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Decolorization of Sulphonated Azodye Metanil Yellow by Newly Isolated Bacterial Strain: *Bacillus* -3330

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Newly isolated *Bacillus sp.* MTCC-3330 was screened for the decolorization of a sulphonated azodye metanil yellow under aerobic conditions. 100% decolorization was observed of Metanil Yellow at 0.01mM, 0.02mM 0.05mM concentrations within 30h, 35h and 45h respectively. Decolorization was confirmed by UV-VIS spectrophotometer. The initial dye solution showed highest peak at the wavelength of 437 nm (λmax of the Metanil Yellow). The decolorized dye showed disappearance of peak, which indicated that the decolorization is due to dye degradation. The bacterium sp. also decolorized the another azodye Methyl Orange.

Key words : *Azodye, Bacillus sp., Metanil Yellow, decolorization, toxicity, biosorption*

ntroduction

In textile industries, during dye processing, upto 10-5% of total textile dyes remain untreated and directly lost nto the effluents[1, 2], which is a complex mixture of substance onsisting of textile dyes and heavy metals associated with lye and some other auxiliaries used during dyeing process. These effluents are highly alkaline, have a fairly high tempera-ure, suspected to be carcinogenic and can produce toxic com-ounds[3, 4] which create lot of environmental problems[5-8]. Azo yes constitute the largest and most important class of syn-hetic dyes[9], are characterized by the presence of one or more zo bonds (-N=N-), which are responsible for their coloration. : is reported that effluents which are a major source of water ollution have been produced by more than 10000, structur-lly different azodyes currently used [10, 11] in textile, paper and :ather industries. These dyes are poorly biodegradable. A umber of physical and chemical methods[12, 13] are being used or colour removal from wastewater, but these processes have igh operational costs and limited applicability[14].However, bio-egradation of azo dyes[15-17] are environment friendly and cost ffective.

Metanil Yellow (monosodium salt of 4-m-ılphophenylazodiphenyl-amine) is an acidic azodye (**Fig.1**). has been used extensively in dyeing of textiles and leathers ıd discharged as industrial effluents[16]. It is also used as ɔloring foods and in many other industries such as soap, ɔsmetics, and spirit lacquer and shoe polishes [18]. Goel and ɔ-workers[19] have reported the toxicity of Metanil Yellow to esh water fish channa punctuates. Metanil Yellow exerts :leterious effect on gastric mucin[20] and causes arrest of ıermatogenesis in rats [20-22] mice[23], guinea pigs [24]. Metanil ellow has also tumor producing effects [25] and on oral consumption it can cause toxic methaemoglobinaemia[26] and cyanosis [27]. Thus the effluents bearing azodye Metanil Yellow must be treated before releasing into the natural environment. Keeping this in view, removal of Metanil Yellow from wastewater has been attempted by various researchers[13, 16, 28-30]. The application of microorganisms for the biodegradation of synthetic dyes is an attractive and simple method. Unfortunately, the majority of dyes are chemically stable and resistant to microbiological attack. The isolation of new strains or the adaptation of existing ones to the decomposition of dyes will probably increase the efficacy of microbiological degradation of dyes in the near future. This paper reports complete decolorization of Metanil Yellow by a newly isolated bacterial strain, of Bacillus sp. which has been deposited at the Microbial Type Culture Collection & Gene Bank (MTCC) Centre, Institute of Microbial Technology, Chandigarh, (Punjab) India.

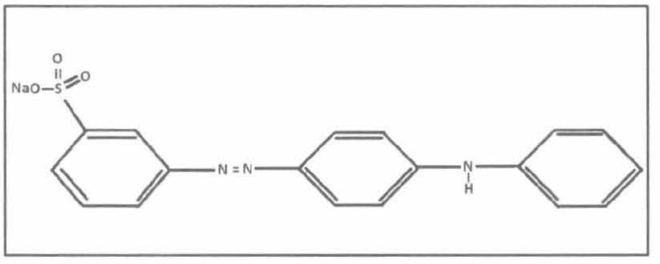

Fig. 1 : Structure of Metanil Yellow

Materials and methods

Beef extract (bacteriological) and peptone (bacteriological) were obtained from BDH and Qualigens Chemical India Pvt. Ltd. Mumbai. Metanil Yellow dye was obtained from Ranbaxy Laboratories India Pvt. Ltd. All other

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chemicals and reagents used in this research were obtained from either CDH (Mumbai) or from Loba Chemie (New Delhi).

