STM JOURNALS

Journal of
Water Pollution &
Purification Research

ISSN: 2394-7306
Volume 13, Issue 1, 2026
January–April
DOI (Journal): 10.37591/JoWPPR

https://journals.stmjournals.com/jowppr

Research JoWPPR

Bioscouring And Anionization of Waste Cotton Fabric for Efficient Removal of Malachite Green Dye

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Abstract

Adsorption is an efficient method for dye removal but the high cost of adsorbents limits its use, emphasizing the need for sustainable, low-cost alternatives. This study explores the usage of modified cotton fabric for the treatment of malachite green (cationic dye) from wastewater. Cellulase extracted from Raphanus sativus and Daucus carota *were evaluated for bioscouring potential which was confirmed by scanning electron microscopy. Further, cotton fabric was chemically modified using acetic acid, confirmed by Fourier transform infrared spectroscopy. Dyeing parameters (pH, liquor ratio, contact time, temperature) was assessed through dye exhaustion. The specific activity of extract from Colocasia esculenta, Raphanus sativus, Daucus carota was 3.61 U/mg and 8.49 U/mg and 6.04 U/mg respectively using carboxymethyl cellulose as a substrate. Samples treated with extracts of Raphanus sativus showed highest glucose release of 1833.3 µg. SEM analysis showed that fabric treated with Daucus carota extract has significant serrated edges. The optimum parameters showed that exhaustion increases with increasing the acetic acid concentration at pH 6, temperature 95 ºC. Percentage exhaustion of dye with optimum parameters is better with anioised fabric i.e. 42.5% (Colocasia esculenta), 28.2% (Raphanus sativus), 25.6% (Daucus carota). Anionised fabrics, especially those treated with* Colocasia esculenta*, demonstrated improved dye removal efficiency, confirming that fabric anionization is an effective, sustainable approach for cationic dye removal from wastewater.*

Keywords: *Colocasia esculenta, raphanus sativus, daucus carota,* cellulase, anionization, FTIR, adsorption

INTRODUCTION

Dyes are predominantly used in industries like textile, pharmaceuticals, cosmetics, food, and paper for their colouring properties. Around 10,000 dyes are available in the market with an estimated production of 7×10^5 tons per year [1]. Textile industry alone is consuming approximately two-third of the dye production, making them the biggest consumer of synthetic dyes. This leads to the production of huge amount of dye containing effluent which then cause water pollution and have harmful impact on the environment as illustrated in Figure 1 [1–3]. During the dyeing process around 10-15% of the dye could not bind to the fabric and remained in the dyebath which eventually get into the environment and contribute to the pollution [4]. Cationic dyes belong to a class of water-soluble dyes that carry a positive charge when dissolved in water. They are extensively used for dyeing textile fabrics, including cotton, nylon, wool, acrylic, and silk.

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Received Date: March 24, 2026
Accepted Date: April 27, 2026
Published Date: April 30, 2026

Citation: Priyanka Kakkar, Pammi Gauba. Bioscouring And Anionization of Waste Cotton Fabric for Efficient Removal of Malachite Green Dye. Journal of Water Pollution & Purification Research. 2026; 13(1): 53–68p.

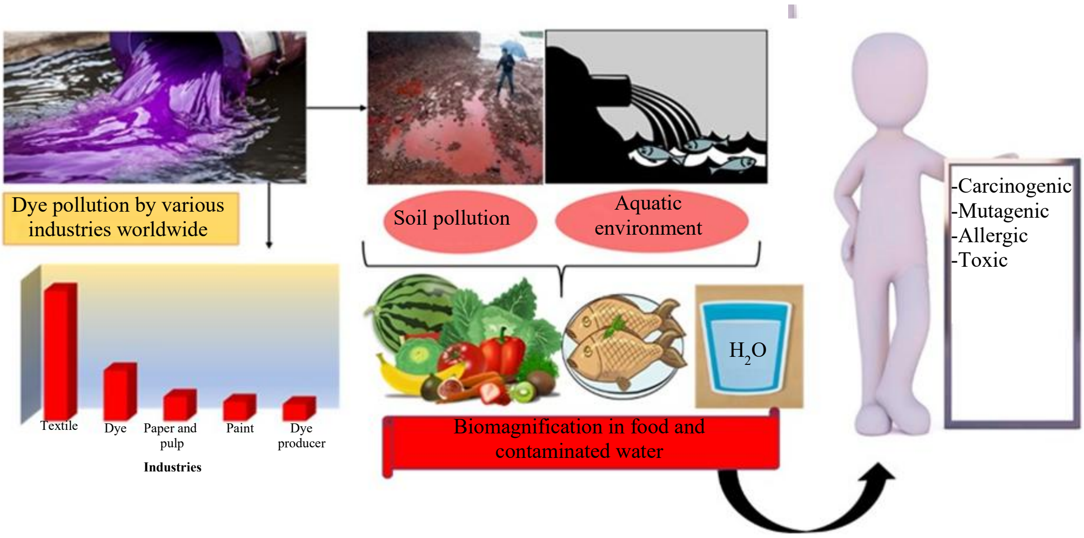

Figure 1. Schematic representation of percentage of dye pollution caused by industries and its on environment [Modified from [48]**]**.

Some examples of cationic dyes are methyl violet, methylene blue, nile blue dye and malachite green [5–7]. Beyond textiles, these dyes also find applications in industries like paper and inkjet printing [7]. Besides their extensive usage they have been associated with potential toxicity, and have harmful effects on the skin, such as skin dermatitis, mutations, and even the development of cancer [5, 8]. Studies suggest that cationic dyes exhibit higher toxicity compared to anionic dyes, with reported tinctorial values of 1 mg/L [9].

Malachite green is a cationic triphenylmethane compound used in industries like textile for colouring textile, food additives and colouring, and for staining cells and tissue samples for microscopic analysis [10]. It has been reported that the structure of malachite green is very complex and often shows resistant to oxidising agents and tend to persist in the environment for longer period of time with a half-life of 12.9-50.34 days. It has negative impact on the environment, aquatic organisms as well as on terrestrial life forms [11–13]. It is considered as multiorgan toxin, cause lesions on the skin, eyes also have negative impacts on spleen, lungs, kidney, fertility rate of rat and mice. In mammalians it is reported to be cytotoxic and have carcinogenic effects below concentration 0.1g/ml [10,11]. Therefore, it is crucial to treat the effluent containing dyes before discharging them into the environment.

