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Investigation of feasibility and potential mechanisms for the bioregeneration of perchlorate laden gel-type anion exchange resin

Arjun Krishna Venkatesan
University of Nevada, Las Vegas

INVESTIGATION OF FEASIBILITY AND POTENTIAL MECHANISMS FOR THE BIOREGENERATION OF PERCHLORATE LADEN GEL-TYPE ANION EXCHANGE RESIN

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Arjun Krishna Venkatesan

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[redacted]
Examination Committee Chair

[redacted]
Dean of the Graduate College

[redacted]
Examination Committee Member

[redacted]
Examination Committee Member

[redacted]
Graduate College Faculty Representative

ABSTRACT

Investigation of Feasibility and Potential Mechanisms for the Bioregeneration of Perchlorate Laden Gel-Type Anion Exchange Resin

by

Arjun Krishna Venkatesan

Dr. Jacimaria Ramos Batista, Examination Committee Chair
Associate Professor, Department of Civil and Environmental Engineering
University of Nevada, Las Vegas

The feasibility and potential mechanisms for the bioregeneration of perchlorate laden gel-type anion exchange resin were investigated in this study. Bioregeneration is a sustainable process when compared to one time use of resin and disposal by incineration. Batch and bench-scale bioregeneration experiments were performed to evaluate the feasibility of the process and to gain insight into potential mechanisms that control gel-type resin bioregeneration. The results of the bioregeneration tests suggested that that the initial phase of the bioregeneration process is controlled by kinetics, while the later phase is controlled by diffusion. Higher perchlorate load in the resin had a positive effect on perchlorate degradation rates, while varying microbial concentration did not have a significant effect on perchlorate degradation in gel-type resin. The presence of nitrate suppressed perchlorate degradation initially, but once all nitrate was denitrified, perchlorate degradation took place. Feasibility study showed that direct bioregeneration of gel-type resin was effective in a fluidized-bed reactor, and the resin could be reused and repeatedly regenerated with this method.

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CHAPTER 1

INTRODUCTION

Excessive use of perchlorate (ClO_4^-) as an oxidizer in rocket fuels and munitions over the years have lead to the release of this contaminant in large quantities in surface and groundwaters. Perchlorate has been detected in more than 270 sites all over United States, out of which more than 45 sites are in the National Priority List (EPA, 2008). Perchlorate is highly soluble and stable compound and hence can have an extensive plume in groundwaters (EPA, 2008). Various technologies exist to remove perchlorate from water, out of which biological reduction and ion-exchange technology are prominent. Biological reduction of perchlorate is a well established process for perchlorate removal. There exist several species of microorganisms that are capable of utilizing perchlorate as electron acceptors for their metabolisms (Urbansky and Schock, 1999). Perchlorate has been degraded successfully in various bioreactors using these microorganisms; commonly known as Perchlorate Respiring Bacteria (PRB) (Wallace *et al.*, 1998; Herman and Frankenberger, 1999; Kim and Logan, 2001).

Ion-exchange technology is the most common process that is being used at present to remove perchlorate from drinking water. In this process, perchlorate ions replace other innocuous anions due to its affinity for the functional group of the ion-exchange resin. The major drawback in this process is that the perchlorate ions are only physically

removed from waters but not destroyed. Also, due to the high affinity of perchlorate ions towards ion-exchange resins, the regeneration of the spent resins becomes extremely difficult (Gingras and Batista, 2002).

Resins that are used to remove perchlorate may be classified into selective (or specialty resin) and non-selective resins. Non-selective ion-exchange resins are less efficient in perchlorate removal as other anions like sulfate and nitrate interfere with their exchange (M.C. Gottlieb, 2005). Also these resins are poorly regenerated and require highly concentrated brine solution (Batista *et al.*, 2002). Disposal of these perchlorate concentrated regenerant is another added problem to the issue. The regenerant contains approximately 3-6% brine and experiments show that microorganisms are unable to degrade perchlorate efficiently at such high level of salinity (Gingras and Batista, 2002; Y. Cang *et al.*, 2004; Xu *et al.*, 2003; Okeke *et al.*, 2002). Hence perchlorate cannot be biologically degraded effectively in regenerant with high salinity.

In the last five years, development of ion-exchange technology resulted in the production of selective or specialty resins for perchlorate removal. These resins have very low affinity towards divalent anions such as sulfate and hence have very high efficiency in removing perchlorate ions due to less interference (M.C. Gottlieb, 2005). Unfortunately, most of these resins cannot be regenerated and are disposed by incineration after one time use (Gingras and Batista, 2002). Due to their high cost, disposal of these resins after one time use makes the ion-exchange technology incomplete and economically unsustainable for perchlorate removal.

Bioregeneration of ion-exchange resin is a new concept which involves the direct contact of spent resin with perchlorate reducing bacteria. This process does not require

the use of concentrated brines for regeneration of resins. The bioregeneration technology has been developed recently and is a patented process (Batista and Jensen, 2006). This process consists of a bioreactor containing a perchlorate reducing bacterial culture and a fluidized bed reactor (FBR) containing perchlorate laden ion-exchange resin. The culture is circulated through a Fluidized Bed Reactor (FBR) containing ion-exchange resin loaded with perchlorate (Batista and Jensen, 2006). The perchlorate reducing culture utilizes perchlorate ions as electron acceptors and degrades it to innocuous chloride, when an electron donor is provided. This technology allows the reuse of ion-exchange resin and also destroys perchlorate making it economically and environmentally sustainable. This process has been carried out successfully in bench-scale experiments for macroporous resins (Batista and Jensen, 2006).

Ion-exchange resins can be classified into gel (microreticular) and macroporous (or macroreticular) with respect to their polymeric background (Crittenden *et al.*, 2005). Macroporous resins have larger pore size and higher di-vinyl benzene (DVB) cross-linking when compared to gel-type resins (Metcalf and Eddy, 2003). The pore size of a typical gel type resin is between 5 and 50 A^0 (0.0005 and 0.005 μm) (Pietrzyk, 1969), whereas for a macroporous resin it can be up to 10,000 A^0 (1 μm) with an average value of 600 A^0 (0.6 μm) (K.A. Kun and R. Kunin, 1968). Bioregeneration of macroporous resins have been carried out successfully in the past (Batista *et al.*, 2007). But there exist no past studies on the bioregenerability of gel-type resins. Hence the overall objective of this research is to evaluate the feasibility of bioregeneration process of a gel-type anion exchange resin containing perchlorate.

1.1. Scientific Basis for Research Hypothesis

The maximum amount of perchlorate load in an ion exchange resin is determined by the influent perchlorate concentration in water and the type of resin used. Drinking waters in the United States are generally contaminated with an average 10 ppb perchlorate concentration (EPA, 2004). However industrial sites from manufacturing and laboratory areas like Aerojet General Corp., Rancho Cordova, and NASA Jet Propulsion Laboratory, Pasadena, CA have water contaminated with perchlorate levels ranging between 1500 – 2500 ppb (EPA, 2008). Very high perchlorate concentration of 160,000 and 300,000 ppb was detected in Edward AFB, military base and Kerr McGee site in Henderson, Nevada respectively (EPA, 2008).

The Malcolm Pirnie engineering consulting firm has performed on-site mini-columns and pilot studies with various perchlorate-selective resins with low perchlorate concentration to determine the maximum amount of water that can be processed before a breakthrough of 4 ppb (detection limit) of perchlorate occurs (AWWA, 2006). The influent water that was tested had a perchlorate concentration of 8 – 10 ppb, nitrate of 4000 – 7000 ppb and sulfate of 25,000 – 40,000 ppb (AWWA, 2006). This perchlorate concentration is similar to that present in a contaminated drinking water well (Urbansky, 1998). Results showed that breakthroughs of perchlorate occurred only after 60,000 to 410,000 bed volumes of water when tested with various perchlorate-selective resins (AWWA, 2006). The results from the experiments are summarized in Table 1.1 that includes % of the resin capacity occupied by perchlorate. These values were calculated for this research based on the capacity of the resin used and the number of bed volumes processed to breakthrough.

From Table 1.1 it can be noted that perchlorate load in the resin varies from 0.6 – 6.9 % for an influent perchlorate concentration of 10 ppb. Hence in this research, perchlorate loads of 5.0 % and 8.4 % (3 g/L_{resin} and 5 g/L_{resin}) are used to simulate perchlorate loads in resin that were used to treat drinking water. Higher perchlorate loads of 33.5 % and 50.25 % (20 g/L_{resin} and 30 g/L_{resin}) will also be tested in this research to simulate perchlorate loads from industrial waters. The load of perchlorate into selective resins, for high influent perchlorate concentrations, has been established in the UNLV Environmental Engineering laboratory.

Table 1.1: Perchlorate Loading in Perchlorate Selective Resins

Resin	Resin Capacity (eq/L)	Influent ClO_4^- Conc. (ppb)	Bed Volumes to Breakthrough	ClO_4^- Loaded (g/L_{resin})	Estimated eq/L_{resin} of ClO_4^- loaded	% Total Capacity occupied by ClO_4^-
PWA2[+]	0.6	10	200,000	2.0	0.020	3.4
SIR-110[+]	0.6	10	410,000	4.1	0.041	6.9
PWA-555[+]	1.0	10	60,000	0.6	0.006	0.6
A530E[+]	0.6	10	100,000	1.0	0.010	1.7
SR-7[+]	0.8	10	80,000	0.8	0.008	1.0
A530E[*]	0.6	10,000	~1000	10.0	0.100	16.7
SIR-111[**]	0.635	100,000	270	27	0.271	42.7

[+]AWWA, 2006, [*]Source: Gu and Brown, 2006; ** Source: Column tests performed at UNLV

The biodegradation mechanism for perchlorate, when it is dissolved in water, has been elucidated (Bruce *et al.*, 1999; Coates *et al.*, 1999; Coates and Achenbach, 2004). Figure 1.1 depicts the model of perchlorate degradation by PRBs when the ion is dissolved in water. In this model, perchlorate ion enters the cell via facilitated diffusion

by transmembrane proteins and is degraded to chloride and oxygen by the enzymes (per)chlorate reductase and chlorite dismutase, located in the periplasmic area/outer membrane of PRBs (Kengen *et* al., 1999; Coates *et al.*, 1999; O'Connor and Coates, 2002; van Ginkel *et al.*, 1996). It has been determined that *c*-type cytochrome is present in PRBs that facilitated the reduction of perchlorate (Bruce *et al.*, 1999; Coates *et al.*, 1999). Studies showed that H_2 reduced *c*-type cytochrome of PRBs were readily reoxidized in the presence of chlorate and perchlorate (Coates *et al.*, 1999). It was also shown that these proteins were specific for (per)chlorate and was unaffected by other electron acceptors like sulfate, fumurate etc (Coates *et al.*, 1999). (per)chlorate reductase is found in the periplasmic area while chlorite dismutase is suggested to be loosely bound to the outer membrane of PRB as it was detected both in the soluble fraction (periplasm) and in the outer membrane (Kengen *et* al., 1999; O'Connor and Coates, 2002).

However this mechanism cannot be applied for the degradation of perchlorate attached to the ion-exchange resin. This is because perchlorate ions are strongly attached to functional groups present in the resin and is not freely available to reach the bacterial cell. In this research, to confirm the strong affinity of perchlorate ions to the resin bead, preliminary column tests were performed to investigate whether perchlorate could be detached from the resin by continuous contact with water and sodium chloride (NaCl) solution. Perchlorate selective, macroporous resin, SIR-100 (ResinTech, West Berlin, NJ) was used for the test. Results show that no perchlorate could be detached from the resin bead by using deionized water and only 0.47% of perchlorate could be removed from the resin using 12 % NaCl solution as regenerant. This result confirms the very

high affinity of perchlorate ions from perchlorate-selective resins and disproves the possible dissociation of perchlorate ions from perchlorate-selective resins abiotically.

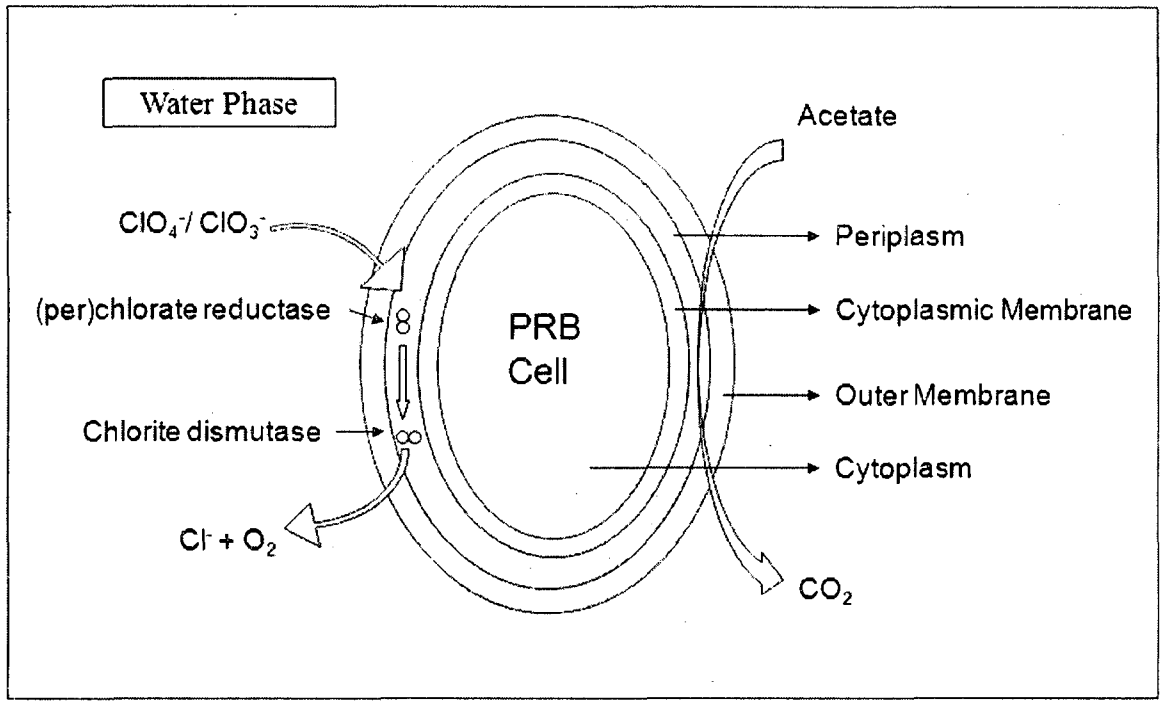


Figure 1.1: Model for Perchlorate Degradation in Water

From the literature it is evident that the pore size in resin beads is of orders of magnitude much smaller than the size of bacterial cells. Gel type resins have pore sizes ranging between 0.005 and 0.04 μm, while macroporous resins has an average pore size of 0.6 μm (Pietrzyk, 1969; K.A. Kun and R. Kunin, 1968). These pore sizes are very small when compared to bacterial cell size, which has an average size of 1 μm (Rittmann and McCarty, 2001). Hence it is only possible for the PRBs to attach to the surface of the resin but not possible to enter ion-exchange resins through pores to utilize perchlorate ions that are attached deep inside the resin bead.

One possible hypothesis to explain the mechanism of bioregeneration process that is taking place inside gel-type ion-exchange resin is made in this research. There may be some substance that is released by PRBs to the bulk liquid, which diffuses through pores in the resin bead and helps in detaching the perchlorate ions from the functional group, thus making it possible for their uptake for cellular metabolism. This hypothesis is depicted schematically in Figure 1.2. This assumption supports the prospect of bioregeneration process in gel-type resin and form the basis for the current study. Once perchlorate ion is detached from the resin bead, it is assumed that the degradation mechanism is similar to that taking place in waters, as shown in Figure 1.1.

Since perchlorate ions are attached to the functional group of the ion-exchange resin, its degradation in resin bead is similar to biodegradation of compounds that are attached to some media in general. Before biodegradation of a substrate can occur, the substrate must be available as a free solute for its uptake and degradation by microorganisms (Gordon and Millero, 1985). Many studies exist that determined the possible mechanism by which these attached substrates become available for biodegradation (Ogram *et al.*, 1985; Subba-Rao and Alexander, 1984; Gordon and Millero, 1985; Guerin and Boyd, 1992). Few of these studies suggested that desorption of substrate from the media may have been facilitated by microorganisms and also that the type of microorganism was important in determining whether the sorbed substrate was bio-available or not (Gordon and Millero, 1985; Guerin and Boyd, 1992). Based on these studies, the mechanism for the biodegradation of perchlorate in ion-exchange resin was hypothesized in this research.

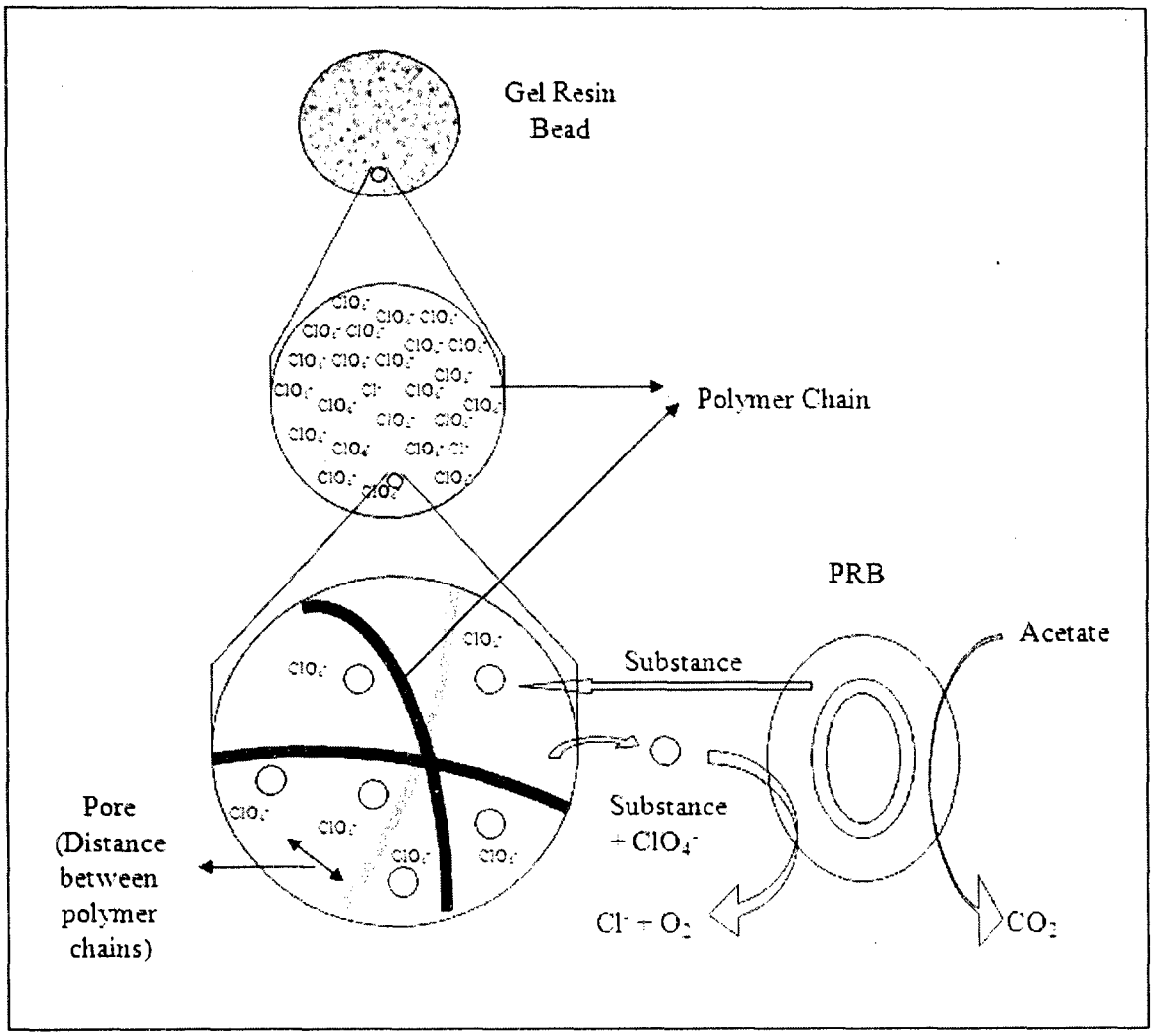


Figure 1.2: Mechanism for Perchlorate Degradation in Gel-type Resin Bead

1.2. Research Objectives and Hypotheses

It is assumed that once perchlorate ions are detached from the resin bead by the detaching substance, the mechanism by which PRBs degrade it to chloride is similar to that taking place in waters. For this reason, all obtained data from this research were compared with those from perchlorate degradation in waters.

Since perchlorate is attached to the resin bead and not freely available, the rate of perchlorate degradation in ion-exchange resin is expected to be slower when compared to

perchlorate degradation in waters. The time that will take for the ' detaching substance' to reach perchlorate sites in resin bead and get perchlorate ion back into the cell is expected to be more when compared to free perchlorate ions in water that diffuse into the cell. Also this time will depend on whether the perchlorate ion is in the surface or deep inside the resin. To reach perchlorate sites inside the resin, the substance needs to diffuse through pores, in which case diffusivity of the substance and pore length may come into play and increase the time.

The two main objectives of this research are (a) to investigate the mechanisms of perchlorate degradation in gel-type anion-exchange resin, (b) to test the feasibility of bioregeneration on a gel-type resin, containing perchlorate, using a fluidized bed reactor. To accomplish the second objective, a bench-scale biodegradation test using a fluidized bed reactor (FBR) and a bioreactor was performed using a gel-type anion-exchange resin (SIR-110-HP, ResinTech, West Berlin, NJ) loaded with perchlorate. The perchlorate-reducing culture was circulated through the column at a known flowrate to foster degradation of perchlorate. The gel-type ion-exchange resin was subjected to three consecutive cycles of bioregeneration to test the feasibility of the process. It is hypothesized in this research that the gel-type anion exchange resin can be bioregenerated based on the hypothesis explained in Figure 1-2.

The first research objective is achieved with batch biodegradation tests. These batch tests are performed to answer the following questions related perchlorate attached to a fixed media (ion exchange resin):

(a) Does the rate of perchlorate degradation depend on the initial amount of perchlorate load in the ion-exchange resin?

(b) How does microbial concentration affect the perchlorate degradation rate in ion-exchange resin beads?

(c) How does the presence of nitrate affect the perchlorate degradation rate in ion-exchange resin?

It is hypothesized in this research that higher initial load of perchlorate in the ion-exchange resin will lead to higher degradation rate. This assumption is based on known half-saturation constant values of the perchlorate-reducing cultures (Table 1.2). Half-saturation constant is the concentration of perchlorate when the growth rate of the microbes is half as fast the maximum specific growth rate (Rittmann and McCarty, 2001). The typical K_s values for perchlorate reducing cultures are shown in Table 1.2. Low perchlorate concentration, below K_s values, will lead to lower degradation rate. All experiments conducted till now on perchlorate biodegradation observed reduced rate with concentration (Herman and Frankenberger, 1999; Kim and Logan, 2000; Brown *et al.*, 2000; Logan and LaPoint, 2002; Gingras and Batista, 2002; Shrout and Parkin, 2006).

Table 1.2: Half-saturation Constant values for PRBs

Culture	K_s (mg/L)	Reference
KJ	33 ± 9	Logan *et al.*, 2001
PDX	45 ± 19	Logan *et al.*, 2001
SN1A	2.2	C.Wang *et al.*, 2008
ABL1	4.8	C.Wang *et al.*, 2008
INS	18	C.Wang *et al.*, 2008
RC1	12	C.Wang *et al.*, 2008

Batch experiments were performed to determine the effect of microbial concentration on the degradation rate of perchlorate. It is hypothesized in this research that higher suspended solids concentration (microbial concentration) will lead to higher degradation rate. This assumption is based on the fact that large number of microbial cells in the culture will result in higher utilization of perchlorate ions. D. Wu *et al.* (2008), conducted experiments to study the effect of different amounts of domestic sludge on perchlorate degradation. Varying amounts of domestic sludge was added to reduce 50 mg/L of perchlorate. It was observed that addition of 0.8-5 g of domestic sludge resulted in 100% removal of perchlorate in 2 days, while only 26.4 and 62.3 % were removed when the additions were 0.3 and 0.5 g respectively (D. Wu *et al.*, 2008). 3.0 g of domestic sludge completely reduced perchlorate from a concentration of 50 mg/L in 12 hours, while 5.0 g reduced the same perchlorate concentration to zero in 6 hours.

This increase in perchlorate degradation can be explained by the substrate utilization model derived from Monod equation. The equation has the following form (C. Wang *et al.*, 2008):

$$\frac{dS}{dt} = -\frac{q_{max} XS}{S + K_s} \tag{1.1}$$

In the above equation, perchlorate utilization (dS/dt) is directly proportional to microbial concentration (X). Hence higher microbial concentration will lead to higher perchlorate degradation. However, due to the limitations of perchlorate degradation in ion-exchange resin explained in the previous section, this relationship between dS/dt and X is not expected to be linear.

Finally, batch experiments were performed to understand the effect of nitrate load on perchlorate degradation rate in the ion-exchange resin. It is hypothesized in this research

that higher nitrate load in the ion-exchange resin will lead to slower rate of perchlorate degradation. This assumption is based on the fact that perchlorate-reducing cultures are capable of carrying out denitrification process by utilizing nitrate as electron acceptors over perchlorate (Xu *et al.*, 2003; Logan and LaPoint, 2002; Chaudhuri *et al.*, 2002; D. Wu *et al.*, 2008; Herman and Frankenberger, 1999).

CHAPTER 2

STATE OF KNOWLEDGE

2.1. Perchlorate as a Contaminant

Perchlorate (ClO_4^-) was first monitored in the year 1997 by the USEPA Drinking Water Program and then by public water systems, which showed high concentrations of this contaminant in surface water, groundwater and soil in western states of USA, including the Colorado River (Urbansky, 1998). Concentrations up to 3700 mg/L of perchlorate have been measured in surface and groundwaters (Logan, 1998). Ammonium perchlorate (NH_4ClO_4) has been used as an energetics booster or oxidant in solid propellant for rockets and missiles. It has been found that high levels of perchlorate in waters resulted due to the discharge from rocket fuel manufacturing plants and military operations. Also, unregulated waste discharge containing ammonium perchlorate in the past increased the concentration level (Urbansky, 1998). Water samples taken from Henderson, Nevada showed perchlorate concentration ranging from 52 to 630 mg/L; Lake Mead samples contained 0.008 mg/L; while the Southern Nevada Water Authority (SNWA) found 0.011 mg/L of perchlorate in tap water (Urbansky, 1998).

Perchlorate salts are highly soluble and stable even in organic solvents and cannot be precipitated by common cations. This nature of perchlorate makes it difficult to remove it from water (Urbansky, 1998). High concentration of perchlorate affects thyroid, bone marrow and muscle tissue. It interferes with iodide accumulation in the thyroid and

inhibits hormone production (Wolff, 1998). The impacts of disrupting thyroid hormone synthesis are greatest on pregnant women and their developing fetuses, infants, children, and individuals who have low levels of thyroid hormones (Wolff, 1998). Perchlorate does not have any federal or drinking water standards set by U.S. Environmental Protection Agency (USEPA), but regulated by individual states (Table 2.1). But an interim health advisory level of 15 ppb has been issued by the EPA recently (EPA, 2009). This advisory level was based on the reference dose recommended by the National Research Council (NRC) of National Academy of Sciences (NAS) (EPA, 2009). The advisory levels for perchlorate as of April, 2005 for the various states are shown below.

Table 2.1: State Perchlorate Limits (EPA, 2005)

State	Level	Regulation Type
Arizona	14 ppb	Health Based Guidance Base
California	6 ppb	Public Health Goal
Maryland	1 ppb	Advisory Level
Massachusetts	1 ppb	Advisory Level
Nevada	18 ppb	Public Notice Standard
New Mexico	1 ppb	Drinking Water Screening Level
New York	5 & 18 ppb	5 – Drinking Water Planning Level 18- Public Notification Level
Texas	17 & 51 ppb	17- Residential Protective Cleanup Level (PCL) 51- Industrial/Commercial PCL

2.2. Perchlorate Removal Methods

2.2.1. Physical Removal

Perchlorate can either be destroyed or physically removed from waters. Physical removal process includes anion exchange, membrane filtration and electrodialysis (Urbansky and Schock, 1999). In membrane filtration, water is forced through a semi-permeable membrane through which the dissolved salts cannot pass and are hence retained. Membrane fouling and damage by microbes are few drawbacks in this process (Urbansky and Schock, 1999). Water is passed through a series of ion-selective membrane in electrodialysis, in the presence of an electric field. Ions get concentrated in every alternate channel, while the remaining channel is filled with deionized water. The concentrate containing perchlorate must be disposed of accordingly (Urbansky and Schock, 1999).

2.2.2. Electrochemical Reduction

Perchlorate can be reduced to chloride by applying an electric current through an electrode at high potential (Urbansky and Schock, 1999). Various cathodes like tungsten carbide, platinum, titanium etc. have been used in experiments. Drawbacks for this process include microbial growth on the electrodes, electrode corrosion and time consumption (Urbansky and Schock, 1999). The oxidation and reduction equations are given below (Shrout and Parkin, 2006; Sawyer *et al.*, 1994):

Reduction reaction: $ClO_4^- + 8\ e^- + 8\ H^+ \longrightarrow 4\ H_2O + Cl^-$ $\Delta G^0 = -94.06$ KJ/e^- eq.

Oxidation reaction: $2\ H_2O \longrightarrow O_2 + 4\ e^- + 4\ H^+$ $\Delta G^0 = 78.14$ KJ/e^- eq.

2.2.3. Ion-exchange

Anion exchange is achieved when the contaminated water is allowed to pass through a resin bed containing high concentration of a replacement ion, usually chloride ions. When in contact, the perchlorate ion replaces chloride ion due to the affinity of perchlorate for the resins (Urbansky and Schock, 1999). This rate of transfer depends on the type of functional group present in the ion-exchange resin. Once the resin bed is spent, it must be regenerated to allow for resin reuse. Regeneration is accomplished by concentrated sodium chloride brine (Urbansky and Schock, 1999). Most of these resins are non-regenerable while few types can be partially regenerated. High concentration of perchlorate will be present in the regenerant solution which must be disposed of accordingly (Urbansky and Schock, 1999). The main drawbacks in this process are the high cost of selective resins, the poor regeneration of non-selective resins and the disposal of perchlorate-containing ion-exchange waste brines.

2.2.3.1. Resin Structure and Classification

Synthetic ion-exchange resins have three-dimensional, cross linked polymer structure as their matrix, having functional groups with fixed ionic charges (Crittenden *et al.*, 2005). The matrix is usually made of vinyl polymers (polystyrene and poly acrylic) and is cross linked with di-vinyl benzene (DVB). Synthetic ion-exchange resins are classified into gel (microreticular) and macroporous (or macroreticular) with respect to their polymeric background (Crittenden *et al.*, 2005). Gel-type resins have lower degree of cross-linking when compared to macroporous resin. Gel-type resins usually have about 4 – 10% DVB cross-linking, while macroporous resins have about 20 – 25 % cross-linking (Crittenden *et al.*, 2005). Gel-type resins are translucent and have high water

content. Hence they lose their pore structure upon drying. If these resins are not in swollen state, the exchange of ions cannot take place (Pietrzyk, 1969).

In comparison, macroporous resins are made by linking microspheres, making them more rigid. They can maintain their pore structure and integrity even when dried (Crittenden *et al.*, 2005). In gel-type resin the pore size is determined by the distance between polymer chains and crosslinks of the swollen gel matrix (Kunin, 1976). These pores are extremely small and hence restrict the uptake of large ions. The pore size of a typical gel type resin is between 5 and 50 A^0 (Pietrzyk, 1969), whereas for a macroreticular resin it can be up to 10,000 A^0 with an average value of 600 A^0 (K.A. Kun and R. Kunin, 1968). Poinescu and Vlad (1996), synthesized macroreticular resins from copolymerization of styrene and di-vinyl benzene monomers in the presence of poly(vinyl acetate). The final products had a mean pore diameter ranging between 3273 A^0 and 8067 A^0 (Poinescu and Vlad, 1996).

Synthetic ion-exchange resins can also be classified based on the functional group that is bonded to the matrix, as: (a) strong-acid cation (SAC), (b) weak-acid cation (WAC), (c) strong-base anion (SBA) and (d) weak-base anion. These classifications are based on the Arrhenius theory of electrolyte strength, which explains the dissociation capability of the functional group to its ionic form at any pH (Crittenden *et al.*, 2005). In general, a low pK value of a resin indicates that the resin give up proton easily, while high pK values indicates that the resin will not give up protons easily (Crittenden *et al.*, 2005).

Strong-base anion exchange resins have quaternary amine group as the functional group. They are classified into Type 1 and Type II quaternary group (Crittenden *et al.*,

2005). Type 1 SBA resins have tri-methyl amine as the functional group, while Type II SBA resins have one ethyl-dimethyl amine as the functional group (Crittenden *et al.*, 2005).. The exchange reactions for both the types are shown below (Crittenden *et al.*, 2005):

$$n[R(CH_3)_3N^+]\ OH^- + A^{n-} \leftrightarrow [nR(CH_3)_3N^+]\ A^{n-} + nOH^- \quad \text{(Type 1)}$$

$$n[R(CH_3)_2(CH_3CH_2OH)\ N^+]\ OH^- + A^{n-} \leftrightarrow \quad [nR(CH_3)_2(CH_3CH_2OH)\ N^+]\ A^{n-} + nOH^- \quad \text{(Type 2)}$$

Where OH^- and A^{n-} are the exchanging anions.

Strong base and weak base anionic resins with styrenic and acrylic matrices have been widely used in the removal of perchlorate ions (Batista *et al.*, 2002). Perchlorate ion is strongly hydrophobic and hence tends to bind tightly with hydrophobic matrices like styrenic group. The affinity of perchlorate to bind with these resins also depends on the functional group that is attached to the matrix (Batista *et al.*, 2002). Higher the affinity, difficult is the separation of perchlorate ions from resins.

Studies show that regeneration of styrenic resin types is poor when compared to acrylic resin types (Batista *et al.*, 2002). Also the efficiency of the resin to remove perchlorate ions depends on the concentration of other anion contaminants present in water as they compete with perchlorate for the resin sites (Batista *et al.*, 2002). Acrylic type resins are suitable to remove perchlorate when sulfate concentration is low since they can be easily regenerated. But at high concentrations of sulfate, styrenic types are preferred (Batista *et al.*, 2002).

2.2.3.2. Resin Selectivity

Ion-exchange resins exhibit preferential selectivity or affinity towards various ions (Crittenden *et al.*, 2005). This selectivity is based on the nature of ion, the nature of the

exchanger, the degree of saturation of the ion exchange complex etc. (Kunin, 1960). Chemical properties of the ion such as the magnitude of the valence and atomic number, and physical property of the ion-exchange resin such as pore size and functional group, also affect the selectivity of the resin. The relative selectivity order for a strong-acid cation exchanger (Amberlite IRN-77) is (Skogley and Dobermann, 1996):

$$Ba^{2+} > Pb^{2+} > Hg^{2+} > Sr^{2+} > Ca^{2+} > Ni^{2+} > Mn^{2+} > Be^{2+} > Cd^{2+} > Cu^{2+} > Co^{2+} > Zn^{2+} =$$

$$Mg^{2+} = Ag^{+} > Cs^{+} > Rb^{+} > Fe2^{+} > K^{+} > NH_4^{+} > Na^{+} > H^{+} > Li^{+}$$

The selectivity order for strong basic anion-exchangers (Amberlite IRN-78) is given as (Skogley and Dobermann, 1996; Crittenden *et al.*, 2005):

$$ClO_4^- > I^- > ClO_3^- > NO_3^- > Br^- > HSO_4^- > NO_2^- = CN^- > Cl^- > HSO_3^- > BrO_3^- > OH^-$$

$$\text{(Type 2 resin)} > HCO_3^- > CH_2COO^- > F^- > SO_4^- > OH^- \text{ (Type 1 resin)} > CO_3^{2-} > HPO_4^{2-}$$

The selectivity coefficient of an ion-exchange resin can be given as (Crittenden *et al.*, 2005):

$$K_B^A = \frac{[A]\{\overline{R}B\}}{[B]\{\overline{R}A\}} \tag{2.1}$$

Where, K_A^B is the selectivity coefficient, [A] and [B] are the concentration of presaturant ion and counter ion in the aqueous phase respectively, $\{\overline{R}A\}$ and $\{\overline{R}B\}$ are the concentration of presaturant ion and counter ion in the resin phase respectively. The value of the selectivity coefficient measures the preference of counter ions to other ions in the solution.

Selectivity of the ion-exchange resin can also be described by another parameter called the distribution coefficient (K_d) (Gu and Brown, 2006). The distribution coefficient for perchlorate ion can be defined as (Gu and Brown, 2006):

$$K_d = [R\text{-}ClO_4^-]/ [ClO_4^-] \tag{2.2}$$

Where [R-ClO_4^-] is the concentration of perchlorate sorbed in the ion-exchange resin and [ClO_4^-] is the concentration of perchlorate in the bulk liquid. K_d has the unit mL/ g of the exchanger.

2.2.3.3. Perchlorate Selective and Non-Selective Resins

Resins can be classified based on their selectivity for perchlorate as perchlorate-selective and non-perchlorate selective resins. Perchlorate selective resins are those which have higher selectivity coefficient for perchlorate ions, while non-perchlorate selective resins have a lower selectivity coefficient for perchlorate. In general, Type-1 polystyrenic strong-base anion exchange resins have higher affinity to perchlorate when compared to Type-II resins (Gu and Brown, 2006). This is because the matrix of Type-1 resin (polystyrene divinylbenzene) is non-polar (or hydrophobic) and has natural affinity towards poorly hydrated anions like perchlorate. Whereas, in the matrix of Type-II resin, one alkyl group in the trialkyl quaternary amine is replaced by ethyl group, thus making it slightly hydrophilic (Gu and Brown, 2006). Hence these resins (Type II) have lower affinity towards perchlorate ions.

Increasing the length of trialkyl chain in the resin from methyl to ethyl, increases the hydrophobic nature of matrix, which in turn increases affinity towards perchlorate ions (Gu and Brown, 2006). One disadvantage in increasing the chain length is that it results in reduced sorption kinetics (Gu and Brown, 2006). These perchlorate-selective resins are the result of a balance between selectivity and absorption kinetics. Few of these resins are bi-functional having two quaternary ammonium groups, one with longer chain length for selectivity while the other is short for higher absorption kinetics (Gu and Brown, 2006). However SIR-110-HP resin (Resintech, West Berlin, NJ), used in this

research is a specialty resin with high selectivity for perchlorate, having tertiary amine as the functional group. Comparison of the distribution coefficient of highly perchlorate-selective and non-selective resins is shown in Table 2.2.

Table 2.2: Ion-exchange Distribution Coefficients of Selective and Non-Selective Resins (Gu and Brown, 2006)

Resin	Type	K_d (mL/g)
Purolite A-830	Non- Selective	180
Purolite A-850	Non- Selective	1800
Purolite 530 E	Selective	627,000
Amberlite PWA2	Selective	920,000

Effective regeneration of Purolite A-520E resin has been achieved by a solution of $FeCl_3$ and HCl (Gu *et al.*, 2002). But most of the other selective resins are non-regenerable and is used just one time (Gu and Brown, 2006). In case of non-selective resins, they are poorly regenerated by brine and require a large volume of the regenerant solution. Hence operational and waste disposal cost is high for non-selective resins when compared to selective resins (B. Gu and D. Coates, 2006). Examples of selective and non-selective resins are given in Table 2.3. Ion-exchange resins in general are costly and hence not economical to use it just once to remove the contaminants. Though they completely remove perchlorate from waters, the biggest challenge faced by the water treatment facilities is to find an efficient way to regenerate and reuse these resins.

Table 2.3: List of Perchlorate selective and non-selective resins

Name	Perchlorate Selective/ Non-selective	Matrix/ Type	Functional Group	Water Retention Capacity (%)
DOWEX PSR-3	Selective	Styrene-DVB, Macroporous	Tri-n-butyl amine $(C_4H_9)_3N$	50-65
DOWEX PSR-2	Selective	Styrene-DVB, Gel	Tri-n-butyl amine $(C_4H_9)_3N$	40-47.5
DOWEX-1	Selective	Styrene-DVB, Gel	Tri methyl amine $(CH_3)_3N$	43-48
A530E	Selective	Polystyrene-DVB, Macroporous	Quaternary Ammonium	50
A532E	Selective	Polystyrene – DVB, Gel	Bifunctional Quaternary amines	36-45
A520E	Non-selective	Polystyrene – DVB, Macroporous	Type 1 Quaternary Ammonium	50-56
A600E	Non-selective	Polystyrene – DVB, Gel	Type 1 Quaternary Ammonium	43-48
A850E	Non-selective	Polyacrylic– DVB, Gel	Quaternary Ammonium	57-62
SIR-110-HP	Selective	Styrene-DVB, Gel	Tri-n-butyl amine $(C_4H_9)_3N$	35-55
SIR-100-HP	Non-selective	Styrene-DVB, Macroporous	Tri ethyl amine $(C_2H_5)_3N$	52-60
PWA-2	Selective	Gel	N/A	34-42
Ionac SR-7	Non-selective	Styrene-DVB	Quaternary Amine	48-52
ASB-1	Non-selective	Styrene-DVB, Gel	Tri methyl amine $(CH_3)_3N$	43-48

(Source: Compiled from the websites of the resin manufacturers: Dow; Purolite; Resintech, Inc; Rohm and Haas; Lanxess.)

2.2.3.4. Ion-exchange Kinetics

Ion exchange process is similar to the adsorption process taking place in activated carbon (W. J. Weber, 1972). The process takes place in several steps. (a) First the exchanging ion reaches the surface of the resin; (b) then it diffuses through a layer of film surrounding the resin particle; (c) pore transport of the ion through the resin bead to reach the active exchange sites; (d) actual exchange of ions; (e) pore transport of the exchanged ion outward through the resin bead; (f) diffusion of the ion through the film; (g) and finally transport of the exchanged ion from the surface of the resin to the bulk liquid (W. J. Weber, 1972). Steps (a) to (c) and (e) to (g) are the rate limiting steps, while step (d) occurs very fast; in other words the diffusion through the hypothetical film around the resin and through the pores are the rate limiting steps in the ion-exchange process (W. J. Weber, 1972).

In general, in batch type-reactors, where the flowrate/stirring are less, the ion exchange rate is determined by film diffusion (W. J. Weber, 1972). The expression for the rate of decrease in concentration is given by (W. J. Weber, 1972):

$$-\frac{dC}{dt} = k_f \frac{a}{V}(C - C_e) \tag{2.3}$$

Where k_f is the film transfer coefficient; a is the effective area for mass transfer; V is the volume of solution in the reactor; C and C_e are the initial and final concentration of the component that is adsorbed by the resin. In systems where there are high flowrate/ stirring of the solution, pore diffusion will be the rate limiting step (W. J. Weber, 1972). In such cases the exchange process expression can be derived from Fick's second law of diffusion and the final expression is given by (W. J. Weber, 1972):

$$\frac{\partial C}{\partial t} = \frac{1}{r^2}\frac{1}{\partial r}\left(r^2 D_l \frac{\partial C}{\partial r}\right) - \frac{\partial q}{\partial t} \tag{2.4}$$

Where r is the resin particle radius; D_l is the diffusion coefficient; q is the concentration of the exchanged ions of the resin in equilibrium with the solution concentration of C.

The amount of perchlorate uptake by the ion-exchange resin can be explained by Langmuir Isotherm (B. Gu *et al.*, 1999):

$$q = \frac{K_{lm} q_{\max} C}{K_{lm} C + 1} \tag{2.5}$$

Where q is the amount of ClO_4^- sorbed by synthetic resins (mg/g), K_{lm} is the sorption coefficient, q_{max} is the maximum quantity or capacity of ClO_4^- sorbed and C is the ClO_4^- concentration in the influent. At low equilibrium concentrations, $K_{lm}C << 1$, and the above equation reduces to a linear form (Zhang *et al.*, 2007 b):

$$q = K_d C \tag{2.6}$$

Where K_d is the distribution coefficient. The Langmuir isotherm equation can also be rearranged to give a linear form:

$$\frac{C}{q} = \frac{1}{K_{lm} q_{\max}} + \frac{C}{q_{\max}} \tag{2.7}$$

2.2.4. Biodegradation

Biological reduction of perchlorate has been carried out successfully in laboratory experiments and is being considered for large-scale treatment of perchlorate contaminated waters. Several genera of microorganisms are capable of using perchlorate as an electron acceptor under anaerobic condition (Kim and Logan, 2001). Perchlorate is degraded through several steps as shown in Figure 2-1.

