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Bioremediation and Detoxification of a Textile Azo Dye-Evans Blue by Bacterial Strain AKIP-2

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ABSTRACT

Increasing industrialization and urbanization result in the discharge of waste to the environment, which in turn creates more pollution. Environmental biotechnology is constantly expanding its efforts in the biological treatment of colored textile effluents, which is an environmental friendly and low cost alternative to physico-chemical decomposition processes. In the present study, effluent samples were collected from various textile and dyeing industries located in and around Kanchipuram, Tamilnadu, India and were exploited for the screening and isolation of bacterial strains that were capable of decolorizing the textile dye, Evans Blue. Optimization of cultural conditions (Temperature, pH, Agitation speeds and Dye concentrations) were carried out to maximize the bacterial growth of E. coli strain AKIP-2 and its decolorization efficiency towards Evans Blue. Both bacterial biomass and decolorization efficiency was found to be optimized at 35°C, neutral pH, after 24 h of incubation. Static conditions proved to be effective in maximizing decolorization. Increase in dye concentration decreased both bacterial growth and decolorization efficiency of E. coli strain AKIP-2.

Keywords
E. coli strain
AKIP-2,
Evans’s Blue,
Textile effluents

Introduction

Textile industry has a major impact not only on the nation’s economy but also on the environmental quality of life in many communities. Textile manufacturing consumes a considerable amount of water approximately 100 litres of H2O kg⁻¹ of textile materials in its dyeing, finishing and manufacturing processes (Tang and Chen, 1996). Considering both the volume generated and the effluent composition, the textile industry wastewater is rated as the most polluting source among all industrial sectors (Koyuncu, 2002). Strong color of the textile wastewater is the most serious problem of textile waste effluent. The disposal of these wastes into receiving water causes damage to the environment (Shyamala et al., 2014). Dyes may significantly affect the photosynthetic activity in aquatic life because of reduced light penetration and may also be toxic to some aquatic life due to the presence of aromatics, metals, chlorides etc, (Husseiny, 2008; Hemapriya et al., 2010). In addition to their visual effect and adverse impact in terms of chemical oxygen demand (COD), many synthetic dyes show their toxic, carcinogenic
and genotoxic effects (Pearce et al., 2003). Traditional wastewater treatment technologies have proven to be markedly ineffective for handling wastewater of synthetic textile dyes because of the chemical stability of these pollutants. Color is one of the most obvious indicators of water pollution and the discharge of highly colored synthetic dyes in textile effluents can be damaging to the receiving water bodies (Nigam et al., 1996; Shyamala et al., 2014). Implementation of physical/chemical methods have inherent drawbacks of being economically unfeasible (more energy consumption and chemical uses), unable to remove the recalcitrant azo dyes and/or their organic metabolites completely, generating a significant amount of sludge that may cause secondary pollution problems (Zhang et al., 2004; Hemapriya and Vijayanand, 2014). The microbial decolorization and degradations of azo dyes has been of considerable interest since it is inexpensive, eco-friendly and produces a less amount of sludge (Kalyani et al., 2008; Saratale et al., 2009). The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms including bacteria, actinomycetes, fungi, yeasts, and algae capable of degrading azo dyes (Shyamala et al., 2014).

Hence, the present investigation was intended to assess the potential of AKIP-2 strain to decolorize the synthetic textile azo dye, Evans Blue under aerobic conditions, and to optimize the culture conditions to maximize the biomass and decolorization efficiency of AKIP-2 Strain.

Materials and Methods

Sampling site and sample collection

The sampling area was the textile industries and dyeing units located in and around Kanchipuram, Tamil Nadu, India. The effluent samples from both textile industries and dyeing units were characterized by its dark color and extreme turbidity.

Azo dye used

The commonly used textile azo dye, Evans Blue used in this study was procured from a local textile dyeing unit. Stock solution was prepared by dissolving 1 g of Evans Blue in 100 ml distilled water. The dye solution was sterilized by membrane filtration, since azo dyes may be unstable to moist-heat sterilization.

