Characterization of various parameters of textile Dyes and their treatment by *Amorphophallus paeoniifolius* crude enzyme extract

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Abstracts

The crude enzyme extracted from *Amorphophallus paeoniifolius* was shown to help lower the pH, BOD, DO, TDS, turbidity, and other textile dyes parameters after 24 hours of treatment on various textile dyes i.e. Yellow CE, Blue CE, and Navy-Blue CE. With the same period of treatment, BOD (55%) DO (40%), pH (5.36%), turbidity (57%) and TDS by (50%) decreased in Yellow CE textile dye whereas BOD (33%) DO (20%), pH (4.7%), turbidity by (50%) and TDS by (50%) decreased in Blue CE. The extracted crude enzyme from *A. paeoniifolius* was found to help in lowering the pH (4.9 %), BOD (33 %), DO (30 %), TDS (50%), turbidity (55%) in Navy Blue CE and other textile water parameters. All the various parameters were also compared with the treated and untreated effluents collected from the textile industry.

Key Word- Textile dyes, *Amorphophallus paeoniifolius*, Crude Enzyme, BOD, DO

Introduction

The oldest and largest sector in India is the textile industry. The sector contributes 14% of the nation's industrial output. 17% of total exports are also generated by this sector. India stands out in the textile sector due to the independent textile industry, the national and international markets, and other aspects. Large amounts of water are used during processing in the textile and dyeing sectors, and this particular effluent comprises 10–40% residual dyestuff that, when released into the environment, causes serious pollution issues [1]. It is not advisable to discharge color-containing effluents into water due to their colours, dyes released, and breakdown products that are toxic and carcinogenic to life, primarily due to the presence of carcinogens like naphthalene, benzamine, and other aromatic compounds. These dyes persist in the environment for a very long time if they are not treated [2]. High-suspended solids, chemical oxygen demand, heat, colour, acidity, and other soluble substances are the main contaminants in the textile waste water. The dissolved oxygen in rivers, ponds, and streams is rapidly depleted by waste from textile industries therefore it is necessary to treat this textile effluent and dye [3]. Generally, three methods are used to treat textile dye and effluent i.e.

physical, chemical, and biological methods. Biological methods contain microorganisms like fungi, bacteria and plant sources, and this method is popular because of it’s a low cost. Several enzymes have been investigated over the past two years for treating different textile sector effluents. Various plant types can treat wastewater effectively as an alternative [4]. *Tyhpa angustifolia, Paspalum scrobiculatum*, and *Salvinia molesta* are a few helpful plant sources for the treatment of textile wastewater [5]. Enzymes can also change a waste's characteristics to make it more treatable or to help bio-convert debris into products with additional value [6]. Many enzymes, including Polyphenol oxidase (PPO) and peroxidase, can be used to clean wastewater. *A. paeoniifolius*, popularly known as Jimikand, is a frequently underutilized plant that offers a number of health advantages [7]. Several novel sources, including *A. peoniifolius,* contain PPO, according to our research team [8].

The goal of our research is to analyse the reduction in parameters like pH, turbidity, TDS, BOD, DO from waste water and dyes and comparative analysis of the treated water (by industry method) and treated water with enzyme was also done. This work will help to treat textile effluents and solve the environmental problems by employing enzymes from rare plant sources.

Material and method

Amorphophallus paeoniifolius corm was purchased from the local market in Ghaziabad, India. Three dye samples Yellow CE, Blue CE, and Navy-Blue CE and treated water and untreated water were collected from the Sky Lark textile industry in Greater Noida, India. The samples were collected in pre-washed autoclaved bottles. Chemicals like sodium thiosulfate, manganous sulphate, alkali iodine solution, starch indicator, sulfuric acid, sodium phosphate, and monosodium phosphate were all procured from CODON biotech Ltd. Noida.

Preparation of crude enzyme extract

The crude enzyme extract was prepared by using method given by Kagalkar et.al; 2009 [9]. The corms of *Amorphophallus paeoniifolius* was cleaned with running water, peeled, cut into small pieces as shown in Fig 1 (A). Cut pieces of corms were homogenized for 20 minutes at 8481 rpm at 4°C in a 50 mM potassium phosphate buffer (pH 7) solution. Whatman No. 1 filter paper was used to filter the supernatant. The isolated raw enzyme (Fig. 1 B) was then kept at -20°C till further processing.

