Effect of poly(vinyl acetate–acrylamide) microspheres properties and steric hindrance on the immobilization of \textit{Candida rugosa} lipase

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\textbf{HIGHLIGHTS}

- Carrier hydrophobicity/hydrophilicity could affect lipase immobilization.
- To enhance lipase activity, lipase/carrier should be rationally designed.
- The optimal immobilized lipase held higher specific activity than free lipase.

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\textbf{ABSTRACT}

Poly(vinyl acetate–acrylamide) microspheres were synthesized in the absence or presence of isooctane via suspension polymerization and utilized as carriers to immobilize \textit{Candida rugosa} lipase. When the hydrophobic/hydrophilic surface characteristics of the microspheres were modified by changing the ratio of vinyl acetate (hydrophobic monomer) to acrylamide (hydrophilic monomer) from 50:50 to 86:24, the immobilization ratio changed from 45\% to 92\% and the activity of the immobilized lipase increased from 202.5 to 598.0 U/g microsphere. Excessive lipase loading caused intermolecular steric hindrance, which resulted in a decline in lipase activity. The maximum specific activity of the immobilized lipase (4.65 U/mg lipase) was higher than that of free lipase (3.00 U/mg lipase), indicating a high activity recovery during immobilization.

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1. Introduction

Lipases are widely used for esterification, transesterification and ester hydrolysis in aqueous or organic solution (Houde et al., 2004), but their poor stability and reusability limit applications in industrial production. In attempts to improve lipase catalytic properties and operational stability, immobilization on supports such as polyglycidylmethacrylate beads (Zhao et al., 2011), silica aerogels (Kharrat et al., 2011) or sol–gel supports (Uyanik et al., 2011) have been investigated.

\textit{Candida rugosa} is a lipase with high activity and broad specificity (Uyanik et al., 2011; Dominguez de Maria et al., 2006; Yilmaz et al., 2011) whose activity is increased when bound to an interface (Dizge et al., 2008; Chiu and Wu, 2004). Accordingly, access of substrate to lipase immobilized on hydrophobic carriers is facilitated; however, a strongly hydrophobic environment may affect lipase structure due to a negative impact on enzyme hydration (Zhang et al., 2012). Therefore, designing carriers with appropriate hydrophobic/hydrophilic properties is necessary for improving lipase performance.

So far, the reported quantity of immobilized protein onto carrier via simple adsorption methods is often quite low. For example, <1 wt.% cytochrome \textit{c} was encapsulated in mesoporous molecular sieves (Glimon-Kinsel et al., 1998), in comparison with large quantities immobilized onto less rigid, organic matrixes such as membranes (Gole et al., 2000; Rao et al., 2002). Generally, when a large amount of enzyme is loaded onto carriers, the immobilized enzymes very often show reduced specific activity (Tischer and Kasche, 1999). Even in case where the activity is high (Han et al., 1999), enzyme loading was extremely low, possibly due to multisite attachment and steric hindrance resulting from protein overload.

In this study, \textit{C. rugosa} lipase (CRL) was chosen as a model lipase to prepare immobilized lipase, and the purpose was to investigate the effect of carrier properties and steric hindrance on lipase activity. Vinyl acetate and acrylamide were used as hydrophobic monomer and hydrophilic monomer, respectively, in the suspension copolymerization owing to their good biological compatibility. Firstly, poly(vinyl acetate–acrylamide) microspheres with different...