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European Journal of Experimental Biology, 2015, 5(7):36-40

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Biodegradation of dyes using consortium of bacterial strains isolated from textile effluent

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ABSTRACT

The textile industries use different types of dyes in their processing units which are liberated in natural water bodies through wastewater. Disposal of these dyes into the environment causes serious damage. There is the need of era to investigate new bacterial dye degraders that have potential for use in a various textile industries to treat effluents. The present study deals with the degradation of Methyl red (MR) and Carbol fuchsin (CF) by bacteria and its consortium isolated from textile effluents. Three different bacterial spp. were isolated and subjected to dye decolorization assays. The isolated bacteria were capable of decolorizing 0.002 gm/L MR and CF dye. The isolates were identified as Proteus spp., Pseudomonas spp. and Acinetobacter spp. Instead of pure bacterial isolates their consortium was exhibited the highest potential in decolorizing MR and CF up to 100% and 96%, respectively within 24 h. TheComplete degradation of dyes was confirmed by FT-IR analysis.

Keywords: Carbolfuchsin, Consortium, Decolorization, Methyl red, Textile effluent.

INTRODUCTION

Azo dyes are the most commonly used synthetic dyes in the textile, food, leather, cosmetic industries [1]. During dyeing process, 5-10% of dyes are released in textile wastewater streams which is ultimately reach to the receiving natural water bodies [2]. Synthetic dyes are made up of abundant class of organic compounds characterized by the presence of unsaturated groups such as –C=C- , -N=N-, -C=N- which are responsible for the dye color and fixation to fibers [3]. These dyes are extremely stable under exposure to light and washing. Thus they retain their color and structural integrity for a long time in the environment [4]. In addition, the strong color of discharge dyes even at vey small concentration has a huge impact on the aqauatic environment. It results in reduction in light penetration and photosynthesis thus release of such products has long lasting effect on the ecological balance of the system[5].

The concern and great attention for the treatment of industrial effluents from textile and manufacturing units is steadily given throughout India[6]. There are different physical, chemical and biological methods are used for the treatment of textile waste water, Such as adsorption, chemical precipitation, photolysis, chemical oxidation and reduction etc. (7). These methods are less efficient, costly, of limited applicability and produce wastes, which are difficult to dispose off. The treatment systems based on using microorganisms capable of degrading dyes have received increasing interest, Owing to their cost effectiveness, ability to produce less sludge and environment benignity [8]. It is now known that several microorganisms including fungi, bacteria, yeasts and algae can decolorize and even completely mineralize many azo dyes under certain environmental conditions. Bacteria cleave the azo bond by azoreductase enzyme, results in decolorization of dye [9].

The present study on the decolorization of MR and CF dyes by the bacteria isolated from local industrial waste. It can be useful in providing an alternative method to accomplish dye degradation of a wide range of dyes in an ecofriendly manner.

MATERIALS AND METHODS

2.1 Sample collection

The untreated textile effluent samples were collected from small dyeing industries which are located in Kalyan-Dombivali MIDC area, Dist.: Thane. The samples were collected in sterile plastic bottles, transported to the laboratory within 1 h and stored at 4°C.

2.2 Chemicals and media

Textile dyes MR,CF, microbiological media and medium ingredients were purchased from Himedia laboratories, Mumbai (MH, India).

2.3 Enrichment of sample

The collected samples were enriched by inoculating 5 ml of sample into 100ml of Nutrient broth with 0.002 gm/L MR and CF. The flasks were incubated at R.T. for 48 h under shaking conditions (140 rpm).

2.4 Isolation of dye decolorizing bacteria

After 48h of incubation, 1 ml of culture broth was diluted and plated on nutrient agar plates. Plates were incubated at 37°C for 24 h. The morphologically distinct bacterial isolates showing clear zone around their colonies due to decolorization of dye were selected for further studies. The pure cultures were stored below 10°C on nutrient agar slopes.

2.5 Dye Decolorization experiment

Decolorization study of MR and CF was carried out by using 0.002 gm/L of dye in 250ml Erlenmeyer flask containing 100 ml of Nutrient broth. 3 ml of 24 h old culture of isolated bacteria (O.D. 0.6 at 600 nm) was inoculated in medium. All the studies were carried at 37°C with pH 7.

After complete decolorization of dyes, 10 ml of aliquot of the culture broth was collected and centrifuged at 5000 rpm for 15 min. The supernatant was removed and analyzed Spectrophotometrically at wavelength 410 nm and 510 nm for MR and CF respectively. The uninoculated medium with MR and CF dye was served as blank. Percentage of decolorization was calculated by using the formula reported earlier [10].

$$\% \text{ Decolorization} = \frac{(\text{Initial OD} - \text{Final OD})}{\text{Initial OD}} \times 100$$

2.6 Identification of bacterial isolates

The isolates were identified by Gram staining, morphological and biochemical tests using the taxonomic scheme of Bergey's manual of determinative Bacteriology.