(A)

(B)

Figure 1. (A) Fine cubes of *A. paeoniifolius* and (B) extracted crude enzyme from *A. paeoniifolius*

Sample treatment

Before and after treating each dye sample with *A. paeoniifolius* crude enzyme extract, various parameters including pH, turbidity, BOD, DO, and TDS were measured. Briefly, according to selected parameter, measured amount of dye samples were taken in the jar and mixed with *A. paeoniifolius* crude enzyme extract and kept at room temperature for 24 hours for further analysis.

Physicochemical characterization of Yellow CE, Blue CE, and Navy-Blue CE dye samples was performed after treatment with the *A. paeoniifolius* crude enzyme extract and compared with the untreated dye sample on different parameters such as pH, turbidity, TDS, BOD and DO. Not only dyes sample were checked, we have also compared the treated water collected from textile industry with untreated water from the same industry treated with *A. paeoniifolius* extract. All the methods were used as per our previous published data [3]. Treated water data is already published with our group [3].

pH Analysis

5 ml of Yellow CE, Blue CE, and Navy-Blue CE dye samples were mixed with 5ml of *A. paeoniifolius* crude enzyme extract individually and kept for 24 hours at room temperature. All the reading was recorded by using pH metre.

Turbidity Analysis

Using a turbidity metre, the mixture of 5 ml of each dye sample and 5 ml of the crude enzyme extract from *A. paeoniifolius* was monitored for 24 hours at room temperature.

BOD Analysis

For calculating the BOD of all the collected samples, two bottles of each dye sample were filled with the sample. At first, the dissolved oxygen (D. O.) for one BOD bottle was calculated and the rest was remained in the dark condition for three days. After three days, the D. O. for the dark bottles was calculated. Then the BOD level of the sample was determined from the difference between the initial and final D.O. The oxygen level of Yellow CE, Blue CE, and Navy-Blue CE dye samples was analyzed on day 1 and after 3 days incubation in the BOD incubator at 27 °C. Water samples were taken without bubbling in 75 ml glass flasks. 0.6 ml of manganous sulphate and alkali iodide solution were added to the bottom of the bottle, and it was shaken upside-down six times to produce a precipitate that was black in colour. The bottles were kept stationary till precipitate is completely settled down. After that, 1.2 ml of strong sulfuric acid was added to each stoppered vial, and shaken thoroughly to dissolve the brown precipitate's material and titrated with 5 ml of the thiosulphate solution until the color turned pale yellow. Few drops of starch solution were added to the solution resulting in the change of color from light to dark blue color and again titrated with thiosulphate solution until the blue color disappeared. The BOD was calculated by using following formula: [10]

BOD (mg/L) = Initial D. O. – Final D. O

DO Analysis

The amount of dissolved oxygen and the titration of iodine by thiosulphate solution are directly proportional [10]. The formula used to calculate the water's DO (mg/l) was

$$DO = (8*1000*N*v)/V$$

Where v is the sample volume (in millilitres), V is the titrant volume, N is the titrant's normality, and 8 is a constant

TDS Analysis

25 ml of each dye sample was filtered through filter paper and kept on pre-weighted evaporating dish, placed in a hot water bath for an evaporation process. The dried residue was weighed after the evaporation process [10]. The total dissolved solid of Yellow CE, Blue CE, and Navy-Blue CE dye samples was determined by using the following formula:

Calculation done by:

Concentration (mg/L) = ((B - A)/ C) * (1000 mg/g) * (1000 ml/L)

B = weight of dried residue + weight of dish

A = weight of empty dish

C= Volume of sample

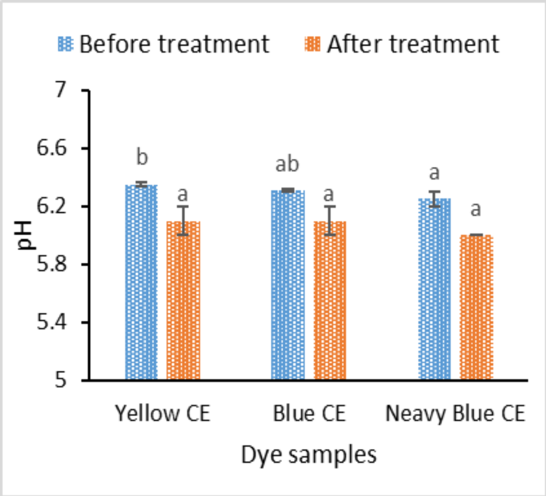
Statistical Analysis

Statistical significance of the variables was evaluated through one-way analysis of variance (ANOVA) at $P < 0.05$ level by using IBM® SPSS® Version 25. Post- Hoc Turkey HSD comparison test was applied for mean comparison. Values are represented as means ± SD of triplicate measurements.

