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Bioremoval of an Industrial Acidic Azo Dye by a Bacterial Strain *Escherichia Coli -K-10*

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Abstract

The removal of dyes from textile waste water by bacterium is one of the environmental friendly method. The decolorization of azodye metanil yellow studied at various concentration by a bacterial sp. *Escherichia coli-K-10* (*E.coli K-10*). The 0.01 mM, 0.02 mM and 0.05 mM concentration of the dyes was 100% decolorized within 40 hrs, 46 hrs and 52 hrs respectively. Decolorization of dyes was monitored by UV/VIS spectrophotometer. Initially, a highest peak of the dye solution was detected at 437nm (λmax of the metanil yellow). The peak disappears from the decolorized solution, indicating that the decolorization of the dye is due to dye degradation. This bacterial sp. has also been used for the decolorization of other azodyes, methyl orange and congo red. The 0.02 mM & 0.04 mM methyl orange became 100% decolorized during 44 hours but the congo red did not.

Keywords: Bioremoval; Metanil yellow; Dye effluents; *E.coli K- 10.*

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Introduction

Azo dyes produces the largest and most important class of synthetic dyes that contain one or more azo bonds (-N=N-). Due to presence of azo bonds, the substance produces colour. Metanil yellow is a monoazoic dye with yellow colour. It is synthesized by the reaction of metanilic acid and diphenyl amine. The chemical formula of metanil yellow is C18H15N3NaO3S. In most of the cases in order to attract people by increasing the colour of prepared food, added excessive quantity of colouring material and ignoring the safe limit of the food colour for use, which became harmful for the people. It has very important use in leather1 textile and paper industries.[2] It is also used in other industries like soap, cosmetics, sprit liaquer, shoe polishes[3] and in the food items.[4] The unused dye flows into the environment as industrial effluents.[5] Studies revealed that the dye has a toxic effect on various physiological systems. Metanil yellow is get absorbed from the intestine through food and enters in the blood and in various organs and interferes, the various cellular metabolic processes. The maximum limit of metanil yellow permissible color to be added in any food shall be 100 mg Kg^{-1} or liter-1 of food consumed.[6] Majority of synthetic food colors are highly toxic synthetic chemicals and banned by the Central and State administration. Metanil yellow is banned dye, as per PFA Act (1954) , by Government of India, because it has been found carcinogenic and tumor producing effects.[7]

Therefore, it is essential to treat the effluents bearing azo dye, before releasing into the environment. Photocatalitic degradation of various industrial dyes were studied in the presence of catalytic substances.[8-11] Biodegradation studies of metanil yellow was also reported by bacterial mixed culture.[12] The remediation of synthetic

dyes from the dye waste waters by the use of microorganisms is simple and low cost method. There are various physical and chemical methods[13] for color removal from waste water, but these processes have high operational costs and limited applicability. This research manuscript reports complete decolorization of metanil yellow by *E.coli-K-10.*

Material and Methods

Metanil yellow dye obtained from Ranboxy Laboratories India Pvt. Ltd., beef extract (bacteriological) and peptone (bacteriological) were obtained from BDH and qualigens chemical India Pvt. Ltd. All other chemical and reagents were obtained from CDH (Mumbai) or from Loba Chemie (New Delhi). The *E.coli Hfrc K-10,* a derivative of Strain K-12 was gifted by R.A. Cooper, Department of Biochemistry , University of Leicester, Leicester, LE/7RH, U.K and was maintained in nutrient agar.

Decolorization study of metanil yellow by E.coli-K-10 was done in sterilized liquid culture medium containg 0.5 g pepton, 0.3 g beef extract and 0.5g NaCl in 100 ml water in 250 ml culture flasks. The concentration of metanil yellow were varied (0.02 mM to 0.05mM) in culture flasks. 1 ml of bacterial suspensions O.D.= 1.55 at λ =540 nm was aseptically inoculated in each culture flasks. 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 degree centigrate and 130 rpm. The bacterial suspension was prepared by transferring bacterium from agar slants above and growing the culture under similar conditions till O.D 540 reaches a value of 1.55.

