Chapter 1

Introduction

Pollution and manipulation of our waterways is a serious problem in the modern world. As industry grows, so can levels of harmful contaminants such as perchlorate (ClO₄⁻), heavy metals, non-biodegradable substances and bacteria rises challenging health concern to new generations. Stemming from industrial by-products, maltreated waste, runoff and poor water quality can lead to major health problems for the public and stressful living conditions for aquatic life. However, in recent years, use of an anthropogenic environmental pollutant known as perchlorate has become wide spread because of its ability to act as an exceptional oxidizer in rocket fuels and other explosives in which a synergetic oxidant is required. Perchlorate was first used in industry in nineteen fifties as ingredient in solid rocket fuel and medical community begins using it at very high levels to treat hyperthyroidism. Chilean nitrate fertilizer containing perchlorate has been widely used since 1923. Although it has been identified as a toxic chemical and environmental pollutant, its development and usage followed the expansion of many industries in many parts of the world. Nevertheless, it has not been recognized or added to drinking water Contaminants Candidate List (CCL) in India. Kannan et al, (2009) reported that, the water and human saliva samples from six states (Tamil Nadu, West Bengal, Bihar, Maharashtra, Karnataka, Pondicherry) in India, serve as an example of the implications that this pollutants can have on our environment.

1.1 PERCHLORATE DEMAND

In recent years, use of various forms of perchlorate has become wide spread because of its extensive applications in Industries and chemical research laboratories. To meet the rising demand, a great deal of attention has been focused on usage of various perchlorate salts and its related activities since the early 1980s. The production of perchlorate or even the capacity for production is difficult to estimate because perchlorate salts are classified as strategic materials. As per US EPA, (2002) the production of perchlorate in US is around 0.0091 million tonnes per year. Over 90% of it is used by the aerospace and defense industry for rocket and missile fuel
munitions. Around 7% were used for explosive use in mining and demolition of buildings etc. 1% for pyrotechnics like fireworks, smoke pots, smoke grenades, road flares and marine safety flares etc. Others constitute the charges that deploy airbags, lubricating oils and paints. Around this time the usage capacity in India (ammonium perchlorate experimental plant, Aluva, Kerala, India) is approximately close to 300-500 tons per year (Subramanian, 2004). However, the production rate is difficult to estimate due to the fact that it is considered as strategic chemicals. Because of its wide range of applications, various forms of perchlorate have been bio-accumulated into the environment and it creates serious health implications to human being.

To meet the rising demand and lack of paucity in identifying an alternative substance, it is challenging us to treat the contaminated sites and waste water polluted with perchlorate. In fact, in India contains extensive armament, cracker manufacture and perchlorate production facilities, the environmental contamination by perchlorate has received little attention by researchers. The occurrence of perchlorate in surface waters and drinking water has also been documented in Korea (Quinones et al, 2007; Her et al, 2011), Japan (Kosaka et al, 2007), Canada (Backus et al, 2005) and China (Shi et al, 2007). Overall, only limited information on perchlorate pollution is available in Asia. Despite the compound’s widespread occurrence and potential health effects, perchlorate level in India is not well understood. The presence of perchlorate in Indian was confirmed by the reports submitted by Kannan and his co-workers through the human saliva samples collected from urban and rural locations in India (Kannan et al, 2009). They confirmed that perchlorate concentrations in water samples from India are one to two orders of magnitude lower than the concentrations reported for the US. Sugimoto et al, (2012) study reconfirms the occurrence of perchlorate contamination in groundwater in 6 cities (Sivakasi, Chennai, Trichy, Mumbai, Madurai and Kolkata) in India. Overall, it infers that the perchlorate contamination in India is wide spread and it will leads to potential health hazards to the people.
1.2 STANDARD PERMISSIBLE LIMIT OF PERCHLORATE

