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PERCHLORATE: HEALTH EFFECTS AND TECHNOLOGIES FOR ITS REMOVAL FROM WATER RESOURCES

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ABSTRACT: Perchlorate has been found in drinking water and surface waters in the United States of America, Canada, and India. It is primarily associated with the release of perchlorate from defense and military operations where rocket and satellite operation production takes place. Natural sources of perchlorate contain certain fertilizers and potash ores. Although it is a strong oxidant, perchlorate is very persistent in the environment. At high concentrations perchlorate can affect the thyroid gland by inhibiting the uptake of iodine. As per WHO gives a maximum tolerable daily intake of 0.01mg/kg body weight for perchlorate. Above 15 ppb intake of perchlorate, it changes normal to an abnormal condition of a living being. Perchlorate is measured in environmental samples primarily by ion chromatography. It can be removed from water by anion exchange or membrane filtration, biological and chemical processes. It remains stable for long times in water and soil in normal atmospheric conditions.

INTRODUCTION: Perchlorate ion occurs in natural and man-made anion or in both ways, which is coordinated tetrahedral with four oxygen atoms and bonded to the central chlorine atom. It is made of in the form of perchloric acid and its salts such as ammonium, potassium, and lithium or sodium perchlorate. Ammonium perchlorate has been used to manufacture rocket propellants, fireworks, weapons, automobiles, airbags, and safety flashboards for highway construction [1]. Due to its high oxidizing power, the major sources of perchlorate ions were from historical disposal practices of rocket fuel and explosives by the aerospace and chemical industries due to its high oxidizing power [2]. Naturally occurring perchlorate is also found in the hyper-arid Atacama Desert, in Chilean nitrates and their derived nitrate fertilizer, and in southeastern California caliche nitrate deposits [3-4].

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Perchlorate is difficult to degrade in normal atmospheric conditions, and it is very stable and stays for a long time in atmospheric conditions. It is shown that it inhibits perchlorate uptake by the thyroid gland [5]. The thyroid is important for normal growth, development, and metabolism in the human body and the effects can be significant in the case of pregnant women and fetuses. Terrible disease like cancer, thyroid aliments and mental disorders which is caused by the consumption of perchlorate present in water [6]. Therefore, perchlorate removal from drinking water sources is critically important to public health. Several perchlorate treatments have been developed, including activated carbon adsorption[7-8], chemical reduction [9], membrane filtration [10] and ion exchange [11]. Except for ion exchange process, all other processes require high cost and time consumable. The ion exchange process is a commercially available method to remove perchlorate from drinking water [12]. Four sites in kerala selected in this study water sample found ammonium perchlorate near (1) experimental plant (APEP) at Aluwa in Ernakulam district. (2) Thumba near Vikram sarabhai space center in Thiruvanthapuram district (3) the other two locations were Kannur and Palakkad district (4) Tube well found near ISRO found water contaminated.

1. Properties of Perchlorate:

1.1. Introduction for Perchlorate: Perchlorate (ClO_4^-) and chlorate (ClO_3^-) have been produced on a large scale by the chemical industry for use in a wide range of applications. The improper storage and/or disposal of these oxyanions have led to harmful concentrations in surface and groundwater supplies. As they are extremely soluble and not significantly broken down in the environment, these characteristics make them persistent and problematic environmental pollution of drinking waters. Moreover, ClO_4^- and chlorate are also a health concern, as they can cause serious diseases, such as cancer. In the medium-term, the removal of ClO_4^- from drinking water will become necessary in order to protect the environment and human health.

The increasing interest in perchlorate pollution includes recent advances in analytical chemistry and a better understanding of perchlorate's health impacts. The advances and developments of chemical methods have allowed the detection of concentrations at low part-per-billion (microgram per litre) µg/L 13. The toxicological research has suggested that such concentrations may be a potential risk for developing foetus and infants [14-15]. Perchlorate inhibits iodide uptake by the thyroid disrupting normal thyroid function, which can lead to a number of serious health problems, especially pertaining to early neurological development [16]. There have been several high-profile cases of perchlorate contamination of surface waters and drinking water supplies in major metropolitan areas [17] and the parties such as U.S. Department of Defence (DOD) responsible for the events had to quickly respond to the regulatory and public demand to prevent further exposures and clean up contaminated sites [18]. In January 2009, the EPA issued a health advisory to assist state and local officials in addressing local contamination of perchlorate in drinking water. The interim health advisory level of 15 micrograms per liter (µg/L), or ppb, is based on the reference dose recommended by the National Research Council (NRC) of the National Academy of Science (NAS) [10]. The most recent technologies for remediation of perchlorate in groundwater are in the group of phytoremediation, in situ bioremediation with the application of Genetic Algorithms (GAs) and ion exchange technology.

1.2 General Chemistry of Perchlorate: Perchlorate originates in the environment as solid salts of ammonium, Potassium or Sodium Perchlorate. The Perchlorate ion (ClO_4^-) can be visualized as a tetrahedron shown in **Fig. 1**, with four oxygen molecules at the tetrahedral corners and a chlorine atom at the center and carries a negative charge.

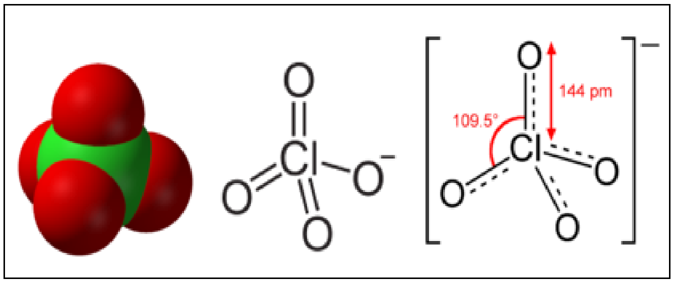

FIG. 1: PERCHLORATE ION STRUCTURE AND DIMENSION

The negative charge is dispersed evenly over the four oxygen atoms and is responsible for the inherent stability of the perchlorate species [19]. The Oxidation state of chlorine in the perchlorate is +7. Hence, it is a strong oxidizing agent. Perchlorate salts are ionic and dissociate completely when dissolved in water. The resultant ClO_4^- anion is very mobile in aqueous systems. Perchlorate is highly resistant to bonding with other matter and moves very freely within bodies of water and does not easily biodegrade. As a result, it can spread widely and remain in water supplies for decades. Perchlorate is measured in water samples primarily by ion chromatography. It can be removed by anion exchange or membrane filtration or be destroyed by biological and chemical properties.

1.3. Properties of Perchlorate:

1.3.1. Physical properties of Perchlorate: Perchlorate is a white or colorless powder that most commonly originates when perchlorate salts dissolve in water. The water solubility of ammonium perchlorate is 200 g/L at 59°F (Schumacher, [12]). An aqueous solution in equilibrium with solid ammonium perchlorate has an estimated density of 1.11 g/cm^3, substantially denser than fresh water and seawater.

The alkali metal perchlorates are decomposed when heated at 482°F. Perchlorate does not absorb well to most surfaces, but exceptions include tetraphenyl arsonium perchlorate and some quaternary ammonium perchlorate salts, forming stable ion pairs. Sometimes these ion pairs remain soluble and are used as phase-transfer catalyst agents. Perchlorate has a relatively low charge density. Therefore, it does not generally form complexes with metals like other anions. Perchlorate is employed as a counter ion in the synthesis of metal compounds when the presence of a non-complexing anion is required [19].

