1.1 GENERAL

Perchlorate (ClO$_4^-$) is one of the largest manufactured chemical substances which are widely used for industrial and other wide range of applications (Khwaja et al 2005, Urbansky and Edward 1998). Perchlorate anion consists of tetrahedral arrangement of four oxygen atoms and one central chlorine atom and it possess as a strong oxidation agent due to chlorine ion as shown in Equation (1.1).

$$\text{ClO}_4^- + 8\text{H}^+ + 8\text{e}^- \rightarrow \text{Cl}^- + 4\text{H}_2\text{O} \quad E_0 = 1.287 \text{ V} \quad (1.1)$$

Perchlorates react very slowly when compared to most permanganate reducing agents such as dichromate and permanganate and it reduces only in the presence of strong concentrated acids. Perchlorate are relatively low charge density, extremely soluble substances, it does not form any complexes with metal ions and any improper discharge of these oxyanions leads to contaminations of surface, ground water supplies, and soil (Coleman et al 2003). Because of its extreme solubility, perchlorates are not significantly broken down to its mineral compounds in the environment and makes perchlorate as a persistence compound (Medina et al 2006). There are four commonly used perchlorate compounds used for various purposes and its physical and chemical properties are tabled in Table 1.1.
Table 1.1 Physical and chemical properties of various perchlorate compounds

<table>
<thead>
<tr>
<th>S.No</th>
<th>Properties</th>
<th>Ammonium perchlorate (NH₄ClO₄)</th>
<th>Sodium perchlorate (NaClO₄)</th>
<th>Potassium perchlorate (KClO₄)</th>
<th>Perchloric acid (HClO₄)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Molecular Weight</td>
<td>117.49</td>
<td>122.44</td>
<td>138.55</td>
<td>100.47</td>
</tr>
<tr>
<td>2</td>
<td>Color</td>
<td>White</td>
<td>White</td>
<td>Colorless</td>
<td>Colorless</td>
</tr>
<tr>
<td>3</td>
<td>Form</td>
<td>Orthorhombic Crystal</td>
<td>Orthorhombic Crystal</td>
<td>Crystalline Powder</td>
<td>Oily Liquid</td>
</tr>
<tr>
<td>4</td>
<td>Melting point</td>
<td>Decomposes /Explosive</td>
<td>480 °C</td>
<td>525 °C</td>
<td>-112 °C</td>
</tr>
<tr>
<td>5</td>
<td>Density</td>
<td>1.95 g/CC</td>
<td>2.52 g/CC</td>
<td>2.53 g/CC</td>
<td>1.768 g/CC</td>
</tr>
<tr>
<td>6</td>
<td>Solubility at 25 °C</td>
<td>200g/L of water</td>
<td>209.6g/100mL of water</td>
<td>15g/L of water</td>
<td>Miscible I cold water</td>
</tr>
</tbody>
</table>

1.2 USES AND OCCURRENCE OF PERCHLORATE

Ammonium perchlorate represents approximately 90% of all perchlorates manufactured in the globe and it is used in manufacturing of rocket fuels, missiles, explosives for various military purposes, fireworks, airbags, lubricating oil, inflators and paints (Farhan et al 2009, Logan et al 2001). Perchlorate is naturally found in deposit of Atacama desert in Chile, Death valley, Mission valley Eocene in California USA, Saskatchewan in Canada, Bolivian playas and remote places in Antarctica (Sturchio et al 2012, Kucharzyk et al 2010, Rajagopalan et al 2009). Many researchers proved that perchlorate can be formed by reacting chloride aerosol with high concentration of ozone in the presence of high electrical discharge.
In many provinces of USA perchlorates are found in concentration ranging from 0.005 g/L to 4.5 g/L and some worst contaminated place like Lavages, Nevada where perchlorate was manufactured for more than 50 years which contains ground water contamination level of 630,000 µg/L to 3,700,000 µg/L (Coates and Achenbach 2004, Christian 2003). Perchlorates were found in beverage in Europe at a concentration of 50 ppb and it was detected in ground water as well as surface waters, which are linked for the use of fertilizer from Chilean origins. Some researchers reported that in some places of Portugal, tap water and bottled water contains perchlorate at a concentration level of 5ppb and Aribi and his co researchers found that perchlorate was detected in Chile apricots and green grapes at concentration of 145 ppb and 40 ppb, Mexican tomatoes and Mexican asparagus at a concentration of 63 ppb and 22 ppb (Aribi et al 2006). Perchlorate was detected at peak concentration of 3900 µg/L in Japan (Kosaka et al 2007). In India perchlorate was detected in six different states in surface, drinking and ground water with concentration ranging from 0.02 to 6.9 µg/L (Kannan et al 2009) and in southern parts of Tamilnadu, the concentration of perchlorate was found to be 64 µg/L and 75 µg/L in groundwater.

