Development and Characterization of Laccase Based Crosslinked Enzyme Aggregates (Cleas)

Muhammad Qureshi, Asma Ansari*, Nadir Naveed Siddiqui, Afsheen Aman
Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan
*Email: asma.ansari@kibge.edu.pk

ABSTRACT

Azo dyes are being largely used in textile, pharmaceutical, food and leather industries. These hazardous chemical compounds are continuously added to the environment where they are not easily degraded and they remain persistent in the environment for a longer period of time. Laccase is one of the enzymes that belongs to oxidoreductase family which have the capability to degrade a variety of substrate molecules including phenols and their derivatives as well as naturally occurring compounds similar to lignin. This makes laccase a suitable enzyme for the degradation of phenolic compounds. Laccase based enzyme aggregates were prepared which was produced by *Trametes pubescence* using solid state fermentation. The effects of temperature, pH and various buffers were analyzed on the enzyme kinetics. The results suggested that the developed aggregates can effectively work at higher temperatures and can be utilized repeatedly in a reaction mixture for continuous reacting system.

Keywords: Laccase, Crosslinked Enzyme Aggregates (CLEAs), Biodegradation, Fungal species, Biocatalyst.

INTRODUCTION

Laccase [EC 1.10.3.2] is a *p*-diphenol oxidoreductase that belongs to the multi-copper oxidase family and is generally produced by a wide variety of organisms. Enzyme can catalyze broad range of substrates that give the enzyme vast application in biodegradation and in bioremediation of various phenolic compounds and dyes being utilized in commercial processes [1]. Nevertheless, the major concern is the susceptibility of enzyme to unfavorable conditions and reutilization of enzyme since soluble enzyme cannot be retrieved from a system. Therefore, to minimize aforementioned concerns and to improve the catalytic performance, immobilization is a suitable approach [2]. One of the types of immobilization is matrix free or carrier free immobilization where enzyme in the presence of a crosslinker forms insoluble aggregates called as Crosslinked Enzyme Aggregates (CLEAs) [3]. Development of CLEAs is of great interest due to their higher stability against pH and temperature. Crosslinked aggregates of laccase have multiple applications in waste water treatment, decolorization of dyes, in leathery and textile industries and in agricultural waste management system [4-7]. Keeping all the aforementioned considerations in view, the aim of the proposed research is to produced and characterize the developed enzyme aggregates and comparatively analyze the thermal stability and reusability of free and insolubilized enzyme.

OBJECTIVES

The objectives of the proposed study are:

- Production of laccase.
- Development of laccase-based aggregates.
- Comparative analysis of free and laccase based CLEAs.

METHODOLOGY

Production of Laccase

Fungal strain was cultivated on solid state fermentation using sugar cane bagasse as matrix for the production of enzyme. Culture was incubated for 15 days at 25℃. Enzyme was extracted using specific buffer and kept at shaking for 2.0 hours for maximum extraction.

Partial Purification and Synthesis of Laccase CLEAs

Enzyme was partially purified ammonium sulphate (*w/v*) and for the aggregate formation the partially purified enzyme was crosslinked using glutaraldehyde as a crosslinker molecule.

Comparative analysis of Free and Aggregated Laccase

Enzyme activity was performed using ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6sulfonic acid)] at 420nm for specific time. Various parameters were analyzed for both free enzyme and CLEAs.

RESULTS/CONCLUSIONS

Developed enzyme aggregates have higher operational stability as compared to free or soluble form of enzyme. Since CLEAs are insoluble in the reaction mixture they are retrievable from the reaction mixture and remain active for multiple cycles retaining their catalytic activity, however, at higher temperature reduction in activity was observed but in case of soluble enzyme loss of catalytic potential was observed at higher temperature. Laccase aggregates can be used in industrial setup for the effective treatment of effluent which may reduce the use of chemical compounds in waste treatment and in decolorization process in textile industry.

Figure 1. Developed Laccase CLEAs.

ACKNOWLEDGEMENT

The authors greatly acknowledge the indigenous support and funding by Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi.

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