NEW METHOD OF DISCHARGE PRINTING ON COTTON FABRICS USING HORSERADISH PEROXIDASE

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Abstract:

Textile discharge printing is the most versatile and important of the methods used for introducing design to textile fabrics. In discharge styles, the pattern is produced by the chemical destruction of the original dye in the printed areas. The discharging agents used can be oxidising or reducing agents, acids, alkalis and various salts. However, the most important methods of discharging are based formaldehyde sulphoxylates and thiourea dioxide. Recently, environmental and industrial safety concerns have increased the potential for the use of enzymes in textile processing to ensure eco-friendly production. Formaldehyde sulphoxylate ($NaHSO_2.CH_2O.2H_2O$) is one of the most powerful discharging agents; however, it is quite toxic and produces formaldehyde, a known human carcinogen associated with nasal sinus cancer and nasopharyngeal cancer. In this work, a hazardous chemical has been replaced with eco-friendly horseradish peroxidase enzyme in textile discharge printing. Enzymatic discharge printing was carried out with a phenol oxidising enzyme system such that the reactive dye was selectively discharged from the cotton fabric in selected areas, creating a printed surface. The effects of enzyme concentration, pH of the printing paste, treatment time and the temperature of enzymatic treatment were studied. The optimum conditions for enzymatic discharge printing were found to be pH 8.5 at 70 °C with a dye concentration of 80 g/L and 60 min as the treatment time.

Key words:

Horseradish peroxide, discharge printing, cotton, hydrogen peroxide, sodium per borate, vinyl sulphone reactive dyes, OKO- Tex 100.

Introduction

Biotechnology has dramatically increased the scope of the application of enzyme systems in all areas of textile processing. Enzymes can be tailored to implement specific reactions, such as decomposition, oxidation and synthesis, for a variety of purposes. There is a growing recognition that enzymes can be used in many remediation processes to target specific purposes in the textile industry. In this direction, recent biotechnological advances have allowed for the production of cheaper and more readily available enzymes through better isolation and purification procedures.

Horseradish peroxidase is a protein with a molecular weight of about 40,000 Da which contains a single protoporphyrin IX hemegroup. This enzyme catalyses the oxidation of a variety of substrates with hydrogen peroxide. The present work aimed at using the horseradish peroxidase enzyme instead of a toxic reducing agent to create a discharge style on cotton fabric dyed with vinyl sulphone reactive dyes.

Figure 1. Horseradish plant (Source: General history of plants)

Structural features of horseradish peroxidase

The enzyme horseradish peroxidase (HRP), found in horseradish (Figure 1), is used extensively in biochemistry applications primarily for its ability to amplify a weak signal and increase the detection ability of a target molecule.

Horseradish peroxidase enzymes belong to class III (the 'classical' secretory plant peroxidases) of the plant peroxidase superfamily, which includes peroxidases of bacterial, fungal and plant origin. The remaining two classes comprise yeast cytochrome c peroxidase, gene-duplicated bacterial peroxidases and ascorbate peroxidases (class I) and fungal peroxidases (class I).

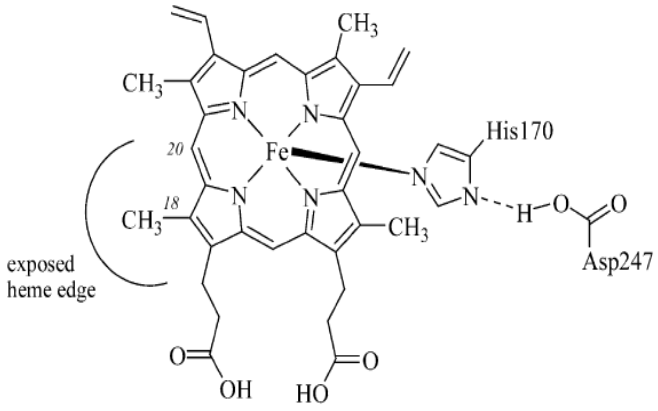

Figure 2. Structural features of the active site of the horseradish peroxidase enzyme

HRP contains two different types of metal centre, the iron (III) protoporphyrin IX (usually referred to as the 'heme group') and two calcium atoms (Figure 2). Both are essential for the structural and functional integrity of the enzyme. The heme group is attached to the enzyme at the proximal histidine residue by a coordinate bond between the histidine side-chain atom and the heme iron atom. The second axial coordination site is unoccupied in the resting state of the enzyme but available to hydrogen peroxide during enzyme turnover.

