Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

A novel strategy to immobilize enzymes on microporous membranes via dicarboxylic acid halides

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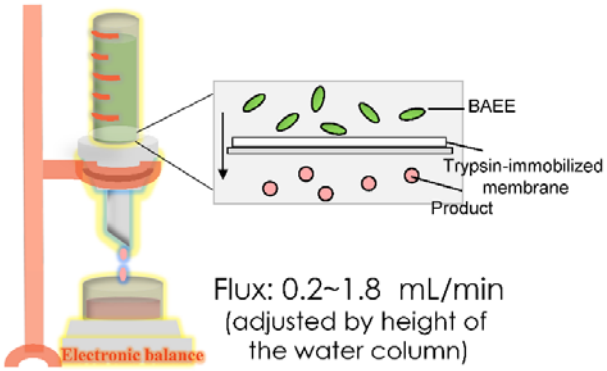

Fig. S1. Experimental setup for the permeability measurement and activity determination in filtration mode for EMBRs.

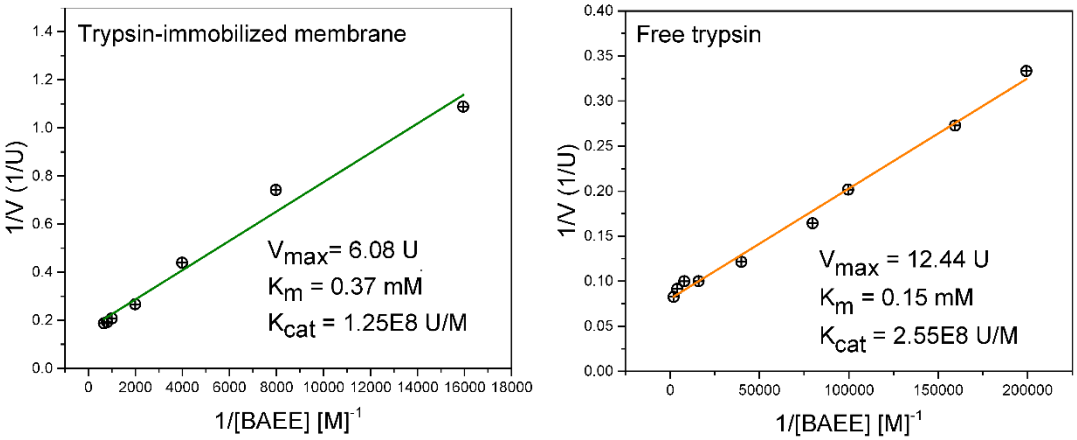

Fig. S2. Lineweaver-Burk plots of the trypsin-immobilized membrane and free trypsin in the soaking mode for the kinetic parameter determination.

Activity determination method for lipase

0.125g p-NPP was dissolved in 25 mL of EtOH and then mixed with an equal amount of 50 mM PBS (pH 7). The mixture was used as substrate solution. 125 μL of lipase solution or a lipase-immobilized membrane with 20 mm diameter was mixed with 2.5 mL of the substrate solution, and stirred at 37℃. After 10 min reaction, 2 mL of 0.5 M Na_2CO_3 was added to terminate the enzymatic reaction. The supernatant was diluted four times with deionized water and the absorbance was measured at 410 nm. Deionized water was used as the blank. A p-NPP unit (U) was defined as the amount of lipase required to produce a ΔAbs_{410nm} of 0.001 per minute with a pH 7 p-NPP substrate at 37 ºC in a reaction volume of 2.625 mL. The specific activity (*SA*) was calculated as follows:

For immobilized lipase:

$$SA\ (\mathrm{U/cm^2}) = 1000\ [\Delta Abs_{410nm(sample)} - \Delta Abs_{410nm(blank)}]/\ (10 \cdot S).$$

For free lipase:

$$SA_{free}\ (\mathrm{U/\mu g}) = 1000\ [\Delta Abs_{410nm(sample)} - \Delta Abs_{410nm(blank)}]/\ (t \cdot A_{free}).$$

S (cm^2) was the membrane area. A_{free} (μg) was the amount of free lipase.

