Visualization of Fluidic and Reaction Dynamics in Microchannels

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ABSTRACT

An easy-to-use and low cost microreactor made of polymethylmethacrylate was mechanically fabricated with a microchannel (200 μm x 200 μm). The laminar flow behavior was visualized by using CCD camera images of red and green aqueous solutions. Numeric Data of red and green components in the images could reveal the fluidic behavior in the microchannel because the spatial spectroscopic information corresponds to the fluidic dynamics of the color solution. Effects of corner shapes in a turn, flow rate and surface roughness were observed on the mixing of the laminar flows. The progress of an enzyme reaction, hydrolysis with β-galactosidase, was also visualized by using the absorption of the product. Nonuniform distributions of the reaction occurrence led to an apparent enhancement of the reaction.

Keywords: microchannel, PMMA, laminar flow, enzyme reaction, galactosidase.

1 INTRODUCTION

Miniaturized chemical analytical systems, that is micro total analytical systems (μTAS), have been attracted interest owing to the advantages of fast analysis time, reduction of reagent consumption, portability and disposability [1, 2]. Miniaturized synthesis systems, that is microreactors, have been also developed and have advantages in preventing from explosibility and toxicity. The precise temperature control and the simple piling-up of the chips for the mass production are also practical feature of the microreactor [3]. An organic synthesis reaction has been demonstrated with better performance in the reaction time and yield [4]. These profitable aspects are mostly resulting from the rapid mass transfer in micro-scale and the large specific interfacial area to volume. The acceleration of enzyme-catalyzed reaction was also reported [5 - 7]. The flow mixing containing the enzyme and substrate enhanced the reaction efficiency in the microchannel with a few hundred micrometer dimensions in comparison with batch reactions. Miyazaki et al. demonstrated a 20-fold increase of the reaction rate in a trypsin-catalyzed hydrolysis [5]. Kanno et al. reported the enhancement of basic carbohydrate-related reactions which are one of the most important chemo-enzymatic reactions [7]. In these simple reactions, it is difficult to consider an increase of the intrinsic reaction rates. Therefore, effects of specific fluidic behavior in the microchannel might be taken into account such as disorder of the interface and mixing between the laminar flows.

For practical applications of the microreactor in the analysis and synthesis, the development of easy-to-fabricate and low cost microfluidic devices is very important. Polymethylmethacrylate (PMMA) is one of most attractive material because of hardness and transparency. Miyazaki et al. [5] observed the acceleration of the enzymatic reaction in a PMMA microreactor with in a glass-fabricated one [6]. However, effects of the PMMA surface still may be evaluated with respect to its roughness and hydrophobicity. To study the microfluidic behavior, the flow should be visualized with simple spectroscopic technique and analyzed quantitatively [8].

In this paper, we observe color solutions in the microchannel with a color CCD camera and simply convert the color information into the fluidic behavior in the channel configuration of the PMMA microreactor. The hydrolysis reaction of β-galactosidase is also measured in the microchannel by using the high resolution CCD camera. The product distribution imaging could reveal the reaction dynamics in details.

2 EXPERIMENTAL

2.1 Fabrication of the Microchannels

The microreactor with the microchannel (200 μm in width, 200 μm in depth, 40 cm in length) was mechanically fabricated on a PMMA plate (3 cm x 3 cm x 5 mm) using Robodrill α-T14As (FANUC, Japan) equipped with a flat end mill (ϕ 100 μm). The top plate assembly of PMMA was covered by adding pressure (2 kg) and heat (120 °C and 4 hour). The machining precision was measured by a three dimensional profile microscope VK-8500 (Keyence, Japan) with the resolution of 0.03 μm. The accuracy was less than 3 % for specified dimensions. The channel length of 40 cm needed the 18 turns in the small plate. The turn with a round, square and acute (5.5 degree) shape was also machined in the same accuracy. The machining speed strongly affected the roughness of PMMA surfaces, as shown in Figure 1. The roughness was less than 0.7 μm

and 40 μm at the machining speed of 10 mm/min and 200 mm/min, respectively.

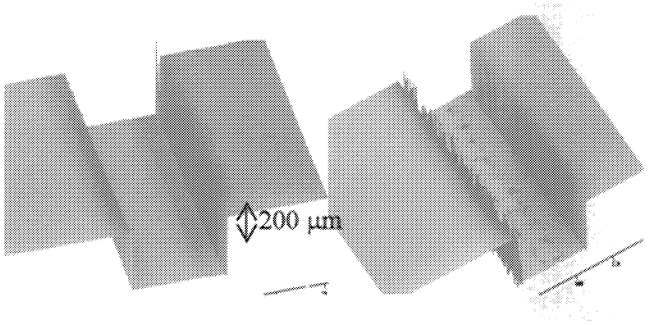

Figure 1: Roughness of PMMA channel surfaces; machining speed of 10 mm/min (left), 200 mm/min (right).