To study the removal of metanil yellow from the liquid culture medium, 2 ml sample was taken out from the culture medium and centrifuged on 4000 gravity for 20 minutes with the help of a centrifuge [Sigma (Germany), modal 3K-30] at different time intervals. The absorbance measurements of the supernatant solution was carried out at λ =437nm with the help of UV/VIS spectrophotometer Hitachi (Japan) model U-2000. The absorbance vs time curves showed that metanil yellow removed from the culture medium.

Decolorization activity was calculated as follows:

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

In order to confirm metanil yellow has been completely removed from the culture medium, the UV/VIS spectras of the supernatant solutions prepared by withdrawing culture medium at the start and at the end of decolorization of metanil yellow were scanned in the range of 190-800 nm. UV/VIS spectrum of the supernatant solution of the culture medium in which no bacterium was inoculated also scanned.

For correlating the growth of the bacterium and decolorization of metail yellow, two parallel set of experiments were carried out, one only for the growth of the bacterium monitored at λ =540

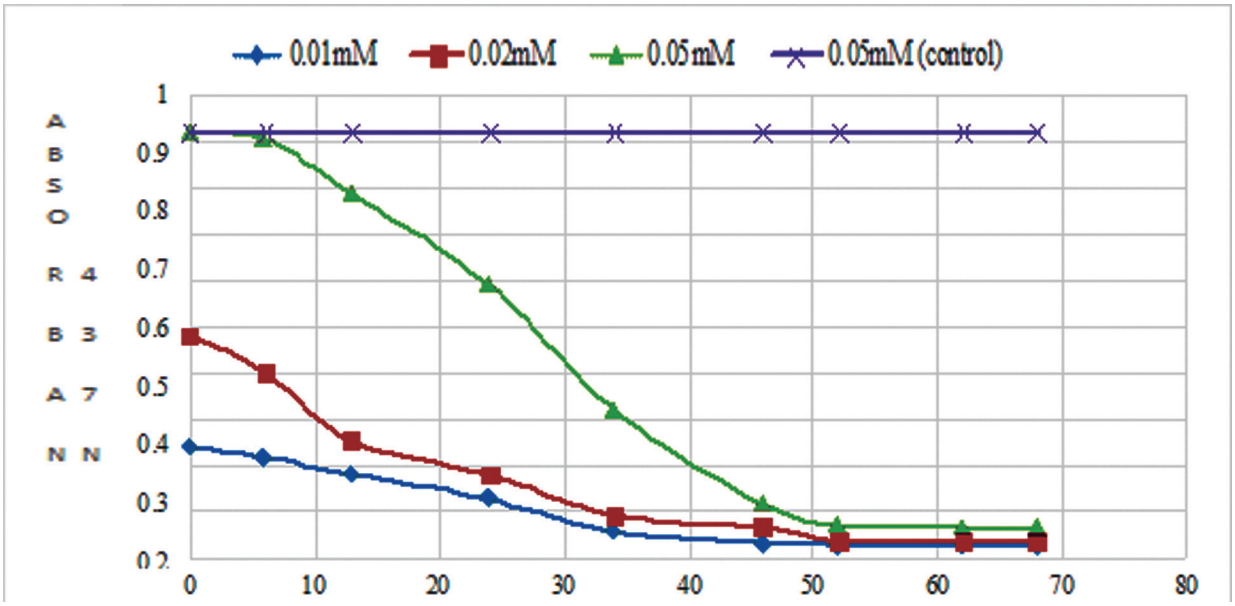

Fig. 1: Variation of absorbance at 437nm of supernatant solution prepared by withdrawing aliquots at different time intervals from the growth medium containing metanil yellow of different concentrations.

nm and the other one for the decolorization of dyes monitored at λ =437 nm by withdrawing an aliquot from the culture medium containing 0.05 mM concentration of dye and bacterium were removed by centrifugation using Sigma (Germany) refrigerated centrifuge model 3K-30 for 20 minutes at 4 degree centigrate at 4000g (~16000rpm).

