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# Bioremediation of an Industrial Effluent Containing Monocrotophos

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**Abstract.** Almost 30% of the precious agricultural output of India is lost owing to pest infestation. In India, pesticide consumption for protecting crops is about 3% of the total world consumption. Monocrotophos (MCP), an organophosphorus insecticide, is widely used to control insects on crops. Being readily water soluble and highly toxic, its removal from wastewater generated during manufacture becomes inevitable. Bioremediation of wastewater containing MCP by *Arthrobacter atrocyaneus*, *Bacillus megaterium*, and *Pseudomonas mendocina* was highest at pH 8.0, but maximum reduction in Chemical Oxygen Demand (COD) was at pH 7.0. Removal of MCP and reduction in COD by *B. megaterium* and *Ps. mendocina* were highest at 35°C, while with *A. atrocyaneus*, it was maximum at 30°C, under aerated culture condition and inoculum density of $10^8$ cells/ml. Use of pure cultures for bioremediation of effluent containing MCP appears to be the first such attempt.

India is primarily an agro-based country with more than 60–70% of its population dependent on agriculture. However, 30% of its agricultural produce is lost owing to pest infestation. There is an urgent need for the development of an effective pest control strategy. In the absence of a better alternative, deployment of pesticides becomes inevitable despite their known hazardous effects. Utilization of pesticides in India is about 3% of the total world consumption and is increasing at the rate of 2–5% per annum.

MCP (dimethyl (E)-1-2-methylcarbamoylvinylphosphate), is an organophosphorus pesticide widely used to control a variety of insects on crops such as cotton, sugar cane, peanuts, ornamentals, and tobacco. MCP is highly toxic and has contact, systemic and residual activity [8]. Today, MCP occupies a prime position in pest management in India, and its consumption in India is estimated at 6000 metric tons per annum [1].

MCP is soluble in water and easily gains entry into the wastewater generated during its manufacture. Removal of MCP from industrial effluent is essential, since MCP is a human health hazard and may cause irritation of eyes and skin on contact, inhibition of acetyl cholinesterase, sweating, muscular weakness, blurred vision, and a risk of death owing to respiratory failure [8]. Efforts are, therefore, required for the treatment and safe disposal of the effluent.

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There are very few reports on the biological treatment of MCP effluent. Xie and Shi [11] and Xie et al. [12] have studied the treatment of MCP wastewater by physicochemical and biochemical technologies. Reports on the use of pure cultures of bacteria for treatment of industrial effluent do not seem to exist.

The present investigation was undertaken to study bioremediation of MCP effluent by the use of pure cultures of bacteria.

## Materials and Methods

**Collection and characterization of effluent from MCP manufacturing unit.** Waste effluent generated during the manufacture of MCP was collected from a factory (situated near Pune, India) manufacturing MCP. Five liters of composite effluent samples were collected weekly and stored at 10°C. Ten samples were collected and characterized with respect to (i) pH measured with digital pH meter (Control Dynamics, Model APX-175 EK); (ii) color; (iii) total solids; (iv) ammoniacal nitrogen; and (v) Chemical Oxygen Demand (COD), as described by Greenberg et al. [3]. MCP content was estimated by HPLC by using a Hinditron (Agaram-LC 2010) solvent delivery pump equipped with UV detector (Agaram-LC 2020) set at 210 nm. A 250 mm × 4 mm stainless steel column packed with Lichro sphere 100 RP-18, end capped (5 μm) in Lichro CART (Merck), was used. The operating conditions were as follows: column temperature, ambient (28 ± 2°C); mobile phase, 20% acetonitrile in water; and flow rate, 0.5 ml/min. Standard aqueous solutions of MCP (concentrations ranging between 0.01 and 100 mg/L)

Table 1. Characteristics of the industrial effluent from MCP manufacturer

| Parameters studied | Range | Mean | Standard deviation (SD) |
|---|---|---|---|
| Color | Orange-brown | – | – |
| pH | 1.25–7.01 | 3.237 | 2.293 |
| Total solids, mg/L | 360–2850 | 1210 | 660.54 |
| Ammoniacal nitrogen, mg/L | 125–644 | 294.2 | 153.6 |
| COD, mg/L | 1257–9500 | 4659.5 | 3299.7 |
| MCP, mg/L | 0–125 | 27.5 | 45.34 |

were used to determine the minimum detectable concentration with a standard linear response calibration curve.