Many physiochemical (solvent extraction, adsorption, photocatalysis, oxidation, membrane-based separation, reverse osmosis), nuclear treatment, electromagnetic processes and biological (aerobic, anaerobic, microbial and phytoremediation) techniques are used for the treatment of industrial runoffs [5, 14–16]. Physiochemical processes like oxidation, coagulation-flocculation, chemical precipitation have many limitations like high cost, works only at specific concentrations, incomplete removal of substances, toxic by-products, high energy requirements, production of secondary sludge, high investment, non-selectiveness [11, 13, 17–18]. Adsorption is a commonly used physiochemical method for dye removal with several benefits including its simplicity, efficiency, design flexibility, ease of operation, and lack of harmful by-products and adaptability [19–21]. However, economic considerations, including the cost and energy associated with using and regenerating adsorbents, must be considered. The large amount of adsorbent requirement, high costs of adsorbents, and the challenge of separating adsorbent from adsorbates [22]. There is a need to shift towards the circular economy model for the development of innovative and sustainable adsorption material with high adsorption capacity, high selectivity, and easy.

Circular economy model is based on the concept of 10Rs: reuse, rethink, repurpose, refurbish, recycle, recreate, repair, reduce, remanufacture and recovery. This is an economy model that believes in minimizing waste and keeping the material in use for longer time by using 10Rs [23]. The rapid growth of the global population has led to an increased demand for food, particularly fruits and vegetables. Consequently, nearly 30–40% of total production is lost or discarded as waste. Numerous studies have explored the potential of utilizing these by-products as raw materials for the development of value-added products [24]. This study investigates vegetable as sustainable sources of enzymes and modification of waste cotton fabric for removal of malachite green dye from water. Cotton fabric is a strong functional material that has the potential to be used in no. of applications, like water treatment, heavy metal removal and dyes, packaging, and biomedical items (drug delivery, scaffolds, biosensors, antimicrobial) [25, 26]. Cotton fabric is made up of cellulose, a natural polymer, which is biodegradable, renewable and a sustainable choice for modification [27]. Recent studies have reported, cellulose fabric can be modified by replacing the hydroxyl group at the surface with other functional groups like carboxyl groups for increasing the hydrophilicity and adsorption capacity for dye removal as depicted in Figure 2 [28,29]. Modification of cotton fabric was done through enzymatic and anionization method. Fourier transform infrared attenuated total reflectance (FTIR-ATR) spectroscopy was done to analyse the structural changes and functional group identification after the acetic acid treatment. In our previous study, we observed the presence of cellulase activity in six different crops including *Colocasia esculenta*, *Raphanus sativus*, and *Daucus carota*. The impact of *Colocasia esculenta* on cotton fabric treatment has been previously documented, but the effects of *Raphanus* and *Daucus* on cotton fabric treatment remain unexplored in our research [30]. Surface modification through anionization is done to introduce anionic groups onto the cotton fabric. The main use of anionization method is to adsorb dye from the solution by forming ionic bonds between the cationic dyes and the anionic groups on the fibre [19]. It is done to explore the possibility of modified cotton fabric as a potential adsorbent for efficient removal of cationic dyes from wastewater.

MATERIALS AND METHODS

Raphanus sativus (R), Daucus carota (C), Colocasia esculenta (CE) were purchased from Safal store, a retail chain of fresh fruits, vegetables and other item at Ghaziabad, owned by Mother Dairy. Plain woven 100% raw cotton fabric with 130 gsm (grams per square meter) was procured from a local supplier. Bioscoured cotton fabric was prepared in our institutional biotechnology laboratory, Malachite green dye, acetic acid, potassium sodium tartrate (Rochelle salt solution) was purchased from Central Drug House (P) Ltd. Carboxymethyl cellulose was purchased from Sigma-Aldrich.

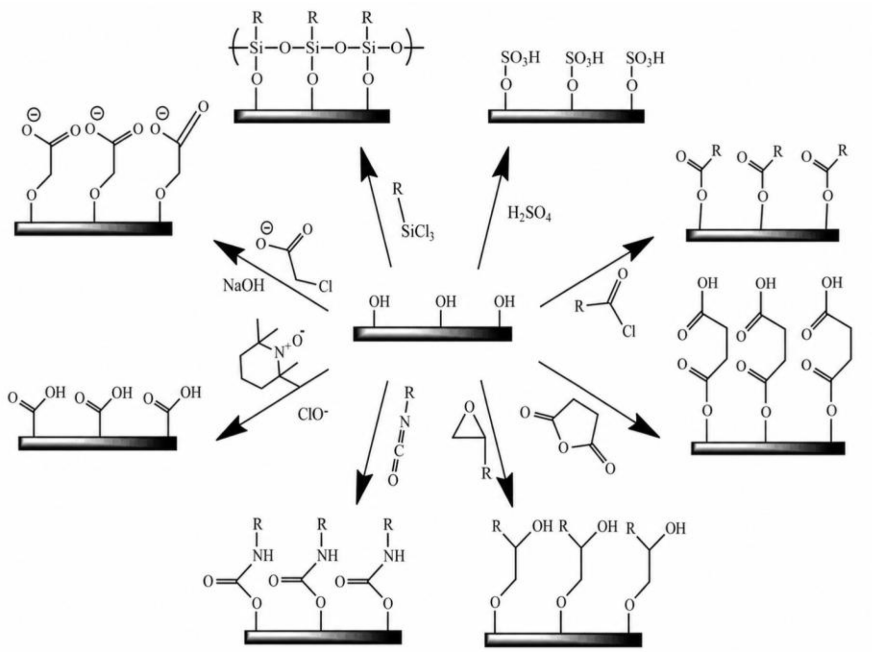

Figure 2. Various ways of cellulose modification [Adapted from [28]]

Extraction of Cellulase from Underground Roots

Cellulase was extracted and characterized from CE, R and C. 400 g of each sample was grounded with 0.1 M citrate buffer, pH 5.0, in a ratio of 1:3 separately. The resulting homogenate was passed through a cheesecloth to obtain the crude enzyme extract. Bradford method was used for the determination of protein concentration using Bovine serum albumin as standard [30, 31].