Mechanism of horseradish peroxidase with hydrogen peroxide

Hydrogen peroxide reacts with ferrous horseradish peroxidase and converts it to oxyperoxidase in a sequence of two reactions. The first is the reaction of ferrous peroxidase with H_2O_2 to form Compound II; the second is the reaction of Compound II with H_2O_2 to form oxyperoxidase.

Most reactions catalysed by HRP and other horseradish peroxidase enzymes can be expressed by the following equation, in which AH2 and AH* represent the reducing substrate and its radical product, respectively. Typical reducing substrates include aromatic phenols, phenolic acids, indoles, amines and sulphonates.

$$H_2O_2 + 2AH_2 \xrightarrow{HRP\ C} 2H_2O + 2AH^{\bullet}$$

Enzymatic catalytic mechanism

Important features of the catalytic cycle are illustrated in Figure 3 below with ferulic acid as the reducing substrate. The generation of radical species in the two single-electron reduction steps can result in a complex profile of reaction products, including dimers, trimers and higher oligomers that may themselves act as the reducing substrate in subsequent turnovers.

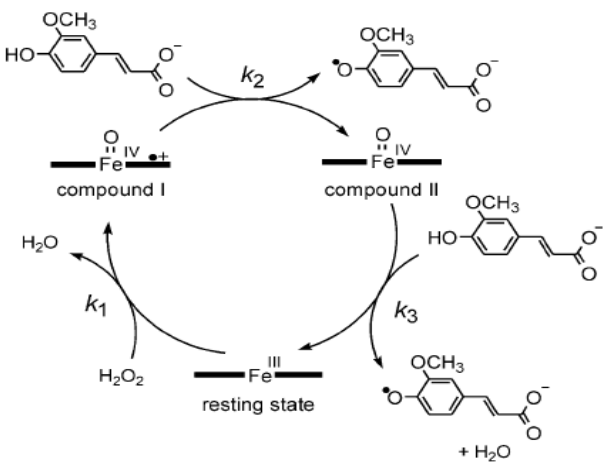

Figure 3. Catalytic cycle of horseradish peroxidase

The first step in the catalytic cycle is the reaction between H_2O_2 and the Fe (III) resting state of the enzyme to generate compound I. (Nigel C. Veitch, 2004). The first single-electron reduction step requires the participation of a reducing substrate and leads to the generation of compound II, an Fe (IV) oxoferryl species that is one oxidising equivalent above the resting state. Both compound I and compound II are powerful oxidants, with redox potentials estimated to be a close to +1 V. The second single-electron reduction step returns compound II to the resting state of the enzyme. The bio-discharge printing concept is derived from this enzymatic catalytic mechanism (oxidation of dyes).

Materials and methods

Materials

Woven cotton fabric (fully bleached) with a plain weave was used as the substrate for dyeing and enzymatic discharge printing. The geometrical properties of the fabric are given in Table 1.

Table 1. Geometric parameters of 100% cotton fabric

Fabric	Ends/cm	Picks/cm	Gm/m^2	Warp Count (Ne)	Weft Count (Ne)
100% cotton	34	33	140	40	40

Dyes and chemicals

The following chemicals were used for dyeing and enzymatic discharge printing on the cotton fabric. The details of the dye and the chemicals used are given in Table 2.

Table 2. Functions of the dye and chemicals used

Sl No.	Dye and Chemicals	Functions
1	Reactive Black CLS (Commercial name)	Dyeing
2	Glauber Salt	Exhaustion agent
3	Sodium Carbonate (Na_2CO_3)	Fixing agent
4	Horseradish Peroxidase (Hi Media RM)	Discharging agent
5	Sodium Alginate	Thickener
6	Hydrogen Peroxide	Oxidising agent
7	Sodium Perborate	H_2O_2 precursor

Methods

Dyeing

The fabric was dyed with reactive dye using the procedure recommended by the dye manufacturer. Exhaust dyeing was carried out at liquor ratio of 1:30. Dyeing of fabric was carried out at 60oC for 60 min. Fixation was conducted for 20 min using 6 to 8 gpl of Na_2CO_3 and 0.01 to 0.5 gpl of caustic lye. The process conditions for dyeing are given in Table 3.

Table 3. Process conditions for dyeing

Parameter	Value
Percentage Dye (OWF) %	5 to 7
Dyeing temperature	60 ^{O}C
Glauber's salt	60 gpl
Na_2CO_3	20 gpl
pH	10-11
Time of dyeing	60 min
Fixation time	20 min.

