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MICROBIOLOGICAL PROCESS FOR THE REMOVAL OF Cr(VI) FROM CHROMATE-BEARING COOLING TOWER EFFLUENT

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Summary:

A microbiological process using *Pseudomonas mendocina* was developed for the removal of Cr(VI) from cooling tower effluent. The process, when carried out in a 20 liter continuous stirred tank reactor removed 25-100 mg chromate/l in 4.5-8 h with >99.9% efficiency in the presence of sugarcane molasses as nutrient. The process could sustain wide variations in pH (6.5-9.5) , temperature (25°C-40°C) and was unaffected by commonly used biocides.

Introduction :

Chromate compounds [containing Cr(VI)] have been used on a large scale in the cooling towers of fertilizer and other heavy industries in India owing to their excellent corrosion inhibiting and biocidal properties. Consequently, effluents discharged from such industries contain large quantities of Cr(VI) (Nriagu, 1988). Cr(VI) is highly toxic and is known to cause lung cancer, chromate ulcer, perforation of nasal septum and kidney damage in humans. According to Indian Standards IS:2296 and IS:2490, the statutory limit for the discharge of Cr(VI) in the inland surface waters is 0.1mg/l.

Different physico-chemical methods such as reduction precipitation, ion exchange, reverse osmosis and electrodialysis, can be used for the treatment of chromate-containing waste waters. However, it has been observed that these processes are costly and relatively unreliable (maximum achievable chromate removal efficiency is 90-95% which may not be sufficient to attain the desired treated effluent quality for disposal, Mahajan, 1985). Therefore, many industries in India using chromate in cooling towers have shifted to highly expensive chemical anodic inhibitors. If a cost-effective and reliable process for the treatment of Cr(VI) bearing effluents could be developed, the industry could possibly revert back to the time-tested, dependable and less expensive chromate based maintenance of cooling towers.

The present study deals with a new microbiological process developed for the removal of Cr(VI) from chromate-bearing cooling tower effluent.

Materials and Methods :

Chromate reducing culture :

The chromate reducing bacterial culture used in the present study was isolated from a sewage sample by enrichment culture technique. The isolate was identified as *Pseudomonas mendocina* as per Bergey's Manual of Systematic Bacteriology (1984) and deposited in the MACS Collection of Microorganisms (Ref. No. MCM B-180).

Effluent sample:

A typical cooling tower effluent (pH 8.5) containing chromate and biocides (Quat-2-C, methylene bis thiocyanate, sodium pentachlorophenate, chlorine and chloramine, either singly or in combination) at desired concentrations was simulated in laboratory and used.

Analyses:

Estimations of Cr(VI) by diphenyl-carbazide method, total chromium content by atomic absorption spectrophotometry (Perkin Elmer, USA Model 2380) and Biological Oxygen Demand (BOD) by Winkler's azide method were carried out as described in APHA (1985).

Factors affecting chromate reduction:

The factors affecting chromate reduction were investigated in batch culture experiments. For this purpose, sterile rubber stoppered conical flasks (100 ml volume) were filled with 50 ml nutrient medium (containing 100 mg chromate/l and 1000 mg/l BOD in the form of sugarcane molasses, unless specified otherwise), inoculated with 18 h old culture of *P. mendocina* MCM B-180 (10% v/v) and incubated at 30°C for 24 h. Chromate reduction was evaluated at different pH values (5.5.-10.5), temperatures (20°C-45°C at pH 7.5), BOD levels (100-800 mg/l at pH 8.5), chromate concentrations (0.5 mM-40 mM) and biocide concentrations (as specified in Table 2).

Chromate reduction in Continuous Stirred Tank Reactor (CSTR) :

A stainless steel reactor vessel with conical base and 20 liter working volume was used. The contents of the reactor were agitated at 8-10 rpm with a step-down, top-driven motor. The chromate-bearing cooling tower effluent with nutrients was added at the bottom of the reactor through a port using a peristaltic pump (Ismatec, Switzerland Model MCP 552). The treated effluent was removed from the top at the same flow rate and was collected in a settler where chromic hydroxide sludge settled. The clarified effluent was discharged.

