Biodegradation of reactive orange 16 (RO-16) dye in packed bed bioreactor using seeds of Ashoka and Casuarina as packing medium

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The main objective of this work was to evaluate the performance of Ashoka and Casuarina (abundantly available in India as agrowaste) seeds as packing material with immobilized mixed culture of microorganisms for biodegradation of reactive orange dye (RO-16) in the continuous packed bed bioreactor. The percent removal of RO-16 was increased with time in both cases and attained constant value on the 10th d of operation with maximum removal 63.5±5% and 69.27±5%, respectively for Ashoka and Casuarina seeds, at inlet flow rate of 1.0 LPH and initial concentration of 500 ppm. The second order of kinetics applied and it fitted well for both packing materials. The results demonstrate that the selected seeds have potential as to be used as packing material for biodegradation application in bioreactors.

Keywords: Ashoka, bioreactor, Casuarina, FT-IR, mixed culture, reactive orange 16

Introduction

Synthetic dyes are integral ingredient of textile, paper, cosmetics, food and pharmaceutical industries because of their ease of production, fastness and variety in colour compared to natural dyes. Some of them are dangerous to living organisms due to their possible toxicity and carcinogenicity. The residual dye in the effluent stream from different sources (textile industries, paper and pulp industries, dye and dye intermediates industries, pharmaceutical industries, tannery, Kraft bleaching industries etc.) contains wide variety of organic pollutants, which create ecological problems and health hazards all over the world. Azo (-N=N-) dyes constitute the largest family of synthetic dyes. Its disposal from dyestuff synthesis and textile processing industry into the water bodies causes reduction in water transparency, oxygen solubility, negative impact on germination and growth of plant species, resulting in imbalance of the ecological function. Due to high stability, the discarded dyes stay in the environment for long-term and thus accumulate leading to bio-magnification. The breakdown of wastewater containing dye produces several kinds of intermediates, which may be toxic, mutagenic and carcinogenic to the living bodies and therefore should not discharge directly into the environment.

Several treatment technologies are available for the treatment of textile industrial effluents like Fenton’s method, electro-chemical oxidation, photochemical oxidation and adsorption. These technologies have many drawbacks, such as, sludge formation, applicability to narrow spectrum of dyes, generation of intermediates, cost-effectiveness and difficult to scale up (physical, chemical or biological methods). Recently, the decolonization of dyes using biological techniques has gained momentum, as these techniques are cost-effective and eco-friendly. Various researchers have studied biodegradation of textiles dyes using several kinds of microbial species like fungi, bacteria, algae and actinomycetes.

For the treatment of dye containing wastewater, free and immobilized cell systems have been used. In the free cell systems, microorganisms are directly inoculated into the bioreactor; while in the immobilized systems, the microorganisms are encapsulated or immobilized in/on some kind of packing medium to provide support to microbes for their growth. Immobilized systems have reported to be better in comparison to free cell systems because of the benefits like improved efficiency, better control of process parameters and high cell retention during the process. Kudlich et al. have reported the complete mineralization of azo dyes by the microorganism, which involved two-step reactions: anaerobic phase (formation of aromatic amines), followed by aerobic phase (degradation of aromatic amines). Slow rate of
degradation is most important challenge, which prohibits the application of biological techniques at industrial scale for bioremediation of dyes. However, the researchers are working to increase the rate of biodegradation of dyes by isolation and application of efficient microbial species having ability to degrade wide spectrum of textile dyes (reactive azo, anthraquinone, direct dyes), selection of most efficient reactor system and optimization of process parameters for the maximization of degradation rate.

The present study focuses on the biodegradation of reactive orange 16 (RO-16) dye in bioreactors packed with agrowastes, namely, Ashoka and Casuarina seeds, and immobilized mixed culture of bacterial species isolated from a site contaminated with dye containing wastewater.

Materials and Methods

Chemicals and Mineral Salt Medium Used

Analytical grade RO-16 (>99.0% purity) was procured from Sigma Aldrich, India. The NaCl-yeast extract medium (NY medium) containing 4% NaCl and 0.5% yeast extract along with trace elements was used for the preparation of inoculum.

Enrichment and Immobilization of Microbes on Packing Media

Dye contaminated wastewater composite sample was collected from the contaminated water/soil nearby the carpet industries of Varanasi, Uttar Pradesh, India. Then the contaminated sample was enriched in NY medium to enhance the colonies of bacterial species in the sample. After enhancing the colonies, the sample was mixed with RO-16 dye solution (50 mg/L) in the ratio of 1:10 and kept for 24 h with the objective to retain the bacterial colonies capable of withstanding RO-16 dye. The resultant solution was again enriched using NY medium to enhance the colonies capable of degrading RO-16. The solution was then diluted with sterile distilled water to make resultant stock solution containing 100×10^6 CFU/mL of bacterium. The seeds of Saraca asoka (Ashoka) and Casuarina equisetifolia (Casuarina) were washed thoroughly with distilled water and dried in an oven at 60°C and then soaked in the solution containing 100×10^6 CFU/mL of bacterium for 36 h to acclimatize the bacteria on the solid surface.