The decolorization of Metanil Yellow by the *Bacillus sp.* was studied in sterilized liquid culture medium containing 0.5g peptone, 0.3g beef extract and 0.5g NaCl to 100 mL water in 250 mL culture flasks in which concentrations of Metanil Yellow were varied (0.02 to 1.0 mM). 1 mL of bacterial suspension optical density (O.D.) 1.55 at λ=540 nm was aseptically inoculated in each culture flask. The controlled culture flasks were not inoculated with bacterial suspensions. Triplicates of culture flasks were set for each concentration of Metanil Yellow and culture flasks were incubated in an orbital shaker maintained at 30°C and 130 rpm. The bacterial suspension was prepared by transferring bacterium from agar slants to the liquid culture medium mentioned above and growing the culture under similar conditions till O.D. at λ=540 nm reaches a value of 1.55.

In order to study the removal of Metanil Yellow from the culture medium, an aliquot of 2 mL was withdrawn at different time intervals, bacterium was removed by centrifugation using Sigma (Germany) refrigerated centrifuge model 3K-30 for 20 minutes at 4°C and 4000×g (~ 16000 rpm) and absorbance of the supernatant solution was determined at λ=437 nm (λ_{max} for Metanil Yellow) using UV/VIS spectrophotometer Hitachi (Japan) model U-2000. In cases where initial O.D. was very high, the supernatant solution was appropriately diluted, in order to bring concentrations in the range that follow Lambert-Beer's law. Metanil Yellow obeys Lambert-Beer's law, till 0.06 mM concentrations and corresponding O.D. value is 1.10. The absorbance verses time curves were drawn to show the removal of Metanil Yellow from the culture medium.

Decolorization activity was calculated as follows:

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance-Observed absorbance}}{\text{Initial absorbance}}$$

In order to confirm Metanil Yellow has been completely removed from the culture medium, the UV/VIS spectra of the supernatant solutions prepared by withdrawing culture media at the start and at the end of decolorization of Metanil Yellow were scanned in the range of 190-800 nm. Supernatant solution of culture medium with the bacterium alone was analyzed UV/VIS spectrophotometer. In order to observe correlation between growth of bacterium and decolorization of Metanil Yellow, the growth of the bacterium was followed at 540 nm and decolorization of Metanil Yellow was followed at 437 nm by withdrawing aliquot from culture medium containing 0.05 mM concentration of Metanil Yellow, at regular time interval and removing the bacterium by centrifugation using Sigma (Germany) refrigerated centrifuge model 3K-30 for 20 minutes at 4°C and 4000×g (~ 16000 rpm) In order to observe decolorization of other azodyes by *Bacillus-3330*, same procedures were used, as used for the decolorization of Metanil Yellow. The different morphological physiological and biochemical tests on the isolated bacterium were done at MTCC Centre, Institute of Microbiology Technology, Chandigarh, India, utilizing their services.

Results and discussion

Under morphological examination the bacterial colon has been found to be of round configuration, wavy margi and convex surface. The bacterial strain is Gram positive, ro shaped and gives positive endo-spore test. The bacterial strai grows between 22 °C to 55 °C in the pH range 5.0 -9.0 and ca tolerate NaCl up to 5.0%. Under bio-chemical tests, th bacterial strain gives positive results of starch hydrolysi ortho- nitrophenyl-â-galactoside (ONPG) hydrolysis, Nitra reduction, Cytochrome oxidase test, Catalase test and Gelati liquefaction.

