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Cotton dyeing with indigo using alkaline pectinase and Fe(II) salt

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An ecofriendly reducing agent, viz. alkaline pectinase alongwith iron (II) salt, has been used in place of sodium hydrosulphite for dyeing of cotton with indigo. Dyed samples are then characterized by ATR-FTIR, SEM and XRD. It is observed that the alkaline pectinase alongwith iron (II) salt reducing system is also capable of producing almost identical level of reduction potential in dye baths, reduction bath stability, dye strength and colour fastness properties of dyed cotton to those obtained with sodium hydrosulphite.

Keywords: Alkaline pectinase, Cotton, Dyeing, Dye strength, Fe(II) salt, Indigo dye, Sodium hydrosulphite

1 Introduction

Indigo is one of the oldest dyes to produce attractive blue shade on denim following the '6 dip 6 nip' technique using sodium hydrosulphite ($Na_2S_2O_4$) as reducing agent and sodium hydroxide (NaOH) as alkali[1]. Sodium hydrosulphite is thermally, hydrolytically and oxidatively unstable, can be oxidized by atmospheric oxygen and produce perilous by-products due to decomposition of sodium hydrosulphite such as sulphur compounds (e.g. Na_2S, NaHS, etc.), which pollute atmosphere through formation of hydrogen sulphide. At the same time, salts of sulphur in the form of sulphates and sulphites (Na_2SO_3, $NaHSO_4$, Na_2SO_4, $Na_2S_2O_3$) contaminate sewage, lowering its *p*H and causing the concrete sewage pipes to become corroded[2].

The shortcomings associated with the use of sodium hydrosulphite have led to the need for alternate non-hydrosulphite based reducing systems. These include the application of iron (II) salts alongwith gluconic acid and NaOH at 60°C[2-4], iron (II) salts in combination with tartaric or citric acid, triethanolamine and NaOH at 25-30°C[5-7]. All these reducing systems showed results comparable to sodium hydrosulphite with some limitations.

In recent years, the application of enzymes in processing of textile has increased due to their ecofriendly and non-toxic nature[8-10]. Enzymes belonging to oxidoreductases and hydrolases classes have been found to work as reducing agent in alkaline media and may catalyze the reduction of dye[11,12]. One of those enzymes (pectinase) has been found to be a potential reducing agent for sulphur dyes[13]. It is being used in various industrial sectors, such as food, agriculture, textile and environmental[14-16]. Commercially, acidic pectinase is used in fruit juice and wine industry, whereas alkaline pectinase is used for retting and degumming of fibre crops, bioscouring, elimination of vegetable impurities in wool and treatment of wastewater from fruit juice industry[15, 17].

An attempt has been made, in this work, to substitute sodium hydrosulphite with alkaline pectinase alongwith iron (II) sulphate for reduction and solubilization of indigo followed by dyeing of cotton through '6 dip 6 nip' with intermediate air oxidation method. Box behnken response surface design is used to analyze the alkaline pectinase reduction system to achieve optimized parameters. The performance of both reduction systems are compared in terms of pH and reduction potential (mV) at various stages of dyeing, dye strength (K/S) of dyed cotton, stability of reduced bath and fastness properties of dyed cotton. Characterization and surface morphology of dyed cotton have been studied by ATR-FTIR, SEM and XRD.

2 Materials and Methods

2.1 Materials

Thoroughly pretreated plain woven cotton fabric (EPI 124, PPI 80, warp 40[s], weft 33[s] and GSM 130) was used in this study.

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2.2 Chemicals

Alkaline pectinase (EC 3.1.1.11, activity 10,000 U/G) and indigo [C.I. Vat Blue 1] were supplied by Verma Chemicals, New Delhi and Dystar, Mumbai respectively, whereas iron (II) sulphate (AR, 99.5%), sodium hydroxide (AR, 96%), sodium hydrosulphite (87-88%) and hydrochloric acid were procured from S D Fine chemicals.

2.3 Preparation of Pad Liquor

2.3.1 Sodium Hydrosulphite and NaOH System

A stock vat and dilution liquor were used to prepare the required concentration of dye solution. For preparing the stock vat, required amounts of sodium hydroxide (NaOH) and dye were added in 100 mL water and heated at 50℃. Required amount of sodium hydrosulphite ($Na_2S_2O_4$) was added and the solution was kept for 15-20 min to complete dye reduction. For preparing the dilution liquor, the required amounts of NaOH and $Na_2S_2O_4$ were added in one litre water at 30°C and stirred well till clear solution was obtained.

