Bacterial Decolorization of Textile Dye Effluents

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(Received: 02 August 2011; accepted: 15 September 2011)

The decolorization of textile dye effluent samples containing red dye was studied by using some potential bacterial strains (Bacillus sp, Pseudomonas sp, Staphylococcus sp and Enterobacter sp) isolated from the effluent sample. The isolated bacterial strains was inoculated separately on to the nutrient broth containing the supernatant of dye effluent sample obtained by centrifugation and incubated under controlled conditions for several days (up to 14 days). The decolorization in the sample by the inoculated organism was measured spectrophotometrically at regular intervals at 530 nm. Also the chemical oxygen demand for the effluent sample before and after inoculation with the test isolates were measured and the extent of reduction in the organic contaminant (dye) in the sample was calculated.

Key Words: Azo dyes, Decolorization, Dye degrading bacteria.

Dyes are widely used in textile, paper, printing, colour photography, pharmaceuticals, cosmetics and many other industries. These are coloured organic compounds which contain certain unsaturated groups responsible for colour and these are not easily degradable. Decolorization or degradation of dyes by microbes is an eco-friendly and cost-competitive alternative to chemical and physical methods. There are few known microorganisms that have the ability to reductively cleave azo bonds under aerobic conditions. The colour and dyes of the effluent persist in aquatic bodies and affect the aesthetic value of the environment. In addition, they form a thin film on the surface and inhibit the photosynthetic activity and affect the aquatic biota. Dye composition might be an important factor causing unstable decolorization because the textile effluents contain a wide range of structurally diverse dyes. The degree of decolorization depends on the type of the dye, molecular weight and substitution groups of the dye molecule. Treatment of waste water containing dyes – usually involves physical and chemical methods. Over the past decades, biological decolorization has been investigated as a method to transform, degrade or mineralize azo dyes because of its eco-friendly and cost-effective characteristics.

Globally about 7×10^5 tonne of dyes are produced annually of which 10-15% flow out in effluents during dyeing process. These dyes released into aquatic and terrestrial environment through the effluents from textile and dye stuff industries and are not normally treated by conventional waste water treatment system.
This study includes the investigation for the presence of potential bacteria from the textile industry effluents, their isolation, identification and study for their decolorizing activity on the effluent sample containing red dyes.

**MATERIALS AND METHODS**

**Collection of Sample**

The discharged textile effluent samples containing reactive textile dye red ASBO dye were collected from textile industry in Sankarankovil, Tirunelveli District.

**Isolation and Identification of effluent adapted bacteria**

The effluent samples from textile industry were collected, processed and tested to isolate the effluent adapted bacterial strains. The sample was serially diluted and plated onto nutrient agar medium. The colonies formed on agar plates were further studied and confirmed by standard biochemical tests. The organisms identified include *Bacillus sp*, *Pseudomonas sp*, *Staphylococcus sp*, *Enterobacter sp*, and these are confirmed by plating them onto respective selective agar medium plates. The isolated organisms were further studied for decolorization of dye in the collected textile effluents.

**Measurement of Dye Discolouration**

The sample dyes (10ml) were centrifuged at 11,000 rpm for 10 minutes and the supernatant of the sample mixed with 25 ml of nutrient broth. The initial absorbance of this dye sample along with nutrient broth were measured at the maximum absorption of 530 nm. Then 0.1 ml of the isolated bacterial cultures were added separately with the nutrient broth containing dye sample and incubated at 37°C. At regular intervals up to 14 days, the dye concentrations were measured using visible spectrophotometer. Based on the absorbance (OD) values obtained at different intervals, the extent of decolorization of the initial dye concentration present in the effluent sample were determined.

\[
\text{Decolorization\%} = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100
\]

**Estimation of Chemical Oxygen Demand**

The value of chemical oxygen demand depicts the amount of chemical contaminants in the particular sample. By this measurement the concentration or the extent of dye contaminant in the textile effluent samples can be calculated. The COD values of the sample was compared before inoculating the organisms onto the effluent dyes (Initial COD) and finally after the period of decolorization. The COD values were determined by the following equation.

\[
\text{COD (mg/l)} = \frac{(A-B) \times N \times \text{Equivalent weight of oxygen \times 1000}}{\text{Volume of sample}}
\]

**RESULTS**

**Spectral Analysis of Dye Discoloration**

The initial absorbance of textile effluent sample was determined at 530 nm. This dye sample was inoculated separately with the four isolated bacterial strains (*Bacillus sp*, *Pseudomonas sp*, *Staphylococcus sp*, *Enterobacter sp*) and inoculated under controlled conditions of definite temperature and pH. The absorbance values of the inoculated samples was periodically observed (at 2nd, 4th, 6th, 8th, 10th, 12th and 14th day) and the extent of dye degradation by four different organisms was noted separately (Table 1).