Determination of Cellulase Activity in Underground Roots

Dinitrosalicylic acid (DNS) method with some modification was used for the determination of cellulase activity in extracts of CE, R and C [32]. Carboxymethyl cellulase assay (CMCase) and Filter paper assay are the two most common assay used for the endoglucanase activity and total cellulase activity respectively. For both the assays, 0.9ml of 0.1M sodium citrate buffer (pH 5), 0.1ml sample and 1ml of 1% (w/v) CMC or 25 mg Whatman no. 1 filter paper soaked in 1 ml of citrate buffer was used as reaction mixture and incubated at 50 °C for 1 h. Further, 2ml of 3,5- DNS was added to terminate the reaction and the test tubes were then set in a water bath at 95°C for 10 min and 1ml of 40% Rochelle salt solution was added after it cooled down. Finally, 6ml of distilled water was added and absorbance was taken at 540nm. A single unit (U) of enzyme activity was defined as the quantity of enzyme capable of generating 1 μmol of glucose equivalent within a minute [33].

Enzymatic Treatment of Cotton Fabric

Small pieces of cotton fabric were placed in 2 litre of reagent bottle having crude extract from of CE, R and C in 1:2 ratio (675ml buffer and 1350 ml enzyme) with 0.1M sodium citrate buffer. Bioscouring of cotton fabric was done at pH 5, at 50 °C for different time intervals (24, 48, 72, 96 hours). Control treatment was also carried out similarly without enzyme addition. All samples were rinsed with distilled water at room temperature and air dried.

Microscopic Analysis of Fabric

Structural changes after the enzymatic treatment of cotton fabric was analysed by scanning electron microscopy (SEM) (model: Zeiss (MA EVO -18 Special Edition)) at Amity Institute of Advanced Research and Studies. Both raw fabric and enzymatically treated sample were first subjected to gold plating as they were non-conducting samples. The analysis of fiber surfaces were recorded at magnification 5000X.

Protox-II

Toxicity analysis of phenolic compounds was done using a free online software named Protox-II available at http://tox.charite.de/protox_II. This is a predictive model built on range of algorithms from data of invitro assays (Tox21) and invivo cases. In-silico toxicity predictions are necessary because it is hard to test all chemicals in lab, as screening is time taking, high cost and has ethical concerns related to animals [34]. Canonical structures of the compounds were taken from the PubChem and uploaded in the tox prediction, canonical smiles tab and then start the prediction. It provides LD50 (lethal dose) value and different levels of toxicity like organ toxicity, oral toxicity, toxicity targets, toxicological pathways and endpoints [35].

Surface Modification of Cotton Fabric Through Anionization

Once the enzymatic treatment is done, the fabrics were soaked in different concentration (0.5%,1%, 1.5%, 2%, 2.5%, 3%) of acetic acid solution for 1 hour and dried at 50 °C for 15 mins to add carboxyl group to the surface of fabric.

Fourier Transform Infrared Spectra- Attenuated Total Reflectance

Enzymatically treated fabric and anionised fabric i.e. fabric treated with enzyme and acetic acid both, was further characterized by FTIR-ATR spectroscopy (Model: Perkin Elmer's Spectrum two FTIR) at wavelength range of 400 to 4000 cm^{-1} at the Nothern India Textile Research Association (NITRA), Ghaziabad.

Droptest

A droplet test was conducted to assess the adsorption characteristics of a colored droplet on cotton fabrics. This evaluation involved measuring the diffusion zone and the time required for the fabric to completely adsorb the droplet. Malechite green (1% w/v) was prepared as a standard dye stock. 50µl of malachite green was dropped onto the raw fabric, enzymatically treated and anionised cotton fabric. A stopwatch was started simultaneously and stopped once the dye was completely adsorbed, and the adsorption time was recorded [27].

Exhaust Dyeing

Dyeing of enzymatically treated and anionised cotton fabric was done using dyebath with liquor ratio 150:1. Dyebath was prepared in test tubes each containing 200 µl of malachite green dye (1% w/v), 100 mg fabric, 14.7ml water making it 15ml total volume of dyebath. The water bath was set at 95°C, 15 mins prior to the addition of dyebath. The dyebath was covered with polythene and incubated at 95°C for 2 hours. After dyeing is completed, cotton fabric is removed from the bath and washed with warm distilled water and air dry [36].

Estimation of Percentage Exhaustion (%E)

The quantity of dye absorbed by the fabric is ascertained using the percentage exhaustion method, which involves measuring the initial and final absorbance of the dye bath during the dyeing process at a wavelength of 619nm as shown in equation 1 [36, 37].

$$\%E = \left(\frac{A_0 - A_t}{A_0}\right) \times 100 \quad (1)$$

Where A_0 is absorbance of the dyebath before dyeing, A_t is absorbance of the dyebath after dyeing. Partition Co-efficient (K) is defined as ratio between the dye absorbed by the fabric and the dye content left in the dye bath at equilibrium as shown in equation 2 [36].

$$K = \frac{dye\ absorbed\ by\ fabric}{amount\ of\ dye\ left\ in\ dyebath} = \frac{liquor\ ratio * \%E}{Q} \quad (2)$$

$$liquor\ ratio = \frac{volume\ of\ dyebath}{mass\ of\ fabric}, Q = 100 - \%E$$

Optimization of Dyeing Parameters

This study investigates the optimal conditions for maximizing the removal of malachite green dye from aqueous solution using surface-modified cotton fabric. It involves changing various factors like liquor ratio, dyebath pH, dyeing temperature, dyeing time. Different liquor ratio was examined from 30:1, 70:1, 110:1, 150:1, 170:1. The pH range of 2.0–10 was examined by adjusting the pH values using hydrochloric acid (0.1 M) and sodium hydroxide (0.1 M). Different temperatures (RT, 30 ºC ,50 ºC ,70 ºC ,90ºC) were employed to investigate their impact on dye removal. Additionally, contact time was varied from 20 to 180 minutes to evaluate its influence on dye removal efficiency [10].

RESULTS AND DISCUSSION

Determination of Enzyme activity

Extracts of *CE, R and C* were studied for cellulase activity by DNS method. It is based on measurement of reducing sugars produced after the enzyme substrate reaction. Total cellulase activity involves three enzymatic activities endoglucanases, exoglucanases, and β-D-glucosidases and can be measured using Whatman filter paper, bacterial cellulose, cotton linters [38]. Carboxymethyl-cellulase (CMCase) assay and filter paper assay are used to determine the endoglucanase and total cellulase activity respectively. Protein concentration of various extracts are depicted in Table 1. The specific activity of extract from Colocasia esculenta, Raphanus sativus, Daucus carota was 3.61 U/mg and 8.49 U/mg and 6.04 U/mg respectively using CMC as a substrate while it was 4.7 U/mg and 9.85 U/mg, 7.02 U/mg respectively using filter paper as a substrate.

