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# Investigation of potential pathways and multi-cycle bioregeneration of ion-exchange resin laden with perchlorate

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# INVESTIGATION OF POTENTIAL PATHWAYS AND MULTI-CYCLE BIOREGENERATION OF ION-EXCHANGE RESIN LADEN WITH PERCHLORATE

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ABSTRACT

**Investigation of Potential Pathways and Multi-cycle Bioregeneration of Ion-Exchange Resin Laden with Perchlorate**

by

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Ion-exchange (IX) is possibly the most feasible technology for perchlorate removal and perchlorate-selective and non-selective IX resins are commercially available for this purpose. The use of both resins has shortcomings. Selective resins are incinerated after one time use, and non-selective resins produce a regenerant waste stream that contains high concentration of perchlorate. A process involving directly contacting of spent IX resin containing perchlorate with perchlorate-reducing bacteria (PRB) to bioregenerate the resin has been developed and proven recently. In this process PRB biodegrade perchlorate ions which are attached to the functional groups of the resin.

Although its feasibility has been proven, there are two issues related to resin bioregeneration technology that deserve attention and were addressed in this research. The first issue relates to the investigation of the mechanisms responsible for resin bioregeneration. It was envisioned that the bioregeneration process involves four steps. First, resin-attached perchlorate (RAP) ions are desorbed from their original functional groups by chloride ion. Second, perchlorate ions are diffused through the pores of the resin. It was expected that this diffusion is affected by both resin bead size and structure.

Third, perchlorate ions are transferred through the liquid film surrounding the resin to the bulk liquid. Forth, perchlorate ions are utilized by the PRB present in the bulk liquid.

The results of the bioregeneration experiments suggested that chloride, the waste product of perchlorate biodegradation, is more likely the desorbing agent of RAP, and increasing the concentration of chloride enhances the bioregeneration process. For commercially available resins, both film and pore diffusion were found to affect the rate of mass transfer. In addition, macroporous resins were found to be more effective than gel-type resins in the bioregeneration process. Bioregeneration rates were faster for resins of smaller bead diameter. The outcome of this study implies that in resin bioregeneration, the use of macroporous resin with relatively smaller bead size in presence of chloride would be preferred. Chloride concentration, however, should be kept below the inhibitory level for PRB microbial activities.

The second issue of bioregeneration process is the possibility of multi-cycle bioregeneration of IX resin. The results of the experiments revealed that capacity loss, which is the main concern in multi-cycle bioregeneration process, stabilized after a few cycles of bioregeneration indicating that the number of loading and bioregeneration cycles that can be performed is likely greater than the five cycles tested. The results further indicated that as bioregeneration progresses, clogging of the resin pores results in the decrease in mass transfer flux from the inner portion of the resin to the bulk microbial culture contributing to stronger mass transfer limitation in the bioregeneration process. Perchlorate buildup, resulting from un-degraded perchlorate accumulation in the inner portion of the resin, after each bioregeneration cycle is a major drawback that limits the number of bioregeneration cycles that can be performed.

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# CHAPTER 1

# PROBLEM STATEMENT

## 1.1. Introduction and Objectives

Perchlorate contamination can occur both naturally in the environment and through manufacturing and use (Rao, et al., 2007; Dasgupta, et al., 2005; ITRC, 2007). Perchlorate mostly has been used as ammonium perchlorate ($NH_4ClO_4$) in the rocket manufacturing industry. Toxicity of perchlorate contamination is well-known in the course of its interference with iodide uptake by thyroid gland (Kirk, 2006; Wolff, 2002, Stoker et al., 2006). Iodide is the most necessary element in the production of thyroid hormones by thyroid gland. Iodide shortage causes mental retardation in children and hypothyroidism in adults (Kirk, 2006; Wolff, 2002).

Perchlorate contamination has been found and reported at approximately 400 surface water and groundwater supplies and soils in 35 states (Tikkanen, 2006), and in at least in 44 states there are some release potentials (Lehman, et al., 2008). Perchlorate contamination has become a serious concern in the southwestern region of the United States. The National Academy of Science (NAS) has recommended the oral reference dose (RfD) of 0.0007 mg/kg/day (Tikkanen, 2006), which corresponds to the interim health advisory level of 15 µg/L in drinking water (Hristovski et al., 2008).

The most promising technologies to treat water contaminated by perchlorate are bioremediation and ion-exchange (Logan et al., 2001; Batista et al., 2002; Okeke et al., 2002; Velizarov et al., 2005; Lehman et al., 2008). Bioremediation is only practical for water with high concentration of perchlorate, due to the relatively slow degradation kinetics of small perchlorate concentrations (Logan et al., 2001; Waller et al., 2004).

Perchlorate also can be removed from water by perchlorate-selective and perchlorate-non-selective resins. Both perchlorate-selective and non-selective resins can be either gel-type or macroporous-type. Although ion-exchange is a well-known technology to water utilities, it has some deficiencies in perchlorate removal. Firstly, it only separates perchlorate from water and it does not destroy the perchlorate ions. Secondly, there are some issues regarding the regeneration process. In the case of non-selective resins, the regenerant waste stream, contains high concentration of perchlorate, and should be dealt with. In the case of selective resins, the resin cannot be effectively regenerated and they are incinerated after one-time use. One-time use of ion-exchange resins constitutes a major cost and environmental challenge for water utilities.

A new concept in ion-exchange technology has been developed and patented (Batista and Jensen, 2006). This concept is based on directly contacting perchlorate-containing ion-exchange resin with a perchlorate reducing microbial culture under anoxic/anaerobic conditions. The process consists of a fermenter, which holds the bacterial culture, and a fluidized bed reactor (FBR), which holds the perchlorate-containing resin (Figure 1.1). In this process, first fresh resin is used to treat perchlorate contaminated water as it is shown in the left hand side of Figure 1.1. After the capacity of the resin is exhausted, the resin is transferred to a FBR. A perchlorate-reducing bacteria (PRB) culture is pumped from the fermenter to the FBR upward. This process can potentially be used for perchlorate-selective and non-selective ion-exchange resins, leading to the conversion of perchlorate loads on the resin to innocuous chloride. The process can also be used for resins contaminated with nitrate. In this case, nitrate is converted to nitrogen gas.

The feasibility of direct bioregeneration of ion-exchange resins loaded with perchlorate was first reported in 2006 (Batista and Jensen, 2006). The concept of bioregeneration was developed and tested on perchlorate and nitrate selective resins (Batista et al., 2007b; Venkatesan et al., 2010). Bioregeneration of selective and non-selective resins, in the presence of small concentrations of NaCl, has also been reported (Wang et al., 2008b; Wang et al., 2009).

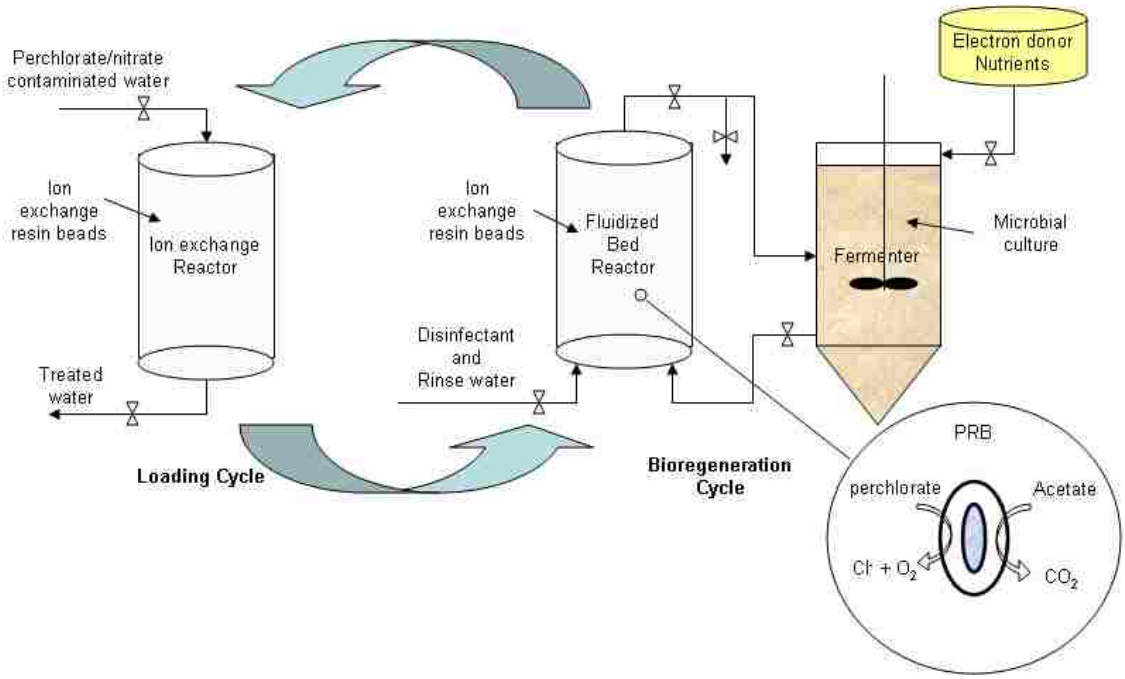


Figure 1.1. Schematic of the Resin Bioregeneration Technology (Left: Loading Cycle; Right: Bioregeneration Cycle Using PRB)

The feasibility of the resin bioregeneration process has been proven. However, there are issues that have not been investigated thus far:

Issue 1: Although the biodegradation of free perchlorate ions dissolved in water have been intensely investigated, there has been no research to explore the mechanism of biological degradation of perchlorate ions attached to ion–exchange resins. Thus, the first objective of this research is to understand the mechanisms of degradation of resin-attached perchlorate ions.

Issue 2: In order to be economically and environmentally sustainable, bioregeneration should be repeatable in several cycles for the same resin. Thus, the second objective of this research is to investigate the feasibility of bioregeneration of ion-exchange resin for several exhaustion-bioregeneration cycles.

## 1.2. Research Issues

Biodegradation of perchlorate ions dissolved in water has been well studied. All the perchlorate reducing bacteria known to date are gram-negative bacteria (Waller et al., 2004; Shrout et al., 2005). It has been proven that the perchlorate-reduction pathway goes from perchlorate ($ClO_4^-$) to chlorate ($ClO_3^-$), then to chlorite ($ClO_2^-$), and finally to chloride ($Cl^-$) (Rikken et al., 1996; Logan, 1998). More importantly, it has been shown that the perchlorate reducing bacteria use two distinct enzymes to reduce perchlorate to chloride (Rikken et al., 1996; Logan, 1998). Conversion of perchlorate ($ClO_4^-$) to chlorate ($ClO_3^-$) and then to chlorite ($ClO_2^-$) are two energy-yielding enzymatic reactions performed by perchlorate reductase (Rikken et al., 1996; Logan et al., 2001); then reduction of chlorite ($ClO_2^-$) to chloride ($Cl^-$) and oxygen ($O_2$) is a non-energy yielding reaction performed by chlorite dismutase (van Ginkel et al., 1996; Rikken et al., 1996). Finally, the oxygen molecule ($O_2$) produced from the dismutation of chlorite ($ClO_2^-$) is

reduced to water ($H_2O$) through oxidation of the electron donor which is an energy yielding reaction (Rikken et al., 1996; Logan et al., 2001).

Studies have indicated that perchlorate reductase and chlorite dismutase are located in the periplasmic area of the cell (Kengen et al., 1999). In the biodegradation of perchlorate, free perchlorate ions in water are transferred across the outer membrane of the cell to the periplasmic area, where perchlorate reductase and chlorite dismutase are located. Although the outer membrane of gram-negative bacteria is a lipid bilayer, it is relatively permeable to small hydrophilic molecules, due to presence of proteins called porins that function as channels for the entrance and exit of these small molecules (Madigan and Martinko, 2005). Transport of perchlorate and chloride ions across the outer membrane is most probably due to existence of porins.

The mechanism of perchlorate degradation described above applies to free perchlorate ions that are dissolved in water. In the case of ion-exchange resin, perchlorate ions are strongly attached to the functional groups of the resin beads (Lehman et al., 2008).

Ion-exchange resins are porous media and can be categorized into gel-type and macroporous-type. The average pore size and water retention in macroporous resins are considerably higher than those of gel-type resins (Sherman et al., 1986). The average pore size of macroporous resins is 0.6 µm, while gel-type resins have an average pore size of about 0.0005 µm (Kun and Kunin, 1968; Dale et al., 2001). Water retention of macroporous resins is approximately 11% higher than that of gel-type resins (Du et al., 2010). The use of macroporous resins compared to gel-type resins is expanding due to

their stability, resistance to oxidation, and less vulnerability to fouling (Weber, 1972; Li and SenGupta, 2000).

From an economical point of view, resin bioregeneration is practical only if the process can be repeated for several consecutive exhaustion-bioregeneration cycles. Biological fouling and capacity loss of the resin are the main concerns when performing several consecutive exhaustion-bioregeneration cycles. Venkatesan et al. (2010) studied the feasibility of bioregeneration process for a perchlorate-selective gel-type ion-exchange resin. The results of this study demonstrated the feasibility of bioregeneration for gel-type perchlorate-selective resin for 3 consecutive exhaustion-bioregeneration cycles (Venkatesan et al., 2010).

### 1.3. Knowledge Gaps and Hypotheses

Figure 1.2 shows a typical curve found for perchlorate degradation on a resin bead with time. Initially, degradation is very fast but it slows down with time. This degradation curve has been observed several times for both perchlorate and nitrate selective resins (Batista and Jensen, 2006; Batista et al., 2007a). Although it has been demonstrated that perchlorate attached to the resin can be biodegraded, to the best of my knowledge, the mechanism for utilization of adsorbed perchlorate ions by bacteria has not been elucidated thus far.

The pore size of ion-exchange resin beads does not allow the PRB, with average cell sizes ranging between 1.0 µm x 3.0 µm and 1.5 µm x 7.0 µm (Wolterinket al., 2002), to penetrate into the pore matrix of the resin, which has a complex network of pores. Thus, it is obvious that perchlorate ions, located on the functional groups inside the beads,

should travel through the fine pore network, and enter the bacterium cell suspended in liquid phase of the culture.

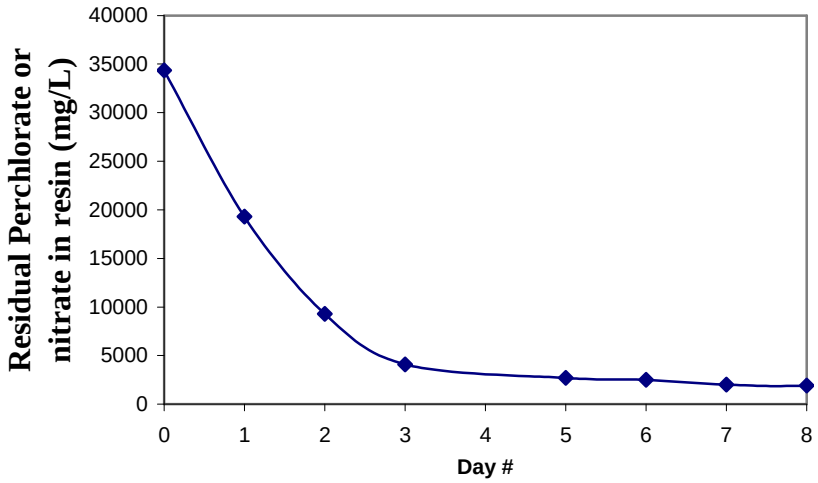


Figure 1.2. A Schematic Curve Resulting from the Bioregeneration of Resins Contaminated with Perchlorate

The known involvement of *c*-type cytochrome(s) in the respiratory electron transfer chain (Coates et al., 1999; Bender et al., 2005) and the strong attachment of perchlorate ions to the active functional groups of the ion-exchange resin beads specify that somehow resin-attached perchlorate ions should be detach from the resin and reach the PRB in the outside of the cell. It is hypothesized in this research that perchlorate ions attached to resin beads: a) desorb from their original functional group located inside the resin bead matrix, b) diffuse from the inside region of the resin bead to the resin surface through the pores, c) transfer through an imaginary liquid film, covering the surface of resin bead, to the outside of the bead, and d) transfer into the periplasmic region of the PRB to be

degraded. Figure 1.3 depicts a conceptual diagram of bioregeneration process for a resin bead. Based on this conceptual model, desorbed perchlorate ions should diffuse through the resin pore matrix and a liquid film surrounding the bead, to the outside of the resin bead where the PRB are located.

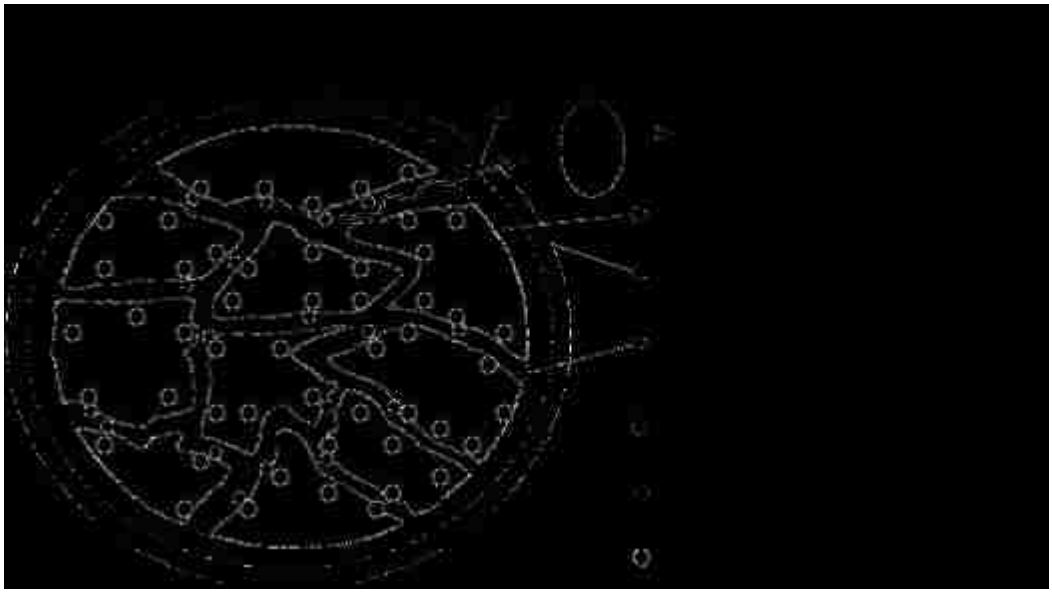

Figure 1.3. Conceptual Model of Resin-attached Perchlorate Degradation Mechanism in the Bioregeneration Process

Three hypotheses were developed to attempt to understand the mechanism of biodegradation of perchlorate ions attached to resin beads. In the first hypothesis, it is envisioned that there is a desorption mechanism involved in the detachment of the resin-attached perchlorate ions. The second and third hypotheses envisioned that mass transfer of desorbed perchlorate ions, from the inner portion of the resin bead to the PRB, is the controlling step in the bioregeneration process rather than biodegradation kinetics of

desorbed perchlorate. Mass transfer process of resin bioregeneration is affected by both resin bead size and the resin structure.

It is well known and accepted that PRB reduce perchlorate to chloride ion. Stoichiometrically, one chloride is released for each perchlorate reduced (Rikken et al., 1996). The bioregeneration results shown in Figure 1.2 were generated in a system where the PRB were grown using perchlorate as the electron acceptor resulting in the accumulation of chloride ions in the microbial solution. Additionally, the solution was continuously re-circulated through the fermenter-FBR set-up. Therefore chloride ions accumulated in the system. Several studies have been published on the negative effects of chloride (i.e. salinity) on perchlorate degradation (Logan et al. 2001; Gingras and Batista, 2002; Okeke et al., 2002). These studies show that significant reduction in perchlorate degradation occurs at salinity levels greater than 5000 mg/L. Therefore, in bioreactors used for resin bioregeneration, chloride levels should be kept below toxic levels to PRB.

In this research, it is thought that chloride resulting from perchlorate biodegradation exchanges with the resin-attached perchlorate and release perchlorate. Larger monovalent and more hydrophobic ions such as perchlorate have more potential to bind to the IX resin compared to smaller and less hydrophobic ions such as chloride (Diamond, 1963; Xiong et al., 2007; Lehman et al., 2008).

The relative affinity of chloride and perchlorate ions to exchange with the resin's functional group, which is commonly expressed as selectivity coefficient, is 1 to 150 for non-selective styrenic resins (Crittenden et al., 2005). For common styrenic perchlorate-selective resins it is 1 to 1300 (Tripp and Clifford, 2000). Highly selective resins may

have selectivity coefficients of about 3500 (ResinTech, West Berlin, NJ). Although perchlorate attaches very strongly to the functional groups of perchlorate-selective resins compared to chloride ion, in presence of chloride a small portion of resin-attached perchlorate ions may be exchanged by the chloride ions instantaneously (Lehman et al., 2008). However, a significant part of the exchanged perchlorate will re-exchange and re-attach to the resin (Lehman et al., 2008), because perchlorate is a monovalent anion with high selectivity (Crittenden et al., 2005; Sodaye et al., 2007). Thus, it is likely that the chloride generated from perchlorate degradation acts as a desorbing agent for the attached perchlorate ions. My first hypothesis is that resin-attached perchlorate is exchanged by chloride, transferred to the PRB cells, and immediately consumed by the cells before re-exchange occurs.

Previous studies showed that the degradation rate of resin-attached perchlorate in IX resin is high in the first days, but it decreases with time (Figure 1.2) (Batista and Jensen, 2006; Batista et al., 2007b; Venkatesan et al., 2010). The reason for this observation may be diffusion or reaction rate control. In a kinetics-controlled process, lower concentration of perchlorate remaining in the resin results in a lower biodegradation rate, because of the high value of the half saturation constant for perchlorate degradation. In a diffusion-controlled process, perchlorate located deep in the resin is degraded slower. It is thought that transfer of desorbed perchlorate ions involves pore diffusion within the resin and film diffusion in the liquid layer surrounding the resin. Film diffusion mostly depends on flow rate, turbulence, and viscosity (Helfferich, 1962; Weber, 1972; Lahav and Green, 2000; Xiong et al., 2007). Pore diffusion is influenced by viscosity, concentration gradient, resin bead size, degree of crosslinking in the structure of resin, and resin pore

size (Helfferich, 1962; Xiong et al., 2007). In this research, it is hypothesized that resin bioregeneration is controlled by mass transfer of desorbed perchlorate ions from the inner portion of the resin bead to the PRB located in the bulk microbial liquid. If the bioregeneration process is controlled by mass transfer, reducing the resin bead size would accelerate the diffusion process and consequently bioregeneration would be faster (Helfferich, 1962).

As it was mentioned earlier, degree of crosslinking and resin pore size affect the pore diffusion in the resin bead. The degree of crosslinking is different for gel-type and macroporous resins. In average, gel-type resins have 8% divinylbenzene crosslinking, while, macroporous resins have about 20-25% percent divinylbenzene crosslinking (Crittenden et al., 2005). Increasing the degree of crosslinking decreases diffusion (Weber, 1972). Ion exchange resins have two types of pores; micropores or the pores within the gel structure of resin and macropores or the pores between the microspheres of macroporous resins (Crittenden et al., 2005). Gel-type resins have only micropores, while macroporous resins have both micropores and macropores resulting in more water content in macroporous resins (Du et al., 2010). Although the degree of crosslinking in macroporous resins is greater than in gel-type resins, it is expected that the overall mass transfer flux in macroporous resins is higher than that of gel-type resins. This is due to presence of macropores and higher water content in macroporous resins. My third hypothesis is that bioregeneration of macroporous ion-exchange resins is faster than gel-type resins due to higher water content and larger pore sizes which result in faster mass transfer process in macroporous resins compared to gel-type resins.

Venkatesan at al. (2010) showed that a gel-type selective resin could be loaded and bioregenerated for 3 cycles. However, the resin capacity loss during the cycles was not studied. Observation of the resin beads after 3 cycles of exhaustion-bioregeneration and fouling removal showed that biofouling of the resin after 3 cycles was not very significant. The use of macroporous resins compared to gel-type resins is expanding due to their stability, resistance to oxidation, and less vulnerability to fouling (Weber, 1972; Li and SenGupta, 2000), hence, it is more preferable to use macroporous resins in the bioregeneration process. In this research, the feasibility of bioregeneration and the resin capacity loss for several consecutive cycles is investigated using a perchlorate-selective macroporous resin. My hypothesis is that the bioregeneration process can be performed on perchlorate-selective macroporous anion-exchange resins for several consecutive exhaustion-bioregeneration cycles, because the fouling would be less significant than that for gel type resins. Therefore, there would not be a significant capacity loss in the resin.

# CHAPTER 2

# STATE OF KNOWLEDGE

## 2.1. Perchlorate in the Environment

Perchlorates are the salts that are derived from perchloric acid. Perchlorates can occur naturally in the environment (Rao, et al., 2007; Dasgupta, et al., 2005) and much contamination has occurred due to perchlorate manufacturing and use (Batista et al., 2002; USEPA, 2005; ITRC, 2007). In the United States (U.S.), extensive production of perchlorate started in the mid 1940's. Perchlorate mainly has been employed as ammonium perchlorate ($NH_4ClO_4$) in the rocket manufacturing industry. Perchlorate usage expanded by employing this salt in the other industries such as manufacturing of fireworks, explosives, matches, batteries, air bags, lubricating oils, leather, paints, refined aluminum, electronic tubes, mordant for fabrics and dyes, and nuclear reactors (Wu, et al., 2008).

Perchlorate also can occur naturally in soils in arid and semi-arid climates where marine seabeds were and climates exposed to lightning storms (Sellers, et. al., 2007). Electrical discharge of chloride aerosol in the atmosphere potentially can produce perchlorate (Dasgupta, et al., 2005). Also, exposing aqueous chloride to high concentrations of ozone can lead to perchlorate production; rain and snow then release it to the environment (Rao, et al., 2007; Dasgupta, et al., 2005). The largest known natural source of perchlorate is located in the Atacama Desert. This desert supplies Chilean nitrate fertilizer for the entire world for many years (Sellers, et. al., 2007).

Toxicity of perchlorate is well-known through its interference with iodide uptake by thyroid gland through functioning of the sodium ($Na^+$)/iodide ($I^-$) symporter in the gland

(Kirk, 2006; Wolff, 2002, Stoker et al., 2006). Iodide is the most essential element in the production of thyroid hormone. Iodide shortage causes cretinism (mental retardation) in children and hypothyroidism in adults (Kirk, 2006; Wolff, 2002).

Perchlorate's effects on the thyroid gland have been studied since the 1950s, when perchlorate was used a medication to limit iodine uptake in the treatment of hyperthyroidism (Graves' disease) (Strawson et al., 2004; Wolff, 1998; Charnley, 2008). Greer et al. (2002) reported that a perchlorate dosage of 0.16 mg/kg-day, which is equivalent to 11 mg/day for a 70-kg person, corresponds to 50% inhibition of iodide uptake in the thyroid gland.

There are two forms of thyroid hormones; thyroxine (T4) and tri-iodothyronine (T3). They are vital to the regulation of protein synthesis, growth and development, brain and nerves functioning, and repair organs (Saatcioglu et al., 1994; Anderson, 2001; Cooper, 2003; Kirk, 2006). When perchlorate competes with iodine, thyroid hormone production reduces. Deficiency of thyroid hormones especially in newborns has profound damages to the development process and metabolism control. Surviving infants borne form mothers suffering hypothyroidism will more probably experience lower IQ even if the disease is controlled from birth (Kirk, 2006).

### 2.2. Perchlorate Contamination in the U.S.

Perchlorate contamination was first detected in groundwater wells in California in 1985 (Tikkanen, 2006). In 1997, due to the advancements in perchlorate measurement techniques, the U.S. Environmental Protection Agency (USEPA) and the California Department of Health Services (CDHS) started sampling and monitoring some drinking water sources. Perchlorate has been found and reported at almost 400 surface water and

groundwater supplies and soils in 35 states (Tikkanen, 2006), and in at least in 44 states there are some release potentials (Lehman, et al., 2008). Perchlorate contamination has become a concern in areas of southern California, Nevada, and Utah. More than 50% of the known perchlorate contaminated sites (about 224 sites) are located in California and Texas and some of these sites have the highest reported concentrations (Wang et al., 2008a). In 2005, the U.S. Government Accounting Office (GAO) analyzed the available data through the country and reported that about 65% of the perchlorate contamination in waters in the U.S. is related to defense and aerospace activities (Tikkanen, 2006). Unregulated Contaminant Monitoring Rule (UCMR), which is set by USEPA, required 3,900 public water systems to monitor perchlorate level between 2001 and 2003, and 1.9% of the total 28,179 sample taken nationwide showed detectable perchlorate levels (i.e. concentrations higher than 4 µg/L) (USEPA, 2005). The UCMR to monitor perchlorate has been renewed by USEPA for 4 years from 2007 to 2011 (USEPA, 2005).

Risk assessment studies have been performed to establish the oral reference dose (RfD) for perchlorate. The RfD is the daily perchlorate dose which is established to be protective of human health due to any type of exposure (USEPA, 2002). In 2002, a RfD draft of 0.00003 mg/kg/day was released by USEPA (USEPA, 2002). In 2005, the National Academy of Science (NAS) released the results of an independent research and recommended the RfD of 0.0007 mg/kg/day (Tikkanen, 2006). According to this report, USEPA revised the RfD to 0.0007 mg/kg/day, which corresponds to the interim health advisory level of 15 µg/L in drinking water (Hristovski et al., 2008).

Although perchlorate does not have a Maximum Contaminant Level (MCL) regulated by USEPA, it has been regulated by some states. Table 2.1 shows the advisory levels for perchlorate in 9 states (Tikkanen, 2006; Sellers et al., 2007).

Table 2.1. Summary of State Drinking Water Advisory Levels for Perchlorate (Compiled from: Tikkanen, 2006; Sellers et al., 2007)

| State | Limit | Level, µg/L |
|---|---|---|
| AZ | Health-based guidance level | 14 |
| CA | Public health goal notification level | 6 |
| MA | Drinking water MCL | 2 |
| MD | Health-based guidance level | 1 |
| NJ | Health-based MCL | 5 |
| NM | Action level | 1 |
| NV | Public notice standard | 18 |
| NY | Drinking water planning level / notification level | 5 / 18 |
| OR | Action level | 4 |
| TX | Drinking water action level | 4 |

## 2.3. Perchlorate Removal Technologies

Perchlorate is a highly soluble non-volatile salt with little sorption affinity for most natural materials except some oxides. Because of its high solubility, perchlorate cannot be removed from aqueous environment by using conventional treatment methods such as coagulation, sedimentation, filtration, or adsorption by activated carbon (Batista et al., 2002; Urbansky and Brown, 2003; Min et al., 2004). Physiochemical methods such as ion exchange, reverse osmosis, nanofiltration, electrodialysis, enhanced activated carbon removal, and new adsorption technologies have been developed to apply for perchlorate removal (Roquebert et al., 2000; Urbansky, 2000; Gu et al., 2001; Batista et al., 2002; Parette and Cannon, 2005; Velizarov et al., 2005). In all of the above-mentioned

physiochemical methods used for perchlorate removal, perchlorate ion is not destroyed, but is physically separated from the contaminated water. A few chemical reduction methods also have been investigated. In the chemical reduction method, transition metals are used as either catalysts (e.g. Pt as hydrogenation catalyst) or direct electron donor (e.g. $Ti^{3+}$ and $Ru^{2+}$) (Urbansky, 1998; Urbansky and Schock, 1999; Mahmudov et al., 2008). In chemical reduction methods, perchlorate is destroyed and reduced to chloride.

In addition to the physiochemical and chemical removal methods, biotechnological removal methods have been investigated for perchlorate remediation. Biological reduction using perchlorate-degrading-bacteria (Logan et al., 2001; Batista et al., 2002; Gingras and Batista, 2002; Brown et al., 2003) and phytoremediation (Urbansky et al., 2000; Susarla et al., 2000) have been investigated. Similar to chemical reduction, biological reduction and phytoremediation also destroy perchlorate. Each method has some advantages and limitations. High capital and operation/maintenance cost, regulatory issues, and waste management are the most common issues involving perchlorate removal technologies. Advantages and limitations of the available technologies are listed in Table 2.2 (compiled from: Roquebert et al., 2000; Urbansky and Schock, 1999).

It is commonly thought that bioreduction and ion exchange technologies are the most promising processes to remove perchlorate anion from contaminated aqueous environments (Batista et al., 2002; Logan and LaPoint, 2002; Min et al., 2004). Although there are some limitations, ion exchange is a well-known, reliable method with reasonable cost; and biological reduction is a method that destroys perchlorate with very low operating cost.

Table 2.2. Advantages and Limitations of Different Perchlorate Removal Process (Compiled from: Roquebert et al., 2000; Urbansky and Schock, 1999)

| Technique | Advantages | Limitations |
|---|---|---|
| Ion Exchange | Reasonable cost<br>Highly effective<br>Fast reaction<br>Easy implementation | Brine disposal (increase of regenerable resins)<br>Resin disposal (in case of non-regenerable resins)<br>Competition with other ions (e.g. nitrate) |
| Biological Reduction | Low operating cost<br>Fast reaction<br>Possible to perform in existing biofilters<br>Destruction of perchlorate as well as some other contaminations | Low reduction rates at low perchlorate concentrations<br>Regulatory and/or public acceptance<br>Unknown byproducts<br>Oxygen competition |
| Electrochemical Reduction | Low maintenance<br>No toxic byproducts | High capital and operation cost<br>Not proven for drinking water<br>Safety |
| Enhanced Activated Carbon Removal | Fast reaction | High capital cost<br>High O&M cost<br>Maintenance |
| Membrane Separation | Available technology<br>High effectiveness<br>Fast<br>Low cost | Waste disposal<br>Scale problem<br>Not selective<br>Maintenance |

## 2.4. Ion-Exchange Process for Perchlorate and Nitrate Removal

Ion exchange materials are non-soluble solid materials that contain exchangeable anionic or cationic functional groups (Helfferich, 1962), which are covalently bonded to a main structure (Szlag and Wolf, 1999). Basically, ion exchange technology is based on exchange of contaminant ion existing in aqueous phase with an innocuous ion attached to the ion exchange materials. The ion, attached to the functional groups of the resin in its original form, is called counter-ion. Figure 2.1 is a schematic ion exchange reactor that uses chloride counter-ion to treat perchlorate/nitrate contaminated water.

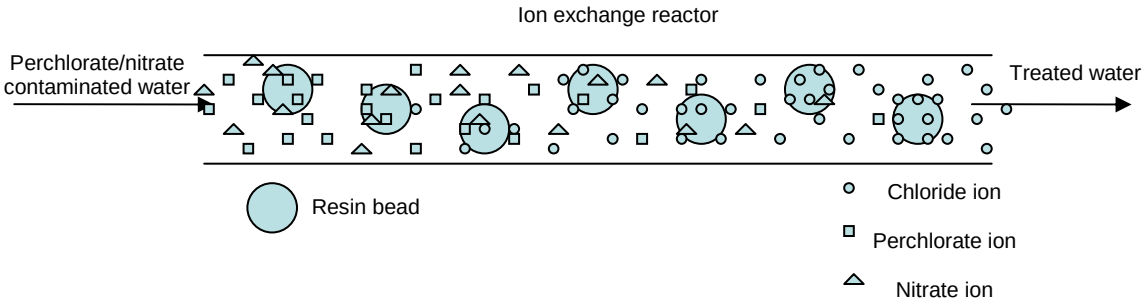


Figure 2.1. Schematic Ion-Exchange Reactor; Contaminated Water Enters from the Left Side and Treated Water is Produced through the IX Process

Since ion exchange technology has been used in water utilities and homes for many years and water utilities are familiar with this technology, this is the strongest candidate to be used by water utilities to remove perchlorate from drinking water, compared to biological perchlorate degradation.

### 2.4.1. Ion Exchange Materials

Ion exchange process can occur naturally in soils, activated carbon, coal, humus, cellulose, wool, lignin, metallic oxides, and even living tissues of plants, animals, algae, and bacteria (Weber, 1972). Historically, ion exchange using natural materials such as clay was employed to improve drinking water quality by the ancient Greeks (Weber, 1972).

In addition to some natural ion exchange materials, different synthetic ion exchange resins have been designed and produced. First synthetic ion exchange resin was developed in 1935 by Adams and Homes (Girolamo and Marchionna, 2001). Today, acrylic and styrenic polymers with divinylbenzene as a cross-linking group are the common synthetic ion exchange resins in the market.

Ion exchange resins can be classified according to the molecular structure and different ionic functional groups attached to the main matrix. Ion exchange resins are either cationic or anionic based on the charge of the ion (i.e. counter ion) bonded to the functional group. Therefore, cationic resins are the resins with positive charged counter-ion, and anionic resins are the resins with negative charged counter-ion. Synthetic resins are categorized as acrylic and styrenic. Acrylic ion exchange resins are the resins that have an aliphatic (i.e. open carbon chain) matrix, and styrenic ion exchange resins are the resins that consist of aromatic polymers in their main structure (Szlag and Wolf, 1999). The styrenic resins are the most common synthetic resins in the market (Simon, 1991). Although the amount of employed divinylbenzene, as a cross-linking group between the main structural polymer chains, is small (i.e. usually less than 12% wt), divinylbenzene is essential for the three-dimensional structure of resins to keep the polymeric chains of the main structure in their specified position (Simon, 1991; Weber, 1972).

Basically, the most important factor to characterize resins is the difference between functional groups. Based on the functional group type, synthetic resins can be classified as strong-acid, weak-acid, strong-base, and weak-base. This classification is based on dissociation constant (i.e. p*K)* of the functional groups bond to the polymer matrix. Resins with p*K* value higher than 13 are called strong-base resins, and resins with very low p*K* value (i.e. <0) are called strong-acid resins. The resins with moderate p*K* values are weak-base and weak-acid (Crittenden et al., 2005).

However a very wide variety of functional groups can be attached to the synthetic structure of polymeric resins (Szlag and Wolf, 1999). There are few functional groups used to design and produce ion exchange resins for water treatment industry (Crittenden

et al., 2005). Table 2.3 summarizes the common types of resins focusing on the employed functional group structure of each type. A brief discussion of structure and functional groups attached to the main matrix of synthetic resins is presented in the next section.

Table 2.3. Common Types of Ion-Exchange Resins and the Resin Structure

| Type of resin | Functional group | Resin structure |
|---|---|---|
| Strong-base anionic type I | Trimethyl-amine | $[\overline{R(CH_3)_3N^+}]Cl^-$ |
| Strong-base anionic type II | Dimethylethanol-amine | $[\overline{R(CH_3)_2(CH_3CH_2OH)N^+}]Cl^-$ |
| Weak-base anionic | Tertiary amine | $[\overline{R(CH_3)_2N}]HOH$ |
| Strong-acid cationic | Sulfonate | $[\overline{RSO_3^-}]H^+$ |
| Weak-acid cationic | Carboxylate | $[\overline{RCOO^-}]H^+$ |

*2.4.1.1. Strong-Base Resins*

Strong-base ion exchange resins are categorized as type I and type II. The term "strong" is not related to the strength of the resin matrix. Fundamentally, the complete dissociation of the functional groups at any practical pH, based on Arrhenius theory of dissociation, is the reason to define the strength level of this type of resin. The functional group in strong base resins is positive-charged quaternary amine which is attached to the main polymeric matrix (Crittenden et al., 2005). The hydroxide counter ion is connected to functional group and will be exchanged with available anions in water. For type I, the exchange reaction can be written as (Crittenden et al., 2005)

$$n[\overline{R(CH_3)_3N^+}]OH^- + A^{n-} \leftrightarrow [\overline{nR(CH_3)_3N^+}]A^{n-} + nOH^- \quad (2.1)$$

The over-bar in the above exchange chemical reaction indicates the immobile part of the resin structure.

Type II exchange reaction is slightly different from type I, because of the structure of the functional group as written below (Crittenden et al., 2005)

$$n[\overline{R(CH_3)_2(CH_3CH_2OH)N^+}]OH^- + A^{n-} \leftrightarrow [\overline{nR(CH_3)_2(CH_3CH_2OH)N^+}]A^{n-} + nOH^- \quad (2.2)$$

The main difference between type I and type II is presence of an ethanol group in type II quaternary amine. Ethanol group is added to type II because it reduces the resin's affinity for hydroxide ion. Type I strong base ion exchange resin has higher chemical stability than the type II does. Having stronger matrix, type I strong base resin does not react with the chemicals in its environment and it does not dissolve in water. In contrast, type II has higher capacity and regeneration efficiency than type I (Crittenden et al., 2005).

$OH^-$ as the counter-ion has typically larger hydrated radius than other anions in water. Hydrated radius is the true radius (i.e. effective size of an ion plus its associated water molecules in solution) of the ion in solution. Therefore, the resin shrinks after the exchange process with the ions with larger hydrated radius (Crittenden et al., 2005). The use of strong base ion exchange resins is increasing to remove nitrate, arsenic, and perchlorate (Baciocchi et al., 2005). In these cases, the resin is used in chloride form.

*2.4.1.2. Weak-Base Resin*

A tertiary amine group, which does not have a permanent positive charge, is the functional group in weak base ion exchange resins. Tertiary amine functional groups that are available in freebase form have a water molecule (HOH) which can be dissociated

and then absorb anions without exchanging any negative ions with the solution (Helfferich, 1962). The exchange reactions to uptake an anion for weak base ion exchange resin can be written as (Crittenden et al., 2005)

$$[\overline{R(CH_3)_2N}]HOH + H^+ + A^- \leftrightarrow [\overline{R(CH_3)N}]HA + HOH \quad (2.3)$$

Weak base resins also can adsorb free mineral acids (e.g. HCl and $H_2SO_4$) using their tertiary amine functional groups. Thus, sometimes weak base synthetic ion exchange resins are called acid adsorbers (Crittenden et al., 2005). In this case HOH molecule is released to the solution as a result of exchange process (Crittenden et al., 2005):

$$[\overline{R(CH_3)_2N}]HOH + HA \leftrightarrow [\overline{R(CH_3)N}]HA + HOH \quad (2.4)$$

Weak base resins work properly in the solutions with the pH between 6.7 and 8.3 at 25°C (Crittenden et al., 2005). NaOH, $NH_4OH$, or $Na_2CO_3$ can be used to regenerate these resins. The regeneration efficiency for weak base resins is much higher than that of either type I or type II of strong base resins.

*2.4.1.3. Strong-Acid Resin*

The functional group in strong acid synthetic ion exchange resins is a charged sulfonate group. Sulfonate functional group normally is dissociated completely at any pH of the solution (Crittenden et al., 2005). The chemical equation of exchange reaction for strong acid resin is as below (Crittenden et al., 2005).

$$n[\overline{RSO_3^-}]H^+ + M^{n+} \leftrightarrow [\overline{nRSO_3^-}]M^{n+} + nH^+ \quad (2.5)$$

Since the hydrated radius of $H^+$ is much larger that other cations, strong acid resin shrinks after the exchange process (Crittenden et al., 2005). The shrinkage is about 7% for gel type resin and 3 to 5% for macroreticular type resin (Crittenden et al., 2005).

*2.4.1.4. Weak-Acid Resin*

In weak acid ion exchange resins, the functional group is normally a carboxylate group. This type of resins has a high affinity to hydrogen ion and does not exchange it easily at a pH less than 6. The exchange reaction in weak acid ion exchange resin is described below (Crittenden et al., 2005).

$$n[\overline{RCOO^-}]H^+ + M^{n+} \leftrightarrow [\overline{nRCOO^-}]M^{n+} + nH^+ \quad (2.6)$$

Weak acid ion exchange resin has higher affinity for proton ion compared to strong acid resin. Thus, regeneration of weak acid resin requires less acid (i.e. HCl or $H_2SO_4$) compared to strong acid resin.

In practice, weak acid ion exchange resin usually is used to treat the cations in waters containing low dissolved carbon dioxide and high alkaline species (e.g. $CO_3^{2-}$, $OH^-$, and $HCO_3^-$) (Szlag and Wolf, 1999). Combinations of strong acid and weak acid resins can be used to reduce the volume of regenerant, and the product water has the same quality as just using strong acid resin alone in the process.

### 2.4.2. Physical Properties of Synthetic Ion-Exchange Resin

Physical properties (e.g. hydration and swelling, density, resistance to osmotic shock, diffusion, and pore size) of synthetic ion exchange resins are the properties related to structural matrix of the polymer (Simon, 1991). The amount of employed divinylbenzene in the polymerization process is the most important factor determining the physical properties of ion exchange resins (Szlag and Wolf, 1999).

The density of ion exchange resin is one of the important physical properties related to degree of cross-linking. In practice, the amount of cross-linking can vary between 2% and 16%, and as a result, density will increase from 90 g/L to 500 g/L for resins in the

hydrogen form, respectively (Simon, 1991). The resin may be damaged during the treatment operation when the amount of cross-linking is low. In contrast, high degree of cross-linkage results in higher resin manufacturing costs (Simon, 1991).

Swelling of synthetic resins depends on the amount of divinylbenzene cross-linkage, nature of functional groups and the hydrated radius of the contaminant ion which is exchanged with the original counter ion. Higher degree of divinylbenzene cross-linkage will decrease the amount of water in the resin bead, and as a result, the percentage of swelling will be lower.

Osmotic shock in synthetic ion exchange resin is due to submission of the resin media to different concentration of solutes, due to normal cycles of exhaustion/regeneration. During the regeneration cycle, high concentrations of solute in the solution will attract water inside the beads and make the resin to shrink. In contract, during the operation, the resin will swell due to reverse concentration gradient. The resin structure should have enough structural support to be able to tolerate several cycles of swelling and shrinking without developing structural fractures (Simon, 1991).

In order to be exchange with the original counter ion of a functional group located inside the bead, an ion should diffuse through the liquid boundary layer and the solid resin phase to reach to the target functional group. In addition, the exchanged ion should diffuse in the opposite direction to reach the liquid phase of the system. Both resin bead size and cross-linking degree are important physical properties to determine the speed of exchange process (Simon, 1991). Increasing the divinylbenzene cross-linking will decrease the diffusivity and the mass transfer rate (Simon, 1991), and as a result more time is needed in order to exchange specified amount of the ions in solution. The effect

of these physical properties in the kinetics of exchange has been discussed in detail in the "kinetics of ion exchange process" section.

*2.4.2.1. Gel-type and Macroporous-type Resins*

Synthetic resins can be categorized according to their polymeric matrix to gel (microreticular) resins and macroporous (macroreticular) resins (Crittenden et al., 2005). Conventional synthetic resins are homogeneous gel-type resins in which the pore is the distance between polymeric chains (Kun and Kunin, 1964; Kun and Kunin, 1968). In contrast, macroporous resins in addition to gel-type porosity have considerable non-gel porosity. The channel network between the tiny sphere-shaped particles that constitute macroporous resin is called the non-gel porosity (Kun and Kunin, 1964)

Initially, the reason to develop macroporous resins was irreversible fouling problem in the gel type resins (Simon, 1991). Normal ions in the ion exchange process are relatively small (i.e. < 10 Å or 0.001 µm). Removal of large natural organic acids with the molecular size of 100 Å (0.01 µm) and larger, which exchange with counter ion of basic synthetic resins, is very difficult (Simon, 1991). This exchange is often irreversible. To be able to accommodate the operation in presence of organic acid molecules, macroporous resins have been developed (Simon, 1991).

The cross-linking degree is different between gel-type and macroporous resins. In average, gel-type resins have 8% divinylbenzene, ranging from 4-10%, while macroporous resins have about 20-25% percent divinylbenzene cross linking (Crittenden et al., 2005). High water content and significant amount of shrinking/swelling are the characteristics of the gel-type resins. In contrast, macroporous resins do not shrink or swell similar to gel-type, due to the ion exchange process. Gel type resin looses its

porous structure, after shrinking when it is exposed to a drying situation (Crittenden et al., 2005). The measured surface area for each gram of gel-type resin has been reported about 2 $m^2$, while, the surface area for each gram of macroporous resin has been measured about 7 to 600 $m^2$ (Crittenden et al., 2005).

Pore size of gel-type and macroporous resins have been measured and reported in the literature. Pietrzyk (1969) measured the pore size of three different gel-type resins, and the average pore size for all three was reported 5 to 50 Å (0.0005 to 0.005 µm). In the same article, the average pore size of three different macroporous resins was measured and reported as 290, 205, and 90 Å (0.029, 0.0205, and 0.009 µm) (Pietrzyk, 1969). While using macroporous resins to treat the waste effluent Kunin (1976) has measured the pore size of five macroporous ion exchange resins, and in all resin samples the pore size has been reported to be smaller than 250 Å (0.025 µm) (Kunin, 1976). Pore diameter changes during the styrene-divinylbenzene polymerization step throughout the macroporous resin manufacturing process (Kun and Kunin, 1964).

There are two common methods for the preparation of macroporous synthetic ion exchange resins (Simon, 1991). In the first method, a monomer with a known molecular weight is added to styrene-divinylbenzene during the polymerization stage. After the polymerization stage, the foreign polymer is converted to a soluble organic electrolyte. This soluble organic electrolyte is washed from the main structure during the extraction stage and leaves the macro-pores (Simon, 1991). The second method, which is the most common method, is known as phase separation. In this method, first a solvent is added to the styrene-divinylbenzene mixture. The solvent should have special characteristic in order to be soluble in the monomers. The solvent should precipitate during the

polymerization stage. After the polymerization stage, the solvent is removed from the pores of the main matrix using extraction technique (Simon, 1991). During the second method of macroporous resin manufacturing, the average pore size of the resin media first decreases, next increases, and then decreases (Kun and Kunin, 1964). The final average pore size has been reported as 600 Å (0.06 µm), and sizes range from 130 Å to 10,000 Å (0.013 to 1 µm) (Kun and Kunin, 1964). However, production of macroporous resins with larger pore size is possible. Poinescu and Vlad (1996) have performed twenty experiments on macroporous resin production and reported average pore size of 15 to 198 nm (150 to 1980 Å) for macroporous resins. It seems that the average pore size of macroporous ion exchange resin is mostly in ångstrom (Å) level, and even in macroporous resins with large pore size, it is in nanometer level.

