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Bioremediation of color and COD from dye effluent using indigenous and exogenous microbes

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ABSTRACT

Dye effluents pose severe environmental hazards. Devising cost-effective and eco-friendly techniques for treating wastewater containing dye effluent is the need of the day. The intention of the present study is to expose a technique for decolorizing the dye effluents, for degrading the contaminants present in the effluent and for minimizing the COD below standard limits. The dye effluent samples collected for the present study characterize a high COD of 972 mg/L, pH 8.6 and highly objectionable color (990 Hazen Units). The predominant indigenous organisms present in the samples were isolated and identified as *Aspergillus niger* and *Aspergillus flavus*. Potential exogenous organisms *Trichoderma viride* and *Aspergillus fumigatus* were obtained, and these organisms were used to conduct treatability studies. An up-flow immobilized column reactor (UFICR) was designed and used to treat the textile effluent. The shaker flask trials give maximum decolorization efficiency of 91% and COD reduction of 84%. The reactor studies showed elevated efficiencies of 92.7% and 93% efficiency in decolorization and COD reduction, respectively. The present study is developed as an efficient strategy to replace the less eco-friendly physicochemical approaches and also to provide a better insight into the field of bioremediation and its role in the treatment of dye effluent.

Keywords: Decolorization; Exogenous organisms; Dye effluent; Eco-friendly; Environmental hazards

1. Introduction

Due to hasty industrialization, there has been a momentous magnification in the exploitation and discharge of chemicals, including heavy metals in the environment [1]. Many dyes are noticeable in water at concentrations as low as 1 mg/L [2]. Textile industry wastewater, a character with dye content in the range 10–200 mg/L, is therefore generally highly colored and is discharged in the open land, and hence, it pollutes the environment [3]. Chemical and physical methods for the treatment of dye wastewater are very expensive, and they face disposal problems [4]. Green technologies for solving the cited problems include the adsorption of dyestuffs on bacterial and fungal biomass [5].

Bioremediation is one of the promising technologies that are predicted to play a vital role in the degradation of the toxic contaminants in an inexpensive way [6]. Fungi, isolated from dye-contaminated wastewater, was capable of decolorizing several direct textile azo dyes, under dissimilar surroundings [7,8]. The microorganisms may be indigenous to the wastewater, or they may be isolated from elsewhere and brought for the treatment studies [9]. Contaminant compounds are transformed by living organisms through the reactions taking place as a part of their metabolic process [10]. A bioreactor is a container that mediates the biological reaction between the microbes and their food (contaminants), so that the contaminants are degraded and

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the harmless water obtained as the output of the biological reaction on the wastewater can be re-used or left open in the environment [11]. The wastewater to be treated flows upward at an unwavering flow rate through a column containing the fungi that are immobilized to a suitable medium [12]. This helps in preventing the escape of the fungi along with the treated matter and thus extends the bed life and reduces the cost of recycling the biomass [13]. The present study aims at identifying the bioremediation potential in the removal of color, COD from the dye effluent. Immobilization technique was used for degradation of pollutants in wastewater making use of indigenous and exogenous microbes, and in order to optimize the reactor parameters, bench-scale bioreactor was designed [1]. The goal of this research is to replace the costly and technically complex physicochemical strategies with efficient biological treatment techniques and to minimize quality parameters present in the dye effluent.

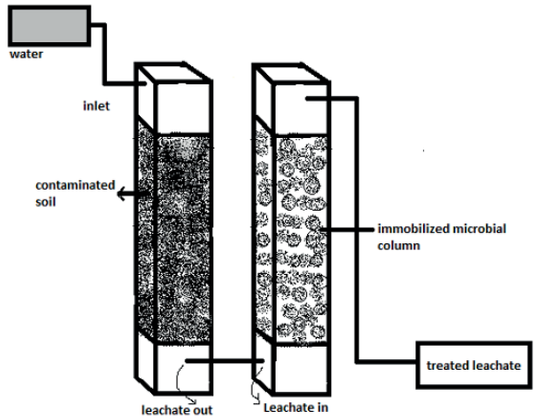

Fig. 1. Schematic diagram of up-flow immobilized column reactor.

