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The removal of perchlorate from waters by a membrane-immobilized biofilm

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THE REMOVAL OF PERCHLORATE FROM WATERS

BY A MEMBRANE-IMMOBILIZED BIOFILM

by

Jian Liu

Bachelor of Science
East China University of Science and Technology, Shanghai, China
1993

A thesis submitted in partial fulfillment
of the requirements for the

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ABSTRACT

The Removal of Perchlorate from Waters by a Membrane-Immobilized Biofilm

by

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This thesis investigated the feasibility of using a membrane-immobilized biofilm reactor to remove perchlorate from waters. Perchlorate (ClO_4^-) is easily reduced under anaerobic conditions to harmless chloride (Cl^-). In this system, the perchlorate-contaminated water is physically separated from the biofilm by the membrane. Perchlorate diffuses, without the need of external energy, to the degrading biofilm, thereby minimizing the contamination of the product water by microbes. A mixed microbial culture capable biologically of reducing perchlorate was enriched and attached to the membrane surface. The results demonstrated the feasibility of using a membrane-immobilized biofilm reactor to remove perchlorate from waters. In addition, the results showed that nitrate and salinity negatively affect perchlorate biodegradation, while sulfate does not have a major effect on perchlorate biodegradation.

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CHAPTER 1

STATEMENT OF THE PROBLEM

Introduction

The American Water Association Research Foundation (AWWARF, 1998) estimated that within the past three years, perchlorate (ClO_4^-) has been detected in water supplies of over 15 million people in the western part of the United States. Perchlorate has also been found in ground and surface water systems in the states of Arizona, Iowa, Indiana, Kansas, Maryland, New Mexico, New York, Pennsylvania, Texas, Utah, and West Virginia. Perchlorate contamination in the United States is associated with the manufacturing and use of ammonium perchlorate (NH_4ClO_4), an important component of rocket fuel and explosives.

In Nevada, perchlorate contamination is the result of the manufacturing of ammonium perchlorate and the discharge of perchlorate-containing wastes into unlined ponds by the Kerr McGee Corporation and the Pacific Engineering & Production Company of Nevada (PEPCON). In other states, perchlorate contamination is associated with the disposal of composite rocket propellants that have to be replaced periodically with fresh perchlorate, due to the short shelf life of perchlorate.

In California, perchlorate has been detected in several drinking water wells and 12 perchlorate occurrences are associated with the manufacturing and testing of solid rocket

fuels for DoD or NASA (EPA, 1998a). Several wells in the San Gabriel Valley, California, have been closed due to perchlorate contamination. Perchlorate has also been detected in water supplies near Magna, Utah; in springs and streams in McGregor, Texas; in wells near East Camden, Arizona; and in water supply wells on Long Island, New York (EPA, 1998a). Perchlorate has been detected widely and in high concentrations in the Las Vegas Valley in Nevada. Several wells in the Basic Management Industrial (BMI) complex, the Las Vegas Wash, and Lake Mead contain perchlorate concentrations as high as 3700 parts per million (ppm). In the Las Vegas Wash, a stream that discharges into Lake Mead and the Colorado River, perchlorate has been detected at concentrations varying from 500 to 1000 parts per billion (ppb). Lake Mead is the primary drinking water source for approximately 1.2 million residents of Las Vegas, and the Colorado River is a water source to millions of people in Arizona and California.

The toxicology data available to evaluate the potential health effects of perchlorate are extremely limited (EPA, 1999a). However, it is proven that perchlorate competes with iodine uptake by the thyroid gland, interfering with the ability of the thyroid to produce thyroid hormones required for normal body growth and development. Studies are currently underway to determine whether perchlorate can affect the thyroid of the developing fetuses and young children (CDHS, 1998). There is no primary drinking water standard for perchlorate. However, the Environmental Protection Agency (EPA) reviewed available toxicology data and set a provisional level for perchlorate in drinking waters as 32 μg/L (ppb). The states of California and Nevada established 18 μg/L as their provisional action level for perchlorate in drinking waters (EPA, 1999a). Studies are underway to evaluate additional health effects of perchlorate on humans. The results

of these studies will be used by the EPA to make a decision regarding a reference dose for perchlorate (EPA, 1999a).

Several treatment technologies, including biological and physicochemical treatment methods, are currently being examined for their potential to remove perchlorate from waters. In 1998, several universities, including the University of Nevada, Las Vegas, were awarded grants from the American Water Works Association Research Foundation (AWWARF) to investigate the potential of ion-exchange, biodegradation, membrane filtration, and ozone/GAC systems to remove perchlorate from waters. The research data available to date points to ion-exchange and biological reduction as the two most promising technologies to treat perchlorate-contaminated wastewaters.

Perchlorate (ClO_4^-) is easily reduced biologically under anaerobic conditions to harmless chloride (Cl^-). Perchlorate-reducing microbes use perchlorate as an electron acceptor in the presence of an electron donor (e.g, organic carbon or hydrogen), nutrients, and minerals. Although perchlorate biodegradation by several strains of bacteria has been reported since the 1960's, only in the last two years have studies on the development of bench and pilot-scale bioreactors for perchlorate removal been initiated. To date, most bioreactor types investigated for perchlorate reduction are attached growth systems with fixed or fluidized beds, using either activated carbon or sand as the media (Catts, 1999; Kim and Logan, 1999; Greene, 1999; Giblin, 1999; and Wallace, 1998). In these systems, the perchlorate-contaminated water is in direct contact with the perchlorate-reducing microbes. Bacterial cells, soluble microbial products and excess organic carbon, used as an electron donors, can contaminate the treated water and may cause undesirable microbial growth in the water distribution system.

An attractive alternative to fixed film bioreactors, in which the microbes are in direct contact with the treated water, may be a membrane-immobilized biofilm reactor. In this reactor set-up, the water to be treated is separated from the microbes by a microporous membrane, on the surface of which a biofilm is grown. Perchlorate is allowed to reach the perchlorate-reducing biofilm by diffusing through the membrane. This process is not energy-intensive for it is solely based on diffusion. The side of the membrane to which the biofilm is attached is in contact with the electron donor, nutrient and minerals needed for microbial growth. The membrane and biofilm also act as a barrier to prevent the carbon source from mixing with the water being treated, minimizing the contamination of treated water by carbon source, bacterial cells, and soluble microbial products.

This thesis evaluates the feasibility of using a membrane-immobilized biofilm reactor to remove perchlorate from waters. Furthermore, the influence of several ions on perchlorate biodegradation will be investigated.

Objectives

The specific objectives of this research are

1. To select perchlorate resistant microporous membrane types that would allow perchlorate to move, solely by diffusion, to the membrane side on which a biofilm is attached.
2. To determine the diffusion coefficients of perchlorate and other anions through the selected microporous membrane types.
3. To enrich for a mixed microbial culture capable of reducing perchlorate.

4. To generally characterize the perchlorate-reducing mixed enrichment culture.
5. To immobilize a biofilm on the surface of the selected microporous membranes.
6. To determine, with batch tests, the minimum ratio of carbon to perchlorate needed to obtain perchlorate biodegradation at reasonable kinetic rates.
7. To investigate the biodegradation of perchlorate by the membrane-immobilized biofilm reactor.
8. To determine the influence of different levels of nitrate, sulfate, and salinity on perchlorate biodegradation in the membrane-immobilized biofilm reactor.

CHAPTER 2

BACKGROUND

History of Perchlorate Synthesis

The first perchlorate compound, named "oxygenated potassium chlorate", was synthesized by Count Friederich Von Stadion in 1816 (Schilt, 1979). G.S. Serullas, who prepared ammonia perchlorate, a number of metal perchlorates, and a solid form of perchloric acid, made important contributions to the development of perchlorates in the early 1830's. He also coined the term "perchlorate" to replace Stadion's term "oxychlorate" (Schilt, 1979).

In 1893, the first commercial perchlorate plant was constructed at Mansbo, Sweden. By 1904, ammonium perchlorate could be produced in an efficient, regular basis in the plant (Schilt, 1979). Because ammonium perchlorate is useful in solid rocket propellants, its commercial growth increased greatly in the 1950's.

Chemical Properties of Perchlorate

The perchlorate anion (ClO_4^-) has been determined to have a tetrahedral geometry, with the chlorine atom at the center and four oxygen atoms at the vertices (Figure 2.1). The perchlorate ion is a powerful oxidizing anion and has a strong oxidizing potential. Thus, perchlorates have been used industrially as oxidizers in

propellants and explosives for over 50 years (Lamm, 1999). It is the most highly oxidized species among the oxychloride series, including hypochlorite (ClO^-), chlorite (ClO_2^-), and chlorate (ClO_3^-).

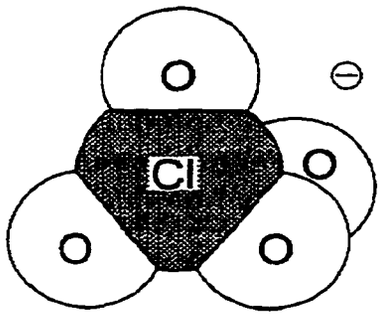


Figure 2.1 Structure of perchlorate ion (Source: EPA, 1998a)

In perchlorate, chlorine has the highest oxidation state (+7), while, the lowest oxidation state is found in chloride (-1), the most stable chlorine species. The ΔG^0 for the reduction of perchlorate to chloride is -1073.79 KJ/mole (Gurol and Kim, 2000). Thus, thermodynamically, perchlorates are favored to undergo chemical reduction by reacting readily with reduced compounds. However, at ambient conditions and in the absence of a catalyst, perchlorate reduction is kinetically slow. One factor affecting perchlorate kinetics include is its structure. The configuration of the perchlorate ion shows that the chlorine atom is surrounded by four oxygen atoms, accounting for all eight electrons filling the outer electron shell. In addition, the tetrahedral packing structure of perchlorate allows for an even distribution of the charge around its relatively large surface area. The strength of the chlorine-oxygen bonds is another factor, which can inhibit the reactivity of perchlorate. The length of the chlorine-oxygen bond, which is an

indicator of bond strength, is the shortest in perchlorate among all the oxychlorides. The average bond distance of chlorine-to-oxygen is 1.42 picometer (pm), and the oxygen-to-oxygen distance is 2.43 pm (Schilt, 1979). Therefore, in order for the reduction process to proceed, a great amount of activation energy is needed to disrupt the stable structure of perchlorate. Common reducing agents, such as thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3^{2-}) or elemental metals (e.g. Fe, Zn, Cu), do not reduce perchlorate (Urbansky, 1999).

Since ammonium perchlorate is widely used as an oxidant in solid rocket propellants, it has been studied extensively. Ammonium perchlorate is stable up to 110 °C, decomposes at 130 °C, and explodes at 380 °C (Schilt, 1979). Its density is 1.95 g/cm^3 at 20°C (MELE, 1998). The following equation is used to describe the decomposition reaction of ammonium perchlorate below 300 °C:

$$4\ NH_4ClO_4 \longrightarrow 2\ Cl_2 + 8\ H_2O + 2\ N_2O + 3\ O_2$$

Above 400 °C, most of the nitrogen is evolved as nitric oxide (Schilt, 1979).

Physical Properties of Perchlorate

Perchlorate is very soluble (Table 2.1) and non-volatile (MELE, 1998, EPA, 1999a). Perchlorate salts can easily dissolve in water to form perchlorate anion. The high solubility of perchlorate increases its mobility in the natural environment. Contamination of waters by perchlorate salts is generally the result of the dissolution of ammonium, potassium, magnesium, or sodium perchlorate. Ammonium perchlorate is very soluble in water and dissociates completely to ammonium and perchlorate ions.

Table 2.1

Solubility and density of perchlorate salts

(modified from Schilt, 1979)

Chemicals	Molecular Weight (g/mole)	Density	Solubility (g/L water)
$LiClO_4$	106.40	2.429	597.1
NH_4ClO_4	117.49	1.952	249.22
$NaClO_4$	122.44	2.499	2096
$KClO_4$	138.55	2.5298	20.62
$RbClO_4$	184.92	2.9	13.38
$CsClO_4$	232.36	3.327	20.00

Health Effects of Perchlorate

The primary health effect of perchlorate relates to its capacity to interfere with the thyroid gland's ability to properly utilize iodine. Iodine is an essential element in the production of thyroid hormones. Iodine deficiency can lead to hypothyrodism in adults, and cretinism (mental retardation) in children (Cortez et al., 1999). The thyroid gland is located in the front of the neck and it is the neck's largest gland. An important function of thyroid tissue is to concentrate iodide from the surroundings, promoting efficient hormone (known as triiodothyronine T3 and thyroxine T4) synthesis (Wolff, 1998). Iodine combines with tyrosine (aminoacid) and produces thyroxine (T4) and triiodothyronine (T3). T4 is the primary hormone, produced by the thyroid gland, and

occupies about 90% of total released thyroid hormone. T3 occupies about 10% of total released thyroid hormone, and it is produced when T4 is secreted into the bloodstream and comes into contact with certain proteins in the body (Cortez, 1999). These hormones are vital, controlling metabolism, growth and development, brain functioning, nerves, and reproductive organs. The production of T3 and T4 hormones is stimulated by another hormone, TSH (thyroid-stimulating hormone).

When perchlorate competes with iodine and because of decreased thyroid hormone production, the pituitary gland releases more thyroid-stimulating hormone (TSH) to stimulate production of thyroid hormones (EPA, 1999a). Prolonged stimulation may result in thyroid neoplasia, particularly in rodents known to be sensitive. Several animal studies found that benign tumors have been introduced to the thyroids of male Wistar rats and female BALB/C mice treated with repeated, high dose exposure of potassium perchlorate (EPA, 1999a).

Perchlorate's effect on the thyroid gland is known from its use as an iodine inhibitor to treat Graves' disease (hyperthyrodism) in the late 1950s and early 1960s (Wolff, 1998). Graves' disease is characterized by hyperthyroidism (thyroid hyperactivity) due to diffuse hyperplasia of the thyroid, infiltrative ophthalmopathy, and infiltrative dermopathy (localized myxedema) (Cooper, 1991). The probable cause of Graves' disease is a defect in organ-specific suppressor T-cell that results in the production of thyroid-stimulating antibodies (TSab) -- antibodies to the thyroid stimulating hormone (TSH) receptor on the thyroid follicular cell membrane. These antibodies behave like TSH and activate the TSH receptor to actively pump iodide, causing increased thyroid hormone synthesis and release as well as thyroid gland growth.

Therapy for Graves' disease is directed to destroying thyroid tissue, inhibiting thyroidal thyroxine (T4) and triiodothyronine (T3) synthesis and release and ameliorating the impact of the hormones on peripheral tissues, alone or in combination (Cooper, 1991). Perchlorate intake is a therapy that directly inhibits the synthesis and released of T3 and T4 hormones.

The United States was the first country to use perchlorate to treat thyrotoxicosis. In the late 1950s and early 1960s, potassium perchlorate ($KClO_4$) was extensively used pharmacologically as an anti-thyroid agent in the treatment of Graves' disease. Potassium perchlorate affects the iodide-trapping mechanism of the thyroid and reduces thyroid hormone synthesis by competitive inhibition of thyroidal iodide uptake. Perchlorate has no effect on the iodination process itself. It is concentrated by the thyroid tissue in a manner similar to iodide but is not significantly metabolized in the gland or peripherally (Wolff, 1998). Goodman (1999) reported that levels of perchlorate in human serum on the order of 0.1 mg/L would be expected to inhibit iodide uptake by about 50%. The sodium-iodide (Na^+/I^-) symporter, a membrane-bound protein located on the basolateral (blood-contact) side of the thyroid folliculare cell, is the site of this inhibition. This inhibition prevents further synthesis of thyroid hormone and results in a decreased production of T3 and T4 thyroid hormones.

It has been reported that people who have been treated with perchlorate suffered gastrointestinal irritation, skin rash, and hematological effects including agranulocytosis, aplastic anemia, and lymphoadenopathy (TERA News, 1997; CDHS, 1998). When perchlorate doses larger than 1,000 mg/day are used, severe hematological effects are more likely to occur (Wenzel, 1984). During 1961 and 1966, seven cases of fatal aplastic

anemia occurred as the result of potassium perchlorate treatment. The use of perchlorate anion was abandoned, except for single dose-use in perchlorate discharge tests and as an adjunct to pertechnetate scanning (Wolff, 1998). In the early 1980s, potassium perchlorate was used again for the treatment of Graves' disease with the lessons learned from earlier reactions kept well in mind (CDHS, 1998). No serious side effects occur as long as the dose is kept below 1,000 mg/day (CDHS, 1998). Cooper (1991) also reported that potassium perchlorate has been used again recently to treat hyperthyroidism with success and without toxicity with doses of 40 to 120 mg/d.

Currently, there is no National Primary Drinking Water Regulation (NPDWR) for perchlorate. In 1992 and 1995, U.S.EPA Superfund Technical Support Center issued a provisional oral reference dose (RfD) for perchlorate, which corresponds to an acceptable range of perchlorate in drinking water of 4 to 18 part per billion. The RfD is an estimate of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious non-cancer health effects during a lifetime. In 1997, the California Department of Health Services (CDHS) established a limit of 18 ppb as its provisional action level to protect against adverse health effects of perchlorate exposures (EPA, 1999a). However, the U.S. EPA National Center for Environmental Assessment (NCEA) has recently announced a new RfD for perchlorate of 0.0009 mg/kg-day, which corresponds to an action level of 32 μg/L in drinking water (Standards for Perchlorate in Drinking Water, 1999; Renner, 1999, Ladd, 2000). Perchlorate was placed on the Office of Water (OW) Contaminant Candidate List (CCL) in March 1998, with note that additional research and information are required before regulatory determinations can be made (Ladd, 2000).

Sources of Perchlorate Contamination and Applications

The major source of perchlorate contamination is man-made and it relates closely to its application. Perchlorate salts have been used widely in the chemical, commercial, aerospace and defense industries because of their strong oxidizing properties. The use of ammonium perchlorate as an ingredient in various explosive mixtures was first described in 1897 (Schilt, 1979). Inorganic perchlorate explosives generally consist of mixtures of either ammonium or potassium perchlorate as oxidant with sulfur and/or various organic materials as fuels. Perchlorate explosives afford certain advantages over dynamite and other nitroglycerine explosives. They are safer to handle, less sensitive to shock, considerably less affected by freezing, free from exudation in warm climates, and relatively nontoxic (Schilt, 1979). The predominant source of perchlorate contamination is ammonium perchlorate. Ammonium perchlorate (NH_4ClO_4) is a strong oxidizer and is widely used as an oxidant in solid rocket propellants, composing up to 70% of the material by weight in some cases (Attaway, 1993). The production of perchlorate salts on a large scale began in the mid-1940's in the United Sates. Because of the limited shelf life of the propellants, as a part of regularly scheduled maintenance operation, composite propellants containing ammonium perchlorate must be periodically washed out of the missile and rocket and replaced with a fresh supply. Currently, the large solid rocket motor disposal inventory has 55 million pounds of propellant ready for treatment. This amount is expected to increase to 164 million pounds of perchlorate-containing solid rocket propellant targeted for disposal over the next 8 years (Wallace, 1998). Thus, large volumes of ammonium perchlorate have been disposed at sites in Nevada, California, Utah, and other states since the 1940s. Much of this waste material had been burned in

open pits and residual propellants were washed to the surrounding soils (MELE Associates, 1998). Perchlorate salts have also been manufactured for use as primary ingredient in rocket motors, missiles, fireworks, manufacture of matches, and in analytical chemistry (Schilt, 1979). Perchlorate salts are also used on a large scale as a component of air bag inflators. Other uses of perchlorate salts include in nuclear reactors, and electronic tubes; as additives in lubricating oils, in tanning and finishing leathers, as a fixer for fabrics and dyes, in electroplating, in aluminum refining, in rubber manufacture, and in the production of paints and enamels (Schilt, 1979).

Until recently, it was thought that no natural sources of perchlorate existed. In late 1900's, Beckurts and Sjollema reported the presence of perchlorate in Chilean nitrate (also known as Chile saltpeter) (Schilt, 1979). In 1958, Baas-Becking, Haldane, and Izard reported the presence of low levels of perchlorate in seawater. However, later studies by Greenhalgh and Riley indicated no detectable concentration of perchlorate in seawater. It appears that perchlorate is not an important natural component of seawaters (Schilt, 1979). Recently, Susarla et al. (1999d) reported the presence of perchlorate in fertilizers. It is believed that Chilean nitrate is one of the sources of perchlorate for the fertilizers. Some damage has been observed in rye crops and has been attributed to the potassium perchlorate present as a contaminant in the saltpeter. It was reported that Chilean nitrate caused leaf rugosity in soybean plants when applied as a fertilizer (Susarla, 1999d). The United States uses 75,000 short tons of Chilean nitrate annually and total annual fertilizer usage is 54 million short tons (Renner, 2000). Perchlorate concentrations detected in fertilizers vary dramatically between fertilizers and between

production lots, which indicate that there are either differences in raw materials or production methods (Renner, 2000).

Perchlorate Contamination in the USA

The primary sources of perchlorate contamination are industrial and military operations that use perchlorate as an oxidizing agent. Within the last two years, perchlorate has been found in the water supplies of over 15 million people in CA, NV, and AZ and in surface or groundwater throughout the United States, including AR, IA, IN, KS, MD, NM, NY, PA, TX, UT, WV (EPA,1999b). EPA estimates that perchlorate has been either manufactured or used in 44 states, and it has been measured in surface or groundwater in 13 states (Renner, 1999). Based on EPA reports, the confirmed perchlorate manufactures and users distribute all over the United States, except in six states--- Alaska, Hawaii, Maine, Vermont, Connecticut, and Rhode Island (Renner, 1999).

Drinking water contaminated with perchlorate was first discovered in Rancho Cordova, California in 1983. This contamination is associated with the Aerojet Liquid Rocket Testing Facility. The California Department of Health Service (CDHS, 2000) also reported perchlorate contamination in drinking water wells in eastern Sacramento County with concentrations up to 260 μg/L. During the late 1980s, perchlorate was detected in concentrations up to 8,000 μg/L in groundwater system beneath the Aerojet General's Aerospace facility near Sacramento, California. In Feb. 1997, Aerojet General

found that perchlorate concentration in offsite drinking water wells was up to 280 μg/L (Okamoto, 1999).

In April 1997, a new analytical method to detect low concentrations of perchlorate of 4 ppb was developed by the California Department of Health Services (CDHS), which led to the discovery of the chemical at various manufacturing sites and some drinking water supply wells of communities in California, Nevada, and Utah (Renner, 2000).

Perchlorate contamination in groundwater, drinking water, and soils has been detected, mainly in the southwestern United States at levels that range from 8 to 3700 mg/L. The highest levels are found in wells located near the Kerr-McGee Chemical Corporation, an ammonium perchlorate manufacturer, located in Henderson, Nevada (Renner, 2000). Perchlorate has also been found in ground water at six Superfund hazardous waste sites in California, at six other California non-Superfund waste sites, in one site in Utah, and in the discharge to a creek in Texas (EPA, 1998b). Water suppliers in both northern and southern California, and the Southern Nevada Water Authority have found perchlorate in their water supplies generally at levels less than 18 ppb but as high as 280 ppb, with several in the 100—200 ppb range (EPA, 1998b). Perchlorate has also been detected at low levels (5 to 9 ppb) in the Colorado River (EPA, 1998b). The CDHS (2000) also found perchlorate contamination up to 29 ppb in some Riverside drinking water well, up to 325 ppb in some San Bernardino County drinking water wells, and up to 221 ppb in 24 agricultural wells.

Las Vegas has the largest reported perchlorate contamination problem. The largest known point source for perchlorate is the seepage in the Las Vegas Wash that

contains 100,000 ppb ClO_4^- (Vieira, 2000). The contamination is the result of the leaching of perchlorate residuals deposited into unlined ponds. Perchlorate contamination of Las Vegas drinking water supply, Lake Mead and the Colorado River mainly stems from the seepage in the Las Vegas Wash. Perchlorate concentrations in the groundwater below the Kerr McGee Corporation area are very high and a recent sample was found to have 1.5 million parts per billion of perchlorate (Rogers, 1998).

Perchlorate Removal Technologies

Because perchlorate is highly soluble and non-volatile in water, it cannot be removed by conventional filtration, activated carbon adsorption, sedimentation, and air-stripping technologies. Several treatment technologies, including ion-exchange, membrane separation, and biological reduction, are under investigation to determine the most effective method for perchlorate removal. Current perchlorate treatments under investigation can be divided into physicochemical and biological treatments.

Physicochemical Treatment

Physicochemical removal processes include ion exchange and membrane filtration. Since these methods do not destroy perchlorate, but physically separate perchlorate from the contaminated water, they require post-treatment processes for the disposal of perchlorate-containing wastes.

Ion-Exchange Method

In the ion exchange process, ion-exchange resins are used to sorb perchlorate from the contaminated water. Ion-exchange resins are non-soluble solids covalently bonded to ionized functional groups that are chemically treated (Vieira, 2000). In the

process, the resins are placed in the column and perchlorate-contaminated water passes through the resin, where the perchlorate ion replaces an innocuous anion (e.g. chloride) that is attached to the resin. The effluent from the column is perchlorate-free water. Eventually, the resin reaches an equilibrium concentration where no more perchlorate can be exchanged with the water; at this point, the resin is saturated and must be regenerated. Regeneration is normally accomplished by passing a high concentration of sodium chloride (NaCl) or sodium hydroxide (NaOH) through the saturated resin bed. The regeneration process generates a perchlorate-rich waste brine that requires further treatment or disposal.

Recently, Vieira (2000) investigated several types of ion-exchange resins to remove perchlorate from waters. He found that styrenic strong base resins had high perchlorate removal efficiency, but low regeneration efficiency. In addition, he found that acrylic strong-base resins showed satisfactory perchlorate removal efficiency and better regeneration efficiency. Acrylic weak-base resins appeared to have satisfactory perchlorate-removal efficiency and very high regeneration efficiency. However, the perchlorate removal efficiency, for all the tested resins, was negatively affected by the presence of humic substances and sulfate.

Tripp and Clifford (2000) also investigated the removal of perchlorate by ion-exchange and found that the more hydrophobic resins exhibited higher separation factors for perchlorate and that the highest separation factors were for polystyrene resins with triethyl and triprophyl functional groups. In addition, they found that the separation factor for perchlorate, for several resins, could be decreased by increasing the temperature to 40 °C.

Membrane Filtration Method

Membrane filtration techniques include reverse osmosis (RO) and nanofiltration. Water is forced through a semi-porous polymer membrane; while dissolved salts are unable to penetrate the membrane. Manufacturers can adjust the membrane permeability towards different anions and cations to a certain degree (Urbansky, 1999). However, the cost of construction, operation and waste disposal for treating large volumes of water for low perchlorate concentrations might be very high right now (Mayer, 1997), and membrane fouling by alkaline earth and transition metal compounds can also present a problem. Yoon et al. (2000) investigated perchlorate removal by two polyamid nanofiltration membranes. They found that membranes with a negative charge can reject perchlorate, which can be explained by the Donnan Equilibrium equation. They found that perchlorate rejection is expected to increase with increasing pH, decrease with increasing ionic strength, and decrease in the presence of mono-and divalent anions and cations.

Chemical Reduction Method

Chemical reduction uses certain reducing agents to reduce perchlorate's chlorine atom, oxidation state +7, to chloride, oxidation state of -1. Although perchlorate is thermodynamically favored to undergo chemical reduction processes by reacting readily with reduced compounds, it does not exhibit its oxidizing properties under the conditions found in contaminated raw and treated waters. Thus, it cannot be reduced by common agents, such as thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3^{2-}), or elemental metals—Fe, Zn, or Cu (Urbansky, 1999). The rate of chemical reduction is too slow to be used practically. Although more exotic reducing agents, such as titanium, vanadium, molybdenum, or

ruthenium, may react with perchlorate, these chemicals are likely to be too unstable or toxic to be practical for water treatment (EPA, 1999a). Donnelly (1997) states that unless safe new catalysts become available, the chemical reduction of perchlorate will play no role in drinking water treatment in the near future. Besides, the toxicity of the by-products produced in the chemical reduction process have to be considered.

Urbansky (1998) reported that electrochemical reduction, which reduce perchlorate to chloride using an electric current, applied directly to the water by a cathode at high potential, appears to be more promising than chemical reduction. The advantage of this method is that the control over kinetics can be achieved by control of the operating potential. Several factors can affect the electrode reduction kinetics, including diffusion of the ions from the bulk water to the electrode surface, and the time required for these ions to associate with the surface and activation past the overpotential required to reach the transition state. Electrode corrosion, surface passivation and natural organic matter (NOM) adsorption to the surface present technological difficulties. The cathodes of many different materials have been tried, including platinum, tungsten carbide, ruthenium, titanium, aluminum and carbon doped with chromium (III) oxide or aluminum oxide. Although electrochemical technologies are well established for other industries (e.g., electroplating of metals, electrolysis of brine), they have not yet found a place in drinking water treatment (Urbansky, 1998).

Biological Treatments

Perchlorate-Biodegrading Microbes

Although the chemical reduction of perchlorate is thermodynamically favorable, it does not occur naturally, since perchlorate behavior is dominated by its slow kinetics.

Fortunately, microorganisms are capable of producing enzymes that can overcome the high activation energy needed for perchlorate reduction. Several studies involving perchlorate biodegradation have been performed (Yakovlev, 1971; Korenkov, 1976; Attaway, 1994; Rikken, 1996; Hurley, 1996, Wallace, 1996). The biological reduction of perchlorate, biodegradation, has been reported by several researchers and provides the prospect for biological perchlorate removal treatment systems. Mixed and pure bacterial cultures have been demonstrated to reduce perchlorate biologically. In the reduction process, which occurs under anaerobic conditions, perchlorate is used as an electron acceptor, and is reduced to innocuous chloride when an electron donor, nutrient and minerals are provided.

Little is known about the biochemical pathways of perchlorate reduction by microorganisms under anaerobic conditions. However, it is currently accepted that perchlorate is first reduced to chlorate, then to chlorite, and finally to oxygen and chloride as shown below:

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

Rikken (1996) isolated and characterized the perchlorate-reducing pure culture-strain GR-1. In this study, based on chlorooxo compounds and on what is known about nitrate reduction by denitrifying bacteria, Rikken and his coworkers provided a hypothetical pathway for perchlorate biodegradation. First, biodegradation could proceed via chlorate (ClO_3^-) and chlorite (ClO_2^-), then via hypochlorite (ClO^-) or dichlorooxide (Cl_2O^-). The disappearance of perchlorate and the stoichiometric production of chloride in the experiment suggested that there was no accumulation of intermediates and that acetate oxidation was coupled to the reduction of perchlorate and chlorate. The reduction

of perchlorate and chlorate was also accompanied by biomass production, indicating that the microbial reduction of perchlorate or chlorate is coupled to energy-yielding reactions. The analysis also revealed that there was no production of potential intermediates such as chlorite and hypochlorite by strain GR-1. Chlorite and hypochlorite are toxic to microbes, and the detoxification mechanism that protects the microbial cell from their effects has not been fully investigated to date. Van Ginkel (1996) found that strain GR-1 contained a heme iron enzyme named chlorite dismutase that effectively catalyzed chlorite to chloride and oxygen. Wallace, et al. (1996) isolated a strain of *Wolinella succinogenes* (coded HAP-1), which demonstrated a chlorite dismutase activity that was at least 1000-fold greater than that of perchlorate or chlorate-reductase. Thus, the production of chlorite during perchlorate or chlorate reduction should not accumulate to toxic levels (Herman, 1998). Rikken's study showed that Strain GR-1 could not grow with chlorite since the energy released by the dismutation reaction cannot be used for biosynthesis, which indicated that chlorite transformation to oxygen and chloride by strain GR-1 is independent of the presence of acetate, i.e., chlorite is directly reduced to chloride and oxygen. However, oxygen can inhibit perchlorate and chlorate reduction, thus, the isolated pure culture of GR-1 has to use perchlorate or chlorate and oxygen at the same time (Rikken, 1996). Van Ginkel (1996) also reported that oxygen was generated in the last step of perchlorate biodegradation, and that oxygen is a preferred electron acceptor and will inhibit perchlorate reduction. However, dissolved oxygen does not accumulate in solution and its production during perchlorate degradation does not inhibit the overall reaction. Based on the results of their experiment, Rikken et al. (1996) proposed a three-step mechanism of perchlorate reduction by strain GR-1 (Figure 2.2)

using acetate as an electron donor, in which chlorate and chlorite are intermediate products, bicarbonate and chloride are end products. In this process, the reduction of perchlorate to chlorate is the rate-limiting step. Eight electrons are transferred from perchlorate to chloride, of which four are provided by acetate and another four electrons were involved when Strain GR-1 dismutates chlorite to form oxygen and chloride.

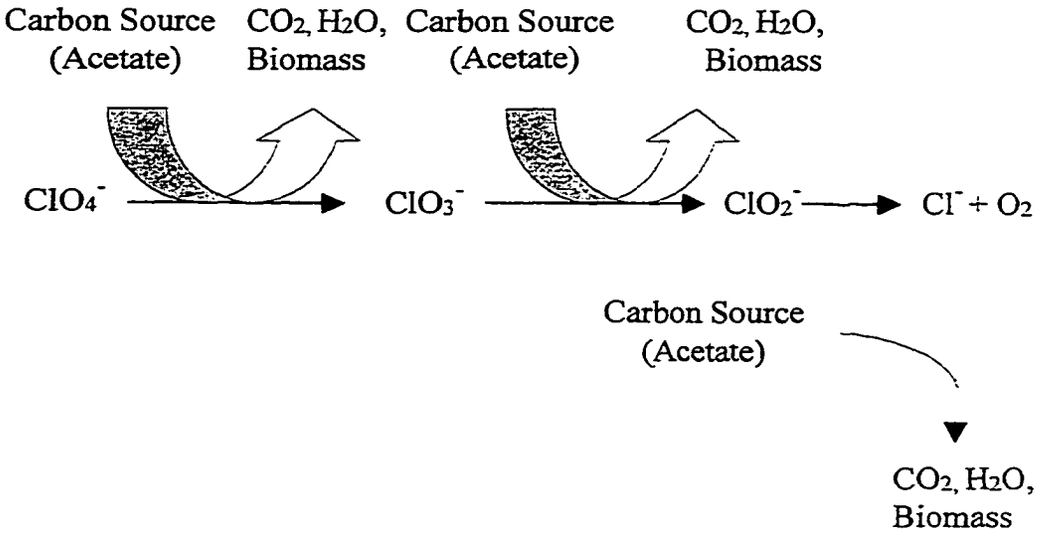


Fig. 2.2 Perchlorate biodegradation pathway by strain GR-1
(Modified from Rikken, 1996)

Attaway and Smith (1993) pointed out that the redox potential needed for perchlorate biodegradation to occur should be lower than –110 mV. Mulvaney (1999) also reported a study in which a perchlorate-reducing mixed culture turned resazurin, a redox indicator, from pink to clear at –110 mV, indicating that oxygen is released from perchlorate biodegradation. However, very little is known regarding the minimum oxygen concentration required to inhibit perchlorate biodegradation. More studies are needed in this area.

Although perchlorate biodegradation has been recognized to be a promising and important technology for treating perchlorate-contaminated water and soils, very little is known about the microorganisms that carry on perchlorate biodegradation.

To date, only a few perchlorate-reducing microorganisms have been isolated and characterized. However, information is available from earlier studies on chlorate biodegradation that is a major step in perchlorate biodegradation. Table 2.2 summarizes the current information available on perchlorate- and chlorate-reducing microorganisms.

Table 2.2 Summary of perchlorate- and chlorate-reducing microorganism studies

Types of Microbes	Characteristics	Electron Acceptor		Electron Donor		Reference
		Positive	Negative	Positive	Negative	
Source: municipal sludge	Mixed culture Reduction rate = 12 mg ClO_4^-/hr-L	ClO_4^- Cr^{6+}		Sludge		MELE (1998) Yakovlev (1971)
Vibrio dechloraticans Cuznesove B-1168 Source: municipal sludge	G^-, single cells, size 0.8-1 x 0.5-0.4 μ, mobile with one flagella of 1.5-2μ, bent-vibrio, sporeless, well-colored with fuchsione and non-colored with methylene blue. Reduction rate = 70 mg ClO_4^-/g of biomass-hour	ClO_4^- ClO_3^- NO_3^-		acetate ethanol sugar with acetate	lactose starch, oxalic acid citric acids	Korenkov (1976)
Strain GR-1 Source: Activated sludge	G^-, oxidase$^+$, motile rod-shaped. No indole formation from tryptophan, no acidification of glucose, no β–glucosidase, no β–galactosidase and no protease. Belong to the β subdivision of the *Proteobacteria* according to 16S rDNA.	ClO_4^-, ClO_3^- NO_3^- Mn(IV) O_2	sulfate iodate bromate chlorite selenate Fe(III)	acetate propionate caprionate Malate succinate lactate	glycine, formate glycolate glucose	Rikken (1996)
Wolinella succinogenes (HAP-1) Source: Anaerobic sewage	G^-,sporeless, rod, 0.5 x 2-8 μm, sporeless, motile, Strictly anaerobic colonies are clear,circular, and mucoid catalase$^-$. pH=6.5-8.0 (Opt. 7.1) T=20-40 °C (Opt.40° C) Reduction rate = 221 mg ClO_4^-/hr-L	ClO_4^-, ClO_3^- NO_3^- fumarate	ClO_2^-, SO_4^{2-} NO_2^- sulfite thiosulfate iron oxide manganese dioxide magnesium oxide	Mixture of H_2 and acetate, aspartate fumarate, lactate malate, pyruvate, succinate Whey powder peptone yeast extract	glucose fructose galactose lactose sucrose starch butyrate citrate formate propionate ethanol methanol 1-propanol	Attaway (1994)

Table 2.2 Summary of perchlorate- and chlorate-reducing microorganism studies

Types of Microbes	Characteristics	Electron Acceptor		Electron Donor		Reference
		Positive	Negative	Positive	Negative	
				brewers' yeast Casamino acids and Cottonseed protein	benzoate	
Strain CKB Source: Paper mill waste	G$^-$, cells 0.5x2 μm, motile, single polar flagellum, facultative anaerobe, completely oxidizing, non-fermentative. Optimum T = 35 °C Optimum pH =7.5 Optimum salinity = 1% NaCl No bacteriochlorophyll Close to the phototroph *Rhodocyclus tenuis* according to 16S rDNA	ClO_4^- ClO_3^- O_2		Acetate, propionate, butyrate, lactate, succinate, fumarate, malate or yeast extract, Fe(II) or sulphide or the reduced form of the humic substances analogue 2,6-anthrahydroquinone disulphonate	Hydrogen glucose	Bruce (1999)
Perclace Source: Biosolids from wasterwater treatment plant	G$^-$, curved rod, facultative anaerobe. T=20-40°C (optimum=25-30°C) PH=5.5-8.5 (optimum=7.0-7.2) A member of the β subdivision of the *Proteobacteria* by its 16S rDNA analysis. Similar to strain GR-1, however, GR-1 can reduce Mn(IV), while perclace could not.	ClO_4^- NO_3^- O_2	Fe(III), Mn(IV) SO_4^{2-}	Acetate, fumarate, propionate, succinate, casamino acids, nutrient broth, peptone, tryptic soy broth and yeast extract	citrate formate glucose lactose sucrose fructose starch methanol ethanol	Herman, (1999)
PDX, D8, KJ, KJ3, KJ4 Source:	G$^-$, rod, motile, facultative anaerobes. PDX: 2.1±0.14x0.55±0.05 μm; KJ: 1.6±0.13x0.74±0.05 μm	ClO_4^- ClO_3^- NO_3^-	SO_4^{2-}	Acetate, lactate, succinate, pyruvate	Saturated hydrocarbon decane (KJ and	Mulvaney (1999)

Table 2.2 Summary of perchlorate- and chlorate-reducing microorganism studies

Types of Microbes	Characteristics	Electron Acceptor		Electron Donor		Reference
		Positive	Negative	Positive	Negative	
Municipal wastewater	D8: 1.7±0.13x0.78±0.04 μm PDX and KJ is similar to isolate GR-1.			Dicarboxylic amino acids glutamic and aspartic acids PDX can also use citrate butyrate and H_2	PDX)	
Ideonella dechloratans Source: Activated sludge	G^-, motile rod shaped, polarly flagellated, catalase$^+$, oxidase$^+$, chemoorganotrophic, Belong to β subgroup of *Proteobacteria*	ClO_3^- NO_3^- O_2		acetate alanine asparagine butyrat fructose glucose lactate propionate pyruvat succinate	glucose aminobenzoate phenol phenylalanine	Malmqvist (1994)
Acinetobacter thermotoleranticus Source: Wastewater from a match factory	G^-, coccoid cells 0.7 to 1.2 μm in diameter, cells could grow as rods or filaments up to 60 μm in length. No flagella, fimbrae were found only in certain strains, oxidase$^-$, catalase$^+$, facultative anaerobes.	ClO_3^- SO_4^{2}	nitrate	Xylose Ethanol n-alkanes (C_9 – C_{18})	benzoic acid pyruvic acid salicin inositol fructose sucrose lactose raffinose sorbose	Stepanyuk (1993), Logan (1998)
Note: G^-: Gram-negative; oxidase$^+$: oxidase-positive; oxidase$^-$: oxidase-negative; catalase$^+$: catalase-positive						

Early studies found that nitrate-reducing microbes have the ability to reduce both perchlorate and chlorate (Rikken, 1996). Hackenthal (1965) reported the reduction of nitrate and chlorine-36 labeled perchlorate by cell-free extracts obtained from nitrate-adapted cells of *Bacillus cereus*. He concluded, from this study, that the same enzyme was used in the reduction of nitrate and perchlorate, because the reduction of nitrate was inhibited by perchlorate and vice versa. Attaway (1993) reports a variety of heterotrophic microorganisms which contain nitrate reductase and are capable of reducing perchlorate to chloride. He suggested that the reduction of perchlorate should be linked to nitrate reductase activity. Attaway (1993) also reports that perchlorate biodegradation in these cultures was inhibited by the presence of nitrate, and that repeated cultivation on perchlorate caused the loss of perchlorate- and nitrate-reducing ability. Korenkov (1976) reported that the perchlorate-reducing strain *Vibrio dechloraticans Cuznesove* B-1168 had the ability to reduce nitrates to nitrites, indicating that an enzyme such as nitrate reductase is present in the microbial cells. However, although most perchlorate or chlorate strains may be denitrifying facultative anaerobes, not all denitrifiers are chlorate reducers (Logan, 1998). Wallace (1996) found, for the isolated strain *Wolinella succinogenes* HAP-1, that the presence of nitrate did not affect perchlorate reduction, which suggests that the enzymes involved in perchlorate reduction were not necessarily the same as those involved in nitrate reduction. Wallace's report is in disagreement with what has been reported by other researchers. However, the recent study by Coates (1999a), who isolated (per)chlorate-reducing bacteria (CIRB) from diverse environments, revealed that not all of the CIRB isolated can use nitrate, which also suggests that the chlorate reduction pathway and the nitrate reduction pathway may be unrelated.