Isolation and screening of bacterial strains decolorizing evans blue

Effluent samples were serially diluted and spread over basal nutrient agar medium containing 50 ppm of Evans Blue. Colonies surrounded by halo (decolorized) zones were picked and streaked on nutrient agar plates containing Evans Blue. Different colonies of dye decolorizing bacteria were picked and re-streaked several times to obtain pure cultures.

Decolorization assay

A loopful of bacterial culture AKIP-2 was inoculated in 100 ml of nutrient broth and incubated at 150 rpm at 37°C for 24 h. Then, 1 ml of 24 h old culture was inoculated in 100 ml of nutrient broth containing 50 ppm of Evans Blue and re-incubated at 37°C till complete decolorization occurs. Suitable control without any inoculum was also run along with experimental flasks. 1.0 ml of sample was withdrawn every 12 h and centrifuged at 10,000 rpm for 15 min. Decolorization extent was determined by measuring the absorbance of the culture supernatant at 590 nm using UV-visible spectrophotometer (Hitachi U 2800), according to Hemapriya et al., (2013).
Decolorization efficiency (%) = Dye (i) - Dye (r) / Dye (i) × 100

Where, Dye (i) refers to the initial dye concentration and Dye (r) refers to the residual dye concentration. Decolorization experiments were performed in triplicates.

**Bacterial strain and culture conditions**

Bacterial strain that showed maximum decolorization percentage on Evans Blue was aerobically cultured in nutrient broth containing 50 ppm of Evans Blue. The pH was adjusted to 7.0. For frequent use, the culture was maintained by transfer to a fresh medium at 24 h intervals. When required for prolonged periods, it was maintained by sub-culturing once every 7 days on slants, prepared by solidifying the above mentioned medium with 2.0 (w/v) agar.

**Optimization of various culture conditions for bacterial biomass and evans blue decolorization by AKIP-2**

**Effect of temperature, pH, agitation rates and dye concentrations**

The effect of temperature, pH, agitation rates and dye concentration on both bacterial biomass and dye decolorizing ability of AKIP-2 strain was studied. This was carried out by incubating the bacterial strain at different temperatures (20-60°C), different pH values of the medium (pH 4.0-10.0), different agitation speeds (0-200 rpm) and various dye concentrations (200-1000 ppm). Bacterial biomass and decolorization percentage was measured at optimum growth (24 h).

**Results and Discussion**

**Effect of incubation time**

Incubation time played a significant role in maximizing both bacterial growth and dye decolorizing ability of *E.coli* strain AKI-2. Results of the present study revealed that the dye decolorizing ability of the isolate was dependent on the bacterial growth. The bacterial cells started multiplying within 4 h and reached their maximum growth within 24 h and thereafter started to decline, due to the depletion of nutrients and accumulation of toxic metabolites (Fig 1 and 2). In contrast, decolorization of Methyl orange by *Bacillus* sp. strain TVU-M4 was achieved after 32 h of incubation (Shyamala *et al*., 2014).

**Effect of temperature**

The results shown in Fig 3 and 4 revealed that *E.coli* strain AKI-2 showed strong decolorizing activity and highest bacterial growth from 30-40°C, with optimum being 35°C after 24 h of incubation. The incubation at 30, 50 and 60°C was found to decrease both bacterial biomass and dye decolorizing ability of the bacterial strain; however the decolorization percentage of the isolate was found to be greatly inhibited at temperature below 30°C. Decolorization of Congo Red by *Bacillus* sp. VT-II was maximized at 40°C (Sawhney and Kumar, 2011).

**Effect of pH**

*E. coli* strain AKI-2 grew well in a broad range of pH (5.0-10.0) and its decolorizing ability does not have strict pH requirement. Bacterial biomass and dye decolorizing ability was found to be optimized at neutral pH (7.0) (Fig 5 and 6). Similarly, neutral pH was found to be effective in maximizing both bacterial growth and dye decolorization efficiency of many bacterial strains.