1.3.2. Chemical Properties of Perchlorate: Perchlorate is a strong oxidizing agent, shown by reaction but slightly weaker than dichromate or permanganate. Moreover, Perchlorate reduction is extremely slow and can usually be observed only in strong concentrated acid.

In fact, the redox behaviour of perchlorate is so rarely observed in chemical systems that sodium perchlorate is used to adjust the ionic strength of solutions prior to electrochemical or other laboratories studies. In 0.1-4.0M acid, perchlorate is not reduced by common reagents such as thiosulphate, sulphite or iron (II) [19].

$$ClO_4^- + 8H^+ + 8e^- \rightarrow Cl^- + 4H_2O,\ E° = 1.287\ V \ldots\ldots\ldots (1)$$

Perchlorate can be reduced by air-sensitive metal cations, such as titanium (III) or rullenium (II) [20] other than some bacterial sytems, Perchlorate reduction is not generally observable. When concentration acid meets a reducing agent, such as organic matter, the resulting reaction is explosive at high temperature.

For this reason, perchloric acid wet-washing is always preceded by nitric acid wet-washing. Some transition metal perchlorate salts and most organic perchlorates (esters) decompose violently or explosively; some detonate under shock loads as light as torch[19].

1.4. Perchlorate as a Pollutant:

Properties: As an anion, ClO_4^- consists of a central chlorine atom surrounded by a tetrahedral array of four oxygen atoms. As the oxidation state of the chlorine is +7, the species is an oxidizing agent (1).

$$ClO_4^- + 8H^+ + 8\ e^- \leftrightarrow Cl^- + 4H_2O,\ E^O = 1.287V \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Nevertheless, ClO_4^- is slightly weaker than dichromate ($Cr_2O_7^{2-}$) or permanganate (MnO_4^-) and its redox reaction is extremely non-labile, *i.e.*, reacts slowly with most reducing agents. The reduction of ClO_4^- can only be observed in strong concentrated acids. In 0.1 to 4.0M acid solution, ClO_4^- is not reduced by common reagents, such as thiosulphates, sulphites, or iron (II).

In fact, the redox behaviour of ClO_4^- is so rarely observed in chemical systems that sodium perchlorate is used to adjust the ionic strength of solution prior to electrochemical or other laboratory studies.

This behaviour results from the high strength of the chlorine-oxygen bonds and the requirement that reduction must proceed initially by oxygen atom abstraction rather than a direct involvement of the central chlorine atom. The kinetic behaviour is illustrated in **Fig. 2**.

The conversion of perchlorate to chlorate is generally the first step in the perchlorate reduction pathway. The reaction is thermodynamically favoured as shown by $\Delta E<0$, *i.e.*, the products have lower internal energy than the reactants.

However, the reaction rate is controlled by the kinetic barrier of the high activation energy E_A of the transition state.

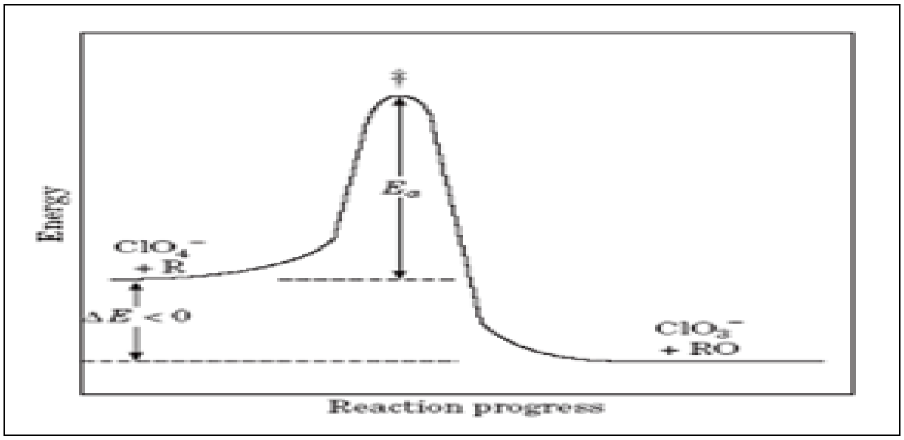

FIG. 2: ENERGY PROFILE FOR THE RATE-LIMITING STEP IN PERCHLORATE REDUCTION, ABSTRACTION OF THE FIRST OXYGEN ATOM [21]

In addition to its resistance to reduction, ClO_4^- has a relatively low charge density. Consequently, it does not generally form complexes with metals as the other anions do. Perchlorate is routinely employed as a counter-ion in the synthesis of metal compounds when a non-complexing anion is required [19]. Some physical and chemical properties of some perchlorate compounds are summarized in **Table 1.**

TABLE 1: SOME PHYSICAL AND CHEMICAL PROPERTIES OF SOME PERCHLORATE COMPOUNDS

Property	Ammonium perchlorate (NH_4ClO_4)	Potassium perchlorate ($KClO_4$)	Sodium Perchlorate ($NaClO_4$)	Perchloric acid ($HClO_4$)
Formula Weight	117.49	138.55	122.44	100.47
Colour/Form	White, Orthorhombic crystals	Colourless Orthorhombic crystal or white crystalline powder, Slightly salty	White orthorhombic deliquescent crystal	Colourless, Oily liquid
Melting Point	Decomposes/explodes	480°C	525°C	-112°C
Taste/Odour	Odourless	Slightly salty	Odourless	Odourless
Density/Specific	1.95 g/cm^3	2.53g/cm^3	2.52g/cm^3	1.67g/cm^3
Solubility	200 g/L water at 25°C	15 g/L water at 25°C	2096 g/L of water at 25°C	Miscible in cold water
Sorption Capacity	Very low	Very low	Very low	Very low
Volatility	Non Volatile	Non Volatile	Non Volatile	Volatile
Octanol/H_2O	-5.84	-7.18	-7.18	-4.63
Partition coefficient(log k_w) Vapor density air = 1	No information	4.8	No information	3.5
pH of aq.solution	5.5 to 6.5	6.0 to 8.5	7.0	Highly acidic

1. 5. Perchlorate Environmental Occurrence: As a strong oxidizing agent, ClO_4^- is mostly used as ammonium perchlorate (NH_4ClO_4) in the manufacturing of solid rocket fuel, missiles, and explosives for various military ammunitions and also in industrial products (*e.g.*, fireworks, airbag inflators, and paint). Its production began in the United States in the mid – 1940's, large amounts of perchlorate waste effluents have been released into the environment. The presence of ClO_4^- in water supplies and soils is also linked to the earlier use of chilean nitrate as fertilizer [22] and recently, its natural formation was reported [23]. Naturally occurring perchlorate is also found in the hyperarid Atacama desert in chilean nitrates and their derived nitrate fertilizer and in southeastern California caliche nitrate, deposits [22]. was shown that ClO_4^- is readily formed by a variety of simulated atmospheric processes; for example, it is formed from chloride aerosol by electrical discharge and by exposing aqueous Chloride to high concentrations of ozone. An interesting fact about perchlorate occurrence is that its presence is not only limited to Earth but occurs on Mars. Perchlorate salts (sodium and magnesium) have been detected in considerable amounts (~1 wt %) in Mar's arctic [24]. The various perchlorate concentrations from different continents, countries, and even producers within the same region added dimension to the complexity of human exposure to perchlorate. Given that some of the levels of perchlorate found in drinking water and food products are relatively high, it could be of health concern when considering all dietary sources.

