Comparison of Bacterial Decolourization of Reactive Textile Dyes under Static and Shaking Conditions

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Abstract: Water pollution control is at present one of the major areas of scientific activity. While coloured organic compounds generally impart only a minor fraction of the organic load to wastewater, their colour renders them aesthetically unacceptable. Colour is one of the most obvious indicators of water pollution and discharge of highly coloured synthetic dye effluents can be damaging to the receiving water bodies. The decolourization of Reactive dyes by bacterial isolates and bacterial consortium under static and shaking condition was investigated in the present study. The decolourisation of reactive dyes was maximum in shaking condition when compared to the static condition. Maximum decolourisation of Reactive orange - 16 was observed by the bacterial consortium under shaking condition (98.65%) followed by Bacillus odyssey (72.92 %), Bacillus thuringiensis (68.81 %), Bacillus subtilis (66.21 %), Bacillus cereus (63.03 %), Alcaligenes sp. (55.40 %) and Nocardiopsis alba (51.38 %). Maximum decolourisation of Reactive Black – B was observed by the bacterial consortium under shaking condition (97.80 %) followed by Bacillus odyssey (68.85 %), Bacillus thuringiensis (64.39 %), Bacillus subtilis (63.77 %), Bacillus cereus (62.12 %), Alcaligenes sp. (58.68 %) and Nocardiopsis alba (56.72 %). Maximum decolourisation of Reactive Yellow – MR was observed by the bacterial consortium under shaking condition (96.30 %) followed by Bacillus odyssey (67.55 %), Bacillus thuringiensis (66.60 %), Bacillus subtilis (60.00 %), Bacillus cereus (55.00 %), Alcaligenes sp. (49.52 %) and Nocardiopsis alba (45.15 %). It was concluded that the decolourization of reactive dyes was maximum in shaking condition when compared to the static condition.

Key words: Reactive Dyes • Shaking Condition • Static Condition • Bacteria And Decolourization

INTRODUCTION

Textile dyes are one of the most prevalent type chemicals in use today. Around 10,000 different dyes with an annual production of more than 7105 metric tonnes worldwide are commercially available [1]. Two percent of dyes that are produced are discharged directly in aqueous effluent and 10 % are subsequently lost during the textile colouration process [2]. Some of the azo dyes, xanthene dyes and anthraquinone dyes are known to be very toxic and mutagenic to living organisms. With the increasing use of a wide variety of dyes, pollution by dye–waste water is becoming increasingly alarming. The two major sources of release of dyes into the environment are the textile and dyestuff manufacturing industries [3]. Reactive dyes, including many structurally different dyes, are extensively used in the textile industry because of their wide variety of color shades, high wet fastness profiles, ease of application, brilliant colors and minimal energy consumption. The three most common groups are azo, anthraquinone and phthalocyanine dyes [4] most of which are toxic and carcinogenic [5]. Disposal of these

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dyes into the environment causes serious damage, since they may significantly affect the photosynthetic activity of hydrophytes by reducing light penetration and also they may be toxic to some aquatic organisms due to their breakdown products [6].

In the textile industry different structures of synthetic dyes are often used during fiber processing and therefore the effluents produced are markedly variable in chemical composition, including organics, nutrients, sulphur compounds, salts and different toxic substances.

In biological treatment processes, various physicochemical operational parameters, such as the level of agitation, oxygen, temperature, pH, dye structure, dye concentration, supplementation of different carbon and nitrogen sources, electron donor and redox mediator, directly influence the bacterial decolorization performance of azo dyes. Thus, to make the process more efficient, faster and practically applicable, prior determination of the effect of each factor on the bacterial decolorization of azo dyes is essential [7].

The ability of bacteria to metabolize azo dyes has been investigated by a number of research groups. Under aerobic conditions azo dyes are not readily metabolized. Although, Kulla [8] reported the ability of Pseudomonas strains to aerobically degrade certain azo dyes. However, the intermediates formed by these degradative steps resulted in disruption of metabolic pathways and the dyes were not actually mineralized. Under anaerobic conditions, such as anoxic sediments, many bacteria gratuitously reduce azo dyes reportedly by the activity of unspecific, soluble, cytoplasmic reductases, known as azoreductases. These enzymes are reported to result in the production of colourless aromatic amines which may be toxic, mutagenic and possibly carcinogenic to animals.

