Decolorization of the leather industry dyes by newly isolated bacterial strains

Guven Ozdemir[1], İhsan Yasa[1], Baris Pazarbası[2], Esra Ersoy[1], Ismail Karaboz[1], Bahri Basaran[3], Behsat Oral Bitlisli[3], Ozcan Sari[3]

1. Ege University, Faculty of Science, Department of Biology, Basic and Industrial Microbiology Section 35100 Bornova-İzmir/TURKEY
2. Celal Bayar University, Faculty of Arts and Sciences, Department of Biology, Manisa/TURKEY
3. Ege University, Faculty of Engineering, Leather Engineering Department, 35100 Bornova-İzmir/TURKEY

Corresponding author: Guven OZDEMİR
e-mail: guven.ozdemir@ege.edu.tr
Fax: +90 232 3881036
Tel: +90 232 3884000/1519

Abstract

Biological oxidation of organic dyes is important for leather industry wastewater treatment. They are generally considered as xenobiotic compounds that are very recalcitrant against biodegradative processes. Nevertheless, during the last few years it has been demonstrated that several microorganisms are able, under certain environmental conditions, to transform azo dyes to non-colored products or even to completely mineralize them. The bacterial metabolism of azo dyes is initiated in most cases by reductive cleavage of azo bond, which results in the formation of (usually colorless) amines.

The present of study deals with the decolorization of some azo dyes (Acid Black 24, Acid Black 210, Acid Brown 165, Acid Green 20, Acid Yellow 36, Acid Blue 7) used in leather industry by the selected three bacteria isolated from aerobic bioreactors treating leather industry and yeast industry wastewater.

In the screen test, because the best results were showed against acid black 24, decolorization procedures were maintained on this dye. After the screening test, three of the bacteria (*Proteus vulgaris, Providencia rettgeri* and *Aeromonas hydrophila*) have significant better effect on dye decolorization. Under static conditions within 336 h, decolorization of acid-black 24 was 95%, 94.5% and %94.5; COD removal was 41.6 %, 69.4% and 66.6% On the other hand, under shaking conditions, decolorization was 74%, 61% and 90%; COD removal was 52.7 %, 83.3% and %83.3 by *Proteus vulgaris*, *Providencia rettgeri* and *Aeromonas hydrophila*, respectively.

Introduction

Synthetic dyes have a wide application in the food, pharmaceutical, textile, leather, cosmetics and paper industries due to their ease of production, fastness, and variety in colour compared to natural dyes. More than 100,000 commercially available dyes are known and close to one million tons of these dyes are produced annually worldwide (Adedayo *et al.,* 2004).

Azo dyes are the largest group of dyes used in textile industry constituting 60-70% of all dyestuffs produced. They have one or more azo groups (R_1-N=N-R_2) having aromatic rings mostly substituted by sulfonate groups. These complex aromatic substituted structures make conjugated system and are responsible for intense color, high water solubility and resistance to degradation of azo dyes under natural conditions. Color in the effluent is one of the most obvious indicators of water pollution and the discharge of highly colored synthetic dye effluents is aesthetically displeasing and can damage the receiving water body by impeding penetration of light. Moreover, azo dyes as well as their breakdown products are cytotoxic or carcinogenic (Khehra *et al.,* 2006). A number of physico-chemical methods, such as adsorption, coagulation, precipitation, filtration and oxidation, have been used to treat dyestuff effluents, but these methods have many disadvantages and limitations. It is, therefore, important to develop efficient and cost-effective methods for the decolorization and degradation of dyes in industrial effluents and contaminated soil (Bhatt *et al.,* 2000).

In the present study, acid black 24 (C.I. No.26370, $C_{36}H_{25}N_5Na_2O_6S_2$), a water soluble, benzidine based azo dye(Figure 1), was selected for carrying out microbial decolorization studies.

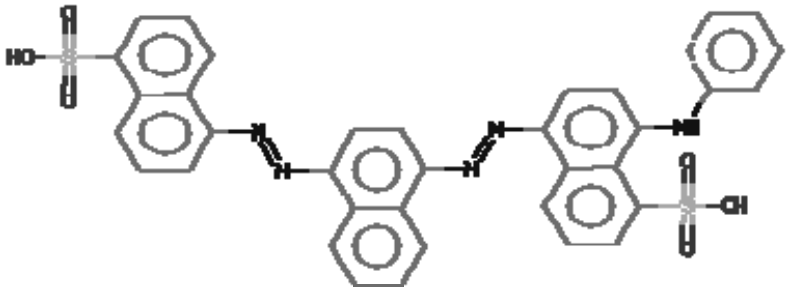

Figure 1. Structure of Acid Black 24 dye

(http://chembank.broad.harvard.edu/compounds/display.htm)

The objective of this work was to study degradation of an azo dye (acid black 24) by bacteria from activated sludge to isolate and identify some of the most active bacterial species, and to evaluate their performance under static and shaking conditions according to COD removal and decolorization of Acid Black 24.