1.6. Health Effects: Perchlorate competitively blocks thyroid uptake and inhibits normal thyroid hormone production, leading to metabolic problems in adults and anomalous development in children [25]. Preliminary toxicological studies have demonstrated that ClO_4^- directly affects iodine uptake by the thyroid gland at concentration of 6 mg per kg of body weight per day, resulting in fatal bone marrow disease [25]. However, the long-term health effects of low levels of ClO_4^- have not yet been established. Chlorate is also a potential chlorine oxyanion pollutant used as an herbicide in agriculture. It is used for the on-site generation of the bleaching agent chlorine dioxide (ClO_2) in the paper and pulp industry [26]. It can also be formed through the ozonization of drinking waters treated with chlorine [27].

When fed to rats and mice in their drinking waters, chlorate and chlorite (ClO_2^-) causes oxidative damage to red blood cells, resulting in haemolytic anaemia and methemoglobin formation [28], shown in the following flow chart.

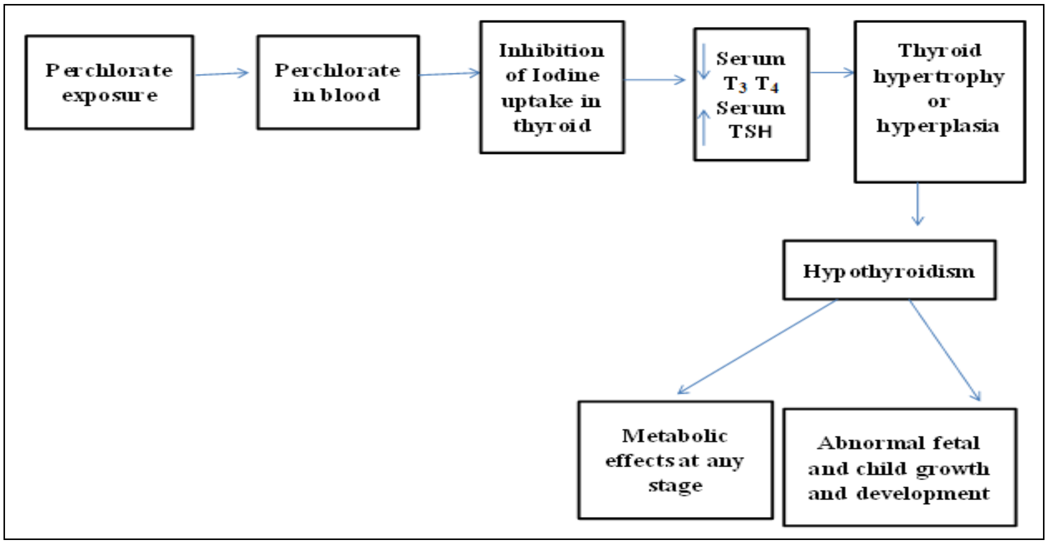

FIG. 3: MODE OF ACTION FOR PERCHLORATE TOXICITY (ADAPTED FROM NR (2005a)

1.7. Public Health Goal for Perchlorate in Drinking Water (Mechanism of Perchlorate Disruption Thyroid): Many adverse effects observed in test animals and humans exposed to perchlorate are related to the disruption of thyroid hormone regulation. The principal hormones secreted by the thyroid are thyroxine (T_4) and triiodothyronine (T_3); both hormones are iodine containing amino acids. While T_4 is produced only by the thyroid gland, about 80% of T_3 is formed in the peripheral tissues by de-iodination of T_4.T_4 and T_3 influence the growth and maturation of tissues, cell respiration and total energy expenditure and the turnover of essentially all substrates (including carbohydrates, cholesterol and proteins),vitamins and hormones (including the thyroid hormones themselves). The major substrates for thyroid hormone synthesis are iodide and tyrosine. Generally, Tyrosine is generally not the rate-limiting substrate. Iodine is a trace element and can be rate-limiting in thyroid hormone synthesis. Ingestion is the main route of iodine intake. Once ingested, iodine is reduced to iodide (I^-) in the gastrointestinal tract and is readily absorbed into the bloodstream through either passive diffusion or active transport. Thyroid tissue can concentrate iodide selectively from the surroundings where the concentration is very low (10^{-8} to 10^{-7} M). The thyroid can actively transport iodide into the organ such that intrathyroidal iodide concentrations can be several hundred folds higher than those of the external medium. Such concentrations are presumably required to promote efficient hormone synthesis because patients lacking the ability to concentrate iodide have goitre and are hypothyroid [29]. A protein model has been proposed of the manner in which sodium and iodide ions are actively transported into the thyroid gland.

The molecule that is responsible for this transport has been named the sodium-iodide symporter (NIS). The structure and regulations of NIS have been characterized [30]. Recently mouse symporter molecule has been cloned and expressed into normally non-iodide-transporting cells; after the modification, these cells showed perchlorate–sensitive iodide accumulation capability [31]. These researchers found evidence to indicate that sodium-iodide symporter is also present in extra-thyroidal tissues, such as the stomach and lacting mammary gland and to lower extent in small intestine, skin and brain. In humans, a majority of T_4 and T_3 in plasma is protein-bound.

In normal plasma, the T_4 binding distribution is 80 % of T_4 binding to thyroxine-binding globulin 15% to transthyretin, and 5 percent to albumin and lipoproteins. For T_3 the distribution is 90% bound to thyroxine-binding globulin and the rest to albumin and lipoproteins with little binding to transthyretin. Very small proportions of T_4 and T_3 are free in plasma, 0.03%, and 0.3%, respectively. Only the free hormone enters cells, exerts its biologic action, and determines thyroid physiologic status [32]. Control of T_4 and T_3 concentrations in blood is mainly regulated by negative feedback involving three glands: the thyroid gland, which produces thyroid hormones, and the pituitary gland and hypothalamus, which respond to and help maintain optimal levels of thyroid hormones **Fig. 4**.

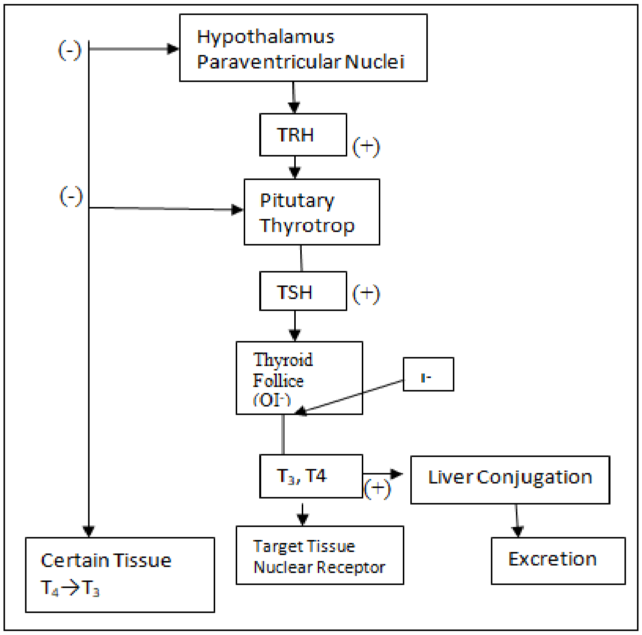

FIG. 4: HYPOTHALAMIC-PITUITARY-THYROID AXIS (FROM U.S. EPA, 1998b)

