Acid green dye decolorizing bacteria from Yamuna water and textile effluents
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ABSTRACT
Dye is a major pollutant in textile effluents. Biological methods are considered cheap and ecologically safe to remove such pollutants. Present study demonstrated 11 bacterial cultures that were isolated from Yamuna water and textile effluent (6 and 5 cultures respectively) as an important weapon to be used in biological system. Of these, 7 cultures were Gram negative cocci, 3 cultures were Gram negative bacilli and only one culture was Gram positive bacilli. All these cultures were showed decolorization capacity for Acid Green dye (9.3% to 97.1%). The most promising cultures (S.2.1 and S.3.2) for decolorization were from Yamuna water (>90%). The other efficient decolorizing cultures (S.4.2, S.4.3 and S.5.1) were from textile effluent (83.7% to 86.1%). Thin layer chromatography of cultures (S.2.1 and S.3.2) did not show any dye specific spot in comparison to dye sample suggested possibility of biodegradation.

Keywords: Bacteria, pollutants, TLC, spectroscopy, biodegradation.

1. Introduction
Indian textile Industry is one of the leading textile industries in the world. It is also one of the highly polluting industries in the state having potential for creating pollution of water and air. Taking into account the volume and composition of effluent, the textile wastewater is rated as the most polluting among all in the industrial sectors (Awomeso et al., 2010; Vilaseca et al., 2010).

In general, the wastewater from a typical textile industry is characterized by high values of BOD, COD, color and pH (Yusuff and Sonibare, 2004; Tufekci et al., 2007). It induces persistent color coupled with organic load leading to disruption of the total ecological/symbiotic balance of the receiving water stream (Puvaneswari et al., 2006). Incomplete use and the washing operations give the textile wastewater a considerable amount of dyes (Mathur et al., 2005).

The dyes are xenobiotic in nature and in some cases are mutagenic and carcinogenic (Daneshvar et al., 2007; Dafale et al., 2010). Allergic effects of these dyes have also been reported by several scientists (Saunders et al., 2004; Sasaki et al., 2008). Without adequate treatment the dyes can remain in the environment for a long period of time.

Traditionally, both physical and chemical methods such as coagulation, ozonation (Lin and Lin, 1993), precipitation, adsorption by activated charcoal, ultrafiltration, nanofiltration (Akbari et al., 2002), electrochemical oxidation, electrocoagulation (Koby et al., 2003;
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Alinsafi et al., 2005) etc were used in the treatment of the textile industrial effluents (Vilaseca et al., 2010). But both methods have many shortcomings (Lorimer et al., 2001; Babu et al., 2007; Andleeb et al., 2010). In situ degradation of the effluent is a novel method under the biodegradation process. In this method, the microorganisms isolated from the site of pollution can be used for the treatment of the effluent (Olukanni et al., 2006; Puvaneswari et al., 2006).

So, present study was designed to isolate efficient dye decolorizing bacterial strains from the Yamuna water as well as textile effluents. Since the bacterial isolates were originated from the dye contaminated textile wastewater of local industry, so they can easily adapt to the prevailing local environment. Therefore, such bacteria can be used to develop an effective biological treatment system for the wastewaters contaminated with dyes.

2. Material and methods

2.1 Isolation and purification of bacteria from Yamuna water and textile effluents

The bacterial cultures were isolated from 3 Yamuna water and 2 textile effluent samples by serial dilution method and purified by repeated streaking. Each of the pure bacterial culture was maintained in the slants as a Pure or Stock culture.

2.2 Characterization of bacterial cultures

The pure cultures were characterized on the basis of colony morphology (Color, Shape, Elevation and Optical Characteristics) and Gram’s staining reaction.

2.3 Screening of dye decolorizing bacterial cultures

2.3.1 Decolorization in solid culture media

Nutrient agar supplemented with Acid Green dye at the concentration 100 mg/L was poured into petriplates and inoculated with 24 hr. old bacterial culture by simple streaking and incubated at 37°C for 14 days. Uninoculated petriplates were also incubated and treated as control and then observed for disappearance/color change.