Several new perchlorate- and chlorate-reducing microorganisms were recently isolated by John Coates and his colleagues (1999a). They isolated 13 perchlorate- or chlorate- reducing bacteria (CIRB) from very diverse environments, including pristine and hydrocarbon-contaminated soils, aquatic sediments, paper mill waste sludges, and farm animal waste lagoons. All of the CIRB isolates were oxidative, gram-negative, sporeless, nonfermenting facultative anaerobes. Most of the isolates were short motile rods with 0.5 μm in diameter and 2 μm long. Some of the isolates were spirilla with 0.2 μm in diameter and 7 μm long. Acetate and other simple dicarboxylic acids were the carbon source for these isolates. None of the CIRB isolates could use hydrogen or hydrocarbons as electron donors. Some of them could use Fe(II) as an electron donor. The bacteria could use perchlorate, chlorate, and oxygen as electron acceptors, some of the isolates could also use nitrate for anaerobic growth (Coates, 1999a). Coates and his colleagues found that all of the isolates were members of the class *Proteobacteria* by analyzing their 16S rDNA sequences. The majority of the isolates were closely related to each other and to phototrophic *Rhodocyclus* species. Their studies increased the number of perchlorate- or chlorate-reducing isolates previously described, and indicated that perchlorate- and chlorate-reducing bacteria are much more prevalent in the environment than previously suspected. (Coates, 1999a and 1999b).

Phytoremediation of Perchlorate

Phytoremediation is the use of plants to clean soil and water contaminated with organic or inorganic pollutants (Susarla et al., 1999b). Plants may take up and assimilate contaminants (phytoaccumulation), volatilize the contaminants into the atmosphere (phytovolatilization), or degrade the contaminants within plant tissues using enzymes

(phytodegradation) (Nzengung et al., 1999). To date, there have been five reports on perchlorate removal by phytoremediation (Susarla et al., 1999a, 1999b, 1999c, Nzengung et al., 1999 and Nzengung et al., 2000). The objective of these studies were a) to investigate the ability of selected plants to remove perchlorate; b) to examine the role of nutrients on perchlorate removal by plants; c) to determine whether perchlorate is accumulated in plant tissue or broken down to chloride by plant mediation; d) to determine the distribution of perchlorate in the different parts of the plants; e) to investigate the factors that influence perchlorate removal by plants.

Susarla et al. (1999a and 1999c) reported that the aquatic plant, parrot-feather (*Myriophyllum aquaticum*), which has been used successfully to treat trinitrotoluene (TNT), trichloroethylene (TCE) and perchloroethylene (PCE) contaminated soils, could take up perchlorate and then transform it to chloride. However, they found that the concentration of perchlorate, the growth substrate (sand versus aqueous), and the presence of chloride affected the depletion of perchlorate by parrot-feather.

Besides parrot-feather, twelve other vascular plant species were also investigated by Susarla and coworkers (Susarla et al., 1999b). Four were trees, one was an herbaceous upland species, four were herbaceous wetland species, and four were herbaceous aquatic species (including parrot-feather). The result of their experiment showed that perchlorate was depleted from solution in the presence of all but two species (waterweed and duckmeat). However, none of the tree species nor the herbaceous upland species tested presented a high perchlorate depletion rate (>= 1000 g perchlorate depleted /kg wet weight). Wetland and aquatic plants such as blue-hyssop, perennial glasswort, and parrot-feather accomplished the highest perchlorate depletion rate. Perchlorate, or

transformation metabolites (chlorate, chlorite, chloride) were observed in all selected plant tissues (roots, stems and leaves). Susarla et al. (1999b) found that factors influencing perchlorate depletion by plants include type of plant, perchlorate concentration, growth substrate, the presence or absence of nutrients, stage of plant maturity, and the presence of chloride ions. In addition, Susarla et al. (1999b) observed that fungal pathogens present in the donor plants might interfere with the depletion of perchlorate by these plants.

Nzengung et al. (1999) reported that woody plants were capable of decontaminating water polluted with perchlorate in sand bioreactors and hydroponic systems under laboratory conditions. Three woody plants were investigated in their experiment. They found that willow trees were the most effective plants for removing perchlorate from solution and thrived well under the experimental conditions. In addition, the researchers identified two phytoprocesses involved in the remediation of perchlorate-contaminated water: (a) uptake and phytodegradation of perchlorate in the tree branches and leaves and (b) rhizodegradation. They found that rhizosphere-associated microbes mediated the degradation of perchlorate to chloride. They also observed that perchlorate temporally accumulated in the leaves of the trees tested. However, the effectiveness of phytoremediation of perchlorate-contaminated environments may depend on the concentration of competing terminal electron acceptors, such as nitrate. Nitrogen present in the nutrient solution also affected perchlorate removal from contaminated water by woody plants. Compared to nitrate, ammonium and urea favored and enhanced rhizodegradation and minimized uptake into the plant.

In another study, Nzengung and Wang (2000) reported that selected woody, edible, and aquatic plants and microbial mats could be used to remove perchlorate from environments. The results showed that initial uptake and transformation of perchlorate in the plant tissues (phytodegradation) were slow. After several days, depending on plant physiology and environmental conditions, the removal of perchlorate from solution by rhizodegradation was very rapid. Nzengung and Wang (2000) suggest that any perchlorate taken up into the green plants was not simply accumulated, but was slowly transformed.

Biological Reactors for Perchlorate Removal

Recently, there have been several reports on biological reactors to remove perchlorate from waters. The Air Force Research Laboratory, Materials and Manufacturing Directorate began development of a biological treatment process for high level perchlorate-contaminated wastewater (concentration from 1000 to 10,000 ppm) eight years ago (EPA Perchlorate, 1999). An up-flow anaerobic fixed bed reactor able to treat up to 1,000 gal/day of effluent was designed, fabricated, and successfully tested at Tyndall Air Force Base, Florida in 1995 by Wallace and his colleagues (Wallace, 1998). In this process, a mixed anaerobic bacterial culture containing 29%--48% *Wolinella succinogenes* HAP-1 was used for perchlorate biodegradation. The mixed culture formed a biofilm on the packing material (diatomaceous earth pellets), and reduced perchlorate to chloride at the rate of 1 g of perchlorate reduced per hour per liter at the residence times of 1.17 and 0.46 hour.

Applied Research Associates developed for the Thiokol Corporation a prototype reactor to treat wastewater containing perchlorate salts, corrosion inhibitors and other

contaminants. In addition to perchlorate, nitrate, nitrite, chlorate, and chromium VI could also be reduced by this process (Coppola, 1998).

Greene (1999) reported that GenCorp Aeroject Corportation developed an anoxic fluidized-bed reactor (FBR) bio-treatment system to remediate groundwater contaminated with ammonium perchlorate (approximate 8 ppm) at a site in Rancho Cordova, California. The full scale FBR system was designed based on the pilot-scale tests of an anaerobic fluidized bed bioreactor completed in 1996. It could treat 4,000 gal/min ground water, with perchlorate loading rate of approximately 0.7 kg/m^3-day. The pilot FBR used granular activated carbon (GAC) as bed media, ethanol as carbon source and electron donor to promote perchlorate reduction. When the carbon source changed to methanol or acetate, the biofilm growth was reduced and its characteristic changed. The full scale FBR system could reduce perchlorate from 8 ppm to less than 18 ppb.

Catts (1999) presented a biological treatment technology for perchlorate biodegradation for the Baldwin Park Operable Unit (BPOU) Steering Committee in San Gabriel Basin, California. This technology was a fixed film bioreactor using granular activated carbon operated as a fluidized bed. He showed that this reactor could effectively remove perchlorate from concentrations of 100 ppb to less than 4 ppb. The bioreactor could also remove nitrate from 50 ppm to 0.1 ppm. However, performance of the bioreactor was strongly dependent upon the dissolved oxygen in the influent, the hydraulic retention time in the bioreactor, and the ethanol concentration in the reactor influent.

Kim (1999) developed fixed-bed columns to treat perchlorate-contaminated waters. Sand or granular activated carbon (GAC) was used as column support media. A

perchlorate-biodegrading biofilm was developed in the column by inoculating with a perchlorate-biodegrading acclimated consortium for one day. The column was then operated in continuous flow and pumped with artificial groundwater containing 20 mg/L of perchlorate, 100 mg/l of acetate, nutrient and trace minerals. Kim found that perchlorate reduction was achieved in the fixed-bed column, under anoxic condition for residence times varying from 13-48 minutes. Acetate was successfully used as carbon source, and the molar ratio of acetate to perchlorate was found to be 2.9 (±0.9). However, when GAC was used as a medium for the fixed-bed column, perchlorate first adsorbed onto the GAC, but it quickly desorbed, returning back into the water and causing sporadic spikes in the effluent. She concluded that GAC seemed not a proper column support medium for perchlorate biodegradation in upflow fixed-bed columns.

Table 2.3 summarizes major characteristics of some reactors tested for biological perchlorate removal. Notice that for most studies, the source of perchlorate-degrading microbes was municipal wastewater. Both organic carbon and hydrogen were successfully used as the electron donor for perchlorate biodegradation. Only a few of the reactors tested dealt with removing perchlorate from drinking waters. Most reactors were built to remove very high concentrations of perchlorate from waste streams.

Table 2.3 Characteristics of Bioreactor Systems Used to Remove Perchlorate from Waters

Reactor Type	Type of Water	Influent Conc. of ClO_4^-	Effluent Conc. of ClO_4^-	Source of Microbes/ Microbe Type	Electron Donor	Reference
Anaerobic tank Retention time = 4.5-9 hrs	Municipal sludge	142-424 mg/L	3 mg/L	Municipal sludge	N/A	Yokovlev, 1971
Up-flow fixed-bed reactor with diatomaceous earth pellets media. Retention time was 0.46 – 1.17 hrs.	Rocket fuel motor washout waste stream	500-1500 mg/L	< 100 mg/L	Municipal anaerobic digester containing *Wolinella succinogenes* HAP-1.	Brewer's Yeast extract	Wallace, 1998
Laboratory = CSTR Pilot Scale = Unknown	Demilitarization wastewater	About 4000 – 11000 mg/L	Variable, from 0-5000 mg/L	Municipal anaerobic digester containing *Wolinella succinogenes* HAP-1.	cheese whey and yeast	Coppola, 1999
Fluidized bed reactor with 0.7 kg/m^3-day loading. Media was sand and activated carbon	Drinking water well	6-7 mg/L	< 4-40 μg/L	Not reported	acetate methanol ethanol	Greene, 1999

Table 2.3 Characteristics of Bioreactor Systems Used to Remove Perchlorate from Waters

Reactor Type	Type of Water	Influent Conc. of ClO_4^-	Effluent Conc. of ClO_4^-	Source of Microbes/ Microbe Type	Electron Donor	Reference
Fixed film fluidized bed with activated carbon as a medium	Groundwater	40 μg/L	4 μg/L	Wastewater from baby food processing plant	Ethanol	Catts, 1999
Fixed film reactor packed with celite.	Groundwater	0.7 mg/L	< 4 μg/L	Not reported	Hydrogen	Giblin, 1999
Fixed film reactor with activated carbon as media. Hydrogen-oxidizing reactor	Synthetic water	35 mg/L 240 mg/L	< 4 μg/L 3-8 mg/L	Municipal wastewater	Acetate Hydrogen	Logan and Kim, 1999
Hollow-fiber membrane-immobilized biofilm	Synthetic water	1-2.5 mg/L	30-50 μg/L	Denitrifying mixed culture	Hydrogen	Rittmann, 2000
Membrane-immobilized biofilm	Synthetic water		< 5 μg/L	Return activated sludge from municipal wastewater	Lactate	Liu and Batista, 2000a, 2000b

Biofilms and Membrane-Immobilized Biofilm Treatment

Biofilms form when microorganisms attach to exposed surfaces or proliferate in aqueous ecosystems, excrete extracellular polysaccharides, which aid adhesion to the submerged surfaces, and form gelatinous layers. Many factors, including nutrient availability, nutrient concentration, pH, temperature, electrolyte concentration, the flux of materials, and the surface itself, can influence the attachment of bacteria to a surface. Lappin-Scott (1992) cited several advantages that adhesion to surfaces provides for bacteria, including: a) Protection from anti-microbial agents, including antibiotics, biocides, and host defense mechanisms, b) Increased availability of nutrients for growth. The chemical nature of the polyanionic exopolysaccharide matrix surrounding the adherent bacteria allows it to act like an ion exchange column concentrating nutrients and ions, particularly cations from the surrounding fluid, c) Increased binding of water molecules, reducing the possibility of desiccation, d) Formation of consortia within biofilms, which enhance the advantages of a, b and c, e) Greater phenotyphic plasticity, 7f) Gradation of metabolic activity, and g) Proximity to progeny and other bacteria, facilitating plasmid transfer.

Biofilms can be detrimental in medical and industrial systems by, for example, forming extensive biofilms on the endoprosthetic implantable devices, causing infection problem, reducing heat transfer fluxes, increasing fluid frictional resistances, and corroding inside pipes. However, biofilms can also be beneficial for industrial applications. Presently, biofilms are widely used in the wastewater treatment. Bryers (1990) listed several advantages of biofilm systems for water and wastewater system: a) retaining biomass and mechanically preventing washout, i.e., reactor operation is

independent of physiological restrictions on growth rate, b) cost-effective, available support material (i.e., sand, quartz, plastic), c) high reactor biomass concentrations, d) increased overall substrate conversion rates due to higher biomass concentrations, e) biomass recovery can occur at high solids concentrations, f) reduced reactor volumes, g) reduced susceptibility to shocks or transients (e.g., temperature, inhibitors), h) possible elimination of a clarification / separation stage.

Bryers (1990) also classified biofilm systems for wastewater treatment into fixed biofilms systems, biomass support particles systems and artificially immobilized whole cell systems.

Fixed biofilms systems depend on the natural tendency of mixed microbial populations to adsorb to surfaces and to accumulate in biofilms. These kinds of reactors are currently used in most wastewater treatment plants. The reactor geometries in this system include completely mixed reactor systems, where the biofilm is exposed to a uniformly mixed bulk liquid such as a rotating biological contactor (RBC); fixed bed or packed bed reactor systems, which have been used for more than a century and known as "trickling filters"; and fluidized bed reactors, where influent passes through a bed of particles coated with biofilm at sufficient velocity to fluidize the bed. Other biofilm systems include packed or fluidized bed systems operated with some degree of effluent biomass recycle.

Opposed to fixed biofilms systems, biomass support particles systems (BSP) can control the thickness of the biofilm. Like the fixed biofilm reactors, BSP also depend on the natural tendency of microbes to adsorb or aggregate. BSP are composed of various

materials, including three dimensional lattice of polyester foam, polypropylene lattice, stainless steel wire and fritted glass particles.

Artificially immobilized whole cell systems do not depend on the natural tendency of microbes to adsorb to surfaces. Instead, immobilization is an engineered process, in which cells are captured within or bound to a support by a variety of physical and/or chemical techniques. Cell growth and replication of the immobilized cells is not necessary and is often undesirable.

To date, almost all the published studies on perchlorate biological removal focus on fixed film bioreactor (Catts, 1999; Kim and Logan, 1999; Greene, 1999; Giblin, 2000; Wallace, 1998; and Rittmann et al., 1999). However, with biological packed-or fluidized-bed reactors, the perchlorate-contaminated water passes directly over the perchlorate-reducing culture. Bacterial cells and soluble microbial products can contaminate the treated water. Therefore, the biological packed or fluidized bed reactors may be found uneconomical because of the costly post-treatment processes, such as disinfection. In addition, in the packed- or fluidized-bed processes, a carbon source (e.g., acetate, lactate, and ethanol) is normally added to the water to be treated as a source of carbon for microbial growth. Excess carbon sources may stimulate undesired microbial growth in the water distribution system. However, hydrogen can be used as an electron donor instead of organic carbon, minimizing the presence of excess organic carbon in the treated water. Rittmann et al. (2000) and Logan (1999) are currently investigating the use of hydrogen as an electron donor in perchlorate removal.

An attractive alternative to a fixed film bioreactor, in which the microbes are in direct contact with the treated water, may be a membrane-immobilized biofilm

bioreactor. In this reactor set-up, the water to be treated is separated from the microbe by a microporous membrane, on the surface of which a biofilm is grown. Perchlorate is allowed to diffuse from the contaminated water to the perchlorate-reducing biofilm by passing through the membrane. The side of the membrane to which the biofilm is attached is in contact with the electron donor, nutrient and minerals needed for microbial growth. The membrane and biofilm also act as a barrier to prevent the carbon source from mixing with the water being treated, minimizing the contamination of treated water by carbon source, bacterial cells, and soluble microbial products.

To the best of the author's knowledge, to date, there are only two membrane-immobilized biofilm reactors being investigated for perchlorate removal (Rittmann, 2000; Liu and Batista, 2000). However, this type of reactor has been investigated for their effectiveness to convert nitrate to nitrogen gas (denitrification). In the early 1980s, Nilsson and coworkers began the work on denitrification by immobilized cells (Nilsson, 1980 and Nilsson, 1982). Later on, Lemoine and coworkers (Lemoine, 1988 and 1991a) used sandwiched immobilized cells between microporous membranes to separate the immobilized culture from the water being treated. In another experiment, they performed tests with a two-cell reactor using a membrane to isolate the cells from the water being treated, and were able to greatly decrease the concentration of organic in the treated waters (Lemoine, 1991b).

However, the use of immobilized cells or gel-immobilized cell denitrification systems present two problems: a) culture stability: the deficiency of localized substrate can affect the bacterial stability, deteriorate the microbial activity, break down the immobilized cell culture and leak into the water being treated, b) limited denitrification

rates (Mattiasson, 1981, Lemoine, 1988). The use of sandwiched gel structure between two membranes seemed to enhance culture life, solve the problems of culture instability and cell leakage. However, introduction of microporous membranes enclosing the gel-entrapped cells appears to lower nutrient diffusion rates to the bacterial cells thereby reducing denitrification rates (McCleaf, 1995, and Reising, 1996).

In an effort to solve the above-cited problems encountered with gel-immobilized cells, McCleaf and Schroeder (1995) proposed a system for denitrification using a microporous membrane-immobilized biofilm. In the proposed system, a denitrifying biofilm is established on one side of a microporous membrane by adding organic substrate and other nutrient to the biofilm side of the membrane, while nitrate diffuses through the membrane from the "clean" water side. The microporous membrane separates the denitrification reaction completely from the water being treated. Therefore, this system solves the problem of product water contamination with microorganisms, substrate, and metabolites. McCleaf and Schroeder (1995) pointed out that the microbial activity of immobilized bacterial cells, such as cells contained behind a permeable membrane, could be considered to be influenced to a greater degree by substrate and waste product transport limitations rather than by microbial activity of a suspended bacterial culture, such as activated sludge. Conversely, the microbial activity of bacterial cells immobilized by a permeable membrane can be considered to be influenced to a lesser degree by mass transport limitations than that of a fixed biological film. McCleaf and Schroeder (1995) found out, as expected, that the nitrate diffusion coefficient through the biofilm-membrane composite is lower than that of the membrane without biofilm. In addition, they found that: a) the nitrate diffusion coefficients through the membrane,

biofilm-membrane composite, and biofilm were all smaller than the nitrate diffusion coefficient in water, b) denitrification rate in this system was more than four times greater than that of the gel-membrane reactor, due to the relative thinness of the biofilm-membrane composite and the higher nitrate diffusion coefficient of the membrane compared with the reported thickness and membrane diffusion coefficient of the gel-membrane reactor (McCleaf, 1995).

McCleaf and Schroeder (1995) observed during their experiment that some carbon source diffused from the reaction side to the clean water side of the reactor. As a result, some microbial growth was observed on the "clean water" side of the reactor. They suggested the use of a smaller pore sizes membrane or the use of ultraviolet (UV) disinfection of the water, prior to treatment, to avoid microbial growth on the "clean water" side of the reactor.

Reising and Schroeder (1996) conducted an experiment to address the problems noted by McCleaf and Schroeder. One 0.02 μm microporous membrane was used in the system to minimize microbial contamination of the product water, both biofilms and suspended growth cultures were performed to study optimization of the nitrate removal rate, and the diffusion of carbon source was controlled during the experiment. The results showed that the nitrate removal rates with the suspended culture systems were greater than those with biofilms, indicating that microbial fouling minimization would be an additional advantage of such a system. The average carbon source (methanol) requirement was 1.4 g organic carbon/g N removed, while the methanol requirement in McCleaf and Schroeder's research was reported to be 2.2 g organic carbon, more than

twice the stoichiometric value. Removal rates followed first-order pseudo-transport controlled models for both the biofilm and suspended-culture systems.

Although no previous studies on perchlorate reduction by a membrane-immobilized biofilm have been published, the work of McCleaf and Schroeder (1995) and Reising and Schroeder (1996) on denitrification in immobilized biofilm reactors provides valuable guidance to the investigation of perchlorate biodegradation by a membrane-immobilized biofilm.

CHAPTER 3

EXPERIMENTAL METHODS AND MATERIALS

Set-Up of the Membrane-Immobilized Biofilm Reactor

Figure 3.1 shows a schematic representation of the set-up of membrane-immobilized biofilm bioreactor used in all perchlorate removal experiments described in this work.

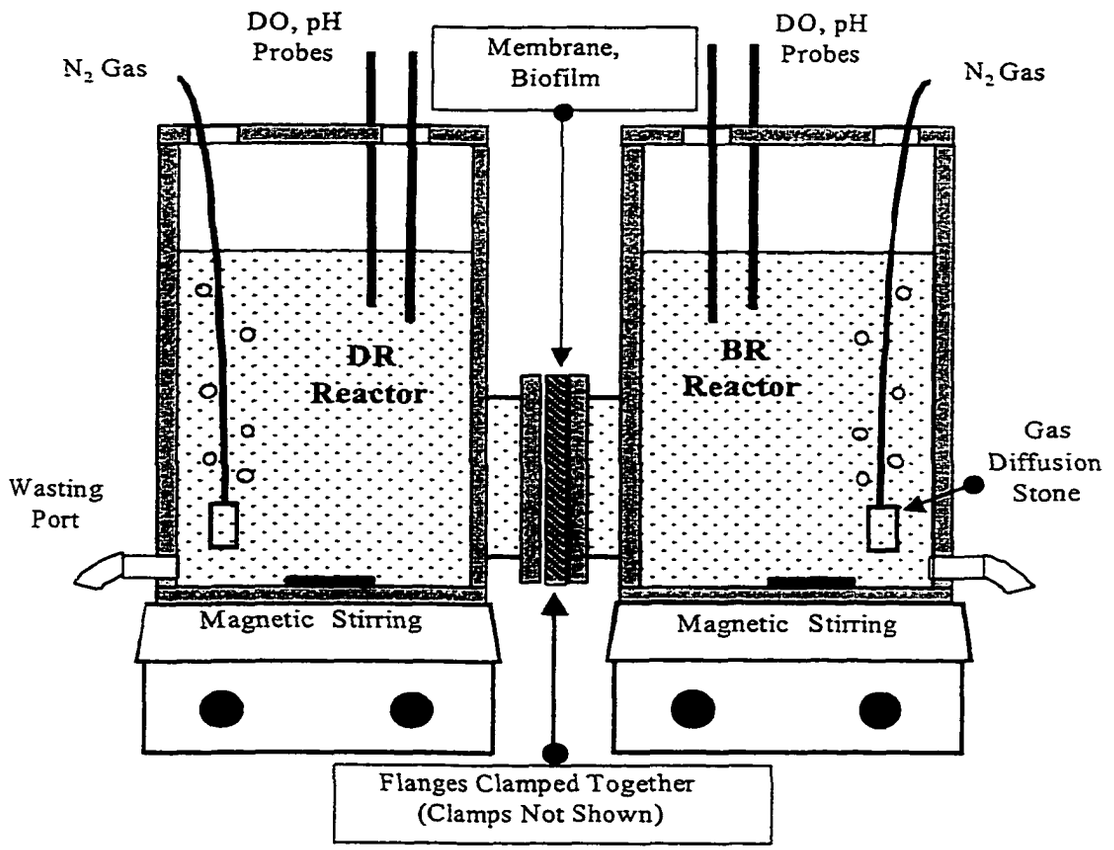


Figure 3.1 Schematic representation of the membrane-immobilized biofilm reactor

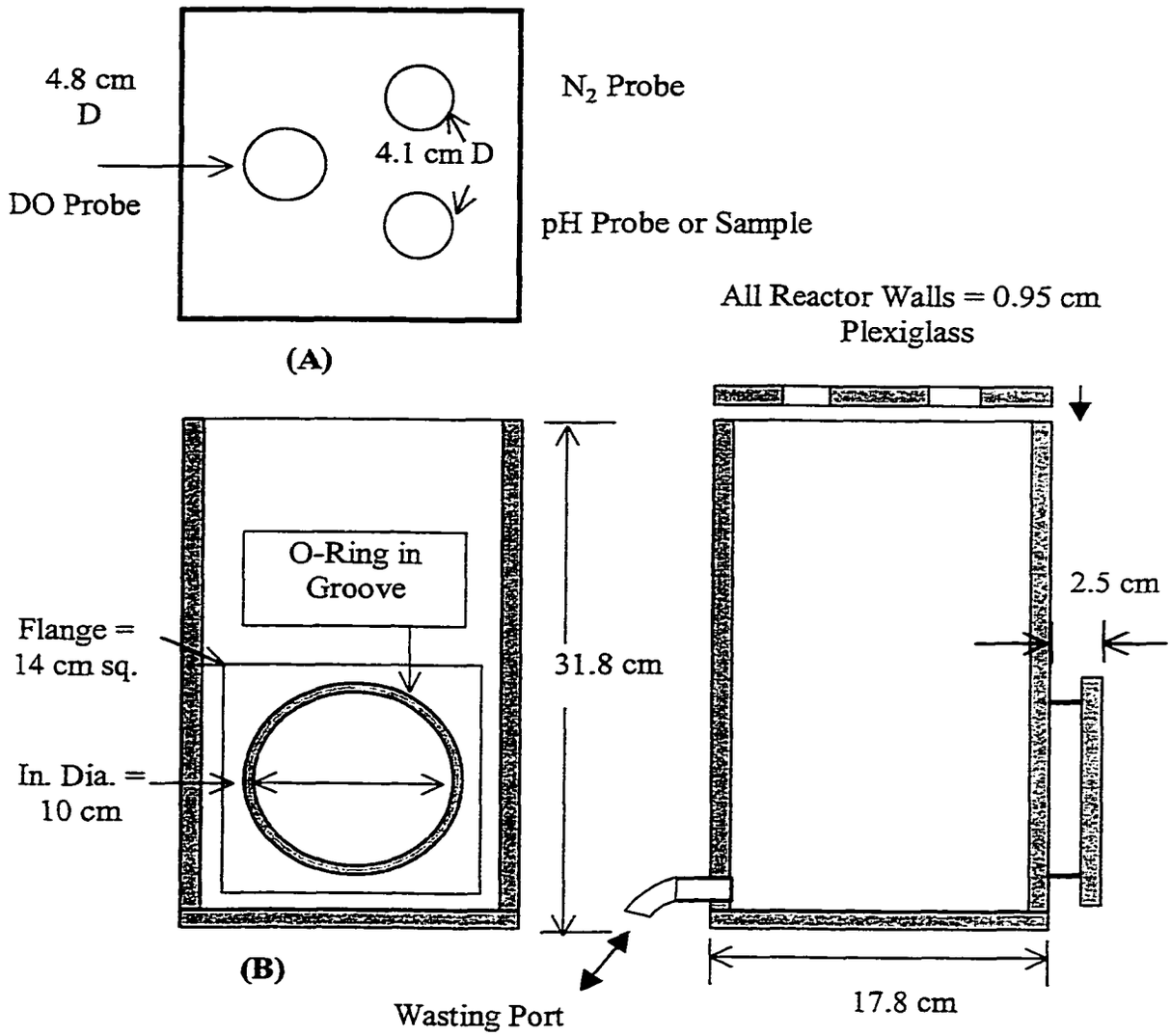


Figure 3.2 Dimensions of the membrane-immobilized biofilm reactor showing: (A) Top cover with three openings for sampling and monitoring; (B) Side view showing connecting plate and membrane opening.

The reactor consists of two five-liter plexiglass reactors, the diffusion reactor (DR) and the biological reactor (BR), separated by a plexiglass plate holding a 10cm-diameter circular microporous membrane, to which a biofilm is attached. The two reactors and the plate are held tightly together either by four stainless steel screws or by clamps and are sealed with o-rings placed on grooves cut in the plexiglass plates. The

reactors are covered by two plexiglass plates, which contain three ports used for sampling and monitoring. When these ports are not in use, they are covered by size 9 or 10 rubber stoppers. There is one waste port at the bottom of each reactor. This port is kept closed by a clamp and is used to empty the reactor. In this set-up, perchlorate diffuses from the DR reactor to the BR reactor, and it is biodegraded by the biofilm attached to the microporous membrane in the BR reactor. The final product of perchlorate biodegradation, chloride, can diffuse back into the DR reactor. There is no need to maintain a pressure difference across the membrane because the transport of ions is solely based on diffusion, and therefore the resulting process is not energy intensive.

Anaerobic conditions are maintained by covering the reactors with plexiglass covers and by purging the reactor with the compressed nitrogen gas using fine-bubble ceramic diffusing stones. Adhesive weather-strip foam is placed around the edges of the covers to completely seal the reactor. A YSI 54A dissolved oxygen meter is used to monitor the anaerobic condition in the reactor. The pH in the BR reactor is kept around neutral by phosphate buffer addition and is monitored by a Corning 450 pH/Ion meter. Complete mixing in both reactors is achieved by magnetic stirring.

Figure 3.2 shows a side view of the microporous membrane connecting the DR and the BR reactors, detailing the attachment of the membrane to a plexiglass plate which has a 10cm-diameter opening for membrane.

Microporous Membranes Selection

Several membrane samples were obtained from Ionics, Inc., Hydranautics, Koch Membranes, Osmonics, Millipore, and US Filter/ Memcor. Some of these membranes

were deemed unsuitable for the experiments due to their pore size or sensitivity to perchlorate. Therefore, it was decided to search for membranes that would be perchlorate resistant. The pore size of the microporous membrane has to be selected so that perchlorate could diffuse into the BR chamber while the back transport of microbial cells, carbon source, and macromolecules into the DR chamber is minimized.

Three membranes were chosen as potential candidates for the immobilization of a biofilm for perchlorate biodegradation. The selected membranes were Memcor BTS-55, Memcor PVDF, and Millipore FGLP membrane. All these three types of membranes were employed in the biodegradation and diffusion tests of the membrane-immobilized biofilm reactor. The characteristics of the selected membranes are shown in Table 3.1.

Table 3.1

Characteristics of the selected microporous membranes used in the membrane-immobilized biofilm reactor

Membrane Name	BTS-55	PVDF	FGLP
Manufacturer	Memcor	Memcor	Millipore
Pore Size, μm	0.2	0.45	0.2
Thickness, μm	125	99	220
Mean Pore Fraction, %	70	70	70
Materials	Polysulfone	N/A	Polytetrafluoroethylene with polyethylene backing

Determination of Diffusion Coefficient for Perchlorate, Nitrate, and Sulfate

The diffusion coefficient of perchlorate through these three membranes, prior to biofilm growth, was experimentally determined to evaluate the migration of perchlorate from the DR reactor (diffusion reactor) to the BR reactor (biological reactor). The testing was performed by attaching, with a water-proof aluminum plumbing tape, a membrane (10 cm diameter) to a square plexiglass plate containing a 10cm-diameter opening. The plate to which the membrane was attached was placed between the DR and BR reactors. The DR reactor (diffusion reactor) was then filled with 5 liters of DI water containing 1000 mg/L perchlorate, while BR reactor (biological reactor) was filled with 5 liters of perchlorate-free DI water. Both reactors were purged with nitrogen gas to remove dissolved oxygen to undetectable levels before adding perchlorate to the DR reactor. The dissolved oxygen (DO) level in the reactor was monitored by a YSI 54A DO meter. Fifteen- milliliter samples were taken, at the same time, from both reactors every 15 minutes for a 4-hour testing period. Perchlorate concentration in the BR reactor was measured by a Dionex 120 ion chromatograph. One of the selected membranes, Millipore FGLP membrane, which is hydrophobic, had to be pre-treated before the testing by completely soaking in 1-propanol for 15 minutes and thoroughly rinsing with DI water.

The diffusion coefficient was calculated using Fick's law as represented in Equation 3.1 (McCleaf and Schroder, 1995). The theoretical diffusion coefficient of perchlorate in water was also calculated using the Wilke-Chang Method (LaGrega, 1994).

$$V_{BR}(\partial C_{BR}/\partial t) = [-(D_M A_M)/(\Delta L_M)](C_{DR}-C_{BR}) \quad (3.1)$$

Where : V_{BR} = volume of BR reactor (cm^3)

$A_M = \pi r^2 \varepsilon$ = membrane pore area (cm^2)

r = membrane radius (cm)

ε = mean pore fraction (0.7 for all membrane tested)

ΔL_M = membrane thickness (cm)

D_M = perchlorate diffusion coefficient through the membrane (cm^2/s)

C_{DR0} = initial concentration of perchlorate in diffusion reactor (mg/L)

C_{DR} = concentration of perchlorate in the diffusion chamber at time t (mg/L)

C_{BR} = concentration of perchlorate in the biological reactor (mg/L)

t = elapsed time (s)

Since the two reactors contained the same volume of water, one can write:

$C_{DR} = C_{DR0} - C_{BR}$

Integration of the above differential equation yields:

$$\ln\frac{C_{DR0}-2C_{BR}}{C_{DR0}-2C_{BR0}} = -\frac{2A_M D_M}{V_{BR}\Delta L_M}t \quad (3.2)$$

Equation 3.2 is the equation of a straight line and plotting $\ln\frac{C_{DR0}-2C_{BR}}{C_{DR0}-2C_{BR0}}$ versus time (t) yields a slope of k equals $\frac{2A_M D_M}{V_{BR}\Delta L_M}$. Therefore, the diffusion coefficent D_M can be obtained because A_M, V_{BR}, and ΔL_M are known.

The test of each membrane was performed in duplicate, and diffusion coefficients were determined by least-square regression (R^2) of the transformed experimental data.

In order to test the interference of nitrate and sulfate on perchlorate biodegradation, the diffusion coefficients of nitrate and sulfate through the selected membrane, in the absence of the biofilm, were also determined. It is important to perform membrane diffusivity tests for nitrate and sulfate, in the absence of a biofilm, to eliminate the possibility of diffusion controlled biodegradation. In other words, if the diffusion of sulfate and nitrate through the membrane is found to be of the same order of magnitude as that of perchlorate, all the anions will be available to the biofilm at approximately the same time.

The diffusion coefficient tests for nitrate and sulfate were performed in the same manner as that for perchlorate. These diffusion tests, performed after perchlorate biodegradation testing with membrane-immobilized biofilms, deemed two of the pre-selected membranes (PVDF and FGLP) unsuitable for biofilm immobilization. Thus, only the BTS-55 membrane was used in the tests. The concentrations of nitrate and sulfate used in the diffusion tests were both 1000mg/L. Nitrate and sulfate concentrations were determined by a Dionex 120 ion chromatograph. Similar to perchlorate, the diffusion coefficients of nitrate and sulfate were calculated by Fick's law.

For comparison purpose, the diffusion coefficients of perchlorate, nitrate, and sulfate in water, in the absence of a membrane, were estimated using the Wilke-Chang method (Equation 3.3) (Reid, 1987).

$$D = \frac{5.06 \times 10^{-7} \times T}{\mu V^{0.6}} \qquad (3.3)$$

Where, T = temperature (°K)

μ = viscosity of water (centipoises, cP)

V = molar volume of anions (cm^3/mol)

D = diffusion coefficient (cm^2/sec)

By using the method of LeBas (LaGrega, 1994), the molar volume of perchlorate, nitrate and sulfate were calculated as 54.2 cm^3/mol, 40.5 cm^3/mol, and 58.8 cm^3/mol, respectively.

Development of Perchlorate Degrading Enrichment Cultures

One-Liter Master Culture Reactor (MCR)

A pure perchlorate-reducing culture isolated from the Penn State wastewater treatment plant was shipped to the UNLV laboratory. In the UNLV laboratory this culture demonstrated very slow kinetics and was then deemed not suitable for the development of membrane-immobilized biofilms. A perchlorate-degrading mixed enrichment culture was then developed from the returned activated sludge (RAS) taken from the Clark County Sanitation District (CCSD) wastewater treatment plant in Las Vegas. The CCSD treatment train contains an activated sludge system preceded by anoxic zones for biological phosphorous removal. The RAS for the enrichment culture was collected in the first anoxic zone where the RAS is mixed with the influent wastewater. The rationale was that perchlorate-reducing microorganisms would be more likely to be found in anoxic environments.

The RAS sample was used to inoculate a one-liter bottle containing buffer, nutrient/minerals, perchlorate, and a carbon source (lactate). The composition and concentration of the buffer and nutrient/minerals media used, as modified from van

Ginkel (1995), are shown in Table 3.2. The bottle was completely sealed with a rubber stopper and kept mixed by a magnetic stirrer at 23±2°C. The ratio of lactate to perchlorate added to the bottle was 5:1 (600 mg/L lactate and 120 mg/L perchlorate). The initial concentration of microbes in the bottle was 295 mg/L. Samples were withdrawn daily through a small opening on the rubber stopper using a 4-inch needle attached to a syringe. The opening on the rubber stopper was sealed with heavy-duty duct tape to maintain anaerobic conditions. The concentrations of perchlorate, chloride, and lactate of the sample were determined by ion chromatography. The background concentration of chloride in the bottle was about 51 mg/L. Background chloride concentration is the result of the addition of the RAS seed which has a very high concentration of total dissolved solids (TDS). The background concentration of chloride in the mineral/nutrient solution is approximately 1 mg/L.

Table 3.2

Concentration of buffer, nutrients & minerals used in the MCR media

Buffer			Nutrients & Minerals		
Component	FW (g/mole)	Concentration in the MCR (g/L)	Component	FW (g/mole)	Concentration in the MCR (mg/L)
K_2HPO_4	174.18	1.55	$MgSO_4.7H_2O$	246.47	100
NaH_2PO_4	119.95	0.85	EDTA	372.24	3
$NH_4H_2PO_4$	115.03	0.5	$ZnSO_4.7H_2O$	287.5	2
			$CaCl_2.2H_2O$	147	1
			$FeSO_4.7H_2O$	278.02	4
			$Na_2MoO_4.2H_2O$	241.95	0.4
			$CuSO_4.5H_2O$	249.7	0.2
			$CoCl_2.6H_2O$	237.9	0.4
			$MnCl_2.4H_2O$	197.91	1
			$NiCl_2.6H_2O$	237.71	0.1
			$NaSeO_3$	149.95	0.1
			H_3BO_3	61.83	0.6

(Source: modified from van Ginkel, 1995)

Four-Liter Master Culture Reactor (MCR)

A second MCR was developed, with a four-liter capacity, using seed microbes from a biofilm growth reactor that was seeded with the first MCR reactor. In this reactor, the culture developed a reddish coloration with time and was named "BALI", for Batista and Liu. Initially, perchlorate (200 mg/L) and lactate (1,000 mg/L) were added to the bottle daily without wasting. Later, a portion of culture was periodically wasted to

prevent accumulation of chloride in the bottle and to decrease the suspended solids concentration. A high suspended solids concentration implies the addition of large amounts of perchlorate and lactate to keep a microbial culture alive.