### 2.4.3. Fouling; A Common Issue in Ion-Exchange Process

Fouling is a problem to all surfaces exposed to aqueous environments that contain inorganic substances, organic substances, or microorganisms. In the water industry, fouling is a common problem in ion exchange beds, electrodialysis systems, and membrane units (Park et al., 2003; Park et al., 2005; Lee et al., 2009). In cation exchange resin systems, hydrous oxides of iron, copper, magnesium, manganese, aluminum, calcium sulfate, grease, oil, and suspended matter are the most common foulants (Pelosi and McCarthy, 1982). The foulants that most often plague anion exchange resins are high molecular weight organics and colloidal silica (Baker et al., 1979; Pelosi and McCarthy, 1982; Lee et al., 2009). High molecular weight organics could be natural organic matter (NOM) molecules or soluble microbial products (SMP). Fouled resin is

usually darker in color compared to the fresh resin. Low pH and high conductivity of the treated water are the indicators of organic fouling (Pelosi and McCarthy, 1982).

The most common fouling in strong base anion exchange resins is organic fouling, which is due to high-molecular-weight NOM and/or SMP molecules (Pelosi and McCarthy, 1982; Humbert et al., 2007; Kabsch-Korbutowicz et al., 2008; Nkambule et al., 2009a). NOM molecules are the molecules resulting from the break down of plants and animals in the environment (Jarvis et al., 2008; Nkambule et al., 2009b), while SMP molecules are the organic molecules released during bacterial cell lysis or lost during synthesis (Rittmann and McCarty, 2001). In general, NOM can be divided into two main categories of organic molecules: identified bio-polymers and humic substances (Humbert et al., 2007; Kabsch-Korbutowicz et al., 2008; Nkambule et al., 2009b). Identified bio-polymers consist of polysaccharides, proteins, and amino sugars that are introduced to water due to degradation of animal and plant tissues (Kabsch-Korbutowicz et al., 2008; Nkambule et al., 2009b). Humic substances are the organic molecules that are originated from a complex chemical process called humification. The nature of humic substances has not been well defined (Humbert et al., 2007). Humic-like substances have higher molecular weight and are more easily degraded under aerobic conditions, while identified bio-polymers have lower molecular weight and are more readily degradable under anaerobic conditions (Park et al., 2005). High-molecular-weight organics cause problems in ion exchange systems because of both their large molecular size and the presence of many functional groups (i.e. phenol, hydroxyl, carbonyl, and carboxyl) in their molecular matrix (Pelosi and McCarthy, 1982; Boyer and Singer, 2008; Kabsch-Korbutowicz et al.,

2008). Since each of these molecules has several functional groups, it can occupy several functional group sites of the ion exchange resin.

There are two main mechanisms responsible for the adsorption of organic molecules from water by strong base ion exchange resins: ion exchange adsorption and van der Waals' type forces (Baker et al., 1979). On the one hand, organic material is normally negatively charged. This negative charge results in anionic exchange phenomenon. On the other hand, relatively hydrophobic and rougher surfaces have higher selectivity for organics compared to hydrophilic and smoother surfaces (Park et al., 2005). Strong base anionic resins are mostly made of polystyrene which is a hydrophobic polymer (Baker et al., 1979). Hydrophobicity of polystyrene anion exchange resins makes them more selective for some anions (e.g. perchlorate) (Yoon et. al., 2009). Van der Waals' type forces that result in adsorption to the anion exchange resin are due to the hydrophobicity of the structure of the resin and the dissolved hydrophobic organic material presented in the environment (Baker et al., 1979).

Fouling leads to weakening of resin bed performance. Capacity loss is the most important problem in ion exchange systems. In general, foulants can coat the resin beads and/or occupy the functional groups on and within the bead, preventing the proper flow and ion exchange phenomenon (Pelosi and McCarthy, 1982; Park et al., 2003). The diffusion of exchanging ions through the pores of the resin is hindered by the slow-diffusing large organic molecules (Kabsch-Korbutowicz et al., 2008). Precipitation and/or adsorption of these large organic molecules within the resin matrix confine the free-flow area of the resin pores (Lindstrand et al., 2000b). Precipitation is affected by solubility of the substance, and adsorption is governed by electrostatic and hydrophobic

interactions between the substance and the resin surface (Lindstrand et al., 2000a). Hence, the resin bed will not service to its theoretical capacity.

Leakage of some foulants that are slowly eluted from the bed after regeneration steps is another problem that may occur due to resin fouling (Park et al., 2003). For instance, high-molecular-weight organic acids, colloidal silica, magnesium hydroxide, calcium carbonate, and calcium sulfate are the foulants that may have continuous leakage during the service cycles. In severe cases, channeling due to accumulation of adsorbed foulants to the resin beads may occur.

The most common reagent to remove organic fouling from anion exchange resin is brine solution (DOW, 2009; Keller, 2009). Increasing the pH of brine using a caustic solution (e.g. NaOH) promotes the efficiency of brine solutions to remove the foulants from the resin (Keller, 2009). Studies have shown that monovalent species such as NaCl and NaOH have better efficiency for fouling removal of organic matter than polyvalent oxygenated ions (e.g. $CaCl_2$ or $Ca(OH)_2$) (Wilson, 1959). Moreover, the chloride ion has more efficiency than the hydroxyl ion for organic removal (Wilson, 1959; Tilsley, 1975; Keller, 2009). The benefit of using sodium chloride and sodium hydroxide solutions for organic fouling removal is that these reagents do not degrade the resin backbone (Dow, 2002; Dow, 2009). Studies have shown that increasing the concentration and contact time of fouling removal reagents increases the efficiency of the fouling removal process (Wilson, 1959; Dow, 2009). Increasing the temperature of the reagent also helps to increase the efficiency of the fouling removal process. However, the temperature should be no more than 95 to 100ºF (35 to 38ºC) to avoid resin damage (Wilson, 1959; Purolite, 1999). Several methods using sodium chloride and sodium hydroxide solutions for

organic fouling removal in different concentrations, contact times, bed volumes, and temperatures have been developed (Purolite, 1999; Dow, 2002; Dow, 2009; Envirotech, 2009).

There are some alternatives for organic fouling removal form ion exchange resin. A common substitute for using NaCl is sodium hypochlorite (NaOCl) (Dow, 2009). Using sodium hypochlorite has another benefit: it also disinfects the resin bed and prevents microbial growth. It has been shown that sodium hypochlorite solution is slightly more effective than sodium chloride solution to remove humic acid fouling (Keller, 2009). The concentration of sodium hypochlorite should be monitored to control the amount of disinfection by-product production and to reduce the risk of resin oxidation that results in reduction of resin capacity (Keller, 2009; Kemper et al., 2009; Lee, 2009). Sodium hypochlorite solutions of 0.01% to 3% with contact time of 20 to 30 min have been used for organic fouling removal purposes (Wilson, 1959; Dow, 2002). The Dow Chemical Company has a detailed protocol for organic fouling removal from anion exchange resins. In this procedure concentration of 0.1-0.25% at the pH of 9.0 with the average contact time of 25 min has been suggested as the appropriate concentration at ambient temperature (Dow, 2009).

Other organic fouling removal reagents such as peracetic acid (PA) and hydrogen peroxide also have been employed for organic fouling removal purposes (Purilite, 1999; Dow, 2002; Park, 2002; Envirotech, 2009; Dow, 2009). Peracetic acid (PA) and hydrogen peroxide have shown an effective treatment on both cationinc and anionic exchange resins. Peracetic acid solutions at concentrations of >1000 (mg/L) have been applied and showed significant bactericides effects at ambient temperature have been

shown (Envirotech, 2009). The contact time of one hour has been suggested for peracetic acid (Purilite, 1999). The spent solutions resulted from cleaning and/or disinfection process are biodegradable (Envirotech, 2009). The Dow Chemical Company has developed a detailed procedure of fouling removal from ion exchange resin systems that uses hydrogen peroxide to treat biological foulants. In this procedure the suggested concentration of hydrogen peroxide is 2% with the contact time of 20-30 min (Dow, 2009). Hydrogen peroxide also has been used in concentrations of 0.05% with the contact time of 2 hours and 0.05% with the contact time of 24 hours (Dow, 2002).

### 2.4.4. Selectivity of Ion-Exchange Resin

The affinity of resin for a certain ion in aqueous solution is called selectivity. According to this affinity, the direction of exchange reaction is determined. Resin selectivity depends on physical and chemical characteristics of the exchanging ions. The magnitude of the valence and the atomic number of the ion are important factors in selectivity (Crittenden et al., 2005). The selectivity increases with larger magnitude of the mentioned chemical properties. Pore size distribution and the type of functional groups on the polymer chain are also determinant factors for the selectivity of the resin. The molality of the ions in the solution also affects the selectivity (Crittenden et al., 2005).

Amount of swelling or pressure within the bead is also important for resin selectivity. In an aqueous environment, all the free ions and also resin-phase ions are surrounded by water molecules. This radius varies for different ions. Normally, when the size of the ion increases, the radius of hydration becomes smaller (Crittenden et al., 2005). When ions with large hydration radius enter the resin bead, the water molecules surrounding

them also enter that space, and cross-linking structure that holds the chains together resist for the swelling forces. Thus, entering the ions with large hydration radius causes some swelling forces to the overall resin structure. Therefore, ions with smaller radius of hydration are more preferred by the resin because they decrease the swelling pressure of the resin. These ions make a tight bound with the resin.

Sulfate is the main competitor of perchlorate in the IX process. Sulfate ions exhaust most of the capacity of resin. Hence, the resin cannot remove perchlorate only after a few bed volumes of water pass through the IX column (Clifford and Weber, 1986). Selective resins have high affinity for the ions of interest (e.g. nitrate or perchlorate) wand very low affinity for competitor ions, particularly sulfate. The most important factor affecting the selectivity of IX resins is the spacing of functional groups (Clifford and Weber, 1986). The distance between active functional groups affects the divalent / mono-valent selectivity of the IX resin. A divalent ion requires two adjacent active functional groups to connect to and to satisfy electroneutrality of the exchange chemical reaction. Therefore, increasing the distance between active functional groups decreases the selectivity for a divalent ion (e.g. sulfate) (Crittenden et al., 2005).

The order of preference for some alkali metals, alkaline earth metals, and anions are presented below (Crittenden et al., 2005).

$Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$ (Crittenden et al., 2005)

$Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+} > Be^{2+}$ (Crittenden et al., 2005)

$ClO_4^- > I^- > ClO_3^- > NO_3^- > Br^- > NO_2^- > BrO_3 > Cl^- > CH_3COO^- > HCO_3^- > OH^- > F^-$

(Crittenden et al., 2005; Sodaye et al., 2007)

As it is apparent from the selectivity of the above-mentioned anions, perchlorate ion has the most affinity for anionic resins among the listed anions (Crittenden et al., 2005; Sodaye et al., 2007; Lehman et al., 2008).

Ionic strength of the solution is an important factor for selectivity. When the ionic strength of the solution increases, the preference of the resin for divalent ions compared to mono-valent ions decreases. This is the main reason why very high saline solutions are used in the regeneration cycles. The use of regenerant solutions with high concentrations in the regeneration cycle enhances the efficiency of the process. However, in some cases such as perchlorate, even using high strength saline solution is not able to regenerate the resin (Lehman et al., 2008).

The law of mass can be applied to the ion exchange reaction when the exchange reaction is assumed as a stoichiometric reaction. Thus, the following generalized equation can represent the ion exchange process (Crittenden et al., 2005).

$$n[\overline{R^{\pm}}]A^{\pm} + B^{n\pm} \leftrightarrow [n\overline{R^{\pm}}]B^{n\pm} + nA^{\pm} \tag{2.7}$$

where $\overline{R^{\pm}}$ is the functional group of the ion exchange resin, A and B are exchanging ions, and n is the valence of the ion.

The equilibrium coefficient can be written for the above reaction where $K_B^A$ is called selectivity coefficient for ion A exchanging with ion B. Selectivity coefficient is a coefficient that compares the affinity of a resin to a certain ion compared to a reference ion which is $Cl^-$ in anionic resins and $Na^+$ in cationic resins. Selectivity coefficient can be expressed as the following equation.

$$K_B^A = \frac{[A^{\pm}]^n\{\overline{R^{\pm}}B^{n\pm}\}}{\{\overline{R^{\pm}}A^{\pm}\}^n[B^{n\pm}]} \tag{2.8}$$

$[A^{\pm}]$ and $[B^{\pm}]$ are the concentration of counter-ion and contaminant ion, respectively. And $\{\overline{R^{\pm}}A^{\pm}\}$ and $\{\overline{R^{\pm}}B^{n\pm}\}$ are the activities of resin-phase counter-ion and contaminant ion, respectively. The selectivity coefficient for different ions has been well-studied and reported in many textbooks and articles.

Selectivity coefficient is not constant and is affected by several factors (Crittenden et al., 2005). The following factors may influence the selectivity coefficient of resins: size and charge of exchangeable ions; resin properties such as particle size, cross-linking amount, type of functional groups; water characteristics including concentration of different ions and organic compounds; and temperature (Crittenden et al., 2005).

Selectivity coefficient is a very practical factor to express the selectivity of a contaminant ion compared to the counter-ion. Table 2.4 shows some typical separation factors for commercial resins. The higher the selectivity coefficient, the greater the affinity of the resin for the target ion.

Generally, type I strong-base ion exchange resins with quaternary amine functional groups attached to the aromatic styrenic chain with divinylbenzene cross-linking bridges are considered to be hydrophobic compared to polyacrylic resins (Gu and Brown, 2006). Therefore, type I strong-base ion exchange resins have high affinity for poorly hydrated anions such as perchlorate (Gu and Brown, 2006). Replacement of one of the trialkyl groups with an ethanol increases the hydrophilic characteristics. Thus, type II strong-base ion exchange resins are less selective for perchlorate anion. Studies showed that increasing the length of trialkylic group from methyl to butyl or hexyl increases the hydrophobic characteristics in addition to charge separation distance (Gu and Brown, 2006). Thus, the selectivity of the resin for perchlorate increases by increasing the length

of trialkylic group (Gu and Brown, 2006). Increasing the length of functional group from methyl to ethyl and then to propyl affects the selectivity coefficient significantly from 125 to 1100 and finally to 1500, respectively (Batista et al., 2002). Although increase in the length of functional group increases the selectivity of the resin, these long-chain functional groups were found to have slower exchange kinetics (Batista et al., 2002). To address this issue, a bi-functional resin consisting of quaternary amine groups with large (C6) and small (C2), which has a high selectivity and acceptable kinetics, has been manufactured and tested by Oak Ridge National Laboratory (ORNL) (USEPA, 2004).

Table 2.4. Some Typical Selectivity Coefficient for Strong-acid and Strong-base Resins (Crittenden et al., 2005)

| Strong-Acid Cation Resins Polysterene divinylbenzene matrix with sulfonate functional groups | | Strong-Base Anion Resins Polysterene divinylbenzene matrix with type-I functional groups | |
|---|---|---|---|
| Cation | Selectivity, $K^{i}_{Li^+}$ | Anion | Selectivity, $K^{i}_{Cl^-}$ |
| $Li^+$ | 1.0 | $HPO_4^{2-}$ | 0.01 |
| $H^+$ | 1.3 | $CO_3^{2-}$ | 0.03 |
| $Na^+$ | 2.0 | $OH^-$ (type I) | 0.06 |
| $NH_4^+$ | 2.6 | $F^-$ | 0.10 |
| $K^+$ | 2.9 | $SO_4^{2-}$ | 0.15 |
| $Rb^+$ | 3.2 | $CH_3COO^-$ | 0.2 |
| $Mg^{2+}$ | 3.3 | $HCO_3^-$ | 0.4 |
| $Zn^{2+}$ | 3.5 | $OH^-$ (type II) | 0.65 |
| $Cu^{2+}$ | 3.8 | $BrO_3^-$ | 1.0 |
| $Be^{2+}$ | 4.0 | $Cl^-$ | 1.0 |
| $Mn^{2+}$ | 4.1 | $CN^-$ | 1.3 |
| $Ca^{2+}$ | 5.2 | $NO^-$ | 1.3 |
| $Ba^{2+}$ | 11.5 | $HSO_4^-$ | 1.6 |
| $Ra^{2+}$ | 13.0 | $Br^-$ | 3.0 |
| | | $ClO_4^-$ (for polyacrylic resin) | 5.0 |
| | | $ClO_4^-$ (for polystyrene resin) | 150 |

Instead of concentration of ions, the equivalent fractions can be used in the equilibrium equation. The preference of the resin for one ion over another can be represented as separation factor (Crittenden et al., 2005).

$$\alpha_j^i = \frac{Y_i X_j}{X_i Y_j} \tag{2.9}$$

where $X_i$ and $X_j$ are equivalent fraction of counter-ion and contaminant ion in aqueous phase, respectively, and $Y_i$ and $Y_j$ are resin-phase equivalent fraction of counter-ion and contaminant ion in aqueous phase, respectively. Like selectivity coefficient, the higher relative separation factor, the greater affinity of the resin for the target ion.

### 2.4.5. Common IX Resins and Their Application to Remove Perchlorate

According to the selectivity coefficients, ion exchange resins used to remove perchlorate from water can be categorized in two groups: perchlorate selective resins and perchlorate non-selective (conventional) resins.

Non-selective resins have relatively lower affinity for perchlorate, thus other anions can compete with perchlorate and occupy most of the functional group sites. Therefore, the breakthrough point occurs more frequently, and as a result the resin bed should be regenerated more often. The disadvantage of using these resins is more frequent regeneration cycles due to the low affinity of these resins for perchlorate, and production of waste brine with high perchlorate concentration.

Selective resins have high efficiency in perchlorate removal and can remove perchlorate for several bed volumes without leakage. However, the regeneration of these resins using the conventional methods is almost impractical. Table 2.5 lists the commercially available perchlorate selective and perchlorate non-selective resins used for perchlorate removal.

Table 2.5. Common Available Perchlorate-selective and Perchlorate-non-selective Resins

| Manufacturer | Resin Type | Commercial name | Water Content (%) | Capacity (eq/L) | Resin structure | Functional group |
|---|---|---|---|---|---|---|
| Calgon | selective | CalRes 2103 | 50-70 | NA | NA | NA* |
| DOW | selective | DOWEX PSR-3 | 50 - 65 | 0.6 | Styrene, Macroporous | $(CH_3)_3$ $Cl^-$ ** |
| DOW | selective | DOWEX PSR-2 | 40-47 | 0.65 | Styrene, Gel | $(CH_3)_3$ $Cl^-$ |
| DOW | non-selective | DOWEX NSR-1 | 53-63 | 0.9 | Styrene, Macroporous | Article I. $(CH_3)_2$ $(CH_2)_2$ OH $Cl^-$ *** |
| DOW | selective | DOWEX-1 | 43-48 | 1.4 | Styrene, Gel | $(CH_3)_3$ $Cl^-$ |
| Purolite | non-selective | A520E | 50-56 | 0.9 | Styrene, Macroporous | $(CH_3)_3$ $Cl^-$ |
| Purolite | selective | A530E | ~50 | 0.55 | Styrene, Macroporous | $(CH_3)_3$ $Cl^-$ |
| Purolite | selective | A532E | 36-45 | 0.75 | Styrene, Gel | $(CH_3)_3$ $Cl^-$ |
| Purolite | non-selective | A600E | 43-48 | 1.4 | Styrene, Gel | $(CH_3)_3$ $Cl^-$ |
| Purolite | non-selective | A850E | 57-62 | 1.25 | Acrylic, Gel | $(CH_3)_3$ $Cl^-$ |
| ResinTech | selective | SIR-110-HP | 35-55 | 0.6 | Styrene, Gel | $(C_4H_9)_3Cl^-$ |
| ResinTech | selective | SIR-110HP-MACRO | 58-65**** | 0.6 | Styrene, Macroporous | $(C_4H_9)_3Cl^-$ |
| ResinTech | non-selective | SIR-100 | 50-65 | 1.0 | Styrene, Gel | $(CH_3)_3$ $Cl^-$ |
| ResinTech | non-selective | SIR-100HP | 52-60 | 0.85 | Styrene, Macroporous | $(CH_3)_3$ $Cl^-$ |
| ResinTech | non-selective | SBG-1HP | 43-50 | 1.45 | Styrene, Gel | $(CH_3)_3$ $Cl^-$ |
| ResinTech | non-selective | SBG-2HP | 37-45 | >1.45 | Styrene, Gel | $(CH_3)_3$ $Cl^-$ |
| Rohm & Haas | selective | PWA2 | 34-42 | >0.6 | Gel | NA |
| Rohm & Haas | non-selective | PWA5 | 52-58 | >1.0 | NA | NA |
| Rohm & Haas | non-selective | PWA12 | 57-64 | >1.25 | NA | NA |
| Sybron | non-selective | ASB-1 | 43-48 | 1.4 | Styrene, Gel | $(CH_3)_3$ $Cl^-$ |
| Sybron | non-selective | SR-7 | 52-67 | 0.6 | Styrene, Macroporous | $(CH_3)_3$ $Cl^-$ |

* Not Available
** Type I quaternary amine functional group
*** Type II quaternary amine functional group
**** Estimated based on Ion Exchange chapter (chapter 16, Crittenden et al., 2005).

Tripp and Clifford (2006) performed research on fifteen perchlorate selective and non-selective resins and reported the selectivity of the resins for perchlorate. According to this research, macroporous resins have higher affinity for perchlorate than gel-type resins. In 20ºC, macroporous resins have selectivity coefficient ranging from 5.5 to 134, while, the selectivity coefficient for macroporous resins is between 145 and 1300 (Tripp and Clifford, 2006). This research shows that macroporous resins are preferred to gel-type resins due to their higher selectivity coefficient for perchlorate.

In addition to strong-base resins discussed above, weak-base resins have also been used to remove perchlorate from waters (Batista et al., 2002). While, the acrylic type weak-base resin can be economically regenerated using either NaCl or NaOH solutions, styrenic type weak-base resin cannot be completely regenerated (Batista et al., 2002). But, due to presence of other ions in waters, it is not economical to use the weak-base ion exchange resins in the treatment processes.

Commercial application using ion exchange resins to remove perchlorate started in 1999. The first commercial Calgon ISEP® system with the total capacity of 2500 gpm was installed to treat three wells in La Puente Valley Water District located in Southern California (California EPA, 2004, ITRC, 2007). The system contained Purolite A-850 resin. The influent concentration of perchlorate was ~ 200 ppb and the effluent perchlorate was < 4 ppb. In 2000, Lawrence Livermore National Laboratory started three Sybron Ionic SR-7 plants with the capacity of 5000, 1400, and 1000 gpm. The project target was to reach a perchlorate concentration of < 4 ppb in the effluent, while the influent concentration for three plants was 10 ppb, 10 ppb, and 7.5 ppb, respectively (California EPA, 2004). A 2000 gpm plant using Amberlite PWA2, manufactured by

Rohm and Haas, was designed to be operated in Aerojet, Sacramento, CA, in 2001. The influent concentration in this plant was 50 ppb and the effluent was designed to be < 4 ppb (ITRC, 2007). In 2002, another Calgon ISEP® system with the total capacity of 450 gpm was started to treat water in Kerr-McGee, Henderson, Nevada. This system operated for 6 months and operation was discontinued due to maintenance problems caused by high TDS, hardness, and sulfate (California EPA, 2004). In this system, the influent and effluent perchlorate concentrations were 100 ppb and < 2 ppb.

Several other ion exchange systems to remove perchlorate from drinking water have been designed and installed in the recent five years. Most of these systems are located in California and Arizona with the capacity ranging from 24 gpm to 5000 gpm (ITRC, 2007). The effluent target for most of these ion exchange systems was < 4 ppb. The highest perchlorate influent concentration in the installed ion exchange systems was 200-300 ppm in Kerr-McGee, Henderson, Nevada. This 850 gpm system was replaced by a biological FBR in 2004 (ITRC, 2007).

### 2.4.6. Regeneration of Ion Exchange Resin

After the capacity of ion exchange resin is exhausted, the resin bed is not able to remove the contaminant ions form the aqueous environment any more. Regeneration is a chemical process that uses very high strength solution of the original counter ion to reestablish the exchange capacity. Theoretically, most of the common resins can be 100% regenerated. But, it is very costly to regenerate a resin bed to its initial capacity. Thus, cost-benefit studies are required to establish an efficient schedule for regeneration.

The following regeneration equations show the regeneration reaction for strong-base ion exchange resin with NaCl and NaOH, respectively.

$$[\overline{R(CH_3)_3N^+}]ClO_4^- + NaCl \leftrightarrow [\overline{R(CH_3)_3N^+}]Cl^- + (Na^+)ClO_4^- \quad (2.10)$$

$$[\overline{R(CH_3)_3N^+}]ClO_4^- + NaOH \leftrightarrow [\overline{R(CH_3)_3N^+}]OH^- + (Na^+)ClO_4^- \quad (2.11)$$

Similarly, regeneration equation for weak-base ion exchange resin using NaCl and NaOH can be written as the following equations, respectively.

$$[\overline{R(CH_3)_2N}]HClO_4^- + NaCl \leftrightarrow [\overline{R(CH_3)N}]HCl + NaClO_4 \quad (2.12)$$

$$[\overline{R(CH_3)_2N}]HClO_4^- + NaOH \leftrightarrow [\overline{R(CH_3)N}]HOH + NaClO_4 \quad (2.13)$$

However, perchlorate non-selective resins can be regenerated using brine solution (Gingras and Batista, 2002; Lehman et al., 2008). Regeneration of perchlorate selective resins, using conventional method (i.e. using brine solution as the regenerant) is impractical (Gu et al., 2001). In order to regenerate perchlorate non-selective ion exchange resins, concentrated mono-valent anion solutions can be used. Typical counter-ion for basic anion resins are chloride and hydroxide in approximate concentration of 2-12% by weight (Gingras and Batista, 2002). Batista et al. (2002) showed lower affinity of perchlorate ion for acrylic resin than for styrenic resins. Brine regeneration is very difficult and impractical because of high affinity of perchlorate ion for type I strong-base ion exchange resin (Gu et al., 2001). Because perchlorate is a monovalent anion with high affinity, a significant part of any exchanged perchlorate in the regenerant solution will re-exchange with the functional groups throughout the regeneration cycle. This re-exchange will cause leakage of perchlorate in the next operation cycle because the adsorbed perchlorate ions will exchange for the chloride coming from the influent (Lehman et al., 2008).

Applying $FeCl_3$ and HCl has been tested to regenerate a specific ion–exchange resin developed by ORNL. Nearly 100% recovery of the active sites of the resin has been reported. Tetrachloroferrate ($FeCl_4^-$) anion from $FeCl_3$ and HCl is the anion that is exchanged with perchlorate attached to the functional groups (Gu et al., 2001). But the full scale feasibility of this regeneration method has been questioned because of the production of regeneration waste with very low pH and high concentration of soluble perchlorate (Kim and Gurol, 2004). In order to reduce the soluble perchlorate in the low pH regeneration waste, high pressure and temperature with several hours residence time is required which make the use of this regeneration method very difficult and costly.

### 2.4.7. Kinetics of Ion-Exchange Process

Ion exchange is a process that depends on following relative rates: a) transport of the contaminant ion from bulk solution to the film surface around the resin bead; b) transport of the ion through the film which surrounds the resin bead; c) transport of the ion through the pore of the resin bead inward to reach to the target functional group; d) exchange of the contaminant ion with the original counter-ion; e) transport of the exchanged ion through the pore outward to reach to the surface of the resin bead; f) transport of the exchanged ion through the boundary layer (i.e. film) surrounding the resin particle to the surface of the boundary layer; and, g) transport of the exchanged resin from the external surface of the film to the bulk solution (Weber, 1972; Helfferich, 1962).

Several studies have been performed to determine the rate-limiting step for ion exchange process and the results showed that the overall process does not depend on the actual exchange reaction (i.e. step d). Rather, the process is controlled by diffusion. The controlling steps are the transport of contaminant ion from the bulk solution to the active

site (i.e. steps a to c) and the transport of exchanged ion form active site to the bulk solution (i.e. steps e to g) (Weber, 1972). The rate-determining step in ion exchange process is either diffusion across the liquid boundary layer of solution around the resin particle or inter-diffusion of exchanging ions through the interstitial pore of the resin. The first process is usually called film diffusion and the second process is normally called pore diffusion (Weber, 1972).

The appropriate representation for the time rate of decrease of concentration for ion exchange process for a batch reactor in which film diffusion is the controlling step would be (Weber, 1972):

$$\frac{-dC}{dt} = k_f \frac{a^0}{V}(C - C_e) \tag{2.14}$$

where $C_e$ is the concentration after equilibrium, a° is the total surface of the resin beads in the reactor, V is the volume of the solution in the reactor, and $k_f$ is the film transfer coefficient.

If the controlling step in the process is the pore diffusion, a diffusion model based on Fick's second law can be used considering simultaneous diffusion-reaction process, and would be expressed as (Weber, 1972):

$$\frac{\partial C}{\partial t} = \frac{1}{r^2}\frac{1}{\partial r}(r^2 D_l \frac{\partial C}{\partial r}) - \frac{\partial q_e}{\partial t} \tag{2.15}$$

where $D_l$ is the diffusion coefficient, $q_e$ is the concentration of exchanged ions in equilibrium with the bulk solution, and r is the resin bead radius.

The important operational differences between film diffusion controlled and pore diffusion controlled processes are listed below (Weber, 1972; Helfferich, 1962).

a) Flow rate and/or stirring: change in flow rate or stirring does not affect pore diffusion, while increasing the turbulence of the solution by increasing the flow rate and/or stirring increases the rate of exchange in a film diffusion controlled system.

b) Resin particle diameter: in a film diffusion controlled system, increasing the particle size decreases the rate of exchange, while in a pore diffusion controlled system reduction of exchange rate with increase of particle diameter has a larger effect.

c) Concentration in the bulk liquid: at high concentrations, pore diffusion is more important than film-diffusion, while at low concentrations, film diffusion is more predominant than pore diffusion.

d) Cross-linking degree: change in the cross-linking degree has more effect on pore diffusion systems than film diffusion processes.

## 2.5. Biological Reduction of Perchlorate

### 2.5.1. Perchlorate Biological Reduction Pathway

Perchlorate biodegradation by bacteria has been well-studied and documented. Perchlorate can be used as an electron acceptor by perchlorate-reducing bacteria under anaerobic conditions. In this process, bacteria use organic compounds (such as acetate, lactate, ethanol and methanol) or hydrogen as electron donors (Wallace et al., 1996, Xu et al., 2003, Logan and LaPoint, 2002). In perchlorate degradation via heterotrophic growth, organic compounds can serve as both energy and carbon source for bacteria. In autotrophic growth, hydrogen is used as a source of energy and carbon dioxide is used as carbon source. In order to sustain microbial growth, trace amount of nutrients and

minerals also are required. Fortunately, perchlorate-reducing bacteria have been found in several different environments. The ubiquity of perchlorate-reducing bacteria in the environment makes perchlorate reduction possible (Kim and Logan, 2000; Logan, 1998; Logan et al., 2001; Rikken et al., 1996; Wu et al., 2001; Kesterson et al., 2005). Reactions 2.16 and 2.17 show the general pathways of heterotrophic and autotrophic perchlorate biodegradation, respectively.

$$ClO_4^- + \underset{\text{(electron donor \& carbon source)}}{\text{Organic molecule}} \xrightarrow{\text{Enzymes}} Cl^- + CO_2 + \text{Biomass} \quad (2.16)$$

$$ClO_4^- + \underset{\text{(electron donor)}}{H_2\ \text{gas}} + \underset{\text{(carbon source)}}{CO_2} \xrightarrow{\text{Enzymes}} Cl^- + H_2O + \text{Biomass} \quad (2.17)$$

Although Korenkov et al. (1976), Attaway and Smith (1993), Stepanyuk et al. (1993), and Malmqvist et al. (1994) have reported some bacterial strains capable to reduce perchlorate to chloride, they did not explain the degradation pathway. In 1996, Rikken et al. characterized strain GR-1 microorganism (*Dechlorosoma* sp. GR-1) from activated sludge biosolids, and they hypothesized that the perchlorate degradation pathway goes from perchlorate ($ClO_4^-$) to chlorate ($ClO_3^-$), then to chlorite ($ClO_2^-$), and finally through hypochlorite ($OCl^-$) to chloride ($Cl^-$). In Rikken's study, perchlorate and chlorate reduction were only observed in the absence of oxygen, but the conversion of chlorite to chloride took place under both aerobic and anaerobic environments. Observing the conversion of chlorite under both aerobic and anaerobic environments, Rikken et al. hypothesized that chlorite disproportionates to oxygen and chloride ion. The reduction of

perchlorate and the production of stoichiometric amounts of chloride showed that there was no accumulation of intermediate reaction products such as chlorite.

Rikken et al. (1996) also observed that the transformation of chlorite to oxygen and chloride is a non-energy yielding process because their measurements showed that the transformation of chlorite is not dependent on the presence of acetate as energy source (i.e. chlorite is reduced to molecular oxygen and chloride directly). In their experiments, Rikken et al. (1996) observed the transformation of chlorite to oxygen and chloride in washed cell suspensions without the addition of any reductive substrates. Van Ginkel et al. (1996) proved that oxygen is generated in the last step of biodegradation and it does not accumulate in the bioreactor.

Based on their findings and the knowledge from previous studies, Rikken et al. (1996) developed the enzymatic pathway for perchlorate reduction depicted in Figure 2.2. In this process, first, perchlorate ($ClO_4^-$) is reduced to chlorate ($ClO_3^-$) and then chlorate ($ClO_3^-$) is converted to chlorite ($ClO_2^-$) via two energy-yielding enzymatic reactions. Next, chlorite ($ClO_2^-$) is reduced to chloride ($Cl^-$) via a non-energy yielding reaction, and finally, oxygen molecule produced from dismutation of chlorite ($ClO_2^-$) is reduced to water via an energy yielding process.

Wallace et al. (1996) have tested the accumulation of intermediates in a bioreactor containing *Wolinella succinogenes* HAP-1 strain, and they found that the reduction of chlorite to chloride is about 1000 times faster than the reduction of perchlorate to chlorite. Using this founding and performing some more experiments, Herman and Frankenberger (1998) concluded that intermediates do not accumulate in the reactor and do not reach toxic levels (Herman and Frankenberger, 1998).

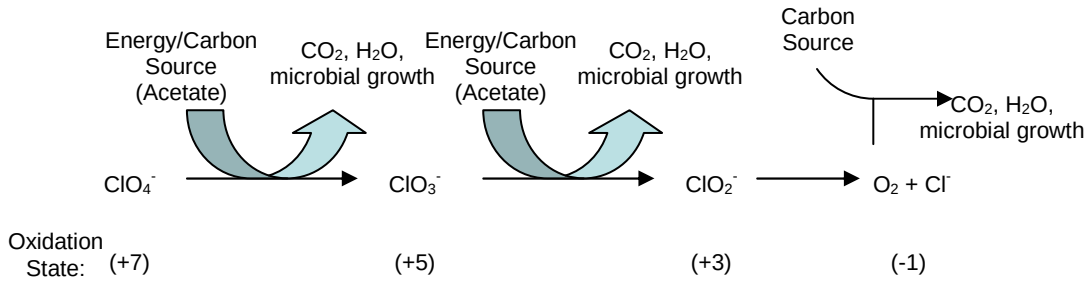


Figure 2.2. The Pathway of Perchlorate Reduction Goes from Perchlorate ($ClO_4^-$) to Chloride ($Cl^-$) (Modified from: Rikken et al., 1996; Logan et al., 2001)

### 2.5.2. Biochemistry of the Perchlorate Reduction

There is very little known about the biochemistry of the bioreduction pathway of perchlorate in the environment. However, studies have revealed some important information about the processes involved in the reduction pathway. Studies have confirmed that there is involvement of c-type cytochrome(s) in the perchlorate reduction pathway (Coates et al., 1999). *C*-type cytochromes are redox active compounds that are commonly involved in respiratory electron transfer chains of various organisms (Coates and Achenbach, 2004). Coates et al. (1999) performed an experiment that used measurement of light absorbance to differentiate between oxidized c-type cytochromes and reduced *c*-type cytochromes. The spectra measurement results show that $H_2$ reduced cytochrome *c* from different perchlorate reducing bacteria willingly reoxidized when perchlorate or chlorate were introduced to the environment, while, it was unaffected by other electron acceptors (i.e. Fe (III), fumarate or sulfate). Bender et al. (2005) presented a model for electron transfer during perchlorate reduction. According to this model, the cytochrome links the periplasmic reductase to the membrane quinone pool, which is the

electron source to reduce perchlorate (Bender et al., 2005). These results indicate that *c*-type cytochrome(s) are specifically involved in the electron transfer of perchlorate reducing bacteria (Coates and Achenbach, 2004; Bender et al., 2005).

### 2.5.3. Enzymes Involved in the Perchlorate Reduction Pathway

The pathway of perchlorate reduction from perchlorate ($ClO_4^-$) to chloride ($Cl^-$) has three main steps (Rikken et al., 1996; Logan et al., 2001), involving perchlorate reductase and chlorite dismutase:

#### *2.5.3.1. Perchlorate Reductase*

Studies show that all perchlorate reducers are able to reduce chlorate as well. This indicates that the reduction pathway for both perchlorate and chlorate ions are identical (Figure 2.3), and the same enzyme (i.e. perchlorate reductase) is responsible for reduction of perchlorate to chlorate and chlorate to chlorite (Kengen et al. 1999). Purified perchlorate reductase can catalyze both reduction processes at the same rate (Kengen et al. 1999). However, there are some species (e.g. members in the genera *Proteus*, *Pseudomonas*, and *Rhodobacter*) that are capable of reducing chlorate and cannot reduce perchlorate (Steinberg et al., 2005).

There are some similarities between perchlorate reductase and other reductases. Perchlorate reductase isolated from strains GR-1 (Kengen et al. 1999), perc1ace (Giblin and Frankenberger, 2001), and chlorate reductase from *Pseudomonas chloritidismutans* strain AW-1 (Wolterink et al., 2003) all have some resemblance to selenate reductase isolated from *Thauera selenatis* and other reductases, heme-proteins, and dehydrogenases (Steinberg et al., 2005). Perchlorate reductase contains selenium (Kengen et al. 1999),

and similarly, formate dehydrogenases extracted from *enterobacteria* or *clostridia* contain selenium (Heider and Bock, 1993).

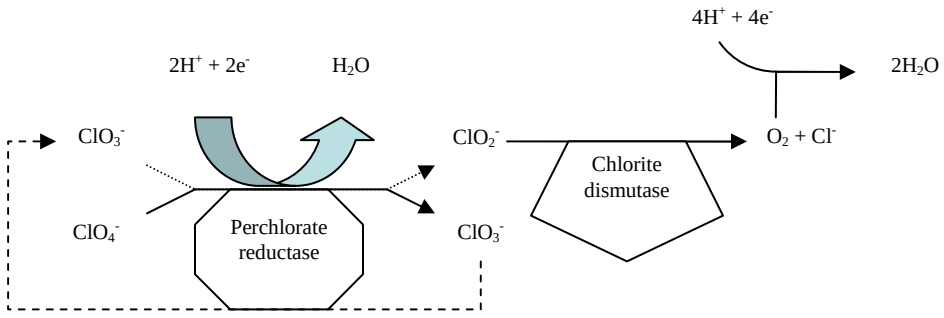


Figure 2.3. The Enzymatic Pathway of Perchlorate Reduction Showing Competition of Chlorate with Perchlorate for Perchlorate Reductase (Modified from: Dudley et al., 2008)

The reduction pathway from perchlorate to chlorate by perchlorate reductase is shown in Figure 2.3 (Dudley et al., 2008). In this pathway the resulting chlorate competes with perchlorate for the active sites of perchlorate reductase molecules. Thus, this competition may slow down the overall pathway. It is likely that increasing the concentration of either perchlorate or chlorate anion results in faster reduction of the anion of which the concentration was increased (Nerenberg et al., 2006; Dudley et al., 2008).

Some species have been identified that accumulate chlorate in the bioreactor (Nerenberg et al., 2002; Dudley et al., 2008). *Dechlorosoma* sp. HCAP-C, also known as *Dechlorosoma* sp. PCC, is a perchlorate reducing bacteria that has been shown to accumulate chlorate. It is reported that *Dechlorosoma* sp. HCAP-C accumulates about 20 percent of the initial perchlorate concentration on a weight basis (Dudley et al., 2008).

In order to study the enzymes involved in the perchlorate reduction pathway, the capacity of different perchlorate-reducing bacteria strains to reduce other potential terminal electron acceptors (i.e. nitrate and oxygen) has been studied. Strain GR-1 and isolate *Ideonella dechloratans* reduce nitrate to nitrogen gas (Rikken et al., 1996; Malmqvist et al., 1994). *Wolinella succinogenes* HAP-1 is able to reduce nitrate only to nitrite (Wallace et al. 1996). But, strain CKB is not capable to reduce nitrate. Simply, it can be suggested that the enzymes responsible for perchlorate and nitrate reduction pathways are more likely independent (Bruce et al., 1999). Additionally, it has been reported that strain perc1ace concurrently reduces perchlorate and nitrate, with no considerable change in utilization rates (Giblin and Frankenberger, 2001). Thus, it can be hypothesized that perchlorate reductase and nitrate reductase are two distinct enzymes involved in the perchlorate and nitrate reduction pathways (Bruce et al., 1999; Giblin and Frankenberger, 2001).

Perchlorate reductase in perchlorate-reducing bacteria, similar to chlorate reductase in chlorate-reducing species, is an oxygen-sensitive enzyme (Kengen et al. 1999, Steinberg et al., 2005). *Azospira* sp. KJ culture loses its ability to reduce perchlorate after 12 hours exposure to air, although the bacteria are still viable (Song and Logan, 2004). Furthermore, extracts from KJ strain have been reported to lose 70% of perchlorate reductase activity and 50% of chlorate reductase activity after 3 days air exposure (Steinberg et al., 2005). However, *Pseudomonas* sp. PDA, a chlorate-respiring bacterium, is able to respire using chlorate under aerobic conditions (Steinberg et al., 2005).

The location of perchlorate reductase has been studied by comparing the results of measurement of the enzyme activity in the whole cell and after lysozyme treatment. Lysosome treatment is a method to separate the periplasmic contents from the cytoplasm and membrane fractions. The results show release of almost all the activity after lysozyme treatment. Thus, it indicates that perchlorate reductase is located in the periplasmic area of the cell (Kengen et al., 1999). It is not apparent how perchlorate reductase is coupled to the membrane while it is soluble in a way that energy is preserved (Kengen et al., 1999). It is expected that perchlorate reductase is a part of the electron transport chain; however, it is located in the periplasmic area and it is soluble (Kengen et al., 1999; Steinberg et al., 2005). In the case of nitrate reductases, soluble periplasmic enzymes have been detected, too, although, most of the nitrate reductases are bound to the inner side of the membrane (Hochstein and Tomlinson, 1988). In contrast, chlorate reductase C from *Proteus mirabilis* and nitrate reductase A, which acts also as chlorate reductase, are cytoplasmic membrane bounded (Steinberg et al., 2005; Oltmann el al., 1976). Chlorate reductase produced by *P. chloritidismutans* has been found in the cytoplasm of the cell (Wolterink et al., 2003). Giblin and Frankenberger (2001) reported that in nitrate grown perchlorate-reducing bacteria, nitrate reductase associated with the membrane/cytoplasmic fraction (not periplasmic fraction) of the lysozyme treated cells has some perchlorate reductase activity. Kengen et al. (1999) also had similar observation about nitrate, bromate, and iodate reduction by perchlorate reductase enzyme. Additionally, Herman and Frankenberger (1998) observed a slight decrease in the perchlorate reduction rate when nitrate was added to the reactor, supporting the idea that perchlorate reductase enzyme is also able to reduce nitrate.

Using the above mentioned observations, it can be concluded that separate reductases are involved in perchlorate-reducing bacteria capable to reduce perchlorate and nitrate, but, both reductases have some ability to reduce other terminal electron acceptor (Giblin and Frankenberger, 2001).

Kinetics of the perchlorate reductase enzymatic activity has been studied and reported. Kengen et al. (1999) reported the half saturation constant ($K_m$) and maximum activity ($V_{max}$) values for perchlorate reductase as 27 µM (2.69 mg $ClO_4^-$/L) and 3.8 U mg protein$^{-1}$ for GR-1 strain, respectively, while 34.5 µM (3.43 mg $ClO_4^-$/L) and 4.8 U mg protein$^{-1}$ have been measured by Okeke and Frankenberger (2003) as the values for $K_m$ and $V_{max}$ for perc1ace strain. Considering the affinity for perchlorate, it seems that the reported $K_m$ values are similar; however, the $V_{max}$ value for perclace strain is slightly higher than that of GR-1 strain (Okeke and Frankenberger, 2003).

Optimum pH and temperature for activity of perchlorate reductase has been studied and reported. Okeke and Frankenberger (2003) reported the optimum temperature of 25ºC to 30ºC with activity range between 20ºC to 40ºC. Okeke and Frankenberger (2003) also reported the optimum pH values of 7.5 to 8.0, while, optima pH for perchlorate and nitrate reductions have been measured and reported by Giblin and Frankenberger (2001) as 8.0 and 9.0, respectively.

*2.5.3.2. Chlorite Dismutase*

The last step in Figure 2.2 (i.e. from chlorite to chloride) is catalyzed by the enzyme chlorite dismutase (van Ginkel et al., 1996). Chlorite dismutase, which is a red-colored enzyme, is a homotetramer with biotechnological and bioremediative application (Streit and DuBois, 2008). Chlorite dismutase dismutases harmful chlorite into harmless

chloride and oxygen. The bioreaction is called dismutation, because chloride and oxygen have been reduced and oxidized at the same time, respectively.

The dismutation process is a non-energy yielding process because measurements show that the conversion is not dependent on the presence of an energy source. It means that chlorite is transformed to the products directly (Rikken et al. 1996).

Chlorite dismutase is a remarkable heme-containing enzyme. First, it decomposes chlorite into chloride and oxygen preventing accumulation of chlorite to toxic levels. Second, it is the only known enzyme for O-O bound formation except for the photosystem II enzymes (Lee et al, 2008; Streit and DuBois, 2008). Third, it produces oxygen that is a negative inhibitor of the perchlorate bioreduction process (Lee et al, 2008).

The production of oxygen by chlorite dismutase does not inhibit the process; however, chloride ($Cl^-$) is a mixed inhibitor with low binding affinity to both free enzyme and enzyme-substrate complex in the enzymatic process (Streit and DuBois, 2008). Chloride irreversibly inactivates the chloride dismutase enzyme after approximately $1.7\times10^4$ turnovers (per heme). The half-life of the enzyme has been measured at about 0.39 min, due to the bleaching of heme chromophore (Streit and DuBois, 2008).

Similar to perchlorate-reducing bacteria, denitrifiers also are able to reduce chlorate to chlorite using nitrate reductase enzyme, but since they are unable to reduce chlorite to chloride due to lack of chlorite dismutase, the toxicity of the environment because of chlorite accumulation would kill the denitrifies (Oltmann et al., 1976). Chlorite dismutase has no function in the nitrate-reduction pathway from nitrate to ammonium and nitrogen gas (Streit and DuBois, 2008). Thus, chlorite dismutase is an enzyme only

presented in chlorate-reducing and perchlorate-reducing bacteria (Xu and Logan, 2003). In contrast with perchlorate reductase, which is able to reduce chlorate, nitrate, iodate and bromate, chlorite dismutase is highly specific for chlorite and alternative analogous anions cannot be served as substrate for this enzyme (Coates and Achenbach, 2004; Coates et al., 1999).

Chlorite dismutase activities have been measured in nitrate-grown perchlorate-reducing bacteria (Xu and Logan, 2003). These basal chlorite dismutase activities, which have been detected in all tests, even under nitrate-reducing condition, may be essential for perchlorate-reducing bacteria survival (Xu and Logan, 2003).

Chlorite dismutase location in the cell structure has been studied (Kengen et al., 1999). Alike perchlorate reductase, chlorite dismutase is located in periplasmic area of the cell due to high enzymatic activity in the whole cell and release of nearly all the activity after lysozyme treatment (Kengen et al., 1999).

Kinetics of the chlorite dimutase enzymatic activity have been studied and reported. $V_{max}$ and $K_m$ values for chlorite dismutase have been measured and reported for perchlorate-reducing bacteria. $V_{max}$ and $K_m$ for chlorite dismutase have been reported as 2,200 U mg protein$^{-1}$ and 170 µM (11.47 mg $ClO_2^-$/L), respectively, for GR-1 strains (van Ginkel et al., 1996). Although, the $K_m$ value for chlorite dismutase is higher than the $K_m$ value for perchlorate reductase, which has been reported as 27 µM (2.69 mg $ClO_4^-$/L) for strain GR-1 (Kengen et al., 1999), the $V_{max}$ value for chlorite dismutase is three orders of magnitude larger than the $V_{max}$ value for perchlorate reductase, which has been measured as 3.8 U mg protein$^{-1}$ for GR-1 strain (Kengen et al., 1999). This shows that although the

half saturation constant for chlorate reductase is relatively higher, the rate of activity for perchlorate reductase enzyme governs the entire reduction process.