Bio-discharge printing

The cotton samples were printed with a printing paste using a hand screen printing technique according to the recipe below (white discharge printing):

Printing Recipe
Horseradish Peroxidase: 30, 50, 70, 80 and 90 g / Kg
Sodium Alginate: 20 g
Hydrogen peroxide: 20 ml
Water: Yg
Total: 1000g

The printed cotton samples were allowed to dry at ambient conditions and were left in an oven for 30 minutes at different temperatures.

Washing

The printed fabrics were rinsed with cold water followed by washing in the presence of sodium perborate at 60°C for 30 min. After washing, the incubated fabrics were again washed with ECE detergent (4 g/L) at 60°C for 30 min. Finally, samples were kept in an air drying room.

Testing Methodology

Table 4. Testing methods and instruments used

No.	Property	Standards	Instrument used
1	Colour change evaluation	ISO 105 JO3:2009	X- Rite Spectrophotometer
2	Formaldehyde content	ISO 14184-1:1998	UV- Vis Spectrophotometer
3	Absorbency tests	AATCC 79:2007	Absorbency tester
4	Crocking fastness to washing	ISO 105 C06-B2S(50℃)	Laundr-O- Meter
5	Colour fastness to rubbing	AATCC 08:2005	Crock meter
6	Bursting strength	ISO 13938-2	Pneumatic Bursting strength Tester
7	Tensile strength	ASTM D5034-2009	Instron tensile strength tester
8	Colour fastness to water	ISO 105 E01:2010	Perspirometer
9	Abrasion resistance	ISO 12947-2 ;1999	Martindale Abrasion tester

Results and discussion

The horseradish peroxidase enzyme was used in this study instead of a toxic reducing agent, i.e. formaldehyde sulphoxylate, which can release formaldehyde, known to be human carcinogen, under a variety of conditions. The effect of enzyme concentration, pH, treatment time and temperature on white discharge printing performance was studied.

Colour change difference testing (ISO 105 J03) was used in which the ΔL* value was considered to evaluate the efficiency of the discharging effect of horseradish peroxide under various conditions. Various parameters were studied in this project to optimise enzyme activity in discharge printing.

Effect of pH

It has been reported that most enzymatic activities are extremely sensitive to pH. Different printing pastes containing the enzyme with the highest concentration were prepared, and the pH values were adjusted to 7, 7.5, 8, 8.5 and 9. The difference in the lightness value between the printed image and the surrounding fabric (base) indicates the effectiveness of the discharge of the dye by the peroxidase enzyme. The results are presented in Table 5 and Figure 4.

Table 5. pH vs. ΔL*value of printed samples

pH	ΔL*
7.0	33.12
7.5	35.00
8	37.02
8.5	41.20
9	39.00

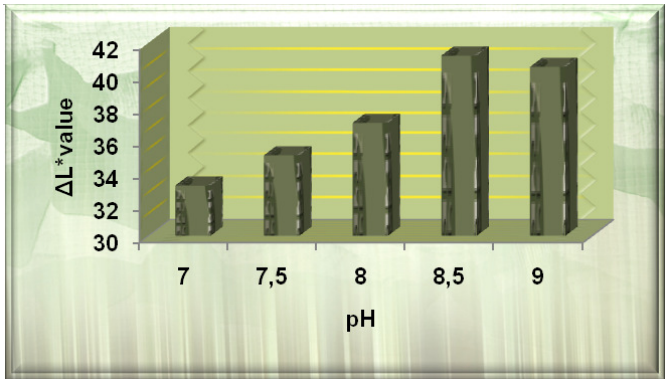

Figure 4. Effect of pH

The current data reveals that the peroxidase enzyme has its optimum oxidising activity at pH 8.5, i.e. the highest ΔL*. Enzymes, being proteins, exhibit Zwitter ion properties. The proton donating or proton accepting groups in enzymatic catalytic sites are at their required state of ionisation at the selected pH, at which the enzyme exhibits its optimal activity. A variation in pH during the course of the reaction may bring about an alteration of the protein structure with a denaturing effect on the enzyme or a change to the ionisation of the active site.

Effect of enzyme concentration

A series of white printing pastes containing different enzyme concentrations (30, 50, 70, 80 and 90 g/kg) was prepared. The cotton fabrics were then printed with these pastes using the screen printing technique. The results are presented in Table 6 and Figure 5.

