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Mixing and Enzyme Reactions in a Microchannel Packed with Glass Beads

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Abstract−Mixing behavior in a microchannel was investigated by means of a spectrophotometer equipped with optical fiber probes. Microreactors with a Y-shaped channel were fabricated on poly(methyl methacrylate) substrates. Glass beads were packed in the intersection and the downstream regions to improve mixing performance. An NaOH solution and a BTB solution as a pH indicator were fed to the microchannel with a syringe pump. As the mixing progressed, the color of the mixed solution changed to blue. The degree of mixing was evaluated by the change in absorbance at 623 nm of the mixing solution. The packed glass beads strongly enhanced the mixing performance in the microchannels, especially in the case of packing in the intersection. The effect of mixing in the microchannel on an enzyme reaction was also investigated. The hydrolysis of o-nitrophenyl-β-D-galactopyranoside by β-galactosidase was used as a model reaction. The results showed that the enzyme reaction was enhanced in the microreactor with glass beads compared with a batch reactor. The microreactor packed with glass beads gave the highest reaction rate.

Key words: Microchannel, Mixing, Enzyme Reaction, Glass Beads, o-Nitrophenyl-β-D-galactopyranoside

INTRODUCTION

Liquid flow in a microchannel is characterized by a low Reynolds number. When two or more miscible liquids are mixed in a microchannel under laminar flow conditions, mixing is controlled essentially by molecular diffusion, and it is difficult to achieve a uniform mixing state within a short period of time. Therefore, mixing is a key factor in microreaction technology. A number of micromixers have been developed to enhance the mixing in microchannels [Ehrfeld et al., 2000]. Splitting and recombining of liquid streams result in rapid mixing due to a decrease in the diffusion distance. Elaborate microstructures have been fabricated in microchannels to produce multiple flow splitting and recombination [Hinsman et al., 2001; Bertsch et al., 2001; Schwesinger et al., 1996; Hessel et al., 2003].

Reaction systems with a packed bed in a liquid stream through microchannels have recently been reported. The hydrogenation of cyclohexanes was investigated for a gas-liquid-solid reaction system in a microfabricated packed bed reactor [Losey et al., 2001]. A bead bed immunoassay system was structured on a microchip for cancer diagnosis [Sato et al., 2001]. Sequential enzyme reactions can be carried out immobilizing catalysts on microbeads, which were packed into two microchannel reaction zones [Seong et al., 2002]. Although the results indicated that microbeads promoted mixing, no quantitative evaluation of mixing was reported.

In this study, an attempt was made to improve the mixing performance in microchannels by disturbing the flow with glass beads packed in the channel. In order to evaluate the degree of mixing in the microchannel, the change in absorbance during the mixing of NaOH and BTB solutions was determined by *in-situ* spectrometry by means of optical fiber techniques.

EXPERIMENTAL SETUP

1. Mixing

Microreactors with a microchannel (400 μm in width, 200 μm in depth) were mechanically fabricated on poly(methyl methacry-

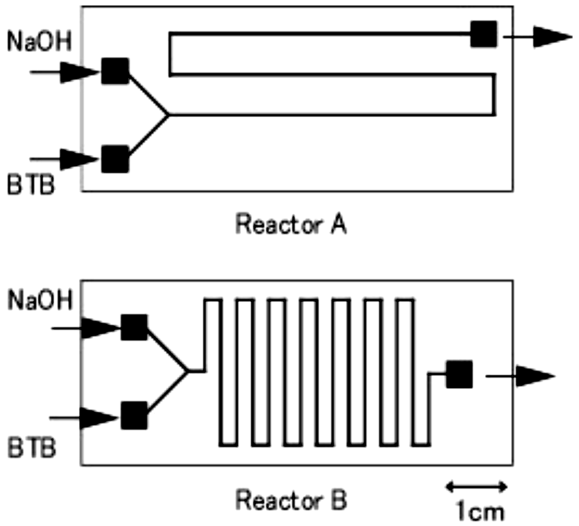

Fig. 1. Schematic of microchannels (400 μm in width and 200 μm in depth) with and without hairpin loops.