Optimum pH and temperature for activity of chlorite dismutase has been studied, as well. van Ginkel et al. (1996) reported that the optimum pH for purified chlorite dismutase is narrow with maximum activity at pH 6.0. However, Xu and Logan (2003) reported the maximum pH for chlorite dismutase activity for the cell suspension at pH 6.0, but, their pH profile is not as narrow as van Ginkel et al. (1996) profile. This finding may show the ability of the cells to buffer external pH changes (Xu and Logan, 2003). Chlorite dismutase is most active at the temperature of 30ºC (van Ginkel et al., 1996), while the chemical reduction of chlorite occurs at a temperature above 200ºC (Taylor et al., 1940).

### 2.5.4. Microorganism Involved in Perchlorate Biodegradation

More than 70 years ago, Aslander (1928) reported that oxyanions of chlorine (such as chlorate and perchlorate) can be biologically reduced under anaerobic conditions. Some unknown soil microorganisms were assumed to be responsible for rapid reduction of chlorate that was used as a herbicide for Canada thistle (Aslander, 1928). First, it was hypothesized that chlorate reduction is performed by nitrate reducing bacteria in nature (de Groot and Stouthamer, 1969). Additional investigations showed that membrane-bound respiratory nitrate reductases and assimilatory nitrate reductases are enzymes that can reduce chlorate as an alternative to nitrate (Rikken et al., 1996; Malmqvist et al., 1994). Although nitrate reducing bacteria are capable of reducing perchlorate or chlorate to chlorite, there is no evidence that these bacteria will have sustainable growth if they

use perchlorate (Oltmann et al., 1976; Coates and Achenbach, 2004). This is because of accumulation of chlorite in the environment due to chlorite dismutase deficiency.

Several dissimilatory perchlorate/chlorate reducing bacteria have been identified, isolated, and grown as a pure culture. To date, the number of perchlorate reducing bacteria that exists in pure culture is well above 50, and it is speedily increasing (Coates and Achenbach, 2004; Nirmala and Jae-Ho, 2008). Some of the strains that have been identified and studied are discussed in this section.

Perchlorate reducing bacteria are found in different environments, including perchlorate/chlorate contaminated and non-contaminated soils and sediments. Even soil samples collected from Antarctica contain perchlorate reducing bacteria (Bender et al., 2004). The reason why perchlorate reducing bacteria occurs in different environments could be the diversity in metabolism pathways of the bacteria. Studies indicated that perchlorate reducing bacteria have diverse metabolism pathways which use different substrates (Coates et al., 1999). So far, all of the studied perchlorate reducing bacteria are facultative anaerobic or microaerophilic. This is reasonable because oxygen is generated as a transient intermediate of the perchlorate/chlorate reduction pathway (van Ginkel et al., 1996). All of the known perchlorate reducing bacteria are able to respire using chlorate. However, there are some chlorate reducing bacteria that are not able to use perchlorate as the electron acceptor source (Xu et al., 2004). Some of the perchlorate reducing bacteria are able to respire using nitrate (Bruce et al., 1999). Almost all of them prefer using nitrate to perchlorate. However, Nirmala and Jae-Ho (2008) reported that isolate *Dechlorospirillum anomalous* prefers perchlorate to nitrate. There are diverse

electron acceptors and electron donors can be utilized by perchlorate reducing bacterial strains (Xu et al., 2004).

The perchlorate reducing bacteria that are isolated so far are all members of four subclasses (i.e. α, β, γ and ε) of the total five subclasses of *Proteobacteria* (Coates et al., 1999; Kesterson et al., 2005). *Proteobacteria* is an important group of bacteria. This group includes several pathogens such as *Salmonella* and *Vibrio* (Madigan and Martinko, 2005). Nitrogen fixing bacteria are also members of this group. *Proteobacteria* are all gram-negative and their outer membrane is mostly composed of lipopolysaccharides. Most of them are motile using flagella, but some are non-motile (Madigan and Martinko, 2005). The *β-proteobacteria* sub-class consists of numerous bacteria that have high adaptability for degradation capacity (Madigan and Martinko, 2005). Some of the *Proteobacteria* genera (such as *Pseudomonas* and *Wolinella*) were studied previously for other purposes; however, their capability to respire using perchlorate has been recognized during the recent decade (Wallace et al., 1996).

Most of the known perchlorate reducing bacteria are closely related to each other (Coates and Achenbach, 2004). However, their known close relatives, such as *Rhodocyclus tenuis* and *Ferribacterium limneticum*, based on 16S rDNA sequence similarity, do not respire using perchlorate (Coates and Achenbach, 2004); therefore, phylogenetic relatedness alone cannot guarantee any conclusion on metabolic functionality of these bacteria. Even with 99% similarity in 16S rDNA sequence and only 1% divergence, some of the perchlorate reducing bacteria relatives exhibit distinct physiologies. For example, *Ferribacterium limneticum* is an obligate anaerobic, non-fermenting, dissimilatory Fe(III) reducer; and *Rhodocyclus tenuis* is a phototrophic, non-

sulfur, purple bacteria containing bacteriochlorophyll (Lee at al., 2002). Thus far, there is no perchlorate reducing bacteria that can grow either by Fe(III) reduction or phototrophy (Coates and Achenbach, 2004).

Perchlorate reducing bacteria in the environment are mostly members of $\beta$ subdivision of *proteobacteria*, consisting of two novel genera with monophyletic origin, which are *Dechloromonas* and *Azospira* (formerly called *Dechlorosoma*) (Achenbach et al., 2001; Coates and Achenbach, 2004). Based on signature nucleotide sequence analysis of the 16S rRNA, *Dechloromonas* genus can be divided into CKB and RCB types (Coates and Achenbach, 2004). Perchlorate reducing bacteria from *Dechloromonas* and *Azospira* genera are ubiquitous and have been found in almost all environmental samples that have been screened (Coates et al., 1999; Bender et al., 2004). Other species that can usually be found in contaminated sites and are common in the groundwater-treating bioreactors are *Dechlorospirillum* species (Coates and Achenbach, 2004). *Dechlorospirillum* species belongs to α subdivision of *proteobacteria*.

Chlorate reducing bacterial species, which utilize chlorate using chlorate reductase, are mainly members of γ subclass of *proteobacteria.* These members of *proteobacteria* have some similarities to *Escherichia coli* genus (Achenbach et al., 2001).

### 2.5.5. Thermodynamics, Kinetics and Stoichiometry of Perchlorate Reduction

#### *2.5.5.1. Thermodynamics of Perchlorate Reduction Pathway*

From a thermodynamic perspective, the standard reduction potentials for the half reactions from perchlorate to chloride and perchlorate to chlorate have been investigated and reported as reactions 2.18 and 2.20 as shown below (Gurol and Kim, 2000):

$$ClO_4^- + H_2O + 2e^- \rightarrow ClO_3^- + 2OH^- \qquad E^0 = 0.360\ V \qquad (2.18)$$

$$ClO_4^- + 2H^+ + 2e^- \rightarrow ClO_3^- + H_2O \qquad E^0 = 1.189\ V \qquad (2.19)$$

$ClO_4^- + 8H^+ + 8e^- \rightarrow Cl^- + 4H_2O$ $E^0 = 1.389$ V (2.20)

The principle of Le Chatelier can clarify the difference between $E^0$ of the reactions 2.18 and 2.19. In reaction 2.19, due to the presence of 1.0 M of $H^+$ in the left hand side, the pH of the environment would be 0.0. Thus $H^+$ acts as the reactant, and the reaction is driven to the right. In contrast, in reaction 2.18, since the $OH^-$ ion is the product, it reduces the driving force of the reaction to take place.

The standard enthalpy of formation ($\Delta H^0_f$) of perchlorate in dilute aqueous solution at 25°C is -30 ± 0.07 kcal/mole (Matyushin et al., 1985), which is equivalent to -125.574 ± 0.293 kJ/mole. Sawyer et al. (2002) reported -129.3 kJ/mole, -99.2 kJ/mole, -66.5 kJ/mole, and -167.20 kJ/mol as standard enthalpy of formation for perchlorate, chlorate, chlorite, and chloride, respectively. From a thermodynamic point of view, negative standard enthalpy of formation shows that the perchlorate formation occurs spontaneously.

The standard Gibbs free energy of formation ($\Delta G^0_f$) for perchlorate and chloride have been reported as -8.5 kJ/mol and -8.0 kJ/mole, respectively. Similarly, Sawyer et al. (2002) reported -8.60 kJ/mol, -3.40 kJ/mol, 17.10 kJ/mol, and -131.30 kJ/mol as the Gibbs free energy of formation for perchlorate, chlorate, chlorite, and chloride, respectively.

$\Delta G'_s$ of perchlorate reduction reaction and related other competitive reactions (i.e. nitrate and oxygen) have been reported in the literature. Reaction 2.21 through 2.30 in Table 2.6 shows some of the $\Delta G'_s$.

The large negative value of $\Delta G^0$ from perchlorate to chloride (reaction 2.24) shows that the reaction is very favorable. However, this is not observed because the reaction is kinetically controlled by a large initial activation energy. Presence of catalyst, enzyme,

heat or light is needed to be able to reduce perchlorate (Gurol and Kim, 2000). Different reductants can be employed to reduce perchlorate, and depending on the reductant, the final product would be different, and can be either chlorate or chloride (Urbansky, 1998). In order to reduce perchlorate to chlorate, ruthenium(II) can be used while vanadium(III), vanadium(II), molybdenum(III), dimolybdenum(III), chromium(II), and titanium(III) can reduce perchlorate to chloride (Urbansky, 1998).

Table 2.6. Stoichiometric Reactions of Perchlorate Reduction and Other Competitive Reactions

| Reaction | $\Delta G^{0'}$ | Reference | Reaction |
|---|---|---|---|
| $ClO_4^- + H_2O + 2\ e^- \rightarrow ClO_3^- + 2\ OH^-$ | -69.59 (kJ/mol) | Gurol and Kim, 2000 | (2.21) |
| $ClO_4^- + 2\ H^+ + 2\ e^- \rightarrow ClO_3^- + H_2O$ | -229.77 (kJ/mol) | Gurol and Kim, 2000 | (2.22) |
| $ClO_4^- + 4\ H^+ + 4\ e^- \rightarrow Cl^- + 2\ H_2O + O_2$ | -437.60 (kJ/mol) | Shrout and Parkin, 2006 | (2.23) |
| $ClO_4^- + 8\ H^+ + 8\ e^- \rightarrow Cl^- + 4\ H_2O$ | -752.48 (kJ/mol) | Shrout and Parkin, 2006 | (2.24) |
| $ClO_4^- + CH_3COO^- \rightarrow Cl^- + H^+ + 2HCO_3^-$ | -966 (kJ/molacetate) | Rikken et al., 1996 | (2.25) |
| $4/3\ ClO_3^- + CH_3COO^- \rightarrow 4/3\ Cl^- + H^+ + 2\ HCO_3^-$ | -1015 (kJ/molacetate) | Rikken et al., 1996 | (2.26) |
| $ClO_4^- + ½\ CH_3COO^- \rightarrow ClO_2^- + ½\ H^+ + HCO_3^-$ | -801 (kJ/molacetate) | Rikken et al., 1996 | (2.27) |
| $O_2 + ½\ CH_3COO^- \rightarrow ½\ H^+ + HCO_3^-$ | -844 (kJ/molacetate) | Rikken et al., 1996 | (2.28) |
| $ClO_2^- \rightarrow O_2 + Cl^-$ | N/Aa | Rikken et al., 1996 | (2.29) |
| $3/5\ NO_3^- + CH_3COO^- + 13/5\ H^+ \rightarrow 4/5\ N_2 + 4/5\ H_2O + 2\ HCO_3^-$ | -792 (kJ/molacetate) | Rikken et al., 1996 | (2.30) |

[a] The dismutation of chlorite ion dose not produce energy for biosynthesis.

To have complete perchlorate reduction to chloride and water, eight electrons are needed. Comparing reactions perchlorate/chloride + oxygen and perchlorate/chloride + water, clearly indicates that four electrons are required to reach from perchlorate to chloride and oxygen; and another four electrons provided by the electron donor are used to reduce the produced oxygen to water (Rikken et al., 1996).

Comparing the $\Delta G^0$'s for the utilization of acetate as electron donor with oxygen, nitrate, and perchlorate (Table 2.6) it can be concluded that oxygen and nitrate are preferred electron acceptors over perchlorate (Rikken et al., 1996). Although there are some exceptions (Nirmala and Jae-Ho, 2008), laboratory experiments show that most of the perchlorate reducing bacteria prefer nitrate to perchlorate. This preference is supported by the Gibbs free energies shown in Table 2.6. During the bioreduction process, first perchlorate is reduced to chlorite and then chlorite is dismutated to chloride and oxygen through a non-energy yielding mechanism (Kengen et al., 1999). The energy produced from reduction of perchlorate to chlorite ($\Delta G_0^{'}$= -801 kJ/mole acetate) is significantly lower that the energy produced by utilization of oxygen as electron acceptor ($\Delta G_0^{'}$= -844 kJ/mole acetate). This shows the thermodynamically preference of oxygen utilization over perchlorate. However, the energy production from perchlorate reduction through chlorite dismutation ($\Delta G_0^{'}$= -801 kJ/mole acetate) has a narrow difference with energy produced from nitrate reduction ($\Delta G_0^{'}$= -792 kJ/mole acetate), nitrate is still thermodynamically more attractive than perchlorate for the bacteria (Nirmala and Jae-Ho, 2008). Accordingly, metabolic reduction of oxygen and nitrate are thermodynamically dominant over perchlorate (Figure 2.4).

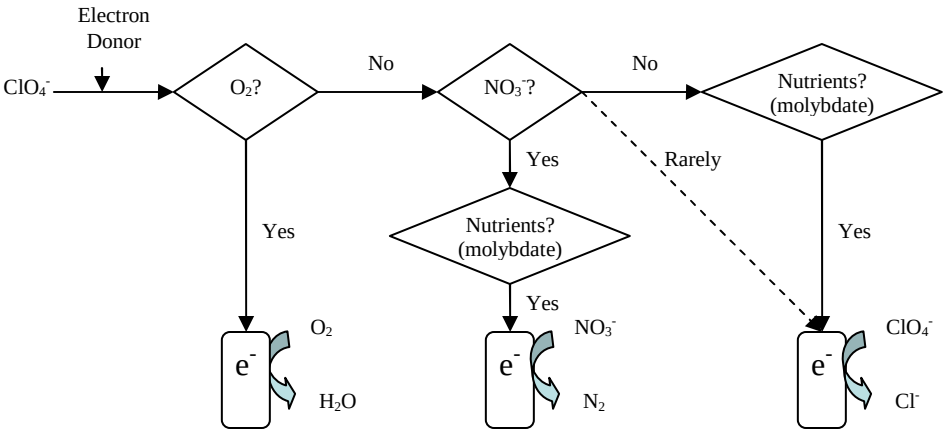


Figure 2.4. Electron Acceptor Preference for Perchlorate Reducing Bacteria (Modified from Coates and Achenbach, 2004)

*2.5.5.2. Kinetics of Perchlorate Reduction Pathway*

In order to be able to model perchlorate reducing bioreactors, kinetic parameters of different perchlorate reducing bacteria have been investigated and reported. Kinetic parameters for some of the perchlorate reducing bacteria that have been studied to date are listed in Table 2.7.

The kinetic parameters listed in Table 2.7, include the maximum substrate utilization rate ($q_{max}$), half saturation constant ($K_S$), biomass yield (Y), and maximum growth rates ($\mu_{max}$). The $q_{max}$ ranges from 6.0 $mgClO_4^-$/mgDW-day to 1.68 $mgClO_4^-$/mgDW-day when the electron acceptor is perchlorate. It means that each gram of dried biomass (DW) can utilize a maximum 6.0 grams of perchlorate per day. In the presence of chlorate, $q_{max}$ has slightly greater value. Observed maximum growth rates ($\mu_{max}$) for perchlorate have been reported to be in the range of 0.067 $h^{-1}$ to 0.24 $h^{-1}$.

Table 2.7. Reported Kinetic Parameters for Perchlorate Reducing Bacteria*

| Pure or mixed culture | $q_{max}$ ($mgClO_4^-$ /mgDW-day) | $K_S$ (mg/L) | Electron Donor | Electron Acceptor | $\mu_{max}$ (1/h) | Y ($g/g_{donor}$) | Reference |
|---|---|---|---|---|---|---|---|
| Vibrio dechloratans | 1.68 | NA | acetate | perchlorate | NA | NA | Korenkov et al. (1976) |
| Wolinella succcinogenes HAP-1 | 2.57 | NA | acetate | perchlorate | NA | NA | Wallace et al. (1996) |
| GR-1 | 5.65 | NA | acetate | perchlorate | 0.1 | 0.24 | Rikken et al. (1996) |
| KJ | 1.32 | 33 | acetate | perchlorate | 0.2 | 0.5 | Logan et al. (2001) |
| PDX | 0.41 | 12 | acetate | perchlorate | 0.24 | NA | Logan et al. (2001) |
| HCAP-C | 4.4 | 76.6 | hydrogen | perchlorate | NA | 0.41 $g/g_{ClO4}$ | Dudley et al. (2008) |
| PC1 | 3.1 | 0.14 | hydrogen | perchlorate | NA | 0.23 $g/g_{ClO4}$ | Nerenberg et al. (2006) |
| RC1 | 6.00 | 12 | acetate | perchlorate | 0.085 | 0.34 | Waller et al., (2004) |
| INS | 4.35 | 18 | acetate | perchlorate | 0.067 | 0.37 | Waller et al. (2004) |
| ABL1 | 5.43 | 4.8 | acetate | perchlorate | 0.086 | 0.38 | Waller et al., (2004) |
| SN1A | 4.60 | 2.2 | acetate | perchlorate | 0.069 | 0.36 | Waller et al., (2004) |
| Perc1ace | NA | NA | acetate | perchlorate | 0.07 | NA | Herman and Frankenberger (1998) |
| Mixed culture | 0.49 | <0.1 | acetate | perchlorate | 0.15 | 0.20 | Wang et al. (2008a) |
| GR-1 | 7.48 | NA | acetate | chlorate | NA | NA | Rikken et al. (1996) |
| PC1 | 6.3 | <0.014 | hydrogen | chlorate | NA | 0.22 $g/g_{ClO3}$ | Nerenberg et al. (2006) |
| HCAP-C | 8.3 | 58.3 | hydrogen | chlorate | NA | 0.34 $g/g_{ClO3}$ | Dudley et al. (2008) |

*all of the kinetic parameters are based on simple Monod, and competitive inhibition has been neglected.

As it is shown in Table 2.7, the half saturation constant ($K_s$) ranges from 0.14 mg/L to 76.6 mg/L. Interestingly, both high and low values for half saturation constants (0.14 mg/L to 76.6 mg/L) are for the strains that utilize hydrogen gas as their electron donor

(i.e. autotrophic growth). In the case of heterotrophic growths, although, Wang et al. (2008a) have reported the half saturation constant for a mixed culture as <0.1 mg/L, most of the studies that have been performed on pure cultures show the half saturation constants range from 2.2 mg/L to 33 mg/L. The perchlorate degradation process follows first-order kinetics under typical concentrations of perchlorate in the environment, which is in the part-per-billion range (Logan et al., 2001). Between the strains listed in Table 2.7, SNA1 and ABL1 are members of *alpha-proteobacteria* subclass, with the half saturation constants of 2.2 mg/L and 4.8 mg/L, which are comparatively lower that the constants found for the rest of the strains. These data may indicate that *Azospirillum*-type perchlorate reducing bacteria have a higher affinity to reduce perchlorate compared to the other strains due to the lower $K_S$ value (Waller et al., 2004).

Growth threshold concentration, $S_{min}$, has been calculated for some of the perchlorate reducing bacteria strains. Dudley et al. (2008) calculated the $S_{min}$ for the strain HCAP-C as 2.2 mg/L and concluded that in order to sustain perchlorate reducing bacteria in the system, alternative electron acceptors (i.e. oxygen and nitrate) are needed in low concentrations, when the perchlorate concentration is lower than $S_{min}$. Nerenberg et al. (2006) had the same conclusion for $S_{min}$ of 14 mg/L for strain PC1.

The decay constant (b) has not been studied as well as the other kinetic parameters. For an autotrophic growth, Nerenberg et al. (2006) reported the decay constant as 0.055/day. Wang et al. (2008a) reported similar value for decay constant as 0.05/day for a mixed culture grown under heterotrophic condition.

*2.5.5.3. Stoichiometry of Perchlorate Reduction*

The stoichiometry of overall reaction of perchlorate utilization can be calculated knowing the observed stoichiometry of moles electron donor per mole of electron acceptor (i.e. perchlorate). Studies have been performed and the stoichiometric ratio of electron donor to perchlorate has been published. Chaudhuri and Coates (2002) reported that pure culture of *Dechlorosoma suillum* strain PS utilizes 1.65 ± 0.24 mole of acetate per each mole of perchlorate. Therefore, the fraction of acetate (i.e. the fraction of eight electrons to reduce perchlorate) which was used to degrade perchlorate, $f_e$, was 1/1.65 or 0.61. Accordingly, the fraction of electrons for biomass synthesis was $f_s = 1\text{-}f_e = 0.39$ (Chaudhuri and Coates, 2002). Wang et al. (2008a) reported the cell yield of 4.7 g protein/mole acetate for strain JB116. They also reported 1.72 mole of acetate was utilized to reduce one mole of perchlorate. Thus, the $f_e$ and $f_s$ in their study would be 0.58 and 0.42, respectively. Consequently, the following overall reaction was reported (Wang et al., 2008a):

$$ClO_4^- + 1.72\ CH_3COO^- \rightarrow$$
$$Cl^- + 0.288\ C_5H_7O_2N + 0.28\ CO_2 + 1.14\ H_2O + 1.72\ HCO_3^- \quad (2.31)$$

Waller et al. (2004) reported the observed stoichiometry of 1.7 mole acetate/mole perchlorate. Accordingly, they calculated the $f_e$ and $f_s$ as 0.59 and 0.41, respectively, which corresponds to biomass yield of 0.31 g cells/g acetate through the following calculations (Rittman and McCarty, 2001; Waller et al., 2004).

$$\frac{0.41\,e^-eq\ cells}{e^-eq\ acetate} \times \frac{5.65\,g\ cells}{e^-eq\ cells} \times \frac{8\,e^-eq}{mole\ acetate} \times \frac{1\,mole\ acetate}{59\,g\ acetate} = 0.31 \frac{g\ cells}{g\ acetate} \quad (2.32)$$

The calculated yield based on observed acetate/perchlorate stoichiometry (0.31 g cells/g acetate) is similar to the measured yield which was 0.36 g cells/g acetate,

indicating the accuracy of the measurements. Waller et al. (2004) used this stoichiometry ratio to present the overall perchlorate reduction reaction using acetate as electron donor and ammonium as nitrogen source:

$$ClO_4^- + 1.7\ CH_3COO^- + 0.28\ NH_4^+ \rightarrow$$

$$Cl^- + 0.28\ C_5H_7O_2N + 0.58\ CO_2 + 1.42\ H_2O + 1.7\ HCO_3^- \quad (2.33)$$

In addition to the above mentioned studies that all have used acetate as the electron donor in pure cultures, Shrout and Parkin (2006) have used lactate as the electron donor in mixed culture. In their study, the approximate optimal lactate/perchlorate ratio was reported ~1.2 g COD/g $ClO_4^-$.

Consequently, according to the studies performed to date, it can be concluded that the stoichiometric ratio of acetate as the electron donor to perchlorate ranges from 1.65 to 1.72 mole acetate/mole perchlorate. It indicates that to degrade one mole of perchlorate at least 1.7 moles of acetate (equivalent to 1.08 g COD/g $ClO_4^-$) are needed.

### 2.5.6. The Controlling Parameters of Perchlorate Reduction

#### *2.5.6.1. Micronutrients*

Studies performed on perchlorate reducing bacteria pure cultures that are members of *Dechloromonas* and *Azospira* genera have demonstrated that these isolates can grow in a wide range of environmental conditions (Coates et al., 1999). Although most of the experimental studies on pure cultures of perchlorate reducing bacteria have been performed on media supplemented defined or undefined vitamin sources (e.g. biotin, folic acid, pyridoxine HCl, riboflavin, thiamine, nicotinic acid, pantothenic acid, vitamin B12, p-aminobenzoic acid, and thioctic acid), it has been proven that at least one strain, *Dechloromonas agitate*, does not require vitamin supplementation for its growth (Bruce

et al., 1999). Zhang et al. (2002) used yeast extract for the autotrophic strain HZ and found that yeast extract improved growth of the culture; however, it was not needed for growth. Both phosphate-buffer system and bicarbonate-buffer system have been used for growth of perchlorate reducing bacteria, but, there is no evidence that shows of the preference of one system to the other (Xu et al., 2003).

*Dechloromonas* and *Azospira* genera are normally not particular in their nutrition requirements, but Bender, et al. (2002) reported that perchlorate reductase requires molybdenum, and selenium as cofactors. Molecular genetic studies on some of the perchlorate reducing bacteria isolates indicate presence of molybdenum dependent genes encoding chlorite dismutase and perchlorate reductase (Chaudhuri et al., 2002). Kengen et al., (1999) reported 1 mole of heterodimeric molecules obtained from perchlorate reductase enzyme purified from strain GR-1 contain 1 mole of molybdenum, 11 moles of iron and 1 mole of selenium, indicating that trace amount of molybdenum, iron and selenium is important for perchlorate reducing bacteria. Studies showed that growth and perchlorate reduction of *Dechloromonas aromatica* were completely inhibited when the culture was transferred to molybdenum free medium. Also, similar inhibition was observed when the *Dechloromonas agitate* culture depleted the molybdenum content (Chaudhuri et al., 2002). Thus, it seems that molybdenum is a requirement for all perchlorate reducing bacteria. Bioavailability of molybdenum can be an important issue in the bioremediation implication strategies. Specially, in low-pH soils adsorption reduces the availability of molybdenum; therefore, it can be an often limiting nutrient in many soils (Chaudhuri et al., 2002).

*2.5.6.2. pH*

Although, some studies have reported the acidity range to have optimum growth and maximum perchlorate degradation rate, the effect of pH on biological perchlorate reduction has not been investigated systemically. Perchlorate degradation involves quite complex enzymatic activity (Rikken et al., 1996), that pH is potentially one of the effective environmental parameters affecting the activity of the involved enzymes. In general, acidity of the environment can affect the activity of an enzymatic system via three possible mechanisms: (i) pH variation can change the three dimensional shape of the enzyme; (ii) changes in pH of the environment can change the ionic form of basic and acidic groups of the active sites of the enzyme molecules; and (iii) pH variation can affect the acidic and basic groups on the substrate molecules and as a result the affinity of the substrate for the for the enzyme can be altered, potentially (Wang et al., 2008a).

Scientists have reported pH ranges and optimum pH for perchlorate reduction by different perchlorate reducing bacteria. pH ranges of 6.6-7.5 (optimum pH of 7.1) and 5.0-9.0 (optimum pH of 7.0) have been illustrated as the pH ranges that heterotrophic mixed cultures of perchlorate reducing bacteria are capable to reduce perchlorate (Attaway and Smith, 1993; Wang et al., 2008a). For heterotrophic pure culture of perc1ace, Coates and Achenbach (2004) have reported pH of 6.5-8.5 (optimum pH of 7.0-7.2) as the pH range that perchlorate reduction can occur. In addition to pH ranges for perchlorate reduction, appropriate acidity for bacteria growth also has been investigated and reported. Bruce et al. (1999) have showed the pH range of 6.5-8.0 (optimum pH of 7.1) for HAP-1 strain as the appropriate pH range for bacteria growth.

Similarly, pH range of 6.5-8.5 (optimum pH of 7.5) has been reported for strain CKB (Herman and Frankenberger, 1998).

It can be concluded that pH range of 6.5-8.0 is the appropriate pH range for perchlorate reducing bacteria for both perchlorate reduction and growth. In order to keep the pH in this pH range, in most of the studies either phosphate buffer system or bicarbonate-buffer system are used to control the acidity and keep it in the optimum range (Xu et al., 2003).

*2.5.6.3. Salinity*

The effect of salinity on conventional microbial systems used in wastewater treatment is well known (Lefebvre and Moletta, 2006). Inhibition of microbial processes is generally attributed to the osmotic pressure effects between inside and outside the cell wall (Lefebvre and Moletta, 2006).

Different bacterial genera have different tolerance levels for salinity. In general, the tolerance levels of the halophilic bacteria for salinity can be categorized into three classes: mild (10-60 g NaCl/L), moderate (60-150 g NaCl/L) and extreme or extremophiles (150-300 g NaCl/L) (Madigan and Martinko, 2005). In addition to the difference tolerance levels between different microorganisms, halotolerance is also linked to the growth phase of the culture. The very old or very young bacteria are more vulnerable in saline environments (McAdam and Judd, 2008; Cang et al. 2004).

Because of the high affinity of ion exchange resins, which are developed to remove perchlorate from water, to perchlorate ion, very strong NaCl solutions (70-120 g NaCl/L) are needed to regenerate the regenerable resins. Thus, perchlorate contamination occurs along high salinity in the regenerant wastes (Logan et al., 2001). The perchlorate

contamination level in the 7% regenerant waste ranges from 2000 to 500,000 µg/L, depending on the ion exchange influent perchlorate concentrations (Gingras and Batista, 2002).

Halophilic bacteria have been obtained by screening various sites and used in order to degrade perchlorate at relatively high levels of salinity. It has been found that not all locations contain halophilic bacteria capable to reduce perchlorate at salinity levels of 3% and higher (Logan et al., 2001). Logan et al. (2001) showed that perchlorate reduction occurred only in three of the total six samples collected from saline environments at salinity of 3%. Although, perchlorate reduction rates have not been reported, growth rates were measured while the salinity ranges from 1% to 15%. The maximum growth rate reported as 0.060±0.003/day for the sample obtained from Great Salt Lake, UT, at a salinity level of 5% (Logan et al., 2001). Growth rate reduced about 40% when the salinity was increased to 11%, and the culture did not have any growth at salinity of 13% and higher (Logan et al., 2001).

In addition to the growth rates, Gingras and Batista (2002) reported the reduction rates on a non-halophilic perchlorate reducing culture enriched from an activated sludge sample. It has been reported that salinity level as low as 0.5% decreased the perchlorate reduction rate to 30% of initial rate while the growth coefficient reduced by 32%, and salinity greater than 1% reduced the rate to 40%, while the growth coefficient decreased more than 40% (Gingras and Batista, 2002).

Salt tolerant bacterial species capable to reduce perchlorate in saline environments have been identified and reported. *Haloferax denitrificans* and *Paracoccus halodenitrficans* as two denitrifying halophilic bacteria and *Citrobacter* sp. have been

reported as the bacteria capable to reduce perchlorate in presence of salinity (Okeke et al., 2002). *Citrobacter* sp. has been classified as an effective salt tolerant culture that was able to reduce about 21% and 18% of the initial perchlorate amount in 1 week at salinity levels of 2.5% and 5%, respectively (Okeke et al., 2002).

Thus, it can be noted that although halophilic perchlorate reducing species can have growth in saline environments containing NaCl as high as 11%, most of the known perchlorate reducing bacteria, which are non-halophilic species, cannot grow and reduce perchlorate in the normal salinity levels of the ion exchange regenerant wastes.

*2.5.6.4. Oxidation-Reduction Potential*

Oxidation-reduction potential of the bioreactor can be used as an indicator to explain the behavior of the perchlorate reducing bacteria. Shrout and Parkin (2006) reported that perchlorate reduction increased with decreasing oxidation-reduction potential in their mixed culture. They achieved 100% removal at oxidation-reduction potential of -220 mV. It has been illustrated that achieving oxidation-reduction potential as low as the levels necessary for sulfate reduction and methanogenesis are not required for perchlorate bioreduction (Attaway and Smith, 1993). Attaway and Smith (1993) noted that perchlorate degradation does not occur at oxidation-reduction potential above -110 mV. Interestingly, a study in 2006 showed 32% perchlorate reduction, by a culture enriched from an anaerobic digester, with an oxidation-reduction potentials as high as +180 mV (Shrout and Parkin, 2006). This is the only report that shows that a perchlorate reducing culture is able to degrade perchlorate in oxidized condition.

*2.5.6.5. Presence of Other Competitive Electron Acceptors*

Other electron acceptors such as oxygen and nitrate can compete with perchlorate to receive electrons. Attaway and Smith (1993) showed the inhibition of perchlorate bioreduction in the presence of oxygen. Oxygen is a preferred electron acceptor for perchlorate reducing bacteria, since the bacteria can obtain more energy consuming oxygen than it can obtain from perchlorate. It has been proven that perchlorate reducing bacteria yields more biomass when oxygen is used to oxidize the available electron donor (Coates et al., 1999; Coates and Anderson, 2000).

The effects of oxygen exposure time on perchlorate reduction by *Dechloromonas* sp. KJ were studied in 2004 (Song and Logan, 2004). It was observed that exposure time more than 12 hours inhibits the ability of the cells to reduce perchlorate. In contrast, cells that were exposed to dissolved oxygen for less than 12 hours could quickly recover the oxidation-reduction potential of the bioreactor to negative values (-127 to -337 mV), and as a result, they were able to reduce perchlorate (Song and Logan, 2004). This study showed that exposure to oxygen for less than 12 hours due to the backwashing cycle of the biofilm layers in perchlorate degrading reactors would not change the ability of the cells to reduce perchlorate (Song and Logan, 2004).

Considering the thermodynamic preference of oxygen to perchlorate by perchlorate reducing bacteria alone may be an oversimplification (Chaudhuri et al., 2002). Studies indicate that genetic regulation plays a significant role in the reduction of perchlorate. Perchlorate reduction is dependent on the presence of chlorite dismutase, and inhibition of chlorite dismutase occurs even with oxygen concentrations of < 2 mg/L (Chaudhuri et al., 2002). Rikken et al. (1996) demonstrated that strain GR-1 has higher chlorite

dismutase activity when the bacteria are grown using perchlorate than the same bacteria grown in aerobic conditions, indicating that chlorite dismutase was induced when the culture was grown without oxygen (Rikken et al., 1996). Chlorite dismutase measurements on *Dechloromonas agitata* pure culture showed the enzyme chlorite dismutase was expressed only when the biomass was grown using perchlorate (O'Connor and Coates, 2002). Similar results were obtained for chlorite dismutase activity of *Dechloromonas suillum* culture (Chaudhuri et al., 2002), indicating the genetic regulation of enzymatic activity of chlorite dismutase.

Another set of experiments on *Dechloromonas suillum* culture were performed by Chaudhuri et al. (2002) to determine whether anoxic condition alone can induce the production of chlorite dismutase. In these experiments, nitrogen gas was introduced to an actively metabolizing aerobic *Dechloromonas suillum* culture. Samples taken after 3.5, 5, and 7 h of incubation did not show any chlorite dismutase activity, pointing that anaerobic condition alone was not sufficient to induce chlorite dismutase activity (Chaudhuri et al., 2002). In addition, Chaudhuri et al. (2002) showed that the existence of perchlorate in the presence of even low levels of dissolved oxygen was not sufficient to initiate the expression of perchlorate reductase. Thus, it can be concluded that both anaerobic/anoxic condition and the presence of perchlorate are necessary to initiate perchlorate reduction enzymatic activity.

Oxygen also results from the dismutation of chlorite by chlorite dismutase. Although, the bioavailability of the produced molecular oxygen is not known (Shrout and Parkin, 2006), studies showed that addition of chlorite to perchlorate reducing culture grown using perchlorate, produced molecular oxygen outside the cells (Rikken et al.,

1996; Coates et al., 1999). This release of oxygen by the bacteria might be due to protection of oxygen sensitive enzymes (Kengen et al., 1999; Shrout and Parkin, 2006). Immediately after production and without accumulation, oxygen is utilized by the cell (Rikken et al., 1996; Logan et al., 2001; Xu and Logan, 2003). The final product of the reaction of oxygen with an electron donor is carbon dioxide and water (Rikken et al., 1996).

Nitrate is another electron acceptor that can be reduced by most of the perchlorate reducing bacteria. However, some perchlorate reducing bacteria such as *Dechloromonas agitata* strain CKB cannot grow on nitrate (Chaudhuri et al., 2002). Other experiments were performed on *Dechloromonas suillum* culture that can grow either on perchlorate or nitrate medium to investigate the preference of the culture for electron donor under different growth conditions. The experiments demonstrated that, in all cases, neither chlorite dismutase activity nor perchlorate degradation was detected until all nitrate ions were utilized (Chaudhuri et al., 2002). Although strain CKB could not grow on nitrate medium, when the culture was transferred into a medium containing both nitrate and perchlorate, it had lower perchlorate reduction rate than the culture incubated in the absence of nitrate. This observation indicates that nitrate acts as competitive inhibitor for perchlorate reduction. More interestingly, although the strain CKB could not utilize nitrate as electron donor source, nitrate was reduced to nitrite in the presence of perchlorate. It suggests that nitrate ions were co-reduced by perchlorate reductase (Chaudhuri et al., 2002). Herman and Frankenberger (1998) also reported similar co-reduction of nitrite by the perclace culture; however, since the perclace culture was unable to grow on nitrite medium, it co-reduced nitrate to nitrogen gas.

*2.5.6.6. Effect of Different Electron Donors on the Reduction Process*

Perchlorate reducing bacteria can utilize various electron donors as the energy source. The electron donor can be either an organic molecule (e.g. acetate, ethanol, lactate, etc) or an inorganic molecule (i.e. hydrogen gas). Several studies have been performed to determine characteristics of the known strains of perchlorate reducing bacteria. The most common electron donor used in these studies is acetate. The other common organic electron donors are propionate, lactate, and ethanol. In addition to organic electron donors, some perchlorate reducing bacteria are also able to utilize hydrogen as energy source (Miller and Logan, 2000; Zhang et al., 2002; Shrout et al., 2005). Only a few autotrophic perchlorate reducing bacteria, those that use hydrogen as the electron donor and carbon dioxide as the carbon source, have been identified and isolated (Zhang at al, 2002; Shrout et al., 2005; Nerenberg et al., 2006; Dudley et al. 2008) and they are listed in Table 2.7.

The ratios of electron donor to perchlorate needed for perchlorate degradation have been studied during the last decade. As discussed in the stoichiometry section, Chaudhuri and Coates (2002), Waller et al. (2004), and Shrout and Parkin (2006) have reported optimal COD to perchlorate ratio as ~1.2 g COD/g $ClO_4^-$. Shrout and Parkin (2006) also noted that perchlorate degradation was faster with lactate compared to acetate, without giving an explanation. Attaway and Smith (1993), Song and Logan (2004), and Shrout and Parkin (2006) observed that the lack of electron donor in the bioreactor results in a more oxidized oxidation/reduction potential.

Activity level of the involved enzymes in the perchlorate reduction pathway (i.e. perchlorate reductase and chlorate dismutase) is affected by the electron donor type.

Shrout et al. (2005) showed that chlorite dismutase activity level in the cells, which were grown utilizing hydrogen, was higher than in the cells grown with organic electron donors. Shrout et al. (2005) concluded that it may be due to the production of alternate dismutase enzymes under autotrophic conditions. There are similar observations such as production of alternative nitrogenase enzymes by nitrogen-fixing bacteria utilizing different electron donors (Harwood and Parales, 1996).

## 2.6. Biological Reduction of Nitrate

Denitrification is the dissimilatory reduction of nitrate ($NO_3^-$) or nitrite ($NO_2^-$) to nitrogen gas ($N_2$) (Rittman and McCarty, 2001). It is an important pathway of the biogeochemical nitrogen cycle. The nitrogen cycle is shown in Figure 2.5. Nitrogen in this cycle exists in the biosphere in several oxidation states, shown in Figure 2.5, from +V in nitrate to –III in ammonia. Denitrification, which is the conversion of nitrate (the most oxidized form of nitrogen) to nitrogen gas, passes through four enzymatic reactions: nitrate ($NO_3^-$) to nitrite ($NO_2^-$), nitrite to nitric oxide (NO), nitric oxide to nitrous oxide ($N_2O$), and finally nitrous oxide to nitrogen gas ($N_2$). Denitrification is an energy yielding process for denitrifying bacteria that use the positive redox potential of $NO_3^-/NO_2^-$ ($E^{0'}$=+0.43 V), $NO_2^-/NO$ ($E^{0'}$=+0.35 V), $NO/N_2O$ ($E^{0'}$=+1.175 V), and $N_2O/N_2$ ($E^{0'}$=+1.355 V) reactions (Einsle and Kroneck, 2004).

In environmental biotechnology, denitrification is used when complete nitrogen removal is necessary. The presence of nitrate as a water contaminant is a common issue in many drinking water sources. Nitrate competes with other electron acceptors to utilize the available electron donor source, and this competition makes this anion an important

factor in anaerobic/anoxic treatment systems (e.g. phosphate removal and perchlorate removal systems).

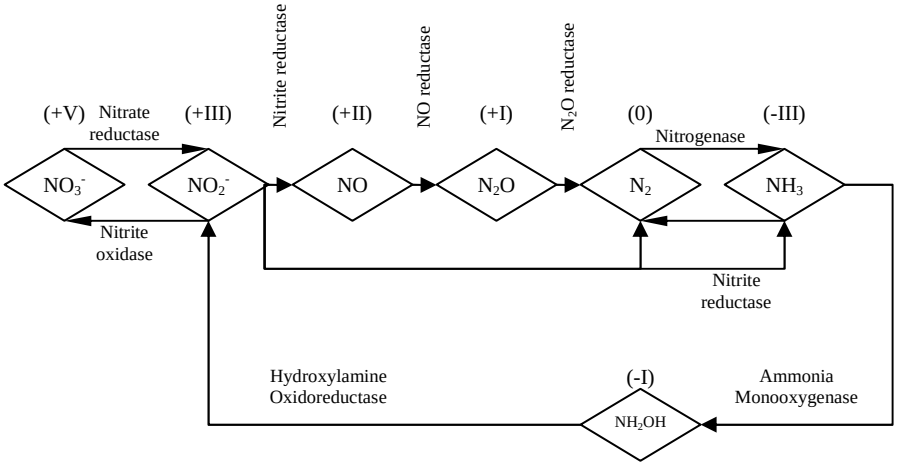


Figure 2.5. The Biological Nitrogen Cycle Pathway, Oxidation States and the Enzymes Involved in the Processes (Modified from: Einsle and Kroneck, 2004)

Dissolved oxygen (DO) controls the denitrification pathway via two distinct mechanisms (Rittman and McCarty, 2001). First, oxygen acts as a repressor for genes responsible for nitrogen-reduction. Studies show that DO concentrations greater than 2.5 to 5 mg/L repress the responsible genes. Second, oxygen acts as an inhibitor for the activity of the denitrification enzymes. Dissolved oxygen above a few tenths of mg/L slows down the activity of the involved enzymes (Rittman and McCarty, 2001). It can be concluded that inhibition of the enzymes by DO is a more sensitive mechanism compared to gene repression by oxygen molecules.

### 2.6.1. Biochemistry of the Nitrate Reduction

Throughout the denitrification metabolic pathway, all steps are catalyzed by complex multi-site metalloenzymes with unique spectroscopic and structural characteristics. Enzymes involved in the stepwise reduction pathway are described below.

#### *2.6.1.1. Nitrate Reductase*

The first step which is a conversion of nitrate to nitrite is performed by nitrate reductase. All of the bacterial and eukaryotic nitrate reductases are molybdenum dependent enzymes. The molybdopterin cofactor in nitrate reductases is in the form of two molybdopetrin-guanine dinucleotide (MGD) molecules which are located in the molybdenum center (Einsle and Kroneck, 2004). Nitrate reductases are responsible for the following reaction. Reaction 2.34 shows that two electrons are needed to reduce nitrate to nitrite.

$$NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O \qquad (2.34)$$

In general, there are four types of nitrate reductases: eukaryotic nitrate reductase and three distinct prokaryotic nitrate reductases, including cytoplasmic assimilatory (Nas), membrane bound (Nar), and periplasmic nitrate (Einsle and Kroneck, 2004). Most of the nitrate reductases are bound to the inner side of the membrane (Hochstein and Tomlinson, 1988). Many bacteria produce more than one type of the above mentioned nitrate reductses (Einsle and Kroneck, 2004). As an example, *E.coli* contains a periplasmic nitrate reductase (Einsle and Kroneck, 2004). However, when it is grown on nitrate under anaerobic conditions, it develops a membrane-bound nitrate reductase, the active sites of which face the cytoplasm of the cell (Jormakka et al., 2002). Also, there are some bacteria containing nitrate reductases with unknown physiological functions.

For instance, *Alcaligenes eutrophus* contains periplasmic nitrate reductase, the physiological function of which is not clear for the microbiologists (Siddiqui et al., 1993).

As discussed in perchlorate reductase section, membrane-bound respiratory nitrate reductases and assimilatory nitrate reductases are also able to reduce perchlorate/chlorate to chlorite, as an alternative for nitrate (Oltmann et al., 1976; Giblin and Frankenberger, 2001). However, denitrifying bacteria are inactivated when they reduce perchlorate to chlorite because of the lack of chlorite dismutase enzyme and accumulation of produced chlorite (Oltmann et al., 1976), which is a disinfectant.

*2.6.1.2. Nitrite Reductase*

Nitrite reductase is an enzyme that reduces nitrogen in nitrite to a lower oxidation state. It is the second involved enzyme in the denitrification process. Certain types of nitrite reductases can reduce nitrite to ammonia through a six-electron transfer process. This process is called nitrite ammonification (Simon, 2002). Denitrificatory nitrite reductases, which reduce nitrite to nitrous oxide, could be either homotrimer copper enzyme or cytochrome $cd_1$ enzymes (Moura and Moura, 2001). Copper nitrite reductase enzymes are categorized into two (i.e. green and blue) subclasses based on absorbance characterization (Einsle and Kroneck, 2004). Cytochrome *cd1* nitrite reductase enzymes are soluble homodimer protein molecules and are located in the periplasm area (Einsle and Kroneck, 2004). Each subunit of these soluble homodimers consists of one heme *c* and one heme *d1* (Moura and Moura, 2001). The optimum temperature for nitrite reductase enzymes has been studied and reported as 35ºC to 38ºC (Peng and Zhu, 2006). The reaction which is catalyzed by denitrificatory nitrite reductase requires one electron from the electron donor and can be shown as the following reaction.

$$NO_2^- + e^- + H^+ \rightarrow NO + H_2O \quad (2.35)$$

*2.6.1.3. Nitric Oxide Reductase*

Nitric oxide reductase enzymes, which are responsible for the third step of the denitrification process to reduce nitric oxide (NO) to nitrous oxide ($N_2O$), belong to the cytochrome oxidases family (Hendriks, et al., 2000). This enzyme has an interesting function, since the N-N bond configuration of $N_2$ gas in nature is mainly due to nitric oxide reductase during denitrification (Moura and Moura, 2001). In fact, nitric oxide reductase regenerates the N-N bond in the nitrogen cycle. Accumulation of nitric oxide is toxic to the cells. Nitric oxide reductases reduce nitric oxide immediately after it is generated. In bacteria, nitric oxide reductase is a membrane protein complex, belonging to the haem/copper cytochrome oxidases, whereas in fungi, nitric oxide reductase is a soluble enzyme that belongs to the category of cytochrome-type proteins (Moura and Moura, 2001).

*2.6.1.4. Nitrous Oxide Reductase*

The last step in the denitrification process is conversion of nitrous oxide ($N_2O$) to nitrogen gas ($N_2$). This is a two-electron reduction process, which is catalyzed by nitrous oxide reductases:

$$N_2O + 2e^- + 2H^+ \rightarrow N_2 + H_2O \quad (2.36)$$

Nitrous oxide reductase is a homodimeric copper-containing metalloenzyme, which consists of two subunits (Moura and Moura, 2001). In this enzyme, copper ion exists in both two centers. Zink, iron, and nickel also have been detected in the structure of this enzyme (Simon, 2002).

Enzyme location, optimum pH and temperature for the activity, and molecular mass of the enzymes involved in the nitrate reduction pathway has been compared to the enzymes involved in the perchlorate reduction pathway in Table 2.8.

### 2.6.2. Microorganisms Involved in Nitrate Biodegradation

Nitrate reduction is a common pathway that can be found in both autotrophic and heterotrophic bacteria. Most of the denitrifier bacteria can use other electron acceptor sources, such as oxygen, perchlorate, and sulfate, as a substitute for nitrate respiration (Hendriks, et al., 2000; Giblin and Frankenberger, 2001). In prokaryotes, denitrification can be detected in some bacteria and even some archaea. Denitrification is common in genera within the *Proteobacteria* group such as *Pseudomonas, Thiobacillus, Alcaligenes* and *Paracoccus*, which are all gram negative bacteria (Rittman and McCarty, 2001). In addition to gram negative bacteria, denitrification can be found in some gram-positive genera including *Bacillus* (Suharti, et al., 2001; Suharti and de Vries, 2005). All denitrifiers are facultative anaerobic bacteria (Rittman and McCarty, 2001). Some halophilic archaea such as *Halobacterium* are also able to perform denitrification (Rittman and McCarty, 2001).

Diversity of microorganism in a denitrification system depends on the conditions of the reactor. Identification of denitrifying bacteria in a denetrification batch reactor, fed with acetate and methanol as external carbon sources, has been studied and α and β subclasses of *Proteobacteria* have been reported as the dominant denitrifying cells (Osaka et al., 2006). Using acetate as the external carbon source, the 16S rRNA sequence analysis illustrated genes of *Comamonadaceae* (e.g., *Comamonas* and *Acidovorax*) and *Rhodocyclaceae* (e.g., *Thauera* and *Dechloromonas*) of the β-

*proteobacteria* group and *Rhodobacteraceae* (e.g., *Paracoccus* and *Rhodobacter*) of the α*-proteobacteria* group in the reactor (Osaka et al., 2006). With methanol as the carbon source, *Methylophilaceae* (e.g., *Methylophilus*, *Methylobacillus*, and *Aminomonas*) and *Hyphomicrobiaceae* were identified and reported (Osaka et al., 2006). These results show that carbon source type has some effects on the genus and diversity level of denitrifiers in the system.