Table 1. Determination of protein concentration and enzyme activity of underground roots.

Sample	Total protein (mg)	Enzyme Activity		Specific Activity (U/mg)		Total Activity	
		CMCase	*Filter Paper*	*CMCase*	*Filter Paper*	*CMCase*	*Filter Paper*
Colocasia esculenta	2861.27	793.77	1034.55	3.61	4.7	1031918	1344921
Raphanus sativus	1764.7	999.85	1158	8.49	9.85	1499792	1737094
Daucus carota	2615.73	1069.9	1243.4	6.04	7.02	1572814	1827810

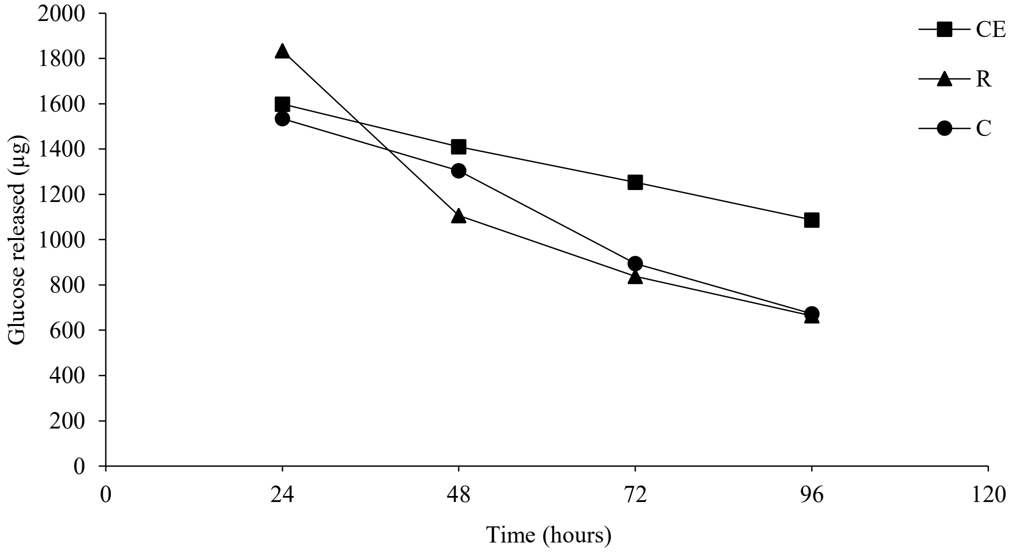

Figure 3. Release of glucose during the enzymatic treatment of cotton fabric at different time intervals.

Enzymatic Treatment of Cotton Fabric

Bioscouring of cotton fabric was done at different time interval (24,48,72, 96 hours) to analyse the time for treatment as shown in Figure 3. Samples treated with extracts of Raphanus sativus showed highest glucose release of 1833.3 µg followed by Colocasia esculenta 1581.6 µg and Daucus carota 1536.66 µg which gradually decreased with time in all the samples. Cotton fabric, composed of cellulose polymers, can be degraded using cellulase to yield glucose. Numerous studies have demonstrated the conversion of this glucose into ethanol after fermentation, making it a viable source for biofuel production. This process falls under the second generation of biofuel production, which incorporates sustainable raw materials such as sawdust, household waste, paper waste, seed waste, and agricultural industry by-products [39]. A study utilizing cellulase derived from Bacillus subtilis F3 demonstrated its effectiveness in the bio-polishing of cotton fabric, highlighting its potential as an efficient enzymatic agent for surface refinement in textile processing. Application of cellulase on cotton fabric allows it to penetrate the structure and breaks the fibres of inner layer and degrade the fibres in yarn. The enzymatic action opens up the fiber structure slightly helping dyes penetrate more evenly [40].

Microscopic Analysis of Fabric

Scanning electron microscopy (SEM) was conducted to assess potential structural alterations following 96 hours of enzymatic treatment applied to the cotton fabric. The examination was carried out at a magnification level of 5000x, with a gold plating on the surface for enhanced imaging. Maximum structural changes are seen in fabric treated with extract of Daucus carota as depicted in Figure 4. This morphological change is indicative of effective bioscouring, wherein the outer cellulose microfibril layers become partially exposed.

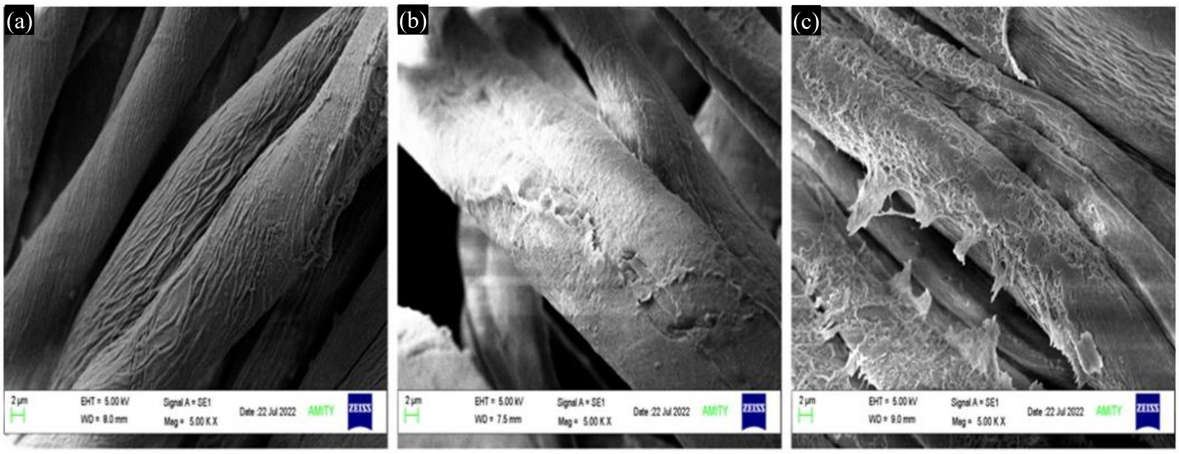

Figure 4. Scanning electron microscope images for bioscoured cotton fabric with a magnification of 5000x (a) untreated (b) treated with *Raphanus sativus* (c) treated with *Daucus carota.*

Malachite Green Toxicology Profile
LD50 Dose - 80mg/kg
Toxicity class - 3

	Prediction and Probability
Carcinogenicity	0.90
Immunotoxicity	0.87
Mutagenicity	0.71
Cytotoxicity	0.74
Aryl hydrocarbon Receptor (AhR)	1.0
Androgen Receptor (AR)	0.99
Androgen Receptor Ligand Binding Domain (AR-LBD)	1.0
Aromatase	1.0
Estrogen Receptor Alpha (ER)	0.78
Estrogen Receptor Ligand Binding Domain (ER-LBD)	1.0
Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	1.0
Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	1.0
Heat shock factor response element (HSE)	1.0
Mitochondrial Membrane Potential (MMP)	0.98
Phosphoprotein (Tumor Supressor) p53	0.51
ATPase family AAA domain-containing protein 5 (ATAD5)	0.97

Figure 5. Computational toxicity prediction of malachite green dye using Protox-II software.

