ABSTRACT

Present work deals with studies on microbial degradation of perchlorate from industrial effluent and other sources. Perchlorate (ClO$_4^-$) is identified as an anthropogenic environmental pollutant leads to hormone deficiency in human beings is one of the most challenging contaminant to treat and regulate in recent times. It has extreme mobility and persistent in ground water and surface water. In the present study a microbial mediated treatment technique has been employed to treat industrial effluent contaminated with ClO$_4^-$ using bioreactors both experimentally and theoretically. Overall pure/mixed culture studies were carried out in batch reactor and stirred tank bio-reactor within optimum growth conditions of Perchlorate Respiring Bacteria (PRB). This research addressed some of the limitations by conducting metabolic and physiologic studies on the PRB enriched in either mixed or pure populations. Our findings depicted that perchlorate can be reduced in contaminated water by native microbial community and can potentially be remediated in-situ or ex-situ. The experimental work consisted of eight parts: (i) a survey analysis conducted by samples collected from various locations in south India (ii) phytotoxicity studies on the effects of perchlorate in four plant systems (iii) perchlorate degradation studies in batch reactor (BR) using mixed culture consortium developed from a waste water effluent sludge (iv) isolation and characterization of perchlorate degrading bacteria from synthetic effluent (SE) and real effluent (RE) (v) studies on the effect of environmental parameters on perchlorate degradation in BR (vi) growth curve and degradation kinetic studies of pure cultures in BR and finally (vii) studied the applicability of biodegradation of perchlorate in a lab scale STBR system for the treatment of RE and SE.

The ClO$_4^-$ level was studied in southern parts of India, the concentrations of ClO$_4^-$ in drinking water, ground water, surface water and effluent water was found to be in the range- 0.004-126.08 μg L$^{-1}$, 1.70-830.61 μg L$^{-1}$, 0.59-1110 μg L$^{-1}$ and 3258.4-93500.0 μg L$^{-1}$ respectively. Experiments were carried out in four plant seeds and investigated the growth inhibitory effect of perchlorate in seed germination and root elongation process. Results show that growth retardation effect was observed on plant systems namely: Lycopersicum esculentum, Vigna mungo, Vigna radiate and Zea mays. The ClO$_4^-$ concentrations greater than 25 mg L$^{-1}$ is observed to be toxic against plant growth and the phytotoxic effect was more in Z. mays. Overall a growth
inhibitory effect is observed, however, phytoremediation act as an effective remediation for removing ClO₄⁻ from the environment.

The isolation and characterization of perchlorate respiring bacteria studied using standard culture techniques under anaerobic culture environment. The microbial screening studies identified six perchlorate reducers namely: Proteobacterium ARJR SMBS, Methylphaga sp. LMN, Pseudomonas aeruginosa strain LMN SMBS, Exiguobacterium sp. LMNARJR, Proteus sp. LMNCRE and Bacillus sp. ARJR exhibited different ClO₄⁻ degrading potential in the nutrient medium. PRBs are observed to be facultative anaerobes and nitrate reducers. Physico-chemical, biological and growth kinetics of the isolates was studied in batch bioreactor (250 ml volume). A kinetic equation is arrived that predicts the relationship between specific growth rate, cell density and ClO₄⁻ degradation rate based on experimental data.

Experiments were carried out in batch reactor (100 ml volume) to study the effect of acetate, nitrate, pH and salinity on ClO₄⁻ degradation by mixed microbial consortium. Batch study on acetate level in the medium infers that acetate act as a good carbon source and electron donor for perchlorate degradation, provided the acetate exceeded the stoichiometric demand of the perchlorate present in the medium. Studies on effect of nitrate on perchlorate degradation revels that nitrate act as an electron acceptor competes with available perchlorate in the medium. Further, studies showed that though the microbial ClO₄⁻ reduction by a unit mass of mixed and heterotrophic bacteria occurred throughout the pH range from 4.0 to 10.0 and optimum reduction obtained at pH 8.0. Salinity is always reported to be an important factor in the perchlorate bio-degradation mechanism. Present work reveal that the bacteria responsible for reductive respiration were active below 2% w/v NaCl salinity and saline tolerance would be a limiting factor in the biodegradation mechanism.

The bio-degradation of ClO₄⁻ using Proteobacterium ARJR SMBS in stirred tank bioreactor system has been studied both experimentally and theoretically. The effect of varying ClO₄⁻ concentration on strain ARJR SMBS was studied by analyzing the microbial growth kinetics and perchlorate degradation rate. The overall experimental data reveal that the degradation was more dynamic in the case of synthetic effluent (SE) than with real effluent (RE). The overall mass balance calculations prove that for one mole of perchlorate a stoichiometric amount of 0.49 mole of chloride is produced. Overall simulation studies confirm that microbial
mediated reduction mechanism in STBR shows the transition from zero-order to first-order kinetics as the reaction proceeds. The maximum observed anoxic growth rate in SE of ARJR SMBS (0.054 h⁻¹) was considerably higher than the growth rate obtained in RE (0.037 h⁻¹). The values estimated for $K_s$ in SE and RE differed slightly, however, found to 76 mg L⁻¹ in SE and 84 mg L⁻¹ for the same substrate and organism in RE. Overall results obtained in this study reveal that *Proteobacterium* ARJR SMBS can be employed for perchlorate degradation in a stirred tank bioreactor system with an average degradation rate of 17.24 mg L⁻¹ over a period of 24 h. From this investigation we have demonstrated that microbiological chemical analysis and a suitable bioreactor design are useful in improving the biodegradation of perchlorate-enriched effluent sludge. The above findings on these bacteria are potentially useful for the development of perchlorate remediation systems under real-field conditions.

**Keywords:** Perchlorate, microbial degradation, perchlorate respiring bacteria, phytotoxicity, microbial kinetics, stirred tank bioreactor.