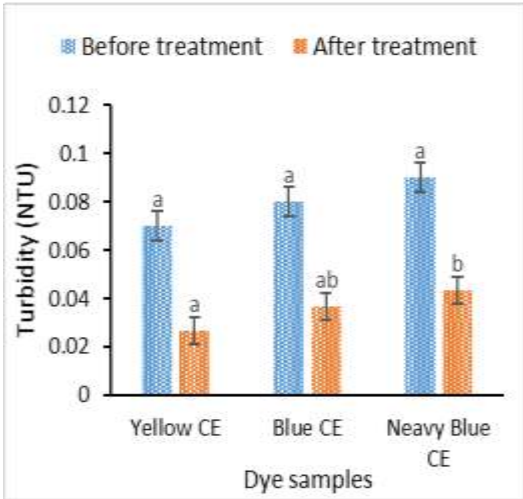
Result and discussion

The pH values of all samples were found to be lower than those that had previously been reported. Use of *A. paeoniifolius* crude enzyme extract on the textile dyes Yellow CE, Navy Blue CE, and Blue CE dye was successful in lowering a range of textile water parameters, including pH, turbidity, BOD, DO, and TDS as shown in Fig. 2.

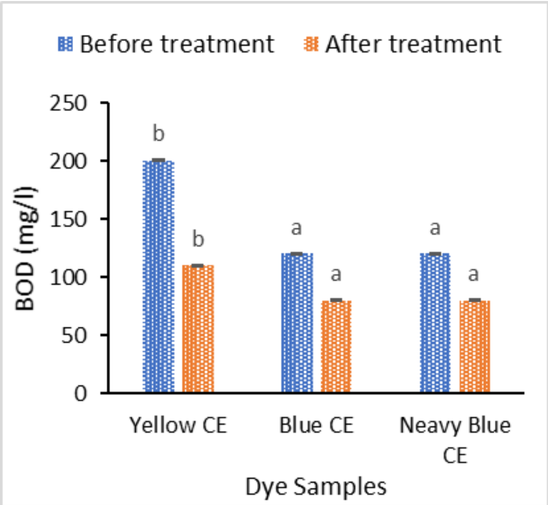
The pH value of Yellow CE, Navy Blue CE and Blue CE was found to be almost similar (6.1± 0.01) after treating with *A. paeoniifolius* crude enzyme extract and has been observed that pH was significantly reduced in all the dye samples and maximum reduction was found in Yellow CE dye sample after treatment as shown in Fig 2 (a). The pH values of all samples were discovered to be lower than those that had previously been reported [11]. The pH of the released effluents has an impact on some chemical processes, including solubility and metal toxicity. The effluents' pH also contributes to the maintenance of the equilibrium between free CO_2, HCO_3, and CO_3^- [12]. Low turbidity implies the cleanliness of the sample. The significant reduction in the turbidity of all the sample was observed ranges 0.043±0.05 to 0.026±0.05 after treatment as shown in Fig 2 (b). The biological oxygen demand method quantifies how much oxygen is consumed by microorganisms during the biological process in water [11]. The BOD of Yellow CE, Blue CE and Navy-Blue CE was found to be 110.15±0.13, 80.23±0.25 and 80.25±0.27 respectively after treatment as shown in Fig 2 (c). The significant reduction in DO of Yellow CE, Blue CE and Navy-Blue CE was observed and found to be 240 ± 0.1, 320.1 ± 0.1 and 280.1 ± 0.1 respectively after treating with *A. paeoniifolius* crude enzyme extract as shown in Fig 2 (d). Due to discharge into irrigation water, textile dyeing effluents with high TDS values may cause salinity issues [13]. After treating with *A. paeoniifolius* crude enzyme the significant reduction in the TDS of Yellow CE, Blue CE and Navy-Blue CE dye sample was observed and found to be 2000.36±0.3, 1000.43±0.4 and 1000.36±0.3 respectively as shown in Fig 2 (e). Textile industry waste water and the treated water from industry before discharge was also collected and all the parameters were tested and compared as shown in Table 1. Untreated water treated with *A. paeoniifolius* crude enzyme extract shows the data somehow similar data as treated with chemical methods by industry, therefore *A. paeoniifolius* extract can be used for treatment of waste water and textile dyes.