The end product from the complete degradation of perchlorate is innocuous chloride. It is believed that the rate limiting step for the above conversion is the perchlorate reduction to chlorate (Kim and Logan, 2001). Perchlorate respiring bacteria (PRB) are ubiquitous in natural environment and members of the class *Proteobacteria* of the *Bacteria* (Coates *et al.*, 1999). They are found to inhabit rivers, soils and even wastewater treatment plants (Xu *et al.*, 2003). The suspicion about perchlorate degradation by bacteria was derived from the knowledge of biodegradation of chlorate by Chlorate Reducing Bacteria (CRB). All PRBs are capable of chlorate reduction while all CRBs need not reduce perchlorate (Xu *et al.*, 2003).

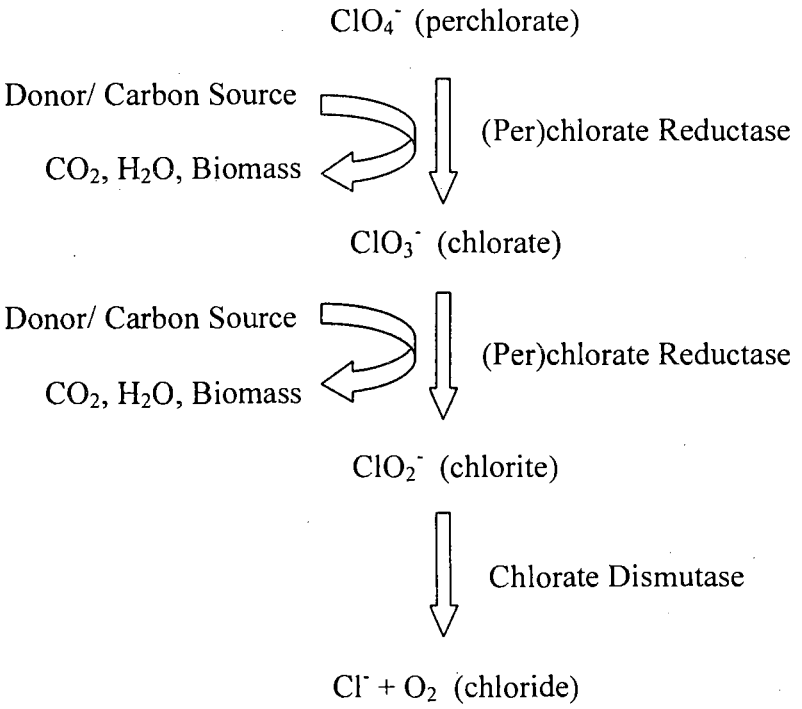


Figure 2.1: Perchlorate degradation pathway (Xu *et al.*, 2003)

Some of the bacterial species that respire perchlorate are listed in Table 2.4. These bacteria produce an enzyme which reduces the activation energy of perchlorate reduction and the energy is used for cellular metabolism (Urbansky and Schock, 1999). Some of these bacteria are pathogens and/or facultative anaerobes. Hence even when low dissolved oxygen content is present in the system, they tend to respire oxygen instead of perchlorate (Urbansky and Schock, 1999).

Table 2.4: Common Perchlorate- Reducing Cultures (Xu *et al.*, 2003)

Isolate	Reference
Vibrio dechloraticans Cuznesove B-1168	Korenkov *et al.* (1976) Wallace *et al.* (1996)
Wolinella succinogenes HAP-1	Bruce *et al.* (1999)
Dechloromonas agitata CKB	Coates *et al.* (1999)
Dechloromonas sp. SIUL	Coates *et al.* (1999)
Dechloromonas sp. MissR	Coates *et al.* (1999)
Dechloromonas sp. CL	Coates *et al.* (1999)
Dechloromonas sp. NM	Coates *et al.* (1999)
Dechloromonas sp. SDGM	Coates *et al.* (1999);
Dechloromonas sp. PS	Miller and Logan (2000)
Dechloromonas sp. JM	Zhang *et al.* (2002)
Dechloromonas sp. HZ	Michaelidou *et al.* (2000)
Dechlorospirilium anamolous wd	Logan *et al.* (2001c)
Dechloromonas sp. KJ	Logan *et al.* (2001c)
Dechloromonas sp. PDX	Rikken *et al.* (1996)
Dechloromonas sp. GR-1	Herman and Frankenberger (1999)
Dechloromonas sp. perc lace	Okeke *et al.* (2002)
Citrobacter sp. IsoCock1	Shrout and Parkin (2002)
Dechloromonas sp. JDS5	

The conversion of perchlorate to chlorate is catalyzed by the enzyme (per)chlorate reductase (PR). As the concentration of chlorate increases due to the reduction of perchlorate, it is believed that the same enzyme (per)chlorate reductase also catalyzes the

conversion of chlorate to chlorite (Dudley *et al.*, 2008). All bacteria capable of degrading perchlorate can also utilize chlorate as electron acceptor, but not all chlorate-respiring bacteria can degrade perchlorate (Coates and Achenbach, 2004). It was found that (per)chlorate reductase activity was found maximum in the periplasmic area in many PRB isolates (L.M. Steingberg *et al.*, 2005; Okeke and Frankenberger, 2003). This lead to the confirmation that (per)chlorate reductase is present as soluble fractions in periplasm.

Chlorite dismutase (CD) is the central enzyme in PRB which causes about the dismutation of chlorite to innocuous chloride (Xu *et al.*, 2003). This enzyme is of particular interest in the perchlorate degradation pathway because of the toxicity of chlorite to bacteria, which otherwise will get accumulated in the absence of the enzyme (Xu *et al.*, 2003). CD is an enzyme which is highly specific for chlorite and has a specific activity of approximately 1928 μmol of chloride/mg protein/min with a molecular mass of 120 kDa (Coates *et al.*, 1999). When compared to nitrate reductase, which has a specific activity between 0.05 and 5 μmol of nitrite/mg protein/min (W. G. Harrison, 1973), CD has high activity and hence very efficient in destroying chlorite. Also the molecular mass of CD is much less than that of nitrate reductase, which has a molecular mass of 375 kDa (L. P. Solomonson *et al.*, 1986). According to studies, CD has been isolated from both soluble fractions (periplasm) and outer membrane of PRBs (O'Connor and Coates, 2002; R. A. Bruce *et al.*, 1999). This result suggested that CD may be loosely bound to the outer membrane of PRB (O'Connor and Coates, 2002).

2.2.4.1. Kinetics of Perchlorate Degradation

The kinetics for perchlorate degradation and bacterial growth can be established by the substrate-utilizing and cellular maintenance model (C. Wang *et al.*, 2008). Both these models are based on the Monod equation.

Substrate-utilizing Model:

$$\frac{dS}{dt} = -\frac{q_{max} XS}{S + K_S} \tag{2.8}$$

Cellular Maintenance Model:

$$\frac{dX}{dt} = \mu_{max}\left[\frac{S}{S + K_s}\right]X - bX \tag{2.9}$$

Where, S is the perchlorate concentration (mg/L); t the time (h); μ_{max} is the maximum specific growth rate (h^{-1}); q_{max} is the maximum specific substrate removal rate (h^{-1}); K_s is the half saturation constant (µg/L); X is the microbial concentration; and b is the endogenous decay rate (h^{-1}) (C. Wang *et al.*, 2008). Typical kinetic parameters from literature are summarized in Table 2-5. The kinetic parameters vary with the type of bacterial isolate. KJ and PDX have higher growth rate of 0.14 and 0.21 h^{-1} and lower perchlorate utilization rate (0.055 and 0.017 h^{-1}) when compared to other isolates like SN1A, ABL1 etc. The half saturation constant varies widely from a value as low as 0.14 mg/L to 470 mg/L.

The observed yield coefficient can be given as (C. Wang *et al.*, 2008):

$$Y = \frac{dX}{-dS} = \frac{dX/dt}{-(dS/dt)} \tag{2.10}$$

$$= \frac{\mu_{max}\left[\frac{S}{S+K_s}\right]X - bX}{q_{max}XS/(S+K_s)} = \frac{\mu_{max}}{q_{max}} - \frac{b(S+K_s)}{q_{max}S} \tag{2.11}$$

$$= \left(\frac{\mu_{max}}{q_{max}}\right)\left(1 - \frac{b(S+K_s)}{\mu_{max}}\right) \tag{2.12}$$

When $S >> K_s$, the maximum observed yield (Y_{max}) is given by (C. Wang *et al.*, 2008):

$$Y_{max} = \left(\frac{\mu_{max}}{q_{max}}\right)\left(1 - \frac{b}{\mu_{max}}\right) \tag{2.13}$$

The above equation indicates that the observed yield is a constant when excess amount of substrate is present. Integrating the above equation gives a linear relation between the biomass concentration and the substrate (C. Wang *et al.*, 2008).

$$X = X_0 + S_0Y_{max} - Y_{max}S \tag{2.14}$$

Where, X_0 and S_0 are the initial biomass and perchlorate concentration respectively. Substituting $\mu_{max} = q_{max}Y_{max} + b$ in the cellular maintenance model, we get the perchlorate reduction kinetics in terms of bacterial growth (C. Wang *et al.*, 2008):

$$\frac{dX}{dt} = (q_{max}Y_{max} + b)\left(\frac{S}{S+K_s}\right)X - bX \tag{2.15}$$

Significance of the substrate utilizing and cellular maintenance model (Equation 2.8 and 2.9) can be understood from Figures 2.2 and 2.3. These graphs were plotted using average values for various parameters from Table 2.5. From the Figure 2.2 it is understood that increasing the substrate concentration S, dX/dt and dS/dt do not increase indefinitely. After about 250 mg/L influent substrate concentration, the curve flattens,

indicating any further increase in substrate concentration does not contribute to significant microbial production or substrate utilization. Figure 2.3 is a plot between biomass concentration (X) and substrate utilization (-dS/dt). From the figure, substrate utilization increases linearly with increasing biomass concentration.

Table 2.5: Typical Kinetic Parameters for Perchlorate-Reducing Bacteria

Culture	Kinetic Parameters				Electron Donor	Reference
	μ_{max} (h^{-1})	q_{max}(h^{-1})	Y_{max}	K_s (mg/L)		
KJ	0.14	0.055	0.5 ± 0.08	33 ± 9	Acetate	Logan *et al.*, 2001
PDX	0.21	0.017	-	45 ± 19	Acetate	Logan *et al.*, 2001
SN1A	0.069	0.192	0.36	2.2	Acetate	C.Wang *et al.*, 2008
ABL1	0.0858	0.226	0.38	4.8	Acetate	C.Wang *et al.*, 2008
INS	0.067	0.181	0.37	18	Acetate	C.Wang *et al.*, 2008
RC1	0.085	0.250	0.34	12	Acetate	C.Wang *et al.*, 2008
PC 1	-	0.129	-	0.14	Acetate	Waller *et al.*, 2004
HCAP-C	-	0.183	-	76.6	Acetate	Dudley *et al.*, 2008

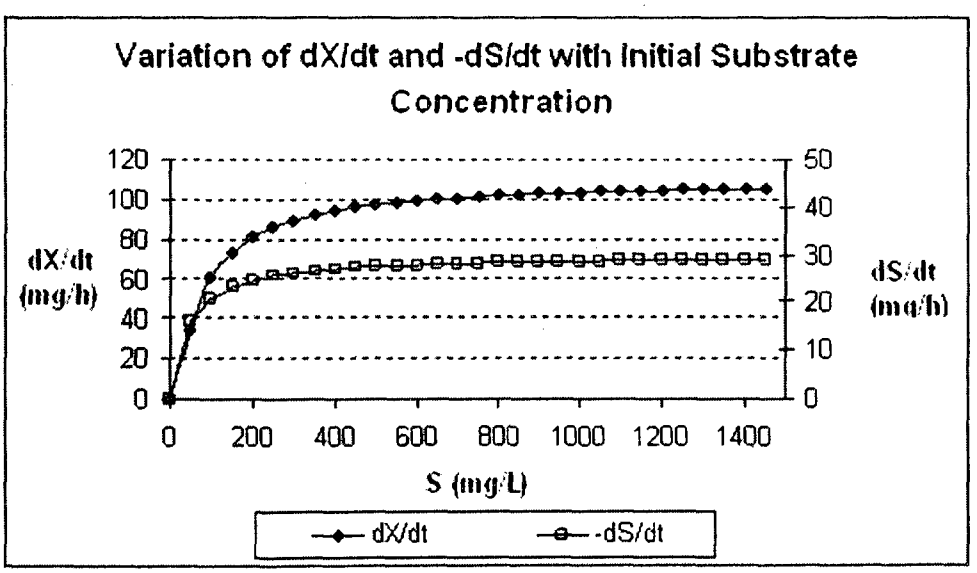


Figure 2.2: Significance of Cellular Maintenance and Substrate Utilizing Model

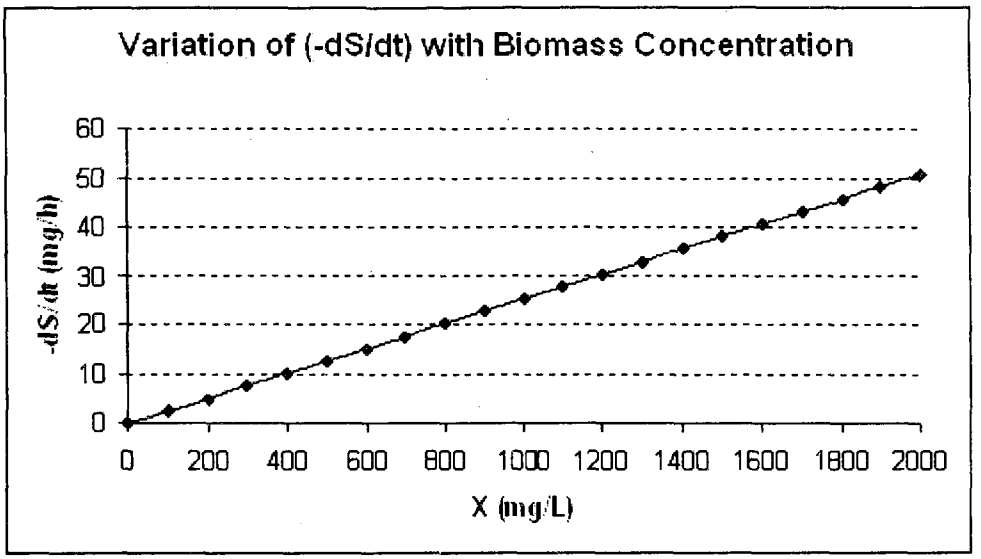


Figure 2.3: Variation of Substrate Utilization with Biomass Concentration

2.2.4.2. Factors Affecting Biodegradation of Perchlorate

Biodegradation of perchlorate is affected by various parameters. Since PRBs are facultative aerobes, even small changes in the environmental condition affect perchlorate degradation. Also an electron donor should be present to carry out the conversion of perchlorate to innocuous chloride. Studies show that during active perchlorate degradation, no molecular oxygen is produced. When chlorite is added to a PRB culture, molecular oxygen is produced outside the cell (Shrout and Parkin, 2006). This clearly shows the capability of these microbes to change their metabolism so as not to produce oxygen while degrading perchlorate because of their enzyme's sensitivity to oxygen. It has been shown that perchlorate degradation is sensitive to redox potentials and does not take place above -110 mV (Shrout and Parkin, 2006).

Shrout and Parkin (2006) carried out experiments to analyze the influence of electron donor, oxygen and redox potential on the biodegradation of perchlorate. In their

experiments, lactate was used as the electron donor, and three different electron donor-to-perchlorate ratios were tested (1:1, 2:1 and 4:1 mass ratios). Mixed lactate enrichment culture (LEC) was used to degrade perchlorate. Results showed that 1:1 ratio degraded perchlorate at a slower rate when compared to ratios of 2:1 and 4:1 (Shrout and Parkin, 2006). Also complete degradation of perchlorate was not observed when the lower ratio of 1:1 was used. During the test it was also noted that the redox potential changed with different ratios of electron donor-to-perchlorate. It was observed that the redox potential increased when the ratio was less than 1.1 and decreased when the ratio was more than 1.5. It was also observed that excessive supply of electron donor did not improve the degradation kinetics any further because of either lack of electron acceptor (perchlorate) or insufficient microorganisms for its utilization (Shrout and Parkin, 2006).

To further understand the influence of redox potential on perchlorate degradation, Shrout and Parkin (2006) carried out experiments at four different potentials (-220 mV, -50 mV, +180 mV and +390 mV). Results showed that 100% perchlorate degradation was observed only at a potential of -220 mV and the lowest degree of perchlorate degradation was observed at +390 mV (Shrout and Parkin, 2006). Also about 30-35 % of perchlorate degradation was observed at potentials -50 mV and +180 mV, which disagree with previous studies showing an upper limit of -110 mV for perchlorate degradation (Shrout and Parkin, 2006). In general, presence of oxygen inhibits perchlorate degradation (Kim and Logan, 2001). But in the experiments conducted by Shrout and Parkin (2006), addition of oxygen did not lead to reduced degradation. They introduced oxygen into the system when active perchlorate degradation was taking place. They even observed perchlorate degradation when the dissolved oxygen level was 4.8 mg/L (Shrout

and Parkin, 2006). Hence they concluded that the presence of oxygen alone does not affect perchlorate degradation, but at higher redox potentials the rate of degradation reduces significantly.

C.Wang *et al.* (2008) carried out experiments to find the effect of pH on perchlorate degradation and correlate it to the degradation kinetics. It was seen that perchlorate degradation took place in the pH range of 5.0-9.0. But the rate at which the degradation took place was significantly different for different pH (C.Wang *et al.*, 2008). The maximum degradation was seen at a neutral pH (7.0). The reduction in the degradation rate with the deviation from pH neutrality is given by the following equation which was obtained by statistical analysis for the sensitivity of q_{max} to pH changes (C.Wang *et al.*, 2008):

$$q_{\max,pH} = q_{\max,pH\,7.0} \exp\left[-\frac{(pH-7.0)^2}{2\sigma^2}\right] \tag{2.16}$$

Where, $q_{max,pH}$ and $q_{max,pH7.0}$ are the maximum substrate utilization rate at a specific pH and at pH 7.0 respectively; σ is the standard deviation.

Effect of temperature changes on perchlorate degradation was studied by D. Wu *et al.* (2008). Results showed that perchlorate degradation was faster at a temperature of 40 ^{0}C when compared to a temperature of 30 ^{0}C. One hundred percent perchlorate degradation was completed in 24 hours at 40 ^{0}C temperature, while it took 48 hours at a temperature of 30 ^{0}C (D. Wu *et al.*, 2008).

Nitrate ions are common co-contaminants that are present along with perchlorate. Studies show that perchlorate reducing bacteria are capable of utilizing nitrate ions as electron acceptors and carryout denitrification process (Xu *et al.*, 2003). There exist several studies which show reduced perchlorate removal rates in the presence of nitrate

(Logan and LaPoint, 2002; Chaudhuri *et al.*, 2002; D. Wu *et al.*, 2008; Herman and Frankenberger, 1999).

Logan and LaPoint (2002) studied the effect of nitrate on perchlorate removal rates in water. In the absence of nitrate, about 30 % perchlorate removal was observed in 5 days when the influent (artificial groundwater) concentration of perchlorate was around 79 ppb to the autotrophic reactor. But in the presence of nitrate (20 ppm as NO_3-N in the influent), perchlorate removal was reduced to an average of 17 % for a 7-day period. However, when contaminated groundwater from Redlands, CA was introduced into the system on day 118, there was no appreciable change in perchlorate removal rates (Logan and LaPoint, 2002). Hence it was summarized by Logan and LaPoint (2002) that, effect of nitrate on perchlorate may depend on the water source and nitrate exposure time.

Chaudhuri *et* al (2002) introduced a perchlorate-grown inoculum into an equi-molar nitrate-perchlorate medium to investigate the effect of nitrate on perchlorate degradation. They observed nitrate reduction prior to perchlorate reduction without any lag period. When a nitrate-grown inoculum was introduced into the same medium, they observed an extended lag period of 40 h, after which nitrate was consumed prior to perchlorate degradation (Chaudhuri *et al.*, 2002). Overall they observed a lower rate of perchlorate utilization in the presence of nitrate.

D. Wu *et al.* (2008) observed perchlorate degradation at various carbon-to-nitrogen (C/N) ratios. They observed only 20.7 % perchlorate removal when C/N ratio was 5. Nitrate concentration also reduced indicating simultaneous reduction of nitrate and perchlorate by PRBs (D. Wu *et al.*, 2008). However, no significant change was observed

at higher ratios of 10, 20 and 30. Also no accumulation of nitrite was observed in the medium indicating the denitrifying capability of PRBs (D. Wu *et al.*, 2008).

Herman and Frankenberger (1999) conducted experiments to study the denitrifying capability of perclace. They observed no significant difference in perchlorate degradation at equimolar concentration of perchlorate and nitrate in the medium. But when the concentration of perchlorate was less by 10, 100 and 1000 folds than nitrate, presence of nitrate decreased the rate of perchlorate degradation (Herman and Frankenberger, 1999). In the absence of nitrate, it took < 24 h to reduce a concentration of perchlorate of 0.089 mg/L to less than detection limit. However in the presence of nitrate (62 mg/L), 36 h was required to achieve the same perchlorate removal (Herman and Frankenberger, 1999).

2.2.5. Biodegradation of Perchlorate in Regenerant Solution and Ion-exchange Resins

Ion exchange is a highly effective process in removing perchlorate from waters especially when the contaminant is present at low concentrations (Xu *et al.*, 2003). However, the regenerant solution from the process contains high concentrations of perchlorate. Perchlorate biodegradation of regenerant solution depends on the nature of the regenerant (Batista *et al.*, 2002). The optimum pH range for the Perchlorate Reducing Bacteria (PRB) is between 5 and 9. When the regenerant used is a caustic solution like ammonium or sodium hydroxide, the pH is usually greater than 11. Hence an acid must be added to neutralize the pH for optimal growth of PRBs (Batista *et al.*, 2002).

Presence of sufficient electron donors is important in the biodegradation process. PRBs tend to take up oxygen and nitrate as electron acceptors over perchlorate. Hence

while providing an electron donor or carbon source (like acetate), it should be provided in excess according to the concentration of nitrate present in the regenerant. Very few microorganisms survive at high saline conditions. The salinity present in the regenerant solution is about 3-6 % salt (Gingras and Batista, 2002). At such high salinity levels perchlorate degradation does not take place efficiently (Okeke *et al.*, 2002). Hence for this process to be feasible, it is required to isolate halo-tolerant PRBs, (which can withstand high salinity) and/or dilute the regenerant solution to lower concentrations (Batista *et al.*, 2002).

Bioregeneration of ion-exchange resin is a new concept which involves the direct contact of spent resin with perchlorate reducing bacteria. This process does not require the use of concentrated brines for regeneration of resins. The bioregeneration technology has been developed recently and is a patented process (Batista and Jensen, 2006). This process consists of a bioreactor containing a perchlorate reducing bacterial culture and a fluidized bed reactor (FBR). The culture is circulated through the FBR containing ion-exchange resin loaded with perchlorate (Batista and Jensen, 2006). The perchlorate reducing culture utilizes perchlorate ions as electron acceptors and degrades it to innocuous chloride, when an electron donor is provided. This technology allows the reuse of ion-exchange resin and also destroys perchlorate making it economically and environmentally sustainable. This process has been carried out successfully in bench-scale experiments (Batista and Jensen, 2006).

C. Wang *et al.* (2008) studied the feasibility and kinetics of the direct bioregeneration of perchlorate laden resin. Batch sorption of resin was performed prior to bioregeneration test with an influent perchlorate concentration of 200- 2000 mg/L

(C.Wang *et al.*, 2008). The spent resins from the batch sorption test were subjected to bioregeneration using perchlorate-reducing cultures. Acetate and sodium chloride were added to the batch-reactors to aid the bioregeneration process (C.Wang *et al.*, 2008). Bioregenerated resins were then subjected to long-term filtration tests to evaluate the capacity of the bioregenerated resins (C.Wang *et al.*, 2008). 100 % breakthrough of perchlorate was seen only after 30,000 bed volumes, indicating successful bioregeneration of the resin.

C.Wang *et al.* (2009) investigated the rate-limiting step for the bioregeneration of ion-exchange resin. They added NaCl and a microbial culture to perchlorate loaded resin. In non-selective resins, NaCl promoted the desorption of perchlorate that was then used up by the bacteria. However, for selective resins, NaCl was able to remove only a small fraction of the sorbed perchlorate. When higher NaCl concentrations were added, perchlorate biodegradation was inhibited by high salinity. C.Wang *et al.* (2009) concluded that since the regeneration process is slow compared to the degradation of perchlorate in waters, desorption of perchlorate ions from resin bead could possibly be the rate-limiting step in the bioregeneration process (C.Wang *et al.*, 2009).

2.3. Bench-scale and Pilot-scale Perchlorate Reduction Processes

Complete perchlorate removal has been achieved in both fixed-bed and fluidized-bed bioreactors (Wallace *et al.*, 1998; Herman and Frankenberger, 1999; Kim and Logan, 2001). Many systems can reduce high concentration of perchlorate to low levels. But systems that can remove low perchlorate concentration in water are of more importance

to the drinking water treatment and bioremediation studies. Different bench-scale and pilot-scale treatment processes are discussed in this section.

2.3.1. Fixed-bed Reactors

Wallace *et al.* in 1998 developed an up-flow anaerobic fixed-bed reactor inoculated with a culture containing *Wolinella succinogens* HAP-1 (Wallace *et al.*, 1998). This reactor was designed to treat high concentration of perchlorate and was packed with diatomaceous earth pellets. The system reduced the perchlorate to < 300 mg/L from an influent concentration of 1500 mg/L with a hydraulic retention time (HRT) of 43 days (Wallace *et al.*, 1998). Sand media bioreactors inoculated with perclace was able to reduce perchlorate to very low levels. Herman and Frankenberger (1999) were able to reduce perchlorate concentration of 130 μg/L to less than 4 μg/L with an HRT of 3 hours in this media (Herman and Frankenberger, 1999). Also a sand-packed reactor having mixed culture was able to reduce perchlorate from 20 mg/L to non-detectable levels (Kim and Logan, 2001).

The result from the sand-packed reactor was compared to a granular-activated carbon (GAC) packed bio-reactor (Xu *et al.*, 2003). GAC packed reactor removed perchlorate completely the first 4 days of operation. But after backwashing on day 6, effluent perchlorate fluctuated between significant amounts. This was attributed to desorption of perchlorate from GAC (Xu *et al.*, 2003). The effect of nitrate on perchlorate removal was examined by Brown *et al.* (2000). The reactor was packed with biologically active carbon (BAC) filters to remove low influent concentrations of perchlorate. Perchlorate (52 μg/L) was removed to below detection level when the nitrate concentration in the influent was 1.4 mg/L. But when the nitrate concentration was increased to 4.5 mg/L,

there was a significant decrease in the perchlorate degradation (Xu *et al.*, 2003). This problem was solved by operating two fixed-bed reactors in series, first one with an HRT of 1 h, while the second one with 10 hours. Complete perchlorate removal was achieved even in the presence of high influent nitrate concentration (Xu *et al.*, 2003).

Pilot-scale fixed bed reactor was operated in Texas for perchlorate removal. The reactor was 5 ft in diameter and 18 ft high, and was made of steel. The reactor was able to remove perchlorate from 20 mg/L to below detection level (Xu *et al.*, 2003). Also two pilot scale bioreactors were operated in California for 7 months. The reactors were 7 ft tall and had two rectangular packings, one of sand and the other of plastic. *Dechlorosoma* sp. KJ was cultured in the reactor, which was able to degrade perchlorate below detection level. Regular backwashing was required for consistent performance throughout the operation (Xu *et al.*, 2003). Microbial treatment processes for perchlorate degradation is summarized in Table 2.6.

2.3.2. Fluidized-Bed Reactors

Successful perchlorate removal is also achieved in fluidized-bed reactor (FBR). Fluidized-bed reactor with sand or GAC packing fed with a mixture of ethanol and methanol removed perchlorate from 25 mg/L to below detection limit (Xu *et al.*, 2003). Three fluidized-bed reactors were tested California for perchlorate removal. First one was in Aerojet facility, Sacramento in the year 1996 (Xu *et al.*, 2003). The reactor was able to reduce perchlorate from 7000-8000 μg/L to < 400 μg/L. In 1998, the second FBR was tested in the San Gabriel Basin. Groundwater was fed to the reactor at the rate of 30 gpm, supplied with ethanol and nutrients. Perchlorate was reduced from 50-100 μg/L to below detection limit. 90% perchlorate removal was achieved when the dissolved oxygen

content was less than 1 mg/L (Xu *et al.*, 2003). Another FBR system was operated in Rancho Cordova, which contained four FBRs 14 ft in diameter. Perchlorate removal from 6-8 mg/L to below detection limit was achieved. Reduced degradation occurred when the system was fed with insufficient amount of ethanol (Xu *et al.*, 2003). California Deparment of Health Service has approved the use of FBRs for treatment of perchlorate-contaminated waters (Xu *et al.*, 2003).

Fluidized-bed reactor (FBR) is a combination of a packed-bed and a stirred tank continuous flow reactors. They are very important in chemical industries as they have excellent mass and heat transfer characteristics. A typical fluidized-bed reactor is shown in Figure 2-4. The substrate to the reactor is fed from the bottom of the reactor with a high velocity so that it carries the media and causes fluidization. Due to the fluidization of the media, excellent mass and heat transfer occurs between the fluid and the solid particles. This reactor can be used for highly exothermal processes as it does not create any hot spots (Werther and Hartge, 2003). However, if fluidization continues for a long duration it may lead to attrition of particle media and erosion of the reactor material.

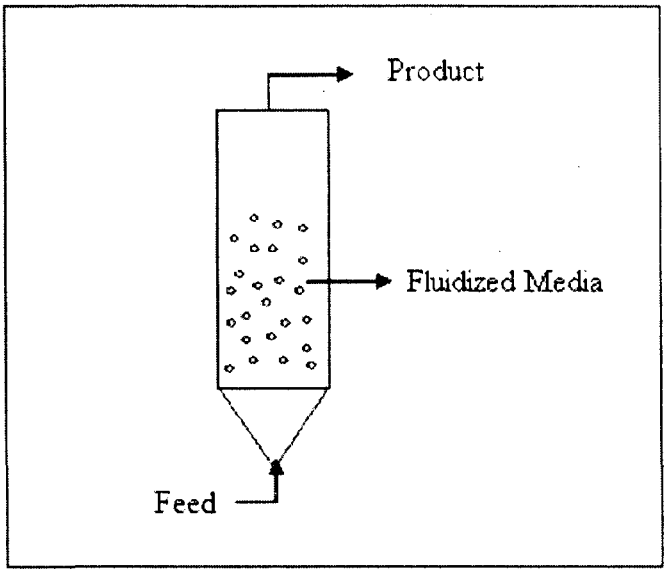


Figure 2.4: Schematic of Fluidized-bed Reactor

Though this reactor type has huge applications in the chemical industries, its use as a bioreactor in treating wastewater is a novel concept which came into being 20 years ago (Shi Hi and Ping, 1994). Fluidized-bed bioreactors (FBBR) are being used at present to remove ammonia and nitrate from wastewaters (S. Soyupak *et al.*, 1990). Sand, expanded clay, activated carbon etc. have been used as particle media in FBBR in the past (Prasanna and Setty, 2008).

Table 2.6: Microbial Treatment Processes for Perchlorate Degradation (Xu *et al.*, 2003)

Reactor Type	Packing Material	Inoculum	Reactor Size	Influent Perchlorate	Effluent Perchlorate	HRT	Degradation Rate
Fixed-bed (Bench-scale)	Diatomaceous earth pellets (20 μm)	*Wolinella succinogens* HAP-1	1.17 m length; 7.6 cm dia	500-1500 mg/L	< 300 mg/L	1.17 h (1500 mg/L) 0.46 h (500 mg/L)	69.7 mg ClO_4^- /min
Fixed-bed (Bench-scale)	Sand (40-70 mesh size)	Perclace	14 cm length; 2.8 cm dia	130 μg/L	< 4 μg/L	3 h	0.7 μg ClO_4^- /min
Fixed-bed (Bench-scale)	Sand	Mixed culture	28 cm length; 2.5 cm dia	20 mg/L	< 4 μg/L	18 – 51 min	0.39 – 1.11 mg ClO_4^- /min
Fixed-bed (Bench-scale)	BAC	Tap water	-	52 μg/L	< 4 μg/L (nitrate 1.4 mg/L)	-	-
Fixed-bed (Pilot/Full-Scale)	Sand/ Plastic	*Dechlorosoma* sp. KJ	7 ft Length; Rectangular beds:2 ft x 1 ft	76 μg/L	< 4 μg/L	-	-
Fluidized-bed (Bench-scale)	Sand/ GAC	Biological solids from anaerobic reactor	-	25 mg/L	< 4 μg/L	-	-
Fluidized-bed (Pilot/Full-Scale)	Activated carbon (10 x 30 mesh)	Sludge from food processing industry	20 in height; 15 ft length	50-100 μg/L	< 4 μg/L	-	-
Fluidized-bed (Pilot/Full-Scale)	GAC	Mixed culture	14 ft diameter	6-8 mg/L	< 4 μg/L	-	-

A typical FBBR is similar to what is shown in Figure 2.2, and wastewater is pumped from the bottom at high velocity. After sufficient fluidization occurs, bio-film starts developing on the inert media to such concentrations, which has been showed to be greater than that present in an activated sludge process (Shieh *et al.*, 1982). Bed expansion is maintained to such levels so that microbial particles are not lost due to excess fluidization. Micro-organisms present in these bio-films make use of the organics in wastewater to remove contaminants (Shi Hi and Ping, 1994). Aerobic or anaerobic environment can be maintained in FBBRs, depending on their applications. Performance of FBBRs was compared to conventional activated sludge process by Shieh *et al.* (1982), which is summarized in Table 2.7. In this research, a FBR will be used to degrade perchlorate/ nitrate loaded ion exchange resin.

Table 2.7: Comparison of activated sludge process with FBBR

(Shieh *et al.*, 1982)

Parameter	Activated Sludge Process	FBBR
Biomass concentration (mg/L)	2000-3000	12000-20000 (BOD removal) 8000-12000 (Nitrification) 30000-40000 (Denitrification)
Loading rate (lbs BOD/ 1000 ft^3 of reactor volume)	30-75	500-1000

2.4. Biodegradation of Substrates Attached to a Media

Perchlorate ions are attached to the functional group of the ion-exchange resin. Hence its degradation in resin bead is similar to biodegradation of compounds that are attached to some solid media in general. Before biodegradation of a substrate can occur,

the substrate must be available as a free solute for its uptake and degradation by microorganisms (Gordon and Millero, 1985). Many studies exist that determined the possible mechanism by which these attached substrates become available for biodegradation (Ogram *et al.*, 1985; Subba-Rao and Alexander, 1984; Gordon and Millero, 1985; Guerin and Boyd, 1992). Ogram *et al.* (1985) assumed three models and found that the model which best fit the experimental data was one that assumed that sorbed substrate (2,4-(dichlorophenoxy) acetic acid) was not degraded. This model also assumed instantaneous desorption of 2,4- (dichlorophenoxy) acetic acid and that both suspended and attached microbes were able to degrade the desorbed substrate (Ogram *et al.*, 1985).

Subba-Rao and Alexander (1984) found that benzylamine biodegradation was independent of desorption from montmorillonite initially. However, at later stages, after most aqueous-phase benzylamine was degraded, it was suggested that biodegradation may be desorption limited and that sorption prevented complete biodegradation (Subba-Rao and Alexander, 1984). These studies suggested reduced degradation rate or no degradation of substance occurred when it was attached to a media.

Experiments conducted by Gordon and Millero (1985), showed that low molecular weight organic acids and sugars adsorbed on hydroxypatite were still available for biodegradation. They also concluded that adsorbed substrate was less available to microbes when compared to free suspended substrates, and hence reduced rate was observed (Gordon and Millero, 1985). They suggested that desorption of attached substrate may have been facilitated by the microorganisms.

Guerin and Boyd (1992) suggested that the type of microorganism was important in determining whether the sorbed naphthalene was bio-available or not. In their experiment, one culture showed initial naphthalene degradation was proportional to the liquid phase naphthalene concentration, indicating the rate was determined by the dissolved substrate (Guerin and Boyd, 1992). However, another type of microorganism was able to degrade both sorbed and free naphthalene. In conclusion, they suggested that organism-specific process may enhance substrate desorption resulting in higher rates of degradation (Guerin and Boyd, 1992).

Based on these studies, the mechanism for the biodegradation of perchlorate in ion-exchange resin, presented in Chapter 1, was hypothesized in this research. From suggestions made by Gordon and Millero (1985); Guerin and Boyd (1992), it was hypothesized that a substance released by PRB is responsible for desorption of perchlorate ions form the resin bead. The desorbed perchlorate ions are then reduced to innocuous chloride by the known mechanism of perchlorate degradation in waters.

CHAPTER 3

MATERIALS AND METHODS

3.1. Culture Enrichment

Perchlorate-degrading culture for the experiments was taken from two seed enrichment cultures, BALI 1 and BALI 2 grown in the UNLV Environmental Engineering laboratory. These two seed cultures were enriched from samples taken from Lake Mead and the Las Vegas Wash, and was kept alive by feeding and wasting the culture weekly. Five liters of the seed culture were taken and grown in a separate 20 gallon master bioreactor (Figure 3.1). This bioreactor is equipped with monitoring probes for Dissolved Oxygen (DO), Oxidation Reduction Potential (ORP) and pH (Figure 3.1). The bioreactor was sealed completely to ensure anaerobic environment for the culture. The reactor was also purged with nitrogen gas to remove any oxygen present in the head space. The culture was mixed by a stirrer to keep the bacteria in suspension.

The microbial culture was grown progressively to a volume of twenty liters by feeding it with perchlorate, acetate and nutrients. The nutrient mixture used is shown in Table 3.1. Acetate was used as the carbon source and electron donor for the bacterial culture. An acetate-to-perchlorate mass ratio of 3:1 was maintained in the bioreactor for sufficient supply of electrons. The pH in the culture was maintained between 7 and 8 using phosphate buffer, which is the optimum range for PRB growth (Table 3.1). The culture was monitored daily by measuring DO, ORP, pH, Optical Density (OD_{600}),

Turbidity, Conductivity, Suspended solids and COD (as a measure of acetate content). Table 3.1 summarizes the nutrients and their concentration used to prepare the microbial media, electron donor and buffer stock. Table 3.2 and Figures 3.2 and 3.3 show the variation of monitored parameters in the 20 gallon bioreactor for a ten-day period.

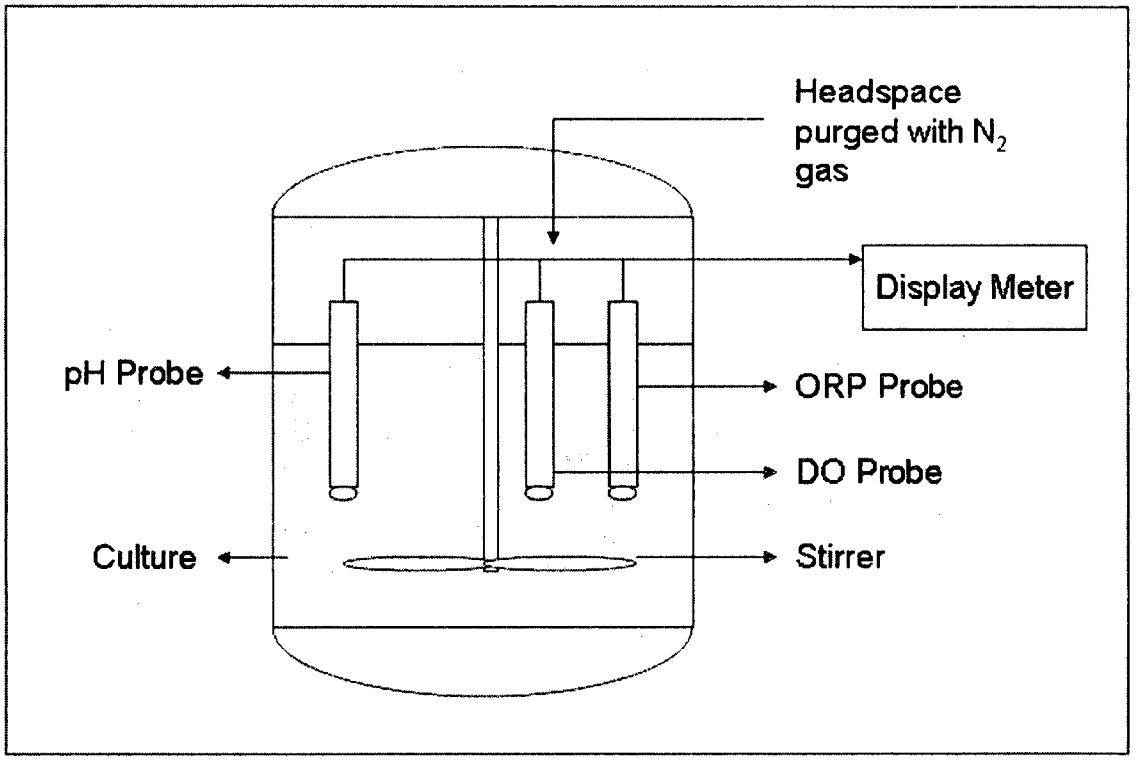


Figure 3.1: Twenty Gallon Master Bioreactor

Table 3.1: Stock solution for culture enrichment and feeding

Solution Name	Components	Concentration of stock (g/L)
Electron donor/ carbon source	CH_3COO^- (Sodium form)	120
Buffer	K_2HPO_4	155
	$NaH_2PO_4.H_2O$	97.783
	$NH_4H_2PO_4$	50
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

Table 3.2: Variation of Parameters in 20 Gallon Bioreactor

Date	Day	COD (mg/L)	Suspended Solids (mg/L)	% OD	Turbidity (NTU)	pH	Conductivity (mS)	ORP	DO
10-Aug	1	4950	2891	1.2	1576	7.70	22.6	-456	0
11-Aug	2	4590	2243	2.5	1326	7.35	22.5	-460	0
12-Aug	3	3885	1866	2.5	1189	7.67	22.48	-469	0
13-Aug	4	3750	1489	3.4	952	7.73	22.7	-470	0
14-Aug	5	3640	1320	4	870	7.81	22.7	-490	0.1
15-Aug	6	3460	1573	3.2	964	7.68	22.41	-480	0
16-Aug	7	5052	1308	4.1	861	7.71	22.46	-484	0
17-Aug	8	4560	2221	1.1	1362	7.47	22.69	-467	0.1
18-Aug	9	4116	2085	2.4	1257	7.62	22.92	-471	0.1
19-Aug	10	3732	1969	2.7	1168	7.66	22.97	-476	0
20-Aug	11	3660	1760	3.1	1108	7.91	23.73	-488	0

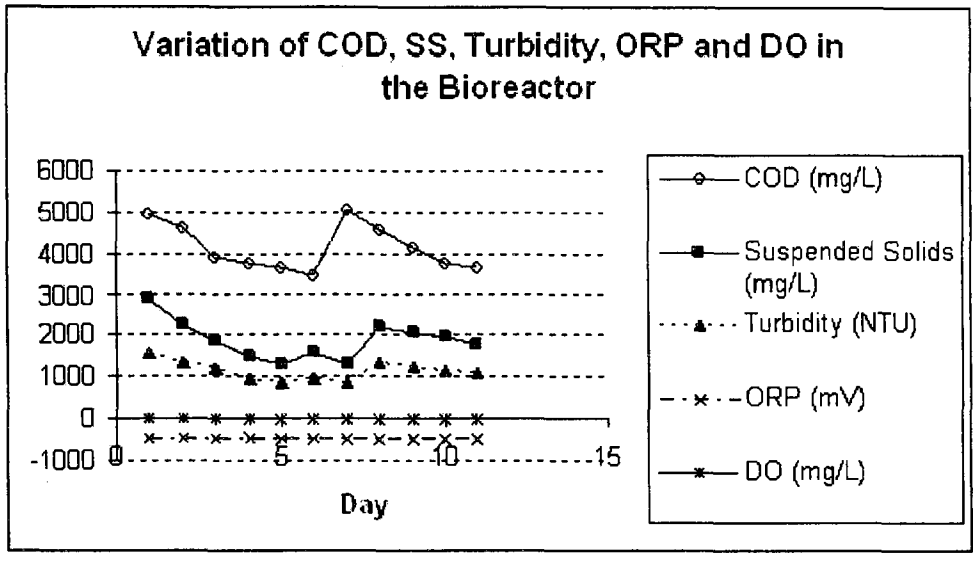


Figure 3.2: Variation of COD, SS, Turbidity, ORP and DO in Bioreactor

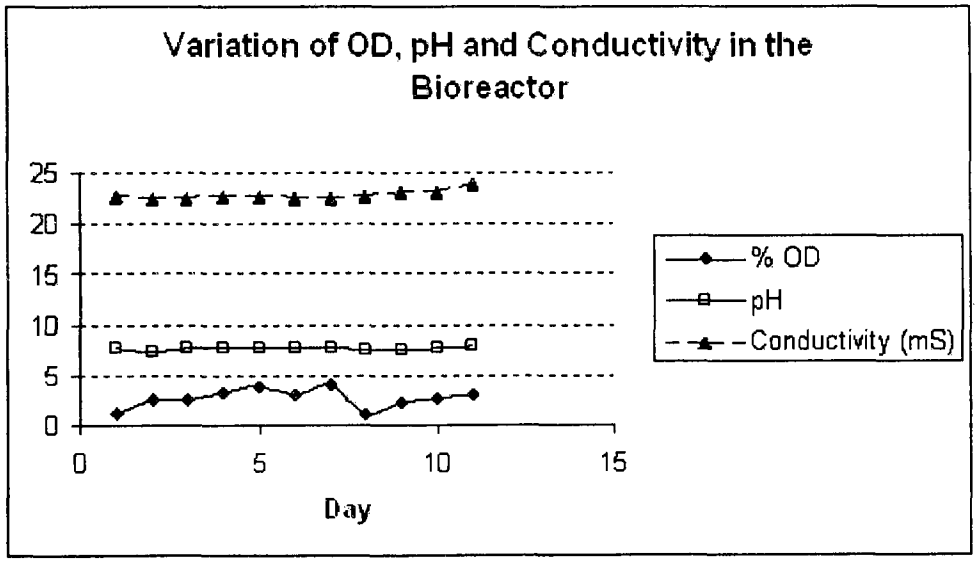


Figure 3.3: Variation of OD, pH and Conductivity in the Bioreactor

To assure that the culture was active, samples were taken on a daily basis for residual perchlorate evaluation. Two ml of samples were taken and centrifuged for 15-20 minutes at a speed of 5000 rpm using a TOMY MC-150 high speed micro-centrifuge (Fremont,

CA). The supernatant was used for measuring perchlorate using Dionex ICS 2000 ion chromatography (Dionex, Sunnyvale, CA). A small amount of culture was wasted on a regular basis to maintain the conductivity below 25 mS and also to maintain the solids retention time (SRT) of the microbes. High conductivity value indicates high chloride concentration in the culture, which is not desirable as it will lead to the net movement of water outwards from within bacterial cells due to osmotic effect, resulting in the death of PRBs.

