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Decolorization and Biodegradation of basic violet dye by fungal-bacterial consortia

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ABSTRACT

The present study was aimed to test the ability of Penicillium *citrinum MTCC* 8009, Aspergillus *terreus MTCC* 3006, *Bacillus cohnii* and their consortia to decolorize basic violet dye. Different parameters such as initial dye concentration, dye to inoculum ratio and period of incubation were studied for the decolourization of the dye. The developed fungal- bacterial consortia exhibited maximum percent decolorization (92%) ability when compared to the treatment of dyes by individual microbes. Percent decolorization of basic violet dye (92%) was more efficient using fungal-bacterial (*Penicillium citrinum* and Bacillus *cohnii*) consortia than with individual cultures. Phyto-toxicity results indicated that bacterial-fungal consortia (*Penicillium citrinum* and *Bacillus cohnii)* treatment was believed to degrade the dyes to non-toxic intermediates. The FTIR analysis also revealed that decolorization of basic violet dyes was due to its degradation.

Indexing terms/Keywords

Basic violet dye, microbial remediation, fungal bacterial consortia, phyto-toxicity, FTIR.

1. INTRODUCTION

Dyes are coloured substances, which are used along with a mordant to impart colour to fibres. There are about 10,000 different dyes that are used globally [18]. The textile industry releases about 10% of the above-mentioned value as their effluents in fresh water which was toxic to the environment [15]. The dyes from textile dyeing effluents pose a serious threat to environment even at a very low concentration (1mg/L). So, these effluents must be treated before they reach the environment [27]. Various treatment technologies have been investigated extensively such as the photochemical oxidation [20], membrane [28], chemical coagulation [1,24], adsorption [13,16,19] aerobic and anaerobic biological processes [11,14], nano filtration [8,26], electrocoagulation, ultra-sonic decomposition [3], pre-dispersed solvent extraction [10], ozonation [4,22], colloidal gas aphrons [21] and liquid- liquid extraction [6,12]. Although the aforesaid physico-chemical methods are economical, they generate sludge which are difficult to dispose-off and these sludges are secondary pollutants. There are viable technologies to treat the sludge. Biological treatments are being extensively investigated due to their low cost and efficient degradation process. Adsorption is one such biological method in which the microbial cells take-up the dyes on their external cell surface, and further they absorb the dyes into the cell. After this, biotransformation of the dye which involves enzymatic degradation takes place and as a result of this process the dye loses its toxicity. Several microorganisms belonging to different taxonomic groups like bacteria, fungi, yeast, algae, actinomycetes etc. have shown their capability to degrade dyes. Despite their great promise, both the bacteria and fungi have faced lot of challenges with respect to their ability to decolourise the dyes individually. On the other hand, some of them have resulted in the release of carcinogenic and mutagenic metabolites. The use of fungi in bioremediation of textile effluent was limited because of their slow growth rate and greater hydraulic retention time to decolorize the dyes completely.

An enhanced degradation and detoxification of the textile dyes could be possible with the synergetic actions of fungi and bacteria consortia which provide a better alternative technology for the removal of pollutants in the water [7,17,23]. In addition, rapid rates of decolorization proved that these synergetic consortia might be a powerful weapon to attack the dyes and completely mineralise them into non-toxic substances. Fungi despite of its slower growth rate is known to secrete various extra cellular enzymes like manganese peroxidase, lignin peroxidase and laccase which are non-specific to the dye molecules. These enzymes upon acting on the dye deforms the dye structure thereby reducing its toxicity. Thus, the fungal-bacteria consortia coordination could be an additional advantage as they have inductive effects on various enzymes which could have improved action than in individual system. Fungal-bacterial consortia system could be further exploited in depth for the eco-friendly remediation of the textile effluents.

2. MATERIALS AND METHODS

2.1 Chemicals

Veratryl alcohol, methyl red, ABTS, Nutrient Medium (NM), toluene, ethyl acetate, methanol and potato dextrose broth (PDB) were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai. Basic violet dye was obtained from TIFAC-CORE department, Kumara guru College of Technology, Coimbatore. All chemicals used were of highest purity available and of an analytical grade. UV–Visible Spectrophotometer (Shimadzu, UV-1800) was used for measuring the absorbance of the solutions.