2.2 Spectroscopic measurements

In the fluidic behavior measurements, red and green fountain-pen inks were diluted and these color aqueous solutions were introduced through a Y-shape inlet using microsyringe pumps to visualize laminar flows. Two colored flows were observed under a color CCD camera with a magnifying lens, HI-SCOPE DH-2700 (HIROX, Japan). The microchannel was illuminated with a ring light integrated in the lens head. Images from the CCD camera were stored digitally in a JPEG format (8 bit in each red(R), green(G), blue(B) color) and the resolution of 640 pixels x 480 pixels. Distribution information for each color was extracted from the JPEG data by using the numerical analysis software, Igor Pro (WaveMetrics, OR), on a personal computer.

In the enzyme reaction analysis, β-galactosidase from *E. Coli* and *p*-nitrophenyl-β-D-galactopyranoside as the substrate were used. The enzyme (50 Units / 10 ml) and substrate (0.32 mM) were dissolved in the same phosphate buffer (pH 8) and these solutions were introduced through a Y-shape inlet separately. A microscope equipped with an objective lens (x5 or x20), an optical filter (400-nm band pass) and a cooled black-white 16-bit resolution CCD camera (BS-30L, Bitran, Japan) was used. The visualization of the reaction dynamics was realized by monitoring the photoabsorption of *p*-nitrophenol as an enzymatic reaction product. The image data were also analyzed by the same procedure as the fluidic analysis except to keep the 16-bit resolution.

3 FLUIDIC BEHAVIOR

Mixing of the laminar flows is evaluated by observing the distribution of the color solutions. Because the color pigment particles in the solutions are very large and those diffusion coefficients are very small, the diffusion mixing of the pigments is negligible to the fluidic one. The R and G components in the CCD image should straightly correspond to the red and green solution distributions, respectively, as shown in Figure 2. These images show the area around the first turn from the inlet. Unfortunately, the G component lines show additional peaks from the darkness at the channel edges.

Effect of the roughness of the PMMA surfaces resulting from the machining speed could be evaluated quantitatively by comparing the shape areas. Attached numbers in Figure 2 present the area ratios between right and left halves; this number approaches unity when the mixing occurs. The mixing progresses in the microchannel with the rough surface even after a 2-cm linear flow from the inlet because the ratio at the intersection C is larger than that at A. The very strong mixing occurs in a right angle turn with the rough surface because the attached number is 0.9 at the intersection D.

Effect of the flow rate on the laminar flow mixing is not observed in the linear channel in the range of 20 – 200 μl/min. However, the corner shape complicates the fluidic behavior because the occurrence of transversal flows to the laminar flow along the flow axis. The constant volumetric flow rate produces the different velocities in the inner and outer flows at the corner and some inertia works to the liquid in the microchannel. This effect appears clearly in the fluidic behavior between first and second turns, as shown in Figure 3.

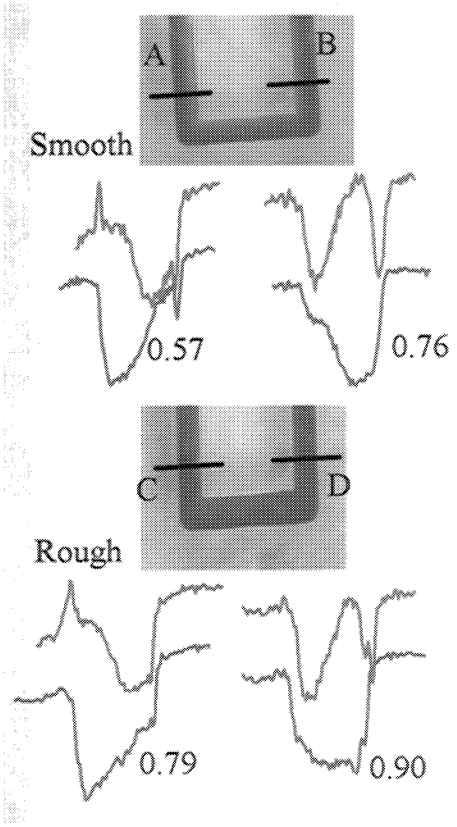

Figure 2: Color resolving of CCD images in RGB mode along intersectional lines (A – D). Effect of roughness of PMMA surfaces appear the distribution of green (upper lines) and red (lower lines) solutions.

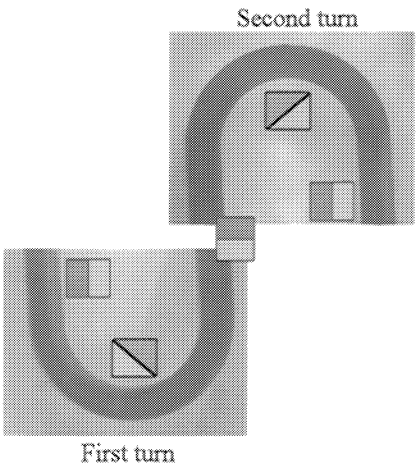

Figure 3: Effect of transversal flow at the corner. Diagrams show the typical flow patterns in the microchannel.

The transversal flows should disorder the laminar flows and promote the mixing at the turns. We fabricated the microchannels with three (round, square, acute) types of turn shape and observed its effect on the mixing. Table 1 presents the number of turns which is required to merge the red and green flows completely. The square turn mixes the flows most easily. The round turn suppresses the mixing efficiently at the high flow rate but the contribution of the transversal flow is smallest in the acute turn.