SRT Studies for the Master Culture Reactor (MCR)

The objective of these studies was to determine an appropriate feed rate for the master culture, so that a desired biomass concentration can be kept. This also allows for developing an enriched mixed culture that is more homogeneous and provides some initial data on the kinetics of perchlorate biodegradation by the enriched mixed culture. This decision was made because it was noticed that microbial composition of the master culture, when observed under the microscope, was changing with time. To keep such culture it is necessary to waste biomass, so that the cost of chemicals to maintain the reactors is minimized. An SRT of 5 days was chosen as the target SRT to be maintained in the master reactor. The reactor was operated on a semi-continuous mode. Daily, 200 ml mixed culture were withdrawn from the one-liter master bottle and 200 ml of solution containing an equivalent amount of perchlorate, lactate, nutrient and buffer was added to the bottle to complete the 1L volume. After the new solution was added, a 20-ml sample was taken for analysis. A portion of the solution taken out of the reactor was filtered through a 1.2 μm glass fiber filter (Whatman, GF/C) for suspended solids (SS) analysis. SS analysis followed Standard Method (Method #2054, APHA, 1995). A portion of resulting filtrate was then filtered though a 0.45 μm membrane filter prior to analyzing for perchlorate, lactate, and chloride. After one SRT cycle was completed, the initial suspended solids concentration and the lactate to perchlorate ratios were varied.

Development of Membrane-Immobilized Biofilms

To investigate the biodegradation of perchlorate by an immobilized biofilm, it is necessary to first establish a biofilm on the surface of the membrane. As the research progressed, the way the biofilm was developed on the membrane was changed to obtain reliable results and for ease of operation. The purposes of the modifications were (a) to decrease the disturbance of the biofilm resulting from emptying and filling up the BR reactor prior to initiating biodegradation studies, (b) to attempt to develop a biofilm of same thickness for the different tests, (c) to minimize the amount of time needed to switch from the biofilm growth mode to the biodegradation experiments, (d) to maintain relatively stable microbial composition of the biofilm for the different tests.

In the beginning, a membrane was placed between the two reactors. Two sets of reactors (4 tanks) were available to facilitate duplication and concomitant biofilm growth and biodegradation studies. Five liters of DI water containing 1000 mg/L lactate, 200 mg/l perchlorate (lactate/perchlorate ratio of 5:1), nutrients/minerals and buffer solutions (Table 3.2) and seed microbes (0.1 g/L) from the MCR were added to the BR reactor. Five liters of DI water were also added to the DR reactor to keep the hydraulic pressure on both sides of the membrane the same. A YSI 54A oxygen meter and a Corning 450 pH/Ion meter were placed in the BR to continuously monitor the oxygen concentration and the pH. Deoxygenation in the BR reactor was obtained and kept by purging with nitrogen gas. Oxygen levels were kept undetectable at all times. The biofilm was established on the surface of a membrane inside of the BR reactor over a one-week period. Biofilm growth was observed not only in the membrane, but also in the walls of the tank. However, given the hydraulics of the reactor, a thicker biofilm developed in the

membrane than in the walls of the tank (Figure 3.3). After the biofilm was grown on the surface of the membrane, these reactors were cleaned, then switched to the perchlorate biodegradation testing.

Figure 3.3 Biofilm established on the Microporous membrane at UNLV

After the biofilm was grown on the membrane, two plexiglass plates were placed in each reactor against the membrane to protect the biofilm. Next, the contents of the reactors were withdrawn through the waste port, the reactor walls were scrubbed with a plastic brush and thoroughly rinsed with DI water. The reactors were then dried with paper towels and used in the perchlorate biodegradation testing. Washing the reactor by the method described above was very cumbersome. Thus, a modification was made to improve the operation. The modification consisted of growing the biofilm in a separate set of reactors from that used for the biodegradation testing. The growth reactor set was used exclusively to grow the biofilm on the membrane. A clean membrane was attached

to the plexiglass plate and the plate was held between the two 5-liter reactors by seven stainless steel clamps. Next, perchlorate-reducing culture, nutrients/minerals, buffer, perchlorate, and lactate were added to one side of the reactor and DI water was added to the other side. After the biofilm was developed on the membrane surface, the membrane containing the biofilm was then removed from the growth reactor and transferred to a clean biodegradation reactor set. However, sometimes, the membrane-containing biofilm was tore up when the clamps were removed to transfer the plate containing the membrane to the biodegradation testing. To overcome this problem, it was decided to grow the biofilms on a manner that would not require clamping of the membrane.

In this modification, a large, rectangular glass container fitted with a plexiglass grid to support the membrane attached to plates, was used to develop the biofilm on the surface of the membrane (Figure 3.4). The glass container was filled with 16 liters of DI water containing buffer, nutrient/minerals, perchlorate, lactate, and perchlorate-reducing microorganisms. The plates containing the membrane were placed membrane-side down on the plexiglass support so that the media containing microbes contacted the surface of the membrane. At the same time, three biofilms could be grown in this reactor assuring homogenous thickness. The reactor was purged with nitrogen gas to ensure the anaerobic condition. The DO level was monitored by a DO meter.

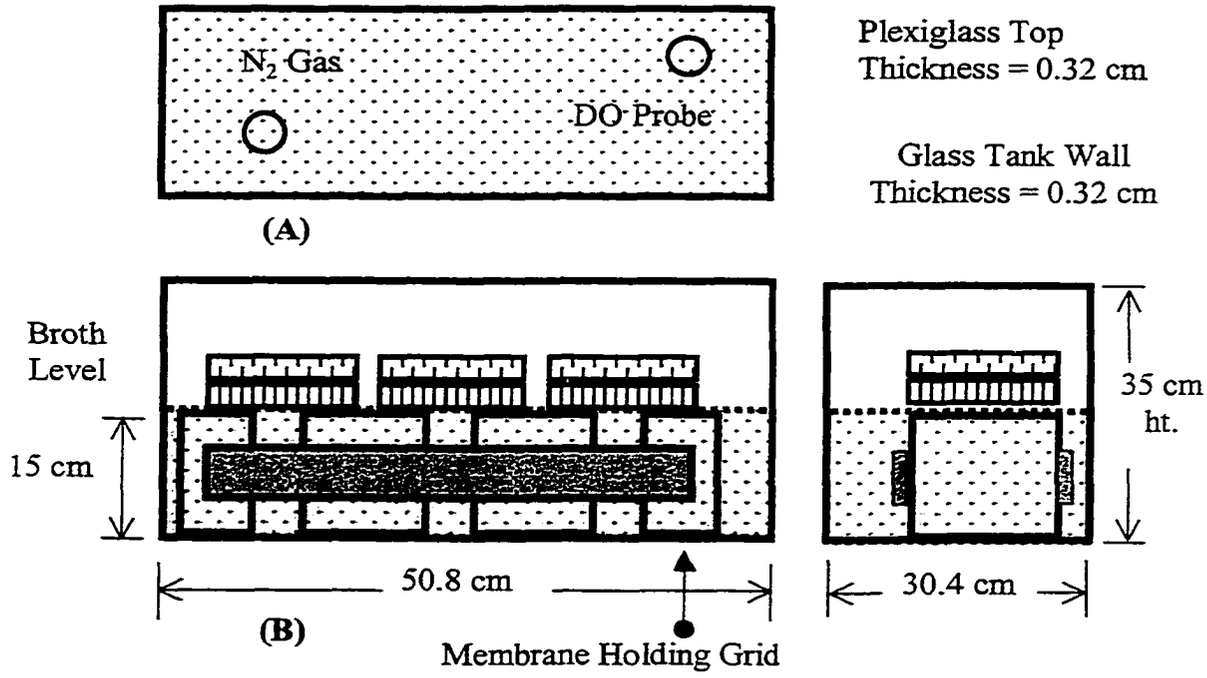


Figure 3.4 Construction of modified biofilm growth reactor

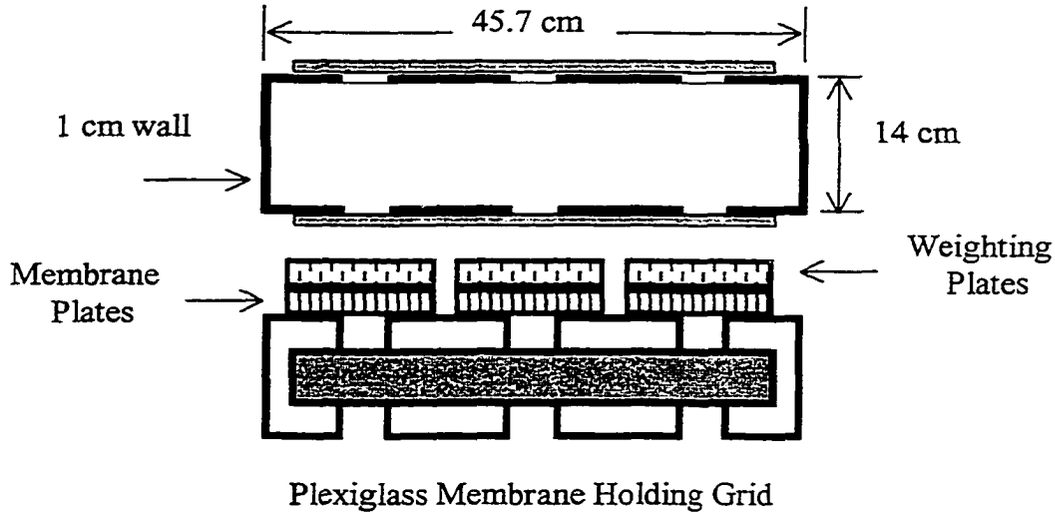


Figure 3.5 Membrane biofilm growth chamber showing: (A) Top view with DO and nitrogen ports; (B) Front view showing holding grid and membrane plate, and (C) Side view showing membrane plate and holding grid.

Perchlorate Biodegradation by Immobilized Biofilms

The testing of perchlorate biodegradation by the membrane-immobilized biofilm was performed after the biofilm was formed on the surface of a membrane. Since modifications were made on the way the biofilm was grown on the membrane, the procedure of perchlorate biodegradation testing was also changed slightly. For the experiments in which the biofilm growth and the biodegradation testing took place in the same reactor set, the following procedure was used: after establishment of the biofilm in the BR reactor side of the membrane, a Plexiglass® plate was held against the internal surface of the reactor to minimize the contact of the biofilm with oxygen. Next, the contents of the BR and the DR reactors were discarded through the waste port and the reactors were scrubbed and rinsed well with DI water. Five liters of solution containing lactate, nutrients/minerals and buffer were then added to the BR reactor. Five liters of solution containing perchlorate were added to the DR reactor on the other side of the biofilm. Last, the Plexiglass® plate was removed and both reactors were immediately closed and kept oxygen-free with nitrogen gas. The concentrations of perchlorate, lactate, chloride, and suspended solids were monitored in both the BR and the DR reactors. The disadvantage of this procedure is that the reactors could not be thoroughly cleaned when switching from the growth to the perchlorate biodegradation mode.

For the case where the biofilm was grown on a separate reactor set from that in which the biodegradation testing took place, the following procedure was used: After a biofilm was developed on the membrane, the clamps were removed and the plate containing the membrane-immobilized biofilm was quickly transferred to the biodegradation reactors. Perchlorate was added to the diffusion reactor (DR). Lactate,

nutrient and buffer were added to the other reactor (BR), which contained the biofilm side of the membrane. As perchlorate diffused from the DR to the BR reactor, it was biodegraded by the biofilm that used lactate as a carbon source (electron donor) and perchlorate as an electron acceptor. The concentrations of perchlorate, lactate, and chloride were monitored daily. Sometimes, the membrane was damaged by the clamps when the membrane was switched from the biofilm development reactor set to the biodegradation reactor.

For the case in which the biofilm was grown on the suspended grid, the plate containing the membrane-immobilized biofilm was carefully removed from the suspended growth grid reactor and clamped to the reactors for biodegradation testing. The other steps of the biodegradation test were the same as those described above.

Figure 3.6 The membrane-immobilized biofilm reactor during the biodegradation mode.

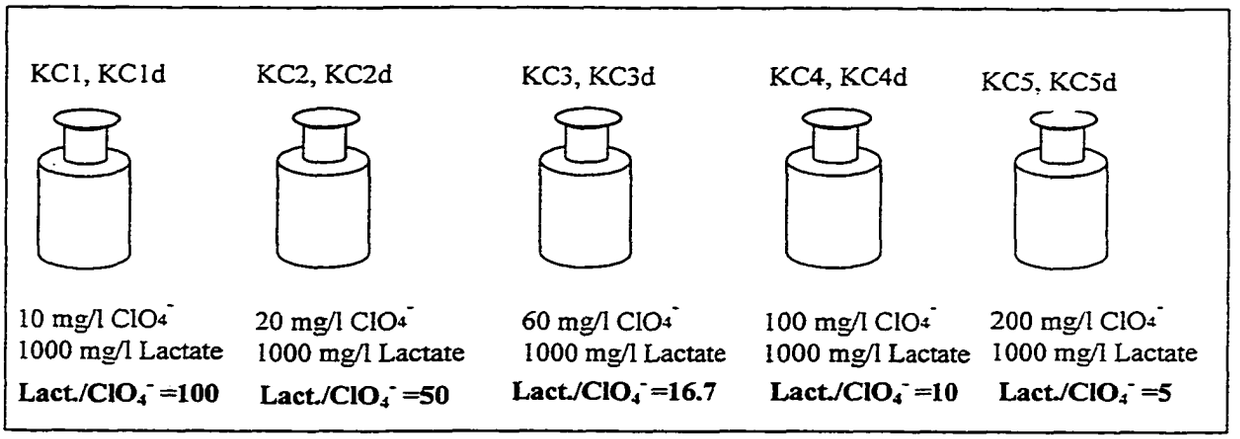
Preliminary Kinetics Study of the "BALI" Culture

Preliminary kinetics studies were performed in the enrichment "BALI" culture. The objectives of these experiments were (a) to determine the limiting carbon to perchlorate ratios needed for perchlorate biodegradation by this culture. This information will be used to determine the amount of carbon to be added to the 5-L membrane-immobilized reactor, so to minimize excess carbon in treated water. (b) to determine the degradation rate of perchlorate by this culture. One set of tests was performed under perchlorate limited condition while the other set was performed under carbon (lactate) limited condition. In the perchlorate-limited tests, the lactate concentration was 1,000 mg/L and perchlorate concentrations varied from 10 to 200 mg/L. For the lactate limited tests, the perchlorate concentration was constant and equal to 100 mg/L; lactate concentrations varied from 20 to 300 mg/L.

The tests were performed in 125-ml serum bottles in duplicate. The perchlorate limited bottles and their duplicates were labeled KC1, KC2, KC3, KC4, KC5, KC1d, KC2d, KC3d, KC4d, and KC5d, respectively. The lactate limited bottles and their duplicates were labeled KL1, KL2, KL3, KL4, KL5, KL1d, KL2d, KL3d, KL4d, and KL5d, respectively. The desired amounts of perchlorate, lactate, buffer and nutrient/minerals were added to the individual bottles. The same amount of microbes ("BALI") from the master reactor, for which the total suspended solids concentration was known, was added to each bottle so to obtain a suspended solids (SS) concentration of approximately 3.5 mg/L. Next, the bottles were sealed with butyl rubber caps and crimped with aluminum rings to assure anaerobic conditions. The bottles were then placed on a rotary shaker (30 rpm) at $20 \pm 2\ ^{0}C$. Five milliliters samples were withdrawn

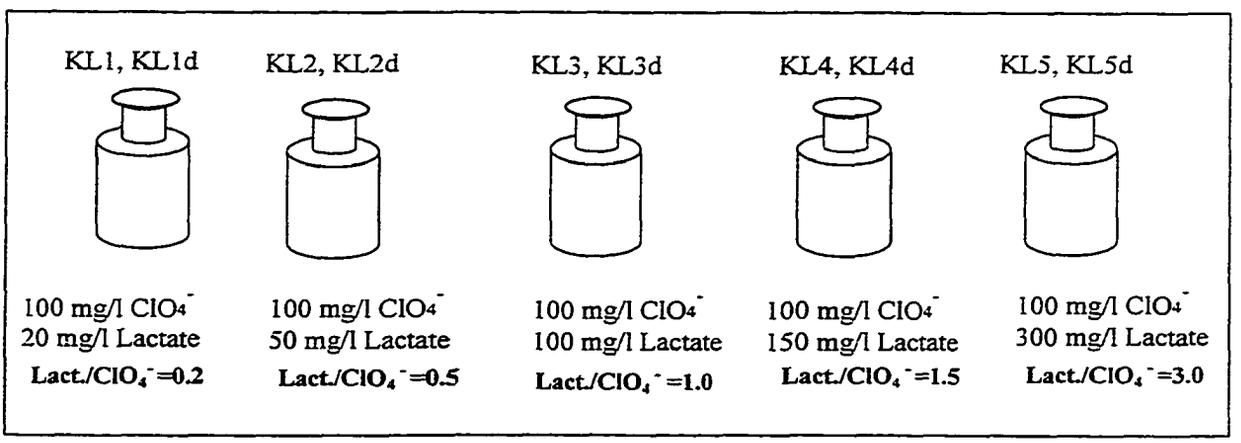
by using a needle attached to a syringe. The samples were filtered through 0.45 μm syringe filters. The solids were determined gravimetrically after drying the filter in a drying chamber containing activated silica gel for about 15 hours. The filtrate was analyzed for perchlorate, lactate, and chloride. The concentrations of lactate and perchlorate added to each bottle are shown in Figure 3.7.

Perchlorate Limited Bottles



(A)

Lactate Limited Bottles



(B)

Figure 3.7 Preliminary kinetics study with the "BALI" culture performed under perchlorate and lactate limited conditions

Microscopic Studies

The "BALI" culture, the reddish culture enriched at the UNLV laboratory from an activated sludge wastewater treatment plant, was studied microscopically. Gram-stain and wet mount slides of the culture were prepared and observed under a Nikon Labophot-2 microscope. Gram stain was performed by spreading a small amount of the mixed culture on a glass slide and allowing it to dry. Next, the culture was attached to the glass slide by heat fixing with a Bunsen Burner. The standard gram-staining procedure was carried out using Crystal Violet (Primary Stain), Gram's Iodine (Mordant), 95% Ethanol (Decolorizer) and Safranin (Counterstain). Wet mounts were prepared by spreading a small amount of the culture on a glass slide and observing it with a bright field microscope.

Influence of Nitrate and Sulfate on Perchlorate Biodegradation by a Membrane-Immobilized Biofilm

Prior to testing the effects of sulfate and nitrate on perchlorate biodegradation by the membrane-immobilized biofilm, batch tests were performed to gather preliminary data on the effects of these co-anions on perchlorate biodegradation. In the batch testing of nitrate interference, about 100 mg/L of perchlorate, 500 mg/L of lactate, buffer, nutrient/minerals and nitrate (0ppm, 10ppm, 30ppm and 60ppm) were added to four 125-ml serum bottles. In the batch testing of sulfate interference, about 10 mg/L of perchlorate, 150 mg/L of lactate, buffer, nutrient/minerals, and sulfate (0ppm, 20ppm, 100ppm and 500ppm) were added to four 125-ml serum bottles. All the tests were performed in duplicate. Because the background sulfate concentration in the

nutrient/minerals is about 41 mg/L and because it was not known at that time whether sulfate was a preferred electron acceptor to perchlorate, a larger amount of lactate was added to assure that the system was not limited by the lack of lactate.

The results of the batch tests, on the influence of nitrate or sulfate on perchlorate biodegradation, were used to guide the testing in the immobilized-biofilm reactor. The Memcor BTS-55 membrane-immobilized biofilm was used for the diffusion testing since diffusion coefficients for perchlorate, nitrate and sulfate, have been determined for this membrane. The nitrate and sulfate interference tests were performed in the same manner as those for perchlorate, and tested in duplicate.

Four tests were performed on the interference of nitrate on perchlorate biodegradation by a BTS-55 membrane-immobilized biofilm. Two concentrations of nitrate (10 mg/L and 50 mg/L) and two perchlorate concentrations (about 10 mg/L and 50 mg/L) were investigated. The perchlorate and nitrate concentrations tested are shown in Table 3.3.

Table 3.3

Perchlorate and nitrate concentrations tested

	Test 1	Test 2	Test 3	Test 4
Perchlorate Concentration (mg/L)	50	50	1	1
Nitrate Concentration (mg/L)	10	50	50	10
Lactate Concentration (mg/L)	200	200	5 (initial) added 200 later	5 (initial) added 280 later

For the sulfate interference testing, the perchlorate and sulfate concentrations are shown in Table 3.4.

Table 3.4

Perchlorate and sulfate concentration tested

	Test 1	Test 2	Test 3	Test 4
Perchlorate Concentration (mg/L)	10	10	0.1	50
Sulfate Concentration (mg/L)	10	200	50	50
Lactate Concentration (mg/L)	220	1100	420	630

A BTS-55 membrane, containing a previously grown biofilm, was placed between the diffusion reactor (DR) and biological reactor (BR). The DR reactor was filled with 5 liters of DI water containing the desired concentration of perchlorate and either nitrate or sulfate. The BR reactor contained 5 liters medium containing lactate, nutrients/minerals and buffer. Both reactors were flushed with nitrogen gas to remove dissolved oxygen to undetectable levels. The DO levels in the reactors were monitored using a YSI 54A DO meter. Samples were taken from each reactor at determined time intervals. Samples were then filtered through a 0.2 μm pore size membrane filter and analyzed for perchlorate and nitrate or sulfate.

Influence of Salinity on Perchlorate Biodegradation by the "BALI" Culture

Studies were performed to determine the influence of salinity levels on the perchlorate biodegradation by the "BALI" enrichment culture. The objective of these experiments was to determine whether high TDS levels would affect the kinetics of biodegradation. Culture tube testing was used for the studies. Microbial activity was assessed by measuring, with a Spectrophotometer (Spectronic 20), the changes in optical density, at 600 nm, due to microbial growth.

In the culture tube testing, the tubes, caps, nutrient/minerals, buffer, lactate, and perchlorate solutions were autoclaved prior to their use in the experiments in order to avoid contamination by other microorganisms. The buffer and nutrient/mineral media were of the same composition as those used in the membrane reactor studies throughout this project. The microbial inoculum was the "BALI" culture, which is the enrichment mixed culture used in all perchlorate biodegradation testing performed with the membrane-immobilized biofilm.

The autoclaved medium containing desired amounts of nutrients, buffer, perchlorate, and lactate were filled to the 8.5 mL culture tubes using a micro-pipette. Next, the desired volume of a suspension of the "BALI" culture was added to each tube. Microbial concentration was measured gravimetrically by filtering the samples through a 0.45 μm acetate membrane filter (Osmonics Inc.). The tubes were then capped and placed in the dark at 23 ± 2 $^{\circ}$C for incubation. Triplicate tubes were prepared for each perchlorate/salinity level combination. Salinity was established by adding desired amounts of NaCl to the growth medium. The salinity levels investigated ranged from 0% to 6%.

In addition to experimental tubes, two sets of control tubes were added to the experiment; one containing no microbes (abiotic control) and another containing no lactate (electron donor control). The concentrations of perchlorate, lactate, and microbes in each tube are shown in Table 3.5. At prescribed intervals, the increase in turbidity (decrease in transmittance) was measured directly in the tubes at 600 nm using a spectrophotometer (Spectronic 20, Bausch and Lomb, Rochester, New York). Tubes were mixed well before each measurement.

Table 3.5

Contents of the culture tubes in the salinity test

	Salinity Testing	Abiotic Control	Electron Donor Control
Biomass (mg/L as SS)	6.5	No microbes	6.5
Perchlorate (mg/L)	500	500	500
Lactate (mg/L)	1500	1500	No lactate

Analytic Methods

The analytical procedures used in this research follows the QA/QC plan submitted to American Water Works Association Research Foundations (Logan and Batista, 1997).

Perchlorate, Nitrate, Sulfate, Lactate, and Chloride Analysis

Perchlorate, nitrate, sulfate, lactate, and chloride were measured using a DX-120 ion-chromatograph with a Dionex IonPac AS11 4mm (10-32) separation column and IonPac AG-11 4mm (10-32) guard column. Varying concentrations of sodium hydroxide (NaOH) were used as the eluent for all analysis; 49 mM for perchlorate; 5mM for nitrate and sulfate; and either 5mM or 1mM for chloride and lactate. The procedure

used for perchlorate analysis is similar to that developed by the California Department of Health Services (Appendix A). The procedures for the analysis of lactate, chloride, nitrate and sulfate were developed in the UNLV Environmental Engineering laboratory and use the same columns used for perchlorate, but the eluent concentrations are different. Several trials were needed to determine the eluent concentration that allowed the detection of the anions at the desired levels and produced calibration curves with R^2 greater than 0.995.

Calibration curve for all anions used either five or seven different concentrations. After the calibration procedure was completed, R^2 values (from linear least squares analysis) of 0.997 or greater for perchlorate, and 0.995 or greater for the other anions were achieved. A mid-standard and QC check consisting of a calibration standard and a sample prepared by another member of the research group, respectively, were added after every 20 samples. Samples with anions concentrations outside the calibration curve range were diluted with DI water so that the analysis were performed within the calibration curve range. A sample calibration curve for each of the anions analyzed is shown in Table 3.6 - 3.10 and Figure 3.8 - 3.12.

Table 3.6

Calibration curve for perchlorate

Perchlorate Concentration (ppb)	Peak Area
5	6833
10	14864
15	21675
30	42888
60	87623
80	118565
100	149887

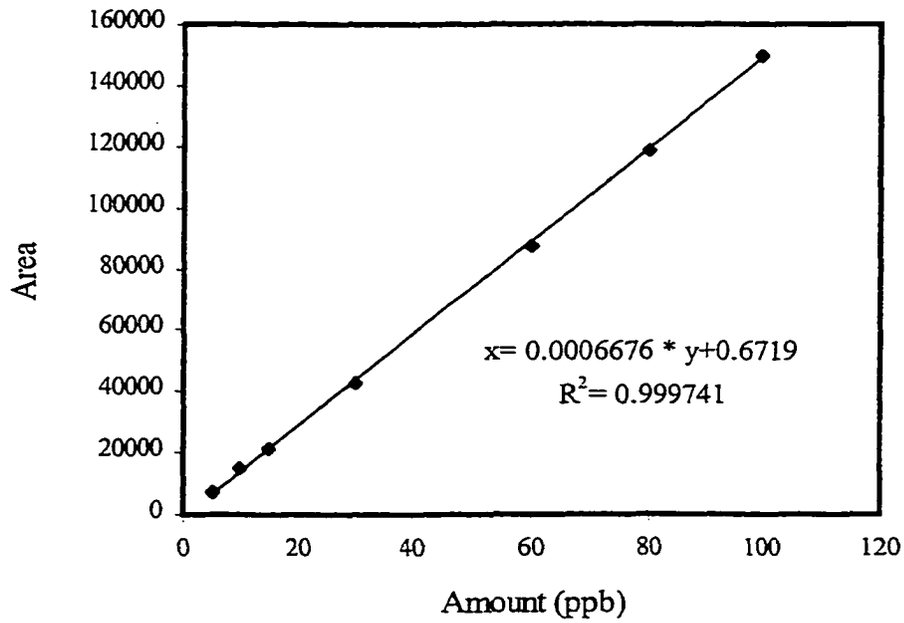


Figure 3.8 Calibration curve for perchlorate

Table 3.7

Calibration curve for lactate

Lactate Concentration (ppb)	Peak Area
30	1533
50	2404
60	3336
120	6547
200	11504
400	23807
600	35380

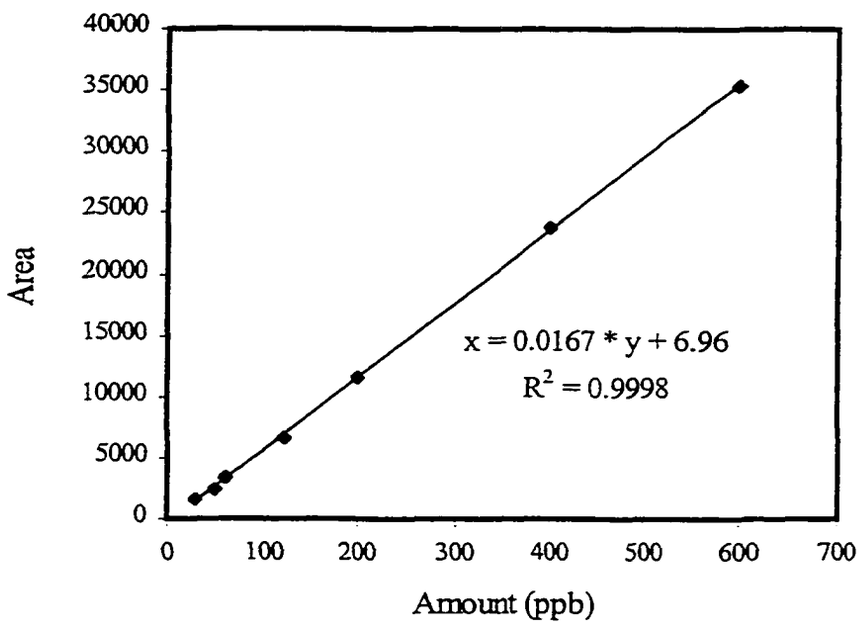


Figure 3.9 Calibration curve for lactate

Table 3.8

Calibration curve for chloride

Chloride Concentration (ppb)	Peak Area
20	2937
25	3347
30	4177
50	7416
100	13698
150	20467
200	27431

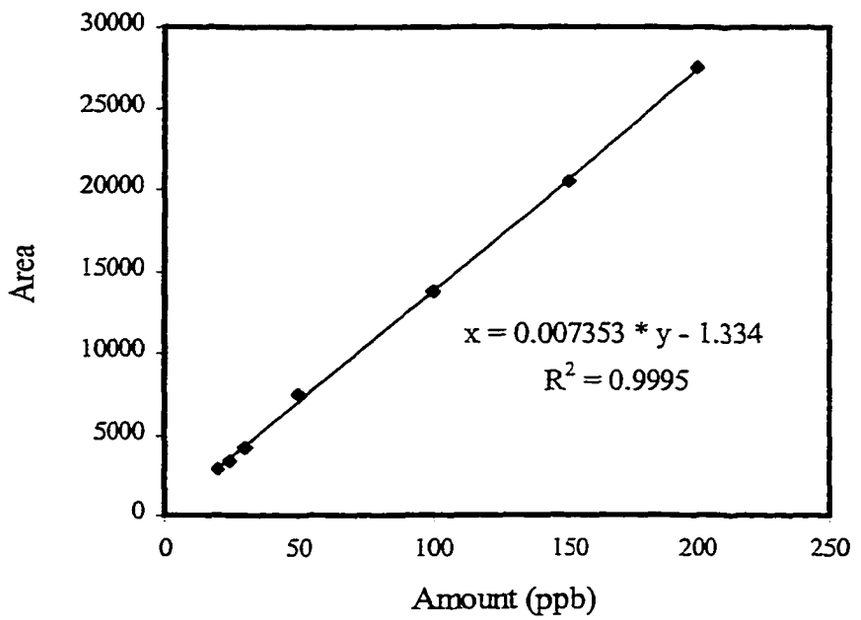


Figure 3.10 Calibration curve for chloride

Table 3.9

Calibration curve for nitrate

Nitrate Concentration (ppb)	Peak Area
25	1199
30	1625
50	2987
100	6423
300	20844
500	35822
1000	73613

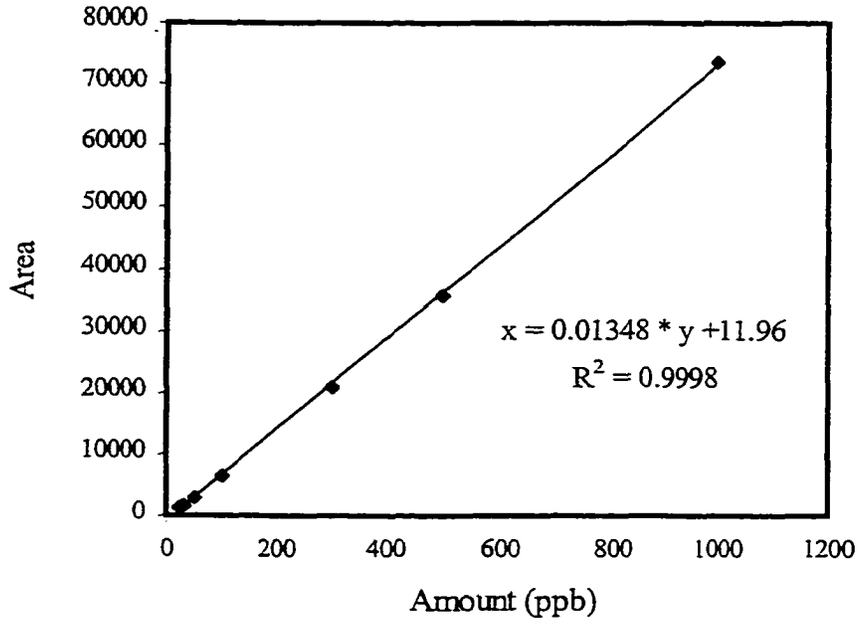


Figure 3.11 Calibration curve for nitrate.

Table 3.10

Calibration curve for sulfate

Sulfate Concentration (ppb)	Peak Area
30	2471
50	4646
80	6513
100	9009
500	49512
300	28880
1000	102032

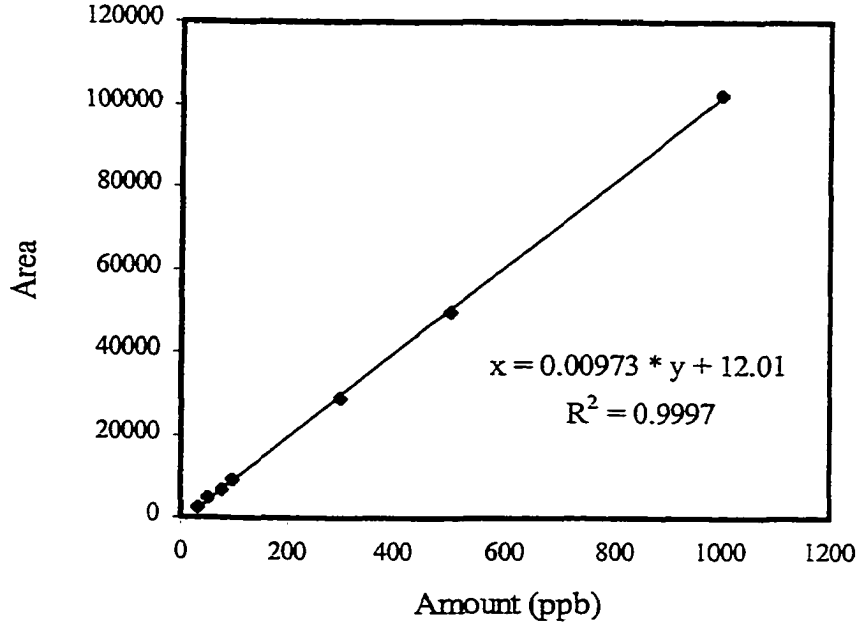


Figure 3.12 Calibration curve for sulfate

Preparation of the Eluent

Prior to measuring the anions, it was necessary to prepare different concentrations of NaOH solutions to be used as the eluent. Properly degassing the DI water to be used in the eluent and adding the correct amount of NaOH are very important steps to obtain a good calibration curve. The following procedure was used to prepare the eluent solutions for anions analysis:

- Four liters of DI water were added into an eluent bottle.
- A desired amount of DI water (depending on the eluent concentration—Table 3.11) was withdrawn from the bottle using a pipette .
- The DI water contained in the bottle was then purged with helium gas for about one hour.
- After the water was degassed, 50% (w/w) NaOH was added to the bottle (Table 3.11).
- Next, the bottle was capped tightly and turned upside down gently at least three times to assure the solution was well mixed.

Table 3.11

Amounts of DI water and NaOH withdrawn and added to the eluent bottles for eluent preparation

Desired Molarity of Eluent	DI water withdrawn (ml)	50% (w/w) NaOH added (mL)
1 mM NaOH	0.2092 mL	0.2092
5 mM NaOH	1.048 mL	1.048
10 mM NaOH	2.096 mL	2.096
49 mM NaOH	24 mL	10.4

Ion Chromatograph Operating Conditions

For all the anion analyses, the IC operating flow rate was 1 mL/min and it operated with external water mode for auto suppression. The system operating pressure was maintained below 1500 psi.

Total Suspended Solids Measurement

Suspended solids analysis was performed gravimetrically to determine the concentration of microbes in the reactors. It was performed by using a filtration apparatus consisting of erlenmeyer flask, filter support, a funnel and a vacuum pump. For some tests, a GF/C Whatman glass-fiber filter (APHA Standard Method) was used, and for others, 0.45 μm and 0.2 μm membrane filters were used. The filter type and size used in every experiment is described in each individual experiment procedure.

Calibration of the DO Meter

The DO meter was calibrated before being used to monitor anaerobic conditions in the reactors. Calibration was performed as follows:

- The YSI 54A Dissolved Oxygen Instrument (YSI Models 54 ARC and 54 ABP) was switched to the "OFF" position and the meter mechanical zero was adjusted.
- Next, the instrument was turned on, and the knob was adjusted to RED Line.
- Then, the probe (YSI 5720) was plugged into the instrument for 10-15 minutes for probe polarization.
- The instrument was switched to the "ZERO" position and adjusted to "0" on the mg/L scale.

- The instrument was switched to "TEMP" and the probe (YSI 5720) was placed in a BOD bottle, containing about 1 inch of water, for about ten minutes for stabilization.
- The temperature on °C scale was obtained from the instrument.
- Calibration values were determined by using the probe temperature and the true local atmospheric pressure or feet above sea level. The altitude of the UNLV Environmental Engineering Laboratory is 2000 ft (the correction factor is 0.93).
- The instrument was switched to the 0-10 mg/L D.O. range and was adjusted with the "CAL control" until the calibration value determined in above step was reached.

The membrane of the DO meter probe (YSI 5720) was changed before every experiment. Model 5775 Oxygen Probe Service Kit containing membrane booklets and one bottle of O_2 probe Solution were used to replace the membrane.

Calibration of the pH Meter

The pH meter used in the experiment was a Corning pH/ion meter 450 with an Orion combination pH 91-56 probe. Three points calibration (pH = 4.0, 7.0 and 10.0) was used.

CHAPTER 4

RESULTS AND DISCUSSION

Diffusivity Tests through Membranes without a Biofilm

Perchlorate Diffusivity Testing

Figure 4.1 and Table 4.1 show the results of duplicate tests for perchlorate diffusion through the BT-55 membrane. The plot of the data resulted in two straight lines, both with R^2 values greater than 0.99 and the same slope of –0.0007. The slope of the lines, as per Equation 4.1, equals $-\frac{2A_M D_M}{V_{BR}\Delta L_M}$. Since A_M, V_{BR}, ΔL_M are known, the diffusion coefficient (D_M), can be calculated.

$$\ln\frac{C_{DR0}-2C_{BR}}{C_{DR0}-2C_{BR0}} = -\frac{2A_M D_M}{V_{BR}\Delta L_M}t \qquad (4.1)$$

For a slope of -0.0007, the diffusion coefficient for perchlorate is calculated as:

$$\text{slope} = -0.0007 = -2 * A_M * D_M/(V_A * L_M)$$

$$A_M = 2 * 3.14 * r^2 * \varepsilon$$

$$\varepsilon = 70\ \% = 0.7, \qquad L_M = 125\ \mu m$$

$$V_{BR} = 5\ \text{liter} = 5 * 10^{-3}\ \ m^3$$

$$So, D_M = K * V_{BR} * L_M / (2*A_M)$$

$$D_M = 0.0007 * 5 * 10^{-3} * 125 * 10^{-6} / (2* 3.14*0.05^2*0.7)$$

$$D_M = 3.98 * 10^{-8} \ m^2/min = 6.64 * 10^{-6} \ cm^2/sec$$

For the diffusion coefficient data through the PVDF membrane (Figure 4.2 and Table 4.2) some variability was found in the duplicate tests. Both R^2 values were greater than 0.99, but the slopes were different and equal to -0.0004 and -0.0006 for tests 1 and 2, respectively. The calculated diffusion coefficients were found to be 3.0 $x10^{-6}$ and 4.5 x 10^{-6} cm^2/sec for test 1 and 2, respectively. The average diffusion coefficient for this membrane is then 3.75 x 10^{-6} cm^2/sec. For the FGLP membrane (Figure 4.3 and Table 4.3), data for both tests were very similar with R^2 values greater than 0.97. The slope was the same (-0.0004) for both tests and a diffusion coefficient of 6.67 x 10^{-6} cm^2/sec was calculated.

