Biobleaching Of Wood Pulp Using Pseudomonas Stutzeri: Elimination Of The Cholorinated Compounds Before Their Generation

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Introduction

Chemical bleaching is a key process to any mill in order to get the paper white. This process uses chlorine and other chemicals to whiten the pulp. Unfortunately, chemical bleaching produces a lot of toxic chlorinated hydrocarbons, which get released into the environment with the wastewater from the mills. These chemicals grab onto other chemicals besides hydrogen, thus creating highly toxic chemicals called “organochlorines”, which are basically poisons, such as dioxins etc. Biotechnology can play a major role in establishing the new, technologically advanced, effective and economic processes in this industry[1,3]. In general, biotechnology offers process-integrated routes for enhancing industrial environmental performance. On the other hand, the biotechnological processes, i.e. biocatalysts, generally work under mild circumstances, are highly selective and use little additional chemicals. So far, this promising potential seems to have been undervalued and the pulp-and-paper sector may be a case in point.

“Biobleaching” as the name suggests is the process of achieving bleaching of pulp by biological means. It relies on the capability of some enzymes or microorganisms to depolymerize lignin and hemicellulose directly. The addition of ligninolytic bacteria or enzymes, which result in the breakdown of the specific bonds between lignin and hemicellulose or lignin and cellulose and thereby release lignin which can come out through washing leaving the pulp white.

Because of their capabilities of catalyzing the oxidation of phenols, laccases are receiving increasing interest as potential industrial enzymes in various applications such as deligninification and detoxification. Identification of bacterial laccases for which genetic tools and biotechnological processes are well established may be of significant importance.

Materials and Methods

Present study exploits the capability of laccase secreting bacterium, *Pseudomonas Stutzeri,* isolated from a forest site (Roorkee, India) to bleach the wood pulp biologically. Because of their capabilities of catalyzing the oxidation of phenols, laccases are receiving increasing interest as potential industrial enzymes in various applications such as deligninification and detoxification. Identification of bacterial laccases for which genetic tools and biotechnological processes are well established may be of significant importance.

A plate assay method was developed to screen the isolated bacteria for laccase secretion (Figure 1). This method was based on the oxidation of 2,2 -azinobis-(3-ethylbenzthiazoline-6-sulfonate)(ABTS), most widely used substrate for laccase. Green zone forming colonies were chosen as laccase positive colonies.

The isolated bacterium *Pseudomonas Stutzeri* produced laccase as the predominant extra cellular phenoloxidase. Among 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS), syringaldazine, veratryl alcohol and vanillic acid; syringaldazine (0.20mM) was found as the best inducer for laccase induction. Laccase activity in the crude extra cellular medium and purified sample was assayed by monitoring the oxidation of 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate) at 420 nm (Figure 2).

In a trial to bleach the wood pulp biologically, extra cellular medium of the culture of *Pseudomonas Stutzeri* was concentrated 20 times and unbleached wood pulp (8% consistency) was incubated in it for 2 hours at 37^0C following by 2 hours incubation at 70^0C for alkali extraction.

Results and Discussion

Use of laccase secreted by *Pseudomonas stutzeri* has been found effective to bleach the wood pulp to an extent of 8 % brightness as measured spectrophotometrically. Bio-bleaching eliminates the use of chlorine in bleaching process of pulp mills and thus stops the generation of chlorinated toxic pollutants. Bleaching of wood pulp could be observed when

unbleached pulp was incubated at 37^0C for two hours in the concentrated extra cellular medium containing enzyme and mediator, 1-hydroxybenzotriazole (HBT)[2].

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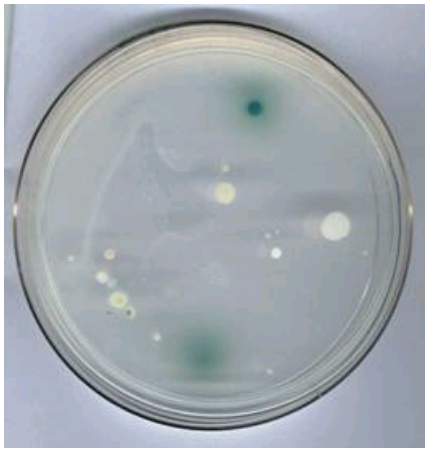

Figure 1: Screening of laccase secreting *Pseudomonas Stutzeri* on ABTS containing plate.

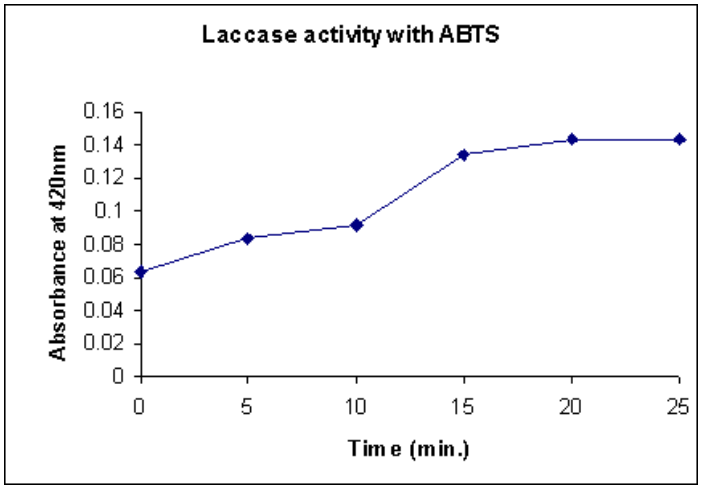

Figure 2: Assaying laccasespectrophotometrically at 420nm using ABTS as substrate