Protox II

Although malachite green dye has found its application in different industries, it poses serious threat to both human health and aquatic environments. Prolonged exposure to this dye can cause cancer, mutations and developmental issues in humans. Furthermore, it exhibits inhibitory effects on vital plants such as wheat, pulses, and rice [41]. Protox is used for the analysis of adverse effect of malachite green dye on the humans. In Figure 5, the toxicity profile of malachite green dye is depicted, including its target name, prediction, and probability. The predicted LD50 dose for malachite green is 80mg/kg, categorizing it as toxicity class 3. There is a 90% probability of it being carcinogenic, an 87% chance of immunotoxicity, and an impact on various pathways, including mitochondrial membrane potential, nuclear receptor signalling pathways (Aryl hydrocarbon receptor (AhR), Androgen Receptor (AR)), stress response pathways, heat shock factor, ATPase family AAA domain containing protein 5 (ATAD5) with probability in a range of 98% to 100%. Previous studies and Protox analysis demonstrate that Malachite green dye poses significant environmental toxicity.

Fourier Transform Infrared Attenuated Total Reflectance (FTIR-ATR) Spectra

This technique is surface sensitive and allows the identification of chemical compounds structure, molecular interactions and bonding. In this study it was used to characterize the chemical changes induced on the fabric surface after treatment with acetic acid. The FTIR-ATR spectrum of the fabric surface exhibited several prominent peaks, which were subsequently compared with the FTIR of acetic acid. This comparative analysis allowed for a comprehensive assessment of the chemical changes induced by the acetic acid treatment on the fabrics surface. Acetic acid structure has a broad O–H stretch between 3200–3500 cm^{-1}, C=O stretch around 1700–1725 cm^{-1}, and prominent C–O stretch at 1200–1300 cm^{-1} [42]. These signature peaks were closely mirrored in the treated cotton samples. The broad peak at a region of 3000-3500 cm^{-1}, indicated OH stretching vibration, which shows presence of hydrogen bonding. The peak at approximately 1670 cm-1 present in the all the samples except in enzymatically treated fabric is indicative of the carbonyl (C=O) stretching vibration, which is characteristic of carboxylic acids, C–O stretching at 1203–1206 cm^{-1}, and O–H bending between 1426–1432 cm^{-1} all of which align with the functional groups present in acetic acid. The peak around wavelength 1499 cm^{-1} are complex bands of C=O and O-H vibrations. Additionally, a peak at around 1028 cm-1 corresponds to the C-O stretching vibration, further confirming the presence of the carboxylic acid group [43,44]. Table 2 and Figure 6 presents the FTIR-ATR spectra of enzymatically treated and anionised cotton fabric treated with extracts of Colocasia esculenta (CE), Raphanus sativus (R), Daucus carota (C), along with 1% acetic acid, utilized for surface modification. The presence of the carbonyl (C=O) and C-O stretching vibrations in the FTIR-ATR spectrum confirms the presence of acetic acid on the cotton fabric surface. This indicates a successful surface modification achieved through the application of acetic acid, offering potential opportunities for adsorption of cationic dyes.

Table 2. Functional groups present in the sample based on FTIR.

S.N.	Frequency range (cm–1)	Functional group	Band of control	Band of treated fabric		
				CE	***R***	***C***
1	3400–3200	Hydroxyl compound	3335.58	3336.18	3334.51	3334.07
2	2854–2926	Methyl group.	2904	2907.8	2904	2907.8
3	1625–1745	Carbonyl compounds		1646	1649.1	1646
4	1432–1621	Aromatic ring		1432		
5	1400-1450	Carboxylic Acid	1426	1432	1426	1426
6	1200-1300	Carboxylic Acid		1203		1206.1
7	1150–911	C–O–C group	1053/900	1054/903	1054	1054

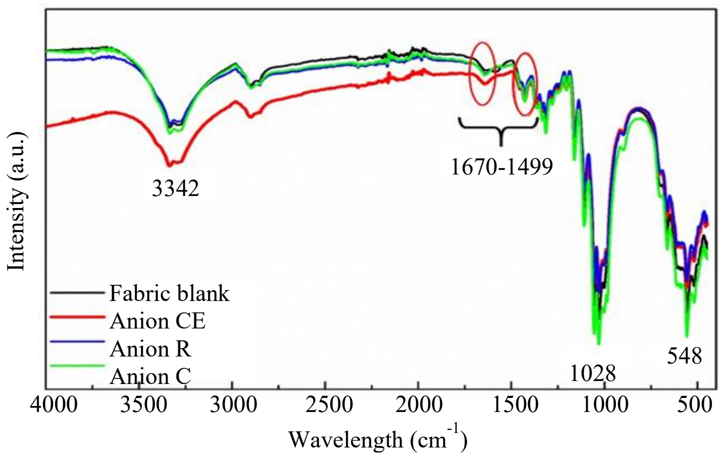

Figure 6. FTIR-ATR spectra of enzymatically treated and anionised cotton fabric treated with extracts of *Colocasia esculenta* (CE)*, Raphanus sativus* (R)*, Daucus carota* (C).

