Stabilization and utility of  $\beta$  galactosidase immobilized on thiolated silica nanoparticles

<sup>1</sup>Shakeel Ahmed Ansari\* and <sup>2</sup>Rukhsana Satar

<sup>1</sup>Department of Biochemistry, Medicine Program, Batterjee Medical College, Jeddah-21442, Saudi Arabia <sup>2</sup>Department of Biophysiology, Division of Biochemistry, Medicine Program, Ibn Sina National Medical College, Jeddah, Saudi Arabia

\*Corresponding Author

Associate Professor Mobile: 00966-581482720 E-mail: shakeel.ansari@bmc.edu.sa

## ABSTRACT

This research demonstrates the synthesis of silica nanoparticles (Si-NPs) via sol-gel method, followed by tetra-ethyl-orthosilicate and mercaptopropyl tri-methoxy-silane mediated thiolation to promote covalent binding of Aspergillus oryzae  $\beta$ -galactosidase. Higher enzyme immobilization yield of 89% was achieved on the developed nanobiocatalyst. Soluble and immobilized enzyme exhibited similar pH and temperature optima at pH 4.5 and 50 °C, respectively. However, β-galactosidase bound to thiolated Si-NPs (IβG) exhibited significant enhancement in activity under extreme temperature and pH variations, as compared to the soluble  $\beta$ -galactosidase (S $\beta$ G), by improving its tolerance towards harsh pH ranges and limiting the thermal movement of the enzyme at the higher temperatures. It was observed that the immobilized enzyme retained 84% activity at pH 6.0 in contrast to 49% by the soluble enzyme under similar condition. Moreover,  $\beta$ -galactosidase retained 70% activity at 60 °C as compared to 91% by the free enzyme under identical exposure. It was further observed that the immobilized enzyme retained 58% activity at 5% galactose concentration even after 1 hour. However, under similar experimental conditions, SBG showed 23% activity. Reusability of immobilized enzyme revealed that it retained 63% activity even after sixth repeated use, and hence could be recovered easily by centrifugation for repeated use in biotechnological applications. The batch reactor experiment indicates that the immobilized enzyme displayed 86% and 79% lactose hydrolysis at 50°C and 60°C, respectively as compared to 71% and 60% lactose hydrolysis by soluble enzyme under identical conditions after 8 h. Future research of the developed nanobiocatalyst is required for analyzing its stability in producing lactose-free dairy in continuous reactors products and in the production of galacto-oligosaccharides.

**Keywords**:  $\beta$ -galactosidase; biotechnological applications; silica nanoparticles; surface modification

**Abbreviations:** Si-NPs, silica nanoparticles; S $\beta$ G, soluble  $\beta$ -galactosidase; I $\beta$ G,  $\beta$ -galactosidase covalently bound to thiolated Si-NPs