Congo Red (Azo dye) decolourization by local isolate VT-II inhabiting dye effluent exposed soil

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ABSTRACT

A variety of samples were collected to isolate Congo red decolourizing bacteria from Baddi (H.P). Total 7 strains were obtained with potential for Congo red decolourization. On primary screening, VT-II, an aerobic gram positive bacillus (Bacillus sp.) was found to have maximum observable Azo dye decolourization activity. The isolate on secondary screening exhibited 70% decolourization. However, under optimal conditions of pH (7.0) and temperature (40°C), maximum decolourization percentage was 85%. Further studies are in progress to explore the possibilities of evolving authentic and commercially viable strain to be used for bioremediation of azo dye containing effluents.

Key words: Decolourization, azo dye, Congo red, bioremediation, Screening.

1. Introduction

Azo dyes (-N = N group) form the largest class of synthetic dyes with a variety of colour and structure (Minussi et al., 2001; and Gharbani et al., 2008). These dyes account for approximately 60-70% of all dyes used in food and textile manufacture. The worldwide production of these dyes is currently estimated at 4,50,000 tons/year with almost 50,000 tons/year lost in effluent during application and manufacture. At the time of production and application about 2-50% of these dyes are lost as waste effluents (Sokmen et al., 2001; and Olukanni et al., 2009).

Congo red (sodium salt of benzidinediazo-bis-1- naphtylamine-4 sulfonic acid) has been reported to be a carcinogenic direct diazo dye used for colouration of paper products (Cripps et al., 1990; and Jaladoni-Buan et al., 2010). The dye infested soils are detrimental to the growth of plants also. Extensive work has been carried out on the pollution problems associated with the discharge of dye effluent from industries. It has been documented that the safe method for azo dye biodegradation is combined aerobic treatment (Mabrouk and Yusef, 2008; and Olukanni et al., 2009). Many organisms such as Bacillus, E. coli, Klebsiella, Enterobacter, Pseudomonas and a group of fungi, yeast have been studied for their decolorization of congo red dye (Chen et al., 2003; and Jaladoni-Buan et al., 2010). However, with increasing industrialization, the magnitude of the problem is constantly on rise and needs a plausible microbial solution. Thus, screening of microflora with effective decolourizing ability could evolve new indigenous strains to be used as bioremediation tools for removal of azo dyes.

Keeping in view, the above cited facts, the present study was carried out to isolate and screen microbial strains for their ability to decolorize azo dyes aerobically and optimize the pH and temperature required for effective decolourization of the isolate.
2. Materials and Methods

2.1 Collection of Samples

The soil samples were collected at random in duplicate from four different sites in and around Baddi, Distt. Solan (H.P), India under aseptic conditions in sterile plastic bottles. Baddi is an industrial town located in Himachal Pradesh, India (30°57’ 31.08”N, 76° 47’ 17.87” E) at 1375 ft. above sea level.

2.2 Enrichment and isolation of Congo red decolourizing bacteria

Bacteria were isolated from the soil samples. Total 7 different isolates were obtained using standard method of cultivation.

For the enrichment of Congo red decolourising bacteria, mineral salts medium (Cohen-Bazire et al., 1957) with 1% congo red was used. The flasks containing the 48 cultures were incubated under shaking condition (120 rpm) in REMI-CIS-24BL at 37°C.

The decolorizing cultures were further enriched by transferring aliquots of enriched cultures into fresh media and processed as earlier. This step was repeated once more.

The isolations were done from the final enriched culture on nutrient agar by streak plate method followed by sub culturing for pure culture isolation. The organisms were identified tentatively on the basis of microscopic characteristics.

2.3 Screening for decolourization activity

10 ppm congo red dye solution in distilled water was scanned spectrophotometrically (Systronics 2202) to find out maximum absorbance ($\lambda_{\text{max}}$) for congo red dye.

Decolourization experiments were done in 100 ml conical flasks containing 50 ml of the nutrient broth with 50 ppm. Congo red. Three flasks each for an isolate were inoculated with approximately 20 mg dry cell mass (Biomass measured using OD 600). The inoculated flasks were incubated at 37°C. The decolourization was measured spectrophotometrically at $\lambda_{\text{max}}$ (495.2 nm). Three millilitre sample was collected at 72 h, centrifuged at 4000 g for 15 minutes to exclude biomass. Percentage decolourisation was calculated as per method documented. (Olukanni et al., 2006).

$$\text{Decolourization} \% = \frac{A_0 - At}{A_0} \times 100$$

Where,

$A_0$ = Absorbance of the blank (dye solution)

$At$ = Absorbance of the treated dyes solution at specific time.
2.4 Percentage decolourisation at different pH and temperature

Congo red- nutrient broth was prepared with 50 ppm of dye concentration. pH of the broth was adjusted from 2.0 to 10.0 using NaOH or HCl as per requirement. Approximately 20 mg dry cell mass of VT-II was added to 50 ml. of dye containing presterilised broth in conical flasks. The experiments were performed in triplicate. The flasks were incubated at 37°C for 24 hrs. The absorbance at 24 hr. incubation at 495.2nm was recorded to calculate percentage decolourisation.

The percentage decolourisation by VT-II was also determined at different temperatures (20°C, 25°C, 30°C, 37°C, 40°C, 45°C and 50°C) to find out optimal conditions of temperature for maximal decolorization using same standard method.