2.2 Microorganism and media

Penicillium citrine MTCC 8009 and *Aspergillus terreus* MTCC 3006 were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. Fungal cultures were periodically sub-cultured on Czapek-Dox agar slants and are stored at 4°C. *B.cohnii* was obtained from Bioprocess Lab, KCT and was maintained on nutrient agar slants at 4℃. The composition of potato dextrose broth [PDB] used for decolourization studies was (g/l): potatoes infusion 200, dextrose 20 and yeast extract 5.

2.3 Degradation of dyes by microbial consortia

The fungal cultures from the stock were inoculated into 250 ml of Erlenmeyer flasks containing 100 ml of PDB and incubated for 8 days at 30°C under shaking condition. One loopful of 24h old grown *Bacillus cohnii* was inoculated into 100 ml of nutrient broth [NB] and incubated at 37°C for 24h under shaking condition. *Penicillium citrinum* and *Bacillus cohnii* consortia was prepared aseptically by transferring the 50 ml of 8 days grown *Penicillium citrinum* into 250 ml
Erlenmeyer flasks containing 50 ml of log phase cells of *Bacillus cohnii*. Similar method was followed for consortia PA *(Penicillium citrinum* and *Aspergillus terreus* consortia) and consortia AB (*Aspergillus terreus* and *Bacillus cohnii* consortia). The pre-grown individual cultures and its developed consortia were then used as inoculums for further degradation studies. The dyes were subjected to degradation studies considering different parameters such as initial dye concentration, dye to inoculum ratio and the period of incubation.

2.4 Decolorization experiment

Decolorization of basic violet dye was carried out under shaking condition with 100 ml of PDB containing *Aspergillus terreus* and *Penicillium citrinum* in PDB, and *Bacillus cohnii* in NB. Initial dye concentrations used in the investigation were 20, 40, 60, and 100 mg/l of dye. A condition of 50% dye to inoculum ratio was prepared by aseptically transferring equal volumes of dye and inoculum. Similarly, for 75%, it was 37.5 ml of dye and 13.5 ml of inoculum or the developed consortia.

Dye to inoculum ratio (100%) was also prepared aseptically by transferring the loopful of culture or its developed consortia into the prepared dye solution. Aliquots of the cultured supernatant were withdrawn at regular intervals of time and % decolorization were calculated.

$$\% \text{ decolorization} = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100$$

2.5 Enzyme assay

The ligninolytic enzymes such as lignin peroxidase [25], laccase [2] and manganese peroxidase [9] were determined as reported in the literature.

2.6 Metabolite analysis

After decolorization of basic violet dye, the fungal mycelia were removed by filtration through Whatman filter paper no.1 while bacterial cells were removed by centrifugation at 10,000 rpm for 20 min. Similarly, the *Penicillium citrinum-Bacillus cohnii* consortium biomass were removed by filtration using mesh cloth followed by centrifugation of the filtrate at 10,000 rpm for 20 min. The supernatant thus obtainedwas subjected to TLC and FTIR analysis.

2.7 UV-Visible analysis of degraded products of basic violet dye

The maximum wavelength of the authentic and its degraded products were checked using UV-Visible spectrophotometer. UV-Visible spectra of authentic dye were compared with original dye to find the extent of degradation process.

2.8 FTIR analysis

FTIR analysis was performed in order to investigate the changes in surface functional groups of the degraded dye products before and after microbial treatment. FTIR analysis was done using Shimadzu spectrophotometer.

2.9 Toxicity studies

To determine the toxicity of the degraded samples, phyto-toxicity test was carried out on *Vigna radiata.* Ten healthy seeds of *Vigna radiata* were separately sowed into plastic pots containing 20 g of washed and oven dried sand. The toxicity study was carried out at room temperature 27°C by daily watering 5 ml of degraded dye solution. Simultaneously, control set was carried out at the same time by daily watering it with the dye solution that was not treated. Tests were done and the results are presented as an average.

