Introduction

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Multi copper oxidases are a group of enzymes that are able to couple the oxidation of a variety of different substrates concomitantly with dioxygen reduction to water. This protein family comprises laccases (p-diphenol: dioxygen oxidoreductase, EC1.10.3.2), metalloxidases, ascorbate oxidases and ceruloplasmin. They are found in prokaryotes, eukaryotes as well as in archea. In plant they have been associated with lignin formation, in fungi with pigment formation, lignin degradation and detoxification processes, in yeast and mammals with iron metabolism,in bacteria with copper homeostasis and manganese oxidation (Bento *et al.*, 2010).

Laccase is a copper – containing oxidase, which is unspecific and has a wide range of substrates. It is able to catalyze one–electron oxidation of various dyes, phenols, aromatic substrates, and a series of other oxidizable compounds (Dai *et al.,* 2010). Laccases are N-glycosylated blue multi copper oxidases that are versatile mineralizers of lignin (Asgher *et al.,* 2011).

Laccases are wide spread in fungi, plants, animals and bacteria (Sivakumar *et al.*, 2010). Laccases are generally monomeric or more rarely, homo – hetero– dimericho or homo– tetrameric glycoproteins, whose activity is dependent on four copper ions, distributed among three different highly conserved binding sites (Pezella *et al.*, 2009).

The one cysteine and ten histidine residues, involved in binding the four copper ions distributed among three different highly conserved,

together with a small region around each of the four regions in which the copper ligands are clustered (Sarnthima and Khammauang, 2008).

Currently, the catalytic properties of laccases are being exploited for a range of biotechnological applications like pulp bleaching, dye decolourisation and detoxification of environmental pollutants. The ever increasing demand for this potential enzyme in the industrial sector requires large quantities of this enzyme from microbial source (Laxmi and Khan, 2010).

For laccase application in industrial processes large amount of enzymes are required. Laccase synthesis is known to be influenced by the culture conditions, including variations in the types and concentration of nutrients available (Ding *et al.*, 2012). Laccase production could be improved using new stratergies of enzymatic production such as the use of inducers and substrate limitation conditions .Carbon and nitrogen sources have been promised to play a significant role in enhancing the production of laccase (Tarvares *et al.*, 2005).

Laccase is generally produced in appreciable concentrations during the idiophase, where growth remains static due to decrease in available substrate, but may be significantly enhanced by adding inducer compounds (Strong, 2011).

Although laccases are considered relatively stable enzymes, prevention of inactivation under industrial conditions remains a priority. Besides the temperature and pH of the medium, other factors may also lower laccase activity. The laccase reaction is usually performed in organic solvents as many toxic compounds of interest are hydrophobic. Although many laccases remain stable in organic media, denaturation also occurs in

addition to changes in the enzyme - substrate interaction. Furthermore, the solvent may also down the reaction and have an influence on intermediate formation. Inactivation of laccases by organic solvents is strain specific but, higher concentrations of organic solvent inhibit laccase activity. Organic solvents can also restrict environmental use of laccase (Majeau *et al.*, 2010)

Laccase encoding genes have been found in gram-negative and gram-positive bacteria, including species living in extreme habitats. Early reports of laccases in actinomycetes were based on rather non-specific substrates reactions, but have been verified for some bacteria of genera *Streptomyces.* Role attributes to bacterial laccases include copper homeostasis, sporulation, or pigmentation of spore to confer resistance to stress factors such as UV radiation or hydrogen peroxide (Piscitelli *et al.*, 2010).

Microorganisms are important sources for enzyme production. Selection of the right organism plays a key role in high yield of desirable enzymes. The yield of extracellular enzymes is significantly influenced by physical and chemical conditions; hence physical parameters must be optimized for the maximum production of enzymes through efficient optimization technique (Dacheva *et al.*, 2009).

Microorganisms do not gain energy from lignin degradation but the degradation enables efficient utilization of carbohydrates. Thus microorganisms utilize polysaccharides often possess lignolytic capability and lignin is finally degraded to CO_2, water and humus (Burra *et al.*, 2011).

Fermentative Process optimization is required to maximize productivity and minimize costs. Statistical experimental design can be used in biological processes to evaluate the effects and interactions of the different parameters that rule a biochemical system (Kumar *et al.*, 2010).

Biological pretreatment involves the use of whole organisms or enzymes in pretreatment of LCW. Both fungi and bacteria are used for biotreatments of LCW. Commercial preparations of fungal and bacterial hydrolytic and oxidative enzymes are widely used instead of these microorganisms (Godliving *et al.*, 2009).

The main limitation for the extensive industrial application of microbial enzymes is their highest cost, since the nutritive substances employed in the culture medium results in increase of total production costs the reduction in the substrate expenses would thus increases the productivity of the processes (Kumar *et al.,* 2010).

During recent years, immense efforts are made to develop strategies to maintain the process under optimum conditions, which can significantly increase the enzyme production. For effective laccase expression, it is highly essential to optimize all the culture conditions and compositions for production media which further facilitates economic design of the full scale fermentation operating system (Kumar and Mishra, 2011).

Plackett-Burman Design has been widely applied in many fields such as medium optimization, formulations of multi component and so on (Naveena *et al.*, 2005). This approach not only allows fast screening of a large range of experimental conditions but also reflects the role of each of the components. Multivariate experiments are designed to significantly reduce the number of experimental runs necessary in the optimization process and to produce more precise results (Rajendran *et al.*, 2011).

The Successful application of laccase in various fields requires production of the enzyme in high amounts. Several studies have been undertaken to produce laccase from different sources. But there are only few

reports on the optimization of laccase production from the environmental samples. Thus in order to achieve optimal laccase production, the bacterial species isolated from the textile effluents samples were selected and subjected to different environmental conditions namely change in incubation period, carbon source, nitrogen source, metal cofactors and inducers. The optimization of the physicochemical conditions were performed using the most powerful statistical tool namely Plackett - Burman Design.

OBJECTIVES

- To formulate a suitable production medium for maximum laccase production from the isolated bacterial strain.
- To optimize the medium using statistical design of experiments.