Padding liquor of 3 g/L indigo was prepared from these two solutions. Dilution liquor 567 mL was added in 100 mL of reduced stock vat to prepare 667 ml padding liquor solution. The concentrations of dye, NaOH and $Na_2S_2O_4$ (as per guidelines of BASF) required to prepare control are shown below:

Stock vat	Dilution liquor	Padding liquor
Dye : 20 g/L	Dye : Nil	Dye : 3 g/L
NaOH : 20 g/L	NaOH : 1.35 g/L	NaOH : 4.416 g/L
$Na_2S_2O_4$: 20 g/L	$Na_2S_2O_4$: 2.0 g/L	$Na_2S_2O_4$: 4.698 g/L

2.3.2 Alkaline Pectinase and Iron (II) Sulphate System

In this case, dye liquor was prepared in the same way as used for hydrosulphite system, except the use of alkaline pectinase alongwith iron (II) sulphate in place of sodium hydrosulphite.

2.4 Dyeing of Cotton with Indigo

Cotton was dyed with reduced indigo from both the reduction systems using ‘6 dip 6 nip’ padding operation. The steps included dipping of cotton in dye liquor for 30 s followed by padding at 1 kg/cm^2 pressure for 75-80% dye pick up and airing for 1 min to complete ‘1 dip 1 nip’ cycle. Cotton fabric was dyed using six such consecutive cycles with the final airing for 3 min, converting the reduced dye on the fabric to its oxidized form. Dyed samples were thoroughly washed in hot water.

2.5 Statistical Analysis of Dyed Cotton

Box Behnken response surface design was used to analyze and optimize the process parameters. This included identifying the best suitable combinations of parameters and their levels, resulting in *K/S* equivalent to that obtained in hydrosulphite system. Five dyeing parameters were studied, viz. concentrations of $FeSO_4$, NaOH, alkaline pectinase & indigo and temperature as factors and their coded values as levels (-1,0,1). Using these five parameters (factors), each with three levels, a 3^5 Box-Behnken design was run to get set of data (run), consisting of total 46 runs with six replicates at the central point. Results were analyzed with response surface plots and equations were derived for response at 95% confidence interval. Response surface figures were analyzed for understanding the effect of individual parameter (factor) on dye strength (*K/S*). Regression equation was formed accordingly. All design formations and statistical analyses were carried out using Design Expert 7 software. A quadratic polynomial was used to analyze the relationship of dye strength (*K/S*) (response) with five independent variables (factors) for Box-Behnken design runs. The accuracy of the model was checked using coefficient of determination (R^2) as the measure of goodness of fit to the model. When R^2 approaches unity, the empirical model fits the actual data. The P-values of less than 0.05 were considered to be statistically significant. Lack of fit test was analyzed to check the adequacy of the model. Two techniques, viz. response surface figures technique and regression equation technique, were used to predict the optimized combination of the factors that will give maximum colour strength (*K/S*).

2.6 Evaluation of Dye Bath and Dyed Cotton

Surface colour strength (K/S) of dyed cotton was evaluated using computer color match (Datacolor Check, Datacolor International, US), and colour fastness properties, viz. light, wash and rubbing, were evaluated through AATCC test methods 16-2004 (light), 61-2007 (wash), 8-2007 (rubbing) respectively. The reduced dye baths were evaluated for *p*H and reduction potential using digital *p*H cum ORP meter (Century Instruments, Chandigarh, India) at various stages of dyeing, i.e. before and after reduction of dye as well as after completion of dyeing.

2.7 Estimation of Dye Uptake

To study quantity of indigo uptake on cotton after each dip and nip, known weight of dyed cotton was dissolved in dimethyl sulfoxide (DMSO) to extract indigo from dyed cotton. The extract was analyzed in UV-Vis spectrophotometer (Perkin Elmer) to evaluate absorbance and subsequently dye uptake per 100 g of cotton.

2.8 Stability of Reduced Dye Bath

Reduction baths in sodium hydrosulphite and alkaline pectinase were prepared in absence of dye and stored for specific period (0-24 h) at 30°C, after which pH and reduction potential (-mV) were noted down. Dye was added and after reduction and solubilization of dye, cotton was dyed. Similarly, for studying the stability of reduced bath in presence of dye, dye baths were prepared and stored for specific period (0-24 h) at 30°C; *p*H and mV were noted down followed by dyeing of cotton in these baths.

2.9 Attenuated Total Reflection- Infrared (ATR-IR) Spectroscopy

FTIR spectra of white as well as dyed cotton fabric were obtained on Alpha eco-ATR spectrometer equipped with a zinc selenide (ZnSe) crystal using 16 scans /sample in the range of 4000 - 500 cm^{-1}, at a resolution of 16 cm^{-1}.