Droptest

A droptest was performed to evaluate the adsorption properties of a coloured droplet on enzymatically treated and anionised cotton fabric. The zone of diffusion and the time taken for the fabric to fully adsorb the droplet was measured as shown in Table 3 and Figure 7. On comparing the enzymatically treated and anionised cotton fabric it was observed that time taken by 50µl dye to adsorb on the fabric was decreased in the anionised fabric. The fabric modified by Raphanus sativus in combination with anionization exhibited the highest adsorption rate at 53.6%. This was followed by the modified fabrics using Colocasia esculenta and Daucus carota, which showed adsorption rates of 49% and 38.8%, respectively. In addition to the improved adsorption properties, the anionised fabric also displayed an increase in the adsorption area. The enzymatically treated fabric had an adsorption area of 1.2, whereas the anionised fabric modified with Raphanus sativus exhibited an area of 1.3. Similarly, the anionised fabric modified with Colocasia esculenta showed an adsorption area of 1.4. The highest increase in the adsorption area was observed in the anionised fabric modified with Daucus carota, reaching an area of 1.6. These findings highlight the positive impact of anionisation treatment, especially in conjunction with the enzymatic modifications, in expanding the adsorption area of the fabric.

Table 3. Droptest: Comparison of unmodified and modified fabric on adsorption properties.

Sample	**Unanionised (Enzymatically treated)**		**Anionised (Enzymatic + Anionizaion treatment)**	
	Time (sec)	***Zone (cm)***	***Time (sec)***	***Zone (cm)***
Blank	10.14	1.3cm	3.51	1.3
Colocasia esculenta	5.2	1.2	2.65	1.3
Raphanus sativus	5.61	1.2	2.6	1.4
Daucus carota	3.17	1.1	1.94	1.6

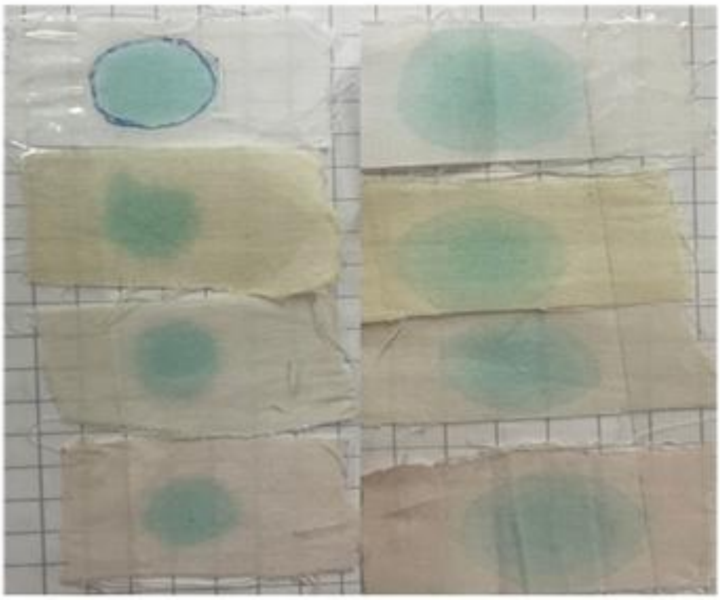

Figure 7. Droptest: Comparison of Enzymatically treated and Anionised fabric on adsorption properties of fabric.

Optimization

Effect of Acetic Acid Concentration

Increase in the percentage exhaustion was examined in three different enzymatically treated cotton fabric (Colocasia esculenta (CE), Raphanus sativus (R), Daucus carota(C)) at five different acetic acid concentrations involving 0.5%,1%,1.5%,2%,2.5%,3%. It was found that percentage of exhaustion was proportional to the concentration of acetic acid i.e. exhaustion increased with increasing acetic acid concentration as shown in Figure 8 (a). This study includes the experiments with 1% acetic acid concentration which is cost effective as compared to high anionization dosage.

Effect of Temperature

It is clearly evident from Figure 8 (b) that the change in temperature of the dyebath majorly effects the exhaustion process. To determine the optimum temperature of the dyeing process the experiment was conducted at different temperature i.e. RT, 30ºC, 50ºC, 70ºC, 90ºC. In Colocasia (CE), and Daucus (C) the rate of the dyeing increased with increasing temperature (90 ºC >70 ºC >50 ºC >30 ºC >RT) while in Raphanus (R) highest exhaustion was seen at room temperature (RT>30 ºC >50 ºC >70 ºC >90ºC). The reported that at high temperature the molecular vibrations in the fiber and the dye increases, providing favorable fixation kinetics [36].

Effect of pH

The pH of dye bath has great influence on the charge present on dye and fabric surface. Cationic dyes, like malachite green, carrying a positive charge and anionised fabric, carrying a negative surface charge may get affected by pH of dyebath leading to different interactions between them. The maximum removal of malachite green dye was observed at pH 6, where the anionized cotton fabric (CE, R, C) exhibited the highest exhaustion due to favourable electrostatic attraction. However, in acidic and highly alkaline pH conditions there was less dye exhaustion indicating the repulsion between dye molecules and fabric surface as shown in Figure 8(c). This response is closely linked to the adsorbent's pH at the point of zero charge (pH_pzc), which represents the pH at which the materials surface is electrically neutral. When the solution's pH is lower than the pH_pzc, the sorbent surface becomes positively charged, thereby favoring the adsorption of anionic dyes. This suggest that excess H^+ ions at acidic pH protonated the negatively charged carboxylic groups on the fabric resulting in diminishing electrostatic attraction with the cationic malachite green dye [45]. Maximum exhaustion at pH 6 indicates an optimal charge balance where the anionized cotton fabrics exhibited strong electrostatic attraction with malachite green. At highly alkaline pH, although the fabric remains negatively charged, malachite green may undergo structural changes that affect its solubility and availability [46]. Our results are in line with those of Aragaw and Alene, who investigated the adsorption properties of reactive, basic and acidic dyes on kaolin adsorbent. Their study demonstrated that increasing the pH of the solution makes the adsorbent surface more negatively charged, thereby enhancing the adsorption of cationic dye, Basic Yellow 28. Conversely, adsorbents surface become more positively charge at lower pH values, favouring the adsorption of anionic dyes such as Congo Red [47].

Effect of Liquor Ratio

The choice of liquor ratio can affect the dye adsorption and removal efficiency. Various liquor ratios (30:1, 70:1, 110:1, 150:1, 170:1.) was examined to find the optimum ratio for the dyeing process. The weight of the fabric was changed in each tube (500mg, 214mg, 136 mg, 100mg, 88mg) while volume of the dyebath remains constant. In all the modified fabric (CE, R, C), 30:1 liquor ratio with a higher fabric weight of 500mg resulted in the highest percentage removal, indicating the highest adsorption efficiency. However, the optimum liquor ratio was determined to be 150:1, where a lower fabric weight of 100mg still allowed for significant adsorption and a high percentage removal as shown in Figure 8 (d). The increase in E% at both 30:1 and 150:1 liquor ratio can be attributed to different influencing factors. At a liquor ratio of 30:1, the higher fabric weight (500 mg) offers more surface area and active sites for dye adsorption, leading to enhanced dye uptake. At high liquor ratio of 150:1, the fabric weight was low (100 mg), but the volume of the dye bath was significantly higher. This results in better dye dispersion and allows sufficient space for dye molecules to move freely and interact with the fiber.

