SUMMARY AND CONCLUSIONS

The results obtained during the course of the present investigations could be briefly summarized as follows.

A "two-stage selection" method was devised for the isolation of chromate reducing microorganisms. The technique employed the selection of chromate tolerant cells in the first stage and subsequently microorganisms capable of utilizing chromate as the terminal electron acceptor instead of oxygen were selected. The method devised stands out as one of the simplest ways of isolating chromate reducing microorganisms without using any laborious anaerobic culture methodology. Using this technique a mixed microbial culture capable of reducing hexavalent chromium was isolated. Seven bacterial cultures and one fungal culture could be isolated in pure form. The seven bacterial cultures were identified using standard morphological, cultural and biochemical characteristics as *Pseudomonas pseudoalcaligenes, Pseudomonas alcaligenes, Pseudomonas delafieldii, Pseudomonas marginata, Pseudomonas stutzeri, Pseudomonas mendocina* and *Enterobacter sakazakii* while the fungal culture was identified using morphological characteristics as an *Aspergillus* sp.

The bacterial cultures were studied with respect to their pH and temperature optima for chromate reduction, tolerance to chromate, ability to use different organic carbon compounds during reduction and the ability to reduce other metals. The cultures isolated, differed, both in their tolerance to chromate as well as their chromate reduction efficiency. This was probably a manifestation of the natural diversity. Further, the bacterial cultures were capable of reducing chromate under oxygen-limiting conditions while the fungal culture was found to do so aerobically. When the cultures were compared on the basis of their chromate reduction efficiencies it was observed that *Pseudomonas mendocina* could reduce 2 mM chromate with an efficiency >99.7% in 24 h, whereas the *Aspergillus* sp. could reduce 2mM chromate with the same efficiency, but

in a period of 6 days. Selection of the most efficient chromate reducing microorganism was done taking into consideration the efficiency of reduction as well as some other factors like range of substrates utilized, pH and temperature optima and the overall acceptability of the process. Thus from among the cultures isolated by enrichment, ***Pseudomonas mendocina* was selected for further studies**. This culture was deposited in the MACS Collection of Microorganisms (MCM) and designated as *P. mendocina* MCM B-180.

Two cultures isolated, viz., *Pseudomonas alcaligenes* and *Pseudomonas pseudomallei* had the ability to reduce metals like Mn^{4+}, Fe^{3+}, Mo^{6+}, Te^{4+} and Se^{6+} in eventhough they did not reduce chromate with high efficiency. Therefore, ***P. alcaligenes* and *P. pseudomallei* could be useful in bioremediation of mixed-metal waste waters**.

Studies on the growth characteristics revealed that the generation time of *P. mendocina* MCM B-180 was 3.3 h under aerobic conditions. It nearly doubled in the presence of chromate under oxygen-limiting conditions during which 2 mM chromate was reduced at an efficiency exceeding 99.7%. As against this, under aerobic conditions only 32 % of the added chromate was reduced.

During growth under oxygen-limiting conditions chromate was reduced to its trivalent form which was confirmed using EPR spectra. The increase in pH of the medium during growth facilitated precipitation of the chromic hydroxide formed upon reduction.

The culture was found to tolerate anions like chlorides and sulfates and cations like iron, lead, cadmium, zinc and copper which are normally encountered in the industrial effluents. Another observation was that, the activity of **chromate reduction by *P. mendocina* MCM B-180 was unaffected in the presence of commonly used biocides like Quat-2-C, methylene-bis-thiocyanate etc.** Chromate reduction efficiency exceeding 99.7% was obtained when molasses was used as a carbon and nitrogen

supplement (at a BOD of 400 mg/l). **Molasses was the nutrient supplement of choice** due to its ready availability, low cost and ease of storage. These characteristics were beneficial attributes of the culture for its use on the industrial scale for the removal of chromate from waste waters.

In India, chromate compounds are largely used as biocides in waters circulated in cooling towers and as an electroplating chemical where the quantities of effluents produced are sufficiently large. **A microbiological process for the reduction of chromate using *P. mendocina* MCM B-180 was developed in a 2 l and 20 l capacity Continuously Stirred Tank Reactor (CSTR) for the treatment of waste waters from the chrome plating plant and cooling towers.**

The 2 l capacity CSTR was a specially fabricated three necked borosilicate bottle. The contents were agitated with a magnetic stirrer. The waste water was added at the bottom while treated effluent was removed at the top. After treatment the chromic hydroxide sludge was allowed to settle in a settler and the clarified effluent was discharged. The performance of this reactor was studied for a period of 100 days. At an Hydraulic Retention Time(HRT) of 24 h, 100 mg/l Cr^{6+} could be removed with an efficiency exceeding 99.7%. Strict anaerobiosis was not essential. The pH of the treated effluent was in the range 7.5 to 8.5. This increase in pH favored the rapid precipitation of the reduced chromium as chromic hydroxide.