2.3.2 Decolorization in liquid culture media

20 mL of nutrient broth supplemented with acid green dye at the concentration 100 mg/L was poured into culture bottles and inoculated with 24 hr old bacterial culture (5% inoculum). Uninoculated culture bottles were also incubated and treated as control. The inoculated and control bottles were incubated at 37°C for 14 days and then observed for disappearance/color change. After completion of the incubation period 10 mL of sample was withdrawn from each of the culture as well as control tube and centrifuged at 10,000 rpm for 10 min. The supernatant were utilized for Spectrophotometric analysis and TLC.

2.3.2.1 Spectrophotometric analysis

Absorbance of the supernatant obtained from the culture as well as the control tubes were recorded at two dye specific wavelengths: 428nm and 602nm (Visible range). Sterilized nutrient broth was used as blank. Three observations were considered for the calculations and the results, are presented as mean values along with standard deviation values. Absorbance values of samples and control at 602nm were utilized to calculate Percent
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decolorization (a measure of decolorization efficiency) for the bacterial cultures according to the following formula:

\[
\text{Percent decolorization} = \frac{(A_0 - A) \times 100}{A_0} \quad (1)
\]

Here

\[A_0 = \text{Absorbance of control at the } \lambda_{\text{max}} \text{ (nm) of the dye (602nm)}.\]
\[A = \text{Absorbance of culture supernatant after incubation period (14 days) at the } \lambda_{\text{max}} \text{ (nm) of the dye (602nm)}.\]

2.3.2.2 TLC analysis

1. TLC analysis was carried out only for the cultures (S.2.1 and S.3.2) showing more than 90% decolorization, after the incubation period in comparison to the dye sample. The distance to the center of the spots and to the solvent front was measured and the Rm value for each spot was calculated according to the following formula:

\[R_m \text{ (relative mobility)} = \frac{\text{Distance substance travels}}{\text{Distance mobile phase (solvent) travels}}\]

3. Result and conclusion

3.1 Isolation and purification of bacteria from Yamuna water and Textile effluents

From all samples of Yamuna water six bacterial cultures were isolated whereas from textile effluents five cultures could be obtained.

In total 11 bacterial cultures could be purified and their respective names are given in Table-1.

### Table 1: No. of bacterial cultures isolated from Yamuna water and Textile effluent samples

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Type of cultures isolated</th>
<th>Name of isolated cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. (Yamuna)</td>
<td>1</td>
<td>S.1.1</td>
</tr>
<tr>
<td>2. (Yamuna)</td>
<td>1</td>
<td>S.2.1</td>
</tr>
<tr>
<td>3. (Yamuna)</td>
<td>4</td>
<td>S.3.1, S.3.2, S.3.3, S.3.4</td>
</tr>
<tr>
<td>4. (Textile)</td>
<td>3</td>
<td>S.4.1, S.4.2, S.4.3</td>
</tr>
<tr>
<td>5. (Textile)</td>
<td>2</td>
<td>S.5.1, S.5.2</td>
</tr>
</tbody>
</table>

3.2 Characterization of bacterial cultures

On the basis of colony morphology it was observed that the cultures from Yamuna water were either white or pale yellow in color, whereas cultures from Textile effluent were orange, yellow, white or colorless. On the basis of the remaining parameters (shape, elevation and optical characteristics) the cultures were showing variable morphology.

All cultures from Yamuna water were Gram negative (Table-2), in which four were cocci in shape while two were bacilli. Majority of the cultures from Textile effluent were also Gram negative in which three were cocci and one was bacilli while only one culture was Gram positive bacilli. On the basis of the above analysis we conclude that there is no variation in
pollution level from 1997 to 2010. As the P-value is quite low at 5% level of significance so
the effect due to three pollutants in three stations is different.