Result and Discussion

Fig. 1 illustrates the decolorization of metanil yellow in culture medium at varying concentration (a) 0.01mM (b) 0.02mM (c) 0.05mM (d) 0.05mM (controlled). From the fig-1, it is obvious that 100% decolorization of metanil yellow was observed at 0.01mM, 0.02mM and 0.05mM concentrations within 40 hours, 46 hours and 52 hours respectively . It has been shown that beyond 0.05mM concentration of dye, reduced the decolorization effeiciency of bacterial species and 1.0 mM concentration of metanil yellow completely inhibits the growth of the bacteria. These results indicate toxicity of metanil yellow at higher dye concentration.

Fig. 2: Shows the UV/VIS spectrum of supernatant solution corresponding to just after inoculation of the bacterium in culture medium containing 0.05mM of metanil yellow [Fig 2(a)], after 52 hours growth of the bacterium [Fig 2(b)] and controlled experiment in which no bacterium has been innoculated [Fig 2(c)].

The absorbance was analysed in the range of 200 nm to 800 nm. The initial dye (just after innoculation) showed high peak at wave length 437nm (λmax of metanil yellow), whereas after decolorization, there is no peak observed at 437nm [Fig 2(b)]. The comparsion study of spectra [Fig 2(b)], and [Fig 2(c)] , indicates completely disappearance of metanil yellow from culture medium.

