Development of a Dextransucrase Based Hybrid System Using Superparamagnetic Nanocomposite of Iron Oxide

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ABSTRACT

Utilization of superparamagnetic iron oxide nanoparticles (SPIONs) in different biotechnological processes is an emerging approach towards achieving efficient operational stability of wide variety of enzymes. Recent advancements in the field of magnetic nanotechnology is because of the unique properties of magnetized nanoparticles such as their superparamagnetism and large surface area to volume ratio which makes them accessible to separate easily in the presence of a magnetic field. In this study, a hybrid system was developed which consists of an iron oxide nanocomposite amalgamated with a biocatalyst. The developed nanocomposite was further characterized by several advanced analytical techniques followed by the analysis of catalytic performance of the enzyme. This hybrid system increased the operational stability and recycling efficiency of the amalgamated biocatalyst. The developed hybrid system might be an efficient approach towards the reusability of enzyme for enhanced production of bioproducts of commercial importance.

Keywords: Biocatalyst, Nanocomposite, Nanotechnology, Superparamagnetic iron oxide nanoparticle.

INTRODUCTION

Superparamagnetic iron oxide nanoparticles (SPIONs) have been explored widely for wide variety of enzyme immobilization. These SPIONs have gained the considerable attention because of the unique properties of magnetic nanoparticles such as their superparamagnetism, small size, low toxicity and large surface area to volume ratio and their easy separation from the reaction system. (Magro *et al*., 2018) Naked superparamagnetic iron oxide nanoparticles have been earlier used for different biotechnological applications (Hudson *et al*., 2014) which possess the rapid oxidation of nanoparticles. To overcome this issue, SPIONs can be coated with a biocompatible biopolymer such as chitosan and dextran (Tingirikari *et al*., 2017). Chitosan is a natural biopolymer which exhibits remarkable properties of hydrophilicity, biocompatibility and biodegradability.

Enzyme purification is a time consuming and a rigorous procedure with less operational stability of biocatalyst. To minimize the purification procedures crosslinking of enzyme with SPIONs is a useful approach for their reusability and operational stability. Immobilization is a frequently used technique on which enzymes are confined, cross-linked or adsorbed using a wide variety of matrices that shows easy recovery of their product with greater purity levels (Eş *et al*., 2015). Dextransucrase is an extracellular enzyme which catalyzes the synthesis of glucans. This enzyme has been previously immobilized on different synthetic and non-synthetic matrices in order to improve its stability and recycling efficiency (Miljković *et al*., 2018; Graebin *et al*., 2016). Up till now this enzyme has not been immobilized previously on superparamagnetic iron oxide nanoparticles and the extensive use of this enzyme need an utmost demand for the immobilization of dextransucrase.

OBJECTIVES

The objectives of the study are as follows;

- Hybridize a super paramagnetic iron oxide nanoparticle using a natural biopolymer.
- Characterize and functionalize this nanocomposite.
- Covalent crosslinking of enzyme (dextransucrase) with this complex hybrid system.

METHODOLOGY

Production and Purification of Enzyme

The microbial strain *Leuconostoc mesenteroides* AA1 with higher enzyme production was selected for the production of extracellular dextransucrase and purified further with specific precipitating agent.

Development of Hybrid System

Iron nanoparticles was coated with a biopolymer chitosan to form a superparamagnetic iron oxide nanocomposite and enzyme was covalently attached to the nanocomposite with the help of a crosslinker molecule.

Characterization and Optimization of Hybrid System

The developed hybrid system was characterized using the following analytical techniques and optimization parameter

- Fourier Transform Infra-Red Spectroscopy (FTIR)
- Atomic force Microscopy (AFM)
- Vibrating Sample Magnetometer (VSM)
- X-Ray Diffraction Spectroscopy (XRD)
- Thermogravimetric Analysis (TGA)
- Operational Stability
- Storage Stability
- Recycling Efficiency

CONCLUSIONS/RESULTS

Superparamagnetic iron oxide nanoparticles (SPIONs) showed the particle size of around 50-100nm which exhibits the property of superparamagnetism through which it can be easily retrieved in the presence of magnetic field. The hybrid system was developed successfully with the coating of SPIONs with a biopolymer which makes it stable and can prevent from the oxidation of nanoparticle. (Figure **1**). The characterization of hybrid system by Fourier Transform Infrared Spectroscopy (FTIR) confirms the presence of amine bonds of chitosan with FeO bond of iron oxide surface which revealed the hybridization of biopolymer with SPIONs.

The microbial strain *Leuconostoc mesenteroides* AA1 produced extracellular enzyme dextransucarse in the presence of inducer sucrose. The microbial strain showed maximum enzyme activity of 600 DSU/ml/hour upon partial purification with precipitating agent. The purified enzyme was then crosslinked with the developed hybrid in order to increase its catalytic performance and reusability for the longer period of time. The crosslinked enzyme showed approximately 650 DSU/ml/hour for about 7 cycles with a slight decrease of about 50-60 units of dextransucarse for further cycles (Table **1**). These results showed that SPIONs is an excellent hybrid system for the increased operational stability with maximum reusability of enzyme.

Figure 1. Superparamagnetic iron oxide nanoparticles coated with a naturally occurring biopolymer.

Table 1: Operational Stability of Enzyme Based Hybrid System.

Number of Cycles	**Operational Stability (DSU/ml/hour)**
1	650 DSU/ml/hour
2	649 DSU/ml/hour
3	650 DSU/ml/hour
4	646 DSU/ml/hour
5	645 DSU/ml/hour
6	649 DSU/ml/hour
7	650 DSU/ml/hour
8	645 DSU/ml/hour
9	600 DSU/ml/hour
10	598 DSU/ml/hour
11	590 DSU/ml/hour
12	586 DSU/ml/hour

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