Journal of Ecobiotechnology 2011, 3(8): 29-32
ISSN: 2077-0464
www.scholarjournals.org

www.journal-ecobiotechnology.com

JEBT-Microbial Biotechnology

Decolourization of Textile Azo Dyes by using Bacteria Isolated from Textile Dye Effluent

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Article Info

Article History

Received : 29-06-2011
Revised : 27-08-2011
Accepted : 27-08-2011

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Abstract

The present study was carried out to isolate dye decolourizing bacteria and to study the dye decolourizing ability. Different bacterial isolate such as, *Bacillus* sp., *Escherichia coli* and *Pseudomonas fluorescens* were isolated from textile dye effluent sample and used for the decolourization study. It was noticed that there was a decrease in the OD in all the three species of all the five dyes as the incubation period increased. *Pseudomonas fluorescens* was more effective followed by *Bacillus,* and *Escherichia coli.* It was found that all the isolated bacteria were efficient decolourizers of Orange 3R. The decolourization of dye amounted to 59, 77, 79 respectively within 16 days. Yellow GR was recalcitrant to decolourization, the O.D. value from an initial value of 0.6912 was reduced only to 0.303 and from 0.746 to 0.218, 1.236, to 1.33 by *Pseudomonas fluorescens, Bacillus* sp. and *Escherichia coli* respectively. Percentage decolourization was 43%, 15%, 90% respectively. The percentage of decolourization of Orange GR is slightly higher than Blue 3R and the percentage decolourization of Black RL is similar to T Blue.

Key Words: Decolourization, Azo dyes, Textile dye effluent and Bacteria

Introduction

Our biosphere is under constant threat from constituting environmental pollution. Water pollution is a state of deviation from pure conditions partially, wholly or largely as a by-product of human activity through direct or indirect effects of changes in energy patterns, chemical and physical composition in nature and abundance of organisms. The function and properties affecting the quality of water is of vital concern for humanity, since it is directly linked with human welfare. Water supply sources like ground water and surface water are related and interconnected by the hydrological cycles. The quality of life in earth is linked inextricably to the overall quality of the environment. Whether water is used as a habitat or to meet drinking and irrigation demands, the maintenance of quality of water is crucial for the survival of life.

The textile industry is one of the industries that generate a high volume of waste water. Strong colour of the textile waste water is the most serious problem of the textile waste effluent. The disposal of these wastes into receiving water causes damage to the environment. Dyes may significantly affect photosynthetic activity in aquatic habitat because of reduced light penetration and may also be toxic to some aquatic life due to the presence of aromatics, metals, chlorides and other toxic compounds (Husseiny, 2008).

Azo dyes are the largest group of dyes. More than 3000 different varieties of azo dyes are extensively used in the textile, paper, food, cosmetics and pharmaceutical industries (Maximo *et al*., 2003). Azo dyes are characterized by the presence of one or more azo groups – N = N -, which are responsible for their colouration and when such a bond is broken the compound loses its colour. They are the largest and most versatile class of dye, but have structural properties that are not easily degradable under natural conditions and are not typically removed from water by conventional waste water system. Azo dyes are designed to resist chemical and microbial attacks and to be stable in light and washing.

A number of azo dyes including reactive dyes are used in textile dying operations. This leads to effluent streams containing intense colour due to the presence of azo dyes. The removal of azo dyes from effluents is important due to their mutagenicity and carcinogenicity together with their intense colouration. Both physicochemical and biological methods for the removal of dyes have been investigated widely. The physicochemical dye removal techniques such as flocculation–coagulation, adsorption, electrochemical oxidation, photocatalytic oxidation, electro- Fenton oxidation appear to face several technical and economic limitations (Rao *et al*., 2006). On the other hand, biological methods such as activated sludge process and anaerobic treatment have been applied to control pollution of aquatic environment. Lower cost of treatment and amenability to scale up easily are the merits of biological methods. The present study was focused on decolourization of textile azo dyes and biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent.

Materials and Methods

Sample collection and preservation

The dye house effluent was collected from a Junior processing dye industry, Arulpuram, Tirupur region (Tamil Nadu, India). The sample was collected in a brown bottle. Prior to the collection the sample bottle was rinsed thoroughly with the sample water. Then the sample was brought to the

laboratory as early as possible and was subjected for various microbiological studies.

Dyes

The dye samples were collected from the Columbia dye Kem processing industry situated at Palladam area. Direct azo dyes used in this research are, Orange 3R (λm = 493 nm), Blue 3R (λm = 572 nm), Yellow Gr (λm = 413 nm), Black RL (λm = 574 nm) and T blue (λm = 600 nm).

Isolation and identification of dye decolourizing bacteria

Pour plate technique was used for the isolation of dye decolourizing bacteria. Well grown bacterial colonies were picked and further purified by streaking. The isolated strains were maintained on Nutrient agar slants and stored at 4°C. Identification of the bacterial isolates was carried out by the routine bacteriological methods *i.e*., By the colony morphology, preliminary tests like Gram staining, capsule staining, endospore staining, motility, catalase and oxidase, plating on selective medias and performing biochemical tests.