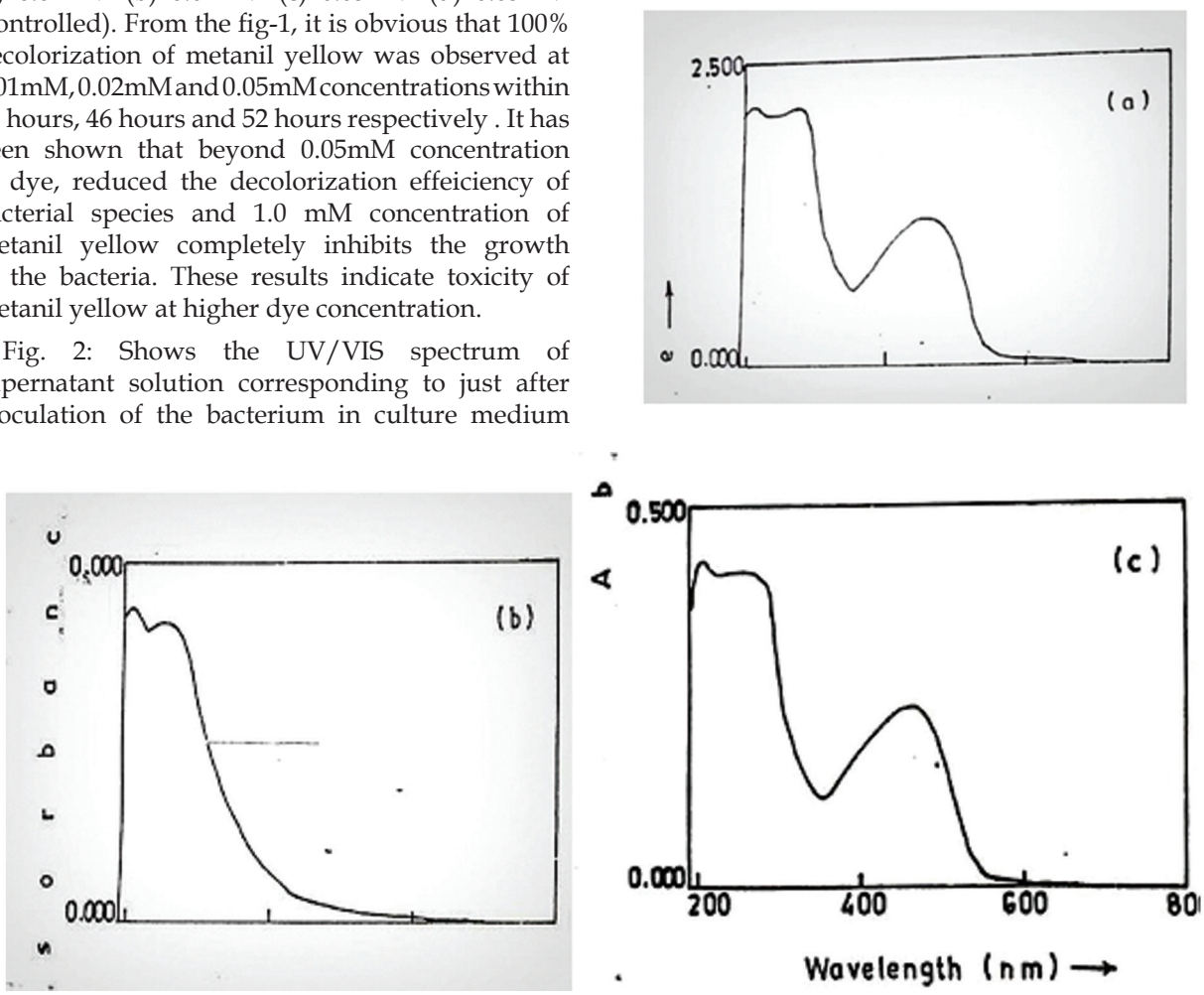

Fig. 2: UV/VIS spectrum of supernatant solutions prepared by withdrawing aliquots from the culture medium containing metanil yellow at different time intervals.

(a) Just after inoculation of the bacterium.
(b) After 52 hours of inoculation when metanil yellow was completely decolorized.
(c) Control experiment

Fig. 3 shows the correlation between growth of the bacterium and decolorization of metanil yellow which indicates that as bacterium is growing, the absorbance of dye decreases and on reaching maximum growth of the bacterium, metanil yellow completely disappears from culture medium.

It indicates that metanil yellow is used as carbon and energy source[14] for the growth of the bacterium.

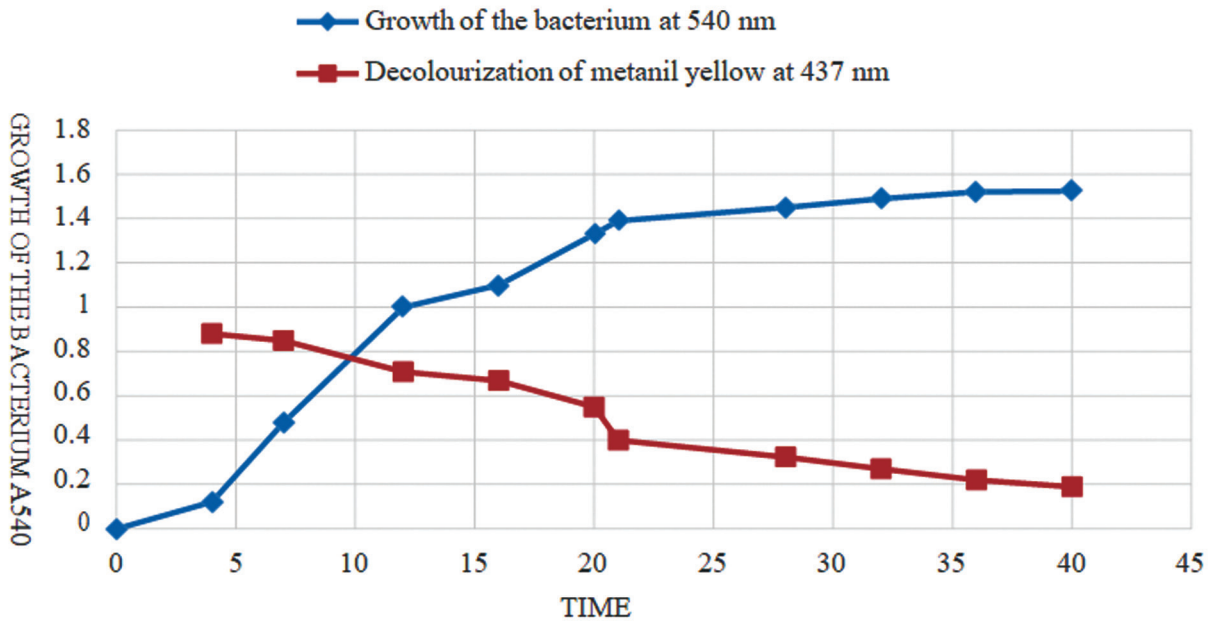

Fig. 3: Correlation between the growth of *E. coli K-10* and decolorization of metanil yellow.

