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Degradation of Textile Dyes Mediated by Plant Peroxidases

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Abstract

The peroxidase enzyme from the plants *Ipomea palmata* (1.003 IU/g of leaf) and *Saccharum spontaneum* (3.6 IU/g of leaf) can be used as an alternative to the commercial source of horseradish and soybean peroxidase enzyme for the decolorization of textile dyes, mainly azo dyes. Eight textiles dyes currently used by the industry and seven other dyes were selected for decolorization studies at 25–200 mg/L levels using these plant enzymes. The enzymes were purified prior to use by ammonium sulfate precipitation, and ion exchange and gel permeation chromatographic techniques. Peroxidase of *S. spontaneum* leaf (specific activity of 0.23 IU/mg) could completely degrade Supranol Green and Procion Green HE-4BD (100%) dyes within 1 h, whereas Direct Blue, Procion Brilliant Blue H-7G and Chrysoidine were degraded >70% in 1 h. Peroxidase of Ipomea (*I. palmata* leaf; specific activity of 0.827 U/mg) degraded 50 mg/L of the dyes Methyl Orange (26%), Crystal Violet (36%), and Supranol Green (68%) in 2–4 h and Brilliant Green (54%), Direct Blue (15%), and Chrysoidine (44%) at the 25 mg/L level in 1 to 2 h of treatment. The *Saccharum* peroxidase was immobilized on a hydrophobic matrix. Four textile dyes, Procion Navy Blue HER, Procion Brilliant Blue H-7G, Procion Green HE-4BD, and Supranol Green, at an initial concentration of 50 mg/L were completely degraded within 8 h by the enzyme immobilized on the modified polyethylene matrix. The immobilized enzyme was used in a batch reactor for the degradation of Procion Green HE-4BD and the reusability was studied for 15 cycles, and the half-life was found to be 60 h.

Index Entries: Peroxidase; plant peroxidase; dye degradation; bioremediation; textile; dyes; azodyes; reactive dyes; *Saccharum*; *Ipomea*.

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Introduction

Dye contamination of water bodies in developing nations is a great problem. Dyes are released to the environment from two major sources: the textile and dyestuff industries *(1)*. Necessary criteria for the use of synthetic dyes are that they are highly stable to light and washing and resistant to microbial attack *(2)*. These dyes are therefore not readily degradable and are not removed from the effluent by the conventional wastewater treatment methods *(3)*.

Azo dyes are the largest class of dyes with the greatest variety of colors *(4)*. They also exhibit great structural variety and, therefore, as a group are not uniformly susceptible to microbial attack *(1)*. Azo dyes constitute 84% of all the dyes used, of which sulfonated azo dyes predominate. Azo dyes constitute basic, acid, mordant, salt, direct, pigment, and developing dyes. They are the main class of dyes in the reactive dye group, which are currently being used for dyeing cotton fabrics. Textile and dyestuff industrial wastes having different dyes are generally treated by physicochemical methods. These methods include adsorption by activated carbon, ultrafiltration, coagulation, and irradiation, as well as by oxidation (with chlorine, hydrogen peroxide, and ozone), reduction, precipitation, electrochemical treatment, and ion-pair extraction.

However, none of these methods have been found to be very suitable, because some methods are expensive and produce toxic pollutants, and the commonly used chemical precipitation produces a large quantity of sludge, which creates disposal problems.

Bioremediation is an ecofriendly process and has thus become increasingly popular. Enzymes produced by microorganisms, plants, and animals can be used in bioremediation. Both anaerobic and aerobic methods are used in the bioremediation of dyes *(5)*. The anaerobic process has a major disadvantage in that the reduction of azo dyes generates colorless but potentially mutagenic and carcinogenic aromatic amines. The reactive dyes, especially azo dyes, are reported to be resistant to aerobic degradation by bacteria. Bioremediation using the (aerobic culture of) white-rot basidiomycetes has become successful to a greater extent *(6)*. These fungi produce three major classes of enzymes designated as lignin peroxidase, manganese-dependent peroxidase, and laccase, which are important in the fungal degradation of lignin *(7,8)*.