Material and Methods

Chemicals

Commercially important and commonly used azo dye for leather and textile dyeing Acid Black 24, Acid Black 210, Acid Brown 165, Acid Green 20, Acid Yellow 36, Acid Blue 7 were obtained from locally markets. The stock solution of dyes was prepared by membrane filtration. All other chemicals used were of analytical grade.

Isolation and cultivation of bacteria efficient in decolorization

Activated sludge samples were obtained from a leather industry wastewater and a yeast industry wastewater in Izmir, Turkey. All samples were transported at 4^0C to the laboratory and used immediately to do the work presented in this article.

All bacterial isolations were carried out in Luria Bertani medium (LB), Bushnell and Hass Mineral medium and Mineral Basal medium but because of the fact that the best results for decolorization were obtained in LB medium, containing (g/liter) 0.05 yeast extract, 0.1 tryptone, 0.1 NaCl, 0.1 dye was selected in the study. The activated sludge samples (2.5 ml) were inoculated into 250 ml flasks containing 50 ml LB medium and dye. Each flask was cultivated at 27^0C on a rotary shaker at 150 rpm for 24 h. After 24 h of cultivation, by taking 1 ml of suspension, sterile LB broth with dye was inoculated and then was cultivated at 27^0C and 150 rpm. This step was carried out three times during 72 h. The last cultures were cultivated during 5 days and this mixed bacterial culture was streaked onto LB agar containing 100 ppm dye. Separate colonies of the predominant types of microorganisms were purified by re-streaking on the same medium. The purified isolates were examined microscopically to check their purity. Obtained pure cultures were maintained on LB agar at 4^oC (in refrigerator) (Hayase *et al.,* 2000, Kumar *et al*., 2005 and Chen, 2002).

Identification of Selected Decolorizing Bacteria

A pure colony of the isolates was identified presumptively on the basis of the following features: colony morphology, colonial pigmentation, cell morphology, Gram-staining reaction, oxidase positivity, O/F tests (Gerhardt *et al*., 1981). Isolates were further characterized biochemically using the API 20E and API 20NE kits (Analytical Profile Index, bioMérieux, Marcy l'Etoile, France).

Static and Aerobic batch decolorization operations

Precultured cells were inoculated at 1% (v/v) into 500ml flasks containing 200 ml LB medium with dye (100 ppm). The cultures were incubated at 27^0C in rotary shakers running at 150 rpm (shaking condition) or without shaking (static condition).

Analytical methods

Color measurements in clarified samples from synthetic wastewaters were performed in a spectrophotometer (Varian UV- Vis carry 300) in the UV-Visible range against a baseline defined by sterile clarified samples from dye-free synthetic wastewater. Calibration graphs of absorbance versus dye concentration were constructed from solutions of the dye in synthetic wastewater for the calculation of the individual dye concentrations. At each sampling time, the samples (2 ml) were centrifuged at 11,000 rpm for 10 min and the absorbance values of supernatants were determined. Absorbance of the sample was measured at the maximum absorption wavelength (at 573 nm) of acid black 24. COD was measured according to a standard procedure (APHA, 1992).

Results and Discussion

Isolation and identification

A number of bacterial cultures were isolated from a leather industry wastewater and a yeast industry wastewater. Three strains were isolated as the most active azo dye-decolorizing bacteria. The strains were identified as *Aeromonas hydrophila*, *Proteus vulgaris* and *Providencia rettgeri* using standart microbiological procedures, API 20E and API 20NE.

Decolorization of Acid Black 24 and COD removal

Because, the isolates showed the highest decolorization capability against acid black 24, this dye was further studied for decolorization.

Experiments on decolorization using Acid Black 24 under laboratory conditions were performed. Three strains were incubated under static and shaking conditions. The results obtained from treatment of Acid Black 24 are shown in Fig. 2, Fig. 3 and Fig. 4. Under static conditions within 336 h, decolorization of acid-black 24 was 95%, 94.5% and %94.5; COD removal was 41.6 %, 69.4% and 66.6% by *Proteus vulgaris*, *Providencia rettgeri* and *Aeromonas hydrophila*, respectively. On the other hand under shaking conditions, decolorization was 74%, 61% and 90%; COD removal was 52.7 %, 83.3% and %83.3.