1.3 HEALTH EFFECTS OF PERCHLORATE

Thyroid hormone syntheses from iodine in the thyroid gland are responsible for the regulation of mammalian metabolism. Perchlorate inhibits thyroid hormone synthesis by competition with iodine uptake by binding into the sodium–iodide symporter, long term reduction of iodine intake will lead to Hypothyroidism (O'Connor et al 2002, Narayanan et al 2003). Perchlorate is found in breast milk leached from bioaccumulation in breast tissues and high concentration exposure of perchlorate leads to mental retardation and fatal bone marrow diseases in fetuses and infants (Yoon et al 2009). Condie (1986)
reported that high exposures of chlorate and chloride in rat and mice, damages red blood cells which results in hemolytic anemia and methemoglobin formation.

Due to severe health hazards possessed by this compound, the United States Environmental Protection Agency (US EPA) added perchlorate as a hazardous substance in drinking water contamination list and recently issued a Reference dosage (RfD) of 0.7 µg /kg body weight /day which is equal to a drinking water equivalent level (DWEL) of 25 µg/L (Bruce et al 1999), in addition to that Massachusetts and Californian states recently issued a Maximum drinking water level (MCL’s) of 2 and 6 µg/L respectively (Coates et al 2004, Zhang et al 2002). There is no regulatory standard by World Health Organization, European environmental agency, central pollution control board of India and state pollution control board of Tamilnadu. This leads to discharge of untreated release of perchlorate contaminated wastewater into the environment. Perchlorate remains in the food even after cooking, and Furthermore, perchlorate does not observe to a significant extend to soils and sediments and in the absence of any biological interaction its mobility and fate are largely influenced by the hydrology of the environment. Due to various exposure, sources of perchlorate in drinking and food products and unavailability of long term health effects data for low concentration of perchlorates exposures, perchlorate is considered as a growing contaminant concern. Due to uncertainty in the toxicology of perchlorate, the allowable concentration may vary from 2-18 mg/L and the health hazards caused by perchlorate attracted attention of researchers for various perchlorate treatment technologies.
1.4 PERCHLORATE TREATMENT TECHNOLOGIES

The treatment technology for perchlorate is classified into physical, chemical, biological methods. The physical and chemical methods of perchlorate treatment removes perchlorate from one phase to another phase which includes Reverse osmosis, Electro dialysis, Ion exchange, Adsorption and Electrochemical treatment, and in contrast the biological methods degrade perchlorate into its mineral compounds. Details of these treatment technologies are discussed in the literature review section, however in this section the significance of biological methods of perchlorate are discussed below.