Germination (%) = (No of seeds germinated / No. of seeds sowed) * 100

3. RESULTS AND DISCUSSION

Table 1: % dye decolorization of dye (20 mg/l) at different time intervals

	No. of days	Day 2			Day 6			Day 10		
	Dye to inoculum ratio (%)	50	75	100	50	75	100	50	75	100
Microorgaminism	*P.citrinum*	38	26	7	56	42	36	72	62	60
	A.terreus	33	30	22	42	32	27	56	41	40
	B.cohnii	17	20	16	37	26	23	52	36	32
	B+P	56	48	38	82	63	58	84	82	80
	B+A	62	42	30	78	59	63	92	84	78
	P+A	48	37	26	68	52	48	86	80	78

It was evident from Table 1 that on day 2, basic violet dye was degraded to 62% by *Penicillium citrinum* and *Bacillus cohnii* consortia at 50% dye to inoculums ratio while *Bacillus cohnii-Aspergillus terreus* decolorized the dyes to 48% and 38% at 75% and 100%dye to inoculum ratio, respectively. It was clear from Table 1 that on day 6, *Bacillus cohnii-Aspergillus terreus* consortia at 50% and 75% dye to inoculums ratio degraded the basic dye to 82% and 63%, respectively, while *Penicillium citrinum* and *Bacillus cohnii* consorti decolorized the dyes to 63%. It also apparent from Table 1 that on 10th day, *P. citrinum* and *Bacillus cohnii* consortia at 50% and 75% dye to inoculums ratios degraded the basic dye to 92% and 84%, respectively. *Bacillus cohnii-Aspergillus terreus* consortia decolorized the dyes to maximum (80%) at 100% dye to inoculum's ratio.

Table 2: % Dye decolourization for 60mg/l of initial dye concentration

	No. of days	Day 2			Day 6			Day 10		
	Dye to inoculum ratio (%)	50	75	100	50	75	100	50	75	100
Microorgaminism	*P.citrinum*	28	26	17	39	34	25	45	43	29
	A.terreus	26	20	15	31	28	25	37	39	35
	B.cohnii	10	8	5	22	19	10	30	50	42
	B+P	48	36	30	63	48	41	79	70	49
	B+A	40	52	48	69	59	55	70	62	63
	P+A	32	36	32	53	43	35	61	55	57

The percentage decolourization of basic violet dye was evident from the Table 2 that on day 2, *B. cohnii and A. terreus* decolorized the dye to 52% and 48% at 75% and 100% dye to inoculum ratio whereas *P. citrinum and B. cohnii* degraded the basic violet dye to 48% at 50% dye to inoculum ratio, respectively. Then on day 6, it is clear that *B. cohnii and A. terreus* decolorized the dye to 69% and 59% at 50% and 75% dye to inoculum ratio whereas *P. citrinum and B. cohnii* removed the basic violet dye to 63% at 50% dye to inoculum ratio, respectively. It was apparent from Table 2 that on day 10, *B. cohnii and A. Terreus* decolourized the dye to 70% and 62% at 50% and 75% dye to inoculum ratio. Thus, it is clear from the Table 2 data that on day 10, the percentage decolourization was found to be maximum i.e., 79% when the dyes were treated with *Penicillium citrinum* and *Bacillus cohnii* consortia.

Table 3: % Dye decolourization for 100 mg/l of initial dye concentration

	No. of days	Day 2			Day 6			Day 10		
	Dye to inoculum ratio (%)	50	75	100	50	75	100	50	75	100
Micr oorg	*P.citrinum*	17	15	12l	23	22	17	28	24	22
	A.terreus	15	10	10	14	13	10	18	16	12

	B.cohnii	5	4	4	10	8	8	14	12	10
	B+P	42	30	20	52	38	28	68	48	38
	B+A	35	42	39	42	42	40	48	46	40
	P+A	27	29	26	34	32	30	40	38	32

From the Table 3 on day 2 basic violet dye was degraded to 42% dye by *P. Citrinum and B. cohnii* consortia at 50% dye to inoculums ratio while *Bacillus cohnii and Aspergillus terreus* decolorized the dyes to 42% and 39% at 75% and 100% dye to inoculum ratio, respectively. On day 6, table 3 data showed that *Penicillium citrinum and Bacillus cohnii* consortia at 50% dye to inoculum ratio decolourized the dye to 52% whereas the degradation was achieved only upto 42% and 40% by *Bacillus cohnii and Aspergillus terreus* consortia at 75% and 100% dye to inoculum ratio, respectively. It is also evident from the Table 3 that the percent decolourization decreases with an increase in dye concentration in all the days using *Penicillium citrinum* and *Bacillus cohnii* consortia. *Penicillium citrinum* and *Bacillus cohnii* consortia. showed maximum percent (68 %) decolorization on 10th day with 50% dye to inoculum ratio.

