofiber coated monolith was demonstrated to be suitable for enzyme immobilization and its enzyme stability was quite high. The polyaniline nanofiber coating was very thin (10 μm thicknesses) and contained many nanopores. However, enzyme immobilization on the monolith only was unstable and its activity rapidly decreased after 1 day as a result of enzyme detachment. The conversion of glucose by glucose oxidase was 83% with a residence time of 2.0 min. The level of conversion did not increase with increasing residence time due to substrate mass transfer limitations.

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