2.10 Scanning Electron Microscopic (SEM) Study

Surface morphology of dyed cotton was characterized using SEM (Zeiss EVO 50, Germany) at a voltage of 10 kV and 5k magnification.

2.11 X-ray Diffraction

The diffractograms were collected using Malvern Pan analytical XRD. The small sample was clamped into a sample holder on a goniometer (radius 240 mm) in the scanning range 10-60° with a step size of 0.008° and X-ray radiation wavelength (λ) of 1.5406 A° using Cu K_{α}. The X-ray generator was operated at 40 mA and 45 kV.

2.12 Tensile Strength of Cotton Fabric

Universal testing machine (Aimil Ltd., Delhi) was used for measuring tensile strength of cotton through ASTM D5035 test method after dyeing in both reducing systems.

3 Results and Discussion

3.1 Dyeing of Cotton with Sodium Hydrosulphite

Cotton was dyed with indigo using '6 dip 6 nip' padding technique followed by oxidation and washing. *K/S* of dyed cotton was obtained as 22.40 at λ_{max} 590 nm. The range of *p*H and mV were 12.2 - 12.8 and from -640 to -780 respectively at various stages of dyeing.

3.2 Dyeing of Cotton with Alkaline Pectinase

Attempts were made to dye cotton with indigo in the same way using alkaline pectinase in place of sodium hydrosulphite with neither generation of required reduction potential nor any dye strength (*K/S*). The reduction potential of bath was in the range of (-340 to -390) mV. In contrast, indigo requires a reduction potential of around -660 mV and more for reduction. It is learnt that enzyme activity is enhanced with addition of metals in bath[18,19]. Because of this, indigo baths are formulated with alkaline pectinase along with iron (II) sulphate as reducing agent. Cotton is dyed in this bath following the same procedure that is used in hydrosulphite system. It is observed that the reduction potential of the bath is raised to around (-720) mV with reduction of indigo and consequently cotton is dyed with lower *K/S*. To improve the *K/S*, levels of dyeing parameters were varied and reduction baths are prepared with dyeing of cotton from each bath to attempt parallel dye strength on cotton against that obtained in hydrosulphite system. It is observed that the concentration ranges are $FeSO_4$ (20-25g/L), NaOH (7.5-12/5g/L), alkaline pectinase (1-2g/L), indigo (7-8g/L) and temperature (60-80°C). The actual values of levels are formed accordingly (Table 1).

Putting these levels in Box Behnken design results in 46 separate runs. indigo baths are prepared based on these sets of parameters and levels, cotton is dyed and *K/S* is evaluated. The results are shown in Table 2.

It is observed that the range of *K/S* is 12.9 - 24.5 and maximum K/S is observed for the bath run 24, i.e. $FeSO_4$ (22.5g/L), NaOH (12.5g/L), alkaline pectinase (2g/L), indigo (7.5g/L) and temperature (70°C). It is to be noted that a 3 g/L indigo bath results in a *K/S* of 22.40 in hydrosulphite system.

Table 1 — Process parameters and their coded levels

Coded factor	Factors (Independent variables)	Levels		
		-1 (low)	0 (medium)	1 (high)
A	Ferrous sulphate, g/L	20	22.5	25
B	Sodium hydroxide, g/L	7.5	10	12.5
C	Pectinase, g/L	1.0	1.5	2.0
D	Indigo, g/L	7.0	7.5	8.0
E	Temperature, °C	60	70	80