Before treatment
After treatment
pH
7
6.6
6.2
5.8
5.4
5
b
a
ab
a
a
a
Yellow CE
Blue CE
Neavy Blue CE
Dye samples

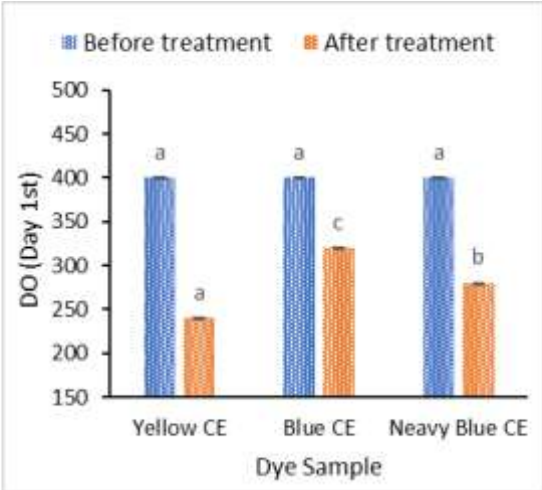
(a)


Before treatment
After treatment
Turbidity (NTU)
0.12
0.1
0.08
0.06
0.04
0.02
0
a
a
a
ab
a
b
Yellow CE
Blue CE
Neavy Blue CE
Dye samples

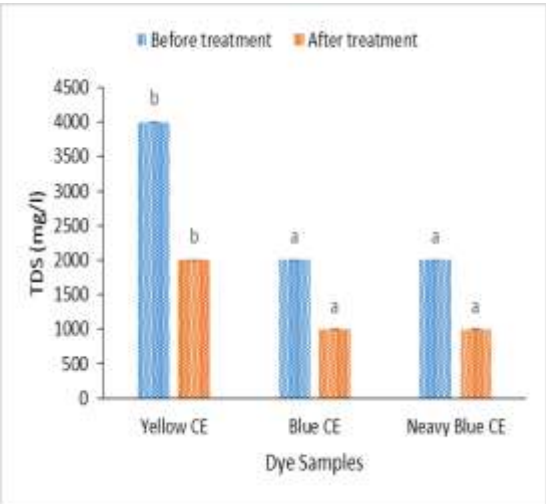
(b)


Before treatment
After treatment
BOD (mg/l)
250
200
150
100
50
0
b
b
a
a
a
a
Yellow CE
Blue CE
Neavy Blue CE
Dye Samples

(c)


Before treatment
After treatment
DO (Day 1st)
500
450
400
350
300
250
200
150
a
a
a
c
a
b
Yellow CE
Blue CE
Neavy Blue CE
Dye Sample

(d)


Before treatment
After treatment
TDS (mg/l)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
b
b
a
a
a
a
Yellow CE
Blue CE
Neavy Blue CE
Dye Samples

(e)

Figure 2 Physicochemical characterization (a) pH (b) Turbidity (c) BOD (d) DO (e) TDS of Yellow CE, Blue CE, and Navy-Blue CE dye before and after treatment. **Note:** With different alphabets, the means before and after treatment show a significant difference at p 0.05, respectively. TDS: Total Dissolved solid; BOD: Biochemical oxygen Demand; COD: Chemical Oxygen Demand; DO: Dissolved oxygen; TSS: Total suspended solids; mg: Milligram; ml: Milliliter; hr: Hours

Table 1: Comparative data of various parameters of textile effluents

S.No.	Parameters	Samples	
		(A) Treated effluents from Industry	**(B)** Untreated water (After treatment with *A. peoniifloius* crude extract)
1	pH	7.43	7.1
2	Turbidity	0.44 NTU	0.43 NTU
3	TDS	1000mg/l	800mg/l
4	BOD	80 mg/lit	40mg/lit
5	DO	Day 1- 200 mg/lit	Day 1 -320 mg/lit

Sample(A): Treated waste water collected from industry before discharge, Sample (B): Same Waste water treated with *A. paeoniifolius* crude extract

Percentage reduction after treating with *A. paeoniifolius* crude enzyme extract

The percentage reduction of pH, turbidity, BOD, DO and TDS in Yellow CE, Blue CE, and Navy-Blue CE dye sample was shown in table 2 and all the dyes various parameters were compared and shown in Figure 3. The percentage reduction was calculated after treating the dye sample with *A. paeoniifolius* crude enzyme extract and it was observed that maximum reduction of pH, turbidity and DO was found in Yellow CE compared to Blue CE and Navy-Blue CE. The % reduction of BOD was least in yellow CE and equally reduced in Blue CE and Navy-Blue CE. It was also observed that Yellow CE decolorizes more effectively than Blue CE and N. Blue CE.