The hypothalamus stimulates the pituitary through thyrotropin-releasing hormone (TRH) to produce thyroid-stimulating hormone (TSH), which prompts the thyroid gland to produce the thyroid hormones T_4 and T_3. The stimulated thyroid actively transports inorganic iodide into the follicular cell. It converts it to an organic form and then into thyroid hormones molecules, which can influence target organs throughout the body. The secreted T_4 and T_3 are metabolized in the liver and other tissues. Some thyroid hormone derivates are excreted in the bile, and some of the iodine is reabsorbed. Cells in the hypothalamus and pituitary gland respond to levels of circulating thyroid hormones, *i.e.*, when hormone levels are high, there is a signal to reduce TRH and TSH output. Similarly, the pituitary is prompted to deliver more TSH to the thyroid gland to increase thyroid hormone output when thyroid hormone levels are low. This negative feedback loop helps the body respond to varying demands for thyroid hormone and maintain hormone homeostasis. Circulating T_4, T_3, and TSH can be readily monitored in the serum of experimental animals and humans by radioimmunoassay and serve as biomarkers of exposure effects of agents that disrupt thyroid-pituitary status [33-35].

1.8. Ion Exchange for Perchlorate Remediation

Principles: Ion exchange (IX) is a process that involves the replacement of ions from a fluid (usually an aqueous solution) with dissimilar ions of a similar charge using a solid matrix with the opposite charge. The solid is usually a porous polymer that contains permanently bound functional groups [30]. Most IX resins are made of cross-linked styrene (STY) and divinylbenzene (DVB), Shown in **Fig. 5**, which imparts insolubility and toughness to the resin 31, the higher the DVB amount, the higher the toughness, which is defined as the ability of the material to withstand a load before fracturing.

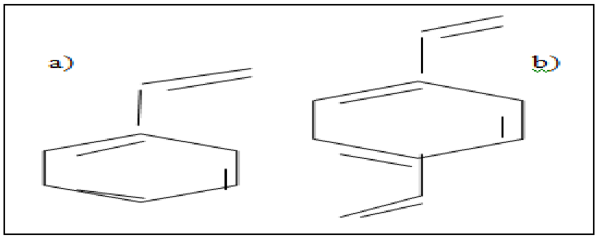

FIG 5: (A) STYRENE (STY) AND (B) DIVINYLBENZENE (DVB) STRUCTURES

Fig. 6 Represents a scheme of polystyrene cross-linking; the link is made by introducing DVB into the structure. As the amount of DVB increases, the sizes of the pores within the resin decrease, which can negatively affect how large ions enter the resin and thus their removal, as in a gel-type ion exchange resin. Macroporous resins overcome this issue by having artificial porosity in the tri-dimensional matrix created during the polymerization process.

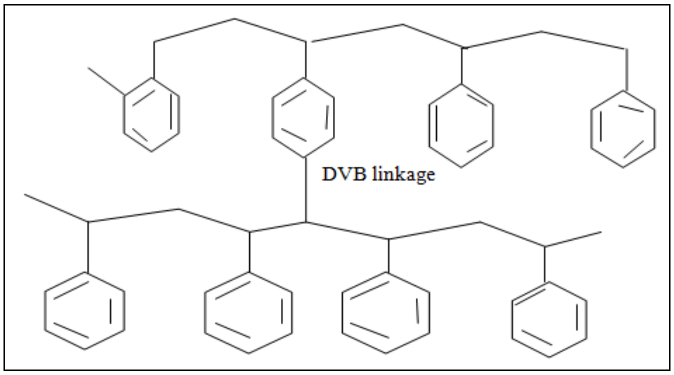

FIG. 6: CROSS-LINKED POLYSTYRENE

Fig. 7 represents the two types of structure: gel and macroporous. Gel-type resins have 4% to 10% cross-linking, and macroporous have 20% to 25%. As a result, macroporous resins possess large surface areas (7 to 600 m^2/g) and porosity of 20 % to 60 % (MWH, 2005). Ion exchange selectivity is also a function of the resin porosity. In general terms the higher the ion valence, the higher the removal by a resin. However, the degree of cross-linking changes the relative affinity of the resin for certain ions relative to the chloride anion and as functional groups attached to the resin matrix.

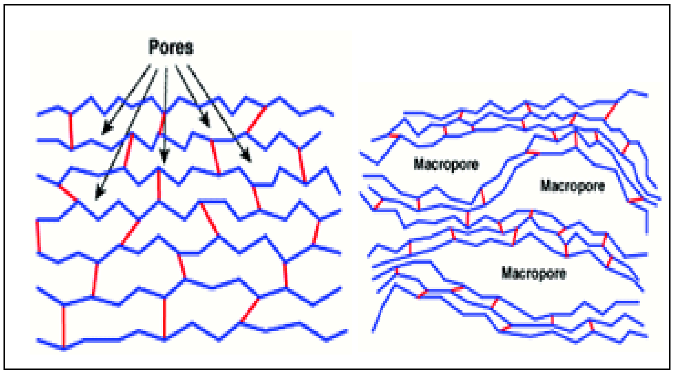

FIG. 7: GEL AND MICROPOROUS STRUCTURES OF ION EXCHANGE RESINS.

IX resins used for perchlorate removal are divided into two categories depending on the functional group attached to the resin matrix: strong base anionic resins (SBA) with quaternary ammonium functional groups and weak base anionic resins (WBA) with secondary or tertiary amines as functional groups [33-34]. SBA resins are further divided into two types based on the functional group. In type I, three alkyls (methyl, ethyl, propyl, and butyl) groups are bonded to the quaternary amine. The type II and ethanol group replaces one of the alkyl groups, as shown in **Fig. 8.**

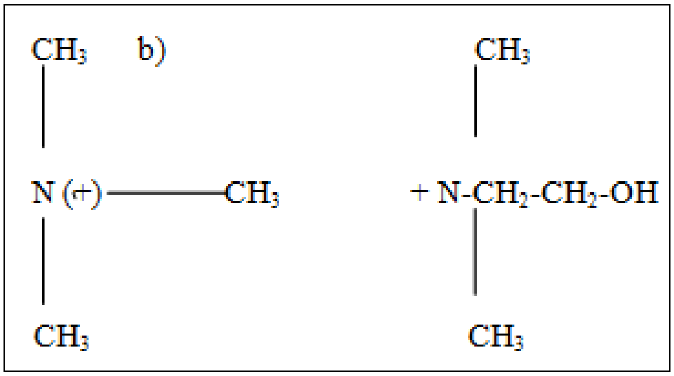

FIG. 8: TYPES OF SBA RESINS A) TYPE I AND B) TYPE II

Type I resins greater chemical stability, and Type II have sightly greater regeneration efficiency and capacity [32.34]. SBA resins are usually operated in the chloride form and regenerated with NaCl. According to work by [35] perchlorate selectivity is significantly controlled by the type of resin matrix and the type of functional group attached to it. They showed that polystyrenic (more hydrophobic) resins present higher affinities for perchlorate than polyacrylic type resins. Perchlorate displays higher affinity for IX resins with macroporous hydrophobic matrices since it is a large ion (58.8 cm^3/g-mole) classified as poorly hydrated [36]. Another important characteristic of IX resins is the particle size. In commercially available resins, the particle diameter ranges from 0.04 to 1.0 mm. This is relevant because IX kinetics are proportional to the inverse of the particle diameter square and smaller particles increase pressure drops across an IX column, which affects the column hydraulics and design.

