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Assessment of Phytotoxicity of Biophysically Treated Disperse Orange Dye on *Vigna Radiata*

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Abstract:

Disperse dyes with less solubility in water is considered be an efficient dye that can be used for the coloration of the polyester, cationic or cellulosic fibers. Amongst them polyester fibers are most difficult to colour because of lack of ionic properties which contributes towards their hydrophobicity. They are grouped under the azo dyes, which is responsible for them being toxigenic in nature. In this study, the toxicity of such dye was analyzed on *Vigna radiata*(moong) plant on the basis of radical and plumule growth and percent germination. A comparison of the above parameters was done with dye, wheat bran treated, acid and alkali modified wheat bran treated dye and also by organism (*Schizophyllum*- S4) treated dye on the plant. The study has revealed that under all the conditions 100% germination of seeds were observed, with around 35% and 15% increase in radical and plumule growth by *Schizophyllum*-S4 treated dye (initial concentration 100mg/L) after 10 days of growth, with only 0.84% and 0.007% reduction of chlorophyll a and chlorophyll b, respectively under the same condition in comparison to the control (plant without aqueous solution of dye). Though with around 7.3% and 16.7% increase in chlorophyll a and chlorophyll b, respectively with dye treated with acid modified wheat bran, depicting that treated dye either with lignocellulosic substrate or with organism can be used for plantation, which aid in the reduction of the toxicity of the dyes used in the textile industries which alone are deleterious for the aquifers.

Introduction

Disperse dyes are synthetic, sparingly soluble azo dyes. The presence of their unrestricted ionizing group contributes towards their lesser attraction towards the polymers like paper, cellulose, cellophane. However, they exhibit good affinity towards polyethylene terephthalate and cellulose acetate13. The conditions required for their usage is high temperature and acidic condition. They are quite cheap in comparison to the natural dyes for which they are having good demand in the textile industries. These dyes poses lots of ill effects to the environment as well as on the users, like allergic responses, like “nylon allergy”, which was dermatitis caused because of the dyes were reported long back in 80’s (1, 2). Fig.1 depicts the structure of Disperse Orange, with an azo group, that contributes towards its recalcitrant property. Various types of remediation methods have been employed for the degradation of such dyes, this study mainly targeted the phytotoxicity study of the dye after various types of treatments such as adsorption on wheat bran (WB), on acid treated and alkali treated wheat bran, organism like a white rot fungus treated dye and also the effect of reusability of the same dye adsorbed bran as an adsorbent in order to compare at which phase a check can be given for the usage of the permeate obtained after the adsorption, so that they can have certain application in the plant growth. The plant taken into consideration was *Vigna radiata*(moon dal), though the application of such treated dyes is

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more appropriately seen in ornamentation plant but still a study was conducted to analyze their effects on such plants.

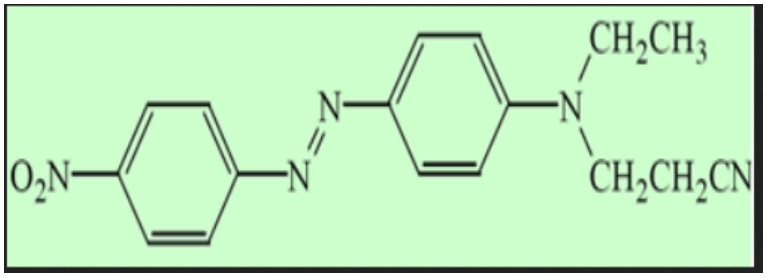

Figure.1 Structure of Disperse Orange25 (Anonymous 1, 2019)

Materials and Methods

Materials

The dye used in the study was of analytical grade obtained from Loba Chemicals, Mumbai, India. The adsorbent used (wheat bran) was procured form the local market from Jalandhar Cantt., Punjab, India. The organism, *Schizophyllum*-S4, was an already isolated and identified strain obtained from the laboratory of Lovely Professional University, Phagwara, Punjab, India.

Methodology

Batchadsorption of the disperse orange was done under on adsorbent, wheat bran (WB)[4], on acid modified wheat bran (AWB) [5]and alkali modified wheat bran [6]. Liquid state decolorization of the dye was carried out under an optimized condition. In 100 mL conical flask 20mL working volume with a concentration of 100mg/Lwas taken in presence of wheat bran.After autoclaving in the laminar air flow the culture was inoculated with 2-3 pieces of button shaped bodies with help of cork borer. The flasks were incubated at 28°C for 7 days and decolourization was monitored by taking optical density after every day for a period of 16 days with a UV- Spectrophotometer [7]. All the experiments were performed in triplicates to reduce the error.