Table 2 — Responses of Box-Behnken analysis

Run	$FeSO_4$ g/L	NaOH g/L	Pectinase g/L	Indigo g/L	Temperature °C	*p*H	*K/S*
1	20	7.5	1.5	7.5	70	12.29	21.49
2	25	7.5	1.5	7.5	70	10.64	12.97
3	20	12.5	1.5	7.5	70	11.41	17.14
4	25	12.5	1.5	7.5	70	11.76	18.95
5	22.5	10	1	7	70	12.41	20.47
6	22.5	10	2	7	70	11.58	18.99
7	22.5	10	1	8	70	12.57	21.43
8	22.5	10	2	8	70	11.55	18.72
9	22.5	7.5	1.5	7.5	60	10.83	14.72
10	22.5	12.5	1.5	7.5	60	11.62	17.23
11	22.5	7.5	1.5	7.5	80	10.98	16.90
12	22.5	12.5	1.5	7.5	80	11.60	18.40
13	20	10	1	7.5	70	12.35	22.00
14	25	10	1	7.5	70	12.70	21.54
15	20	10	2	7.5	70	12.38	20.91
16	25	10	2	7.5	70	11.92	19.83
17	22.5	10	1.5	7	60	11.59	19.59
18	22.5	10	1.5	8	60	11.08	16.81
19	22.5	10	1.5	7	80	11.88	19.86
20	22.5	10	1.5	8	80	12.63	20.45
21	22.5	7.5	1	7.5	70	10.94	16.10
22	22.5	12.5	1	7.5	70	12.63	21.05
23	22.5	7.5	2	7.5	70	10.35	13.50
24	22.5	12.5	2	7.5	70	12.78	24.5
25	20	10	1.5	7	70	12.39	21.13
26	25	10	1.5	7	70	12.75	22.38
27	20	10	1.5	8	70	12.30	20.23
28	25	10	1.5	8	70	12.77	22.63
29	22.5	10	1	7.5	60	12.87	20.48
30	22.5	10	2	7.5	60	12.52	20.47
31	22.5	10	1	7.5	80	12.50	20.25
32	22.5	10	2	7.5	80	11.51	18.47
33	20	10	1.5	7.5	60	12.30	23.86
34	25	10	1.5	7.5	60	12.74	22.85
35	20	10	1.5	7.5	80	11.40	19.77
36	25	10	1.5	7.5	80	11.23	17.16
37	22.5	7.5	1.5	7	70	10.15	12.9
38	22.5	12.5	1.5	7	70	12.63	20.18
39	22.5	7.5	1.5	8	70	10.98	13.64
40	22.5	12.5	1.5	8	70	12.62	23.34
41	22.5	10	1.5	7.5	70	12.59	22.18
42	22.5	10	1.5	7.5	70	12.58	21.98
43	22.5	10	1.5	7.5	70	12.58	21.76
44	22.5	10	1.5	7.5	70	12.56	22.20
45	22.5	10	1.5	7.5	70	12.60	22.34
46	22.5	10	1.5	7.5	70	12.58	21.94

3.2.1 Influence of Dyeing Parameter on K/S

The influence of process parameters, viz. concentrations of $FeSO_4$, NaOH, pectinase & indigo and temperature on *K/S* has been investigated by Box Behnken design and response surface methodology. All the main effect, two factors interaction and cubic effect with R-square 0.96 are obtained by ANOVA (Table 3).

The model is found significant at 95% confidence level, as value of 'Prob>F' is less than 0.05. In this case, $FeSO_4$, NaOH, alkaline pectinase and temperature are the significant model factors. The model equation in coded form is shown below:

$$K/S = +22.05 - 0.64\times A + 0.70\times B - 0.73\times C - 0.18\times D + 0.91\times E + 2.58\times A\times B + 0.29\times A\times D - 0.40\times A\times E + 1.51\times B\times C + 0.61\times B\times D - 0.44\times C\times E + 0.84\times D\times E - 0.067\times A^2 - 3.55\times B^2 - 0.58\times C^2 - 1.07\times D^2 - 1.53\times E^2 - 3.35\times A^2\times E - 1.03\times A\times B^2 + 1.56\times A\times D^2 + 0.95\times B^2\times C + 1.15\times B^2\times D + 3.29\times B\times C^2 + 3.54\times B\times D^2 - 1.46\times C^2\times E$$

Table 3 — ANOVA results for *K/S*

Source	Sum of squares	df	Mean square	F Value	p-value prob>F
Model	366.8477909	25	14.67391164	20.88725085	< 0.0001 (significant)
$FeSO_4$ (A)	3.3282	1	3.3282	4.737451744	0.0417
NaOH (B)	3.9762	1	3.9762	5.65983283	0.0274
Alkaline Pectinase (C)	6.3948	1	6.3948	9.102534828	0.0068
Dye (D)	0.3888	1	0.3888	0.553428652	0.4656
Temperature (E)	6.58845	1	6.58845	9.378181583	0.0061
AB	26.677225	1	26.677225	37.9730984	< 0.0001
AD	0.330625	1	0.330625	0.470620751	0.5006
AE	0.64	1	0.64	0.910993665	0.3512
BC	9.1204	1	9.1204	12.9822291	0.0018
BD	1.4884	1	1.4884	2.118629643	0.161
CE	0.7744	1	0.7744	1.102302335	0.3063
DE	2.839225	1	2.839225	4.041431233	0.0581
A^2	0.039763636	1	0.039763636	0.056600658	0.8144
B^2	110.1404182	1	110.1404182	156.7769113	< 0.0001
C^2	2.9106	1	2.9106	4.143028378	0.0553
D^2	10.0386	1	10.0386	14.28922032	0.0012
E^2	20.45193333	1	20.45193333	29.11184642	< 0.0001
A^2E	29.97135	1	29.97135	42.66204685	< 0.0001
AB^2	2.842816667	1	2.842816667	4.04654371	0.0579
AD^2	6.468816667	1	6.468816667	9.207892194	0.0065
B^2C	2.679075	1	2.679075	3.813469302	0.065
B^2D	3.9675	1	3.9675	5.64744901	0.0276
BC^2	28.7766	1	28.7766	40.96140673	< 0.0001
BD^2	33.32326667	1	33.32326667	47.43325756	< 0.0001
C^2E	5.70375	1	5.70375	8.118875184	0.0099
Residual	14.05059167	20	0.702529583	-	-
Lack of fit	13.84045833	15	0.922697222	21.95504177	0.0015 (significant)
Pure error	0.210133333	5	0.042026667	-	-
Cor total	380.8983826	45	-	-	-