The above samples were analyzed to obtain the mean values and standard deviations as per the procedure described by Greenberg et al. [3].

**Microorganisms used for the studies.** Three cultures used for the studies were isolated from soil that had been previously exposed to MCP. The cultures identified according to the Bergey's Manual of Systematic Bacteriology [1984, 1986], were *Arthrobacter atrocyaneus* [5], *Bacillus megaterium* [2], and *Pseudomonas mendocina* [7]. These are deposited at MACS Collection of Microorganisms (MCM), designated MCM B-423, MCM B-425, and MCM B-424 respectively.

**Inoculum development.** The cultures were inoculated on effluent agar (industrial effluent of pH 7.0 solidified with 2% Difco agar) in Roux bottles (to obtain sufficient quantity of inoculum) and were incubated for 48 h at 30°C. The bacterial growth from these Roux bottles was suspended in sterile saline. The Optical Density (OD) was checked at 600 nm as measured on Beckmann DU-8B UV-Vis Spectrophotometer USA. The exact count in the suspension was determined by comparing the OD with standard Brown's opacity tubes.

**Bioremediation of MCP effluent under different environmental conditions.** The effect of the following environmental conditions on bioremediation of MCP effluent by the three bacteria— *Arthrobacter atrocyaneus*, *Bacillus megaterium*, and *Pseudomonas mendocina*— was studied in order to determine the optimum conditions, by changing one condition at a time and keeping all others constant.

**Initial pH of the effluent.** The pH of the effluent was adjusted to 6.0, 7.0, and 8.0 with 40% NaOH and then dispensed into 100-ml aliquots in 250-ml flasks. Three flasks for each pH were inoculated with the three cultures separately at an inoculum density of $10^6$ cells/ml and were incubated at 30°C for 48 h under shake culture condition (150 rpm) along with an uninoculated control for the respective pH values.

**Temperature of incubation.** Three sets of three flasks each, containing effluent (pH 7.0) in 100-ml aliquots in 250-ml flasks, were inoculated with the three cultures separately at an inoculum density of $10^6$ cells/ml. The sets were incubated at 25°, 30°, and 35°C for 48 h under shake culture condition (150 rpm) along with an uninoculated control for each temperature.

**Culture conditions.** Three sets of three 250-ml flasks, each containing 100-ml aliquots of effluent (pH 7.0), were inoculated with the three cultures separately at an inoculum density of $10^6$ cells/ml. The flasks were incubated at 30°C for 48 h along with an uninoculated control under stationary condition, shake culture condition (150 rpm), and aerated condition achieved by passing sterile compressed air at a flow rate of 70–80 ml/min into each of the flasks.

**Inoculum density.** Three sets of three 250-ml flasks, each containing effluent (pH 7.0) in 100-ml aliquots, were inoculated with the three cultures separately at an inoculum density of $10^6$, $10^7$, and $10^8$ cells/ml. The flasks were incubated at 30°C for 48 h under shake culture condition (150 rpm) along with an uninoculated control.

All experiments were carried out in duplicate. A one-way ANOVA model [10] was used to determine the statistical significance (F-values) of the response of bioremediation by the isolates under different environmental conditions.

## Results

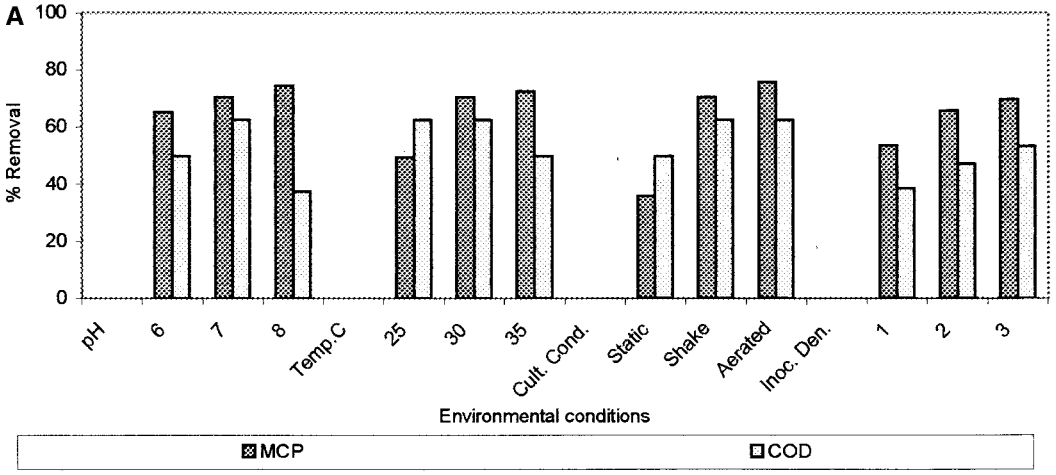
**Characteristics of the effluent.** The characteristics of the industrial effluent are detailed in Table 1. The effluent was acidic to neutral (pH 1.25–7.01) and orange to brown in color. The effluent showed a fairly high level of ammoniacal nitrogen (125–644 mg/L), COD ranging between 1406 and 9262 mg/L and MCP content ranging from 0 to 125 mg/L.