Peroxidase (EC 1.11.1.7; donor: hydrogen-peroxide oxidoreductase) is an enzyme that catalyzes the oxidation of a large number of aromatic structures at the expense of hydrogen peroxide. The enzyme has been identified in all higher plants. Plant peroxidases are present everywhere in the cell and in various isoforms. They have the ability to catalyze the oxidation of a large variety of substrates through a reaction with hydrogen peroxide *(9)*. Peroxidases can act on specific recalcitrant pollutants by either precipitation or transformation into other products, and a reduction in enzyme cost has also been accomplished through enzyme immobilization on vari-

ous supports *(10)*. Some novel applications of the peroxidases include treatment of wastewater containing phenolic compounds and synthesis of various aromatic chemicals *(10)*. Horseradish peroxidases (HRPs) have been studied for their ability to decolorize lignin-containing pulp and paper wastewater *(11–13)* and textile dye wastewater *(6,14)*, and they are also capable of mineralizing a number of sulfonated and nonsulfonated azo dyes such as Remazol Brilliant Blue *(15)*. Treatment of Crystal Violet with commercial HRP type II has been reported to result in a high degree of decolorization *(16)*.

The relation between the biodegradability and molecular structure of the dyes depends on the type, quantity, and position of the substituted groups on the aromatic ring, and the molecular weight of dyes *(17)*. In the case of sulfonated azo dyes, the position and number of the SO_3H group among others affect the degradation rate of the dye molecule *(18)*. The decolorization rate of the azo dyes also depends on their oxidation potential. Decreasing the oxidation potential of an azo dye increases the decolorization rate *(19)*.

A major limitation for the use of plant peroxidase is the low yield and high cost of production compared to the bacterial or fungal enzymes. The cost of the enzymes can be reduced either by reducing the production cost or by extracting from cheaply available plant source and increasing the purification factor and percentage of recovery after purification. The source of commercial plant peroxidase is soybean and horseradish roots, cultivated and harvested in various countries having relatively cool climates. Lin et al. *(20)* have isolated peroxidase from the leaves of *Ipomea cairica*. Nearly 200 plants have been examined for potent peroxidase activity. Among them *Ipomea palmata* and *Saccharum spontaneum* were the best. These two plants are cheaply and abundantly available and have high peroxidase activity. Thus, these two plants were selected for dye degradation.

Immobilization is the most practical technique to overcome the high cost of the enzymes by repeat usage. The stability of carrier-enzyme binding is an important criterion in the application of immobilized enzymes. The covalent immobilization of enzymes onto insoluble supports has been a topic of active research *(21)*. HRP was immobilized on a magnetite support and used to remove chlorophenol from wastewater *(22)*.

The objectives of the present study were to isolate potent peroxidases from easily and abundantly available plant sources, such as *I. palmata* and *S. spontaneum*, and to use them with or without immobilization for the degradation of textile dyes.

Materials and Methods

Sources of Peroxidase Enzyme

High peroxidase activity was found in *I. palmata* and *S. spontaneum*. The plant materials were collected from the campus of Regional Research Laboratory (CSIR), Trivandrum, India.

Table 1
Dyes Used for Degradation

Name of dye	Chemical class	Color index/value	λ_{max} (nm)
Supranol Green	Anthraquinone dye	Acid Green 84	607
Direct Blue	Azo dye	Direct Blue 54, C.I. 27960	574
Chrysoidin	Monoazo, cationic	Basic Orange, C.I. 11270	451
Brilliant Green	Triphenyl methane, cationic	Basic Green 1, C.I. 42040	625
Procion Navy Blue HER	Azo dye	Reactive Blue 171	606
Procion Brilliant Blue H-7G	Pthalocyanine dye	Reactive Blue 3	628
Procion Green HE-4BD	Azo dye	Reactive Green 19	624
Blue M R	Azo dye	Reactive Blue	598
Methyl Orange	Azo dye	Orange III, C.I. 13025	507
Crystal Violet	Triphenyl methane	Basic Violet, C.I. 42555	590
Orange G	Azo dye	Acid Orange 10, C.I. 16230	475
Azo Violet	Azo dye	4-(4-Nitrophenylazo resorcinol)	450
Azo Carmine B	Azo dye	Acid Red 103, C.I. 50090	516
Methylene Blue	Triazine	Basic Blue 9, C.I. 52015	661
Bromophenol Blue	Triphenyl methane	3,3',5,5' Tetrabromophenol sulfonaphthalein	580

