Biodegradation of Bulk Drug Industrial Effluents by Microbial Isolates from Soil

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Bacteria isolated from soils exposed to bulk drug wastes were used for the aerobic treatment of two bulk drug industrial effluents. Shake flask experiments followed by scale up studies using individual bacterial isolate reduced COD by 75 per cent. Supplementation of the native bacterial flora with a specific culture helped in efficiently reducing COD of bulk drug effluent.

Introduction

Bulk drug industrial effluents are generated through raw materials used in the process for the manufacture of various bulk drugs and intermediates. Effluents are typically toxic, colored, inorganic, organic, turbid, with high suspended solids. Many organic compounds that are recalcitrant in nature are produced while manufacturing bulk drugs. Although many of them can be readily treated, some compounds that are poorly degradable are released in the effluent. In order to protect the environment from undesirable toxic material the wastewater must be suitably treated before discharge.

Biological wastewater treatment is the most widely accepted process due to its ease of handling and economic feasibility. An attempt was made to establish a biological treatment process for bulk drug industrial effluents collected from IDA Bollaram, Hyderabad. Cultures were isolated and selected after intensive screening of soil samples that was exposed for prolonged periods to bulk drug wastes.

Biological treatment processes mainly include fractionation of COD and assessment of significant kinetic and stoichiometric coefficients. COD fractionation involves identification of inert and biodegradable COD together with readily biodegradable and slowly biodegradable fractions. Emphasis was laid on COD reductions as a means of monitoring the treatment process.

Wastewater treatment depends on the ability of the microorganism to metabolize carbonaceous organic matter within a specified period of retention in the treatment process. Most organic compounds are vulnerable to microbial attack. Bacteria and fungi are especially capable of producing a wide variety of enzymes that can degrade organic compounds and mineralize the substances. Complex mixture of products are produced during the biodegradation of even a single compound. The breakdown products are dependent upon both the microorganisms present and the environmental circumstances. However, the particular substance will not be degraded if the relevant microbial strains are absent or fail to grow during the treatment.

Materials and Methods

Twenty-liter samples of two bulk drug effluents were collected from IDA Bollaram, Hyderabad. Sample A (COD 11,000 mg/l) was collected from primary sedimentation tank of an effluent treatment plant and Sample B (COD 14,000 mg/l) from neutralization tank of an effluent treatment plant of another industry. Physico-chemical characters of the samples are mentioned in Table 1.

Experiments Conducted

The following experiments were carried out:
1. Isolation and screening of bacterial cultures,
2. Chemical treatment of the effluent, and
3. Biological treatment of effluent with individual microbial isolates.
Table 1 – Physico-chemical characteristics of sample A and B

<table>
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<th>SI No.</th>
<th>Parameter</th>
<th>Sample A (mg/l)</th>
<th>Sample B (mg/l)</th>
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<tr>
<td>1</td>
<td>pH</td>
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<tr>
<td>2</td>
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<td>4</td>
<td>TSS</td>
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<td>1080</td>
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<td>5</td>
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<td>14076</td>
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<tr>
<td>6</td>
<td>BOD</td>
<td>5164</td>
<td>9894</td>
</tr>
<tr>
<td>7</td>
<td>Chlorides</td>
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<td>5320</td>
</tr>
<tr>
<td>8</td>
<td>Sulphates</td>
<td>88</td>
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<tr>
<td>9</td>
<td>Alkalinity</td>
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<tr>
<td>10</td>
<td>Alkalinity (Phenolphthalein)</td>
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<td>20</td>
</tr>
<tr>
<td></td>
<td>Alkalinity (Methyl orange)</td>
<td>210</td>
<td>380</td>
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</tbody>
</table>

Isolation and Screening of Bacterial Cultures

Cultures were isolated from soil samples collected from different sites where bulk drug effluents were being treated. One gram of soil was serially diluted to $10^{-5}$ in Bushnell Haas' broth (without hydrocarbons) of the following composition: 

- $\text{MgSO}_4$ - 0.2g; 
- $\text{CaCl}_2$ - 0.02g; 
- $\text{KH}_2\text{PO}_4$ - 1.0g; 
- $\text{K}_2\text{HPO}_4$ - 1.0g; 
- $\text{NH}_4\text{NO}_3$ - 1.0g; 
- $\text{FeCl}_3$ - 0.05g; 

in one litre of water (pH 7.0 ± 0.2). A 0.25ml aliquot of each dilution ($10^{-5} - 10^{-7}$) was transferred onto the surface of Bushnell Haas agar medium containing 0.05 ml each of benzene and toluene as carbon sources. Using a sterile L-shaped glass rod the sample was spread on the entire surface of the agar. The plates were inverted and a filter paper pad dipped in a mixture of benzene and toluene (1:1) was placed in the lid of the petri plate to maintain an atmosphere of benzene and toluene. The plates were incubated in a BOD incubator at 28°C for 5d.

Colonies that appeared after 120 h, were transferred onto Nutrient agar slants and incubated at 28°C for 48 h. The isolates were screened in the effluent samples A and B to select bacteria capable of reducing COD. A loopful of culture was inoculated into 50ml of Nutrient Broth (Hi-media) in 250ml Erlenmeyer conical flask having following composition:

- Peptone - 5.0g; 
- Beef extract - 1.5g; 
- Yeast extract - 1.5g, 
- NaCl - 5.0g and distilled water one litre (pH 7.4 ± 0.2). The flasks were incubated at 28°C for 48 h, on a rotary shaker at 200 rpm (stroke 1.5 in). Microbial characteristics viz. growth, shape and motility were observed under a phase contrast microscope (Olympus CH-2).

