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Original Research Paper

# Biotransformation of Yellow 4G and Orange 2R Textile Dyes by Acclimatized Aerobic Bacteria

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**Key Words:**
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## ABSTRACT

Degradation of the textile dyes viz., Yellow 4G and Orange 2R was carried out using acclimatized soil bacterial isolates. The microbial flora from soil was acclimatized to the dyes and six promising organisms were isolated, which could decolorize 1000 µg/mL of dyes to more than 91.00 % in nutrient medium in less than 24 hours. The percent decolorization of the dyes was determined by spectrophotometry. The six isolates reduced COD more than 75%. The degradation products formed after degradation were analysed by GC-MS technique and it was found that these cultures together degraded Yellow 4G and Orange 2R to the products having molecular weights 149, 65, 60, 57, 43, 41 and 271, 159, 145, 107, 102, 91, 81, 61, 55, 43 and 41 respectively.

## INTRODUCTION

Textile dyes constitute a major class of environmental pollutants. These dyes may form aromatic amines which are recognized as possible human carcinogens (Banat et al. 1996, Weisburger 2002), so their degradation is the only solution for safe disposal. Various physicochemical methods such as adsorption, coagulation, precipitation, filtration and oxidation have been used for treatment of dye-stuffs, but they have many limitations like cost effectiveness, residue (sludge) formation, etc., and hence it is important to develop a cost effective and efficient method for decolorization and degradation of these textile dyes. Biotransformation by microorganisms can be used for decolorization and degradation of dyes. Studies have been focused on the microorganisms that are able to degrade the dyes which have been proved to be the most effective and cost competitive alternative for the dye waste treatment (Casieri et al. 2008).

Aerobic treatment of the textile dyes has proven ineffective in most of the cases, but is often the typical method of treatment used today (Edward 2000, Yang et al. 1998). Aerobic microorganisms cannot reduce the linkage and their ability to destroy the chromogen is less than anaerobic microorganisms. However, aerobic sludge has been successfully used to stabilize the dye metabolites (Brown & Laboureur 1983) and also it does not generate any toxic by-products.

Present study involves, biotransformation of Yellow 4G and Orange 2R dyes by using acclimatized microorganisms isolated form soil. The ability of the isolates to degrade the dye was observed in nutrient medium. The decolorization observed was determined by spectrophotometer. Percent COD reduction after the decolorization was also determined. The degradation of dyes was determined by GC-MS analysis.

## MATERIALS AND METHODS

**Acclimatization:** Soil samples from area nearby waste disposal sites of textile industries, sewage,

Table 1: Molecular weight of the degraded products.

| Sr. No. | Dyes | Molecular weights of degradation products |
|---|---|---|
| 1. | Yellow 4G | 149, 65, 60, 57, 43, 41 |
| 2. | Orange 2R | 271, 159, 145, 107, 102, 91, 81, 61, 55, 43 and 41 |

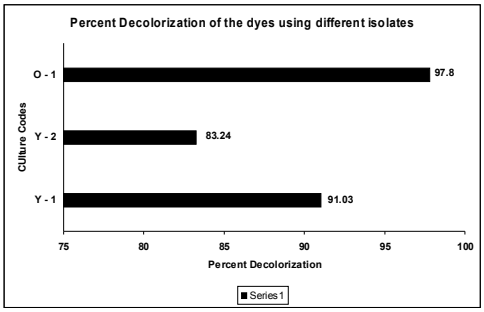


Fig. 1: Percent decolorization of the textile dyes by using different isolates.

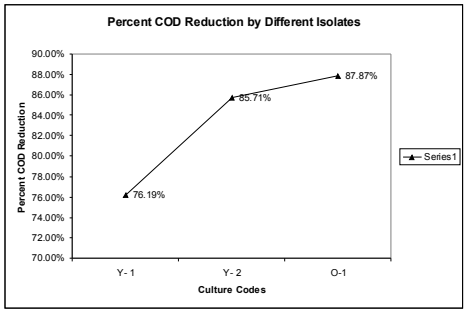


Fig. 2 Percent COD Reduction of the textile dyes by using different isolates.

sludge and ETP along with compost were collected and homogenized properly. The microflora from the samples was acclimatized in the dyes Yellow 4G and Orange 2R (1%) for the period of one month. One gram of acclimatized soil was inoculated in the nutrient broth and after incubation isolation was carried out on nutrient agar incorporated with the same dye concentration. The colonies showing good decolorization were selected for further studies.