Before using the culture for biodegradation tests, it was made sure that perchlorate was zero and sufficient suspended solids were present. The residual perchlorate was measured using Ion Chromatography (IC) technique. IC was performed using Dionex ICS-2000 fitted with an AS40 automated sampler. IonPac AS16 (4 X 250 mm) column was used for the analysis. Detailed procedure is displayed in Appendix A.

3.2. Multi-Cycle Bioregeneration of Gel-type Anion Exchange Resin

3.2.1. Resin Loading

SIR-110-HP (Resin Tech, West Berlin, NJ) gel-type anion exchange resin was used in the bench-scale experiment. Some characteristics of the resin are shown in Table 3.3. Five hundred ml of the resin was used for the first cycle. The resin was loaded with perchlorate, nitrate, sulfate, chloride and bicarbonate. Concentrations of ions loaded in the ion-exchange resin are shown in Table 3.4.

Table 3.3: Characteristics of SIR-110-HP

Characteristics	Details
Polymer Structure	Styrene with DVB
Functional Group	Tri-n-butyl amine $(C_4H_9)_3N$
pH Range	0 - 14
Ionic Form	Chloride
Water Retention	35 – 55 %
Solubility	Insoluble
Swelling	~ 12 %
Temperature Range	35 – 104 ^{0}F
Total Capacity	0.6 meq/mL

Table 3.4: Concentration of Anions for Resin Loading

Component	Concentration before loading (mg/L)	Concentration after loading (mg/L)	Resin load g/L_{resin}	% Capacity occupied in the ion-exchange resin
Perchlorate	31910	2.46	31.91	53.45
Nitrate	605.6	2.65	0.602	1.62
Sulfate	651.7	304	0.348	1.21
Chloride	663.55	5374	-	-
Bicarbonate	500	683	-	-

One liter of resin was mixed with one liter of stock solutions of desired concentration of perchlorate, nitrate, sulfate, chloride and bicarbonate ions (Table 3.4) in a three liter jar using a rotary mixer (Associate Design & Mfg. Co., Alexandria, VA). The contents were mixed for 16 hours to ensure complete loading of the ions to the ion-exchange resin. Concentrations of anions before and after the loading process were measured using Dionex ICS 2000 IC (Dionex, Sunnyvale, CA). The perchlorate content in the ion-exchange resin was measured using oxygen combustion bomb (Parr Instruments, Moline,

IL). Detailed procedure for the determination of perchlorate in resin samples using oxygen bomb is given in Appendix B.

After 16 hours of mixing, the solution was decanted and the resin was washed ten times with 2 liters deionized water to remove residual ions. The loaded resin was transferred to a labeled glass container and stored in refrigerator until the start of the experiment.

3.2.2. FBR Resin Bioregeneration

Three cycles of bioregeneration were performed and each cycle was run for a period of 21-25 days. Experimental set up for the bioregeneration experiment is showed in Figure 3.4 (a) and (b). A Fluidized bed reactor (FBR), 30 inches in length and 2 inches diameter plexi-glass column was used for the bioregeneration test. A self priming pump was used to circulate the perchlorate-reducing culture from the 20 gallon master reactor through the FBR. The loaded resin was then transferred into FBR and was connected to the 20 gallon master reactor through a self priming pump. The culture was then pumped through the FBR at a flowrate of 210 ml/ min (~2.5 gpm/ ft^2 of column). Five ml resin samples were taken daily via valves placed on the side of FBR. The resin samples were rinsed six times thoroughly with 50 ml deionized water and stored in refrigerator for further analysis.

Fifty ml culture sample from the 20 gallon master reactor was taken daily to monitor COD, %OD, turbidity and suspended solids. pH, ORP and DO were monitored directly from the probes placed inside the bioreactor. Throughout the bioregeneration process, the suspended solids was maintained around 1500- 2000 mg/L. Whenever the suspended solids dropped below 1500 mg/L, concentrated centrifuged culture was added to the

reactor to increase suspended solids. The concentrated culture contained significant amounts of acetate and hence increased the acetate concentration as well in the bioreactor. Cycle 1 of the column test was run for 21 consecutive days. After cycle is complete, the bioregenerated resin was subjected to bio-fouling removal and disinfection. Bio-fouling removal procedure is explained in section 3.2.4.

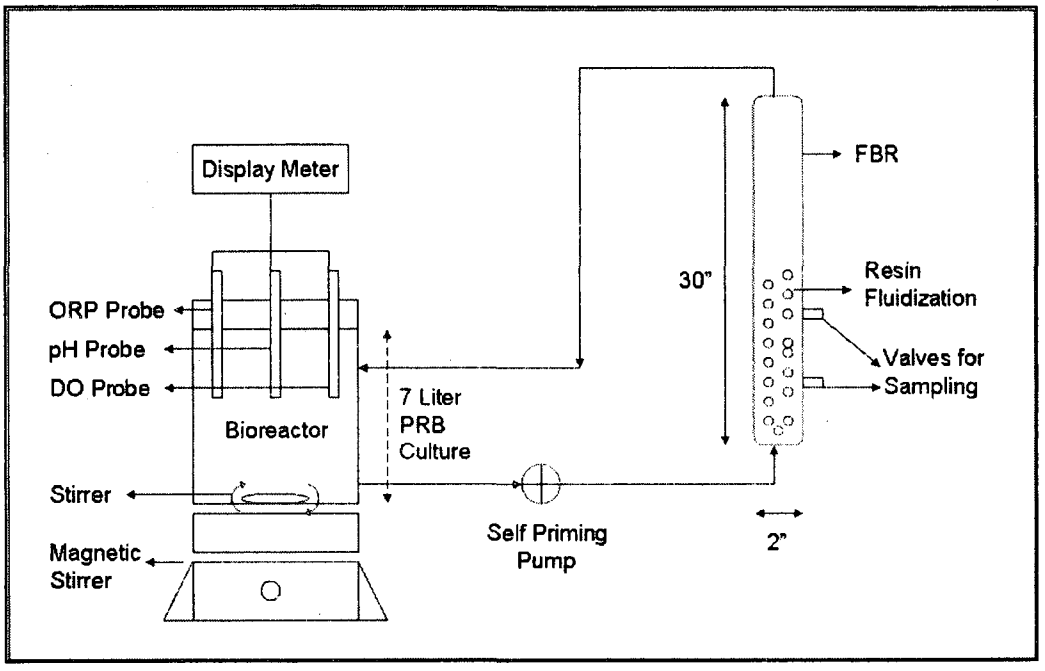


Figure 3.4 (a): Experimental Set-up for Bioregeneration of Gel-type Resin

The bioregenerated resin from cycle 1 was again loaded with the same amount of perchlorate, nitrate, sulfate, chloride and bicarbonate for cycle 2. Notice that the amount of resin to be regenerated in subsequent cycles was smaller than that used in cycle 1 because resin samples were taken from each cycle. A resin sample of 360 ml and 250 ml were loaded for cycles 2 and 3, respectively. For cycle 2 and cycle 3 bioregeneration test, a seven liter glass bioreactor was used instead of the 20 gallon master reactor for

perchlorate-reducing culture enrichment. This reactor was placed on a magnetic stirrer and was sealed properly to maintain anaerobic conditions.

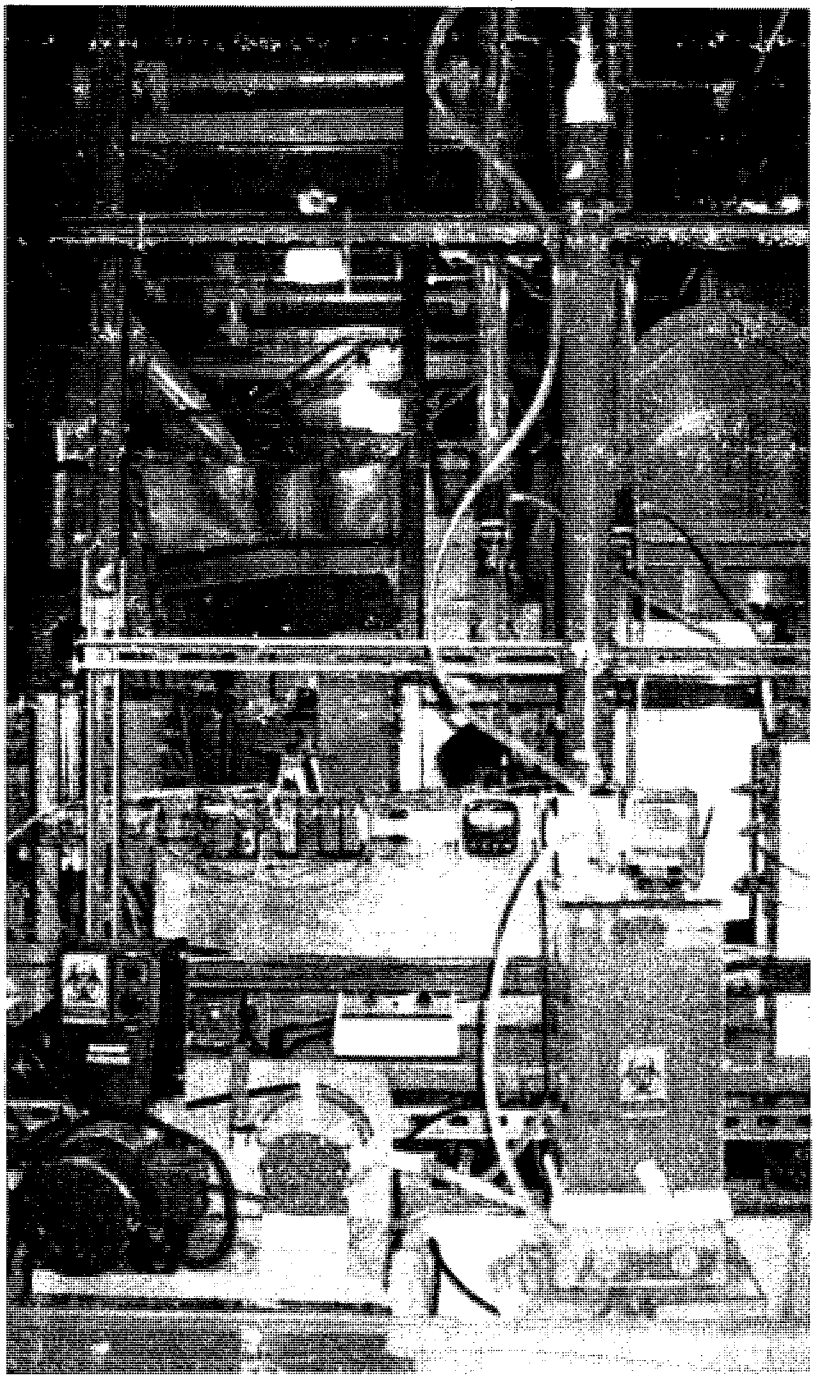


Figure 3.4 (b): Experimental Set-up for Bioregeneration of Gel-type Resin

3.2.3. Perchlorate Analysis in Resin

Residual perchlorate in the resin sample was analyzed using an oxygen combustion bomb 1108 (Parr Instruments, Moline, IL). This procedure was developed at the UNLV Environmental Engineering laboratory. Currently there are no published or standardized methods to measure perchlorate attached to ion-exchange resins. In this process, the resin samples are burnt inside the combustion bomb, thus converting the residual perchlorate ions to chloride ions.

$$ClO_4^- \longrightarrow 2O_2 + Cl^-$$

Both chloride and perchlorate are available in the resin sample during bioregeneration. It is therefore important to eliminate the presence of chloride in the sample before it is subjected to the parr bomb. A pre-treatment strategy was developed at UNLV, where the resin sample is first contacted with an excessive amount of nitrate (10,000 mg/L). The nitrate ions replace any chloride present in the resin. After treatment with nitrate, the resin samples are rinsed six times thoroughly with 500 ml of deionized water to remove any chloride present. The pre-treated resin sample is then subjected to parr bomb. Any chloride present in the parr bomb buffer is provenient from the reduction of perchlorate to chloride.

Two ml of resin sample was mixed with 250 ml of 10,000 mg/L nitrate solution for 15 hours in an Erlenmeyer flask placed on a Cole-Palmer orbital shaker (Vernon Hills, IL). The treated resin was then rinsed six times with 500 ml deionized water and dried at 105^0C for one hour to remove moisture content. About 100 mg of the dried resin was weighed in the parr bomb crucible for perchlorate analysis. Four hundred mg of paraffin oil was used as a combustion aid. The crucible was placed inside the parr bomb and

burnt in the presence of oxygen and parr bomb buffer solution (10 ml of 35 mM NaOH and 3 ml of 3% H_2O_2). Detailed procedure for the oxygen combustion bomb is given in section 3.7.2.

After 20 minutes, the bomb calorimeter was opened and the residual liquid present in bottom cylinder was diluted to 500 ml in a volumetric flask. The collected solution which contains chloride resulting from perchlorate reduction was then analyzed for chloride using ICS 2000 Ion Chromatography (Dionex, Sunnyvale, CA). QA/QC protocol was used in this research and included analysis of replicate samples, equipment calibration, and proper procedures for sample storage.

3.2.4. Bio-fouling Removal

One of the major concerns about bioregeneration of ion-exchange resins is bio-fouling. The UNLV Environmental Engineering Laboratory has studied resin bio-fouling and has developed a procedure to remove bio-fouling from ion-exchange resins (Batista *et al.*, 2007). In this study, bio-fouling of the resin was observed after the bioregeneration process. The resins became darker in color due to the build-up of organic matter on the resin. Bio-fouling was removed in three steps. Firstly, two bed volumes containing 1:1 mass ratio of 12% NaCl and 2% NaOH solution was circulated through FBR for 15 hours. Next a fresh solution of the same composition (two bed volumes) was again pumped through the FBR for 3 hours. Finally, 2 bed volumes of 12% NaCl was pumped through the FBR for 2 hours.

The resin was rinsed thoroughly with ten bed volumes of deionized water. Complete rinsing of resin was confirmed by measuring the conductivity of effluent, until it equaled that of the deionized water. Finally the resin was disinfected using sodium hypochlorite

solution (1% as chlorine) with a contact time of 15 – 20 minutes. The resin was again rinsed with fifteen bed volumes of deionized water and stored in refrigerator until the start of next bioregeneration cycle.

3.3. Investigation of Mechanism of the Bioregeneration Process for a Gel-type Anion Exchange Resin

3.3.1. Resin Loading

SIR-110-HP resin was loaded with various concentrations of perchlorate and nitrate for the batch experiments. To evaluate the effect of initial perchlorate load on the degradation rate, the resin was loaded with three different concentrations of perchlorate (3 g/L_{resin}, 5 g/ L_{resin}, and 20 g/ L_{resin}). To evaluate the effect of nitrate on perchlorate degradation, resin was loaded with two combination of nitrate and perchlorate (20g/ L_{resin} NO_3^- and 3 g/ L_{resin} ClO_4^-; 20g/ L_{resin} NO_3^- and 20 g/ L_{resin} ClO_4^-).

The amount of resin and stock solution added for resin loading is summarized in Table 3.5 for the three batch experiments. The initial and final concentration of perchlorate and nitrate before and after loading process was measured using Dionex ICS 2000 ion chromatography (Sunnyvale, CA). Measured volume of resin to be loaded was transferred to a 100 ml or 3 liter bottle and was mixed thoroughly with respective concentration of perchlorate and nitrate (as shown in Table 3.5) for 16 hours in a rotary mixer at 25 rpm.

After 16 hours of mixing, the solution was decanted and the resin was washed ten times with 100 ml deionized water to remove residual ions. The loaded resin was

transferred to a labeled glass container and stored in refrigerator until the start of the experiment.

Table 3.5: Resin Loading for Batch Biodegradation Experiments

Total Amount of resin (ml)	Perchlorate Load (g/ L_{resin})	Nitrate Load (g/ L_{resin})	mg of perchlorate needed for loading	Expected Concentration in 100/ 300 ml (mg/ L)	Measured Concentration before Loading (mg/L)	Measured Concentration after Loading (mg/L)
150	3	-	450	1500_{300}	1947	0.2
30	5	-	150	1500_{100}	2069	0.0
150	20	-	3000	10000_{300}	11179	0.54
20	3	20	60	600_{100}	665	0.12
20	20	20	400	4000_{100}	4518	1.25

3.3.2. Effect of Varying Initial Ion Exchange Perchlorate Load on Perchlorate Degradation

Twenty eight batch-bioreactor tubes of capacity 25 ml each were used in the batch experiment. The tubes were divided into three sets each with different perchlorate load in resin: 3 g/ L_{resin}, 5 g/ L_{resin}, and 20 g/ L_{resin}. Each of these reactor tubes contained 2 ml of resin loaded with the respective perchlorate amount, perchlorate-reducing culture with suspended solids of 1000 mg/L, acetate added in the mass ratio of 3:1 acetate-to-perchlorate, buffer and nutrients. Experimental set up of the batch experiment is summarized in Table 3.6 and Figure 3.5 to 3.7.

Table 3.6: Experimental Condition for Evaluating the Effect of Varying Initial Perchlorate Load on the Degradation Rate

Perchlorate Load (g/L_{resin})	Number of tubes	Amount of Resin in each tubes (ml)	Amount of Perchlorate Present (mg)	Amount of Acetate Required (mg)	Conc. of Acetate in 25 ml (mg/L)
3	10	2	6	18	720
5	8	2	10	30	1200
20	10	2	40	120	4800

The culture for the biodegradation experiment was obtained from BALI 1 and BALI 2 reactors. Culture samples were centrifuged and the supernatant was tested for zero perchlorate by using Dionex ICS 2000 (Sunnyvale, CA). Once zero perchlorate was confirmed in the culture, about one liter of culture was centrifuged using Legend RT Sorvall centrifuge (Kendro, Thermo Fisher Scientific, Inc., Waltham, MA). The supernatant was discarded and the concentrated culture was rinsed twice with phosphate buffer solution and centrifuged again. This procedure was followed to remove residual acetate present in the culture.

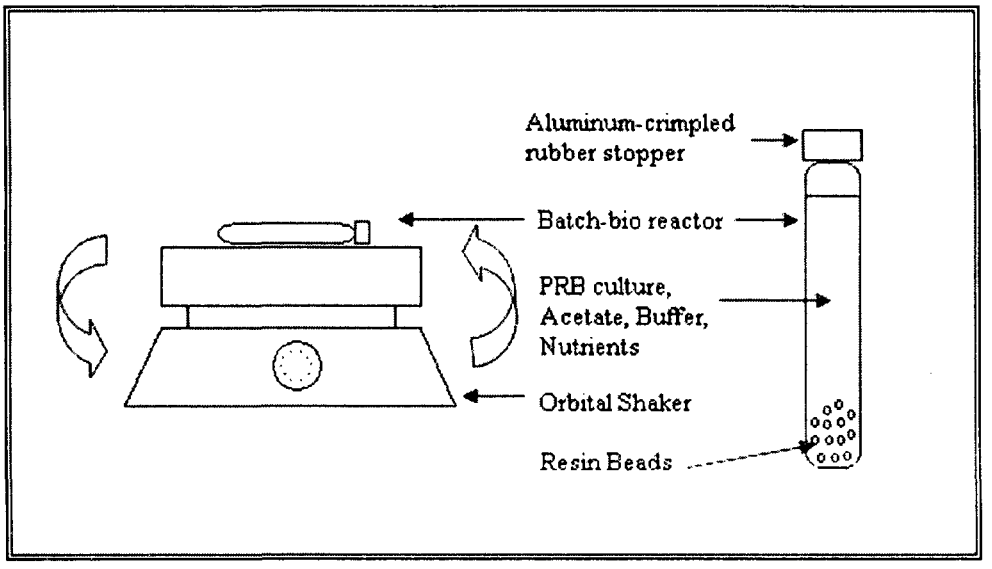


Figure 3.5: Batch Experiment Set-up

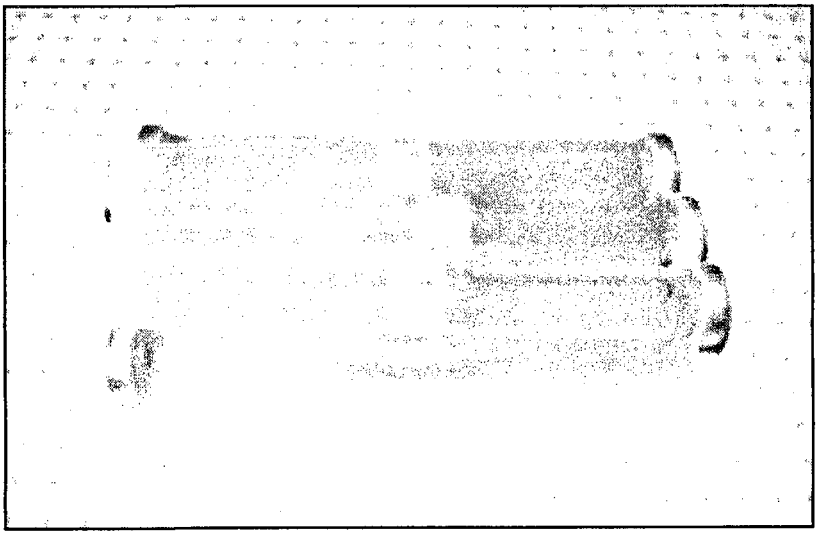


Figure 3.6: Reactor Tubes with Culture and Resins

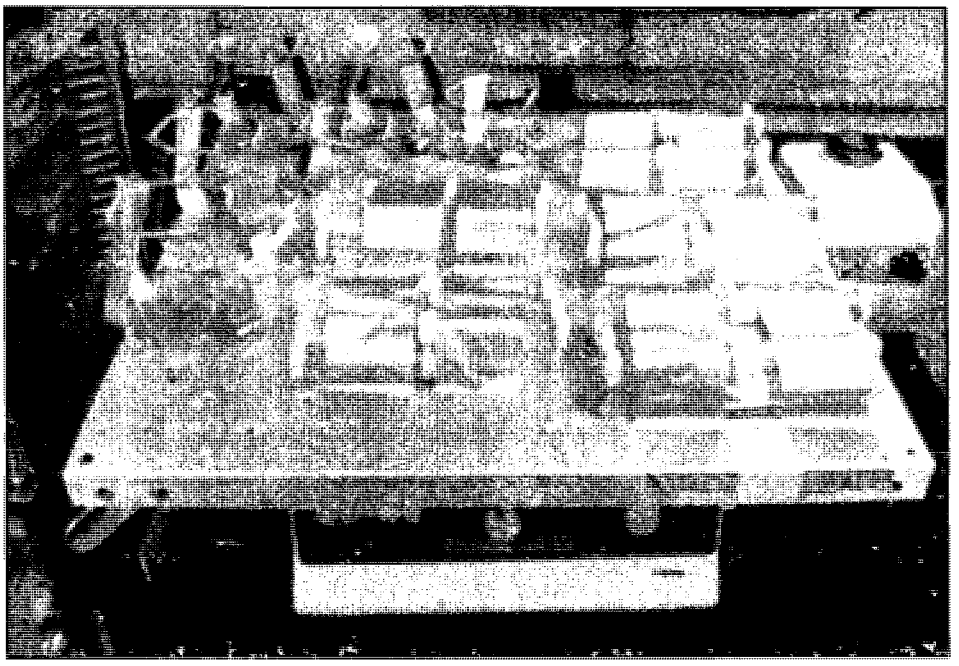


Figure 3.7: Reactor Tubes Taped to Orbital Shaker

The culture was diluted to 1000 mg/L in 25 ml batch-bioreactors by adding acetate, buffer and nutrients. The acetate, nutrients and buffer solutions were purged with nitrogen gas for approximately 20 minutes to decrease the dissolved oxygen content and maintain anaerobic condition in the tubes. Once all the components were added to the bioreactor tubes, they were sealed using aluminum-crimpled butyl rubber stopper (Wheaton Industries, Inc., Millville, NJ) and labeled with respective perchlorate load. The bioreactor tubes were then taped to a Cole-Palmer orbital shaker (Vernon Hills, IL), 51704 series, to maintain the microbes in suspension. The biodegradation experiment was carried out for ten consecutive days and one tube was removed every alternate day to determine perchlorate degradation (Day 2, 4, 6, 8 and 10). Duplicate tubes were provided for day 2, 6 and 10 for the 5 g/L_{resin} reactor set, while duplicates were provided for all the samples in 3 and 20 g/L_{resin} batch-reactor sets.

The culture samples were analyzed for turbidity, pH, OD, COD and suspended solids. Finally, the resin samples were rinsed six times with 25 ml deionized water and stored in refrigerator for further analysis.

3.3.4. Effect of Microbial Concentration on Perchlorate Degradation in Ion Exchange Resin

Biodegradation of perchlorate was tested with three different microbial concentrations (suspended solids): 500 mg/L, 1000 mg/L and 2000 mg/L. Ten glass batch bioreactor tubes were set for each suspended solids concentration, which includes both sample and duplicate tubes. Two perchlorate loads, 3 g/L_{resin} and 20 g/L_{resin} were tested with different suspended solids concentration for bioregeneration. Each of these tubes contained 2 ml of resin loaded with the respective perchlorate amount, perchlorate-reducing culture, acetate added in the mass ratio of 3:1 acetate-to-perchlorate, buffer and nutrients. Experimental set up of the batch experiment is summarized in Table 3.7.

The culture from the 20 gallon master reactor was concentrated using Sorvall Legend RT centrifuge (Kendro, Thermo Fisher Scientific, Inc., Waltham, MA) to obtain the desired high concentration of 2000 mg/L. Dilution of this concentrated culture was performed to obtain the desired lower microbial concentration of 500 mg/L and 1000 mg/L in the 25 ml batch bio-reactor.

Table 3.7: Experimental Condition for Evaluating the Effect of Microbial Concentration on Perchlorate Degradation Rate

Perchlorate Load (g/L_{resin})	Number of tubes	Amount of Resin in each tubes (ml)	Conc. Of Perchlorate in 25 ml (mg/L)	Conc of Acetate in 25 ml (mg/L)	Suspended Solids (mg/L)
3	10	2	240	720	500
20	10	2	1600	4800	500
3	10	2	240	720	1000
20	10	2	1600	4800	1000
3	10	2	240	720	2000
20	10	2	1600	4800	2000

The culture for the biodegradation experiment was obtained from BALI 1 and BALI 2 reactors. Culture samples were centrifuged and the supernatant was tested for zero perchlorate by using Dionex ICS 2000 (Dionex Corporation, Sunnyvale, CA). The culture was then rinsed twice with phosphate buffer to reduce the acetate content. Concentrated culture was diluted to 500, 1000 and 2000 mg/L in 25 ml reactor tubes by adding acetate, buffer and nutrients. The acetate, nutrients and buffer solutions were purged with nitrogen gas for 20 minutes to decrease the dissolved oxygen content and maintain anaerobic condition in the tubes.

Once all the components were added to the reactor tubes, they were sealed using aluminum-crimpled butyl rubber stoppers (Wheaton Industries, Inc., Millville, NJ) and labeled with respective perchlorate load and suspended solids concentration. The bioreactor tubes were then taped to a Cole-Palmer orbital shaker (Vernon Hills, IL),

51704 series, operated at a speed of 250 RPM to keep the microbes in suspension. The biodegradation experiment was carried out for ten consecutive days and one tube was removed every alternate day to determine perchlorate degradation (Day 2, 4, 6, 8 and 10). The tubes were analyzed for turbidity, pH, OD, COD and suspended solids. The resin samples were rinsed six times with 25 ml deionized water and stored in refrigerator for further analysis.

3.3.5. Effect of Nitrate Load on Perchlorate Degradation in Ion-exchange Resin

The resin was loaded with two combinations of nitrate and perchlorate: 20g/ L_{resin} NO_3^- and 3 g/ L_{resin} ClO_4^-; 20g/ L_{resin} NO_3^- and 20 g/ L_{resin} ClO_4^-. Table 3.8 shows the concentration of acetate added to the reactor tubes. Table 3.9 shows the total capacity occupied by nitrate and perchlorate in the resin.

Table 3.8: Experimental Condition for Evaluating the Effect of Nitrate Load on Perchlorate Degradation Rate

Amount of Nitrate Present in 2 ml resin (mg)	Amount of Perchlorate Present in 2 ml resin (mg)	Amount of acetate required for nitrate (mg)	Amount of acetate required for perchlorate (mg)	Total amount of acetate required (mg)	Concentration of acetate in 25 ml (mg/L)
40	6	37.6	18	55.6	2224
40	40	37.6	120	157.6	6304

Table 3.9: Percentage Capacity of Resin Occupied by Perchlorate and Nitrate

Nitrate Load (g/L_{resin})	Perchlorate Load (g/L_{resin})	% Capacity occupied by Nitrate	% Capacity occupied by Perchlorate
20	3	53.76	5.025
20	20	53.76	33.5

The culture for the biodegradation experiment was obtained from BALI 1 and BALI 2 reactors. Culture samples were centrifuged and the supernatant was tested for zero perchlorate by using Dionex ICS 2000 (Dionex Corporation, Sunnyvale, CA). The culture was then rinsed twice with phosphate buffer to reduce the acetate content. The culture was diluted to 1000 mg/L in 25 ml reactor tubes by adding acetate, buffer and nutrients. The acetate, nutrients and buffer solutions were purged with nitrogen gas for 20 minutes to decrease the dissolved oxygen content and maintain anaerobic condition in the tubes.

Once all the components were added to the reactor tubes, they were sealed using aluminum-crimpled butyl rubber stopper (Wheaton Industries, Inc., Millville, NJ) and labeled with respective perchlorate and nitrate loading. The bioreactor tubes were then taped to a Cole-Palmer orbital shaker (Vernon Hills, IL), 51704 series, to keep the microbes in suspension. The biodegradation experiment was carried out for 10 consecutive days and one tube was removed every alternate day to determine perchlorate degradation (Day 2, 4, 6, 8 and 10). Duplicate tubes were provided for day 2, 6 and 10 samples.

The culture samples were analyzed for turbidity, pH, OD, COD and suspended solids. The resin samples were rinsed six times with 25 ml deionized water and stored in refrigerator for further analysis.

3.4. Analytical Methods

3.4.1. Analysis of Perchlorate, Nitrate, Sulfate and Chloride

Concentration of perchlorate, nitrate, sulfate and chloride was determined using Dionex ICS 2000 (Sunnyvale, CA). IonPac AS16 column with 4 X 250 mm dimension was used for the analysis. Chromleon 6.70 (SP2a Build 1871) software was used to run the IC. Dionex RFIC EluGen Cartridge containing sodium hydroxide was used as the eluent. The eluent concentration for the analysis was set to 35 mM and the current was set to 100 mA for perchlorate and low-range chloride analysis, while 30 milli-moles concentration and 110 mA current was used for nitrate, sulfate and high-range chloride analysis.

The instrument was first calibrated with standard perchlorate solution prepared from anhydrous sodium perchlorate salt (EMD Chemicals, Capitol Scienctific Inc., Austin, TX) for perchlorate analysis. Concentration of 5, 10, 25, 50, 75 and 100 ppb were prepared from the standard and a calibration curve was established in the chromleon software. Five ml samples were prepared from the centrifuged culture with appropriate dilution so that the concentration of perchlorate measured falls into the calibrated range (5- 100 ppb). The sample injection volume was 1000 µL. Detailed procedure is described in Appendix A.

3.4.2. Oxygen Combustion Bomb

To measure the amount of perchlorate left in the ion exchange resin oxygen combustion bomb 1108 (Parr Instruments, Moline, IL) was used (Figure 3.8). Two ml of resin sample was first treated with 250 ml of 10,000 mg/L nitrate solution for 15 hours in a 500 ml erlenmeyer flask. This was done to replace all un-exchanged chloride ions present in the resin. The treated resin was then rinsed six times with 500 ml deionized water to remove excess nitrate. The resin was then air dried for 30 minutes and transferred to pre-weighed aluminum dishes (Thermo Fisher Scientific, Waltham, MA). The aluminum dishes were then placed in a gravity oven (Thermo Fisher Scientific, Waltham, MA) maintained at a temperature of 105^0C to remove moisture from the resin sample. The difference in weight before and after drying gives the moisture content of resin.

About 100 mg of the dried resin sample was weighed in the combustion bomb crucible. Four hundred mg of paraffin oil (EMD Chemicals, Inc., San Diego, CA) was added to the resin sample as a combustion aid. The crucible was then placed in the cradle (loop electrode) attached to the parr bomb head. Ten cm of nickel-chromium fuse wire was connected to the two electrodes present in the parr bomb head. Ten ml of 35 mM sodium hydroxide (NaOH) and 3 ml of 3% hydrogen peroxide (H_2O_2) was added to the bottom cylinder of parr bomb as buffer solution. Sodium hydroxide was used in the parr bomb to match the eluent (NaOH) that was used in IC.

The bomb head was fitted into the bottom cylinder and twisted thoroughly to make it air tight. The vent opening on the bomb head was then closed with a screw. Oxygen gas was then filled in the cylinder to a pressure of 30 -35 psi. The whole calorimeter (oxygen

bomb) was then submerged in a bucket of deionized water. The electrodes were then connected to the igniter and the bomb was fired.

After 20 minutes, the bomb calorimeter was opened and the residual liquid present in bottom cylinder was diluted to 500 ml with deionized water in a volumetric flask. This final solution was then used to measure chloride concentration in ICS 2000 ion chromatography (Dionex, Sunnyvale, CA). QA/QC for every three samples was performed to ensure accurate and precise results. Ultimate coal standard sample (Alpha Resources Inc, Stevensville, MI), with 0.13% chloride content, was used for quality assurance.

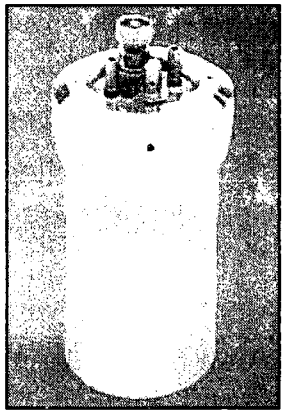


Figure 3.8: Oxygen Combustion Bomb 1108

3.4.3. Suspended Solids

The suspended solids were measured using a filtration apparatus with a 47 mm Whatmann glass microfiber filters (GFC). A vacuum pump was attached to the filtration apparatus to drain the water through the filter paper. Known volume of the culture was pipetted into the filtration apparatus. The amount of solids trapped in the filter paper was weighed and evaluated as suspended solids after drying at 105^0C for one hour.

3.4.4. Chemical Oxygen Demand (COD)

COD analysis was performed using high range, low range and ultra low range HACH COD digestion vials (Hach Company, Loveland, CO) using the diluted supernatant of the culture obtained after centrifuge. Dilution was performed to make sure the COD in the sample was within range. The COD vial was then heated at 150 ^{0}C for 2 hours in an HACH DRB200 Dry Thermostat Reactor. After heating, the COD was measured using HACH DR/3000 spectrophotometer at the desired wavelength.

3.4.5. pH

The pH of the microbial culture was measured using a Fisher Scientific model AR25 pH meter. The pH meter was calibrated daily using standard pH buffers (4.01, 7.0 and 10). Samples were taken daily from the bioreactor and was immediately analyzed.

3.4.6. Turbidity and Optical Density

Turbidity of the culture samples were measured using a Hach turbidimeter (Model 2100 N). Grab samples from the bioreactor was immediately analyzed with optical density at 600 nm wavelength using Hach DR/3000 Spectrophotometer.

3.4.7. Dissolved Oxygen

The dissolved oxygen content of the culture was analyzed daily using YSI Model 58 Dissolved Oxygen meter (YSI, Inc., Warm Springs, OH).

3.4.8. Conductivity

Grab samples from the bioreactor were immediately analyzed for conductivity using YSI (Model # 30/10 FT) conductivity meter (YSI, Inc., Warm Springs, OH).

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

QA/QC for all measurements and sample preparation was carried out to ensure accuracy and precision in the collected data. This minimizes errors conducted by personnel and other over looked apparatus and equipments.

Residual perchlorate, COD, pH, Turbidity and suspended solids were important parameters that were analyzed throughout these experiments. Any mistakes while analyzing these parameters will impact the final results greatly. Hence an effective QA/QC plan was established to reduce such errors. This section will deal the precautions that were taken throughout the experiments.

3.5.1. Sampling Procedures

To prevent contamination among samples, the containers used for sampling were soaked for 24 hours in a 30% mixture of Micro 90 cleaning solution with tap water, then rinsed with tap water and further rinsed with deionized water. All sample containers were labeled with date and initials, and stored in refrigerator for further analysis. The bottles used to store stock chemicals were washed in similar way and stored in refrigerator.

Resin samples collected during bioregeneration test were immediately rinsed six times with 25 ml deionized water to remove any adhering microbes. The sample tubes were then labeled and immediately stored in refrigerator.

Standard and blank solutions for IC analysis were prepared using deionized water. Duplicate samples were run for every six samples analyzed in the IC. In addition, quality control samples of known concentration were analyzed for every ten samples analyzed in the IC.

3.5.2. Calibration of Instruments

The IC, pH meter and turbidity meter were calibrated frequently to ensure accurate results. The calibration was done using blanks and known standard concentration samples every two weeks. For the IC, standard solution for perchlorate, nitrate, sulfate and chloride were prepared using the respective standard salts/ solutions. The R-squared value for the calibration curve was made sure to be above 99.97% for the IC.

The analytical balance in the laboratory is calibrated every six months by Precise Weighing Systems (Santa Clarita, CA). pH meter was calibrated using three buffer standards (4.01, 7.0 and 10.01). The accuracy of the micropipettes was checked by weighing different volumes of deionized water in the analytical balance.

3.5.3. Deionized (DI) Water Quality

Deionized water used for sample preparation and stock chemical preparations was free of impurities, microorganisms and particulate matters. The specific resistance of the DI water was maintained above 17 Mohm-cm. When values dropped below the required resistance value, ion-exchange columns in the DI water machine were replaced.

3.5.4. Colum Test and Batch Test Apparatus Cleaning

The columns and glass bioreactors used for the tests were sterilized with clorox solution and rinsed thoroughly with deionized water. The column tubings were rinsed with clorox and deionized water or replaced with new tubings to prevent contamination.

3.5.5. Quality Control and Quality Assurance

The accuracy of the data analyzed was determined by calibration of the instruments with known standards. The calibration curve best fit was determined by least square analysis. The R-square values that were achieved for the calibration of various

parameters are tabulated in Table 3.10. Accuracy of the measurement was ensured by introducing duplicate samples and the quality assurance was performed by introducing standards between every ten measurements. Also to prevent carry over in IC measurements, the samples were arranged from low to high concentrations and also blanks were introduced between the samples in few cases.

Table 3.10: Analytical Quality Control for Various Parameters

Parameter	Method	R^2	Detection Limit	Calibration Range
Perchlorate	IC	0.9998	5 ppb	5 – 100 ppb
Chloride (High Range)	IC	0.9998	1 ppm	1 – 10 ppm
Chloride (Low Range)	IC	0.9997	100 ppb	100 – 500 ppb
Nitrate	IC	0.9999	1 ppm	1 -10 ppm
Sulfate	IC	0.9997	I ppm	1 – 10 ppm

CHAPTER 4

INVESTIGATION OF POTENTIAL MECHANISMS CONTROLLING THE BIOREGENERATION PROCESS FOR PERCHLORATE LADEN GEL-TYPE ANION EXCHANGE RESIN

4.1. Introduction

Ion-exchange technology is the most common process that is being used at present to remove perchlorate from drinking water. In this process, perchlorate ions replace other innocuous anions due to its affinity for the ion exchange functional group. Development of ion-exchange technology resulted in the production of selective or specialty resins for perchlorate removal. These resins have very low affinity towards divalent anions such as sulfate and hence have very high efficiency in removing perchlorate ions due to less interference (Baruth, 2005). But most of these resins cannot be regenerated and are disposed by incineration after one time use (Gingras and Batista, 2002). Due to their high cost, disposal of these resins after one time use makes the ion-exchange technology incomplete and economically unsustainable for perchlorate removal.

Synthetic ion-exchange resins are classified into gel (microreticular) and macroporous (or macroreticular) with respect to their polymeric background (Crittenden *et al.*, 2005). Gel-type resins have lower degree of cross-linking when compared to macroporous resin. Gel-type resins usually have about 4 – 10% DVB cross-linking, while macroporous resins have about 20 – 25 % cross-linking (Crittenden *et al.*, 2005).

Gel-type resins are translucent and have high water content. Hence they lose their pore structure upon drying. In comparison, macroporous resins are made by linking microspheres, making them more rigid. They can maintain their pore structure and integrity even when dried (Crittenden *et al.*, 2005).