This equation can predict theoretical *K/S* of dyed samples for given dyeing parameters. The influence of various parameters are discussed hereunder:

- **Influence of $FeSO_4$ and NaOH Concentration**

The combined effect of $FeSO_4$ and NaOH concentrations on *K/S* at constant temperature, indigo concentration (medium level) and alkaline pectinase concentration (higher level) is shown in Fig. 1(a). Higher level of NaOH (12.5g/L) and medium level of $FeSO_4$ (22.5g/L) result in the maximum *K/S* of 24.5. Corresponding *p*H of dye bath is found to be in the range of 12.3-12.8 before and after reduction of dye as well as at the end of dyeing. On decreasing the concentration of NaOH, *K/S* of dyed cotton is decreased to 13.5, may be due to partial reduction of indigo. $FeSO_4$ reacts with NaOH to form $Fe(OH)_2$, thus decreasing the concentration of NaOH (lower level) in the dye bath.

- **Influence of $FeSO_4$ and Temperature**

The combined effect of $FeSO_4$ concentration and temperature on *K/S* at constant NaOH, alkaline pectinase concentration (higher level) and indigo concentration (medium level) is shown in Fig. 1(b). It can be seen that the medium level of $FeSO_4$ (22.5g/L) and temperature (70℃) gives the maximum *K/S* of 24.5. On increasing the $FeSO_4$ concentration and temperature, *K/S* of dyed cotton is decreased due to inactivation of pectinase to reduce indigo.

- **Influence of NaOH and Pectinase Concentration**

The combined effect of NaOH and pectinase concentrations on *K/S* at constant $FeSO_4$, indigo concentration and temperature (medium level) is shown in Fig. 1(c). Higher levels of NaOH (12.5g/L) and pectinase concentration (2g/L) result in the

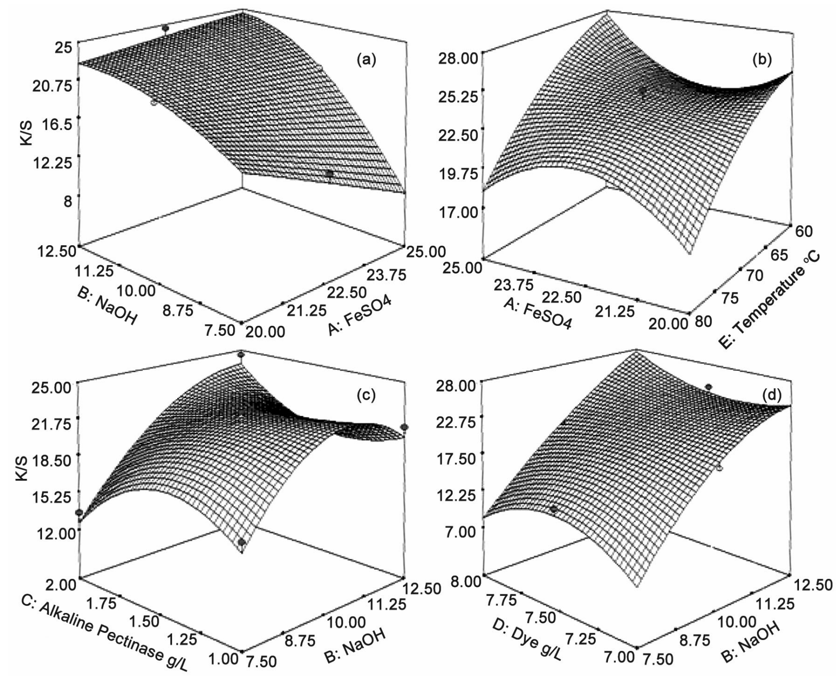

Fig. 1 — Influence of indigo dyeing parameters on dye strength of cotton (a) $FeSO_4$ and NaOH, (b) $FeSO_4$ and temperature, (c) NaOH and pectinase, and (d) NaOH and indigo

maximum *K/S* of 24.5. On decreasing the concentration of NaOH and pectinase, *K/S* of dyed cotton is decreased, may be due to partial reduction and solubilization of indigo in the dyebaths.