**Decolorization of the dyes in the nutrient broth:** The cultures isolated from the soil were inoculated in the tubes containing nutrient broth and 1mL of 10,00 µg/mL of dye. All the tubes were inoculated for 24 hours at ambient temperature and the percent decolorization was determined by using spectrophotometer (Equiptronics Digital Spectrophotometer-EQ-822) at its specific absorbance maxima (λ max) *viz.* Yellow 4G-485nm and Orange 2R-484nm.

**Percent decolorization studies:** Decolorization studies were carried out to measure the percent decolorization shown by the promising isolates of dyes within 24 hrs. The decolorized samples were centrifuged at 10000 rpm for 10 minutes in a cooling centrifuge to separate cell mass. Percent decolorization of the dyes was calculated on spectrophotometer by using λmax of the respective dyes. The % decolorization was calculated using the following equation.

$$\text{Percent Decolorization} = \frac{\text{Final Transmittance - Initial Transmittance}}{\text{Initial Transmittance}} \times 100$$

**Percent COD reduction:** Percent COD reduction was determined by reflux method using $K_2Cr_2O_7$ as a strong oxidizing agent.

**GCMS analysis:** To study the products formed after degradation of dyes Yellow 4G and Orange 2R, the decolorized samples were analysed by GCMS.

The consortium of the isolates was inoculated in 100mL of sterile nutrient broth containing 1000 µg/mL of each dyes *viz.* Yellow 4G and Orange 2R. The broth was then incubated at ambient temperature for 24 hrs in separate flasks. The decolorized broth was then centrifuged at 10,000 rpm for

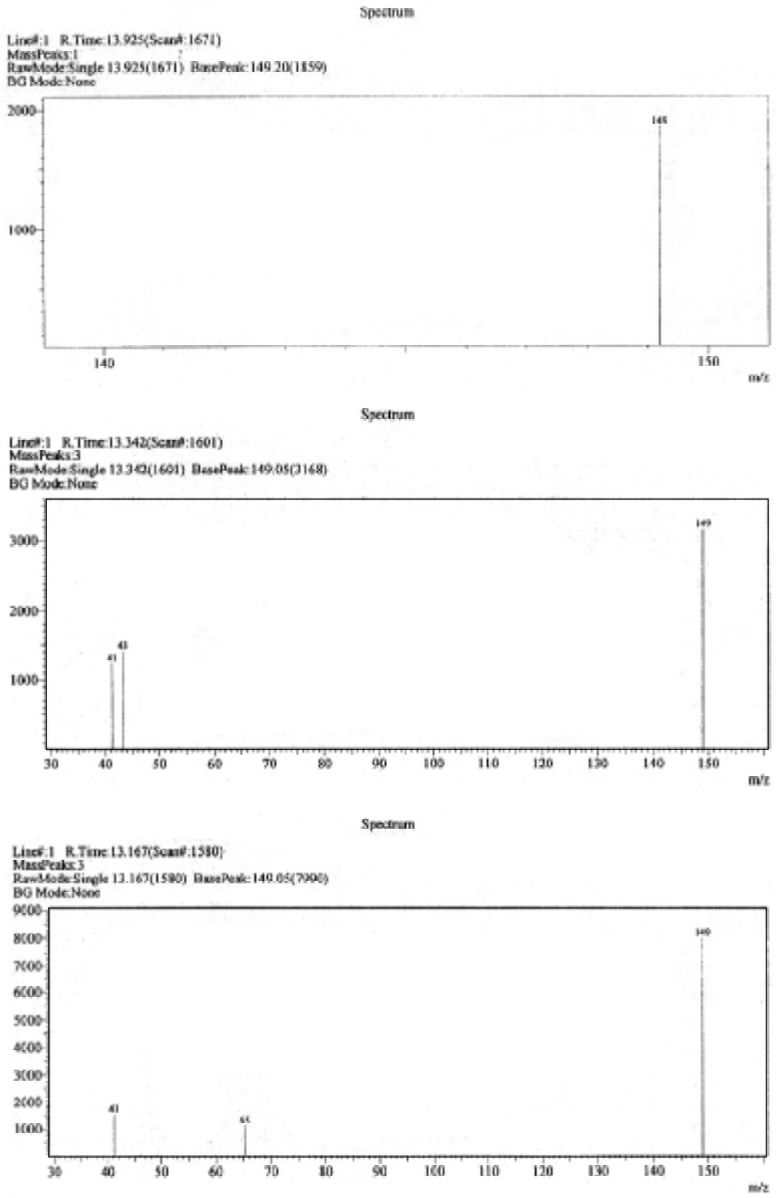


Fig. 3a: GCMS analysis report of degraded products of Yellow 4G dye by microbial consortium.

