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Electronic Supplementary Information:

Improved anti-bacteria performance using hydrogel-immobilized lysozyme as catalyst in water

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Experimental details

Materials

Lysozyme (lyophilized powder, protein ≥90 %, ≥40000 units/mg protein), poly (ethylene glycol) methacrylate (PEGMA, Mn: 575 g/mol), poly (ethylene glycol) diacrylate (PEGDA, Mn: 258 g/mol), acetone (99.8%) and 2-hydroxy-2-methylpropiophenone were purchased from Sigma Aldrich. Ethanol was purchased from Fisher Scientific. All samples with or without lysozyme were separately stored at -20 °C, 4 °C and 25 °C for activity tests.

Synthesis of the hydrogel-immobilized lysozyme

In a typical experiment, 0.5 ml PEGMA and PEGDA (1:10 volume ratio), 0.5g lysozyme powder and 5mg 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (initiator) (GC (area %), > 97.5 %; TCI) were mixed with 4.5ml deionized (DI) water. After stirring at 500 rpm for 10 min, the solution was irradiated under UV with a wavelength of 450 nm for 30 min until it formed the hydrogel.

Lysozyme activity test sample preparation

The UV-Vis 2600 spectrophotometer was used for the activity test. 100 mg lysozyme powder, 0.32 mg/ml lysozyme in DI water and 1.0 g *h*-lysozyme samples were stored under -20, 4, 25 (RT), 60, 65, 70 and 80 °C in a refrigerator and an oven, respectively.

Measurement of lysozyme activity

In a standard assay, whole cells of Micrococcus lysodeikticus in 0.1 M PBS (pH = 6.24) were used as substrate to determine the activity of lysozyme. The initial optical density of the UV–vis adsorption spectrum (UV-Vis 2600) was adjusted to reach approximately 1.3 at 450 nm wavelength by varying the ML concentration. After reacting with lysozyme at 25 °C, the change of optical density upon time was used to calculate the activity of lysozyme. A blank ML assay without lysozyme was used to subtract the background. The average value of three activity measurements was used to calculate. The lysozyme activity was tested every week in 2 months. The relative activity was calculated based on the activity of pure lysozyme powder stored under -20 °C. Since the activity decreases with time, we tested the activity within the first minute reaction[37].

Recycling activity test

The concentration of Micrococcus lysodeikticus was adjusted until the optical density of the UV-vis spectrum at 450 nm reached 1.3. Then 100 ul lysozyme solution (6.4 mg/ml) was added into 900 µl ML solution and started to record the OD_{450} adsorption change in 700 seconds. When the adsorption decreased to almost zero, a desired volume of ML solution was added thus the OD_{450} reached about 1.3 again. During the second cycle, the new OD_{450} adsorption evolution was recorded in 700 seconds. This process was repeated until a minimum adsorption was reached.

Antibacterial measurements

Bacteria

Gram-negative E. coli: W3110 containing the ala-TFPI gene, CMCC# 4807. Gram-positive B. subtilis: ATCC® 6633-MINI-PACK™.

Regrowth of E. coli

The E. coli was cultured to reach a concentration of 10^7 CFU/ml in LB solution. The adsorbent materials were added into the solution and placed into a shaking incubator with 150 rpm speed at 37 °C for 8 hours. Then the OD_{600} value (600nm wavelength) of 1ml sample was measured at 0, 2, 4, 6 and 8 hours. The average value upon three sample measurements was selected for analysis. 3.2 mg/ml UV irradiated lysozyme solution, 3.2 mg/ml lysozyme powder solution and 32 mg/ml hydrogel were used to normalize the lysozyme concentration for comparison. The natural grown E. coli under the same condition was used as control sample.

Inhibition of low concentration bacteria

The E. coli and B. subtilis bacteria suspensions with a concentration of 10^5 CFU/ml were incubated and mixed with the adsorbents at 37 ° C for 4 h at 150 rpm shaking speed, respectively. The adsorbents include control (pure E. coli or B. subtilis), 3.2 mg/ml UV irradiated lysozyme, 3.2 mg/ml hydrogel and 3.2 mg/ml h-lysozyme. The E. coli inhibition with 1 mg/ml lysozyme concentration was also checked. Survival concentration was obtained using the colony forming count method. The bacteria concentration was checked at 1^{st}, 3^{rd}, 5^{th}, 26^{th} and 30^{th} hours, respectively. The average concentration upon three measurements was used.