Fig.2. illustrates the decolorization of Metanil Yello in liquid culture medium at varying concentration (a) 0.01 mM (b) 0.02 Mm, (c) 0.05 mM (d) 1.0 mM and (e) 0.05 ml

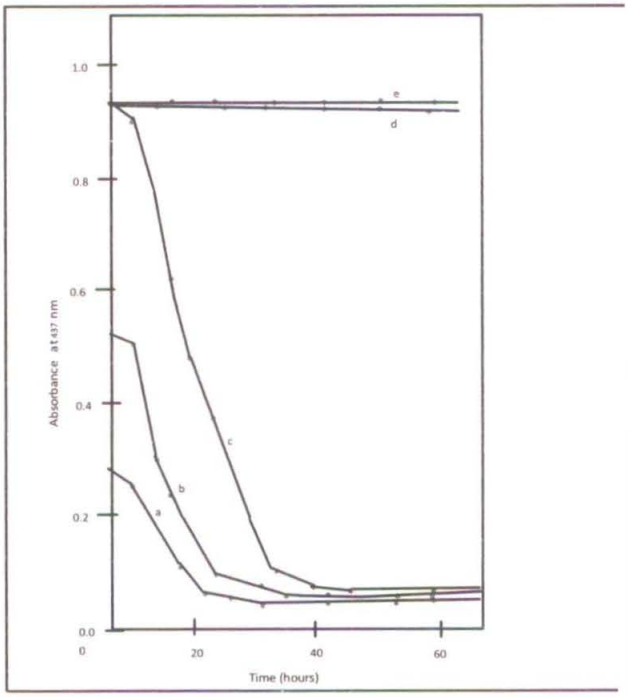

Fig.2 : Variation of absorbance at 437nm for supernata solutions containing Metanil Yellow of differe concentrations : (a) 0.01 mM, (b) 0.02 mM, (c) 0.05 mM, 1.0 mM, (e) 0.05 mM (control) (In case of (d) 20 fold diluti of the supernatants were used)

(controlled). As can be seen 100% decolorization of Metanil Yellow was observed at 0.01 mM, 0.02 mM and 0.05 mM concentrations within 30 h, 35 h and 45 h respectively. It has been shown that beyond 0.05 mM concentration of dye reduced the decolorization efficiency of bacterial sp. and 1.0 mM concentration of Metanil Yellow completely inhibits the growth of the bacterium. These results indicate toxicity of Metanil Yellow at higher dye concentration.

The UV/VIS spectrum of supernatant corresponding to just after inoculation of the bacterium in culture medium containing 0.05 mM of Metanil Yellow [**Fig.3 (a)**], after 45 h growth of the bacterium [**Fig.3 (b)**] and controlled experiment in which no bacterium has been inoculated [**Fig.3 (c)**] whereas [**Fig.3 (d)**] corresponds the UV/VIS spectra of supernatant of the culture medium in which no Metanil Yellow has been added but the bacterium was grown for 45 h and [**Fig. 3(e)**] shows the UV/VIS spectrum of supernatant of only growth medium, containing no Metanil Yellow and no bacterium. The absorbance was analyzed from 200 nm - 800 nm. The initial dye (just after inoculation) showed high peak at wavelength 437 nm (λmax of the Metanil Yellow) whereas after decolorization, there is no peak observed at 437 nm [**Fig.3 (b)**]. The comparison study of spectra [**Fig.3 (b)**], [**Fig.3 (d)**] and [**Fig.3 (e)**] also indicates completely disappearance of Metanil Yellow from culture medium. **Fig.4**. shows the correlation between growth of bacterium and decolorization of Metanil Yellow which indicates that as bacterium is growing the absorbance of dye decreases and on reaching maximum growth of bacterium, Metanil Yellow completely disappears from culture medium (absorbance of dye becomes nearly zero). It