15 minutes in cooling centrifuge. Centrifugate was mixed with equal amount of dichloromethane in separating funnel. Samples were shaken vigorously for 15 minutes and kept for 10-15 minutes to separate solvent and aqueous phases. After separation, aqueous phase was discarded and solvent phase allowed for partial evaporation. Partially evaporated samples were analysed by GCMS technique.

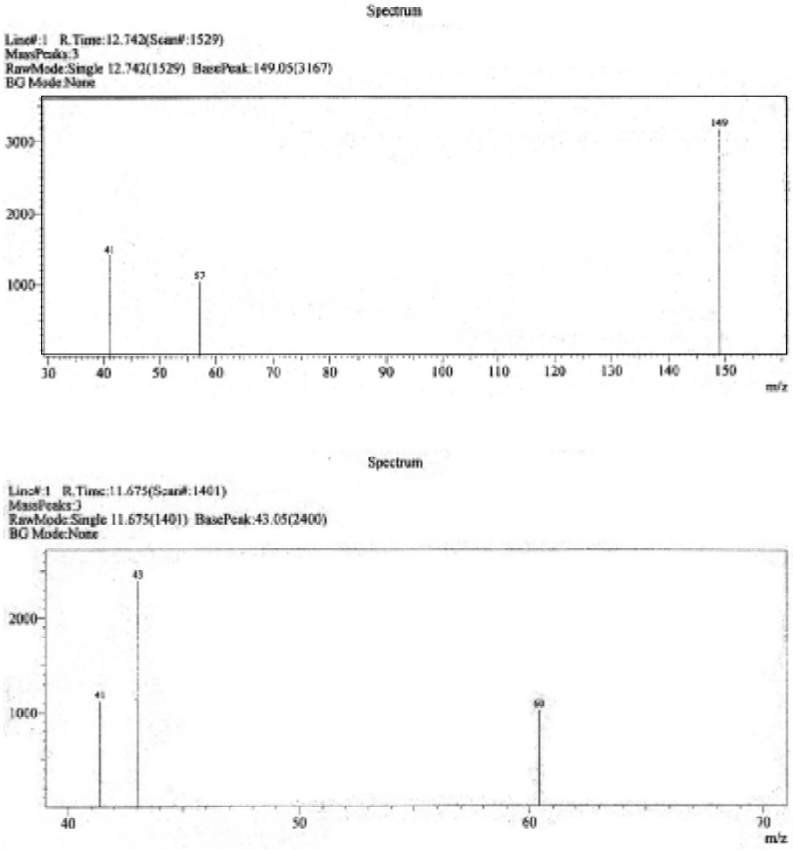


Fig. 3b: GCMS analysis report of degraded products of Yellow 4G dye by microbial consortium.

## RESULTS AND DISCUSSION

**Percent decolorization of the isolates:** The isolates showing efficient decolorization of the dyes Yellow 4G and Orange 2R on the agar medium were selected as the promising isolates, and used for further study. Percent decolorization of the dyes was determined at their respective λmax. In all, 3 isolates Y-1, Y-2 and O-1 were found to decolorize the dyes efficiently so these were selected for further studies. Yellow 4G dye was decolorized to 91.03 % and 83.24 % by the two isolates Y-1 and Y-2. Orange 2R dye was decolorized to 97.80 % by one isolate O-1. The results are given in Fig. 1.

**COD reduction:** The percent COD reduction values were calculated and it was observed that Y-1, Y-2 and O-1 reduced COD to 76.19, 85.71 and 87.87 % respectively. The results are shown in Fig. 2.

**GCMS analysis:** The GCMS analysis reports of the two dyes are shown in Fig. 3a,b and Fig. 4 respectively. The reports showed that the dyes were degraded by the consortium having different molecular weights (Table 1).

