Conclusions
Summary & Conclusion

Microorganisms, because of their smaller genome, short life span, ability to grow faster, as well as their adaptability to diverse environmental conditions, are preferred research material. *Arthrobacter* identified from a polluted site selected in this study, grew on minimal media containing sodium arsenite and has shown PCR amplification for primer pairs of aoxB, and dot blot positive for arsA. Its identification is accomplished using biochemical as well as by PCR and sequencing of 16S rDNA molecule (accession no. 491801). This bacterium is found to retain arsenic in their cell biomass which has been shown in EDX and they transform arsenic which is confirmed by number of techniques like XRD analysis, As (III) and As (V) chemical speciation. Measurement of Eh and pH is recorded while growth of bacteria in media, which gives idea of redox transformation As(III). This is the first report that *Arthrobacter* is capable of oxidizing arsenite, mediated by gene arsenite oxidase. Its gene (partial) responsible for oxidation of arsenite (arsenite oxidase) has been cloned and sequenced; accession no (AM 492534). The enzyme arsenite oxidase which is a heterodimer consisting of large subunit of 80 kDa and small subunit 14 kDa is purified and subjected to MALDI-TOF identification. MALDI-TOF further confirms the presence of peptide in bacteria. The enzyme has been purified by standard purification method to homogeneity and characterized by observing effect of various metal and organic compounds, effect of Heat, effect of temperature etc. Km, Vmax values for the enzyme were determined. Inhibition of enzyme in presence of inhibitor DEPC increased the Km value from 26μM to 66.6μM. Minimum inhibitory concentration of As(III) along with other metal is determined in this study which shows that *Arthrobacter* sp.15b has highest MIC (85mM) among other arsenite oxidizing bacteria known till date. In order to set up a pilot plant study using the enzyme, preliminary experiments were carried out using immobilization methods. Cells were entrapped in calcium alginate beads and their involvement in oxidation has been studied. The cell free extract of the bacteria is also entrapped in beads and its sorption capability of arsenite as well as arsenate has been determined. Since intact cell and its cell free extract binds to arsenite and oxidizes it, this process could be potentially applied to develop a strategic filter to remove arsenic from contaminated system. The product of an enzyme mediated oxidation of arsenite to arsenate, thus it can be trapped into other chemical like charcoal or alumina which are known to have high affinity for As(V). In
order to show the wide distribution of genes of arsenite oxidase and arsenate reducta-
 among bacteria which grew on sodium arsenite supplemented nutrient agar plate, we
performed dot blot hybridization studies. This finding suggests that inclusion of sodium
arsenite alone in media might trigger the expression both of genes. We would like to
conclude this study with following remarks-

- The partial (aoxB) gene cloned can be utilized to fish out the full length gene of
  arsenite oxidase of *Arthrobacter* as well as other bacteria grew in nutrient media
  containing sod.arsenite.
- The techniques like SEM, EDX and XRD can be employed to see biotransformation capability of isolate.
- Arsenite oxidase, which has specificity for arsenite can be immobilized on
electrode, and thereby substrate enzyme interaction can be studied. This would
facilitate designing of a sensor for arsenite. The detected amount of arsenite can
be directly correlated with bioavailable arsenite.
- The enzyme arsenite oxidase has been purified to its homogeneity having specific
  activity 16 $\mu$MAs(III)/min/mg protein $K_m$ 26 $\mu$M, $V_{max}$ 2.4 and is of mol.wt ~94
  kDa having large sub unit 80 kDa and Small sub unit 14 kDa.
- Full length gene can be cloned and expressed. Resulting recombinant protein can
  be immobilized and further oxidation of arsenite can be studied at bioreactor
  scale.
- We therefore, envisage that this study has a potential to develop it as a
  bioremediation technique for arsenic contamination although its standardization is
  labor intensive. High level of intellectual input is needed to understand and
  optimize the behavior of cells and the biomolecules involved in the process.