Bioregeneration of ion-exchange resin is a new concept which involves the direct contact of spent resin with perchlorate reducing bacteria. This process does not require the use of concentrated brines for regeneration of resins. The bioregeneration technology has been developed recently and is a patented process (Batista and Jensen, 2006). This process consists of a bioreactor containing a perchlorate reducing culture, in which dissolved oxygen (DO), Oxidation Reduction Potential (ORP) and pH are monitored. The culture is circulated through a Fluidized Bed Reactor (FBR) containing ion-exchange resin loaded with perchlorate (Batista and Jensen, 2006). The perchlorate reducing culture utilizes perchlorate ions as electron acceptors and degrades it to innocuous chloride, when an electron donor is provided. This technology allows the re-use of ion-exchange resin and also destroys perchlorate making it economically and environmentally sustainable. This process has been carried out successfully in bench-scale experiments for macroporous (Batista and Jensen, 2006).

Mechanism by which bacteria degrade perchlorate that is present in ion-exchange resins have not been elucidated to date. From past studies, it is only apparent that the bacteria utilize perchlorate ions as electron acceptors for their metabolism and convert perchlorate to innocuous chloride. There exist no studies which describe the exact process by which these bacteria reach and utilize the perchlorate ion attached to the functional group of ion-exchange resin. Hence in this research batch biodegradation

experiments will be performed to understand some of the mechanisms involved in the bioregeneration process of a gel-type resin. These batch experiments are performed to answer the following questions related to perchlorate attached to a fixed media (ion exchange resin):

(d) Does the rate of perchlorate degradation depend on the initial amount of perchlorate load in the ion-exchange resin?

(e) How does microbial concentration affect perchlorate degradation rate in ion exchange resin?

(f) How does the presence of nitrate affect perchlorate degradation rate in ion-exchange resin?

4.2. Scientific Basis for Research Hypotheses

The maximum amount of perchlorate load in an ion exchange resin is determined by the influent perchlorate concentration in water and the type of resin used. Drinking waters in the United States are generally contaminated with an average 10 ppb perchlorate concentration (EPA, 2004). However industrial sites from manufacturing and laboratory areas like Aerojet General Corp., Rancho Cordova, and NASA Jet Propulsion Laboratory, Pasadena, CA have water contaminated with perchlorate levels ranging between 1500 – 2500 ppb (EPA, 2008). Very high perchlorate concentration of 160,000 and 300,000 ppb was detected in Edward AFB, military base and Kerr McGee site in Henderson, Nevada respectively (EPA, 2008).

The Malcolm Pirnie engineering consulting firm has performed on-site mini-columns and pilot studies with various perchlorate-selective resins with low perchlorate

concentration to determine the maximum amount of water that can be processed before a breakthrough of 4 ppb (detection limit) of perchlorate occurs (AWWA, 2006). The influent water that was tested had a perchlorate concentration of 8 – 10 ppb, nitrate of 4000 – 7000 ppb and sulfate of 25,000 – 40,000 ppb (AWWA, 2006). This perchlorate concentration is similar to that present in a contaminated drinking water well (Urbansky, 1998). Results showed that breakthroughs of perchlorate occurred only after 60,000 to 410,000 bed volumes of water when tested with various perchlorate-selective resins (AWWA, 2006). The results from the experiments are summarized in Table 4-1 that includes % of the resin capacity occupied by perchlorate. These values were calculated for this research based on the capacity of the resins and the number of bed volumes processed to breakthrough.

From Table 4.1 it can be noted that perchlorate load in the resin varies from 0.6 – 6.9 % for an influent perchlorate concentration of 10 ppb. Hence in this research, perchlorate loads of 5.0 % and 8.4 % (3 g/L_{resin} and 5 g/L_{resin}) are used to simulate perchlorate loads in resin from treating contaminated drinking water. Higher perchlorate loads of 33.5 % and 50.25 % (20 g/L_{resin} and 30 g/L_{resin}) will also be tested in this research to simulate perchlorate loads from industrial waters. The load of perchlorate into selective resins, for high influent perchlorate concentrations, has been established in the UNLV Environmental Engineering laboratory.

Table 4.1: Perchlorate Loading in Perchlorate Selective Resins

Resin	Resin Capacity (eq/L)	Influent Perchlorate Concentration (ppb)	Bed Volumes to Break-through	Perchlorate Loaded (g/L_{resin})	Estimated eq/L_{resin} of perchlorate loaded	% Total Capacity occupied by Perchlorate
PWA2[+]	0.6	10	200,000	2.0	0.020	3.4
SIR-110[+]	0.6	10	410,000	4.1	0.041	6.9
PWA-555[+]	1.0	10	60,000	0.6	0.006	0.6
A530E[+]	0.6	10	100,000	1.0	0.010	1.7
SR-7[+]	0.8	10	80,000	0.8	0.008	1.0
A530E[*]	0.6	10,000	~1000	10.0	0.100	16.7
SIR-111[**]	0.635	100,000	270	27	0.271	42.7

[+]AWWA, 2006, [*]Source: Gu and Brown, 2006; ** Source: Column tests performed at UNLV

The biodegradation mechanism for perchlorate, when it is dissolved in water, has been elucidated (Bruce *et al.*, 1999; Coates *et al.*, 1999; Coates and Achenbach, 2004). Figure 4.1 depicts the model of perchlorate degradation by PRBs when the ion is dissolved in water. In this model, perchlorate ion enters the cell via facilitated diffusion by transmembrane proteins and is degraded to chloride and oxygen by the enzymes (per)chlorate reductase and chlorite dismutase, located in the periplasmic area/outer membrane of PRBs (Kengen *et* al., 1999; Coates *et al.*, 1999; O'Connor and Coates, 2002; van Ginkel *et al.*, 1996). It has been determined that *c*-type cytochrome is present in PRBs to facilitate the reduction of perchlorate (Bruce *et al.*, 1999; Coates *et al.*, 1999). Studies showed that H_2 reduced *c*-type cytochrome of PRBs were readily reoxidized in the presence of chlorate and perchlorate (Coates *et al.*, 1999). It was also shown that

these enzymes were specific for (per)chlorate and was unaffected by other electron acceptors like sulfate, fumurate etc (Coates *et al.*, 1999). (per)chlorate reductase is found in the periplasmic area while chlorite dismutase is suggested to be loosely bound to the outer membrane of PRB as it was detected both in the soluble fraction (periplasm) and in the outer membrane of PRB (Kengen *et* al., 1999; O'Connor and Coates, 2002).

The mechanism described above cannot be applied for the degradation of perchlorate attached to the ion-exchange resin. This is because perchlorate ions are strongly attached to functional groups present in the resin and is not freely available to reach the bacterial cell. In this research, to confirm the strong affinity of perchlorate ions to the resin bead, preliminary column tests were performed to investigate whether perchlorate could be detached from the resin by continuous contact with water and sodium chloride (NaCl) solution. Perchlorate selective, macroporous resin, SIR-100 (ResinTech, West Berlin, NJ) was used for the test. Results show that no perchlorate could be detached from the resin bead by using deionized water and only 0.47% of perchlorate could be removed from the resin using 12 % NaCl solution as regenerant. This result confirms the very high affinity of perchlorate ions from perchlorate-selective resins and disproves the possible dissociation of perchlorate ions from perchlorate-selective resins abiotically.

From the literature it is evident that the pore size in resin beads is of orders of magnitude much smaller than the size of bacterial cells. Gel type resins have pore sizes ranging between 0.005 and 0.04 μm, while macroporous resins has an average pore size of 0.6 μm (Pietrzyk, 1969; K.A. Kun and R. Kunin, 1968). These pore sizes are very small when compared to bacterial cell size, which has an average size of 1 μm (Rittmann and McCarty, 2001). Hence it is only possible for the PRBs to attach to the surface of the

resin but not possible to enter ion-exchange resins through pores to utilize perchlorate ions that are attached deep inside the resin bead.

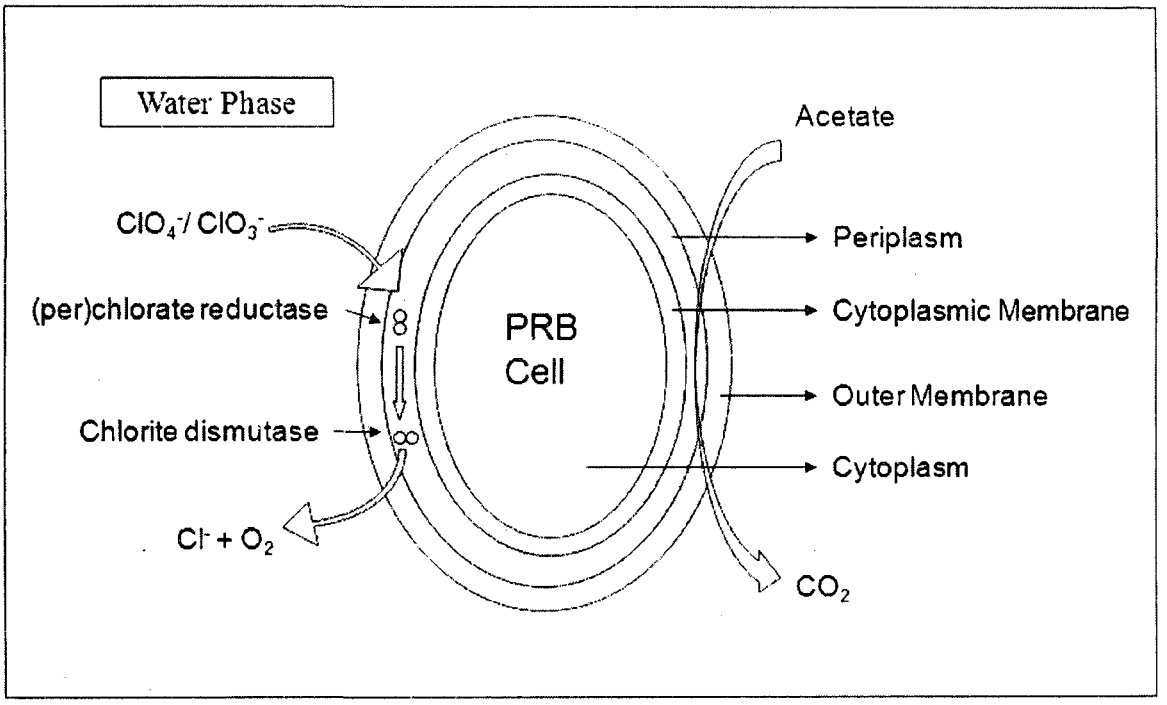


Figure 4.1: Model for Perchlorate Degradation in Water

One possible hypothesis to explain the mechanism of bioregeneration process that is taking place inside gel-type ion-exchange resin is made in this research. There may be some substance that is released by PRBs to the bulk liquid, which diffuses through pores in the resin bead and helps in detaching the perchlorate ions from the functional group, thus making it possible for their uptake for cellular metabolism. This hypothesis is depicted schematically in Figure 4.2. This assumption supports the prospect of bioregeneration process in gel-type resin and form the basis for the current study. Once perchlorate ion is detached from the resin bead, it is assumed that the degradation mechanism is similar to that taking place in waters, as shown in Figure 4.1.

Perchlorate ions are attached to the functional group of the ion-exchange resin. Hence its degradation in resin bead is similar to biodegradation of compounds that are attached to some media in general. Before biodegradation of a substrate can occur, the substrate must be available as a free solute for its uptake and degradation by microorganisms (Gordon and Millero, 1985). Many studies exist that determined the possible mechanism by which these attached substrates become available for biodegradation (Ogram *et al.*, 1985; Subba-Rao and Alexander, 1984; Gordon and Millero, 1985; Guerin and Boyd, 1992). Few of these studies suggested that desorption of substrate from the media may have been facilitated by microorganisms and also the type of microorganism was important in determining whether the sorbed substrate was bio-available or not (Gordon and Millero, 1985; Guerin and Boyd, 1992). Based on these studies, the mechanism for the biodegradation of perchlorate in ion-exchange resin was hypothesized in this research. It is assumed that once perchlorate ions are detached from the resin bead by the 'substance', the mechanism by which PRBs degrade it to chloride is similar to that taking place in waters.

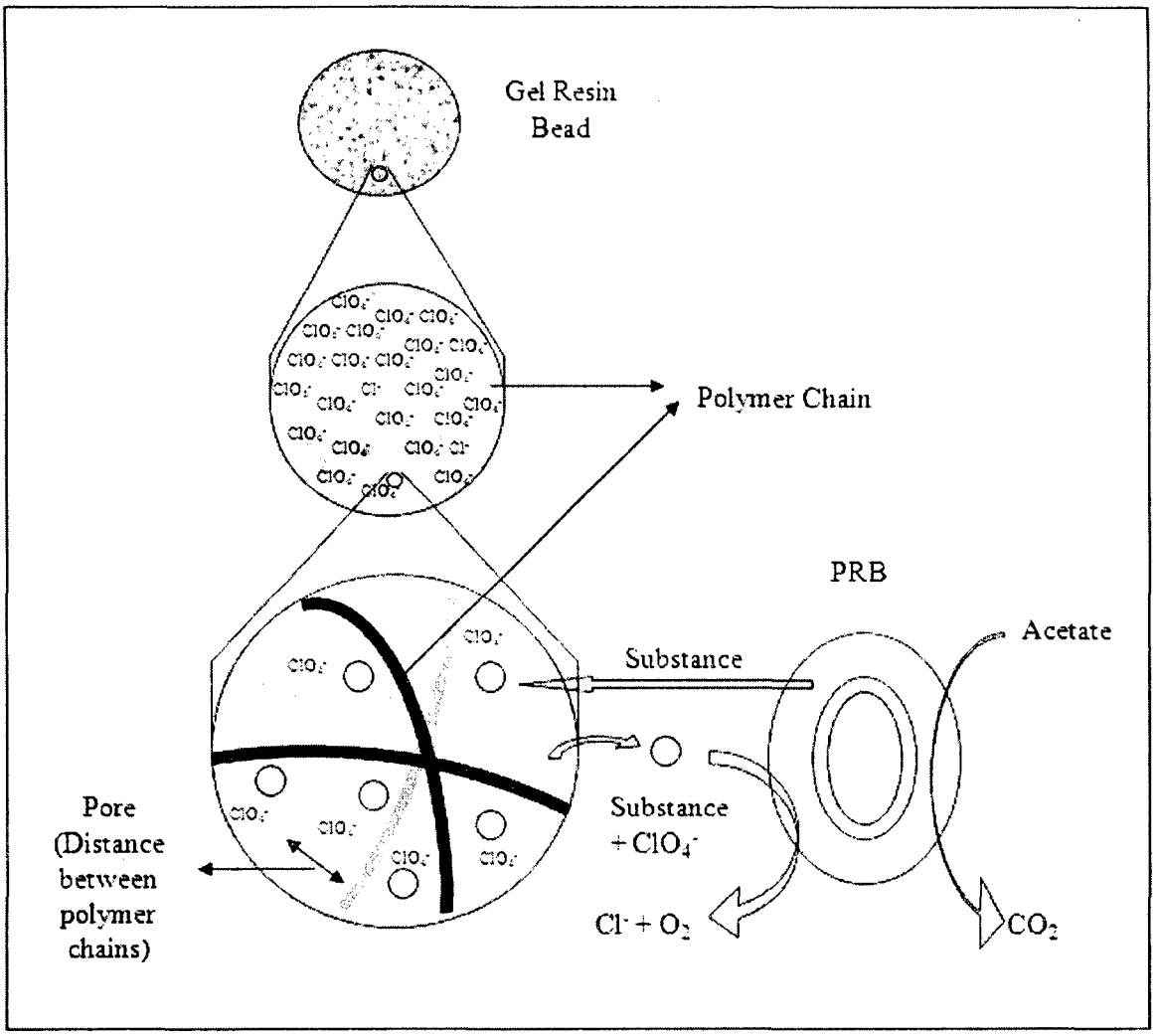


Figure 4.2: Mechanism for Perchlorate Degradation in Resin Bead

Since perchlorate is attached to the resin bead and not freely available, the rate of perchlorate degradation in ion-exchange resin is expected to be slower when compared to perchlorate degradation in waters. The time that will take for the 'substance' to reach perchlorate sites in resin bead and get perchlorate ion back into the cell is expected to be more when compared to free perchlorate ions in water that diffuse into the cell. Also this time will depend whether the perchlorate ion is in the surface or deep inside the resin. To reach perchlorate sites inside the resin, the substance needs to diffuse through pores, in

which case diffusivity of the substance and pore length may come into play and increase the time.

4.3. Materials and Methods

4.3.1. Perchlorate-Reducing Culture

Perchlorate-reducing culture for the experiments was taken from two enrichment seed cultures, BALI 1 and BALI 2, grown in the UNLV Environmental Engineering Lab. Two liters of the culture was rinsed with phosphate buffer and centrifuged using Legend RT Sorvall centrifuge (Kendro, Thermo Fisher Scientific, Inc., Waltham, MA). This procedure was followed to remove excess acetate from the culture prior to biodegradation experiment. The centrifuged concentrated culture was then diluted with required amounts of acetate and nutrients in the batch-bioreactor tubes.

4.3.2. Resin Loading

SIR-110-HP resin was loaded with various concentrations of perchlorate and nitrate for the batch experiments. To evaluate the effect of initial perchlorate load in resin on the degradation rate, the resin was loaded with three different concentrations of perchlorate (3 g/L_{resin}, 5 g/ L_{resin}, and 20 g/ L_{resin}). To evaluate the effect of nitrate on perchlorate degradation, resin was loaded with two combination of nitrate and perchlorate (20g/ L_{resin} NO_3^- and 3 g/ L_{resin} ClO_4^-; 20g/ L_{resin} NO_3^- and 20 g/ L_{resin} ClO_4^-).

Thirty or 150 ml of resin to be loaded was transferred to a 100 ml or 3 L bottle and was mixed thoroughly with the desired concentration of perchlorate and nitrate for 16 hours in a rotary mixer (Associate Design & Mfg. Co., Alexandria, VA) at 25 rpm. After 16 hours of mixing, the solution was decanted and the resin was washed ten times with

100 ml deionized water to remove residual ions. The initial and final concentration of perchlorate and nitrate during resin loading was measured using Dionex ICS 2000 ion chromatography (Sunnyvale, CA). Table 4.2 shows the resin load in the resin. The loaded resin was transferred to a labeled glass container and stored in refrigerator until the start of the experiment.

Table 4.2: Resin Loading for Batch Biodegradation Experiments

Total Amount of resin (ml)	Required Perchlorate Load (g/ L_{resin})	Nitrate Load (g/ L_{resin})	mg of perchlorate needed for loading	Expected Concentration in 100/ 300 ml (mg/ L)	Measured Concentration before Loading (mg/L)	Measured Concentration after Loading (mg/L)	Actual Perchlorate Load in Resin (g/L_{resim})
150	3	-	450	1500_{300}	1947	0.2	3.8
30	5	-	150	1500_{100}	2069	0.0	6.8
150	20	-	3000	10000_{300}	11179	0.54	22.3
20	3	20	60	600_{100}	665	0.12	3.3
20	20	20	400	4000_{100}	4518	1.25	22.5

4.3.3. Resin Bioregeneration

The culture for the biodegradation experiment was obtained from BALI 1 and BALI 2 reactors. Culture samples were centrifuged and the supernatant was tested for zero perchlorate by using Dionex ICS 2000 (Sunnyvale, CA). Once zero perchlorate was confirmed in the culture, about two liter of culture was centrifuged using Legend RT Sorvall centrifuge (Kendro, Thermo Fisher Scientific, Inc., Waltham, MA). The supernatant was discarded and the concentrated culture was rinsed twice with phosphate buffer solution and centrifuged again. This procedure was followed to remove residual acetate present in the culture.

The culture was diluted to 500 mg/L or 1000 mg/L or 2000 mg/L in 25 ml batch-bioreactors by adding acetate, buffer and nutrients. 1000 mg/L of suspended solids were used during evaluation of the effect of varying initial perchlorate load and nitrate load on the degradation rate. 500 mg/L, 1000 mg/L and 2000 mg/L suspended solids were used to determine the effect of microbial concentration on perchlorate degradation rate.

Table 4.3: Experimental Condition for Evaluating the Effect of Varying Initial Perchlorate Load on the Degradation Rate

Perchlorate Load (g/L_{resin})	Number of tubes	Amount of Resin in each tubes (ml)	Conc. of Perchlorate in 25 ml (mg/L)	Conc. of Acetate added in 25 ml (mg/L)
3	10	2	240	720
5	8	2	400	1200
20	10	2	1600	4800

The acetate, nutrients and buffer solutions were purged with nitrogen gas for approximately 20 minutes to decrease the dissolved oxygen content and maintain anaerobic condition in the tubes. Once all the components were added to the bioreactor tubes (Tables 4.3 to 4.5), they were sealed using aluminum-crimpled butyl rubber stopper (Wheaton Industries, Inc., Millville, NJ) and labeled accordingly. The bioreactor tubes were then taped to a Cole-Palmer orbital shaker (Vernon Hills, IL), 51704 series, to maintain the microbes in suspension. The biodegradation experiment was carried out for

ten consecutive days and one tube was removed every alternate day to determine perchlorate degradation (Day 2, 4, 6, 8 and 10).

The culture samples were analyzed for turbidity, pH, OD, COD and suspended solids. Finally, the resin samples were rinsed six times with 25 ml deionized water and stored in refrigerator for further analysis.

Table 4.4: Experimental Condition for Evaluating the Effect of Microbial Concentration on Perchlorate Degradation Rate

Perchlorate Load (g/L_{resin})	Number of tubes	Amount of Resin in each tubes (ml)	Conc. Of Perchlorate in 25 ml (mg/L)	Conc of Acetate in 25 ml (mg/L)	Suspended Solids (mg/L)
3	10	2	240	720	500
20	10	2	1600	4800	500
3	10	2	240	720	1000
20	10	2	1600	4800	1000
3	10	2	240	720	2000
20	10	2	1600	4800	2000

Table 4.5: Experimental Condition for Evaluating the Effect of Nitrate Load on Perchlorate Degradation Rate

Amount of Nitrate Present in 2 ml resin (mg)	Amount of Perchlorate Present in 2 ml resin (mg)	Amount of acetate required for nitrate Degradation (mg)	Amount of acetate required for perchlorate Degradation (mg)	Total amount of acetate added (mg)	Concentration of acetate in 25 ml (mg/L)
40	6	37.6	18	55.6	2224
40	40	37.6	120	157.6	6304

Table 4.6: Percentage Capacity of Resin Occupied by Perchlorate and Nitrate

Nitrate Load (g/L_{resin})	Perchlorate Load (g/L_{resin})	% Capacity occupied by Nitrate	% Capacity occupied by Perchlorate
20	3	53.76	5.025
20	20	53.76	33.5

4.3.4. Perchlorate Analysis in Resin

Residual perchlorate in the resin sample was analyzed using an oxygen combustion bomb 1108 (Parr Instruments, Moline, IL). The resin samples were burnt inside the combustion bomb, thus converting the residual perchlorate ions to chloride ions.

$$ClO_4^- \longrightarrow 2O_2 + Cl^-$$

Two ml of resin sample was first treated with 250 ml of 10,000 mg/L nitrate solution for 15 hours in a 500 ml erlenmeyer flask. The treated resin was then rinsed six times with 500 ml deionized water to remove excess nitrate. The resin was dried in a gravity oven (Thermo Fisher Scientific, Waltham, MA) maintained at a temperature of 105^0C to remove moisture content. About 100 mg of the dried resin sample was used for the analysis. Four hundred mg of paraffin oil (EMD Chemicals, Inc., San Diego, CA) was added to the resin sample as a combustion aid. Ten ml of 35 mMoles NaOH and 3 ml of 3% H_2O_2 was added to the bottom cylinder of parr bomb as buffer solution. Oxygen gas was then filled in the cylinder to a pressure of 30 -35 psi and the bomb was fired inside a bucket of deionized water. The residual solution in the bottom cylinder was then analyzed for chloride concentration using ICS 2000 Ion Chromatography (Dionex,

Sunnyvale, CA). QA/QC for every three samples was performed to ensure accurate and precise results. Ultimate coal standard sample (Alpha Resources Inc, Stevensville, MI), with 0.13% chloride content, was used for quality assurance.

4.3.5. Analytical Methods

Concentration of perchlorate and nitrate was determined using Dionex ICS 2000 (Sunnyvale, CA). IonPac AS16 column with 4 X 250 mm dimension was used for the analysis. Chromleon 6.70 (SP2a Build 1871) software was used to run the IC. Dionex RFIC EluGen Cartridge containing sodium hydroxide was used as the eluent. The eluent concentration for the analysis was set to 35 mM and the current was set to 100 mA for perchlorate and chloride analysis.

The suspended solids were measured using a filtration apparatus with a 47 mm Whatmann glass microfiber filters (GFC). COD analysis was performed using high range HACH COD digestion vials (Hach Company, Loveland, CO). pH of was measured using a Fisher Scientific model AR25 pH meter. Optical density at 600 nm wavelength and turbidity of the culture was measured using Hach DR/3000 Spectrophotometer and Hach turbidimeter (Model 2100 N) respectively.

4.3.6. Statistical Analysis

Statistical analysis was performed to determine whether there is a significant difference in perchlorate biodegradation with varying initial perchlorate load, microbial concentration and nitrate load. The statistical significance of the differences was determined using single factor ANOVA test.

4.4. Results and Discussions

4.4.1. Effect of Initial Perchlorate Load on Perchlorate Degradation Rate in Ion-Exchange Resin

Figure 4.3 shows perchlorate degradation during bioregeneration process when the initial perchlorate loads are 3, 5 and 20 g/L_{resin}. Average perchlorate degradation rate expressed as mg of perchlorate degraded per mg of suspended solids per day ($mg_p/mg_{ss}/d$), when initial perchlorate load was 3, 5 and 20 g/L_{resin} is about 0.021, 0.025 and 0.11 $mg_p/mg_{ss}/d$ respectively. The degradation rate for 20 g/L_{resin} initial load is about 5.5 times higher than the degradation rate for 3 and 5 g/L_{resin}. The result indicates that higher perchlorate load in resin has a positive effect on the degradation rate in ion-exchange resin. The perchlorate degradation rates are summarized in Table 4.7.

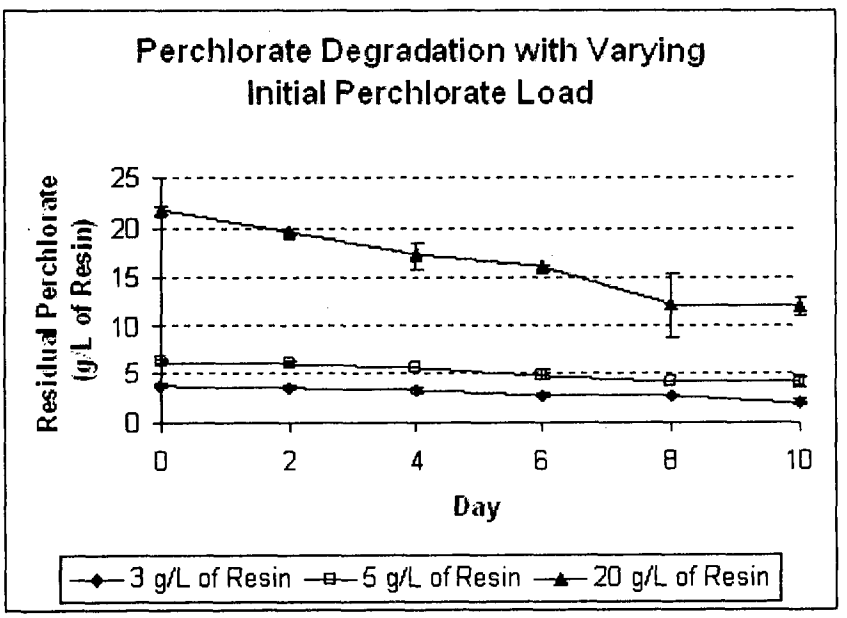


Figure 4.3: Perchlorate Degradation with Varying Initial Perchlorate Load

(Error bar = standard deviation)

Table 4.7: Perchlorate Degradation Rates with Varying Initial Perchlorate Load

Initial Perchlorate Load in Resin (g/L_{resin})	Initial Perchlorate Concentration in Bioreactor Tubes ($mg/L_{culture}$)	Average Perchlorate Degradation Rate ($mg_p/mg_{ss}/d$)	Maximum Rate Observed ($mg_p/mg_{ss}/d$)
3.76	300.8	0.021	0.044
6.37	509.6	0.025	0.035
21.96	1756.8	0.11	0.19

Even though the concentration of perchlorate in reactor tubes for all perchlorate loads was higher than the average K_s value, lower rates were observed. The substrate utlizing model developed based on the Monod equation is given as (C. Wang *et al.*, 2008):

$$\frac{dS}{dt} = -\frac{q_{max} XS}{S + K_s} \tag{4.1}$$

Where, S is the perchlorate concentration (mg/L); t the time (d); q_{max} is the maximum specific substrate removal rate (d^{-1}); K_s is the half saturation constant (mg/L); and X is the microbial concentration (C. Wang *et al.*, 2008). Dividing both sides of the equation by microbial concentration (X), we can get the rate of substrate utilization in terms of mg of substrate/ mg of microbes/ day as shown below:

$$\frac{1}{X}\frac{dS}{dt} = -\frac{q_{max} S}{S + K_s} \tag{4.2}$$

A plot between the rate and varying initial substrate concentration (S) is shown in Figure 4.4. Theoretical degradation rates were calculated at average values for K_s = 45 mg/L and q_{max} = 1.2 d^{-1}. The degradation rate drops when the substrate concentration is

less than 200 mg/L as shown in Figure 4.4. However, any concentration above 200 mg/L does not increase the rate significantly. The observed degradation rates for the three perchlorate loads are much lower than the calculated theoretical degradation rates. The concentration of perchlorate found in all reactor tubes is much higher than 200 mg/L and still very low degradation rates were observed as shown in Table 4.7. These results indicate that the reduced perchlorate degradation in resins may not be due to kinetics.

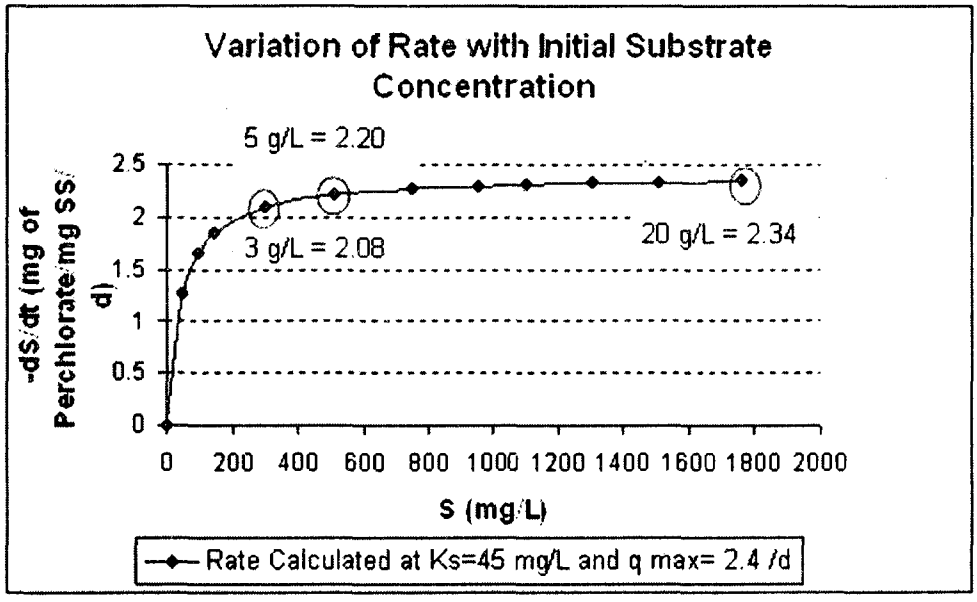


Figure 4.4: Variation of Degradation Rate with Initial Substrate Concentration

Diffusion might possibly be the reason for the observed reduction in perchlorate degradation. PRBs can utililize perchlorate attached to the surface and outer region of the resin bead relatively easily when compared to perchlorate ions attached deep inside the resin. This is because, the 'substance' released by the PRB need to diffuse through pores and reach perchlorate ions that are deep inside the resin bead. Amount of time for this diffusion will depend on pore length, pore tortuosity and how deep the perchlorate

ion is present in the bead. Assuming homogeneous distribution of perchlorate ions in a spherical resin bead, a relationship between perchlorate content and the radius of the resin bead was developed in this research. Figure 4.5 and 4.6 shows the distribution of perchlorate (% and μg), with resin bead radius.

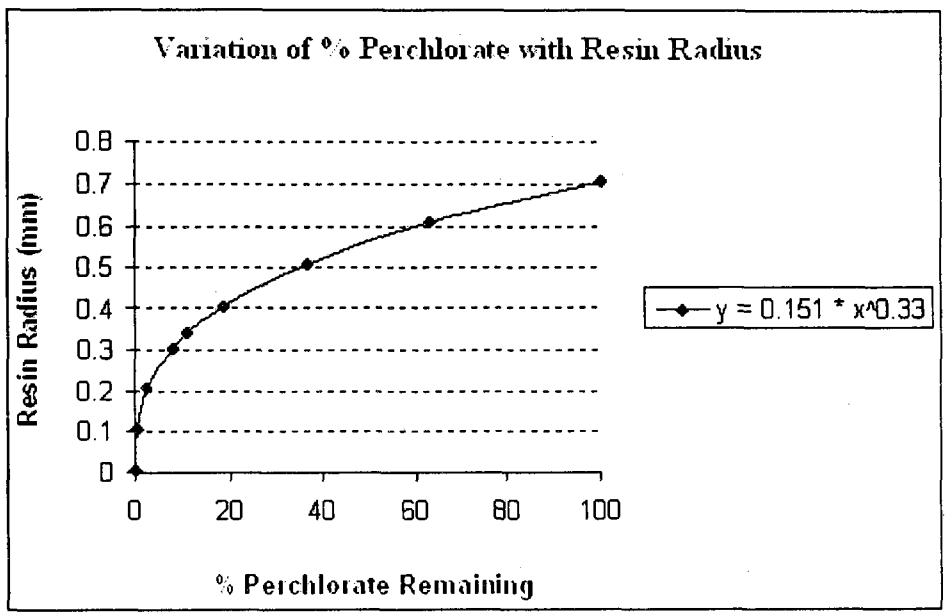


Figure 4.5: Variation of % Perchlorate with Resin Radius

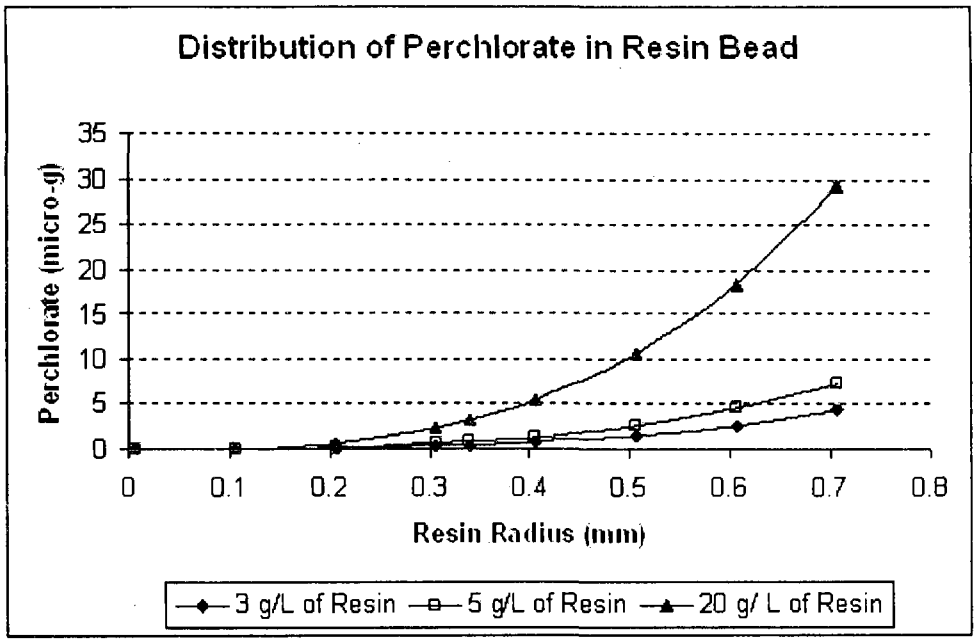


Figure 4.6: Distribution of Perchlorate for Various Load in a Resin Bead

Perchlorate content in the resin bead increases significantly with increase in radius. Considering 'r' to be the radius of the resin bead, the perchlorate content of the bead at a distance of 0.5r from the centre of the bead is only about 10 % of the total perchlorate content of the resin. Hence 90 % of the loaded perchlorate in the resin bead is present at a radius greater than 0.5r. With this relationship between perchlorate distribution and resin radius, the reduction of unused resin core with time is plotted for the three perchlorate loads as shown in Figure 4.7. Perchlorate degradation slows down when the unused core reaches a radius of 0.57 mm for 20 g/L_{resin} load and a radius of about 0.61 mm for 3 and 5 g/L_{resin} load. Overall, the degradation of perchlorate slows down when the unused resin core radius reaches an average of 0.6 mm.

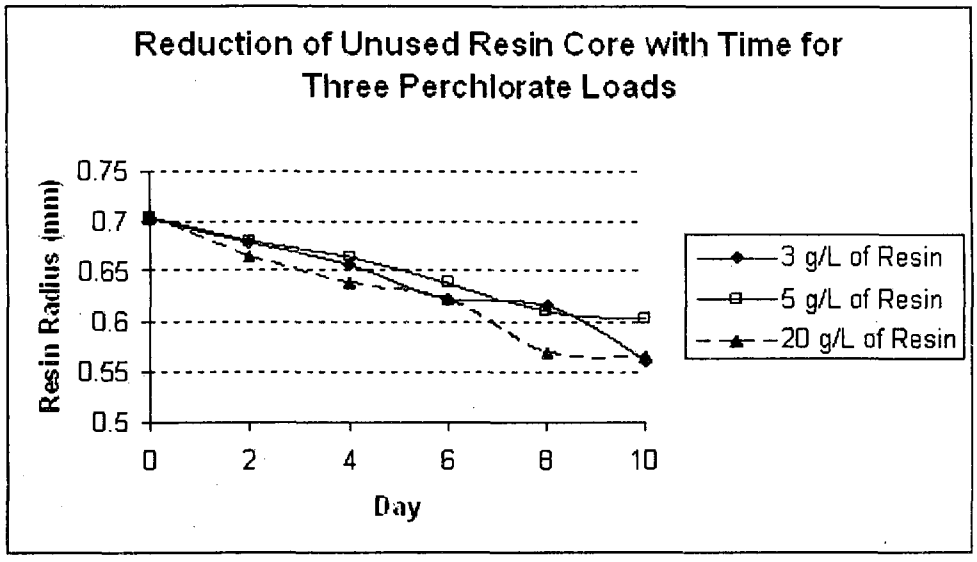


Figure 4.7: Reduction of Unused Resin Core with Time for Three Perchlorate Loads

However, if we compare the amount of perchlorate present for the different loads from Figure 4.6, 20 g/L_{resin} load has high perchlorate content (29.2 μg) when compared to the other two loads (7.3 and 4.4 μg for 5 and 3 g/L_{resin} load respectively). Hence even though the degradation slows down at a radius of about 0.6 mm, the amount of perchlorate degraded varies with different loads, highest being for the 20 g/L_{resin} load. Hence higher degradation rates are observed for higher perchlorate loads.

Suspended solids concentration dropped in the reactor tubes for all perchlorate loads (Figure 4.8). This is due to the production of insufficient biomass during perchlorate degradation to compensate for biomass decay. However, the reduction of suspended solids was less in higher perchlorate load (20 g/L_{resin}) when compared to lower loads (3 and 5 g/L_{resin}). This is due to higher degradation rates of 20 g/L_{resin} producing more biomass for compensating the decay when compared to lower loads.

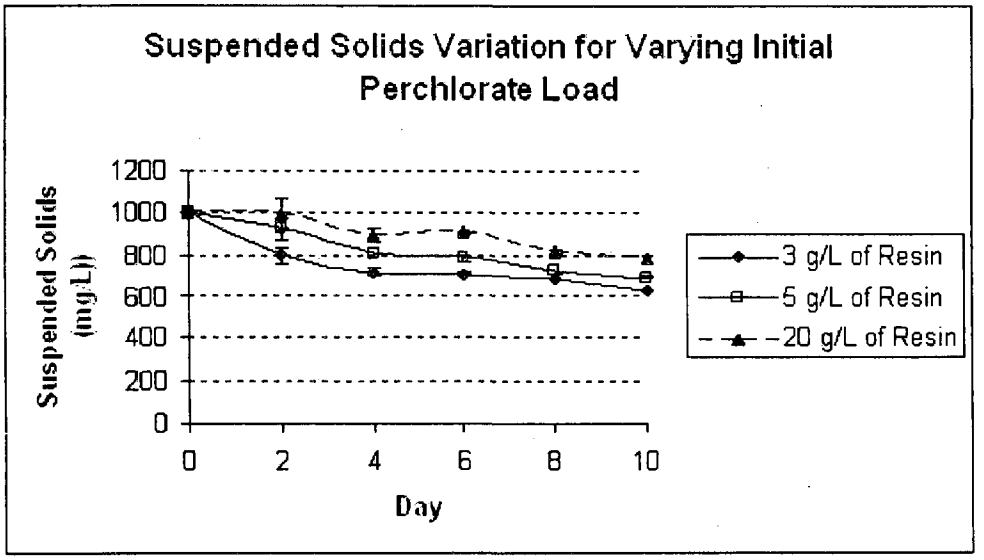


Figure 4.8: Suspended Solids Variation With Varying Initial Perhclorate Load

(Error bar = Standard Deviation)

4.4.2. Effect of Microbial Concentration on Perchlorate Degradation Rate in Ion-exchange Resin

Perchlorate degradation curves for resins loaded with 3 and 20 g of perchlorate/L_{resin} when subjected to 500 mg/L, 1000 mg/L and 2000 mg/L suspended solids concentration are shown in Figure 4.9 and 4.10 respectively.

The average perchlorate degradation rate when expressed as mg of perchlorate degraded per day (mg_p/d), when 3 g/L_{resin} perchlorate loaded resin is subjected to 500 mg/L, 1000 mg/L and 2000 mg/L suspended solids, is about 0.47, 0.35 and 0.43 mg_p/d respectively. These rates are very similar and when single factor ANOVA test was performed for the three sets of data, no significant difference was observed ($p = 0.944$). However, when the rates were expressed as mg of perchlorate degraded per mg of suspended solids per day (mg_p/mg_{ss}/d), lower rates were observed with increasing

microbial concentration (0.054 $mg_p/mg_{ss}/d$ for 500 mg/L SS, 0.022 $mg_p/mg_{ss}/d$ for 1000 mg/L SS and 0.012 $mg_p/mg_{ss}/d$ for 2000 mg/L SS). The degradation rates for both 3 g/L_{resin} and 20 $g/_{resin}$ load is summarized in Table 4.8.