2. Materials and methods

2.1. Sample collection

The dye effluent samples were collected from Veerapandi common effluent treatment plant, Tirupur. 25 L of effluent were collected in clean plastic cans. The samples were true representative samples of dyeing and bleaching effluents from 72 units located in Tirupur. The physicochemical parameters like COD, pH, TDS, total hardness, TSS and color of the effluent were taken note of before the treatment process. This helps not only to compare the treatment efficiency but also to estimate the required percentage of treatment to be achieved in conformation with the proposed government standards.

2.2. Enrichment and isolation of dye degrading fungus

The potato dextrose agar medium was prepared and sterilized. The serially diluted samples were placed on potato dextrose agar plates, and the plates were incubated at room temperature for 3–5 d. The incubated plates were observed for the predominant types of organisms. The screening of organisms was done on the basis of survival in a concentrated dye environment [14]. 3 mL of direct malachite green dye was added to 100 ml of PDA agar. After autoclaving, the dye added agars were solidified in Petri dishes. The isolated organisms were separately streaked on these plates and then incubated at 37°C. The results were observed after 24 h for bacteria and after 48 h for fungi. The fungal colonies that were screened by survival in the dye concentrated medium were compared with the morphological characteristics of different microbes. Two exogenous microbes such as *Trichoderma viride* and *Aspergillus fumigates* were obtained from Thiagarajar College of Engineering, Madurai [15,16].

2.3. Up-flow immobilized column reactor for dye

The anoxic up-flow immobilized reactor has been used efficiently in various dye related studies. Kitchen scrubbers were cut into small pieces (2 mm × 3 mm). These pieces were added to suitable nutrient medium (15 pieces per 250 mL of nutrient medium) and inoculated with the required fungal spores. After 48 h of incubation, the immobilized media were used for column reactor studies. The reactor having the dimensions 36 inch × 3 inch × 3 inches (split into three layers) was fabricated using acrylic material shown in Fig. 1, and the effluent flows upward in the up-flow immobilized column reactor (UFICR) [1]. The color and COD of the effluent were reduced, and the treated effluent is collected in the collection flask.

3. Results and discussion

Surfacing of the zone of acceptance around microbial growth on the potato dextrose agar with the quantity of dye reflects the existence of dye degrading capability of microbes. The dye degradation ability of four fungal isolates was analyzed by frequent subculturing, isolation and purification ways.

3.1. Identification of fungal sample

Approximate identification of fungal sample was made by the slide culture technique and various morphological studies under microscope by staining. Identification studies were further confirmed at Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. The results reveal that the fungal isolate were *Aspergillus niger* and *Aspergillus flavus*.

3.2. Assessment of efficiency by shaking flask method

3 mL of organism inoculum ([1.5 + 1.5] mL in case of combinations) was inoculated into 100 mL effluent samples and incubated in a shaking condition using orbital shaker. Different treatment trials were made by changing the organism and the concentration of the effluent. Effective organisms were identified and used in the bioreactor studies.

From the various trials, the organisms show a minimum efficiency at 100% concentration of the effluent. Maximum efficiency was shown when the effluent was diluted

to 60% concentration. The organism trials show maximum efficiency in dye decolorization process rather than in the process of COD reduction. It was found that, at 60% concentration, *Aspergillus niger* shows the maximum efficiency of 91%. The efficiency of all the organism trials was represented graphically in Fig. 2, which also obviously implies the efficiency of *Aspergillus niger.* With 100% concentration of the dye effluent, the *Trichoderma viride* trial shows maximum efficiency in COD reduction.

In the trials using indigenous organisms, *Aspergillus niger* showed 91% efficiency in decolorization. In the trials using exogenous organisms *Trichoderma viride* showed 84.2% efficiency in COD reduction. From this observation, it is evident that a combination of these two organisms can be used in bioreactor studies to reduce COD and color of dye effluent to the maximum. Decolorization and fungal growth were also observed after 72 h of incubation, and duly recorded by UV-visible spectrophotometer at 543 nm.