Table 2.8. Typical Characteristics of Enzymes Involved in Nitrate and Perchlorate Reduction

| Enzyme name | Enzyme location | Molecular mass (kDa) | Optimum pH | Optimum temperature (ºC) | Characteristic |
|---|---|---|---|---|---|
| Perchlorate reductase | periplasm (Kengen et al., 1999) | 420 (Steinberg et al., 2005) | 7.5-8.0 (Okeke and Frankenberger, 2003) | 25-30 (Okeke and Frankenberger, 2003) | Selenium containing heme-protein (Steinberg et al., 2005) |
| Chlorite dismutase | Periplasm (Kengen et al., 1999). | 140 (van Ginkel et al., 1996) | 6.0 (van Ginkel et al., 1996) | 30 (van Ginkel et al., 1996) | Heme-containing O-O bound former (Lee et al, 2008) |
| Nitrate reductase | cytoplasm, membrane-bound, and periplasm (Einsle and Kroneck, 2004) | 230 (Polcyn, 2008) | 9.0 (Giblin and Frankenberger, 2001) | 70-80 (Morozkina and Zvyagilskaya, 2007) | molybdenum dependent (Einsle and Kroneck, 2004) |
| Nitrite reductase | membrane-bound, and periplasm (Einsle and Kroneck, 2004) | 120 (Moura and Moura, 2001) | NA | 35-38 (Peng and Zhu, 2006) | Heme-containing protein (Moura and Moura, 2001) |
| Nitric oxide reductase | Membrane-bound (Hendriks, et al., 2000) | 160-180 (Barton, 2004) | NA | NA | Oxidoreductase enzyme (Moura and Moura, 2001) |
| Nitrous oxide reductase | Periplasm (Rasmussen et al., 2005) | 120 (Simon, 2002) | 7.5 (Rasmussen et al., 2005) | NA | Multi-copper enzyme (Moura and Moura, 2001) |

# CHAPTER 3

# METHODOLOGY

## 3.1. Work Plan for Issue One

The first objective of this research, which involves three hypotheses, was to understand the mechanism of degradation of resin-attached perchlorate ions. In the first hypothesis, it was envisioned that there is a desorption mechanism involved in the detachment of the resin-attached perchlorate ions. The second and third hypotheses assumed the diffusion of desorbed perchlorate, from the inside region of the resin bead to the surface, is the rate-controlling step in the resin bioregeneration process. The hypotheses posed for this objective were:

Hypothesis 1: The first hypothesis was that resin-attached perchlorate exchanges instantaneously with chloride, generated from the degradation of perchlorate. The perchlorate ion thereby is free to be taken up by the PRB. However, this hypothesis did not explain how the first resin-attached perchlorate ions are biodegraded.

Hypothesis 2: The second hypothesis was that the bioregeneration of ion-exchange resin is controlled by diffusion rather than by the rate of resin-attached perchlorate desorption.

Hypothesis 3: The third hypothesis was that bioregeneration of macroporous ion-exchange resins is faster than gel-type resins due to higher water content and larges resin pore size, which result in a faster diffusion rate in macroporous resins compared to gel-type resins.

### 3.1.1. Experimental Approach

All the experiments were performed in the Environmental Engineering Laboratory at UNLV. Hypotheses 1, 2, and 3 were tested through a series of batch bioregeneration experiments. In the tests associated with hypothesis 1, a series of batch bioregeneration tests were performed using different concentrations of chloride. Chloride was hypothesized to be the reason for desorption of resin-attached perchlorate. It was expected that resin-attached perchlorate desorption and bioregeneration can be observed only in the presence of chloride ion.

In the tests associated with hypothesis 2, the ion exchange resin beads were sorted to obtain resin samples with different particle sizes. In one of series of batch bioregeneration tests, resin beads were crushed to reduce the resin-bead size. The batch bioregeneration experiments were performed using resin samples with different bead sizes. It was expected that the PRB bioregenerate the smaller diameter resins at a higher rate than that for the larger resins due to the increase in specific surface area per volume of resin and as a result enhancement in the mass transfer.

To test hypothesis 3, batch bioregeneration tests were performed using gel-type and macroporous ion-exchange resins, which include perchlorate-selective and non-selective resins. It was expected that the bacteria bioregenerate the macroporous resins in higher rates compared to the gel-type resins since the macroporous resins have higher water content and larges resin pore size than the gel-type resins.

### 3.1.2. Experiments

#### *3.1.2.1. Resin Characteristics*

Two perchlorate-selective resins (SIR-100HP and SIR-110HP-MACRO) and two non-selective resins (ONAC ASB-1 and IONAC SR-7) were used in the batch bioregeneration experiments (Table 3.1). These resins all have a styrenic matrix. Table 3.1 presents the characteristics of the selected resins used for this research.

Table 3.1. Characteristics of the IX Resins Used in Batch Experiments to Test Hypotheses 1-3

| Hypothesis | Commercial name | Resin Type | Water Con. (%) | Capacity (eq/L) | Resin structure | Functional group |
|---|---|---|---|---|---|---|
| Hypothesis 1<br>Hypothesis 2<br>Hypothesis 3 | SIR-110HP (ResinTech) | $ClO_4^-$-selective | 35-55 | 0.6 | Styrene, Gel | $(C_4H_9)_3^+Cl^-$ |
| Hypothesis 1<br>Hypothesis 3 | IONAC ASB-1 (Sybron) | non-selective | 43-48 | 1.4 | Styrene, Gel | $(CH_3)_3^+ Cl^-$ |
| Hypothesis 3 | SIR-110HP-MACRO (ResinTech) | $ClO_4^-$-selective | 58-65 | 0.6 | Styrene, Macroporous | $(C_4H_9)_3^+Cl^-$ |
| Hypothesis 3 | IONAC SR-7 (Sybron) | non-selective | 48-52 | 0.8 | Styrene, Macroporous | $(CH_3)_3^+ Cl^-$ |

#### *3.1.2.2. Resin Loading*

The resin samples were loaded fully to eliminate the original counter ion (i.e. chloride) of the functional groups of the resins. Presence of chloride ion was controlled very carefully in all the experiments dealing with hypothesis 1 since the chloride ion was assumed as the desorbing agent of resin-attached perchlorate ions in the resin-bioregeneration process. Table 3.2 shows the total capacity of the resins as eq/l and g/l

and the concentrations of stock solutions. All the resins were loaded with sodium perchlorate.

Table 3.2. Resin Loadings Used for the Batch Bioregeneration Experiments for Issue 1

| Hypothesis | Resin name | Capacity (eq/L) | Capacity (g/L) | Concentration of Stock Loading Solution |
|---|---|---|---|---|
| Hypothesis 1 | SIR-110HP | 0.6 | 59.7 | 120,000 (mg-$ClO_4$/L) |
| Hypothesis 1 | IONAC ASB-1 | 1.4 | 139.3 | 120,000 (mg-$ClO_4$/L) |
| Hypothesis 2 | SIR-110HP | 0.6 | 59.7 | 120,000 (mg-$ClO_4$/L) |
| Hypothesis 3 | SIR-110HP-MACRO | 0.6 | 59.7 | 120,000 (mg-$ClO_4$/L) |
| Hypothesis 3 | SIR-110HP | 0.6 | 59.7 | 120,000 (mg-$ClO_4$/L) |
| Hypothesis 3 | IONAC SR-7 | 0.8 | 79.6 | 120,000 (mg-$ClO_4$/L) |
| Hypothesis 3 | IONAC ASB-1 | 1.4 | 139.3 | 150,000 (mg-$ClO_4$/L) |

The resin samples were loaded batchwise. Batch loading was selected to shorten the time period required to load the resin. Furthermore, in the batch loading, the ions are distributed more homogeneously through the resin particles. For each milliliter of the resin sample, 1 mL of the loading solution (Table 3.2) was prepared and added to a glass bottle. The bottle was then placed on a rotary mixer (Associate Design Mfg. Co., Alexandria, VA) at 40-50 rpm and 22±2ºC. After 24 hours, the mixer was stopped, and the resin was separated from the liquid supernatant using a filter paper. The supernatant was sampled for residual perchlorate analysis and the rest of it was decanted. Perchlorate

concentrations of the added and residual solutions were then measured using Ion Chromatography (IC) analysis.

The loaded resin was then rinsed 12 times with 1 L of de-ionized (DI) water for 6 hours to remove all the residual unattached perchlorate ions and the rinsate solutions were submitted for perchlorate analysis using IC. After 12 times rinsing, no perchlorate was detected in the rinsate solution. The resins were then dried at room temperature (i.e. 22±2ºC) for 6 hours, labeled, and stored in the refrigerator.

*3.1.2.3. Resin Size Reduction*

Loaded SIR-110HP resin particles were sorted to different sizes for the batch bioregeneration experiment evaluating the effect of resin particle size on the bioregeneration process (i.e. hypothesis 2 of issue 1). The normal SIR-110HP resin size ranges between 1.19 to 0.297 mm, which corresponds to US mesh number 16 and 50 respectively. In order to obtain very small size of SIR-110HP, the loaded resin particles were crushed using a mortar and pestle. A U.S. standard (Das, 2001) stainless-steel series of sieves (Fisher Scientific, Inc., Waltham, MA) with mesh sizes of 0.853, 0.710, 0.599, 0.500, 0.150, and 0.106 mm were used for size distribution analysis and size separation of the dried resin particles (Table 3.3).

Five different particle sizes of 0.924, 0.778, 0.652, 0.547, and 0.128 mm were used as the mean particle sizes for SIR-110HP in the experiment evaluating the effect of particle size on the bioregeneration process. The specific surface area per volume was calculated by determine the total surface area per unit of solid volume of the resin (Sepaskhah et al., 2010). For the experiment examining the performance of gel-type and macroporous resins during bioregeneration process (i.e. hypothesis 3), only resin particles with the

representative particle size of 0.778 mm (i.e. remained on the sieve #25) were used. Size distribution of SIR-110HP and ASB-1 resins was not changed for the batch bioregeneration experiment evaluating the effect of initial chloride on bioregeneration process.

Table 3.3. Standard Sieve Numbers Selected to Perform the Sieve Analysis to Separate the Resin Beads with Different Diameters

| Sieve Number | Opening Size (mm) | Mean size (mm) | Specific surface area per volume, 1/mm |
|---|---|---|---|
| 18 | 1.000 | NA | NA |
| 20 | 0.853 | 0.924[a] | 6.476 |
| 25 | 0.710 | 0.778 | 7.677 |
| 30 | 0.599 | 0.652 | 9.167 |
| 35 | 0.500 | 0.547 | 10.919 |
| 100 | 0.150 | NA | NA |
| 140 | 0.106 | 0.128 | 46.785 |

[a] $0.924 = (1.000 \times 0.853)^{0.5}$

*3.1.2.4. Perchlorate Reducing Master Culture*

Perchlorate-reducing microbial culture for this research was taken from two master seed cultures, called BALI I and BALI II. These microbial cultures were enriched from samples taken from the Las Vegas Wash and Lake Mead in Nevada. These areas have been contaminated with ammonium perchlorate for the past five decades and were presumed likely sources of PRB (Gingras and Batista, 2002). The culture was enriched under anaerobic conditions by providing perchlorate as the electron acceptor, acetate as the electron donor, and a mineral/nutrient/buffer broth for the seed (Table 3.4). The culture has been identified using two different molecular methods, Restriction Fragment

Length Polymorphism (RFLP) and 16S rRNA sequencing (Kesterson, 2005). The characterization results indicate the culture is composed of at least six isolates, two of which are able to degrade perchlorate as the electron acceptor source. All six isolates are gram-negative, facultative anaerobe bacteria. The bacterial species that have been identified in the culture include *Pseudomonas*, *Azospira* (formerly called *Dechlorosoma*), *Dechloromonas*, *Aeromonas*, and *Rhizobium*, which are typically present in soil and water (Kesterson, 2005).

The master seed cultures were maintained by feeding/wasting and monitoring for total suspended solids (TSS), perchlorate residual, conductivity, turbidity, chemical oxygen demand (COD), pH, oxidation reduction potential (ORP), and dissolved oxygen (DO) on a weekly basis schedule. During the growth, the reactors were sealed completely to ensure anaerobic condition for the microorganisms. The culture was mixed using a magnetic stirrer to keep the biomass in suspension.

*3.1.2.5. Stock Solutions for Perchlorate Reducing Culture*

The stock solutions for feeding the seed master cultures were the electron acceptor (i.e. perchlorate), electron donor (i.e. acetate), and mineral/nutrient/buffer solutions. The concentration of the electron acceptor and electron donor solutions was 40,000 mg-$ClO_4$/L and 120,000 mg- $CH_3COO^-$/L. Table 3.4 shows the electron acceptor, electron donor, buffer, and mineral/nutrient stock solutions.

The observed stoichiometry of ~1.7 mole acetate / mole perchlorate (i.e. ~1.01 g acetate / g perchlorate) has been reported in the previous studies (Chaudhuri and Coates, 2002; Waller et al. 2004; Wang et al., 2008a). Hence, a mass ratio of 2 for acetate/perchlorate (i.e. 2000 mg/L / 1000 mg/L) was used to feed the master cultures.

Table 3.4. Electron Donor, Buffer, and Nutrient Stock Solution Used for Feeding the Master Seed Cultures

| Solution Name | Components | Concentration of stock (g/L) |
|---|---|---|
| Electron donor/ carbon source (40X)* | $CH_3COO^-$ (Sodium form) | 120.00 |
| Electron Acceptor/ Perchlorate (40X) | $NaClO_4$ | 40.00 |
| Buffer (100X) | $K_2HPO_4$ | 155.00 |
| | $NaH_2PO_4.H_2O$ | 97.783 |
| | $NH_4H_2PO_4$ | 50.000 |
| Mineral/Nutrients (100X) | $MgSO_4.7H_2O$ | 5.500 |
| | EDTA | 0.300 |
| | $ZnSO_4.7H_2O$ | 0.200 |
| | $CaCl_2.2H_2O$ | 0.100 |
| | $MnCl_2.4H_2O$ | 0.100 |
| | $FeSO_4.7H_2O$ | 0.400 |
| | $Na_2MoO_4.2H_2O$ | 0.040 |
| | $CuSO_4.5H_2O$ | 0.020 |
| | $CoCl_2.6H_2O$ | 0.040 |
| | $NiCl_2.6H_2O$ | 0.010 |
| | $NaSeO_3$ | 0.010 |
| | $H_3BO_3$ | 0.060 |

* 40X: need to be diluted 40 times

*3.1.2.6. Cell Extraction from Master Microbial Culture*

For the series of batch bioregeneration experiment that was performed using different concentrations of chloride, two liters of BRP microbial culture obtained form the master seed cultures were rinsed five consecutive times using phosphate buffer solution (Table 3.4) to eliminate the presence of chloride ion in the liquid phase. The culture was centrifuged using a Legend RT Sorvall centrifuge (Kendro, Thermo Fisher Scientific, Inc., Waltham, MA) at the rotational speed of 3850 rpm for 45 minutes. The supernatant containing chloride was discarded and phosphate buffer solution was added to the concentrated biomass. The mixture of the concentrated biomass and phosphate buffer

solution was then blended to reach a SS concentration of 1500 mg/l using a bench-top orbital shaker (Cole-Parmer, Series 51704) for 20 min at 80-100 rpm. The culture was centrifuged and rinsed five times to ensure the elimination of chloride from the culture. A sample of rinsed culture was then submitted for chloride analysis by IC and no chloride was detected.

*3.1.2.7. Batch Testing Procedure*

All the bioregeneration experiments were performed in batch-bioreactor tubes with a capacity of 25 mL. For each experiment a series of batch-bioreactor tubes were used so that a tube was sacrificed at the given time intervals for sampling (Table 3.5). The culture was diluted to 1000 mg/L in the batch-bioreactor tubes by adding nutrient, buffer, and acetate media (Table 3.4) and DI water. Initial concentrations of 0, 500, 1000, and 5000 mg/L of sodium chloride (NaCl) were used for the series of bioregeneration experiments evaluating the effect of varying initial chloride concentration on bioregeneration process. A concentration of 9000 mg/L of acetate was used for all of the batch-bioreactor tubes.

Approximately 1.2 g of resin (i.e. about 2 ml) was added to each batch-bioreactor tube. All the solutions (i.e. nutrient, buffer, and acetate stock solutions, and DI water) were purged with nitrogen gas for 30 minutes prior to start the experiment to eliminate dissolved oxygen in the solutions. After all the solutions were added to the batch-bioreactor tube, they were sealed using aluminum-crimpled butyl rubber-stopper (Wheaton Industries, Inc., Millville, NJ), labeled, and placed on a rotary mixer at 22±2ºC and 40-50 rpm.

Table 3.5. Experimental Design Used for Batch Bioregeneration Experiments

| Resin name | Resin particle size, mm | Amount of Resin in each tube, g | Initial conc. of NaCl, mg/L | Bioregeneration length, days |
|---|---|---|---|---|
| Series #1: $Cl^-$ variation (hypothesis 1) | | | | |
| SIR-110HP | Original distribution | 1.2 | 0 | 8 |
| SIR-110HP | Original distribution | 1.2 | 500 | 8 |
| SIR-110HP | Original distribution | 1.2 | 1000 | 8 |
| SIR-110HP | Original distribution | 1.2 | 5000 | 8 |
| ASB-1 | Original distribution | 1.2 | 0 | 8 |
| ASB-1 | Original distribution | 1.2 | 500 | 8 |
| ASB-1 | Original distribution | 1.2 | 1000 | 8 |
| ASB-1 | Original distribution | 1.2 | 5000 | 8 |
| Series #2: Size variation (hypothesis 2) | | | | |
| SIR-110HP | 0.924 | 1.2 | NA[a] | 8 |
| SIR-110HP | 0.778 | 1.2 | NA | 8 |
| SIR-110HP | 0.652 | 1.2 | NA | 8 |
| SIR-110HP | 0.547 | 1.2 | NA | 8 |
| SIR-110HP | 0.128 | 1.2 | NA | 8 |
| Series #3: Gel-type resin vs. Macroporous resin (hypothesis 3) | | | | |
| SIR-110HP | 0.778 | 1.2 | NA | 8 |
| SIR-110HP-MACRO | 0.778 | 1.2 | NA | 8 |
| ASB-1 | 0.778 | 1.2 | NA | 8 |
| SR-7 | 0.778 | 1.2 | NA | 8 |

[a] Initial concentration of chloride was not changed (i.e. it was the same concentration as the master seed cultures).

Each series of bioregeneration experiment contained 5 bioreactor-tubes (i.e. days 1, 2, 4, 6, and 8), 2 replicates for quality control (i.e. day-1-QC and day-8-QC), an abiotic control tube (i.e. resin and nutrient/buffer/acetate medium, no microbial cell), and a replicate for abiotic control tube (Figure 3.1). For the series of bioregeneration experiment evaluating the effect of varying initial chloride concentration (i.e. hypothesis 1), two other control tubes were prepared (Figure 3.1): (a) an abiotic tube containing resin and DI water (i.e. no microbial cells, no nutrient/buffer/acetate medium), and (b) a bioreactor-tube containing resin, microbial cells, and buffer solution (i.e. no

nutrient/acetate medium). The sealed abiotic reactor-tubes were autoclaved immediately after preparation of tubes to avoid any microbial activity. All the control tests were performed in duplicate.

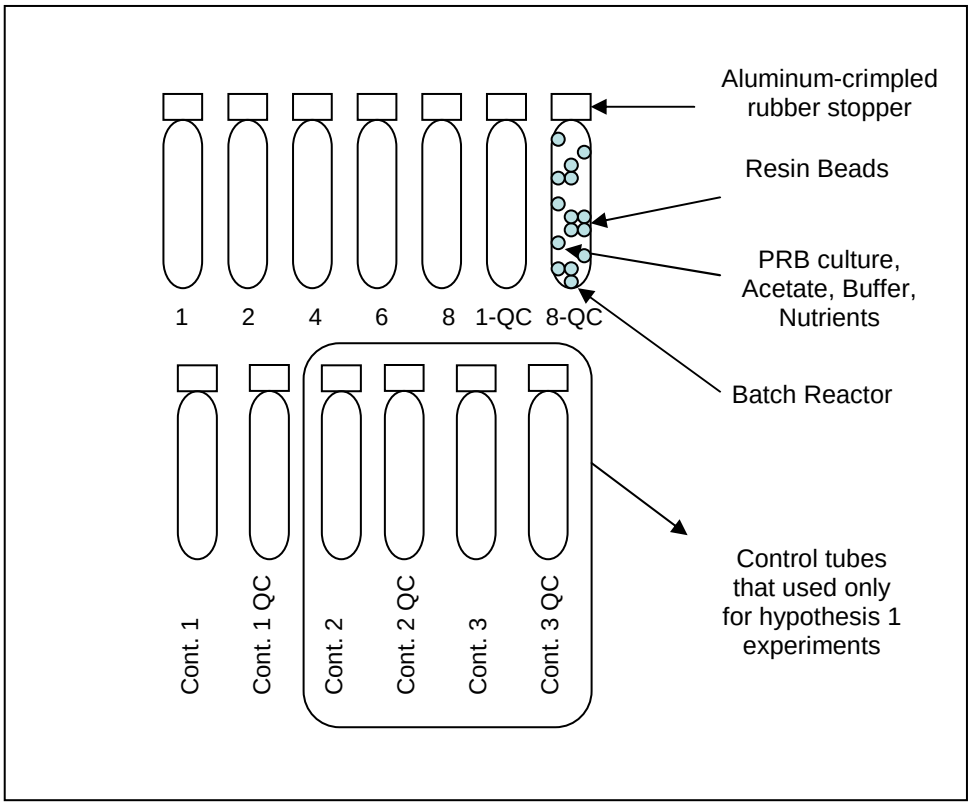


Figure 3.1. Batch Experiment Setup Used for Resin Bioregeneration Experiments for Hypothesis 1-3

Bioreactor-tubes were collected form the mixer on days 1, 2, 4, 6, and 8. The resin was rapidly settled and the microbial culture above the settled resin was collected and tested for COD, pH, SS, and ORP. The resin sample was then rinsed 5 times with 5 mL of DI water to remove the remaining microbial cells and organics which might remain in the resin. Preliminary experiments by measuring % transmittance of the rinsate solution

showed that after 5 time rinse, the % transmittance of the rinsate solution before and after rinsing does not change. The resin sample was then submitted to resin-attached residual perchlorate analysis.

All the control tubes were collected after 8 days from the mixer. In the abiotic tests, the supernatant was submitted for perchlorate analysis by IC to determine the amount of desorbed perchlorate. The resin from the control tubes containing resin, microbial cells, and buffer solution was rinsed 5 times with 5 mL of DI water to remove the remaining microbial cells and organics and submitted to resin-attached residual perchlorate analysis.

For the experiment investigating the resin attached perchlorate desorption kinetic, 1.2 g of 0.924 and 0.128 mm SIR-110HP resin was added to a 25 mL batch-reactor tube containing 23 mL of 2000 mg/L NaCl solution and placed on a rotary mixer at 22±2ºC and 40-50 rpm. One hundred µL (100 µL) samples were collected from the batch-reactor tubes using a micropipette after prescribed time intervals and submitted to perchlorate analysis by IC. The desorption kinetic experiments were performed in duplicate.

*3.1.2.8. Batch Test Design to Test Hypothesis 1-3 of Issue One*

Testing hypothesis 1 involved using two different gel-type resins (i.e. SIR-110HP and ASB-1) and four different initial chloride concentrations (0, 500, 1000, 5000 mg-NaCl/L). Both resins were loaded with perchlorate (section 3.1.2.2). In total, 8 sets of experiments were performed to study hypothesis 1 (Table 3.6).

Testing hypothesis 2 involved using a gel-type perchlorate-selective resin (i.e. SIR-110HP) that was sorted in five different sizes. To obtain the smallest size (i.e. 0.128 mm), the resin beads were crushed using mortar and pestle. In total, 5 sets of experiments were performed to study hypothesis 2 (Table 3.7). For hypothesis 3,

perchlorate-selective and non-selective resins in the form of gel-type and macroporous-type were used to perform four sets of batch bioregeneration tests.

*3.1.2.9. Investigation of Diffusion and Reaction Control of Bioregeneration*

The shrinking core model (Arevalo et al., 1998; Pritzker, 2005) can be used to describe perchlorate desorption and utilization in IX resin bead. Based on the shrinking core model, about 90% of perchlorate load is located between $r_0/r = 0.5$ and $r_0/r = 1.0$. Reducing the resin bead size will accelerate the diffusion process and a result bioregeneration of resin, if the bioregeneration process is controlled by pore diffusion (Helfferich, 1962). According to the shrinking core model, reducing the resin bead size exposes more surface to the liquid phase, and if the bioregeneration process is pore diffusion controlled, it will enhance the process.

In addition to pore diffusion, it is thought that film diffusion is also involved in the bioregeneration process. Film diffusion limitation increases with decreasing resin bead size. While, decreasing resin bead size results in increase of pore mass transfer flux. The rate controlling step in mass transfer process can be mathematically identified (Helfferich, 1962):

$$\frac{X\overline{D}\delta}{CDr_0}(5+2\alpha_B^A) << 1 \quad \text{pore diffusion control} \tag{3.1}$$

$$\frac{X\overline{D}\delta}{CDr_0}(5+2\alpha_B^A) \approx 1 \quad \text{pore / film diffusion control} \tag{3.2}$$

$$\frac{X\overline{D}\delta}{CDr_0}(5+2\alpha_B^A) >> 1 \quad \text{film diffusion control} \tag{3.3}$$

where:

X = concentration of fixed ionic group ($eq_{perchlorate}/L_{resin}$)

C = concentration of solution ($eq_{chloride}/L_{solution}$)

$\overline{D}$ = pore diffusion coefficient ($cm^2/s$)

D = film diffusion coefficient ($cm^2/s$)

$r_0$ = mean particle radius (mm)

$\delta$ = assumed liquid film thickness (cm)

$\alpha_B^A$ = selectivity coefficient

The Thiele modulus is an understood measure of the comparison of diffusion limited to kinetic limited reactions (Thiele, 1939; Helfferich, 1962; Hong et al., 1999). In the bioregeneration process, Thiele modulus can be used to examine whether the process is controlled by diffusion of perchlorate ion thought the resin pores or by the biological reduction of perchlorate in the bulk liquid.

The Thiele modulus for a process involving reaction and diffusion can be calculated mathematically as following (Helfferich, 1962; Hong et al., 1999):

$$M_T = \frac{r_0}{3}\sqrt{\frac{k}{D_e}} \tag{3.4}$$

where:

$M_T$ = Thiele modulus

$r_0$ = mean particle radius (mm)

$D_e$ = pore / film diffusion coefficient ($cm^2/s$)

k = kinetic coefficient (1/sec)

## 3.2. Work Plan for Issue Two

The second objective of this research was to investigate the possibility of multi-cycle ion-exchange resin bioregeneration. The hypothesis posed for this objective was:

Table 3.6. Summary of the Batch Experiments Performed to Test Hypothesis 1- Issue One

| | Resin Name | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SIR-110HP (Selective) | | | | IONAC ASB-1 (non-selective) | | | |
| Resin load | $ClO_4^-$ | $ClO_4^-$ | $ClO_4^-$ | $ClO_4^-$ | $ClO_4^-$ | $ClO_4^-$ | $ClO_4^-$ | $ClO_4^-$ |
| NaCl Conc. (mg/l) | 0 | 500 | 1000 | 5000 | 0 | 500 | 1000 | 5000 |
| mL of NaCl solution (200 g/l) | 0 | 0.057 | 0.115 | 0.575 | 0 | 0.057 | 0.115 | 0.575 |
| mL of Culture (1500 mg/L) | 15.33 | 15.33 | 15.33 | 15.33 | 15.33 | 15.33 | 15.33 | 15.33 |
| mL of Acetate (120 g/L) | 1.725 | 1.725 | 1.725 | 1.725 | 1.725 | 1.725 | 1.725 | 1.725 |
| mL of Nutrients (100X) | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 |
| mL of Buffer (100X) | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 |
| mL of DI water | 5.485 | 5.431 | 5.370 | 4.910 | 5.485 | 5.431 | 5.370 | 4.910 |
| Resin mass (g) | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 |
| Total volume of tube (mL) | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 |
| # of sample tubes / # of replicate tubes | 5 / 2 | 5 / 2 | 5 / 2 | 5 / 2 | 5 / 2 | 5 / 2 | 5 / 2 | 5 / 2 |
| Days of sampling | 1, 2, 4, 6, and 8 | 1, 2, 4, 6, and 8 | 1, 2, 4, 6, and 8 | 1, 2, 4, 6, and 8 | 1, 2, 4, 6, and 8 | 1, 2, 4, 6, and 8 | 1, 2, 4, 6, and 8 | 1, 2, 4, 6, and 8 |
| replicates for main tubes | D1QC & D8QC | D1QC & D8QC | D1QC & D8QC | D1QC & D8QC | D1QC & D8QC | D1QC & D8QC | D1QC & D8QC | D1QC & D8QC |
| # of control tubes (all replicated) | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Total No of tubes | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 |

Table 3.7. Summary of the Batch Experiments Performed to Test Hypothesis 2- Issue One

| | Resin Name | | | | |
|---|---|---|---|---|---|
| | SIR-110HP | | | | |
| Resin Size (mm) | 0.924 | 0.778 | 0.652 | 0.547 | 0.128 |
| NaCl Conc. (mg/l) | NA | NA | NA | NA | NA |
| mL of NaCl solution (200 g/l) | NA | NA | NA | NA | NA |
| mL of Culture (1500 mg/L) | 15.33 | 15.33 | 15.33 | 15.33 | 15.33 |
| mL of Acetate (120 g/L) | 1.725 | 1.725 | 1.725 | 1.725 | 1.725 |
| mL of Nutrients (100X) | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 |
| mL of Buffer (100X) | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 |
| mL of DI water | 6.060 | 6.060 | 6.060 | 6.060 | 6.060 |
| Resin mass (g) | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 |
| Total volume of tube (mL) | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 |
| # of sample tubes / # of replicate tubes | 5 / 2 | 5 / 2 | 5 / 2 | 5 / 2 | 5 / 2 |
| Days of sampling | 1, 2, 4, 6, and 8 | 1, 2, 4, 6, and 8 | 1, 2, 4, 6, and 8 | 1, 2, 4, 6, and 8 | 1, 2, 4, 6, and 8 |
| replicates for main tubes | D1QC & D8QC | D1QC & D8QC | D1QC & D8QC | D1QC & D8QC | D1QC & D8QC |
| # of control tubes (all replicated) | 1 | 1 | 1 | 1 | 1 |
| Total No of tubes | 9 | 9 | 9 | 9 | 9 |

Table 3.8. Summary of the Batch Experiments Performed to Test Hypothesis 3- Issue One

| | Resin Name | | | |
|---|---|---|---|---|
| | SIR-110HP | SIR-110HP-MACRO | IONAC ASB-1 | IONAC SR-7 |
| Resin Size (mm) | 0.778 | 0.778 | 0.778 | 0.778 |
| NaCl Conc. (mg/l) | NA | NA | NA | NA |
| mL of NaCl solution (200 g/l) | NA | NA | NA | NA |
| mL of Culture (1500 mg/L) | 15.33 | 15.33 | 15.33 | 15.33 |
| mL of Acetate (120 g/L) | 1.725 | 1.725 | 1.725 | 1.725 |
| mL of Nutrients (100X) | 0.23 | 0.23 | 0.23 | 0.23 |
| mL of Buffer (100X) | 0.23 | 0.23 | 0.23 | 0.23 |
| mL of DI water | 6.060 | 6.060 | 6.060 | 6.060 |
| Resin mass (g) | 1.20 | 1.20 | 1.20 | 1.20 |
| Total volume of tube (mL) | 25.0 | 25.0 | 25.0 | 25.0 |
| # of sample tubes / # of replicate tubes | 5 / 2 | 5 / 2 | 5 / 2 | 5 / 2 |
| Days of sampling | 1, 2, 4, 6, and 8 | 1, 2, 4, 6, and 8 | 1, 2, 4, 6, and 8 | 1, 2, 4, 6, and 8 |
| replicates for main tubes | D1QC & D8QC | D1QC & D8QC | D1QC & D8QC | D1QC & D8QC |
| # of control tubes (all replicated) | 1 | 1 | 1 | 1 |
| Total No of tubes | 9 | 9 | 9 | 9 |

Hypothesis: The bioregeneration process can be performed on perchlorate-selective macroporous anion-exchange resins for several consecutive exhaustion-bioregeneration cycles. It was expected that bioregeneration does not affect the capacity of ion-exchange resin significantly.

### 3.2.1. Experimental Approach

A perchlorate-selective resin was subjected to several cycles of loading and bioregeneration. The loading cycles were performed batch-wise, while the bioregeneration cycles were run using a fluidized bed reactor (FBR), which was connected to a fermenter that contained the perchlorate reducing culture.

To be economically feasible, the bioregeneration process must be practical to be performed for several loading-bioregeneration cycles. The number of bioregeneration cycles that must be performed depends on the virgin resin price in the market, and bioregeneration operation and maintenance costs. For this research, the resin was subjected to five cycles of the loading-bioregeneration process, due to the time and experiment costs limitations.

### 3.2.2. Experiments

#### *3.2.2.1. Resin Characteristics*

SIR-110-MP, which is a perchlorate-selective macroporous resin, was used in the experimental tests (Table 3.9). This resin is not commercially available and it was manufactured exclusively for this research. Table 3.9 presents the characteristics of SIR-110-MP resin.

Table 3.9. Characteristics of the Resin Used in the Experiments to Study Issue Two

| Resin Name | Resin Type | Water Con. (%) | Capacity (eq/L) | Resin structure | Functional group |
|---|---|---|---|---|---|
| SIR-110-MP (ResinTech) | $ClO_4^-$-selective | 58-65 | 0.6 | Styrene, Macroporous | $(C_4H_9)_3^+Cl^-$ |

*3.2.2.2. Resin Loading*

Batch loading was selected to shorten the time period required to load the resin. In the batch loading process, the ions are distributed homogeneously through the resin beads. A loading of 10 grams of perchlorate per liter of resin was selected (Table 3.11). This loading was chosen to mimic the amount of perchlorate that would be present in the resin in industrial applications. Industrial applications include waters that are contaminated with high concentrations of perchlorate (i.e. >50 ppb).

Preliminary studies performed to estimate the amounts of perchlorate load in industrial and domestic water treatment applications. Studies showed that with an influent perchlorate concentration of 10 ppb, SIR-110HP, which is a gel-type perchlorate-selective resin, can be loaded up to 410,000 bed volumes (BVs) (Seidel et al., 2006). Also, studies performed in the Environmental Engineering Laboratory at UNLV, showed that with the influent perchlorate concentration of 100,000 and 1000 ppb, SIR-110-MP resin can be loaded up to 270 and 6,120 bed volumes, respectively. Table 3.10 shows the total amount of perchlorate ion per liter of resin in both industrial and domestic applications.

Table 3.10. Estimation of Perchlorate Loads in Perchlorate Selective Resins for Industrial and Domestic Applications (Seidel et al., 2006; Studies performed at UNLV)

| Resin | Perchlorate, ppb | Bed Volumes | Total grams of $ClO_4^-$ / L of resin | Capacity (eq/L) | % capacity occupied with $ClO_4^-$ |
|---|---|---|---|---|---|
| SIR-110HP | 10 | 410,000 | 4.1 | 0.6 | 6.9% |
| SIR-110-MP | 1,000 | 6120 | 6.12 | 0.6 | 9.7% |
| SIR-110-MP | 100,000 | 270 | 27.00 | 0.6 | 42.7% |

Table 3.10 shows that in perchlorate-selective resin, depending on the influent concentration, the loading ranges from 4 to 27g perchlorate per liter of resin. The loading combination chosen to load the SIR-110-MP resin is presented in Table 3.11. The selectivity coefficients for these ions are listed in Table 2.4.

For each milliliter of the resin sample, 1 mL of the loading solution (Table 3.11) was added to a 2-L glass container and the contained was then placed on a rotary tumbler (Associated Design MFG Co., Alexandria, VA) with the rotational speed of 30 rpm. After 24 hours, the resin was separated from the liquid portion using a coffee filter paper, rinsed three times with 5 BVs of deionized (DI) water, air dried for 2 hours, and stored in the refrigerator. The liquid portion was then submitted to perchlorate, nitrate, sulfate, and chloride analysis by IC, and the amount of adsorbed ions were calculated by subtracting the residual ion in the liquid portion from the initial values. The amount of perchlorate adsorbed in the resin was then confirmed by the residual perchlorate measurement analysis using the oxygen Parr Bomb.

Table 3.11. Loading Combination Used to Load the SIR-110-MP Resin for Issue Two of This Research

| Component | Concentration of Stock Loading Solution (mg/L) |
|---|---|
| Perchlorate | 10,000 |
| Nitrate | 460 |
| Sulfate | 500 |
| Chloride | 500 |
| Bicarbonate | 500 |

*3.2.2.3. Perchlorate Reducing Master Culture*

Perchlorate-reducing microbial culture for this part of the research was taken from two master seed cultures, called BALI I and BALI II. The characteristics of the master seed culture have been described in detail in section 3.1.2.4.

*3.2.2.4. Fermenter Start-up and Monitoring*

A 10-gallon (37.8 L) HDPE plastic fermenter connected to a 3-inch diameter (7.6 cm) x 50 inch (127 cm) tall plexiglass fluidized bed reactor (FBR) was used in this research. The fermenter was covered with a HDPE lid and sealed with a weather strip to avoid oxygen entry. The fermenter was instrumented with a +GF+ Signet pH/ORP meter (J.L. Wingert Co., Garden Grove, CA) and a Omega dissolved oxygen meter (Omega Engineering Inc., Stamford, CT). The microbial culture in the fermenter was monitored for SS, pH, DO, COD, ORP, conductivity, and perchlorate residual level. A stirrer was used to mix the bacterial enrichment culture in the fermenter at 45 rpm rotational speed. The fermenter contained three ports. The first port was used for nutrient feeding using a peristaltic pump. The second port was used to waste the culture from the bottom of the fermenter when it was needed. The third port was used as a vent fitted with a “U” shape

tube that is connected to a 1-L HDPE bottle that contained a 10% NaOH solution. This set-up served as a scavenger of the gases produced in the fermenter. Figure 3.2 shows the schematic design of the fermenter that contains microbial culture and the FBR that contains the loaded resin.

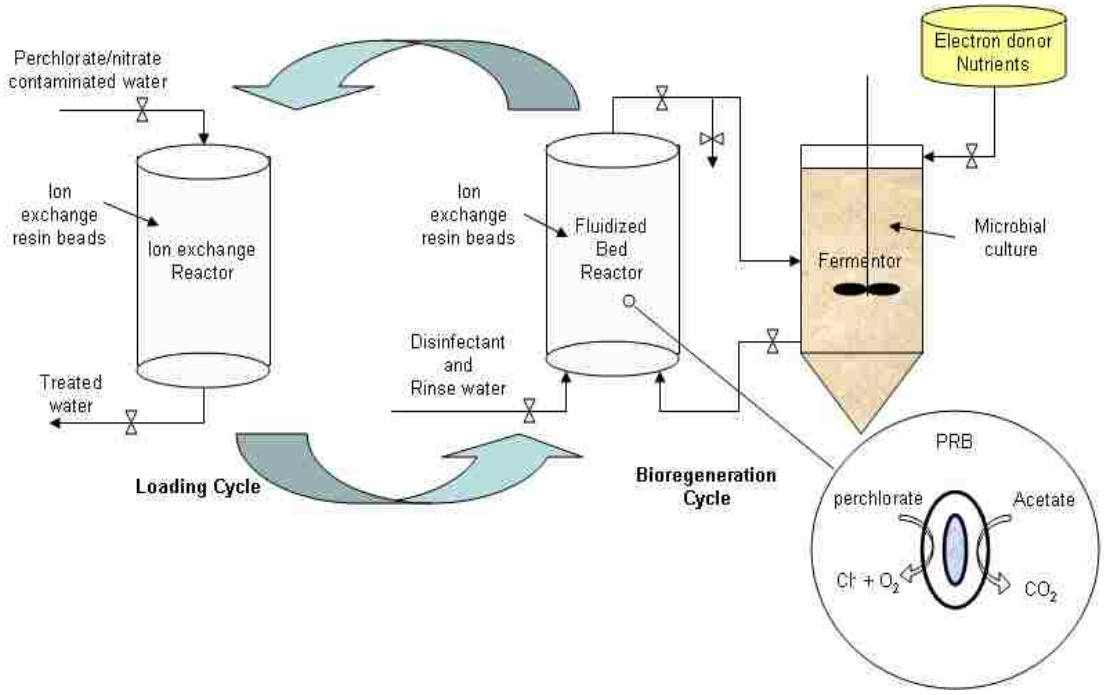


Figure 3.2. Schematic Design of the Fermenter and the FBR

At start-up, approximately 4-L of seed culture, was taken from the master microbial reactor and transferred into the 10-gallon fermenter. Perchlorate, acetate, nutrient, and buffer solutions were then added to the fermenter to foster microbial growth. The composition of the nutrient and buffer solutions used is depicted in section 3.1.2.5. Perchlorate degradation was monitored with time, and more perchlorate, acetate, nutrient,

and buffer solutions were added until 30-L of microbial solution with a SS of about 1500 mg/l was achieved. The culture was not wasted during the start-up period.

Prior to starting the resin bioregeneration cycles, all perchlorate remaining in the fermenter was allowed to biodegrade, so that no perchlorate was present in the microbial solution.

*3.2.2.5. Bioregeneration Procedure for FBR Reactor*

One thousand five hundred mL (1500 mL) of loaded SIR-110-MP resin (procedure shown in section 3.2.2.2) was transferred to the FBR column. The bacterial culture contained in the fermenter was then fed up-flow using a peristaltic pump with a flow rate of 1250 mL/min to have 30 to 40% expansion in the resin bed. Then the microbial culture was returned back to the fermenter. The culture was monitored for SS, pH, DO, COD, ORP, conductivity, and perchlorate residual, daily. The minimum amount of acetate in the bioreactor was kept above 1500 mg/L. The pH of the fermenter was maintained between 7.0 and 8.0 using a phosphate buffer. ORP and temperature variations also were controlled. DO was kept below 0.2 mg/L.

Daily, a 4 mL resin sample was taken from the FBR ports located along the FBR column. The resin was then rinsed with 1 BV of DI water 5 times to remove microbial cells. Then, the resin sample was transferred to a 15-mL plastic container with about 10-mL of DI water on top, labeled, and stored in the refrigerator. Finally, the resin samples were subjected to residual perchlorate analysis using an oxygen Parr Bomb (Appendix A).

Resin bioregeneration process was continued until the residual perchlorate concentration in the resin remained constant. Bioregeneration processes ran for a period

of 9-14 days. After completion of bioregeneration process, the microbial culture in the FBR was transferred from the bottom of FBR to the fermenter using a peristaltic pump. The resin was then rinsed 5 times with 1 BV of DI water to remove the remaining microbial cells and organics which might remain in the resin. To ensure the rinsing procedure is effective, % transmittance of the rinsate solutions were measured. The resin was then submitted to the fouling removal process (section 3.2.2.6). This procedure for loading-bioregeneration of the resin was repeated for five consecutive times. Since there was not any electron acceptor to maintain the microbial culture growth while the resin was under defouling and reloading processes, the fermenter was fed directly with perchlorate and acetate solutions. Phosphate buffer was added to the fermenter to control pH variation. Suspended solids (SS) of 1500 mg/l and zero remaining perchlorate in the fermenter were needed to start the next bioregeneration cycle.

*3.2.2.6. Defouling and Disinfection of the Resin*

Defouling was performed using a NaOH/NaCl mixture and disinfection was carried out using a sodium hypochlorite solution. Preliminary investigations showed that NaOH/NaCl mixture remove the fouling resulted from the bioregeneration process with an acceptable efficiency by visual observation. Furthermore, sodium hypochlorite was shown to have better disinfection and defouling effect compared to the other common disinfectants (Batista et al., 2007a). Preliminary investigations showed that 1.5 bed volumes of 100 mg/L of total chlorine residual is sufficient to reach the required *E.Coli* and heterotrophic plate counts (HPC) (i.e. <1 MPN/100mL for *E.Coli* and <500 CFU/mL for HPC) after resin disinfection. In addition, preliminary suggested that if excessive amount of sodium hypochlorite is used, more disinfection by-product (i.e. NDMA) will

be produced as a result of the presence of high free-chlorine. The results of the preliminary tests were used to develop the following procedure for fouling removal and disinfection of bioregenerated ion-exchange resins. The fouling removal and disinfection procedure is summarized in Table 3.12.

Defouling of the resin was performed in 4 steps:

1) Soaking for 16 hours in 1.5 BV of the 6% NaCl/ 0.5% NaOH solution,
2) Soaking for 2 hours in 1.5 BV of the 6% NaCl/ 0.5% NaOH solution,
3) Soaking for 2 hours in 12% NaCl solution, and
4) Rinsing with six bed volumes of DI water.

All the solutions were pumped up-flow with a small flow rate of 80 mL/min. Chemical oxygen demand (COD) of the rinsate solutions after each step of fouling removal procedure was measured.

Table 3.12. - Fouling Removal and Disinfection Procedure Used after Bioregeneration Process

| | Fouling removal reagent | Applied volume | Retention time |
|---|---|---|---|
| Fouling removal procedure | 12% NaCl + 2% NaOH | 1.5 BV | 12 hours |
| | 12% NaCl + 2% NaOH | 1.5 BV | 4 hours |
| | 12% NaCl | 1.5 BV | 2 hours |
| | DI water rinse | 3 BV | N/A |
| Disinfection procedure | 100 mg/L free chlorine using sodium hypochlorite | 1.5 BV | 15-20 min |
| | DI water rinse | until no residual chlorine is detected in the rinsate | |

The resin was disinfected with 1.5 BV of 100 mg/l sodium hypochlorite solution. The disinfectant agent was pumped up-flow to the column with a small flow rate of ~80 mL/min. The residence time was 15 to 20 minutes. The resin was then rinsed with six BVs of DI water. Total Coliform test using IDEXX Quanti-Tray method (IDEXX Laboratories, Inc., Westbrook, ME) was performed on the rinsate solution after disinfection step. The bioregenerated and disinfected resin was then loaded batch wise again (section 3.2.2.2) to commence the new bioregeneration cycle. The process was repeated five times consecutively.

*3.2.2.7. Resin Capacity Measurement*

It was expected that bioregeneration would result in decreased resin capacity after each cycle. Therefore, the total capacity of fresh and bioregenerated resin was measured. To measure the capacity, 15 mL of wet resin was placed in a pipette filled with a stopcock. One L of 4.0% HCl was passed through the resin bed to convert the resin to the chloride form. Next the resin was rinsed with 1 L of DI water to rinse interstitial chloride. One L of 1.0 N $NaNO_3$ solution was then passed through the resin to replace the chloride ions with nitrate. The effluent from the $NaNO_3$ rinse was collected and titrated with $AgNO_3$ to measure the chloride concentration. Theoretically, each mole of detected chloride in the effluent corresponds one mole of nitrate exchanged by the active functional groups. The resin capacity in equivalents/L is then calculated using the chloride measurements.

## 3.3. Analytical Methods

### 3.3.1. Analysis of Perchlorate, Nitrate, and Chloride

All perchlorate concentrations and low concentrations of chloride was measured using Dionex ICS-2000 Ion Chromatograph (IC) (Dionex Corporation, Sunnyvale, CA), consisting of an Ion Suppressor-ULTRA II (4 mm), IonPac AS16 (4 mm) analytical, AG16 (4 mm) guard columns, and an AS16 autosampler. For perchlorate, EPA method 314.0 was used with a current of 100 mA and a NaOH concentration of 35 mM with a flow rate of 1.0 mL/min. A calibration curve was established using perchlorate standard solutions with concentrations between 5 and 100 µg/L (i.e. 5, 10, 25, 50, 75, and 100 µg/L). A coefficient of determination of 99.97% was used for calibration. Similarly, a current of 100 mA and a NaOH concentration of 35 mM with a flow rate of 1.0 mL/min were used to measure low-concentration chloride ion. Calibration curve for low-concentration chloride was plotted with standard solutions with concentrations between 100 and 500 µg/L (100, 200, 300, 400, and 500 µg/L) using a coefficient of determination of 99.97%. For nitrate, sulfate, and high concentrations of chloride anions, IonPac AS20 (4 mm) analytical and AG16 (4 mm) guard columns were used on the same IC with a current of 110 mA and a NaOH concentration of 30 mM and a flow rate of 1.0 mL/min. The calibration curve for nitrate, sulfate, and high concentrations of chloride anions measurement was prepared for concentrations between 1 and 10 mg/L and a 99.99% coefficient of determination.

### 3.3.2. Residual Perchlorate Measurement (Oxygen Combustion Bomb Method)

The amount of perchlorate left in the resin sample was analyzed indirectly using Oxygen Parr Bomb apparatus (Parr Bomb 1108, Parr Instruments, Moline, IL). Parr

Bomb (oxygen combustion bomb) was used to ignite the resin sample and convert the residual perchlorate in the resin sample to chloride ions. Parr Bomb, as it is shown in Figure 3.3, is a closed stainless steel cylinder. This stainless steel cylinder can tolerate an inside pressure of 100 psi. The detailed Parr Bomb procedure, developed by UNLV, is shown in Appendix A.