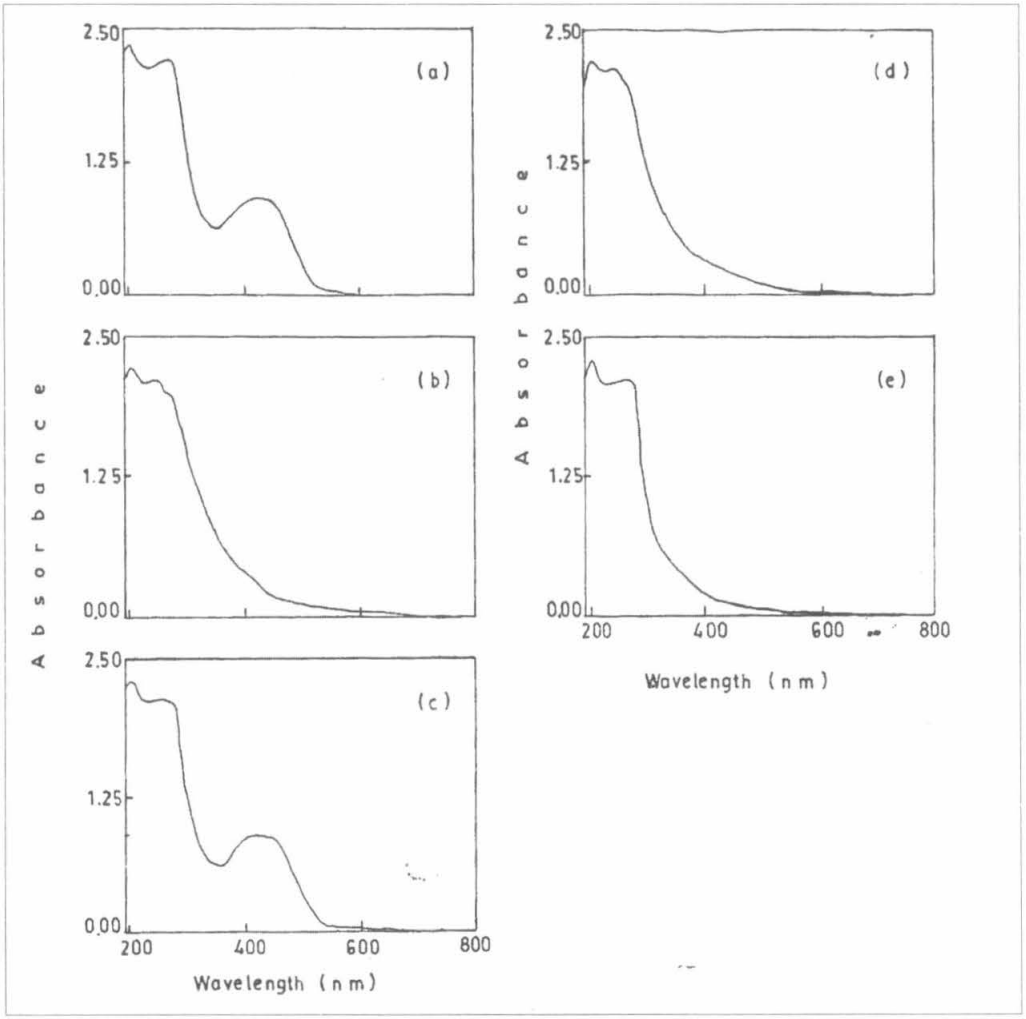

Fig. 3 : UV/VIS spectrum of supernatant solutions prepared by withdrawing aliquots from the culture medium containing Metanil Yellow at different time interval : (a) Just after inoculation of the bacterium; (b) After 45 hrs of inoculation when Metanil Yellow was completely decolorized ; (c) Control experiment ; (d) After 45 hrs of the growth of the bacterium containing no Metanil Yellow ; (e) Growth medium containing no Metanil Yellow and no bacterium.

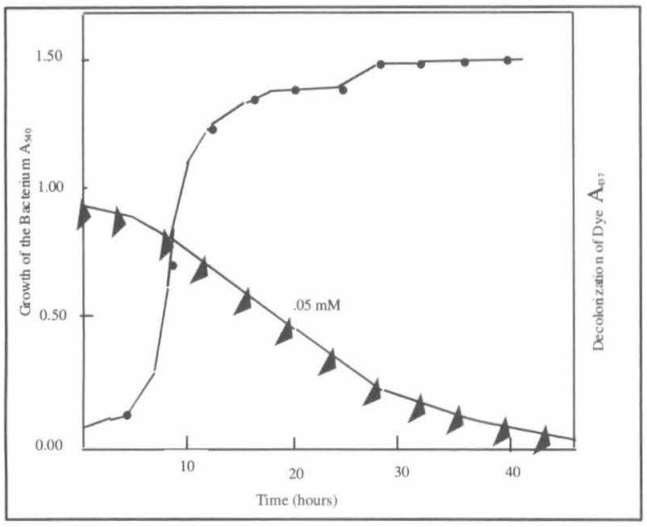

Fig. 4: Correlation between the growth of *Bacillus sp. and* decolorization of Metanil Yellow. Growth of the bacterium (●) Decolorization of Metanil Yellow (▲)