Table 4.1

Perchlorate diffusion testing by a BTS-55 membrane

TEST 1			TEST 2 (duplicate test)	
Time (min)	CA [Conc. of BR (mg/L)]	$\ln[(C_{B0}-2C_A)/(C_{B0}-2C_{A0})]$	CA [Conc. of BR (mg/L)]	$\ln[(C_{B0}-2C_A)/(C_{B0}-2C_{A0})]$
5	1.88	-0.003767087	1.444	-0.002892178
15	5.615	-0.011293533	4.927	-0.009902872
30	9.212	-0.018595836	9.966	-0.020133322
45	15.13	-0.030727285	15.104	-0.030673663
60	N/A	N/A	18.3	-0.037286585
75	18.69	-0.038096545	24.775	-0.050819722
90	30.46	-0.062854606	30.345	-0.062609716
105	31.15	-0.064325211	34.63	-0.07177531
120	N/A	N/A	40.375	-0.084197159
135	45	-0.094310679	45.55	-0.095520202
150	N/A	N/A	51.12	-0.107852507
165	52.49	-0.110909215	54.51	-0.115433298
180	N/A	N/A	60.99	-0.130085907
195	61.51	-0.131271092	63	-0.134674903
210	N/A	N/A	68.28	-0.146830868
225	70.84	-0.152778289	81.07	-0.176904257
240	74.9	-0.162283663	N/A	N/A
255	N/A	N/A	82.42	-0.180131956

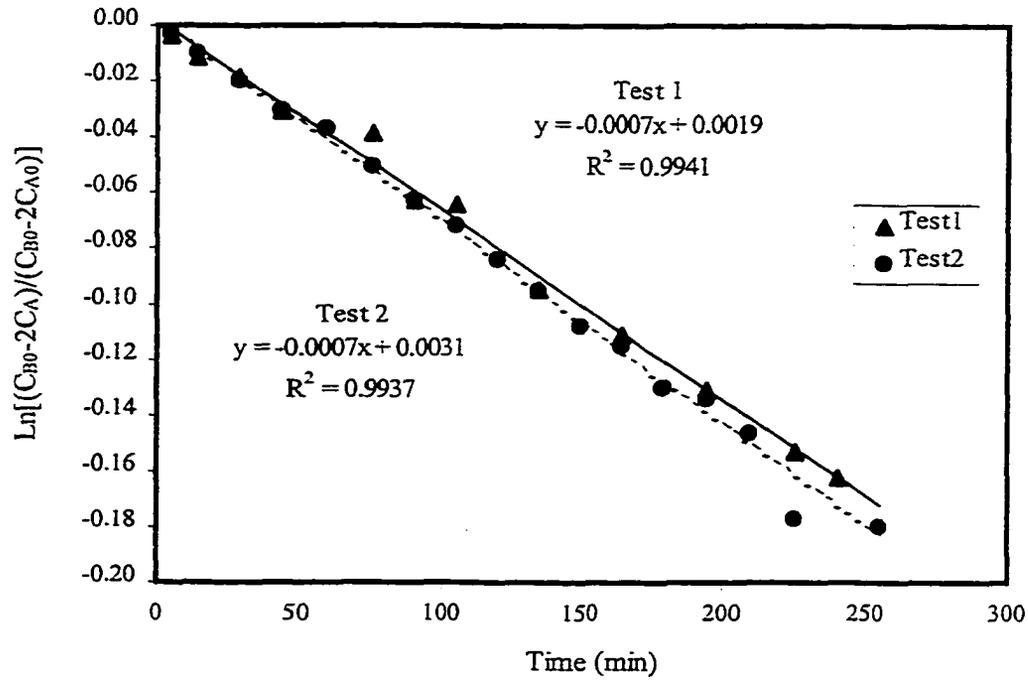


Figure 4.1 Perchlorate diffusion coefficient by a Memcor BTS-55 membrane

Table 4.2

Perchlorate diffusion testing by a PVDF membrane

Test 1			Test 2 (duplicate test)	
Time (min)	C_A[conc. of BR (mg/L)]	ln[(C_{B0}-2C_A)/(C_{B0}-2C_{A0})]	CA[conc. of BR (mg/L)]	ln[(C_{B0}-2C_A)/(C_{B0}-2C_{A0})]
5	0.718	-0.001437032	1.449	-0.002902207
15	3.063	-0.006144841	4.543	-0.009127529
30	6.306	-0.012692206	9.198	-0.018567311
45	8.98	-0.018123238	13.68	-0.027741255
60	11.395	-0.023053706	18.085	-0.036840348
75	N/A	N/A	22.095	-0.04519613
90	N/A	N/A	27.275	-0.056094274
95	19.74	-0.040280475	N/A	N/A
105	22.135	-0.045279833	30.485	-0.062907851
120	26.355	-0.054150002	34.35	-0.071173819
135	27.33	-0.056210628	40.00	-0.083381609
150	29.67	-0.061173522	43.28	-0.090537587
165	34.74	-0.072011709	48.05	-0.101036544
180	38.61	-0.080364426	51.74	-0.109234677
195	39.39	-0.0820564	55.27	-0.117140742
210	39.7	-0.082729648	61.21	-0.13058716
225	43.15	-0.090252989	63.24	-0.135224253
240	45.1	-0.094530484	65.86	-0.141241036
255	49.78	-0.104871746	N/A	N/A

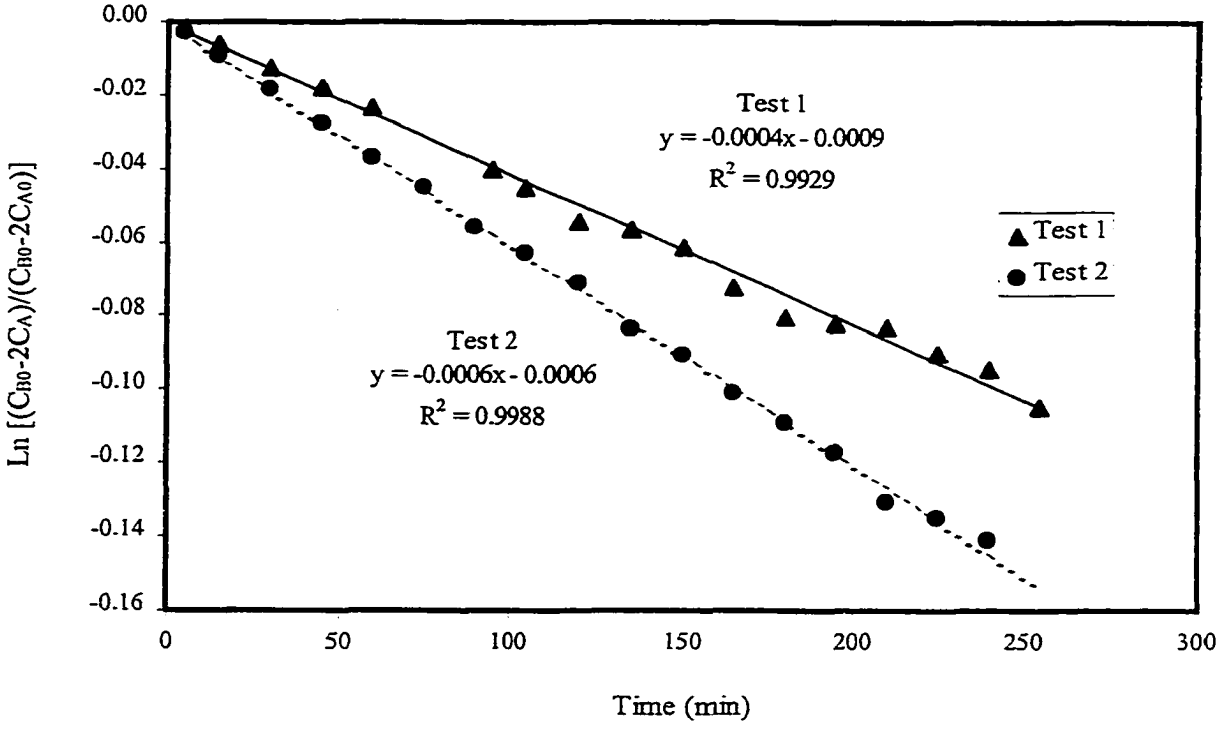


Figure 4.2 Perchlorate diffusion through a Memcor PVDF membrane

Table 4.3

Perchlorate diffusion testing by a FGLP membrane

	Test 1		Test 2 (duplicate test)	
Time (min)	CA[conc. of BR (mg/L)]	$\ln[(C_{B0}-2C_A)/(C_{B0}-2C_{A0})]$	CA[conc. of BR (mg/L)]	$\ln[(C_{B0}-2C_A)/(C_{B0}-2C_{A0})]$
5	0.8828	-0.001767161	0.7306	-0.001462269
15	2.7045	-0.005423682	2.437	-0.004885917
30	4.881	-0.009809961	5.467	-0.010994216
45	8.296	-0.016731189	5.084	-0.010220047
60	10.275	-0.020764089	11.51	-0.023289098
75	12.83	-0.02599496	12.795	-0.025923119
90	16.695	-0.033960174	15.64	-0.031779667
105	19.53	-0.039843307	19.775	-0.040353354
120	22.58	-0.046211492	22.9	-0.046881986
135	24.33	-0.049883762	25.76	-0.052894576
150	29.07	-0.059898635	28.01	-0.0576503
165	30.04	-0.061960514	27.43	-0.056422214
180	32.31	-0.066802415	32.77	-0.067786457
195	37.34	-0.077615655	29.14	-0.060047289
210	36.42	-0.075629129	37.6	-0.078177781
225	41.96	-0.087651582	44.13	-0.092400417
240	44.67	-0.093585668	47.3	-0.099378444

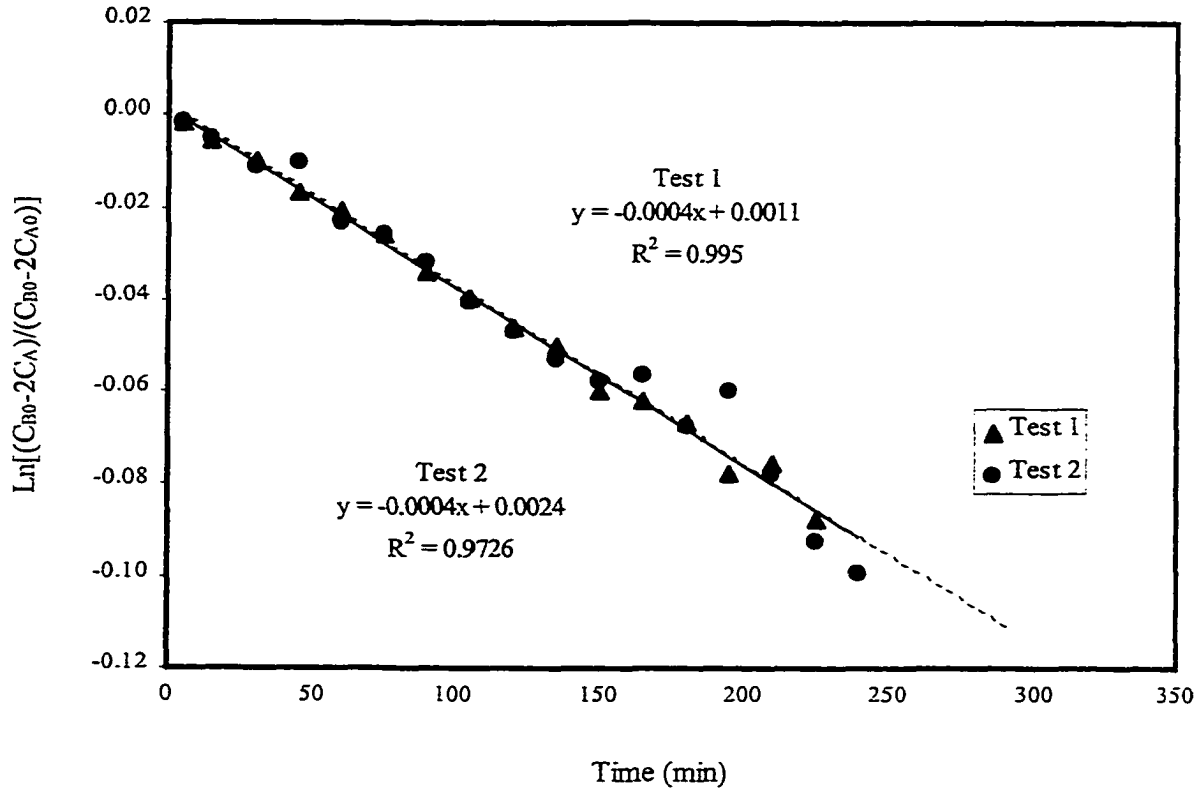


Figure 4.3 Perchlorate diffusion through a Millipore FGLP membrane

The membrane characteristics as well as the calculated diffusion coefficients for all three membranes are summarized in Table 4.4.

Table 4.4

Experimental determination of diffusion coefficient for perchlorate through three types of membranes

Membrane Type	Memcor BTS-55	Memcor PVDF	Millipore FGLP
Pore Size, μm	0.2	0.45	0.2
Thickness, μm	125	99	220
Pore Fraction, %	70	70	70
Diffusion Coefficient, cm^2/sec	**6.64×10^{-6}**	**3.75×10^{-6}**	**6.67×10^{-6}**

Notice in Table 4.4 that the 0.2μm membranes have similar diffusion coefficients. The FGLP has a slightly larger diffusion coefficient and it is thicker than the BTS membrane. The smallest diffusion coefficient was found for the PVDF membrane that has the largest pore sizes, but it is considerably thinner than the other membranes.

For comparison, the diffusivity of perchlorate in water, without a membrane, was calculated by using the Wilke-Chang Method, in which the molar volume of perchlorate was calculated using the Method of LeBas (Reid, 1987). The diffusion coefficient of perchlorate calculated by the Wilke-Chang method (Reid, 1987) was found to be 1.53×10^{-5} cm^2/sec. Therefore, the diffusivity of perchlorate through the microporous membranes tested is significantly smaller than that in water. This result confirms the hypothesis that perchlorate would migrate through semi-permeable membranes by diffusion. The diffusion coefficient will allow for the calculation of the perchlorate flux through the membrane without the biofilm for different perchlorate concentrations. In addition, it will provide insight into the effects of the biofilm thickness on the transport of

perchlorate to the BR reactor. All three membranes were tested for biofilm immobilization.

Nitrate Diffusivity Testing

Tables 4.5 and Figure 4.4 show the results of duplicate diffusivity testing (test 1 and test 2) for nitrate through the Memcor BTS-55 membrane (pore size 0.2 μm; 125 μm thick). For test 1, the initial concentration of nitrate in the DR reactor was 1066.96 ppm, while for test 2, the initial concentration of nitrate in the DR reactor was 1031.74 ppm. Figure 4.4 was generated by plotting the last columns of Tables 4.5 against time (Equation 4.1). The slopes of the generated lines were calculated and the diffusivity was determined by Fick's law. Notice that the duplicate tests provided very similar results and the same slope (-0.0005) with R^2 values greater than 0.996. Therefore, the calculated diffusion coefficient of nitrate through the BTS-55 membrane is 4.74×10^{-6} cm^2/sec. McCleaf and Schroeder (1995) found diffusion coefficient for nitrate of the same order of magnitude (3.4×10^{-6} cm^2/sec) for a membrane with a 0.2 μm pore size and 37.5 μm thickness.

Table 4.5

Nitrate diffusivity testing by a BTS-55 membrane

	Test 1		Test 2 (duplicate test)	
Time (min)	C_{BR} [Conc. of BR reactor (mg/L)]	Ln [(C_{DR0}-$2C_{BR}$)/(C_{DR0}-$2C_{BR0}$)]	C_{BR} [Conc. of BR reactor (mg/L)]	Ln [(C_{DR0}-$2C_{BR}$)/(C_{DR0}-$2C_{BR0}$)]
0	0.629		0.34	
15	5.58	-0.009925011	4.82	-0.009057768
30	10.17	-0.01865786	8.56	-0.016402944
45	14.02	-0.026042072	12.64	-0.024477879
60	18.04	-0.033810978	16.67	-0.032518384
75	22.6	-0.042697154	20.26	-0.039735874
90	24.85	-0.047111046	24.12	-0.047554744
105	28.14	-0.053600412	26.92	-0.053264966
120	31.59	-0.06045091	31.04	-0.061726887
135	33.12	-0.063504043	34.03	-0.067913092
150	38.44	-0.074193315	38.91	-0.078092571
165	40.35	-0.078059052	42.12	-0.084845445
180	46.26	-0.090116116	45.58	-0.092175675
195	48.94	-0.095631895	47.48	-0.096223919
210	51.34	-0.100597354	49.11	-0.099709994
225	56.63	-0.111629907	56.39	-0.115429786
240	59.84	-0.118384343	60.10	-0.123536905

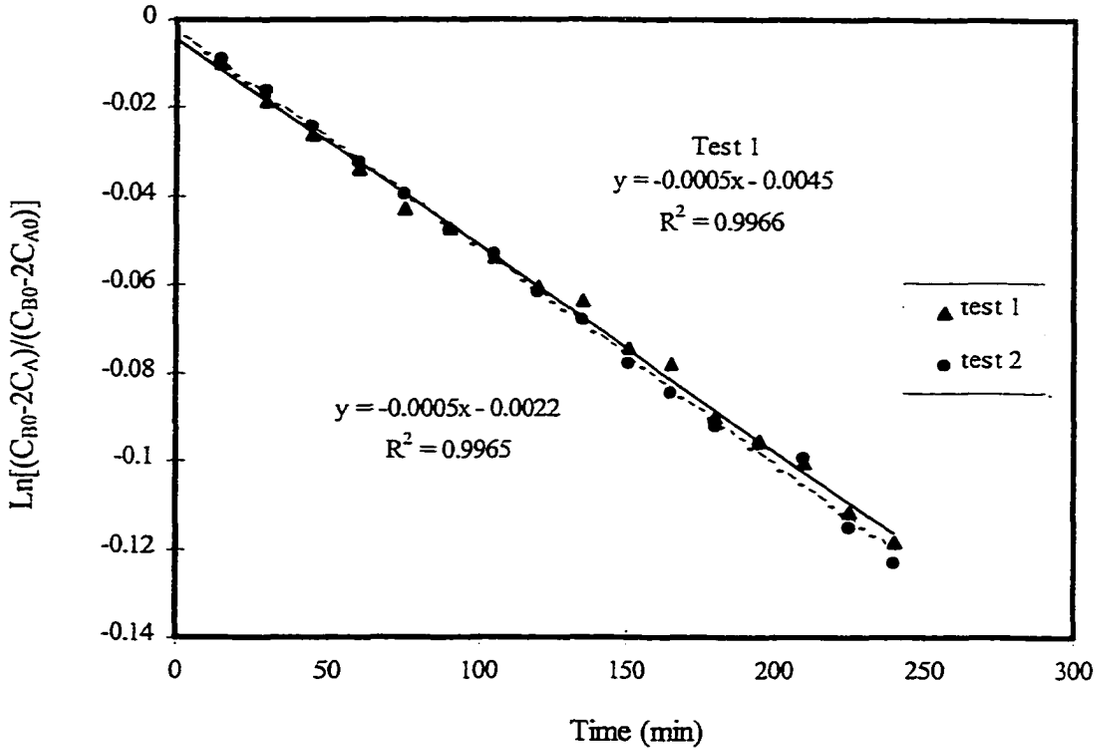


Figure 4.4 Nitrate diffusivity testing through Memcor BTS-55 Membrane

Sulfate Diffusivity Testing

Tables 4.6 and Figure 4.5 show the results for duplicate diffusivity tests (test 1 and test 2) for sulfate through the Memcor BTS-55 membrane. For test 1, the initial concentration of sulfate in the DR reactor is 949.76 ppm; while for test 2, the initial concentration of sulfate in the DR reactor is 973.26 ppm. The data on the last columns of Table 4.6 were used to build Figure 4.5, according to Equation 4.1. For test 1, the slope was found to be –0.0004 with R^2 value of 0.988. For test 2, the slope was –0.0004 with R^2 value of 0.995. The calculated diffusion coefficient of sulfate through the BTS-55 membrane is therefore 3.79×10^{-6} cm^2/sec.

Table 4.6

Sulfate diffusivity testing by a BTS-55 membrane

Test 2			Test 2 (duplicate test)	
Time (min)	C_{BR} [Conc. of BR reactor (mg/L)]	Ln [$(C_{DR0}-2C_{BR})/(C_{DR0}-2C_{BR0})$]	C_{BR} [Conc. of BR reactor (mg/L)]	Ln [$(C_{DR0}-2C_{BR})/(C_{DR0}-2C_{BR0})$]
0	0		0	
15	3.77	-0.007970528	2.65	-0.005460497
30	4.72	-0.009989078	5.08	-0.010494013
45	7.45	-0.015812536	8.13	-0.01684787
60	10.69	-0.022768189	10.93	-0.022716677
75	13.1	-0.027973553	12.92	-0.026908761
90	12.15	-0.02591841	15.96	-0.033346869
105	17.75	-0.038094326	18.59	-0.038950318
120	22.42	-0.048362779	20.72	-0.043511598
135	24.58	-0.053148113	26.35	-0.055669081
150	27.98	-0.060727285	25.93	-0.054757009
165	29.22	-0.063505811	30.96	-0.065735217
180	31.35	-0.068296697	31.91	-0.067822236
195	34.20	-0.074743152	37.18	-0.079479468
210	39.94	-0.08785405	37.73	-0.080703935
225	40.06	-0.088129988	40.52	-0.086938522
240	41.15	-0.09063992	43.17	-0.092896475

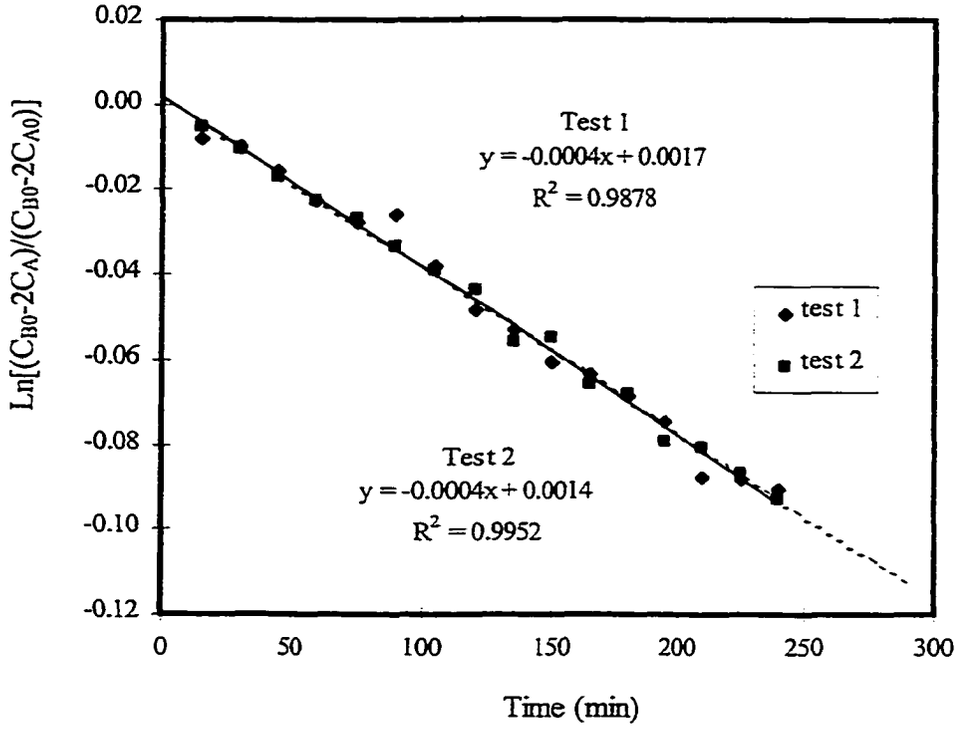


Figure 4.5 Sulfate diffusivity testing by a Memcor BTS-55 Membrane

Table 4.7 shows the experimentally determined diffusion coefficients for nitrate, sulfate, and perchlorate through the Memcor BTS-55 membrane. In addition, the diffusivity of these compounds in water, as estimated by the Wilke-Chang method (at 25°C) are shown in the table. The molar volume of nitrate (31.9 cm^3/g.mole), sulfate (58.8 cm^3/g.mole), and perchlorate (54.2 cm^3/g.mole) were calculated using the Method of LeBas. Notice that for the three compounds, as expected from the values of their molar volumes, the diffusivity in water is from the fastest to the slowest: Nitrate > perchlorate > sulfate. However, the experimentally determined diffusivity values through the membrane did not show the same relationship for perchlorate. For these, the diffusivity through the BTS-55 membrane follows: Perchlorate > Nitrate > sulfate. Thus, through

the membrane in question, the diffusivity of nitrate is higher than that of sulfate, expected from their diffusivities in water. However, perchlorate diffusivity is larger than those for nitrate and sulfate. The implication of these findings to the performance of the membrane-immobilized biofilm reactor is that sulfate and nitrate contained in perchlorate-contaminated waters may not reach the biofilm (located on the BR side of the reactor) as fast as perchlorate and their effects on perchlorate biodegradation may be reduced.

Table 4.7

The Diffusion coefficients of perchlorate, nitrate and sulfate in water and through Memcor BTS-55 membrane

	Perchlorate	Nitrate	Sulfate
With BTS-55, cm^2/sec (Testing Data)	6.64×10^{-6}	4.74×10^{-6}	3.79×10^{-6}
Without BTS-55, cm^2/sec (Calculated by Wilke-Chang Method)	1.53×10^{-5}	2.12×10^{-5}	1.47×10^{-5}

Development of One-Liter Master Culture Reactor (MCR)

Table 4.8 shows the data obtained when acclimating the CCSD enriched culture to degrade perchlorate. The data presented in Table 4.8 clearly indicate that activated sludge cultures can be easily acclimated to biodegrade perchlorate. The results show that within four days about half of the perchlorate was reduced anaerobically by the biomass. After five days of incubation, perchlorate concentration decreased to undetectable levels. The concentration of chloride in the reactor was initially high (51 mg/L) due to the high

TDS of the waste activated sludge sample used as the inoculum. The suspended solids concentration remained high in the presence of perchlorate and started decreasing rapidly when perchlorate was used up. The decreased suspended solids indicates the enrichment of the culture with perchlorate-reducing microbes and the decay of the microbes that cannot use perchlorate as an electron acceptor.

Table 4.8

Perchlorate biodegradation in the first master culture reactor

Day	ClO_4^- (mg/L)	Cl^- (mg/L)	Lactate (mg/L)	SS (g/L)
1	120	51.0	600	0.295
2	**	**	**	**
3	**	**	**	**
4	66.04	110.4	153.7	0.39
5	< 5 ppb	122.7	194.3	0.26
6	< 5 ppb	139.4	204.6	0.29
7	< 5 ppb	143.2	168.0	0.19

** Not sampled

Development of Immobilized Biofilms on the Membranes

The FGLP membrane showed the largest diffusion coefficient for perchlorate (Table 4.4), and it was, therefore, selected to be tested first for biofilm development. Table 4.9 shows the concentrations of perchlorate, lactate, and chloride in both reactors during biofilm growth on the Millipore FGLP membrane.

Table 4.9

Biofilm development on a Millipore FGLP membrane

	BR Reactor					DR Reactor		
Day	pH	SS, (g/L)	ClO_4^-, (mg/L)	Lactate, (mg/L)	Cl^-, (mg/L)	ClO_4^-, (mg/L)	Lactate, (mg/L)	Cl^-, (mg/L)
1	6.576	0.1	203.5	1122	51.3	0	0.196	0.275
2	6.673	0.11	203.3	825.9	59.1	0.022	0.238	0.191
3	6.957	0.13	208.3	676.8	65.5	0.070	0.253	0.226
4	6.916	0.21	136.9	232.7	120.0	0.129	0.295	0.218
5	7.105	0.07	< 5 ppb	195.2	169.4	0.181	0.141	0.228
6	7.277	0.09	< 5 ppb	64.6	193.2	0.198	0.470	0.265

Notice in Table 4.9 that the initial perchlorate (203.5 mg/L), chloride (51.3 mg/L), and lactate (1122 mg/L) concentrations are higher than the concentration of these components added to the process. This occurs because these components were present in the MCR from which the seed microbes were taken.

As shown in Table 4.9 and Figure 4.6, 200 mg/L perchlorate was completely biodegraded by the enrichment culture (about 0.2 g/L SS) very quickly. Notice that the bulk of the perchlorate biodegradation did not occur until day-three and in day-five all perchlorate had been consumed by the microbes. A lactate to perchlorate ratio of about 5.2:1 was consumed. A thick biofilm was developed in the membrane separating the two reactors and in the walls of the BR reactor as well.

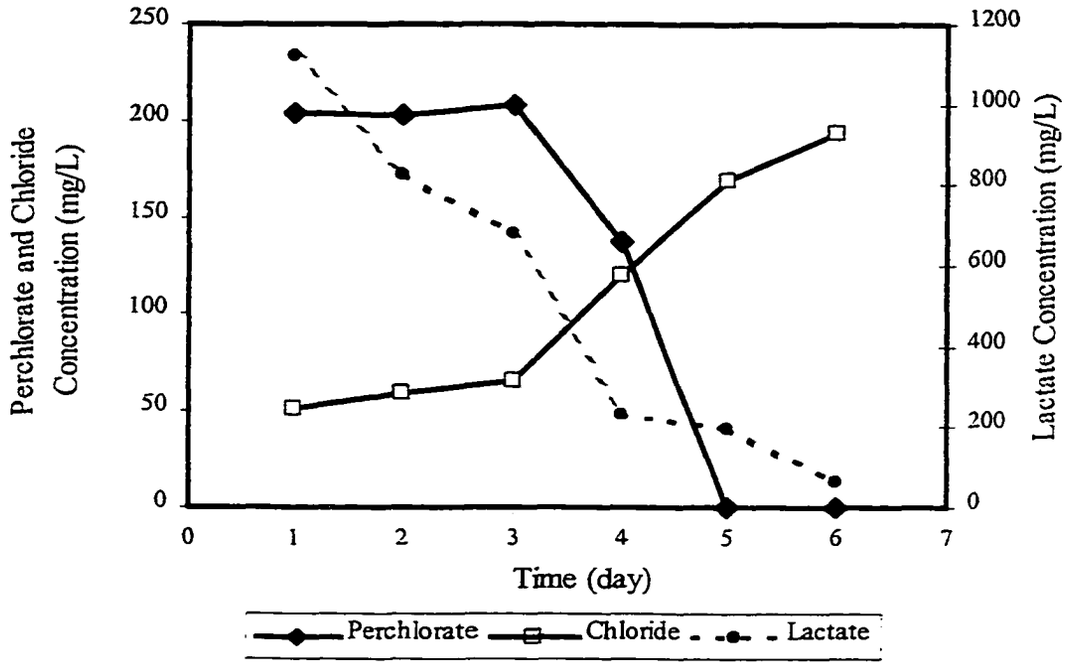


Figure 4.6 Anaerobic consumption of perchlorate and lactate in the BR

The chloride concentration in the reactor increased with increasing perchlorate biodegradation, but it was not proportional to perchlorate degradation. It seems that there is a lag between perchlorate biodegradation and chloride formation. Notice in Table 4.9 that the SS concentration in the reactor quickly decreased when perchlorate was no longer available.

In the DR reactor, where only DI water was added, the concentrations of perchlorate, lactate, and chloride were monitored (Table 4.9 and Figure 4.7). Notice that only small amounts (μg/L range) of perchlorate, lactate, and chloride diffused to the DR reactor. The concentrations of perchlorate and lactate increased with time, but the chloride concentration varied somewhat during the six-day period. It was also observed that after the fifth-day that the volume in the BR reactor had increased about 0.5 L due to osmosis. This movement of water from the DR to the BR reactor due to the difference in

concentrations promoted dilution in the BR reactor. The concentration values shown in Table 4.9 are the raw numbers and have not been corrected for the dilution caused by osmotic pressure.

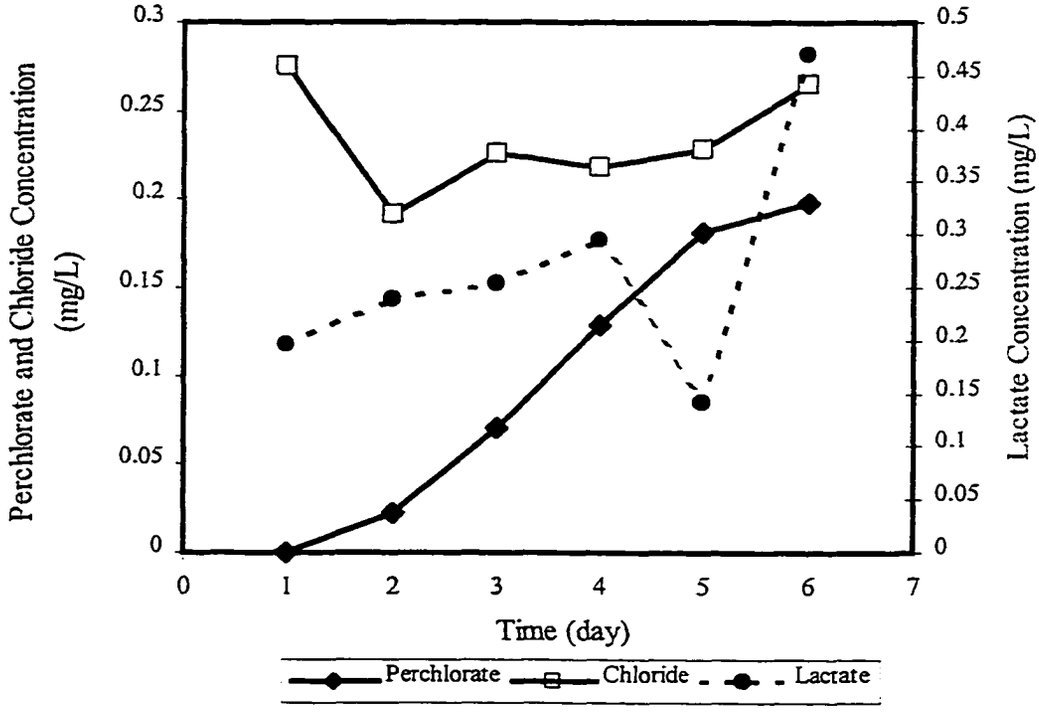


Figure 4.7 Anaerobic consumption of perchlorate and lactate in the DR

Biodegradation of Perchlorate by Membrane-Immobilized Biofilms

Biofilms were developed in all three selected microporous membrane and perchlorate biodegradation by the membrane-immobilized biofilms was investigated. Table 4.10 shows the testing schedule and the number of biodegradation cycles performed for each membrane. One cycle consists of a run from an initial perchlorate concentration to a lower desired perchlorate level in the DR reactor.

Table 4.10

The time sheet of perchlorate biodegradation testing

Testing Cycle	Testing Period for Different Membrane Types		
	FGLP	BTS-55	PVDF
First Cycle	6/7/99—6/13/99	6/28/99—7/12/99	9/7/99-9/24/99
Second Cycle	6/17/99—7/10/99	7/13/99—7/23/99	
Third Cycle		7/23/99—8/6/99	

Testing of Biofilm Immobilized on the FGLP Membrane

In the first cycle, 223 mg/L perchlorate was added to the DR reactor separated by a biofilm immobilized on Millipore FGLP membrane (Table 4.11). In the BR reactor 1100 mg/l lactate, nutrients/minerals and buffer were added. Notice that in nine days only about 37 mg/L was biodegraded. Perchlorate diffusion was very poor through the biofilm immobilized in this membrane. This was unexpected, since this membrane showed the largest diffusion coefficient in the diffusion tests without a biofilm. It is worth mentioning that the biofilm developed in this membrane was very thick. This happened because this membrane has a plastic backing that favors microbial attachment. Therefore, transport of perchlorate to the BR reactor was limited by diffusion. Also notice that the chloride concentration in the BR and in the DR reactors increased with time, indicating perchlorate biodegradation. (Figure 4.8) The results show that chloride migrated easily from the BR to the DR reactor with time, due to its small molar volume.

Table 4.11

Perchlorate biodegradation by a biofilm immobilized on a Millipore FGLP membrane (1st cycle)

	DR Reactor (ClO_4^- only)			BR Reactor (lactate, nutrients/minerals, buffer)		
Day	ClO_4^-, (mg/L)	Cl^-, (mg/L)	Lactate (mg/L)	ClO_4^-, (μg/L)	Cl^-, (mg/L)	Lactate (mg/L)
1	223.2	0.346	< 30 ppb	< 5	1.32	1076
2	217.3	0.65	< 30 ppb	< 5	1.69	1030.
3	214.8	1.78	<30 ppb	< 5 ppb	2.01	266.1
4	205.2	3.22	<30 ppb	< 5 ppb	2.07	249.7
5	199.4	3.77	<30 ppb	< 5 ppb	2.73	191.9
6	190.8	5.42	<30 ppb	< 5 ppb	2.99	89.28
7	202.6	6.28	<30 ppb	< 5 ppb	3.45	151.7
8	1932.	6.56	<30 ppb	< 5 ppb	3.68	164.2
9	186.5	7.13	<30 ppb	< 5 ppb	4.02	161.7

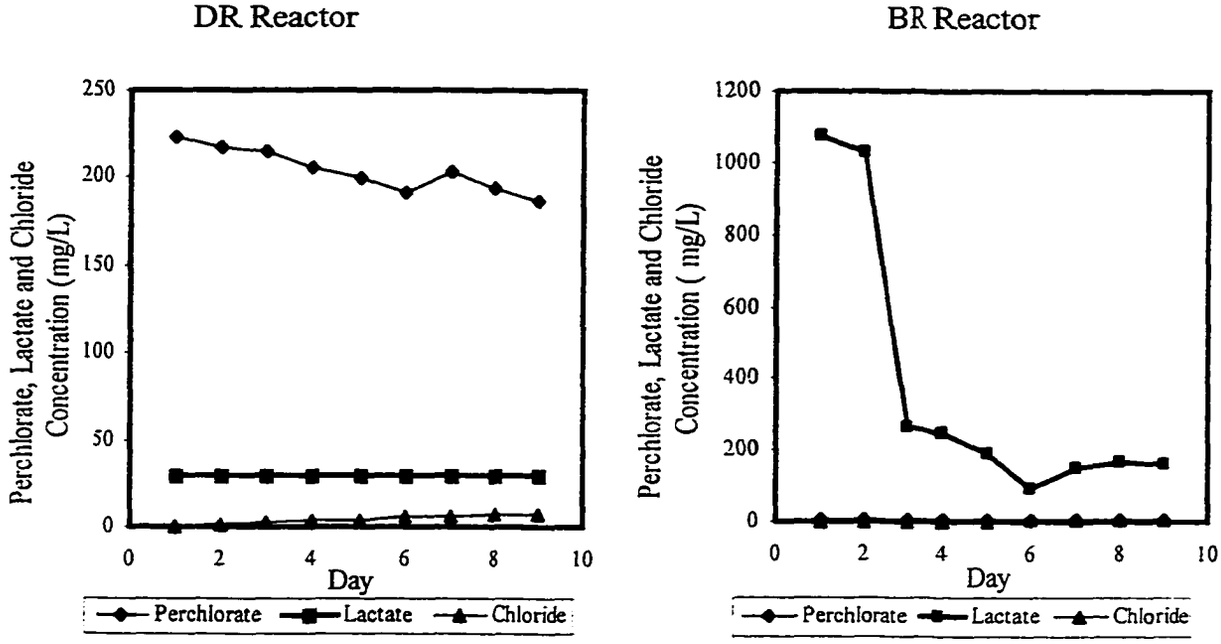


Figure 4.8 Perchlorate, lactate, and chloride concentrations in the DR and BR reactors during biodegradation of perchlorate by a biofilm immobilized on a FGLP Membrane (1st cycle)

Although only a small amount of perchlorate was biodegraded, a large amount of lactate was used in the BR reactor. Since the work is being perfomed using a mixed-biological culture, it is likely that lactate was used as a carbon source by microbes in the biofilm which are not dependent on perchlorate as an electron acceptor

In a second trial with the FGLP membrane (Table 4.12) 223.16 mg/L perchlorate was added to the DR reactor and 1099.8 mg/l lactate, nutrient and buffer were added to the BR reactor. As shown in Figures 4.9, only very small amounts of perchlorate were able to diffuse to the BR reactor. Thus, biodegradation of perchlorate was again limited by diffusion through the thick biofilm. It was concluded from the above results that the FGLP membrane was not suitable for perchlorate degradation.