3. Results

Total 7 local microbial isolates were screened for Congo red (azo) dye decolourization. Among all isolates, the maximum decolourization was observed for VT-II (Bacillus sp.). The isolates VT-I, V, VI followed VT-II with almost same results.

On secondary screening of isolates for percentage azo dye decolourization it was observed that VT-II had maximum decolourization (70%) of Congo red followed by VT-I (68.4%) at λmax (495.2 nm)

The studies on effect of different pH on percentage decolourization of azo dye was also evaluated. VT-II showed maximum activity (70.86%) at pH 7.0. (Table-1).

<table>
<thead>
<tr>
<th>pH</th>
<th>Initial Absorbance at (495.2 nm)</th>
<th>Final Absorbance at (495.2 nm)</th>
<th>Decolourization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Test</td>
<td>Control Test pH ± S.D.</td>
<td>Control Test ±S.D</td>
</tr>
<tr>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.97 ± 0.0282</td>
</tr>
<tr>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>1.83 ± 0.0141</td>
</tr>
<tr>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>3.92 ± 0.0282</td>
</tr>
<tr>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.04 ± 0.0360</td>
</tr>
<tr>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
<td>6.28 ± 0.0424</td>
</tr>
<tr>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
<td>7.64 ± 0.050</td>
</tr>
<tr>
<td>8.00</td>
<td>8.00</td>
<td>8.00</td>
<td>7.88 ± 0.0223</td>
</tr>
<tr>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
<td>8.84 ± 0.0640</td>
</tr>
<tr>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>9.95 ± 0.0424</td>
</tr>
</tbody>
</table>

It was also observed that the decolorization showed approximately four fold rise with increasing pH 2.0 to 7.0. Further increase in pH of the media led to decline in decolourization percentage with minimum activity (7.0%) at pH. 10. (Figure 1)
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The influence of different temperatures on percentage decolourization of Azo dye was also carried out at optimized pH 7.0. (Table-2).

**Table 2: Percentage decolourization of Azo dye by VT-II at different temperatures**

| Tempt . (°C) | pH | Absorbance (495.2 nm) | Decolour-  
<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Final</th>
<th>S.D.</th>
<th>Density (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
<td>Control</td>
<td>Test ± S.D.</td>
</tr>
<tr>
<td>20°C</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
<td>7.97 ± 0.2404</td>
</tr>
<tr>
<td>25°C</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
<td>8.05 ± 0.2121</td>
</tr>
<tr>
<td>30°C</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
<td>8.11 ± 0.0141</td>
</tr>
<tr>
<td>35°C</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
<td>7.73 ± 0.2404</td>
</tr>
<tr>
<td>37°C</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
<td>7.61±0.05</td>
</tr>
<tr>
<td>40°C</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
<td>7.07±0.0921</td>
</tr>
<tr>
<td>45°C</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
<td>7.40±0.3748</td>
</tr>
<tr>
<td>50°C</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
<td>6.71±0.2969</td>
</tr>
</tbody>
</table>

Maximum activity (85%) was recorded at 40°C. The percentage decolourization increased with increasing temperatures till 40°C.

The increase in temperature beyond 37°C led to decline in decolourization activity of the strain (Figure 2).
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Figure 2: Plot of Temperature Vs decolourization (%) of Congo red

4. Discussion

The bacterial isolates exhibited variable decolourization ability for Congo red. VT-II strain was observed to show maximum decolourization at pH 7.0 and temperature 40°C.

The influence of temperature and pH was also observed. A large number of researchers have carried out similar work to investigate effect of pH and temperatures on decolourization of dyes by microorganisms (Raghukumar et al., 1996; Shedbalkar et al., 2008; Jadhav et al., 2008; Wang et al., 2009; and Saratale et al., 2009). The decolourization activity was pH and temperature dependent. The optimal conditions for decolourisation by this isolate were found to be at pH 7.0 and temperature 40°C. Since, each microbial strain and its enzymes are highly specific to pH and temperature, so decrease or increase in decolourization extent of dye might have been due to variation in pH and temperature.

Nosheen et al., 2010 documented that behaviour of each strain varied for dye decolourization with variation in pH. Similar studies have been carried out on other azo dye and bacterial combinations to determine optimal conditions for maximum decolourization efficiency (Maier et al., 2004; and Olukanni et al., 2009). Our work corroborated with earlier findings that favourable pH for dye decolourisation by bacterial strains has been found to be 7-8. Suwannawong et al., 2010 observed that optimal pH for congo red decolourization was at 6.0-7.0. The significant suppression of decolourizing activity at different pH and temperatures might be due to loss of cell viability or deactivation of enzymes responsible for decolourization.

Thus, it is evident that the isolate VT-II had maximum decolourization activity at pH 7.0 and temperature 40°C and could be an effective bioremediation tool under these conditions for treatment of Congo red containing industrial effluent.

5. Conclusion

The study led us to conclude that the isolate had adequate potential to decolourize the azo dye. Thus, the isolate could be exploited for its bioremediation ability. Moreover, further studies on it could explore new tools and techniques to evolve advanced and effective microbial solutions for treatment of dye industry effluents.
Acknowledgement

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6. References


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