Chemicals

All chemicals were obtained as pure commercial products. HRP type II (salt free) (170 Purpurogallin unit/mg of solid), ABTS (2,2'-azino-bis[3-ethylbenz-thiazolin-6-sulfonic acid]), glutaraldehyde, hexamethylene diamine, and bovine serum albumin (BSA) were purchased from Sigma, St. Louis, MO, and H_2O_2 from BDH, England.

Dyes Selected for Decolorization Studies

Dyes that are currently used in the textile industry were used in our study and are listed in Table 1. A variety of dyes such as acid, basic, and direct that include the chemical classes of azo, triphenyl methane, and anthraquinone dyes were chosen.

Supranol Green was purchased from Dystar, India; Direct Blue, Procion Navy Blue HER, and Procion Green HE4BD were procured from Atul India Pvt.; Chrysoidin, Brilliant Green, and Azo Violet were purchased from Aldrich (Milwaukee, WI); Bromophenol Blue, Methyl Orange, and Orange G were procured from Sigma (St. Louis, MO); Crystal Violet and Azo Carmine B were procured from Himedia (India); Procion Brilliant Blue H-7G was purchased from Atic, India; and Methylene Blue was from

Merck (Darmstadt, Germany). Blue M R was obtained from a dyeing factory in Tirupur, India.

Assay Procedure for Peroxidase Enzyme

Peroxidase activity was assayed by the method of Bergmeyer *(23)* in both the pellet and supernatant. The following reaction mixture was employed: 0.004 m*M* ABTS, 0.002 m*M* H_2O_2, 0.067 *M* sodium phosphate buffer (pH 6.0), enzyme extract-100 µL for a total volume of 2.4 mL. Enzyme activity was evaluated by measuring the change in optical density at 420 nm. The volume activity was calculated from the change in absorbance per minute, $\Delta E/\Delta T$, and the extinction coefficient for the system is (2.645×10^3 mmol/cm).

Assay Procedure for Immobilized Peroxidase Enzyme

The activity of the immobilized *Saccharum* peroxidase was determined using the procedure for immobilized enzymes *(24)*. The enzyme matrix was kept outside the light path with stirring, aliquots of the reaction mixture were introduced into the cuvet at definite time intervals, and the increase in absorbance per minute was monitored. The activity of the immobilized enzyme was calculated from the following formula:

$$\text{Activity retention (\%)} = \frac{\text{Immobilized enzyme activity}}{\text{Initial activity of enzyme solution}} \times 100$$

Degradation of Dyes Using Enzymes

The oxidative degradation of the textile dyes was standardized for each plant peroxidase. To obtain maximum degradation, the parameters pH, enzyme, and H_2O_2 concentration were standardized for each dye by trial-and-error method. The spectrum of each dye was scanned by a UV spectrophotometer (UV2100; Shimadzu) in the range of 270–700 nm. At the beginning of decolorization, a decrease in absorbance of the dye in the visible range was followed by an increase in absorbancy in the UV owing to the presence of intermediate degradation products, but at the end of decolorization, absorbance in the UV range was also decreased. The percentage of degradation was calculated from the difference between the initial and final λ_{max} values of each individual dye.