3.1 Enzyme activities

Fig. 1: The fungal bacterial consortia (*Penicillium citrinumand Bacillus cohnii*) showed maximum lignin peroxidase activity (1.8 U/ml) with 20 mg/l of dye concentration.

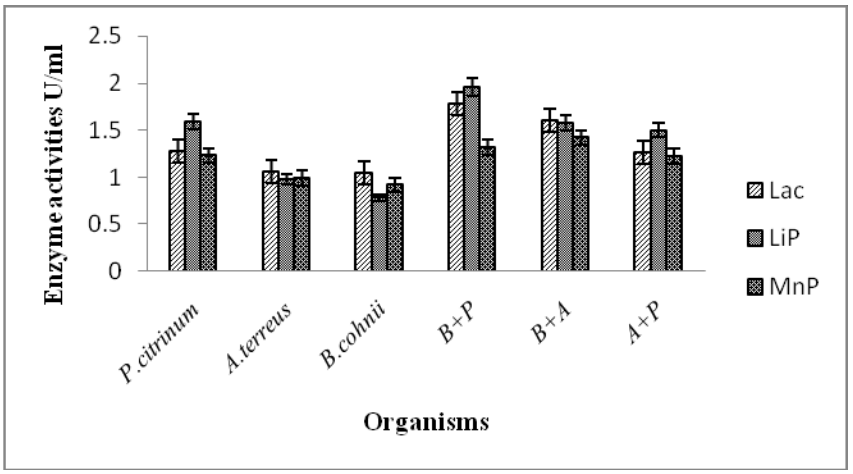


Fig. 2: The fungal bacterial consortia (*Penicillium citrinum and Bacillus cohnii*) showed lignin peroxidase activity of (2.3 U/ml) with 60 mg/l of dye concentration.

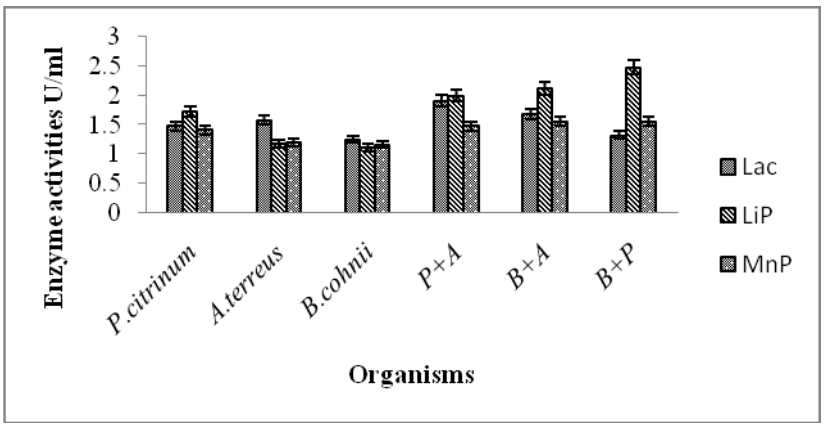


Fig. 3: The fungal bacterial consortia (*Penicillium citrinum and Bacillus cohnii*) showed lignin peroxidase activity of (2.7 U/ml) with 100 mg/l of dye concentration.