1.9. Ion Exchange Systems for Perchlorate Removal: Currently, there are two types of IX systems available for removing perchlorate from drinking water, regenerable and single-use. **Fig. 9** represents a flow chart that depicts the regenerable and single-use type of systems.

The regenerable system uses perchlorate selective WBA or non-selective SBA resins. The advantages of this system are the capability of reusing the resin and the requirement for small regenerant volumes (< 0.02% of treated water) due to the fact that WBA is pH-dependent. At low pH, they are protonated and can exchange ions, but the resin becomes uncharged at higher pH and can be regenerated.SBA can be regenerated with high concentrations of NaCl.

The disadvantages of this system are the use of sodium chloride brine of concentration ranging from 6% to saturation or the need to adjust the pH of the treated water before and after IX by the addition of chemicals. Additionally, the disposal of spent regenerant solution becomes a challenge because of the high sodium chloride concentration and the presence of perchlorate, nitrate, sulphate, and even arsenic anions [37-39].

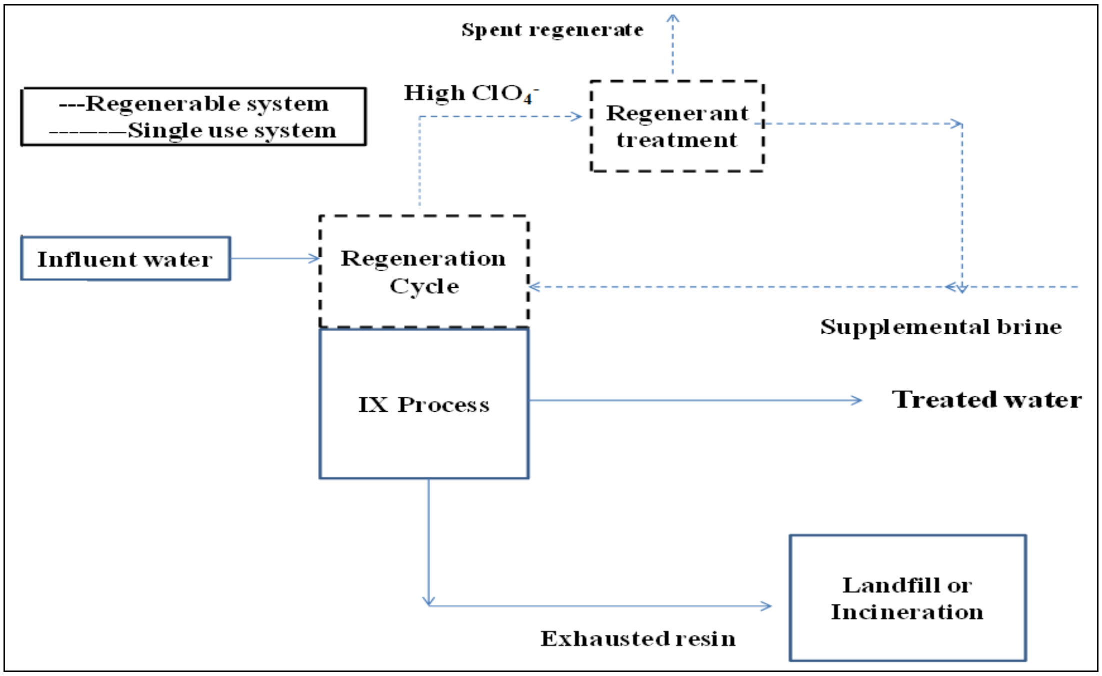

FIG. 9: ION EXCHANGE SYSTEMS USED FOR PERCHLORATE REMOVAL FOR DRINKING WATER

Single-use IX systems use highly selective SBA resins that bind perchlorate so tightly that is not feasible to regenerate them. However, the large number of bed volumes of water treated at very low perchlorate concentrations before breakthroughs makes them an attractive choice [39-40]. The disadvantage of the single-use IX resins is the management of spent resins, including landfill and incineration. Landfill disposal is not widely used since it requires analysis for waste classification, and perchlorate is not destroyed. Incineration by thermal destruction eliminates perchlorate and the costly IX resin after a single use, making the process unsustainable [39].

2. Perchlorate Treatment Technologies: Ideally, technology should be able to handle concentrations ranging from ≤ 5 μgL^{-1} all the way to ~10 gL^{-1}.The existing water treatment technologies for removing oxyanions like ClO_4^- can be divided into physical, chemical, and biological technologies. Physical technologies are considered as a removal technology and chemical and biological as a destruction process. Within the first group, anions[41] and membrane processes, such as electrodialysis 42, nanofiltration or reverse osmosis 43, are commonly used. However, ClO_4^- is accumulated in brine solution or a concentration stream, which has to be treated after disposal. Destruction is generally regarded as a preferable process because it eliminates the need for subsequent disposal of removal material, which is regarded as a hazard in this case.

2.1 Physical Processes: Physical removal process works exactly as the name suggests. They physically separate ClO_4^- ion from water. As these techniques do not destroy the ClO_4^-, they create a subsequent need for disposal and treatment of the ClO_4^- and any waste products of the process. In addition, all of these techniques currently suffer from a lack of selectivity. Along with the ClO_4^-, they tend to remove or replace unacceptably large quantities of beneficial dissolved salts or their components parts. Although these technologies are all well-established, they will be difficult to use in large systems, mainly because of the low concentration of ClO_4^- in the source water and the lack of selectivity. Moreover, their use is limited even in small water systems by pre-treatment and post-treatment.

2.2 Anion Exchange: Anion exchange is a technology frequently used to remove ClO_4^- from drinking water, groundwater, surface water and environmental media at full scale. Anion exchange resins are usually packed into a column and as contaminant ions are exchanged for other ions, such as chlorides or hydroxides in the resin **Fig.10.**

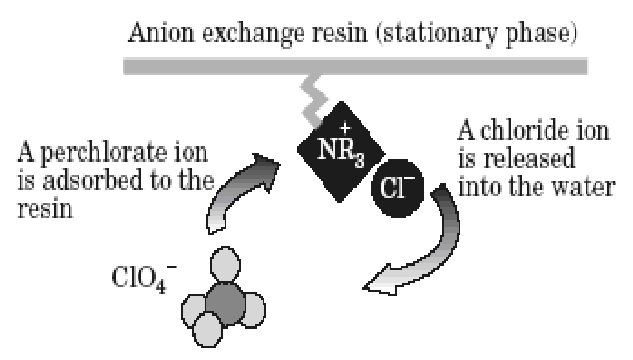

FIG. 10: MECHANISM OF ANION–EXCHANGE-CHLORIDE FOR PERCHLORATE

The most commonly used anion exchange media for ClO_4^- removal are synthetic and strongly basic resins. This technology has been used to reduce ClO_4^- concentrations to less than 3 µg/L [41]. Its effectiveness is sensitive to a variety of untreated water contaminations and characteristics. It has also been used as a polishing step for other water treatment processes, such as biological treatment of ClO_4^-. In general, ion exchange is often preceded by treatments, such as filtration and oil-water separation to remove organics, suspended solids, and other contaminants that can foul the resins and reduce their effectiveness, The main drawback of this technology is the periodic generation or resins to remove the adsorbed contaminations and replenish the exchanged ions. The regeneration process results in a backwash solution, a waste regeneration solution, and waste rinse water needs to be treated afterward; the lack of selectivity should also be considered a disadvantage of this process.