Table 6. Concentration vs. ΔL*value of printed samples

Enzyme concentration (g/Kg)	ΔL*Value
30	33.29
50	35.18
70	36.99
80	41.53
90	39.16

The ΔL* value increased while concentration of enzyme increase up to 80g but it seems as irregular since in 90 g usage ΔL* value get reduced. The above observations indicate that the optimum conditions for white discharge printing, i.e. the highest ΔL*, was dependent on the nature of the reactive dye used. The variation in the amount of enzyme required to obtain the high difference in lightness value depended on

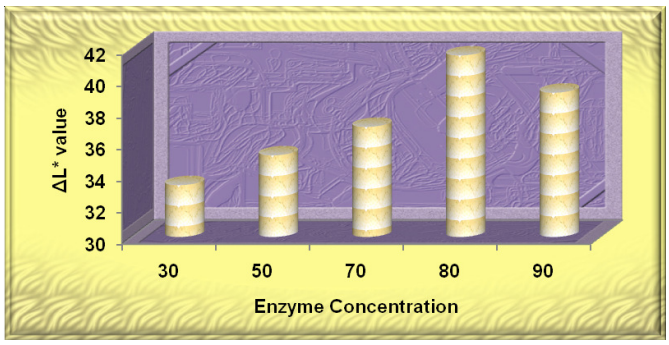

Figure 5. Effect of enzyme concentration

chemical structure, reactivity, structural configuration and the bonding energy of reactive dyes with the cotton fabric.

Effect of Temperature

It has been reported that enzymes their exhibit maximum activity in a specific temperature range. Hence, it is of great interest to investigate the effect of drying temperature on reactive dye colour discharge by horseradish peroxidase enzyme. Cotton fabric samples were printed with the enzymatic white paste; after printing, the samples were subjected to drying at different temperatures, i.e. at 40, 50, 60, 70 and 80°C. Finally, the fabrics were washed according to the procedure mentioned earlier. The results are presented in Table 7 and Figure 6.

Table 7. Temperature vs. ΔL*value of printed samples

Temperature °C	ΔL* Value
40	33.45
50	35.20
60	37.86
70	41.20
80	40.45

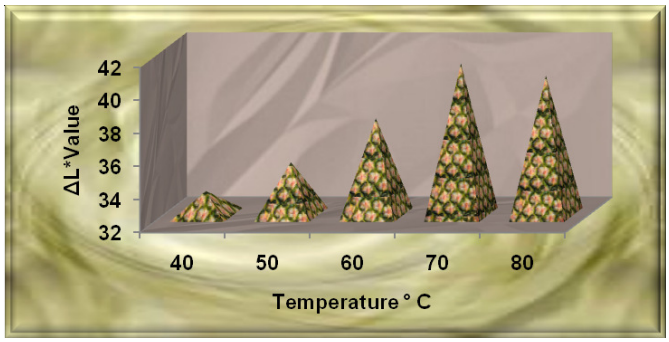

Figure 6. Effect of temperature

It can be observed in the table that, as the drying temperature increased from 70°C to 80°C, the ΔL* value decreased. The enzyme exhibited its maximum activity at a specific temperature and the above observation indicates that 70°C is the optimum temperature.

Effect of treatment time

It has been reported that the initial period of time the amount of substrate which has been transformed is directly proportional to the length of treatment time. After this initial period, the rate of reaction begins to decrease and the yield of the reaction is no longer directly proportional to the treatment time. Provided the substrate is present in excess, one explanation for this phenomenon is the progressive loss of enzyme activity after a period of time. This may be due to the effect of heat on the tertiary structure of the enzyme or due to the formation of some product or side product of the reaction which inhibited the enzyme.

Therefore, the printed cotton samples were subjected to a drying process for various intervals of time in order to determine the optimum time necessary for attaining maximum dye removal. The treatment was carried out at 70°C for 15, 30, 45, 60 and 75 min followed by washing and drying.

Table 8. Treatment Time vs. ΔL*value of printed samples

Time	ΔL* Value
15	27.45
30	31.45
45	34.26
60	39.55
75	39.60

Table 8 shows that colour removal increased by increasing the treatment time until it reached a maximum at 60 min. Increasing the drying time beyond 60 min caused a slight increase in the ΔL* value . This may be due to the progressive loss of enzyme activity.

Formaldehyde content determination

The printed samples were evaluated using UV-Vis spectrophotometry according to the ISO 14184-1:1998 standard test method: Determination of formaldehyde (free and hydrolysed formaldehyde - water extraction method).