2.7 Effect of microbial consortium on dye degradation

Three selected culture as consortium was tested for their ability to decolorize MR and CF and its percentage decolorization was measured Spectrophotometrically.

2.8FT-IR analysis

Decolorized culture medium by microbial consortium was centrifuged at 7000 rpm for 20 min. the metabolites present in the culture supernatant were extracted using equal vol. of Ethyl acetate. The FT-IR analysis of metabolites was carried out using JASCO FT/IR 4100.

1 µl of sample was run using KBr pellets. The spectra collected within a scanning range of 400-4000cm^{-1}.

3. Results and discussion

3.1 Isolation of dye degrading bacterial strains

A total of 6 isolates showing clear zone around their colony were isolated from collected textile effluent samples. All the six isolates show distinct morphological characteristics. The isolates were labeled as Ia, Ib, Ic, Id, Ie and If and stored at 4°C on Nutrient agar slopes. These bacteria were isolated, since it has adaptability towards the dye as they exist in the stress.

3.2 Dye decolorization experiment

The selected isolates were tested separately to decolorize both azo dyes. The percentage of degradation was noted in table no. 1. The results suggest that, all the bacterial strain was able to decolorize both the dyes. Ia, Id and If isolates showed decolorization of both dyes (90-100%) and thus selected for further studies.

Table 1: MR and CF decolorization activity of isolated bacteria (%)

Sr.no.	Isolates	% of MR decolorization	Time of decolorization (h)	% of CF decolorization	Time of decolorization (h)
1	Ia	92	24	90	72
2	Ib	86	48	71	96
3	Ic	95	24	85	72
4	Id	98	24	91	48
5	Ie	98	24	79	96
6	If	99	24	90	72

Moreover, it was observed that MR dye decolorization percentage and time require for decolorization was less than that of CF decolorization. After centrifugation all bacterial strains pellets retained its original color and was not deeply colored because of adsorbed dyes. This indicates that, color removal was due to degradation and not by adsorption. The isolates decolorize the dye substrate and decolorizing efficiency was dependent on growth of the isolate in the flask. There was neither growth nor decolorization in the control flasks was observed. This showed that the decolorization was due to the metabolic activity of the organism.

3.3 Identification and Effect of microbial consortium on decolorization

The selected pure bacterial isolates Ia, Id and If were identified as *Proteus spp., Pseudomonas spp.* and *Acinetobacter spp*. respectively.

Along with pure cultures isolates, consortium of all the isolates was tested for their ability to decolorize MR and CF. Consortium showed 100% and 96% decolorization of MR and CF respectively. Mix consortia of the cultures showed maximum decolorization percentage within minimum time (24h) compared to individual colonies. Higher degree of biodegradation and mineralization can be expected when co metabolic activity within a microbial community complement each other. One organism may be able to cause a biotransformation of the dye which consequently render it more accessible to another organism that otherwise is unable to attack the dye.

3.4Infrared spectrum analysis

FT-IR spectrum of Fig.1 and 2 displayed peaks of control dye and their degraded metabolites test