	Flow rate	
	200 μl/min	20 μl/min
round	15 turns	6 turns
square	6 turns	3 turns
acute	10 turns	8 turns

Table 1: Number of turns to merge the color flows in various corner shapes.

4 ENZYME REACTION

Kanno et al. reported that the hydrolysis of β-galactosidase was enhanced in the microchannel compared with the batch reaction in the respect of both reaction rate and yield [7]. They measured an amount of product by collecting the first fraction from the outlet of the microreactor and applying an HPLC analysis. The CCD visualization in this paper could reveal the on-line reaction dynamics in the microchannels. The reaction time was controlled by changing both the flow rate and the observing point under the microscope. Figure 4 presents the time courses of the hydrolysis at the distance of 10 cm and 30 cm from the merging point in the Y-shape inlet, in which the intensity is normalized at 38 min. The absorption of the reaction product was calculated from the summation of the digital image data in target areas (100 mm x 100 mm). Simple Bouguer - Beer low is approved in the concentration range of 0 mM - 0.2 mM (absorption of 0 - 0.15) comparing the intensities between water and the reaction solutions. The digital image with low noise and high resolution could provide the product distributions in the lowest concentration of 10 μM. After long time passes, the amount of the product in the on-line spectroscopic measurement is almost same as that in the batch reaction as following the Michaelis - Menten mechanism. This fact means the intrinsic rate and yield of the reaction do not vary in the microchannel. However, the reaction at the closer point to the inlet start quickly compared with at distant one. This fact indicates that the reaction does not proceed uniformly along the microchannel.

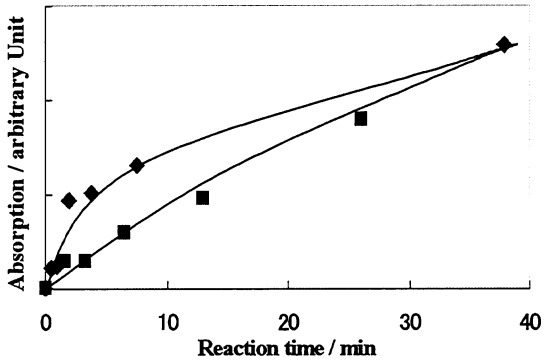

Figure 4: Time courses of the enzyme reaction at the distance of 10 cm (◇) and 30 cm (□) from the inlet.

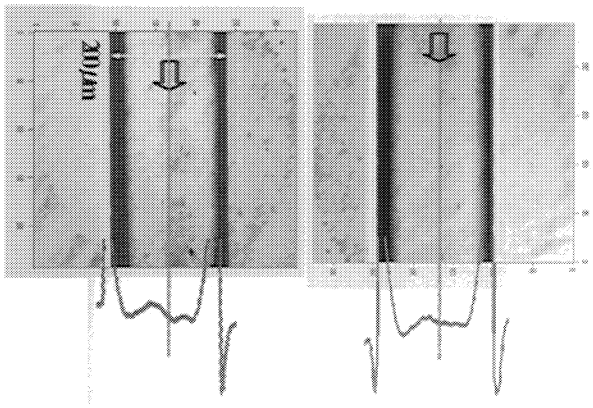

Figure 5: The CCD images of the enzyme reaction in the microchannels; reaction point around 10 cm (left) and around 30 cm (right). Lines at the bottom present the summations of the blocky digital data along the flows.

The visualization of the reaction distribution could reveal the reason of the unhomogeneity. The blocky images from the CCD camera correspond to the product distribution, as shown in Figure 5. The CCD images in this figure are enhanced the contract to identify the distributions by eyes. Therefore, the summations of the blocky digital data along the flows are plotted as the intersectional lines at the bottom. The closer reaction point at 10 cm from the inlet shows a nonuniform distribution. Dark parts which indicate the hydrolysis occurs efficiently appear in the enzyme flow side. On the other hand the distant point at 30 cm shows more uniform distribution except the slight darkness at the center region. This observation can be explained by the large difference of mobility between the enzyme and substrate molecules. Typical diffusion coefficient is 10^{-13} m^2/s and 10^{-9} m^2/s for protein and small molecules and it takes 10^5 s and 10 s to move for 100 μm, respectively.

The transversal diffusion of the substrate dominantly progresses against the laminar flow in the early stage of the enzyme reaction in the microchannel. After two laminar flows merge completely, the enzyme reaction occurs homogeneously in the microchannel. The laminar flow brings the reaction product efficiently in the center region because the linear velocity distribution of the flow is parabolic and the product moves fast in the center. Therefore, the collection-analysis method to evaluate the reaction efficiency in the previous report might provide the high enhancement of the reaction in the microreactor.

5 SUMMARY

The simple spectroscopic and image analysis has been applied for the observation of the microfluidic behavior in the PMMA microreactor. The color solution injection could easily and quickly characterize the flow in the microchannel. This approach is very useful for designing a prototype of the microreactor. Furthermore, the visualization of the enzyme reaction could evaluate the effect the design of the microreactor for the reaction efficiency.

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