Initially, 2 liter culture of *P. mendocina* MCM B-180 was inoculated (2.0×10^8 cells/ml) in 18 liter chromate-bearing (0.5 mM) effluent supplemented with sugarcane molasses (final BOD 800 mg/l). The reactor was operated as a batch process for the first three days and subsequently run on a continuous mode. The samples of the treated effluent from the reactor were collected daily and analyzed for Cr(VI) content, pH and total viable count.

Results and Discussion :

Factors affecting chromate reduction:

P. mendocina MCM B-180 showed high level of resistance to chromate (30mM). It was also able to reduce chromate at high concentrations in batch culture (150 mg chromate/l

was reduced to less than 0.1 mg/l in 18 h at 30°C). Optimum pH for chromate reduction was found to be 6.5-9.5. Optimum temperature for the same was 25°C-30°C. Chromate reduction was not observed in uninoculated controls under identical conditions confirming that reduction of chromate was carried out by *P. mendocina*. The reduced chromate was found to precipitate in the medium as chromic hydroxide. It is known that chromium exists in only two stable forms viz. trivalent and hexavalent forms (Bell and Lott, 1966), of which trivalent chromium readily precipitates as chromic hydroxide at pH 7.0 or above (Bopp and Ehrlich, 1988).

Table 1:
Effect of BOD on cell density and chromate reduction efficiency :

BOD* (mg/l)	Cell density (cells/ml)	Chromate reduction (%)
100	4.0×10^6	66.5
200	1.6×10^7	91.5
400	7.2×10^7	99.7
800	2.0×10^8	100

* Dosage was adjusted using molasses having 1,000,000 mg /l BOD; e.g. 0.1ml molasses was added to 1 liter medium to get 100 mg/l BOD.

The present investigation was directed towards developing a microbial process for chromate removal from industrial effluents, especially, cooling tower effluent. Such effluents contain very low quantities of organics. Therefore, external addition of nutrients is essential to support the microbial growth. In the batch experiments carried out to determine the minimal nutritional requirement for chromate reduction of >99.9% it was observed that increase in the molasses concentration (estimated in terms of BOD dosage) increased the cell density, which, in turn, improved chromate reduction efficiency (Table 1). A minimum of 800 mg/l BOD was essential to support >99.9% reduction of 100 mg chromate/l.

Table 2:
Effect of biocides on MCM B-180 mediated chromate reduction process :

Biocides	Concentration (µg/ml)	Chromate reduction (%)
Quat-2-C	20	0
	18	65
	15	100
Methylene bis-thiocyanate	20	0
	15	15.8
	13	26.32
	10	100
Sodium penta-chlorophenate	150	10.53
	100	26.32
	75	100
Chloramine	100	65
	75	100
	50	100
Chlorine	30	100
	20	100
	10	100

Although *P. mendocina* MCM B-180 was capable of utilizing a variety of carbon and nitrogen sources, molasses was chosen in the present study due to its ready availability, low cost and ease of storage. Moreover, minute quantities of molasses could satisfy the nutritional requirement of the culture because of its high BOD (1,000,000 mg/l) content.

Various biocides are used in cooling towers to inhibit microbial growth. Such biocides, when discharged along with the cooling tower effluent, may have a pronounced levelling effect on the microbial chromate reduction process. The minimal inhibitory and sub-inhibitory concentrations of five commonly used biocides are listed in Table 2. Quat-2-C, methylene-bis-thiocyanate and chlorine are normally used in cooling towers at a concentration of 10 mg/l; while sodium pentachlorophenate and chloramine are used at 50 mg/l and 100 mg/l level respectively. Interestingly, *P. mendocina* MCM B-180 mediated chromate reduction remained unaffected in the presence of biocides at these concentrations with the exception of chloramine.

