P-062 **Encapsulation of lipase in alkyl-silicate on silicate nanofibers for transesterification in organic solvents**

Sakai S.[1*] Liu Y.[2] Taya M.[1] and Kawakami K.[3]
[1]Chem Eng, Mat Sc & Eng, Osaka Univ, Osaka, Japan [2]Collaborative Res Cent Energy Eng, Inst Indust Sc,UnivTokyo, Japan [3]Chem Eng, Fac Eng, Kyushu Univ, Fukuoka, Japan
sakai@cheng.es.osaka-u.ac.jp

INTRODUCTION AND OBJECTIVES

In recent decades, nanostructured and mesoporous constructs have attracted attention as carriers of enzymes because of their very high surface area-to-volume ratio resulting in a high content of enzymes in a given volume of reactor (Kim 2007). Silicate is one of the frequently investigated materials for the carriers. A high tolerance to organic solvents and ease of fabricating nanostructured and mesoporous constructs are attractive points of silicate (Kim 2007). In this study we aimed to use electrospun silicate nanofibers as carriers of lipase for reactions in organic solvents. Electrospinning is a simple and versatile method for producing nanofibers that are exceptionally long in length, uniform in diameter and diversified in composition. Features of electrospun fibers compared with other nanostructured carriers are high porosity and interconnectivity. Silicate nanofibers have been prepared via the electrospinning process (Sakai 2006). Lipases catalyze several processes in organic media including transesterification, esterification, and thiotransesterification as well as the hydrolysis of triglycerides in aqueous media. An emerging application of lipase is in fatty acid-esters, biodiesel production. A unique feature of this enzyme is that the catalytic activity in organic solvents containing a slight amount of water can be enhanced by tuning hydrophobicity of surrounding environment. For example, encapsulating the enzyme in hydrophobic organically modified silicate obtained from alkyl-substituted silicon alkoxides has been reported as an effective way for enhancing the catalytic activity (Reetz 1996; Furukawa 1998). In this study, the lipase molecules immobilized on electrospun silicate nanofibers were encapsulated in alkylsilicate.

MATERIALS AND METHODS

Lipase encapsulation and immobilization

Typically, tetramethoxysilane (18.9 μL, TMOS), *n*-butyltrimethoxysilane (99.3 μL, BTMOS), distilled water (75 μL), and 40 mM HCl solution (5 μL) were mixed in a glass tube in an ice bath. Then, the mixture was heated at 90 °C for several minutes for allowing hydrolysis reaction of TMOS and BTMOS until the mixture became a homogeneous sol. Then the resultant sol was cooled in an ice bath for 5 min. Phosphate buffer solution (1.8 mL, 100 mM, pH7.5) was added to the cooled sol. After that, 1.0 mL phosphate buffer solution containing lipase at 150–300 mg lipase regent powder was slowly poured into the sol solution and mixed using a magnetic stirrer for 20 s. Silicate fibrous mats, 300 mg and about 700 nm in diameter of individual fibers, cut into small pieces of 3 mm × 3 mm square were added into the sol containing lipase. After 1 min of mixing, resultant sol containing silicate fibers was lyophilized for 1 day. The amount of alkyl-silicate deposition for a unit mass of lipase protein was controlled by changing the ratio of TMOS + BTMOS mixture to water. The amounts of lipase and water were fixed in all conditions.

Catalytic activity measurement

The catalytic activity of the immobilized lipase was determined from the initial transesterification rate of rapeseed oil and *n*-butanol resulting in fatty acid butyl-esters as follows: The fabrics immobilizing 100 μg lipase-protein (BSA standard) or reagent lipase powder containing 100 μg lipase-protein was placed in a 20 mL screw-capped glass vial containing 4.2 mL of a mixture of rapeseed oil, *n*-butanol and water at 8000 ppm. The molar ratio of triglyceride and alcohol was 1 : 3. The vessel was mixed at 1300 rpm at 40 °C using a temperature controlled shaker. Initial transesterification rates were determined from the increase in concentration of butyl ester during the first 2 h of reaction, with measurements taken every half hour. The concentration of C18 butyl esters was determined using a gas chromatograph.

Stability measurement

The fabric (760 mg) immobilizing 1000 μg lipase protein was filled in a glass tube of 1 cm inner diameter and 10 cm length. Both ends of the glass tube were sealed with caps equipped with plastic tube connected to a pump. Reaction medium kept at 40 °C was flowed at 0.25 cm/min during 15 days of operation. The medium expelled from the outlet of the glass tube was collected for determining the degree of conversion. The reason we adopted this flow rat was that it gave around 30% of conversion yield.

RESULTS AND DISCUSSION

Figure 1b shows the electrospun silicate fibers immobilizing the lipase encapsulated in alkylsilicate prepared from TMOS and BTMOS. Difference in the smoothness of the surfaces of fibers compared to the fibers non-containing lipase (a) clearly indicates a deposition of alkylsilicate on individual fibers shown in Figure 1a. The preparation condition of the lipase immobilizing fibers was 0.7 mg-alkylsilicate/μg-lipase protein. The clearly visible space between the fibers decreased with increas-

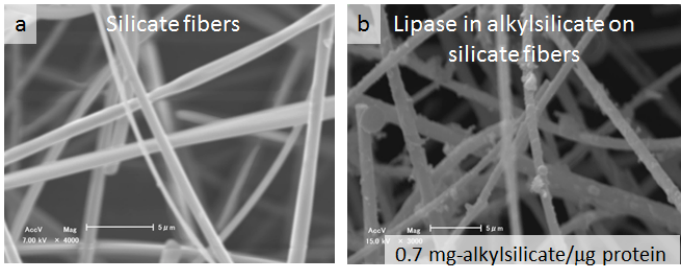

Figure 1. SEM images of silicate fiber (a) before and (b) after immobilizing lipase in alkylsilicate.

ing the amount of alkylsilicate.

