Silanized Maghemite for Cross-linked Enzyme Aggregates of Recombinant Xylanase from *Trichoderma reesei*

Shalyda Md Shaarani[a, b], Jamaliah Md Jahim[c], Roshanida A. Rahman[a], Ani Idris[a], Abdul Munir Abdul Murad[d], Rosli Md Illias[a,]

[a] Department of Bioprocess & Polymer Engineering, Faculty of Chemical & Energy Engineering, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia

[b] Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang, 26300, Gambang, Kuantan, Pahang, Malaysia

[c] Department of Chemical and Process Engineering, Universiti Kebangsaan Malaysia, 43600, UKM Bangi, Selangor, Malaysia

[d] School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600, UKM Bangi, Selangor, Malaysia

ABSTRACT

Numerous state-of-the-art technologies and new types of carriers have been developed recently to improve enzyme immobilization. Cross-linked enzyme aggregate (CLEA) technology is a lucrative prospect, as several robust biocatalysts have been generated using this simple method of carrier-free immobilization with the possibility of using semipurified enzyme. However, the low lysine content in the enzyme remains a challenge for effective crosslinking. In this work, maghemite (γ- Fe_2O_3), a recently sought after nanoparticle, was silanized with (3-aminopropyl) triethoxysilane (APTES) for use in the preparation of cross-linked enzyme aggregates of recombinant xylanase from*Trichoderma reesei* (Xyl-CLEA-silanized maghemite). Prior screening revealed ethanol to be the best precipitant, and a 0.2: 1 (v:v) glutaraldehyde to enzyme ratio was essential to form active CLEAs. The Xyl-CLEA-silanized maghemite succeeded in increasing the activity recovery 1.66- and 1.5-fold compared to conventional Xyl-CLEAs and Xyl-CLEA-BSA, respectively. The silanization of maghemite with APTES was proven feasible when a 0.0075:1 (v:v) maghemite to enzyme ratio was able to achieve a 78% activity recovery of the xylanase aggregates, whereas the non-silanized maghemite only achieved a 47% activity recovery. At an elevated temperature of 60 °C, Xyl-CLEA-silanized maghemite retained approximately 50% of its initial activity, compared to the free enzyme, for which the activity recovery had plummeted to 20%. Conversely, Xyl-CLEA suffered a total loss of activity at this temperature, whereas Xyl-CLEA-BSA retained only a 6% activity. Xyl-CLEA-silanized maghemite also successfully retained more than 50% of its activity after up to 6 cycles, whereas Xyl-CLEA retained approximately 10% of its initial activity after only 5 cycles. The surface morphology, particle size and loading of silanized maghemite were confirmed by field emission scanning electron microscopy (FESEM) and Fourier transform infrared (FTIR) spectroscopy. The combination of the effective surface modification, high enzyme activity recovery, improved stability and enhanced reusability of Xyl-CLEA-silanized maghemite presents an attractive process for xylanase immobilization and provides a promising catalyst for the biomass industry.

KEYWORDS: Cross-linked enzyme aggregates; Silanized maghemite; Recombinant xylanase; Immobilization; Surface modification

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