Performance of Continuous Stirred Tank Reactor (CSTR):

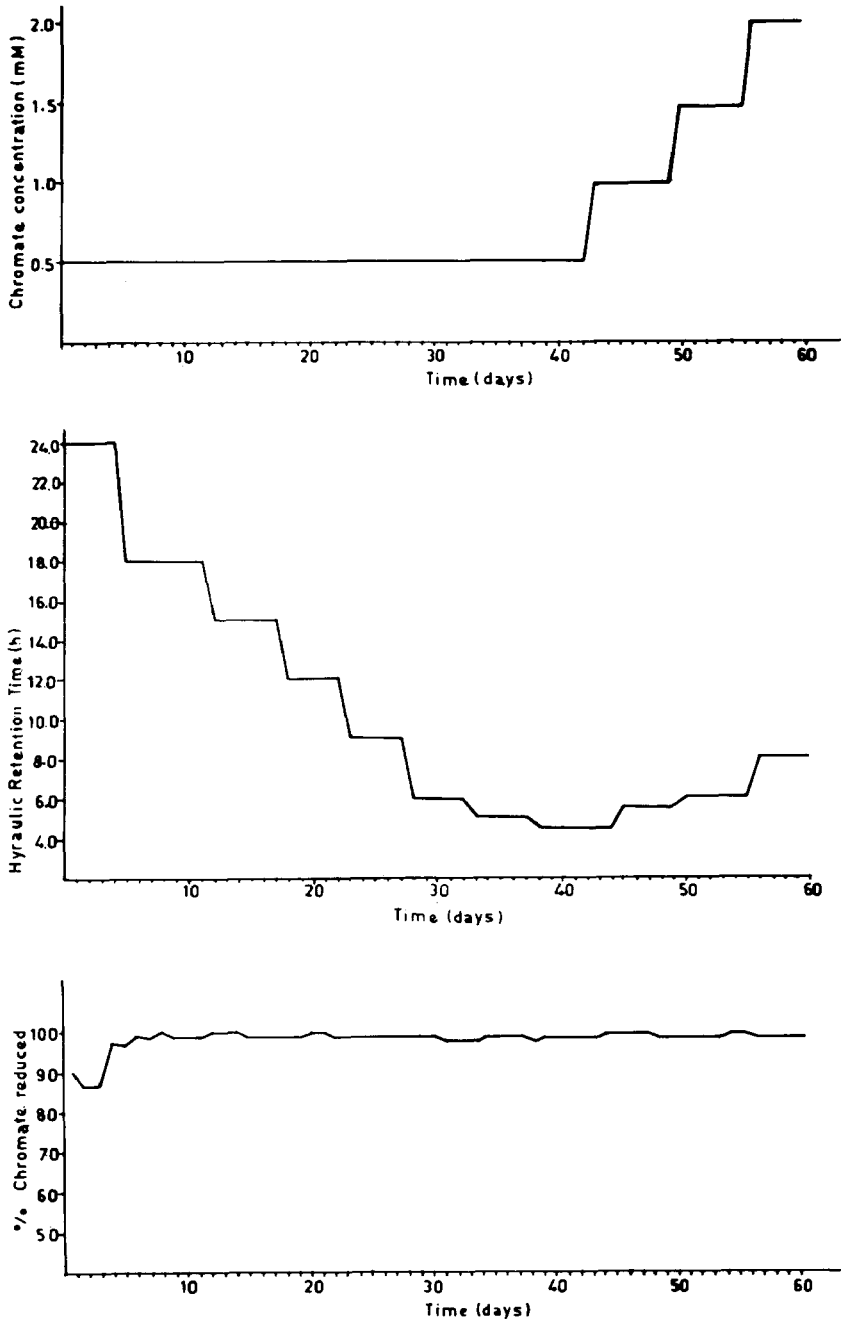
The performance of chromate reduction process in a CSTR using *P. mendocina* MCM B-180 over a period of 60 days is shown in Fig. 1. It depicts a typical profile of chromate reduction efficiency and hydraulic retention time (HRT) for a period of 60 days at various input chromate concentrations of 0.5 to 2.0 mM. It was observed that the total viable count in the effluent coming out of the reactor was ca. 1×10^6 cells/ml whereas the cell density in the reactor was ca. 3×10^9 cells/ml, indicating high retention of the biomass in the reactor. The biomass retention achieved was due to low agitation speed of 8-10 rpm and the inherent adhesion properties of the culture.