Figure 3.3: Oxygen Combustion Bomb 1108 (Parr Instruments)

### 3.3.3. Total Suspended Solids (TSS)

The total suspended solids (TSS) was measured using a filtration apparatus (Thermo Fisher Scientific, Waltham, MA). A glass microfiber filter with an average pore diameter of 0.45 µm (Whatmann glass microfiber filters (GFC)) was placed in the filtration apparatus as the filter paper. The initial weight of a microfiber filter paper and an aluminum dish (Thermo Fisher Scientific, Waltham, MA) was measured and recorded. A known volume of the sample was measured using a micropipette and passed through the filter paper. Then the filter paper in the aluminum dish was dried in 105ºC oven (Thermo

Fisher Scientific, Waltham, MA) for 60 min. The amount of TSS was then calculated by subtracting the initial weight from the final weight divided by the volume of the sample.

### 3.3.4. Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) was measured using Hach COD digestion vials (Hach Company, Loveland, CO) in three different renges: high range (0-1500 mg/l), low range (0-150 mg/l) and ultra low range (0-40 mg/l). Based on the strength of the sample, the appropriate Hach COD vial was selected and used. Dilutions performed to ensure the COD of the sample is in these ranges, when it was needed. Two mL (2 mL) of sample was pipetted and placed in the COD vial. The Hach COD vial was then placed in a Hach DRB-200 Dry Thermostat COD digester (Hach Company, Loveland, CO) for 120 min at the constant temperature of 150ºC. After 120-min digestion time, the vial was cooled down for 20 min at the room temperature. Lastly, the COD of the vial was measured using a Hach DR-3000 spectrophotometer (Hach Company, Loveland, CO).

### 3.3.5. pH

The pH of the samples was measured using a Fisher Scientific model AR25 pH meter (Thermo Fisher Scientific, Waltham, MA). Every day the pH meter was calibrated according to the pH meter operation instructions. Two standard pH solutions of 7.00 and of 10.01 (Thermo Fisher Scientific, Waltham, MA) were used to perform the calibration procedure. If the slope was above 90%, the pH meter was considered calibrated.

### 3.3.6. Conductivity

The conductivity of the samples was measured using YSI (Model # 30/10 FT) conductivity meter (YSI, Inc., Warm Springs, OH). The temperature of the samples was 22±2ºC for the conductivity analysis.

### 3.3.7. Dissolved Oxygen (DO)

The dissolved oxygen (DO) of the samples was measured using YSI Model 58 Dissolved Oxygen meter (YSI, Inc., Warm Springs, OH). The Dissolved Oxygen meter was calibrated daily according to the operation instructions. Barometric pressure and room temperature were used to calibrate the DO meter. Barometric pressure and room temperature was measured using Princo barometer model 453 (Princo Inc., Southampton, PA).

### 3.3.8. Turbidity

The turbidity of the samples was measured using a Hach turbidimeter Model 2100N (Hach Company, Loveland, CO). The turbidimeter was calibrated before every measurement. According to the operation instructions, five company-sealed Formazin standard turbidity vials were used to calibrate the turbidimeter.

### 3.3.9. Absorbance and Optical Density (OD)

The absorbance and optical density of the samples were measured using a Hach DR 3000 Spectrophotometer (Hach Company, Loveland, CO). DI water was used to calibrate the spectrophotometer before each measurement. The samples were scanned for all wavelengths, and the maximum hits were selected to use as the wavelength of measurement.

### 3.3.10. Scanning Electron Microscopy (SEM)

Fresh and bioregenerated resin samples were rinsed with 5 BVs of DI water and air-dried for 24 hours at 22±2ºC. Scanning electron microscopy imaging of the resin samples was performed using Jeol JSM-7500F SEM (JEOL Ltd., Tokyo, Japan) employing secondary electron detector at 1.00 kV.

## 3.4. Analysis of Data

### 3.4.1. Introduction

In this research, several bioregeneration experiments were performed. Chemical analyses were conducted to evaluate the performance of the bioregeneration tests. The main chemical analysis was the determination of residual resin-attached perchlorate in the resin sample with time for which an oxygen Parr bomb method was developed. Other analyses included perchlorate, nitrate, chloride, and sulfate measurements by IC, COD, TSS, and optical density (OD). The experimental data were analyzed statistically in order to interpret the obtained results.

### 3.4.2. Bioregeneration Data Analysis – Issue One

#### *3.4.2.1. Data Analysis for Hypothesis 1*

Hypothesis 1 of issue one involved batch bioregeneration experiments using different amounts of chloride. In the batch bioregeneration experiments, resin samples were taken according to the schedule (Table 3.6), and residual resin-attached perchlorate was analyzed in the samples. For each set of the batch experiments (Table 3.6), residual resin-attached perchlorate was plotted against time (i.e. day) to observe the bioregeneration of perchlorate throughout the days. For each resin type (i.e. perchlorate-selective and non-selective resins), four curves representing four initial chloride

concentrations were plotted. Each set of experiments contain 5 data points, 2 of which have replicates (i.e. day 1 and day 8). The data obtained from replicate samples were compared using independent t-test assuming a 95% confidence interval ($p<0.05$) in Excel spreadsheet. The null hypothesis was that the difference in residual resin-attached perchlorate concentration between replicates is zero. The alternate hypothesis was that the residual resin-attached perchlorate concentration between the replicate samples is different. Up to 5% deviation per the average of the replicate values was acceptable, otherwise the residual resin-attached perchlorate analysis were repeated.

To evaluate the effect of chloride between four data sets of bioregeneration experiments and to observe whether the data sets are significantly different, two-way analysis of variance (i.e. ANOVA) using the F statistical test was performed on the replicated data points (i.e. day-1 and day-8) (Table 3.13). SPSS Statistics, version 16.0, (SPSS Inc., Chicago, IL) was used to perform the ANOVA test.

The confidence interval was 95% for statistical analysis ($p<0.05$). The null hypothesis of the test was that the biodegradation in all four initial chloride concentrations are similar. The alternate hypothesis was that the residual resin-attached perchlorate degradation pattern associated with different initial chloride concentrations among the data sets is significantly different.

*3.4.2.2. Data Analysis for Hypothesis 2*

Hypothesis 2 of issue one investigated the controlling step in the bioregeneration envisioned model by evaluating the effect of resin particle size on the bioregeneration process (Table 3.7). The collected data (i.e. residual resin-attached perchlorate

concentration) were plotted against time (i.e. day). In total, 5 bioregeneration curves were plotted.

The data obtained from replicate samples of each bioregeneration test were compared using an independent t-test assuming a 95% confidence interval ($p<0.05$). The null hypothesis was that the difference in the residual resin-attached perchlorate concentration between replicates is zero. The alternate hypothesis was that the residual resin-attached perchlorate concentration between the replicate samples is different. Up to 5% deviation per the average of the replicate values was acceptable, otherwise the residual resin-attached perchlorate analysis were repeated

Table 3.13. Data Input Arrangement for SPSS to Perform ANOVA Test for the Experiment Evaluating the Effect of Initial Chloride Concentration

| Initial chloride concentration (mg/L) | Day-1 | Day-8 |
|---|---|---|
| 0 | D1-0 | D8-0 |
| 0 | D1-0-QC | D8-0-QC |
| 500 | D1-500 | D8-500 |
| 500 | D1-500-QC | D8-500-QC |
| 1000 | D1-1000 | D8-1000 |
| 1000 | D1-1000-QC | D8-1000-QC |
| 5000 | D1-5000 | D8-5000 |
| 5000 | D1-5000-QC | D8-5000-QC |

To evaluate the effect of resin size among five data sets of bioregeneration experiments, two-way analysis of variance (i.e. ANOVA) using the F statistical test was

performed on the replicated data points (i.e. day-1 and day-8) assuming 95% confidence interval (Table 3.14). The null hypothesis of the test was that the biodegradation data in all five experiments with different resin sizes is similar. The alternate hypothesis was that the biodegradation experiments among the data sets associated with different resin sizes are different.

*3.4.2.3. Data Analysis for Hypothesis 3*

In the experiments associated with hypothesis 3, perchlorate-selective and non-selective resins in the form of gel- and macroporous-type were subjected to bioregeneration process (Table 3.8). The obtained data (i.e. residual resin-attached perchlorate) were plotted against time (i.e. day). In total, 4 bioregeneration curves representing two gel-type resins (i.e. perchlorate-selective and non-selective resins) and two macroporous resins (i.e. perchlorate-selective and non-selective resins) were plotted.

Table 3.14. Data Input Arrangement for SPSS to Perform ANOVA Test for the Experiment Evaluating the Effect of Resin Bead Size

| Resin particle size (mm) | Day-1 | Day-8 |
|---|---|---|
| 0.924 | D1-0.928 | D8-0.928 |
| 0.924 | D1-0.928-QC | D8-0.928-QC |
| 0.778 | D1-0.778 | D8-0.778 |
| 0.788 | D1-0.778-QC | D8-0.778-QC |
| 0.652 | D1-0.652 | D8-0.652 |
| 0.652 | D1-0.652-QC | D8-0.652-QC |
| 0.547 | D1-0.547 | D8-0.547 |
| 0.547 | D1-0.547-QC | D8-0.547-QC |
| 0.128 | D1-0.128 | D8-0.128 |
| 0.128 | D1-0.128-QC | D8-0.128-QC |

All data obtained from replicate samples were compared using an independent t-test assuming a 95% confidence interval ($p < 0.05$). The null hypothesis was that the difference in the residual perchlorate concentration between replicates is zero. The alternate hypothesis was that the residual perchlorate concentration between the replicate samples is different. Up to 5% deviation per the average of the replicate values was acceptable, otherwise the residual resin-attached perchlorate analysis were repeated

To evaluate the effect of resin structure (i.e. gel-type vs. macroporous resin) among four data sets of bioregeneration experiments, two-way analysis of variance (i.e. ANOVA) using the F statistical test was performed on the replicated data points (i.e. day-1 and day-8) assuming 95% confidence interval. The null hypothesis of the test was that the biodegradation data in all four experiments with different resin sizes is similar. The alternate hypothesis was that the two biodegradation experiments for gel-type resins (i.e. ASB-1 and SIR-110HP) are different with the two biodegradation experiments for macroporous resins (i.e. SIR-110HP-MACRO and SR-7).

### 3.4.3. Bioregeneration Data Analysis – Issue Two

Issue two of this research involved batch loading and FBR bioregeneration of SIR-110-MP resin for five consecutive cycles. Daily collected data (i.e. residual resin-attached perchlorate concentration) were plotted against time (i.e. day) to observe the bioregeneration rate of the FBR bioregeneration process. In total, 5 bioregeneration curves were plotted.

Descriptive statistical analyses, including mean, standard error, standard deviation, variance, sample range, and skewness, on each data set were performed. The broader sample range (i.e. the difference between first day and last day of bioregeneration

process) indicated the more efficiency in perchlorate biodegradation. In addition, the higher skewness in the data sets indicated that the bioregeneration curve is steeper at first.

The resin capacity loss for each bioregeneration cycle was calculated by subtracting the final resin capacity after each bioregeneration cycle from the initial capacity of the resin before that particular cycle. Descriptive statistical analyses, including mean, standard error, and standard deviation on the resin capacity loss data were performed. Lastly, the total capacity loss for the resin bed was calculated by subtracting the final capacity of the resin from the initial capacity of the virgin resin.

## 3.5. Quality Assurance/Quality Control (QA/QC)

### 3.5.1. Introduction

The quality assurance / quality control evaluation is essential to guarantee the quality of the results collected during the experimental phase. Minimizing the systematic errors (i.e. procedure, instrumental, and human error) assures the quality of the analysis, while checking the accuracy, precision, and detection limits of the employed methods control the quality of the collected data.

### 3.5.2. Quality Assurance

The critical parameters being analyzed in this research were the residual resin-attached perchlorate concentration, chloride, perchlorate, COD, optical density (DO), TSS, pH, DO, and conductivity. Residual resin-attached perchlorate analysis was directly involved in determination of the bioregeneration process' performance. TSS, DO, COD, pH, conductivity, chloride, and perchlorate analyses were critical to maintain the fermentor / master seed culture in an appropriate condition. The goal was to collect all the data through the experimental phase accurately. However, error can occur through

data collection. The main sources of errors in the posed experimental procedures were: human error, lack of balance calibration, lack of Ion Chromatograph (IC) calibration, lack of pipette calibration, lack of turbidimeter calibration, lack of pH meter calibration, lack of DO meter calibration, and inappropriate resin sample rinsing and/or preservation procedure. To minimize the error sources, the following precautions were taken:

1) The IC that was used to measure chloride, perchlorate, nitrate, and sulfate was calibrated weekly. The calibration curve for perchlorate with standard solutions was performed between 5 and 100 ppb (i.e. 5, 10, 25, 50, 75, and 100 ppb) using 99.97% coefficient of determination. For low-concentration chloride, the calibration curve was between 100 and 500 ppb (100, 200, 300, 400, and 500 ppb) using 99.97% coefficient of determination. For nitrate, sulfate, and high concentrations of chloride the calibration curve was between 1 and 10 ppm (i.e. 1, 2.5, 5, 7.5, and 10 ppm) using 99.99% coefficient of determination.

2) To prevent carry over in the IC measurements, the prepared samples were measured from low to high concentrations. Furthermore, blank samples (i.e. DI water) were introduced between the samples to assure there is no carry over.

3) The calibration of the analytical balances, which was used to weigh the chemicals for the solutions and the resin samples, was checked weekly. Five grams (5 g) and 50 g standard weights were used to calibrate the balances every week. In the Environmental Engineering Lab at UNLV, the balances were also calibrated every six months by a contractor.

4) The micropipettes were calibrated every week. Various volumes of water were weighed using an analytical balance. If the weight of the water in grams was the same as the volume of transferred water, the micropipette considered as calibrated, otherwise, it was sent to the outside contractor for calibration.

5) The appropriate pipette tips as it is recommended by the micropipette manufacture were used.

6) The spectrophotometer was calibrated before every use.

7) The turbidimeter was calibrated before every use using standard Formazin solutions.

8) A thermometer was maintained in the oven, and it was monitored every week to ensure the consistent temperature.

9) All resin samples were rinsed at least six times with DI water to ensure that all the cells have been rinsed off from the sample and there is no electron donor / electron acceptor is in contact with the resin sample.

10) All the resin and culture samples were stored in the refrigerator immediately.

11) All glass micro-fiber filter papers, which were used to measure TSS, were stored in the desiccators prior to use to avoid moisture interference.

12) Aluminum dishes which were used for TSS analysis were preignited at 550ºC for about an hour to avoid weight loss during the TSS analysis.

13) DO and pH meters were calibrated every day. Two standard pH solutions of 7.00 and of 10.01 were used perform the calibration procedure. If the slope was above 90%, the pH meter was considered as calibrated. DO meter was

calibrated using 100% air saturation method. For this method, the barometric pressure and temperature were measured using Princo barometer.

14) All the stock solution were capped properly and stored in the refrigerator to avoid chemical/biological reactions and also evaporation.

15) Deionized (DI) water was used to prepare solutions, rinse the glassware, and rinse the resin samples. The resistance of the DI water was monitored daily to be above 17 Mohm-cm. When the resistance values dropped below 17 Mohm-cm, the IX column of the DI water system was replaced.

16) The temperature of the COD digester was monitored using a thermometer every time to ensure the digester is at 150ºC.

17) A stop-watch was used to record the time intervals for all of the proposed experimental procedure specially for the residual perchlorate analysis.

18) All the Parr bomb ignitions were cooled down in a bucket of DI water for 20 minutes.

19) All components (except the resin) of the control tubes of the experiments associated with issue one, were autoclaved to ensure that there is no bacteria present in the blank tubes.

20) All the parameters measured for the QA/QC purpose were recorded in a bound logbook.

21) Glassware were soaked in soap for at least six hours and washed with tap water and triple rinsed with DI water.

### 3.5.3. Quality Control

Accuracy, precision, detection limit, and the coefficient of determination ($R^2$) of the chemical analyses that were used in this research are listed in Table 3.15. The accuracy of the obtained data was determined by using the known standard solutions. The precision was determined by performing replicate measurements. For the Parr bomb method, the precision was repetition of the residual resin-attached perchlorate concentration measurement by replication the whole procedure for every five samples.

Table 3.15: Accuracy, Precision, Detection Limit, and the Coefficient of Determination of the Chemical Analyses of Various Parameters

| Parameter | Method | $R^2$ | Detection Limit | Precision[a] | Calibration Range |
|---|---|---|---|---|---|
| Perchlorate | IC | 0.9997 | 5 ppb | 95% confidence limit | 5 – 100 ppb |
| Chloride (High Range) | IC | 0.9999 | 1 ppm | 95% confidence limit | 1 – 10 ppm |
| Chloride (Low Range) | IC | 0.9997 | 100 ppb | 95% confidence limit | 100 – 500 ppb |
| Nitrate | IC | 0.9999 | 1 ppm | 95% confidence limit | 1 -10 ppm |
| Sulfate | IC | 0.9999 | 1 ppm | 95% confidence limit | 1 – 10 ppm |
| COD (High Range) | HACH Manganese III | NA | 0.1 ppm | 95% confidence limit | 0 – 1500 ppm |

[a] Replicate samples were run every five samples.

# CHAPTER 4

# INVESTIGATION OF POTENTIAL MECHANISMS OF ION-EXCHANGE RESIN BIOREGENERATION

## 4.1. Abstract

Ion-exchange (IX) is possibly the most feasible technology for perchlorate removal. Perchlorate-selective and non-selective IX resins are commercially available. Selective resins are incinerated after one time use, and non-selective resins produce a regenerant waste stream that contains high concentration of perchlorate. A process involving directly contacting spent IX resin containing perchlorate with perchlorate-reducing bacteria (PRB) to bioregenerate the resin has been recently developed. In this process PRB biodegrade perchlorate ions that are strongly attached to the functional groups of the resin. In this study, the potential mechanisms for bioregeneration of resin-attached perchlorate (RAP) were envisioned and investigated. It was envisioned that the bioregeneration process involves four steps. First, RAP ions are desorbed from their original functional groups promoted by chloride ion. Second, perchlorate ions are diffused through the pores of the resin. It is expected that this diffusion is affected by both resin bead size and structure. Third, perchlorate ions are transferred through the liquid film surrounding the resin to the bulk liquid. Forth, perchlorate ions are utilized by the PRB present in the bulk liquid. It is hypothesized that mass transfer is controlling the bioregeneration process. In this research, batch bioregeneration experiments were performed using resin samples loaded with perchlorate. In the batch tests, different initial chloride concentrations were used to investigate the effect of chloride as the desorbing agent. Different resin bead sizes were used to examine the effect of resin bead size on the

pore diffusion (i.e. second step). Gel-type and macroporous resins with uniform bead size were used to study the effect of resin structure on pore diffusion, as well. The results of the bioregeneration experiments suggested that chloride, the product of perchlorate biodegradation, is more likely the desorbing agent of RAP, and increasing the concentration of chloride enhances the bioregeneration process. For commercially available resins, both film and pore diffusion found to be effective in the rate of mass transfer. Also, macroporous resins were found more effective than gel-type resins in the bioregeneration process. The outcome of this study implies that in resin bioregeneration, the use of macroporous resin with relatively small bead size in presence of higher chloride concentration would be preferred. Chloride concentration, however, should be monitored and kept below the inhibitory level for PRB microbial activities.

## 4.2. Introduction

Perchlorate is both a man-made and a naturally occurring contaminant, and is a national drinking water concern because of its widespread use in aerospace and defense industries (USEPA, 2003; Rao, et al., 2007). Perchlorate has been on the United States Environmental Protection Agency's (USEPA's) drinking water Contaminant Candidate List (CCL) since 1998 (Brandhuber and Clark, 2005) and is a regulated drinking water contaminant in nine states in the United States (US) (Tikkanen, 2006; Sellers et al., 2007). Toxicity of perchlorate is well-known through its interference with iodide uptake by thyroid gland through functioning of the sodium ($Na^+$) / iodide ($I^-$) symporter in the gland, resulting in deficiency of thyroid hormones (Kirk, 2006; Wolff, 1998, Stoker et al., 2006). The most effective technologies to remove perchlorate from waters are biological reduction and IX (Logan et al., 2001; Gingras and Batista, 2002; Lehman et al., 2008).

Biodegradation is mostly practical for waters with high concentrations of perchlorate, due to the relatively high half-saturation constant for perchlorate degradation (Logan et al., 2001; Waller et al., 2004). Biological reduction of perchlorate dissolved in water has been well studied. All the PRB known to date are gram-negative bacteria (Waller et al., 2004; Shrout et al., 2005). It has been proven that the perchlorate-reduction pathway goes from perchlorate ($ClO_4^-$) to chlorate ($ClO_3^-$), then to chlorite ($ClO_2^-$), and finally to chloride ($Cl^-$) (Rikken et al., 1996; Logan, 1998). Stoichiometrically, 1 mole (M) chloride is released as the reduction waste product of 1 M perchlorate (Rikken et al., 1996). Hence, chloride ion is always present in a bioreactor, where PRB are grown using perchlorate as the electron acceptor. Additionally, it has been shown that the PRB use two distinct enzymes located in the periplasmic area of the cell to reduce perchlorate to chloride (Rikken et al., 1996; Kengen et al., 1999; Logan, 1998), and c-type cytochrome(s) is(are) involved in the respiratory electron transfer chain (Coates et al., 1999; Bender et al., 2005). Free perchlorate ions in water are transferred across the outer membrane of the PRB cell to the periplasmic area, where the enzymes required for perchlorate reduction are located. Therefore, the current knowledge dictates that the perchlorate ion is biodegraded inside PRB cell.

Ion-exchange is currently the technology of choice to remove perchlorate from drinking waters contaminated with low concentrations of perchlorate (Gingras and Batista, 2002; Lehman et al., 2008). Perchlorate can be effectively removed from water by perchlorate-selective and perchlorate-non-selective resins. Based on their structure, both perchlorate-selective and non-selective resins can be categorized into gel-type or macroporous-type. The average pore size and percentage water retention in macroporous

resins are considerably higher than those of gel-type resins (Sherman et al., 1986). The average size of micropores in gel-type resins is about 0.0005 µm, while macroporous resins in addition to have micropores, contain macropores with the average size of 0.6 µm (Kun and Kunin, 1968; Dale et al., 2001). Water retention of macroporous resins is approximately 11% higher than that of gel-type resins (Du et al., 2010). The use of macroporous resins compared to gel-type resins is expanding due to their stability, resistance to oxidation, and less vulnerability to fouling (Weber, 1972; Li and SenGupta, 2000).

Although IX is a well-known technology to water utilities, it has some deficiencies. First, it only separates perchlorate from water and it does not destroy it. Second, in the case of non-selective resins, the regenerant waste stream contains a high concentration of perchlorate that must be treated and disposed of. In the case of selective resins, the resin cannot be effectively regenerated and therefore it is incinerated. This constitutes a major challenge and cost for perchlorate removal with IX process.

Resin bioregeneration as a new concept in IX technology has been developed and patented (Batista, 2006). This concept is based on directly contacting perchlorate-containing IX resin with a PRB culture under anoxic/anaerobic conditions. Although the biological reduction of free perchlorate ions in water has been well studied (Logan, 1998; Coates and Achenbach, 2004), the biological reduction of RAP ions has only recently been initiated (Wang et al., 2009; Venkatesan et al., 2010). Perchlorate-selective and non-selective IX resins can be directly bioregenerated (Batista and Jensen, 2006; Batista et al., 2007b; Wang et al., 2008b), leading to the conversion of RAP on the resin to innocuous free chloride ions. However, the mechanisms involved in resin

bioregeneration have not yet been fully elucidated. In this research, potential mechanisms responsible for resin bioregeneration are investigated.

### 4.2.1. Potential Mechanism for Ion-Exchange Resin Bioregeneration

It has been shown that, typically, perchlorate-reducing bacteria isolates are rod-shape with cell sizes ranging between 1.0 µm x 3.0 µm and 1.5 µm x 7.0 µm (Wolterink et al., 2002), while the average pore size of gel-type and macroporous resins are 0.0005 µm and 0.6 µm, respectively (Kun and Kunin, 1968; Dale et al., 2001). Hence, it is not possible for PRB to enter the pores of IX resin beads during the bioregeneration process. The known involvement of c-type cytochrome(s) in the respiratory electron transfer chain (Coates et al., 1999; Bender et al., 2005) and the strong attachment of perchlorate ions to the active functional groups of the IX resin brings to question the mechanisms by which RAP can be degraded. In this research, it is envisioned that the degradation of RAP ions involves four steps as following: (1) Desorption of perchlorate from the resin's functional groups. It is hypothesized that perchlorate desorption is promoted by chloride ions which are the waste product of perchlorate biodegradation and are available in all PRB reactors fed perchlorate as the electron acceptor (Rikken et al., 1996); (2) Diffusion of the desorbed perchlorate from the interior of the resin bead to the resin bead surface through the resin pores. Pore diffusion is controlled by both resin bead size and the resin structure. If the bioregeneration process is controlled by pore diffusion, reducing the resin bead size would accelerate the diffusion process (Helfferich, 1962). Additionally, macroporous resins, which have higher water content and larger pore sizes compared to gel-type resins, are expected to have higher diffusion rates and as a result better performance in the bioregeneration process; (3) Diffusion of perchlorate ion through a

liquid film on the surface of resin bead to the bulk microbial fluid; and (4) biodegradation of the perchlorate ion by PRB in the bulk microbial fluid, where nutrients and electron donors are present. Perchlorate ions should enter the periplasmic region of the PRB where the enzymes responsible for perchlorate reduction are located. The envisioned conceptual model for biodegradation of RAP is shown in Figure 4.1. The bioregeneration mechanisms hypothesized above assumes that some initial chloride concentration is already present in the microbial fluid used in the biodegradation process.

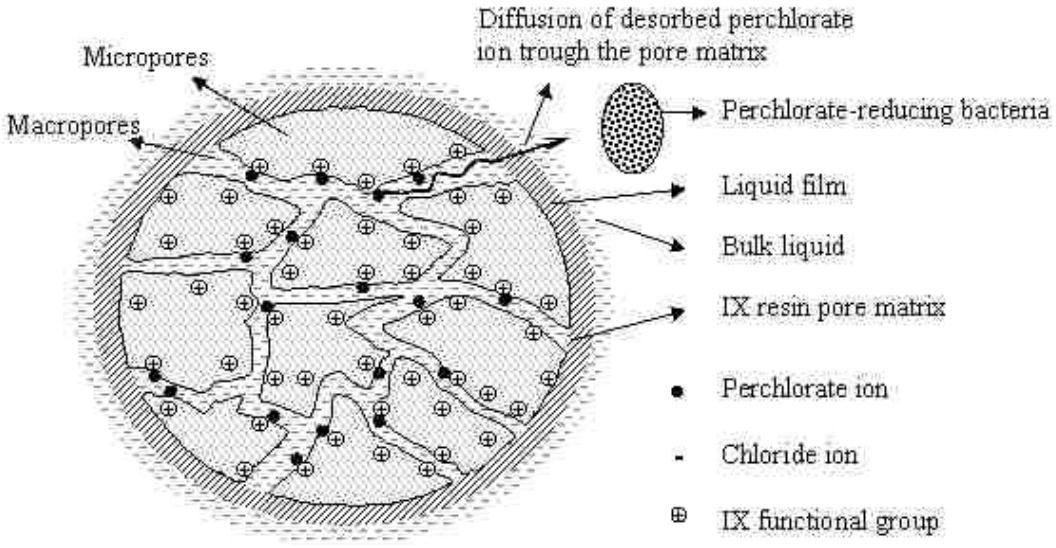


Figure 4.1. Conceptual Model for Biodegradation of Resin-attached Perchlorate in the Bioregeneration Process. It Is Envisioned that, to be biodegraded, RAP ions Should: 1) Desorb from the Functional Group Located in the Resin Bead Matrix, 2) Diffuse from the Resin Bead to the resin Surface through the Resin Pores, 3) Transfer through a liquid fim on the resin surface to the Bulk Liquid, and 4) Enter the Periplasmic Region of the PRB to Be Biodegraded.

It is essential to mention that although chloride is thought to be the desorption agent for perchlorate attached to the resin, at higher concentrations it negatively affects

perchlorate biodegradation. Several studies have been published on the negative effects of chloride (i.e. salinity) on perchlorate degradation (Logan et al. 2001; Gingras and Batista, 2002; Okeke et al., 2002). These studies show that significant reduction in perchlorate degradation occurs at salinity levels greater than 5000 mg/L. Therefore, in bioreactors used for resin bioregeneration, chloride levels should be kept below toxic levels to PRB.

Recent research has shown that biodegradation of RAP ions has a slower degradation rate compared to biological degradation of free perchlorate ions (Venkatesan et al., 2010). Figure 4.2 shows typical perchlorate degradation curve during the bioregeneration process obtained through preliminary experiments of this research. At first the biodegradation rate is fast and then it slows down and stabilizes with time. This pattern can be thought as being either kinetic or diffusion controlled (Batista and Jensen, 2006; Venkatesan et al, 2010). In the case of kinetic control, the slower degradation rate could be explained by the slower degradation of perchlorate for low perchlorate concentrations as compared to initially higher concentrations. In the case of diffusion control, it is envisioned that it takes longer for perchlorate ions located deep into the bead to reach the surface; that is, perchlorate ions located in the outer portion of the resin bead are degraded first. In this research, we will explore whether the bioregeneration process is controlled by kinetics or by pore diffusion.

### 4.2.2. Mechanism of Desorption

Ion exchange involves diffusion of ions through the IX resin porous matrix and exchange reaction, in the functional group, between the counter ion (i.e perchlorate ion in this research) and the free ion (i.e chloride ion in this research) soluble in the liquid

phase. It is widely accepted that in ion exchange, the controlling step of the whole process is diffusion rather than the actual chemical exchange reaction in the functional group (Helfferich, 1962; Helfferich, 1965; Nkedi-Kizza et al., 1984). Ion exchange reaction rate constants may be defined for IX processes, but in reality these numbers do not represent the actual exchange reaction rates (Helfferich, 1962). Since the actual exchange reaction is instantaneous, pore diffusion of the involved ions is determining the rate of ion exchange.

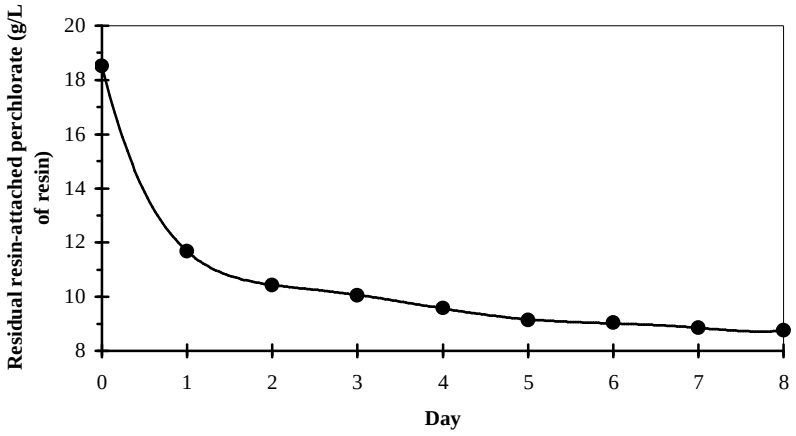


Figure 4.2. Typical Perchlorate Degradation during the Bioregeneration Process

In this research, it is thought that chloride resulting from perchlorate biodegradation exchanges with the RAP ions in the resin bead pores, releasing them. It is known that the larger and more hydrophobic ions, such as perchlorate, have more potential to bind to the IX resin compared to smaller and less hydrophobic ions such as chloride (Diamond, 1963; Xiong et al., 2007; Lehman et al., 2008). The affinity of the ions to be adsorbed to

the functional groups of the resin is commonly expressed as either selectivity coefficient or separation factor. Selectivity coefficient of chloride compared to selectivity coefficients of perchlorate in common non-selective styrenic resins used for perchlorate removal are about 1 to 150 (Crittenden et al., 2005) and 1 to 1300 for common perchlorate-selective styrenic resins (Tripp and Clifford, 2000). High selective perchlorate resins may have selectivity coefficients of about 3500 (ResinTech, West Berlin, NJ). Given the high affinity of perchlorate for ion-exchange, regeneration efficiency of IX resins containing perchlorate has been found to be limited particularly for styrenic-type resins (Batista et al, 2000) and very high concentrations of chloride are needed to perform the regeneration (Sodaye et al., 2007). Although perchlorate attaches very strongly to the functional groups of perchlorate-selective resins, in the presence of chloride a small portion of RAP ions may exchange by the chloride ions instantaneously (Lehman et al., 2008). However, a significant part of the exchanged perchlorate will re-exchange and re-attach to the resin (Lehman et al., 2008), because perchlorate is a monovalent-hydrophobic ion with high selectivity (Crittenden et al., 2005; Sodaye et al., 2007).

### 4.2.3. Mechanisms of Diffusion

It is thought that transfer of chloride ions from the bulk liquid to the functional groups of resin and release of RAP involves pore diffusion within the resin and film diffusion in the liquid layer surrounding the resin. Film diffusion mostly depends on flow rate, turbulence, and viscosity, which affect the thickness of the diffusion boundary layer (Helfferich, 1962; Weber, 1972; Lahav and Green, 2000; Xiong et al., 2007). Pore diffusion is influenced by viscosity, concentration gradient, resin bead size, degree of

crosslinking in the structure of resin, and resin pore size (Helfferich, 1962; Xiong et al., 2007). The degree of crosslinking is different for gel-type and macroporous resins. On average, gel-type resins have about 8% divinylbenzene crosslinking, while, macroporous resins have about 20-25% percent divinylbenzene crosslinking (Crittenden et al., 2005). Increasing the degree of crosslinking decreases the diffusion (Weber, 1972). Ion exchange resins have two types of pores; micropores, the pores within the gel structure of resin, and macropores, the pores between the microspheres of macroporous resins (Crittenden et al., 2005). Gel-type resins have only micropores, while macroporous resin have both micropores and macropores, resulting in higher water content in macroporous resins (Du et al., 2010) (Figure 4.1). In addition, the resin capacity in macroporous resins is smaller than gel-type resins due to more water content in macroporous resins compared to gel-type resins (Crittenden et al., 2005). Although the degree of crosslinking in macroporous resins is higher than gel-type resins, it is expected that the overall mass transfer rate in macroporous resins is higher than that of gel-type resins due to presence of macropores.

Resin bead size is also an important parameter in diffusion process in IX resins. Increasing the resin size decreases the overall rate of ion exchange in the resin (Helfferich, 1962; Weber, 1972). The shrinking core model (Arevalo et al., 1998; Pritzker, 2005), in which the counter ions in the outer region of the resin bead are desorbed and diffused to the bulk liquid prior to the counter ions that are located deep inside the resin bead, has been shown to be an appropriate model to describe the effect of resin bead size in the diffusion process in IX resins (Venkatesan et al., 2010).

Mass transfer control by either pore diffusion or film diffusion for IX resins can be expressed mathematically as (Helfferich, 1962):

$$\frac{X\overline{D}\delta}{CDr_0}(5+2\alpha_B^A) << 1 \quad \text{pore diffusion control} \tag{4.1}$$

$$\frac{X\overline{D}\delta}{CDr_0}(5+2\alpha_B^A) \approx 1 \quad \text{pore / film diffusion control} \tag{4.2}$$

$$\frac{X\overline{D}\delta}{CDr_0}(5+2\alpha_B^A) >> 1 \quad \text{film diffusion control} \tag{4.3}$$

where: X is concentration of fixed ionic group in eq/L, C is the concentration of the free ion in solution in eq/L, $\overline{D}$ is the pore diffusion coefficient in $cm^2/s$, D is the film diffusion coefficient in $cm^2/s$, $r_0$ is the mean resin bead radius in cm, $\delta$ is the liquid film thickness in cm, and $\alpha_B^A$ is the selectivity coefficient for the involved ions. Xiong et al. (2007) has calculated the pore diffusion coefficient for perchlorate ion in IX process for different resins. Four granular macroporous resins and a fibrous resin were used in their experiments. The macroporous resins were A-530E, DOW 66, IRA 900, and IRA 958, which were all granular, with water content of 50-57%, 40-46%, 58-64%, and 66-72%, respectively, and the fibrous (i.e. not granular) resin was Smopex-103x with water content of less than 5%. The pore diffusion coefficient for granular macroporous resins has been reported to vary from $4.67 \times 10^{-9}$ to $1.67 \times 10^{-8}$ $cm^2/s$ (Xiong et al., 2007). Although the pore diffusion coefficient for the fibrous resin was not reported, their results showed that the porosity of the IX resin has a direct effect on accessibility of functional groups and diffusion rate of the involved ions. The film diffusion coefficient in IX processes has been reported as $10^{-5}$ $cm^2/s$ (Helfferich, 1962; Lahav and Green, 2000). The liquid film thickness in a well-mixed condition has been reported as $10^{-3}$ cm

(Helfferich, 1962; Lahav and Green, 2000; Crittenden et al., 2005). The selectivity coefficient for highly perchlorate-selective resin has been determined to be > 3500 (ResinTech, West Berlin, NJ). Table 4.1 shows the values of the above-mentioned parameters involved in the mass transfer control calculation.

Table 4.1. Mass Transfer Parameters for IX Resins

| Parameter | Value | Unit | Reference |
|---|---|---|---|
| δ, liquid film thickness | $10^{-3}$ (well-stirred condition) | cm | Helfferich, 1962; Lahav and Green, 2000 |
| D, film diffusion | $10^{-5}$ | $cm^2/s$ | Helfferich, 1962; Lahav and Green, 2000 |
| $\overline{D}$, pore diffusion | $1.67 \times 10^{-8}$ - $4.67 \times 10^{-9}$ | $cm^2/s$ | Xiong et al., 2007 |
| $\alpha_B^A$, selectivity coefficient | 3500 | NA | ResinTech, West Berlin, NJ |

### 4.2.4. Perchlorate Biodegradation Kinetics

Perchlorate degradation follows first-order kinetics under typical concentrations of perchlorate in the environment, which is in the part-per-billion range (Logan et al., 2001). The observed biodegradation rate of perchlorate by PRB varies between 0.0007 to 20 mg/L.min (Logan et al., 2001). A broad range of perchlorate biodegradation rates occures because of the differences in the concentration of perchlorate in the bioreactor. Since biodegradation obeys first-order kinetics under typical concentrations of perchlorate in the environment, which is in the part-per-billion range (Logan et al., 2001), higher concentration of perchlorate results in higher observed biodegradation rate. The kinetic parameters for some perchlorate reducing bacteria that have been studied to date are listed in Table 4.2.

Table 4.2. Kinetic Parameters for Perchlorate Reducing Bacteria

| Culture | Kinetic parameters | | Reference |
|---|---|---|---|
| | $q_{max}(d^{-1})$ | $K_s$ (mg/L) | |
| PDX | 0.41 | 12 ± 4 | Logan et al., 2001 |
| KJ | 1.32 | 33 ± 9 | Logan et al., 2001 |
| INS | 4.34 | 18 | Waller et al., 2004 |
| ABL1 | 5.42 | 4.8 | Waller et al., 2004 |
| SN1A | 4.60 | 2.2 | Waller et al., 2004 |
| RC1 | 6.00 | 12 | Waller et al., 2004 |
| PC 1 | 3.09 | 0.14 | Nerenberg et al., 2006 |
| HCAP-C | 4.39 | 76.6 | Dudley et al., 2008 |
| Mixed culture | 0.49 | <0.1 | Wang et al., 2008a |

### 4.2.5. Comparison of Kinetic and Diffusion Control

The Thiele modulus, a dimensionless number which is an acceptable measure of the comparison of diffusion-limited to kinetic-limited reactions, can be employed to determine whether resin bioregeneration is diffusion or kinetically controlled (Thiele, 1939; Helfferich, 1962). As discussed earlier, IX processes which occur in the IX resin bead are diffusion-controlled (Helfferich, 1962; Nkedi-Kizza et al., 1984). In the case of bioregeneration process, the Thiele modulus can be used to determine whether the bioregeneration process is controlled by biological degradation or pore diffusion. The Thiele modulus for processes involving reaction and diffusion in ion-exchange resins can be calculated mathematically as follows (Helfferich, 1962; Hong et al., 1999):

$$M_T = \frac{r_0}{3}\sqrt{\frac{k}{D_e}} \tag{4.4}$$

where $M_T$ is Thiele modulus (dimensionless number), $r_0$ is the mean resin bead radius in cm, $D_e$ is the diffusivity coefficient in $cm^2/s$, and k is the reaction kinetic coefficient in 1/sec. Diffusivity coefficient for perchlorate in IX process is shown in Table 4.1.

According to Helfferich (1962), low Thiele modulus ($M_T < 3$) indicates that the reaction kinetics are not the controlling step in the bioregeneration process, and the process is mass transfer limited.

Although the feasibility of IX bioregeneration has been proven, the mechanism of RAP reduction has not been elucidated thus far. The specific objectives of this research were to: (1) investigate the effect of different initial concentrations of chloride as the desorbing agent for RAP release in the IX resin bioregeneration process, (2) study the influence of resin bead size and different resin structures (i.e. gel-type and macroporous) in the resin bioregeneration process, and (3) investigate the controlling step in the envisioned conceptual model of IX resin bioregeneration process.

## 4.3. Materials and Methods

### 4.3.1. Experimental Approach

Batch resin bioregeneration tests were performed to test the envisioned resin bioregeneration mechanism. The tests were performed in batch-bioreactor tubes containing desired amounts of resins loaded with perchlorate, PRB culture, micro nutrients and buffer, and acetate as the electron acceptor. For each experiment, a series of batch-bioreactor tubes was prepared and a tube was removed and sacrificed at desired time intervals to determine perchlorate biodegradation rates. Resin samples from the bioreactor tubes were submitted to an oxygen Parr bomb procedure, developed for this research, to determined remaining perchlorate in the resin with time. Three series of batch bioregeneration experiments were performed using: (a) different initial chloride concentrations to test the effect of chloride on the desorption of RAP, (b) different resin bead sizes to examine whether pore diffusion is the controlling step in the

bioregeneration process, and (c) gel-type and macroporous type selective and non-selective resins to evaluate the effect of resin structure on the bioregeneration process.

### 4.3.2. Resin Characteristics

Two perchlorate-selective resins, SIR-100HP and SIR-110HP-MACRO (Resin Tech, West Berlin, NJ), and two non-selective resins, IONAC ASB-1 and IONAC SR-7 (Lanxess Sybron Chemicals Inc., Birmingham, NJ), were used in the batch bioregeneration experiments (Table 4.3). The perchlorate-selective macroporous resin, SIR-110HP-MACRO, was manufactured specially for this research (Resin Tech, West Berlin, NJ). All resins have a styrenic matrix. SIR-110HP-MACRO and IONAC SR-7 have macroporous structure, while SIR-100HP and IONAC ASB-1 are gel-type (Table 4.3).

### 4.3.3. Resin Loading

The resin samples were loaded batch-wise instead of in a column. Batch loading was selected to shorten the time period required to load the resin, and to assure homogeneous distribution of the ions through the resin beads. Sodium perchlorate ($NaClO_4$) salt was used to load the resin. For each milliliter of resin sample, 1 mL of loading solution (Table 4.4) was prepared and added to a 2-L glass bottle. The bottle was then placed on a rotary mixer (Associate Design Mfg. Co., Alexandria, VA) at 40-50 rpm and 22±2ºC. After 24 hours, the mixer was stopped, and the resin was separated from the liquid using a paper filter. Preliminary experiments showed that the contact time should be at least 6 hours to reach equilibrium in the loading step. The liquid phase was sampled for residual perchlorate analysis.

Perchlorate was measured in the original solution, before resin addition, and after exchange took place. The difference between initial and final concentrations in solution was assumed to be the perchlorate loaded to the resin (i.e RAP). Perchlorate concentrations were measured using Ion Chromatography (IC) analysis.

Table 4.3. Characteristics of the Resins Used in Batch Bioregeneration Experiments

| Commercial name (manufacturer) | Resin Type | Water Content. (%) | Capacity (eq/L) | Resin structure | Functional group |
|---|---|---|---|---|---|
| SIR-110HP (ResinTech) | $ClO_4$-selective | 35-55 | 0.6 | Styrene, Gel | Tri-n-butyl-amine $(C_4H_9)_3^+Cl^-$ |
| SIR-110HP-MACRO[a] (ResinTech) | $ClO_4$-selective | 58-65 | 0.6 | Styrene, Macroporous | Tri-n-butyl-amine $(C_4H_9)_3^+Cl^-$ |
| IONAC SR-7 (Sybron) | $NO_3$-selective | 52-67 | 0.8 | Styrene, Macroporous | Quaternary ammonium $(CH_3)_3^+ Cl^-$ |
| IONAC ASB-1 (Sybron) | Non-selective | 43-48 | 1.4 | Styrene, Gel | Quaternary ammonium $(CH_3)_3^+ Cl^-$ |

[a] specially made to this research

The resin samples were loaded fully to their capacity to eliminate the original counter ion (i.e. chloride) from the functional groups of the resins. Table 4.4 shows the initial and final concentrations of perchlorate in solution and the percent capacity of the resin occupied by perchlorate after loading.

Table 4.4. Resin Loading for Batch Bioregeneration Experiments

| Resin name | Volume of resin, mL | Volume of loading solution, mL | Added concentration, g-$ClO_4/L_{solution}$ | Residual concentration, g-$ClO_4$/ $L_{solution}$ | Capacity occupied, g-$ClO4/L_{resin}$ | Percent capacity occupied, % |
|---|---|---|---|---|---|---|
| SIR-110HP | 500 | 500 | 119.68 | 39.48 | 80.19 | 134.3% |
| SIR-110HP-MACRO | 100 | 100 | 119.68 | 33.97 | 85.71 | 143.5% |
| IONAC SR-7 | 100 | 100 | 119.68 | 32.86 | 86.82 | 109.1% |
| IONAC ASB-1 | 500 | 500 | 149.26 | 20.97 | 128.28 | 92.1% |

After loading, the resin was then rinsed 12 times with 1 L of de-ionized (DI) water for 30 minutes to remove all the residual unattached perchlorate ions. Rinsing solutions were submitted to perchlorate analysis using IC. After 12 times rinsing, no perchlorate was detected in the rinsing solution. The resins were then air-dried at room temperature (22±2ºC) for 6 hours, labeled, and stored in the refrigerator.

### 4.3.4. Resin Size Separation

The bead sizes of commercially available for gel-type SIR-110HP resin ranges from 1.19 to 0.297 mm, which corresponds to U.S. screen mesh number 16 and 50, respectively. To evaluate the effect of resin bead size (i.e. bead diameter) on the bioregeneration process, loaded SIR-110HP resin beads were sorted into different sizes (i.e. 0.924, 0.778, 0.652, and 0.547 mm). A U.S. standard stainless-steel series of sieves (Fisher Scientific, Inc., Waltham, MA) with mesh sizes of 0.853, 0.710, 0.599, 0.500, 0.150, and 0.106 mm was used for size separation of the resin beads (Table 4.5). Resin was screened for 20 minutes. To obtain a smaller resin size (i.e. 0.128 mm), the loaded

resin beads were crushed using a mortar and pestle. Therefore, it is expected that these crushed bead pieces will behave differently, but it is not possible to obtain smaller bead size by a different manner. Scanning electron microscopy (SEM) imaging of the crushed resin was performed using Jeol JSM-7500F SEM (JEOL Ltd., Tokyo, Japan) and the resin particles appear rough in shape. The SEM image of the crushed resin is shown in Figure 4.3. However, all the resins used in this study were assumed to be spherical.

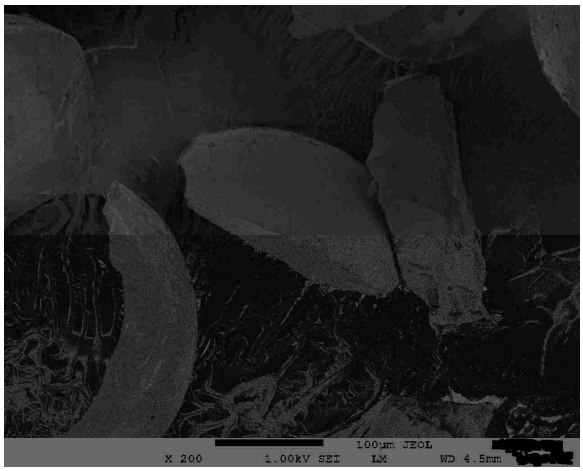


Figure 4.3. SEM Image of the Crushed Resin

Five different bead sizes of 0.924, 0.778, 0.652, 0.547, and 0.128 mm were used in the experiment evaluating the effect of bead size on the bioregeneration process. These bead sizes are geometric means of the top and bottom sieve sizes and account for sizes equal or larger than the retaining bead size (Table 4.5).

For the experiment comparing biodegradation in gel and macroporous resin types, resin beads with the representative bead size of 0.778 mm (i.e. retained on 0.710 mm

sieve) were used in. For the experiments evaluating the effect of initial chloride on the bioregeneration process, SIR-110HP and ASB-1 resins were not sieved and used as it comes from manufacturer.