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late) substrates (3 cm×7 cm×5 mm) by using a compact numerically-controlled drilling machine (MicroMC, PMT Corp., Japan) with a flat end mill (400 μm in diameter). The top plate assembly was prepared by thermo compressing bonding at 120 °C under a vacuum for 4 hours. Fig. 1 shows the shapes of the microchannel in two microreactors (A and B). The lengths of the microchannels, L, in Reactor A with two hairpin loops and Reactor B with fourteen hairpin loops were, respectively, 180 mm and 384 mm. A 6.4 mmol/L NaOH solution and 6.4 mmol/L bromothymol blue (BTB) solution, as a pH indicator, were introduced into a Y-shaped inlet region using a microsyringe pump. As the mixing progressed, the color of the mixed solution changed to blue. The absorbance at 623 nm, A, was measured spectrophotometrically (Shimadzu, MultiSpec-1500) with optical fiber probes (320 μm in diameter). The two probes located on and underneath the microreactor were configured to move concurrently along the microchannel. By moving the probes in a direction parallel to flow, the average value of the absorbance in an incompletely mixed flow could be determined.

Absorbance of the BTB solution and an equivalent mixture of BTB and NaOH solution were used to define the degree of mixing. When no mixing occurs, half of the channel is filled with the BTB solution and the other half with transparent liquid, and thus the absorbance across the microchannel can be written as $A_{min}/2$, where A_{min} is the absorbance of the BTB solution. When the mixing is complete, the absorbance becomes equal to that of equivalent mixture of BTB and NaOH solutions. The degree of mixing, M, of the fluids in the microchannel was defined by the following equation.

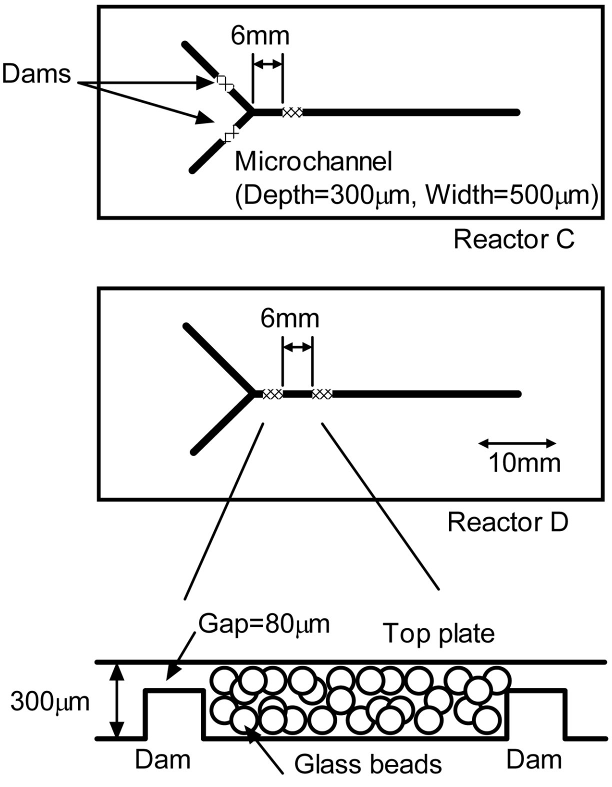
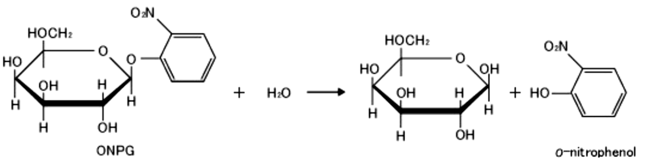


Fig. 2. Schematic of a microchannel packed with glass beads (top: Reactor C, bottom: Reactor D).



Scheme 1. Hydrolysis of o-nitrophenyl-β-D-galactopyranoside with β-galactosidase.

$$M = \frac{A - A_{min}/2}{A_{max} - A_{min}/2} \qquad (1)$$

where A is the absorbance across the microchannel, and A_{max} is the absorbance of the equivalent mixture of BTB and NaOH solutions.