With increasing level of perchlorate in soil and water, there is a serious need of well defined $\text{ClO}_4^-$ standards around the world. As such, in India there is no standard recommendation level for perchlorate that is present in the effluent water, drinking water, surface water and also the soil. The US Environmental Protection Agency (US EPA) is considering a standard of 1 ppb, which would be equivalent of a grain of salt in an Olympic-sized pool. In 1997, with the development of new analytical method to detect low concentrations of $\text{ClO}_4^-$, US EPA added $\text{ClO}_4^-$ to drinking water CCL (Urbansky and Schock, 1999; ITRC, 2005; Pontius et al, 2000). The reference dose (RfD) according to National Academy of Science (NAS) review is that, daily ingestion of $\text{ClO}_4^-$ up to 0.0007 mg/kg of body weight can occur without adversely affecting the thyroid and iodide uptake (US EPA, 2005a). The National Research Council (NRC) concluded that this RfD would protect the health of even the most sensitive populations (Urbansky and Schock, 1999; Perciasepe, 1998). This RfD is elevated from the provisional RfD of 0.0001 ng/kg suggested by the US EPA, (2002). The higher RfD translates to Maximum Contaminant Level (MCL) of 24.5 μg L\(^{-1}\) perchlorate in drinking water (US EPA, 2002). Further, US EPA issued a safety standard that perchlorate less than 24.5 ppb in drinking water is safe and this was much higher than the public health goal set in California (6 ppb).

The acute lethal oral dose of perchlorate for an adult human is estimated to be 214 mg/kg for a 70-kg person (Von Burg, 1995). According to $\text{ClO}_4^-$ fact sheet issued by Massachusetts Department of Environmental Protection (DEP), promulgated a drinking water standard or MCL of 2 μg L\(^{-1}\) (US EPA, 2005a). The selected MCL was determined to provide the best overall protection to public health, considering the benefits of disinfections, while retaining a margin of safety to account for uncertainties in the data used for defining the standards. Several states in the US have set advisory levels of perchlorate in the range of 1–18 μg L\(^{-1}\) (US EPA, 2005a). In 2005, the New Jersey DEP recommended that drinking water with $\text{ClO}_4^-$ concentration above 5 μg L\(^{-1}\) should be avoided (Buffler et al, 2006).
1.3 CHEMISTRY OF PERCHLORATE

Perchlorate is an oxygen-rich chemical which readily dissolve in water, generating the perchlorate anion (ClO$_4^-$). It consists of a tetrahedral array of four oxygen atoms around a central chlorine atom as shown in Figure 1.1. The tetrahedron structured symmetry of perchlorate anion infers that it is thermodynamically strong oxidizing agent and a kinetically sluggish species. Its reduction is generally very slow and rendering common reductants ineffective. It can combine with sodium, potassium or ammonium to form complex salts and ammonium perchlorate is the most prevalent form used in rocket fuel. Due to its relatively low charge density, perchlorate does not form complexes with metals in the same manner as other anions in its ionic state and has little tendency to adsorb to environmental, mineral media or organic surfaces (poor nucleophile, poor coordinating ability) (Urbansky, 1998).

![Figure 1.1: The structure and dimensions of perchlorate anion.](image)

Perchlorate salts are formed in a two step electrochemical process in which sodium chloride is first oxidized to sodium chlorate, and then sodium chlorate is oxidized to sodium perchlorate as showed in equation (1.1) and (1.2) (Mendiratta et al, 1996). The potential for oxidation of chlorate to perchlorate is close to the potential for oxidation of water to oxygen.

\[ \text{ClO}_4^- + 2 e^- + 2 H^+ \rightarrow \text{ClO}_3^- + H_2O \ E = +1.226 \ \text{V} \] (1.1)

\[ O_2 + 4 e^- + 4 H^+ \rightarrow 2H_2O \ E = +1.272 \ \text{V} \] (1.2)

According to Lewis structure concept the preliminary structure of perchlorate anion has a formal charge of 3+ while each oxygen must absorb one more electron than is necessary to neutralize the positive charge of its nucleus resulting in a formal charge of 1+ on each oxygen atom. Lewis structures predict that 7 bonds are shared over 4 positions for a 1 and 3/4 bond character. In fact, a delocalization of the extra electrons...
on oxygen towards the highly electronegative chlorine atom makes perchloric acid a strong acid and the chlorine atom has an oxidation state of +7, which makes the molecule a strong oxidizing agent. It is highly soluble and mobile in aqueous systems with a molecular mass of 99.45 amu. It will persist in the environment for many decades in ground and surface water because of its resistance to react with other available constituents present in water or soil. Due to this combination of solubility, stability, and mobility creates both localized and area-wide impacts of toxicological interest. The solubility of sodium perchlorate, ammonium perchlorate and potassium perchlorate is 2110, 220 and nearly 10 g L⁻¹ respectively at 20°C (Motzer, 2001). It is kinetically inert to exchange of oxygen atoms with water and the half-life for exchange at room temperature is estimated to be greater than 100 years (Hoering et al, 1958). It has long half-lives with ordinary reactive metals such as Ru⁺² (3.6 days), Ti⁺³ (61.25 days), V⁺² (11.3 years) (Espenson and Abu-Omar, 1997).