Table 4.12

Perchlorate biodegradation by a biofilm immobilized on a FGLP 14250 membrane (2nd cycle)

	DR Reactor (ClO_4- only)			BR Reactor (Lactate, Nutrients/Minerals, and Buffer)		
Day	ClO_4^- (mg/L)	Lactate (mg/L)	Cl^- (mg/L)	ClO_4^- (mg/L)	Lactate (mg/L)	Cl^- (mg/L)
1	223.16	0	0.32	0	1099.8	1.32
2	217.32	0	0.69	0	929.6	1.69
3	214.76	0	1.78	0	287.11	2.01
4	205.24	0	3.38	0	249.73	2.07
5	199.48	0	4.00	0	191.9	2.73
6	190.8	0.013	5.42	0	89.28	2.99
7	202.65	0.016	6.28	0	151.7	3.45
8	192.96	0.012	6.56	0	164.2	3.68
10	188.36	0.021	8.18	0.036	148.38	2.14
11	197.44	0	9.47	0.024	No data	No data
12	186.24	5.67	8.88	0	35.77	4.35
13	178.08	0	8.99	0	38.12	6.79
14	193.84	0	10.02	0.04	20.81	6.81
15	187.44	0	10.72	0.25	1.75	4.67
16	185.08	0	10.78	0.83	0	8.79
17	187.88	0	7.42	0	0	9.36
18	183.4	0	10.3	0.34	0	10.3
19	182.08	13.32	9	0.65	0	11.6
20	156.56	7.91	10.3	0.93	0	12.78
21	178.22	13.92	11.59	0.78	0	15.22
22	176.56	0	13.21	0.13	0	13.83
23	169.76	0	11.46	0.82	0	15.37
24	169.96	0	11.75	1.77	0	16.4

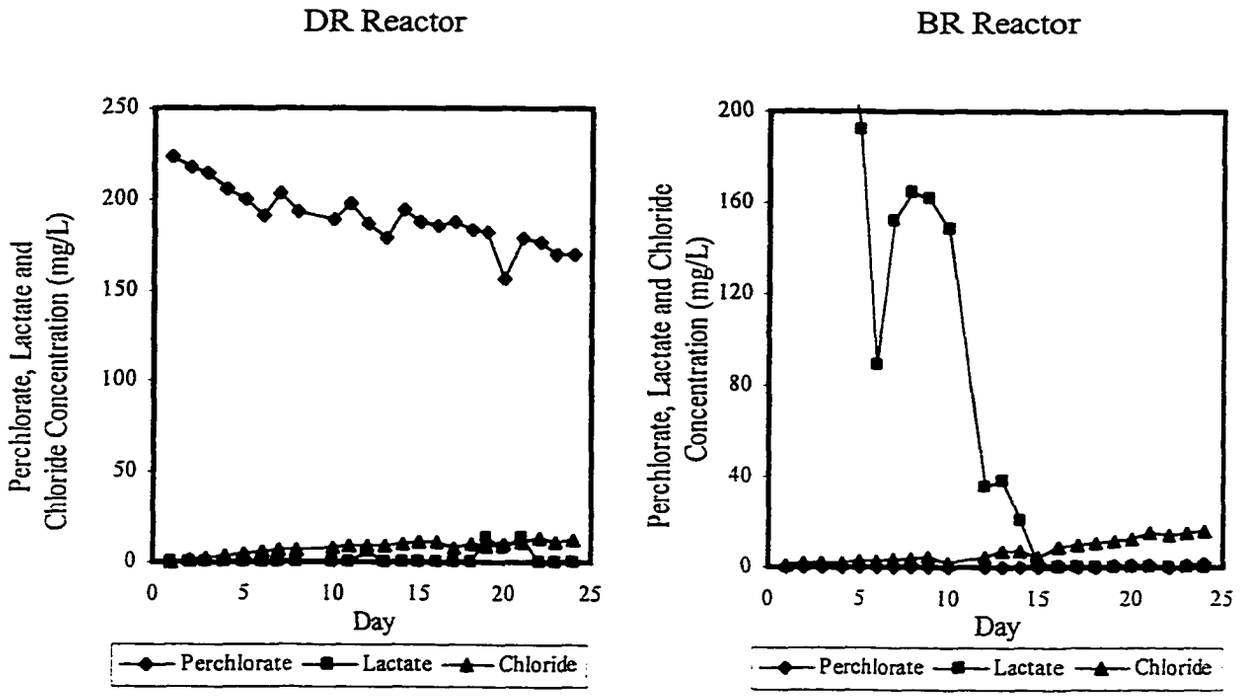


Figure 4.9 Perchlorate, lactate, and chloride concentrations in the DR and BR reactors during biodegradation of perchlorate by a biofilm immobilized on a FGLP membrane (2nd cycle)

Testing of Biofilm Immobilized on the BTS-55 Membrane

Three testing cycles were performed with this membrane. These tests involved two steps, the growth of the biofilm on the membrane and the biodegradation experiment. To grow the biofilm, lactate, perchlorate, nutrient, buffer, and microorganisms from the enriched culture were added to the BR reactor and DI water was added to the DR reactor. After the biofilm reached a certain thickness, the biofilm was protected by a sliding plate and the tanks were emptied and rinsed with DI water. Next, lactate, nutrient, and buffer were added to the BR reactor and perchlorate was added to the DR reactor. The plate was then removed from the biofilm and the reactors were sealed and kept anaerobic by purging with nitrogen gas.

Table 4.13 and Figures 4.10 show that, after 5 days, practically all perchlorate diffused to the BR reactor where it was biodegraded. Notice that the concentration of perchlorate in the BR reactor, at all times, was very small. The lactate in the BR reactor decreased proportionally to perchlorate in the DR reactor. However, lactate kept decreasing although no more perchlorate was available. It is suspected that lactate was fermented, since the work is being performed using a mixed culture.

The small concentrations of chloride and perchlorate in the first day of the experiment in the BR reactor are the result of the previous biofilm growth experiments. When the plate is held against the biofilm to keep it anaerobic during rinsing of the reactors, a small amount of growth solution is retained in the system and will be part of the BR reactor when the plate is removed. Because of this inconvenience, the experimental set-up was modified. In the new set-up, one set of reactors is used to grow the biofilm on a mobile plate that is removed, after the biofilm is developed, and attached to a new set of reactors by metal clamps.

An investigation of Figure 4.10 and Table 4.13 shows that, contrary to perchlorate and lactate, chloride concentrations change significantly from one reactor to another. This is due to two factors: (a) diffusion of chloride through the membrane due to its small size, (b) movement of water from the DR reactor (smaller concentration of ions) to the BR reactor (higher concentration of ions) by osmotic pressure. In all the tests performed with this membrane, migration of water from the DR to the BR reactor was observed. That means, with time, the volume of water in BR increased. As can be observed in Table 4.13 (last row), the chloride concentration stabilizes when both reactors contain about the same concentration of chloride. The fact that water is transferred by osmotic

pressure to the BR reactor may have implications in the design of full-scale membrane-immobilized biofilm systems. Product water (perchlorate free) could migrate to the BR reactor decreasing the total volume of treated water produced. In the experiments, DI water was used in the DR reactor. However, natural waters contaminated with perchlorate contain several other ions and the difference of ionic gradient may not be sufficiently high to cause the water migration from the DR to the BR reactor.

Table 4.13

Perchlorate biodegradation by a biofilm-immobilized on a BTS-55 membrane (1st cycle)

	DR Reactor (ClO_4- only)			BR Reactor (Lactate, nutrient/mineral, and buffer)		
Day	ClO_4^- (mg/L)	Lactate (mg/L)	Cl^- (mg/L)	ClO_4^- (mg/L)	Lactate (mg/L)	Cl^- (mg/L)
1	231.12	0	0.58	0.194	1101.13	12.09
2	171.4	14.52	10.19	26.39	240.18	2.63
3	109.45	74	33.29	4.19	215.03	25.2
4	3.21	0.744	93.36	0	208.5	23.99
5	0.025	0	90.42	0	115.19	32.97
6	0	14.91	72.13	0.078	114.1	41.92
7	0	24.74	74.94	0	108.7	36.87
8	0	12.45	90.84	0	35.97	36.77
9	0	15.01	65.84	0	29.41	38.54
10	0	0	65.08	0	5.2	37.05
11	0.028	0	64.43	0.034	5.55	37.4
12	0.019	15.17	61.15	0	0.27	53.87
13	0	13.69	60.55	0.088	0	49.22
15	0	0	56.05	0	0	57.31

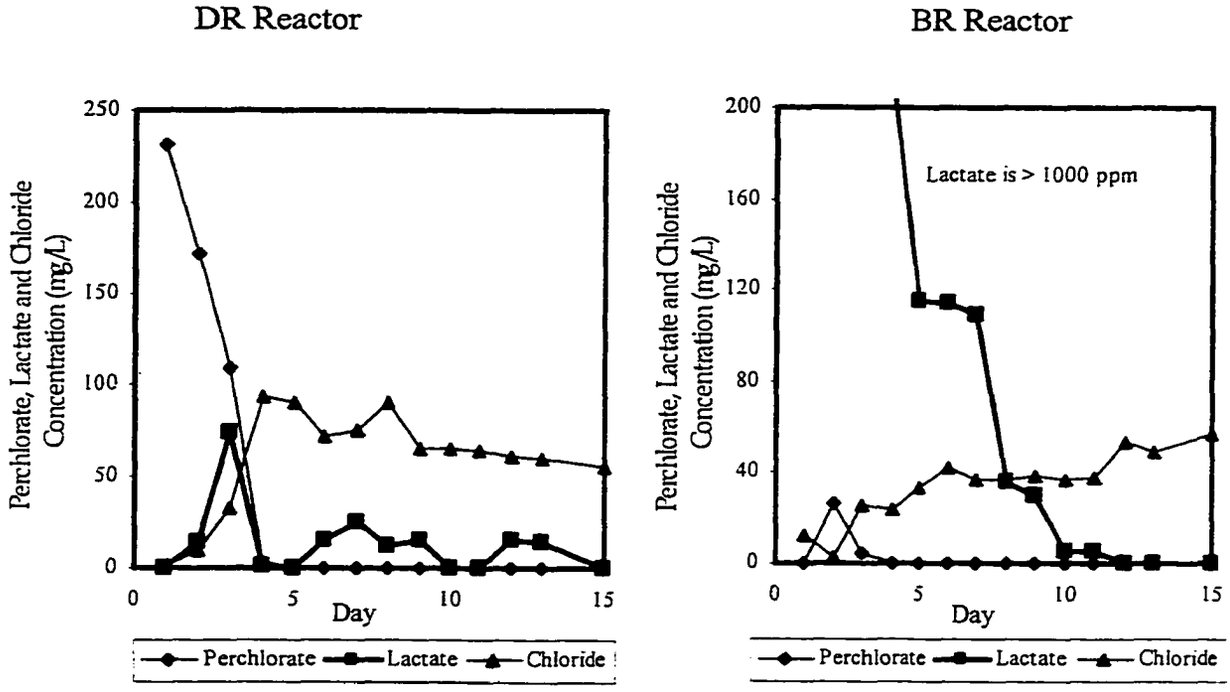


Figure 4.10 Perchlorate, lactate, and chloride concentrations in the DR and BR reactors during biodegradation of perchlorate by a biofilm immobilized on a BTS-55 Membrane (1st cycle)

Table 4.14

The molar ratio of perchlorate to chloride (1st cycle)

Moles ClO_4^- / Moles Cl^-	0.82
Moles ClO_4^- biodegraded/m^2-day (for first 6 days)	0.25

Table 4.14 shows the molar ratio of perchlorate biodegraded to that of chloride formed to be 0.82 for this run. Theoretically, each mole of perchlorate would generate one mole of chloride, thus this ratio is somewhat smaller than expected. Table 4.14 also shows that about 0.25 moles of perchlorate were biodegraded by the biofilm per day per

were considered, since the system was perchlorate-limited after the sixth day. An example calculation for the perchlorate biodegradation rate is shown in Appendix B (Example 1).

For the second cycle and third cycles, more lactate, nutrient, and buffer were added to BR reactor and more perchlorate was added to the DR reactor. The addition was made to the resulting solutions from the previous cycles. Table 4.15, 4.17 and Figure 4.11, 4.12 show the data obtained from these tests. The high concentrations of chloride in the first day are the result of perchlorate biodegradation from the previous cycles. As can be seen in Figures 4.11 and 4.12 perchlorate was easily biodegraded by the biofilm as indicated by the decrease in perchlorate degradation and the increase in chloride concentration.

Investigation of Tables 4.15 and 4.17 indicates that perchlorate degradation in the second cycle (0.11 moles ClO_4^- per day per m^2 of the membrane area) was slower than in the first cycle (0.25 moles ClO_4^- per day per m^2 of the membrane area) (Table 4.14) and third cycles (0.22 moles ClO_4^- per day per m^2 of the membrane area) (Table 4.18). Possible reasons for the differences in degradation rates in the three cycles include: (a) difference in biofilm thickness. It is not possible to measure the thickness of the biofilm between cycles, without terminating the experiment, but visually the biofilm seems thicker with time. (b) change in the microbial ecology of the reactor due to lactate degradation in the absence of perchlorate. In several runs, it was observed that when perchlorate was absent, lactate degradation still proceeded. It is possible that lactate is been fermented by other microorganisms, since the work is been performed with a mixed-culture. As mentioned above, in the first cycle perchlorate biodegraded at a rate of

0.25 moles/m^2-day. After the fifth day, all perchlorate was degraded, but lactate biodegradation proceeded for ten more days. In the second cycle, perchlorate biodegradation rate was only 0.11 moles/m^2-day (Table 4.16), but in cycle three, the perchlorate biodegradation was 0.22 moles/m^2-day (Table 4.18), similar to that of the first cycle. It is possible that fermentation occurred at the end of the first cycle, negatively affecting perchlorate biodegradation in the second cycle.

After the third cycle the reactors were carefully emptied and the membrane-immobilized biofilm was carefully weighed together with the membrane. The thickness of the membrane without the biofilm was originally measured using a micrometer. However, this method was found to be inadequate to measure the thickness of the membrane and biofilm together, since the biofilm was very fragile. The amount of microbes present in the biofilm was then estimated gravimetrically. This was done by carefully removing the microbes from the membrane with DI water, filtering through a 0.45μm sterilized membrane filter and drying at 105 °C for 24 hrs. The estimated thickness of the biofilm, based on a microbial density of 1.07 g/ml, was calculated to be 24.7 μm (Table 4.19). This value is very close to the thickness of a biofilm (27.5 μm) reported by McCleaf and Schroeder (1995), measured using a microscope with a calibrated ocular micrometer, on a denitrification study using a membrane-immobilized biofilm.

Table 4.15

Perchlorate biodegradation by a biofilm immobilized on a BTS-55 membrane (2nd cycle)

	DR Reactor (ClO_4^- only)			BR Reactor (Lactate, Nutrients/Minerals, and Buffer)		
Day	ClO_4^- (mg/L)	Lactate (mg/L)	Cl^- (mg/L)	ClO_4^- (mg/L)	Lactate (mg/L)	Cl^- (mg/L)
1	246.96	0.94	49.89	0.19	1058.9	44.71
2	187.08	12.73	61.6	0.35	266.54	64.86
3	134.21	0	93.22	0.055	204.93	72.14
4	117.74	0	93.25	0.024	172.05	78.91
5	105.58	0	86.69	0.003	99.22	80.00
6	96.42	0	95.37	0	33.66	84.65
7	89.24	0	89.58	0	0	88.47
8	79.82	0	80.99	3.09	0	69.02
9	67.6	0	80.3	1.43	5.44	74.7
10	57.88	0	80.81	2.26	7.06	75.16
11	50.07	0	82.73	3.25	9.28	79.12

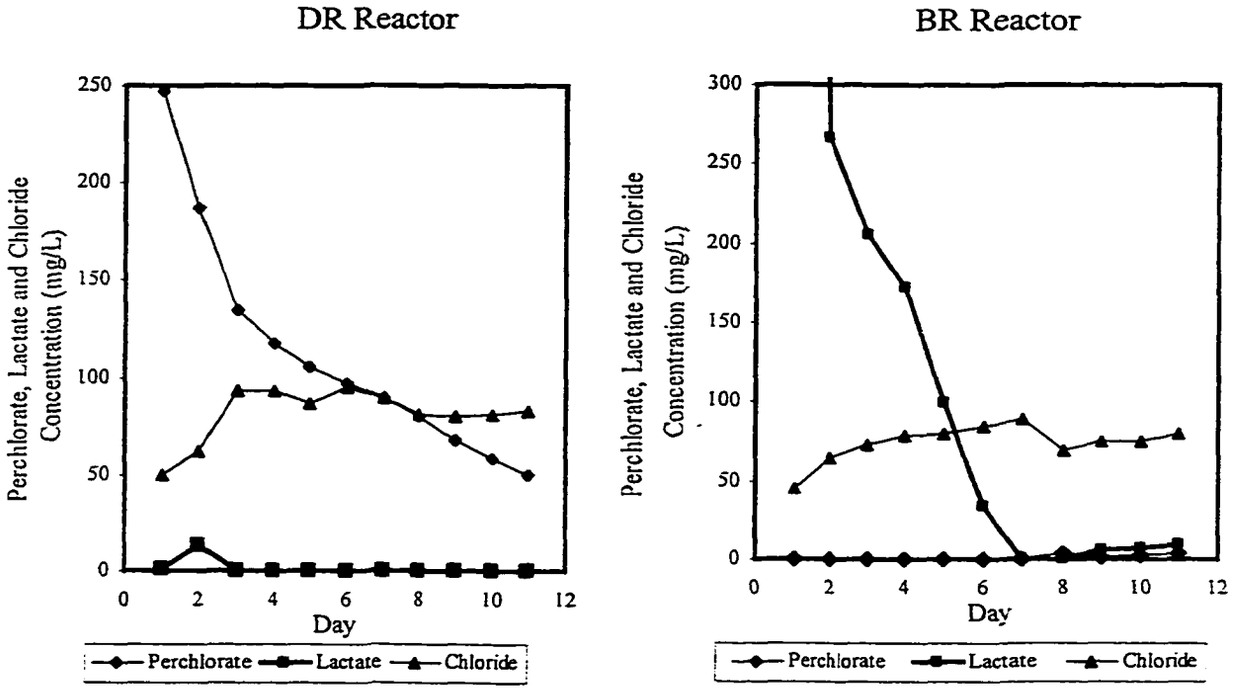


Figure 4.11 Perchlorate, lactate, and chloride concentrations in the DR and BR reactors during biodegradation of perchlorate by a biofilm immobilized on a BTS-55 Membrane (2^{nd} cycle)

Table 4.16

The molar ration of perchlorate to chloride (2^{nd} cycle)

ClO_4^- / Cl^-	1.03
Moles ClO_4^- biodegraded/m^2-day	0.11

Table 4.17

Perchlorate biodegradation by a biofilm immobilized on a BTS-55 membrane (3rd Cycle)

	DR Reactor (ClO_4- only)			BR Reactor (Lactate, Nutrients/Minerals, and Buffer)		
Day	ClO_4^- (mg/L)	Lactate (mg/L)	Cl^- (mg/L)	ClO_4^- (mg/L)	Lactate (mg/L)	Cl^- (mg/L)
1	323.72	0	82.47	3.42	1037.84	71.08
2	248.6	0	89.58	0	373.64	80.48
3	212.42	0	100.84	0	286.16	83.39
4	188.42	0	106.47	0	247.34	92.35
5	159.04	0	115.48	0	253.31	90.01
6	124.38	0	139.62	0	255.1	97.72
7	89.94	0	148.77	0	260.39	98.5
8	49.57	0	164.75	0	243.88	106.18
9	12.64	0	177.84	0	223.41	106.92
10	0	8.8	175.97	0	240.09	114.08
11	0	16.6	170.58	0	212.95	115.85
12	0	0	161.42	0	211.07	125.32
13	0	11.27	157.83	0	184.19	127.91
14	0	11.27	151.09	0	192.1	128.3
15	0	11.27	155.12	0	148.09	134.32

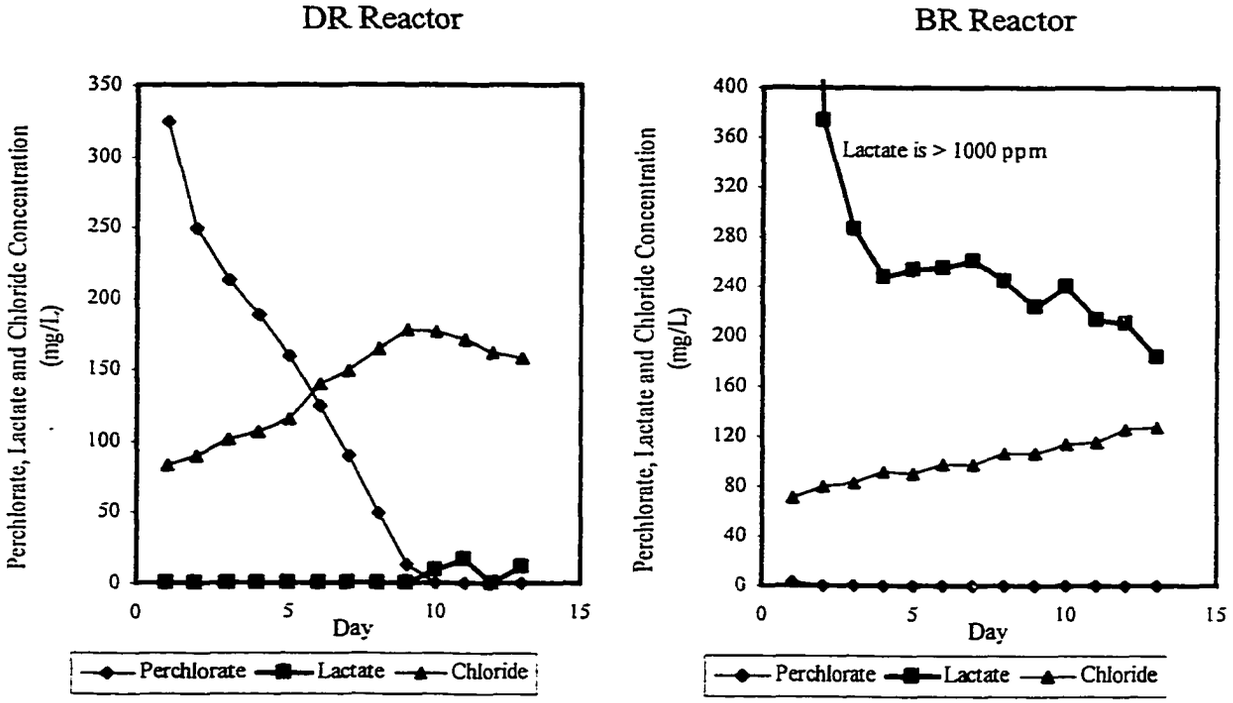


Figure 4.12 Perchlorate, lactate, and chloride concentrations in the DR and BR reactors during biodegradation of perchlorate by a biofilm immobilized on a BTS-55 Membrane (3rd cycle)

Table 4.18

The molar ratio of perchlorate to chloride (3rd cycle)

ClO_4^- / Cl^-	0.86
Moles ClO_4^- biodegraded/m^2-day (from the first to the 9th days)	0.22

Table 4.19

Estimation of thickness of biofilm immobilized on BTS-55 membrane

Wet weight of membrane +biofilm+o-ring, g	12.7258
Wet weight of membrane + o-ring, g	8.2616
Wet weight of microbes, g	4.4642
Dry weight of microbes, g	0.2072
Membrane diameter, cm	10.00
Membrane surface area, cm^2	78.50
Density of microbes, g/cm^3	1.07
Biofilm thickness, μm	24.7

Tables 4.15 and 4.17 show the perchlorate to chloride ratios for the second and third cycle runs. Stoichiometrically, a ratio of 1.0 is expected, since each mole of perchlorate biodegraded generates one mole of chloride. For the second cycle a ratio of 1.03 (Table 4.16) was found and for the third cycle a ratio of 0.86 (Table 4.18) was found. These are very satisfactory ratios, considering the size of the reactor-set (10 liters) used in the study.

Testing of Biofilm Immobilized on the PVDF Membrane

This was the first test performed after the experimental set-up was modified. The amount of lactate added to the BR reactor in this experiment was lower (about 700 mg/L) as compared to the other experiments performed with the BTS-55 and FGLP membranes (about 1100 mg/l lactate).

Table 4.20 and Figure 4.13 show the biodegradation of perchlorate by the biofilm immobilized on the PVDF membrane. Notice that the biodegradation rate (0.06 moles of

perchlorate per day per m^2 of the membrane area) is much smaller than the values found for the BTS-55 membrane (0.11 to 0.25 moles of perchlorate per day per m^2 of the membrane area). The PVDF membrane has the smallest diffusion coefficient from all three membranes tested. Therefore, the lower biodegradation rate is the result of low diffusion of perchlorate through the membrane. Notice in Table 4.20, that perchlorate biodegradation proceeded after the lactate concentration was undetectable. The perchlorate to chloride ratio for this test was found to be 0.99 (Table 4.21). This is a very good mass balance of the moles of chloride generated by the biodegradation of perchlorate.

Table 4.20

Perchlorate biodegradation by a biofilm immobilized on a PVDF membrane

	DR Reactor (ClO_4- only)			BR Reactor (Lactate, Nutrients/Minerals, and Buffer)		
Day	ClO_4^- (mg/L)	Lactate (mg/L)	Cl^- (mg/L)	ClO_4^- (mg/L)	Lactate (mg/L)	Cl^- (mg/L)
1	207.68	0	0	0.23	761.95	5.85
2	188	6.4	6.63	16.57	702.06	6.53
3	143.84	21.58	19.55	13.57	656.7	12.18
4	106.68	0	30.24	0.17	375.2	19.55
5	93.6	0	34.03	0.16	105.53	21.71
6	83.04	0	37.74	0	7.82	25.34
7	73.7	0	36.94	3.9	0	26.04
8	67.88	0	37.35	6.56	0	27.18
9	59.46	0	37.66	7.65	0	29.95
10	54.44	0	39.19	9.19	0	30.15
11	49.62	0	39.51	9.97	0	32.57
12	46.65	0	40.43	11.77	0.27	33.53
13	31.86	0	40.63	13.74	0	34.43
14	40.21	0	40.45	14.37	0	34.78
15	39.14	0	36.85	15.74	0	30.96
16	32.15	0	31.95	16.46	0	28.78
17	31.56	0	32.21	17.55	0	29.33
18	30.04	0	33.73	18.35	0	29.7

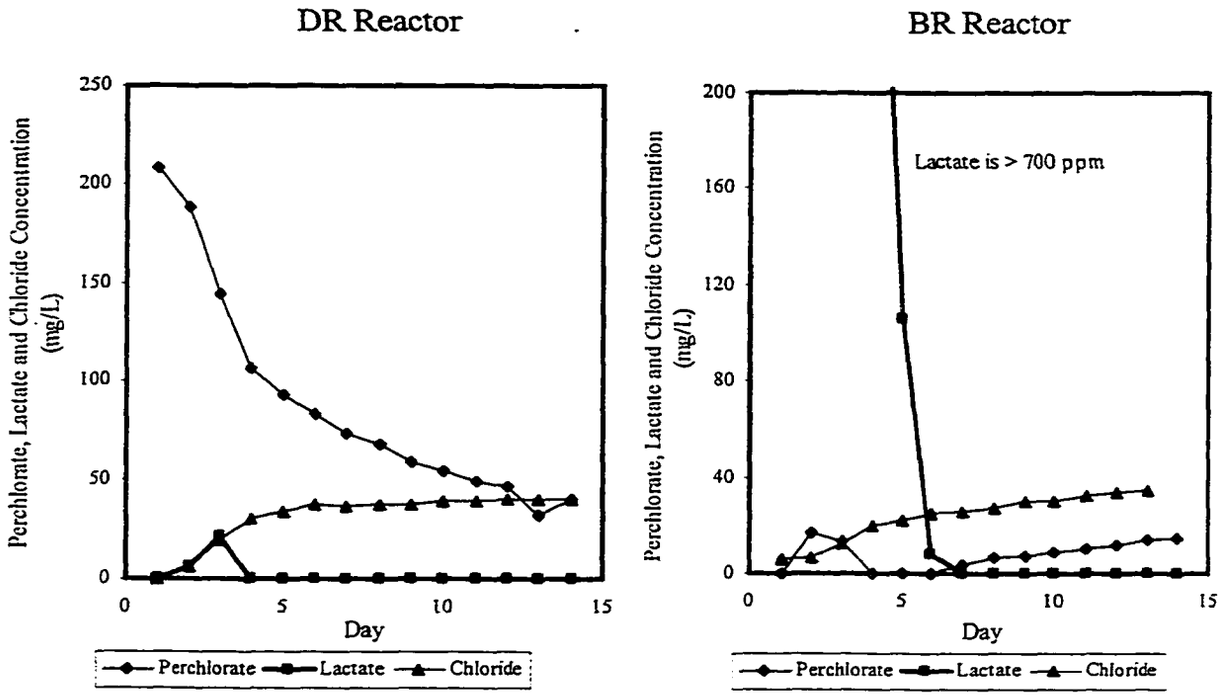


Figure 4.13 Perchlorate, lactate, and chloride concentrations in the DR and BR reactors during biodegradation of perchlorate by a biofilm immobilized on a PVDF Membrane

Table 4.21

The molar ratio of perchlorate to chloride (1st cycle)

ClO_4^- / Cl^-	0.99
Moles ClO_4^- biodegraded/m^2-day	0.06

Table 4.22

Summary of relevant parameters calculated for the biofilms immobilized on the BTS-55 and PVDF Membranes

Membrane	Parameters	1st cycle	2nd cycle	3rd cycle
BTS-55	ClO_4^- / Cl^-	0.82	1.03	0.86
	Moles ClO_4^- biodegraded/m^2-day	0.25	0.11	0.22
PVDF	ClO_4^- / Cl^-	0.99		
	Moles ClO_4^- biodegraded/m^2-day	0.06		

Interesting Observations Regarding the Enrichment Culture

As reported earlier (Table 4.10), the BTS-55 membrane reactor was run for forty days. At the end of the third cycle, it was observed that the reactor's discharge tubing acquired a red coloration. Further observation indicated that the red color was due to the presence of red microbes in the system. A decision was then made to transfer the contents of the reactor to serum bottles and further feed the culture to investigate whether the red microbes would further dominate the environment. After several days, the culture became more enriched with the red microorganisms (Figure 4.14). The culture has been named BALI (for Batista and Liu). Interestingly, a recent article by Bruce et al. (1999) reports of a perchlorate degrading strain, isolated from a paper mill waste, which is pink in color.

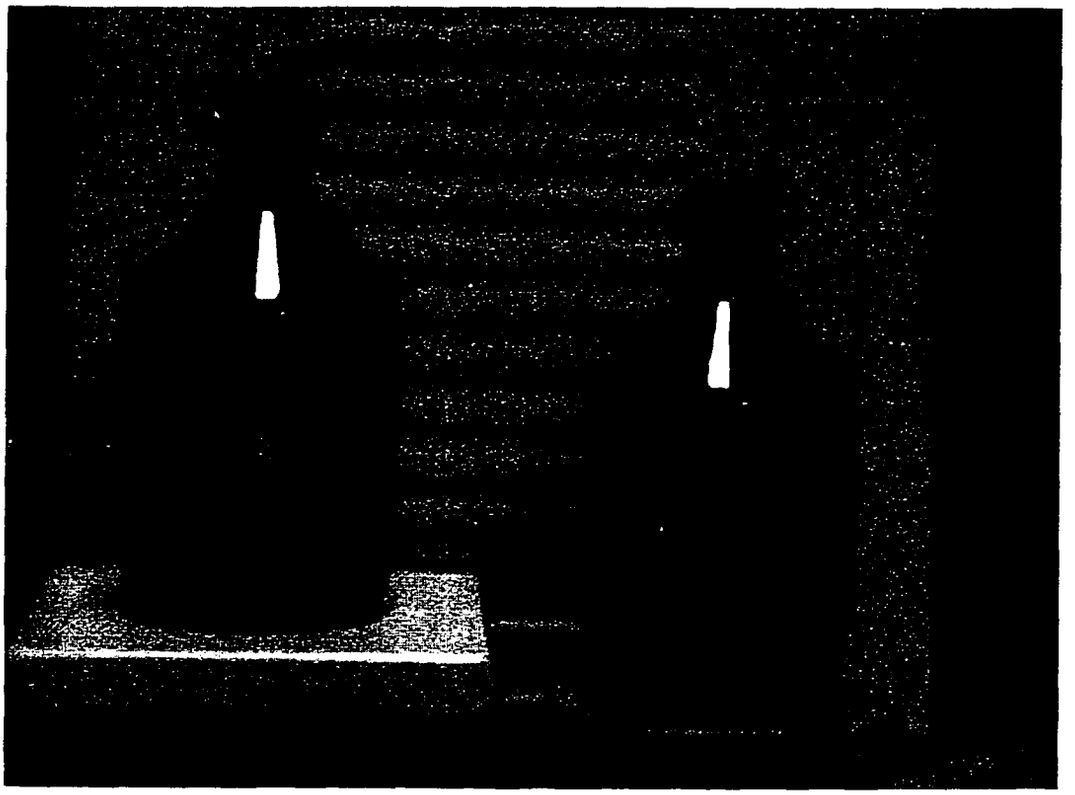


Figure 4.14 Master culture reactors containing the BALI red culture(on the right side) and another perchlorate degrading culture which does not have the red coloration (on the left side)

SRT Studies

Two cycles of SRT studies were performed in the master culture reactor. Shown below are mass balances for perchlorate, lactate, and chloride, and volatile suspended solids (VSS) for the different cycles:

First Cycle

Table 4.23 shows the mass balance of volatile suspended solids in the master reactor for the first cycle. The daily increase in biomass as well as the average biomass present in the reactor has been calculated from daily VSS measurement. The average VSS concentration was used to calculate the grams of perchlorate biodegraded and the

grams of chloride formed by each gram of biomass present in the reactor. The daily biomass increase will be used to estimate the yield coefficient, that is, the grams of biomass formed per gram of lactate biodegraded. Table 4.24 shows the mass balance for perchlorate for the 5-day period. Notice that in the first day only 18.54 mg of perchlorate were degraded. This is the result of the acclimation period needed by the microbes to adjust to the new conditions. In the second day, the culture was already acclimated and perchlorate was biodegraded as indicated by the consumption of lactate (Table 4.25) and the increase in chloride concentration. (Table 4.26). In the fourth and fifth days the amount of perchlorate biodegraded with time increased significantly and perchlorate concentrations went to undetectable level. In the second and third days 113.82 mg and 135.82 mg of perchlorate (Table 4.24) were biodegrade and the mg ClO_4^-/mg VSS was calculated to be 0.38 and 0.43 respectively. For the fourth and fifth days, 54.08 and 55.12 mg of perchlorate were biodegraded and the mg ClO_4^-/mg VSS was calculated to be 0.19 and 0.21 respectively. These results show that each milligram of the enriched culture present was capable of biodegrading an average 0.4 mg of perchlorate per day, when the system was not perchlorate limited. In fourth and fifth days, about 55 mg perchlorate were degraded and the ratio of perchlorate to biomass for both days were practically the same (0.2 mg of perchlorate per day).

Table 4.26 shows the mass balance of chloride in the master reactor and Table 4.27 shows the daily chloride to perchlorate ratios. The data show some of the perchlorate to chloride ratios somewhat above the expected value (one). However, some of them are very close to the theoretical value. The somewhat higher values could be due to analytical accuracy. Although the calibration curves for chloride, and lactate always

meet an $R^2 = 0.995$, the IC sensitivity for very low chloride and lactate levels is low and precision of measurements are within ± 20%. Notice in Table 4.24, that similar to the perchlorate mass balance, the mass of chloride in the second and third days are roughly the double of those in the fourth and fifth days, reinforcing again the relationship between perchlorate biodegradation and chloride formation.

Table 4.25 shows the mass balance for lactate in the reactor. Notice that the ratio of the mass of VSS generated and the mass of lactate consumed varied considerably. Because the system was perchlorate limited in the fourth and fifth days and because the work was performed using a mixed-culture, it is believed that the cell yields do not reflect the exclusive use of perchlorate as an electron acceptor. As it was shown before, there is indication, from the experimental results, that lactate may have been utilized by the mixed-culture through fermentation.

Table 4.23

Mass balance of VSS in the reactor (1st cycle)

day	VSS measured (mg)	VSS left (mg)	Average VSS(mg)	VSS Increase (mg)
0	265			
1	312	249.6	288.5	47
2	352	281.6	300.8	102.4
3	352	281.6	316.8	70.4
4	284	227.2	282.8	2.4
5	292	233.6	259.6	64.8

Note:

VSS measured = the VSS mass present in the 1L bottle each day.

VSS left = mass of biomass present in the bottle after daily withdrawal of 200 ml from the master reactor

Average VSS = the average microbial mass present in the reactor.

VSS Increase = the daily increase in biomass in the master reactor.

Table 4.24

Mass balance for perchlorate (1^{st} cycle)

Initial (mg)	Final (mg)	Biodeg. *(mg)*	Out (mg)	In (mg)	Th (mg)	MS (mg)	VSS Avg.(mg)	(mg) ClO_4^-/ (mg) VSS
233.12	214.58	*18.54*	42.916	40	211.66	210.84	288.5	0.06
210.84	97.02	*113.82*	19.404	40	117.62	135.12	300.8	0.38
135.12	0	*135.12*	0	40	40.00	54.08	316.8	0.43
54.08	0	*54.08*	0	40	40.00	55.12	282.8	0.19
55.12	0	*55.12*	0	40	40.00	51.88	259.6	0.21

Note:

The initial suspended solids concentration was 265 mg/l

Initial = Initial mass of perchlorate in the reactor

Final = Final mass of perchlorate in the reactor after 1 day

Biodeg. = Mass of perchlorate biodegraded after 1 day

Out = Mass of perchlorate taken out in the 200 ml volume

In = Theoretical increase in perchlorate mass when 200 ml of fresh solution was added

Th = Theoretical total mass of perchlorate in the reactor

MS = measured perchlorate mass in the reactor

VSS = Average biomass in the reactor

$mgClO_4^-/mgVSS$ = mg of perchlorate is biodegraded per mg of biomass present in the reactor

Table 4.25

Mass Balance Lactate (1^{st} cycle)

Initial (mg)	Final (mg)	Biodeg. *mg*	Out (mg)	In (mg)	Th (mg)	MS (mg)	VSS Increase(mg)	(mg) VSS/ (mg) Lactate
1763.52	1621.12	*142.4*	324.224	200	1496.90	1543.01	47	0.33
1543.01	1230.64	*312.37*	246.128	200	1184.51	1256.48	102.4	0.33
1256.48	764.7	*491.78*	152.94	200	811.76	789.09	70.4	0.14
789.09	656	*133.09*	131.2	200	724.80	692.83	2.4	0.02
692.83	482	*210.83*	96.4	200	585.60	573.82	64.8	0.31

Note:

Initial = Initial mass of lactate in the reactor

Final = Final mass of lactate in the reactor after 1 day

Biodeg. = Mass of lactate biodegraded after 1 day

Out = Mass of lactate taken out in the 200 ml volume

In = Theoretical increase in perchlorate mass when 200 ml of fresh solution is added

Th = Theoretical total mass of perchlorate in the reactor

MS = measured lactate mass in the reactor

VSS Increase = The increase of biomass in the reactor after one day

mgVSS/mg Lac. = mg of biomass generated per mg of lactate consumed

Table 4.26

Mass balance for chloride (1st cycle)

Initial (mg)	Final (mg)	*Produced (mg)*	Out (mg)	In (mg)	Th (mg)	MS (mg)	VSS Avg.(mg)	(mg) Cl^-/ (mg) VSS/
204.43	186.32	*-18.11*	37.26	0	149.06	155.97	288.5	-0.06
155.97	194.73	*38.76*	38.95	0	155.78	158.71	300.8	0.13
158.71	203.04	*44.33*	40.61	0	162.43	161.66	316.8	0.14
161.66	176.55	*14.89*	35.31	0	141.24	135.68	282.8	0.05
135.68	151.09	*15.41*	30.22	0	120.87	121.12	259.6	0.06

Note:

Initial = Initial mass of chloride in the reactor

Final = Final mass of chloride in the reactor after 1 day

Produced = Mass of chloride produced after 1 day

Out = Mass of chloride taken out in the 200 ml volume

In = Theoretical increase in chloride mass when 200 ml of fresh solution is added

Th = Theoretical total mass of chloride in the reactor

MS = measured chloride mass in the reactor

Avg. VSS.= The average biomass present in the reactor

mgCl^-/mgVSS = mg of chloride generated per mg of VSS present in the reactor

Table 4.27

The daily molar ratio of perchlorate to chloride (1st cycle)

Day	1	2	3	4	5
ClO_4^- / Cl^-	-0.37	1.05	1.09	1.30	1.28

Second Cycle

Table 4.28

Mass balance of VSS in the reactor (2^{nd} cycle)

day	VSS measure (mg)	VSS left	average VSS	VSS increase
0	155	124		
1	232	185.6	193.5	77
2	236	188.8	210.8	50.4
3	224	179.2	206.4	35.2
4	244	195.2	211.6	64.8
5	208	166.4	201.6	12.8

Table 4.29

Mass balance for perchlorate (2^{nd} cycle)

Initial (mg)	Final (mg)	Biodeg. (mg)	Out (mg)	In (mg)	Th (mg)	MS (mg)	VSS Avg.(mg)	(mg) ClO_4^- / (mg) VSS
214.72	0	214.72	0	40	40.00	36.66	193.5	1.11
36.66	0	36.66	0	40	40.00	43.59	210.8	0.17
43.59	0	43.59	0	40	40.00	43.76	206.4	0.21
43.76	0	43.76	0	40	40.00	46.29	211.6	0.21
46.29	0	46.29	0	40	40.00	46.59	201.6	0.23

Table 4.30

Mass balance for lactate (2^{nd} cycle)

Initial (mg)	Final (mg)	Biodeg. *(mg)*	Out (mg)	In (mg)	Th (mg)	MS (mg)	VSS Increase(mg)	(mg) VSS/ (mg) Lac.
1051.4	289.42	*761.98*	57.884	200	431.54	409.16	77	0.10
409.16	111.08	*298.08*	22.216	200	288.86	214.56	50.4	0.17
214.56	63.53	*151.03*	12.706	200	250.82	221.07	35.2	0.23
221.07	26.46	*194.61*	5.292	200	221.17	198.12	64.8	0.33
198.12	0	*198.12*	0	200	200.00	221.77	12.8	0.07

Table 4.31

Mass balance for chloride (2^{nd} cycle)

Initial (mg)	Final (mg)	*produced (mg)*	Out (mg)	In (mg)	Th (mg)	MS (mg)	VSS Avg.(mg)	(mg) Cl^-/ (mg) VSS/
45.22	115.87	*70.65*	23.17	0	92.70	91.53	193.5	0.37
91.53	108.32	*16.79*	21.66	0	86.66	80.14	210.8	0.08
80.14	97.96	*17.82*	19.59	0	78.37	78.82	206.4	0.09
78.82	92.38	*13.56*	18.48	0	73.90	72.46	211.6	0.06
72.46	90.2	*17.74*	18.04	0	72.16	69.43	201.6	0.09

Table 4.32

The daily molar ratio of perchlorate to chloride (2^{nd} cycle)

Day	1	2	3	4	5
ClO_4^- / Cl^-	1.08	0.78	0.87	1.15	0.93

Table 4.29 shows the mass balance of perchlorate in the master bottle in the second cycle. Notice that the ratio of perchlorate biodegraded to biomass present, in the second, third, fourth and fifth days are similar and in agreement with the results found in the first cycle (Table 4.24). The perchlorate to chloride ratios for the second cycle varied from 0.87 to 1.15. Again, these ratios are very reasonable considering the sensitivity of the analytical method.