Table 2: Gram staining of different bacterial cultures

<table>
<thead>
<tr>
<th>Culture Name</th>
<th>Gram’s stain</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.1.1</td>
<td>Gram negative</td>
<td>cocci</td>
</tr>
<tr>
<td>S.2.1</td>
<td>Gram negative</td>
<td>cocci</td>
</tr>
<tr>
<td>S.3.1</td>
<td>Gram negative</td>
<td>bacilli</td>
</tr>
<tr>
<td>S.3.2</td>
<td>Gram negative</td>
<td>bacilli</td>
</tr>
<tr>
<td>S.3.3</td>
<td>Gram negative</td>
<td>cocci</td>
</tr>
<tr>
<td>S.3.4</td>
<td>Gram negative</td>
<td>cocci</td>
</tr>
<tr>
<td>S.4.1</td>
<td>Gram negative</td>
<td>bacilli</td>
</tr>
<tr>
<td>S.4.2</td>
<td>Gram negative</td>
<td>cocci</td>
</tr>
<tr>
<td>S.4.3</td>
<td>Gram positive</td>
<td>bacilli</td>
</tr>
<tr>
<td>S.5.1</td>
<td>Gram negative</td>
<td>cocci</td>
</tr>
<tr>
<td>S.5.2</td>
<td>Gram negative</td>
<td>cocci</td>
</tr>
</tbody>
</table>

3.3 Screening of dye decolorizing bacterial cultures: Decolorization of liquid culture media:

All the cultures were showing good growth in the dye supplemented liquid culture media
(NB). On the visual analysis of the culture tubes, 4 cultures (S.1.1, S.3.3, S.4.1, S.5.2 ) were
not showing any color change in comparison to control and remaining 7 culture tubes (S.2.1, S.3.1, S.3.2, S.3.4, S.4.2, S.4.3, S.5.1) were showing change in color from green to blue
(Figure- 2a-2c). After centrifugation when the pellets of the bacterial cultures were observed
visually, it was found that the pellet of all the bacterial cultures was Green in color suggesting
decolorization mainly by adsorption.
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Figure 1: a) Control (Green) b) Culture tube showing no color change (Green) c) Culture tube showing color change from green to blue.

3.3.1 Spectrophotometric analysis

Spectrophotometric analysis showed that all the cultures were showing absorbance values lower than the control, at both the dye specific wavelength i.e.; 428nm and 602nm (Figure-2).

Figure 2: Absorbance values of control and culture supernatant at dye specific wavelengths.

All the 11 cultures were showing decolorization, although to variable extent 9.3% to 97.1%. The culture no.S.1.1, S.3.3, S.4.1, S.5.2 were not showing any color change in comparison to control (Figure-2) but on the basis of spectrophotometric analysis they were showing slight decolorization (9.3% to 44.8%) (Table-3). The cultures showing color change from green to blue in comparison to control included culture no.S.2.1, S.3.1, S.3.2, S.3.4, S.4.2, S.4.3, and
S.5.1. These cultures were showing decolorization in the range from 43.0% to 97.1% (Table-3). Two cultures S.2.1 and S.3.2 were showing more than 90% decolorization at 602nm and considered as the most promising cultures. Cultures S.4.2, S.4.3 and S.5.1 could be also considered as good cultures since they were showing more than 80% decolorization at 602nm.

Table 3: Percent decolorization of 11 cultures at 602nm:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Culture No.</th>
<th>% decolorization at 602nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>S.1.1</td>
<td>41.4%</td>
</tr>
<tr>
<td>2.</td>
<td>S.2.1</td>
<td>91.7%</td>
</tr>
<tr>
<td>3.</td>
<td>S.3.1</td>
<td>44.3%</td>
</tr>
<tr>
<td>4.</td>
<td>S.3.2</td>
<td>97.1%</td>
</tr>
<tr>
<td>5.</td>
<td>S.3.3</td>
<td>44.8%</td>
</tr>
<tr>
<td>6.</td>
<td>S.3.4</td>
<td>43.1%</td>
</tr>
<tr>
<td>7.</td>
<td>S.4.1</td>
<td>9.3%</td>
</tr>
<tr>
<td>8.</td>
<td>S.4.2</td>
<td>83.8%</td>
</tr>
<tr>
<td>9.</td>
<td>S.4.3</td>
<td>83.7%</td>
</tr>
<tr>
<td>10.</td>
<td>S.5.1</td>
<td>86.1%</td>
</tr>
<tr>
<td>11.</td>
<td>S.5.2</td>
<td>28.9%</td>
</tr>
</tbody>
</table>