In the dye decolorization studies, 25–200 ppm (25–200 mg/L) of dye in 1 mL of glycine-HCl (0.2 *M*) and phosphate (0.2 *M*) buffer at different pH in the range of 3.0–8.0 at ambient temperature (28 ± 2°C) was incubated with the plant enzyme of an activity range from 0.028 to 1.2 IU along with H_2O_2. The concentration of H_2O_2 was standardized separately for each enzyme and individual dye for optimum degradation, which ranged from 8.8×10^{-5} to 2×10^{-3} *M*.

Preparation of Modified Polyethylene Matrix

Five grams of polyethylene was melted at 120–130°C until it became brown, and to this 2 mL of toluene was added with constant stirring to form a gel. To introduce amine groups on the surface of the matrix, 1 mL of hexamethylene diamine was added to the gel with stirring at the same temperature (120–130°C). The modified matrix was kept for 5 h to solidify and was made into 1-mm pieces.

A 0.5-g sample of the blended polyethylene was taken and treated with 5% glutaraldehyde (5 mL in 0.2 *M* sodium phosphate buffer, pH 7.0) for 2 h in a shaker at 100–110 rpm. The matrix was kept on a Buchner funnel and washed 10 times with distilled water to remove loosely bound glutaraldehyde.

Before coupling the matrix with the enzyme, the enzyme was first immobilized with BSA to reduce the denaturing effects of excess glutaraldehyde and to give a better simulated biologic environment for the enzyme *(25)*. Twenty milligrams of BSA in 5-mL of phosphate buffer (0.2 *M*) at pH 7.0 was treated with the glutaraldehyde-treated matrix at 40°C in a water bath for 4 h, and then it was heated to 60–70°C to denature the coupled BSA. The matrix was washed several times with distilled water and finally with sodium phosphate buffer (0.2 *M*), pH 7.0, and dried at 40–50°C.

The BSA-treated matrix was taken and treated with the enzyme from *S. spontaneum* in phosphate buffer (0.2 *M*), pH 7.0, for 16 h at 4°C. It was washed with the same buffer, then with distilled water, and finally with 0.5 *M* NaCl solution, and was stored at 4°C. The assay of the immobilized enzyme was done by the procedure mentioned earlier.

Results and Discussion

The crude extract was purified by ion exchange and gel permeation chromatography. After purification, the *Ipomea* and *Saccharum* peroxidase had a specific activity of 34.7 IU/mg of protein (purification factor 77.8) and 86.22 IU/mg of protein (purification factor 77.8), respectively. This purified enzyme was used for the dye degradation studies.

There was great difference in the properties of both the enzymes. The pH optimum for *Ipomea* enzyme was 4.0 whereas for *Saccharum* it was 3.5. The optimum temperature for *Ipomea* was lower (70°C) than for *Saccharum* (80°C). It was found that *Saccharum* enzyme had a pH (3.0–9.0) and temperature (40–50°C) stability for 100 h, whereas *Ipomea* enzyme was stable only for 5 h at the same pH and temperature conditions. The thermal stability of the peroxidase enzyme is reported to be owing to the large number of cysteine residues in the polypeptide *(9)*. The inhibition of these two enzymes was studied at a concentration of 1 m*M*, with an enzyme activity of 0.028 U and an incubation period of 100 min. *Ipomea* enzyme was found to be inhibited by ferrous salts and *Saccharum* enzyme by NaN_3, EDTA, and $HgCl_2$. There was no inhibition of both the enzymes with $CaCl_2$,

Table 2
Degradation Dyes Mediated by *I. palmata* Peroxidase

Name of dye	Dye conc. (mg/L)	Enzyme conc. (IU)	H_2O_2 conc. (*M*)	pH	Duration (h)	Reduction (%)[a]
Methyl orange	50	1.2	2×10^{-3}	6.0	2	26.2 ± 1.2
Chrysoidine	25	0.04	2×10^{-3}	8.0	1	44 ± 3.0
Supranol Green	25	0.04	2×10^{-3}	4.0	4	84 ± 5.0
	50	0.04		4.0	4	68 ± 2.0
Brilliant Green	25	0.04	2×10^{-3}	8.0	1	54 ± 2.0
Direct Blue	25	0.04	2×10^{-3}	4.0	2	15 ± 0.23
Crystal Violet	50	1.2	2×10^{-3}	6.0	2	36.3 ± 0.7

[a]Values are the average of four independent experiments.