For primary screening process, 20 ml of the above seed was inoculated into 80ml of effluent A and B and incubated at 28°C on a rotary shaker at 200 rpm. After 48 h incubation, the flasks were checked microscopically for their characteristics and compared with that observed in the initial inoculum. Cultures with relatively good growth / motility in the effluent sample were selected and incubated for 240 h and checked for their ability to reduce COD.

The cultures selected from primary screen were subjected to secondary screening process for confirmation of COD reductions. Five cultures showing good COD reductions were shortlisted and allotted DRCC numbers, as follows:

1. DRCC - 165, gram +ve coccobacilli,
2. DRCC - 166, gram -ve cocci,
3. DRCC - 167, gram -ve cocci,
4. DRCC - 168, gram +ve motile curved bacteria, and
5. DRCC - 169, gram +ve coccobacilli (*DRCC : Dr Reddy's Culture Collection).

COD Analysis

Closed reflux colorimetric method⁹(E.Merck) was adopted for analysis. Sampling was carried out at 0, 48, 120, 192 and 240 h, respectively. 5 ml of the sample was centrifuged at 10000 rpm for 10 min and the supernatant was filtered through 0.45 μ filter¹⁰ for the estimation of soluble COD. Merck’s COD solution A and B were added in the manner prescribed, followed by the addition of suitably diluted sample making the total volume up to 5 ml. The Merck’s digestion tubes were placed in Merck’s thermoreactor at 148°C and refluxed for 2 h. After cooling, optical density of the sample was read at 585 nm using Spectrophotometer (Spectronic 20D) with 1cm diam cuvette. The resulting OD multiplied by a factor of 4600 gave the COD in mg/l.
**Chemical Treatment**

100 ml of sample A and B were treated with 200 mg each of alum at pH 7.0, ferrous sulphate at pH 6.0, and lime for 30 min under constant stirring. At the end of each process, the samples were centrifuged at 10,000 rpm for 15 min to remove the floc formed and the supernatant was taken up for subsequent treatments. The resultant samples were analysed for COD, TS, TDS, and TSS.

**Biological Treatment Using Soil Isolates**

Biological treatment was initiated using five individual DRCC cultures. Twenty per cent inoculum of each culture grown in nutrient broth was added to the effluent taken in 500 ml Erlenmeyer flasks. These were then incubated at 28 ± 0.5°C on a rotary shaker for 240 h. Control flask without DRCC cultures was also run for the same period. pH was maintained between 7.2 - 7.5 and evaporation losses were made up every 24 h with demineralised water. COD analysis was carried out at 0 h and after 48, 120, 192 and 240 h of treatment, respectively.

**Scale Up Studies**

10 l scale-up of the process was attempted in a 12 l plastic carboy. Air was sparged through a ring sparger and agitation (100 rpm) carried out with an overhead stirrer having two, four bladed impellers. Isolate that gave high COD reduction in shake flask was selected for the scale up. Seed inoculum was developed in two stages: 100 ml of NB in 500 ml Erlenmeyer conical flask was inoculated with a loopful of culture and incubated for 24 h (stage I) which was then transferred to 2 l NB in a 5 l Aspirator bottle (stage II). pH of the effluent was adjusted to 7.4. Aeration and agitation was started 30 min before inoculation. 100 ml samples were withdrawn into a conical flask after inoculation and incubated on shaker. 10 l control effluent without any added culture was also run in parallel. pH was maintained between 7.2 - 7.5 and evaporation losses were made up every 24 h with demineralised water. COD analysis of the shake flask and scale up samples was carried out at 0, 120, and 240 h, respectively.

**Results and Discussion**

Different biochemical mechanisms are responsible for the microbial degradation of compounds present in the effluent. The concentration and types of constituents present in the effluent play an important role in the response of microorganisms to the mixture of organic compounds. The results of chemical treatment compared to untreated effluent are presented in Table 2.

The TDS of the sample A and B increased by about 15 - 19 per cent after chemical treatment with concomitant decrease in TSS, however, no significant decrease in soluble COD was observed. Results of biological treatment of sample A and B with individual bacterial isolates are illustrated in Figure 1 and Figure 2 (average of three experiments).

DRCC 165, a gram positive coccobacilli lowered COD by 75 per cent in both sample A and B. While COD reductions of 63 - 70 per cent in sample A and B were obtained with other cultures. Control (uninoculated), containing native bacterial flora recorded a decrease in COD of 20 - 25 per cent. The
experiment was terminated at 240 h as no further significant COD reductions were noticed. Kinetics of COD reduction with reference to control is shown in Figure 3 and Figure 4.

Figure 5 and 6 represent the results of 10 l scale-up study. COD reduction of 72 per cent was achieved in sample A, as compared to 74 per cent in shake flasks, run in parallel, while in sample B it was 64.8 per cent as compared to 66 per cent in shake flask. Thus the results of shake flask were reproduced in scale-up for both sample A and sample B.

The efficiency of biodegradation of the effluents depends mainly on its physico-chemical characteristics, concentration of the chemicals and the specific bacteria capable of withstanding the adverse conditions such as high TDS, COD, presence of solvents, antibiotics, etc. Non-uniformity of the effluents generated is, a major problem faced by the bulk of drug industry. The nature and the characteristics of the effluent changes depending on the type and quantity of product being manufactured and also the process adopted. This is further complicated by the use of different intermediates in the process.
Our experiments indicate that DRCC 165 was the best soil isolate capable of reducing COD up to 75 per cent of sample A and B. Thus the native organisms present in the effluents may not be sufficient to effectively degrade it. Results of the biological treatment indicate that the effluents need to be supplemented with DRCC 165 in order to establish an efficient treatment process.

Further, COD reduction of the effluents, to meet permissible limits may be possible by using a different group of bacteria. Work is in progress for identification and characterization of DRCC 165.

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References