Table 9. Results of formaldehyde content test

S. No	Sample Code	Description	Formaldehyde content in ppm
1	COS	Cotton original sample	4
2	CDS	Cotton dyed sample	10.2
3	CEPS	Cotton enzymatic printed sample	10.3
4	CCVS	Cotton conventionally printed sample	90

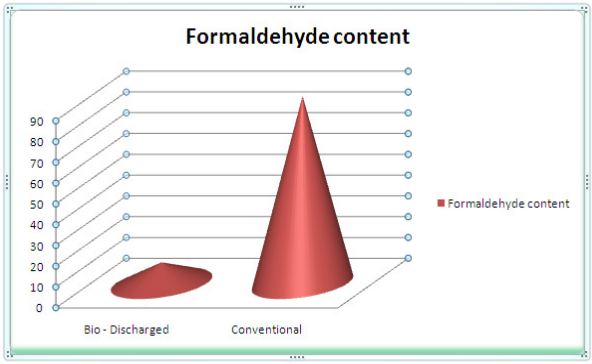

Figure 7. Bio-discharged vs. conventionally discharged printed sample

It is clear from the Table 9 that the CEPS and CDS samples showed the same formaldehyde content, whereas the COS sample indicated a content of 4 ppm, which is the detection

limit equipment, hence there was no formaldehyde present. But, in case of the CCVS fabric, formaldehyde was present in the range of 90 ppm. From that, we observed that the formaldehyde content in conventionally discharge printed fabric was beyond the acceptable limit according to OKO- Tex 100 norms (skin contact: 75 ppm).

Tensile strength

The printed samples were tested using the Instron tensile strength tester; the results of these tests on the fabric tensile strength (warp) of the samples are given in Table 10.

Table 10. Tensile strength result - warp

S. No	Sample code	Mean tensile strength in N (warp)	Loss or gain over original	Percentage of loss or gain
1	COS	351.2	0.0	0.0
2	CDS	349.0	-2.2	- 0.62
3	CEPS	348.0	-3.2	- 0.91
4	CCVS	328.3	-22.9	-6.52

The breaking strength test of printed cotton samples were carried out in the warp direction using a tensile strength tester. The breaking strength of the conventionally discharged printed fabric showed greater strength loss compared with the bio-discharge printed fabric in the warp direction.

Abrasion resistance

The printed samples were tested for abrasion resistance by using a Martindale abrasion tester; the results of fabric abrasion resistance value of the samples are given in Table 11.

Table 11. Abrasion resistance value

S. No	Sample code	Mean abrasion resistance in rubs	Loss or gain over original	Percentage of loss or gain
1	COS	19100	0.0	0.0
2	CDS	18600	- 500	- 2.6
3	CEPS	18500	-600	- 3.14
4	CCVS	17000	- 2100	- 11.0

The abrasion resistance value of the conventionally discharged printed fabric was poorer compared with the bio-discharged printed fabric.

Bursting strength

Printed specimens were clamped over an expansive diaphragm by means of a circular clamping ring. Increasing compressed air pressure was applied to the underside of the diaphragm, causing distension of the diaphragm and the fabric. The pressure was increased smoothly until the test specimen bursts. The results are presented in Table 12. The bursting strength of the conventionally discharged printed fabric was poorer compared with the bio-discharge printed fabric

Colour Fastness and absorbance properties

All samples had excellent colour fastness to washing and water. In terms of colour fastness to wet rubbing, the result was slightly higher due to it is black colour sample. The absorbency properties of all samples were the same which reveals that the printing process had no effect on this property.

Table 12. Bursting strength result

S. No	Sample code	Mean bursting strength in kPa	Loss or gain over original	Percentage of loss or gain
1	COS	347.0	0.0	0.0
2	CDS	342.4	- 4.6	- 1.32
3	CEPS	341.2	- 5.8	- 1.67
4	CCVS	305.0	- 42.0	- 12.1

Table 13. Colour fastness and absorbency properties

Samples	Absorbency	Washing		Water		Crocking	
						Dry	Wet
	Sec	Colour change	Staining	Colour change	Staining	Staining	Staining
CDS	2	4-5	4-5	4-5	4-5	4	3
CEPS	2	4-5	4-5	4-5	4-5	4	3
CCPS	2	4-5	4-5	4-5	4-5	4	3

Conclusion

Bio-engineering is an indispensible tool in modern industry where environmental concerns play a critical role in sustaining in the competitive market. Innovative methods using formulations with horseradish peroxidase and H2O2 in discharge printing of textiles can be carried out successfully. Formaldehyde liberation can be fully avoided in this kind of bio-discharge printing. The optimum conditions for using the horseradish peroxidase formulation were found to be pH 8.5, 70 °C, a dye concentration of 80 g/L and 60 min of treatment. By using the peroxidase enzyme discharge printing method, the following advantages were observed:

– Elimination of formaldehyde,
– Energy saving,
– Environmentally friendly,
– Reduction of strength loss.

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