Screening of bacterial isolates for textile direct azo dye degradation

Inoculum preparation

The suspension of 2 days old cultures of bacteria were used to investigate their abilities to decolourize dyes. They were prepared in saline solution (0.85% sodium chloride). A loopful of bacterial cultures were inoculated into 50 ml of saline and incubated at 37°C for 3 hours (Benson, 1994).

Decolourization experiment

Fifty milliliter of Nutrient agar sterile medium was amended separately with each of the textile dyes (200 mg/l) and subsequently inoculated with 2% bacterial suspension. The suspension contained 2.5 x 10^6 cfu/mL (colony forming unit) spores. The flasks were kept in mechanical shaker and incubated at 30±1°C for 8 days. Samples were drawn at 2 days intervals for observation. Samples were centrifuged at 10000 rpm for 10 minutes. Decolourization was assessed by measuring absorbance of the supernatant with the help of spectrophotometer at wave length maxima (λm) of respective dye. Two control flasks (dye + medium without inoculums and medium with inoculums without dye) were maintained.

Decolourization assay

Decolourization assay was measured in the terms of percentage decolourization using UV-Spectrophotometer. The percentage decolourization was calculated from the following equation,

$$\% \text{ Decolourization} = \frac{\text{Initial OD - Final OD}}{\text{Initial OD}} \times 100$$

Results and Discussion

In Tamil Nadu many of the districts are known for textile industries. Tirupur is one among them. These industries discharge the coloured effluents with dyes and toxic compounds into the open environment.Textile and dyeing industry are among those which contribute much to water and soil pollution. They consume substantial volumes of water and chemicals. Further, about 10,000 different dyes and pigments are being used. Among these azo-dyes are widely used. Apart from chemicals nearly 10-15% of the dye is lost as effluent during the dyeing process (Jothimani *et al.*, 2003).

Biodegradation of commercially available textile dyes namely Orange 3R, Blue 3R, Yellow Gr, Black RL and T blue were studied against five bacterial isolates which have been isolated from the dye effluent sample by Pour plate method and percentage decolourization was shown in the figures accompanying the results. Three different bacteria were isolated from the textile dye effluent. Based on preliminary tests, plating on selective media and biochemical tests, they were identified as *Bacillus* sp., *Escherichia coli* and *Pseudomonas fluorescens*.

Olukanni *et al*., (2005) isolated eighteen textile effluent adapted bacterial isolates belonging to the genera, *Bacillus, Acinetobacter, Staphylococcus, Legionella* and *Pseudomonas* were investigated for the potential of textile effluent adapted bacteria in decolourizing it. *Bacillus* and *Legionella* were found to have use in effluent treatment.

Ajibola *et al*., (2005) checked the ability of *Staphylococcus aureus, Bacterioides fragilis, Bacillus subtilis, Bacillus cereus, Clostridium perifringens, Escherichia coli* and *Peptostreptococcus* sp. to reduce and stabilize textile effluents containing predominantly Indigo Blue.

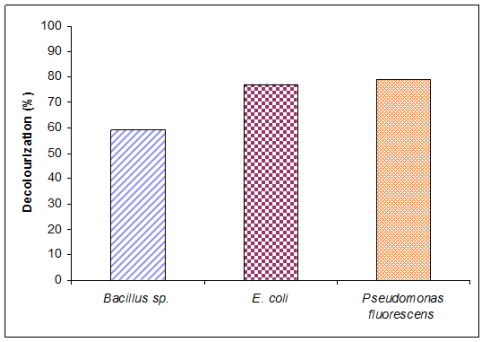

Fig. 1. Decolourization percentage of Orange 3R by bacterial isolates

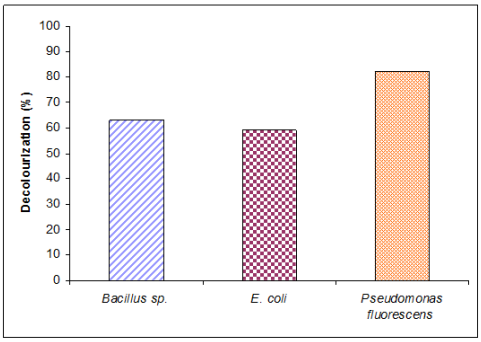

Fig. 2. Decolourization of *Antroquinone* Blue 3R by bacteria

The decolourization efficiency of *Bacillus* sp., *Pseudomonas fluorescens,* and *Escherichia coli* was studied by measuring the optical density after 0, 4, 8, 12, 16 days of incubation and the results were showed in Figure – 1, 2, 3, 4 and 5. It is noticed that there was a decrease in the OD in all the three species in all the five colours as the incubation period increased. *Pseudomonas fluorescens* was more effective followed by *Bacillus* sp., and *Escherichia coli.* The percentage of decolourization of colours by the bacteria was also

calculated. It was found that all the isolated bacteria were efficient decolourizers of Orange 3R. The decolourization of dye amounted to 59, 77, 79 respectively within 16 days. Yellow GR was recalcitrant to decolourization, the O.D. value from an initial value of 0.6912 was reduced only to 0.303 and from 0.746 to 0.218, 1.236, to 1.33 by *Pseudomonas fluorescens, Bacillus* sp., and *Escherichia coli* respectively. Percentage decolourization was 43%, 15%, 90% respectively. The percentage of decolourization of Orange GR is slightly higher than Blue 3R and the percentage docolourization of Black RL is similar to T Blue.