1.4 SOURCES AND APPLICATION

Perchlorate has been released into the environment for over half a century primarily due to the use of ammonium perchlorate as the propellant in missiles, military ordinance and rockets (Silva, 2003). Ammonium perchlorate is the most important propellants because it has high oxygen content and decomposes to the gaseous phase’s products water, HCl, N₂, O₂ and leaving no residue. Studies showed that perchlorate could be formed naturally in the atmosphere from chlorine species (Urbansky, 1998; Logan et al, 2001a) and lightening may also play a role in the creation of some atmospherically produced ClO₄⁻ (Jackson et al, 2006). Apparently, perchlorate is reached to the environment both by natural and manmade activities. US EPA reported that atmospheric fallout from fireworks consists of fine particles of burnt black powder, paper debris and residue. Perchlorate in paper debris ranges from 302 to 34,200 µg kg⁻¹ (US EPA, 2002). Following in the atmospheric creation, ClO₄⁻ return to the earth surface in dissolved form. Perchlorate compounds are used in a number of operation such as air bag initiators for vehicles, ejection seats, leather and tanning industries, oxygen generators, paints and enamel industries, electroplating, electro polishing, engine oil testing, flash powder for photography, chemical laboratories in analytical testing, chemical explosives, match industries, fertilizers,
household bleach, road flares etc (Damian and Pontius, 1999; Retskin, 1997; Urbansky et al, 2001). Motzer, (2001) reported that the incomplete combustion of the rocket fuel during take-off of rockets, space shuttles and firework displays leads to the unburned perchlorate which becomes dispersed in the atmosphere and then settles on the land or in water.

It is noteworthy that, potassium perchlorate has been used in medical practice for the treatment of thyroid disorders to suppress the overproduction of hormones for treating hyperthyroidism resulting from Grave's disease (Chiovato et al, 1997; Cooper, 1996). It has been used to treat thyrotoxicosis without toxicity at doses ranging from 40 to 120 mg day⁻¹ (Cooper, 1996). Perchlorate acid an acidic form of perchlorate is used to treat medical devices for heart operations (Gregory, 1939; MADEP, 2005). Since 2002, it is estimated that around 75,000 tons of Chilean nitrate fertilizer containing 0.01% perchlorate used annually in US. Commonly used perchlorate compounds and its characteristics are summarised in Table 1.1.
Table 1.1: Summary of characteristics and uses of some commonly used perchlorate compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Molecular weight (g mol(^{-1}))</th>
<th>Density (g cm(^{-3}))</th>
<th>Physical appearance</th>
<th>Aqueous solubility at 20(^{\circ}) C (10(^{3}) x mg L(^{-1}))</th>
<th>Decomposition temperature (°C) / Reaction</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium perchlorate</td>
<td>NH(_4)ClO(_4)</td>
<td>117.488</td>
<td>1.952</td>
<td>Colourless or white orthorombhic and regular crystals</td>
<td>217-220</td>
<td>150</td>
<td>Energetic booster in rocket fuel</td>
</tr>
<tr>
<td>Sodium perchlorate</td>
<td>NaClO(_4)</td>
<td>122.439</td>
<td>2.02-2.499</td>
<td>Hygroscopic/deliquescent, white orthorombhic</td>
<td>2010</td>
<td>492</td>
<td>Strong oxidizing agent used in explosive and chemical industries</td>
</tr>
<tr>
<td>Potassium perchlorate</td>
<td>KClO(_4)</td>
<td>138.547</td>
<td>2.5298</td>
<td>Colourless crystal to white crystalline powder, hygroscopic</td>
<td>7.5 to 16.8</td>
<td>440</td>
<td>Solid oxidant for rocket production</td>
</tr>
<tr>
<td>Lithium perchlorate</td>
<td>LiClO(_4)</td>
<td>106.3906</td>
<td>2.428-2.429</td>
<td>Deliquescent, white crystal</td>
<td>375</td>
<td>&lt; 250-400</td>
<td>Electrolyte in voltaic cells, synthesis of organic chemicals</td>
</tr>
<tr>
<td>Perchloric acid</td>
<td>HClO(_4). 2H(_2)O</td>
<td>223.21</td>
<td>2.21</td>
<td>White, hygroscopic powder</td>
<td>Very soluble</td>
<td>250</td>
<td>Analytical, oxidizing and dehydrating agent</td>
</tr>
</tbody>
</table>
1.5 HEALTH EFFECTS