Table 2: Percentage reduction of different parameters after treating with *A. paeoniifolius* crude enzyme

Dye sample	pH	Turbidity	BOD	DO	TDS
Yellow CE	5.36 %	57 %	20 %	40 %	50 %
Blue CE	4.7 %	50 %	33 %	20 %	50%
Navy-Blue CE	4.9 %	55 %	33 %	30 %	50 %

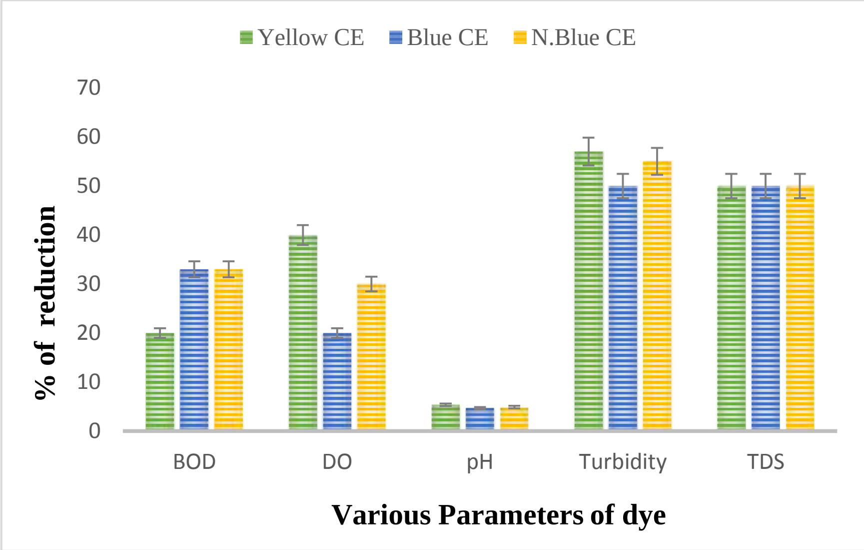


Figure 3 Comparative percent reduction in various parameters of dye Yellow CE, Blue CE, N. Blue CE after treatment with *A. paeoniifolius* crude enzyme extract.

Enzymes from plant sources can potentially decolorize and break down a wide range of contaminants. Wastewater treatment uses enzymes including oxidoreductase, transferase, hydrolases, and lignin mineralizing enzyme [14]. In a previous study, *I. hederifolia* was also successful in treating a dye mixture and textile effluent, lowering BOD by up to 65 and 63% and COD by up to 62 and 68%, respectively, in 60 to 96 hours [15]. For the treatment of textile industry effluent, plants like *Typhonium flagelliforme*, *Blumea malcolmii*, and *Phragmites australis* have been used, and they have proven significant in reducing BOD, TDS, TOC, COD, and TSS [16,17]. According to research by [18], *Typha angustifolia* and *Bouteloua dactyloides* both decolorize textile effluent by up to 70% within six days and up to 92% within 24 hours, respectively [19]. Similarly, it has been observed that the aquatic plant *Eichhornia* spp. was able to remove several dyes from waste water, containing Black HY, Congo Red, and direct Blue 6B [20].

As illustrated in Figure 4, visible colour changes were also reported in the samples. These colour changes further need to be monitored with the use of a spectrophotometer for a percent decolorization study and our group is working for the same (Unpublished data). Utilization of *A. paeoniifolius* enzyme extract can be economical and further this plant not only contain PPO but also contain cellulase and some other enzymes which can also be further checked [21].

(a)Yellow CE Before treatment (b) Yellow CE After treatment

(a) N. Blue CE Before treatment (b) N. Blue CE After treatment

Figure 4 Dye Yellow CE and N. Blue CE (Before and after treatment)

Conclusion

A suitable biological treatment of textile effluents can be used to address the environmental problem of dye removal from textile industry. As a result, treating textile wastewater *with A. paeoniifolius* is effective, economical, and environmentally beneficial. Additionally, these colour changes must be observed using a spectrophotometer to conduct a percent decolorization study. The utilisation of enzyme systems that work better under different physiological temperature and pH settings, leading to higher efficiency with less energy use and reduced costs. Enzymes from this underutilized corm *A. paeoniifolius* can be utilized to make remediation processes that are less damaging to the environment than conventional ones in wastewater treatment.

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