$CoNO_3$, $MgSO_4$, $MnSO_4$, $ZnSO_4$, $BaCl_2$, $MnCl_2$, NaCl, and KCl. *Ipomea* enzyme was not inhibited by EDTA at the concentrations used for the experiment.

Degradation of Dyes with Plant Peroxidases

Fourteen dyes were taken for the decolorization studies at 25–200 mg/L levels using the purified enzymes. The degradation studies using the plant enzyme gave very promising results. *I. palmata* peroxidase and *S. spontaneum* peroxidase degraded different dyes in different degrees. The end product analysis by thin-layer chromatography (TLC) and high-performance liquid chromatography revealed the absence of amines and confirmed the mediation of these enzymes in the oxidative degradation of the dyes.

Peroxidase of *Ipomea* (*I. palmata* leaf; specific activity of 0.827 U/mg) degraded the dyes substantially. The optimized conditions for the various dyes were standardized. The H_2O_2 concentration was 2×10^{-3} *M* for all the dyes (25–50 mg/L), and degradation was carried out at ambient temperature for 1–4 h. The pH optimum for degradation of acid dyes was between 4.0 and 6.0 and for the basic dyes between 6.0 and 8.0 (Table 2). Methyl Orange, an acidic mono azo dye at 50 mg/L, was degraded to 26% at pH 6.0 in 2 h of treatment and the basic mono azo dye Chrysoidine at 25 mg/L initial concentration was degraded by 44% at pH 8.0 in 1 h. The green dyes degraded better with this enzyme. The anthraquinone dye, Supranol Green, was degraded by 84% at a concentration of 25 mg/L and 68% at 50 mg/L in 4 h at pH 4.0. Brilliant Green, a basic triphenyl methane dye at a concentration of 25 mg/L, was degraded by 54% in 1 h at pH 8.0. Direct Blue, a diazo dye was, degraded only to 15% after 2 h in the same reaction conditions, and even after 18 h, only 30% decolorization was obtained. Crystal Violet, a basic triphenyl methane dye, at a concentration of 50 mg/L, was degraded by 36% at pH 6.0 in 2 h. For Procion Brilliant Red, a reactive dye, no significant decolorization was observed at any of the selected pH values.

Table 3
Degradation of Dyes Mediated by *S. spontaneum* Peroxidase

Name of dye	Dye conc. (mg/L)	Enzyme conc. (IU)	H_2O_2 conc. (*M*)	pH	Duration	Reduction (%)[a]
Chrysoidine	50	0.028	8.8×10^{-3}	6.0	0.5 h	70 ± 2.0
Blue M R	100	0.028	8.8×10^{-5}	3.5	1 h	60.35 ± 1.15
	200	0.028		3.5	1.5 h	53.5 ± 1.06
Procion Brilliant Blue HER	50	0.028	8.8×10^{-5}	3.5	1 h	76.49 ± 2.2
Supranol Green	100	0.028	8.8×10^{-4}	3.0	20 min	99 ± 1.0
Procion Green HE-4BD	50	0.028	8.8×10^{-5}	3.0	1 h	99 ± 1.0
Direct Blue	50	0.028	8.8×10^{-5}	3.0	0.5 h	70 ± 4.0

[a]Values are the average of five independent experiments.