The pH tolerance of the decolorizing bacteria is quite important because the reactive azo dyes bind to cotton fibers by the addition or substitution mechanisms under alkaline conditions (Aksu *et al*., 2007).
Fig. 1 Effect of incubation time on bacterial biomass of *E. coli* strain AKI-2

Fig. 2 Effect of incubation time on decolorization of evans blue by *E. coli* strain AKI-2

Fig. 3 Effect of temperature on bacterial biomass of *E. coli* strain AKI-2
**Fig. 4** Effect of incubation time on decolorization of evans blue by *E. coli* strain AKI-2

**Fig. 5** Effect of pH on bacterial biomass of *E. coli* strain AKI-2

**Fig. 6** Effect of pH on decolorization of evans blue by *E. coli* strain AKI-2
Fig. 7 Effect of agitation speed on bacterial biomass of *E. coli* strain AKI-2

![Effect of Agitation Speed](image)

Fig. 8 Effect of agitation speed on decolorization of Evans blue by *E. coli* strain AKI-2

![Effect of Agitation](image)

Fig. 9 Effect of dye concentration on bacterial biomass of *E. coli* strain AKI-2

![Effect of Dye Concentration](image)
Fig.10 Effect of dye concentration on decolorization of evans blue by E. coli strain AKI-2

**Effect of dye concentrations**

The influence of different dye concentrations (0-1000 ppm) were analyzed on bacterial biomass and decolorization ability of E.coli strain AKI-2. The results shown in Fig 7 and 8 revealed that the decolorization rate increased linearly with increase in initial dye concentration upto 100 ppm. As the dye concentration increased in the culture medium, a decline in color removal was attained. At high concentration (1000 ppm), Evans blue greatly suppressed both bacterial biomass and decolorization ability.

**Effect of agitation speeds**

Microorganisms vary in their oxygen requirement. The effect of various agitation speeds (0-250 rpm) on the bacterial growth and color removal capacity of E.coli strain AKI-2 was studied at 35°C after 24 h of incubation. The decolorization ability of the isolate was found to be maximized at static conditions. Shaking conditions highly repressed the decolorizing ability of E.coli. In contrast, the bacterial biomass was found to be maximized when incubated at 200 rpm (Fig 9 and 10).

**References**


Koyuncu, I. (2002). Reactive dye removal in

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Ecofriendly Bioremediation of Malachite Green, a Triphenylmethane Dye by Textile Effluent Acclimatized Bacterial Strain - Chromohalobacter sp. Strain IAK-7

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Abstract

Environmental pollution has been recognized as one of the major problems of the modern world. The increasing demand for water and the dwindling supply has made the treatment and reuse of industrial effluents as an attractive option. 7 bacterial isolates, designated as IAK-1 to IAK-7 were found to be effective in decolorizing Malachite Green. The superior strain with the highest decolorization efficiency was identified as Chromohalobacter sp. Strain IAK-7. Dye decolorization of Malachite Green was found to be maximized after 24 h of incubation. Increase in dye concentration was found to be inversely proportional to the decolorization of Malachite Green. HPLC and FTIR analysis revealed the degradation of the parental dye molecule.

Introduction

At present scenario, environmental pollution is a major socio-economic and also a health problem. Among the various types of environment pollution, water pollution is a major concern. Nowadays, water pollution has become a matter of great concern in our society. Most of the water pollutions are related to the industrial effluents. Water is life but now a-days due to the advancement in industrialization, it is spoiling a lot. Environmental pollution has been recognized as one of the major problems of the modern world. The increasing demand for water and the dwindling supply has made the treatment and reuse of industrial effluents as an attractive option. Textile effluents are of global concern because they color the drains and ultimately the receiving water bodies (Olukanni et al., 2006). Textile industries consume a considerable amount of water in their manufacturing processes. Considering both the volume and the effluent composition, the textile industry is rated as the most polluting among the industrial sectors. Therefore treatment of industrial effluent containing aromatic compounds becomes necessary prior to their final discharge into the environment (Shyamala et al., 2014).