The amount of deposited alkylsilicate per amount of lipase protein also influenced the catalytic activity of lipase, expressed as initial reaction rate (Figure 2). Even the immobilization of lipase on electrospun silicate fibers via physical adsorption was effective for increasing catalytic activity. Encapsulation in alkylsilicate was effective for further enhancing catalytic activity: By increasing the amount of alkylsilicate to 0.7 mg-alkylsilicate/μg-lipase protein, the catalytic activity increased to 15-times and 1.8-times compare with those detected for lipase powder and physically adsorbed lipase, respectively. The enhancement of catalytic activity by immobilizing via physical adsorption was interpreted as a consequence of the high dispersity of lipase on electrospun silicate fibers. Reagent lipase containing dextrin as stabilizing agent does not dissolve in organic reaction media, thus the enzyme molecules exist in/on the dextrin particles. The enhancement of catalytic activity by enclosing in alkylsilicate can be explained by a possible interaction between the lipophilic domains of the lipase and the hydrophobic regions of the alkylsilicate. Interestingly, catalytic activity of encapsulated lipase decreased further increased the amount of alkylsilicate deposition to 1 and 1.7 mg-alkylsilicate/μg-lipase protein. This result can be explained by the plugging between fibers resulting in hindrance of substrates supply to immobilized lipase.

According to the above mentioned results showing the

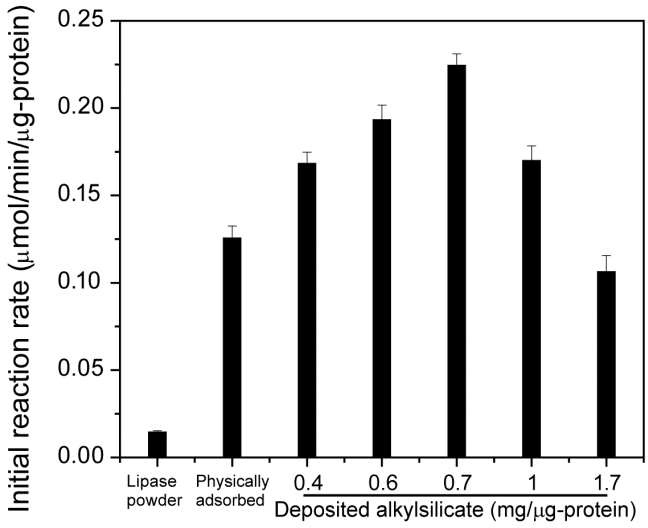

Figure 2. Effect of the amount of deposited alkylsilicate on catalytic activity of lipase.

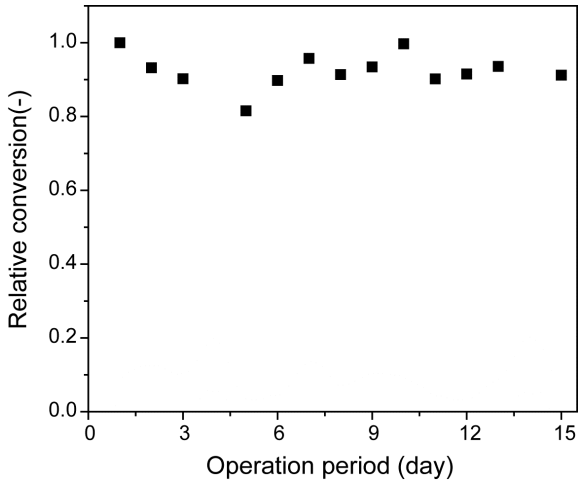

Figure 3. Transition of conversion in a flow-through reactor. Relative conversion yield is a value normalized against the value of day 1.

feasibility of the nanofibrous catalysts, we evaluated catalytic activity of the catalysts immobilizing lipase in alkylsilicate at 0.7 mg-alkylsilicate deposition/μg-lipase protein. The relative conversion yield at 15 days of operation of a flow-through reactor was 90% of that of the day after starting operation (Figure 3).

CONCLUSION

We developed the silicate nanofibrous catalyst immobilizing lipase encapsulated in alkylsilicate for reactions in organic solvents. The catalytic activity prepared under the optimal condition of this study was 15-times higher than that of reagent lipase powder, non-immobilized on fibers. In addition, it was twice higher than that immobilized on silicate nanofibers via physical adsorption. The catalyst was stable more than 2weeks: The catalytic activity at 15 days of operation was about 90% of that detected at day 1. These results indicate the feasibility of the catalyst for reactions in organic solvents.

REFERENCES

- Furukawa SY et al. (1998) *Characterization of Candida rugosa lipase entrapped into organically modified silicates in esterification of menthol with butyric acid*. J Ferment Bioeng **85**, 240-242.
- Kim MI et al. (2007) *Crosslinked enzyme aggregates in hierarchically-ordered mesoporous silica: a simple and effective method for enzyme stabilization*. Biotechnol Bioeng **96**, 210-218.
- Reetz MT et al. (1996) *Effecient immobilization of lipases by entrapment in hydrophobic sol-gel materials*. Biotechnol Bioeng **49**, 517-534.
- Sakai S et al. (2006) *Prospective use of electrospun ultra-fine silicate fibers for bone tissue engineering*. Biotechnol J **1**, 958-962.