2.3. Reverse Osmosis: Reverse osmosis (RO) is a membrane technique also used for ClO_4^- removal [13]. Reverse osmosis is a physical separation method based on the principles of osmosis. In this technology, high pressure is applied to reverse the osmosis process and force water molecules to pass through the semi-permeable membrane out of the perchlorate contaminated water **Fig. 11**. As a result, two water channels are formed in the reverse osmosis system. One is treated water from the freshwater side of the system, and the other is concentrated or salty water containing ClO_4^- which is subject to further treatment before disposal.

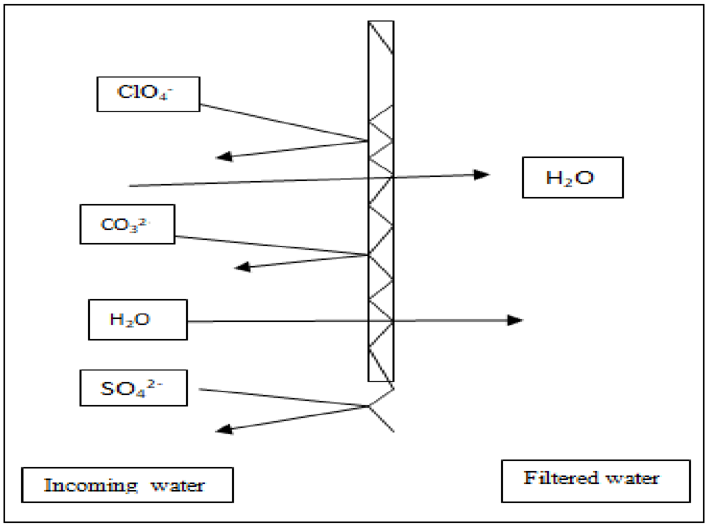

FIG. 11: REVERSE OSMOSIS (RO) THE INFLUENT WATER IS FORCED THROUGH A MEMBRANE THAT IS IMPERMEABLE TO DISSOLVED SALTS

RO membranes can remove 80% or more of the ClO_4^-. Membrane filtration point-of-use devices can be practical options for homeowners, small businesses, or isolated users. Over again, the lack of selectivity and the concentrate disposal are the most disadvantages of the process. Membrane corruption should also be considered as trouble in this technology.

2.4. Electrodialysis: Electrodialysis is another physical method for removing ClO_4^-.This technology applies an electric current to remove ClO_4^-.Perchlorate contaminated water is exposed to an electric current as it passes through a semi-permeable **Fig. 12**. This separates ClO_4^- ions from contaminated groundwater and surface water. The technology produces alternate channels of nearly deionized water (the dilute of dialyzate) and salty water (the concentrate). The dilute is used, and the concentrate undergoes further treatment before disposal [42, 44].

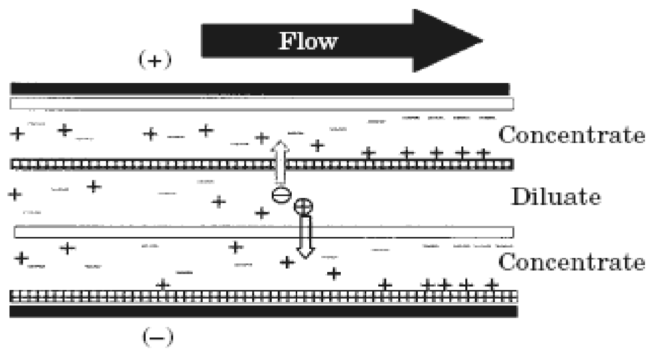

FIG. 12: ELECTRODIALYSIS WATER FLOWS THROUGH ALTERNATE SEMI-PERMEABLE WHILE UNDER THE INFLUENCE OF AN ELECTRIC FIELD

2.5. Chemical Process:

Chemical and Electrochemical Reduction: From the description of the oxidation-reduction reactions of ClO_4^- above. It is clear that chemical reduction will play no role in drinking water treatment shortly. Chemical reduction is simply too slow. Unless safe new catalysts become available, this appears unlike to change. Common reductants like iron metal, thiosulphate, sulphite, iodide, and ferrous ions do not react at any observable rate, and the more reactive species are too toxic (and still to sluggish).In addition, any reductant will necessarily have oxidized by-products. The toxicity of the by-products must be considered, and consequently, there is more hope for electrochemical reduction; a decided advantage of electrochemical reduction is a large amount of control over kinetics that results from control of operating potential. Although electrochemical technologies are well established for other industries, such as electroplating metals and electrodialysis of brine, they have not yet found a place in drinking water treatment. Nevertheless, the electricity consumption and the high operation costs of this technology should also be considered.

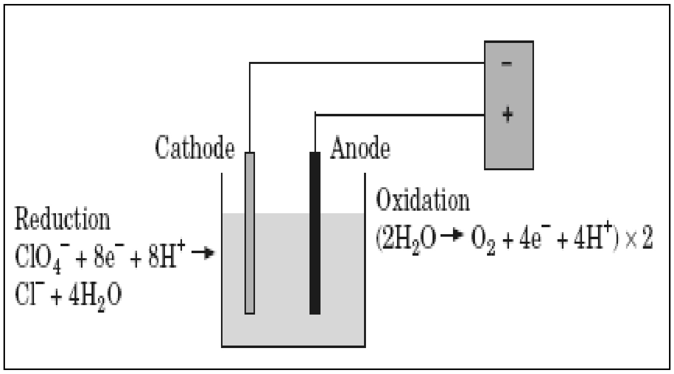

FIG. 13: SAMPLE ELECTROLYTIC CELL OF THE REDUCTION OF PERCHLORATE

2.6. Biological Process: The high reduction potential of chlorate and perchlorate (ClO_3^-/ Cl^-) E^o=1.03V, ClO_4^-/Cl^-, E^o=1.287V) makes them ideal electron acceptors for microbial metabolism. The biological reduction appears to hold the most promise for large-scale treatment of perchlorate-laden waters since ClO_4^- can be biologically degraded under suitable conditions. Some technology currently used for this purpose is the bioreactor and membrane bioreactors. The latter combines biological with the physical process of removing ClO4 from contaminated water.

2.7. Bioreactor: A Bioreactor frequently serves as a technology for remaining for removing ClO_4^- from contaminated groundwater and surface water at full scale. This technology uses microorganisms capable of reducing perchlorate into innocuous Chloride and oxygen in the presence of an electron donor and an appropriate medium to support microbial growth; contaminated water is placed in direct contact with microbes that selectively degrade the contaminant concern. Bioreactors have been used at sites to reduce ClO_4^- concentrations to less than 4 µg/L[14]. An example of this system is the fluidized bed reactor. They are made up of this suspended sand or granular activated carbon media that are selected to produce a low concentration effluent (i.e. at parts –per-billion levels) and provide larger surface area for growth of microorganisms. The fluidized bed expands with bio films' increased growth on the media particles. The results of this biological growth are a system capable of additional degradative performance for target contaminants. Normally the treated effluents are suitable for discharge, but when applied for drinking water treatment, the effluent from the bioreactor might require further treatment to remove biosolids present in the effluent.