Physico-chemical methods have major limitations such as economically unfeasible, unable to remove the recalcitrant azo dyes and their metabolites, involves complicated procedures, more energy consumption and chemical usage. Whereas biodecolorization has been proposed as eco-friendly, generates less sludge and less expensive. Biological dye removal techniques are based on the microbial biotransformation of dyes. Many
researchers have demonstrated the partial or complete biodegradation of dyes by pure and mixed cultures of bacteria, actinomycetes, fungi and algae (Stolz, 2001; Dong et al., 2003; Hemapriya et al., 2010). Bioremediation is a pollution control process that uses the biological systems to catalyze the degradation or transformation of various toxic chemicals into less harmful forms. Microorganisms for dye decolorization may be obtained simply by the isolation of existing dye degrading cultures from the textile effluent samples, by the adaptation of promising strains to the conditions present in the textile effluents or by the construction of suitable organisms employing genetic engineering methods (Kandelbauer et al., 2004).

The members of triphenylmethane family are animal carcinogens. The Food and Drug Administration (FDA) nominated Malachite Green, a triphenylmethane dye as a priority chemical for carcinogenicity testing by the National Toxicology Program 1993 (Srivastava et al., 2000). Malachite Green and its reduced form, leucomalachite green, may persist in edible fish tissues for extended periods of time. Therefore there are both environmental and human health concerns about bioaccumulation of Malachite Green and leucomalachite green in terrestrial and aquatic ecosystems (Parshetti et al., 2006). The present study focuses on the ecofriendly bioremediation of Malachite Green, a triphenylmethane dye by textile effluent acclimatized bacterial strain -

Materials & Methods

Sample Collection and Physico-chemical analysis

The sampling area was the textile industries and dyeing units located in and around Kanchipuram, Tamil Nadu, India. The effluent samples from both textile industries and dyeing units were characterized by its dark color and extreme turbidity. Physico-chemical properties of the effluent samples such as TS, TDS, TSS, BOD, COD, pH and color were analyzed (APHA, 1980).

Azo Dye Used

The commonly used textile azo dye, Malachite Green used in this study was procured from a local textile dyeing unit. Stock solution was prepared by dissolving 1 g of Malachite Green in 100 ml distilled water. The dye solution was sterilized by membrane filtration, since azo dyes may be unstable to moist-heat sterilization. All the chemicals used in this study were of the highest purity available and of an analytical grade.

Isolation and Screening of Bacterial Strains

Decolorizing Azo dyes

Effluent samples were serially diluted and spread over basal nutrient agar medium pH was adjusted to 7.0 before autoclaving and incubated at 37°C for 5 days. Colonies surrounded by halo (decolorized) zones were picked and streaked on nutrient agar plates containing azo dyes. The plates were re-incubated at 37°C for 3 days to confirm their abilities to decolorize Malachite Green.

Decolorization Assay

1 ml of 24 h old culture of IAK-7 strain was inoculated in 100 ml of nutrient broth containing 50 ppm of Malachite Green and re-incubated at 37°C till complete decolorization occurs. Suitable control without any inoculum was also run along with experimental flasks. 1.0 ml of sample was withdrawn every 12 h and centrifuged at 10,000 rpm for 15 min. Decolorization extent was determined by measuring the absorbance of the culture supernatant, using UV-visible spectrophotometer (Hitachi U 2800), according to Hemapriya et al. (2010).

\[ \text{Decolorization efficiency (\%) = } \frac{\text{Dye (i)} - \text{Dye (r)}}{\text{Dye (i)}} \times 100 \]

Where, Dye (i) refers to the initial dye concentration and Dye (r) refers to the residual dye concentration. Decolorization experiments were performed in triplicates.

Optimization of Various culture Conditions for Bacterial Biomass and Azo dye decolorization by Bacterial Isolates

Effect of Incubation Time and Dye Concentrations

The effect of incubation time and dye concentration on both bacterial biomass and dye decolorizing ability of the isolate was studied. This was carried out by incubating the bacterial strains at different incubation time (0-36 h) and various dye concentrations (100-500 ppm).