- **Influence of NaOH and Indigo Concentration**

The combined effect of NaOH and indigo concentration on *K/S* at constant $FeSO_4$ concentration temperature (medium level) and pectinase concentration (higher level) is shown in Fig. 1(d). Here, higher level of NaOH (12.5g/L) and medium level of indigo concentration (7.5g/L) give maximum *K/S* of 24.5. On decreasing the NaOH concentration, *K/S* of dyed cotton is decreased to 13.5, may be due to insolubilization of indigo in dyebaths.

3.3 Indigo Uptake and Dye Strength

The dye uptake [amount of dye (g)/100g cotton] after each dip/nip has been evaluated and is shown in Fig. 2(a). Respective *K/S* after each dip/nip against that of indigo uptake is shown in Fig. 2(b). *K/S* and dye uptake both increase proportionately. Though final *K/S* is found nearly identical in both the reducing systems, total dye uptake is found to be higher in pectinase system in spite of less *K/S* in pectinase system after first dip/nip as compared to that in hydrosulphite. The same *K/S* in both the cases with variation in dye uptake permits more diffusion of dye into cotton in alkaline pectinase system.

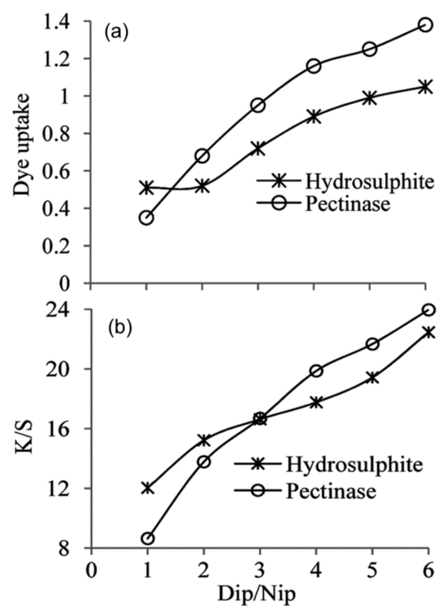

Fig. 2 — Result of dyeing after each dip/nip (a) dye uptake, and (b) *K/S*

3.4 Stability of Reduction Bath

3.4.1 In Absence of Dye

The stability of reduction baths having $Na_2S_2O_4$ and alkaline pectinase with iron (II) salt is also studied in the absence of dye upto 24 h. The blank reduction baths without indigo are stored for specific time after which *p*H and reduction potential of baths are measured succeeded by addition of indigo; the results are shown in Table 4. Both the reducing systems retain their reducing capability upto 24 h. There is a progressive drop in mV and *p*H in both reducing baths with increase of time. *K*/*S* gradually decreases with increase in storage time in both reducing systems [Fig. 3(a)]. Though reduction baths in alkaline pectinase system show good stability over 24 h, the hydrosulphite based baths go on losing stability beyond 4 h at a faster pace. However, maximum surface colour strength (*K/S*) is observed for dyeing at zero hours (just after preparation of baths) in both the reduction systems.

3.4.2 In Presence of Dye

Reduction baths with $Na_2S_2O_4$ and alkaline pectinase with iron (II) salt have been prepared followed by addition of indigo, covered and stored upto 24 h. After storing for specific time, *p*H and reduction potential of the baths are measured (Table 4). Both the reducing systems retained their reducing capability till 24 h. There is a steady drop in mV and *p*H of both reducing baths with increase of time and proportionate fall in *K/S* as well is observed [Fig. 3(b)]. In this case also, reduced dyebaths show maximum dye strength for dyeing at zero hour.

3.5 Characterization

3.5.1 SEM Analysis of Dyed Cotton Fabric

The purpose of SEM analysis is to evaluate morphological differences among undyed and dyed cotton using sodium hydrosulphite/alkaline pectinase alongwith iron (II) sulphate as reducing agents. The SEM image of undyed cotton as well as cotton dyed using sodium hydrosulphite and pectinase system is shown in Figs 4(a) -(c) respectively. The images show minimal damage on the surface of cotton dyed in sodium hydrosulphite system, while this is not