The reactor used for the treatment of cooling tower effluent was a 20 l capacity stainless steel vessel mounted on a rack. The reactor had a conical base and the contents could be agitated with a step-down, top-driven motor. A port for the addition of waste water containing chromium was provided just below the impeller. The outflow was situated at the liquid-head space gas interface in the reactor. Before discharge, the treated effluent was taken to a settler where the chromic hydroxide sludge formed settled easily and could be separated. The performance of this reactor was assessed for a period of 60 days. During a period of 35 days a stepwise reduction in HRT could be successfully

achieved to a minimum of 4.5 h for the removal of 25 mg/l Cr^{6+}. The optimum HRT for the removal of 50 mg/l, 75 mg/l and 100 mg/l was observed to be 6 h, 7 h and 9 h respectively. It was also observed that if the pH of the feed was maintained at 8.5, the pH of the treated effluent coming out of the reactor remained consistently around 7.5. The total chromium content in the outflow (from the settler) never exceeded 2 mg/l. The dissolved oxygen (D.O.) concentration of the treated effluent was 0.9 mg/l and average COD was 230 mg/l. These values indicated that **the treated effluent conformed to the statutory limits,** excepting for D.O. level. The D.O. content of the treated effluent would need to be increased to >5 mg/l by aeration.

The volume of sludge produced in the process was minimal. Therefore, its disposal in dried form as land-fill should not be difficult. It is known that chromic hydroxide is resistant to leaching in soils and hence it is a safe material for disposal by land-filling. The land filled **sludge could also be a potential source of chromium metal since it was found to contain 50% chromium.**

The microbiological process for the reduction of chromium developed in the present investigation had several distinct advantages:

- no chemical additives or aeration required
- no pH adjustments for effluents generated from cooling towers required
- the process produced low volumes of sludge and it contained 50% metallic chromium
- easy to operate and maintain
- unaffected by the presence of commonly used biocides and metal ions
- low capital and operating costs (minimum 8 times cheaper than the conventional treatment methods)

The laboratory-scale process developed was licensed to a reputed environmental engineering company for commercialization in India (Appendix III for details).

P. mendocina MCM B-180 was found to reduce 2 mM chromate completely within a period of 24 h. It could, however, tolerate upto 30 mM chromate in the milieu. Studies carried out on the mechanism of chromate ressitance and reduction showed that this strain harbored a plasmid (designated pARI180) of molecular weight 12 kb. The plasmid could be cured by heat treatment at 42°C for 24 h. It could be transferred in *E. coli* DH5α strain by transformation. Further, it was proved to be a conjugative plasmid. Chromate reduction studies using *P. mendocina* MCM B-180, its cured derivatives as well as *E. coli* DH5α transformants proved that **resistance to chromate was plasmid borne and that the plasmid pARI180 carried the genetic determinants for chromate reduction. Plasmid encoded chromate reduction has not been reported so far.** It was also proved that **reduction of chromium was one of the mechanisms of resistance** in this culture. Preliminary studies carried out also indicated that other mechanisms responsible for chromate resistance could be intracellular synthesis of chromium-binding proteins.

Chromate reduction in *P. mendocina* MCM B-180 was observed to be enzymatic. The **chromium reducing enzyme could be purified** after heat treatment at 70°C for 30 min followed by DEAE Sephacel column chromatography. With this method, about 19.5 fold purification of the enzyme could be achieved. The enzyme had a K_m of 353 μM chromate and a V_{max} of 7.7 μM chromate reduced/min.mg protein.

Thus the investigations carried out could be concluded as follows:

- ***A laboratory-scale microbiological process for the treatment of chromium containing industrial waste waters was developed and standardized using an indigenously isolated bacterial culture P. mendocina MCM B-180. The process was found to be highly efficient and economical.***

- ***The basic studies carried out proved that chromate reduction is a plasmid borne property in P. mendocina MCM B-180 and plays an important role in the chromate resistance of this organism. The results also indicated the existance of additional mechanism(s) of chromate resistance, e.g. synthesis chromium binding proteins.***

- ***In the light of the above facts the present work tends to the advancement of knowledge and it is hoped that these studies would pave a way for future research. The studies also highlight the utility of microbial technology in the abatement of environmental pollution as exemplified by the successful transfer of the process know-how to the industry.***