Table 4.5. SIR-110HP Resin Size Distribution in the Test Evaluating the Effect of Initial Chloride on the Bioregeneration Process

| Sieve number | Sieve opening size (mm) | Geometric mean resin size (mm) | External surface area per volume of resin sphere (1/mm) |
|---|---|---|---|
| 18 | 1.000 | NA | NA |
| 20 | 0.853 | 0.924[a] | 6.494 |
| 25 | 0.710 | 0.778 | 7.712 |
| 30 | 0.599 | 0.652 | 9.202 |
| 35 | 0.500 | 0.547 | 10.969 |
| 100 | 0.150 | NA | NA |
| 140 | 0.106 | 0.128 | 46.875 |

[a] $0.924 = (1.000 \times 0.853)^{0.5}$

### 4.3.5. Perchlorate-reducing Enrichment Culture

Perchlorate-reducing microbial culture was taken from two master seed cultures, called BALI I and BALI II, enriched and grown in the Environmental Engineering Laboratory at UNLV. The sources of perchlorate-reducing bacterial inocula were the Las Vegas Wash and Lake Mead. The culture was enriched under anaerobic conditions by providing perchlorate as the electron acceptor, acetate as the electron donor, and a mineral/nutrient/buffer broth for the seed (Table 4.6). The nitrogen source used to grow

PRB was $NH_4H_2PO_4$. The PRB culture has been characterized using 16S rRNA sequencing (Kesterson et al., 2005).

Table 4.6. Nutrients and Buffer Stock Solution for Feeding the Perchlorate Degrading Culture

| Solution Name | Components | Concentration of stock (g/L) |
|---|---|---|
| Buffer (100X) | $K_2HPO_4$ | 155.00 |
| | $NaH_2PO_4.H_2O$ | 97.783 |
| | $NH_4H_2PO_4$ | 50.000 |
| Nutrients/Minerals (100X) | $MgSO_4.7H_2O$ | 5.500 |
| | EDTA | 0.300 |
| | $ZnSO_4.7H_2O$ | 0.200 |
| | $CaCl_2.2H_2O$ | 0.100 |
| | $MnCl_2.4H_2O$ | 0.100 |
| | $FeSO_4.7H_2O$ | 0.400 |
| | $Na_2MoO_4.2H_2O$ | 0.040 |
| | $CuSO_4.5H_2O$ | 0.020 |
| | $CoCl_2.6H_2O$ | 0.040 |
| | $NiCl_2.6H_2O$ | 0.010 |
| | $NaSeO_3$ | 0.010 |
| | $H_3BO_3$ | 0.060 |

### 4.3.6. Biomass Extraction from Master Seed Culture

For the series of batch bioregeneration experiments that were performed using different concentrations of chloride, two liters of PRB microbial culture obtained from

the master seed cultures were rinsed five consecutive times using 1X phosphate buffer solution (Table 4.6) to eliminate the presence of chloride ion in the liquid phase. The culture was centrifuged using a Legend RT Sorvall centrifuge (Kendro, Thermo Fisher Scientific, Inc., Waltham, MA) at the rotational speed of 3850 rpm for 45 minutes at $22^0$ C. The liquid phase containing chloride was discarded and 1X phosphate buffer solution (Table 4.6) was added to the concentrated biomass to obtain a SS concentration of 1500 mg/L. The biomass was then resuspended using a bench-top orbital shaker (Cole-Parmer, Series 51704) for 20 min at 80-100 rpm. This procedure was repeated five times to ensure the elimination of chloride from the culture. A sample of culture rinsing solution was then submitted for chloride analysis by IC and no chloride was detected. Detection limit for chloride ion in the IC was 100 μg/L.

### 4.3.7. Batch Testing Procedure

All the bioregeneration experiments were performed in batch-bioreactor tubes with a capacity of 25 mL. For each experiment, a series of batch-bioreactor tubes were used so that a tube was sacrificed at desired time intervals for sampling (Table 4.7). The culture was diluted to 1000 mg-SS/L in the batch-bioreactor tubes by adding nutrient, buffer, and acetate media (Table 4.6) and DI water.

Initial concentrations of 0, 500, 1000, and 5000 mg/L of sodium chloride (NaCl) were used for the series of bioregeneration experiments evaluating the effect of varying initial chloride concentration on bioregeneration process. A ratio of 1.7 mole of acetate to mole of perchlorate (Chaudhuri and Coates, 2002) (9000 mg/L of acetate) was used for the batch-bioreactor tubes to supply required electron donor.

Approximately 1.2 g of resin (about 2 mL) was added to each batch-bioreactor tube. All the solutions (i.e. nutrient, buffer, and acetate stock solutions, and DI water) were purged with nitrogen gas for 30 minutes prior to start the experiment to completely remove dissolved oxygen. After all the solutions were added to the batch-bioreactor tube, they were sealed using aluminum-crimpled butyl rubber-stopper (Wheaton Industries, Inc., Millville, NJ), labeled, and placed on a rotary shaker at 22±2ºC and 40-50 rpm.

Table 4.7. Experimental Design for Batch Bioreactor-Tubes

| Resin name | Resin bead size, mm | Amount of Resin in each tube, g | Initial conc. of NaCl, mg/L | Bioregeneration length, days |
|---|---|---|---|---|
| Series #1 (Evaluation of the Effects of Chloride on Biodegradation) | | | | |
| SIR-110HP | Original distribution | 1.2 | 0 | 8 |
| SIR-110HP | Original distribution | 1.2 | 500 | 8 |
| SIR-110HP | Original distribution | 1.2 | 1000 | 8 |
| SIR-110HP | Original distribution | 1.2 | 5000 | 8 |
| ASB-1 | Original distribution | 1.2 | 0 | 8 |
| ASB-1 | Original distribution | 1.2 | 500 | 8 |
| ASB-1 | Original distribution | 1.2 | 1000 | 8 |
| ASB-1 | Original distribution | 1.2 | 5000 | 8 |
| Series #2 (Evaluation of Resin Bead Size on Diffusion) | | | | |
| SIR-110HP | 0.924 | 1.2 | NA[a] | 8 |
| SIR-110HP | 0.778 | 1.2 | NA | 8 |
| SIR-110HP | 0.652 | 1.2 | NA | 8 |
| SIR-110HP | 0.547 | 1.2 | NA | 8 |
| SIR-110HP | 0.128 | 1.2 | NA | 8 |
| Series #3 (Evaluation of Resin Matrix on Bioregeneration) | | | | |
| SIR-110HP | 0.778 | 1.2 | NA | 8 |
| SIR-110HP-MACRO | 0.778 | 1.2 | NA | 8 |
| ASB-1 | 0.778 | 1.2 | NA | 8 |
| SR-7 | 0.778 | 1.2 | NA | 8 |

[a] Initial concentration of chloride was not changed (i.e. it was the same concentration as the master seed cultures).

Each series of bioregeneration experiment contained 5 batch bioreactor-tubes (i.e. days 1, 2, 4, 6, and 8), 2 replicates for quality control (i.e. day-1-QC and day-8-QC), an abiotic control tube (i.e. resin and nutrient/buffer/acetate medium, no microbial culture), and a replicate for abiotic control.

For the series of bioregeneration experiment evaluating the effect of varying initial chloride concentration, two other control tubes were prepared: (a) an abiotic tube containing resin and DI water (i.e. no microbial cells, no nutrient/buffer/acetate medium), and (b) a batch-bioreactor tube containing resin, microbial cells, and buffer solution (i.e. no nutrient/buffer/acetate medium). The abiotic batch-reactor tubes were autoclaved immediately after preparation to avoid any microbial activity, then sealed and labeled. All the control tests were performed in duplicate.

After the desired bioregeneration time had been elapsed, batch bioreactor-tubes were taken from the shaker, opened and sacrificed on days 1, 2, 4, 6, and 8. The resin was allowed to settle and the microbial culture was collected and tested for COD, pH, and SS. The resin sample was then rinsed 5 times with 5 mL of DI water to remove remaining microbial cells and organics which might have remained in the resin. Preliminary experiments by measuring % transmittance of the rinsing solution showed that after 5 times rinse, the % transmittance of the rinsing solution before and after rinsing does not change. The resin sample was then submitted to resin-attached residual perchlorate analysis using an oxygen Parr-bomb.

All the control tubes were removed from the shaker and sacrificed after 8 days. In the abiotic tests, the liquid phase was submitted to perchlorate analysis by IC. The resin from the control tubes containing microbial cells and buffer solution was rinsed 5 times

with 5 mL of DI water to remove remaining microbial cells and organics, and then submitted to resin-attached residual perchlorate analysis using an oxygen Parr-bomb.

### 4.3.8. Residual Perchlorate Analysis

In this research the method of residual RAP measurement developed by Venkatesan et al. (2010) was used. In this method, small samples of resin (i.e. about 100 to 200 mg) are ignited in closed stainless steel oxygen Parr bomb cylinder (Parr Instruments, Moline, IL), to incinerate the polymer structure of the resin and convert the resin-attached residual perchlorate to chloride (resin-$ClO_4^-$ + $O_2$ → $CO_2$ + $Cl^-$). Thus, the resulting soluble chloride can be measured by IC.

Prior to ignition, the resin sample has to undergo preliminary treatment. One mL of resin is placed in 100 mL of nitrate solution (10,000 mg/L as $NO_3^-$) and mixed in a shaker for 24 hours to exchange the chloride ions present in the functional groups of the resin with nitrate. The goal is to eliminate any chloride previously loaded to the resin so that any chloride measured after ignition originated from perchlorate. The resin is then allowed to settle for 3 minutes, and the liquid phase is submitted to perchlorate analysis by IC. Next, the resin is rinsed with DI water 6 times to remove residual anions. The resin sample is then dried at 105ºC for 1 hour in a 1330GM VWR oven (VWR, West Chester, PA). About 100 mg of the dried resin and 400 mg of paraffin oil are measured and placed in the small crucible of the oxygen Parr bomb. Three mL (3 mL) of 3% $H_2O_2$ and 10 mL of 35 mM NaOH are added to the cylinder. Then, the oxygen Parr bomb cylinder is pressurized to about 500 psi (30-35 atm) with oxygen gas. The oxygen Parr bomb is submerged in 5-L of DI water to control the high temperature resulting from the ignition, and the resin is ignited using 10 cm of nickel fuse wire. After ignition, the

cylinder is kept in DI water for 20 minutes for cooling. Then, the Parr bomb cylinder is opened and its content is rinsed with small portions of DI water and transferred to a 250 mL volumetric flask. The resulting solution in then submitted to chloride analysis in the IC. Ultimate coal (Alpha Resources Inc., Stevensville, MI) sample with known chloride content is used for quality assurance. The standard error for replicate coal samples measured in the Parr bomb is 2.4%.

### 4.3.9. Chemicals and Analyses

Sodium perchlorate ($NaClO_4$) and sodium nitrate ($NaNO_3$) salts were ACS grade and obtained from VWR (West Chester, PA). DI water with a resistivity of 17.5 MΩ cm was acquired from a Barnstead water purification system (Dubuque, IA) and used in all steps. The pH values were determined with an Orion 920A+ pH meter (Orion Research, Boston, MA). The % transmittance was measured using a Hach DR 5000 Spectrophotometer at the wavelength of 600 nm. Suspended solids (SS) were measured based on Standard Methods 2540-D (Greenberg et al., 2005). Chemical oxygen demand (COD) was analyzed using high range (0-1500 mg/l) Hach COD digestion vials (Hach Co., Loveland, CO).

Concentrations of perchlorate and low concentrations of chloride were analyzed using a Dionex ICS-2000 ion chromatography (Sunnyvale, CA). The IC system consisted of an ion Suppressor-ULTRA II (4 mm), IonPac AS16 (4 mm) analytical, AG16 (4 mm) guard columns, and an AS16 autosampler. EPA method 314.0 was used for perchlorate analysis with a current of 100 mA and an EGC II KOH eluent cartridge with concentration of 35 mM and a flow rate of 1.0 mL/min. IC calibration was performed using standard solutions of 5, 10, 25, 50, 75, and 100 µg/L with 99.97% coefficient of

determination. For low concentrations of chloride, an IonPac AS16 column with a current of 100 mA, an EGC II KOH eluent concentration of 35 mM, and a flow rate of 1.0 mL/min were used. Coefficient of determination for low chloride concentration analysis (100 – 1000 µg/L) was 99.99%. For high concentrations of chloride, IonPac AS20 (4 mm) analytical and AG16 (4 mm) guard columns were used with a current of 110 mA, an EGC II KOH eluent concentration of 30 mM and a flow rate of 1.0 mL/min. Coefficient of determination for high concentrations of chloride analysis (1-5 mg/L) was 99.99%.

#### 4.3.10. Statistical Analysis

Analysis of variance (ANOVA) was performed to determine whether there was a significant difference in perchlorate biodegradation rates for varying initial chloride concentrations in the microbial medium, resin bead size, and with different resin structure (gel-type vs. macroporous). SPSS Statistics, version 16.0, (SPSS Inc., Chicago, IL) was used to perform the statistical analysis.

#### 4.3.11. Investigation of Diffusion and Reaction Control of Bioregeneration

In this research, it was envisioned that to be degraded, resin-attached perchlorate ions should be: a) desorbed from the functional groups located inside the resin bead, b) diffused from the inside region of the resin bead to the surface through the pore matrices, c) transferred through the liquid film covering the surface of resin bead, and d) degraded by PRB to obtain energy.

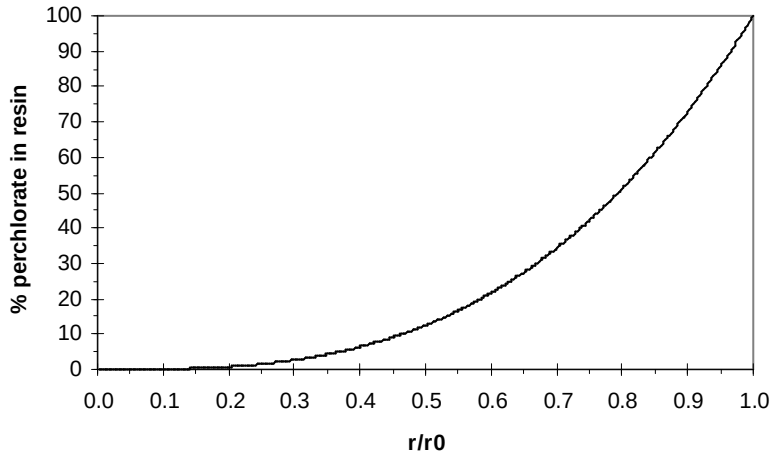


Figure 4.4. Perchlorate Distribution in the Resin Bead Against $r/r_0$ (r: Radius of Core, $r_0$: Radius of Resin Bead) (Modified from Venkatesan et al., 2010)

It is widely accepted that desorption of RAP (i.e. step a) is not the rate controlling step when compared to diffusion of desorbed perchlorate from the inside region of the resin bead to the surface through the pore matrices (Helfferich, 1962; Helfferich, 1965; Nkedi-kizza et al., 1984). It is assumed that RAP ions are homogeneously distributed in the resin bead; thus, the desorbed perchlorate ions which are located in the outer region of resin bead can diffuse out of the resin in a shorter time compared to the ions located deep in the resin. The shrinking core model (Arevalo et al., 1998; Pritzker, 2005) can be used to describe perchlorate desorption and utilization in IX resin bead. Figure 4.4 shows theoretical perchlorate distribution verses radius of resin bead. As shown in Figure 4.4, about 90% of perchlorate load is located between $r_0/r = 0.5$ and $r_0/r = 1.0$. Reducing the resin bead size will accelerate the diffusion process and a result bioregeneration of resin, if the bioregeneration process is controlled by pore diffusion (Helfferich, 1962). According to the shrinking core model, reducing the resin bead size exposes more surface

to the liquid phase, and if the bioregeneration process is pore diffusion controlled, it will enhance the process.

In addition to pore diffusion, it is thought that film diffusion is also involved in the bioregeneration process. Film diffusion limitation increases with decreasing resin bead size, while, decreasing resin bead size results in increase of pore mass transfer flux. The rate controlling step in mass transfer process can be mathematically identified (Helfferich, 1962):

$$\frac{X\overline{D}\delta}{CDr_0}(5+2\alpha_B^A) << 1 \quad \text{pore diffusion control} \tag{4.1}$$

$$\frac{X\overline{D}\delta}{CDr_0}(5+2\alpha_B^A) \approx 1 \quad \text{pore / film diffusion control} \tag{4.2}$$

$$\frac{X\overline{D}\delta}{CDr_0}(5+2\alpha_B^A) >> 1 \quad \text{film diffusion control} \tag{4.3}$$

The experiment to evaluate the controlling step of bioregeneration involved SIR110-HP resin which was loaded with 78 $g_{perchlorate}/L_{resin}$. Chloride concentration detected in the liquid phase of the PRB culture before starting the bioregeneration experiment was approximately 2000 $mg_{chloride}/L_{solution}$. The presence of chloride is a consequence of feeding the PRB stock culture with perchlorate, which is degraded to chloride. The pore diffusion coefficient for perchlorate ion in Table 4.1, which ranges from $4.67 \times 10^{-9}$ to $1.67 \times 10^{-8}$ $cm^2/s$ (Xiong et al., 2007) is for macroporous resins, while SIR110-HP resin used in this study is a gel-type resin. Xiong et al. (2007) has concluded that the porosity of the IX resin has a direct effect on diffusion rate of the involved ions; hence for SIR110-HP resin, the pore diffusion coefficient for perchlorate ion is expected to be less than the reported value in Table 4.1 for macroporous resins. Therefore, the minimum

pore diffusion coefficient in Table 4.1 (i.e. $4.67 \times 10^{-9}$ $cm^2/s$) was used as the pore diffusion coefficient for perchlorate ion in SIR-110HP resin. Hence:

X = concentration of fixed ionic group (78 $g_{perchlorate}/L_{resin}$ or 0.78 $eq_{perchlorate}/L_{resin}$)

C = concentration of solution (approximately 2000 $mg_{chloride}/L_{solution}$ or 0.056 $eq_{chloride}/L_{solution}$)

$\overline{D}$ = pore diffusion coefficient ($4.67 \times 10^{-9}$ $cm^2/s$) (minimum value from Table 4.1)

D = film diffusion coefficient ($10^{-5}$ $cm^2/s$) (Table 4.1)

$r_0$ = mean particle radius (0.924 – 0.128 mm) (Table 4.7)

$\delta$ = assumed liquid film thickness ($10^{-3}$ cm) (Table 4.1)

$\alpha_B^A$ = selectivity coefficient (3500) (Table 4.1)

Regardless of whether pore diffusion or film diffusion is the controlling step in the mass transfer process of bioregeneration, mass transfer of desorbed perchlorate ions (i.e. either step 2 or step 3 of the envisioned conceptual model) can be compared to biodegradation of perchlorate ions in the bulk liquid. The Thiele modulus is an understood measure of the comparison of diffusion limited to kinetic limited reactions (Thiele, 1939; Helfferich, 1962; Hong et al., 1999). In the bioregeneration process, Thiele modulus can be used to examine whether the process is controlled by diffusion of perchlorate ion thought the resin pores or by the biological reduction of perchlorate in the bulk liquid

The Thiele modulus for a process involving reaction and diffusion can be calculated mathematically as following (Helfferich, 1962; Hong et al., 1999):

$$M_T = \frac{r_0}{3}\sqrt{\frac{k}{D_e}} \tag{4.4}$$

where:

$M_T$ = Thiele modulus

$r_0$ = mean particle radius (0.924 – 0.128 mm) (Table 4.7)

$D_e$ = pore / film diffusion coefficient (Table 4.1)

k = first-order kinetic coefficient (1/s)

Pore diffusion and film diffusion coefficients are listed in Table 4.1. If the mass transfer process is pore diffusion controlled, the pore diffusion coefficient should be employed as the $D_e$, and if the mass transfer process is film diffusion controlled film diffusion coefficient should be employed as the $D_e$. Since SIR110-HP resin used in this research is a gel-type resin and the reported values for pore diffusion coefficient in Table 4.1 are for macroporous resins, the minimum diffusion coefficient as of $4.67 \times 10^{-9}$ $cm^2/s$ was used in calculation for Thiele modulus.

In order to find the biodegradation rate constant, k, the data for perchlorate biodegradation in water from Cox et al. (2000), Logan (2001a), and Logan et al. (2005) were used assuming the degradation of perchlorate obeys first-order kinetics, and k value was calculated as $1.61 \times 10^{-5}$, $7.66 \times 10^{-5}$, and $4.69 \times 10^{-6}$ 1/s, respectively. In similar researches, Tan et al. (2004) has reported the perchlorate biodegradation rate constant in sediment ranging between $1.50 \times 10^{-6}$ and $5.32 \times 10^{-6}$ 1/s. Hiremath et al. (2006) has measured the perchlorate biodegradation rate constant in brine solution in 6.8% brine solution using salt-tolerant PRB as of $6.67 \times 10^{-6}$ 1/s. In all the above mentioned studies, perchlorate biodegradation obeys first-order kinetics. In this research, the highest and lowest perchlorate biodegradation rate constant, as of $7.66 \times 10^{-5}$ 1/s and $1.50 \times 10^{-6}$ 1/s were used in this study to calculate the Thiele modulus.

## 4.4. Results

### 4.4.1 Potential Mechanism Involved in the Desorption of Resin-attached Perchlorate

Figures 4.5.a and b show the RAP biodegradation in perchlorate-selective (i.e. SIR-110HP) and non-selective (i.e. ASB-1) resins with varying concentrations of chloride (i.e. 0, 500, 1000, and 5000 mg/L of NaCl) present in the microbial solutions. Table 4.8 shows the biodegradation rates as mg of perchlorate degraded per day ($mg_p$/d) in perchlorate-selective and non-selective resins for day-0 to day-2 and day-2 to day-8 period of times. As shown in Table 4.8, except for the bioregeneration experiments with 0 mg/L of initial chloride, the rate of perchlorate degradation was fast during the first two days of bioregeneration and then decreased significantly after the second day. In the bioregeneration experiments with 0 mg/L of initial chloride, perchlorate biodegradation rate increased for day-2 to day-8 compared to day-0 to day2 in both perchlorate-selective (i.e. SIR-110HP) and non-selective (i.e. ASB-1) resins.

Figure 4.5.a shows that after 8 days of bioregeneration for perchlorate-selective resin (i.e. SIR-110HP), the overall observed perchlorate biodegradation rate was 0.375, 0.497, 0.504, and 0.535 $mg_p$/d for initial NaCl concentrations 0, 500, 1000, and 5000 mg/L, respectively. For non-selective resin (i.e. ASB-1) the biodegradation rate was 0.370, 0.527, 0.549, and 0.583 $mg_p$/d, respectively (Figure 4.5.b).

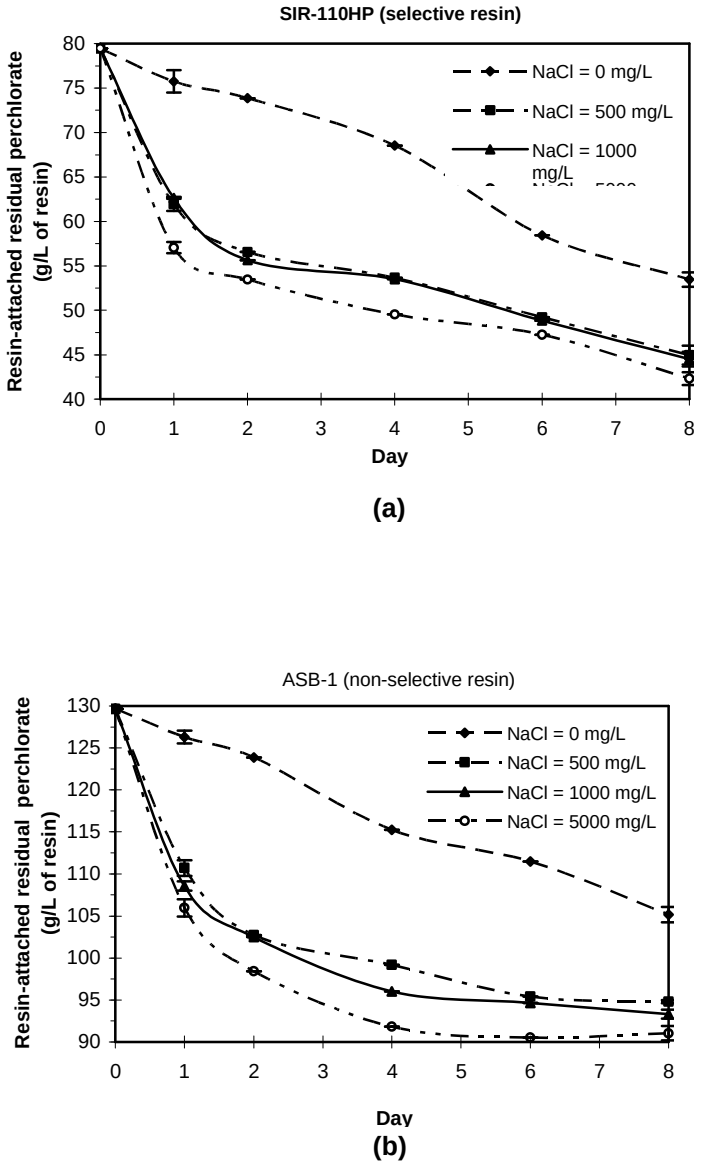


Figure 4.5. Residual Resin-attached Perchlorate Concentration in Presence of Different Initial Concentrations of Chloride Added to Bioregeneration Tubes for: (a) Perchlorate-selective Resin (SIR-110HP), and (b) Non-selective Resin (ASB-1)

Analysis of significance in both perchlorate-selective and non-selective resins at 95% confidence level showed that RAP degradation is significantly affected by the initial NaCl concentration. Analysis of significance showed that in both perchlorate-selective

and non-selective resins the bioregeneration experiment results of initial NaCl concentration of 0 mg/L compared to 500 mg/L, and 500 mg/L compared to 5000 mg/L are significantly different, however, there is no significant difference between initial chloride concentrations of 500 and 1000 mg/L in both perchlorate-selective ($p=0.082$) and non-selective resins ($p=0.141$). The most significant difference in the results in both perchlorate-selective and non-selective resins is found between 0 mg/L of NaCl and the presence of NaCl as a whole. The results suggest that the addition of NaCl had a positive effect on increasing the overall bioregeneration performance in both resins.

Analysis of the control tests with different initial chloride concentrations (Figure 4.6) shows that increasing the amount of initial NaCl concentration increased the amount of RAP desorption into the liquid phase as free perchlorate ions. Figure 4.6 shows that no perchlorate desorption was observed in the tests with DI water and bacteria/buffer solution. However, in the control test with nutrient/buffer/acetate solutions, with 500 mg-NaCl/L, 1000mg-NaCl/L, and 5000 mg-NaCl/L, the percentage of RAP desorbed was 0.02%, 0.31%, 0.49%, and 0.64%, respectively, for (SIR110-HP) resin, and for non-selective resin the amount desorbed RAP was 0.05%, 2.29%, 3.35%, and 7.37%, respectively. Resin-attached perchlorate desorption in all the control tests was less than 8% (Figure 4.6), however, in Figure 4.5 more RAP was desorbed and biodegraded. The reason for smaller desorption in control tests is establishment of equilibrium in the batch control tests, while in the bioregeneration tests, as soon as RAP is released to the bulk microbial fluid, it is degraded, and the system never reaches an equilibrium point.

Table 4.8. Resin-attached Perchlorate Biodegradation Rates for Day-0 to Day-2 and Day-2 to Day-8 of the Bioregeneration Experiments for Perchlorate-selective and Non-selective Resins

| IX Resin | Initial NaCl concentation (mg/L) | Observed perchlorate biodegradation rate for day-0 to day-2 ($mg_p/d$) | Observed perchlorate biodegradation rate for day-2 to day-8 ($mg_p/d$) | Observed overall perchlorate biodegradation rate ($mg_p/d$) |
|---|---|---|---|---|
| Perchlorate-selective (SIR110-HP) | 0 | 0.324 | 0.392 | 0.375 |
| Perchlorate-selective (SIR110-HP) | 500 | 1.321 | 0.223 | 0.497 |
| Perchlorate-selective (SIR110-HP) | 1000 | 1.373 | 0.214 | 0.504 |
| Perchlorate-selective (SIR110-HP) | 5000 | 1.499 | 0.214 | 0.535 |
| Non-selective (ASB-1) | 0 | 0.350 | 0.376 | 0.370 |
| Non-selective (ASB-1) | 500 | 1.625 | 0.160 | 0.527 |
| Non-selective (ASB-1) | 1000 | 1.640 | 0.185 | 0.549 |
| Non-selective (ASB-1) | 5000 | 1.886 | 0.149 | 0.583 |

Although the resin was rinsed well (i.e. 12 times) prior to the experiments to remove all the unattached perchlorate ions to prevent leakage of perchlorate to the liquid phase during the desorption tests, RAP was desorbed in the tests with nutrient/buffer/acetate solutions. The possible explanation for desorption of perchlorate in these tests could be presence of 0.991 mg/L of chloride ion in the components of nutrient medium (i.e. $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CaCl_2.2H_2O$, and $MnCl_2.4H_2O$) (Table 4.6). The effect of phosphate buffer medium can be neglected due to the result of control tests with bacterial cells, and buffer solution (Figure 4.6), showing no RAP was desorbed in the presence of buffer solution. Residual RAP analysis (i.e. Parr-bomb analysis) of the resin collected from the tests with bacterial cells, and buffer solution confirmed that no perchlorate degradation occurred.

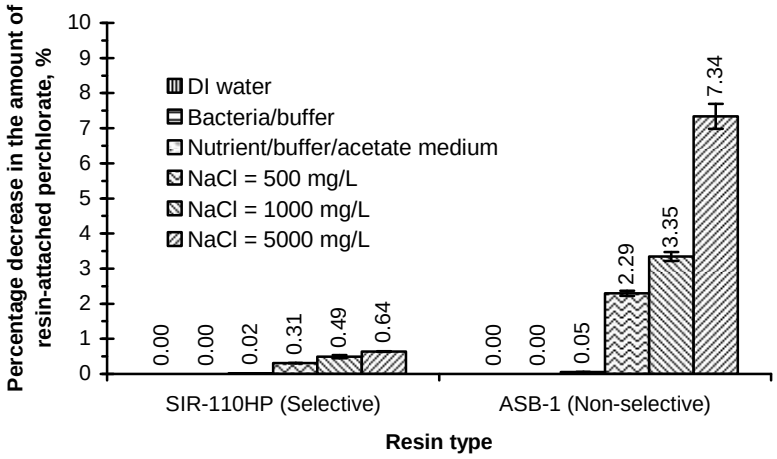


Figure 4.6. Decrease in the Amount of Resin-attached Perchlorate for Perchlorate-selective Resin (SIR-110HP) and Non-selective Resin (ASB-1)

Perchlorate desorption in the bioregeneration process is envisioned to be according to the following reaction:

$[R\text{-}N\text{-}(C_4H_9)_3]\text{-}ClO_4^- + Cl^- \leftrightarrow [R\text{-}N\text{-}(C_4H_9)_3]\text{-}Cl^- + ClO_4^-$

where $[R\text{-}N\text{-}(C_4H_9)_3]$ is the functional group of the resin. According to this exchange reaction, increasing the concentration of chloride in the left side of the reaction makes it to produce more free-perchlorate ion in the right side. Thus, increasing the NaCl concentration has a positive effect on desorption of RAP as shown in Figure 4.6.

Figures 4.5.a and b show that 32% and 18% of the initial RAP in SIR-110HP and ASB-1 resins was biodegraded during 8 days of bioregeneration with initial NaCl concentration of 0 mg/L, respectively. The potential reason for desorption while the initial NaCl concentration was 0 mg/L (Figures 4.5.a and b) is possibly due to the presence of 0.991 mg/L of chloride ion introduced by addition of nutrient/buffer/acetate medium to the batch bioreactor-tubes. Figure 4.7 shows a significant increase of RAP

biodegradation rate while the initial concentration of NaCl in the batch bioreactor-tubes increases. In Figure 4.7, the actual concentration of initial chloride, including initially added chloride and the chloride ion resulted from addition of nutrients medium to batch bioreactor-tubes, is calculated as 1, 304, 608, and 3035 mg-$Cl^-$/L.

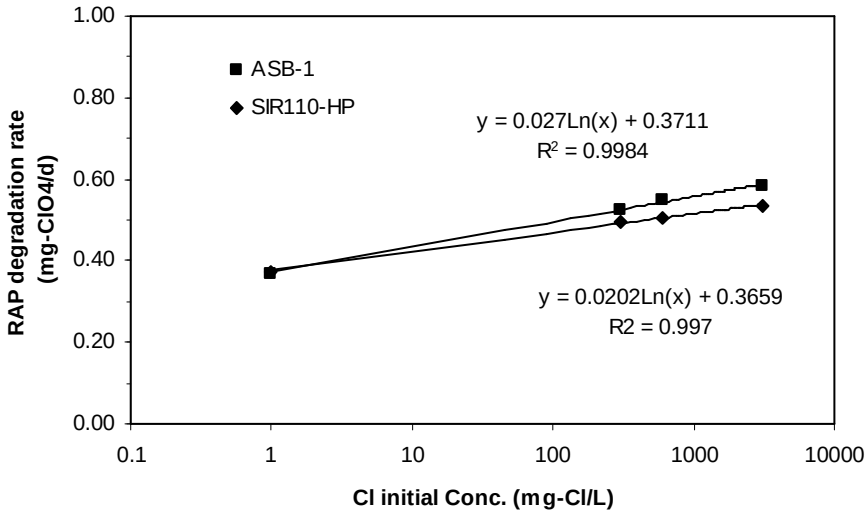


Figure 4.7. Resin-attached Perchlorate Degradation Rate in Presence of Different Initial Concentrations of NaCl for Perchlorate-selective Resin (SIR-110HP) and Non-selective Resin (ASB-1)

Sensitivity analysis on the data in Figure 4.7 shows that even a slight change of the initial chloride ion concentration in the low concentration range (i.e. less than 100 mg/L of chloride ion) noticeably affects the RAP biodegradation rate. A larger change of initial chloride ion concentration in high concentration range (i.e. more than 1000 mg/L of chloride ion) has the same effect on the RAP biodegradation rate.

Employing yield coefficient of 0.5 $g_{SS}/g_{acetate}$ (Logan et al., 2001) and stoichiometry of the overall reaction of free-perchlorate degradation of 1.7 $mole_{acetate}/mole_{perchlorate}$ (Waller et al. 2004), it is estimated that in order to grow 1000 $mg_{SS}/L$ of PRB in a acetate-fed bioreactor, about 2040 $mg/L_{culture}$ of perchlorate should be utilized which contributes to 730 $mg/L_{culture}$ of chloride is the system. Therefore, any PRB culture grown by perchlorate, as the electron acceptor, contains sufficient chloride concentration, and can be used in the IX resin bioregeneration process.

4.4.2. Determination of the Rate Controlling Step in the Resin Bioregeneration Process

Perchlorate degradation curves of SIR-110HP resin for resin bead sizes of 0.924, 0.778, 0.652, 0.547, and 0.128 mm are shown in Figure 4.8. The observed average RAP degradation rate for resin sizes of 0.924, 0.778, 0.652, 0.547, and 0.128 mm through 8 days of bioregeneration process was 0.406, 0.419, 0.441, 0.458, and 0.796 $mg_p/d$, respectively. Statistical analysis showed that the biodegradation curves of the five bead sizes are significantly different, and perchlorate degradation in the 0.128 mm resin is significantly higher than that of the other sizes. However, the bioregeneration curves of 0.547 mm and 0.652 mm resins, 0.652 mm and 0.778 mm resins, and 0.778 mm and 0.924 mm resins are not significantly different in pairs, while the bioregeneration curves of 0.547 mm and 0.778 mm resins are significantly different ($p=0.047$).

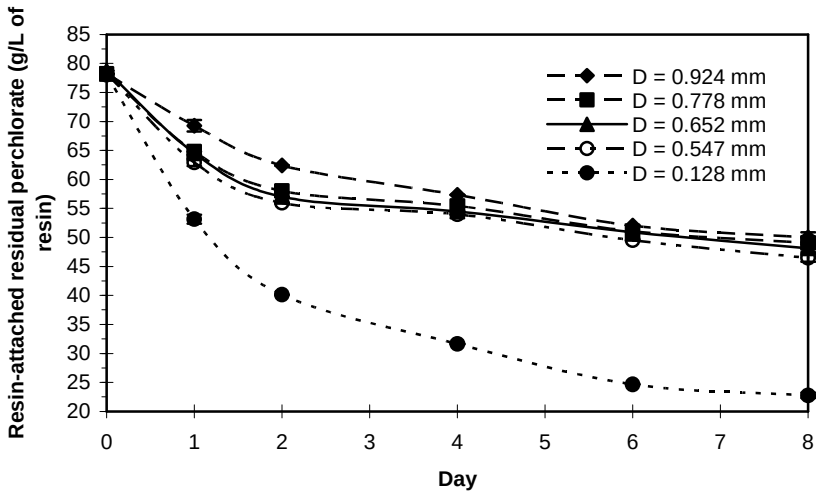


Figure 4.8. Residual Resin-attached Perchlorate Concentration for Different Resin Bead Sizes for Perchlorate-selective Resin (SIR-110HP).

Mass balance calculations showed that if all the RAP content inside the batch bioreactor-tube had desorbed entirely and suddenly, free-perchlorate concentration inside the batch bioreactor-tube would be approximately 7 mg/$L_{culture}$. In Figure 4.8, it takes 8 days for resin size of 0.128 mm to have 71% reduction of the initial perchlorate load. Assuming that the degradation of perchlorate obeys first-order kinetics, using the biodegradation rate constant, k, from the data for perchlorate biodegradation in water from Cox et al. (2000), Logan (2001a), and Logan et al. (2005) as $1.61\times10^{-5}$, $7.66\times10^{-5}$, and $4.69\times10^{-6}$ 1/s, respectively, the time needed to reach 71% reduction of the initial perchlorate load (i.e. 7 mg/L) can be calculated as: $C = C_0 e^{-kt}$, where, C is the concentration at a certain time (mg/L), $C_0$ is the initial concentration (mg/L), k is the biodegradation rate constant (1/s), and t is time (s). Accordingly, about 11 hours is needed to have 71% degradation of initial perchlorate concentration of 7 mg/L.

However, in the bioregeneration experiments a significant amount of perchlorate was still present in the resin after 8 days of bioregeneration (Figure 4.8). Hence, there is a controlling step involved in the bioregeneration process that slows it down as compared to perchlorate degradation in water. The perchlorate degradation rate, however, significantly improved by increasing the ratio between the external surface area per volume of the resin bead $\left[\left(\frac{\pi d^2}{\pi d^3/6}\right)=\frac{1}{mm}\right]$ of resin brad (Figure 4.9), suggesting that resin bead size have a significant effect on biodegradation rates.

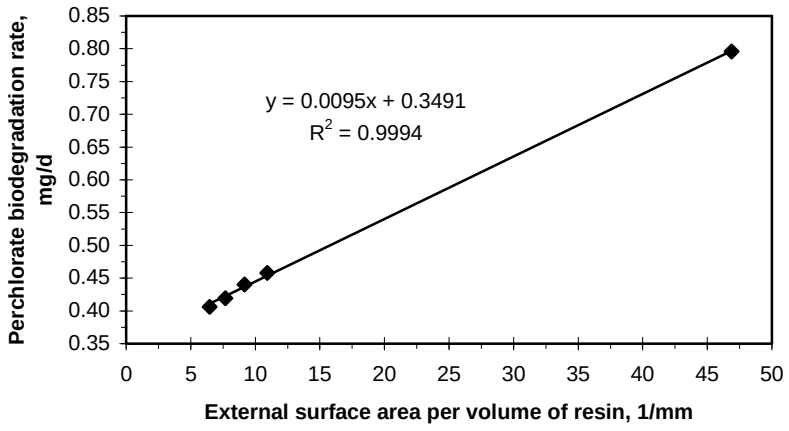


Figure 4.9. The Effect of Increasing External Surface Area per Volume of Resin Sphere on Biodegradation of Resin-attached Perchlorate.

Shrinking core modeling (Arevalo et al., 1998; Pritzker, 2005; Venkatesan et al., 2010) was used to study the effect of resin bead size on the bioregeneration process. Based on the shrinking core model, in the bioregeneration process, RAP ions in the outer

region of the resin are utilized prior to the RAP ions that are located deep inside the resin bead. For resin bead sizes of 0.924, 0.778, 0.652, 0.547, and 0.128 mm, respectively, 36.1%, 37.3%, 38.8%, 40.6%, and 70.8% of the initial perchlorate load was degraded after 8 days of bioregeneration. According to the shrinking core model the unused core containing RAP ions within the resin for theses resin sizes has the radius of 0.398, 0.333, 0.277, 0.229, and 0.042 mm, respectively. Figure 4.10 shows the results of shrinking core model analysis as resins bead size versus radius of unused core within the resin bead. As shown in Figure 4.10, reducing the resin bead size has a direct effect on decreasing the ratio of radius of unused core to the radius of resin bead ($r/r_0$). In Figure 4.10, the volume of outer region of the resin bead, $\left[1-\frac{r^3}{r_0^3}\right]\times 100\%$, in which the perchlorate load is depleted has been calculated as 36.1%, 37.3%, 38.8%, 40.6%, and 70.8% for the resin bead sizes of 0.924, 0.778, 0.652, 0.547, and 0.128 mm, respectively.

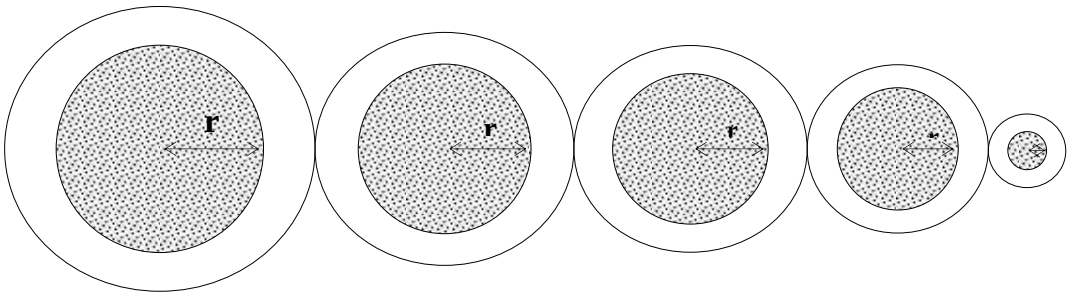


Figure 4.10. Changing the Unused Core Containing Perchlorate within the Resin Bead Based on Shrinking Core Model, r is Radius of Unused Core and $r_0$ is the Total Radius of Resin Bead.

The results of analysis of mass transfer controlling step in resin bioregeneration process are shown in Table 4.9. The results specify that for the resin sizes of 0.924, 0.778, 0.652, and 0.547 mm, pore diffusion is about equally fast as film diffusion since the $\left[\frac{X\overline{D}\delta}{CDr_0}(5+2\alpha_B^A)\right]$ value is about 1. Thus both film and pore diffusion influence the rate of mass transfer. However, bioregeneration process for the resin size 0.128 mm is film diffusion controlled. Hence, for the resin sizes of 0.924, 0.778, 0.652, and 0.547 mm both mass transfer mechanisms (i.e. pore diffusion and film diffusion) influence the rate of mass transfer, and any change in the process that enhances either pore diffusion or film diffusion enhances the overall bioregeneration rate. It is well-established that in an ion exchange process controlled by pore diffusion, decreasing the resin bead size enhances the overall rate of exchange (Helfferich, 1962; Weber, 1972). Accordingly, since the overall performance of the bioregeneration process was improved by increasing decreasing the resin bead size, mass transfer of ions through the pores seems to play an important role in the bioregeneration process. However, decreasing the resin size increases the relative magnitude of film diffusion on the process. Yet, film diffusion is not as significant as pore diffusion, because in the case of a resin bead size of 0.128 mm, although the process is film diffusion controlled, the overall biodegradation is superior compared to the other sizes (Figure 4.10). Therefore, the influence of pore diffusion is more considerable than the influence of film diffusion in determining the rate of the process.

Table 4.9. Mathematical Expression of the Mass Transfer Controlling Step in the Bioregeneration Process

| X ($eq_p/L_r$) | $\overline{D}$ ($cm^2/s$) | δ (cm) | C ($eq_{Cl}/L_s$) | D ($cm^2/s$) | Resin size (mm) | $r_0$ (cm) | Selectivity Coef. | $\frac{X\overline{D}\delta}{CDr_0}(5+2\alpha_B^A)$ |
|---|---|---|---|---|---|---|---|---|
| 0.78 | $4.67 \times 10^{-9}$ | $10^{-3}$ | 0.056 | $10^{-5}$ | 0.924 | 0.0462 | 3500 | 0.99 |
| 0.78 | $4.67 \times 10^{-9}$ | $10^{-3}$ | 0.056 | $10^{-5}$ | 0.778 | 0.0389 | 3500 | 1.17 |
| 0.78 | $4.67 \times 10^{-9}$ | $10^{-3}$ | 0.056 | $10^{-5}$ | 0.652 | 0.0326 | 3500 | 1.40 |
| 0.78 | $4.67 \times 10^{-9}$ | $10^{-3}$ | 0.056 | $10^{-5}$ | 0.547 | 0.0273 | 3500 | 1.67 |
| 0.78 | $4.67 \times 10^{-9}$ | $10^{-3}$ | 0.056 | $10^{-5}$ | 0.128 | 0.0064 | 3500 | 7.12 |

The results of calculation of Thiele modulus are shown in Table 4.10. Since the effect of pore diffusion is similar to the effect of film diffusion for the resin sizes of 0.924, 0.778, 0.652, and 0.547 mm, diffusivity coefficient, $D_e$, for both pore diffusion and film diffusion was used to calculate the Thiele modulus. For resin size of 0.128 mm, diffusivity coefficient, $D_e$, for film diffusion was used.

Table 4.10. Calculation of Thiele Modulus for the Bioregeneration Process

| Resin size (mm) | $r_0$ (cm) | $M_T$ for $D_e = 4.67 \times 10^{-9}$ ($cm^2/s$) (pore diffusivity coefficient) | $M_T$ for $D_e = 10^{-5}$ ($cm^2/s$) (film diffusivity coefficient) |
|---|---|---|---|
| 0.924 | 0.0462 | 1.97[a] – 0.28[b] | 4.26E-02[a] – 5.96E-3[b] |
| 0.778 | 0.0389 | 1.66 – 0.23 | 3.59E-02 – 5.02E-3 |
| 0.652 | 0.0326 | 1.39 – 0.19 | 3.01E-02 – 4.21E-3 |
| 0.547 | 0.0273 | 1.17 – 0.16 | 2.52E-02 – 3.53E-3 |
| 0.128 | 0.0064 | NA | 5.90E-03 – 8.28E-4 |

[a] The highest perchlorate biodegradation rate constant as of $7.66 \times 10^{-5}$ 1/s was used to calculate $M_T$
[b] The lowest perchlorate biodegradation rate constant as of $1.50 \times 10^{-6}$ 1/s was used to calculate $M_T$

The results show that bioregeneration process is not governed by kinetics of biodegradation of desorbed perchlorate ions by PRB. The calculation shows that bioregeneration process is controlled by diffusion, since Thiele modulus was found less than 3 (Helfferich, 1962).

4.4.3. Effect of Ion-exchange Resin Structure on the Bioregeneration Process

Perchlorate degradation curves of gel-type (i.e. SIR-110HP and ASB-1) and macroporous (i.e. SIR-110HP-MACRO and SR-7) resins with uniform resin bead size of 0.778 mm are shown in Figures 4.11.a and b. In Figure 4.11.a, the degradation curves start at different initial RAP load because of the differences in the loading stage (Table 4.4).

Table 4.11 shows the average observed resin-attached perchlorate degradation rate for the batch bioregeneration experiments. Figure 4.11.b shows concentration of RAP to initial concentration of RAP ($C/C_0$), since in Figure 4.11.a, initial concentration of RAP in the resin is different in different resins. The average observed resin-attached perchlorate degradation rate for ASB-1 and SIR-110HP resins which are gel-type is 0.607 and 0.419 $mg_p/d$, respectively, while it is 0.688 and 0.681 $mg_p/d$ for SIR-110HP-MACRO and SR-7 macroporous resins, respectively.

The average observed free-perchlorate degradation rate from other studies is also shown in Table 4.11. The average observed resin-attached perchlorate degradation rate is about one order of magnitude lower than the average observed free perchlorate degradation rate. As it was discussed in section 4.4.2, the resin-attached perchlorate degradation is likely limited by mass transfer in the pores of the resin beads. Statistical analysis revealed that perchlorate biodegradation in macroporous resins was significantly

more effective than that of gel-type resins, although all the employed resins had uniform resin bead size (Table 4.7). However, no significant difference in perchlorate biodegradation between perchlorate-selective (i.e. SIR-110HP and SIR-110HP-MACRO) and non-selective (i.e. ASB-1 and SR-7) resins was found.