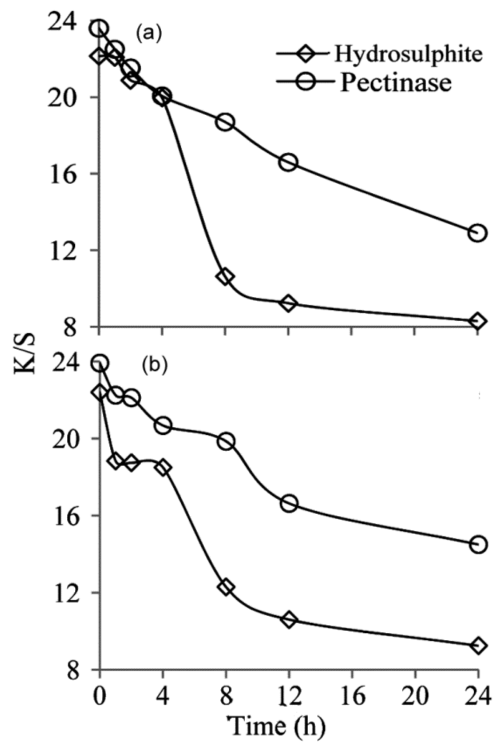

Fig. 3 — Dye strength of cotton (a) in absence, and (b) in presence of dye

Table 4 — Stability of reduction baths in absence and presence of dye

Time h	Before reduction of dye				After reduction of dye				After completion of dyeing			
	*p*H		mV		*p*H		mV		*p*H		mV	
	Hydro	Pec	Hydro	Pec	Hydro	Pec	Hydro	Pec	Hydro	Pec	Hydro	Pec
In absence of dye												
0	12.74	12.79	-772	-736	12.60	12.66	-760	-719	12.30	12.46	-748	-703
1	12.74	12.77	-765	-732	12.38	12.61	-755	-714	12.18	12.42	-750	-698
2	12.73	12.72	-770	-728	12.29	12.58	-767	-710	12.09	12.38	-720	-695
4	12.72	12.78	-782	-725	12.50	12.64	-740	-709	12.10	12.48	-730	-693
8	12.70	12.75	-706	-738	12.30	12.62	-685	-718	12.19	12.46	-640	-680
12	12.72	12.70	-680	-722	12.34	12.55	-660	-706	12.12	12.38	-610	-670
24	12.66	12.68	-655	-720	12.20	12.50	-620	-702	12.05	12.28	-580	-655
In presence of dye												
0	12.51	12.76	-709	-718	12.48	12.55	-701	-707	12.35	12.39	-612	-690
1	12.49	12.78	-716	-736	12.45	12.58	-705	-705	12.35	12.42	-610	-683
2	12.49	12.72	-703	-719	12.45	12.62	-699	-706	12.26	12.45	-605	-670
4	12.48	12.66	-705	-711	12.44	12.48	-695	-705	12.13	12.32	-600	-665
8	12.48	12.79	-718	-709	12.44	12.59	-707	-696	12.04	12.41	-590	-666
12	12.49	12.62	-701	-705	12.42	12.45	-689	-692	11.80	12.35	-550	-652
24	12.48	12.75	-704	-707	12.41	12.60	-685	-690	11.50	12.29	-489	-640

Hydro–Hydrosulphite, and Pec–pectinase.

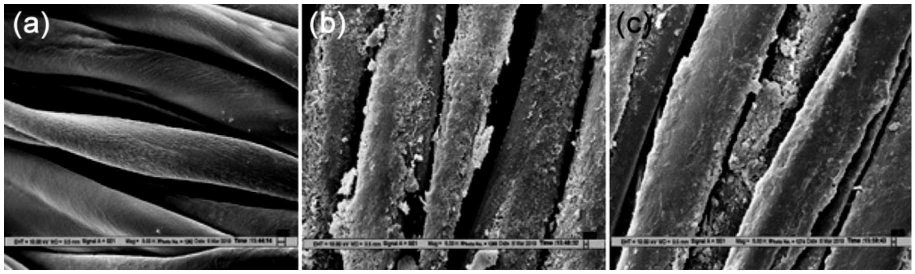

Fig. 4 — SEM images of (a) undyed cotton, (b) cotton dyed using sodium hydrosulphite, and (c) cotton dyed using alkaline pectinase

observed in alkaline pectinase system. The white spots on dyed cotton are those of indigo deposited on surface.

3.5.2 ATR-FTIR of Dyed Cotton

ATR-FTIR spectra are obtained using a brucker spectrophotometer. Spectra of dyed cotton with $Na_2S_2O_4$ and alkaline pectinase are compared with that of undyed cotton (control). The absorption bands are mainly observed in the ranges of 3869 - 2902 cm^{-1} and 1620 - 522 cm^{-1}.