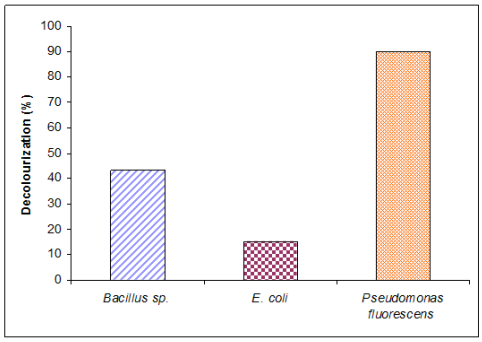

Fig. 3. Decolourization of azo dye Yellow GR by bacteria

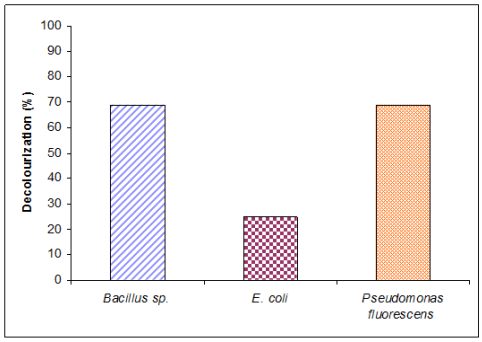

Fig. 4. Decolourization of azo dye Black RL by bacteria

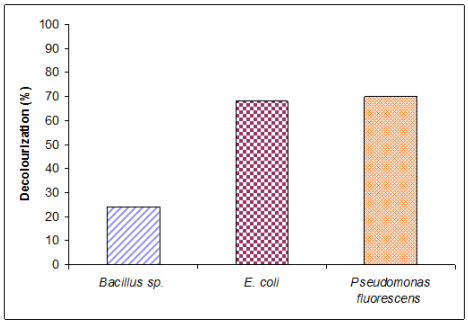

Fig. 5. Decolourization of Copper Phthalocyanine T Blue at 664 nm by bacteria

The decolourization of textile reactive azo dyes by *Clostridium biofermentans* isolated from a contaminated site was studied under aerobic conditions. *Clostridium biofermentans* decolourized the dyes Reactive red 3B-A, Reactive black 5, and Reactive yellow 3B-A, by over 90% after 36 hours post-inoculation spectrophotometric analyses of the reactive dyes showed no distinct peak indicating aromatic amines. The results suggested that *Clostridium biofermentans* was a suitable bacterium for the biological processing of dye-contaminating waste water (Min-Ho Joe *et al*., 2008). Under anaerobic conditions, the decolourization of many azo dyes takes place via reduction of the azo bond for both aerobic as well as facultative anaerobic bacteria (Bragger *et al*., 1997)

Saranraj *et al*., (2010) investigated the decolourization and degradation of Direct azo dyes and biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent. They isolated five different bacterial species from the textile dye effluent sample and the isolates were identified as *Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and *Escherichia coli.* In our study also we have isolated three same genus among five. The bacterial inoculums were inoculated into flasks containing Direct azo dyes (500 mg/l) with trace amounts of yeast extract, glucose and sucrose and then sterilized and incubated for 4 days. In their research, *Pseudomonas aeruginosa* (97.33%) was identified as the best decolourizer of Congo Red. Similar results we also obtained in our present study. The best decolourizer of Direct Green-PLS was *Bacillus subtilis* (99.05%). *Klebsiella pneumoniae* (87.27%) highly decolourized the Direct Violet-BL. *Escherichia coli* (61.56%) was the best decolourizer of Direct Sky Blue-FF. The best decolourizer of Direct Black-E was *Klebsiella pneumoniae* (92.03%). They assessed the bacterial biodegradation of textile dye effluent by physicohemical analysis.

Conclusion

Application of traditional waste water treatment requires enormous cost and continuous input of chemicals which becomes uneconomical and causes further environmental damage. Hence, economical and eco-friendly techniques using bacteria can be applied for fine tuning of waste water treatment. Biotreatment offers easy, cheaper and effective alternative for colour removal of textile dyes. Thus, by this present study I concluded that the bacterial isolates like *Bacillus* sp*., Pseudomonas fluorescens,* and *Escherichia coli* can used as a good microbial source for waste water treatment.

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