The initial absorbance of the dye sample was increased after the inoculation of organisms up to their log phase and then the values decreased constantly at the period of degradation. The dye sample was found to be well degraded by the *Pseudomonas sp* (78.62%) when compared to other three organisms (Fig. 1).

**COD Determination**

The COD was measured by calculating the amount of oxidizing agent (Potassium dichromate) consumed during oxidation of organic matter (non biodegradable) under acidic condition. Table 2 shows the significant decrease in the value of COD from 3800 mg/ml to 1100 mg/ml. this reduction in the COD value indicates the extent of dye degradation, that is the reduction in the concentration of dye in the effluent sample by the degradative action of the inoculated organisms (Fig. 2).
Table 1. Discoloration of red dye using different bacterial strains

<table>
<thead>
<tr>
<th>Time of Observation</th>
<th>Pseudomonas sp</th>
<th>Bacillus sp</th>
<th>Staphylococcus sp</th>
<th>Enterobacter sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before inoculation</td>
<td>0.262</td>
<td>0.262</td>
<td>0.262</td>
<td>0.262</td>
</tr>
<tr>
<td>After inoculation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd day</td>
<td>1.204</td>
<td>2.134</td>
<td>0.992</td>
<td>1.273</td>
</tr>
<tr>
<td>4th day</td>
<td>1.576</td>
<td>2.001</td>
<td>1.131</td>
<td>1.943</td>
</tr>
<tr>
<td>6th day</td>
<td>0.935</td>
<td>1.168</td>
<td>0.984</td>
<td>1.684</td>
</tr>
<tr>
<td>8th day</td>
<td>0.780</td>
<td>1.089</td>
<td>0.380</td>
<td>1.103</td>
</tr>
<tr>
<td>10th day</td>
<td>0.202</td>
<td>0.234</td>
<td>0.208</td>
<td>0.210</td>
</tr>
<tr>
<td>12th day</td>
<td>0.103</td>
<td>0.174</td>
<td>0.114</td>
<td>0.185</td>
</tr>
<tr>
<td>14th day</td>
<td>0.056</td>
<td>0.105</td>
<td>0.075</td>
<td>0.155</td>
</tr>
</tbody>
</table>

Table 2. Estimation of chemical oxygen demand

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Blank (A)</th>
<th>Sample (B)</th>
<th>Initial COD (mg/ml)</th>
<th>COD after Degradation (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas sp</td>
<td>28</td>
<td>12</td>
<td>3800</td>
<td>1100</td>
</tr>
<tr>
<td>Bacillus</td>
<td>14</td>
<td></td>
<td></td>
<td>1380</td>
</tr>
<tr>
<td>Staphylococcus sp</td>
<td>15</td>
<td></td>
<td></td>
<td>1280</td>
</tr>
<tr>
<td>Enterobacter sp</td>
<td>13</td>
<td></td>
<td></td>
<td>1493</td>
</tr>
</tbody>
</table>

Fig. 1. Decolorization (%) of red dye

Fig. 2. Cod determination of red dye
DISCUSSION

Environmental pollution has been recognized as one of the major problems of the modern world. Biological decolorization has been investigated as a method to transform or mineralize azo dyes\(^7\). The capability of microorganisms to decolorize or reduce various kinds of dye residues depends on the characteristics of each microbe\(^8\).

A novel bacterial strain \textit{Pseudomonas sp} capable of decolorizing reactive textile dye Red BL1 isolated from soil sample collected from contaminated sites of textile industry from Solapur, India\(^9\). Bacterial strain of \textit{Enterobacter sp} has been reported with decolorizing ability against both azo and anthroquinone dyes\(^10\). COD reduction of secondary treated tannery waste water at various time intervals was studied and reported with COD removal of 61\%\(^11\).

In this present study \textit{Pseudomonas sp} was found to have more degradative activity on red dye ASBO (78.62\%) (Figure 2) when compared to \textit{Bacillus sp}, \textit{Enterobacter sp} and \textit{Staphylococcus sp}. This is observed by the effective discoloration of the red dye in the sample and its absorbance values at various intervals. Also the values of COD decreased maximally from 3800 mg/ml to 1100 mg/ml in sample inoculated with \textit{Pseudomonas sp} and the minimum degradation from 3800 mg/ml to 1493 mg/ml was shown by the \textit{Enterobacter sp}.

REFERENCES