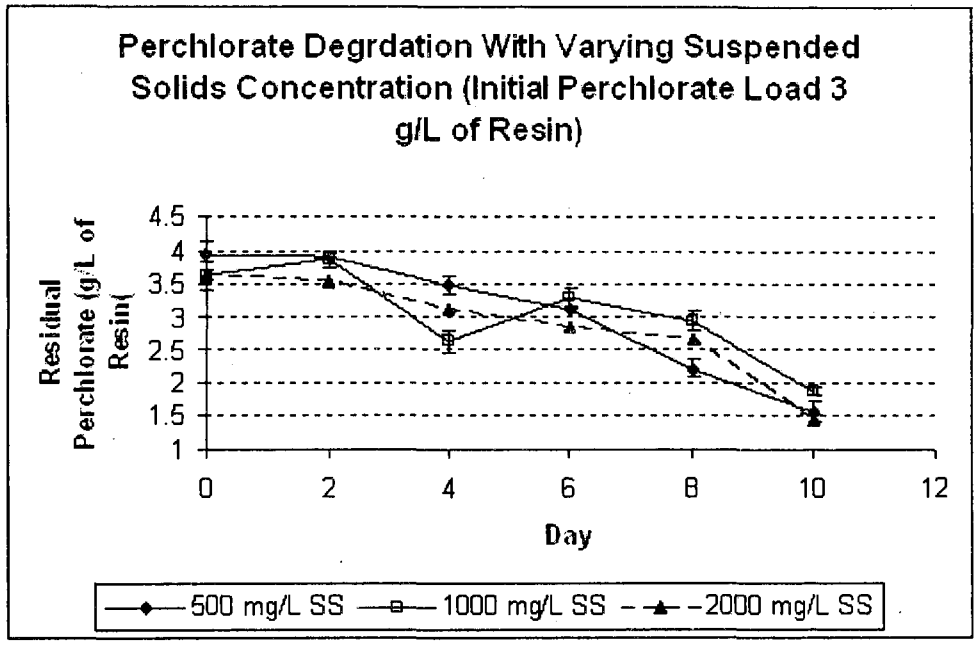


Figure 4.9: Perchlorate Degrdation With Varying Suspended Solids Concentration (Initial Perchlorate Load 3 g/L of Resin)

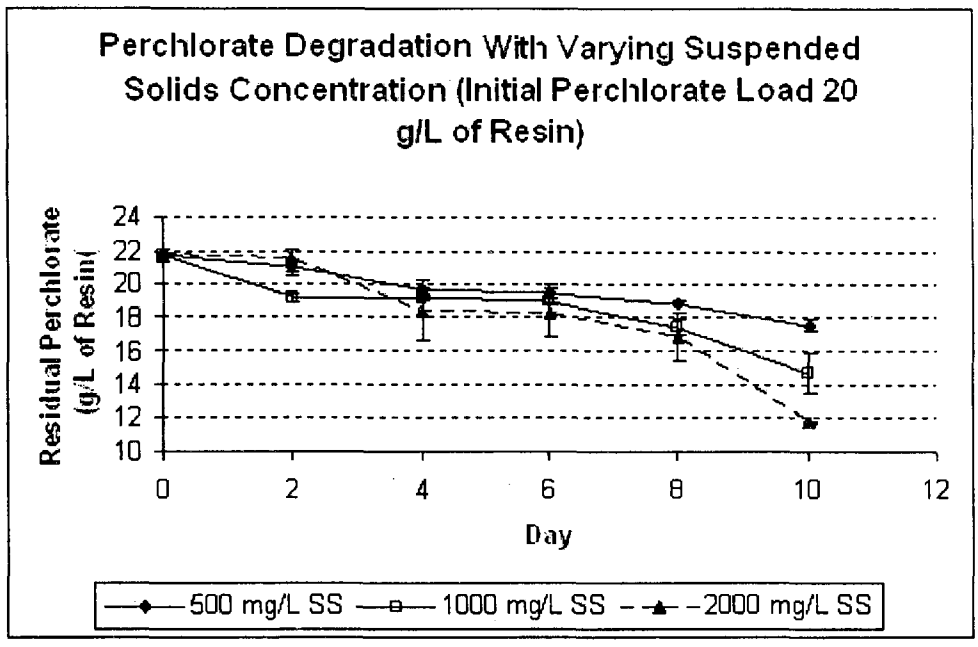


Figure 4.10: Perchlorate Degrdation With Varying Suspended Solids Concentration (Error bar = Standard Deviation) (Initial Perchlorate Load 20 g/L of Resin)

Table 4.8: Perchlorate Degradation Rates with Varying Microbial Concentration

Initial Perchlorate Load in Resin (g/L_{resin})	Suspended Solids Concentration (mg/L)	Average Perchlorate Degradation Rate ($mg_p/mg_{ss}/d$)	Maximum Rate Observed ($mg_p/mg_{ss}/d$)
3	500	0.052	0.11
	1000	0.022	0.07
	2000	0.012	0.04
20	500	0.075	0.13
	1000	0.073	0.15
	2000	0.054	0.15

The average perchlorate degradation rates for the three microbial concentration when the initial perchlorate load was 20 g/L_{resin}, is about 0.84, 1.41 and 2.00 mg_p/d (for 500, 1000 and 2000 mg/L SS respectively). These values indicates higher degradation rates with increasing microbial concentration. However similar to 3 g/L_{resin} load, a decrease in microbial activity was observed when the rates were expressed as $mg_p/mg_{ss}/d$, (0.075 $mg_p/mg_{ss}/d$ for 500 mg/L SS, 0.073 $mg_p/mg_{ss}/d$ for 1000 mg/L SS and 0.054 $mg_p/mg_{ss}/d$ for 2000 mg/L). ANOVA test for the three sets of data shows no significant difference ($p = 0.352$), however a lower p value was observed when compared to the p value obtained for 3 g/L_{resin} load.

Test of significance suggest that there is no significant effect on perchlorate degradation rate with varying microbial concentration. When the final percentage of perchlorate degraded is calculated for the three suspended solids concentration, we observe an increase in the amount degraded for the 20 g/L_{resin} loaded resin (Figure 4.11). However, there is not much change for the 3 g/L_{resin} loaded resin.

This increase can be explained with the substrate-utlizing model (Equation 4.1). dS/dt is directly proportional to the microbial concentration (X). Hence, higher the microbial concentration, higher is the rate at which the substrate is utilized. However, this is true only for perchlorate degradation in waters, where the perchlorate ions are freely available for microbial uptake. Since the perchlorate ions are attached to functional groups in the ion-exchange resin, their availabiltiy is restricted by pore size and the percentage of microbes which is in contact with the resin bead. Hence a linear relationship is not observed in Figure 4.11.

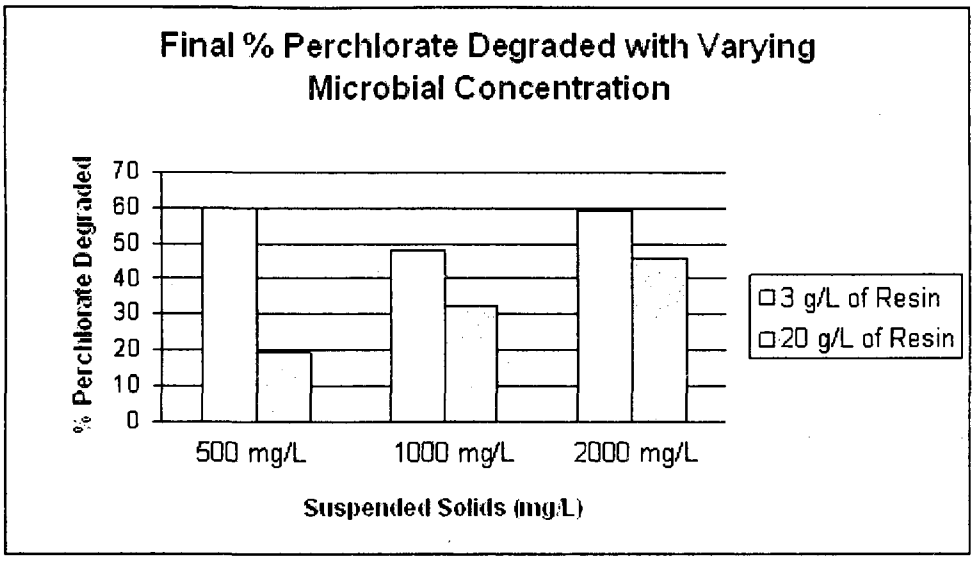


Figure 4.11: Final % Perchlorate Degraded with Varying Microbial Concentration

In case of low resin load (3 g/L_{resin}), the surface area occupied by perchlorate ions in the resin is much less compared to high perchlorate loads (Figure 4.6). Hence, even when exposed to high microbial concentration, the low surface area allows only a limited amount of bacterial cells to reach perchlorate sites and degrade it efficiently. Based on these results, it can be stated that microbial concentration have limited effect on perchlorate degradation rate in ion-exchange resins with high perchlorate loads.

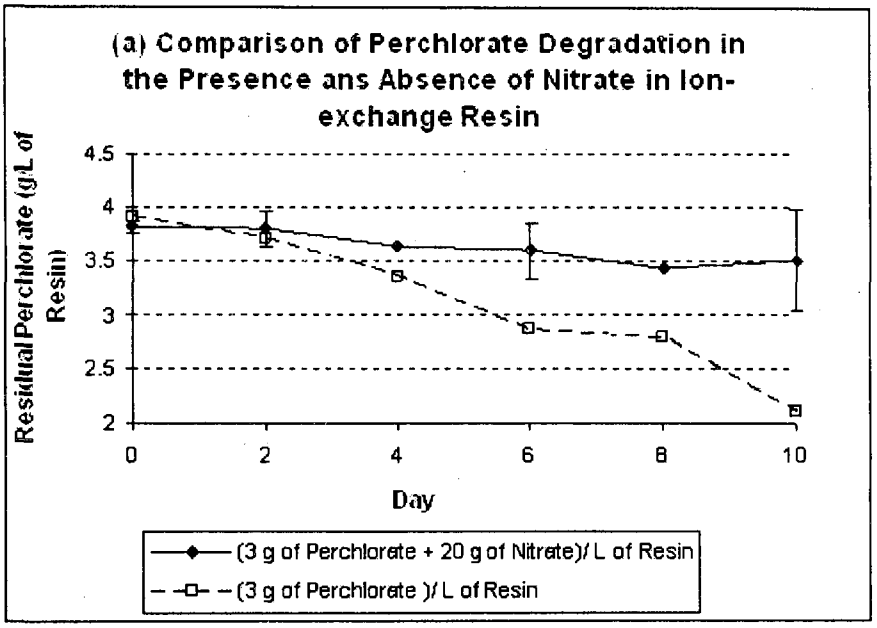
4.4.3. Effect of Nitrate Load on Perchlorate Degradation Rate in Ion-exchange Resin

Comparison of perchlorate degradation curves in the presence and absence of nitrate in ion-exchange resin is shown in Figure 4.12 a and b, for initial perchlorate load of 3g/L_{resin} and 20 g/L_{resin} respectively. Percent of perchlorate degraded is compared in Figure 4.13. Figure 4.12 (a) shows very less perchlorate degradation in the presence of

nitrate. Only 16.25 % perchlorate degradation was achieved in the presence of 20 g nitrate/ L_{resin}, while in the absence of nitrate 46.23 % perchlorate removal was achieved for 3 g/ L_{resin} initial perchlorate load in ten days.

For high perchlorate load (20g/L_{resin}), almost no perchlorate degradation was observed in the first four days (Figure 4.12 b). However after day 4, perchlorate was degraded rapidly by PRBs. Eventually, higher perchlorate removal was achieved (52.33 %) in the presence of nitrate when compared to the perchlorate degradation in the absence of nitrate (45.19 %) in a span of ten days. Perchlorate degradation rates in the presence of nitrate is summarized in Table 4.9.

The 4 day lag period observed in perchlorate degradation is due to the presence of nitrate. PRBs prefer nitrate as electron acceptors over perchlorate, and carry out denitrification process (Xu *et al.*, 2003; Logan, 1998). Denitrification process was confirmed by the presence of nitrogen bubbles in the reactor tubes. These bubbles were not observed in the absence of nitrate. Once all nitrate was denitrified, perchlorate degradation took place rapidy in the reactor tubes. Figure 4.12 (b) confirms nitrate reduction prior to perchlorate reduction similar to that taking place in waters.



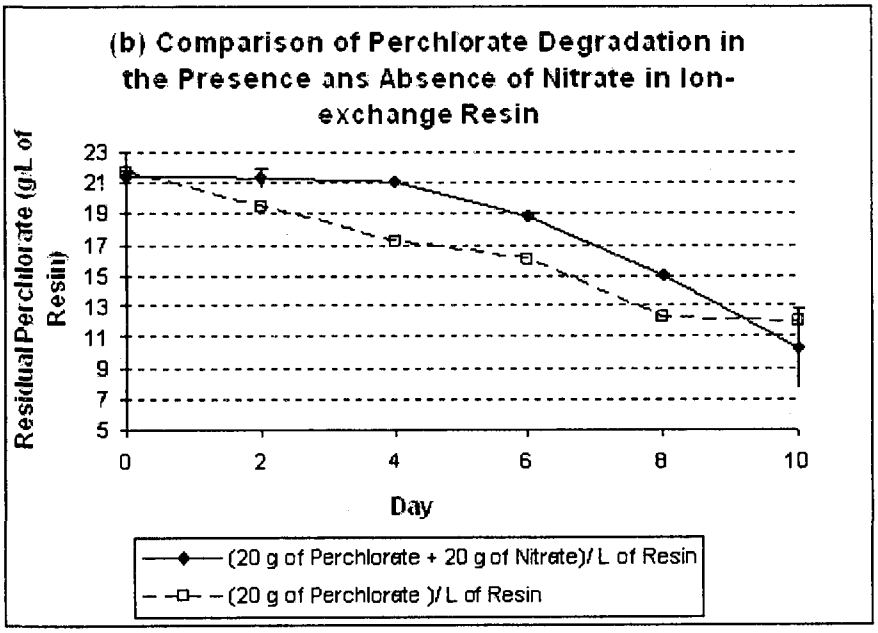


Figure 4.12: Comparison of Perchlorate Degradation in the Presence ans Absence of Nitrate in Ion-exchange Resin: (a) 3g Perchlorate/ L_{resin}; (b) 20g Perchlorate/ L_{resin}

(Error bar = Standard Deviation)

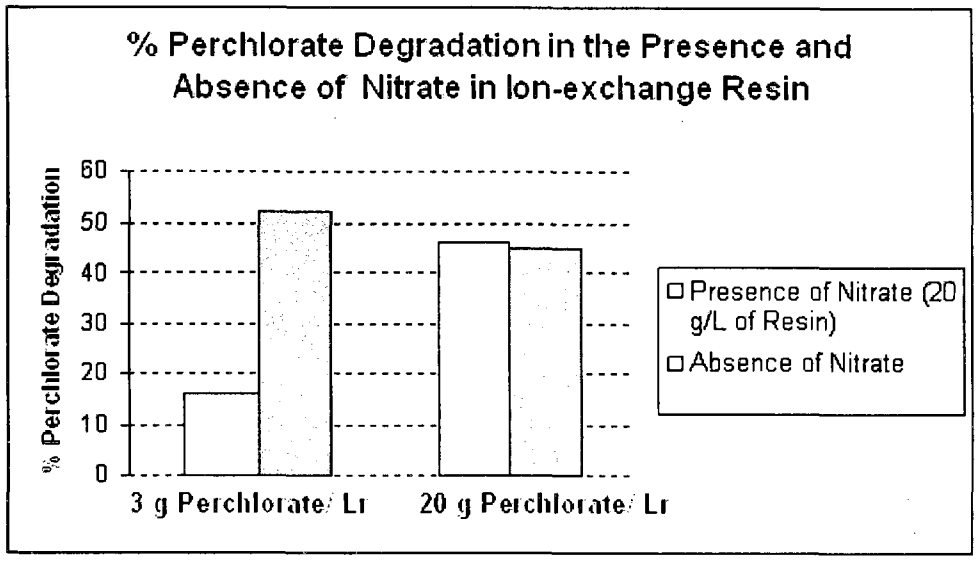


Figure 4.13: % Perchlorate Degradation in the Presence and Absence of Nitrate in Ion-exchange Resin

Table 4.9: Perchlorate Degradation Rates in the Presence of Nitrate

Initial Perchlorate Load in Resin (g/L_{resin})	Initial Nitrate Load in Resin (g/L_{resin})	Day	Perchlorate Degradation Rate (mg_p/d)
3.7	20	2	0.09
		4	0.04
		6	0.22
		8	-
		10	0.26
21.4	20	2	0.14
		4	0.30
		6	2.21
		8	3.86
		10	4.70

During perchlorate degradation in the absence of nitrate, suspended solids in the reactor tubes kept decreasing. This is due to the production of insufficient biomass during perchlorate degradation to compensate for biomass decay. In the presence of nitrate however, suspended solids increased during the first four days. But after four days, the suspended solids started to decrease (Figure 4.14). The increase in suspended solids may be due to the biomass production from denitrification process. The suspended solids concentration increased rapidly from 1000 mg/L to more than 2000 mg/L within four days. This suggest that nitrate ions are preferred by the substance released by PRBs when compared to perchlorate ions in ion-exchange resin. The relative difference may be due to the lower affinity of nitrate ions to the functional group. Also nitrate reduction prior to perchlorate reduction occurs in the resin similar to that taking place in water (Herman and Frankenberger, 1999; Chaudary *et al.*, 2002; D. Wu *et al.*, 2008; Nozawa-Inoue *et al.*, 2005). Higher microbial production resulting from denitrification of nitrate might be the reason for higher perchlorate degradation rate after four days.

Statistical analysis was performed to determine whether there is a significant difference in perchlorate degradation in the presence and absence of nitrate. The results from the single factor ANOVA test at 95% confidence level show that the difference is not significant ($p = 0.39$ for 3g perchlorate/L_{resin} load and $p = 0.54$ for 20 g perchlorate/L_{resin} load).

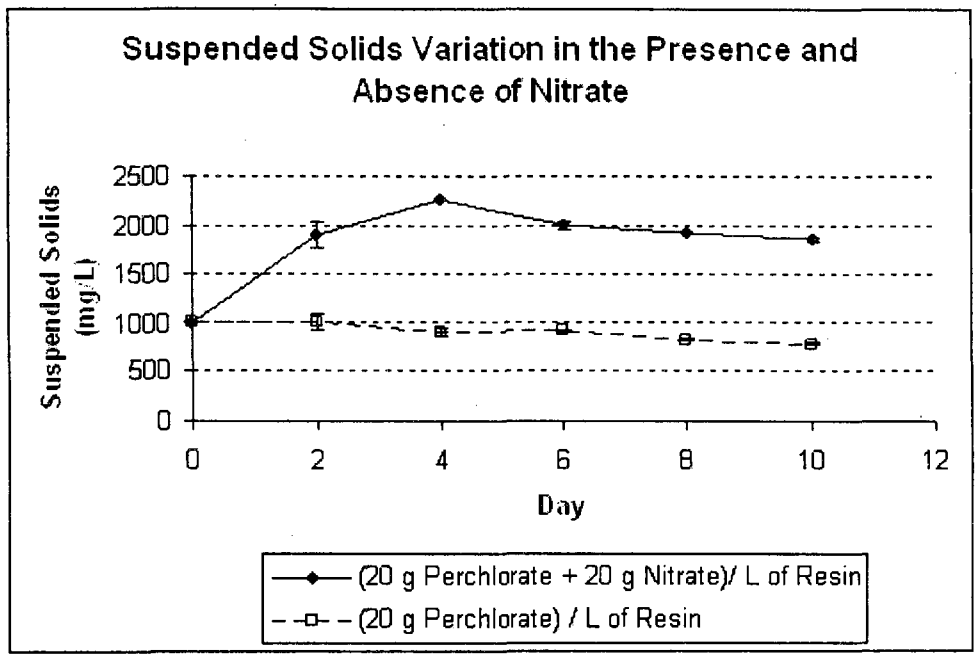


Figure 4.14: Suspended Solids Variation in the Presence and Absence of Nitrate

4.5. Conclusions

This study aimed to investigate some of the mechanisms involved in the bioregeneration of a gel-type anion exchange resin. It is suggested from the results that perchlorate degradation in resin is diffusion limited. The rate of degradation may depend on the diffusivity of the substance released by PRB, pore length and the distribution of perchlorate ions in the resin bead. In general, perchlorate ions present in the outer surface/region of the resin bead is easily available for PRB uptake when compared to ions that are attached deep inside the resin. Hence with time, perchlorate degradation in ion-exchange resin slows down once all the perchlorate ions are utilized from the outer region of the bead. Effect of nitrate on perchlorate degradation in very similar to that taking place in waters. The lag time observed for perchlorate degradation is due to the denitrification process taking place prior to perchlorate degradation. Denitrification

process is confirmed by the presence of nitrogen bubbles in the tube reactors. The key findings of this research are:

1. Higher initial perchlorate load in ion-exchange resin results in higher degradation rate (mg_p/d) similar to that taking place in waters. 19.6 mg of perchlorate was degraded in five days for 20 g/L_{resin} load, while only 3.6 and 4.2 mg removal was achieved for 3 and 5 g/L_{resin} load respectively, under similar conditions. Suspended solids reduced in the reactor due to insufficient production of microbes to compensate for biomass decay.
2. Variation in suspended solids did not have a significant effect on perchlorate degradation. However, in resin with high perchlorate load (20 g/L_{resin}), higher perchlorate degradation was observed in the presence of high suspended solids. 46% perchlorate removal was achieved with 2000 mg/L suspended solids, while only 32% and 19% removal was observed with 1000 and 500 mg/L suspended solids respectively. Hence it can be stated that microbial concentration have limited effect on perchlorate degradation rate in ion-exchange resins with high perchlorate loads.
3. Nitrate has similar effect on perchlorate degradation in ion-exchange resins as in the case of waters. Denitrification occurs prior to perchlorate degradation. Reduced perchlorate removal was observed in resins with low perchlorate load (3 g/L_{resin}) in the presence of nitrate. In high perchlorate loaded resins (20 g/L_{resin}), no perchlorate removal was observed during first four days of the experiment, after which rapid perchlorate degradation was observed. The lag phase is the period during which PRBs carry out denitrification of nitrate.

CHAPTER 5

MULTI-CYCLE BIOREGENERATION OF GEL TYPE ANION EXCHANGE RESIN CONTAINING PERCHLORATE IN A FLUIDIZED BED REACTOR

5.1. Introduction

Excessive use of perchlorate (ClO_4^-) as an oxidizer in rocket fuels and munitions over the years have lead to the release of this contaminant in large quantities in surface and groundwaters. Perchlorate has been detected in more than 270 sites all over United States, out of which more than 45 sites are in the National Priority List (EPA, 2008). Perchlorate is highly soluble and stable compound and hence can have an extensive plume in groundwaters (EPA, 2008). Various technologies exist to remove perchlorate from water, out of which biological reduction and ion-exchange technology are prominent. Biological reduction of perchlorate is a well established process for perchlorate removal. There exist several species of microorganisms that are capable of utilizing perchlorate as electron acceptors for their metabolisms (Urbansky and Schock, 1999). Perchlorate has been degraded successfully in various bioreactors using these microorganisms; commonly known as Perchlorate Respiring Bacteria (PRB) (Wallace *et al.*, 1998; Herman and Frankenberger, 1999; Kim and Logan, 2001).

Ion-exchange technology is the most common process that is being used at present to remove perchlorate from drinking water. In this process, perchlorate ions replace other innocuous anions due to its affinity for the ion exchange functional group. Development

of ion-exchange technology resulted in the production of selective or specialty resins for perchlorate removal. These resins have very low affinity towards divalent anions such as sulfate and hence have very high efficiency in removing perchlorate ions due to less interference (Baruth, 2005). Some commercially available perchlorate selective resins are shown in Table 5.1. But most of these resins cannot be regenerated and are disposed by incineration after one time use (Gingras and Batista, 2002). Due to their high cost, disposal of these resins after one time use makes the ion-exchange technology incomplete and economically unsustainable for perchlorate removal.

Table 5.1: Commercially Available Perchlorate Selective Resins

Name	Matrix/ Type	Functional Group	Water Retention Capacity (%)
DOWEX PSR-3	Styrene-DVB, Macroporous	Tri-n-butyl amine $(C_4H_9)_3N$	50-65
DOWEX PSR-2	Styrene-DVB, Gel	Tri-n-butyl amine $(C_4H_9)_3N$	40-47.5
A530E	Polystyrene-DVB, Macroporous	Quaternary Ammonium	50
A532E	Polystyrene – DVB, Gel	Bifunctional Quaternary amines	36-45
SIR-110-HP	Styrene-DVB, Gel	Tri-n-butyl amine $(C_4H_9)_3N$	35-55
PWA-2	Gel	N/A	34-42

(Source: Compiled from the websites of the resin manufacturers: Dow; Purolite; Resintech, Inc; Rohm and Haas; Lanxess.)

Synthetic ion-exchange resins are classified into gel (microreticular) and macroporous (or macroreticular) with respect to their polymeric background (Crittenden *et al.*, 2005). Gel-type resins have lower degree of cross-linking when compared to macroporous resin. Gel-type resins usually have about 4 – 10% DVB cross-linking, while macroporous resins have about 20 – 25 % cross-linking (Crittenden *et al.*, 2005). Gel-type resins are translucent and have high water content. Hence they lose their pore structure upon drying. In comparison, macroporous resins are made by linking microspheres, making them more rigid. They can maintain their pore structure and integrity even when dried (Crittenden *et al.*, 2005).

In gel-type resin the pore size is determined by the distance between polymer chains and crosslinks of the swollen gel matrix (Kunin, 1976). These pores are extremely small and hence restrict the uptake of large ions. The pore size of a typical gel type resin is between 5 and 50 A^0 (0.0005 and 0.005μm) (Pietrzyk, 1969), whereas for a macroreticular resin it can be up to 10,000 A^0 (1 μm) with an average value of 600 A^0 (0.6 μm) (K.A. Kun and R. Kunin, 1968).

Bioregeneration of ion-exchange resin is a new concept which involves the direct contact of spent resin with perchlorate reducing bacterial culture. This process does not require the use of concentrated brines for the regeneration of resins. The ion-exchange bioregeneration technology has been developed recently and is a patented process (Batista and Jensen, 2006). Bioregeneration of macroporous resins have been carried out successfully in the past (Batista *et al.*, 2007). However, bioregeneration of gel-type resins have not been investigated to date. Gel-type resins have smaller pores than macroporous resins and are more susceptible to biofouling, a major concern in

bioregeneration. Yet, gel-type resins have higher capacity than macroporous resins and most commercially available perchlorate-selective resins are gel-type. From an environmental point of view, resin bioregeneration is more sustainable than one-time use followed by incineration. Resin incineration generates carbon dioxide, a greenhouse gas. However, the costs of resin bioregeneration include carbon source, nutrients, bio-fouling removal and resin rinsing. To be economically viable, resin bioregeneration has to be performed for several cycles. The number of cycles that will make the process viable depends on many factors including fresh resin cost, loading of perchlorate and other anions in the resin, degree of bio-fouling, and resin loss in the process. Another factor that may significantly affect resin bioregeneration is "carbon credits". This is especially important for California, where perchlorate contamination is widespread and new regulations are in place to provide "carbon credits" to firms and agencies, including water utilities that reduce carbon dioxide generation.

The objective of this research is to investigate the feasibility of the bioregeneration process for a gel-type anion exchange resin and to gain insight into potential mechanisms that control gel-type resin bioregeneration. Three cycles of bioregeneration will be performed.

Bioregeneration of gel-type ion-exchange resin will be performed in a Fluidized Bed Reactor (FBR). FBR is a combination of a packed-bed and a stirred tank continuous flow reactors. Due to the fluidization of the media, excellent mass and heat transfer occurs between the fluid and solid particles (Werther and Hartge, 2003). However, if fluidization continues for a long duration it may lead to attrition of resin beads and erosion of the reactor material.

5.2. Materials and Methods

5.2.1. Culture Enrichment

Perchlorate-degrading enrichment culture for the experiments was taken from two seed enrichment cultures, BALI 1 and BALI 2 which have been maintained in the Environmental Engineering Laboratory for over 8 years. These two seed cultures were enriched from samples taken from Lake Mead and the Las Vegas Wash, and was kept alive by feeding and wasting the culture weekly. For this research five liters of the seed culture were taken and grown in a separate 20 gallon master bioreactor. This bioreactor was equipped with monitoring probes for Dissolved Oxygen (DO), Oxidation Reduction Potential (ORP) and pH. The bioreactor was sealed completely to ensure anaerobic environment for the culture. The reactor was also purged with nitrogen gas to remove any oxygen present in the head space. The culture was mixed by a stirrer to keep the bacteria in suspension.

The microbial culture was grown progressively to a volume of twenty liters by feeding it with perchlorate, acetate and nutrients. The nutrient mixture used is shown in Table 5.2. Acetate was used as the carbon source and electron donor for the bacterial culture. An acetate-to-perchlorate mass ratio of 3:1 was maintained in the bioreactor for sufficient supply of electrons. The pH in the culture was maintained between 7 and 8 using phosphate buffer, which is the optimum range for PRB growth. The culture was monitored daily by measuring DO, ORP, pH, Conductivity, Suspended solids and COD (as a measure of acetate content). Table 5.2 summarizes the nutrients and their concentration used to prepare the microbial media, electron donor and buffer stock.

Table 5.2: Stock solution for culture enrichment

Solution Name	Components	Concentration of stock (g/L)
Electron donor/ carbon source	CH_3COO^- (Sodium form)	120
Buffer	K_2HPO_4	155
	$NaH_2PO_4.H_2O$	97.783
	$NH_4H_2PO_4$	50
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

5.2.2. Resin Loading

SIR-110-HP (Resin Tech, West Berlin, NJ) gel-type anion exchange resin was used in this study. Some characteristics of the resin are shown in Table 5.3. Five hundred ml of the resin was used for the first cycle. The resin was loaded with perchlorate, nitrate, sulfate, chloride and bicarbonate to simulate typical resin loading found in industrial water contaminated with perchlorate. Concentrations of ions loaded in the ion-exchange resin are shown in Table 5.4.

Table 5.3: Characteristics of SIR-110-HP

Characteristics	Details
Polymer Structure	Styrene with DVB
Functional Group	Tri-n-butyl amine $(C_4H_9)_3N$
pH Range	0 - 14
Ionic Form	Chloride
Water Retention	35 – 55 %
Solubility	Insoluble
Swelling	~ 12 %
Temperature Range	35 – 104 ^{0}F
Total Capacity	0.6 meq/mL

Table 5.4: Concentration of Anions for Resin Loading

Component	Concentration before loading (mg/L)	Concentration after loading (mg/L)	Resin load g/L_{resin}	% Capacity occupied in the ion-exchange resin
Perchlorate	31910	2.46	31.91	53.45
Nitrate	605.6	2.65	0.602	1.62
Sulfate	651.7	304	0.348	1.21
Chloride	663.55	5374	-	-
Bicarbonate	500	683	-	-

One liter of resin was mixed with one liter of stock solutions of desired concentration of perchlorate, nitrate, sulfate, chloride and bicarbonate ions (Table 5.4) in a three liter jar using a rotary mixer (Associate Design & Mfg. Co., Alexandria, VA). Nitrate, sulfate, chloride and bicarbonates are common contaminants that are present in perchlorate contaminated waters. Hence to simulate typical resin load from contaminated waters, these anions were added during the loading process. The contents were mixed for 16 hours to ensure equilibrium and complete loading of the ions to the ion-exchange resin.

The concentrations of anions in the resin before and after the loading process were measured using Dionex ICS 2000 Ion Chromatography (Dionex, Sunnyvale, CA). The perchlorate content in the ion-exchange resin was measured using oxygen combustion bomb 1108 (Parr Instruments, Moline, IL). After 16 hours of mixing, the solution was decanted and the resin was washed ten times with 2 liters deionized water to remove residual ions. The loaded resin was transferred to a labeled glass container and stored in refrigerator until the start of the experiment.

5.2.3. Resin Bioregeneration in FBR

Three cycles of bioregeneration were performed and each cycle was run for a period of 21-25 days. Experimental set up for the bioregeneration experiment is showed in Figure 5.1. A Fluidized bed reactor (FBR), 30 inches in length and 2 inches diameter plexi-glass column was used for the bioregeneration test. A self priming pump was used to circulate the perchlorate-reducing culture from the 20 gallon master reactor through the FBR. The loaded resin was then transferred into FBR and was connected to the 20 gallon master reactor through a self priming pump. The culture was then pumped through the FBR at a flowrate of 210 ml/ min (~2.5 gpm/ ft^2 of column). Five ml resin samples were taken daily via valves placed on the side of FBR. The resin samples were rinsed six times thoroughly with 50 ml deionized water and stored in refrigerator for further analysis.

Fifty ml culture sample from the 20 gallon master reactor was taken daily to monitor COD and suspended solids. The pH, ORP and DO were monitored directly from the probes placed inside the bioreactor. Throughout the bioregeneration process, the suspended solids was maintained around 1500- 2000 mg/L. Whenever the suspended

solids concentration dropped below 1500 mg/L, concentrated centrifuged culture, taken from the BALI stock culture, was added to the reactor to increase suspended solids concentration. The concentrated culture contained significant amounts of acetate and hence increased the acetate concentration as well in the bioreactor. Cycle 1 of the column test was run for 21 consecutive days. After cycle was complete, the bioregenerated resin was subjected to bio-fouling removal and disinfection.

Bio-fouling of the resin was observed after the bioregeneration process. The resins became darker in color due to the build-up of organic matter on the resin. The bio-fouling was visually much stronger than that observed in macroporous resins. However, it was possible to remove bio-fouling using a three-step procedure. Firstly, two bed volumes containing 1:1 mass ratio of 12% NaCl and 2% NaOH solution was circulated through FBR for 15 hours. Next a fresh solution of the same composition (two bed volumes) was again pumped through the FBR for 3 hours. Finally, a solution of 12% NaCl (two bed volumes) was pumped through the FBR for 2 hours. The resin was rinsed thoroughly with ten bed volumes of deionized water. The resin was disinfected using sodium hypochlorite solution (1% as chlorine) with a contact time of 15 – 20 minutes. After fouling removal the resin appearance was almost like that of fresh resin. After fouling removal, the resin was again rinsed with fifteen bed volumes of deionized water and stored in refrigerator until the start of next bioregeneration cycle.

The bioregenerated resin from cycle 1 was again loaded with the same amount of perchlorate, nitrate, sulfate, chloride and bicarbonate for cycle 2. Notice that the amount of resin to be regenerated in subsequent cycles will be smaller than that used in cycle 1 because resin samples were taken from each cycle. A resin sample of 360 ml and 250 ml

were loaded for cycles 2 and 3, respectively. For cycle 2 and cycle 3 bioregeneration test, a seven liter glass bioreactor was used instead of the 20 gallon master reactor for perchlorate-reducing culture enrichment. This reactor was placed on a magnetic stirrer and was sealed properly to maintain anaerobic conditions.

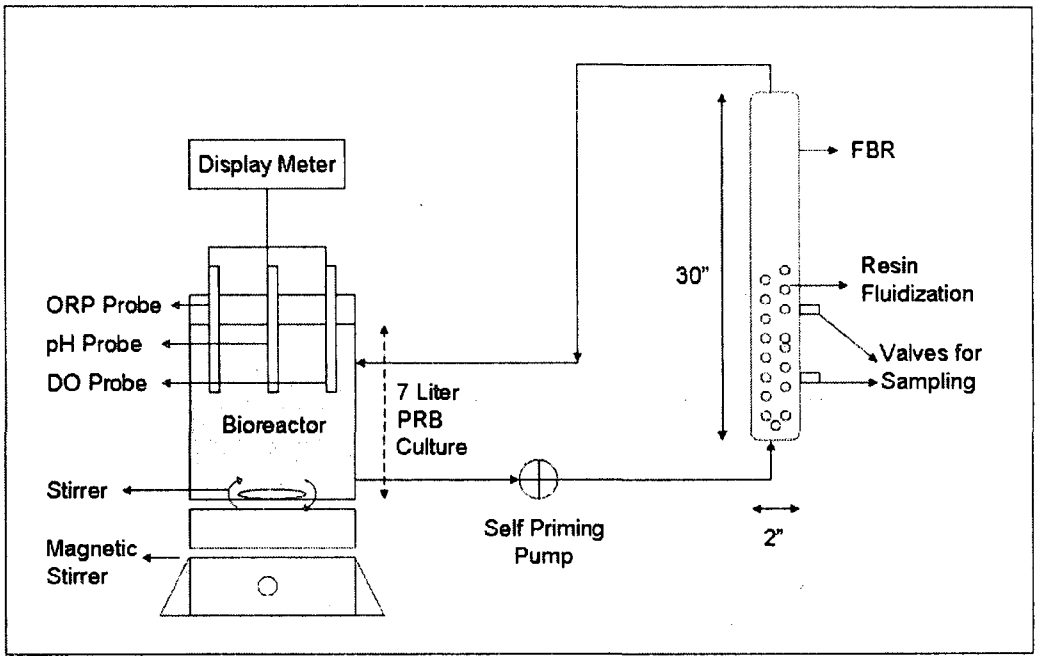


Figure 5.1: Experimental Set-up for Bioregeneration of Gel-Type Resin

5.2.4. Perchlorate Analysis in Resin

Residual perchlorate in the resin sample was analyzed using an oxygen combustion bomb 1108 (Parr Instruments, Moline, IL). The resin samples were burnt inside the combustion bomb, thus converting the residual perchlorate ions to chloride ions.

$$ClO_4^- \longrightarrow 2O_2 + Cl^-$$

Two ml of resin sample was treated with 250 ml of 10,000 mg/L nitrate and rinsed six times with 500 ml deionized water to remove excess nitrate. About 100 mg of the dried

resin sample was used for the analysis. Four hundred mg of paraffin oil (EMD Chemicals, Inc., San Diego, CA) was added to the resin sample as a combustion aid. Ten ml of 35 mMoles NaOH and 3 ml of 3% H_2O_2 was added to the bottom cylinder of parr bomb as buffer solution. Oxygen gas was then filled in the cylinder to a pressure of 30 - 35 psi and the bomb was fired inside a bucket of deionized water. The residual solution in the bottom cylinder was then analyzed for chloride concentration using ICS 2000 Ion Chromatography (Dionex, Sunnyvale, CA). QA/QC protocol was used in this research and included analysis of replicate samples, equipment calibration, and proper procedures for sample storage.

5.2.5. Analytical Methods

Concentration of perchlorate, nitrate, sulfate and chloride was determined using Dionex ICS 2000 (Sunnyvale, CA). IonPac AS16 column with 4 X 250 mm dimension was used for the analysis. Chromleon 6.70 (SP2a Build 1871) software was used to run the IC. Dionex RFIC EluGen Cartridge containing sodium hydroxide was used as the eluent. The eluent concentration for the analysis was set to 35 mM and the current was set to 100 mA for perchlorate analysis, while 30 milli-moles concentration and 110 mA current was used for nitrate, sulfate and chloride analysis.

Suspended solids concentrations were measured using a filtration apparatus with a 47 mm Whatmann glass microfiber filters (GFC) according to Standard Methods. COD analysis was performed using high range HACH COD digestion vials (Hach Company, Loveland, CO). The pH was measured using a Fisher Scientific model AR25 pH meter. The dissolved oxygen content of the culture was analyzed daily using YSI Model 58 Dissolved Oxygen meter (YSI, Inc., Warm Springs, OH). Grab samples from the

bioreactor was immediately analyzed for conductivity using YSI (Model # 30/10 FT) conductivity meter (YSI, Inc., Warm Springs, OH).

5.2.5. Statistical Analysis

Statistical analysis was performed to determine whether there is a significant difference in perchlorate biodegradation rate between the three bioregeneration cycles. The statistical significance of the differences was determined using single factor ANOVA test.

5.3. Results and Discussions

5.3.1. Preliminary Test

To confirm the strong affinity of perchlorate ions to the resin bead, preliminary column tests were performed in this research. A column of 18 inches in length and 0.75 inches diameter was used for the test. Fifty ml SIR-100 resin (Resintech, West Berlin, NJ) loaded with 12 g of perchlorate/ L_{resin} was transferred into the column. SIR-100 is a macroporous resin with high selectivity for perchlorate. SIR-100 was used during the preliminary test due to the unavailability of SIR-110-HP (gel-type) at that time. Autoclaved deionized water was circulated up-flow through the column using a Masterflex peristaltic pump (Cole-Palmer, Vernon Hills, IL) continuously for fourteen days. For the first seven days of the test, 40 % bed expansion was maintained. During the remaining seven days, the flowrate was increased and 80-100 % bed expansion was maintained in the column. Water samples from the column were collected daily and analyzed for perchlorate using Dionex ICS 2000 Ion Chromatography (Sunnyvale, CA). Perchlorate concentration was zero for all the samples tested throughout the fourteen day

period. Also residual perchlorate in SIR-100 was analyzed using oxygen bomb 1108 (Parr Instruments, Moline, IL). No difference was observed between initial and final perchlorate load in the resin. This result confirms that perchlorate ions does not dissociate from resin beads by just using water.

Another column test was performed, this time using 12 % sodium chloride (NaCl) as a regenerant for the resin. SIR-100 resin with 32 g of perchlorate/ L_{resin} load was used to test the regeneration of the resin using 12 % NaCl solution. Fifty ml of the loaded resin was transferred into the column and 500 ml of 12 % NaCl solution was pumped up-flow through the column using Masterflex peristaltic pump (Cole-Palmer, Vernon Hills, IL) maintaining a bed expansion of 50-60 %. The brine solution was pumped continuously for fourteen days, and regenerant samples were taken twice a day for perchlorate analysis. Perchlorate in the brine samples varied between 10 and 19.5 mg/L during the fourteen day period. The final perchlorate in the brine after fourteen days was 14.3 mg/L. This concentration corresponds to the approximate removal of 0.15 g of perchlorate/ L_{resin}. Only 0.47% of perchlorate was removed from the resin using 12% NaCl solution during a period of fourteen days. This result confirms the very high affinity of perchlorate ions to ion-exchange resin and disproves the possible dissociation of perchlorate ions from perchlorate-selective resins abiotically.

5.3.2. Perchlorate Degradation During Three Cycles

Figure 5.2 and 5.3 shows the combined graph of perchlorate degradation for all three bioregeneration cycles. Notice that for all three cycles, perchlorate degradation rate was fast during the first ten days, but then it slowed down significantly after ten days. There are two possible interpretations for these findings: (1) mass transfer control - perchlorate

degradation is controlled by diffusion of perchlorate from the inner portion of the resin bead into the PRB cell or (2) Kinetics control- Perchlorate degradation is concentration dependent.

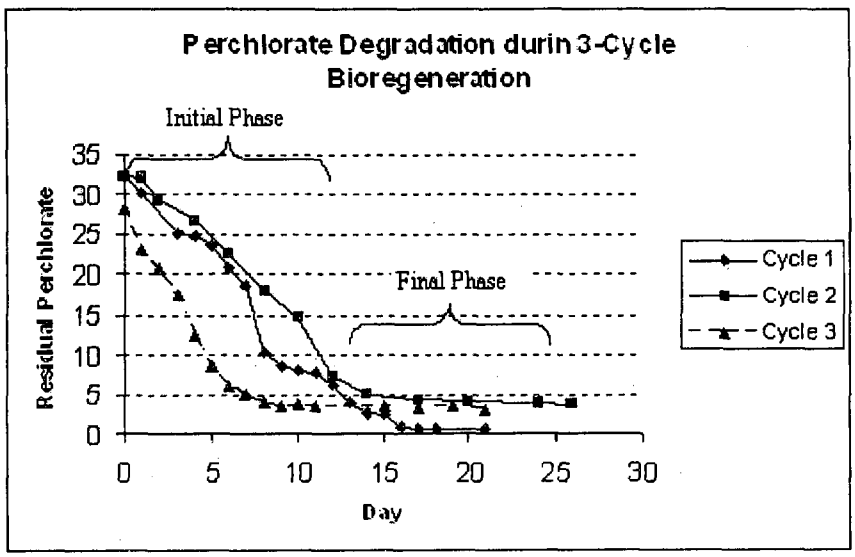


Figure 5.2: Perchlorate Degradation in 3- Cycle Bioregeneration Test

Maximum degradation occurred during the first 12 days for cycles 1 and 2, and during the first 9 days for cycle 3. Initial perchlorate load in the resin at the start of cycles 1,2 and 3 when expressed in terms of concentration (mg/L of culture) is about 10,926 $mg/L_{culture}$, 7782 $mg/L_{culture}$ and 4743 $mg/L_{culture}$ respectively. The average degradation rate expressed as mg of perchlorate degraded per mg of suspended solids per day ($mg_p/mg_{ss}/d$) during the maximum degradation period (phase 1) for cycles 1, 2 and 3 is about 0.5, 0.24 and 0.22 respectively. The observed degradation rates for the three cycles are lower than the published degradation rates for perchlorate degradation in waters (Table 5.5), even when the initial perchlorate concentration during all three cycles were much higher than the initial concentrations shown in Table 5.5.