4. TLC analysis

After TLC analysis the dye sample showed 3 spots having Rm value 0.258, 0.516 and 0.761. (Figure 3) In case of samples S.2.1, S.3.1 no spot was obtained after development. Observations suggest that the acid green dye might have been degraded by the respective bacterial cultures during the incubation period.

Figure 3: After TLC analysis the A). Sample S.2.1 and S.3.2 treated dyes does not show any spot while (B). Untreated dye sample showed 3 spots.

The present study was carried out to isolate bacterial cultures from Yamuna water and textile effluents, having capability to decolorize textile dyes. In totality 11 microbial cultures were purified, which can be termed as native micro flora of the Yamuna and textile effluent samples as no growth was obtained on plates of Nutrient agar, and this ruled out any possibility of the laboratory contamination.

Both Yamuna water and textile effluent contributed nearly equal number of bacterial cultures (6 and 5 cultures respectively). Presence of these cultures in the samples suggests that they are adapted to their polluted environment. Gupta et al., (2013) isolated 26 bacterial cultures.
from textile effluents. No growth of any microorganism was seen in R.O. and tap water this suggests that they were free from microbial contamination.

The 11 bacterial cultures obtained from Yamuna water and textile effluents were characterized on the basis of colony characteristics and Gram’s reaction. When the isolates were compared on this basis of colony characteristics it was found that some isolates from Yamuna were showing quite similar colony characteristics, but most of the isolates from textile effluents were different on this criteria. This result indicates that there is more diversity in the bacterial micro flora of textile effluents. Also it was observed that isolates from the Yamuna and textile effluents were showing quite different colony characteristics, which means that isolates obtained from Yamuna might be different from the isolates of textile effluents.

Yamuna water contributed Gram negative cocci and Gram negative bacilli while textile effluent contributed Gram negative cocci, bacilli and Gram positive bacilli. It might be concluded that Gram negative cocci is more adapted to grow in the polluted environment. Also it can be concluded that the Yamuna and textile effluents were quite distinct in terms of bacterial groups inhabiting there, which is parallel to the results of colony characteristics.

These isolates were further screened for their dye decolorization capabilities. When the bacterial cultures were subjected to the dye decolorization experiment in solid culture medium against the Acid Green dye, it was found that all the 11 isolates were quite ineffective in decolorizing the dye on the solid media. This might be due to the unavailability of the dye molecules for the bacterial culture which were bound in the solid medium.

In case of liquid culture medium it was found that all the cultures were decolorizing the dye Acid green to some extent. Decolorization of dye solution may take place in two ways, either adsorption on the microbial biomass or biodegradation of the dye molecules by the bacterial cells. Dye adsorption may be evident from the inspection of the bacterial growth, those adsorbing the dye will be deeply colored (similar in color to the adsorbed dye), while those degrading the dye will remain colorless. In this experiment all of the isolate were found to be colored with dye Acid green after completion of decolorization experiment, indicating that these isolates were decolorizing the dye mainly by adsorption but the biodegradation also cannot be ruled out.

After spectrophotometric analysis it was observed that the most promising cultures (S.2.1 and S.3.2) showing more than 90% decolorization were from Yamuna water and this might be due to their adaptation for variety of pollutants contributed by various industries. All the other efficient cultures (S.4.2, S.4.3 and S.5.1) showing 83.7% to 86.1% decolorization were from textile effluent suggesting that they developed adaptive capability due to their growth in dye contaminated environment. On TLC analysis for cultures S.2.1 and S.3.2, it was clearly indicated that these cultures decolorized the dye by adsorption as well as biodegradation since no dye specific spot could be obtained after incubation in comparison to dye sample.

Acknowledgment

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5. References
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