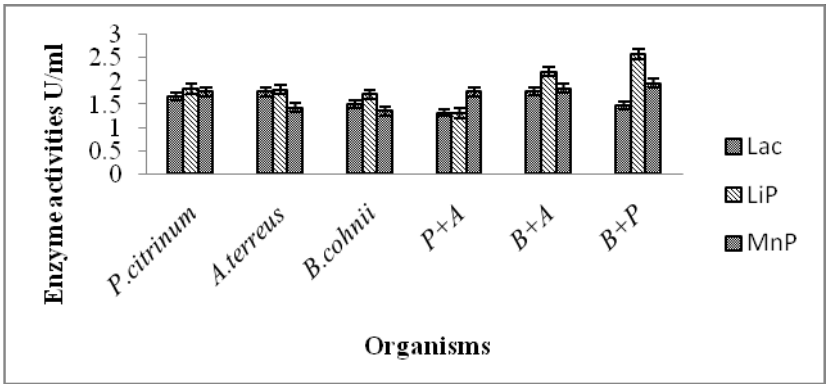


Fig. 4: UV-Visible spectra of basic violet dye and its degraded products.

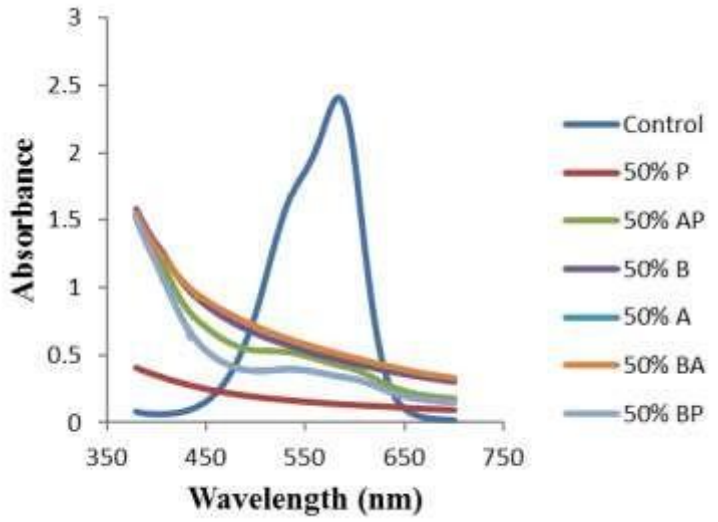
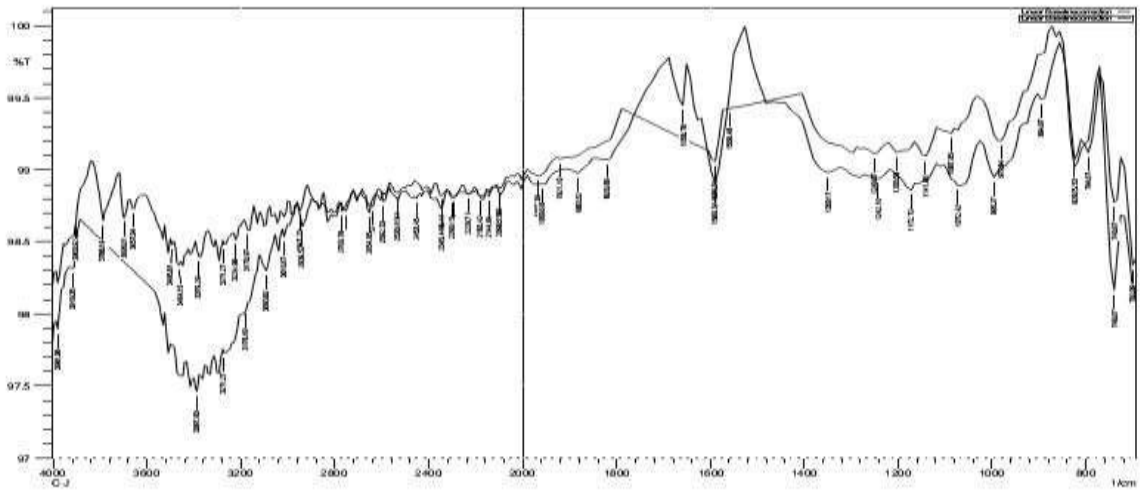