1.4.1 Significance of Biological Removal of Perchlorate

Based on the chemical reaction addressed in Equation (1.2), the perchlorate reduction potential makes perchlorate suitable to serve as electron acceptor for microbial metabolism. The effective treatment strategy used by many of the researchers for perchlorate remediation was the biodegradation of perchlorate into chloride and oxygen with the help of suitable perchlorate reducing microorganism. Further, compare to the abiotic techniques used for removal of perchlorate like ion exchange, reverse osmosis and electro dialysis, the biological reduction of perchlorate converts perchlorate into nontoxic chloride without the generation of high concentrated perchlorate and brains, for the purpose of regenerating ion exchange resigns. In addition to that the biological reduction process of perchlorate possess additional advantages like the simultaneous reduction of nitrate, bromide and other contaminants in the same system. There are various microorganism isolated and kinetic parameters are evaluated in order to predict the rate of perchlorate degradation in natural and engineered systems. The mechanisms of perchlorate
removal by microorganism and bioreactor Technology adopted for perchlorate treatment are discussed below.

1.4.2 Microbiological Reduction of Perchlorate

The respiration pathway used for biological degradation of perchlorate by various bacteria proposed by Rikken et al (1996) is given below:

\[
\text{ClO}_4^- \rightarrow \text{ClO}_3^- \rightarrow \text{ClO}_2^- \rightarrow \text{Cl}^- + \text{O}_2
\] (1.2)

The pathway of perchlorate degradation shown in Figure 1.1 includes three steps

(i) The first and second step involves electron transfer from perchlorate to chloride with the help of enzyme called perchlorate reductase.

(ii) The third or final step involves transfer of chlorite into chloride and oxygen with the help of chlorite dismutase enzyme. This step, doesn’t gain any energy due to non-consumption of electron.

![Figure 1.1](Adapted from Riken et al (1996))
1.4.3 Bioreactor Treatment Method for Perchlorate Degradation

Bioreactor technology is mostly adopted for removing perchlorate from surface and ground water at large scale. This method uses the ability of perchlorate degradation microorganism, with suitable electron donor and growth medium, perchlorate can be converted in to chloride and oxygen and it is used by many researchers to remove perchlorate to a concentration level of less 4 µg/L (Attaway and Smith 1993). There are various mode of operation for perchlorate removal in a bioreactor is adopted such as batch, semi continuous and continuous. In batch mode of bioreactor operation the reactor is initially operated with all the necessary elements and no further addition or removal of any substances. The substrate is added along with the essential nutrient at intermediate time for semi continuous batch reactor in order to prolong the operation and except normal sampling, no material is removed from the reactor. If the reactor is fed with highly concentrated nutrients, then the volume of the reactor will be much more over time due sufficient presence of substrate concentration. While in the continuous mode of bioreactor operation the growth nutrients are continuously pumped in parallel with reduced or depleted medium in which it is continuously removed. In this mode of bioreactor operation the rate of addition of nutrients and the rate of removal of nutrients are equal hence the reactor volume is unchanged. In Perchlorate treatment using bioreactor either fluidized bed reactor method or continuously stirred tank reactor (CSTR) methods or fixed film method are widely adopted (Wallace et al 1998).

In fluidized and fixed film methods, sand or any adsorbent materials are used as a support media for microorganism to treat perchlorate. Compared to the packed bed reactor system, fluidization reactor provides larger surface area on the media particle for microbial growth, this result in rapid biological growth in the system capable of increased efficiency for
target contaminant in a smaller volume, however in general the fluidized bed reactor system requires greater pumping rate than fixed bed reactor system (Sahu et al 2009). Due to longer residential time CSTR technology may not be applicable for treating low perchlorate concentration in ground water.

A packed bed reactor is another type of fixed film bioreactor that has potential to achieve clean effluent even if the perchlorate concentrations are very low (Losi et al 2002). This method achieves very shorter residence time as compared to CSTR and avoid slow growth rate, incomplete substrate oxidation, the need for maintaining bed dynamics and enables to handle shock loads. Therefore an optimized packed bed reactor process has high potential to reduce perchlorate compared to other processes.