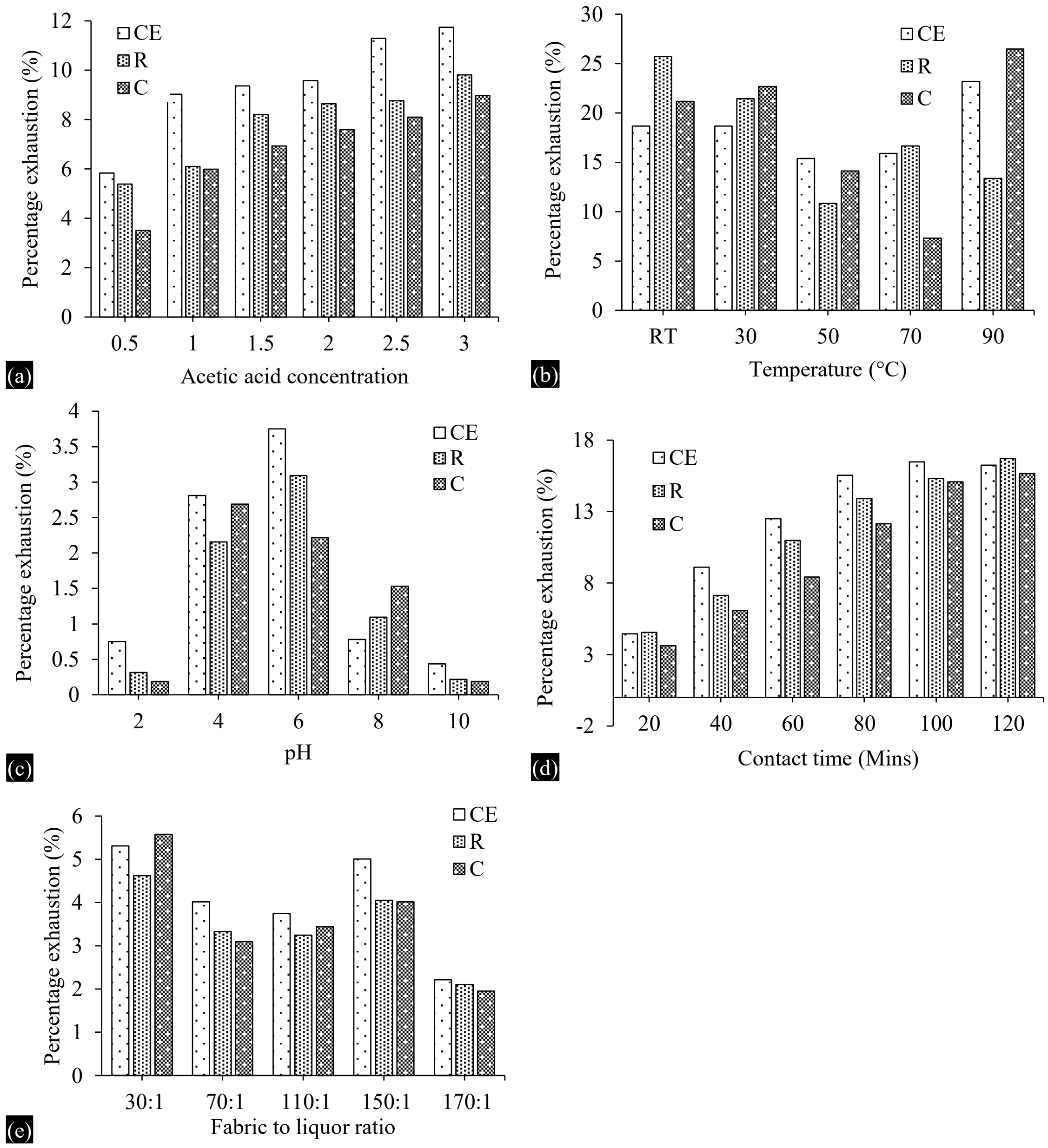

Figure 8. Optimization of (a) acetic acid concentration for anionization; and dyeing parameters (b) temperature, (c) pH (d) liquor ratio (e) contact time.

Effect of Contact Time

It is necessary to ensure appropriate contact time for good adsorption between dye and fabric. The exhaustion increased gradually in all anionised fabric when the time was increased from 20 to 100 min and then become constant up to 120 min as shown in Figure 8 (e). This suggest that initially there were unoccupied active sites on the cotton fabric till 100 min. However, the removal efficiency stabilized after 100 min of contact time indicating lack of active sites [47].

Exhaust Dyeing

Based on the optimal conditions obtained from the above results, exhaust dyeing was carried out. The dyeing process was conducted using 1% acetic acid concentration, a liquor ratio of 150:1, a dyebath pH of 6, and a dyeing temperature of 95ºC for a duration of 2 hours (120 minutes). Percentage exhaustion of dye in the solution is better with anioised fabric i.e. 42.5% (CE), 28.2% (R), 25.6% (C) as shown in Table 4. Dye adsorbed by the fabric, dye left in the dye bath, and partition coefficient was best observed in the anionised fabric as shown in Table 5. The fabric treated with *Colocasia esculenta* (CE) extract followed by anionization exhibited the highest dye exhaustion. This suggests that the cellulase activity present in the CE extract was comparatively more effective than those from *Daucus carota* and *Raphanus sativus*. The higher activity of CE has been reported in our previous study, where we compared the cellulase activity of different extracts [30]. The combination of enzymatic treatment and surface anionization results in the improvement of dye exhaustion. The enzymatic treatment of cotton fabric increases the porosity of the fabric and exposes more hydroxyl group for replacement with carboxylic acid [40]. Studies have reported that cellulose fabric can be modified by replacing the hydroxyl group at the surface with other functional groups for increasing the hydrophilicity and adsorption capacity for dye removal [28, 29]. The change in the dye bath color was visual in Colocasia esculenta treated fabric with anionization as shown in Figure 9. Absorption capacity of the cotton fabric was increased by applying acetic acid as an anionization agent. Therefore, application of the anionization agent on the cotton fabric can improve the uptake of the cationic dyes through exhaustion. This is because the ionic bond formed between the anionised cotton fabric and dye was significantly stronger than any other dye attached directly through Van der Waal forces [36]**.**

Table 4. Percentage Exhaustion of Modified and unmodified cotton fabric.