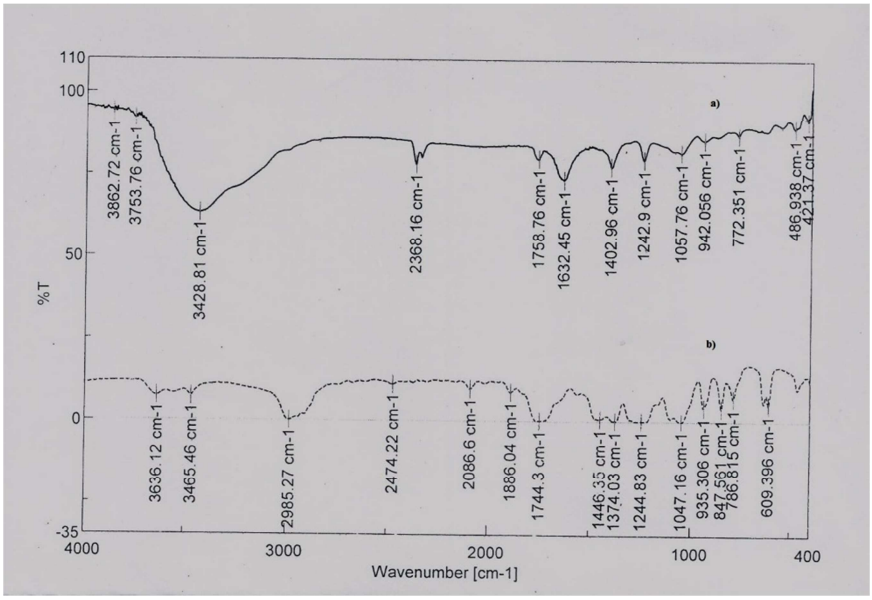

Fig. 1: FT-IR spectrum a) Control MR b) Treated MR

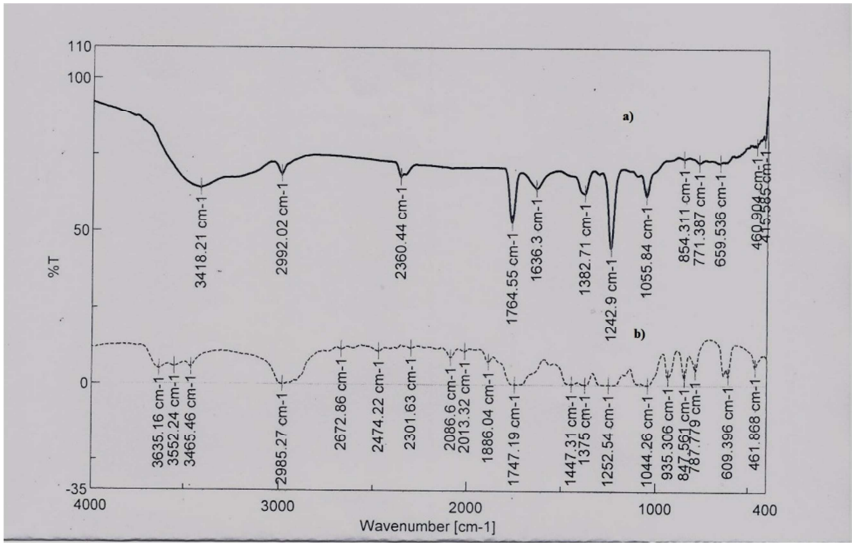

Fig. 2: FT-IR spectrum a) Control CF b) Treated CF

The IR spectrum for MR shows that the vibrations between 3700-3100 cm^{-1} are associated with the –OH (carboxylic acids), -NH- (Amine) is almost changed after degradation by consortia as shown in Fig 1a. The strong absorption at 1632$cm^{-1}$and 1402 cm^{-1} is because of presence of Azo bond (-N=N-). Absence of these peaks after decolorization indicates cleavage of azo bond. In IR spectra of treated sample (Fig.1b), new peaks at 2985cm^{-1}, 1374cm^{-1}, 847cm^{-1} and 609 cm^{-1} were observed which indicates that there may be formation of new compounds originated from the fragmentation of parent MR molecule.

The control IR spectrum of CF shows the following observation. The peaks in between 3700-3100 cm^{-1}shows the presence of –OH,-NH-, =C-H (amides and amines).The peaks in between 2700-2000cm^{-1} responsible of Nitriles, azide compounds in the sample and 800-400cm^{-1} peaks are associated with Aromatic compounds in the sample(Fig. 2a). The IR spectra obtained from treated sample shows the several variations in the region at 3700-3100 cm^{-1}, 2700-2000cm^{-1} and 800-400cm^{-1}as it is compared with control IR spectra of CF (Fig.2b). It may be the because of drastic destruction of CF by microbial consortium.

CONCLUSION

The present concludes that the collected effluent samples were good source of dye degrading bacteria. All isolated bacteria can able to degrade MR and CF dyes. Out of them the three bacterial isolates, *Proteus spp., Pseudomonas spp.* and *Acinetobacter spp.*were shows maximum dye decolorization. Both the dyes tested were decolorized by selected isolates with some difference in decolorization time depending on the dye structure. The use of microbial consortium was very efficient than individual culture in dye decolorization process. The complete degradation of dye could be achieved during study which was confirmed by FT-IR analysis. This suggests that microbial consortium of isolates may have an efficient enzymatic system for the cleavage of parent dye. It could successfully be employed in the treatment of textile effluent. However, further work is needed to identify the genes responsible for this kind of textile azo dyes decolorization.

Acknowledgement
I am grateful to my PHD guide Dr. Prandya Joshi, Asst. Professor and HEAD, Dept. microbiology, Birla College Kalyan, Mumbai University for her guidance and support during the project. My heartfelt thanks to Dr. SandeshJaybhaye, Asst. Professor, and Dept. of chemistry, for providing me an opportunity to do this project work in his department.

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