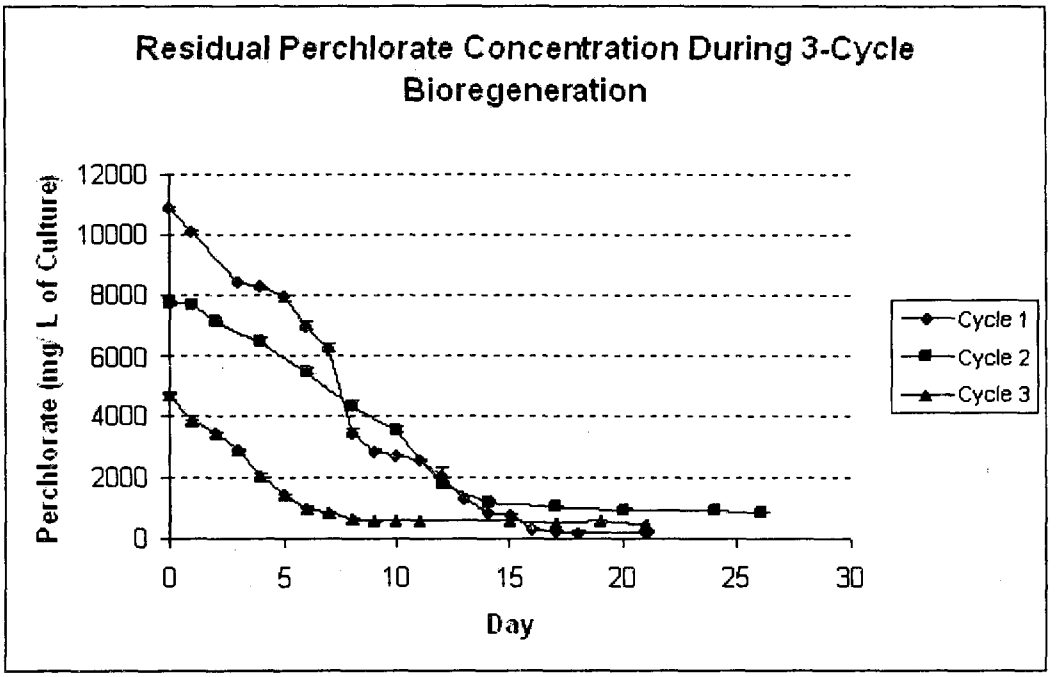


Figure 5.3: Residual Perchlorate Concentration during 3-Cycle Bioregeneration

The concentration of perchlorate in resin after 10 days of bioregeneration in cycles 1,2 and 3 is about 2697 mg/$L_{culture}$, 3540 mg/$L_{culture}$ and 596 mg/$L_{culture}$, and the respective degradation rates from tenth day to the end of the cycle are 0.18 $mg_p/mg_{ss}/d$, 0.06 $mg_p/mg_{ss}/d$ and 0.003 $mg_p/mg_{ss}/d$ respectively. Perchlorate degradation rates for all three cycles are summarized in Table 5.6. The perchlorate degradation rates from tenth day to the end of the cycle (phase 2) are about ten times lower than the published rates of degradation in waters. Again notice that the concentrations are higher than that shown in Table 5.5. Higher degradation rate was observed for cycle 1 when compared to cycle 2 and cycle 3. In cycle 1, 97.8% perchlorate degradation was achieved in a span of 21 days. However in cycles 2 and 3, only 88.3% and 89.7% respectively, was achieved in 21 days. This may be because the resin used in cycle 1 was cleaner. Eventhough bio-

fouling was treated after every cycle, it is very much possible to have some amount of fouling to be present in the resin after treatment, which can affect the mass transfer of perchlorate ions from the resin during bioregeneration process. This might possibly be the reason for the observed reduction in perchlorate degradation rates for cycles 2 and 3.

Table 5.5: Perchlorate Degradation Rates in Water

Initial Perchlorate Concentration (mg/L)	Observed Degradation Rate (mg_p /mg_{ss}/day)	Theoretical Degradation Rate (mg_p /mg_{ss}/day)	Carbon Source	Reference
250	0.64	1.16 - 4.04[+]	Lactate	Shrout and Parkin, 2006
100	0.36/ 1.17	0.3/ 0.99*	Acetate	Logan *et al.*, 2001
204	1.02	1.13 - 3.9[+]	Lactate	AWWA, 2004

[+]Calculated using K_s and q_{max} from Logan *et al.*, 2001 and C.Wang *et al.*, 2008;

* Calculated using K_s and q_{max} from Logan *et al.*,2001.

Table 5.6: Perchlorate Degradation for 3-Cycle Bioregeneration

Cycle #	Initial ClO_4^- Conc. in FBR ($mg/L_{culture}$)	Average ClO_4^- Degradation Rate during first 10 days ($mg_p/mg_{ss}/d$)	Theoretical Degradation Rate ($mg_p/mg_{ss}/d$)	ClO_4^- Conc. in FBR after 10 days ($mg/L_{culture}$)	Average ClO_4^- Degradation Rate from tenth day to the end of cycle ($mg_p/mg_{ss}/d$)	Theoretical Degradation Rate ($mg_p/mg_{ss}/d$)
1	10,926	0.5	1.32 – 4.33*	2697	0.18	1.30 – 4.31*
2	7782	0.24	1.31 – 4.33*	3540	0.06	1.31 – 4.31*
3	4743	0.22	1.31 – 4.32*	596	0.003	1.25 – 4.21*

*Calculated using K_s and q_{max} from Logan *et al.*, 2001 and C.Wang *et al.*, 2008

There have been demonstrations that perchlorate degradation in water follows Monod's kinetics (C. Wang *et al.*, 2008). Some insight can be gained, when evaluating the results obtained from analyzing Monod's equation which is given as :

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

Where, S is the perchlorate concentration (mg/L); t the time (d); q_{max} is the maximum specific perchlorate removal rate (d^{-1}); K_s is the half saturation constant for perchlorate (mg/L); and X is the microbial concentration (C. Wang *et al.*, 2008). Dividing both sides of the equation by the microbial concentration (X), we can get the rate of substrate utilization per unit microbial concentration (rate):

$$\frac{1}{X}\frac{dS}{dt} = -\frac{q_{max} S}{S + K_s} \tag{5.2}$$

A theoretical plot of the rate of degradation versus the initial perchlorate concentration (S) is shown in Figure 5.4. Theoretical degradation rates were calculated at average, low and high values of K_s and q_{max} reported in the literature (Table 5.7), and also from values obtained from Logan *et al.*, (2001) and C. Wang *et al.* (2008) (Figure 5.4 a and b). Figure 5.4 a shows that the maximum perchlorate degradation rates are about 2.4 mg perchlorate/mg SS/day for average values. Perchlorate degradation rates increase with increasing perchlorate concentrations, but it levels off for perchlorate concentrations above 5000 mg/L. Also, perchlorate degradation rate increases with increase in K_s and q_{max} values as shown in Figure 5.4 (a and b). The degradation rates obtained during phase 1, except for cycle 1, are much smaller than those observed for perchlorate degradation in

waters. Phase 2 degradation rates were several orders of magnitude smaller than those for waters.

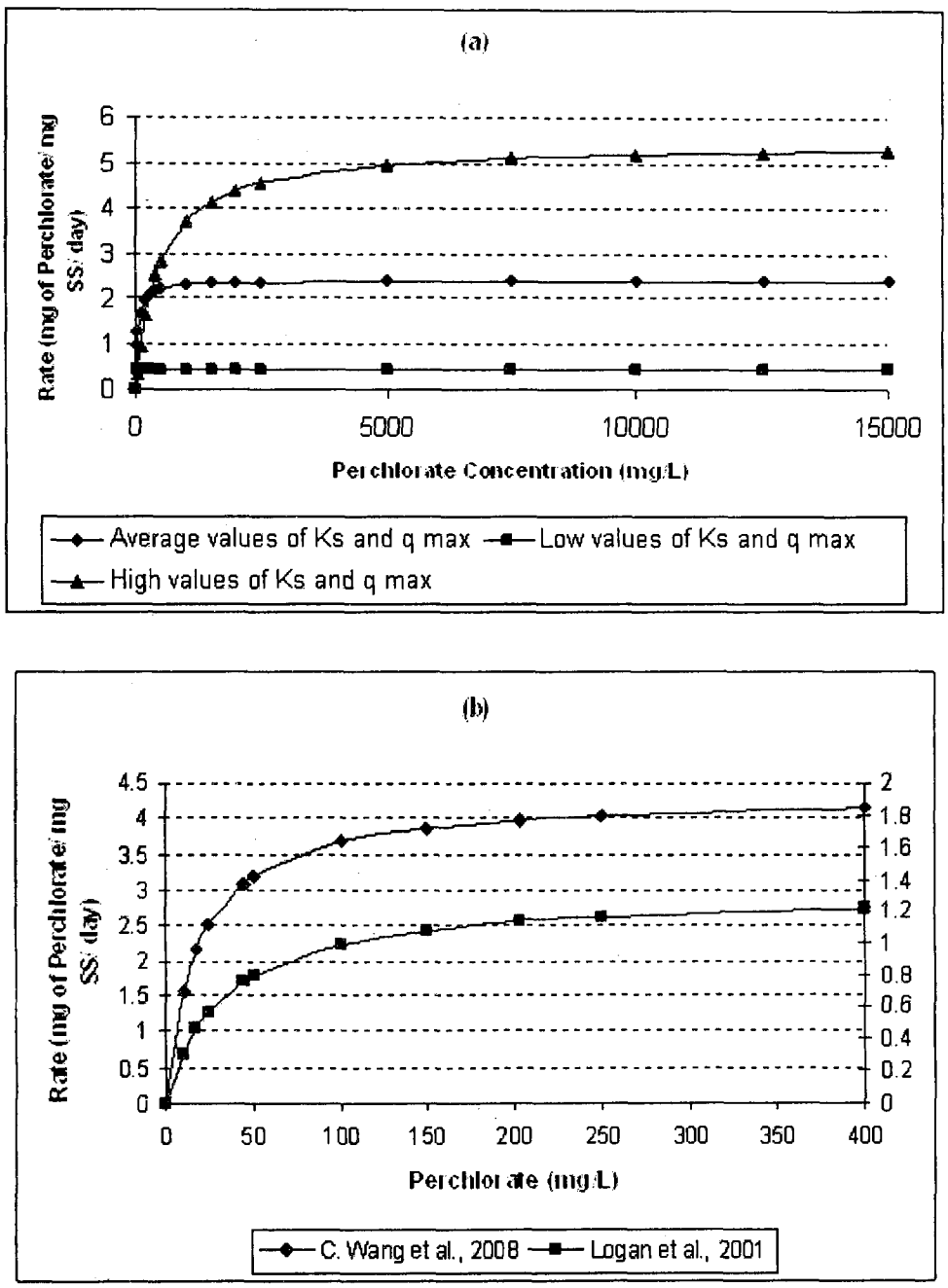


Figure 5.4: Variation of Degradation Rate with Initial Substrate Concentration: (a) Plotted with Average, Low and High K_s and q_{max} values; (b) Plotted with K_s and q_{max} values obtained from Logan *et al.*(2001) and C. Wang *et al.*(2008)

The published values for degradation rates in water also differ from the theoretical values. This is because, the half saturation constant varies within a wide range from 0.14 to 470 mg/L, while q_{max} varies between 0.41 to 5.42 d^{-1} as shown in Table 5.7. Theoretical value range was calculated using the average K_s and q_{max} values, while the published perchlorate degradation rates might have been observed at different K_s and q_{max} values. However the difference in rate between theoretical values and published results are much less compared to the difference between degradation rates in the resin and theroretical values.

Table 5.7: Kinetic Parameters for Perchlorate Degradation in Waters

Culture	Kinetic Parameters		Electron Donor	Reference
	$q_{max}(d^{-1})$	K_s (mg/L)		
KJ	1.32	33 ± 9	Acetate	Logan *et al.*, 2001
PDX	0.41	45 ± 19	Acetate	Logan *et al.*, 2001
SN1A	4.60	2.2	Acetate	C.Wang *et al.*, 2008
ABL1	5.42	4.8	Acetate	C.Wang *et al.*, 2008
INS	4.34	18	Acetate	C.Wang *et al.*, 2008
PC 1	3.09	0.14	Acetate	Waller *et al.*, 2004
HCAP-C	4.39	76.6	Acetate	Dudley *et al.*, 2008

Diffusion might possibly be the reason for the observed reduction in perchlorate degradation during phase 2. If a substance is released by PRBs to detach perchlorate from the resin bead, it would be relatively easy for the substance to reach perchlorate attached to the surface and outer region of the resin when compared to perchlorate ions attached deep inside the resin bead. This is because, perchlorate ions that are deep inside

the resin bead need to diffuse through pores to reach outside the resin and become available for PRB uptake. The amount of time for this diffusion will depend on pore length, pore tortuosity and how deep the perchlorate ion is present in the bead. Assuming homogeneous distribution of perchlorate ions in a spherical resin bead, a relationship between perchlorate content and the radius of the resin bead was developed in this research. Figure 5.5 shows the distribution of perchlorate (%), with resin bead radius. A resin radius of 0.705 mm, which is the radius of the resin beads used in this research, was considered.

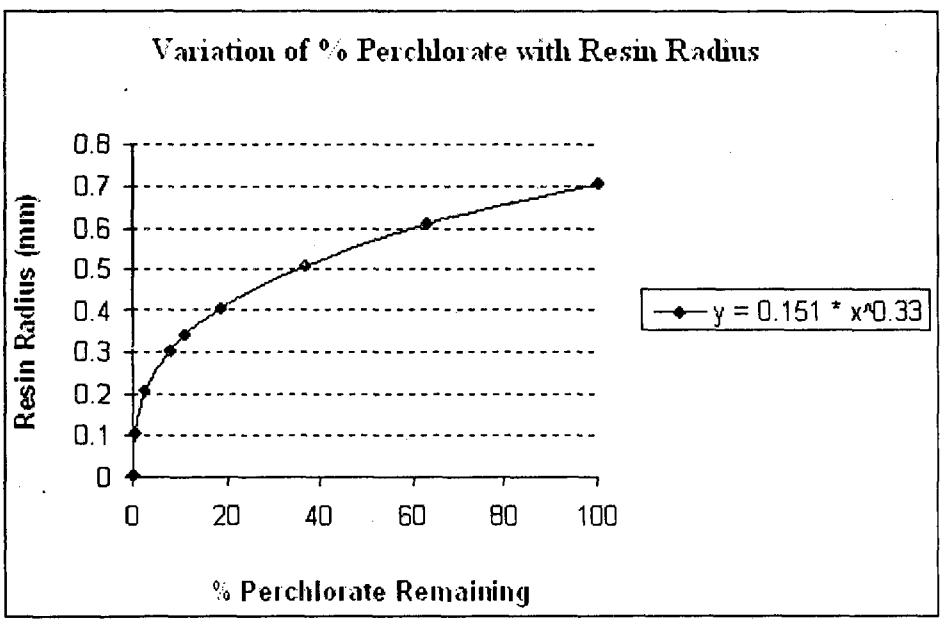


Figure 5.5: Variation of % Perchlorate with Resin Radius

Perchlorate content in the resin bead increases significantly with increase in radius. Considering 'r' to be the radius of the resin bead, the perchlorate content of the bead at a distance of 0.5r from the centre of the bead is only about 10 % of the total perchlorate content of the resin. Hence 90 % of the loaded perchlorate in the resin bead is present at

a radius greater than 0.5r. With this relationship between perchlorate distribution and resin radius, the variation of unused resin core with time is plotted for the three bioregeneration cycles (Figure 5.6). Perchlorate degradation slows down when the unused core reaches a radius of 0.2 mm for cycle 1 and a radius of 0.35 mm for cycles 2 and 3.

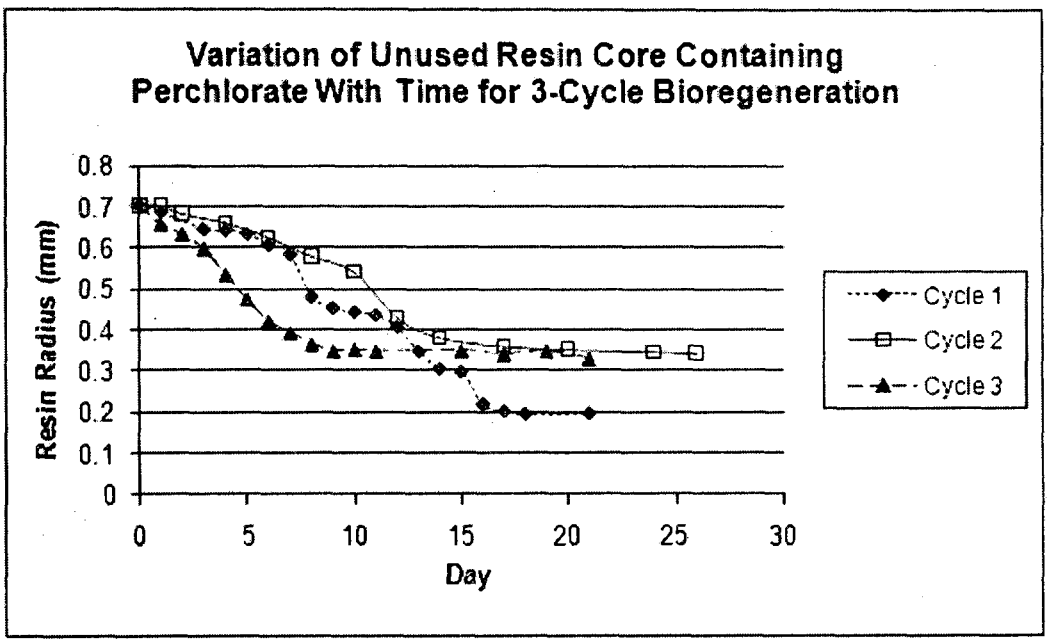


Figure 5.6: Variation of Unused Resin Core Containing Perchlorate with Time for 3-Cycle Bioregeneration

Figure 5.6 suggests that, perchlorate present in between 0.35 and 0.705 mm (total radius of resin bead) radius is more easily utilized by PRBs, while perchlorate attached deep inside the resin (< 0.3 mm radius) needs to diffuse through pores of longer path to reach outside of resin, and hence not available easily. Hence this theory suggests that perchlorate degradation deep inside the resin bead is controlled by diffusion. Due to this

reason, reduced perchlorate degradation was observed during phase 2. Since perchlorate is easily utilized from the outer region of the resin bead, it can be assumed that they will reach the water phase more easily, and hence perchlorate degradation might be kinetics controlled in this case. In general, it can be summarized that the initial phase (phase 1) of the bioregeneration process is controlled by kinetics, while the later phase (phase 2) is controlled by diffusion.

5.3.3. Bioreactor Performance

Figure 5.7 shows the variation of suspended solids concentration along with the degradation of perchlorate for all three bioregeneration cycles. Cycle 1 was started with an initial suspended solids concentration of about 3000 mg/L. Cycles 2 and 3 were started with initial suspended solids of around 2000 mg/L. The suspended solids decreased with time in all the three cycles as shown in Figure 5.7. When the suspended solids dropped to 1500 mg/L, centrifuged culture from the 20 gallon master reactor with high suspended solids was added to the bioreactor to maintain the suspended solids concentration between 1500-2000 mg/L throughout the bioregeneration experiment. The arrows shown in the graph points the days on which concentrated culture was added to the bioreactor to maintain suspended solids concentration. The decrease in suspended solids indicates that the rate of microbial decay is greater than that of microbial growth. Since acetate (electron donor) and nutrients were provided in sufficient amounts in the reactor, the unavailability of perchlorate ions is the reason for the decreased production of suspended solids.

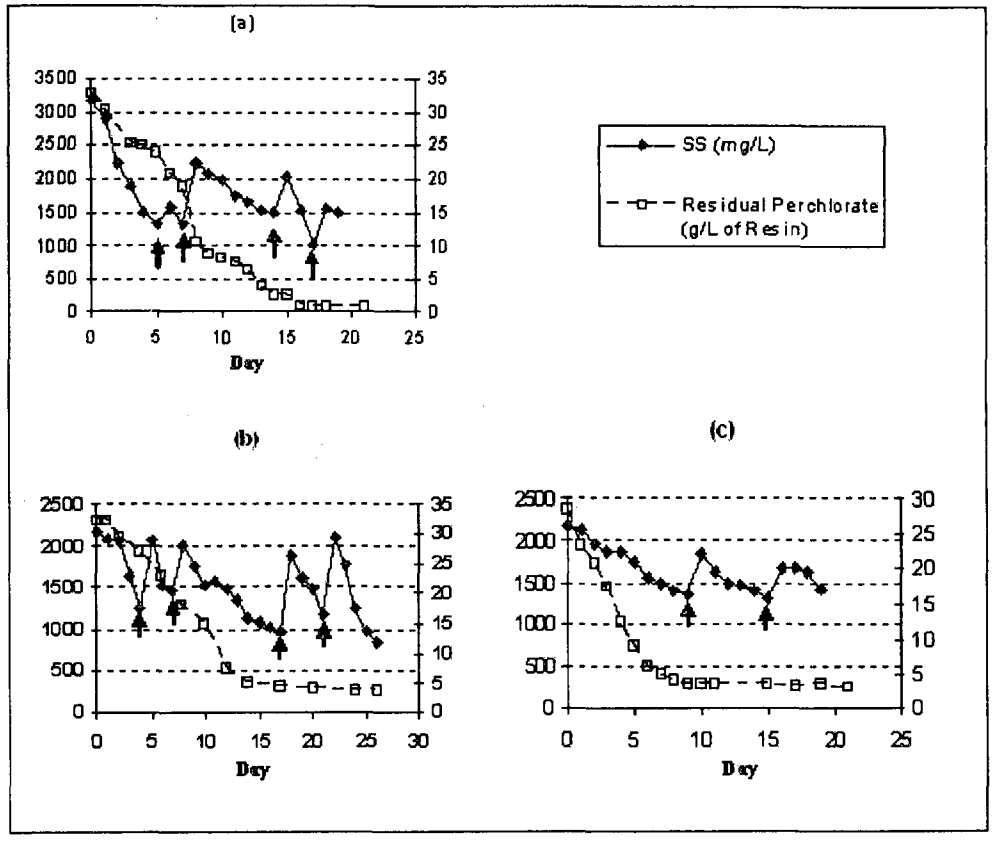


Figure 5.7: Suspended Solids Variation in the Bioreactor for 3 Cycle Bioregeneration (a) Cycle 1; (b) Cycle 2; (c) Cycle 3. The arrow mark indicates the day on which concentrated culture was added to the bioreactor to maintain high suspended solids.

The amount of residual acetate in the bioreactor was measured as Chemical Oxygen Demand (COD), since 1 mg/L acetate is approximately equal to 1 mg/L COD. The variation in COD with time for all three cycles is shown in Figure 5.8. The COD in the reactor was maintained well above the required 3:1 ratio of acetate-to-perchlorate in all the three cycles to make sure that bioregeneration process is not limited by carbon source. By the end of the bioregeneration process in all three cycles, about 3000 to 4000 mg/L of acetate was still present in the culture. Very high initial acetate concentration was present in cycle 2 (8000 mg/L) when compared to cycles 1 and 3 (about 5000 and 3000

mg/L respectively). However when comparing the rates for all three cycles, there was no significant increase in perchlorate degradation rates for cycle 2. This confirms the study from Shrout and Parkin (2006) that excessive supply of electron donor does not improve the degradation kinetics due to lack of electron acceptors and/or insufficient biomass to utilize them. The increase in acetate concentration seen in Figure 5.8 (marked by arrows) is due to the addition of concentrated culture to maintain suspended solids concentration, which contained significant amount of acetate in it. From Figure 5-9 it is clear that acetate was consumed by PRBs during perchlorate degradation.

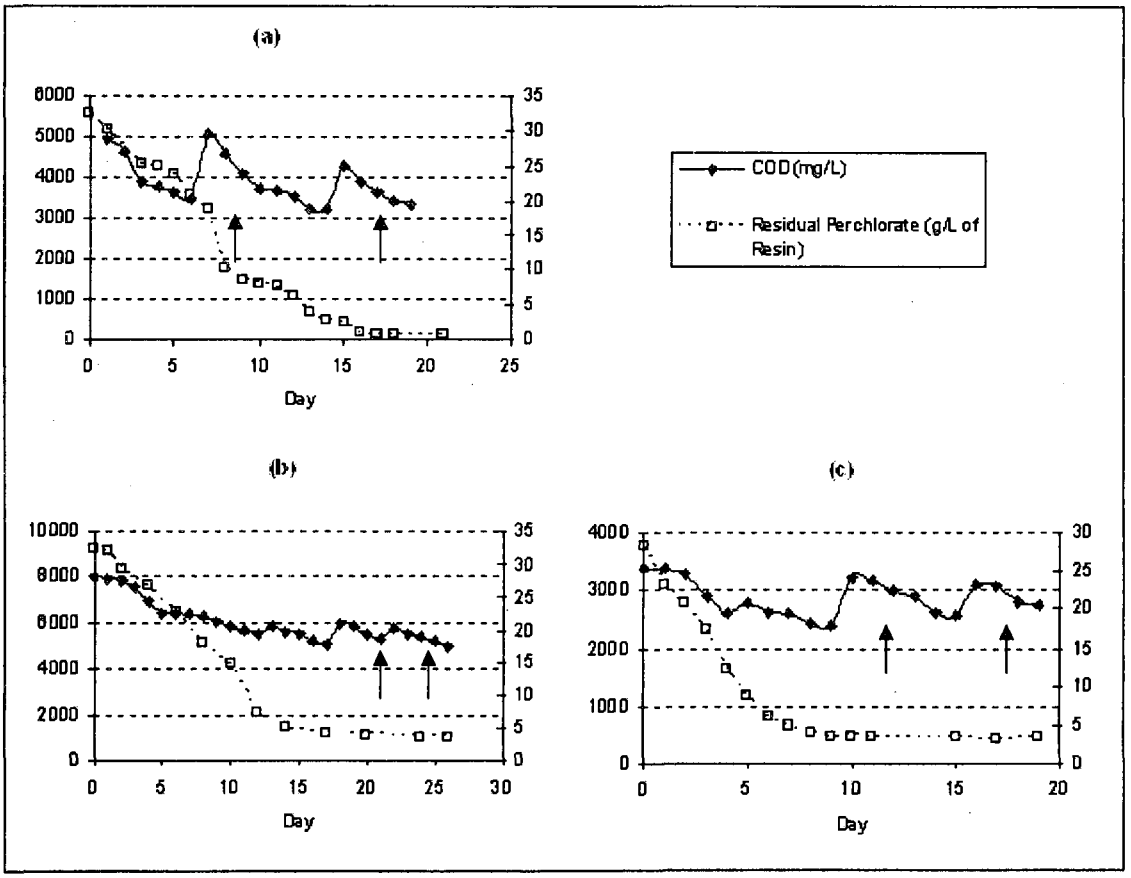


Figure 5.8: COD Variation in Bioreactor During 3 Cycle Bioregeneration

The ORP values in the bioreactor for Cycle 1 and Cycle 2 varied between -500 and -550 mV. The ORP in Cycle 2 varied between -350 and -450 mV (Figure 5.9 b). This ORP range lies well below the reported maximum ORP of -110 mV for perchlorate degradation (Attaway and Smith, 1993). The dissolved oxygen (DO) content in the bioreactor was maintained below 0.1 mg/L. However in Cycle 3, a higher DO level around 0.4 mg/L was observed on day 5 and day 6 but eventually was reduced to < 0.1 mg/L by purging the bioreactor with nitrogen gas. pH in all three bioregeneration cycles varied between 7.1 and 7.9 which is in the optimal range for perchlorate degradation (Figure 5.9 a). The conductivity during bioregeneration process varied between 18.5 and 24.9 mS (Figure 5.9 c). This increase of conductivity is due to the increase in chloride concentration, which is the end product of perchlorate degradation by PRBs.

Statistical analysis was performed to determine whether there is a significant difference in perchlorate degradation between the three cycles during phase 1. The results from the single factor ANOVA test at 95% confidence level show that the difference is significant ($p = 0.009$) for the three cycles. The difference was due to the observed higher degradation rates for cycle 1 when compared to cycles 2 and 3.

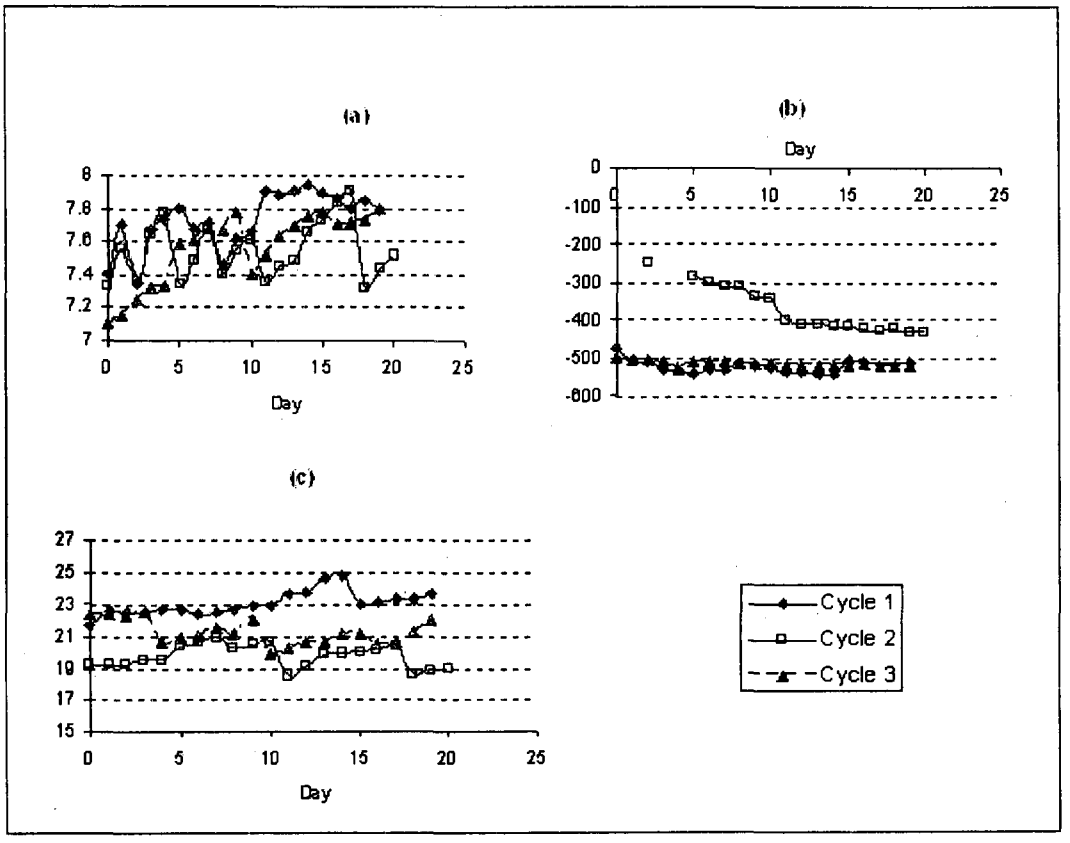


Figure 5.9: pH, ORP and Conductivity Variation in Bioreactor During 3 Cycle Bioregeneration: (a) pH; (b) ORP (mV); (c) Conductivity (mS)

5.4. Conclusions

Results from the current study confirms the feasibility of gel-type anion exchange bioregeneration, similar to the bioregeneration of macroporous anion exchange resin. The direct bioregeneration of gel-type anion-exchange resin was effective in regeneration of perchlorate loaded resin, and the resin could be reused and repeatedly regenerated with the method applied in this research.

A relationship was established between perchlorate distribution and the radius of resin bead, assuming homogeneous distribution of ions in spherical resins. Results from

this relationship suggests that perchlorate ions that are present deep inside the resin bead (< 0.3 mm radius), were not easily available for PRB uptake due to diffusion. However perchlorate present in the outer region of the resin bead were easily utilized by PRBs. Hence it can be summarized that perchlorate degradation is controlled by both kinetics and diffusion depending on the position of perchlorate ion in the resin bead.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

6.1. Conclusions

The two main objectives of this research are (a) to investigate potential mechanisms for the bioregeneration of perchlorate laden gel-type anion exchange resin; and (b) to investigate the feasibility of the bioregeneration process using a fluidized-bed reactor (FBR). To accomplish the first objective, batch biodegradation experiments were performed, while the second objective was accomplished by performing multi-cycle bioregeneration of gel-type resin using FBR. The major conclusions of this study are:

- The bioregeneration process was effective in the regeneration of perchlorate laden gel-type resin, and the resin could be reused and repeatedly regenerated. This makes the ion-exchange process for perchlorate removal economically and environmentally sustainable.
- It was hypothesized in this research that there exist a substance that is released by PRB which induces desorption of perchlorate ions from the ion-exchange resin bead prior to its biodegradation. As expected, results from experiments confirm slower perchlorate degradation rates in ion-exchange resin when compared to perchlorate degradation in waters.

- Results from bioregeneration experiments suggest that the initial phase of perchlorate degradation in ion-exchange resin is more controlled by kinetics while the final phase of bioregeneration is controlled by diffusion.
- The results from batch experiment indicates higher perchlorate degradation rate for higher initial perchlorate load in the resin. The microbial concentration has a limited effect on perchlorate degradation rate for high perchlorate loaded resin.
- The effect of nitrate on perchlorate degradation in resin depends on the perchlorate-to-nitrate ratio in the resin bead. Higher ratio creates a lag phase for perchlorate degradation during which nitrate is denitrified by PRBs. However lower ratio decreases perchlorate degradation rates in the ion-exchange resins.

6.2. Recommendations for Future Work

Much work is still required for understanding the exact mechanism by which perchlorate is degraded in ion-exchange resin. The following are recommendations for future research:

- Experiments are needed to investigate and confirm the presence of the 'substance' released by PRBs that induce desorption of perchlorate ions from the resin bead.
- Since suspended solids in the bioreactor kept decreasing with time, process modification is required to find the exact suspended solids that can be

maintained constant by perchlorate degradation in resin throughout the bioregeneration process.

- The feasibility of bioregeneration process using an FBR was tested in this study. Hence, other reactors like fixed-bed reactors should be investigated for the bioregeneration process in the future.
- The effect of nitrate, which is a co-contaminant that is present with perchlorate in waters, on perchlorate degradation in ion-exchange resin was studied in this research. However, even other co-contaminants (e.g. sulfate) also exist in waters. Hence experiments are needed to investigate the effect of other anions on perchlorate degradation in ion-exchange resin.

APPENDIX A

ANION CONCENTRATION DETERMINATION USING DIONEX ICS 2000 ION CHROMATOGRAPHY

A.1. Instrumentation

Dionex ICS-2000 Ion Chromatographic instrument controlled using Chromeleon software (version 6.70) was used during this research to measure perchlorate, chloride, nitrate and sulfate. IonPac AS16/ AS20 column with 4 X 250 mm dimension and Dionex RFIC EluGen Cartridge containing sodium hydroxide as the eluent was used for the analysis. All the instrumental components were obtained from Dionex (Sunnyvale, CA, USA). Post-column eluent suppression was carried out using an anion self-regenerating suppressor (ASRS-ULTRA II 4 mm). The IC system was fitted with a 1000-µL sample loop that was used to introduce the sample via an AS40 autosampler.

A.2. Standards

All chemicals used were of ACS reagent grade. All standards were prepared using deionized water. Stock solutions of 1000 mg/L were prepared for all standards. Appropriate dilutions of 1000 mg/L standard solution were made for studies of method linearity and the method detection limit. Standards used and conditions for analysis are summarized in Table A-1.

Table A-1: Standards and Conditions

Chemical	Form	Company	Standards	IonPac Column	Current (mA)	Eluent Conc. (mM)
Perchlorate	Sodium Perchlorate Salt	EMD Chemicals, Inc., San Diego, CA	5, 10, 25, 50, 75, 100 ppb	AS16	100	35
Nitrate	Standard Solution	EMD Chemicals, Inc., San Diego, CA	1, 2.5, 5, 7.5, 10 ppm	AS20	110	30
Chloride	Standard Solution	Ricca Chemical Company, Arlington, TX	Low Range: 100, 200, 300, 400, 500 ppb	AS16	100	35
			High Range: 1, 2.5, 5, 7.5, 10 ppm	AS20	110	30
Sulfate	Standard Solution	Ricca Chemical Company, Arlington, TX	1, 2.5, 5, 7.5, 10 ppm	AS20	110	30

A.3. Procedure

Sample preparation

- Centrifuge samples if suspended solids present.
- Use supernatant to prepare 5 ml samples in IC vials. Dilute samples to concentration within measurement range.
- Close vials with IC caps.
- Place blanks (DI water) as first and last samples
- Label vials with sample name.
- Arrange vials with increasing concentration in the vial holder.

Prime Pump

- Fill 2 L eluent bottle with DI water. Close bottle.
- Open helium gas cylinder and check for leaks.
- Open prime valve and select prime in the IC touch screen. Prime pump for 3 minutes at 3ml/min flowrate.
- Switch OFF prime and close valve.

Baseline Run

- Open Chromleon browser.
- Goto Control Panel and open 'ICS-2000-Tradaitional_system_AS40_UNLV.pan'
- Check ICS-2000 pupm 'connected' and click ON
- Set flowrate = 1.00 ml/min
- Change concentration to 30 or 35 mM in ICS-2000 Eluent Generator Setting and select ON.
- Select CR-TC to ON
- Set column temperature to 30 ^{0}C
- Set SRS current to 100 or 110 mA in the ICS-2000 detector.
- Switch 'ON' SRS Mode.
- Select 'Control' and select 'Acquisition on'. Click OK to run the baseline.
- After Signal reaches to ~0 μS, switch 'Acquisition OFF' from 'Control'.

Entering Samples in Chromleon Browser

- Create file in the corresponding folder (Perchlorate, Chloride etc.)
- Enter sample names in the file created.
- Enter dilution factors in the column.
- Select type as 'unknown' for samples and 'standard' for calibration.
- Change all status to 'Single'.
- Create a sample 'stop' after entering all samples. Select 'shutdown' for program.
- Save the changes.

Sample Analysis

- Place sample holders in the automated sampler.
- Press 'Run' button in the sampler.
- Select 'Batch' from the control panel window, and select start.
- Select the name of the file that was created, and click OK.
- Goto chromleon browser and double click on sample name to read concentration after analysis.

APPENDIX B

RESIDUAL PERCHLORATE DETERMINATION IN ION-EXCHANGE RESIN USING OXYGEN COMBUSTION BOMB 1108

For measuring residual perchlorate in resin beads, oxygen combustion bomb 1108 (Parr Instruments, Moline, IL) was used. Combustion of solid/ liquid combustible materials for chemical analysis in a closed Parr bomb has been accepted as a standard method for many years (H.W. Hilton *et al.*, 1972; Sheppard and Rodegkar, 1962). Perchlorate ions in resins are converted to chloride ion after combustion. The inorganic materials remain as ash, while all the other elements can be recovered from bomb washings.

Oxygen bomb 1108 cylinder is made up of columbium-stabilized stainless steel, which has excellent resistance to most chemicals. Separate valves are provided for charging the bomb with oxygen and releasing the gases. The firing circuit is completed by a grounded electrode, which also supports the combustion capsule.

B.1. Operating Procedure

Sample preparation

- Tare capsule, place sample in capsule and weigh.
- Add 50-200 mg of sample.
- Add paraffin oil (400 mg) as a combustion aid. Make sure the sample is fully covered with oil.
- Place bomb head on support stand and place capsule in cradle (loop electrode), snuggly.
- Cut about 10 cm of fuse wire and place one end through hole on straight electrode and the other end through the hole of the loop electrode, lower sleeve over looped wire to ensure the wire will not come lose. Once wire is in place, lower the middle of wire (while both ends are still attached to electrodes) onto the sample or pellet, making sure the wire is not touching the sides of the capsule.
- Add 10 ml 35 mM NaOH and 3 ml 3% H_2O_2 solution to the bottom cylinder.
- Push down on head and twist it to make sure it sits properly in the cylinder. Once the head is inside the cylinder, screw cap on until it stops
- Close outlet valve.

Charging the bomb with Oxygen:

- Place bomb behind face shield and put slip connector on gas inlet fitting, push down as far as it will go.
- Turn on oxygen tank. Open filling connection valve very slowly and fill until it reaches 30-35 PSI. Close valve and open release.

Place bomb in DI water:

- Submerge oxygen bomb in bucket containing DI water.
- Make sure there are no bubbles from the release valve.

Firing the Bomb:

- Connect the bomb to ignition unit with wires.
- Ignite the bomb by pressing the button.

Unloading Bomb

- Take bomb out of bucket after 20 mins.

- Slowly un-tighten the release valve.
- Once the oxygen is out of the bomb take out the head of thebomb.
- Using DI water, rinse the head of the bomb into the cylinder.
- Dilute the sample from cylinder to 500 ml in a volumetric flask.
- Measure chloride in 500 ml sample by IC.

B.2. Parr Bomb Calculator (Spreadsheet)

Parr bomb Calculator

			QC
density of wet resin	g/ml	0.62	0.62
moisture content	%	20	20
shrinkage after loading	%	22	22
weight of dried resin (at 105C for 1 hours)	g	0.1016	0.09996
Conc of Cl^-(from IC, in 500 ml)	mg/l	5.589	5.543
mass of Cl^- in sample	mg	2.7945	2.7715
% dry weight of Cl^-	%	2.750	2.773
weight of wet resin (loaded)	g	0.122	0.120
volume of wet resin (loaded)	ml	0.197	0.193
volume of fresh resin	ml	0.240	0.236
mg Cl^-/ mL of wet resin		14.211	14.325
mg Cl^-/ mL of fresh resin		11.648	11.742
ClO_4^-/ Cl^- = 99.5/35.5		2.803	2.803
mg ClO_4^-/ mL of loaded resin		39.830	40.151
mg of ClO_4^-/ ml of fresh resin		32.648	32.910

Date: 8/16/2008

Sample Description: SIR 110- Cycle 1- Day 0 QC- rinsed with 250 ml of NO3 and 6*500 ml DI

APPENDIX C

OVERALL REACTION FOR PERCHLORATE AND NITRATE BIODEGRADATION

C.1. Perchlorate Biodegradation

Electron acceptor half reaction (R_a) (Shrout and Parkin, 2006):

$H^+ + e^- + 1/8\ ClO_4^- = 1/8\ Cl^- + 1/2\ H_2O$ $\Delta G_a^0 = -94.06$ KJ/e^- eq.

Electron donor half reaction (R_d) (Rittmann and McCarty, 2001):

$1/8\ CO_2 + 1/8\ HCO_3^- + H^+ + e^- = 1/8\ CH_3COO^- + 3/8\ H_2O$ $\Delta G_d^0 = 27.4$ KJ/e^- eq.