Toxicity study

Healthy seeds of moong (*Vigna radiata* or *Phaseolus aureus*) were collected from local store. The seeds were surface sterilized with 1% sodium hypochlorite solution for 5-10 minutes and rinsed thoroughly with distilled water for many times to remove the excess amount of chemical, before use for the experimental work [8].

In this experiment, the effect of treated disperse orange on germination of *V. radiata* was evaluated. The surface sterilized seeds were evenly placed in sterile 10 cm petri dishes containing untreated dye, and laboratory scale treated (wheat bran treated dye (1^{st}, 2^{nd} and 3^{rd} cycle) and organism treated dye) layered with double layer of Whatman filter paper 1 while tap water was taken as control and incubated at 28°C in the dark for germination. The relative seed germination %, relative root growth %, using the plant growth data after 48 hrs and 168 hrs.

The Relative seed germination percentage calculated using the formula:

$$\text{Relative seed germination (\%)} = \frac{\text{Number of seeds germinated in presence of effluent/dye}}{\text{Number of seeds germinated in control}} \quad \text{(Eq.1)}$$

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Pot Study

The phyto-toxicity assay was carried out in triplets using medium sized plastic container. Soil in each container was incubated with 10 seeds of moong (*Vigna radiata* or *Phaseolus aureus*). The assay was checked using untreated, unmodified wheat bran treated (1st cycle, 2nd cycle and 3rd cycle), modified (acid and alkali) wheat bran treated and organism treated effluent/dye samples. The assay was performed at room temperature (30 ± 2 °C) and 5 ml of sample was watered separately per day. At the same time plain water was used to carry out the control set. After 10 days of incubation, length of plumule (shoot), radicle (root), Chlorophyll content (mg/g) and germination (%) was recorded [9,10].

$$\text{Germination \%} = \frac{\text{Number of seeds germinated in presence of effluent/dye}}{\text{Number of seeds germinated in control}} \quad \text{(Eq. 2)}$$

$$\text{Chlorophyll a} = \frac{(12.3 \times D_{663} - 0.86 \times D_{645}) \times V}{D \times 1000 \times W} \quad \text{(Eq.3)}$$

$$\text{Chlorophyll b} = \frac{(19.3 \times D_{645} - 3.60 \times D_{645}) \times V}{D \times 1000 \times W} \quad \text{(Eq. 4)}$$

Whereby, D_{645} is theOptical density at 645 nm, D_{663} is theOptical density at 663 nm, V is the final volume in ml, W is the fresh weight of leaf and D is the path length (cm)

Result and Discussion

After adsorption under pH 5, incubation time 180 mins, adsorbate dosage 100mg/L, adsorbent dosage, 12 gm/L at a temperature of 45°C on WB, AWB and AMWB, the maximum percentage of disperse orange removal obtained was 93.48, 96.41 and 70.24% respectively (process not disclosed). Thus, the permeate obtained from the study after been used for phytotoxicity analysis has shown 33% more radical growth but 20% less plumule growth with dye obtained after adsorption on wheat bran in comparison to the control. Whereas, on reusage, in the third cycle, the dye obtained has shown 20% increase in radicle growth with 23% increase in plumule growth too. In comparison to acid and alkali modified wheat bran acid modified wheat bran has shown 30% increase in radicle growth but only 0.09% plumule growth. However, the best result was shown with that of organism treated dye, with 35% radicle growth and 15% plumule growth (Table1; Table 2; Figure 2 and Figure 3) depicting that organism treated dye can be used for plantation as may be a consequence of degradation of the dye by the enzyme produced by the organism which does not affect the plant adversely. In another similar study it has been shown that Direct Yellow 4 (DY4) treated by citrus POD reduced the toxicity of DY4, indicating that the biodegradation of dyes are quite efficient in reduction of toxicity of the dyes (11). In another study with *Sorghum vulgare* Pers seeds, the effect of acid orange 10 and treated dye has shown that there was also 100%germination in control while the treated dye yielded 27% and untreated dye yielded only 06% germination (12). Another study reported that *Triticum spp*. (Wheat) yieldedcent percent germination of seeds in control and but the seeds treated with dye showed no germination. In the same study with *Vigna radiata* (Moong seeds), 100% germination in control was observed whereas, the seeds treated with dye at 500-1000ppm only 40% and with 5000ppm dye only 20% germination was recorded (13).

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Conclusion

Thus, treated dyes shows better result with respect to phytotoxicity. Thus such treated aqueous dyes can be used for ornamental trees, which can be avoided to be disposed off the environment like that.

Figure 2: Effect of DO (100 mg/L) on plant; A-Control, B-Untreated, C-Unmodified WB treated (1st cycle), D-Unmodified WB treated (2nd cycle), E- Unmodified WB treated(3rd cycle), F- Acid modified WB treated, G-Alkali modified WB treated, H-Organism treated.