**Removal of MCP from the effluent under different environmental conditions.** The results of different environmental conditions on removal of MCP containing effluent are represented in Fig. 1a, 1b, and 1c. The results indicate that, although maximum (65–77%) MCP removal was at pH 8.0, the reduction in COD was highest (62.5%) at pH 7.0. As regards incubation temperatures, *B. megaterium* and *Ps. mendocina* showed maximum removal of MCP (68–76%) and reduction in COD (62–68%) at 35°C, while with *A. atrocyaneus*, MCP removal and COD reduction were maximum at 30°C. Among the different culture conditions, the aerated culture showed maximum removal of MCP (75–80%) and reduction in COD (53–60%) by all three cultures. As to the initial cell load, it was seen that the removal of MCP and reduction in COD increased as the initial cell density increased, maxima of 77% and 60% respectively being reached at a cell density of $1 \times 10^8$ cells/ml.

## Discussion

The composition of the effluent was found to vary widely, as revealed by the large SD values. This variation may be due to the fact that the effluent was not segregated but was from an industrial plant manufacturing several other pesticides. This probably is the reason for the lower reduction in COD compared with removal of MCP. It should also be noted that the cultures used for this bioremediation study were already acclimated to MCP and not to many other constituents that might be present in the effluent.

Cultures of all three bacteria showed very little difference in the amount of MCP removed at all pH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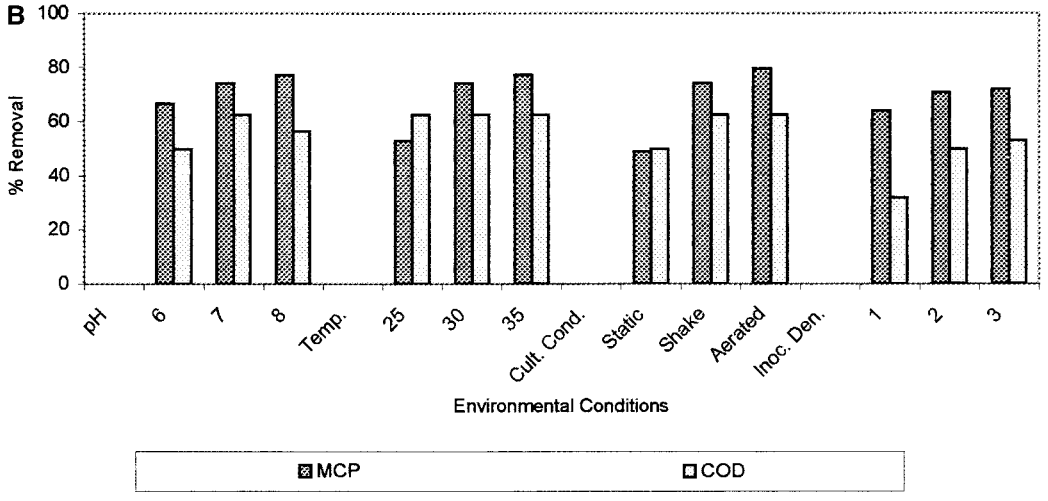
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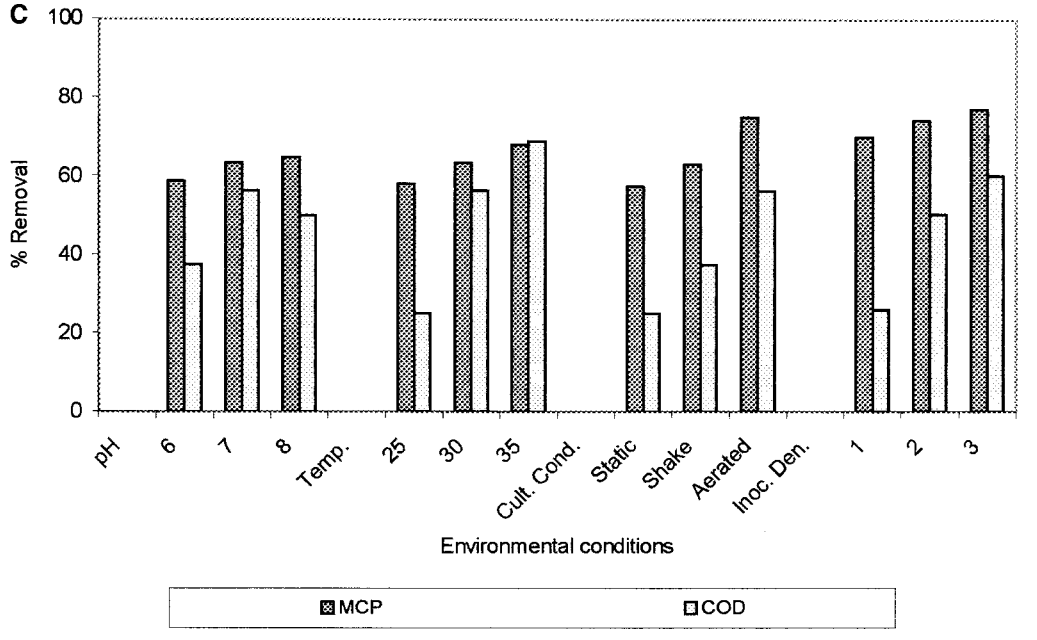
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levels. Statistical analysis showed that the pH was not significant, because the F-value of 1.95 ($p < 0.05$) for MCP removal and the F-value of 1.665 ($p < 0.05$) for reduction in COD were not significant in the bioremediation process. This indicated that fluctuations in pH within the range of 6 to 8 do not affect the bioremediation process.

