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Mediators in biobleaching of pulps by laccases

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ABSTRACT

Laccases oxidize variety of aromatic and non aromatic compounds by using oxygen as the electron acceptor and producing water as by-product. Generally laccases need mediators for catalyzing the removal of lignin from pulp. The potential of laccase mediator system (LMS) to degrade lignin polymer has been demonstrated by many researchers, but still the high cost and possible toxicity of artificial mediators hamper their application at an industrial level of biobleaching of pulps. Current review presents the critical information of how mediators (synthetic or natural) are helpful for bio-bleaching of different pulps.

INTRODUCTION

Irrespective the source (bacteria, fungi and plants) of laccases, their catalytic nature is not specific for a single substrate. They act on a broad range of phenolic as well as non-phenolic substrates [1,2]. Therefore, laccases can be applied to different industrial and laboratory purposes [3]. The catalytic potential of this enzyme can be extended by making its combination with mediators (low molecular weight chemicals that behaves like an 'electron shuttle' between laccase and substrate) [4,5]. Since 1990, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) was served as a laccase substrate mediating or enhancing the enzyme action [6], the range of compounds that can be converted by laccases has increased dramatically. At first, mediators were considered to mean low-molecular-weight laccase substrates whose enzymatic oxidation gave rise to stable high-potential intermediates. The latter took part in chemical (non enzymatic) reactions with other compounds, not oxidizable by laccases alone, following the diffusion controlled kinetics [7]. Ideally without side reactions mediator can perform many cycles without degradation. The oxidized mediator form produced in the course of the enzymatic reaction can non-enzymatically oxidize compounds (including nonphenolic lignin structures) with ionization potentials exceeding the potentials of laccases. True redox mediators include a limited number of compounds, which possess the requirement of multiple reaction cycles in the redox process. The transition metal complexes have high redox potential and can directly oxidize nonphenolic lignin structures at relatively low concentrations in the reaction mixture. However, these compounds have some drawbacks, which limit their industrial use. The most significant of them are high cost as compared to best synthetic mediators and insufficient environmental safety. The organic compound best fitting the term ''redox mediator'' is ABTS. Its use for oxidation of nonphenolic lignin structures gave impetus to search for new laccase mediators. Most of them are not true redox mediators, because they are eliminated from the reaction by secondary chemical transformations after one or few cycles. Search for new laccase mediators was performed by the colorimetric screening in which the efficiency of a series of naturally-occurring substituted phenols related to lignin polymer to act as laccase mediators was demonstrated by Camarero, [8]. Syringaldehyde, acetosyringone, vanillin, acetovanillone, methyl vanillate and p-coumaric acid significantly promoted the oxidation of recalcitrant dyes by laccase. These phenolic compounds are present in herbaceous plants and are subsequently incorporated into the soil organic matter [5]. The application of natural phenolic mediators for enzymatic bleaching and delignification of kraft pulp has also been investigated. First evidence of natural phenols to mediate enzymatic delignification of paper pulps showed syringaldehyde and acetosyringone as promising laccase mediators [9]. Recently the mediator classification model based on decision tree (DT) analysis showed, ionization energy and radical heat are the main descriptors that determine the laccase-mediator activity of phenolic derivatives. The DT model permitted to establish that 2,6-dimethoxyphenol derivatives with a slightly electron withdrawing substituent at the 4-position of the benzene ring are active mediators [10].

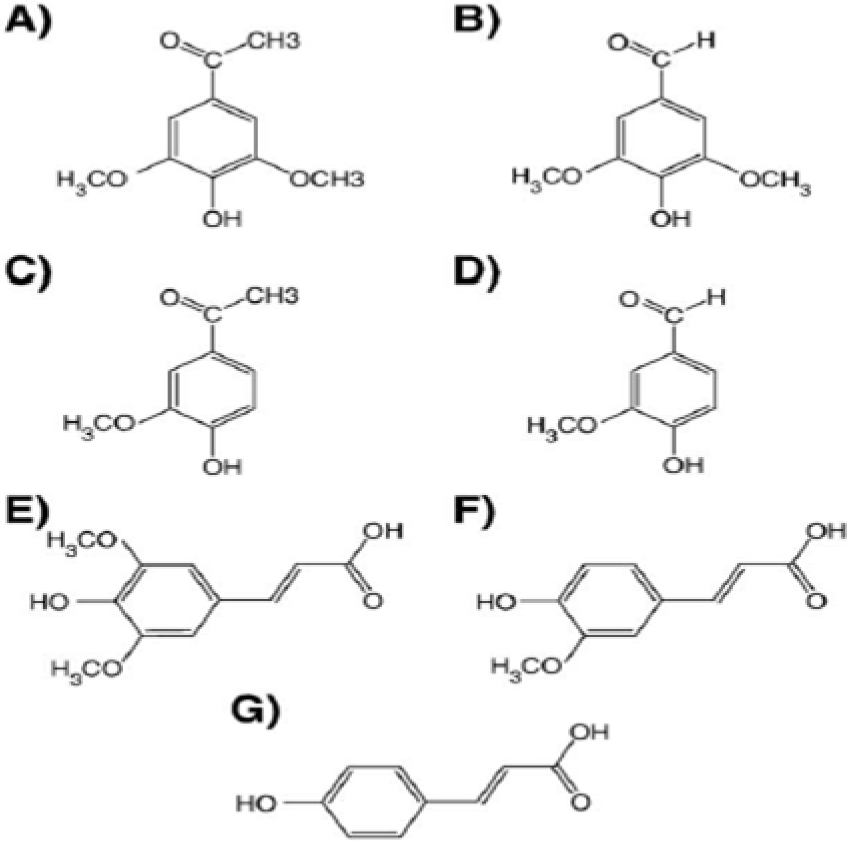

Fig1. Chemical structure of some phenolic compounds [5], widely considered as natural mediators for biobleaching by laccases. A) Acetosyringone, B) Syringaldehyde, C) Vanillin, D) Acetovanillone, E) Sinapic acid, F) Ferulic acid, G) *p*-coumaric acid