In the first day, the ratio of perchlorate biodegraded to biomass present was very high (1.11 mg ClO_4^-/mg VSS) (Table 4.29) and the chloride formation, for that day, was correspondingly high (Table 4.31). The results show that a larger amount of perchlorate than that found in the first cycle, can be biodegraded by the culture.

Similar to the observations for cycle one, the lactate concentration varied considerably (Table 4.30) and they do not seem to correlate to perchlorate biodegradation.

Preliminary Kinetics Study

The results of the kinetic studies clearly show that the "BALI" culture requires a minimum organic carbon to perchlorate ratio for perchlorate biodegradation to occur at acceptable rates. Notice in Figure 4.15 that under lactate limited conditions, no microbial growth was observed, except in bottles KL5 and KL5d in which the lactate to perchlorate ratio was equal to three. For all perchlorate limited bottles, microbial growth (easily identified by the red coloration of the "BALI" culture in Figure 4.15) was observed. The microbial growth was coupled with a decrease in perchlorate and lactate concentrations and an increase in chloride concentration in the bottles.

Figure 4.15 Kinetic studies with the "BALI" culture showing microbial growth in perchlorate limited bottles (left) and no growth in lactate limited bottles (right)

The results indicate that a lactate to perchlorate ratio of at least three is needed for perchlorate biodegradation to occur at acceptable rates. The results of these tests were used to determine the minimum amount of carbon source (lactate) to be added to the BR reactor of the membrane-immobilized biofilm reactor. This is critical, since excess carbon in the effluent of perchlorate-removing reactors may present a challenge to subsequent unit processes (e.g. disinfection).

Figure 4.16 and Table 4.33 show the biodegradation of perchlorate in the perchlorate limited bottles. Notice that a larger acclimation time (3 days) was needed for the bottles containing the highest perchlorate concentrations (100 and 200 mg/L). For the bottles containing lower perchlorate concentrations (10-60 mg/l) only about 2 days were required for complete biodegradation. After acclimation, perchlorate removal from the

lower perchlorate concentration bottles was 100% (KC1, and KC2), 72.4% (KC3), and for the higher perchlorate concentration bottles, it was 52% (KC4), and 28.2 % (KC5). Example calculations for the perchlorate removal after acclimation for bottles KC3 to KC5 are shown in Appendix B (Example 2).

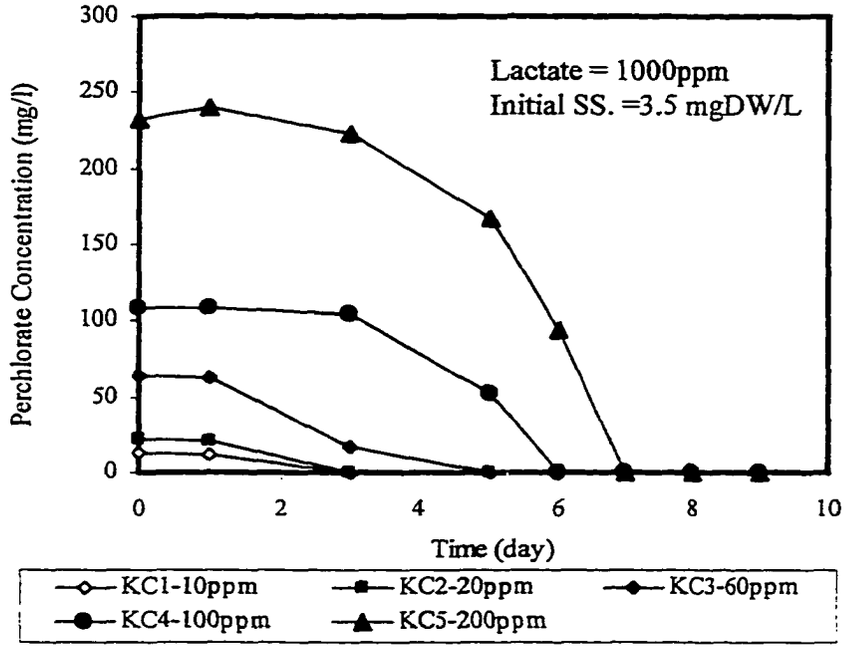


Figure 4.16 Perchlorate biodegradation in perchlorate limited bottles

Table 4.33

Perchlorate biodegradation in perchlorate limited bottles

Time (day)	KC1 (10ppm)	KC2 (20ppm)	KC3 (60ppm)	KC4 (100ppm)	KC5 (200ppm)
	ClO_4^-	ClO_4^-	ClO_4^-	ClO_4^-	ClO_4^-
0	13.09	21.9	63.65	108.72	232.2
1	11.42	20.65	63.09	108.14	239.78
3	0	0	17.54	104.14	222.85
5	0	0	0	52.17	166.68
6	0	0	0	0	93.34
7	0	0	0	0	0

Figure 4.17 to Figure 4.21 and Table 4.34 to Table 4.38 show the concentration of perchlorate, lactate, and chloride in the perchlorate limited bottles. Notice that in all bottles, the perchlorate and lactate concentrations decreased with time while the chloride concentration increased. Notice that the suspended solids (SS) concentrations are not shown. The reason is the 5-ml samples taken daily did not contained sufficient microorganisms to be accurately weighed in a four digit accuracy balance (0.0000 g).

Table 4.34

Perchlorate, lactate and chloride concentration of preliminary kinetics study (KCl)

Time (day)	Perchlorate (mg/L)	Lactate (mg/L)	Chloride (mg/L)
0	13.09	897.07	0.38
1	11.42	816.62	0.63
3	0	95.57	3.74
5	0	104.39	3.48
6	0	95	5.43
7	0	81.78	5.64
8	0	87.55	5.53
9	0	87.03	6.64

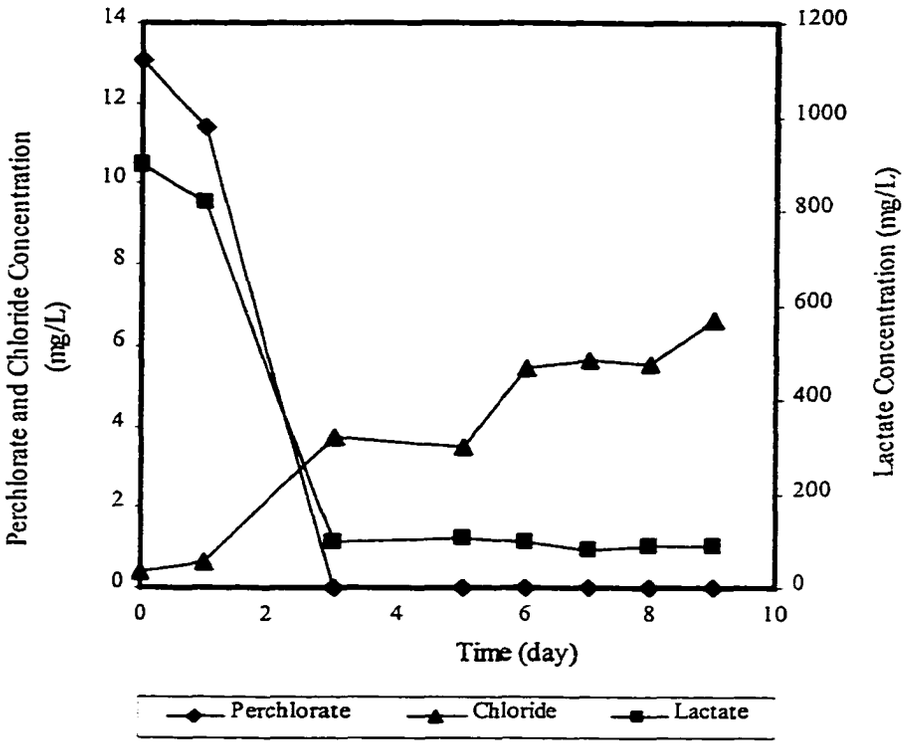


Figure 4.17 Microbial reduction of perchlorate to chloride when lactate/ClO_4^-=100

Table 4.35

Perchlorate, lactate and chloride concentration in preliminary kinetics study (KC2)

Time (day)	Perchlorate (mg/L)	Lactate (mg/L)	Chloride (mg/L)
0	21.9	870.18	0.39
1	20.65	N/A	0.2
3	0	93.43	6.7
5	0	77.22	6.46
6	0	72.7	8.01
7	0	67.48	7.26
8	0	41.64	8.11
9	0	43.89	8.62

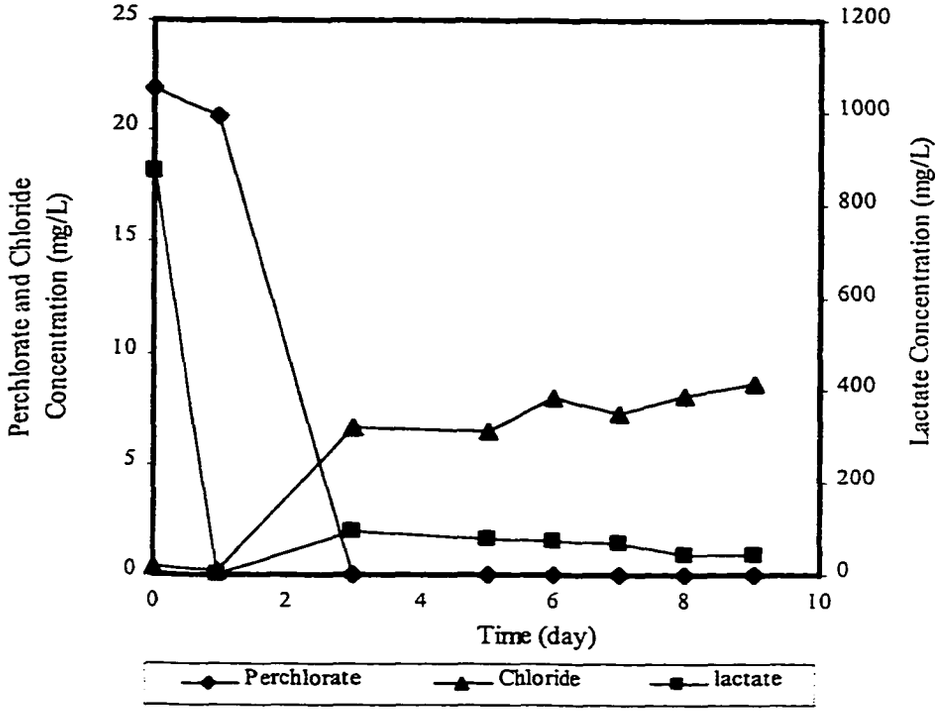


Figure 4.18 Microbial reduction of perchlorate to chloride when lactate/ClO_4^-=50

Table 4.36

Perchlorate, lactate and chloride concentration in preliminary kinetics study (KC3)

Time (day)	Perchlorate (mg/L)	Lactate (mg/L)	Chloride (mg/L)
0	63.65	972.58	0.35
1	63.09	784.61	0.45
3	17.54	103.38	11.46
5	0	110.83	17.11
6	0	105.94	19.01
7	0	95.23	17.52
8	0	N/A	17.62
9	0	74.59	15.96

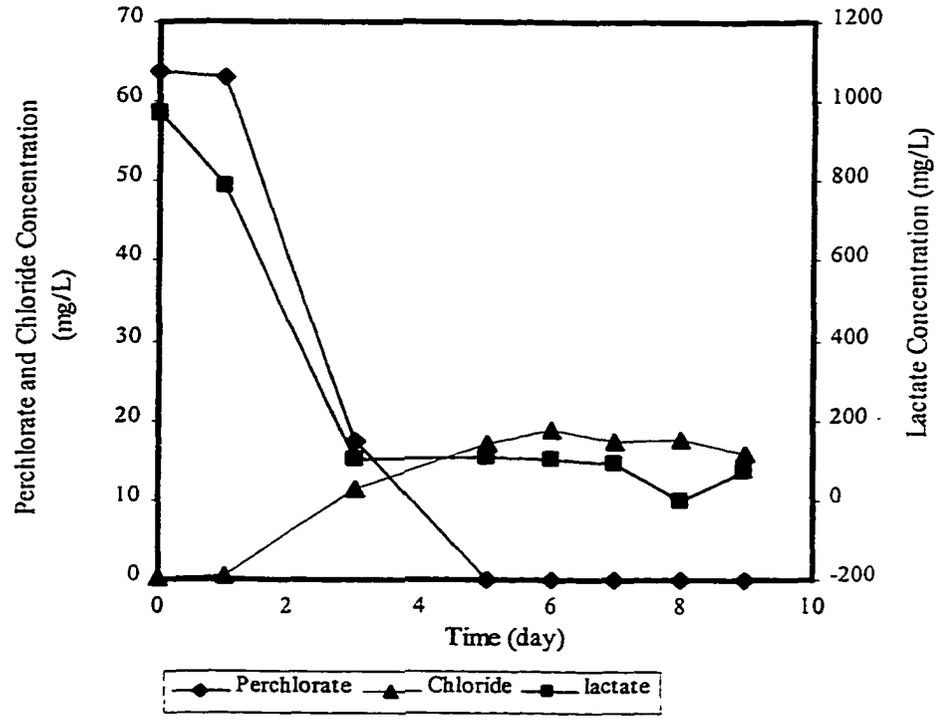


Figure 4.19 Microbial reduction of perchlorate to chloride when lactate/ClO_4^-=16.7

Table 4.37

Perchlorate, lactate and chloride concentration in preliminary kinetics study (KC4)

Time (day)	Perchlorate (mg/L)	Lactate (mg/L)	Chloride (mg/L)
0	108.72	1000	0
1	108.14	N/A	0
3	104.14	690.11	3.1
5	52.17	N/A	20.77
6	0	91.02	36.45
7	0	N/A	34.73
8	0	71.58	25.29
9	0	73.28	33.42

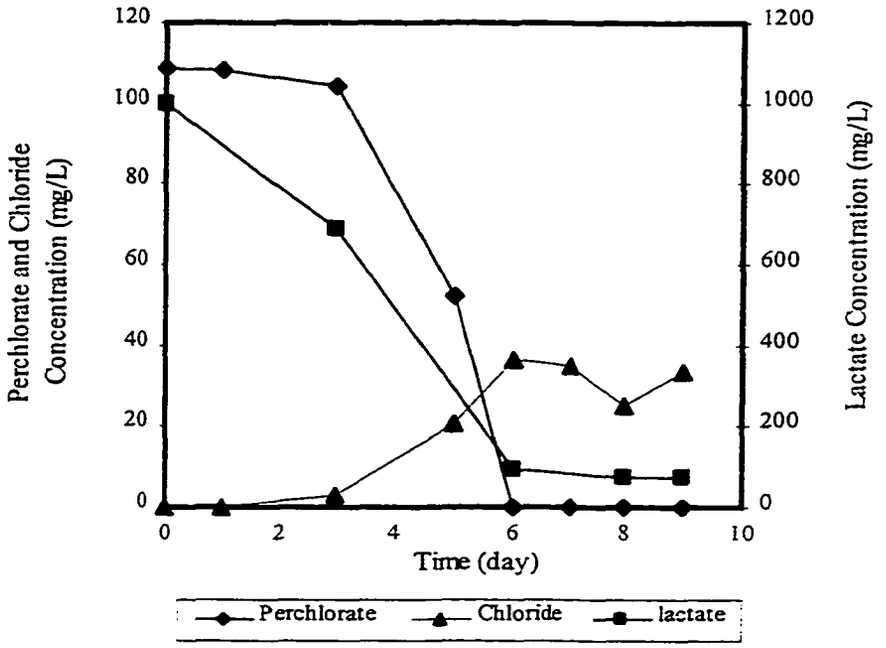


Figure 4.20 Microbial reduction of perchlorate to chloride when lactate/ClO_4^-=10

Table 4.38

Perchlorate, lactate and chloride concentration in preliminary kinetics study (KC5)

Time (day)	Perchlorate (mg/L)	Lactate (mg/L)	Chloride (mg/L)
0	232.2	711.29	0.33
1	239.78	N/A	0
3	222.85	664.01	1.24
5	166.68	94.91	15.93
6	93.34	90.56	38.05
7	0	N/A	72.47
8	0	N/A	50.48
9	0	65.88	71.22

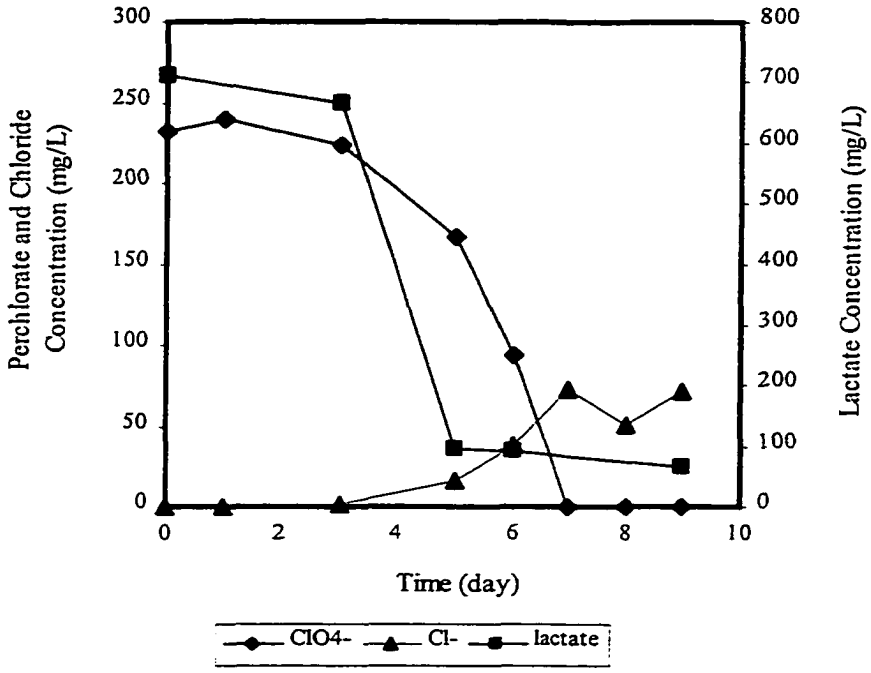


Figure 4.21 Microbial reduction of perchlorate to chloride when lactate/ClO_4^-=5

Figure 4.22 and Table 4.39 show the perchlorate biodegradation for the only bottle of the lactate-limited group in which biodegradation occurred. Notice that lactate and perchlorate concentration decreased with time while the chloride concentration increased. For all the other bottles in which the lactate to perchlorate ratio was smaller than 3.0, biodegradation was not observed.

Table 4.40 shows the perchlorate biodegradation for the lactate-limited group in which perchlorate biodegradation did not occur.

Table 4.39

Perchlorate, lactate and chloride concentration in preliminary kinetics study (KL5)

Time (day)	Perchlorate (mg/L)	Lactate (mg/L)	Chloride (mg/L)
0	103.34	314.51	0.054
1	102.72	0	0.39
3	81.52	0	9.78
5	28.73	37.29	23.3
7	0	0	36.13
9	0	0	32.9
17	0	0	38.11

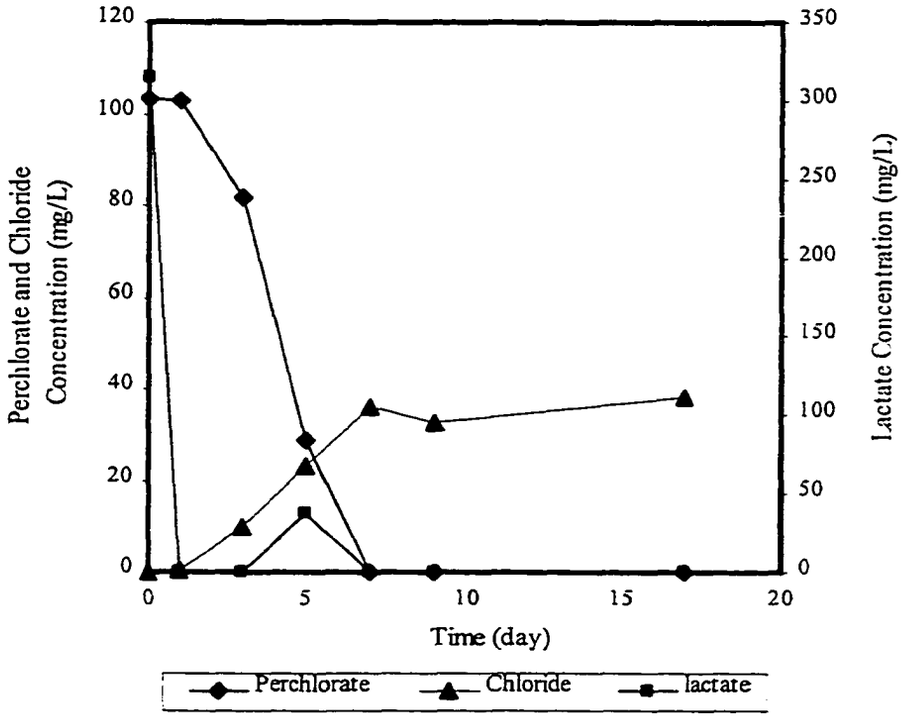


Figure 4.22 Microbial reduction of perchlorate to chloride when lactate/ClO_4 =3

Table 4.40

Perchlorate concentration of preliminary kinetics study

in lactate-Limited bottles (KL1-KL4)

Time	Perchlorate Concentration (mg/L)			
(day)	KL1	KL2	KL3	KL4
0	89.31	84.50	87.50	84.51
9	87.80	87.60	86.44	83.76
12	90.76	85.56	86.62	85.45
17	88.4	85.20	84.38	85.00

Influence of Nitrate on Perchlorate Biodegradation by the Membrane-Immobilized Biofilm

Batch Testing

Figures 4.23 and Table 4.41 show the results for the batch testing on the interference of nitrate on perchlorate biodegradation. The lower three dashed lines show the decrease in the concentration of nitrate with time in the presence of approximately 100 mg/L perchlorate. The top four solid lines show the decrease in perchlorate concentration with time in the presence of about 10 mg/L, 30mg/L and 60 mg/L of nitrate. As shown in Figure 4.23, the rate of nitrate biodegradation is much greater than that of perchlorate. In addition, significant perchlorate biodegradation only started after the nitrate had been almost completely biodegraded. These results show that the enrichment mixed culture ("BALI") prefers nitrate to perchlorate as an electron acceptor. Thus, nitrate negatively affects perchlorate biodegradation.

Table 4.41

The interference of nitrate on perchlorate biodegradation (batch testing)

Time	0 NIT	10 NIT		30 NIT		60 NIT	
(hr)	ClO_4^- (ppm)	ClO_4^- (ppm)	NO_3^- (ppm)	ClO_4^- (ppm)	NO_3^- (ppm)	ClO_4^- (ppm)	NO_3^-(ppm)
0	108.36	100.23	10.2	103	31.95	105.34	64.9
2.5	104.7	99.12	9.82	101.42	32.06	101.72	68.16
22	96.02	92.07	0.61	95.61	0.61	99	25.23
34	0	0	0	0	0	0	0

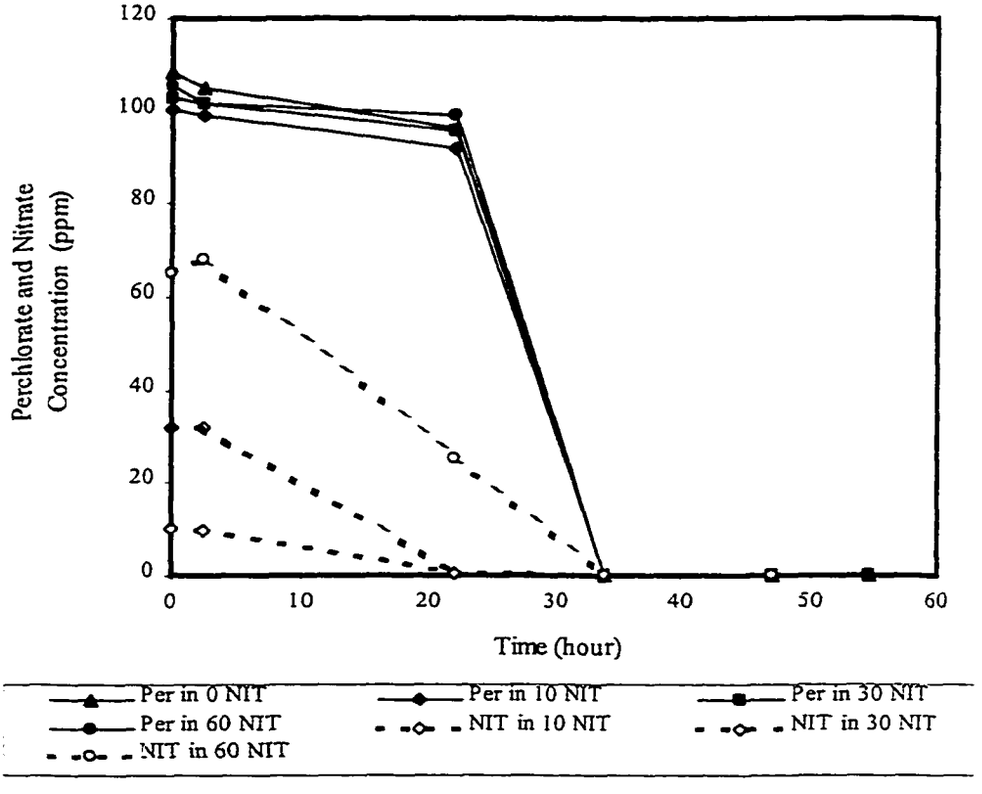


Figure 4.23 Batch testing of nitrate interference on perchlorate biodegradation using "BALI" culture.

Membrane-Immobilized Biofilm Reactor Testing

Figures 4.24, 4.25 and Table 4.42 show the data for the biodegradation of high levels of perchlorate (about 50 mg/L) in the presence of approximately the same concentration of nitrate (50 mg/L). Both the diffusion reactor (to which perchlorate and nitrate were added) and the biological reactor (to which lactate, nutrients and buffer were added) were sampled with time and samples were analyzed for perchlorate and nitrate. Figure 4.25 shows the perchlorate and nitrate biodegradation rate expressed as percentage of nitrate or percentage of perchlorate biodegraded per hour. In the first four hours of the experiment, about the same mass of perchlorate and nitrate were biodegraded. From 4 to about 30 hours, nitrate biodegradation dominated the system and perchlorate biodegradation was hindered. After most of the nitrate had been biodegraded, perchlorate biodegradation resumed. The fast initial perchlorate biodegradation in the first hours of the experiment can probably be attributed to the faster perchlorate diffusion through the membrane. In the diffusion testing, it was reported that diffusion through the permeable membrane used decreases in the order perchlorate>nitrate>sulfate. Thus, with time, when nitrate and perchlorate concentration on the biofilm were the same, nitrate dominated the system. It may also be that the "BALI" culture need some time to acclimate to the nitrate environment. No nitrate was present in the nutrient/mineral solution used to grow the "BALI" culture. Thus, this culture quickly adapts to the nitrate-containing environment. One can say that the bacteria contained in the "BALI" culture are capable of reducing both nitrate and perchlorate.

In Figure 4.24 the concentrations of nitrate and perchlorate in the BR reactor are shown. After about 60 hours, the perchlorate and nitrate diffusing to the BR reactor were

not biodegraded due to carbon limitation. This figure also shows a higher concentration of perchlorate as compared to that of nitrate in the BR reactor, again confirming that the biofilm prefers nitrate to perchlorate as an electron acceptor.

Table 4.42

The interference of nitrate on perchlorate biodegradation (test 1)

	DR Reactor (ClO_4^- and NO_3^-)				BR Reactor (Lactate, Nutrient/Minerals, and Buffer)	
Time (hour)	ClO_4^- (mg/L)	NO_3^- (mg/L)	mg ClO_4^- Biodegraded/hr	mg NO_3^- Biodegraded/hr	ClO_4^- (mg/L)	NO_3^- (mg/L)
[redacted]			0.00	0.00	0.00	0.00
4	46.70	55.50	4.94	3.75	0.00	0.83
17	42.39	27.34	1.66	10.83	N/A	N/A
20	42.30	21.03	0.15	10.52	N/A	N/A
24	39.96	16.90	2.93	5.16	N/A	N/A
26	38.32	14.58	4.10	5.80	N/A	N/A
29	35.19	12.51	5.22	3.45	N/A	N/A
31	34.26	11.50	2.33	2.53	N/A	N/A
40.5	28.70	9.31	2.93	1.15	N/A	N/A
[redacted]			1.69	0.40	N/A	N/A
54	24.33	7.90	1.53	0.68	0.00	0.00
66	21.26	6.67	1.28	0.51	2.03	0.65
73	19.93	6.00	0.95	0.48	N/A	0.73
78.5	19.17	5.72	0.69	0.25	3.59	1.06
89.5	18.37	4.99	0.36	0.33	5.20	1.39
97	17.34	4.29	0.69	0.47	N/A	1.34
102.25	15.57	3.54	1.69	0.71	6.45	1.19
113.5	13.24	2.88	1.04	0.29	7.15	0.78
126	N/A	2.14	N/A	0.30	N/A	0.83

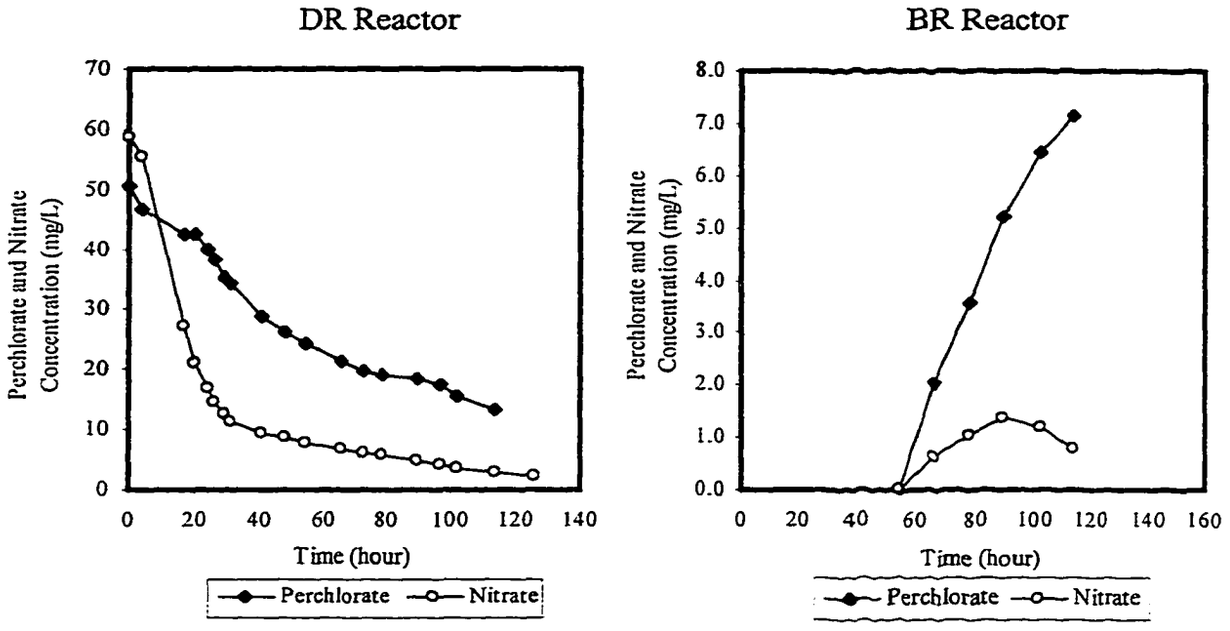


Figure 4.24 The interference of nitrate on perchlorate biodegradation by BTS-55 membrane-immobilized biofilm (test 1)

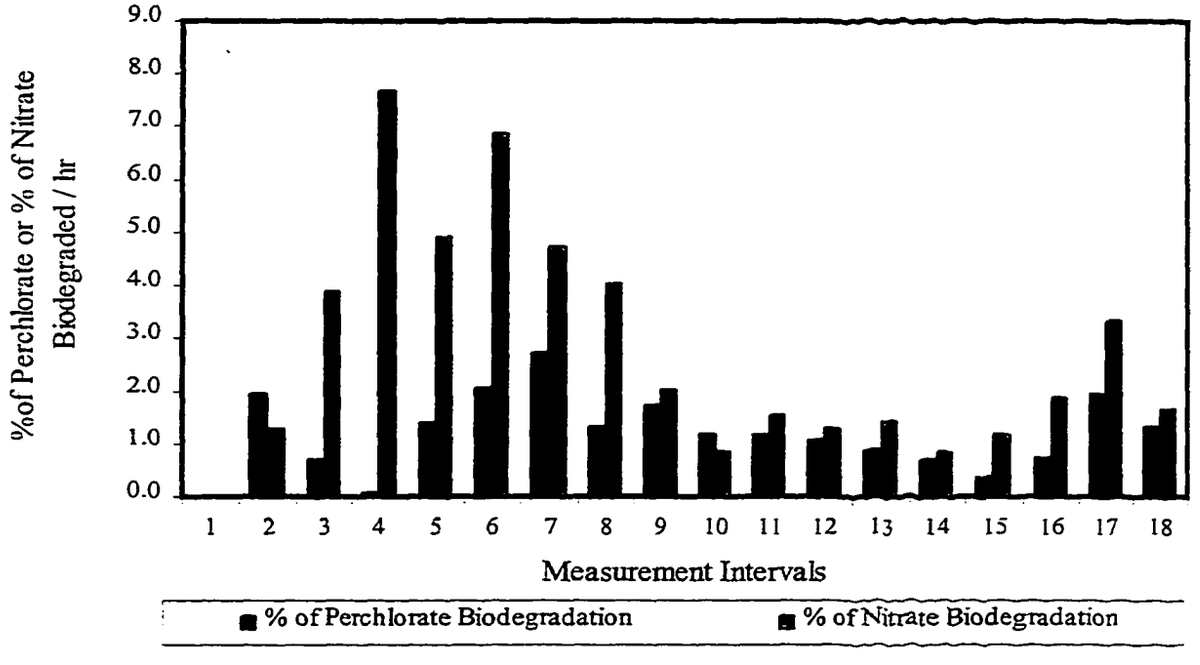


Figure 4.25 Nitrate and perchlorate biodegradation rate (test 1)

Figure 4.26, 4.27 and Table 4.43 present the results of the biodegradation of about 50mg/L of perchlorate in the presence of 10mg/L nitrate by a BTS-55 membrane-immobilized biofilm reactor. This testing was performed to investigate the interference of a lower concentration of nitrate on perchlorate biodegradation. In this test, the initial concentration of perchlorate was kept the same as the test described above. Since the initial concentration of perchlorate was higher than that of nitrate and for comparison purpose, the biodegradation rate of both anions was expressed as the percentage of perchlorate or nitrate biodegradation per hour. Similar to that observed at higher concentration of nitrate (50 mg/L), in the first 4 hours of the experiment, about the same amount of perchlorate and nitrate were biodegraded. After all nitrate had been biodegraded, perchlorate biodegradation resumed until no more carbon was available.

Table 4.43

The interference of nitrate on perchlorate biodegradation (test 2)

	DR Reactor (ClO_4^- and NO_3^-)				BR Reactor (Lactate, Nutrient/Minerals, and Buffer)	
Time (hour)	ClO_4^- (mg/L)	NO_3^- (mg/L)	% of ClO_4^- Biodegraded /hr	% of NO_3^- Biodegraded /hr	ClO_4^- (mg/L)	NO_3^- (mg/L)
			0.00	0.00	0.00	0.00
			1.21	1.40	0.00	0.00
17	43.45	0.00	0.88	7.69	0.00	0.00
20	40.29	0.00	2.42	0	0.00	0.00
24	39.00	0.00	0.80		0.00	0.00
26	38.02	0.00	1.26		0.00	0.00
29	35.35	0.00	2.34		0.00	0.00
31	34.08	0.00	1.80		0.00	0.00
40.5	26.51	0.00	2.34		0.00	0.00
48	21.68	0.00	2.43		0.00	0.00
54	18.45	0.00	2.48		0.00	0.00
66	14.73	0.00	1.68		0.00	0.00
73	13.40	0.00	1.29		0.00	0.00
78.5	12.50	0.00	1.22		0.45	0.00
89.5	11.24	0.00	0.92		1.63	0.00
97	10.38	0.00	1.02		2.03	0.00
102.25	9.19	0.00	2.18		2.46	0.00
113.5	7.62	0.00	1.52		2.67	0.00

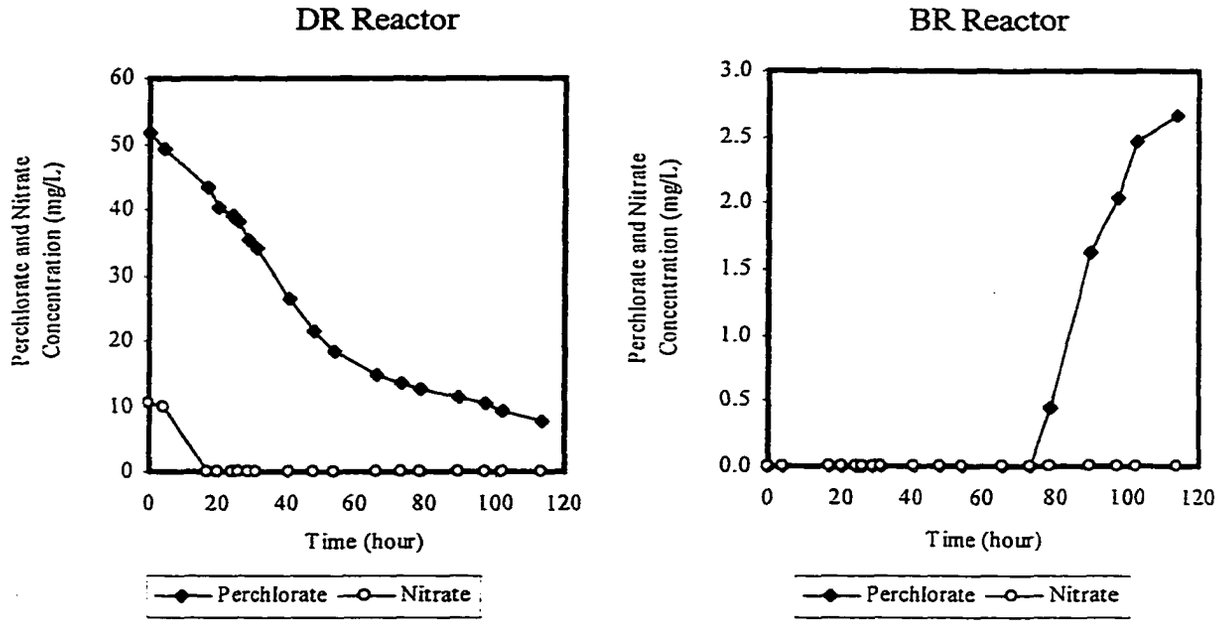


Figure 4.26 The interference of nitrate on perchlorate biodegradation by a BTS-55 membrane-immobilized biofilm (test 2)

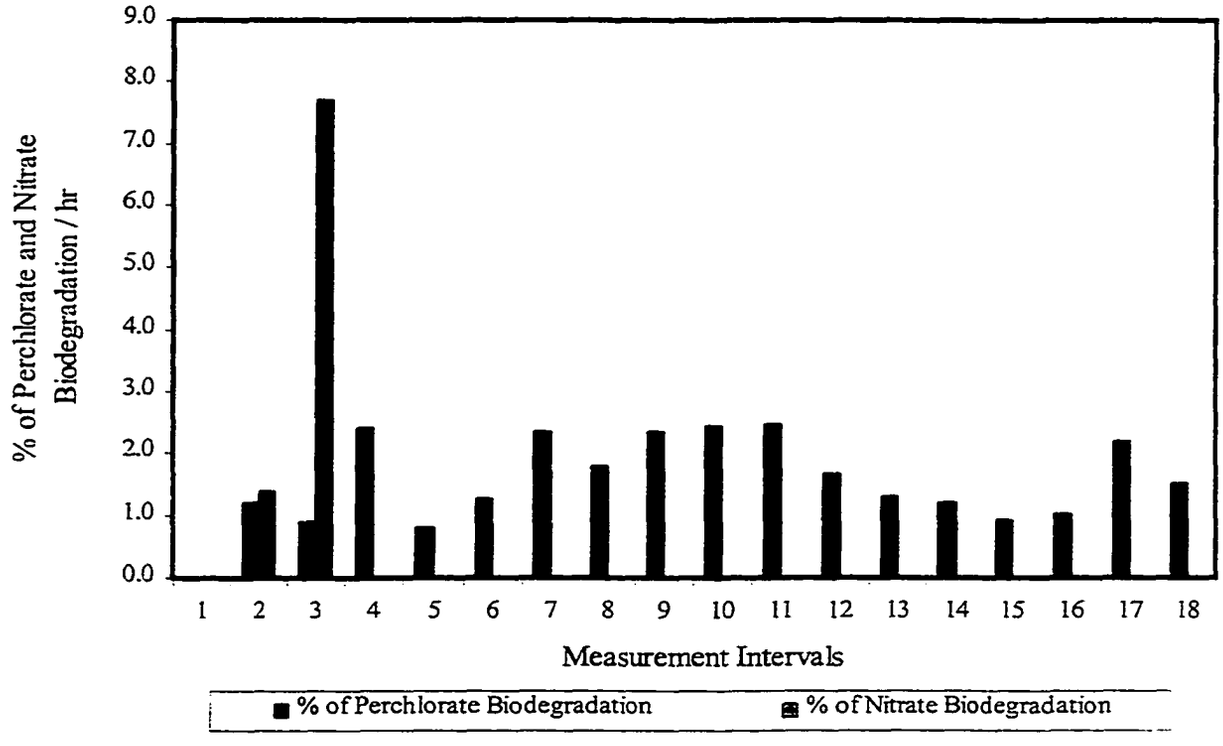


Figure 4.27 Nitrate and perchlorate biodegradation rate (test 2)

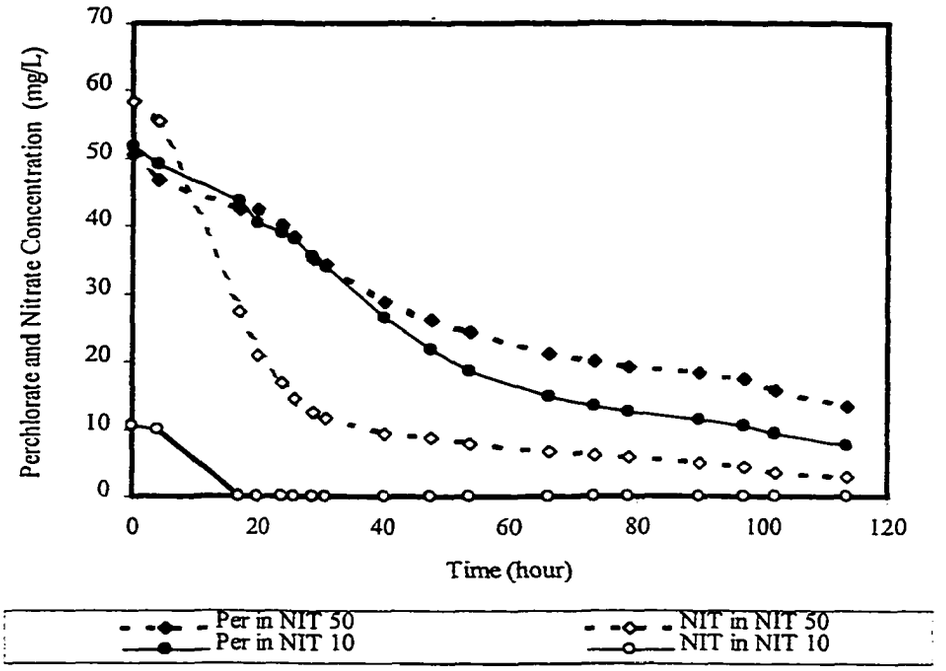


Figure 4.28 Interference of various concentration of nitrate on perchlorate biodegradation in BTS-55 membrane-immobilized biofilm reactors (test 1 & test 2)

Figure 4.28 summarizes the interference of nitrate on the biodegradation of high levels of perchlorate in two sets of experimental data. The dashed lines show the set in which the concentration of perchlorate and nitrate were approximately the same. The solid lines show the set in which the nitrate concentration was five times lower that that of perchlorate. Figure 4.28 shows very clearly that the nitrate biodegradation rates are much faster than that of perchlorate under both conditions. It also shows that at lower nitrate concentration and for the same concentration of lactate (carbon source), perchlorate biodegradation occurred to a greater extent.

