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Polysaccharide conjugated laccase for the dye decolorization and reusability of effluent in textile industry

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ABSTRACT

Nine different polysaccharides were screened for conjugation with laccase and evaluated for pH and thermal stability. All the polysaccharides decreased the thermal and pH stability of laccase at 50 °C and 60 °C, where conjugation with gum Arabic showing the most pronounced effect. Thermal instability of gum Arabic conjugated laccase was affirmed by differential scanning calorimeter while the structural changes in the conjugated laccase responsible for thermal instability was analysed by fluorescence spectrophotometer. The gum Arabic conjugated laccase showed an unusually high tolerance to sodium chloride, thermal instability and lower stability in alkaline conditions. Gum Arabic conjugated laccase was found to decolorize Remazol brilliant blue R in the textile effluent at a slower rate without any microbial growth which was unlike that observed in effluent treated with free laccase. Further, effluent treated with conjugated laccase enabled its reuse as liquor for the dyeing to get desired shade.

1. Introduction

Synthetic dyes are among the pollutants in the waste water produced from textile industries, paper printing and colour photography. In textile processing, 50% of the dye is lost after the dyeing process which is then released as effluent (Vaidya and Datye, 1982). The presence of even small amount of dye is visible and negatively affects the aesthetics, water transparency, solubility of gases in water, and also the safety to aquatic life. Most of these dyes are carcinogenic and hazardous and also pose serious environmental hazards (Swami et al., 2012). Further, the adsorption of light by these textile dyes creates problems for photosynthetic aquatic plants and algae (Singh and Singh, 2006). Hence these dyes need to be removed from the effluent before releasing it in water bodies or on land.

Biological treatments for decolourization of dyes are cost effective and environmentally friendly which make them suitable over many physical and chemical treatments including electro flotation, irradiation, electrochemical destruction, ozonation, absorption and use of activated carbon. Biological treatments involve direct use of microorganisms (Dayaram and Dasgupta, 2008) or the application of purified enzymes isolated from the organisms (Zouari-Mechichi et al., 2006; Hu et al., 2009; Grassi et al., 2011; Wang et al., 2011). Laccase is a multinuclear copper containing oxidase which catalyses the oxidation of variety of phenolic and non phenolic aromatic compounds with concomitant reduction of oxygen to water. It is reported to decolorize many textile dyes by oxidation. It is considered as a blue enzyme for green chemistry. Wide substrate specificity of this enzyme has attracted lot of attention from application scientists in industrial and environmental fields (Riva, 2006). Immobilized laccase is better suited for dye decolorization due to repeated use in the process. Immobilization of laccase on alumina has been shown to enhance thermal stability and tolerance against inhibitors making it suitable for decolorizing dyes in textile effluents (Abadulla et al., 2000).

Textile industries require large volumes of water for processing. Reusing enzymatically decolorized effluent for dyeing reduces the water consumption. However, the enzymes used in the decolorization process remain in the effluent after treatment which makes it rather difficult to reuse the effluent for dyeing. Hence new approaches where textile effluents can be reused for dyeing after dye decolorization despite the presence of enzyme need to be developed.

Many approaches have been used till date for increasing the stability of enzymes. Enzyme-polysaccharide interaction is one such approach which has been shown to have a significant effect on the thermal and pH stability (Gomez et al., 2000; Darias and Villalonga, 2001; Altikatoglu and Kuzu, 2010) of enzymes.

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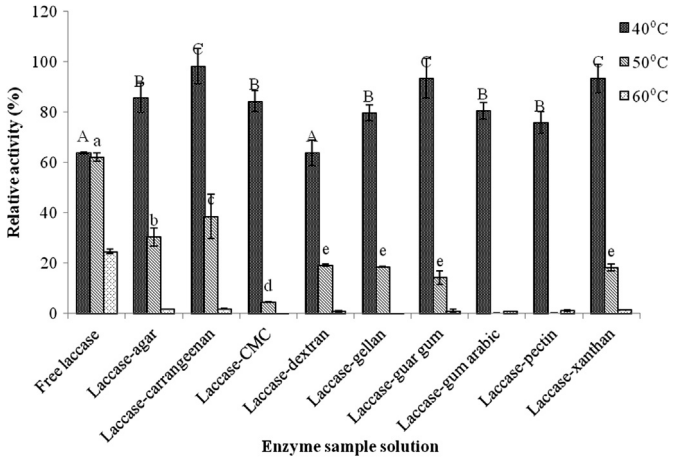

Fig. 1. Profile of free and conjugated laccase with respect to thermal stability. (Values on the bar with different letters are significantly different ($P \leq 0.05$) as measured by student's *t* test). Statistical analysis has been done within the different polysaccharides.

Polysaccharides provide rigidity (Klibanov, 1983) and hydration (Srivastava, 1991) to the enzyme during conjugation through covalent binding. Conjugation of dextran to α-amylase by covalent binding brings about structural changes which may be responsible for increased thermal and pH stability (Jadhav and Singhal, 2012) of the enzyme-polysaccharide conjugate *vis-à-vis* free enzyme. Conjugation of polysaccharide to enzyme can cause structural changes which can both stabilize or inhibit the activity and stability thereof. Enzymes respond differently to the same modification depending on their structural and functional properties. Microencapsulation of glucose oxidase and laccase using poly(ethyleneimine) has shown completely contrasting effect on both the enzymes. At high temperature, poly(-ethyleneimine) chelates copper from active site of laccase and hence decrease its thermal stability whereas same microencapsulation enhances the thermal stability of glucose oxidase (Zhang and Rochefort, 2011).