Analysis of Biodegraded samples by HPLC and FTIR

Biodegradation of Malachite Green was monitored by High Performance Liquid Chromatography (HPLC) and Fourier Transform Infra Red (FTIR) spectroscopy.
HPLC Analysis of Decolorized Sample

Ten ml of decolorized samples were taken after 24 h of incubation, centrifuged at 12,000 g for 30 min, and filtered through 0.45 µm membrane filter (Millipore). The filtrates were then extracted with diethyl ether and flash evaporated in rotary vacuum evaporator in temperature controlled water bath (50°C) and residues were dissolved in 2 ml of HPLC grade methanol and used for analysis. These extracted samples were analyzed by HPLC having a mobile phase of 50:49.6:0.4% (methanol: water: disodium hydrogen phosphate).

FTIR Analysis of Decolorized Samples

The biodegraded azo dye samples were characterized by FTIR spectroscopy. The analysis results were compared with the control dye. The FTIR analysis was done in the mid IR region (400-4000 cm⁻¹) with 16 scan speed. The samples were mixed with spectroscopically pure KBr in the ratio (5:95). The pellets were fixed in sample holder and then analyzed (Saratale et al., 2009; Shyamala et al., 2014).

Results and Discussion

Environmental biotechnology is constantly expanding its efforts in the biological treatment of colored textile effluents, which is an environmental friendly and low-cost alternative to physico-chemical decomposition processes. Wastewater from textile industries pose a threat to the environment as large amount of chemically different dyes are used for various industrial applications such as textile dyeing and a significant proportion of these dyes enter the environment via wastewater (Dayaram and Dasgupta, 2008). The presence of dyes imparts an intense color to effluents, which leads to environmental as well as aesthetic problems (Singh and Singh, 2006). The treatment of azo-dye-containing wastewaters still presents an arduous task and a technical challenge (Pandey et al., 2007).

Physico-Chemical Analysis of Effluent Samples

Textile effluent samples collected from the local Textile Industries at Kanchipuram were bluish black in color with pungent smell. The average temperature at the sampling sites was around 35°C at day time. The physico-chemical characteristics of the effluent samples were shown in the Table 1. The pH value of the effluent samples was found to be alkaline (10.42). Total Dissolved solids of the effluent sample were found to be found to be 9.64 mg/l. BOD value of the sample was found to be 110 mg/l. However, the COD value was maximum in the effluent sample (1032 mg/ml). The electrical conductivity was recorded as 16.69.

Isolation, Screening and Identification of bacterial strains decolorizing textile dyes

Seven bacterial isolates, designated as IAK-1 to IAK-7 were found to be effective in decolorizing Malachite Green. Out of 07 isolates, IAK-7 was found to be the superior strain with the highest decolorization efficiency (Table 2). Based on the morphological, cultural, biochemical characteristics and 16S rRNA sequencing, the bacterial strain IAK-7 was identified as Chromohalobacter sp. Strain IAK-7.

Optimization of dye decolorizing ability of Chromohalobacter sp. Strain IAK-7

Effect of incubation time

Incubation time played a substantial role in maximizing the decolorization of Malachite Green by Chromohalobacter sp. Strain IAK-7. Dye decolorization by the isolate was found to be growth dependent, since considerable dye decolorization was noticed in the fermentation broth as soon as the bacterial strains entered the late exponential phase (~16 h) and the activity reached the maximum level in stationary phase (~24 h). However Bacillus cereus strain DC11 decolorized Acid Blue-25 (an anthraquinone dye), Malachite Green (triphenylmethane dye) and Basic Blue-25 (azo dye) after 6, 4 and 2 h respectively (Deng et al., 2008) (Fig. 2).