2.8. Membrane Bioreactor: Combining physical removal with biological degradation the ion exchange membrane bioreactor (IEMB) is an integrated process that combines the transport of charged pollutants from water streams through an appropriated ion exchange membrane with their simultaneous biodegradation by a suitable microbial culture in a separate compartment **Fig. 14**. This process was successfully tested for drinking water ClO_4^- removal from 100 ppb to 4 ppb of ClO_4^- [40].

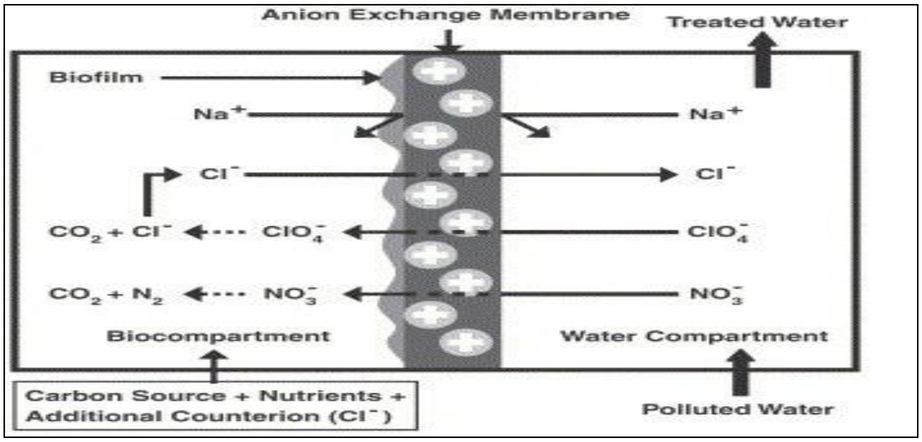

FIG. 14: SCHEMATIC DIAGRAM OF ION TRANSPORT AND BIOREDUCTION IN THE ION EXCHANGE MEMBRANE BIOREACTOR [43]

Biological degradation of ClO_4^- itself or combined with physical removal gathers operational conditions, making this process the most promising technology for ClO4- treatment because of its low operation costs and high selectivity. Several drinking glasses of water, wastewater and *in-situ* treatment systems are being developed to remove ClO4 biologically$^-$. But there is little ongoing research directed towards perchlorate reducing bacteria (PRBs) physiology.

2.9. Biological Perchlorate Reduction: Understanding the respiratory pathways used by bacteria will be important to the long-term operation of biological reactors.

In 1999, Rikken [45] and his colleagues proposed a three-step mechanism of perchlorate reduction.

$$ClO_4^-\rightarrow ClO_3^-\rightarrow ClO_2^-\rightarrow Cl^-+O_2$$

The ClO_4^- reduction pathway consists of three steps: the first two steps via two electrons transfer with (per) chlorate reductase, which sequentially reduces perchlorate to chlorate, then chlorate to chlorite [50, 52]. The third step with chlorite dismutase, which transforms chlorite into chloride and oxygen by disproportionation, does not consume electrons and therefore does not directly produce energy for the cells [49, 53].

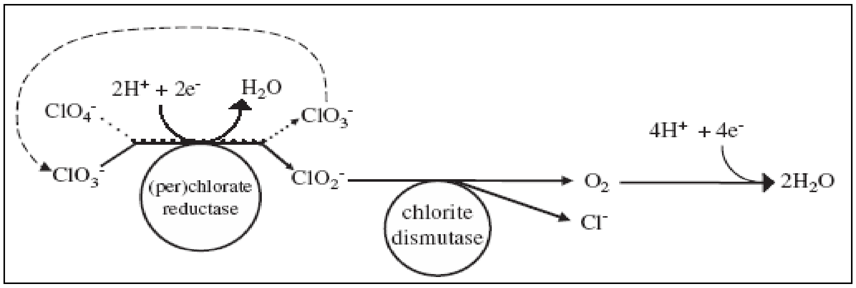

FIG 15: SCHEMATIC OF PERCHLORATE-REDUCING PATHWAY BASED ON ACCEPTED ROLES OF (PER) CHLORATE REDUCTASE AND CHLORITE DISMUTASE ENZYMES [47]

Chlorate produced by the perchlorate reductase should compete with ClO_4^- for the catalytic site of the perchlorate – reductase enzyme, presumably slowing the ClO_4^- reduction rate. While some amount of chlorate accumulation is possible, it only has been reported for a mixed culture growing on nitrate and ClO_4^- [47] and for a pure culture growing on ClO_4^- [48].

3. Enzymes Responsible for Perchlorate Reduction

3.1. Perchlorate Reductase: Perchlorate reductase and chlorite dismutase are the only enzymes in the perchlorate reduction pathway that have been isolated and characterized [46, 50, 52, 54] **Fig. 16**. However, concerning chlorate reducer, a chlorite reductase from a *pseudomonas Chloritidismutans* that can only use chlorate as a terminal electron acceptor was also isolated and characterized [55].

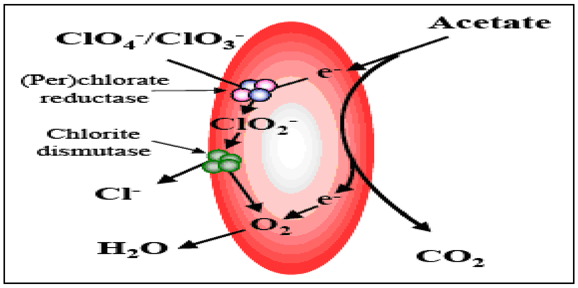

FIG 16: MODEL OF THE PATH WAY INVOLVED IN THE RESPIRATORY REDUCTION OF (PER) CHLORATE BY (PER) CHLORATE REDUCING BACTERIA [56]

To date, few data are available for perchlorate reductase. The first isolated perchlorate reductase belongs to strain GR-1[50].It was found that a single enzyme is able to catalyze both chlorate and perchlorate. The oxygen-sensitive enzyme is located in the periplasm and has an apparent molecular mass of 420kDa, with subunits of 95 kDa and 40kDa in a α3β3 composition. Metal analysis of this enzyme showed the presence of 11 mol of iron, 1 mol of molybdenum, and 1 mol of selenium permol of the hetero dimer. Few years later, another perchlorate reductase was purified and characterized. The molecular analysis from the bacterium perclace revealed two subunits of 35 kDa and 75 kDa, less than reported for the two subunits of strain GR-1, which can be due to post-translational modification of the protein in each bacterium. Very little information has been published on perchlorate reductases' temperature and pH activity/ stability profiles. Perclace perchlorate reductase displayed a wide range of temperature activity (20 to 40 °C) but most active at 25 to 35 °C. The enzyme was also relatively active in a wide range of pH [54] . Genetically, the perchlorate reductase operon (PCR) has recently been identified in the genome of two perchlorate-reducing bacteria, *Dechloromonas agitate* and *Dechloromonas aromatic*. There are four genes in the transcriptional unit, PCR ABCD, encoding two structural subunits (pcr A and pcrB), acytochrome (pcr C) and a molybdenum chaperone subunit (pcr D).Amino acid sequence analysis of the products encoded by the pcr operon indicated similarties to subunits of microbial nitrate reductase, selenate reductase, dimethyl sulphide dehydrogenase, ethyl benzene dehydrogenase and chlorate reductase, all members of the type II DMSO (dimethyl sulfoxide) reductase family.