Biological degradation of Acid Black 24 has been reported recently by Young and Yu (1997). The study carried out by Young and Yu (1997) showed that 98% of Acid Black 24 (9 days) was reduced in color by *P.chrysosporium* and *T. versicolor.*

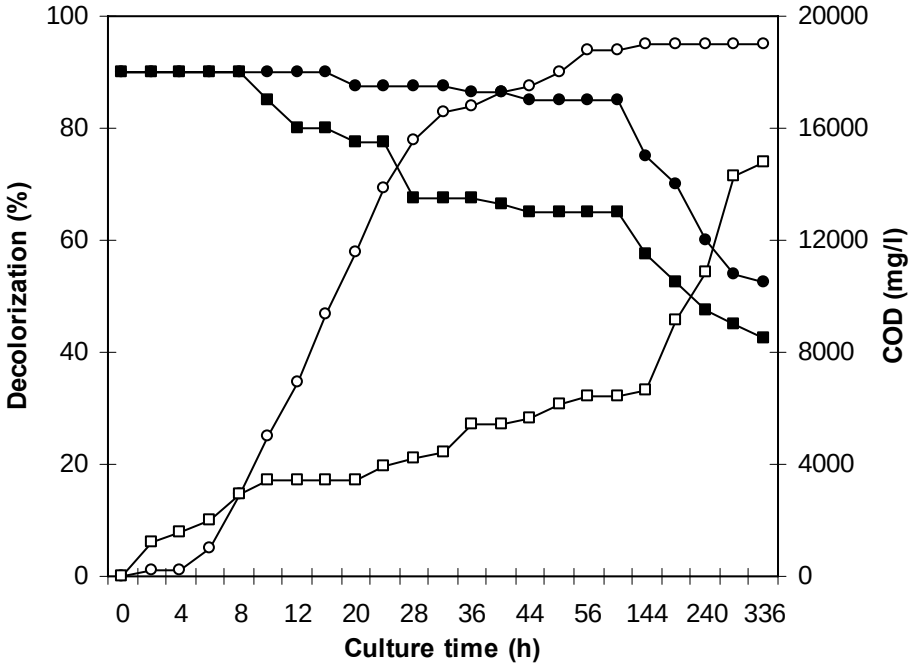

Figure 2. Decolorization (%) and COD removal by *Proteus vulgaris* of synthetic wastewater under static condition (○,●) and shaking condition (□, ■).The Decolorization (%) and COD removal are shown as open and closed symbols, respectively.

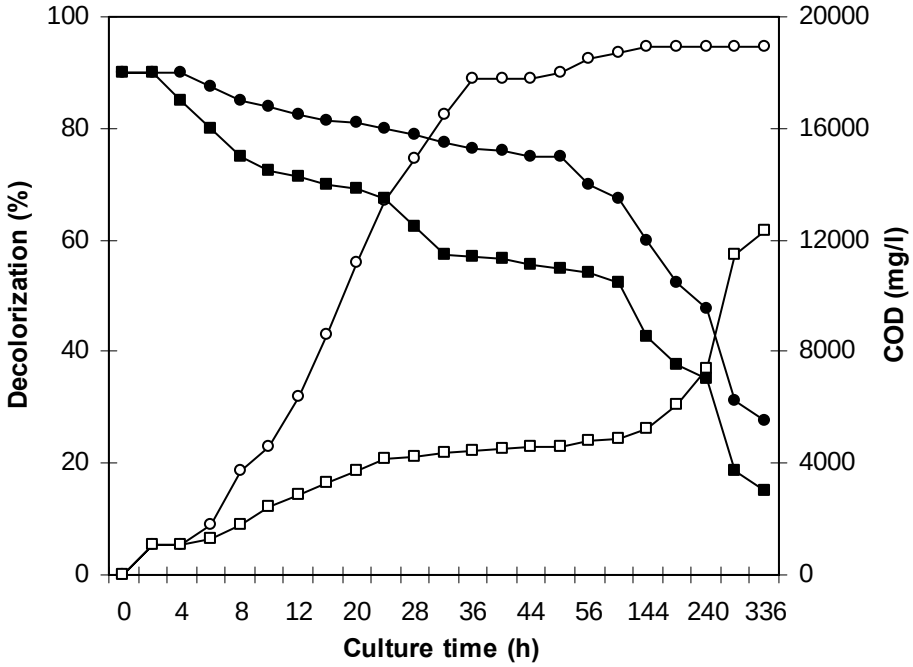

Figure 3. Decolorization (%) and COD removal by *Providencia rettgeri* of synthetic wastewater under static condition (○,●) and shaking condition (□, ■).The Decolorization (%) and COD removal are shown as open and closed symbols, respectively.

Under static conditions in 144 h, decolorization of acid-black 24 reached 95%, 94.5%, but COD removal was obtained 16.6%, 33.3% by *Proteus vulgaris, Providencia rettgeri,* respectively. Although dye reduction was stable between 144 h and 336 h, COD continued to decrease.