Cell synthesis equation for ammonium as nitrogen source (R_c) (Rittmann and McCarty, 2001):

$1/5\ CO_2 + 1/20\ HCO_3^- + 1/20\ NH_4^+ + H^+ + e^- = 1/20\ C_5H_7O_2N + 9/20\ H_2O$

The amount of equivalents of electron donor that must be oxidized to supply the required energy for the cell synthesis (A) is given by (Rittmann and McCarty, 2001):

$$A = \frac{\frac{\Delta G_p}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon}}{\varepsilon \Delta G_r}$$

Where, ΔG_p is the energy required to convert acetate to pyruvate (= 35.09 – ΔG_c^0); ΔG_{pc} is the energy required to convert pyruvate carbon to cellular carbon (=18.8 KJ/e^- eq for Ammonium as nitrogen source); ΔG_r is the energy released per equivalent of donor oxidized for energy generation; ε is the energy transfer efficiency (0.6); n is a constant which accounts for the fact that ΔG_p for some electron donors is negative, meaning

energy is obtained by its conversion to pyruvate (= +1 when ΔG_p is positive and = -1 when ΔG_p is negative).

$$\Delta G_p = 35.09\text{-}27.40 = 7.69 \text{ KJ/e}^- \text{ eq}$$

$$\Delta G_r = \Delta G_a^0 - \Delta G_d^0 = -94.06 - 27.40 = -121.46 \text{ KJ/e}^- \text{ eq}$$

ΔG_p is positive, hence n = +1. Substituting the values in equation for A, we have A = 0.6058. The fraction of donor used for synthesis (f_s^0) is given by (Rittmann and McCarty, 2001):

$$f_s^0 = \frac{1}{1+A} = 0.6227$$

And the fraction of donor used for energy synthesis (f_e^0) is given by (Rittmann and McCarty, 2001):

$$f_e^0 = 1 - f_s^0 = 0.3773$$

The overall reaction (R) is given by (Rittmann and McCarty, 2001):

$$R = f_e^0 R_a + f_s^0 R_c - R_d$$

Solving for the overall equation for perchlorate degradation, we have:

$f_e^0 R_a$: $0.3773\ H^+ + 0.3773\ e^- + 0.0472\ ClO_4^- = 0.0472\ Cl^- + 0.1887\ H_2O$

$f_s^0 R_c$: $0.1245\ CO_2 + 0.0311\ HCO_3^- + 0.0311\ NH_4^+ + 0.6227\ H^+ + 0.6227\ e^- = 0.0311\ C_5H_7O_2N + .02802\ H_2O$

$-R_d$: $0.125\ CH_3COO^- + 0.375\ H_2O = 0.125\ CO_2 + 0.125\ HCO_3^- + H^+ + e^-$

R: $0.0472\ ClO_4^- + 0.0311\ NH_4^+ + 0.125\ CH_3COO^- = 0.0311\ C_5H_7O_2N + 0.0939\ HCO_3^- + 0.0472\ Cl^- + 0.0939\ H_2O$

The above equation for 1 mole of perchlorate is given by:

$$ClO_4^- + 0.6589\ NH_4^+ + 2.648\ CH_3COO^- = 0.6589\ C_5H_7O_2N + 1.9894\ HCO_3^- + Cl^- + 1.9894\ H_2O$$

C.2. Nitrate Biodegradation

Electron acceptor half reaction (R_a) (Rittmann and McCarty, 2001):

$1/5\ NO_3^- + 6/5\ H^+ + e^- = 1/10\ N_2 + 3/5\ H_2O$ $\qquad \Delta G_a^0 = -72.20\ KJ/e^-\ eq.$

Electron donor half reaction (R_d) (Rittmann and McCarty, 2001):

$1/8\ CO_2 + 1/8\ HCO_3^- + H^+ + e^- = 1/8\ CH_3COO^- + 3/8\ H_2O$ $\qquad \Delta G_d^0 = 27.4\ KJ/e^-\ eq.$

Cell synthesis equation for nitrate as nitrogen source (R_c) (Rittmann and McCarty, 2001):

$$1/28\ NO_3 + 5/28\ CO_2 + 29/28\ H^+ + e^- = 1/28\ C_5H_7O_2N + 11/28\ H_2O$$

$$\Delta G_p = 35.09\text{-}27.40 = 7.69\ KJ/e^-\ eq$$

$$\Delta G_r = \Delta G_a^0 - \Delta G_d^0 = -72.20 - 27.40 = -99.6\ KJ/e^-\ eq$$

ΔG_p is positive, hence n = +1. Substituting the values in equation for A, we have A = 0.7387. The fraction of donor used for synthesis (f_s^0) is given by (Rittmann and McCarty, 2001):

$$f_s^0 = \frac{1}{1+A} = 0.575$$

And the fraction of donor used for energy synthesis (f_e^0) is given by (Rittmann and McCarty, 2001):

$$f_e^0 = 1 - f_s^0 = 0.425$$

The overall reaction (R) is given by (Rittmann and McCarty, 2001):

$$R = f_e^0\ R_a + f_s^0\ R_c - R_d$$

Solving for the overall equation for nitrate degradation, we have:

f_e^0 Ra: $0.085\ NO_3^- + 0.51\ H^+ + 0.425\ e^- = 0.0425\ N_2 + 0.111\ H_2O$

f_s^0 R_c: $0.021\ NO_3 + 0.103\ CO_2 + 0.6\ H^+ + 0.575\ e^- = 0.021\ C_5H_7O_2N + 0.231\ H_2O$

$-R_d$: $0.125\ CH_3COO^- + 0.375\ H_2O = 0.125\ CO_2 + 0.125\ HCO_3^- + H^+ + e^-$

R: $0.106\ NO_3^- + 0.11\ H^+ + 0.125\ CH_3C00^- = 0.021\ C_5H_7O_2N + 0.0425\ N_2 + 0.022\ CO_2 + 0.111\ H_2O + 0.125\ HCO_3^-$

The above equation for 1 mole of nitrate is given as:

$NO_3^- + 1.04\ H^+ + 1.18\ CH_3C00^- = 0.2\ C_5H_7O_2N + 0.4\ N_2 + 0.21\ CO_2 + 1.05\ H_2O + 1.18\ HCO_3^-$

APPENDIX D

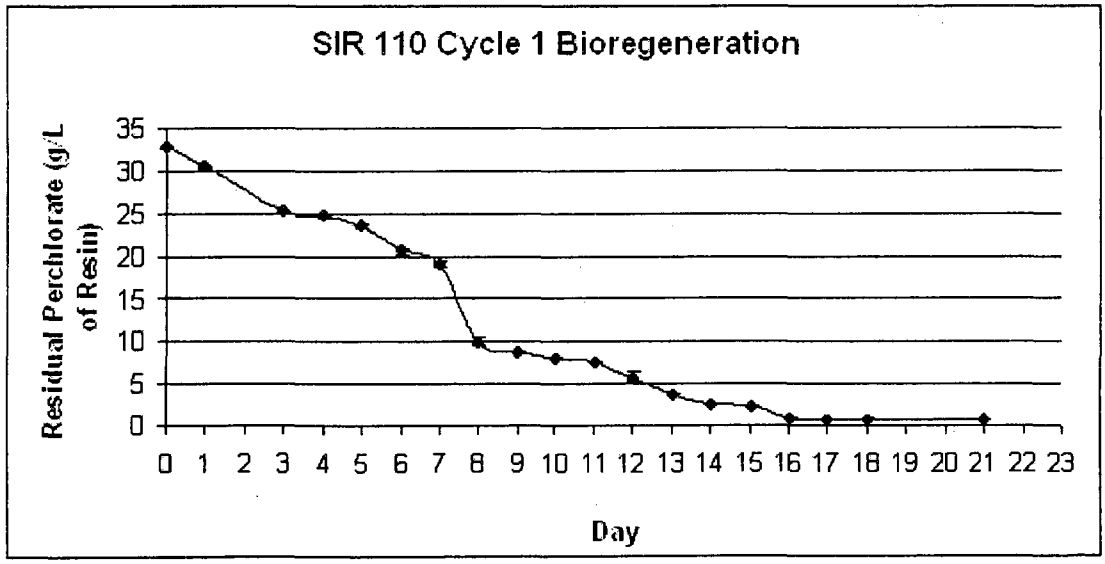
EXPERIMENTAL RESULTS FOR MULTI-CYCLE BIOREGENERATION OF GEL TYPE ANION EXCHANGE RESIN

D.1. Resin Loading

Cycle #	Anion	Measured feed Conc, (mg/L)	QA/QC measured feed conc.	Residual Conc. After Loading (mg/L)	QA/QC measured Residual conc.	Capacity Occupied by Resin (g/L_{resin})	% Capacity Occupied
Cycle 1	ClO_4^-	31910	± 128	2.462	± 0.101	31.91	53.45
	NO_3^-	605.6	± 1.37	2.65	± 0.02	0.603	1.62
	SO_4^{2-}	651.7	± 2.56	304	± 2.0	0.347	1.2
	Cl^-	652.5	± 11.05	5309	± 65.0	N/A	N/A
	HCO_3^-	500	± 1.0	760	± 0.0	N/A	N/A
Cycle 2	ClO_4^-	30960	± 323	2.745	± 0.002	30.96	51.85
	NO_3^-	526.82	± 13.15	13.04	± 0.03	0.514	1.38
	SO_4^{2-}	551.98	± 16.6	173.08	± 13.48	0.379	1.32
	Cl^-	632.6	± 2.01	6130	± 104	N/A	N/A
	HCO_3^-	500	± 2.0	650	± 0.0	N/A	N/A
Cycle 3	ClO_4^-	28900	± 135	2.083	± 0.032	28.89	48.41
	NO_3^-	580	± 6.0	10.37	± 0.67	0.57	1.53
	SO_4^{2-}	479	± 9.0	375	± 9.0	0.104	0.36
	Cl^-	632.6	± 2.03	5100	± 76.0	N/A	N/A
	HCO_3^-	500	± 0.0	608	± 5.1	N/A	N/A

D.2. Bioregeneration Cycle 1 Results

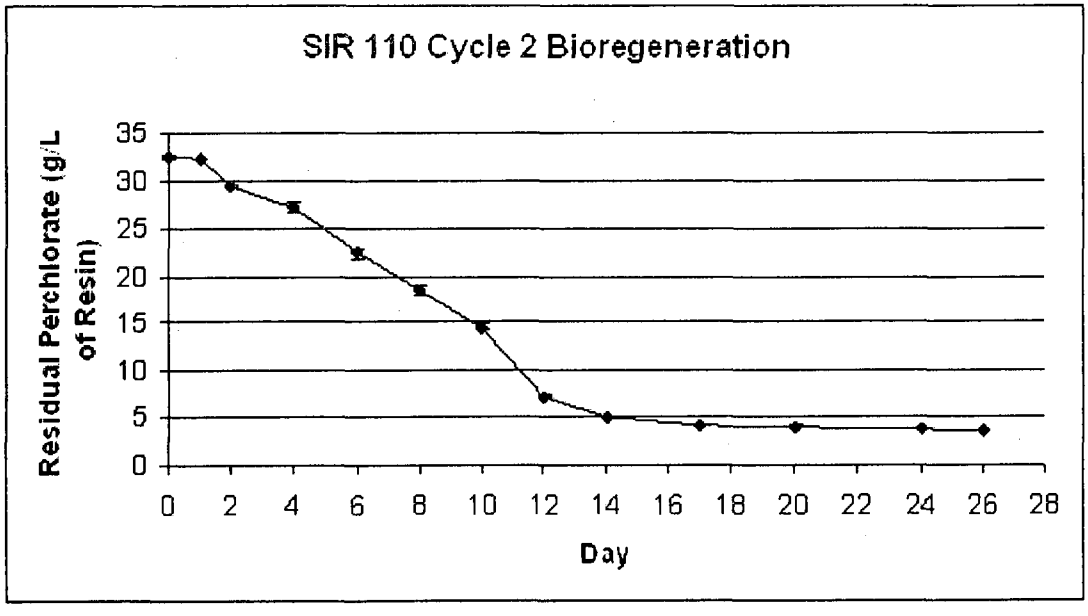
Date of Sampling	Day	Residual Perchlorate (g/L_{resin})	QA/QC	Average	Std. Dev.
8/10/2008	0	32.64793721	32.9104618	32.7791995	0.1856329
8/11/2008	1	30.24773589	30.5148713	30.3813036	0.18889329
8/12/2008	3	25.18893223	-	25.1889322	-
8/13/2008	4	24.78193699	24.5699226	24.6759298	0.14991684
8/14/2008	5	23.80871677	23.3608809	23.5847988	0.31666778
8/15/2008	6	20.92253135	20.3991574	20.6608444	0.37008125
8/16/2008	7	18.70294345	19.2250051	18.9639743	0.36915333
8/17/2008	8	10.35430646	9.695672	10.0249892	0.46572489
8/18/2008	9	8.639075462	8.80327997	8.72117772	0.11611012
8/19/2008	10	8.089761351	7.89426525	7.9920133	0.13823662
8/20/2008	11	7.694980612	7.5105707	7.60277566	0.1303975
8/21/2008	12	6.257915696	5.3318523	5.794884	0.65482571
8/22/2008	13	3.894607986	-	3.89460799	-
8/23/2008	14	2.609425761	-	2.60942576	-
8/24/2008	15	2.428944399	2.35222256	2.39058348	0.05425054
8/25/2008	16	0.953517889	0.83088971	0.8922038	0.08671122
8/26/2008	17	0.74985849	0.69338745	0.72162297	0.03993105
8/27/2008	18	0.675668555	0.86649927	0.77108391	0.13493769
9/2/2008	21	0.692017121	0.75400414	0.72301063	0.04383144



G-1: SIR 110 Cycle 1 Bioregeneration

D.3. Bioregeneration Cycle 2 Results

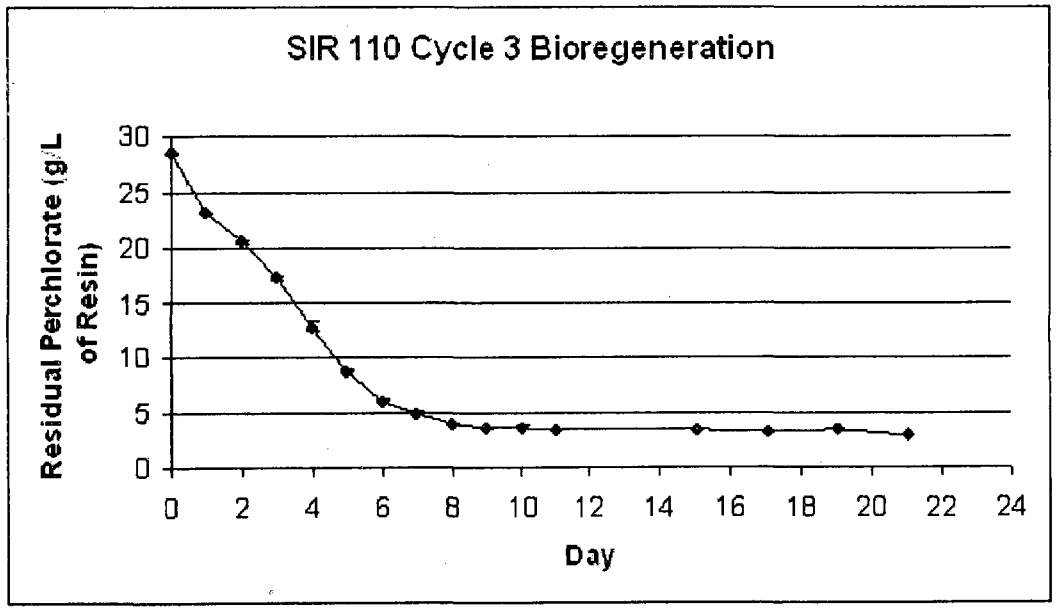
Date of Sampling	Day	Residual Perchlorate (g/L_{resin})	QA/QC	Average	Std. Dev.
18-Sep	0	32.31127701	32.53989	32.42559	0.161656564
19-Sep	1	32.15303812	-	32.15304	-
20-Sep	2	29.4824785	29.2274	29.35494	0.180370591
22-Sep	4	26.86617805	27.60636	27.23627	0.523387472
24-Sep	6	22.78686042	22.02219	22.40452	0.540704113
26-Sep	8	18.06258616	18.89147	18.47703	0.586112129
28-Sep	10	14.75208058	14.28913	14.5206	0.327356614
30-Sep	12	7.332408808	7.235825	7.284117	0.068295325
1-Oct	14	5.072433042	4.973908	5.023171	0.069667671
4-Oct	17	4.282954281	4.236005	4.259479	0.033198438
7-Oct	20	4.003569012	4.2271	4.115335	0.158060403
11-Oct	24	3.814122233	3.855808	3.834965	0.029476579
13-Oct	26	3.676684379	3.699909	3.688297	0.016422636



G-2: SIR 110 Cycle 2 Bioregeneration

D.4. Bioregeneration Cycle 3 Results

Date of Sampling	Day	Residual Perchlorate (g/L)	QA/QC	Average	Std. Dev.
11/8/2008	0	28.38313792	28.5270109	28.45507	0.1017
11/9/2008	1	23.15471225	23.1040226	23.12937	0.0358
11/10/2008	2	20.64617831	20.4383344	20.54226	0.147
11/11/2008	3	17.41553699	17.1384232	17.27698	0.1959
11/12/2008	4	12.49730922	13.0990001	12.79815	0.4255
11/13/2008	5	8.736919524	8.82753197	8.782226	0.0641
11/14/2008	6	6.108431186	6.0211148	6.064773	0.0617
11/15/2008	7	4.984047284	5.00640032	4.995224	0.0158
11/16/2008	8	4.001078699	3.99887199	3.999975	0.0016
11/17/2008	9	3.47457834	3.64087411	3.557726	0.1176
11/18/2008	10	3.572657314	3.72671357	3.649685	0.1089
11/19/2008	11	3.444383696	3.50502084	3.474702	0.0429
11/23/2008	15	3.478577002	3.43049348	3.454535	0.034
11/25/2008	17	3.246578524	3.34075551	3.293667	0.0666
11/27/2008	19	3.463319218	-	3.463319	-
11/29/2008	21	2.933396155	-	2.933396	-



G-3: SIR 110 Cycle 3 Bioregeneration

D.5. Culture Parameter Variation during Cycle 1 Bioregeneration

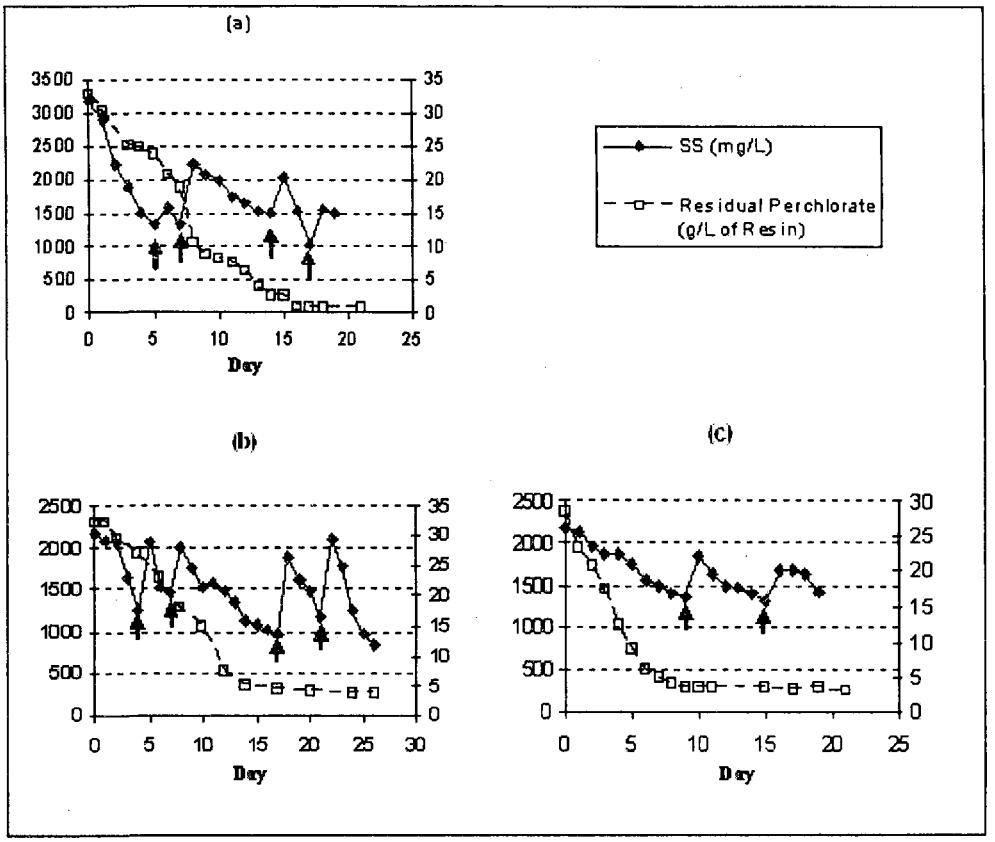
Date	COD (mg/L)	SS (mg/L)	OD600nM %transmittance	Turbidity (NTU)	pH	Conductivity (mS)	ORP (mV)
9-Aug	2555	3169	0.8	2290	7.41	21.6	-477
10-Aug	4950	2891	1.2	2076	7.70	22.6	-508
11-Aug	4590	2243	2.5	1579	7.35	22.5	-510
12-Aug	3885	1866	2.5	1289	7.67	22.48	-529
13-Aug	3750	1489	3.4	1000	7.73	22.7	-535
14-Aug	3640	1320	4	870	7.81	22.7	-540
15-Aug	3460	1573	3.2	1064	7.68	22.41	-530
16-Aug	5052	1308	4.1	861	7.71	22.46	-534
17-Aug	4560	2221	1.1	1562	7.47	22.69	-517
18-Aug	4116	2085	2.4	1457	7.62	22.92	-521
19-Aug	3732	1969	2.7	1368	7.66	22.97	-526
20-Aug	3660	1760	3.1	1208	7.91	23.73	-538
21-Aug	3540	1646	3.5	1120	7.89	23.83	-539
22-Aug	3230	1522	3.9	1025	7.91	24.61	-540
23-Aug	3200	1502	4.1	1010	7.95	24.82	-541
24-Aug	4260	2023	2.6	1410	7.90	23.08	-508
25-Aug	3890	1517	2.8	1021	7.87	23.12	-511
26-Aug	3650	1018	3.4	638	7.81	23.35	-522
27-Aug	3410	1555	3.9	1050	7.86	23.41	-520
28-Aug	3330	1493	4.1	1003	7.80	23.66	-513

D.6. Culture Parameter Variation during Cycle 2 Bioregeneration

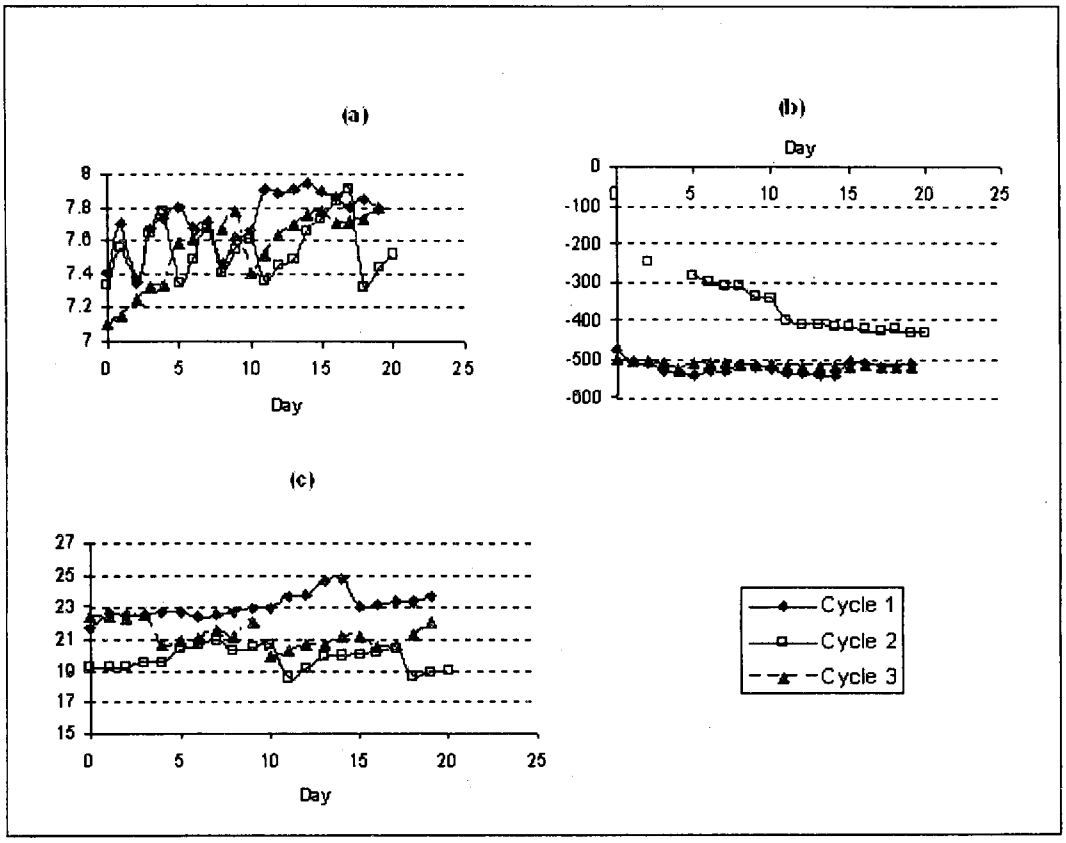
Date	SS (mg/L)	OD600nM %transmittance	Turbidity (NTU)	pH	Conductivity (mS)	ORP (mV)
18-Sep	2165	1.8	1183	7.34	19.22	
19-Sep	2086	1.9	1057	7.56	19.15	
20-Sep	2049	1.8	1430	7.36	19.23	-251
21-Sep	1627	2.5	1106	7.64	19.53	
22-Sep	1258	3.1	822	7.79	19.55	
23-Sep	2052	1.8	1432	7.35	20.35	-285
24-Sep	1536	2.6	1036	7.49	20.53	-302
25-Sep	1471	2.8	986	7.68	20.86	-311
26-Sep	1992	1.9	1386	7.41	20.28	-310
27-Sep	1743	2.2	1195	7.55	20.49	-336
28-Sep	1518	2.7	1022	7.61	20.63	-340
29-Sep	1574	2.6	1065	7.36	18.51	-399
30-Sep	1484	2.7	996	7.45	19.02	-410
1-Oct	1353	2.9	895	7.49	19.85	-408
2-Oct	1139	3.2	731	7.66	19.93	-419
3-Oct	1092	3.5	695	7.73	20.03	-419
4-Oct	1027	3.6	645	7.84	20.11	-422
5-Oct	984	3.9	612	7.92	20.32	-428
6-Oct	1888	1.9	1306	7.33	18.68	-421
7-Oct	1621	2.4	1101	7.44	18.85	-430
8-Oct	1489	2.8	1000	7.52	18.96	-431
9-Oct	1172	3.3	756	7.63	19.01	-444
10-Oct	2091	1.7	1462	7.69	19.23	-442
11-Oct	1762	2.3	1209		19.3	
12-Oct	1264	3.1	827		19.36	-456
13-Oct	983	3.9	611		19.56	-456
14-Oct	836	4.1	498		19.55	-458

D.7. Culture Parameter Variation during Cycle 3 Bioregeneration

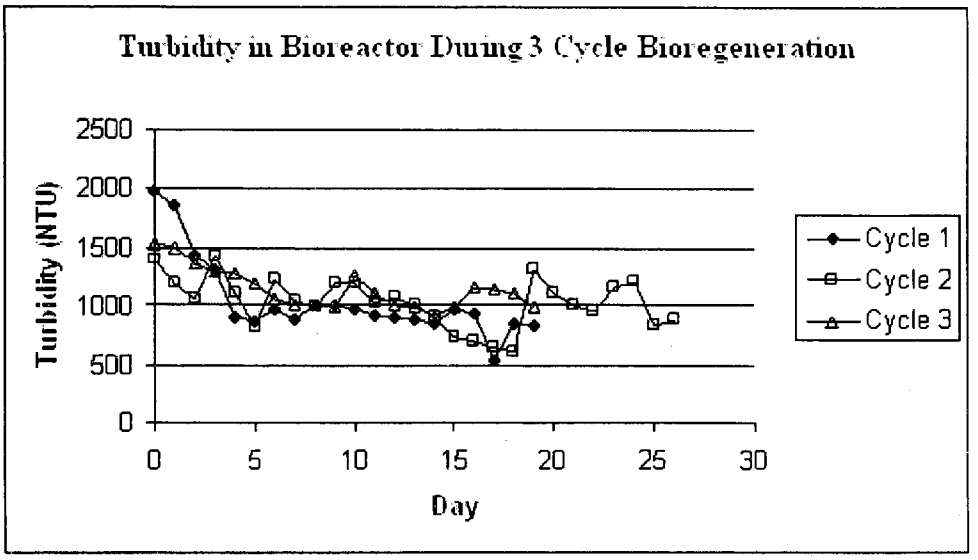
Date	COD (mg/L)	SS (mg/L)	OD600nM %transmittance	Turbidity (NTU)	pH	Conductivity (mS)	ORP (mV)
8-Nov	3370	2187	1.8	1536	7.1	22.44	-501
9-Nov	3360	2129	1.8	1491	7.15	22.42	-508
10-Nov	3280	1958	1.7	1360	7.25	22.33	-508
11-Nov	2890	1866	1.9	1289	7.33	22.45	-511
12-Nov	2590	1859	2	1284	7.34	20.58	-527
13-Nov	2760	1736	1.9	1189	7.59	20.91	-511
14-Nov	2620	1562	2.3	1056	7.61	20.96	-509
15-Nov	2590	1498	2.5	1007	7.68	21.54	-512
16-Nov	2420	1390	2.8	924	7.67	21.13	-514
17-Nov	2390	1345	3.4	889	7.78	22.05	-516
18-Nov	3210	1835	1.9	1265	7.41	19.85	-517
19-Nov	3150	1633	2.1	1110	7.52	20.26	-521
20-Nov	2980	1492	2.3	1002	7.63	20.56	-518
21-Nov	2890	1466	2.6	982	7.69	20.62	-519
22-Nov	2630	1392	2.7	925	7.76	21.11	-519
23-Nov	2560	1302	3.3	856	7.78	21.14	-521
24-Nov	3100	1685	2.1	1150	7.70	20.51	-517
25-Nov	3056	1678	2.1	1145	7.71	20.66	-519
26-Nov	2810	1617	2.2	1098	7.73	21.23	-519
27-Nov	2750	1427	2.6	952	7.81	22.01	-520



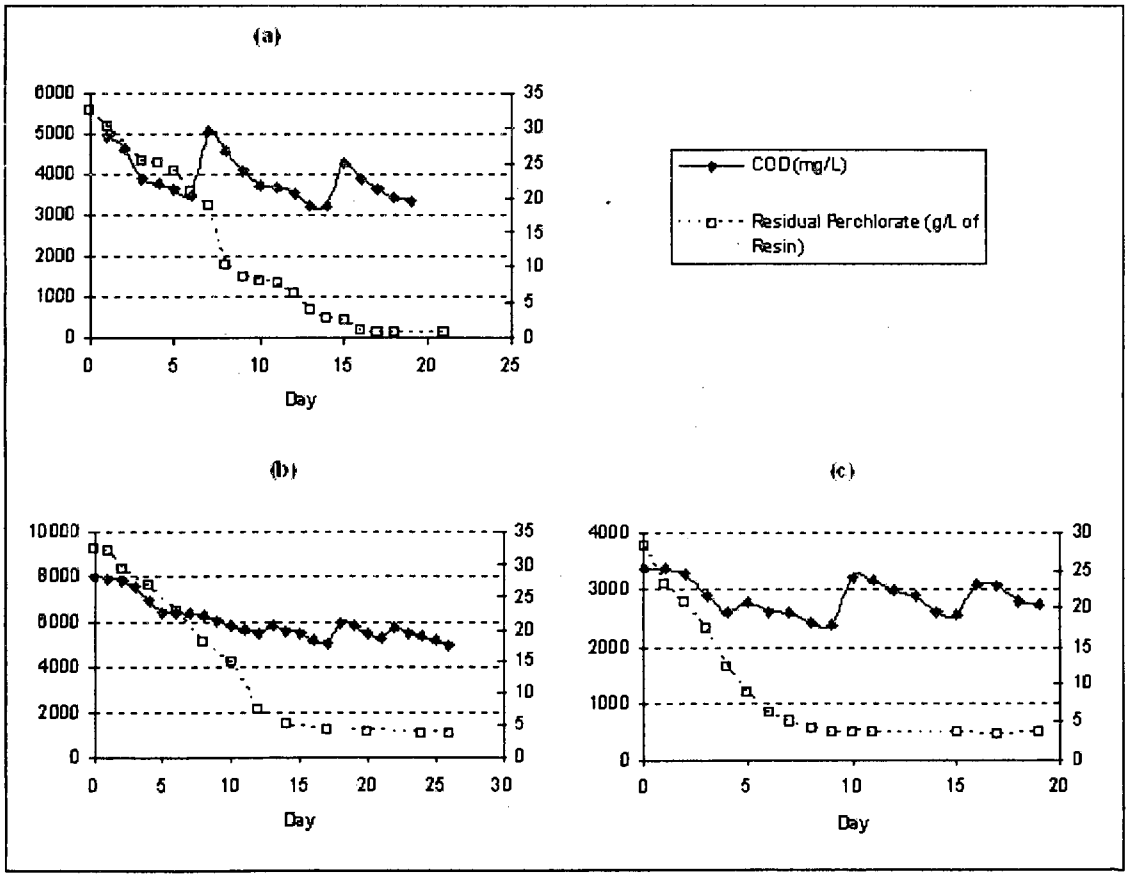
G-4: Suspended Solids Variation in the Bioreactor for 3 Cycle Bioregeneration



G-5: pH, % OD and Conductivity Variation in the Bioreactor During 3 Cycle Bioregeneration: (a) pH; (b) ORP (mV); (c) Conductivity (mS)



G-6: Turbidity in Bioreactor During 3 Cycle Bioregeneration



G-7: COD in Bioreactor During 3 Cycle Bioregeneration

APPENDIX E

EXPERIMENTAL RESULTS FOR INVESTIGATION OF POTENTIAL MECHANISMS OF BIOREGENERATION PROCESS FOR A GEL-TYPE ANION EXCHANGE RESIN CONTAINING PERCHLORATE

E.1. Effect of Initial Perchlorate Load on Perchlorate Degradation Rate in Ion-exchange Resin: (a) 3 g/L_{resin}, (b) 5g/L_{resin} amd (c) 20 g/L_{resin}

(a)

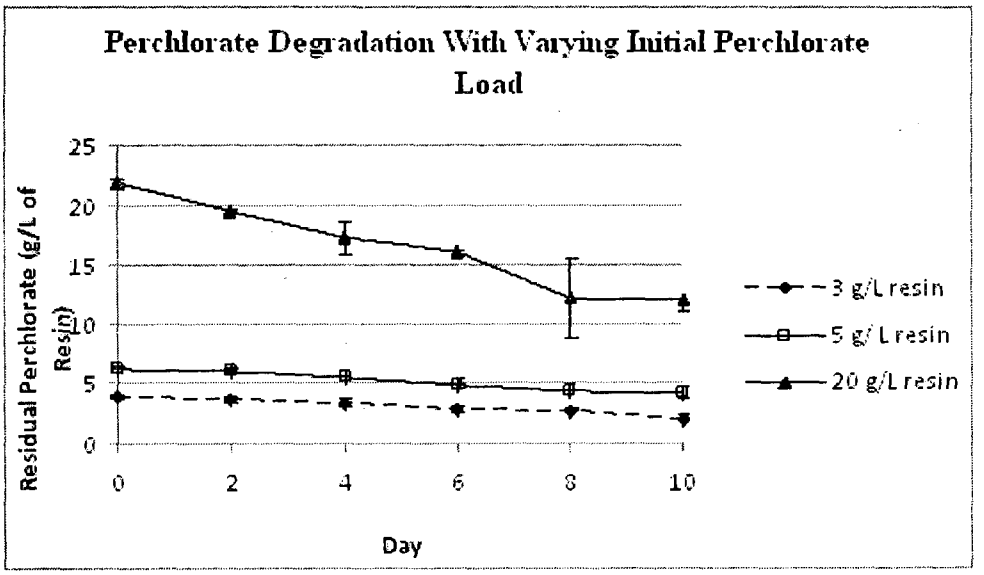
Date of Sampling	Day	Residual Perchlorate (g/L_{resin})	Duplicate	Average	Std. Dev.
19-Feb	0	3.91543159	3.613275	3.764353	0.213657
21-Feb	2	3.7024421	4.064585	3.883514	0.256074
23-Feb	4	3.36018944	2.944214	3.152202	0.369395
25-Feb	6	2.85946634	3.225184	3.042325	0.258601
27-Feb	8	2.78671628	2.777574	2.782145	0.006464
1-Mar	10	2.10521537	2.549369	2.327292	0.314064

(b)

Date of Sampling	Day	Residual Perchlorate (g/L_{resin})	Duplicate	Average	Std. Dev.
19-Feb	0	6.29645801	6.459444	6.377951	0.115249
21-Feb	2	6.03812104	6.319042	6.178582	0.198641
23-Feb	4	5.64163236		5.641632	
25-Feb	6	4.979	5.637021	5.308081	0.465191
27-Feb	8	4.35030535		4.350305	
1-Mar	10	4.19041615	4.838982	4.514699	0.458605

(c)

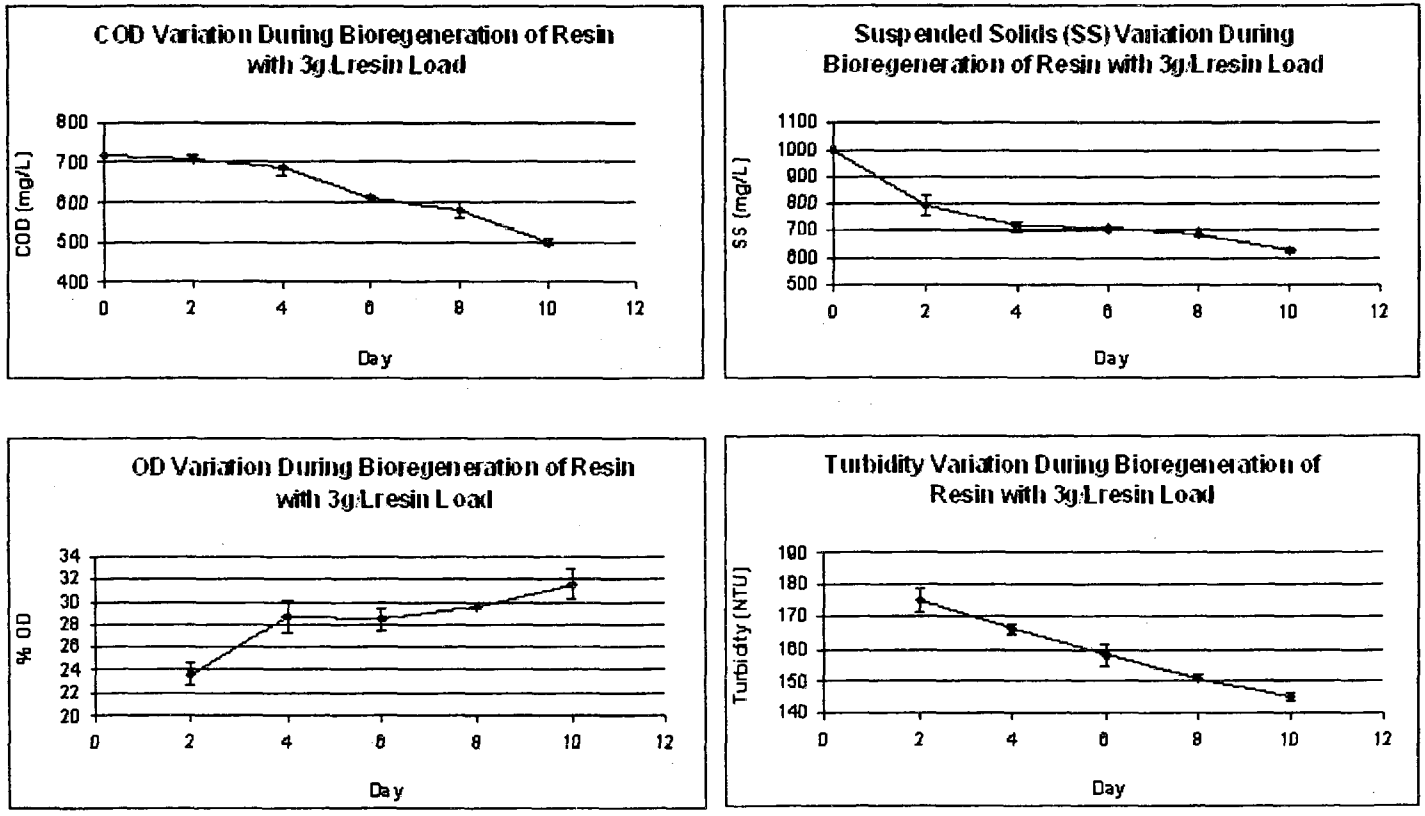
Date of Sampling	Day	Residual Perchlorate (g/L)	Duplicate	Average	Std. Dev.
19-Feb	0	21.7329173	22.19758	21.96525	0.328565
21-Feb	2	19.4289557	19.59783	19.51339	0.119414
23-Feb	4	17.1801229	19.06286	18.12149	1.331299
25-Feb	6	15.9881144	15.56732	15.77772	0.297544
27-Feb	8	12.1774593	16.91095	14.5442	3.347082
1-Mar	10	11.9106011	13.11134	12.51097	0.849052



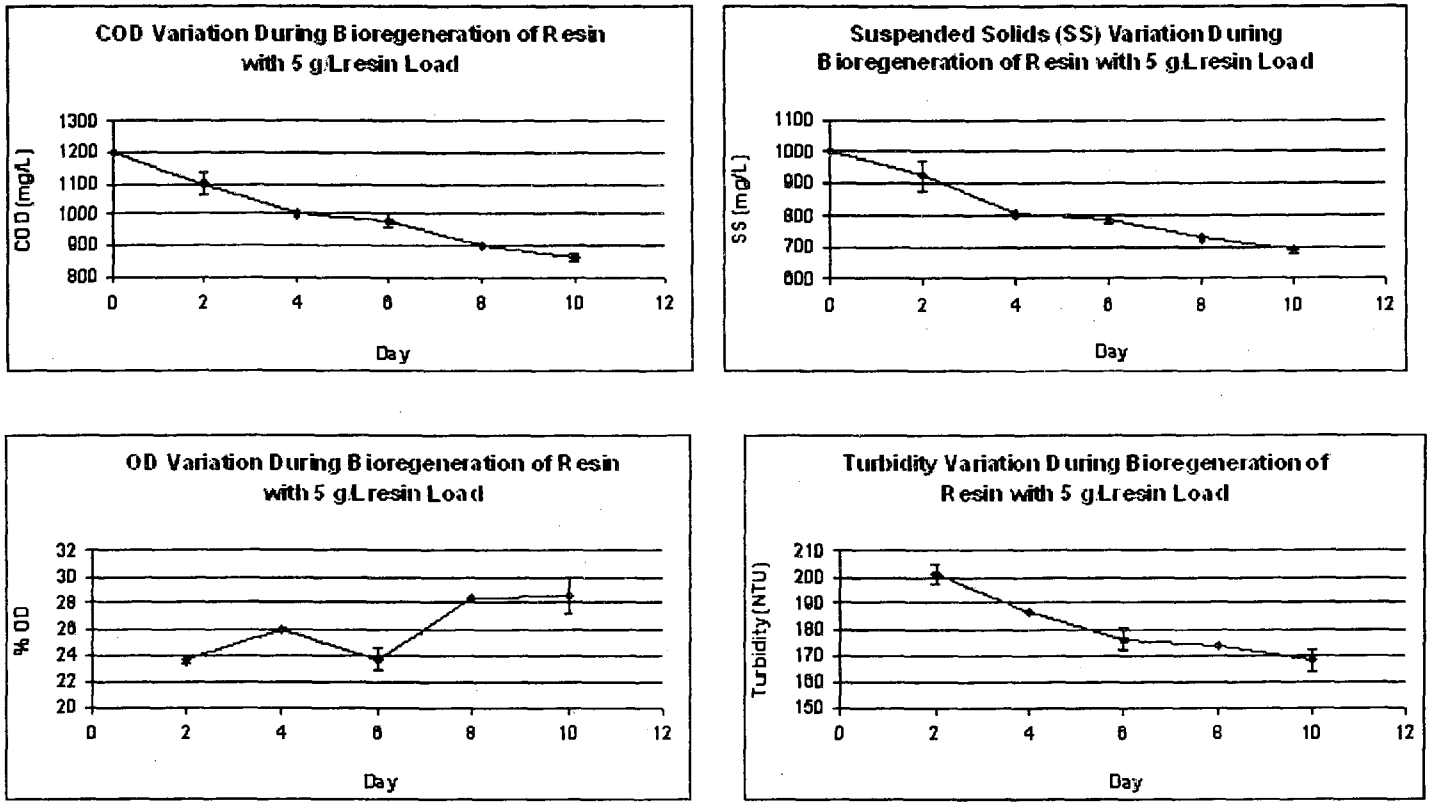
E-1: Perchlorate Degradation with Varying Initial Perchlorate Load