Decolorization of azo dyes occurs under strictly anaerobic, facultative anaerobic and aerobic conditions by different trophic groups of bacteria. It has been reported that under anaerobic conditions the feeding of a carbon source, such as simple substrates to a bacterial culture like glucose, starch, acetate and ethanol and more complex ones, such as whey and tapioca, could affect the decolorization process and that most of the reduction of azo dyes to amines occurs during active bacterial growth. It was also observed that the decolorization of azo dyes was far superior under strictly anaerobic conditions, although it also occurred under semi-anaerobic ones [9]. In the present study, the decolorization of Reactive dyes by bacterial isolates and bacterial consortium was studied under Static condition and Shaking condition.

**MATERIALS AND METHODS**

**Dyes Used:** Reactive azo dyes were used in this study. The dye samples were commercially graded and supplied by the dealers of “SIGMA Aldrich, U.S.A”. Reactive azo dyes used in this research were:

- Reactive Orange – 16 (λm = 480 nm)
- Reactive Black – B (λm = 600 nm)
- Reactive Yellow – MR (λm = 600 nm)

**Bacterial Isolates Selected for the Present Research:** Six different bacterial isolates were isolated and identified from the textile dye effluent. The isolated bacterial isolates were identified and characterized as Bacillus odyssey, Bacillus thuringiensis, Bacillus subtilis, Bacillus cereus, Alcaligenes sp. and Nocardiopsis alba.

**Maintenance of Bacterial Isolates:** 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.

**Effect of Different Conditions on Decolourization of Reactive Textile Dyes:** The Reactive dye decolourization was assessed in different conditions viz., Static condition and Shaking condition. One milliliter of the bacterial cell suspension was inoculated in 250 ml Erlenmeyer flasks containing Nutrient Broth and Reactive azo dyes (500 mg/l). The cultures were incubated at 37 °C for 4 days in a static condition and rotary shaker running at 180 rpm.

Decolourization assay was measured in the terms of percentage decolourization using spectrophotometer. The percentage decolourization was calculated by following the formula of Dafale et al. [10].

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

**RESULTS AND DISCUSSION**

Bacterial biodegradation of non-azo dyes has only recently been studied. It has been observed that several bacteria can degrade anthraquinone dyes [11,12]. Aerobic decolorization of triphenylmethane dyes has also been demonstrated. In phthalocyanine dyes, reversible reduction and decolorization under anaerobic conditions have been observed [13, 14]. Generally, the decolorization of azo dyes occurs under conventional anaerobic, facultative anaerobic and aerobic conditions by different groups of bacteria. The mechanism of microbial degradation of azo
Table 1: Decolourization of Reactive Orange – 16 under different conditions

<table>
<thead>
<tr>
<th>S.No</th>
<th>Bacterial isolates</th>
<th>Static condition</th>
<th>Shaking condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Final OD</td>
<td>% Decolourization</td>
</tr>
<tr>
<td>1.</td>
<td><em>Bacillus odyssey</em></td>
<td>0.350</td>
<td>70.66 %</td>
</tr>
<tr>
<td>2.</td>
<td><em>Bacillus thuringiensis</em></td>
<td>0.394</td>
<td>66.97 %</td>
</tr>
<tr>
<td>3.</td>
<td><em>Bacillus subtilis</em></td>
<td>0.427</td>
<td>64.20 %</td>
</tr>
<tr>
<td>4.</td>
<td><em>Bacillus cereus</em></td>
<td>0.466</td>
<td>60.93 %</td>
</tr>
<tr>
<td>5.</td>
<td><em>Alcaligenes sp.</em></td>
<td>0.560</td>
<td>53.05 %</td>
</tr>
<tr>
<td>6.</td>
<td><em>Nocardiopsis alba</em></td>
<td>0.605</td>
<td>49.28 %</td>
</tr>
</tbody>
</table>