Peroxidase of *S. spontaneum* leaf (specific activity of 0.23 IU/mg) could degrade at the optimized concentration of 0.028 IU of enzyme/50–200 µg of the selected dyes at pH 3.0–6.0 with an H_2O_2 concentration of 8.8×10^{-3} to 8.8×10^{-5} *M* at ambient temperature (Table 3). The green dyes were easily degraded by the enzyme at the optimum pH of 3.0 compared with blue and yellow dyes. Supranol Green, an acid dye with anthraquinone structure, was easier to degrade completely at 100 mg/L within 20 min, whereas Procion Green HE-4BD, a reactive azo dye, was degraded much slower and took 1 h for complete degradation at 50 mg/L. The blue dyes at the optimum pH of 3.0–3.5 were degraded by the enzyme much more slowly. Seventy percent of Direct Blue (50 mg/L), a direct azo dye, was degraded by the enzyme in 30 min. Procion Brilliant Blue H-7G (50 mg/L), a phthalocyanine dye, was degraded by 76.5% in 1 h. The reactive azo dye Blue M R (100 mg/L) was degraded by 60.4% in 1 h, and double the concentration of this dye (200 mg/L) was degraded by 53.5% in 1.5 h. This reactive dye is more difficult to degrade and is used extensively for the dyeing of cotton materials in the Tirupur area in South India. Chrysoidine, a cationic basic azo dye, at 50 mg/L was degraded by 70% by this enzyme at the optimum pH of 6.0 within 30 min.

The results were compared with the commercially available HRP enzyme (Table 4), and the dose of enzyme was 0.1 IU/200 µg of the dyes and the optimum pH was from 5.7 to 7.0 in the presence of 0.04 m*M* H_2O_2. Simple monoazo dyes were degraded better. HRP could degrade 82% Azo Violet (100 mg/L) in 4 h, and 50% Methyl Orange (200 mg/L) in 4 h. Methylene Blue and Azocarmine were degraded by 27–31%, whereas Orange G and Bromophenol Blue degraded to only 3–9.5%.

The degradation of dyes essentially proceeds via a conventional heme peroxidase reaction, by sequential one-electron oxidation by peroxidase compounds 1 and 11 via radical mechanism. The end product analysis by

Table 4
Degradation of Dyes Mediated by HRP Type II

Name of dye	Dye conc. (mg/L)	Enzyme conc. (IU)	pH	Duration (h)	Reduction (%)[a]
Methyl Orange	200	0.1	7.0	4	50 ± 3.0
Orange G	200	0.1	7.0	4	3.2 ± 0.2
Azo Violet	100	0.1	6.0	4	82.3 ± 5.0
Azocarmine	100	0.1	6.5	4	31.0 ± 1.0
Methylene Blue	50	0.1	7.0	4	27.4 ± 1.5
Bromophenol Blue	50	0.1	5.7	4	9.5 ± 0.5

[a]Values are the average of three independent experiments.

TLC showed low molecular weight compounds, mainly oxidative degradation compounds and not amines. However, an earlier work *(25)* reported that the HRP-mediated degradation of Remazol Brilliant Blue R, a vinyl sulfonyl anthraquinone dye, produced oligomers. It is reported that degradation of the azo dyes by fungal peroxidase proceeds via a preliminary oxidative activation of the dyes that results in the formation of cationic species, making the molecule vulnerable to the nucleophilic attack of water *(26)*. Goszczynski et al. *(26)* have postulated two types of hydrolytic cleavage with model sulfonated azo dye compounds: asymmetric splitting gives rise to quinone and diazene derivatives, while symmetric splitting results in the formation of quinone monoimine and nitroso derivatives. These unstable intermediates undergo further redox, oxidation, and hydrolytic transformation, eventually producing organic byproducts and ammonia *(26)*. The aromatic substitution patterns also influence the degradation of azo dyes mediated by fungal peroxidases. Azo dyes with a hydroxyl group on the para position relative to the azo linkage, and at least one or two substitutions of methyl groups, which are electron releasing, in the ortho position relative to the hydroxyl group were found to be preferred substrates for peroxidases with lower oxidation potentials *(27)*. However, the structure of most of the dyes used in our studies was not available, and, hence, one cannot arrive at a definite conclusion regarding the intermediate product formation during the degradation process.