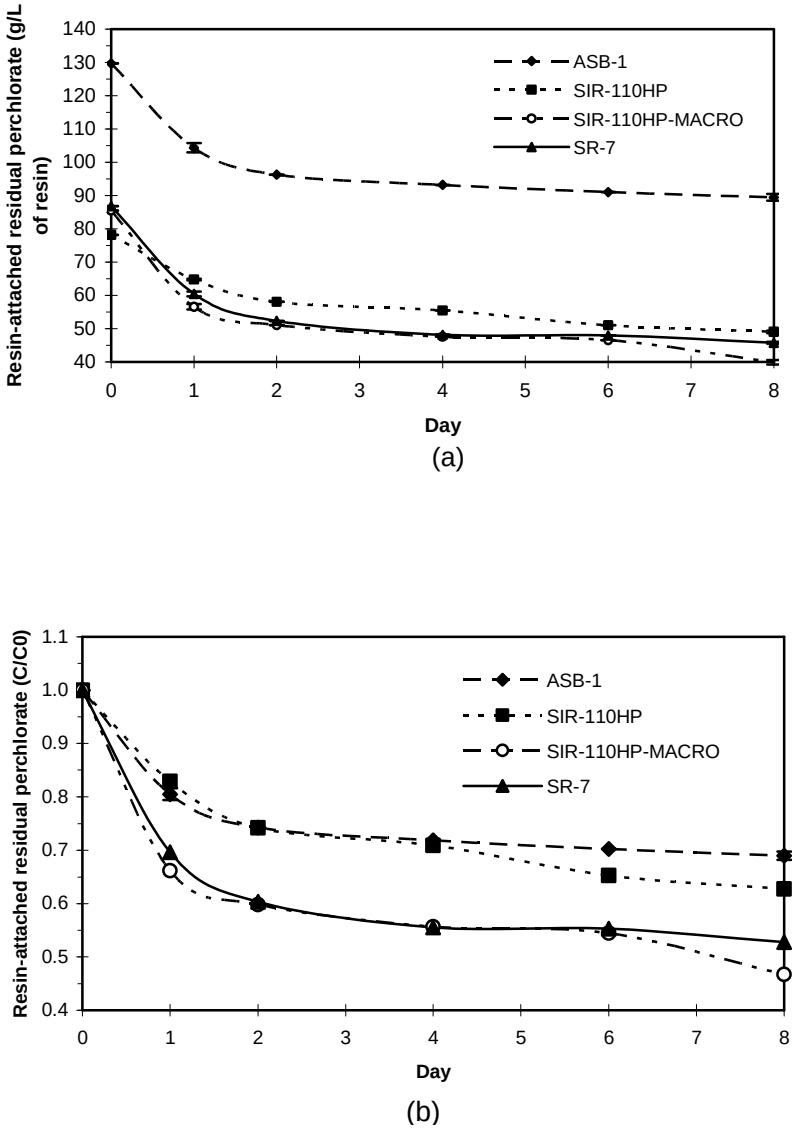


Figure 4.11. Residual Resin-attached Perchlorate Concentration in Gel-type (ASB-1 and SIR-110HP) and Macroporous (SR-7 and SIR-110HP-MACRO) Resins.

Table 4.11. Resin-attached Perchlorate Degradation in Gel-type and Macroporous Resins

| Resin name, structur type | $ClO_4^-$ ion situation | Observed perchlorate biodegradation rate ($mg_p/mg_{ss}/d$) | Observed perchlorate biodegradation rate ($mg_p/d$) | Reference |
|---|---|---|---|---|
| ASB-1, gel | Attached to resin | 0.031 | 0.607 | This Study |
| SIR-110HP, gel | Attached to resin | 0.022 | 0.419 | This Study |
| SIR-110HP-MACRO, macroporous | Attached to resin | 0.035 | 0.688 | This Study |
| SR-7, macroporous | Attached to resin | 0.035 | 0.681 | This Study |
| NA | Free in water | 1.68 | NA | Korenkov et al., 1976 |
| NA | Free in water | 2.57 | NA | Attaway and Smith., 1993 |
| NA | Free in water | 0.36 | NA | Logan et al., 2001 |
| NA | Free in water | 0.64 | NA | Shrout and Parkin, 2006 |

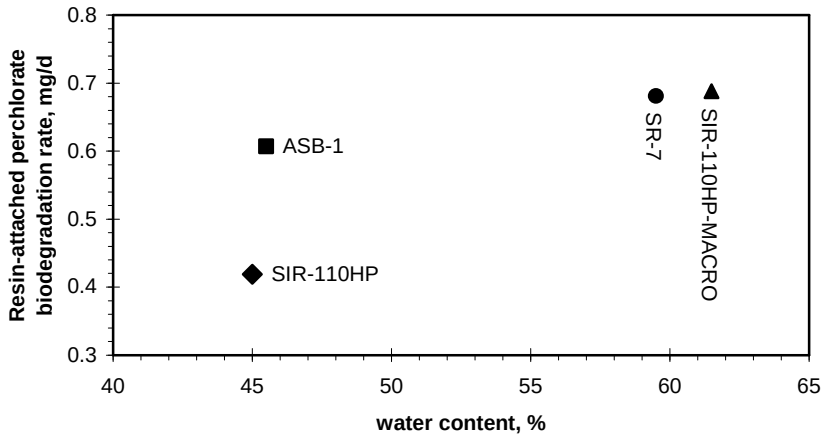


Figure 4.12. Resin-attached Perchlorate Biodegradation Rate Verses Water Content for ASB-1 and SIR-110HP (i.e. Gel-type Resins), and SR-7 and SIR-110HP-MACRO (i.e. Macroporous Resins)

The results of section 4.4.2 suggest the pore diffusion of the desorbed perchlorate ions to the surface of the resin bead is likely the main controlling step in the bioregeneration process. Macroporous resins have macropores which contributes to higher water content compared to gel-type resins (Table 4.3) (Sherman et al., 1986). Considering the overall biodegradation rates of macroporous and gel-type resins and the difference among their water content (Figure 4.12), it appears that mass transfer control is stronger in gel-type resins than that of macroporous resins. Table 4.11 shows the RAP biodegradation rate in tested resins with different water contents. The possible reason for stronger mass transfer limitation in gel-type resins and as a result lower RAP biodegradation rate could be the resin structure. It is likely that smaller pore size in gel-type resins results in stronger pore diffusion limitation, due to less availability of liquid phase to carry hydrated perchlorate ions, and makes the bioregeneration process slower in the case of gel-type resins.

## 4.5. Discussion

A conceptual model for resin bioregeneration process was envisioned in this research involving detachment of perchlorate from the resin functional site, its diffusion through the resin pores and liquid film, and its biodegradation inside the PRB cells. Accordingly, the objectives of this research were defined to be: study the effect of different initial concentrations of chloride as the envisioned desorbing agent for RAP release; investigate the effect of resin size and different resin structures (i.e. gel-type and macroporous) in the bioregeneration process; and explore the controlling step in the conceptual model of resin bioregeneration process.

The results showed that chloride is likely to be the desorbing agent for desorption of RAP ions in the bioregeneration process. Also it was found that increasing the initial chloride concentration significantly improves the bioregeneration performance in both perchlorate-selective (i.e. SIR-110HP) and non-selective (i.e. ASB-1) resins. Based on the results, even in very low concentrations of chloride (i.e. 1 mg/L of chloride) bioregeneration process can be initiated. However, increasing the chloride concentration from 500 mg/L to 5000 mg/L as NaCl only slightly enhances bioregeneration performance. Chloride, as the product of perchlorate biodegradation, is the anion which is always present in all perchlorate-reducing cultures grown using perchlorate. As mentioned before, the minimum of 730 mg/$L_{culture}$ of chloride occurs if a minimum of 2040 mg/L of perchlorate is used as the electron acceptor to grow 1000 mg/L of PRB suspended solids. Therefore, any PRB culture grown in perchlorate as the electron acceptor contains sufficient chloride concentration, and can be used in the IX resin bioregeneration process.

Increasing the chloride concentration by adding NaCl salt to PRB to boost the performance of bioregeneration process can be considered in IX resin bioregeneration plants. However, very high concentrations of chloride have inhibitory effects on the biological path of perchlorate biodegradation (Logan et al., 2001, Gingras and Batista 2002). NaCl concentrations of 1-1.5% (10,000-15000mg/L) can reduce perchlorate biodegradation rates over 90% (Gingras and Batista 2002). Thus, chloride concentration levels should be monitored at all times, especially when NaCl salt is used to boost the performance of the bioregeneration process.

The results indicated that decreasing the resin size from 0.924 mm to 0.128 mm has a significant positive effect on the bioregeneration performance. Except for very small resin size (i.e. 0.128 mm), the effects of pore diffusion were found to be about as equal to film diffusion in the mass transfer process of bioregeneration. For the very fine resin bead size (i.e. 0.128 mm), the mass transfer rate was film diffusion limited.

The bioregeneration process was found to be mass transfer limited rather than biodegradation kinetics limited due to the following four evidences: First, the results of the experiments in this research showed that in the resin size of 0.128 mm, 71% of initial perchlorate load can be degraded after 8 days. However, if the perchlorate load was dissolved in the liquid phase, 71% reduction of initial perchlorate would be obtained after about 11 hours. This evidence shows that the bioregeneration process is not biodegradation kinetic limited. Second, resin size reduction improved the performance of bioregeneration process, showing that pore diffusion was improved while the resin bead size was reduced. Third, macroporous resins were found to be more effective in the bioregeneration process, due to their higher water content and pore size which lead to higher pore diffusion rate in macroporous resins compared to pore diffusion rate in gel-type resins. Forth, the Thiele modulus calculations showed that the process is mass transfer limited rather than biodegradation rate limited.

The typical sizes of ion-exchange resin beads mostly vary between 1.1 mm to 0.3 mm (ResinTech, West Berlin, NJ), however, most commonly used IX resins are about 0.7 mm in size. For bioregeneration, employing smaller resin bead sizes would be preferable due to higher pore mass transfer flux. Using smaller resin beads, however, increases the potential chance of losing resin in the loading-bioregeneration process. Film diffusion

can be controlled by increasing the turbulence in the bioregeneration reactor. Increasing the flow rate in the bioregeneration process results in higher turbulence and a thinner quiescent liquid film (boundary layer) surrounding the resin beads. If bioregeneration is performed in a fluidized bed reactor (FBR) (Venkatesan et al., 2010), higher flow rates constitute a greater expansion of the resin bed, which corresponds to larger FBR volume needed. Additionally, higher flow results in a higher pressure drop since a very fine screen is needed to keep the small resin beads in the FBR, leading to a higher energy consumption in the FBR. Temperature also should be monitored in the bioregeneration process, since even slight temperature drops contribute to increasing the viscosity and higher film diffusion control.

Macroporous resins (i.e. SIR-110HP-MACRO and SR-7) with higher water content and larger pore sizes were found to be more effective in the bioregeneration process when compared to gel-type resin (i.e. SIR-110HP and ASB-1). Mass transfer flux in macroporous resins is known to be higher than that of gel-type resins. Thus, employing macroporous resins is recommended due to higher bioregeneration performance. In addition, macroporous resins are more resistant to oxidation and less vulnerable to fouling when compared to gel-type resins. However, using macroporous resins contributes to higher capital costs due to higher price compared to gel-type resins.

The overall recommendations are as follows: a) use initial NaCl concentrations between 1000 mg-NaCl/L and 5000 mg-NaCl/L, b) monitor salt level in the PRB culture to keep it below the inhibitory level for PRB microbial activities, c) use IX resin with smaller resin bead size to increase pore mass transfer, d) enhance turbulence in the

reactor to minimize negative effects of film diffusion, e) use of macroporous resins to have higher pore mass transfer rates.

## 4.6. Conclusions

The specific conclusions of this work can be summarized as follows:

1) Chloride ion, which is the product of perchlorate biodegradation and available in all PRB cultures fed with perchlorate, is likely the desorbing agent of RAP in IX bioregeneration process.

2) Increasing the concentration of chloride in the microbial solution has a positive effect on perchlorate desorption from the resin. Degradation rates for selective and non-selective resins respectively were 0.375 and 0.370 $mg_p$/d when no chloride was added as compared to 0.535 and 0.583 $mg_p$/d when 5,000 mg/l chloride was present.

3) The results show that decreasing the resin bead size from 0.924 mm to 0.128 mm has a significant positive effect on IX resin bioregeneration process (i.e. 55% improvement in the overall perchlorate biodegradation rate), while, decreasing the resin size from 0.778 mm to 0.652 mm and from 0.652 mm to 0.547 mm was not significant positive effect on IX resin bioregeneration process.

4) Bioregeneration is mass transfer controlled rather than biodegradation kinetic controlled. Both the kinetics of desorption of RAP ions and kinetics of biodegradation of desorbed perchlorate ions were found not to control the overall rate of bioregeneration.

5) For the resin sizes of 0.924, 0.778, 0.652, and 0.547 mm, which are the common sizes of resins available in the market, both mass transfer mechanisms (i.e. pore diffusion and film diffusion) were found to control the bioregeneration process. For small resin size of 0.128 mm, film diffusion was found to be the controlling step in bioregeneration.

6) Macroporous resins were more effectively bioregenerated compared to gel-type resins. This result can be explained by larger water content and larger average pore size in macroporous compared to gel-type resins.

# CHAPTER 5

# MULTI-CYCLE BIOREGENERATION OF SPENT PERCHLORATE CONTAINING MACROPOROUS SELECTIVE ANION EXCHANGE RESIN

## 5.1. Abstract

Ion-exchange (IX) using perchlorate-selective resin is possibly the most feasible technology for perchlorate removal. However, commercially available gel and macroporous resins cannot be regenerated using traditional brine desorption. The use of macroporous resins compared to gel-type resins is expanding because of their stability and greater resistance to fouling. In water treatment applications, selective resins are currently used once and then incinerated, making the IX process economically and environmentally unsustainable. A new concept has been developed involving biological regeneration of resin containing perchlorate. This concept involves directly contacting perchlorate-containing resins with a perchlorate-reducing microbial culture. In this study, the feasibility of multi-cycle loading and bioregeneration of a macroporous perchlorate-selective resin was investigated. Loading and bioregeneration cycles, using a bench-scale fermenter and a fluidized bed reactor (FBR), followed by fouling removal and disinfection of the resin were performed. The results revealed that selective macroporous resin can be successfully employed in a consecutive loading-bioregeneration IX process. Resin capacity loss stabilized after a few cycles of bioregeneration indicating that the number of loading and bioregeneration cycles that can be performed is likely greater than the five cycles tested. The results also revealed that most of the capacity loss in the resin is due to perchlorate buildup in the resin as a consequence of clogging of the pores of the resin beads. Perchlorate buildup was found to be the major drawback of the

bioregeneration process. Perchlorate buildup may limit the reuse of the resin after bioregeneration, if the treated resin cannot produce a water quality that meets the desired standards or if the amount of bed volumes processed is significantly reduced. The results further indicate that as bioregeneration progresses, clogging of the resin pores results in the decrease in mass transfer flux from the inner portion of the resin to the bulk microbial culture contributing to stronger mass transfer limitation in the bioregeneration process.

## 5.2. Introduction

Perchlorate ($ClO_4^-$) contamination has been detected in several surface and groundwater sources in 26 states in the United States, particularly in the arid Southwestern region (USEPA, 2003; Brandhuber and Clark, 2005), and it has been on the United States Environmental Protection Agency's (USEPA's) drinking water Contaminant Candidate List (CCL) since 1998 (Brandhuber and Clark, 2005). Most of the perchlorate contamination in the environment is due to rocket fuel manufacturing and use (Urbansky et al., 2000). In addition, perchlorate is used in other industries to produce explosives, matches, firework supplements, air bags, and other products. (Wu, et al., 2008). Recent studies show that electrical discharge through chloride aerosol in the atmosphere potentially can produce perchlorate and release it to the environment by rain and snow (Rao, et al., 2007, Dasgupta, et al., 2005). Perchlorate in the human body interferes with the natural process of iodine adsorption by the thyroid gland, inhibits thyroid hormone production, and causes iodine accumulation in the gland (Wolff, 1998; Kirk, 2006). The Interim Drinking Water Health Advisory level for perchlorate has been issued as 15 micrograms per liter (μg/L) in 2005 (NRC, 2005).

Biological reduction and IX are the most effective technologies to remove perchlorate from waters (Logan et al., 2001; Gingras and Batista, 2002). Although bioremediation is a cost-effective technology for perchlorate removal, it is not an efficient technology for low concentrations because degradation rates slow down with decreasing perchlorate concentration (i.e. perchlorate biodegradation has relatively high half-saturation constants (Logan et al., 2001; Dudley et al., 2008) (Table 5.1)). Ion-exchange is currently the technology of choice to remove low concentrations of perchlorate from drinking waters (Gingras and Batista, 2002; Lehman et al., 2008). Morphologically, perchlorate-selective resins can be categorized into gel- and macroporous-type resins. The average pore size of gel-type resins is about 0.0005 µm, while macroporous resins have an average pore size of about 0.6 µm (Kun and Kunin, 1968; Dale et al., 2001). The percent crosslinking of the backbone polymer chains in gel-type and macroporous resins varies. Gel-type resins have about 4-10% crosslinking, whereas macroporous resins have 20-25% crosslinking in average (Crittenden et al., 2005). The use of macroporous resins compared to gel-type resins is expanding due to their stability, resistance to oxidation, and less vulnerability to fouling (Weber, 1972; Li and SenGupta, 2000).

There are many pilot and full scale operational and under-construction water supplies treatment plants using IX technology to remove perchlorate from drinking water throughout the United States. These plants have capacities varying from 23 to 55,000 $m^3$/day (4 to 10,000 gpm) and influent perchlorate concentration varying from 7 to 350 µg/L. These plants can remove perchlorate to < 4 µg/L (NASA, 2006). In recent years, IX resins with high affinity for perchlorate ion (i.e. perchlorate-selective) have been manufactured (Seidel et al., 2006). Even though these resins can treat a large number of

bed volumes (BVs) of water before perchlorate breakthrough occurs, regeneration by traditional brine desorption technique cannot be employed, and the spent perchlorate-selective resin is incinerated or disposed in a landfill after one time use. Incineration of the resin produces greenhouse gases, and landfilling presents the potential for re-contamination of the environment with perchlorate. Although a regeneration method has been developed employing $FeCl_3$-HCl solution as the regenerant for one type of perchlorate-selective gel-type resin (Gu et al., 2001), regeneration of most commercially available perchlorate-selective resins using NaCl brine is not feasible and is not currently practiced.

Table 5.1. Half-saturation Constant and Maximum Perchlorate Utilization Rate for Degradation of Free Perchlorate Ion in Water

| Culture | Kinetic parameters | | Reference |
|---|---|---|---|
| | $q_{max}(d^{-1})$ | $K_s$ (mg/L) | |
| PDX | 0.41 | 12 ± 4 | Logan et al., 2001 |
| KJ | 1.32 | 33 ± 9 | Logan et al., 2001 |
| INS | 4.34 | 18 | Waller et al., 2004 |
| ABL1 | 5.42 | 4.8 | Waller et al., 2004 |
| SN1A | 4.60 | 2.2 | Waller et al., 2004 |
| RC1 | 6.00 | 12 | Waller et al., 2004 |
| PC 1 | 3.09 | 0.14 | Nerenberg et al., 2006 |
| HCAP-C | 4.39 | 76.6 | Dudley et al., 2008 |
| Mixed culture | 0.49 | <0.1 | Wang et al., 2008a |

Resin bioregeneration as a new concept in IX technology has been developed and patented (Batista, 2006). This concept is based on directly contacting IX resin containing perchlorate with a perchlorate-reducing microbial culture under anoxic/anaerobic conditions. The feasibility of this concept has been reported on perchlorate-selective and non-selective gel-type resins (Batista and Jensen, 2006; Batista et al., 2007b).

Although the biodegradation of free perchlorate ions in water has been well studied (Logan, 1998; Coates and Achenbach, 2004), the biological reduction of attached-perchlorate ions to a medium such as IX resins has only recently been initiated (Wang et al., 2008b; Wang et al., 2009; Venkatesan et al., 2010). Investigation has shown that biological degradation of resin-attached perchlorate has a slower degradation rate compared to biological degradation of free perchlorate (Venkatesan et al., 2010).

A main concern in resin bioregeneration is capacity loss due to biological fouling of the resin beads (Batista et al., 2007a). Macroporous resins are less susceptible to bio-fouling than gel-type resins. Although resin bioregeneration has several environmental benefits, to be economically viable, the cost of purchasing fresh resin must be equal or greater than costs of resin bioregeneration. Therefore, resin bioregeneration is sustainable only if the process can be repeated for several consecutive exhaustion-bioregeneration cycles.

Bioregeneration of gel-type resins loaded with perchlorate has been investigated recently (Venkatesan et al., 2010). Biodegradation of macroporous resins has not been fully investigated, to date. In this research, the feasibility of multi-cycle bioregeneration of macroporous perchlorate-selective resin, which is thought to be less susceptible to fouling, has been investigated. The specific objectives of this research were to: (1) evaluate the feasibility of multi-cycle bioregeneration of macroporous IX perchlorate-selective resin, (2) estimate the capacity loss after the multi-cycle bioregeneration process, (3) evaluate the influence of resin fouling on mass transfer mechanism of bioregeneration process, and (4) compare the degradation rate of resin-attached perchlorate in the macroporous resin with degradation rate of soluble perchlorate in

water. The findings of this research can be useful in determining potential capacity loss during bioregeneration. Capacity loss is a major factor influencing economics and full-scale application of bioregeneration as a technology for perchlorate treatment.

## 5.3. Materials and Methods

### 5.3.1. Experimental Approach

A bioregeneration system that includes a fermenter and a FBR was designed and built. The resin was loaded batchwise and placed in the FBR (Figure 5.1). A perchlorate-reducing microbial culture present in the fermenter was passed through the FBR containing the resin. At pre-determined time intervals, resin samples were taken from the FBR and residual perchlorate concentration in the resin was determined. An oxygen Parr bomb method was developed to determine the amount of resin-attached-residual perchlorate with time. Following bioregeneration, the resin was defouled and disinfected with sodium hypochlorite and the resin capacity was measured. This procedure was repeated for five consecutive cycles of loading and bioregeneration.

### 5.3.2. Experimental Setup

A small scale system was assembled to bioregenerate the selected resin for five cycles. The set-up consisted of a 10-gallon (37.9 L) polyethylene fermenter and a 3-inch diameter (7.6 cm) x 50-inch (127 cm) tall plexiglass FBR. Three ports were drilled along the FBR column to take resin samples (Figure 1). The fermenter for cultivation of perchlorate-reducing bacteria (PRB) was equipped with automatic oxidation reduction potential (ORP), pH, dissolved oxygen (DO), and temperature probes. A stirrer was used to gently mix the bacterial enrichment culture in the fermenter at 25-30 rpm rotational speed. Before each bioregeneration cycle, the fermenter was purged with nitrogen gas

for 30 minutes to establish anaerobic conditions. Perchlorate loaded resin (section 5.3.4) was placed in the FBR column and the bacterial enrichment culture was fed upflow using a peristaltic pump to achieve 30-40% expansion of the resin bed. The microbial culture was then recirculated back to the fermenter where acetate (i.e. the electron donor), nutrient, and minerals were added to sustain bacterial growth. After the bioregeneration cycle, the resin was rinsed, defouled, and disinfected.

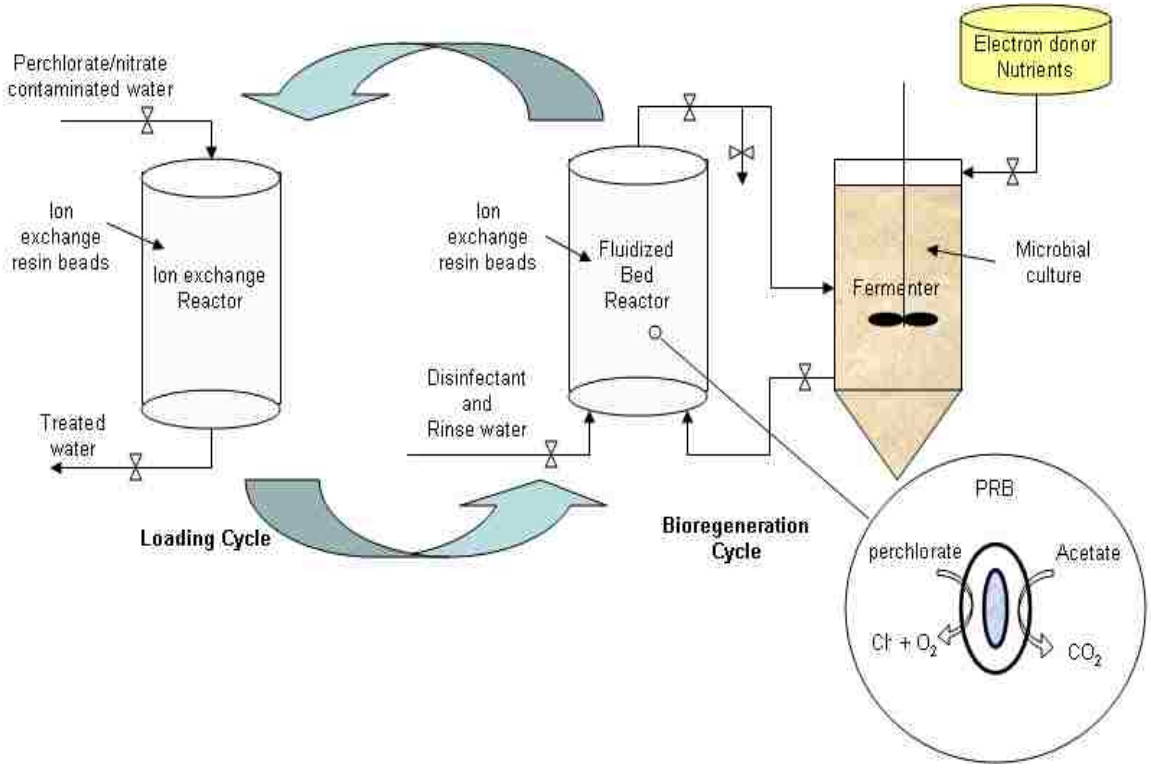


Figure 5.1. Schematic of the Resin Bioregeneration System Technology (Left: Loading of Resin with Perchlorate during Water Treatment; Right: Bioregeneration of Spent Resin Using a Microbial Culture).

### 5.3.3. Composition of the Enrichment Culture

A mixed enrichment bacterial culture was used in this research. The sources of PRB inocula were waters from Lake Mead and the Las Vegas Wash in southern Nevada. These areas have been contaminated with ammonium perchlorate for the past five decades and were presumed likely sources of PRB. The PRB were enriched under anoxic conditions in an acetate, perchlorate, and mineral/nutrient/buffer broth (Table 5.2). Although resin-attached nitrate was available in the FBR as the nitrogen source for PRB, $NH_4H_2PO_4$ was added to the microbial culture to supply nitrogen needed through the bioregeneration process (Table 5.2).

Table 5.2. Nutrient and Buffer Stock Solution for Feeding the Culture

| Stock solution | Components | Concentration of component (g/L) |
|---|---|---|
| Nutrients | $MgSO_4.7H_2O$ | 5.500 |
| | EDTA | 0.300 |
| | $ZnSO_4.7H_2O$ | 0.200 |
| | $CaCl_2.2H_2O$ | 0.100 |
| | $FeSO_4.7H_2O$ | 0.400 |
| | $Na_2MoO_4.2H_2O$ | 0.040 |
| | $CuSO_4.5H_2O$ | 0.020 |
| | $CoCl_2.6H_2O$ | 0.040 |
| | $MnCl_2.4H_2O$ | 0.100 |
| | $NiCl_2.6H_2O$ | 0.010 |
| | $NaSeO_3$ | 0.010 |
| | $H_3BO_3$ | 0.060 |
| Buffer | $K_2HPO_4$ | 155.0 |
| | $NaH_2PO_4.H_2O$ | 97.783 |
| | $NH_4H_2PO_4$ | 50.0 |

A 3:1 molar ratio for acetate/perchlorate was used during the enrichment phase. The PRB enrichment culture was then characterized using two different molecular methods, Restriction Fragment Length Polymorphism (RFLP) and 16S rRNA sequencing (Kesterson et al., 2005).

### 5.3.4. Resin Loading

The resin used in this research was ResinTech SIR-110-MACRO which is a macroporous perchlorate-selective strong base anion-exchange resin has tri-n-butyl-amine ($(C_4H_9)_3N$) functional groups having a nominal resin capacity of 0.6 eq/L (ResinTech, Inc., West Berlin, NJ). The resin bead size ranges from 1.18 mm to 0.30 mm (i.e. from #16 to #50 U.S. standard mesh size). This resin was specially manufactured for this research.

The resin used for the bioregeneration tests was loaded batchwise instead of in a column. Loading was performed batchwise because of the large volume of water needed to load the resin in a column and to shorten the time period required to load the resin. Additionally, in the batch loading process, the ions are distributed homogeneously through the resin beads. The composition of the synthetic solution and residual concentrations used to load the resin for five cycles are given in Table 5.3. The feed water composition was chosen to simulate the amount of anions that would be present in the resin when waters with high perchlorate concentrations, typically found in industrial sites, are treated with IX.

Table 5.3. Resin Loading Data through Five Cycles of the Experiment

| Cycle # | Anion | Added feed conc., mg/L | Residual conc. after 24 hr, mg/L | Capacity occupied, g/L resin | Capacity occupied, meq/Lresin | Percent capacity occupied[a] |
|---|---|---|---|---|---|---|
| Cycle 1 | $ClO_4^-$ | 11061 | 0.257 | 11.06 | 111.16 | 17.5% |
| | $NO_3^-$ | 491 | 12.6 | 0.478 | 7.71 | 1.2% |
| | $Cl^-$ | 514 | >2100 | N/A | N/A | N/A |
| | $SO_4^{-2}$ | 532 | 251 | 0.281 | 5.85 | 0.9% |
| | $HCO_3^-$ | 500 | 800 | N/A | N/A | N/A |
| Cycle 2 | $ClO_4^-$ | 10151 | 0.265 | 10.15 | 102.01 | 16.1% |
| | $NO_3^-$ | 499 | 15.4 | 0.484 | 7.81 | 1.2% |
| | $Cl^-$ | 495 | 2988 | N/A | N/A | N/A |
| | $SO_4^{-2}$ | 532 | 133 | 0.3987 | 8.31 | 1.3% |
| | $HCO_3^-$ | 500 | 750 | N/A | N/A | N/A |
| Cycle 3 | $ClO_4^-$ | 10229 | 0.502 | 10.23 | 102.79 | 16.2% |
| | $NO_3^-$ | 468 | 12.4 | 0.456 | 7.35 | 1.2% |
| | $Cl^-$ | 511 | 2891 | N/A | N/A | N/A |
| | $SO_4^{-2}$ | 506 | 161.2 | 0.344 | 7.17 | 1.1% |
| | $HCO_3^-$ | 500 | 690 | N/A | N/A | N/A |
| Cycle 4 | $ClO_4^-$ | 10082 | 1.305 | 10.08 | 101.31 | 16.0% |
| | $NO_3^-$ | 464 | 12.8 | 0.451 | 7.27 | 1.1% |
| | $Cl^-$ | 508 | 2886 | N/A | N/A | N/A |
| | $SO_4^{-2}$ | 516 | 163 | 0.353 | 7.35 | 1.2% |
| | $HCO_3^-$ | 500 | 635 | N/A | N/A | N/A |
| Cycle 5 | $ClO_4^-$ | 10018 | 1.061 | 10.02 | 100.67 | 15.9% |
| | $NO_3^-$ | 458 | 7.47 | 0.451 | 7.27 | 1.1% |
| | $Cl^-$ | 506 | 2964 | N/A | N/A | N/A |
| | $SO_4^{-2}$ | 495 | 199.2 | 0.296 | 6.16 | 1.0% |
| | $HCO_3^-$ | 500 | 610 | N/A | N/A | N/A |

[a] Total capacity = 0.64 eq/Lresin

A volume of 1300 mL of resin was selected for use through five cycles of loading, bioregeneration, fouling removal, and disinfection. In the loading step, the initial volume of resin was measured and added to a glass bottle. An equal volume of feed solution (Table 5.3) was prepared and added to the bottle. The bottle was then placed in a rotary mixer (Associated Design MFG Co., Alexandria, VA) at 30 rpm and 22±2ºC. After 24 hours, the mixture was allowed to settle for 3 min. The supernatant was sampled for residual analysis of anion and the left over supernatant was decanted. The initial and residual concentrations of anions in the solution were then submitted to Ion Chromatography (IC) analysis. The loaded resin was rinsed 6 times with 2 BVs of de-

ionized (DI) water to remove residual anions. Conductivity measurements of the rinsate solution showed that rinsing six times with 2 BVs of DI water was effective to remove the excess ions from the loaded resin. After the rinsing, the resin was stored in the refrigerator until the start of the bioregeneration experiments.

### 5.3.5. Resin Bioregeneration

The resin bioregeneration process was continued until the residual perchlorate concentration in the resin remained constant. The bioregeneration processes ran for a period of 9-14 days. Daily, 4 mL of resin sample were taken from the ports along the FBR column using a 20-mL syringe. The resin sample was then rinsed 6 times with 5 BVs of DI water to remove microbial cells and excess anions, labeled, and stored in the refrigerator. The residual perchlorate in the samples was then measured.

During the bioregeneration cycles, the effluent line from FBR to the fermenter was monitored for perchlorate daily, and no perchlorate was detected. Chemical oxygen demand (COD), suspended solids (SS), and conductivity analysis were performed on the samples taken form the fermenter on a daily basis.

Through the bioregeneration cycles, the volume of the microbial solution was 30L. The SS in the fermenter was maintained at 1000-2000 mg/L. Whenever the SS dropped too much owing to electron acceptor limitation, PRB cells originating from the stock enrichment culture were concentrated by centrifugation and added to the fermenter to increase the biomass. The amount of electron donor (acetate) in the fermenter was measured as COD and maintained above 1500 mg/L. After the bioregeneration process was complete, the microbial culture in the FBR was transferred from the bottom of FBR to the fermenter using a peristaltic pump. The resin was then rinsed with 1 BV of DI

water 5 times to remove microbial cells. The resin was then submitted to the fouling removal process.

### 5.3.6. Bio-fouling Removal and Disinfection

Biological fouling is a consequence of bioregeneration. The procedure used for fouling removal was developed in the Environmental Engineering Laboratory at University of Nevada Las Vegas (UNLV) (Batista et al., 2007a) (Table 5.4). As shown in Table 5.4, fouling removal procedure includes 4 consecutive steps. First, 1.5 BVs of fouling removal reagent (i.e. 12% NaCl / 2% NaOH mixture) was pumped up-flow to the FBR column containing resin. After 12 hours, the rinsate solution was decanted and sampled. Second, 1.5 BVs of fouling removal reagent (i.e. 12% NaCl / 2% NaOH mixture) was pumped up-flow to the FBR column, and after 4 hours, the rinsate solution was emptied and sampled. Third, 1.5 BVs of fouling removal reagent (i.e. 12% NaCl) was pumped up-flow to the FBR column, and after 2 hours, the rinsate solution was decanted and sampled. Last, the resin was rinsed with 3 BVs of DI water to remove excessive amount of ions. The rinsate solution samples collected after each step of fouling removal were submitted for COD analysis.

Following fouling removal procedure, the resin was disinfected using 1.5 BVs of sodium hypochlorite with the concentration of 100 mg/L as free chlorine which was pumped up-flow to the FBR column. After 15-20 minutes contact time, the solution was decanted. The resin then was rinsed until no residual chlorine was detected in the rinsate solution. Residual chlorine measurement using Capital Controls Series 17T2000 amperometric titrator (Steven Trent Services, Ft. Washington, PA) showed that rinsing with 2 BVs of DI water six times is sufficient to remove to remove all the residual

chlorine from resin. Total coliform analysis using IDEXX Quanti-Tray method (IDEXX Laboratories, Inc., Westbrook, ME) was performed on the DI water rinsate solutions after disinfection step. No coliform bacteria were detected in the rinsate solutions collected from the resin after disinfection procedure. The bioregenerated, defouled, disinfected, and rinsed resin was then submitted to loading process again to initiate the next bioregeneration cycle.

Table 5.4. Fouling Removal and Disinfection Procedure Used after Bioregeneration Process

| | Fouling removal / disinfection reagent | Applied volume | Retention time |
|---|---|---|---|
| Fouling removal procedure | 12% NaCl + 2% NaOH | 1.5 BV | 12 hours |
| | 12% NaCl + 2% NaOH | 1.5 BV | 4 hours |
| | 12% NaCl | 1.5 BV | 2 hours |
| | DI water rinse | 3 BV | N/A |
| Disinfection procedure | 100 mg/L free chlorine using sodium hypochlorite | 1.5 BV | 15-20 min |
| | DI water rinse | until no residual chlorine is detected in the rinsate | |

### 5.3.7. Residual Perchlorate Analysis

For this research, a method has been developed to measure resin-attached residual perchlorate remaining as bioregeneration progresses. In this method, small samples of resin are ignited in an oxygen Parr bomb (Parr Instruments, Moline, IL) and the perchlorate present is converted to chloride ion (resin-$ClO_4^-$ + $O_2 \rightarrow CO_2 + Cl^-$). The chloride concentration is then measured using IC.

Prior to ignition in the Parr bomb, 1 mL of resin sample was placed in 100 mL of concentrated nitrate solution and mixed for 24 hours in a rotary shaker. This was performed to assure that all chloride ions attached to the resin functional groups were replaced by a nitrate ion. Thus, chloride ions detected after ignition are associated with perchlorate load. The resin was then separated from the supernatant by filtering through a filter paper and rinsed 6 times with DI water to remove anions not attached to the resin. Next, the resin was dried in a gravity oven (VWR, West Chester, PA) at 105ºC for 1 hour. Then, 50 to 200 mg of the dried resin sample and 400 mg of paraffin oil were weighed and placed in the Parr bomb. Ten mL (10 mL) of 35 mM NaOH and 3 mL of 3% $H_2O_2$ were also added to the Parr bomb. The Parr bomb was then capped and pressurized with 500 psi (30-35 atm) oxygen gas. The resin sample was then ignited using 10 cm of nickel fuse wire. During the ignition, perchlorate was converted to chloride ion. The Parr bomb cylinder was then opened and its content was rinsed with small portions of DI water and transferred to a 250 mL volumetric flask. Chloride ion was then determined in the resulting ignition solution using ion chromatograph (IC). Because combustion of one mole of perchlorate will produce one mole of chloride, the amount of perchlorate in the resin sample can be calculated. Ultimate coal (Alpha Resources Inc., Stevensville, MI) sample with a known chloride content was used as the chloride standard for quality assurance. In this case a known amount of coal was ignited in the Parr bomb and the chloride concentration in the product solution was measured. Measurements were 99.73% accurate.

### 5.3.8. Resin Capacity Measurement

It was expected that bioregeneration would result in decreased resin capacity after each cycle. Therefore, the total capacity of fresh and bioregenerated resin was measured. To measure the capacity, 15 mL of wet resin was placed in a pipette filled with a stopcock. One L of 4.0% HCl was passed through the resin bed to convert the resin to the chloride form. Next the resin was rinsed with 1 L of DI water to rinse interstitial chloride. One L of 1.0 N $NaNO_3$ solution was then passed through the resin to replace the chloride ions with nitrate. The effluent from the $NaNO_3$ rinse was collected and titrated with $AgNO_3$ to measure the chloride concentration. Theoretically, each mole of detected chloride in the effluent corresponds one mole of nitrate exchanged by the active functional groups. The resin capacity in equivalents/L is then calculated using the chloride measurements.

### 5.3.9. Chemicals and Analyses

All perchlorate concentrations and low concentrations of chloride were measured using a Dionex ICS-2000 IC (Dionex Corporation, Sunnyvale, CA), consisting of an Ion Suppressor-ULTRA II (4 mm), IonPac AS16 (4 mm) analytical, AG16 (4 mm) guard columns, and an AS16 autosampler. For perchlorate, EPA method 314.0 was used with a current of 100 mA and a NaOH concentration of 35 mM with a flow rate of 1.0 mL/min. A calibration curve was established using perchlorate standard solutions with concentrations between 5 and 100 µg/L. A coefficient of determination of 99.97% was used for calibration. Similarly, a current of 100 mA and a NaOH concentration of 35 mM with a flow rate of 1.0 mL/min were used to measure low-concentration chloride ion. Calibration curve for low-concentration chloride was plotted with standard solutions with

concentrations between 100 and 500 µg/L using a coefficient of determination of 99.97%. For nitrate, sulfate, and high concentrations of chloride anions, IonPac AS20 (4 mm) analytical and AG16 (4 mm) guard columns were used on the same IC with a current of 110 mA and a NaOH concentration of 30 mM and a flow rate of 1.0 mL/min. The calibration curve for nitrate, sulfate, and high concentrations of chloride anions measurement was prepared for concentrations between 1 and 10 mg/L and a 99.99% coefficient of determination.

Bicarbonate and suspended solids were_measured according to Standard Methods 4500-CO2-D and 2540-D, respectively (Greenberg et al., 2005). Chemical oxygen demand (COD) was measured using high range (0-1500 mg/l) Hach COD digestion vials (Hach Co., Loveland, CO). The pH values were measured using a Fisher Scientific model AR25 pH meter (Springfield, CO). Conductivity was determined using YSI (Model # 30/10 FT) conductivity meter (YSI, Inc., Warm Springs, OH). The DO in the FBR was measured using YSI Model 58 Dissolved Oxygen meter (YSI, Inc., Warm Springs, OH). The % transmittance was measured using a Hach DR 5000 Spectrophotometer at the wavelength of 600 nm. DI water with a resistivity of 17.5 MΩ cm was obtained from a Barnstead water purification system (Dubuque, IA) and used in all steps, unless otherwise noted. All the used salts were ACS grade and obtained from VWR (West Chester, PA).

### 5.3.10. Scanning Electron Microscopy (SEM)

Fresh resin and bioregenerated resin, sampled at the end of cycle 5, were rinsed with DI water and air-dried for 24 hours at 22±2ºC. The resins were then glued on a metal surface and allowed to air-dry for 20 1 hour. Then, the glued resins were scratched using

soft sandpaper to expose the inner portion of the beads for imaging. The samples then rinsed with DI water air-dried for 24 hours at 22±2ºC. Scanning electron microscopy imaging of the resin samples was performed using Jeol JSM-7500F SEM (JEOL Ltd., Tokyo, Japan) employing secondary electron detector at 1.00 kV. After SEM imaging, the bioregenerated resin sample was then soaked in 100 mg/L sodium hypochlorite solution for 20 minutes. The sample was then rinsed with DI water air-dried for 24 hours at 22±2ºC, and submitted for SEM imaging again.

## 5.4. Results

### 5.4.1. Enrichment Culture Characterization

Previously published characterization of the PRB enrichment culture (Kesterson et al., 2005) revealed that the culture is composed of at least 6 bacterial genera, two of which are able to degrade perchlorate as an electron acceptor. All 6 isolates are gram-negative and facultative anaerobic bacteria. The bacterial genera that have been identified in the culture include *Pseudomonas*, *Azospira* (formerly *Dechlorosoma*), *Dechloromonas*, *Aeromonas*, and *Rhizobium*, which are typically present in soils and waters (Kesterson et al., 2005).

### 5.4.2. Fermenter Performance

Typical values of COD, SS, pH, and conductivity of the microbial enrichment culture present in the fermenter through the bioregeneration cycles appears in Figure 5.2.a-d. The SS varied between 1000-2000 mg/L during five cycles of bioregeneration. As it is shown in Figure 5.2.a and 5.2.c, there was a decrease in the amount of biomass in the fermenter as bioregeneration progressed. The likely reason for this decrease was shortage of electron acceptor (i.e perchlorate) for the microbial culture since the electron

donor (i.e. acetate, measured as COD) was available in the system. At some points during bioregeneration (e.g. day-2 and day-3 in Figure 5.2.a), concentrated PRB cells originating from the master seed cultures were added to the fermenter to increase the biomass. Figure 5.2.a and 5.2.c also show a drop in the COD values that was due to the consumption of acetate as the electron donor source by the bacteria.

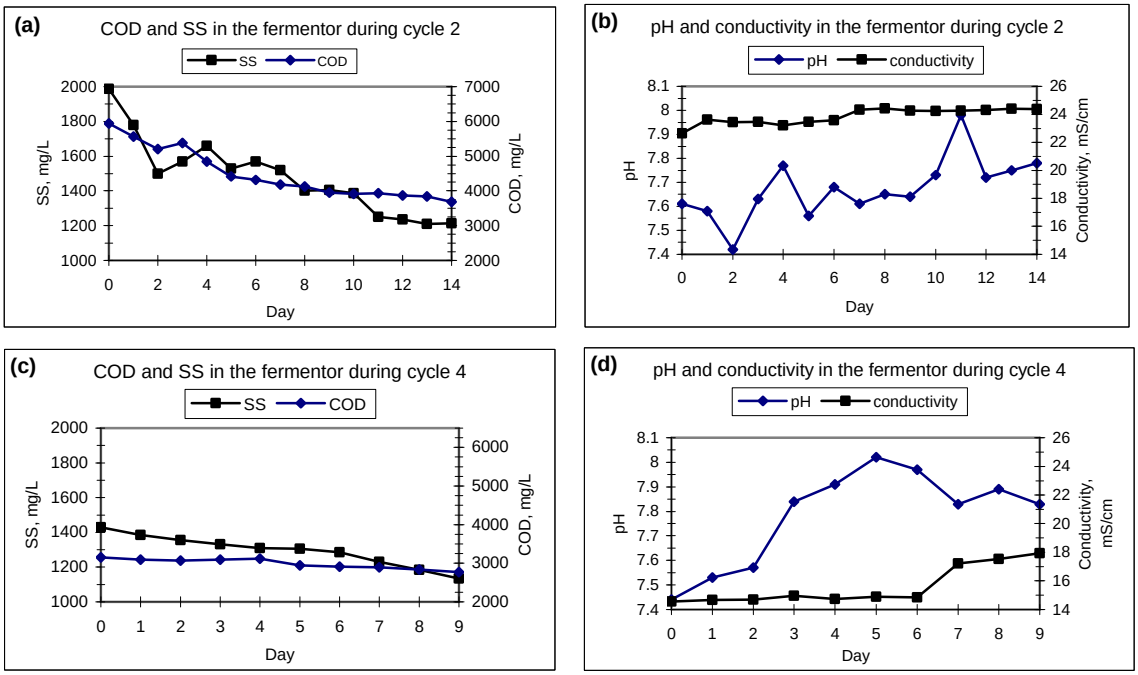


Figure 5.2. Typical Fermenter Operating Conditions: (a) and (c) Decrease of COD and SS during Cycles 2 and 4, (b) and (d) Increase of pH, and Conductivity in the Fermenter during Cycles 2 and 4.

The pH of the fermenter was maintained between 7.1 and 8.0 using phosphate buffer. There was an increase in the pH values as the bioregeneration proceeded (Figures 5.2.b and 5.2.d). The observed increase in pH is likely due to biodegradation of resin-attached

nitrate (i.e denitrification). The loaded resin contained several anions, including nitrate and the bacterial culture used is known to degrade both nitrate and perchlorate. Only perchlorate degradation was monitored in this study. Figures 5.2.b and 5.2.d also show a slight increase of the conductivity in the microbial culture. This increase in the conductivity in the microbial culture was due to the conversion of resin-attached residual perchlorate to chloride during bioregeneration.

ORP and temperature of the fermenter also were monitored. ORP varied from (-)390 mV to (-)470 mV and temperature of the system varied between 22±2ºC. DO concentrations were always below 0.2 mg/L.

### 5.4.3. Resin Bioregeneration

Perchlorate biodegradation for all five cycles of bioregeneration is shown in Figures 5.3.a and 5.3.b. Cycles 1, 2, and 3 were run for 14 days, while cycles 4 and 5 were run for 9 and 8 days, respectively. The stabilization of the residual perchlorate concentration in the resin bed was the criterion to stop the bioregeneration cycle (Figure 5.3.a). The data show that perchlorate biodegradation is fast during the first days, and then it slows down and stabilizes. In Figure 5.3.a, all the data except day-0 are Parr-bomb measurements of resin-attached perchlorate remaining in the resin as bioregeneration progresses. Initial perchlorate concentration (i.e. day-0) for each cycle is the amount of perchlorate loaded to the resin at the beginning of that cycle (Table 5.3) plus the remaining perchlorate in the resin from the previous cycle. For cycle 1 the initial perchlorate concentration is the amount of perchlorate loaded to the resin at the beginning of the cycle. Notice in Figure 5.3.a that the initial perchlorate concentration in the resin for each cycle differs. The reason for that is perchlorate buildup in the resin after each

cycle. At the end of bioregeneration, the remaining perchlorate values in cycles 1-5 are 3.1 $g_{perchlorate}/L_{resin}$, 5.4 $g_{perchlorate}/L_{resin}$, 7.4 $g_{perchlorate}/L_{resin}$, 8.5 $g_{perchlorate}/L_{resin}$, and 8.8 $g_{perchlorate}/L_{resin}$.