Perchlorate can hamper the thyroid gland's ability to take up the essential nutrient iodide (Xu et al, 2004). Therefore, once $\text{ClO}_3^-$ reaches the human body it will inhibit the iodine uptake and it prevents the normal growth and metabolism (Wolff, 1998; Urbansky and Schock, 1999; Xu et al, 2004). The potential health concern associated with increase in $\text{ClO}_3^-$ is that iodide deficiency (hypothyroidism) which results in goiter and developmental defects in fetuses. Hormones required for normal development of the central nervous system of fetuses and infants are secreted by the thyroid gland. These hormones are required for normal skeletal growth and development. In both infants and adults, thyroid gland hormones determine metabolic activity and affect many organ systems. Hence, improper thyroid function may affect metabolic functions, growth, cardiovascular and central nervous systems. Thyroid hormones appear to have their most profound effects on the terminal stages of brain differentiation, including synaptogenesis, growth of dendrites and axons, myelination and neuronal migration. The proper functioning of a mother’s thyroid gland is critical to both the health of the mother and the proper development of fetus (SWRCB, 2002). In pregnant women, thyroid malfunction may distress the fetus (cretinism) and children which result in behavioural changes, delayed development and decreased learning capabilities in new born (Kirk et al, 2005; Blount et al, 2009; Kirk et al, 2005).

Adverse health effects from perchlorate are considered acute, producing a strong or serious short-term effect. Perchlorate ions (larger than that of iodide) are competitive inhibitor and actively deposited into thyroid follicular cells. The pituitary gland responses by secreting more thyroid stimulating hormone (TSH), which in turn causes thyroid hypertrophy and iodine deficiency (causes goiter). These hormones play an essential role in regulating the body's metabolism and physical growth. It helps to control the energy level, temperature, weight, mood and mental performance. Any notable imbalance in thyroid hormones could significantly impair all these functions. In fact, in children, it will hinder their mental development, potentially leading to mental retardation, loss of hearing and speech and motor skill deficits. Pregnant women with low levels of iodine have higher risks of miscarriage (Wolff,
In vivo human studies proved that perchlorate does not metabolize or accumulate and eliminate from the body fairly rapidly, with a half-life of 8 h (Anbar et al, 1959; OEHHA, 2002).

Experiments on rats and fishes proved that ClO$_4^-$ at very high doses, caused thyroid tumours in laboratory rats (Paulus et al, 2007; Park et al, 2007; Bradford et al, 2006). However, it is not certain whether similar effects would occur in humans. Mattie et al, (2006) reported that the primary route of ClO$_4^-$ exposure is through ingestion of water or food contaminated with ClO$_4^-$ and besides skin absorption, direct inhalation can also be considered as secondary route to exposure. Interestingly, potassium perchlorate has been used to treat hyperthyroidism resulting from Grave's disease (Chiovato et al, 1995; Cooper et al, 1996; Urbansky, 1998). Recently, attention has begun to focus on the health effects of low-level perchlorate exposure, because iodine deficiency linked to adverse neurological development and reduction of intelligence quotient in children (OEHHA, 2002). Now the technology continues to improve to find out the propensity of perchlorate for blocking iodide uptake to the thyroid. Thus, our full understanding of health effects from low-level exposure to perchlorate is still emerging.

