ABSTRACT

Perchlorate is the most oxygenated member of series of compounds made of chlorine and oxygen. Ammonium perchlorate is inorganic salt, which is widely used as a strong oxidizer in the solid propellant for rockets and missiles since the mid 1940s. A versatile use of Perchlorate salts for the manufacture of propellants, explosives and pyrotechnic devices by chemical, aerospace and defense industries have made the most out of it. An improper disposal and restricted degradation by the conventional methods have made it a matter of high concern. Perchlorate is of potential health concern because of its interference with the uptake of iodide by the thyroid gland, thus inhibiting the production of thyroid hormone required for normal metabolism.

Ammonium perchlorate is highly soluble in water & readily dissociates to form perchlorate anion, which is highly mobile in aqueous systems and can persist for many decades under typical conditions which are very difficult to remediate with standard methods of water treatment. Biodegradation is the most attractive and promising option for remediation of ammonium Perchlorate. All Perchlorate reducing species reported to date have been heterotrophic, Gram negative, non spore forming, facultative anaerobes. Anaerobic degradation is cost consuming and also needs continuous management.

So our objective of work is that to screen out aerobic bacteria for the degradation and then optimize the bioremediation process for the best result.

The location for soil sample collection was chosen because of possible enrichment of perchlorate reducing microbes as a result of effluence from the perchlorate production and processing industry.
Sampling was done from different regions in order to maintain uniform representation of the micro flora in and around the factory as well as from the Lonar lake having a high salinity.

For initial growth of the micro flora of the five consortia collected (A, B, C, D and E), nutrient broth was identified because of its rich composition coupled with the fact that perchlorate reducers are facultative anaerobes and are capable for utilizing alternative sources of energy.

The selections of ten bacterial isolates were done from the consortium which showed maximum percent degradation of perchlorate. Based on tolerance to diluted effluent further study was focused on isolates labeled as A1, A2, A3.

Bacterial Identification was carried out by 16S rDNA homology. Bacterial DNA is extracted by using Prepman ultra solution (Applied Biosystem) and then identification was carried out by using the 16S rDNA microseq kit (Applied Biosystem) and 3130 genetic analyzer. The sequence is then analyzed with the help of NCBI database. The strains were identified as A1-Pseudomonas stutzuri, A2-Arthrobacter atrocyaneous and A3- Arthrobacter sp. ArthroaeroA3. We were apprehensive about the polymorphic information content that would be generated from the popular RAPD study and therefore chose AFLP as a tool for genome analysis.

Together with ribosomal analysis this section of molecular study assisted in identifying and differentiating these three economically important microbes at a molecular level.

The potent strains identified were further studied to optimize the factors, which will accelerate the process of bioremediation. So we studied the vital factors such as carbon sources, trace elements, pH and temperatures.
For all the isolates under study glucose was found to be suitable carbon source. Degradation was optimum from pH range from 7-7.5. Out of different trace elements tested ferrous was found to inhibit the growth of bacteria in mineral medium. Presence of Magnesium and molybdenum was found to enhance the degradation by the selected bacteria. Residual perchlorate was estimated by Dionex Ion chromatography system (ICS 3000).

Toxicity of the degraded product was carried out as per the OECD guidelines. Fish toxicity and algae growth inhibition tests were performed to check toxic effect of degraded product.

To conclude in this study we could successfully isolates potent perchlorate degrading bacteria from soil samples. They were identified with the help of 16S ribosomal sequencing as- *Pseudomonas stutzeri*, *Arthrobacter atrocyaneus* and *Arthrobacter sp. ArthroaeroA3*. AFLP analysis generated unique genomic signature that can be used for molecular monitoring as well as tracking of genomic variations. All the three potential perchlorate reducers could tolerate perchlorate up to 4000ppm and degrade perchlorate at around 80% of 150ppm. At higher perchlorate concentrations of 4000ppm 8-9% degradation was observed. Standardized mineral medium was designed containing glucose as carbon source and magnesium or molybdenum as trace element. Ammonium perchlorate was used as single nitrogen source. Toxicity study showed no toxic effect on fish thyroid histology and fresh water algae inhibition.