Meanwhile, the activity retention (R) was obtained:

$$R\ (\%) = 100 \cdot\ (SA/D)/SA_{free}$$

where D (μg/cm^2) was the density of lipase on the membrane.

Results of activity measurements of the immobilized lipase

Lipase-immobilized membranes were fabricated with the following condition: 25 ºC reaction temperature, 1,000 ppm lipase concentration, 180 min reaction time, and 1.0 wt % SC concentration.

The values of D and SA were 18 μg/cm^2 and 25 U/cm^2 (1.39 U/μg). The value of SA_{free} was 1.17 U/μg. Using these values, R was calculated as 119 %. These data suggested this method could also improve the catalytic activity of lipase.

Fig. S3 shows the thermal stability of the lipase including the effect of temperature on the activity, the continuous operation capacity, and the reusable performance. From Fig.S3(A), the optimal catalytic temperature for lipase-immobilized membrane and free lipase was 45 ºC and 37 ºC, respectively, indicating the thermal stability of lipase was improved by the immobilization as the same as in the case of trypsin. Fig.S3(B) exhibited the capacity of continuous operation at 37 ºC. We can see that the stability was improved after the immobilization. Fig.S3(C) showed the lipase-immobilized membrane. The remained activity still can achieve to 45 % after the ten-cycle reaction.

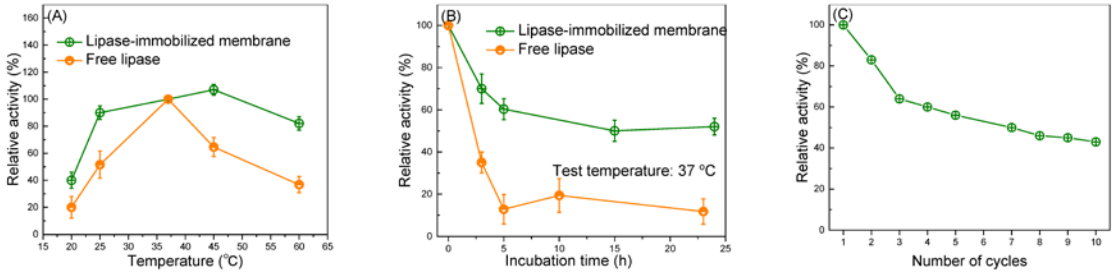

Fig. S3. Evaluation of the stability of the lipase-immobilized membrane and free lipase. (A) The effect of the catalytic temperature on the relative activity of the lipase-immobilized membrane and free lipase. (B) Time courses of the relative activity of the lipase-immobilized membrane and free lipase at 37 °C. (C) The relative activity of lipase-immobilized membrane during ten operation cycles.

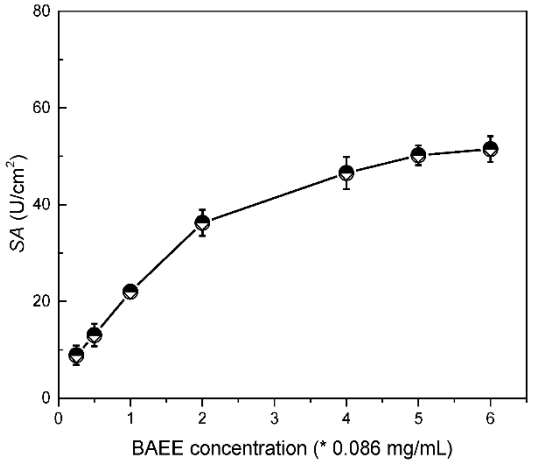

Fig. S4. Effect of the substrate (BAEE) concentration on the enzymatic activity of the trypsin-immobilized membrane.