Initial OD of Reactive Orange – 16 at 480 nm = 1.193

Table 2: Decolourization of Reactive Black – B under different conditions

<table>
<thead>
<tr>
<th>S.No</th>
<th>Bacterial isolates</th>
<th>Static condition</th>
<th>Shaking condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Final OD</td>
<td>% Decolourization</td>
</tr>
<tr>
<td>1.</td>
<td><em>Bacillus odyssey</em></td>
<td>0.419</td>
<td>67.21 %</td>
</tr>
<tr>
<td>2.</td>
<td><em>Bacillus thuringiensis</em></td>
<td>0.479</td>
<td>62.51 %</td>
</tr>
<tr>
<td>3.</td>
<td><em>Bacillus subtilis</em></td>
<td>0.491</td>
<td>61.58 %</td>
</tr>
<tr>
<td>4.</td>
<td><em>Bacillus cereus</em></td>
<td>0.511</td>
<td>60.01 %</td>
</tr>
<tr>
<td>5.</td>
<td><em>Alcaligenes sp.</em></td>
<td>0.553</td>
<td>56.72 %</td>
</tr>
<tr>
<td>6.</td>
<td><em>Nocardiopsis alba</em></td>
<td>0.588</td>
<td>53.99 %</td>
</tr>
</tbody>
</table>

Initial OD of Reactive Black – B at 600 nm = 1.278

dyes involves the reductive cleavage of azo bonds with the help of azoreductase enzymes under anaerobic conditions that resulted in the formation of colorless solutions containing potentially hazardous-aromatic amines [15]. The resulting intermediate metabolites (e.g., aromatic amines) are further degraded aerobically or anaerobically [16].

Many recent studies focus on the utilization of microbial biocatalysts to remove dye from the effluent. Extensive studies have been carried out to determine the role of the diverse groups of bacteria in the decolorization of azo dyes [17]. The bacterial decolorization and degradation of these dyes has been of considerable interest since it can achieve a higher degree of biodegradation and mineralization, is applicable to a wide variety of azo dyes, is inexpensive and environmentally-friendly and produces less sludge [18-20].

The decolourization of Reactive Orange – 16 by bacterial isolates and bacterial consortium under static and shaking condition was investigated and the results were furnished in Table – 1. Among the six bacterial isolates tested, maximum decolourization of Reactive orange - 16 was observed by the bacterial consortium under shaking condition (98.65 %) followed by *Bacillus odyssey* (72.92 %), *Bacillus thuringiensis* (68.81 %), *Bacillus subtilis* (66.21 %), *Bacillus cereus* (63.03 %) and *Alcaligenes sp.* (55.40 %). The bacterial isolate *Nocardiopsis alba* showed minimum decolourization of Reactive Orange – 16 (51.38 %). The decolourization of Reactive Orange – 16 was maximum in shaking condition when compared to the static condition.

The decolourization of Reactive Black – B by bacterial isolates and bacterial consortium under static and shaking condition was studied and the results were given in Table – 2. Among the six bacterial isolates tested, maximum decolourization of Reactive Black – B was observed by the bacterial consortium under shaking condition (97.80 %) followed by *Bacillus odyssey* (68.85 %), *Bacillus thuringiensis* (64.39 %), *Bacillus subtilis* (63.77 %), *Bacillus cereus* (62.12 %) and *Alcaligenes sp.* (58.68 %). The bacterial isolate *Nocardiopsis alba* showed minimum decolourization of Reactive Black – B (56.72 %). The decolourization of Reactive Black – B was maximum in shaking condition when compared to the static condition.