Bioreactor Set Up, Operation and Analytical Methods

Fig. 1 shows the schematic diagram of the packed bed bioreactor. The bioreactor was fabricated using transparent cylindrical Pyrex glass of 10 cm in internal diam and 32 cm in length. It was packed upto the height of 10 cm with acclimatize seeds of Ashoka and Casuarina separately. The total volume and working volume of the bioreactor was 2512 and 960 mL, respectively. From top of the reactor, the dye contaminated waste water was supplied by a peristaltic pump (PP10, India) at a flow rate of 1.0 L/h through a sieve plate for proper distribution of waste stream onto the bed. The experiment was conducted at room temperature (27±5°C) (Table 1). The concentration of the dye was measured using UV-Visible spectrophotometer (Elico SL210). The presence of various functional groups on the surface of seeds was analyzed using FT-IR (Thermo Scientific, Nicolet 5700) within spectrum range of 400-4000 cm⁻¹.

![Fig. 1 — Schematic diagram of bioreactor and experimental setup for RO-16 dye biodegradation.](image)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Average value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height of the packed bed (m)</td>
<td>0.32</td>
</tr>
<tr>
<td>Diameter of column (m)</td>
<td>0.10</td>
</tr>
<tr>
<td>Flow rate (L/h)</td>
<td>1.00</td>
</tr>
<tr>
<td>Total volume (L)</td>
<td>2.51</td>
</tr>
<tr>
<td>Packed volume (L)</td>
<td>0.96</td>
</tr>
<tr>
<td>Effective bed residence time (h)</td>
<td>0.96</td>
</tr>
<tr>
<td>Operating temperature (°C)</td>
<td>27.0±5.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.0±0.3</td>
</tr>
<tr>
<td>Inlet conc. (ppm)</td>
<td>500</td>
</tr>
<tr>
<td>Outlet conc. (Casuarina seed) (ppm)</td>
<td>153</td>
</tr>
<tr>
<td>Removal efficiency (Casuarina seed)%</td>
<td>69.3</td>
</tr>
<tr>
<td>Outlet conc. (Ashoka seed) (ppm)</td>
<td>182</td>
</tr>
<tr>
<td>Removal efficiency (Ashoka seed) (%)</td>
<td>63.6</td>
</tr>
</tbody>
</table>
Kinetic Studies

The kinetic parameters are important indicators of efficacy of bacteria as well as reacting system. Kinetics parameters were obtained by using suitable kinetic equation and fitting the variation of substrate with respect to time. The second order kinetics (Eq. 1) was found to be best fitted the experimental results.\(^1\)

\[
\frac{1}{S} = \frac{1}{S_0} + kt \quad \ldots \quad (1)
\]

In the equation, \(S\) is dye concentration at time \(t\), \(S_0\) is initial dye concentration and \(k\) is second order rate constant.

Results and Discussion

Adsorption property of the packing material helped the biodegradation process in the bioreactor by catching the pollutant on its surface. In the FT-IR results of original packing materials, \(-S-, =C=O, =C=O, =NH\) and \(-OH\) functional groups were found on Casuarina seeds, whereas \(-C=H, =SO, -OH\) (acidic), \(-OH\) (alkali), \(-NH\) and \(-COOH\) groups were present on the Ashoka seed (Fig. 2). The functional groups \(-OH, =NH, =OH\) and \(C=O\) have already been reported by various researchers to play important role in the adsorption of RO-16 dye on solid surface.\(^13-17\)

At the start-up, the bioreactors packed with immobilized seeds of Ashoka and Casuarina were fed with synthetic solution containing low concentration of RO-16 (50 mg/L) mixed with NY medium and trace element solution\(^11,18\) for 1 d in order to acclimate the bed properly with isolated mixed consortium of microorganisms. After which, the dye concentration was increased to 500 mg/L and kept constant for the rest of the experimental period (9 d). The flow rate of dye solution in the reactor was maintained at 1 L/h.

The results show that percent removal of dye increased with time and tended to attain constant value of 63.6 and 69.8%, respectively for the bioreactors packed with Casuarina and Ashoka seeds (Fig. 3). Thus the results demonstrated that Casuarina and Ashoka seeds supported the isolated bacteria well on their surfaces and showed good capability to treat dye-containing wastewater even at very high concentration of dye (500 ppm).