Due to the adsorption of the dyes to the *Aspergillus niger* surface and its metabolic breakdown, the color has been effectively removed. Reference [17] reports the microbial decolorization of azo dyes and dye industry effluent by *Fomes lividus*. It is suggested that the batch mode treatment of *Fomes lividus* is one of the most competent ways for color removal in dye industry effluents.

3.3. UFICR

The operating parameters like HRT and bed life are to be determined for sustaining the treatment process. The bed life was estimated on the basis of both COD reduction and decolorization.

The efficiency in decolorization increases with the increase in HRT, which is graphically represented in Fig. 3. The efficiency interval between 8 and 10 HRT was comparatively very low. Hence, 8 HRT could be an economically efficient time span in case of large scale operations. Reference [18] examined the biodegradation and biosorption in relation to decolorization of synthetic dyes by *Funalia trogii*. The researchers suggested that it is possible to decolorize a high concentration of commercial dyes, with due measures taken for the treatment of dye wastewater.

3.4. Optimum bed life

The optimum life of the immobilized fungi is to be estimated for the periodic re-bloom and change of immobilized fungi as shown in Figs. 4 and 5.

The bed life was estimated with respect to decolorization and COD reduction. At 90 h, the bed life ends for decolorization. At 50 h the bed life ends for COD. Thus, 50-h bed life could be considered as optimum bed life to achieve efficient reduction of both COD and color. The decline of pH at the end of these experiments may be due to the secretion of the organic acid by the fungus itself [7]. The decline in initial pH was observed with decolorization. Reference [7] reports that the fungus creates organic acid salts such as malonate; oxalate during the preliminary growth period, which soon can be decomposed by specific enzymes such as manganese peroxidase enzyme. For the degradation of dyes in dye wastewater, the exploits of microbes rather

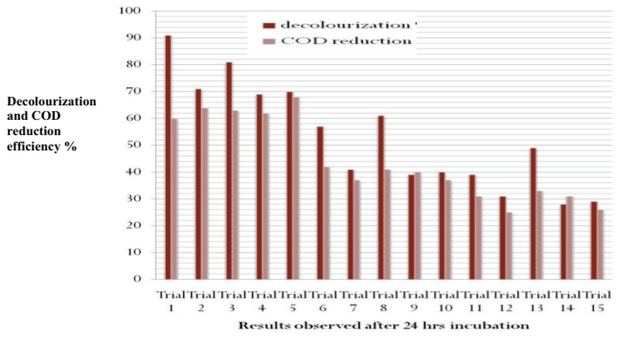

Fig. 2. Efficiency of indigenous and exogenous organisms.

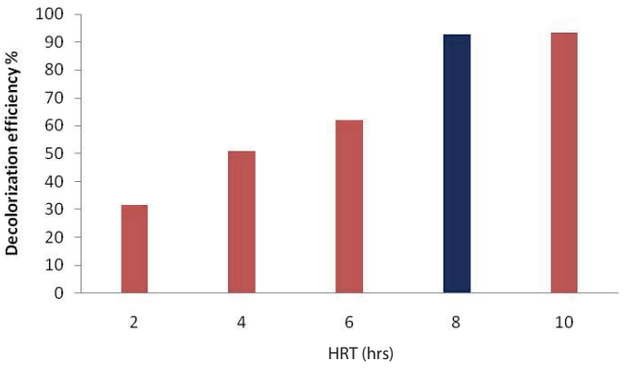

Fig. 3. Optimum HRT of fungal column.

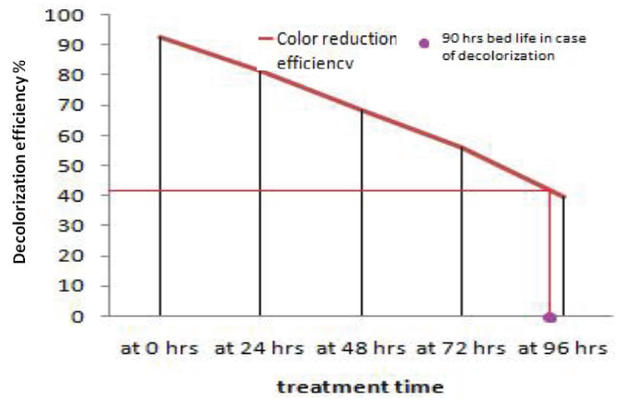

Fig. 4. Optimum bed life of fungal column with respect to decolorization.

than enzymes will be beneficial, since the cost of enzyme purification is annulled and the microbial cell gets protection from the insensitive procedural environment for the enzymes [19].