Temperatures between 30° and 35°C were found to be optimal for the bioremediation of MCP and reduction in COD. This temperature range is the most suitable for growth of the isolates, and both MCP and COD removal are growth-related processes. Statistical analysis showed that the F-value of 13.52 ($p < 0.05$) for MCP removal was higher than the table value (3.68), indicating that temperature is significant for MCP. However, removal of COD was not found to be a temperature-dependent process, since the F-value for reduction in COD was 0.6378 ($p < 0.050$).

Shake culture or aerated culture conditions are better than static culture for growth and removal of MCP and COD. Statistical analysis of the data revealed that the culture condition was significant for MCP removal, with an F-value of 13.85 ($p < 0.05$), but it appeared to be insignificant for COD reduction with an F-value of 2.53 ($p < 0.05$).

It is clear from the figures and statistical analysis that the initial cell density significantly affected the removal of MCP ($F = 5.934$, $p < 0.05$) and reduction of COD ($F = 57.90$ $p < 0.05$). The desired cell density can be maintained either by recycling the cells or by immobilization of the cells on a suitable inert support like brick or pumice stone pieces. Kanekar et al. [4] have also developed a process for bioremediation of phenol and methyl violet from an industrial effluent by immobilization of the *Ps. mendocina* cells on brick media.

Bioremediation of MCP from industrial effluent was optimum at pH 7–8, 30°–35°C, under aerated culture condition, and at an inoculum density of $10^8$ cells/ml. Similar work for optimization has been reported by Sarnaik and Kanekar [9] and Kulkarni and Kanekar [6].

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Fig. 1. (**A**) Effect of different environmental conditions on MCP and COD removal by *A. atrocyaneus*. The culture was grown in effluent under various environmental conditions, by changing one condition at a time and keeping all others constant. (**B**) Effect of different environmental conditions on MCP and COD removal by *B. megaterium*. The culture was grown in effluent under various environmental conditions, by changing one condition at a time and keeping all others constant. Temp., temperature °C. Cult. Cond., culture condition. Inoc. Den., inoculum density (cells/ml): 1, $10^6$ cells/ml; 2, $10^7$ cells/ml; 3, $10^8$ cells/ml. (**C**) Effect of different environmental conditions on MCP and COD removal by *Ps. mendocina*. The culture was grown in effluent under various environmental conditions, by changing one condition at a time and keeping all others constant. Temp., temperature °C. Cult. Cond., culture condition. Inoc. Den., inoculum density (cells/ml): 1, $10^6$ cells/ml; 2, $10^7$ cells/ml; 3, $10^8$ cells/ml.