The results obtained in the present study was compared to the results of similar studies available on biodegradation of RO-16 in the batch as well as continuous systems as shown in Table 2. Due to limited availability of literature on biodegradation of RO-16 in continuous bioreactor, the batch studies were also included for comparison purpose. In most of the studies,\(^3,12,14,19\) the biodegradation experiments were conducted with initial RO-16 concentration ranging from 20 to 200 mg/L and reported removal in
the range of 50-100%. Some researchers\textsuperscript{20-22} also conducted experiments with high initial dye concentration (100-1000 mg/L) and reported removal in the range of 49-95%. The significant variation in the % removal of RO-16 was due to various factors including nature of microorganism, packing media, type of system used (batch, packed batch & continuous), residence time or stay time, operating conditions during the biodegradation experiments and initial concentration of RO-16.

The % removal of RO-16 obtained by other researchers\textsuperscript{20-22} in batch system are better than present study but one of the major reason may be higher stay time (1 to 10 d) of the pollutant in the reactor as compared to the present study in which very low residence time (0.96 h) was used. With low residence time and higher concentration (500 ppm), the removal was found more than 60% in the case of both materials, which is quite high and thus indicate the suitability of selected materials as packing media for biodegradation.

Second order kinetics was used to fit the experimental data. The second order kinetic constant \( k \) was calculated by “\( 1/s \) vs “\( t \)” (Fig. 4) and found to be 0.0391 and 0.0547 L/mg day, respectively for the Ashoka and Casuarina seed. The initial rate of reaction was also calculated and found to be 3925 and 4367 mg/L/d, respectively for the Ashoka and Casuarina (Table 3). The results on kinetic study reported by other workers are also presented in Table 3. The comparison of results clearly indicates that the materials along with immobilized bacterial species used in the present study have better potential to treat the dye containing wastewaters. The rate constant of both seeds were comparable with the results of other studies, even when the present study was carried at high initial concentration of dye, which many times create toxicity and inhibitory conditions in the system.

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**Table 2 — Comparative study for removal of azo dye RO-16 at various operating conditions by different microorganisms**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Conditions</th>
<th>Experimental condition</th>
<th>Inlet conc. (mg/L)</th>
<th>Removal efficiencies (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter sp.</td>
<td>Adsorption on powdered activated carbon (PAC)</td>
<td>pH 7 150 rpm, 30(^\circ)C</td>
<td>20</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>Morganella sp.</td>
<td>Flask studies</td>
<td>pH 7 150 rpm, 30(^\circ)C</td>
<td>20</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>Bacillus cohnii RAPT1</td>
<td>Cells immobilized on polyurethane foam (PUF)</td>
<td>pH 8, 30(^\circ)C Immobilization time 36 h 3 ( \times ) 10(^4) CFU/mL</td>
<td>200</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Irpex lacteus</td>
<td>Rotating drum biological contactor</td>
<td>pH 7 28(^\circ)C</td>
<td>100</td>
<td>50-90</td>
<td>14</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>Batch study</td>
<td>pH 8 37(^\circ)C</td>
<td>50</td>
<td>98</td>
<td>14</td>
</tr>
<tr>
<td>Nocardiosis alba</td>
<td>Batch studies</td>
<td>pH 7, 33(^\circ)C</td>
<td>100-1000</td>
<td>Max. 95.0</td>
<td>20</td>
</tr>
<tr>
<td>Ganoderma sp. En 3</td>
<td>Batch immobilization</td>
<td>pH 7 150 rpm, 28(^\circ)C</td>
<td>100-1000</td>
<td>95.1-49.1</td>
<td>21</td>
</tr>
<tr>
<td>Bacterial extracellular polysaccharide composite Mixed culture</td>
<td>Batch adsorption study Cells immobilized on Ashoka seeds</td>
<td>pH 7 150 rpm, 25(^\circ)C pH 7 27±5(^\circ)C</td>
<td>80-120</td>
<td>92-6</td>
<td>22</td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Cells immobilized on Ashoka seed</td>
<td>pH 7±0.3 27±5(^\circ)C 500</td>
<td>63.5±5</td>
<td>Present study</td>
<td></td>
</tr>
<tr>
<td>Mixed culture</td>
<td>Cells immobilized on Casuarina seed</td>
<td>pH 7±0.3 27±5(^\circ)C 500</td>
<td>69.27±5</td>
<td>Present study</td>
<td></td>
</tr>
</tbody>
</table>

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*Fig. 4 — Kinetic analysis on the biodegradation of RO-16 by immobilized mixed culture of bacteria on Ashoka and Casuarina seeds.*
Despite the present study was conducted at high dye concentration, the initial rate of removal in case of both seeds was found significantly higher compared to those reported by other workers (Table 3).

### Conclusion
In the present study, performance of the continuous bioreactors packed with Ashoka and Casuarina seeds and immobilized with mixed bacterial cultures, isolated from RO-16 dye waste contaminated soil, was evaluated. The steady state % removal was 63.5 and 69.5, respectively for Ashoka and Casuarina seeds at initial RO-16 concentration of 500 mg/L and residence time of 0.96 h. The second order of kinetics applied and it fitted well for both packing materials. The results demonstrate that the selected seeds have the potential to be used as packing material for biodegradation application in bioreactors.

### Acknowledgement
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### References