E.2. Culture Parameter Variation during Batch-1 Experiment

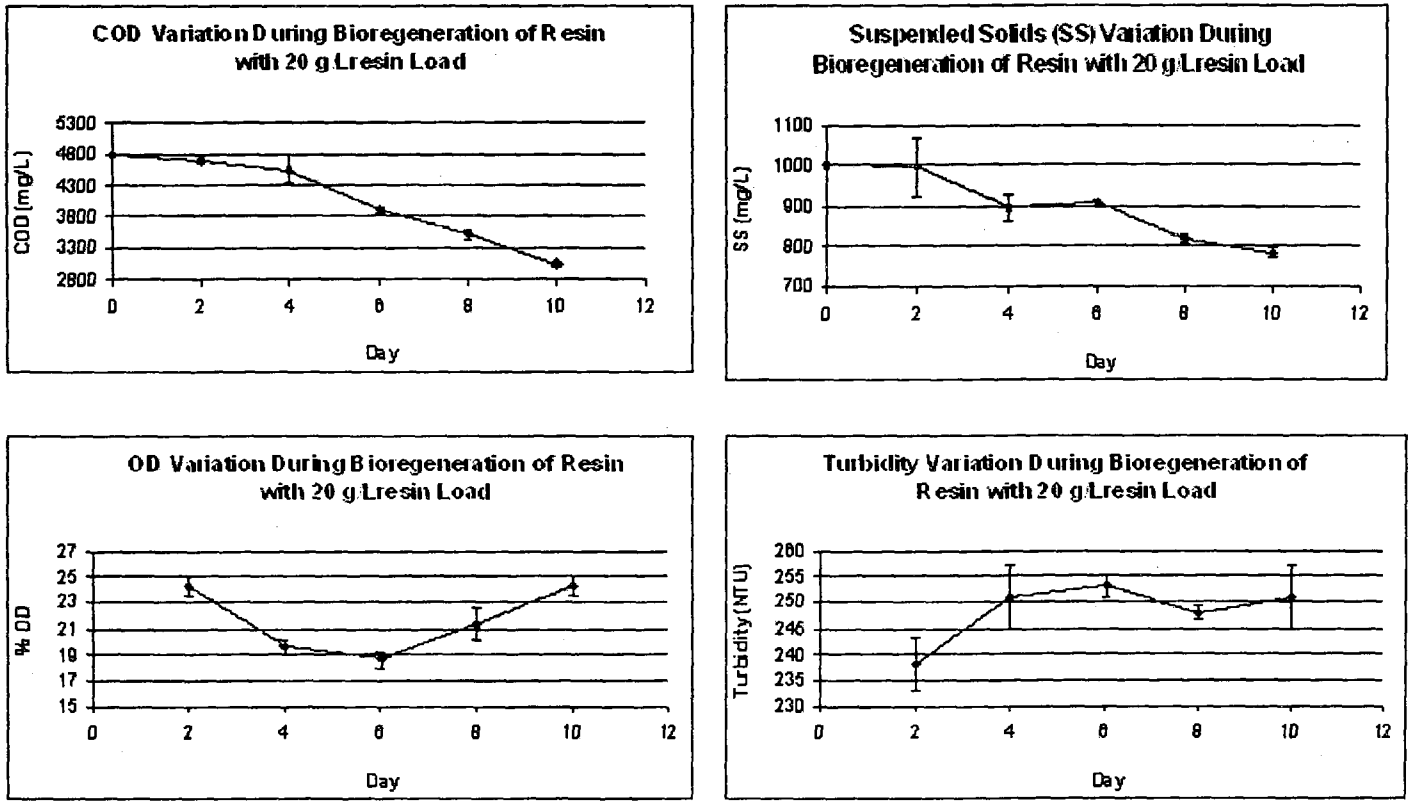
Date	Day	COD mg/l	Duplicate	SS mg/l	Duplicate	OD_{600nM} %transmittance	Duplicate	Turbidity (NTU)	Duplicate	pH	Duplicate
3 g/L_{resin}											
2/19/2009	0	720	720	1000	1000						
2/21/2009	2	710	700	793	846	23.7	25.1	175	170	7.3	7.3
2/23/2009	4	685	660	715	743	28.7	26.8	166	164	7.24	7.24
2/25/2009	6	610	600	706	713	28.4	27.1	158	153	7.26	7.25
2/27/2009	8	580	550	689	671	29.6	30.0	151	150	7.29	7.3
3/1/2009	10	500	490	625	614	31.5	33.4	145	147	7.28	7.29
5 g/L_{resin}											
2/19/2009	0	1200	1200	1000	1000						
2/21/2009	2	1100	1150	926	856	23.6	23.2	201	195	7.29	7.28
2/23/2009	4	1000		804		25.9		186		7.24	
2/25/2009	6	980	950	783	764	23.7	24.9	176	170	7.23	7.21
2/27/2009	8	900		724		28.3		173		7.25	
3/1/2009	10	860	840	689	701	28.5	26.6	168	162	7.24	7.25
20 g/L_{resin}											
2/19/2009	0	4800	4800	1000	1000						
2/21/2009	2	4700	4720	996	893	24.3	23.2	238	245	7.28	7.27
2/23/2009	4	4550	4250	895	850	19.6	20.3	251	242	7.2	7.25
2/25/2009	6	3900	3850	905	893	18.6	19.5	253	250	7.21	7.23
2/27/2009	8	3500	3400	816	804	21.3	23.1	248	246	7.22	7.23
3/1/2009	10	3050	3100	781	763	24.2	25.3	251	242	7.24	7.25



E-2: Culture Parameter Variation during Bioregeneration of Resin with 3 g/L_{resin} Load



E-3: Culture Parameter Variation during Bioregeneration of Resin with 5 g/L_{resin} Load



E-4: Culture Parameter Variation during Bioregeneration of Resin with 20 g/L_{resin} Load

E.3. Effect of Microbial Concentration on Perchlorate Degradation Rate in Ion-exchange

(a) 3 g/L_{resin} Perchlorate Load (b) 20 g/L_{resin} Perchlorate Load

(a)

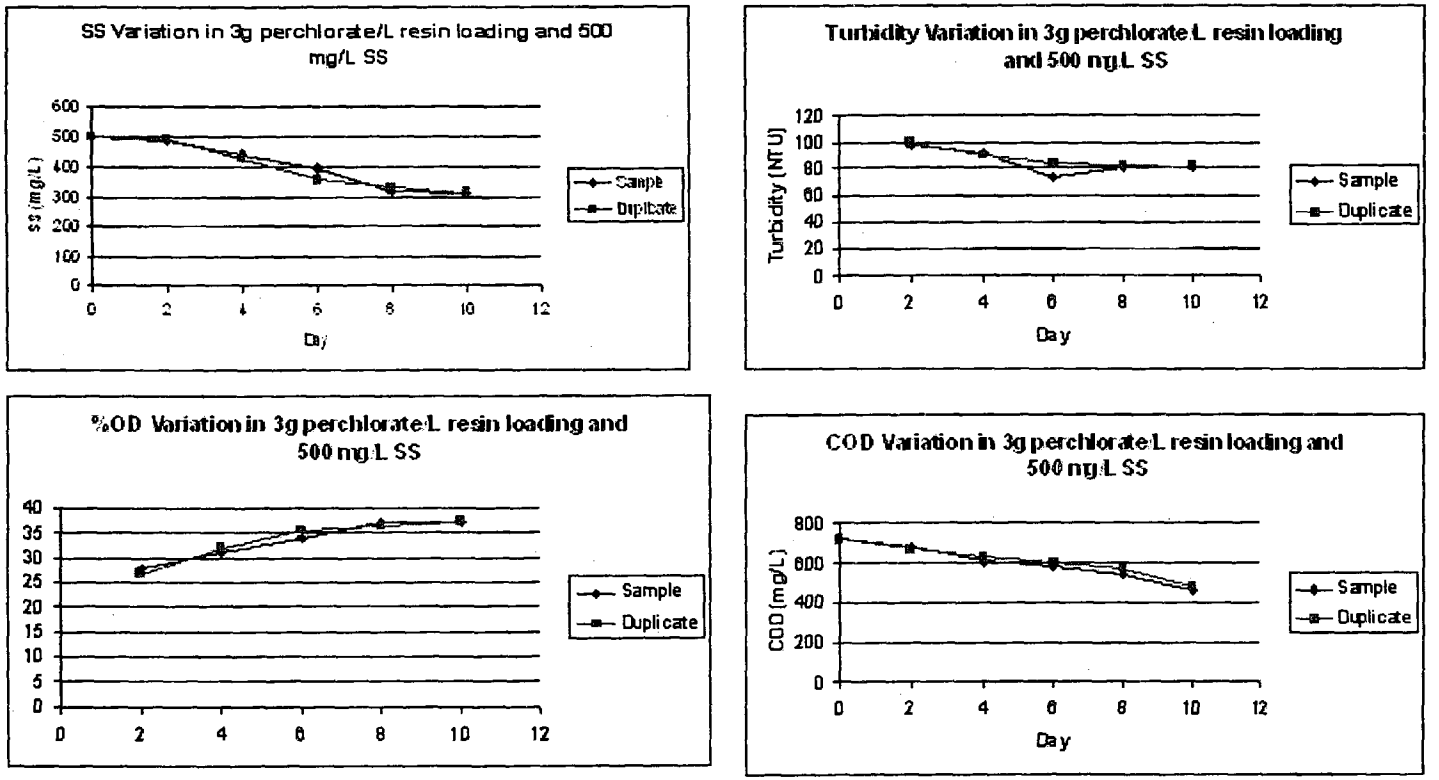
Resin Load 3 g/L									
Day	500 mg/L SS	Duplicate	Std. Dev.	1000 mg/L SS	Duplicate	Std. Dev.	2000 mg/L SS	Duplicate	Std. Dev.
0	3.915432	3.613275	0.213657	3.613275	3.915432	0.213657	3.613275	3.915432	0.213657
2	3.885954	3.767862	0.083503	3.874196	3.700383	0.122905	3.527327	3.613002	0.060582
4	3.484273	3.280464	0.144114	2.620668	2.390349	0.16286	3.106803	3.178761	0.050882
6	3.122635	3.089315	0.023561	3.28049	3.523993	0.172183	2.827329	2.775354	0.036752
8	2.22127	2.03354	0.132745	2.928158	3.129847	0.142615	2.646847	2.652489	0.003989
10	1.569927	1.365976	0.144215	1.864855	1.774464	0.063916	1.463112	1.30197	0.113945

(b)

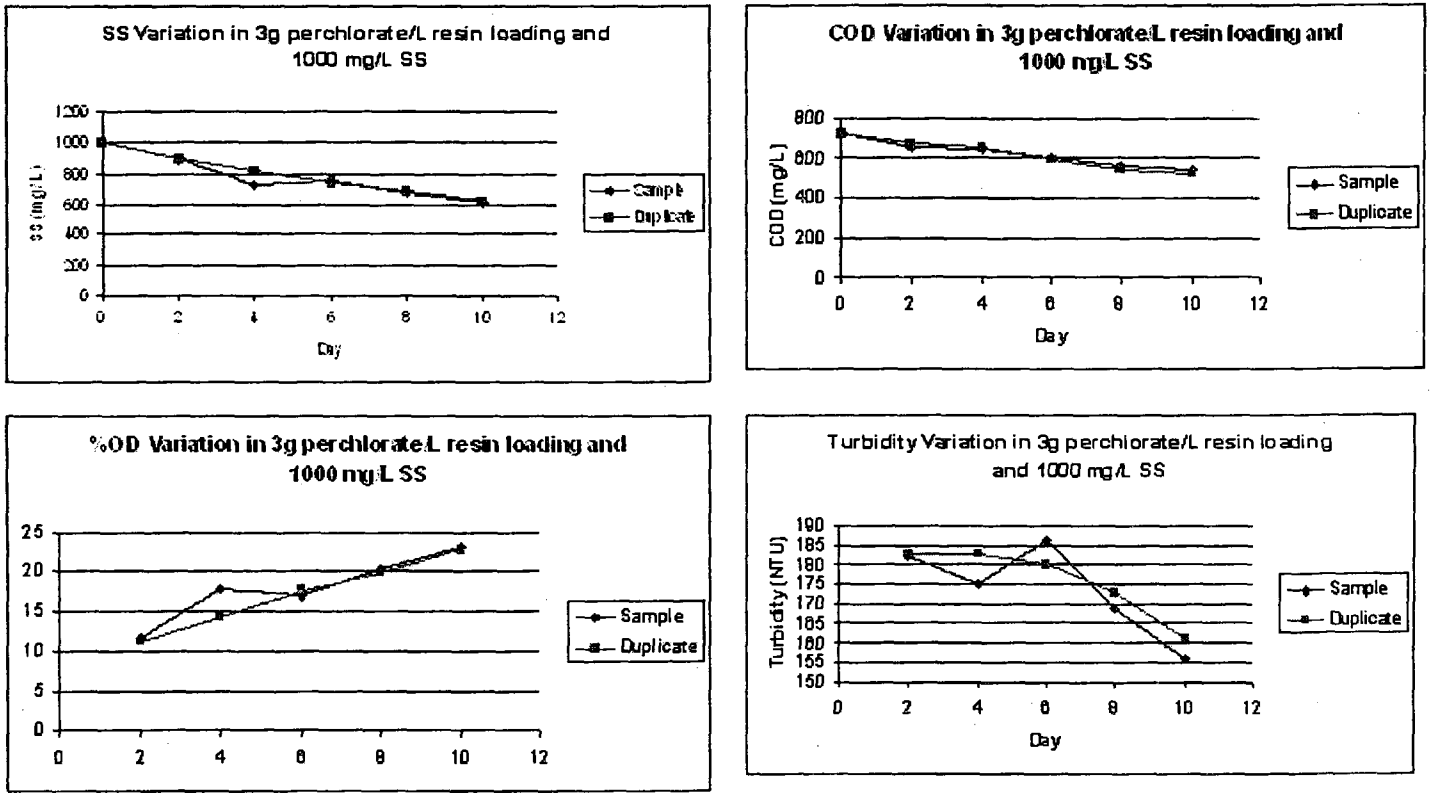
Resin Load 20 g/L									
Day	500 mg/L SS	Duplicate	Std. Dev.	1000 mg/L SS	Duplicate	Std. Dev.	2000 mg/L SS	Duplicate	Std. Dev.
0	21.73292	22.19758	0.328565	21.73292	22.19758	0.328565	21.73292	22.19758	0.328565
2	21.01801	20.40069	0.436513	19.21015	19.50895	0.211283	21.45934	20.53645	0.652579
4	19.67077	19.90959	0.168872	19.21524	18.96947	0.173785	18.42768	15.77813	1.873521
6	19.50904	20.19817	0.487288	19.02641	18.72822	0.210857	18.27265	16.15198	1.499543
8	18.87731	19.1427	0.187664	17.40987	18.04404	0.448423	16.87032	14.85154	1.427495
10	17.54938	18.04366	0.349509	14.70231	12.99408	1.2079	11.73704	11.68788	0.034766

E.4. Culture Parameter Variation during Batch-2 Experiment (3 g/L_{resin} Load)

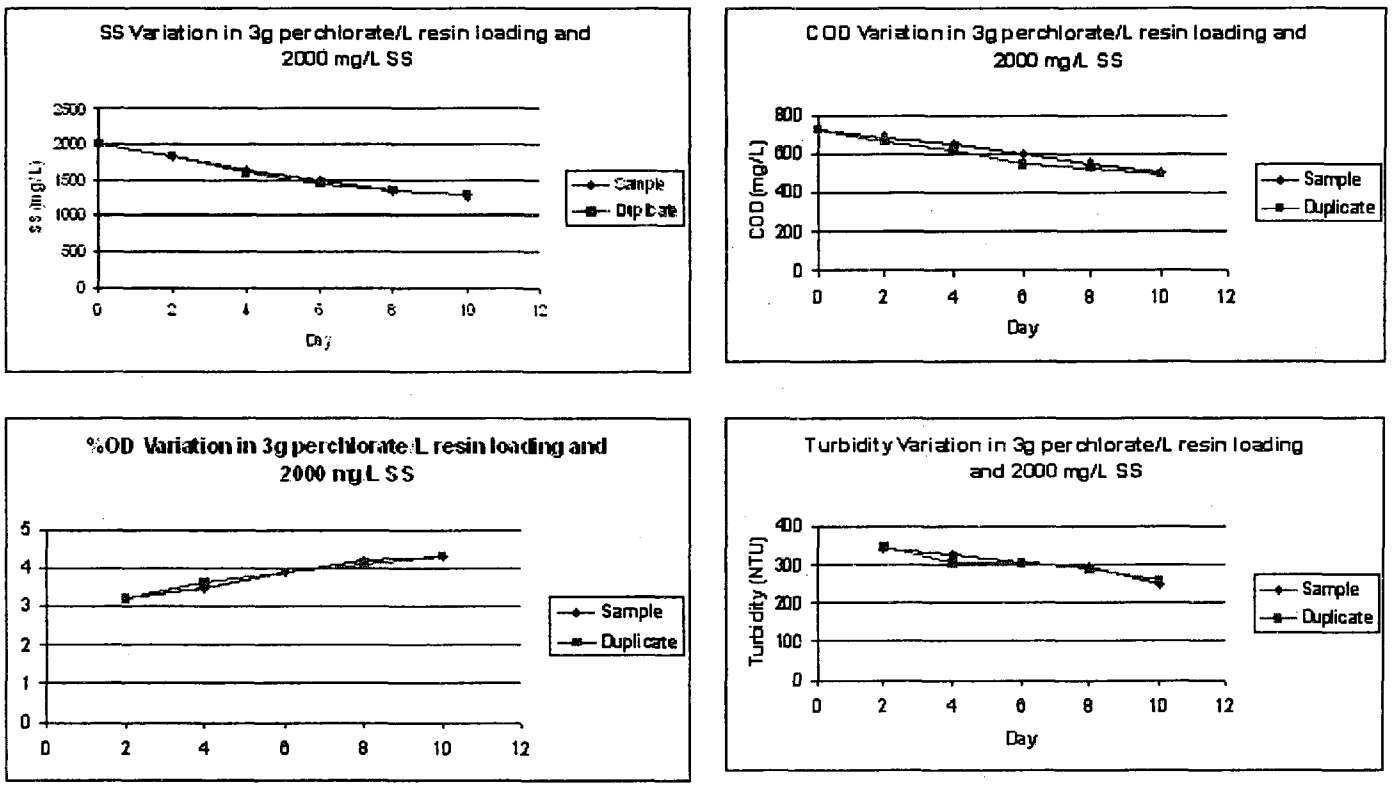
Date	Day	COD mg/l	Duplicate	SS mg/l	Duplicate	OD_{600nM} %transmittance	Duplicate	Turbidity (NTU)	Duplicate	pH	Duplicate
2/4/2009	0	720	720	500	500						
2/6/2009	2	680	670	487	496	27.8	26.7	97.2	99.2	7.05	7.07
2/8/2009	4	610	630	444	427	31.1	32.1	91.3	89.6	7.03	7.04
2/10/2009	6	580	600	396	353	33.8	35.5	72.8	73.6	7.01	7.02
2/12/2009	8	540	570	317	333	37.1	36.5	81.3	82.3	7.01	7.01
2/14/2009	10	460	480	306	314	37.5	37.3	80.6	81	7	7.02
2/4/2009	0	720	720	1000	1000						
2/6/2009	2	660	670	890	896	11.5	11.3	182	183	7.19	7.19
2/8/2009	4	645	650	731	815	17.8	14.4	175	183	7.14	7.16
2/10/2009	6	600	595	754	736	16.9	17.6	186	180	7.2	7.19
2/12/2009	8	560	540	672	680	20.2	19.9	169	173	7.15	7.17
2/14/2009	10	540	520	603	612	23.2	22.8	156	161	7.14	7.14
2/4/2009	0	720	720	2000	2000						
2/6/2009	2	685	667	1836	1845	3.2	3.2	343	345	7.24	7.24
2/8/2009	4	650	650	1643	1605	3.5	3.6	325	303	7.22	7.26
2/10/2009	6	590	580	1478	1470	3.9	3.9	306	302	7.29	7.3
2/12/2009	8	550	530	1364	1355	4.2	4.1	295	290	7.25	7.26
2/14/2009	10	500	490	1289	1305	4.3	4.3	248	261	7.23	7.24



E-4: Culture Parameter Variation during Bioregeneration of Resin with 3 g/L_{resin} Load and Suspended Solids 500 mg/L



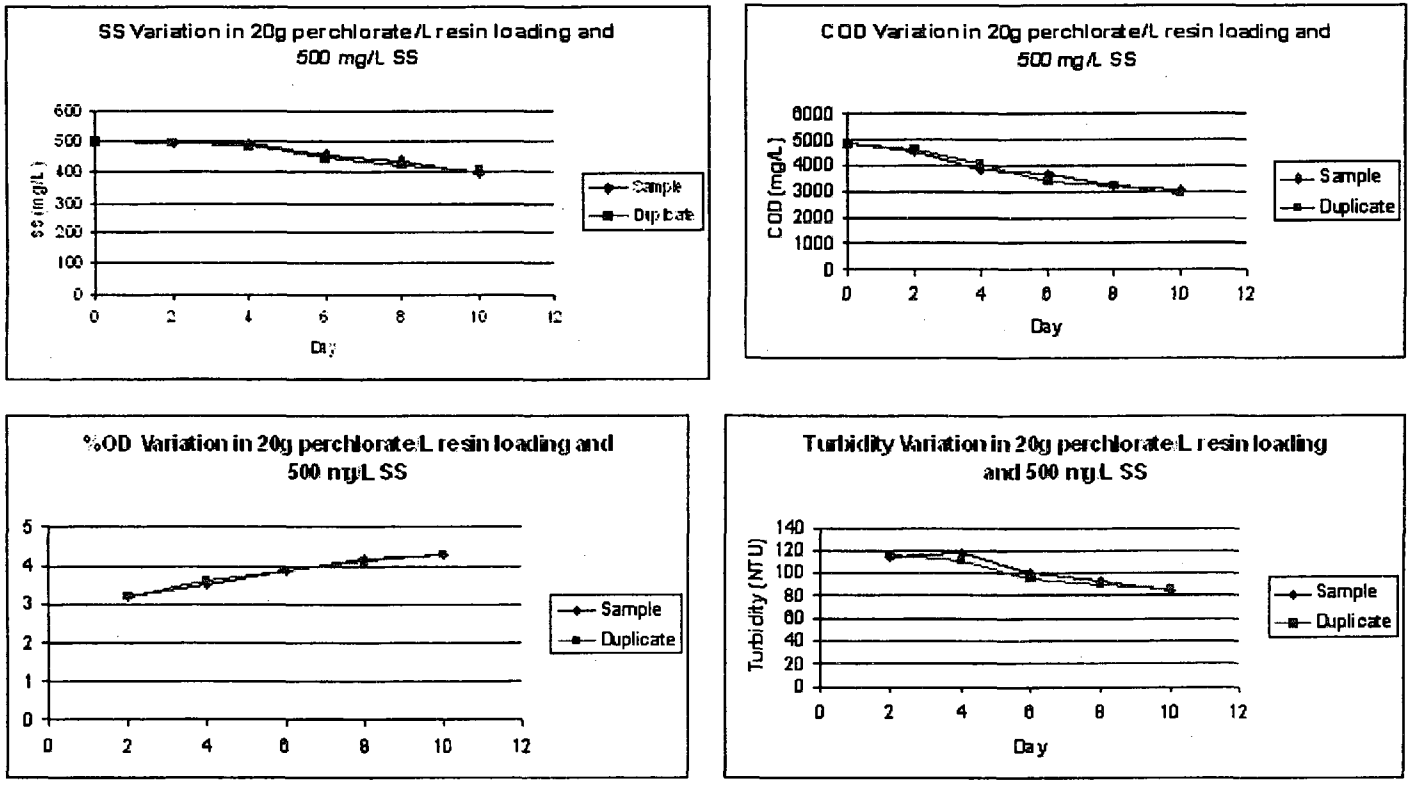
E-5: Culture Parameter Variation during Bioregeneration of Resin with 3 g/L_{resin} Load and Suspended Solids 1000 mg/L



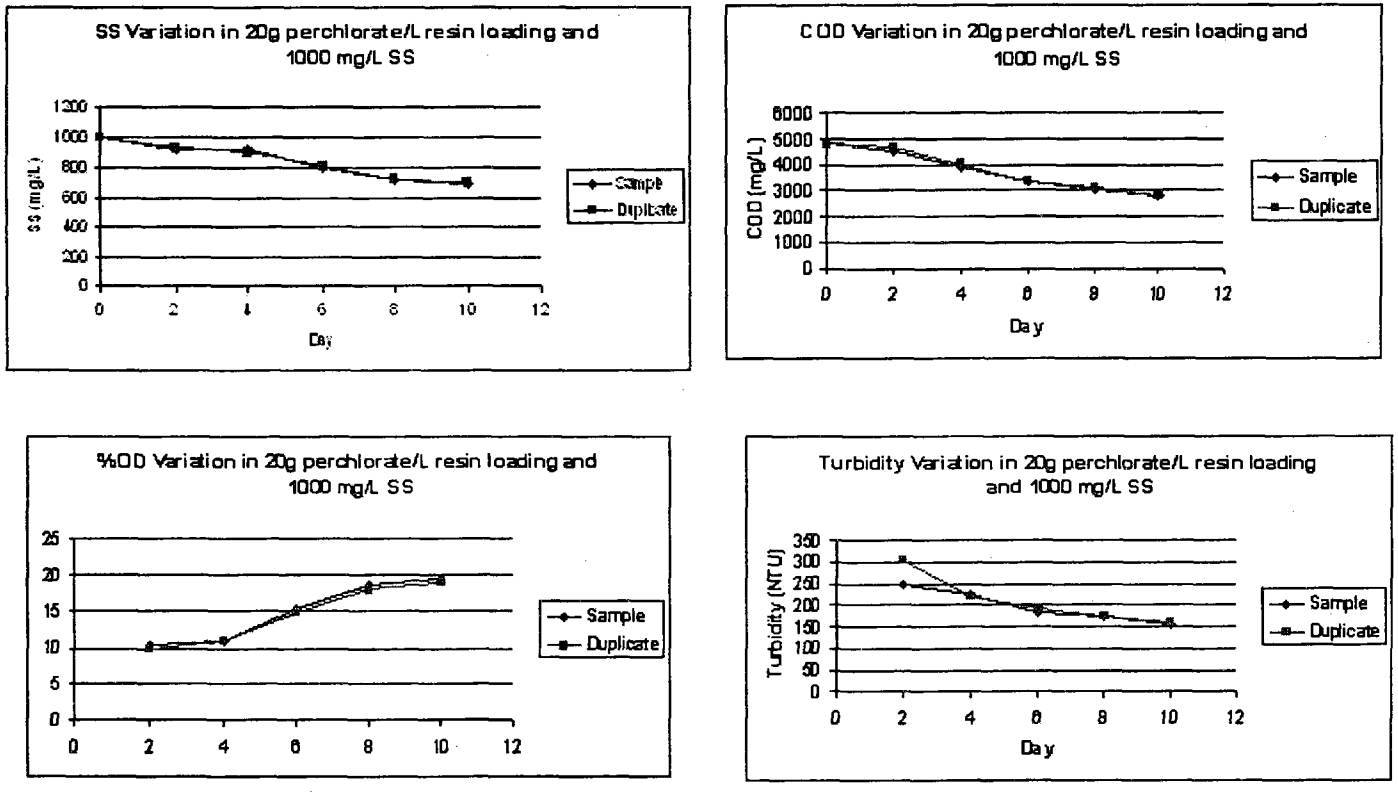
E-6: Culture Parameter Variation during Bioregeneration of Resin with 3 g/L_{resin} Load and Suspended Solids 2000 mg/L

E.5. Culture Parameter Variation during Batch-2 Experiment (20 g/L_{resin} Load)

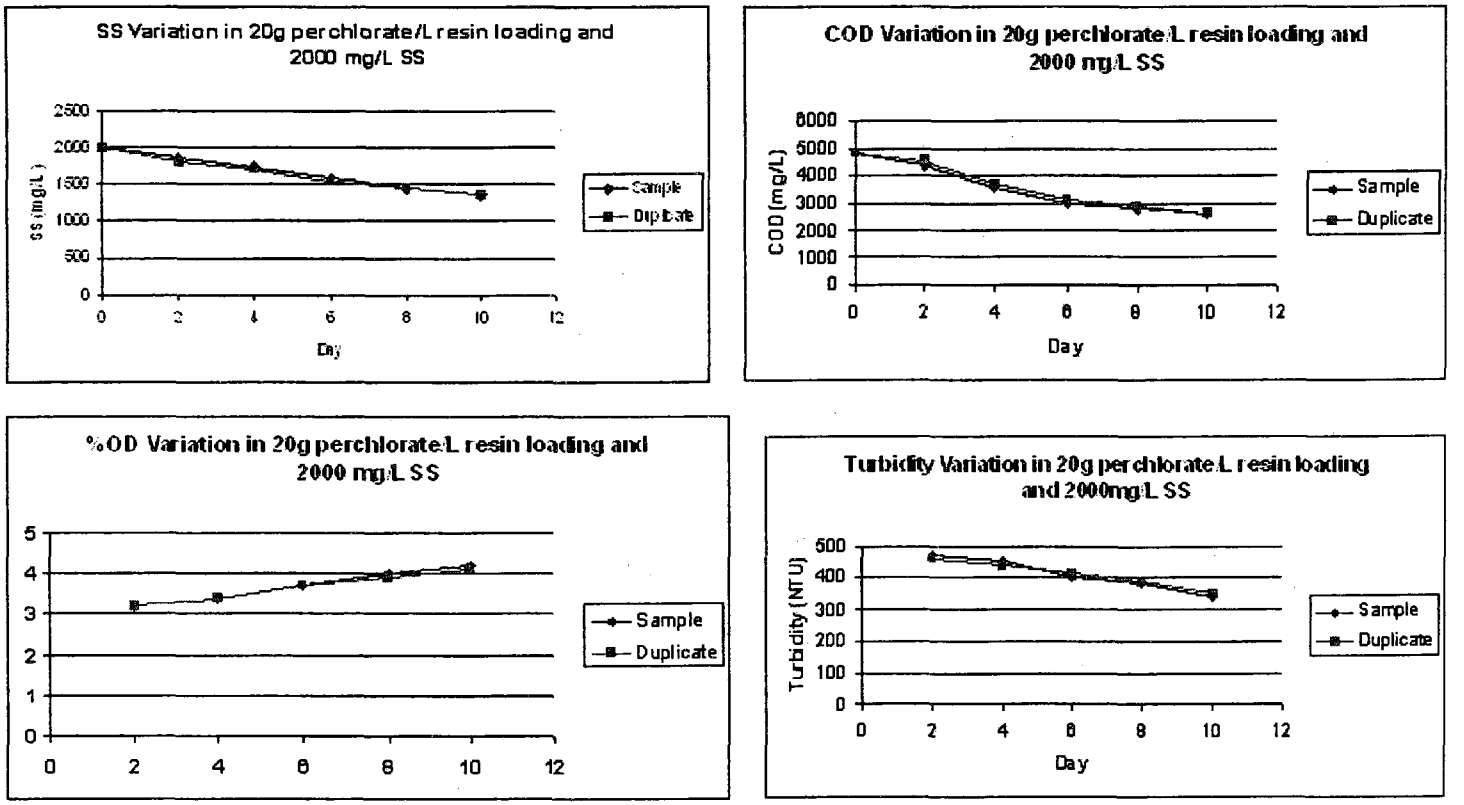
Date	Day	COD mg/l	Duplicate	SS mg/l	Duplicate	OD_{600nM} %transmittance	Duplicate	Turbidity (NTU)	Duplicate	pH	Duplicate
2/4/2009	0	4800	4800	500	500						
2/6/2009	2	4550	4600	493	496	19.4	18.5	114	116	7.1	7.08
2/8/2009	4	3880	4050	495	483	18.1	20.8	117	111	7.08	7.08
2/10/2009	6	3620	3400	454	446	26.5	29.3	99.6	94.5	7.12	7.15
2/12/2009	8	3200	3220	438	421	30.6	31.5	92.3	90.6	7.1	7.09
2/14/2009	10	3050	2900	396	402	34.7	34.2	85.3	86	708	7.07
2/4/2009	0	4800	4800	1000	1000						
2/6/2009	2	4520	4680	920	932	10.4	9.9	250	302	7.18	7.21
2/8/2009	4	3890	4020	909	903	10.8	11.0	226	223	7.15	7.16
2/10/2009	6	3360	3400	795	806	15.2	14.8	185	192	7.2	7.21
2/12/2009	8	3050	3100	715	723	18.5	18.1	172	176	7.18	7.19
2/14/2009	10	2820	2860	692	702	19.4	19.0	153	160	7.16	7.17
2/4/2009	0	4800	4800	2000	2000						
2/6/2009	2	4350	4550	1855	1814	3.2	3.2	475	462	7.28	7.29
2/8/2009	4	3520	3670	1735	1703	3.4	3.4	453	436	7.21	7.21
2/10/2009	6	2980	3120	1589	1549	3.7	3.7	404	413	7.27	7.29
2/12/2009	8	2800	2900	1450	1465	4.0	3.9	386	390	7.26	7.25
2/14/2009	10	2600	2650	1363	1369	4.2	4.1	340	351	7.24	7.25



E-7: Culture Parameter Variation during Bioregeneration of Resin with 20 g/L_{resin} Load and Suspended Solids 500 mg/L



E-8: Culture Parameter Variation during Bioregeneration of Resin with 20 g/L_{resin} Load and Suspended Solids 1000 mg/L



E-9: Culture Parameter Variation during Bioregeneration of Resin with 20 g/L_{resin} Load and Suspended Solids 2000 mg/L

E.6. Effect of Nitrate Load (20 g/L_{resin}) on Perchlorate Degradation Rate in Ion-exchange

(a) 3 g/L_{resin} Perchlorate Load (b) 20 g/L_{resin} Perchlorate Load

(a)

Date of Sampling	Day	Residual Perchlorate	Duplicate	Average	Std. Dev.
27-Nov	0	3.781442779	3.86514625	3.8232945	0.0591873
29-Nov	2	3.684515301	3.91135043	3.7979329	0.1603967
1-Dec	4	3.641148115		3.6411481	
3-Dec	6	3.418004201	3.78233234	3.6001683	0.2576189
5-Dec	8	3.427132168		3.4271322	
7-Dec	10	3.8451751	3.16674588	3.5059605	0.4797219

(b)

Date of Sampling	Day	Residual Perchlorate	Duplicate	Average	Std. Dev.
27-Nov	0	21.442722	20.86572	21.1542212	0.408002128
29-Nov	2	21.2966	20.360794	20.8286968	0.66171437
1-Dec	4	20.994603		20.994603	
3-Dec	6	18.778454	18.451154	18.6148038	0.231436082
5-Dec	8	14.921128		14.921128	
7-Dec	10	10.221417	13.867137	12.0442773	2.577913183

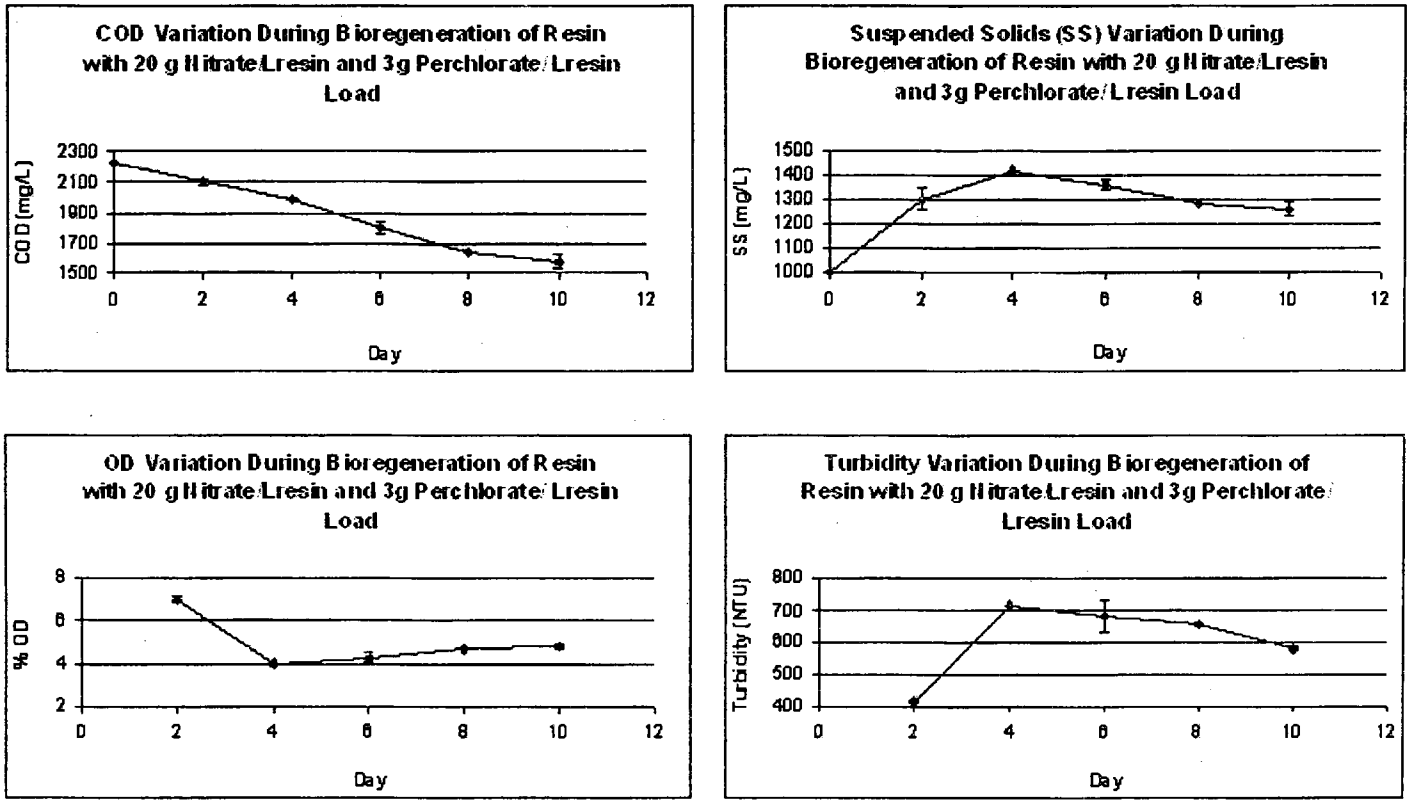
E.7. Culture Parameter Variation During Batch-3 Experiment: (a) 3 g/L_{resin} Load, (b) 20 g/L_{resin} Load

(a)

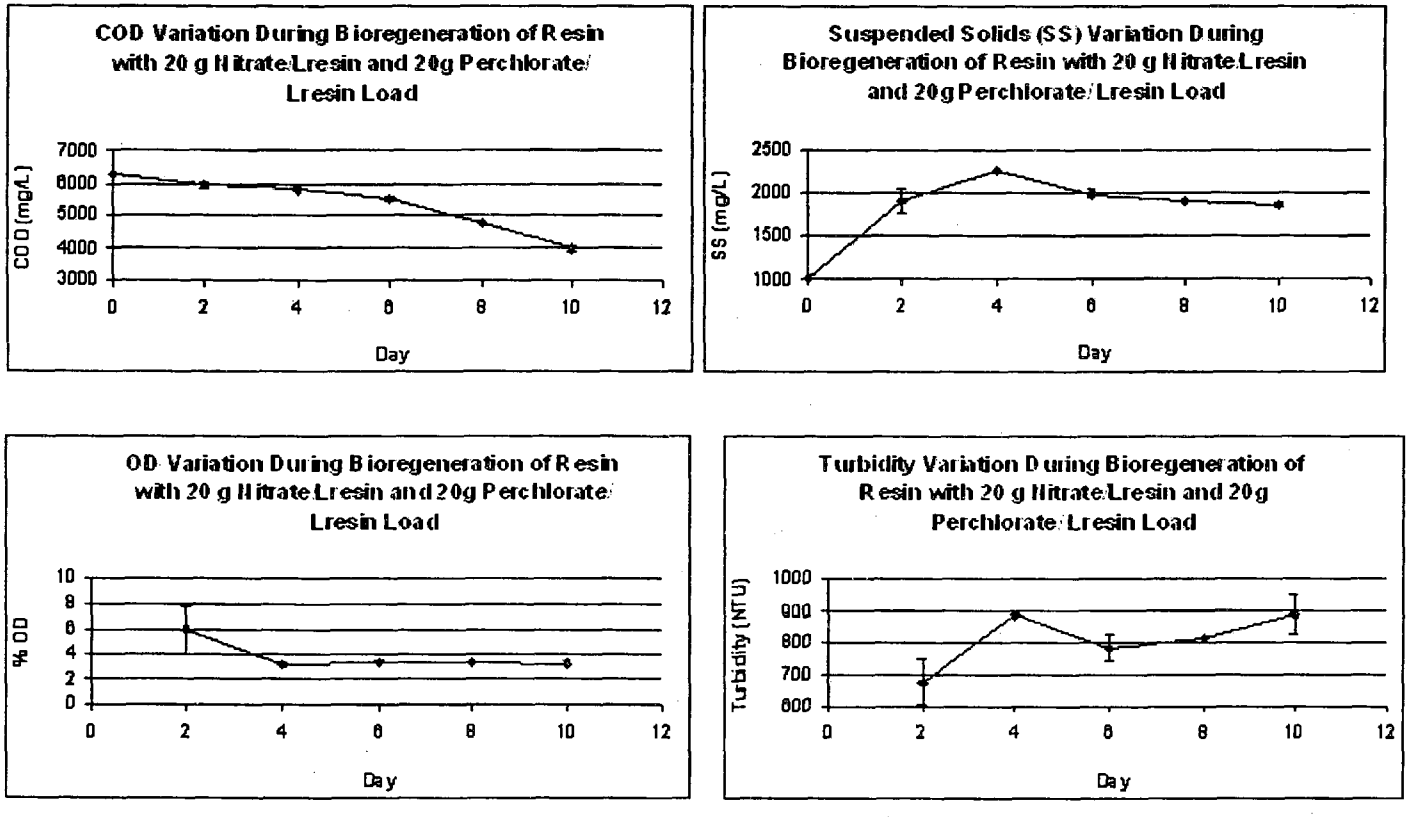
Date	Day	COD mg/l	Duplicate	SS mg/l	Duplicate	OD_{600nM} %transmittance	Duplicate	Turbidity (NTU)	Duplicate	pH	Duplicate
2/19/2009	0	2224	2224	1000	1000						
2/21/2009	2	2100	2080	1300	1370	7	7.2	415	410	7.91	7.86
2/23/2009	4	1980	-	1420	-	4	-	714	-	7.82	-
2/25/2009	6	1800	1850	1360	1385	4.3	4.6	680	610	7.74	7.8
2/27/2009	8	1640	-	1280	-	4.7	-	658	-	7.8	-
3/1/2009	10	1580	1640	1258	1215	4.8	4.9	580	590	7.76	7.77

(b)

Date	Day	COD mg/l	Duplicate	SS mg/l	Duplicate	OD_{600nM} %transmittance	Duplicate	Turbidity (NTU)	Duplicate	pH	Duplicate
2/19/2009	0	6304	6304	1000	1000						
2/21/2009	2	5950	6070	1900	2100	5.9	3.2	677	778	7.85	7.92
2/23/2009	4	5800	-	2250	-	3.1	-	884	-	7.93	-
2/25/2009	6	5500	5400	1986	2043	3.4	3.3	783	839	7.71	7.7
2/27/2009	8	4800	-	1906	-	3.4	-	815	-	7.76	-
3/1/2009	10	3980	4100	1854	1889	3.3	3.5	886	795	7.79	7.8



E-10: Culture Parameter Variation during Bioregeneration of Resin with 20 g/L_{resin} Perchlorate and 3 g/L_{resin} Nitrate



E-11: Culture Parameter Variation during Bioregeneration of Resin with 20 g/L_{resin} Perchlorate and 20 g/L_{resin} Nitrate

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VITA

Graduate College
University of Nevada, Las Vegas

Arjun Krishna Venkatesan

Local Address:
1555 E Rochelle Ave Apt#134
Las Vegas, Nevada 89119

Degrees:
Bachelor of Technology, Chemical Engineering, 2007
Anna University, India

Special Honors and Awards:
James F. Adams/ GPSA Scholarship, 2008

Thesis Title: Investigation of Feasibility and Potential Mechanisms for the Bioregeneration of Perchlorate Laden Gel-type Anion Exchange Resins.

Thesis Examination Committee:
Chair Person, Dr. Jacimaria Batista, Ph.D.
Committee Member, Dr. Sajjad Ahmad, Ph.D.
Committee Member, Dr. Thomas Piechota, Ph.D.
Graduate Faculty Representative, Dr. Eduardo Robleto, Ph.D.