Figure 4.29-4.33 and Table 4.44 and Table 4.45 show the results of interference of high nitrate concentration (about 54 mg/L and 11 mg/L respectively) on the biodegradation of low concentration perchlorate (about 1 mg/L). Notice that presence of

nitrate concentration ranging from 10 to 50 mg/L significantly affected perchlorate biodegradation even when the concentration of perchlorate is very low (1 mg/L).

Table 4.44

The interference of nitrate on perchlorate biodegradation (test 3)

	DR Reactor (ClO_4^- and NO_3^-)				BR Reactor (Lactate, Nutrient/Minerals, and Buffer)		
Time (hour)	ClO_4^- (mg/L)	NO_3^- (mg/L)	% of ClO_4^- Biodegraded/hr	% of NO_3^- Biodegraded/hr	ClO_4^- (mg/L)	NO_3^- (mg/L)	Lactate (mg/L)
0	1.03	54.00	0.00	0.00	0.00	0.00	4.99
2	0.99	52.92	1.94	1.00	0.00	0.00	
4	1.00	51.03	-0.51	1.79	0.00	0.00	
6	0.98	50.50	1.00	0.52	0.00	0.00	
8	0.94	49.88	2.04	0.61	0.00	0.00	
9.5	0.92	47.07	1.42	3.76	0.00	0.00	
22.5	0.88	43.18	0.33	0.64	0.00	0.00	
[redacted]			1.82	-0.18	0.00	0.00	[redacted]
27	0.79	40.20	2.98	3.65	0.00	0.00	
29	0.80	39.92	-0.63	0.35	0.00	0.00	
31	0.76	37.90	2.50	2.53	0.00	0.00	
[redacted]			1.97	3.65	0.00	0.00	[redacted]
46.5	0.35	19.21	3.86	3.36	0.00	0.00	
49	0.35	17.46	0.00	3.64	0.00	0.00	
51	0.35	14.18	0.00	9.39	0.00	0.00	
53	0.31	13.24	5.71	3.31	0.00	0.00	
55	0.31	11.44	0.00	6.80	0.00	0.00	
56.5	0.25	10.59	12.90	4.95	0.00	0.00	101.89
70.5	0.12	3.81	3.71	4.57	0.00	0.00	53.69

Table 4.44 (continued)

	DR Reactor (ClO_4^- and NO_3^-)				BR Reactor (Lactate, Nutrient/Minerals, and Buffer)		
Time (hour)	ClO_4^- (mg/L)	NO_3^- (mg/L)	% of ClO_4^- Biodegraded/hr	% of NO_3^- Biodegraded/hr	ClO_4^- (mg/L)	NO_3^- (mg/L)	Lactate (mg/L)
72	0.1	3.56	11.11	4.37	0.00	0.00	
74	0.093	3.04	3.50	7.30	0.00	0.00	
75	0.086	2.88	7.53	5.26	0.00	0.00	
76	0.082	2.49	4.65	13.54	0.00	0.00	
77	0.075	2.44	8.54	2.01	0.00	0.00	
78.5	0.068	2.24	6.22	5.46	0.00	0.00	
80	0.064	2.00	3.92	7.14	0.00	0.00	43.83
81	0.057	1.83	10.94	8.50	0.00	0.00	
94.5	0.027	0.91	3.90	3.72	0.00	0.00	15.07
97	0.025	0.72	2.96	8.35	0.00	0.00	0

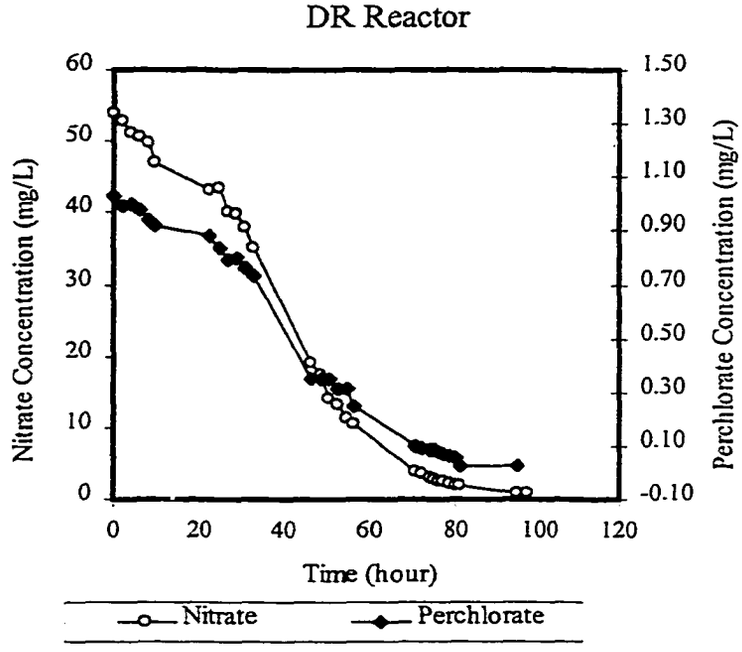


Figure 4.29 The interference of nitrate on perchlorate biodegradation (test 3)

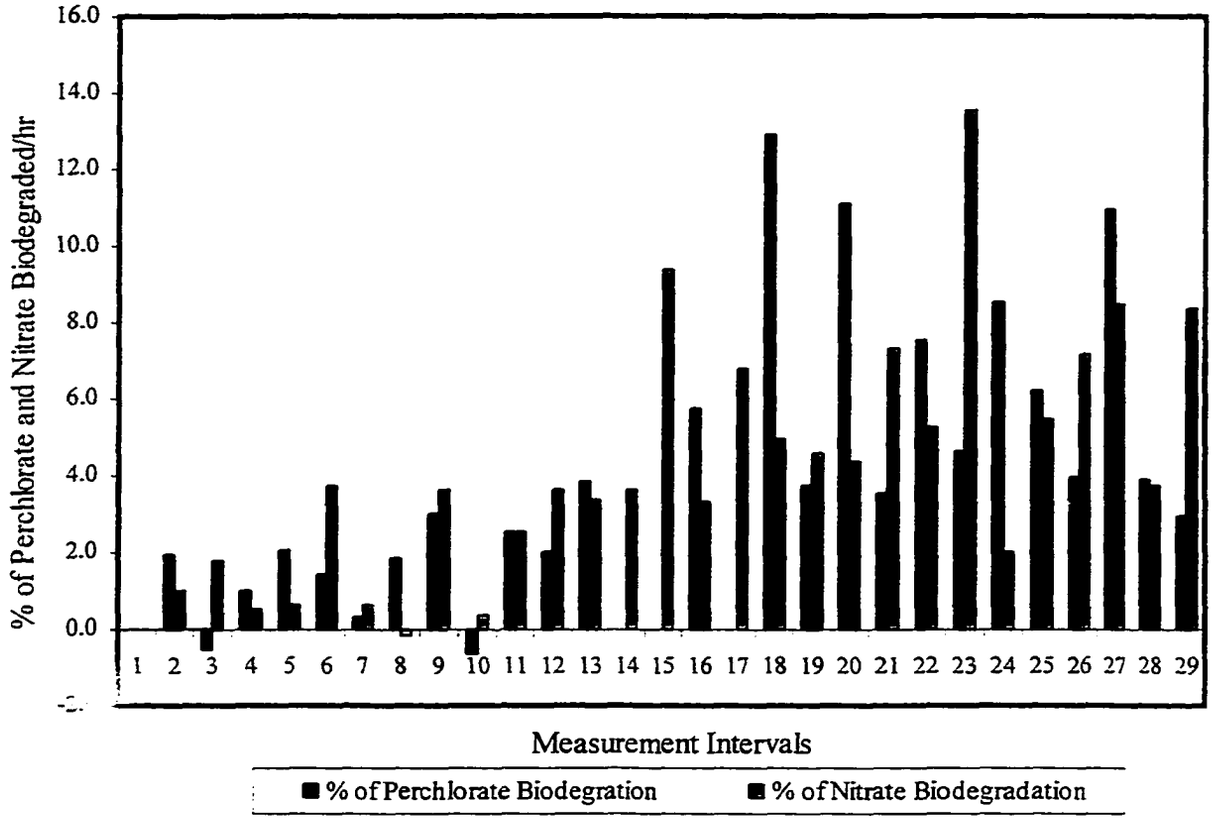


Figure 4.30 Biodegradation rates of perchlorate and nitrate (test 3)

Table 4.45

The interference of nitrate on perchlorate biodegradation (test 4)

	DR Reactor (ClO_4^- and NO_3^-)				BR Reactor (Lactate, Nutrient/Minerals, and Buffer)		
Time (hour)	ClO_4^- (mg/L)	NO_3^- (mg/L)	% of ClO_4^- Biodegraded/hr	% of NO_3^- Biodegraded/hr	ClO_4^- (mg/L)	NO_3^- (mg/L)	Lactate (mg/L)
0	0.96	11.09	0.00	0.00	0.00	0.00	6.73
2	0.94	9.50	1.04	7.17	0.00	0.00	
4	0.91	9.41	1.60	0.47	0.00	0.00	
6	0.92	8.93	-0.55	2.55	0.00	0.00	
8	0.90	8.91	1.09	0.11	0.00	0.00	
9.5	0.89	8.86	0.74	0.37	0.00	0.00	
22.5	0.82	7.87	0.61	0.86	0.00	0.00	
[redacted]			0.49	0.66	0.00	0.00	[redacted]
27	0.73	7.75	4.94	-0.06	0.00	0.00	
29	0.73	7.43	0.00	2.06	0.00	0.00	
31	0.71	6.83	1.37	4.04	0.00	0.00	
33	0.67	6.29	2.82	3.95	0.00	0.00	
46.5	0.44	4.20	2.54	2.46	0.00	0.00	
[redacted]			0.91	1.33	0.00	0.00	[redacted]
51	0.44	4.03	-1.16	0.37	0.00	0.00	
53	0.44	3.98	0.00	0.62	0.00	0.00	1.92
55	0.43	3.83	1.14	1.88	0.00	0.00	1.69
56.5	0.42	3.75	1.55	1.39	0.015	0.00	0
70.5	0.37	3.30	0.85	0.86	0.064	0.00	
72	0.36	3.21	1.80	1.82		0.00	
74	0.35	3.10	1.39	1.71	0.076	1.16	
77	0.35	2.94	0.00	1.72	0.081	1.07	
80	0.35	2.93	0.00	0.11	0.090	1.07	
94.5	0.31	2.51	0.79	0.99	0.120	1.17	
99	0.3	2.48	0.72	0.27	0.130	1.32	
104	0.29	2.43	0.67	0.40	0.140	1.62	
119	0.27	2.07	0.46	0.99	0.160	1.35	0

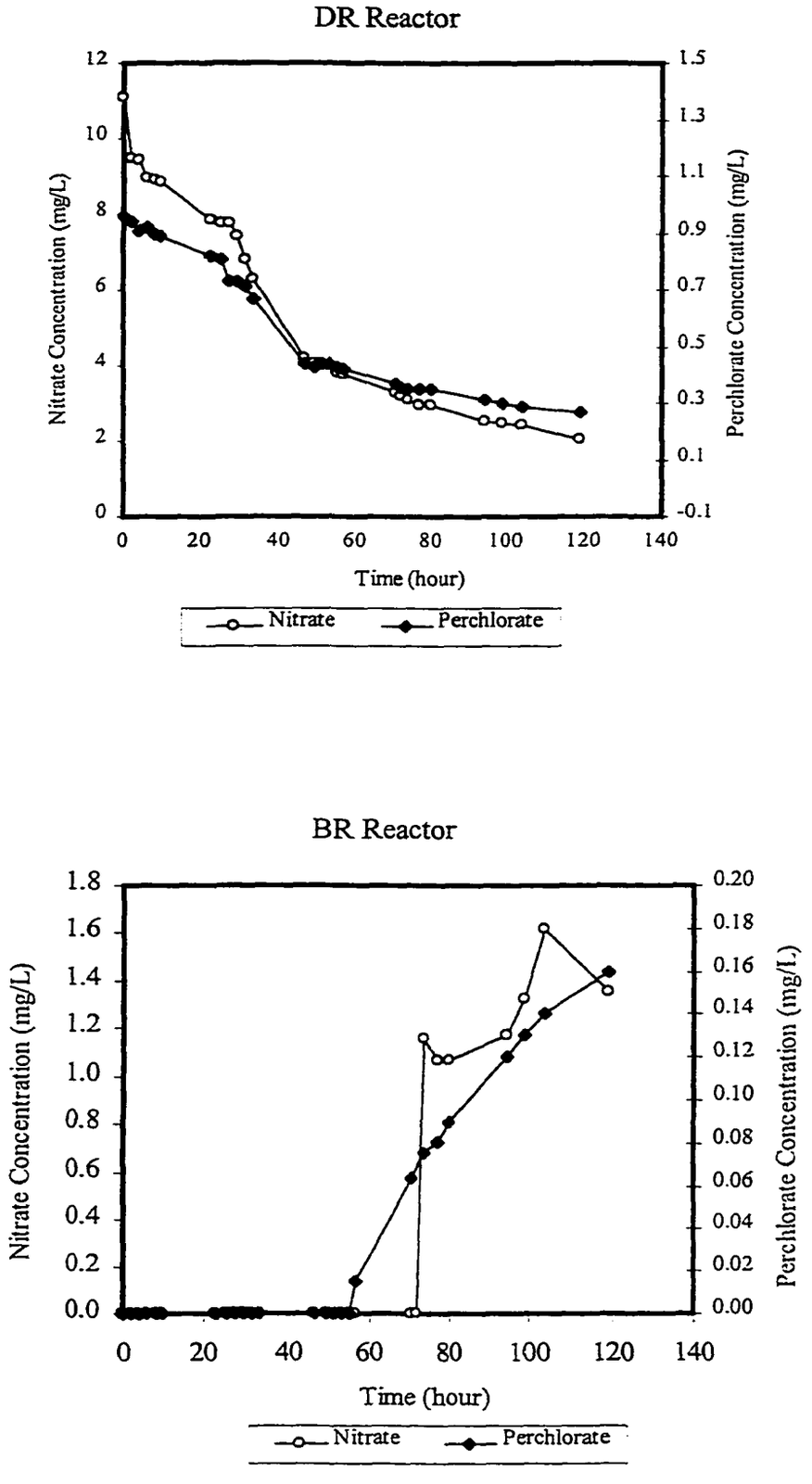


Figure 4.31 The interference of nitrate on perchlorate biodegradation (test 4)

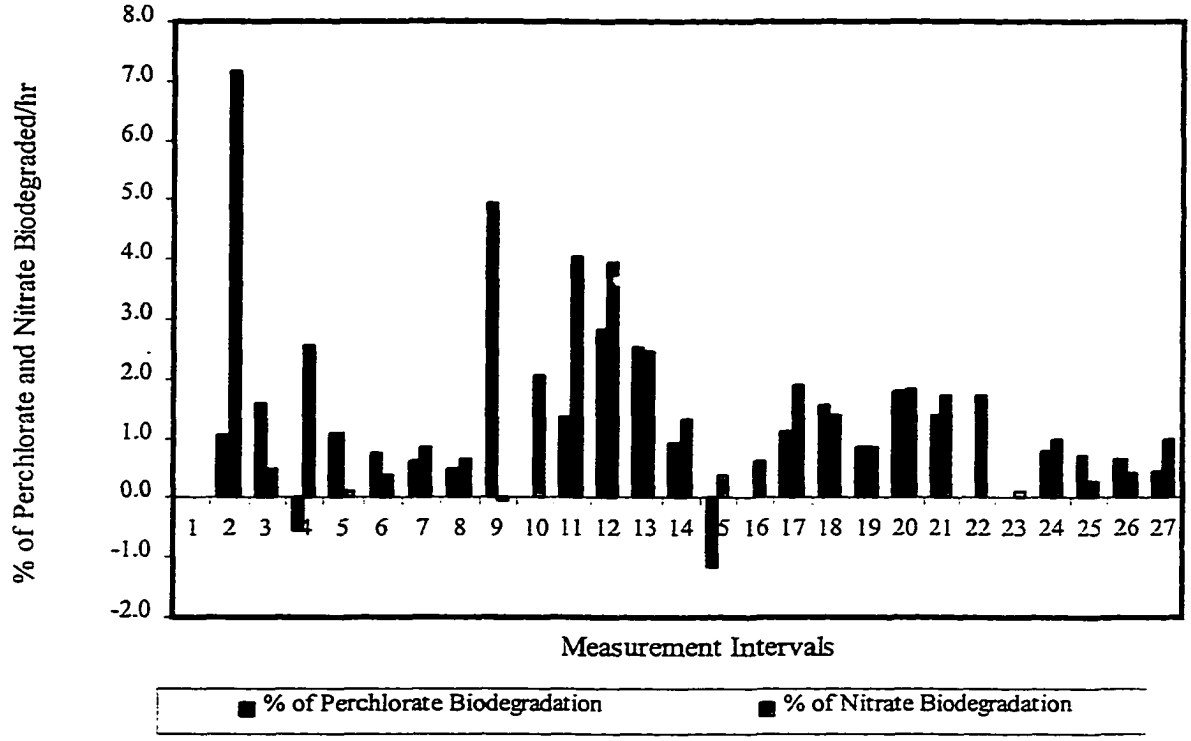


Figure 4.32 Biodegradation rates of perchlorate and nitrate (test 4)

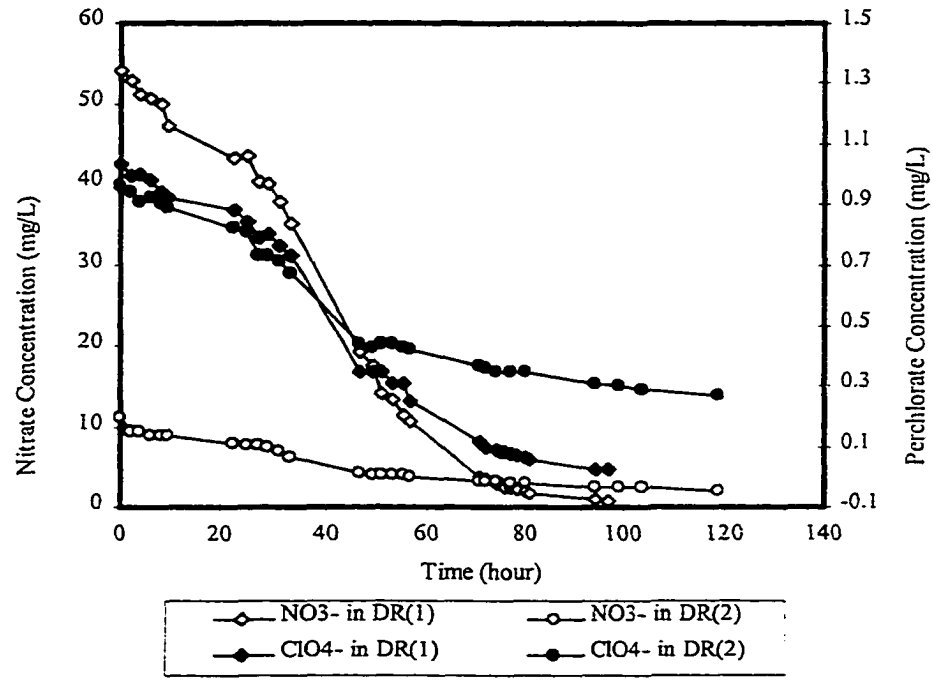


Figure 4.33 Nitrate and perchlorate biodegradation in BTS-55 membrane-immobilized biofilm reactors (test 3 & test 4)

Table 4.46

Summary of nitrate interference on perchlorate biodegradation

Initial Perchlorate Concentration (mg/L)	Initial Nitrate Concentration (mg/L)	Perchlorate Biodegradation Rate (mg/hr)	Nitrate Biodegradation Rate (mg/hr)	Ratio of Nitrate to Perchlorate Biodegradation
50.65	58.50	0.51	1.04	2.04
51.58	10.38	0.48	0.99	2.06
0.96	11.09	0.016	0.15	9.4
1.03	54.0	0.005	0.38	76

Table 4.46 summarizes all the results obtained from the testing of nitrate interference on the biodegradation of lower and higher perchlorate concentrations. The biodegradation rates of perchlorate and nitrate, shown in Table 4.46, were obtained from selected data highlighted in Table 4.42 to Table 4.45 The data selected considered the presence of sufficient lactate to assure that the system was not under carbon (electron donor) limiting conditions.

For higher perchlorate concentration (about 50 mg/L), the presence of 10 or 50 mg/L of nitrate equally affects perchlorate biodegradation. The biodegradation rates for perchlorate (0.51 mg/hr and 0.48 mg/hr) and for nitrate (1.04 mg/hr and 0.99 mg/hr) were found quite similar despite a five-fold difference in nitrate concentration. For lower perchlorate concentrations (about 1 mg/L), a different response to the presence of nitrate is found; the higher the nitrate concentration, the greater the negative effect on perchlorate biodegradation. For the nitrate concentration of 50 mg/L, the perchlorate biodegradation rate was only 0.005 mg /hr, while for a nitrate concentration of 10 mg/L,

the rate of perchlorate biodegradation (0.016 mg/hr) increased 3.2 times. At the same time, the nitrate biodegradation rate decreased from 0.38 mg/hr to 0.15 mg/hr.

Although the biofilms used in the tests described above were developed under very similar conditions, it is still not possible to assure that all the biofilms had the same thickness and the same microbial composition. However, the results described above seem to strongly suggest that the negative effect of nitrate on perchlorate biodegradation is more significant when the perchlorate concentrations are lower. Thus, for drinking waters moderately contaminated with perchlorate, the presence of nitrate will significantly hinder perchlorate biodegradation. This findings imply higher cost for the design of perchlorate removal systems because of increased carbon source (e.g.: lactate) usage and reactor volume needed for biodegradation.

Influence of Sulfate on Perchlorate Biodegradation by the Membrane-Immobilized Biofilm

Batch Testing

Table 4.47 and Figure 4.34 show the result of sulfate interference on perchlorate biodegradation in batch tests. For this test, the initial concentrations of perchlorate were about 10 mg/L, while the initial concentrations of sulfate varied from 40 mg/L to 600 mg/L. The nutrient/minerals broth used for microbial growth contained a background sulfate concentration of approximately 41 mg/L. The results showed that perchlorate biodegraded from about 10 mg/L to undetectable levels within 16 hours, while the sulfate concentration remained practically constant during the same period. Thus, sulfate

concentrations ranging from 40 to 600 mg/L seem not to interfere with perchlorate biodegradation. This is confirmed by the fact that sulfate biodegradation started after all the perchlorate had been biodegraded (Table 4.47). Sulfate was very slowly biodegraded and even after 117 hours, the largest reduction of sulfate observed was about 5.2%.

Table 4.47

Interference of sulfate on perchlorate biodegradation (batch testing)

	SO_4^{2-}-40 test		SO_4^{2-}-60 test		SO_4^{2-}-140 test		SO_4^{2-}-600 test	
Time (hour)	ClO_4^- (mg/L)	SO_4^{2-} (mg/L)	ClO_4^- (mg/L)	SO_4^{2-} (mg/L)	ClO_4^- (mg/L)	SO_4^{2-} (mg/L)	ClO_4^- (mg/L)	SO_4^{2-} (mg/L)
0	10.79	41.68	10.9	59.72	11.03	146.06	11.11	617.5
16	2.97	44.12	1.33	60.07	0	144.4	0	605.79
25	0	52.57	0	59.62	0	139.41	0	606.36
40		N/A		59.95		140.36		601.91
69		41.77		66.89		139.66		614.54
117		40.8		60.39		138.4		593.03
Reduced %	100%	2.1%	100%	~0 %	100%	5.2%	100%	4.0%

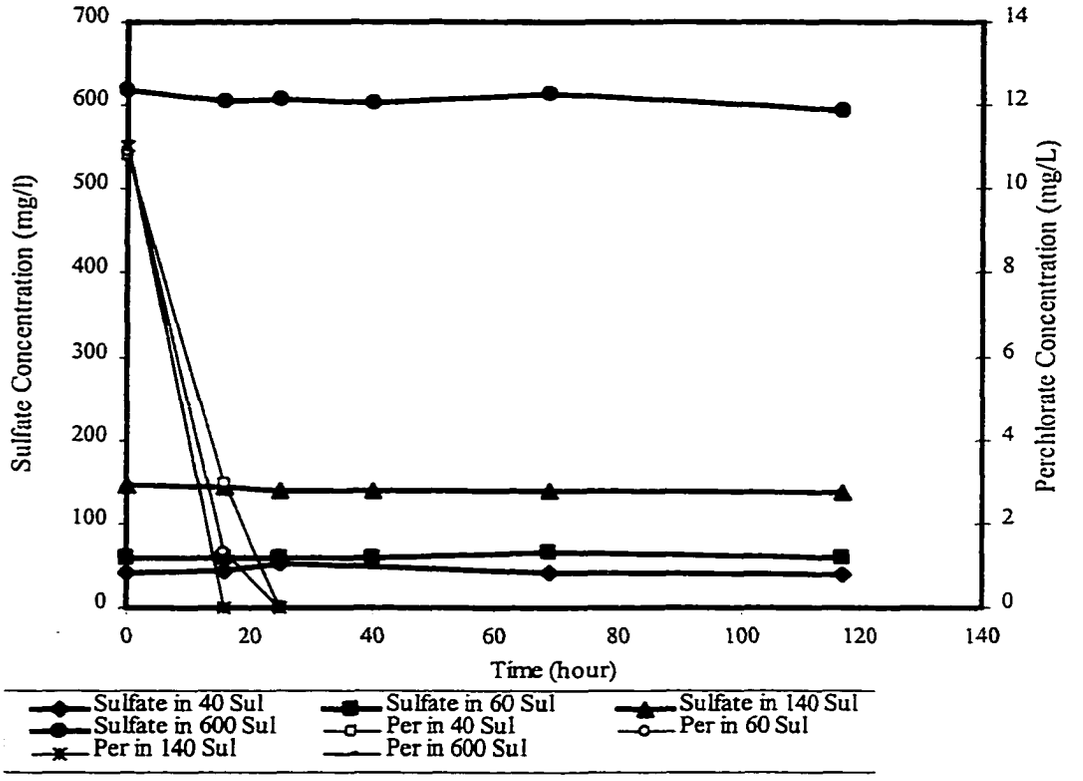


Figure 4.34 Interference of sulfate on perchlorate biodegradation (batch testing)

Figure 4.34 shows the variations in perchlorate and sulfate concentrations with time as biodegradation occurs. In the graph, the legend "Sulfate in 40 Sul" means the concentration of sulfate in the test in which the concentration of sulfate was 40 mg/L and the concentration of perchlorate was 10 mg/L. Similarly, the "Per in 40 Sul" means the concentration of perchlorate in the test in which the concentration of sulfate was 40 mg/L and the concentration of perchlorate was 10 mg/L. The figure clearly shows that sulfate concentration remained almost constant for the experimental period, while for perchlorate, the concentrations decreased sharply to zero after 16 hours.

Membrane-Immobilized Biofilm Testing

Table 4.48 to 4.51 and Figure 4.35 to 4.40 show the results of interference of sulfate on perchlorate biodegradation in BTS-55 membrane-immobilized biofilm reactors. The sulfate concentrations added to the DR reactors, in individual tests, varied from 10 mg/L to 200 mg/L while the perchlorate concentrations varied from 100 μg/L to 50 mg/L. The first test performed indicated a much higher sulfate concentration in the BR reactor than that added to the DR reactor. Investigation of the issue revealed that the nutrient/minerals broth added to the BR reactor contains about 41 mg/L sulfate resulting from the addition of $MgSO_4.7H_2O$, $ZnSO_4.7H_2O$, $FeSO_4.7H_2O$, and $CuSO_4.5H_2O$ (Table 3.2). Thus, all the perchlorate biodegradation experiments described in this report were performed in the presence of about 41 mg/L sulfate. No nitrate is present in the buffer or nutrient/minerals broth.

As seen in Figure 4.35 to Figure 4.40, the perchlorate concentration in the DR reactor decreased with time as a result of biodegradation by the biofilm as it diffused to the BR reactor, in which the perchlorate concentration remained very low. However, the sulfate concentrations in the DR and BR reactor varied considerably. This variation of sulfate concentration is the result of sulfate diffusion, in both directions, from the BR to the DR reactor. Because of this variation, it is not possible to calculate accurately the biodegradation rates for sulfate.

Table 4.52 summarizes perchlorate and sulfate biodegradation rates. The data used in this calculation were bold in Table 4.48 to Table 4.51. The data were selected so that the system was not limited by lactate. In addition, two biodegradation rates were calculated for sulfate, one used the data for which perchlorate was present, and the other

used the data after all perchlorate had been biodegraded. Notice that the higher the initial perchlorate concentration, the higher the perchlorate biodegradation rate, independent of the initial sulfate concentration. The sulfate biodegradation rates, in the presence of perchlorate, were much smaller (or null) than those of perchlorate. In the absence of perchlorate, however, the sulfate biodegradation rates were larger than those in the presence of perchlorate, except for the case in which the perchlorate and sulfate concentrations were about 50 mg/L. The reason for that is not clear from the data available. However, it can be concluded that sulfate does not affect perchlorate biodegradation. Thus, the "BALI" mixed culture prefers perchlorate to sulfate as an electron acceptor.

Table 4.48

The interference of sulfate on perchlorate biodegradation (test 1)

	DR Reactor (ClO_4^- and SO_4^{2-})					BR Reactor (Lactate, Nutrient/Minerals, and Buffer)		
Time (hr)	ClO_4^- (mg/L)	SO_4^{2-} (mg/L)	Lactate (mg/L)	% of ClO_4^- Biodegraded/ hr	% of SO_4^{2-} Biodegraded/ hr	ClO_4^- (mg/L)	SO_4^{2-} (mg/L)	Lactate (mg/L)
0.00	**10.78**	**13.60**	0.00	0.00	0.00	**0.02**	**46.49**	224.40
8.00	10.22	11.80	14.71	0.65	1.65		63.42	225.08
21.25	8.79	16.18	34.88	1.06	-2.80	0.06	51.37	201.51
27.00	**7.42**	**18.71**	32.47	2.71	-2.72	0.00	**40.00**	136.19
45.50	0.00	**13.66**	16.65	5.41	1.46	0.00	**27.01**	82.20
54.00	0.00	9.33	11.46		3.73	0.00	13.68	67.25
70.00	0.00	**10.15**	0.00		-0.55	0.00	**17.58**	19.10
79.00	0.00	18.80	0.00		-9.47	0.00	14.09	0.00
94.00	0.00	18.09	0.00		0.25	0.00	15.85	0.00
118.00	0.00	12.63	0.00		1.26	0.00	13.19	0.00
142.00	0.00	12.71	0.00		-0.03	0.00	13.62	0.00
165.75	0.00	13.10	0.00		-0.13	0.00	14.06	0.00

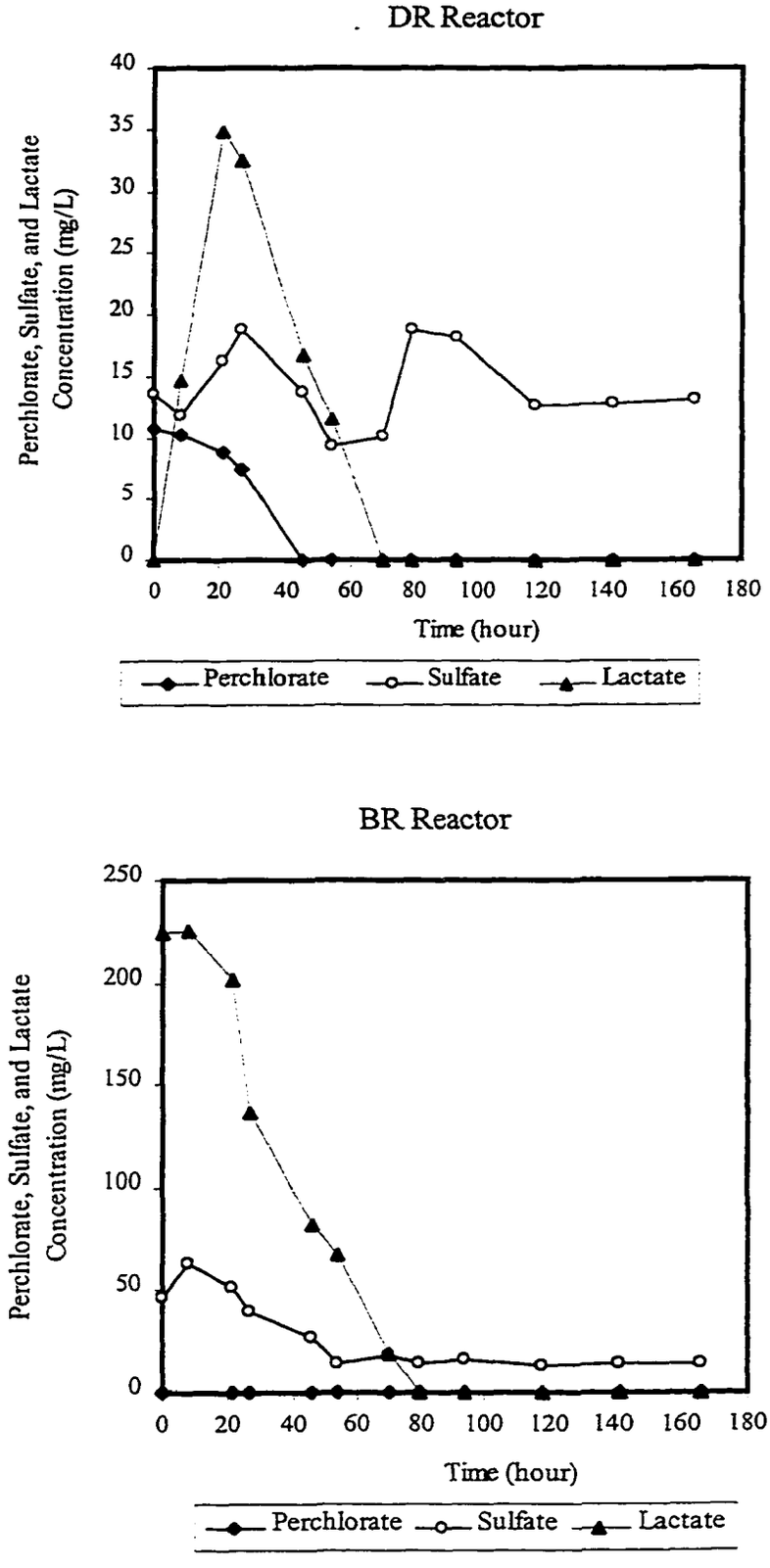


Figure 4.35 The interference of sulfate on perchlorate biodegradation (test 1)

Table 4.49

The interference of sulfate on perchlorate biodegradation (test 2)

	DR Reactor (ClO_4^- and SO_4^{2-})					BR Reactor (Lactate, Nutrient/Minerals, and Buffer)		
Time (hr)	ClO_4^- (mg/L)	SO_4^{2-} (mg/L)	Lactate (mg/L)	% of ClO_4^- Biodegraded /hr	% of SO_4^{2-} Biodegraded/ hr	ClO_4^- (mg/L)	SO_4^{2-} (mg/L)	Lactate (mg/L)
0.00	**9.69**	**213.30**	0.00	0.00	0.00	0.00	**43.37**	1119.84
8.00	8.90	206.05	110.34	1.02	0.42	N/A	52.57	1061.31
21.25	8.01	209.27	228.80	0.75	-0.12	0.80	68.07	1020.54
27.00	**7.09**	**208.34**	222.61	2.00	0.08	0.00	**62.71**	937.34
45.50	0.00	173.70	235.43	5.41	0.90	0.00	64.89	649.77
54.00	0.00	**158.43**	303.53		1.03	0.00	**53.68**	529.96
70.00	0.00	129.83	216.50		1.13	0.00	51.46	220.70
79.00	0.00	113.11	146.22		1.43	0.00	54.01	165.55
94.00	0.00	118.75	129.61		-0.33	0.00	57.16	145.55
118.00	0.00	108.34	78.14		0.37	0.00	61.72	100.77
142.00	0.00	118.23	88.24		-0.38	0.00	72.85	90.58
165.75	0.00	**112.21**	54.01		0.21	0.00	**74.54**	12.56

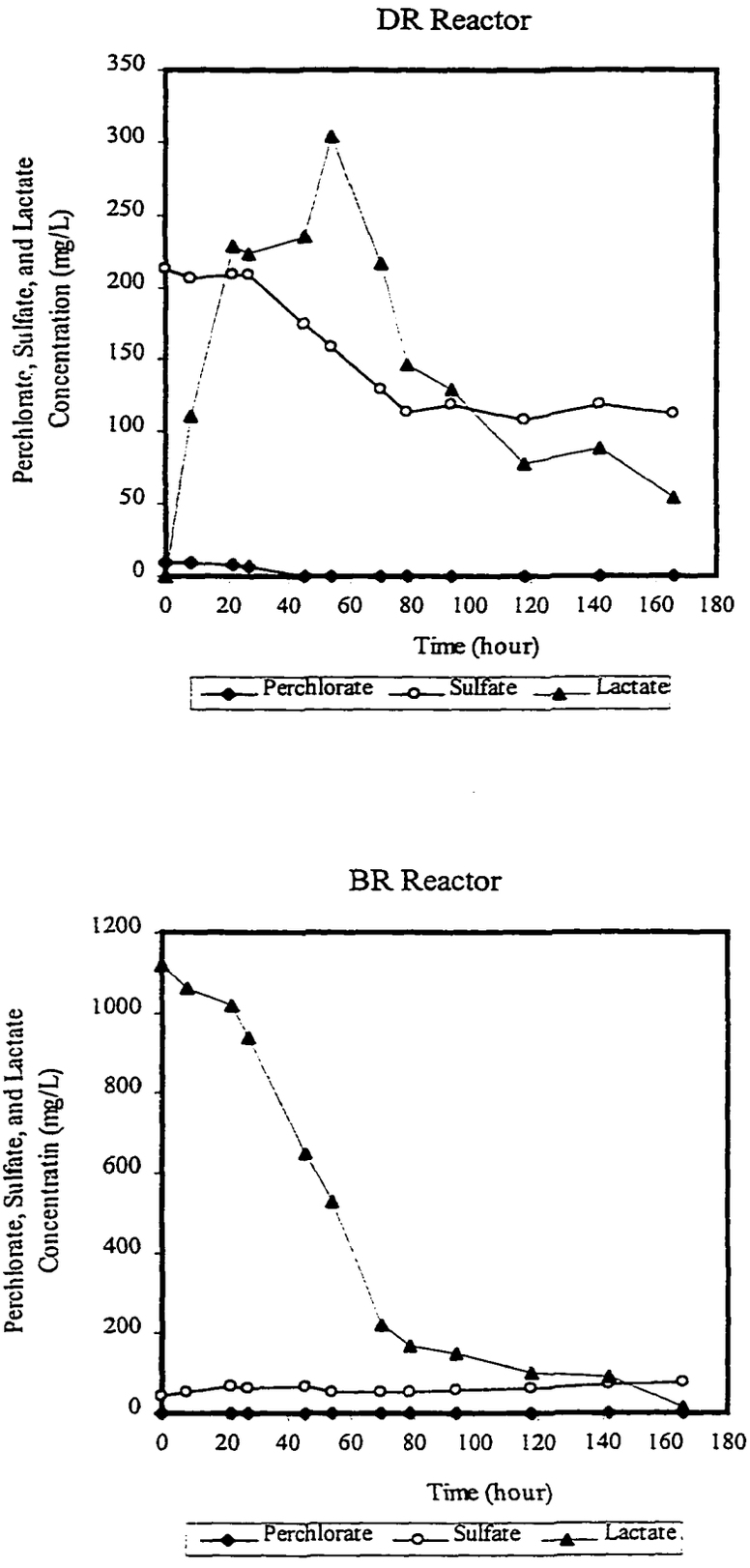


Figure 4.36 The interference of sulfate on perchlorate biodegradation (test 2)