A strong band spectra is found in range of 3869-2900 cm^{-1}, which is due to stretching vibration of O-H and C-H bonds and the band peak around 3258-3252 cm^{-1} is because of stretching vibration of R-OH in cellulose. This peak also includes inter- and intra-molecular hydrogen bond[20-22]. The band peak at around 2911cm^{-1} is due to symmetrical and asymmetrical stretching of -CH_2 groups in cellulose[23, 24]. Typical band in the range of 1623-522 cm^{-1} is also observed. The absorption band at 1620-1619 cm^{-1} is characterized for stretching of C=C[22]. The band peaks at 1459, 1393, 1366, 1310,1161 and 1057 cm^{-1} are characterized for deformation or stretching vibrations of C=O, C–H, C–O–C, C–O, C—N, C=C and N-H groups in cellulose as well as indigo[23-25]. Differences are observed in spectrum of dyed cotton using alkaline pectinase. There are changes in absorption band at 748 cm^{-1} and 699 cm^{-1}, which is assigned to in-plane bending of methyl group and out of plane ring (C=C) bend respectively in pectinase structure[24]. ATR-IR spectra of both sodium hydrosulphite and alkaline pectinase systems are found to be nearly same. Thus, it can be concluded that no chemical changes occur in new proposed reduction system.

3.5.3 XRD of Dyed Cotton

The X-ray diffractograms of undyed and dyed cotton is shown in Fig. 5. The purpose of the XRD is to evaluate the loss in crystallinity index of cotton after dyeing. The degree of crystallinity is one of the most important parameters for crystalline structure

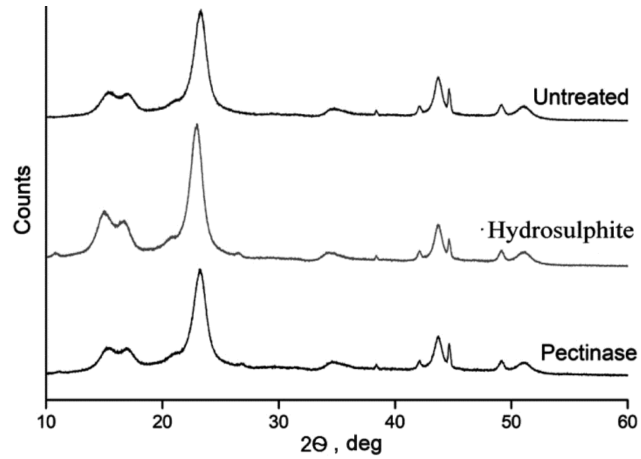

Fig. 5 — XRD diffractograms of undyed and dyed cotton

and is evaluated by Herman's method[19, 26]. According to which, the crystallinity index is calculated by the ratio of crystalline area to the total area of X-ray diffraction curve, as given below.

Crystallinity Index (%) = $A_{Crystalline} / A_{Total}$

Oxidation reaction of indigo may led to a decrease in degree of polymerization of cellulose and may cause loss in tensile strength of cotton. Study reveals that crystallinity of dyed cotton remained unaltered in both the reducing systems. There is marginal fall (7.0-7.5%) in crystallinity of dyed cotton with hydrosulphite, whereas in pectinase system, there is no fall in crystallinity of cotton (Table 5). This indicates that no such significant damage in cotton is occurred in newly proposed alkaline pectinase based reducing systems.

3.6 Fastness Performance

Performance of light, rub and wash fastness of indigo dyed cotton in $Na_2S_2O_4$ and pectinase systems have been evaluated. The light, wash and rub fastness ratings remain in the ranges 6, 4-5 and 4-5 in both $Na_2S_2O_4$ and pectinase reducing systems respectively.

Table 5 — Tensile strength and crystallinity index % of undyed and dyed cotton

Sample	Warp way		Weft way		Crystallinity index, %
	Breaking strength Nf	Loss in strength %	Breaking strength Nf	Loss in strength %	
Untreated	527	-	448	-	70.01
Hydrosulphite	487.8	7.44	417.5	6.8	66.62
Pectinase	527	4.55	425	5.1	68.16

4 Conclusion

The work reported in this study illustrates application of alkaline pectinase alongwith iron (II) salt as reducing agent for dyeing of cotton with indigo. Surface dye strength with 3 g/L indigo in hydrosulphite system shows complete match with that of cotton dyed in pectinase system for an indigo concentration of 7.5 g/L, though dye uptake with increased concentration of indigo is found to be on higher side. The stored baths in both absence and presence of indigo show good stability upto 24 h, but maximum dye strength is obtained at zero hour dyeing. Damage on surface of dyed cotton is less prominent in alkaline pectinase based reducing system. The change in crystallinity index is around 2%, i.e. no vital damage is observed. Loss in tensile strength of dyed cotton using alkaline pectinase is lesser than that with hydrosulphite due to less damage of cotton. Thus, the study confirms viability of alkaline pectinase alongwith iron (II) salt in place of sodium hydrosulphite for indigo dyeing of cotton.

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