The decolourization of Reactive Yellow – MR by bacterial isolates and bacterial consortium under static and shaking condition was tested and the results were presented in Table – 3. Among the six bacterial isolates tested, maximum decolourization of Reactive Yellow – MR was observed by the bacterial consortium under shaking condition (96.30 %) followed by *Bacillus odyssey*
### Table 3: Decolourization of Reactive Yellow – MR under different conditions

<table>
<thead>
<tr>
<th>S.No</th>
<th>Bacterial isolates</th>
<th>Final OD</th>
<th>% Decolourization</th>
<th>Static condition</th>
<th>Final OD</th>
<th>% Decolourization</th>
<th>Shaking condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bacillus odyssey</td>
<td>0.403</td>
<td>65.40%</td>
<td>0.378</td>
<td>67.55%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Bacillus thuringiensis</td>
<td>0.415</td>
<td>64.37%</td>
<td>0.389</td>
<td>66.60%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Bacillus subtilis</td>
<td>0.489</td>
<td>58.02%</td>
<td>0.466</td>
<td>60.00%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Bacillus cereus</td>
<td>0.551</td>
<td>52.70%</td>
<td>0.524</td>
<td>55.00%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Alcaligenes sp.</td>
<td>0.613</td>
<td>47.38%</td>
<td>0.588</td>
<td>49.52%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Nocardiopsis alba</td>
<td>0.670</td>
<td>42.48%</td>
<td>0.639</td>
<td>45.15%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Initial OD of Reactive Yellow – MR at 600 nm = 1.165

(67.55 %), Bacillus thuringiensis (66.60 %), Bacillus subtilis (60.00 %), Bacillus cereus (55.00 %) and Alcaligenes sp. (49.52 %). The bacterial isolate Nocardiopsis alba showed minimum decolourization of Reactive Yellow – MR (45.15 %). The decolourization of Reactive Yellow – MR was maximum in shaking condition when compared to the static condition.

Various groups have reported that bacterial degradation is best under aerobic and shaking conditions [21]. In order to test this was also true for our isolates, a study was done under static and shaking conditions. The degradation of the dye also appeared to be dependent on the shaking culture of the culture.

Some studies have reported that during bacterial degradation of azo dyes both oxidative and reductive enzymes play a role. These earlier results suggest that for efficient color removal aeration and agitation, which increases the concentration of oxygen in the solution, should be avoided [22]. It was also observed that the effect of oxygen on azo reduction is irreversible [17]. Quantitative analysis of the correlation between DO and the decolorization rate in the decolorization of C.I. Reactive Red 22 by Escherichia coli has been studied in detail [23]. In this study under static conditions (No agitation) the DO level in the culture immediately dropped to nearly zero and thus decolorization occurred, whereas under agitation at a rate of 200 rpm the DO level only decreased to 0.5 mg/L and no significant color removal was observed.

The intermediates formed during azo dye reduction reaction, like the simple aromatic compounds, are degraded via hydroxylation and ring-opening in the presence of oxygen [17]. The aerobic condition is required for the complete mineralization of the azo dye molecules. Thus, for the most effective effluent treatment an anaerobic process with subsequent aerobic treatment can be used to decolorize wastewaters containing dyes and improve their biodegradability [24].

### CONCLUSIONS

The conclusions of the present research work were:
- The decolourization of reactive dyes was maximum in shaking condition when compared to the static condition.
- Maximum decolourization of Reactive orange - 16 was observed by the bacterial consortium undershaking condition (98.65 %) followed by Bacillus odyssey (72.92 %), Bacillus thuringiensis (68.81 %), Bacillus subtilis (66.21 %), Bacillus cereus (63.03 %), Alcaligenes sp. (55.40 %) and Nocardiopsis alba (51.38 %).
- Maximum decolourization of Reactive Black – B was observed by the bacterial consortium undershaking condition (97.80 %) followed by Bacillus odyssey (68.85 %), Bacillus thuringiensis (64.39 %), Bacillus subtilis (63.77 %), Bacillus cereus (62.12 %), Alcaligenes sp. (58.68 %) and Nocardiopsis alba (56.72 %).
- Maximum decolourization of Reactive Yellow – MR was observed by the bacterial consortium undershaking condition (96.30 %) followed by Bacillus odyssey (67.55%), Bacillus thuringiensis (66.60 %), Bacillus subtilis (60.00 %), Bacillus cereus (55.00 %), Alcaligenes sp. (49.52 %) and Nocardiopsis alba (45.15 %).

### REFERENCES