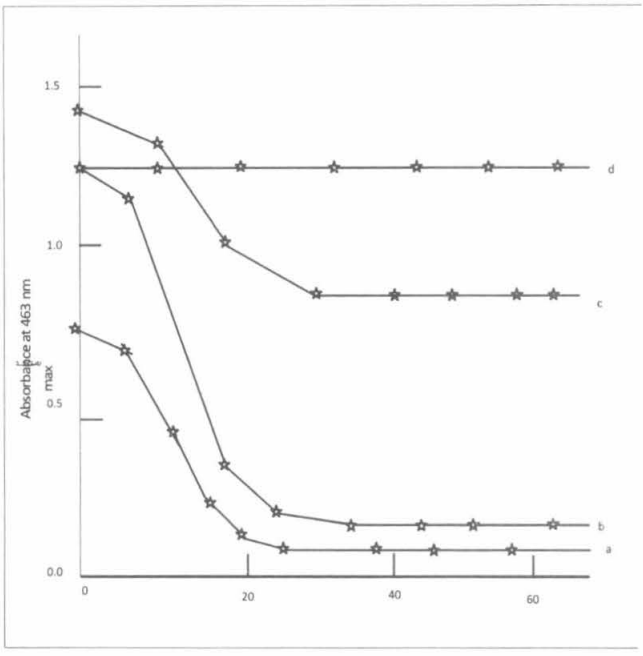

Fig. 5 : Variation of absorbance at 463 nm (λmax for Methy Orange) of supernatant solutions containing differen concentrations of Methyl Orange : (a) 0.02 mM; (b) 0.0 mM; (c) 1.0 mM; (d) 0.04 mM (control). In case of (c) 20 fol dilution of the supernatants were used.

reveals that Metanil Yellow is used as carbon and energy source for the growth of the bacterium and some enzyme(s) produced during the growth of the bacterium which is responsible for the decolorization of dye. These results are supporting that decolorization is most probably due to biosorption[31] of dye in which aromatic ring of dye can take as sole carbon and energy source[32]. Biosorption is a process that utilizes biological materials as adsorbent, and this method has been studied by several researchers as an alternative technique to conventional methods for removal of toxic material from wastewater [33, 34]. Decolorizations of some other azodyes (Methyl Orange and Congo Red) were tested with *Bacillus-3330*. **Fig.5** shows the decolorization of Methyl Orange in liquid culture medium at varying concentrations : (a) 0.02 mM, (b) 0.04 mM, (c) 1.0 Mm and (d) 0.04 mM (contolled). The observations indicate that lower concentration of Methyl Orange (0.02 mM and 0.04 mM) is completely removed but higher concentration (1.0 mM) is not completely removed from the culture medium by the *Bacillus -3330*. No decolorization was observed in case of Congo Red which reveals that it behaves as recalcitrant to aerobic degradation. Similar observation has been reported that most azodyes are recalcitrant to aerobic degradation by bacterial cell [35].

Various anaerobic bacteria have been reported [36] to degrade azodyes. However under aerobic conditions these dyes have been considered as non-biodegradable[37]. *Bacillus sp.* degrades Metanil Yellow under aerobic condition which is an interesting finding and can be used for bioremediation of sites polluted by Metanil Yellow under aerobic conditions which are easier to achieve than the anaerobic condition which require exclusion of oxygen from the growth mediun and are difficult to maintain.

Conclusion

The present study confirms the ability of isolate bacterium sp., *Balcillus-3330* to decolorize the textile as wel as leather dye, Metanil Yellow up to 0.05 mM concentratio with decolorization efficiency of 100%, from the culture mediun The bacterial strain has also decolorized other azodye Methy Orange. This bacterial strain grows in between temperatur 22°C to 55°C and with pH 5.0 to 9.0. Thus, *Bacillus-3330* ca be used fairly at high temperature to decolorize wastewate having either acidic or basic nature, both, polluted wit selective azodyes.

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