The results showed that the isolates from the acclimatized soil have good decolorization and degradation of the dyes Yellow 4G and Orange 2R. The decolorization of Yellow 4G and Orange 2R took place in nutrient medium suggesting that the presence and availability of a co-substrate is nec-

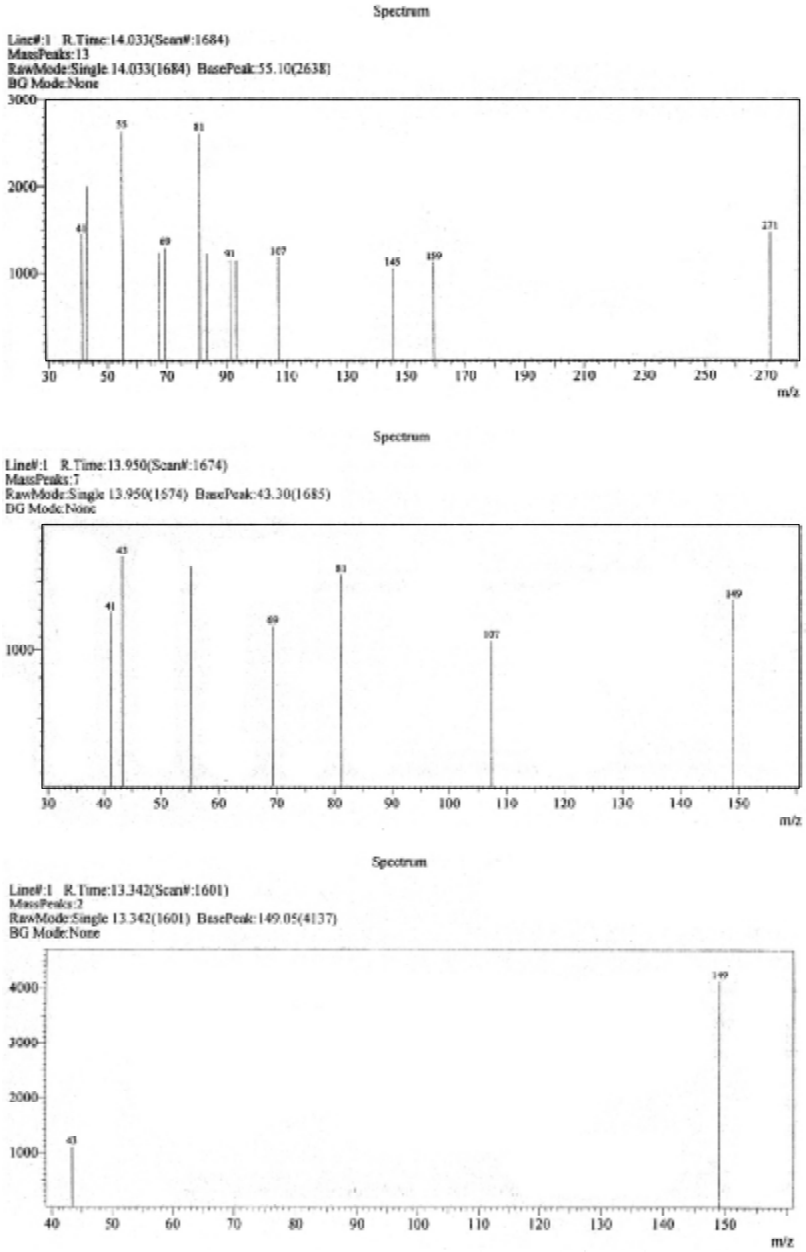


Fig. 4: GCMS analysis report of degraded products of Orange-2R dye by consortium.

essary, because it acts as an electron donor for the azo dye reduction (Nigam et al. 1996).

Confirmation of the biodegradation of the dye Yellow 4G and Orange 2R was done by analysing the samples with GCMS. Earlier reports of Kodam et al. (2005) showed the dye degradation in static condition. The degradation products of the dye were of much lower mass than the original com-

pounds. Elisangela et al. (2009) reported that *Klebsiella* strain VN-31 could decolorize the dyes Reactive Yellow 107, Reactive Red 198, Reactive Black 5 and Direct Blue 71 in presence of the co-substrate glucose and pyruvate which was very important for the microorganisms. Kaushik & Malik (2009) reported decolorization of four dyes Acid Blue 120, Acid Red 88, Acid Blue 89 and Acid Violet 19 in static and shaking conditions, which showed that decolorization of the dyes increased with reduction in time in shaking condition.

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