Sample	Unanionised				Anionised			
	Initial absorbance (A_0)	***Final absorbance (A_t)***	***(A_0) - (A_t)***	***%E***	***Initial absorbance (A_0)***	***Final absorbance (A_t)***	***(A_0) - (A_t)***	***%E***
Blank	0.036	0.035	0.001	2.77	0.035	0.035	0	0
Colocasia esculenta	0.032	0.030	0.002	6.25	0.040	0.023	0.017	42.5
Raphanus sativus	0.044	0.036	0.008	18.18	0.039	0.028	0.011	28.2
Daucus carota	0.038	0.029	0.009	23.68	0.039	0.032	0.007	25.6

Table 5. Dyeing parameters of malachite green dye with and without anionization agent.

	Colocasia esculenta		Raphanus sativus		Dacus carota	
	Unanionised	***Anionised***	***Unanionised***	***Anionised***	***Unanionised***	***Anionised***
Liquor ratio	150 l/g	150 l/g	150 l/g	150 l/g	150 l/g	150 l/g
% E	6.25	42.5	18.18	28.2	23.68	25.6
Dye absorbed by fabric (Liquor ratio* % E)	937.5	6375	2727	4230	3552	3840
Q (dye left in solution)	93.75	57.7	81.82	71.8	76.32	74.4
K (L/kg)	10	110.4	33.32	58.91	46.51	51.61

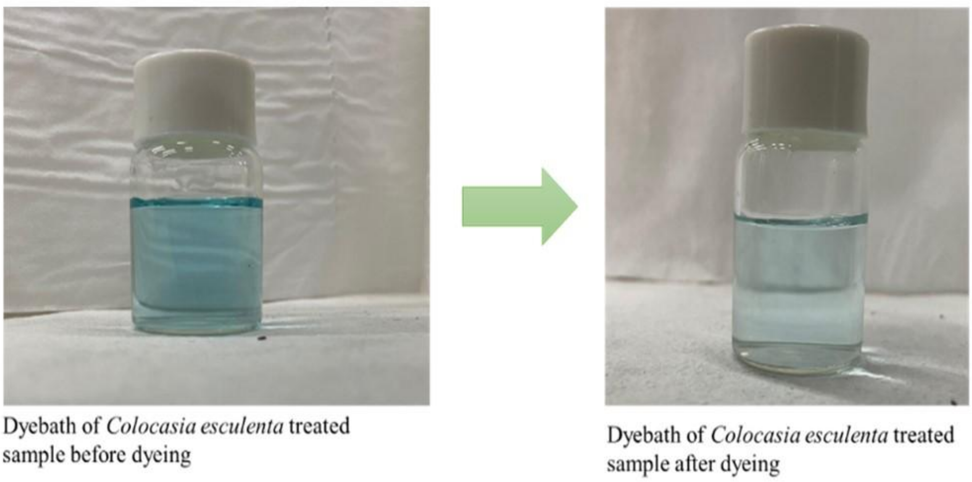

Figure 9. Visualization of dyebath of anionised c*olocasia esculenta* sample before and after dyeing.

CONCLUSION

This study describes the bioscouring of cotton fabric using underground roots as a possible environmentally acceptable source of cellulase. Anionization of cotton fabric is a type of surface modification used to enhance the adsorption capacity of the fabric. The specific activity of extract from Colocasia esculenta, Raphanus sativus, Daucus carota was 3.61 U/mg and 8.49 U/mg and 6.04 U/mg respectively using CMC as a substrate while it was 4.7 U/mg and 9.85 U/mg, 7.02 U/mg respectively using filter paper as a substrate. Samples treated with extracts of Raphanus sativus showed highest glucose release of 1833.3 µg followed by Colocasia esculenta 1581.6 µg and Daucus carota 1536.66 µg which gradually decreased with time in all the samples. SEM analysis showed that fabric degraded with Daucus carota extract has shown significant degradation. The droptest results demonstrated the significant improvement in adsorption properties when comparing the enzymatically treated fabric to the anionised fabric. The dye adsorption time reduced in anionized fabric as compared to the unanionised control fabric. In Colocasia treated sample it went from 5.2 sec to 2.65 secs and for Raphanus 5.6 to 2.6 secs and for Daucus 3.17 to 1.94 secs. The zone (diameter) of the adsorbed dye was also increased in the anionized sample. In Colocasia treated sample it went from 1.2 cm to 1.3 cm and for Raphanus 1.2 to 1.4 and for Daucus 1.1 to 1.6. Optimal conditions for exhaust dyeing was observed at 1% acetic acid concentration, a fabric-to-liquor ratio of 150:1, a dyebath pH of 6, and a dyeing temperature of 95ºC for 2 h (120 min). Percentage exhaustion of dye in the solution is better with anioised fabric i.e. 42.5% (CE), 28.2% (R), 25.6% (C). Modification of the fabric surface lead to improved dye adsorption, presenting significant applications in various industries. These findings demonstrate that vegetable peels can serve as a sustainable source of eco-enzymes for diverse industrial applications, including bioscouring, while waste cotton fabric can be effectively modified to enhance its adsorption capacity. Overall, the results confirm that anionization is an efficient and viable method for improving the adsorption and removal of cationic dyes from wastewater

Acknowledgment

The authors are grateful to the Department of Biotechnology, Jaypee Institute of Information Technology, Noida, Uttar Pradesh, India for providing necessary facilities to execute this work.

- *Conflict of interest:* The authors declare that they have no conflict of interest
- *Funding:* The author declares that no funding was received to assist with this research and preparation of this manuscript.
- *Ethical statement:* This study does not involve any human or animal testing.
- *Data availability:* All data generated or analysed during this study are included in this article
- *Author Contributions:* Priyanka Kakkar Conceptualization, methodology, software, validation, formal analysis, investigation, data curation writing—original draft
- *Pammi Gauba:* Conceptualization, validation, resources, writing—review and editing, visualization, supervision All authors have read and agreed to the published version of the manuscript.

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