The chromate reduction efficiency of the system was found to be 86% for the first three days, which increased to >99% on the fourth day. The reactor was operated on a continuous mode with HRT of 24 h from third day and 18 h from fifth day. It could be seen from the Fig.1 that the HRT could be gradually brought down to a minimum of 4.5 h in 38 days. The total viable count in the reactor at this stage was 1×10^{10} cells/ml. However, further decrease in the HRT adversely affected the chromate reduction efficiency.

After the optimization of HRT at 4.5 h for the reduction of 0.5 mM chromate, Cr(VI) concentration in the effluent was increased step-wise. It was observed that with increase in Cr(VI) concentration from 0.5 mM to 1.0 mM there was a slight drop in the chromate reduction efficiency. However, with subsequent gradual increase in HRT to 6 hours, chromate reduction efficiency was restored to >99.9%. Similarly, the optimum HRT for 1.5 mM and 2 mM chromate reduction was observed to be 7 h and 9 h respectively. Thus, all other factors remaining constant, HRT influenced the chromate reduction efficiency. The pH of the influent waste water was around 8.5 throughout the experiment. It was observed that pH of the effluent coming out of the reactor after chromate reduction was consistently around 7.5 which was well within the statutory limits. It has been reported that microbial chromate reduction process requires anaerobic conditions (Ohtake and Hardoyo, 1992). Gopalan and Veermani (1994) have reported aerobic reduction of Cr(VI) by *Pseudomonas* species. The present investigation revealed that MCM B-180 did not require strict anaerobiosis for Cr(VI) reduction. However, studies with CSTR revealed that chromate reduction efficiency increased significantly with low partial pressures of oxygen in the reactor (D.O. < 1.0 mg/l).

In developing nations like India, industrial waste water treatment has to be a low-cost proposition for its wider acceptance and utilization. At present, waste waters containing chromium are generally treated by a chemical reduction process. However, this process

Figure 1:Performance of molasses-based CSTR using *P. mendocina* MCM B-180



is beset with problems of high capital and operating costs. Moreover, the practical efficiency of the process is around 90% which may not be adequate to obtain the treated effluent of desired quality. Processes for aerobic and anaerobic bacterial reduction of chromate in waste water have been reported by Gopalan and Veermani (1994) and Ohtake and Hardoyo (1992) respectively. In the first case only 81% to 91% chromate was reduced in 36 to 72 hours in a synthetic feed containing 15 to 124 mg chromate/l. In the second case, use of anion-exchange membrane reactor was suggested for removal of chromate from waste water which is unfeasible for the treatment of large volumes of effluents (e.g. typical volumes of cooling tower effluent are in the range of 200 to 800 M^3/h). The process reported in the present investigation has distinct advantages viz.: (i) no chemical additives or aeration required, (ii) no pH adjustment of cooling tower effluent is necessary, (iii) the process produces low volumes of sludge, (iv) the process is easy to operate and maintain, and is capable of handling chromate shockloads (up to 1600 mg/l), (v) it is unaffected by the presence of commonly used biocides, (vi) and it has low capital cost. The process, thus has the potential of becoming an economical and reliable alternative to the conventional processes employed for the treatment of chromate-bearing industrial effluents on a commercial scale.

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