Decolorization of the leather and textile dye by newly isolated bacteria, *Providencia rettgeri*, was performed under laboratory conditions.

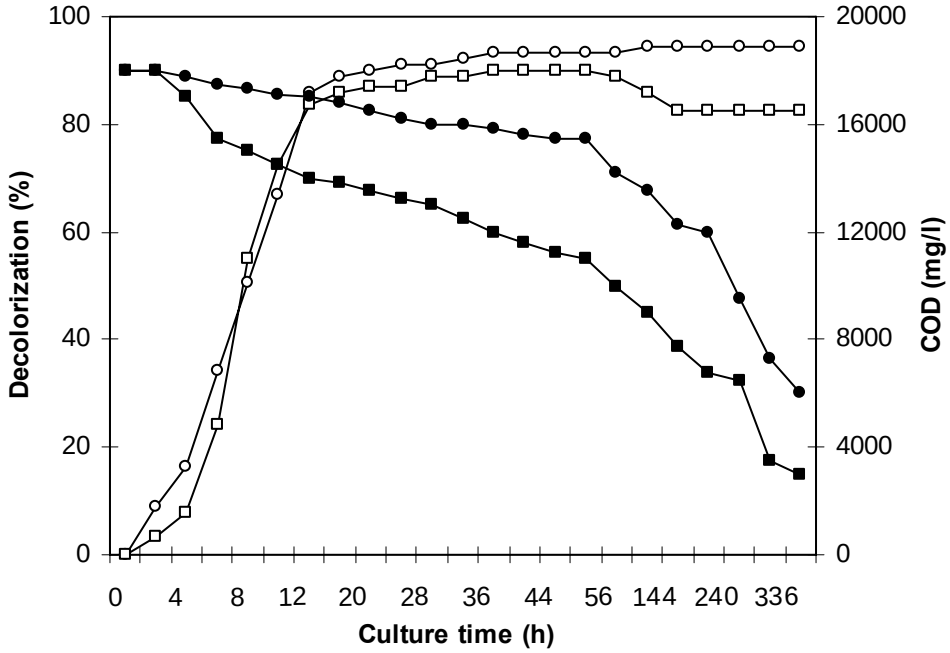

Figure 4. Decolorization (%) and COD removal by *Aeromonas hydrophila* of synthetic wastewater under static condition (○,●) and shaking condition (□, ■).The Decolorization (%) and COD removal are shown as open and closed symbols, respectively.

Under static conditions, 94.5% of Acid Black 24 was reduced in color in 72 h, but COD removal was obtained 25% by *Aeromonas hydrophila*. Although dye reduction was stable between 144 h and 336 h, COD continued to decrease.

Although there was an increase in percentage of decolorization (90%) of *Aeromonas hydrophila* up to 36 h under shaking conditions, the decrease after 36 h was probably the desorbtion of adsorbed dye.

COD removal under static conditions was lower than under shaking conditions. In contrast to results of COD removal, percentage of Acid Black 24 decolorization under static conditions was higher than under shaking conditions.

Anaerobic conditions are usually refered to as being favourable to the reduction step, but it has been also shown that strains of *Bacillus subtilis, Aeromonas hydrophila, Pseudomonas cepacia* and of a *Flavobacterium sp.* can reduce azo dyes in the presence of oxygen. However, the ultimate purpose of complete mineralization of dye molecules can not usually be reached under anaerobic conditions (Martins *et al.,* 1999).

While *Aeromonas hydrophila* showed the best decolorization both under static and shaking conditions, the lowest result of COD removal was obtained from *Proteus vulgaris*.

Aeromonas hydrophila displayed good growth in aerobic or agitation culture, color removal was the best in anoxic or anaerobic culture. More than 90% of RED RBN was reduced in color within 8 days at a dye concentration of 3000 mg/ l (Chen *et al.*,2003). Under anaerobic conditions, the decolorization of many azo dyes via reduction of the azo bond has been shown for anaerobic (e.g., *Bacteroides* sp., *Eubacterium* sp., and *Clostridium* sp.) as well as facultative anaerobic (e.g., *Proteus vulgaris* and *Streptococcus faecalis*) bacteria (Maier *et al.,* 2004). The participation of redox mediators in the decolorization of azo dyes by bacterial species is now generally accepted, and several mediators (usually quinonoid compounds) have been described as effectively enhancing decolorization processes (Romalho *et al.*,2004).

In our experiments after centrifugation cells from static culture was observed colorless whereas cells from shaking culture was observed like color of acid black 24. With respect to these, it was concluded that the decolorization mechanism of *Proteus vulgaris*, *Providencia rettgeri* and *Aeromonas hydrophila* in shaking condition was due to adsorbtion of dye by viable or died cells and its components on the other hand it was due to serve dye as an electron acceptor in static culture.

Acknowledgements

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