Fig. 2 shows the microreactors packed with glass beads (150 μm in diameter). They will be referred to as Reactors C and D in the followings. In order to prevent escape of the beads, dam structures were constructed in the intersection and the downstream regions of the microchannel (500 μm in width, 300 μm in depth). The gap between the dam and the top plate was 80 μm. In Reactor C, the length of the packed region in the downstream of the junction was 6 mm. The distance between the dams was 6 mm for Reactor D. The interval between the dams was filled with the glass beads before the top plate was placed.

2. Enzyme Reaction

The hydrolysis of o-nitrophenyl-β-D-galactopyranoside (o-NPG) by β-galactosidase was used as a model reaction (Scheme 1). The reaction was carried out in Reactors C and D with and without glass bead packing. A teflon capillary tube (500 μm in inner diameter) of a suitable length was connected to the microreactor to ensure the sufficiently long residence time for the reaction. This was necessary since the residence times in Reactors C and D were shorter than 2 seconds, while the time needed for the enzyme reaction was longer than 100 seconds. The length of the teflon tube used was varied from few centimeters to 1 meter according to the residence time of interest. A normal scale batch reaction was also performed in a 100 mL flask for comparison. o-NPG (0.33 mmol/L) in McIlvaine buffer (pH 4.5) and 0.1 mg/mL β-galactosidase from *E. coli* (E.C.3.2.1.23) in the same buffer were introduced into the microchannel by pumping at an identical flow rate of 6 mL/h. The reaction was carried out at 52 °C. When microreactors were used, the reaction mixture was dropped to a 1 mol/L sodium carbonate solution to terminate the reaction. In flask experiments, the reaction was quenched after mixing the reaction mixture with a 1 mol/L sodium carbonate solution at a fixed ratio. The concentration of o-nitrophenol was measured from the absorbance at 420 nm.

RESULTS AND DISCUSSION

A distinct two phase flow of NaOH and BTB solutions was observed at the junction region of the microchannel. The boundary between both solutions became gradually unclear as the solutions moved down the channel due to the mixing and mixed solution near the boundary turned to blue in color. Fig. 3 shows the effect of flow velocity on mixing behavior in Reactor A. The degree of mixing, M, was not influenced by the flow velocity, u, at the same residence time because the mixing behavior in the microchannel was principally controlled by molecular diffusion. However, the degree of mix-

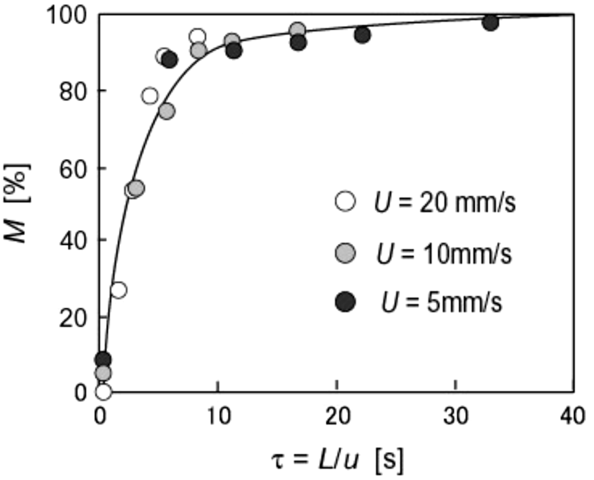


Fig. 3. Effect of flow velocity on the degree of mixing in a microreactor (Reactor A).

ing by molecular diffusion could reach 90% within 10 seconds of residence time (τ=L/u).