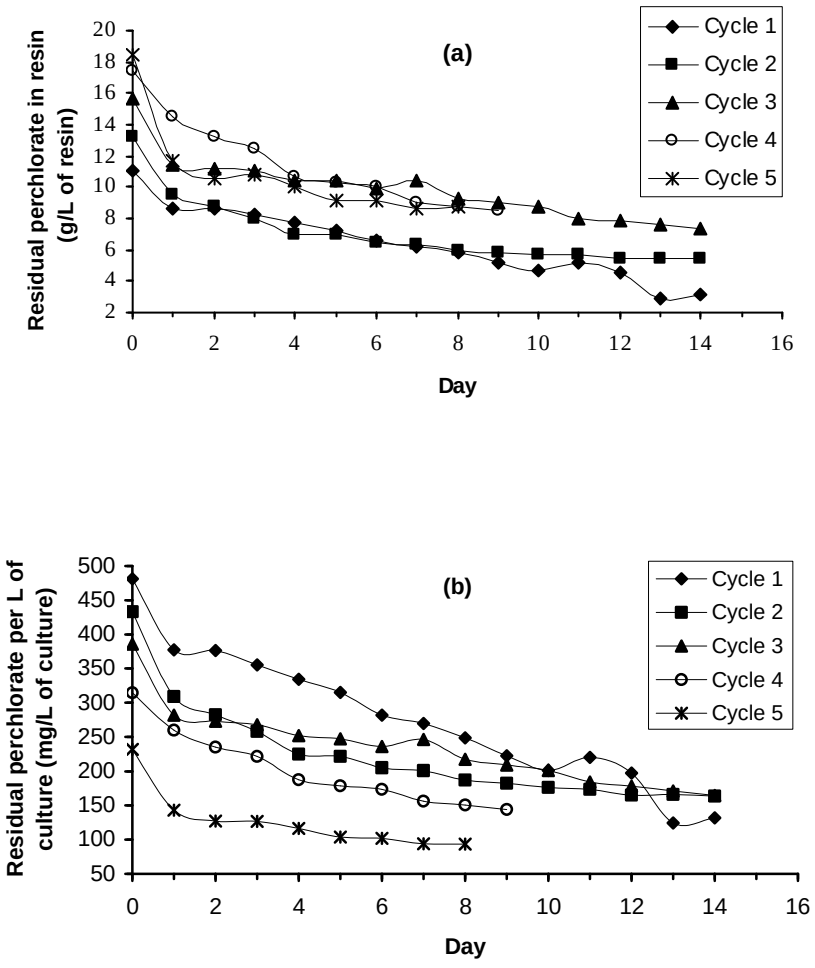


Figure 5.3. Residual Perchlorate Concentration during Five Cycles of Bioregeneration: (a) Residual Resin-attached Perchlorate Concentration, and (b) Residual Perchlorate Content in the Resin per Liter of Microbial Culture.

Table 5.5. Resin-attached Perchlorate and Free Perchlorate Biodegradation Rates

| Cycle # | $ClO_4^-$ ion situation | Initial perchlorate concentration ($mg/L_{culture}$) | Observed perchlorate biodegradation rate ($mg_p/mg_{ss}/d$) | Theoretical (Calculated) biodegradation rate ($mg_p/mg_{ss}/d$) | Reference |
|---|---|---|---|---|---|
| 1 (1st day) | Attached to resin | 422 | 0.096 | 0.362[a] - 5.854[b] | This Study |
| 2 (1st day) | Attached to resin | 361 | 0.066 | 0.357 - 5.838 | This Study |
| 3 (1st day) | Attached to resin | 296 | 0.065 | 0.352 - 5.819 | This Study |
| 4 (1st day) | Attached to resin | 336 | 0.039 | 0.341 - 5.780 | This Study |
| 5 (1st day) | Attached to resin | 177 | 0.066 | 0.321 - 5.704 | This Study |
| NA | Free in water | 298 | 1.68 | 0.338 – 5.768 | Korenkov et al., 1976 |
| NA | Free in water | 915 | 2.57 | 0.383 – 5.922 | Attaway and Smith., 1993 |
| NA | Free in water | 100 | 0.36 | 0.353 - 5.357 | Logan et al., 2001 |
| NA | Free in water | 250 | 0.64 | 0.385 - 5.725 | Shrout and Parkin, 2006 |

[a] Calculated using $K_s$ and $q_{max}$ from Logan *et al*., 2001
[b] Calculated using $K_s$ and $q_{max}$ from Waller *et al*., 2004
[c] Not Applicable

Figure 5.3.b depicts the resin-attached residual perchlorate mass as g of $ClO_4^-$ per liter of microbial culture. In all five cycles depicted in Figure 5.3.b, the highest perchlorate biodegradation rate was obtained in the first days of bioregeneration, and then it was reduced throughout the rest of the experiment. The initial perchlorate load at the start of cycles 1-5 was 481 $mg/L_{culture}$, 433 $mg/L_{culture}$, 385 $mg/L_{culture}$, 314 $mg/L_{culture}$, and 231 $mg/L_{culture}$, respectively (Figure 5.3.b). The reason for the reduction of the initial perchlorate load was the decrease of resin volume from 1305 mL in cycle 1 to 375 mL in cycle 5 due to sampling. Samples were taken during bioregeneration for the Parr-bomb and resin capacity measurement while the volume of the microbial culture in the fermenter was kept constant at 30 L for all five cycles.

The observed degradation rate for resin-attached perchlorate expressed as mg of perchlorate biodegraded per mg of SS per day ($mg_p/mg_{ss}/d$) for day 1 of cycle 1-5 is shown in Table 5.5. For comparison, the degradation rate for free perchlorate ion in water from other studies is also shown in Table 5.5.

Theoretical degradation rates were calculated using Monod's kinetics (Table 5.5) (Rittman and McCarty, 2001), which is an accepted model for perchlorate biodegradation in water (Waller et al., 2004):

$$\frac{dS}{dt} = -\frac{q_{\max} XS}{S + K_s} \tag{5.1}$$

where, S is concentration of perchlorate (mg/ $L_{culture}$); t is the time (d); $q_{max}$ is the maximum perchlorate utilization rate ($d^{-1}$); X is the SS in the system (mg/L); and $K_s$ is the half-saturation constant for perchlorate (mg/L). Nine pairs of $K_s$ and $q_{max}$ from other studies (Table 5.1) were used to calculate the degradation rates and the lowest and highest theoretical rates are reported in Table 5.5.

In Chapter 4, a conceptual model for resin bioregeneration process was envisioned which involves four steps as follows: a) Desorption of resin-attached perchlorate from the functional groups located in the resin bead matrix; b) Diffusion of the desorbed perchlorate from the inner portion of the resin bead to the surface through the resin pores; c) Diffusion of perchlorate through the liquid film covering the surface of resin bead to the bulk microbial fluid; and d) Biodegradation of perchlorate in the periplasmic region of PRB. In all five cycles of bioregeneration, the observed perchlorate biodegradation rate in the first day of the bioregeneration process was one order of magnitude smaller than the smallest calculated theoretical biodegradation rate of free perchlorate (Table 5.5). This observation could be attributed to two potential explanations: (1) kinetics

control: perchlorate biodegradation rate is concentration dependent and it slows down for perchlorate concentrations smaller that the half saturation constant for perchlorate (Cox et al., 2000; Logan, 2001, Tan et al., 2004; Hiremath et al., 2006), and/or (2) mass transfer control: perchlorate biodegradation rate depends on the diffusion of the desorbed perchlorate ions from the original functional groups located in the resin bead to the bacteria located outside the resin bead (i.e. steps c and d of the above-mentioned conceptual model). Based on the results of Chapter 4, both pore diffusion and film diffusion mechanisms are controlling in resin bioregeneration process for perchlorate-selective resins. Hence, the reason for higher observed perchlorate biodegradation rate for free perchlorate compared to resin-attached perchlorate (Table 5.5) is the limitation in the mass transfer flux.

### 5.4.4. Capacity Loss Evaluation

Figure 5.4 depicts the amount of loaded perchlorate to the resin, initial perchlorate (i.e. loaded perchlorate plus remaining perchlorate from the previous cycle), remaining perchlorate in the resin, and biodegraded perchlorate in the resin in each cycle. In cycles 1-5, 72.0%, 59.2%, 52.7%, 51.4%, and 52.5% of the initial perchlorate (i.e. loaded perchlorate plus remaining perchlorate from previous cycle) was degraded during bioregeneration, respectively (Figure 5.4). It is likely that every time the resin was loaded, there was a core in the resin (in the center of the bead) that was not bioregenerated. It seems that the size of this core from cycle 1 to cycle 5 grew, and every time the resin was loaded, perchlorate was replaced in the outer portion of the resin bead, and permanent perchlorate in the center of bead was carried over to the next cycle. Notwithstanding the remaining perchlorate in the resin carried over from the previous

cycles, in cycles 1-5, 72.0%, 77.3%, 80.4%, 89.1%, and 97.0% of the perchlorate, which was loaded to the resin in each cycle (section 2.4), was utilized, respectively. These results show that the amount of perchlorate load, loaded in the loading step, that was being bioregenerated in the bioregeneration process increased from cycle 1 (i.e. 72%) to cycle 5 (i.e. 97%). Although the initial perchlorate load of the resin was almost the same during all five cycles (i.e. 11.06 $g_{perchlorate}/L_{resin}$, 10.15 $g_{perchlorate}/L_{resin}$, 10.23 $g_{perchlorate}/L_{resin}$, 10.08 $g_{perchlorate}/L_{resin}$, and 10.02 $g_{perchlorate}/L_{resin}$ for cycles 1-5, respectively), for cycle 1 and cycle 5, 72.0% and 97.% of the initial perchlorate load was biodegraded, respectively. Figure 5.5 depicts that the undegraded perchlorate load buildup was fast through cycles 1 and 2, and began to stabilize in cycles 4 and 5. The reason for this observation could be that some of the functional groups of the resin located in the center of resin bead could not be regenerated and were permanently occupied by perchlorate throughout the bioregeneration cycles. These functional groups are occupied by perchlorate because the results of Parr bomb measurement of resin samples at the end of bioregeneration process show that perchlorate ion is available in the resin.

Bio-fouling and as a result clogging of the resin pores can be the potential reason for perchlorate permanent load build-up in the resin. A SEM analysis of the rinsed and air-dried fresh resin and bioregenerated resin that was sampled after cycle 5 revealed that some of the pores of the bioregenerated resin were clogged, while the pores in the fresh resin bead were clearly accessible (Figures 5.6.a and 5.6.b). After SEM imaging, the bioregenerated resin sample was soaked in 100 mg/L sodium hypochlorite solution for 20 minutes, rinsed with DI water, and air-dried. A SEM analysis of the treated-

bioregenerated resin sample showed that most the pores were unclogged after treatment with sodium hypochlorite solution (Figure 5.6.c), suggesting that the clogging materials are likely organics that can be removed using sodium hypochlorite solution. Although, the substance that is clogging the pores has not been characterized because it was difficult to remove it from the pores in significant amount for an analysis, it is likely that the pores are clogged by bacterial debris or biodegradation byproducts. The perchlorate load buildup may be due to slowing down of mass transfer in the resin pores because of clogging.

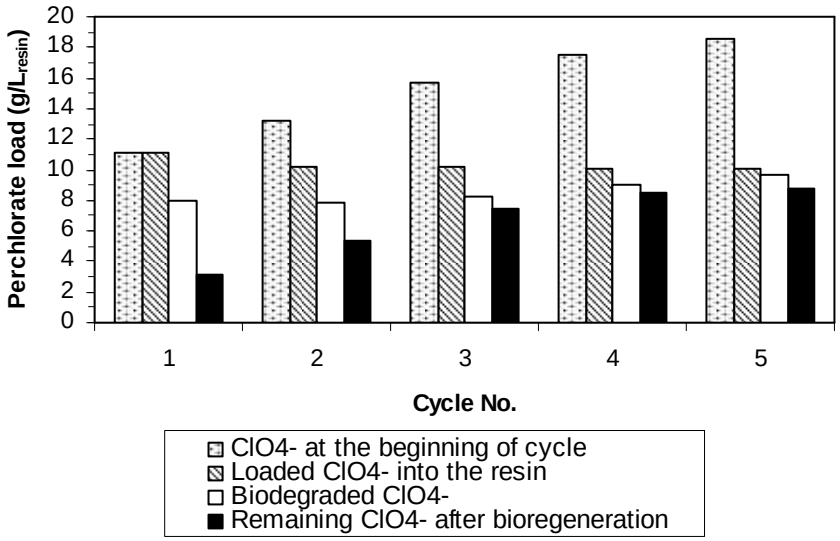


Figure 5.4. Initial Perchlorate (i.e. Loaded Perchlorate plus Remaining Perchlorate from the Previous Cycle); Loaded Perchlorate to the Resin; Biodegraded Perchlorate; and Remaining Perchlorate for Each Cycle of Bioregeneration. However Loaded Perchlorate Stayed Approximately Constant through Different Cycles, Total Initial Perchlorate-Load at the Beginning of Each Cycle Increased from Cycles 1 through Cycle 5 Due to Residual Perchlorate Leftover from the Previous Cycle. The Amount of Biodegraded Perchlorate Also Increased from Cycle 1 to Cycle 5.

The results of Chapter 4 revealed that bioregeneration process is controlled by mass transfer. Since the desorbed perchlorate must somehow diffuse through the pore matrix of the resin bead and reach the bacteria, any decrease in the pore diffusion flux due to clogging affects bioregeneration process. The SEM imaging showed that after the resin was fouled some of the pores in the resin were clogged. Thus, during the establishement of clogging in the resin through the bioregeneration process, pore diffusion flux decreased. It appears that establishment of fouling, and as result, clogging causes lower mass transfer flux compared to mass transfer flux in not-fouled resin. Therefore slower perchlorate biodegradation rate at the end of bioregeneration process can be expected as Figure 5.3.a, since: a) just because of pore diffusion, it takes longer for perchlorate located deep into the bead to arrive to the surface (Venkatesan et al., 2010) independent on pore clogging, and b) if there are clogged pores then the mass transfer slows down even more.

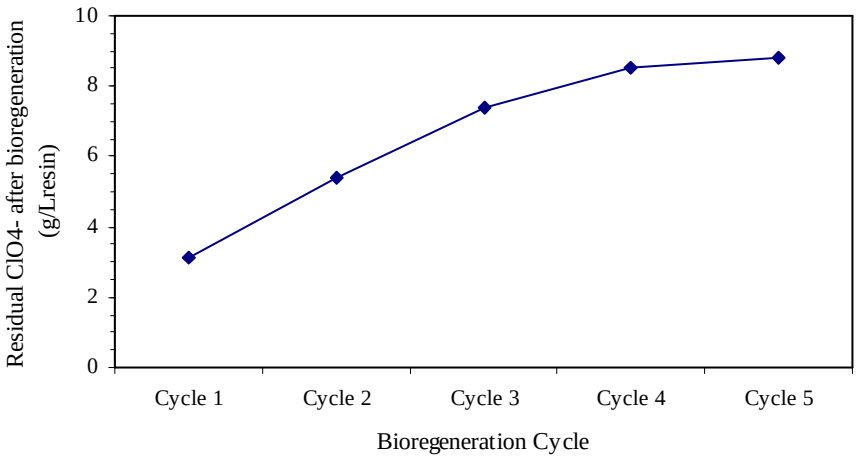


Figure 5.5. Permanent Perchlorate Load Buildup through 5 Cycles of Bioregeneration: The Load Buildup is Stabilized in the Last Cycles

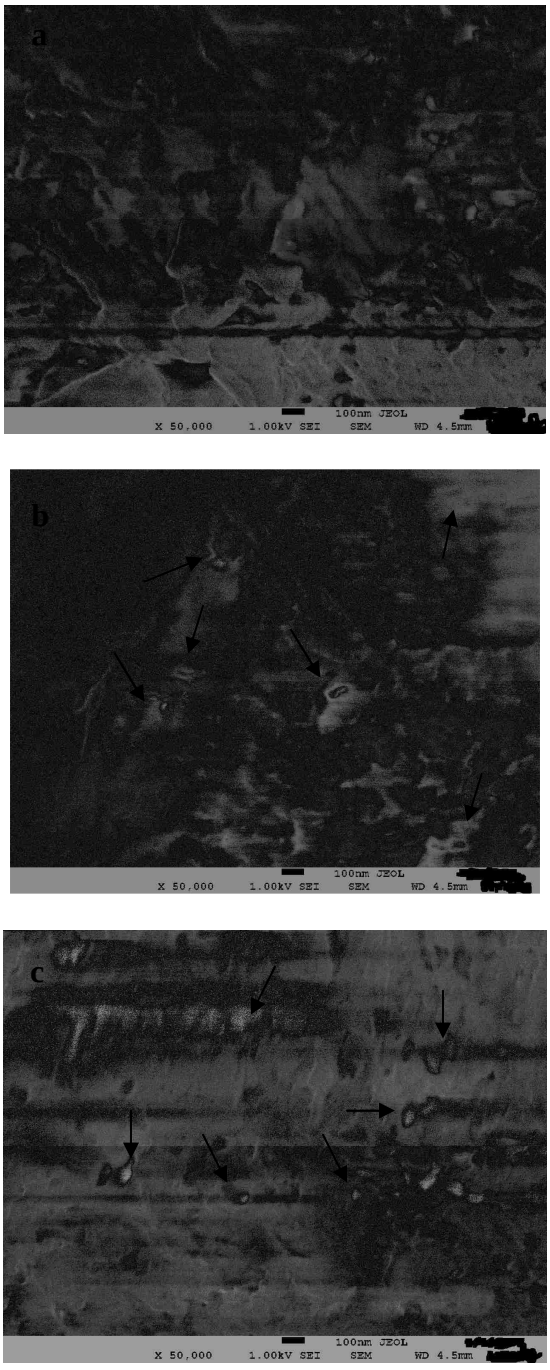


Figure 5.6. SEM Images of the (a) Fresh Resin (with Normal Pores), (b) Bioregenerated Resin Sampled after Cycle 5 (Arrows Show the Clogged Pores), and (c) Bioregenerated Resin Which is Treated with 100 mg/L Sodium Hypochlorite (Arrows Show the Unclogged Pores).

Resin was treated for biological fouling after each bioregeneration cycle (as expressed in Table 5.4), and the COD of the rinsate was measured (Figure 5.7). Prior to the fouling removal procedure, the resin was rinsed with 1 BV of DI water for 5 times to remove the remaining microbial cells and organics which might remain in the resin. Table 5.6 shows the typical data of the % transmittance of the rinsate solutions, indicating that the employed rinsing procedure removed the remaining microbial cells from the resin effectively. Even though the resin was rinsed with 1 BV of DI water for 5 times prior to the bio-fouling treatment, significant amounts of organics were detected in the fouling removal rinsate solutions (Figure 5.7). In cycles 1-5, 3840 mg.COD/$L_{resin}$, 4778 mg.COD/$L_{resin}$, 5745 mg.COD/$L_{resin}$, 4650 mg.COD/$L_{resin}$, and 3465 mg.COD/$L_{resin}$ of organic substances, resulting from biodegradation, were removed from the resin. The nature of the organic substance removed was not investigated, but it is thought to be byproducts of biodegradation.

The resin treated by the fouling removal and disinfection procedure had a yellowish brown color which made the resin look different from the fresh resin. Though applying higher concentrations of the disinfectant agent may be able to retrieve the original light color of the resin, treatment with concentrated disinfectant reagent potentially increases the risk of oxidation of the active functional groups of the resin beads, which may result in capacity loss of the resin.

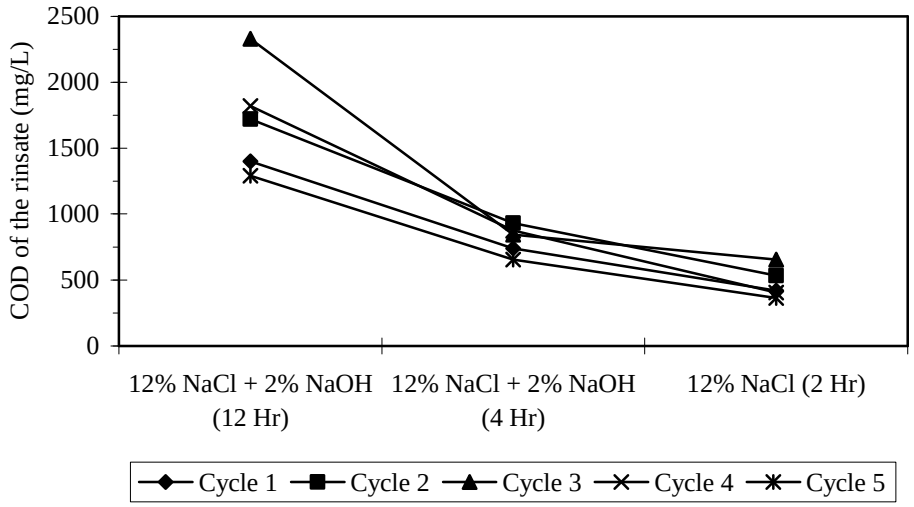


Figure 5.7. COD of Eluate Obtained during Fouling Removal Process Applied on the Resin.

Table 5.6. Typical Data for Rinsing the Resin with 1 BV of DI Water for 5 Times Prior to the Biofouling Removal Procedure

| Number of rinsing | % transmittance of the rinsate at 600 nm |
|---|---|
| 1 | 79.8% |
| 2 | 97.6% |
| 3 | 100.0% |
| 4 | 100.0% |
| 5 | 100.0% |

Resin capacity was measured before bioregeneration, before disinfection, and after disinfection in each cycle. Table 5.7 shows the resin capacity measurements from cycle 1 through cycle 5 of bioregeneration. The resin capacity measurement method (section 2.8) was not able to account for perchlorate load build-up in the resin. The reason is the

method uses $Cl^-$ ion to convert the resin to the chloride form, and due to the higher affinity of the perchlorate-selective resin for perchlorate than chloride, perchlorate cannot be replaced by chloride during the first step of the capacity measurement method. Hence, the observed capacity loss reported in Table 5.7 is due in part to perchlorate load build-up in the resin in addition to other possible factors such as disinfection and microbial activity effects. Capacity measurement at different steps shows that the capacity loss is not cumulative throughout the consecutive loading-bioregeneration cycles. Capacity loss did not increase with each cycle of bioregeneration. Comparing pre-disinfection and post-disinfection values from Table 5.7, it appears that the applied disinfection step had very little effect on the resin capacity.

Table 5.7 indicates that after five cycles of loading, bioregeneration, fouling removal, and disinfection of the resin, the total capacity loss was about 15.6%, which corresponds to 9.93 g $ClO_4^-/L_{resin}$, if the total capacity of the resin is 0.64 eq/ $L_{resin}$. There is 1.13 g $ClO_4^-/L_{resin}$ difference between 9.93 g $ClO_4^-/L_{resin}$ capacity loss (Table 5.7) and the permanent perchlorate load of 8.80 g of $ClO_4^-/L_{resin}$ (Figure 5.4, Cycle 5).

A significant finding of this study is that the bioregeneration process can be performed as a multi-cycle process. The capacity loss was mostly due to remaining perchlorate load, and it was stabilized after a few bioregeneration cycles. Although after cycle 5 there was about 9 g of $ClO_4^-/L_{resin}$ permanent perchlorate load in the resin, it is only about 15% of the total capacity and the resin still can be used in the loading-bioregeneration process.

Table 5.7. Resin Capacity Measurement and Capacity Loss for Five Cycles of Bioregeneration

| Cycle # | Sampling point | Capacity (eq/L) | Capacity loss (%)[a] |
|---|---|---|---|
| Cycle 1 | Pre-bioregeneration | 0.64 | 0.0 |
| | Pre-disinfection | 0.61 | 4.7% |
| | Post-disinfection | NA | NA |
| Cycle 2 | Pre-bioregeneration | 0.52 | 18.8% |
| | Pre-disinfection | NA | NA |
| | Post-disinfection | 0.54 | 15.6% |
| Cycle 3 | Pre-bioregeneration | 0.54 | 15.6% |
| | Pre-disinfection | 0.54 | 15.6% |
| | Post-disinfection | 0.54 | 15.6% |
| Cycle 4 | Pre-bioregeneration | NA | NA |
| | Pre-disinfection | NA | NA |
| | Post-disinfection | 0.56 | 12.5% |
| Cycle 5 | Pre-bioregeneration | NA | NA |
| | Pre-disinfection | 0.54 | 15.6% |
| | Post-disinfection | 0.55 | 14.1% |

[a] Virgin resin capacity was assumed 0.64 eq/L

## 5.5. Discussion

A main concern in resin bioregeneration is capacity loss of the resin. The results showed that there is a perchlorate load buildup, thought to be in the center of resin, through cycle 1 to cycle 5. The perchlorate load buildup is a major reason for resin capacity loss through bioregeneration cycles. The capacity loss resulting from perchlorate load buildup is more significant through the first cycles and stabilizes after a few cycles. It is thought that there is a region in the center of resin bead where some the functional groups of the resin are unavailable and permanently occupied by perchlorate

throughout the bioregeneration cycles. The possible reason for unavailability of the functional groups in that region of resin bead is likely to be clogging of the resin pores, which has been observed by SEM imaging. However, in a broader view, capacity loss of the resin can have another potential reason in addition to clogging of pores in the resin structure which is oxidation of some of functional groups in the resin due to exposure to disinfectant agent (i.e. sodium hypochlorite).

Perchlorate buildup was found to be the major drawback of the bioregeneration process. Evaluation is needed to determine whether bioregenerated resins, containing some residual perchlorate, can be used to produce water that meets the regulatory levels for perchlorate. Clearly, since capacity loss is involved in the bioregeneration process, lower bed volumes of water can be processed to reach breakthrough point. If perchlorate leakage occurs when using bioregenerated resin, another IX column containing fresh resin can be used to polish the effluent. This polishing IX column can last for a very long time since the leakage of the IX column containing bioregenerated resin is expected to be much less than the influent perchlorate concentration to the water treatment plant.

Obviously one has to evaluate the economical feasibility of the bioregeneration process. Two factors play main roles in the economical evaluation of IX resin bioregeneration process. On the one hand, there is cost of purchasing fresh resin in the places where spent resin is incinerated. On the other hand, there are costs associated with resin bioregeneration process. Capital costs include building fermenter, the FBR, pumps, and pipelines. Operating costs include personnel, energy, and purchasing electron donor, nutrients/minerals, and buffer chemical costs. In addition there is a reduction in the volume of processed water because of capacity loss of the resin due to bioregeneration.

Considering the number of loading-bioregeneration cycles and the 15% capacity loss due to bioregeneration process, bioregeneration is feasible only if its costs are less than the costs of replacing the spent resin with fresh resin. The results of economical assessment of the trade-off between bioregeneration and use of fresh resin (Appendix B) showed that from economical point of view employing resin bioregeneration process decreases the cost of perchlorate removal from water about $70. There are also environmental costs to be considered when spent resins are incinerated. Depending on regulations concerning minimizing greenhouse gases, in some states, carbon credits could be awarded to water utilities that stop resin incineration. In the long-term, resin bioregeneration can be the technology of choice for water utilities dealing with perchlorate contamination.

Biodegradation of resin-attached perchlorate ions is slower than biological degradation of free perchlorate ions. It has been shown that resin bioregeneration process is mass transfer controlled. Results of resin bioregeneration from different studies showed that at first the biodegradation rates are fast and then slow down and stabilize. There are two reasons involved in slowing down of the degradation of resin-attached perchlorate ions: a) pore diffusion itself independent on pore clogging: it takes longer for perchlorate ions located deep into the bead to arrive to the surface, and b) pore clogging: SEM imaging showed that as bioregeneration progresses, pore clogging establishes in the resin bead which causes the mass transfer slows down even more.

## 5.6. Conclusions

The objective of this research was to determine whether macroporous perchlorate-selective resins can be bioregenerated for many cycles. The results obtained from this study revealed that macroporous perchlorate-selective IX resins can be bioregenerated

and reloaded for five cycles. However, the capacity loss is significant (i.e. about 15%) but not cumulative after multi-cycle loading-bioregeneration process. Capacity loss is mostly due to permanent perchlorate load buildup in the resin. This perchlorate load buildup is fast through the first two cycles and almost stabilizes through cycles 4 and 5. The following can be concluded from the results of this research:

7) The reason for perchlorate load buildup could be unavailability of some of the functional groups located in the center of resin due to clogging of the pores of the resin. The applied fouling treatment and disinfection methods were effective during the regeneration cycles, and could remove significant amount of organics from the resin.

8) As bioregeneration progresses, establishment of fouling causes lower mass transfer flux in the resin compared to mass transfer flux in unfouled resin.

9) Slower perchlorate biodegradation at the end of the bioregeneration process can be attributed to: a) pore diffusion independent of pore clogging: it takes longer for perchlorate located deep in the bead to arrive at the surface, and b) pore clogging: a combination of slower diffusion due to location within the bead and clogging of the pores by bacteria causes the mass transfer slows down even more.

10) The perchlorate biodegradation rate of resin-attached perchlorate is significantly slower than the biodegradation rate of soluble perchlorate.

# CHAPTER 6

# CONCLUSIONS, IMPLICATIONS, AND RECOMMENDATIONS FOR FURTHER RESEARCH

## 6.1. Conclusions

Resin bioregeneration as a new concept in IX technology has been developed and patented (Batista, 2006). This concept is based on directly contacting perchlorate-containing IX resin with a PRB culture under anoxic/anaerobic conditions. Generally, the process consists of a fermenter, which holds perchlorate-reducing microbial culture, and a fluidized bed reactor (FBR), which holds the perchlorate-laden resin. In this process, first the fresh resin is used to treat water. After the capacity of the ion exchange resin is exhausted, the resin is transferred to the FBR column. Next, the perchlorate-reducing microbial culture is pumped from the fermenter to the FBR upward. In this process, perchlorate-selective and non-selective ion-exchange resins can be directly bioregenerated by perchlorate-reducing bacteria (PRB), leading to the conversion of perchlorate ions to innocuous chloride. An electron donor such as acetate needs to be provided to the bacteria. Although the feasibility of perchlorate-laden IX bioregeneration process has previously been proven (Batista and Jensen, 2006; Batista et al., 2007b; Wang et al., 2008b; Venkatesan et al., 2010), the mechanism of resin-attached perchlorate reduction has not been elucidated thus far. The objectives of this research were:

a) to understand the mechanisms of degradation of resin-attached perchlorate, and
b) to investigate the feasibility of bioregeneration of ion-exchange resin for several exhaustion-bioregeneration cycles.

Bioregeneration tests using both batch and fluidized bed reactor were performed to investigate these two issues. The results of this research revealed that there is a desorption step involved in biodegradation of resin-attached perchlorate (RAP). Chloride ion, which is the product of perchlorate biodegradation present in all PRB cultures grown using perchlorate, is likely the desorbing agent of RAP in the bioregeneration process. Increasing the concentration of chloride in the PRB culture, below the chloride inhibitory level to PRB, enhances the IX resin bioregeneration process. It was also found that macroporous resins, which have higher water contents and larger average pore sizes bioregenerate better than gel-type resins. Larger pore sizes in macroporous resins result in higher pore diffusion. The results further showed that decreasing the resin bead size has a significant positive effect on resin bioregeneration process. Mass transfer of desorbed perchlorate from the original functional group in the resin to the bulk microbial liquid was found to be the controlling step in the IX bioregeneration process.

Another goal of this research was to study the feasibility of multi-cycle bioregeneration and estimate the capacity loss due to resin bioregeneration. A significant finding of this study is that the bioregeneration process can be performed several times. Perchlorate-selective macroporous IX resins can be successfully employed in consecutive loading-bioregeneration IX process. It was found that capacity loss is significant (i.e. about 15%) after five multi-cycle loading-bioregeneration process. However, this loss is not cumulative. Capacity loss is mostly due to permanent perchlorate load build-up. Perchlorate load buildup is fast in the first cycles and it stabilizes through the rest of the cycles. The results showed that bioregeneration results in resin fouling. Although there is no knowledge of the exact composition of the organics that cause fouling, fouling

removal procedure and disinfection methods used in this research were effective to remove significant amount of organics from the resin. Chlorine worked very well as a disinfectant and a fouling removal agent. Establishment of fouling during bioregeneration causes lower mass transfer flux in the resin compared to mass transfer flux in unfouled resin. The results further showed that slower perchlorate biodegradation at the end of bioregeneration process can be attributed to: a) pore diffusion; it takes longer for perchlorate located deep into the bead to arrive to the surface, and b) pore clogging; slower diffusion due to clogging of the pores by bacterial debris causes mass transfer to slow down.

## 6.2. Implications

In general, the results of this research revealed that it is possible to bioregenrate resins laden with perchlorate and that the process is limited by mass transfer. However, the accumulation of un-degraded perchlorate is a serious issue and may lead to economically unfavorable outcome. The results of this research can be applied for future development of IX resin bioregeneration as a new technology in water treatment industry. According to the results, chloride solution can be applied to facilitate bioregeneration. However, designers of such systems should assure that chloride concentration does not exceed toxicity levels for PRB microbial activities (i.e salinity > 1.5%). Smaller resin bead sizes are recommended if resins are to be bioregenerated. However, designers should account for excessive hydraulic head loss in the FBR reactor caused by smaller bead sizes. In addition, operators should be mindful of the negative effects of the boundary liquid film for resin sizes smaller than 0.5 mm that may control the biodegradation rate of resin-attached perchlorate, if the hydraulic loading rate (i.e. fluidization) of the resin bed is not

sufficient. Higher surface loading rate (i.e. higher flow rate) should be used to increase film diffusion. However, potential resin loss due to high hydraulic loading should also be minimized. Macroporous resins should be used to enhance pore diffusion. However, there is a trade-off involved in using macroporous resins which have relatively smaller capacity compared to gel-type resins. Fouling removal is a vital step in bioregeneration and should be incorporated to any bioregeneration design. Fouling can be removed satisfactory by soaking the resins in mixtures of sodium chloride and sodium hydroxide and by rinsing with chlorine solutions.

## 6.3. Recommendations for Future Research

This research could be followed with additional work that would contribute to further understanding of the mechanisms for biodegradation of resin-attached perchlorate:

1. Investigating other monovalent anions such as bromide, and iodide as the desorbing agent of resin-attached perchlorate on the resin bioregeneration process and comparing the results with those of resin bioregeneration process using chloride ion.
2. Investigating the effect of divalent anions such as sulfate as the desorbing agent for resin-attached perchlorate on the bioregeneration process and comparing the results of resin bioregeneration process using monovalent anions.
3. Studying the bioregeneration of resin loaded with nitrate and the effect of initial concentrations of chloride, bromide, sulfate, and iodide on bioregeneration process.

4. Investigating the feasibility of bioregeneration of either gel-type or macroporous resins for more than five consecutive cycles and investigate ways of minimizing accumulation of perchlorate in the resin..
5. Evaluating the economic feasibility of bioregeneration considering cost of purchasing fresh resin, and costs associated with the resin bioregeneration process (i.e. capital costs and operating costs).
6. Determining whether bioregenerated resins, containing some residual perchlorate, can be used to produce water that meets the regulatory levels for perchlorate.
7. Studying the specific composition of organics that cause resin fouling.
8. Studying the changes of resin water content and resin pore clogging (as important factors on mass transfer) during multi-cycle bioregeneration of ion-exchange resins.

## 6.4. Contributions of this Research to the Field of Perchlorate Removal Using Ion-Exchange Process

Providing clean water is one of the major challenges in the world. Sustainable development of communities requires reliable methods to provide clean water. Perchlorate ($ClO_4^-$) ion as a water contaminant is known to affect the functioning of the thyroid gland of humans. Ion exchange is the technology of choice to remove perchlorate from drinking waters and its use for this purpose has been increased significantly during the last decade. Since the spent perchlorate-contaminated ion-exchange resin is not currently regenerable using the common regeneration methods, the incineration/disposal of spent resin is the major challenge and cost to drinking water utilities. In case of incineration, it produces significant amounts of greenhouse gases, and in case of

landfilling, it has potential to re-contaminate the environment. Bioregeneration, which uses perchlorate-reducing bacterial culture, is an environmentally sustainable technology because it allows reusing of ion-exchange resin and presents a major innovation in water treatment technology. The goal of this research was to study the mechanism, controlling factors, and feasibility of multi-cycle bioregeneration of ion-exchange resin. Therefore, the results of this research have direct value to applications of ion-exchange technology in water treatment utilities to treat waters contaminated with perchlorate.

The water treatment industry can employ the results presented here to further develop bioregeneration as a technology for water treatment. If the technology can be developed successfully, the cost of treating perchlorate contaminated waters can be reduced significantly. Furthermore, such a technology would result in less generation of greenhouse gases because it would make incineration of loaded resin unfeasible. Depending on regulations concerning minimizing greenhouse gases, in some states, carbon credits could be awarded to water utilities that stop resin incineration.

# APPENDIX A

# PROCEDURE TO MEASURE RESIDUAL PERCHLORATE IN ION-EXCHANGE SAMPLES USING OXYGEN PARR-BOMB

## Introduction

In the past, UNLV developed a potassium iodine (KI) extraction method to measure perchlorate contained in ion-exchange resins. This method works well for nitrate-selective resins, but it does not remove perchlorate well from gel-type perchlorate-selective resins. In order to measure the residual amount of perchlorate in the ion-exchange resin samples, a procedure has been developed in UNLV Environmental Laboratory using oxygen Parr Bomb. The method is based on conversion of perchlorate to chloride when a resin sample is combusted in a closed combustion chamber. The chloride resulting in the combustion is then measured by ion-chromatography. The method uses an oxygen Parr Bomb apparatus as it is shown in Figure 3.3.

## Materials and Instruments

The materials and instruments needed for the procedure are listed below:

1) Parr Bomb or Oxygen Combustion Bomb apparatus: UNLV Environmental Laboratory uses Parr Bomb apparatus number 101A -C20 09C507 M17210; from the Parr Instrument Company.
2) Ion-chromatograph (IC) system. UNLV uses a Dionex ICS-2000 (Dionex Corporation, Sunnyvale, CA) consisting of an Ion Suppressor-ULTRA II (4 mm), IonPac AS16 (4 mm) analytical, AG16 (4 mm) guard columns, and AS16 auto-sampler.
3) Bench-top orbital shaker (Cole-Parmer, Series 51704)

4) 105ºC oven (Thermo Fisher Scientific, Waltham, MA)

5) 10,000 mg NO3-/L nitrate solution

6) Paraffin oil (EMD Chemicals, Inc., San Diego, CA)

7) Aluminum dish (Thermo Fisher Scientific, Waltham, MA)

8) A 500-ml volumetric flask

9) A 500-ml Erlenmeyer flask

10) 3% hydrogen peroxide solution

11) 35 mM sodium hydroxide solution

12) Compressed pure oxygen cylinder with a regulator

13) A 5-L water bucket that fits the Parr-Bomb

Safety Warning: Heat is generated during combustion. The Parr-Bomb must be placed inside a water bucket during combustion. The Parr bomb uses compressed oxygen. The oxygen cylinder must be securely fastened to a bench to avoid falling.

To operate the Parr Bomb for residual perchlorate measurement the following procedure has been developed:

Step 1: Measure approximately 1 mL of resin sample;

Step 2: Rinse the resin sample gently using DI water to remove suspended solids and organic/inorganic materials (e.g. microbes, silt, etc.);

Step 3: Place the rinsed resin sample in a 500 mL Erlenmeyer and add 100 mL of 10,000 mg $NO_3^-$/L. The goal of this step is to exchange each chloride still remaining in the resin's functional group with a nitrate ion. In this manner, the only chloride ions that will result from the combustion are those related to perchlorate;

Step 4: Place the Erlenmeyer containing the resin sample and the nitrate solution on a bench-top orbital shaker (Cole-Parmer, Series 51704) at 50-60 rpm for 24 hours;

Step 5: After 24 hours, carefully decant the nitrate solution, measure the amount of desorbed perchlorate in the solution, and rinse the resin sample six times with 100 mL of DI water;

Step 6: Drain the residual water from the sample by placing it on a filter paper. Air-dry the sample for 30 minutes;

Step 7: Place the sample in a pre-weighed clean aluminum dish (Thermo Fisher Scientific, Waltham, MA) and weigh the aluminum dish containing resin sample. Record the measurement;

Step 8: Place the aluminum dish in 105ºC ovens (Thermo Fisher Scientific, Waltham, MA) for 60 minutes to completely dry the resin sample;

Step 9: Weigh the aluminum dish containing the dried sample in order to calculate the water content of the resin;

Step 10: Weigh 100 mg of the sample carefully inside the pre-heated Parr-Bomb crucible;

Step 11: Add 400 mg paraffin oil (EMD Chemicals, Inc., San Diego, CA) to the crucible;

Step 12: Place the crucible inside the cradle (loop electrode) which is attached to the bomb cap;

Step 13: connect the two electrodes present in the Parr Bomb cap using a 10 cm of nickel-chromium fuse wire;

Step 14: Add 3 mL of 3% hydrogen peroxide and 10 mL of 35 mM sodium hydroxide solution to the bottom of Parr Bomb apparatus. Sodium hydroxide (NaOH) is used to match the eluent solution (i.e. NaOH) that is used in the Dionex ICS-2000 Ion Chromatograph (IC) (Dionex Corporation, Sunnyvale, CA);

Step 15: Close the Parr Bomb properly make sure it is air tight;

Step 16: Inject the oxygen gas inside the Parr Bomb apparatus up to 500 psi (30-35 atm) pressure;

Step 17: Completely immerse the Parr Bomb apparatus in water in a 5-L water bucket;

Step 18: Ignite the sample. During combustion all attached perchlorate anions are converted to chloride anion. The chloride dissolves into the NaOH solution inside the apparatus;

Step 19: Thirty minutes (30 min) after the ignition, open the cap of the Parr-Bomb and use 250 ml of DI water, in small portions each time, to transfer the contents of the bomb to a 250-ml glass volumetric flask;

Step 20: Measure the concentration of the chloride ion in the rinsate from the Parr-Bomb using Ion-chromatograph (IC) analysis; and

Step 21: Convert the measured concentration of chloride in the solution to residual perchlorate in the resin sample.

# APPENDIX B

# ECONOMIC ASSESSMENT FOR THE TRADE-OFF BETWEEN BIOREGENERATION AND USE OF FRESH RESIN

## Introduction

Two factors play main roles in the economical evaluation of ion-exchange resin bioregeneration process. On the one hand, there is cost of purchasing fresh resin in the places where spent resin is incinerated. On the other hand, there are costs associated with the resin bioregeneration process. Capital costs associated with bioregeneration include design and construction cost for the fermenter and FBR, purchase of pumps, pipelines, and instrumentation. Operating costs include personnel, energy to fluidize the FBR, transportation of resin from the source to the bioregeneration facility, and purchasing of electron donor, nutrients/minerals, and buffer.

Because the resin is not fully regenerated every cycle due to mass transfer limitations and biofouling, bioregenerated resins have less capacity than fresh resins. Therefore, the volume of perchlorate-contaminated water that is processed, when bioregenerated resin is used, will be less than when fresh resin is used. Considering the number of loading-bioregeneration cycles and the 15% resin capacity loss observed in this research, bioregeneration is feasible only if its costs are less than the costs of replacing the spent resin with fresh resin. There are also environmental costs to be considered when spent resins are incinerated. Depending on regulations concerning minimizing greenhouse gases, in some states, carbon credits could be awarded to water utilities that stop resin incineration.

In the following example, operating costs including personnel, energy, transportation, and purchasing chemical costs are compared to the costs associated with purchasing fresh resin. All the calculations are for 1 $ft^3$ of perchlorate-selective resin. Table B.1 shows the total costs to replace perchlorate laden resin with fresh resin.

Table B.1. Total Costs to Replace Perchlorate Laden Resin with Fresh Resin

| Item | Cost |
|---|---|
| Purchasing fresh perchlorate-selective resin | \$250 per $ft^3$ of resin |
| Disposal (incineration) of perchlorate laden resin (typical incineration cost for California from Cameron Environment Inc., Torrance CA) | \$14 per $ft^3$ of resin |
| Total | \$264.00 per $ft^3$ of resin |

Capital costs and operating / maintenance costs are involved in the bioregeneration process. According to a study performed in Civil and Environmental Engineering Department at UNLV, total capital costs to design and build a bioregeneration plant with a capacity of 4900 $ft^3$ of resin per year is approximately \$4,100,000. The expected life of the bioregeneration plant is 20 years. Assuming interest rate of 6% and 20 years investment time period, the bioregeneration plant costs \$358,000 per year. Hence, the capital costs for 1 $ft^3$ of resin would be \$43.06 per $ft^3$ of resin. The other costs associated to bioregeneration process are operating and maintenance costs. Table B.2 shows the operating costs involved in bioregeneration of IX resin.

Table B.2. Total Costs Associated with Bioregeneration of IX Resin

| Item | Cost |
|---|---|
| Transportation to bioregeneration facility | *$0.50 per $ft^3$ of resin* |
| Macro and Micronutrient for bioregeneration | *$0.06 per $ft^3$ of resin*<br>Estimated assuming DAP (diammonium phosphate) and urea as the sources of nitrogen and phosphate and can be purchased from Brenntag Pacific, Santa Fe Springs, CA). Micronutrient media is the same used in this research (Table 4.6) |
| NaCl salt needed for fouling removal | Salt price = \$100 per ton, 1 Bed volume (BV) = 28 L<br>Salt needed for steps 1 and 2 of fouling removal (Table 5.4) = 28 L × 1.5 BV × 60 g/L × 2 = 5040 g of NaCl<br>Salt needed for step 3 of fouling removal (Table 5.4) = 28 L × 1.5 BV × 120 g/L = 5040 g of NaCl<br>Total Salt = 10 kg of NaCl<br>Salt cost = *$1.10 per $ft^3$ of resin* |
| NaOH needed for fouling removal | NaOH price = \$1/L (conc. of 50%), 1 BV = 28 L<br>NaOH needed for step 1 of fouling removal (Table 5.4) = 28 L × 1.5 BV × 20 mL = 840 mL of 50% NaOH<br>NaOH needed for step 2 of fouling removal (Table 5.4) = 28 L × 1.5 BV × 20 mL = 840 mL of 50% NaOH<br>Total NaOH = 1.68 L of NaOH<br>NaOH cost = *$1.68 per $ft^3$ of resin* |
| NaOCl needed for disinfection | NaOCl price = \$0.25/L (conc. of 6%), 1 BV = 28 L<br>NaOCl needed (Table 5.4) = 28 L × 1.5 BV × 16 mL = 700 mL of 6% NaOCl<br>Total NaOCl = 0.7 L of NaOCl<br>NaOCl cost = *$0.175 per $ft^3$ of resin* |
| Water rinsing | Water price = \$0.005/ gal<br>Water needed = 6 BV<br>Total water cost = 6 BV × 28 L/BV × 0.264 gal/L × \$0.005/ gal = *$0.22 per $ft^3$ of resin* |
| Electricity cost | Estimate #1: Electricity as was used in the experimental FBR reactor (Chapter 5)<br>Power needed to obtain 40% expansion per $ft^3$ of resin = 0.9 hp<br>Bioregeneration time = 10 days<br>Electricity price = \$0.1/kw-hr<br>Energy needed to run the pump for 10 days = 0.9 hp × 0.7456 kw/hp × 24 hr/day × 10 day = 160.2 kw-hr<br>Electricity cost = 160.2 kw-hr × 0.1/ kw-hr = *$16.02 per $ft^3$ of resin*<br>Estimate #2: Utilities cost = 0.02 × capital cost (Anderson, 2009)<br>Utilities cost = 0.02 × 4,100,000 = \$82,000 per year<br>Utilities cost per $ft^3$ of resin = 82,000 / 4900 = *$16.73 per $ft^3$ of resin* |
| Maintenance and labor costs | Labor cost = \$76,000 per year (Anderson, 2009)<br>Labor cost = \$76,000 per year/4900 $ft^3$ of resin = *$15.51 per $ft^3$ of resin*<br>Maintenance cost = 0.02 × capital cost (Anderson, 2009)<br>Maintenance cost = 0.02 × 4,100,000 = \$82,000 per year<br>Maintenance cost per $ft^3$ of resin = 82,000 / 4900 = *$16.73 per $ft^3$ of resin* |
| Total operating and maintenance cost | *$53.63 per $ft^3$ of resin* |

As it is shown in Table B.2, operating costs includes transportation to bioregeneration facility site, costs associated with purchasing nutrient chemical, salt (NaCl), sodium hydroxide (NaOH), and sodium hypochlorite (NaOCl), electricity, labor, and maintenance costs.

The results of this research (i.e. Chapter 5) showed that perchlorate-selective resin can be successfully bioregenerated for five consecutive times. The economical feasibility of bioregeneration process here can be estimated using the results of Table B.1 and B.2. Using perchlorate-selective resin, it is expected that 100,000 BV of water contaminated with 50 μg/L of perchlorate can be processed before breakthrough of 4μg/L occurs (Seidel et al., 2006). Two different situations are assumed to treat a water contaminated with 50 μg/L of perchlorate (Table B.3). In situation #1, after the capacity of the resin is exhausted, the resin is transferred to a facility and incinerated. In situation #2, after the capacity of the resin is exhausted, the resin is transferred to a bioregeneration facility.

For situation #2, the bioregeneration process is repeated five times. Although the results of this research showed that after five times loading and bioregeneration, resin capacity loss is about 15%, here it is assumed that after each cycle of bioregeneration, the volume of water that can be treated using the bioregenerated resin reduces by 10,000 BV. It is important to emphasize that column loading of bioregeneration resins was not performed in this research. Therefore, the 10,000 BV reduction is an assumption used for illustration purposes only. Tests should be performed to determine the actual reduction in BVs to breakthrough.

As it is shown in Table B.3, the volume of treated water generated in situation #1 is larger than the volume of water treated using situation #2. However, in situation #1, the

cost of the treatment process is $0.0036 per gallon of treated water, while for situation #2 it is $0.0016 per gallon of treated water, which shows that using bioregeneration, the costs of perchlorate removal reduce by approximately 55%.

Table B.3. Total Costs for 1 $ft^3$ of Perchlorate-Selective Resin in: a) A Process Involving Purchasing New Resin, and b) A Process Involving Resin Bioregeneration

| Situation #1 Resin Incineration | | Situation #2 Resin Bioregeneration | |
|---|---|---|---|
| Volume of treated water, BV | Cumulative cost | Volume of treated water, BV | Cumulative cost |
| 100,000 (cycle 1) | $264 | 100,000 | $250 |
| 200,000 (cycle 2) | $528[a] | 190,000 | $347[b] |
| 400,000 (cycle 3) | $792 | 270,000 | $444 |
| 300,000 (cycle 4) | $1056 | 350,000 | $541 |
| 500,000 (cycle 5) | $1320 | 410,000 | $637 |

[a] $528 = $264 + $264

[b] $276 = $250 + $43.06 + $53.63 ($43.06 is the capital and $12.49 is the operating and maintenance costs associated with the bioregeneration process)

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