1.6 BIOREMEDIATION OF PERCHLORATE

Bioremediation is the use of living organisms, primarily microorganisms to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health or the ecosystem. This results in the breakdown of complex compounds into simpler forms such as carbon dioxide and water. Bioremediation is an efficient technology for the treatment of contaminated water and other sources with perchlorate. Bioremediation of perchlorate contaminated groundwater can occur via bacterial reduction of perchlorate to chloride. Earlier reports proved that all perchlorate-reducing bacterial isolates are either facultative or microaerophiles (Wallace et al, 1998; Coates et al, 1999). Therefore, oxygen produced during perchlorate degradation does not accumulate in pure cultures; it is consumed by facultative microbes faster than it is produced (Bruce, 1999). As on date most of the
perchlorate degrading isolates reported have been heterotrophic (Logan, 1998; Achenbach et al, 2001), and required organic substrates for synthesizing cellular materials. Growth substrates (eg. acetate, lactate etc.) introduced into the water to support the growth of heterotrophic Perchlorate Respiring Bacteria (PRB), can lead to the release of unoxidized organic substrates that may stimulate subsequent microbiological growth in water distribution systems. Studies have been reported that chlorate is a partial reduction product from perchlorate and appears to be more toxic than perchlorate (Jackson et al, 2005). Inorganic pollutants such as nitrate, nitrite and perchlorate have a positive reduction potential and are used as the final electron acceptors in the electron transport chain by some microorganisms. Bioremediation of salty wastes is desirable since bioremediation is considered to be a cost effective treatment technology (Okeke et al, 2002). Although perchlorate reduction has been demonstrated in bacterial pure cultures, little is known about the efficacy of using PRB as inoculants for bioremediation in the field (Zhang et al, 2005).

1.7 SCOPE OF THE WORK

Biological perchlorate removal studies are continuing in many parts of the world. There are few full scale engineered biological systems are available for removing ClO₄⁻ in industrial discharges, drinking water and other contaminated sources. In fact, in India there is not much awareness or treatment technologies are available to treat this anthropogenic environmental pollutant. But, still there are contradictory reports as well as gap in these areas exist. In spite of its importance, the most available research on ClO₄⁻ is focused on determining the effects of ClO₄⁻ on human exposure and its deleterious effects on other species throughout the environment. The discharge from the ClO₄⁻ manufacturing industries are highly saline (300 g L⁻¹ NaCl) in nature and biological reduction of ClO₄⁻ under stressful conditions is challenging us. Therefore, as far as human nature and other ecological effects are concerned, there is a need of efficient and cost effective treatment technologies to treat the contaminated sites with ClO₄⁻ for better field application purposes. With the above background information the purpose of the present investigation emphasis on microbial degradation of ClO₄⁻ and attempted to study the various environmental factors which affects on its biodegradation. The microbial communities (new
organisms) involved in ClO\(_4^-\) reduction are being reported from many natural environments contaminated with ClO\(_4^-\). However, studies on the effect of ClO\(_4^-\) on growth of PRBs and its characteristics are limited. Considering the paucity of previous studies reported in the literature, the following objectives are formulated for the present study.

OBJECTIVES

1. Conduct a survey analysis by samples collected from various locations in south India
2. Study the effects of perchlorate in selected plant systems (a phytotoxicity approach)
3. Perchlorate degradation study in a batch reactor (3L volume) using mixed culture consortium developed from a waste water effluent sludge
4. Isolation and characterization of pure perchlorate degrading isolates
5. Study the effect of environmental parameters on perchlorate degradation in batch reactor (100 ml volume)
6. Growth curve and degradation study of pure cultures in batch reactor (250 ml volume)
7. Microbial growth kinetic studies of pure microbial strains
8. Biodegradation of perchlorate treatment in a lab scale Stirred Tank Bioreactor (STBR) System (5 L Volume)

The following chapter presents a detailed survey of the literature on perchlorate removal methods and the effect of various environmental factors on its biodegradation. Chapter 3 gives a description of the current survey report of India and the occurrence of perchlorate in various water samples collected from southern part of India. Chapter 4 presents the materials and methods followed for analysis. Chapter 5 gives the description of the experimental set-up and the parametric measurement procedures. Chapter 6 presents the results and discussion of phytotoxicity study of perchlorate on four plant systems, isolation and characterization of perchlorate degrading isolates, effects of environmental parameters on ClO\(_4^-\) degradation in batch bio-reactor, degradation kinetics of pure isolates and the treatment of real/synthetic effluent contaminated with perchlorate in a lab scale Stirred Tank Bio-reactor (STBR) system. Chapter 7 the concluding chapter gives the summary and conclusions.