The present work is a report on the conjugation of laccase with different polysaccharides and its effect on thermal and pH stability. Effect of conjugation on the inhibition of laccase by inhibitors has also been evaluated. Free and polysaccharide-conjugated laccase have been used for decolorizing an important textile dye, Remazol brilliant blue R, and enzymatically treated effluent was further used as liquor for dyeing. The treated effluents were analysed for their ability to dye cotton fibres.

2. Materials and methods

2.1. Materials

Laccase from *Trametes versicolor* was purchased from Sigma Aldrich, Mumbai, India. All polysaccharides (agar, carrageenan, carboxymethyl cellulose, dextran, gellan, guar gum, gum Arabic, pectin and xanthan) were purchased from Himedia, Mumbai, India. Sodium metaperiodate was obtained from S. D. Fine Chemicals, Mumbai. Remazol brilliant blue R was purchased from Atul Industries, Gujarat, India. Purity of dye was 50%. Open bath beaker dyeing machine was used for dyeing cotton fibres.

2.2. Preparation of laccase-polysaccharide conjugate

Sodium metaperiodate (0.1M) solution was prepared in 0.1M sodium acetate buffer of pH 5.0 and used as the oxidizing solution. Polysaccharides *viz.* agar, carrageenan, carboxymethyl cellulose (CMC), dextran, gellan, guar gum, gum Arabic, pectin, and xanthan, 20 mg each was oxidized in 10 ml of oxidizing solution in dark for 90 min after which the oxidation was stopped by adding 0.3 ml of ethylene glycol, and kept in dark for 1 h. Oxidized polysaccharide solutions were dialyzed against 0.1 M sodium acetate buffer of pH 5.0 at 4 °C overnight. Laccase solution (0.2 mg/ml) was prepared in buffer of pH 5.0 and mixed with equal volume of each oxidized polysaccharide solution and kept for conjugate formation for 20 h at room temperature (~28 ± 2 °C) as reported in our previous work (Jadhav and Singhal, 2012). Sodium borohydride (20 mg) was then added to 10 ml of conjugate mixture to reduce remaining oxidized sites of polysaccharide and kept for 4 h. Finally, all the prepared conjugate solutions were dialyzed against 50 mM of sodium citrate buffer of pH 5.0 at 4 °C overnight (Srivastava, 1991; Villalonga et al., 1999; Ahmed et al., 2007). These conjugates were used for analyzing the activity and stability of laccase.

2.3. Laccase activity assay

Assay mixture for test consisted of 0.05 M sodium citrate buffer of pH 5.0 (110 μl), enzyme solution (25 μl, appropriately diluted) and reaction was started immediately by adding 0.216 mM syringaldazine in absolute methanol (15 μl). Oxidation of syringaldazine was followed for 2 min by taking absorbance at 530 nm. The assay mixture for the blank consisted of 0.05 M sodium citrate buffer of pH 5.0 (110 μl), distilled water (25 μl) and syringaldazine 0.216 mM in absolute methanol (15 μl). Extinction coefficient (ε) of the oxidation reaction was 65 mM^{-1} cm^{-1} (Elsayed et al., 2012). One unit of enzyme activity was defined as the micromole of substrate oxidized per min per ml of enzyme solution at room temperature

Table 1
Residual activity (%)[a] of free and conjugated laccase after incubation in buffer of various pH for 1 h at room temperature (28 ± 2 °C).

Enzyme sample	pH							
	3	4	5	6	7	8	9	10
Free laccase	22.36[a]	46.81[a]	64.22[a]	60.50[a]	61.22[a]	62.32[a]	61.79[a]	47.04[a]
L accase-agar	56.16[b]	49.35[a]	55.33[b]	61.96[a]	59.36[a]	65.26[a]	70.08[b]	41.70[b]
Laccase-carrageenan	38.50[c]	71.08[b]	75.68[c]	80.86[b]	72.30[b]	70.04[b]	74.61[b]	44.04[a]
Laccase-CMC	37.22[c]	49.18[a]	56.55[b]	55.07[c]	48.68[c]	49.89[c]	56.73[c]	28.44[c]
Laccase-dextran	32.44[c]	47.40[a]	61.78[a]	50.33[d]	54.44[c]	48.09[c]	53.09[c]	31.35[c]
Laccase-gellan	38.40[c]	54.29[a]	64.10[a]	62.20[a]	51.73[c]	58.98[a]	60.27[a]	30.57[c]
Laccase-guar gum	44.61[c]	62.17[c]	74.18[c]	68.64[e]	61.36[a]	36.93[d]	67.44[b]	28.05[c]
Laccase-gum Arabic	42.39[c]	59.08[c]	74.78[c]	67.89[e]	55.59[c]	54.95[c]	65.95[a,b]	40.92[b]
Laccase-pectin	36.78[c]	53.92[a]	65.77[a]	66.14[e]	62.68[a]	57.11[a]	78.13[d]	35.57[c]
Laccase-xanthan	39.39[c]	62.53[c]	79.00[c]	68.85[e]	66.27[b]	58.73[a]	80.51[d]	42.07[b]

[a] Data are result of triplicate analyses and all the standard deviations were less than ±5%. (Values in the each column with different letters are significantly different ($P \leq 0.05$) as measured by student's *t* test).