![Figure 1.2 Typical packed bed bioreactor](image)

Figure 1.2 shows the line diagram of an up flow packed bed bioreactor for perchlorate degradation. The advantage of up flow packed bed bioreactor packed with activated carbon meets the requirement to treat perchlorate contaminated water efficiently. This process utilizes GAC as a media for physical removal of chemicals, further the microorganism started to colonize over the GAC surface and form a significant biomass or biofilm with help of polymer substances.
The information about the biofilm can be obtained by measuring the constituents present in the biofilm and its thickness determines the mass transfer properties like nutrient diffusion, flux from the bulk liquid to the biofilm and frictional resistance. Furthermore the thickness of the biofilm growth can be described using a sigmoidal curve. The activity of the microbial biofilm has been increased upon its adsorption onto the GAC media, and there are several factors which influence the activity of the biofilm. One hypothesis stating that the microbes attached to the GAC surface undergoes physiological changes and the other one stating that the biofilm changes the local concentration of nutrients, oxygen, enzymes and it limits the toxic substances entering into the system, further the absorbed biofilm is less affected by changes in the environmental conditions.

Initially the pollutants are removed by GAC from the liquid and microorganisms are able to colonize on the surface of the GAC media and forms biofilm as they feed on rich supply of adsorbed and entrapped organic matter, and dissolved nutrients and other substances present in the water phase. Biofilm creates a laminar film region towards the GAC surface and this transport is accomplished by molecular diffusion across the cell membranes within dense microbial cell clusters. Biofilm process is capable of processing and biodegrading significant fraction of the entrapped pollutant in the GAC pores. This process offers several advantages like decreasing the frequency of filter backwashing and biological regeneration of exhausted GAC. Even though molecular diffusion is the primary mechanism for pollutant transport, another complex convective transport is reported. As the biofilm grows, the cells and extracellular substances form a porous structure which is separated by interstitial voids and open channels. Biodegradation of a substance is a multistep process, initially the substances adsorbed on to the
surface of the GAC after that it undergoes microbial degradation. This process cycle regenerates and prolong the bed life of GAC.

The microorganisms that are capable of degrading perchlorate are inoculated into the reactor which is having a larger surface area compared to other packed bed media with soil and sand. This leads to high biomass concentration, fixed film microorganism activity over the solid surface, prevention of the suspended biomass washout, faster reaction rate as well as reduction in the frequency of clogging compared to down flow mode. As the electron acceptor passes by the active biofilm layer, it penetrates into the bilayer and eventually biodegraded. The main advantage of using GAC as a carrier medium includes the wider application of GAC in drinking water treatment plants where existing GAC filters can easily be retrofitted to operate as biological active carbon reactors (BAC) and further GAC not only be used to support the growth of biofilms but sorption by GAC has been shown to complement biological removal in BAC reactor’s dynamic loading conditions. It may be assumed that the sorption capability of GAC serves as a temporary sink for contaminants and then allows biological degradation of the sorbed contaminant. In addition to the direct benefits of acting as a temporary sink of target contaminants, the sorptive capacity of GAC should be able to enhance biological perchlorate removal indirectly by lowering the concentration of oxygen, the competing electron acceptor, through chemisorption. Molecular oxygen can be irreversibly removed by interacting with the GAC surface. It also assumed that sorption, intraparticle diffusion and desorption resulted in increased biofilm activity on the GAC compared to non-absorbing carrier media. The present study is aimed at “biodegradation of perchlorate” in an UFPBR.

The overall aim of this study is to remove and degrade perchlorate using microorganism, isolated from contaminated environment attached onto
packed bed media of GAC and to understand the complex interaction between sorption, desorption, biological processes and reaction operation in an up flow packed bed bioreactor.

1.5 OBJECTIVES

The following are the objectives of the study

- To determine perchlorate removal using culture microorganism isolated from contaminated site.
- To determine perchlorate removal using physical adsorption.
- To determine perchlorate removal efficiency using up flow packed bed bioreactor.
- To determine the kinetic parameters of perchlorate removal in packed bed reactor through kinetic modeling