Transcriptional analysis indicated that pcr gene cluster is expressed in the anaerobic perchlorate and chlorate grown culture of *D. agitata*. However aerobic cultures with perchlorate, chlorate or nitrate as the electron acceptor displayed no induction of pcr transcription, indicating the ability of oxygen to completely inhibit expression of the perchlorate reductase operon. Conversely, the *D. agitata* cld gene exhibits basal expression under aerobic conditions, thus further implicating separate regulation of chlorite dismutase and perchlorate reductase.

3.2. Chlorite Dismutase: A central step in the reductive pathway of perchlorate and chlorate that is common to all (per) chlorate reducing bacteria is the dismutation of chlorite into Chloride and molecular oxygen catalysed by chlorite dismutase [51].

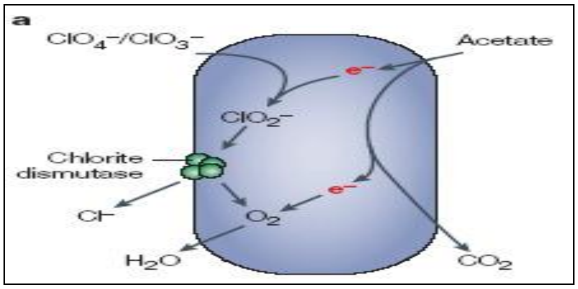

FIG 17: MODEL OF PATHWAY INVOLVED IN THE REDUCTION OF CHLORITE BY PERCHLORATE REDUCING BACTERIA [57]

The oxygen production during (per) chlorate reduction and subsequently chlorite dismutation is the only known biological oxygen generating pathway besides photosynthesis and detoxification of H2O2 by catalases. A logical consequence of this oxygen production is, that (per) chlorate-reducing bacteria are not strictly anaerobic bacteria. The chlorite dismutase from GR-1 has a molecular mass of 140kDa and consists of four 32 kDa subunits, each one containing 0.9 molecules of proto heme IX and 0.7 molecules of iron. In this strain, the enzyme displays maxima for activity at pH 6.0 and 30 °C [52].

Chlorite dismutase from *I. dechloratnas* was also purified and the results revealed a tetrameric enzyme of 115 kDa [58]. The chlorite dismutase gene is present in all (per)chlorate reduced and such as detection of this gene is unable to distinguish between perchlorate reducing bacteria and those that can only reduce chlorate.

The chlorite dismutase gene (*CLD*) was isolated and characterized from *D. agitata* and from *Ideonella dechloratans*. In the case of *D. agitata*, the chlorite dismutase gene is basally expressed under aerobic conditions. In contrasts, chlorite dismutase expression is constituted in the chlorate reducing microorganism pseudomonas strain PDA and *pseudomonas* strain pk [59].

3.3. Chlorate Reductase: Up to now, at least three enzymes that can reduce chlorate have been purified and characterized. A chlorate reductase C had been purified from the denitrifying strain *proteus mirabilis* [60] as well as *Pseudomonas chloritidismutans* [55] and pseudomonas sp. PDA [61]. Comparison with the periplasmic perchlorate reductase of strain GR-1 showed that the cyto plasmic chlorate reductase of *P. chloritidismutans* reduced only chlorate and bromated. Differences were also found in N-terminal sequence, Molecular weight, and subunit composition. However, the metal analysis and electron paramagnetic resonance measurements showed the presence of iron and molybdenum, which are also found in other dissimilatory oxyanions reductase. Regarding genetic studies, the chlorate reductase from PDA had three subunits (60, 48, and 27 kDa); a gene cluster of a chlorate reductase from *I. dechloratans* was also analyzed [62].

Evidence for separate enzymes is provided in directly by the fact that not all CRB are capable of respiration with ClO_4^- although this question will require further research to resolve. Improved understanding of the biological ClO_4^- reduction kinetics and its biochemical mechanisms will lead to better biological remediation processes. Since there are hardly any ClO_4^- kinetic degradation data available, the present work was focused on isolation of possible new ClO_4^- reducing bacteria as well as its kinetic characterization.

3.4. Factors that Interfere with Perchlorate Enzyme Induction: Many environmental factors have been shown to affect microbial (per) chlorate reduction, including trace elements, pH, salt concentration, and presence of other electron acceptors. Several lines of evidence suggest that the optimal pH for perchlorate reduction occurs around neutral pH. The *Dechloromonas* and *Azospira* species generally grow optimally at pH values near neutrality in freshwater environments; concerning salinity, no microorganism isolated has been demonstrated to reduce perchlorate in salinities greater than 2% [44].

This presents a problem for the biological treatment of waste brine concentrated with perchlorate collected by ion-exchange processes, suggesting that the chloride content limits the metabolism.

An enrichment culture from the salt lake was able to carry out perchlorate reduction in salt brines as concentrated as 11% NaCl [63]. But perchlorate removal efficiency was not reported, while another enrichment culture developed from marine sediments was reported to reduce 70 to 90 mgL^{-1}. Perchlorate at 6% NaCl within 24 h [64].

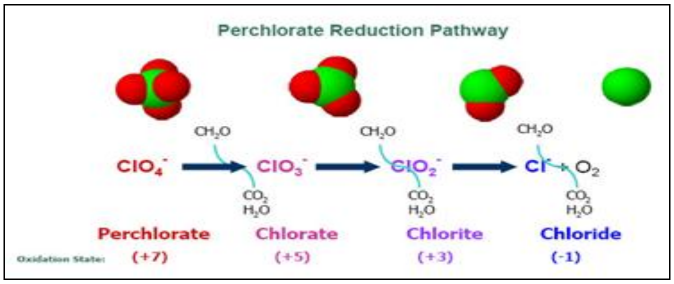

FIG. 18: PERCHLORATE REDUCTION PATHWAY WITH DIFFERENT OXIDATION STATE

Regarding the presence of other final electron acceptors, oxygen and nitrate can be inhibitors of perchlorate reduction [51]. Furthermore, molecular studies focused on identifying the gene encoding the perchlorate reductase demonstrated that the presence of atmospheric oxygen down-regulated its expression.

CONCLUSIONS: Perchlorate has been found to be widespread in drinking water sources in the United States and India. Though the known adverse effect of perchlorate exposure on humans is hypothyroidism, detailed animal toxicity studies conducted on two generations of rats have potential adverse effects on their fetuses.

Perchlorate has been found in cow's milk and mother's milk and hence perchlorate ingestion by infants through maternal intake is high. Therefore, perchlorate can be considered a contaminant of concern in the near future in India.

The most common perchlorate treatments currently in use employ *ex-situ* technologies. Two treatment technologies currently dominate the field of perchlorate treatment: ion exchange and biological treatment.

Research demonstrated that bioreactors, mostly fluidized bed reactors operated under anaerobic conditions, could effectively reduce high concentrations of perchlorate. Selective ion exchange resins of various types have been developed to treat lower perchlorate concentrations.

Although bioreactors have been proven to be effective, the best applicable technology for removing perchlorate remains to be ion exchange. Ion exchange resins are used to treat drinking water supplies and groundwater containing 10 to 100,000 µg/L perchlorate. Research is presently directed toward technologies that could remove perchlorate to less than 10 µg/L from water sources.

Such recent technologies include electrochemical reduction, catalytic reduction and metallic nanoparticles.

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