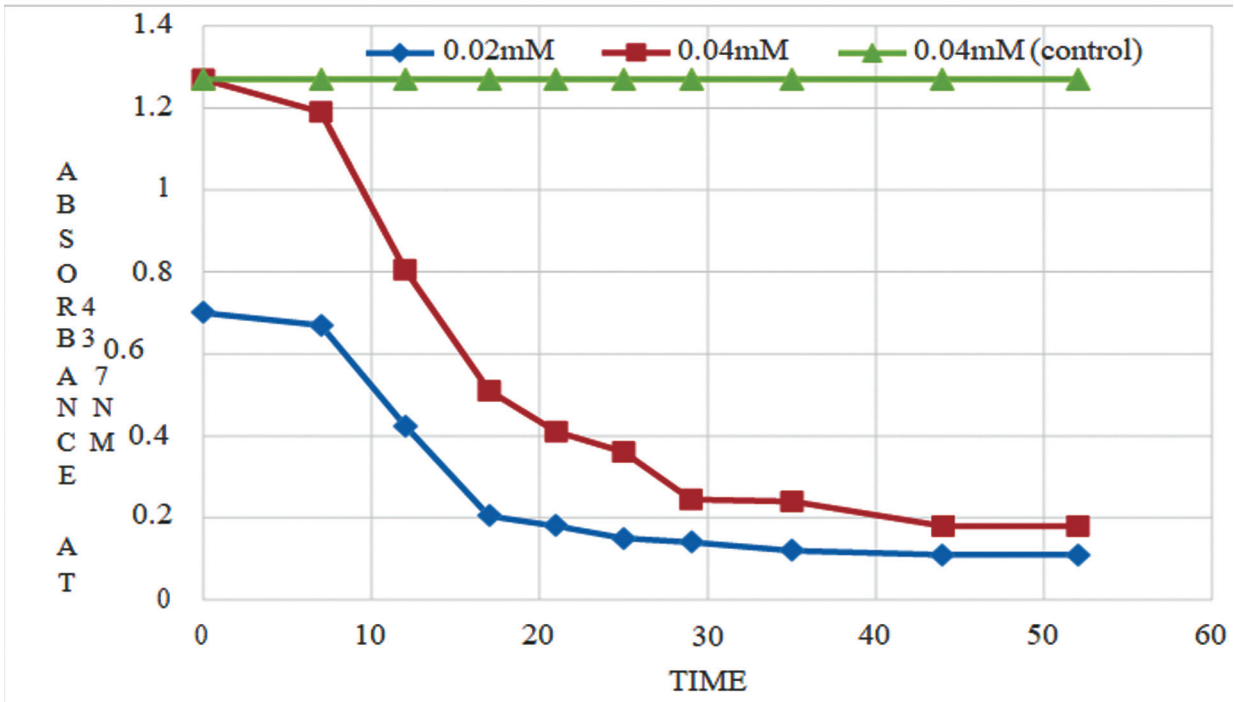

Fig. 4: Variation of absorbance at 463nm of supernatant solutions prepared by withdrawing aliquots at different time intervals from the growth medium containing methyl orange of different concentration.

The decolorization of other acidic azo dyes, methyl orange and congo red were also tested by the reported bacterial strain *E.coli K-10.*

It has been found that 0.02 mM and 0.04 mM, methyl orange is completely decolorized within 44 hours (Fig. 4) , but congo red was not decolorized by this reported strain. It indicate that congo red behave as recalcitrant to aerobic degradation.

Thus *E.coli* species degrades metanil yellow under aerobic conditions 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 conditions.

Conclusion

The study show the ability of *E. coli K-10* to decolorize the textile dyes metanil yellow up to 0.05 mM concentration with 100% effeiciency in culture medium. It is obvious that these bacterial species can be used for bioremediation of sites polluted by metanil yellow under aerobic conditions. This bacterial strain also decolorizes the other azo dyes, methyl orange.

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