It was evident from our studies that *Saccharum* enzyme is superior to HRP and *Ipomea* enzyme in its capacity for mediating dye degradation. Hence, this enzyme was immobilized onto a matrix, to be used in repeated cycles, thus making the process economically feasible.

Decolorization of Selected Dyes Mediated by Immobilized S. spontaneum *Peroxidase on Modified Polyethylene Matrix*

Most of the matrices currently used for the immobilization of enzymes, such as silica, controlled pore glass, polyvinyl alcohol, polyacrylamide, and chitosan beads, could not be used, because of the adsorption of the dyes

onto the matrices, thereby inactivating the enzyme. Hence, a hydrophobic matrix such as modified polyethylene matrix was developed, and the enzyme was successfully immobilized on this matrix.

The retention activity of the enzyme after immobilization was 20%. The four textile dyes Procion Green HE-4BD, Supranol Green, Procion Brilliant Blue H-7G, and Procion Navy Blue HER (50 mg/L) were degraded using half the quantity of enzyme (25 mg of immobilized matrix having 0.0125 IU of enzyme) compared to the soluble enzyme degradation experiments. These dyes degraded completely after incubating with the immobilized enzyme for 6–8 h in 1 mL of 20 m*M* sodium phosphate buffer at pH 3.0. The concentration of H_2O_2 requirement was different for each dye. The matrix did not adsorb the dyes. The results are given in Table 5.

The optimum pH of the immobilized enzyme was 3.0 for all the dye degradation systems. Procion Navy Blue HER and Procion Green HE-4BD (50 mg/L) showed 50% degradation with the immobilized *Saccharum* enzyme in 3 h and complete degradation in 6 h. Procion Brilliant Blue H-7G and Supranol Green degraded slowly and complete degradation was observed in 7 to 8 h. The immobilized matrix was used in a batch reactor for 15 cycles of Procion Green HE-4BD dye degradation. In all, 750 µg of this dye could be degraded, and the immobilized peroxidase had a half-life of 60 h, corresponding to 10 cycles of degradation.

Conclusion

The leaves of *I. palmata* and *S. spontaneum* were found to be a promising source of peroxidase owing to the high content of the enzyme and easy availability. The purified enzymes were used for the oxidative degradation of selected dyes. *Saccharum* enzyme was found to be better than *Ipomea* peroxidase and HRP in that it had high pH and temperature stability (30–80°C) and high specific activity. This enzyme could degrade the green textile dyes Procion Green and Supranol Green completely within 20 min to 1 h at 50–100 mg/L. A hydrophobic matrix was chosen for the immobilization of this enzyme to be used for continuous dye degradation. The immobilized *Saccharum* enzyme on modified polyethylene matrix did not adsorb the dyes to the matrix but completely degraded Procion Navy Blue HER, Procion Green HE-4BD, Procion Brilliant Blue H-7G, and Supranol Green within 6–8 h at pH 3.0. The immobilized enzyme was continuously used to degrade Procion Green HE-4BD dye at 50 mg/L in a batch reactor and it degraded 750 µg of the dye. The half-life of the immobilized enzyme was found to be 60 h. The immobilized enzyme has many more uses apart from the degradation of dyes, including biotransformations, biosensors, bioremediation, and analytical and detergent formulations.

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Table 5
Degradation of Dyes Mediated by Immobilized *S. spontaneum* Peroxidase on Modified Polyethylene Matrix

Name of dye	Dye conc. (mg/L)	Optimum pH for degradation	H_2O_2 conc. (*M*)	Immobilized enzyme conc. for 25 mg (IU)	Time for complete degradation (h)[a]
Procion Navy Blue HER	50	3.0	1.73×10^{-3}	0.0125	6 ± 1
Procion Brilliant Blue H-7G	50	3.0	1.73×10^{-4}	0.0125	8 ± 1
Procion Green HE-4BD	50	3.0	1.73×10^{-5}	0.0125	6 ± 0.5
Supranol Green	50	3.0	1.73×10^{-3}	0.0125	7 ± 0.5

[a]Values are the average of four independent experiments.

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