Effect of dye concentration

The influence of different dye concentrations (100-500 ppm) were investigated on decolorization of Malachite Green by Chromohalobacter sp. Strain IAK-7. The results revealed that the decolorization rate of both the isolates was optimized in the presence of initial dye concentration of 100 ppm (Fig. 3). As the dye concentration increased in the culture medium, a gradual and directly proportional decline in color removal was attained. At high concentration (500 ppm), Malachite Green greatly suppressed decolorization ability of Chromohalobacter sp. Strain IAK-7. Similar results were reported by many researchers (Hemapriya and Vijayanand, 2013; Shyamala et al., 2014).
Table 1 Physicochemical characterization of the textile effluent

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Untreated Effluent sample</th>
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<tbody>
<tr>
<td>01.</td>
<td>pH</td>
<td>10.42</td>
</tr>
<tr>
<td>02.</td>
<td>Temperature (°C)</td>
<td>35</td>
</tr>
<tr>
<td>03.</td>
<td>Electrical conductivity</td>
<td>16.69</td>
</tr>
<tr>
<td>04.</td>
<td>TDS (mg/l)</td>
<td>9.614</td>
</tr>
<tr>
<td>05.</td>
<td>COD (mg/l)</td>
<td>1032</td>
</tr>
<tr>
<td>06.</td>
<td>BOD (mg/l)</td>
<td>110 mg/l</td>
</tr>
<tr>
<td>07.</td>
<td>Color (OD at 600 nm)</td>
<td>0.230</td>
</tr>
</tbody>
</table>

Table 2 Decolorization efficiency of bacterial strains on Malachite Green

<table>
<thead>
<tr>
<th>S.No</th>
<th>Isolate</th>
<th>Decolorization Efficiency (%)</th>
</tr>
</thead>
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<tr>
<td>01.</td>
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<tr>
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<td>IAK-6</td>
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<td>IAK-7</td>
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</tr>
</tbody>
</table>

Fig. 1 Chemical structure of Malachite Green

Fig. 2 Effect of Incubation Time on Decolorization of Malachite Green by IAK-7 strain
Fig. 3 Effect of Dye concentration on Decolorization of Malachite Green by IAK-7 strain

Fig. 4 FT-IR analysis of the Malachite Green (control)

Fig. 5 FT-IR analysis of biodegraded Malachite Green sample
Analysis of biodegraded samples by HPLC and FTIR

Decolorization of dyes may take place either by adsorption (Aravindhan et al., 2007) or degradation (Kumar et al., 2007). In the case of adsorption, dyes are only adsorbed onto the surface of bacterial cells, whereas new compounds come into being when dyes are degraded by bacterial enzymes during the degradation process. The exact mechanism of the dye decolorization, the products of biotransformation of Malachite Green were analyzed by HPLC and FTIR.

HPLC Analysis of decolorized samples

The HPLC analysis of Malachite Green dye sample collected at 0 h incubation showed 1 major peak with retention time of 2.080 min. As the decolorization progressed, the biodegradation of parent compound was observed with 03 detectable peaks (retention time 1.761, 2.098 and 2.738) at 24 h extracted metabolites, however major peak was not observed at 2.080 min (Data not shown), clearly indicating the biodegradation of Malachite Green dye by Chromohalobacter sp. Strain IAK-7.

FTIR Analysis of decolorized samples

FT-IR spectra of Malachite Green parent dye displays peaks at 3321, 2945, 1448 and 1411 cm⁻¹, for -OH and -NH stretching vibration, aromatic -CH stretching vibration, >C=O stretching and -N=N- stretching vibration, respectively. Peak at 619 shows the presence of -Br on the dye (Fig. 4). The FT-IR spectra of degradation product displays peak at 3298 cm⁻¹ for -OH stretching indicating hydroxylation of the product, a peak at 2925 and 1450 cm-1 for –CH and >C=O stretching, for the formation of an intermediate with carbonyl group. The peak of -Br is disappeared in the product indicating the debromination or dehalogenation reaction, clearly indicating the degradation of Malachite Green by Chromohalobacter sp. Strain IAK-7 (Fig. 5). Similar result was reported by Shyamala et al. (2014).

References


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