Use of chemical mediators in biobleaching

ABTS is the most successful mediator that used widely for biobleaching of various pulps at laboratory scale only, because this is cost intensive for industrial purposes at large scale biobleaching of pulps [11]. Yet, delignification of pulp by ABTS is not fully understood, although it is apparent, that enhancement of delignification by ABTS is because laccase initially generates a stable radical cation from ABTS, that acts as an electron shuttle between residual lignin in the kraft pulp fiber wall and the large laccase molecule [12]. Generally, it is considered that laccases possessed lower specific activities and mediators increase the same when implementing for delignification of pulps. Laccase from *Streptomyces cyaneus* has been used for bleaching of kraft pulp in presence of ABTS at laboratory sacle, at pH 5.0, and found increase in brightness of pulp [13]. Singh et al. [14] evaluated the alkalophilic laccase from ɣ-proteobacterium JB for biobleaching of wheat straw rich soda pulp in the presence of ABTS, at pH 8.0 and found there was decrease in kappa number and increase in brightness of pulp. On the other hand, 1-hydroxybenzotriazole (HBT) is also widely reported as chemical based mediator for biobleaching of pulps, but HBT inactivate laccases after some time and possesses high toxicity even at low concentrations [9]. When *Pycnoporus cinnabarinus* laccase was evaluated for totally chlorine free (TCF) bleaching of paper pulp, it was found that inactivation of laccase by HBT (50% in 4 h) decreased 20% in the presence of eucalypt kraft pulp. Although laccase–HBT decreased kappa number by 4 units with respect to the control and increase in brightness by 6% ISO units [15]. The laccase and HBT in combination was found suitable for delignification of pulp and this system reduced the amount of hydrogen peroxide required for subsequent alkaline bleaching by a factor of 3–4 relative to control tests [16]. Laccase was applied to delignify the kraft pulp in presence of polyoxometalate (POM). It was found that sequence of catalytic redox cycles was similar to laccase-mediator system (LMS) where electrons are transferred from the substrate (lignin) via POM and laccase to oxygen. Results of this study showed POM-laccase system decreased kappa number of eucalypt kraft pulp from 13.7 to 8.5. Among different POM used, SiW11V (4.2mM) showed the better results for biobleaching at 60˚C, O_2 pressure of 5 bar at pH 6.3[17].

Role of natural mediators in biobleaching

Natural mediators are basically phenols present in pulping extracts [9, 18], effluent streams of paper industry [19] and some can be extracted from plant materials. Natural mediators have been observed to perform similarly like chemical mediators, with increased enzyme activity and more modest useful rates [5]. Natural mediators cause less inactivation of the laccases as comparison to chemical mediators and represent the promising alternatives for eco-friendly biobleaching of pulps. The first report of natural mediators syringaldehyde and acetosyringone to mediate delignification of eucalypt pulp by laccase from *Pycnoporus cinnabarinus* at pH 4.0 and 50°C [9]. Aracri et al. [20] reported that sinapic acid, ferulic acid, coniferyl aldehyde and sinapyl aldehyde evaluated as laccase mediators, they lead to lower bleaching efficiency for sisal pulp as these phenolic compounds tend to bind to pulp fibers. Fillat, [21] showed that syringaldehyde, acetosyringone and p-coumaric acid acted as natural mediators for laccase of *P. cinnabarinus* at pH 4.0, 50°C to bleach flax pulp. All natural mediators resulted in a reduced kappa number after the subsequent alkaline treatment with hydrogen peroxide. Generally natural mediators increased KN, decreased brightness and changed optical properties of the pulp after the L stage, suggesting that natural mediators tend to couple to fibers during a laccase- mediator treatment [22]. The ability of two natural phenols to act as mediators of the recombinant *Myceliophthora thermophila* laccase (MtL) in eucalypt-pulp delignification was investigated. After alkaline peroxide extraction, the properties of the enzymatically-treated pulps improved with respect to the control. The pulp brightness increased (3.1 points) after the enzymatic treatment with MtL alone, but the highest improvements were obtained after the MtL treatment using syringaldehyde (4.7 points) and especially methyl syringate (8.3 points) as mediators. Likewise, a decrease in kappa number up to 2.7 points was obtained after the MtL–methyl syringate treatment, followed by decreases of 1.4 and 0.9 points after the treatments with MtL–syringaldehyde and MtL alone, respectively [23].

CONCLUSION

The search for eco-friendly natural mediators should be current research priority for biobleaching of pulps [11]. The detection of low-molecular-weight aromatic compounds could open new possibilities for cost effective biobleaching at industrial level. Laccase or LMS treatment can be incorporated into almost every part of the production process of paper products from biopulping to recovery of secondary fibers and mill effluent treatment. In many cases, the application of laccase/LMS during one stage of the production process gives an immediate benefit, while further benefits appear at subsequent parts of the production chain.

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