When two aqueous solutions are brought into contact in a closed vessel (2Y in width), component A in one solution and component B in the other solution begin to diffuse into each other. The differential equation for the diffusion of component A in the unsteady state is as follows.

$$\frac{\partial C_A}{\partial t} = D_A \frac{\partial^2 C_A}{\partial y^2} \tag{2}$$

at t=0, 0<y<Y: $C_A = C_{A0}$
t=0, −Y<y<0: $C_A = 0$
y=±Y: $\partial C_A/\partial y = 0$

Where t is the elapsed time, y is the distance from the center line, C_A is concentration of A and D_A denotes the diffusion coefficient. The average concentration of component A in each phase is solved analytically as

$$\frac{\overline{C_A}}{C_{A0}} = \frac{1}{2} \pm \frac{1}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(n+0.5)^2}\exp[-\{(n+0.5)\pi/Y\}^2 D_A t] \tag{3}$$

When Y=200 μm and $D_A = 1.5\times10^{-9}$ m^2 s^{-1} were assumed, the elapsed time for attaining 90% of mixing ($\overline{C_A}/C_{A0}$=0.45 or 0.55) was calculated to be 25 seconds from Eq. (3). The experimental mixing time was shorter than the calculated one. This can be attributed to the nonlinear relationship between the average concentration and the average absorbance.

In order to prepare a compact microreactor with a long residence time, many hairpin loops of microchannel must be created in the microchannel. Because of this, the effect of hairpin loops on the mixing behavior in a microchannel was investigated. As shown in Fig. 4, hairpin loops greatly affected the mixing behavior in a microchannel. The degree of mixing in Reactor B with fourteen hairpin loops was higher than that in Reactor A at same residence time. Kawazumi et al. [2002] investigated the behaviors of a two phase flow consisting of red and green ink solutions in a microchannel with eighteen hairpin loops and observed that the two fluids mixed after the first turn, but they separated out after the second turn. They argued that this is due to the secondary flow induced by cornering inertia. Yamaguchi et al. [2004] observed laminar flow behavior in a microchannel using a confocal fluorescence microscopy and analyzed it via a computational fluid dynamics simulation. The secondary flow by centrifugal force was found to significantly affect flow behavior at the hairpin loop in a microchannel. As a result, the cross-sectional shape of a two-phase flow was distorted at the loop. Therefore, mixing in the microchannel is promoted by the secondary flow at the hairpin loop region and by an increase in the surface area of the boundary face.

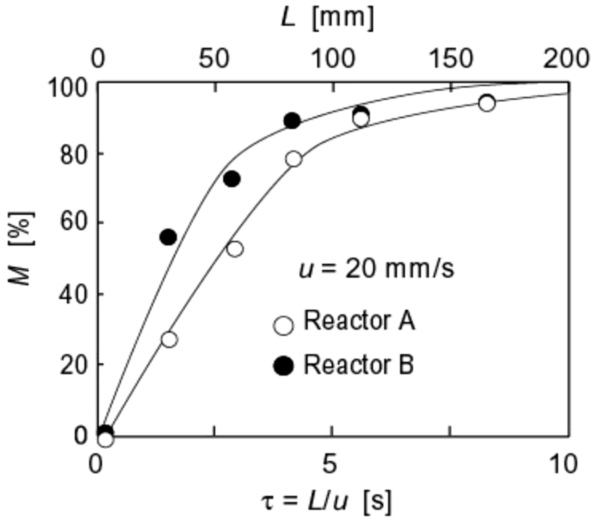


Fig. 4. Effect of hairpin loops on the degree of mixing in a microchannel.

Glass beads were introduced into the microchannel, as illustrated in Fig. 2, to enhance mixing. Fig. 5 shows the effect of glass beads packing on the degree of mixing. When the glass beads were not packed in the channel, the dam structures in microchannel caused a contraction in the flow and an expanding flow, which had little influence on the mixing of the two-phase flow. The packing at the