Removal of textile dyes from industrial effluent was performed formerly by numerous methods, including alum sludge, yeast biomass, anaerobic bacteria, bacterial biomass, fungi and/or ozonation and electroflocculation [7,20].

The use of *A. niger* to remove dyes from textile industry reveals that this fungal strain is competent enough to remove

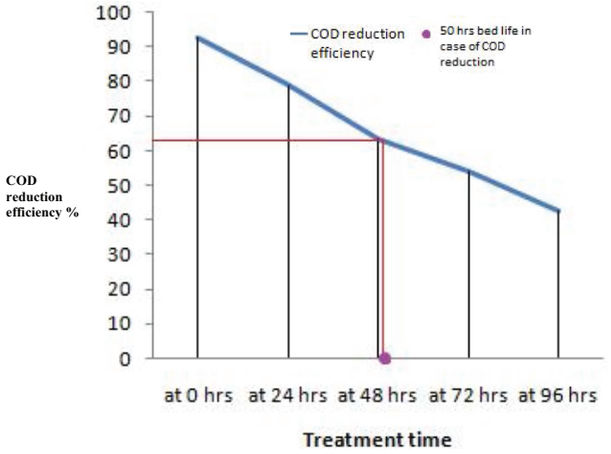

Fig. 5. Optimum bed life of fungal column with respect to COD reduction.

dyes in a relatively short span of time from the media. This means that there is a fast interaction between the fungal mycelium and the dye during the process. This interface might be based on a biosorption of dyes on the integral fungal biomass. This is in synchronization with the research carried out on decolorization of eight textile dyes by the white rot fungus, *Trametes versicolor* [9]. It is reported that during the initial stages of the process of decolorization of textile dyes by *T. versicolor*, the toxicity of the solution remnants is unaffected, declined or even augmented. At the end, it appears to occur with dyes that may be complex to decolorize or those, which require long decolorization times [21]. These conclusions also emphasize the significance of monitoring toxicity modification in any decolorization process as toxicity may enhance.

The decolorization capacity of the chosen dyes ranged between 40% and 90% after 1 d of incubation. These results are in harmony with reference [22], which established decolorization of several dyes (Red HE-8B, Malachite Green, Navy Blue HE-2R, Magenta, Crystal Violet) and an industrial effluent with growing cells of *P. chrysosporium*. All the dyes and the industrial effluent were decolorized to a certain degree with varying percentages of decolorization (10%–60%).

4. Conclusion

The characterization of the dye effluent reveals the risk of releasing the effluent in to the environment without treatment. The indigenous organisms present in the effluent samples that have the potential to degrade dye contaminants were *Aspergillus niger* and *Aspergillus flavus*. The potential exogenous organisms obtained were *Trichoderma viride* and *Aspergillus fumigatus.* Assessment of the efficiency through the use of shaking flasks revealed that *Aspergillus niger* and *Trichoderma viride* can achieve 92% efficiency in decolorization and 83% efficiency in COD reduction, respectively. Therefore, the combination of *Aspergillus niger* and *Trichoderma viride* will be efficient for use in up-flow fungal column reactor studies. In reactor studies maximum efficiency of 92.7% decolorization and 93.2% COD reduction were achieved at an optimum HRT of 8 h. The bed life of the fungal column was estimated at 50 h. After 50 h the immobilized pieces were washed and then suspended in the nutrient medium for re-bloom. Then it will be ready for re-use.

By the use of the up-flow column reactors, the process of remediation of the dye effluent is optimized. But the dye effluent has to be diluted to 60% concentration initially by the addition of water only for starting up the process; in further processes, a portion of the treated effluent can be redirected to dilute the effluent. After the treatment, the effluents can be released to the inland surface water since they comply with CPCB standards.

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