Fig 5 - The FTIR analysis for the dye and its degraded product



The FTIR spectra for basic violet showed specific peaks at 3464.15 cm^{-1}(for OH stretching), 1658.78 cm^{-1}(for –C=C- stretching of alkenes), 15.89.34 cm^{-1} (for C-C stretching if aromatic compounds), 1350.17 cm^{-1} (for C-H vibration of alkanes), 995.27 cm^{-1} (for =C-H bending of alkenes), 825.53 cm^{-1} (for C-Cl stretching of alkyl halides), and at 740.67 cm^{-1} (for the bending of alkynes). After the consortium (B+P) decolorization, a significant reduction in IR peaks was observed in
the 3387 cm^{-1} (for N-H stretching of primary and secondary amines), 1589.34 (for C-C stretching of aromatic compounds), 1141.86 cm^{-1} (for C-N) and at 740.67 cm^{-1}(for the C-Cl alkyl halide stretching). Vanishing of major peaks and formation of new peaks in the FTIR spectrum of metabolites released by *Penicillium citrinum-Bacillus cohnii* consortia suggests the

biotransformation of basic violet dye into distinct non-toxic metabolites. These results also indicated that decolorization of the dye was by virtue of biodegradation.

3.2 PHYTO-TOXICITY STUDIES

Fig.6-Germination of *Vigna radiata* seedling on *Penicillium citrinum-Bacillus cohnii* treated dye solution

Basic violet dye (100 mg/l) strongly inhibited the germination of *Vigna radiata. On* the contrary, degraded products of the dye did not inhibit the germination of the seedlings (Fig.7). Complete germination (100%) as well as significant growth in the plumule and radicals were observed for the plants grown in (*Penicillium citrinum-Bacillus cohnii)* a treated dye consortium metabolites as compared to authentic dye. Results of the present investigation indicates that the dye was toxic to these plants, while the metabolites formed after consortium degradation was less toxic, which signifies the detoxification of dye by *Penicillium citrinum-Bacillus cohnii* consortium. These results also underline the importance of fungal-bacterium synergism for bioremediation of textile effluent in terms of both decolorization and detoxification. Plant bioassays have been used to establish the toxicity levels of dye, and its degraded products on common agricultural crops. The assessment of toxicity of dyes, and its degraded products is often great concern as most of them exert toxic effect on plants and animals when released in stream water. Rubine GFL dye was decolorized to 78% using fungal-bacteria consortia of *Pseudomonas sp. and Aspergillus ochraceus* [5].They also reported that dye degrading enzymes such as azoreductase, veratyl oxidase, tyrosinase, laccase, and NADH-DCIP reductase enzymes in the medium were responsible for the decolorization of Rubine GFL dye. In the present study, microbial consortia consisting of *Penicillium citrinum* and *Bacillus cohnii* showed maximum percent (92%) decolorization for basic violet dye. Ligniniolytic enzymes such as lignin peroxidase, laccase and manganese peroxidase were detected in the medium and these enzymes were believed to be responsible for the degradation of basic violet dye. Further, FTIR analysis for the dye and its degraded product showed the disappearance of major peaks at their functional group and a similar results reported in the literature [5]. Results of phytotoxicity of the present study are in good agreement with their work using fungal-bacterial consortia.

4. CONCLUSION

Fungal-bacterial synergetic consortium was applied for the degradation of basic violet dye in submerged conditions. Results revealed that the synergetic metabolic activities of *Bacillus cohnii* and *Penicillium citrinum* the consortium led to complete decolorization of basic violet dye. An enhanced efficiency of *Penicillium citrinum-Bacillus cohnii* consortia could be due to constitutive levels of ligninolytic enzymes (laccase, lignin peroxidase and manganese peroxidase). Results of the present investigation paves the way for the decolorization of dye with co-culture approach that could alleviate the pollution problems due to synthetic dyes. Thus in this present work the degradation was found to be more efficient i.e., 92 % of degradation in fungal bacterial *(Penicillium citrinum and B. cohnii*) consortia at the day 10 for 50% of dye to inoculum ratio when compared with the individual cultures. An increase in the concentration of dye increases the contact time for efficient degradation. FTIR analysis proved that control effluent showed specific peaks at 3464 cm^{-1} for OH stretching while in degraded product the peaks were obtained at 3387 $cm^{-1}$for N-H stretching. The disappearance of major peaks at the alkane groups further supports the degradation process. Phototoxicity studies conducted for authentic basic violet dye showed an inhibition of germination of *Vigna radiata* seedlings while complete germination was observed for the plants grown in the presence of degraded products which are less toxic or non-toxic to *Vigna radiata*.

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