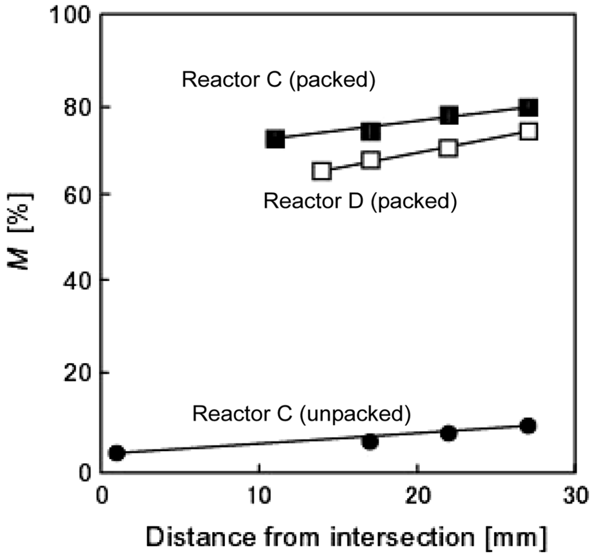


Fig. 5. Mixing behavior of a microchannel packed with glass beads. Flow velocity=22 mm/s.

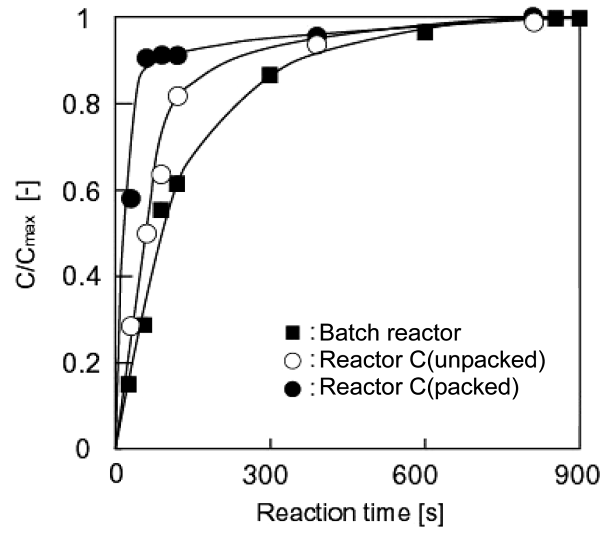


Fig. 6. Comparison of reaction rates in continuous flow-type microreactors and a conventional batch reactor. Flow velocity=11 mm/s.

intersection region was most effective for mixing. Since the apparent flow velocity was fixed at 22 mm/s in this experiment, the degree of mixing in the Reactor C packed with glass beads reached a level of 80% perfect mixing within a residence time of 1 second.

The hydrolysis of o-NPG was carried out in the microchannel illustrated in Fig. 2 and in a batch reactor (100 mL flask). In Fig. 6, the vertical axis presents the normalized concentration of o-nitrophenol by its concentration at equilibrium, C/C_{max}. In each case, the enzyme reaction reached an equilibrium state after a residence time of 600 seconds. In addition, the equilibrium concentrations of o-nitrophenol in these reactors were almost the same. The reaction in the microreactors was faster than that in the batch reactor [Tanaka et al., 2000; Kanno et al., 2002; Miyazaki et al., 2001]. In a flask stirred at an agitation rate of 300 rpm, the complete mixing of a total 20 mL solution can be usually attained within several seconds. On the other hand, Figs. 3 and 4 show that more than 10 seconds is required to attain complete mixing in a microchannel without packing. Accordingly, the mixing behavior in the early stage had little influence on the reaction rate.

Several other hypotheses are suggested as the reason for acceleration of an enzyme reaction in a microreactor. For example, the unidirectional laminar flow in microchannels forms a stable shear force field, which may affect the orientation of the enzyme and its reactivity. Absorption of enzyme molecules on the surface can also be a possible reason. A further investigation is required to clarify the reason for the acceleration.

CONCLUSIONS

The degree of mixing in a microchannel was determined from the change in absorbance caused by neutralization during the mixing process of NaOH and BTB solutions. When glass beads were packed at the intersection region of a microchannel, the mixing rate was greatly enhanced. Enzyme reactions in microchannel reactors are faster than that in a normal scale batch reactor with a stirrer. In the microchannel reactor without glass bead packing, almost complete mixing (the degree of mixing=90%) was attained within 10 seconds. Accordingly, mixing behavior in the early stage had little influence on the apparent reaction rate. However, the microchannel reactor with glass bead packing showed the highest performance for the enzyme reaction.

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