

2009

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Kinunda, G. (2009) Immobilization of invertase enzyme on large pore micelle templated silica, Master dissertation, University of Dar es Salaam. Dar es Salaam.

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Immobilization of invertase enzyme on large pore micelle templated silica

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Large Pore Micelle Templated Silica (LP-MTS) and Dodecylamine templated Micelle Templated Silica (DDA-MTS) have been prepared by co-condensation of tetraethoxysilane and 3-aminopropyltrimethoxysilane using cashew nut shell liquid (CNSL) or dodecylamine (DDA) template. The prepared materials were characterized by different techniques to confirm the functionalization. The incorporation of amine functional groups was confirmed by acid titration and DRIFT spectroscopy. The maximum loading for LP-MTS and DDA-MTS were 3.3 and 2.8 mmol/g, respectively. Modification of these MTS materials for enzyme immobilization was done by treating them with glutaraldehyde resulting into Glu-LP-MTS and Glu-DDA-MTS and then supporting enzyme thus forming Glu-LP-MTS-Enz and Glu-DDA-MTS-Enz. The prepared MTS materials have surface areas ranging from 100 m²/g to 214 m²/g whereas pore diameters range from 3.1 to 25 nm as determined by BJH method. SEM images show that LP-MTS and DDA-MTS materials comprised of roughly spherical particles whereas supported glutaraldehyde shows a rupture of the spherical particles to a fine powder. The covalent immobilized invertase shows an aggregation of fine particles to blocks of large particles while cross-linked invertase shows a cluster of the fine particles. The invertase immobilization efficiency was about 98 % and 32 % on LP-MTS and DDA-MTS respectively. Immobilization shifted the pH for maximum activity from 4.0 to 5.0. The optimum temperature for free and immobilized invertases ranged from 40 - 50°C. LP-MTS immobilized invertase and DDA-MTS immobilized invertase showed higher activity at 45 and 50 °C, respectively. The V_{max} for free, covalent immobilized and cross-linked invertase on LP-MTS were 15636, 15472 and 15415 U, respectively whereas K_m were 0.035, 0.047 and 0.051 M, respectively. DDA-MTS immobilized invertase has V_{max} of 5000 U and K_m of 0.1 M. All immobilized invertases were stored for a month and used in five days interval without noticeable loss in activity.