Plant treatment	Radical (cm)	Plumule (cm)	Germination %
Control	4.3 ± 0.5	12.8 ± 2.21	100
Untreated	0.9 ± 0.1	7.0 ± 0.5	100
Unmodified WB (first cycle) treated	6.1 ± 0.9	10.2 ± 1.4	100
Unmodified WB (second cycle) treated	5.3 ± 0.3	12.9 ± 1.4	100
Unmodified WB (third cycle) treated	5.4 ± 0.8	14.4 ± 1.1	100
Acid modified WB treated	6.2 ± 1.3	11.6 ± 0.7	100

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Alkali modified WB treated	4.6 ± 0.6	9.8 ± 0.5	100
Organism treated	6.6 ± 1.5	15.1 ± 1.8	100

Table 1: Radical length, plumule length and germination % of plant treated with DO (100 mg/L)

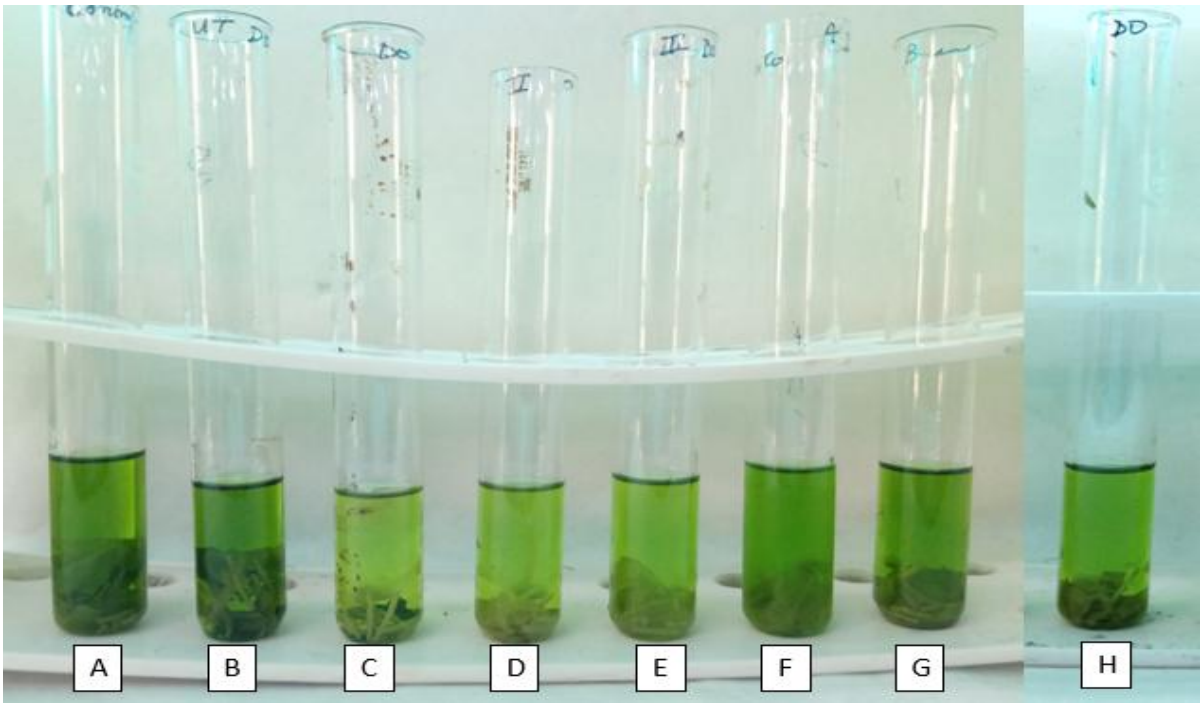

Figure 3: Effect of DO (100 mg/L) on chlorophyll content; A-Control, B-Untreated, C-Unmodified WB treated (1st cycle), D-Unmodified WB treated (2nd cycle), E- Unmodified WB treated(3rd cycle), F- Acid modified WB treated, G-Alkali modified WB treated, H-Organism treated.

Plant treatment	Chlorophyll a	Chlorophyll b
Control	0.945 ± 0.084	0.264 ± 0.016
Untreated	0.919 ± 0.087	0.281 ± 0.017
Unmodified WB (first cycle) treated	0.299 ± 0.028	0.095 ± 0.0049
Unmodified WB (second cycle) treated	0.489 ± 0.036	0.157 ± 0.006
Unmodified WB (third cycle) treated	0.567 ± 0.047	0.187 ± 0.010
Acid modified WB treated	1.020 ± 0.091	0.317 ± 0.027

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Alkali modified WB treated	1.003 ± 0.093	0.299 ± 0.021
Organism treated	0.937 ± 0.058	0.262 ± 0.020

Table 2: Chlorophyll content of plant treated with DO (100mg/L)

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