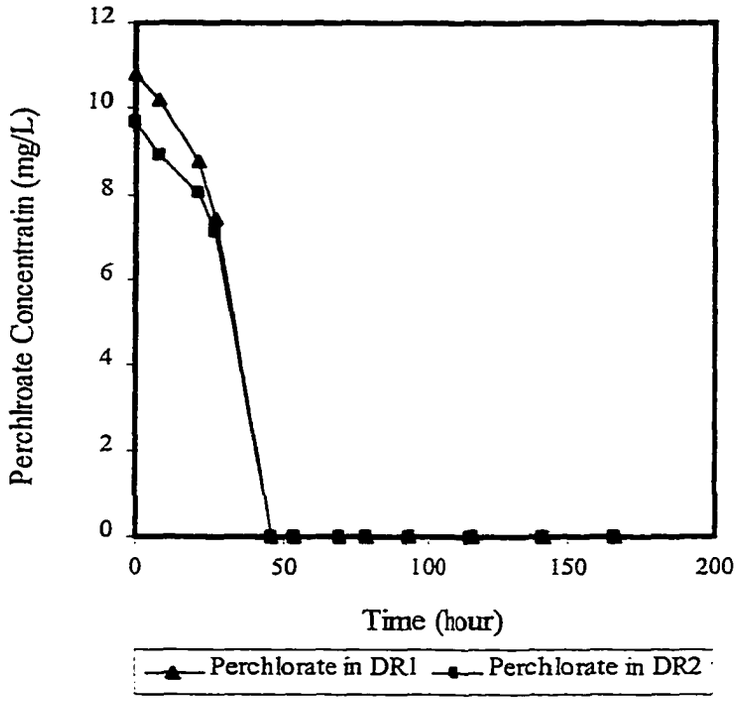


Figure 4.37 The interference of sulfate on perchlorate biodegradation (test 1 & test 2)

Table 4.50

The interference of sulfate on perchlorate biodegradation (test 3)

	DR Reactor (ClO_4^- and SO_4^{2-})					BR Reactor (Lactate, Nutrient, and Buffer)		
Time (hour)	ClO_4^- (µg/L)	SO_4^{2-} (mg/L)	Lactate (mg/L)	% of ClO_4^- Biodegraded/ hr	% of SO_4^{2-} Biodegraded /hr	ClO_4^- (µg/L)	SO_4^{2-} (mg/L)	Lactate (mg/L)
0	**116.63**	**49.77**	0.00	0.00	0.00	0.00	**42.15**	423.92
2.5	109.16	48.60	7.96	2.56	0.94	0.00	42.93	432.78
6	93.01	49.00	20.38	4.23	-0.24	N/A	N/A	N/A
16.5	**40.04**	**52.38**	37.92	5.42	-0.66	0.00	**41.13**	331.94
23.5	0.00	**45.60**	40.83	14.29	1.85	0.00	**37.72**	276.24
30	13.12	45.13	38.34	N/A	0.16	N/A	N/A	N/A
40.5	0.00	40.65	39.55	9.52	0.95	0.00	34.92	124.74
64.5	0.00	42.95	33.07	N/A	-0.24	0.00	34.90	60.01
71	0.00	**49.15**	24.76	N/A	-2.22	0.00	**32.89**	14.98
88.5	0.00	48.33	0.97		0.10	N/A	N/A	N/A
95	0.00	48.26	0		0.02	0.00	36.27	0.00
113	0	46.46	0		0.21	0.00	39.52	1.43
144	0	47.31	0		-0.06	0.00	41.52	0.00
144.5	N/A	N/A	N/A		N/A	0	36.79	257.5 (add)
168.5	0	42.85	0		0.38	0	36.52	27.48
186	0	41.91	0		0.13	N/A	37.57	0

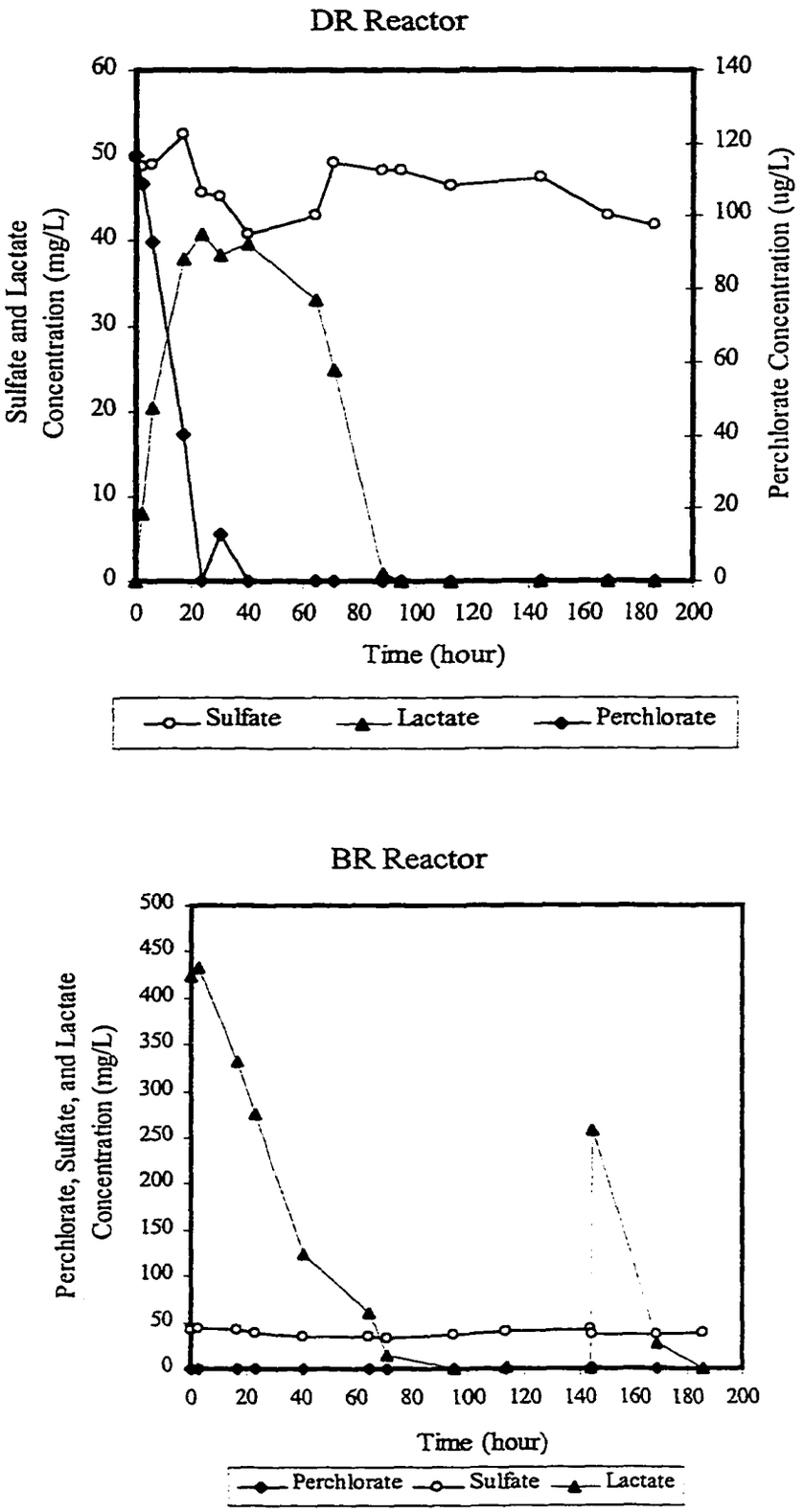


Figure 4.38 The interference of sulfate on perchlorate biodegradation (test 3)

Table 4.51

The interference of sulfate on perchlorate biodegradation (test 4)

	DR Reactor (ClO_4^- and SO_4^{2-})					BR Reactor (Lactate, Nutrient, and Buffer)		
Time (hour)	ClO_4^- (mg/L)	SO_4^{2-} (mg/L)	Lactate (mg/L)	% of ClO_4^- Biodegraded /hr	% of SO_4^{2-} Biodegraded/ hr	ClO_4^- (mg/L)	SO_4^{2-} (mg/L)	Lactate (mg/L)
0	**50.00**	**47.55**	0.00	0.00	0.00	**0.54**	**40.41**	630.86
6	46.96	50.92	23.32	1.01	-1.18	2.10	37.08	612.90
16.5	38.30	46.17	37.63	1.76	0.89	1.44	32.42	531.78
23.5	**12.62**	**40.43**	21.93	9.58	1.78	0.00	**29.27**	489.46
30	0.00	**38.46**	17.48	15.38	0.75	0.00	**22.29**	431.52
40.5	0.00	42.90	22.97		-1.10			
47.5	0.00	39.77	33.97		1.04	0.00	14.90	185.61
71	0.00	**45.18**	32.18		-0.58	0.00	**12.52**	52.90
95	0.00	41.08	34.46		0.38	0.00	16.95	0.00
113	0.00	42.7	17.78		-0.22	0.00	19.67	1.83
144	0	40.31	0		0.18			
144.5						0	24.16	238.68
168.5	0	38.22	0		0.21		2.05	18.71
186	0	39.18	0		-0.14		10.23	0.83

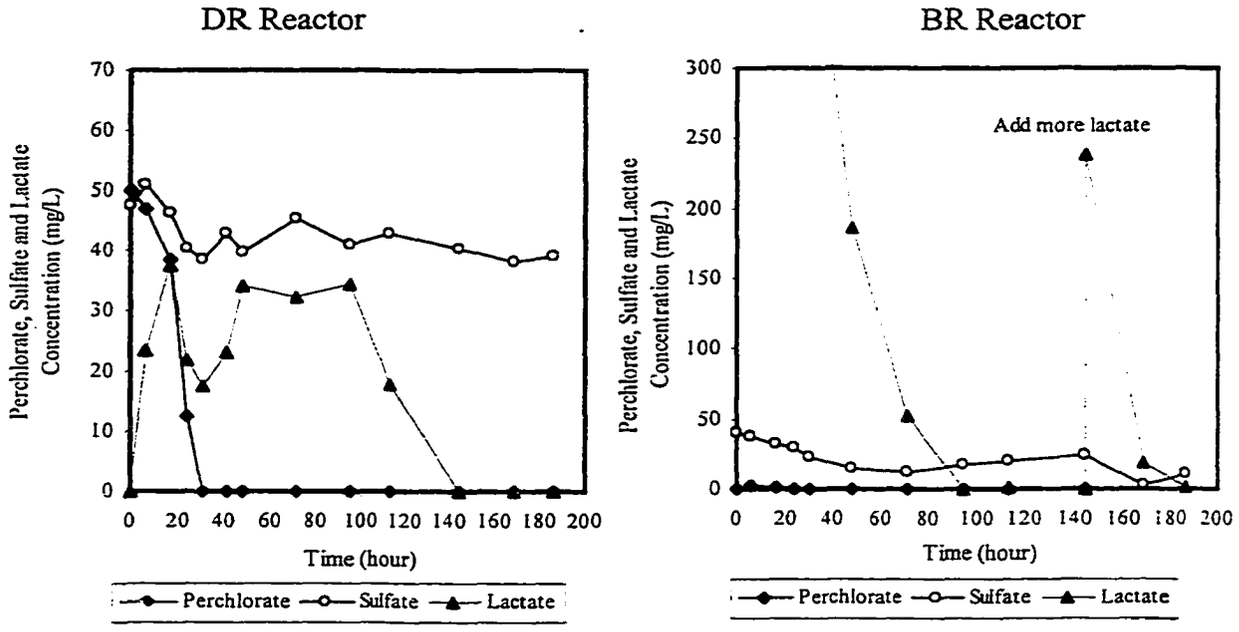


Figure 4.39 The interference of sulfate on perchlorate biodegradation (test 4)

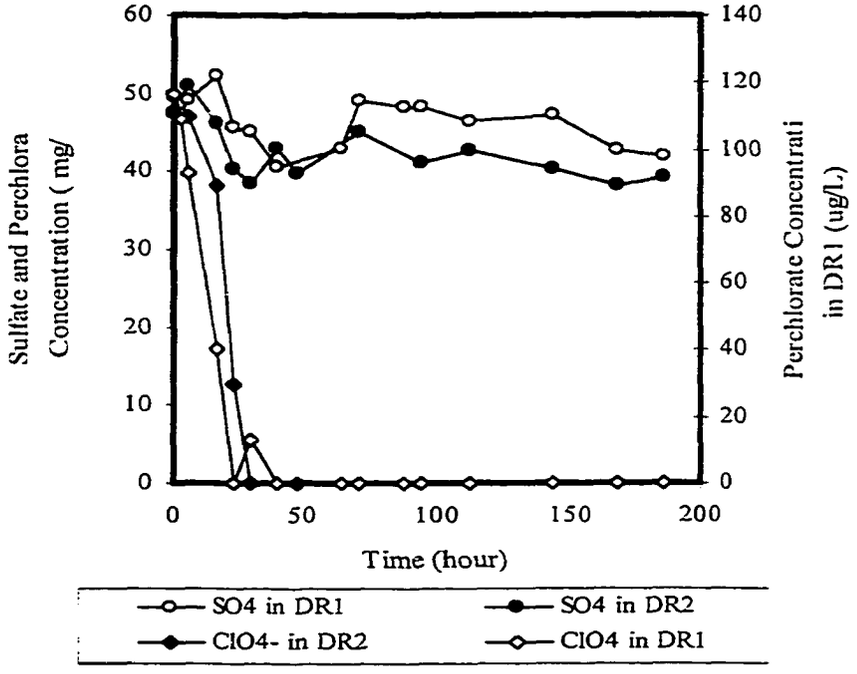


Figure 4.40 The interference of sulfate on perchlorate biodegradation (test 3 & test 4)

Table 4.52

Biodegradation rate for perchlorate in the presence of sulfate

	Test 1		Test 2		Test 3		Test 4	
	ClO_4^-	SO_4^{2-}	ClO_4^-	SO_4^{2-}	ClO_4^-	SO_4^{2-}	ClO_4^-	SO_4^{2-}
Initial Concentration (mg/L)	10.78	13.60	9.69	213.30	0.12	49.77	50	47.55
Biodegradation Rate (mg/hr) (before all ClO_4^- was biodegraded)	0.13	0.05	0.1	0.00	0.005	0.00	1.6	0.78
Biodegradation Rate (mg/hr) (after all ClO_4^- was biodegraded)		0.51		0.23		0.03		0.074

Influence of Salinity on Perchlorate Biodegradation by the "BALI" Culture

Figure 4.41 shows the change in transmittance through the culture tubes with time for different salinity levels. Notice that the higher the salinity level, the higher the transmittance, indicating that the microbes grow much slower at higher salinity levels. Also notice that for each individual salinity level, the transmittance first decreased with time until it reached a minimum, and then it increased slowly with time and reached a constant level. There may be two possible explanations for the increase in transmittance after it had reached a minimum. The first possibility is endogenous respiration – since the test was run for a long time and the lactate to perchlorate ratio was 3:1, it is likely that after the carbon run out, endogenous respiration took place thereby decreasing the concentration of biomass in the tube. The second possibility is co-flocculation. It is

possible that the increase in transmittance is the result of co-flocculation of the microbial cells. In a separate research project taking place in the UNLV Environmental Engineering laboratory, microbial flocculation was also observed in perchlorate biodegradation batch tests at higher salinity levels. Microscopic observations of the batch tests culture used in this experiments showed flocculation. Although the test tubes used in this experiment were shaken well before each measurement, it is likely that the intensity of the shaking was not sufficient to break up all the formed flocs.

Figures 4.42 to 4.51 Show the influence of different salinity levels on perchlorate biodegradation by the "BALI" enrichment mixed culture. In the figures, a, b and c represent triplicates culture tubes tested. Tube d represents a control tube to which no microbes were added, and tube e is a control tube to which no lactate was added. The raw data used to build the figures are shown in Appendix A. For each salinity level tested, three graphs were plotted. The first graph (A) shows the raw transmittance (T) data measured as microbial growth took place in the culture tubes. The second graph (B) is a plot of the absorbance (log 1/T) for the data corresponding to the exponential growth phase. For specific growth rate calculations, only the first portion of the data, those representing the exponential growth phase, are of significance. The exponential growth phase data include the data from the beginning of the experiment to the time where the minimum transmittance was obtained. The third graph (C) is a plot of the logarithmic of the absorbance with time. The slope of this curve represents the specific growth rate (μ) of the culture under consideration. For exponential microbial growth, one can write that the rate of microbial growth is directly proportional to the initial number of microbes present. Thus:

$$\frac{dn}{dt} \propto n \qquad (4.2)$$

The proportionality sign can be substituted by a constant, μ (the specific microbial growth rate), the equation 4.2 can be written as:

$$\frac{dn}{n} = \mu\, dt \qquad (4.3)$$

Integrating from n_0 to n and from t = 0 to t, one gets

$$\ln \frac{n}{n_0} = \mu t \qquad (4.4)$$

Where, n_0 = initial number of microbes

n = number of microbes at time t

μ = specific microbial growth rate ($time^{-1}$)

t = elapsed time

The absorbance is directly proportional to the number of microbes in the tube at time t, thus one can write:

$$\ln A = \ln A_0 + \mu t \qquad (4.5)$$

Where, A_0 = initial absorbance of the culture tube

A = absorbance of the culture tube at time t

The transmittance data show that perchlorate biodegradation was significantly hindered with increasing salinity and at salt concentrations greater than 4% no microbial growth occurred.

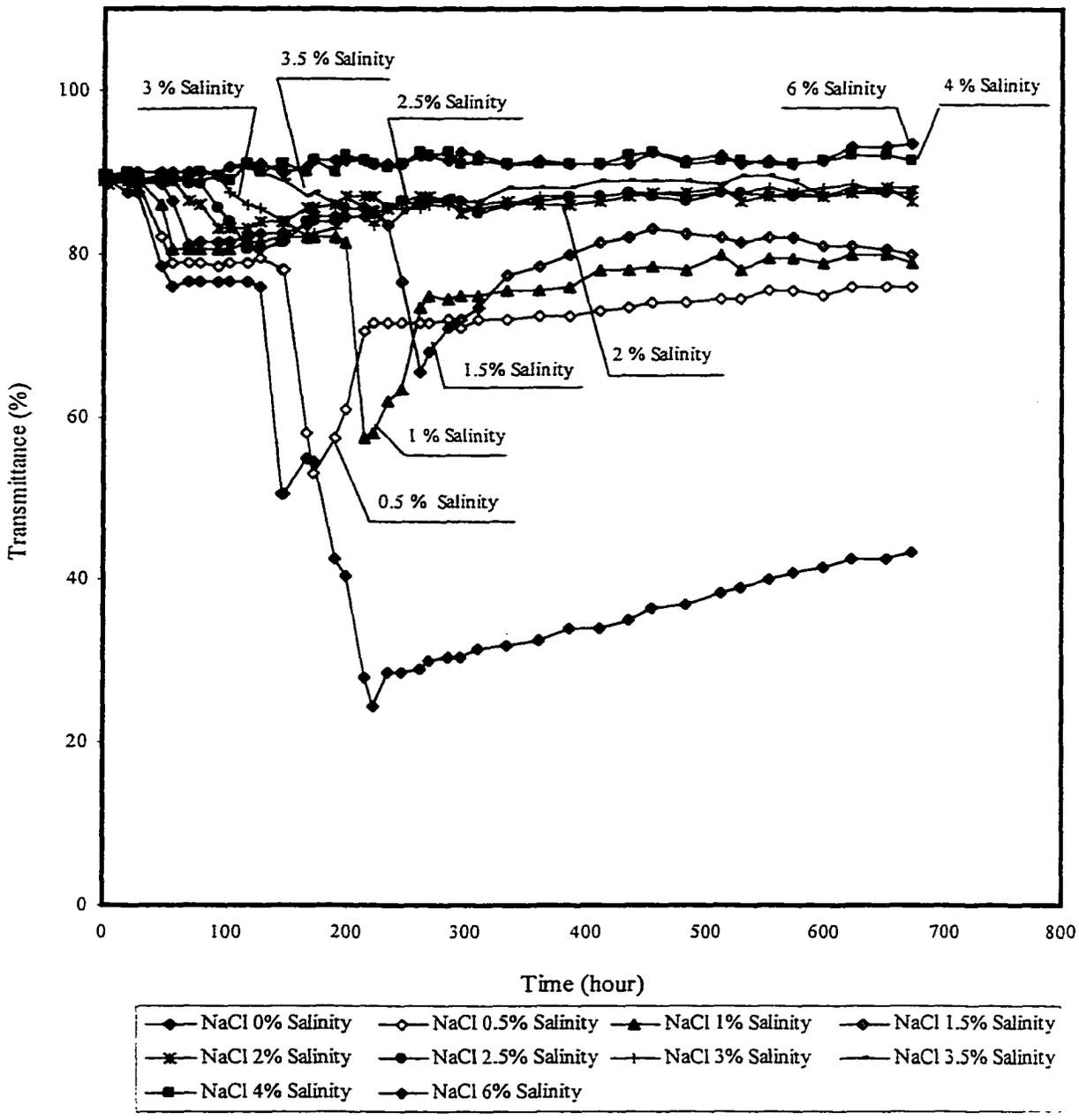


Figure 4.41 Transmittance of different salinity concentration

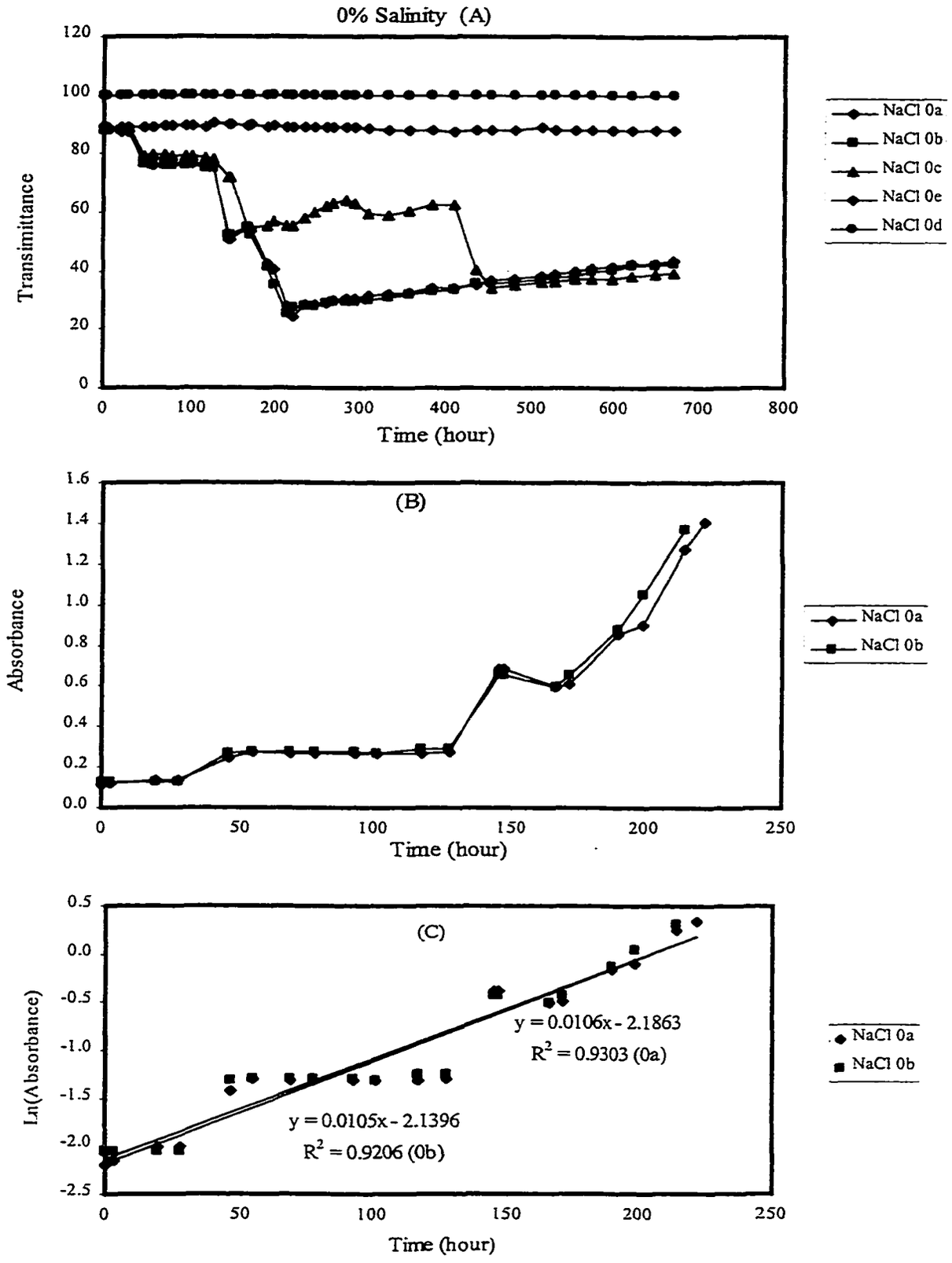


Figure 4.42 Interference of salinity (0%) on perchlorate biodegradation

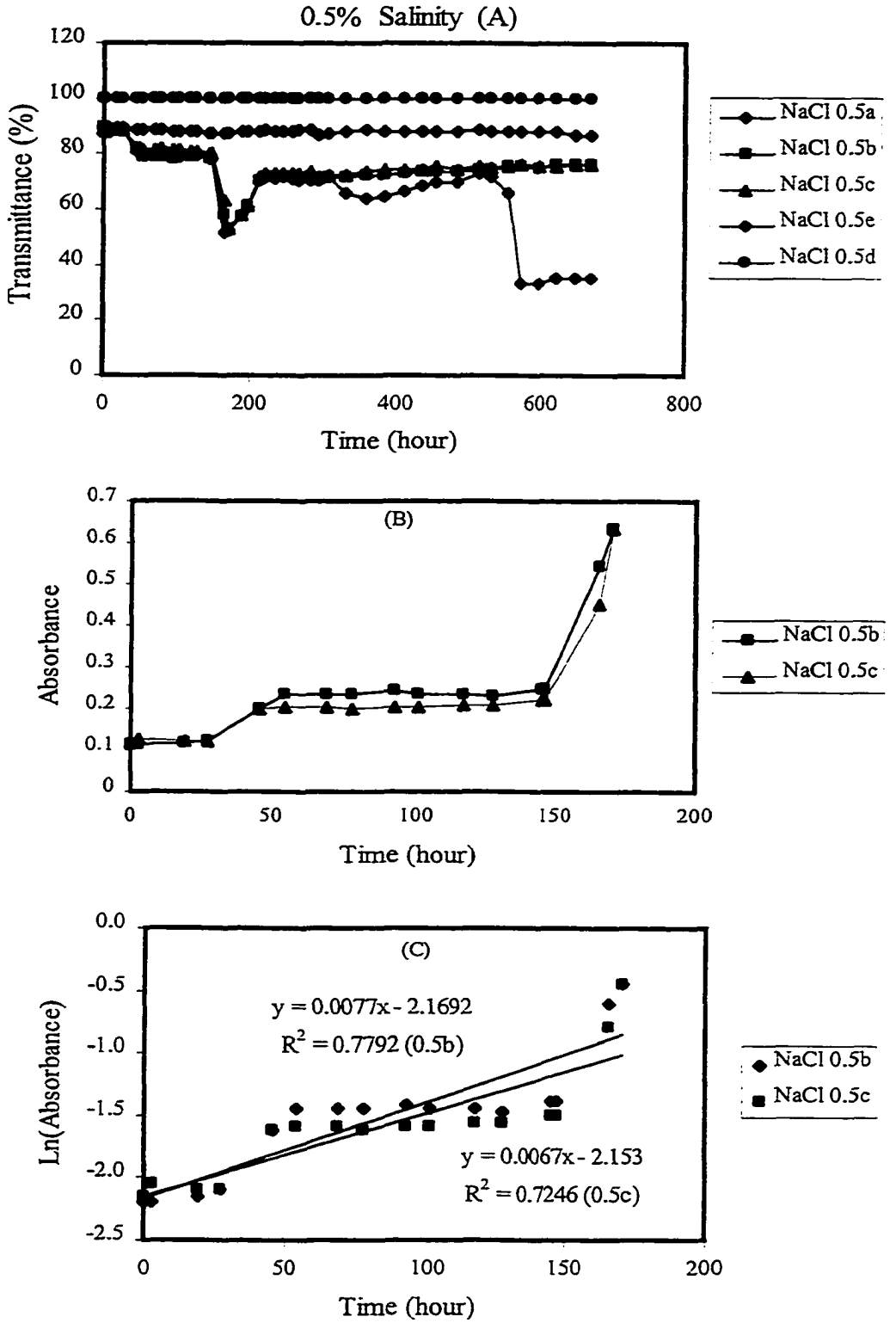


Figure 4.43 Interference of salinity (0.5%) on perchlorate biodegradation

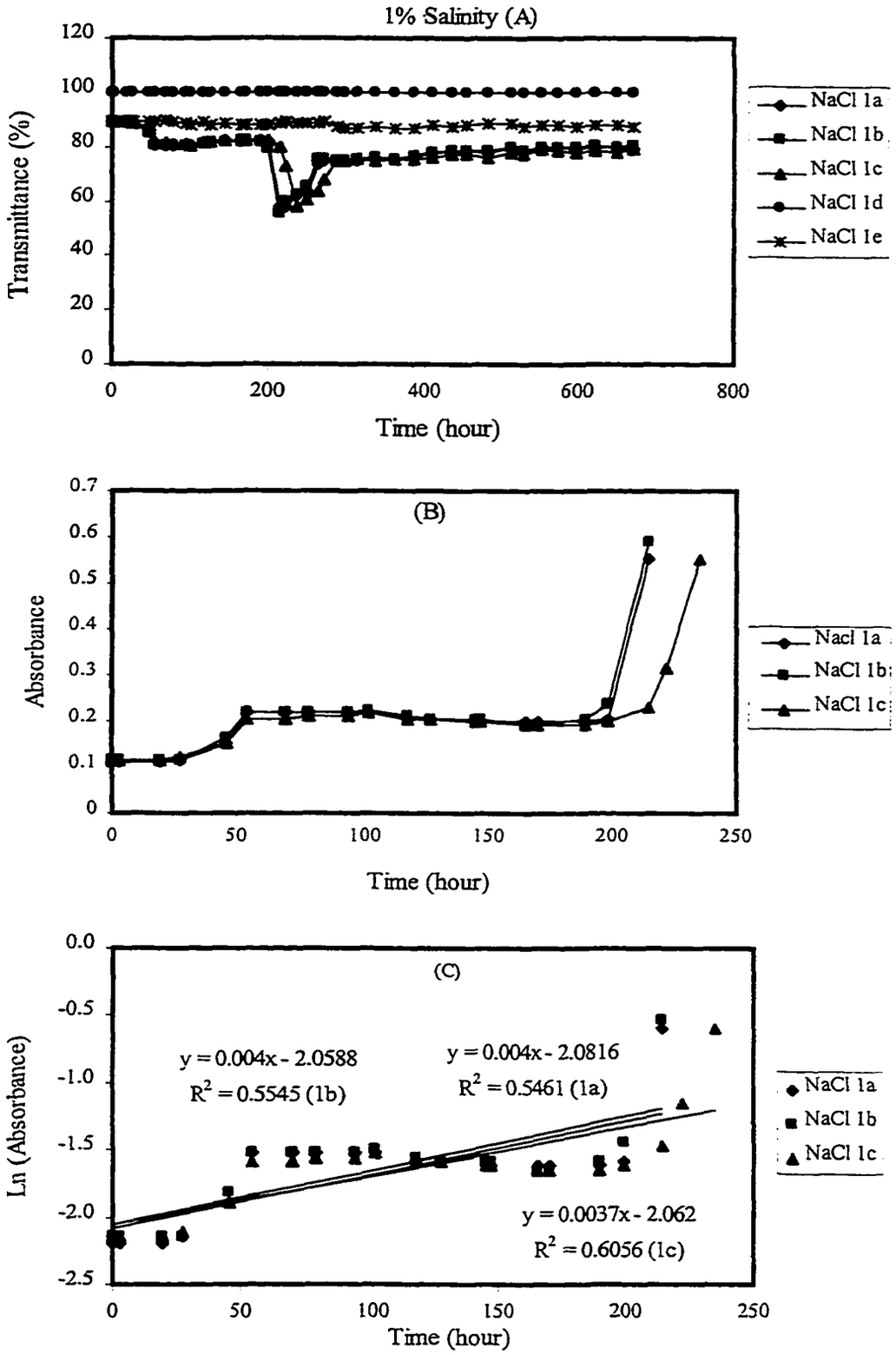


Figure 4.44 Interference of salinity (1.0%) on perchlorate biodegradation

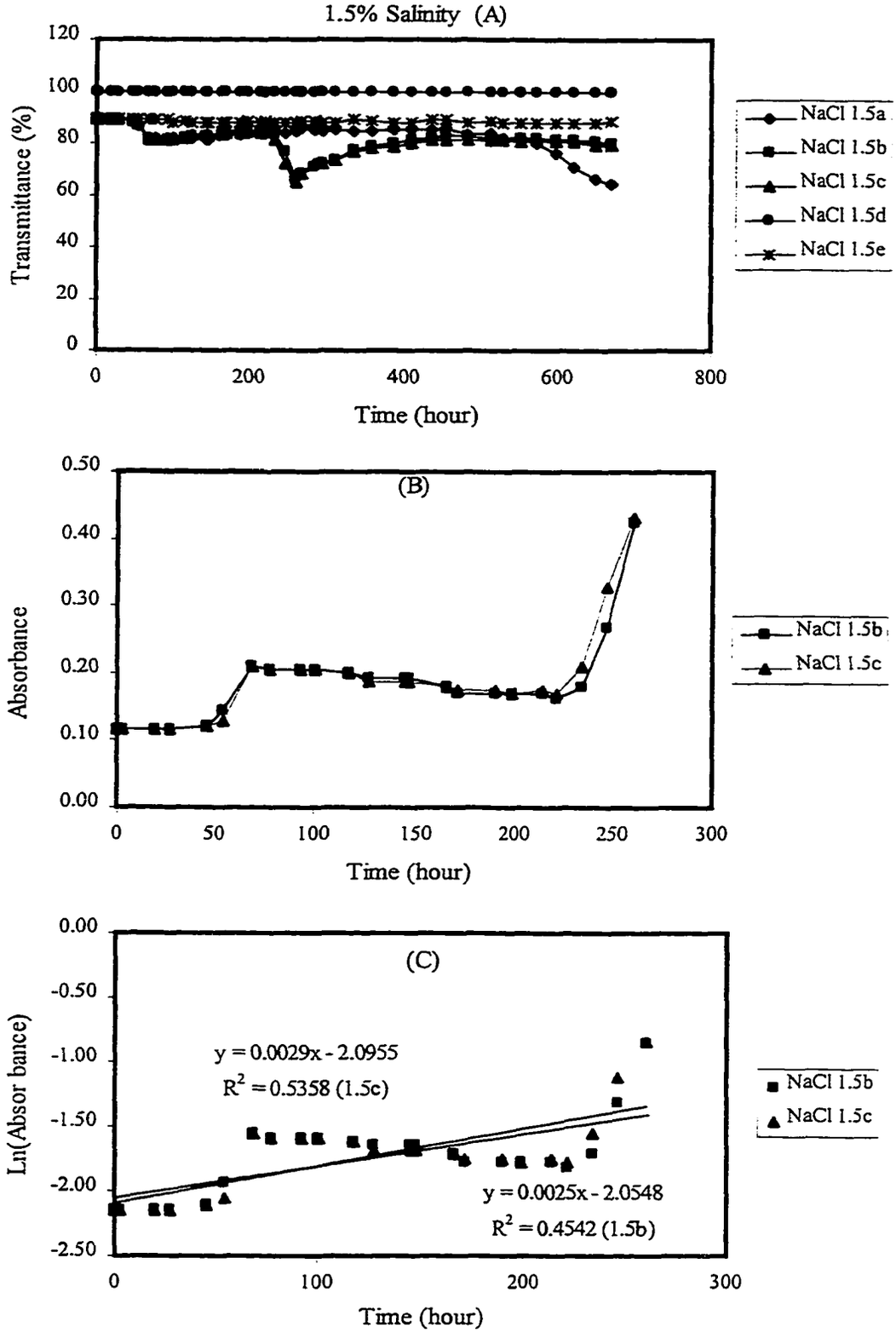


Figure 4.45 Interference of salinity (1.5%) on perchlorate biodegradation

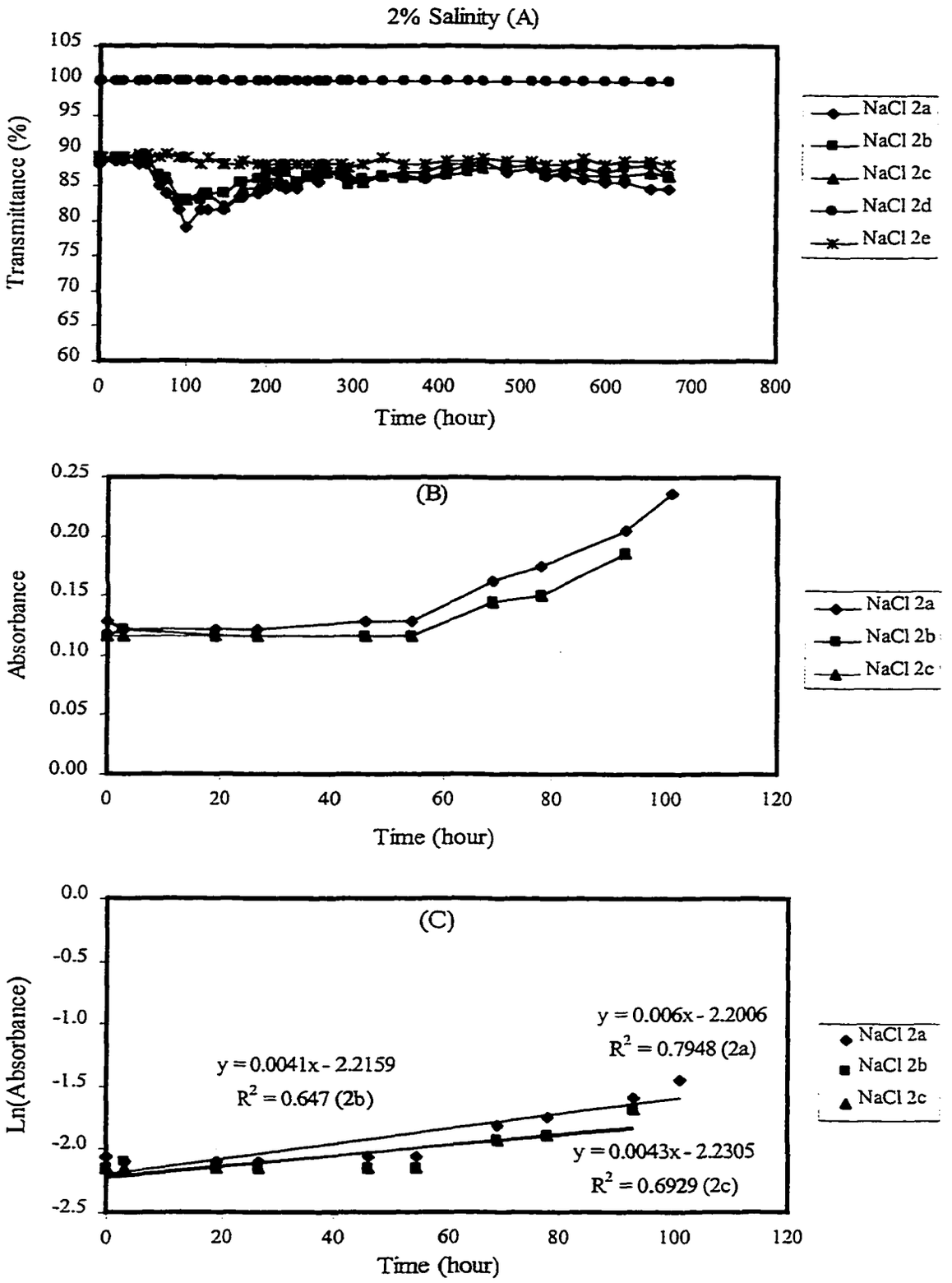


Figure 4.46 Interference of salinity (2.0%) on perchlorate biodegradation

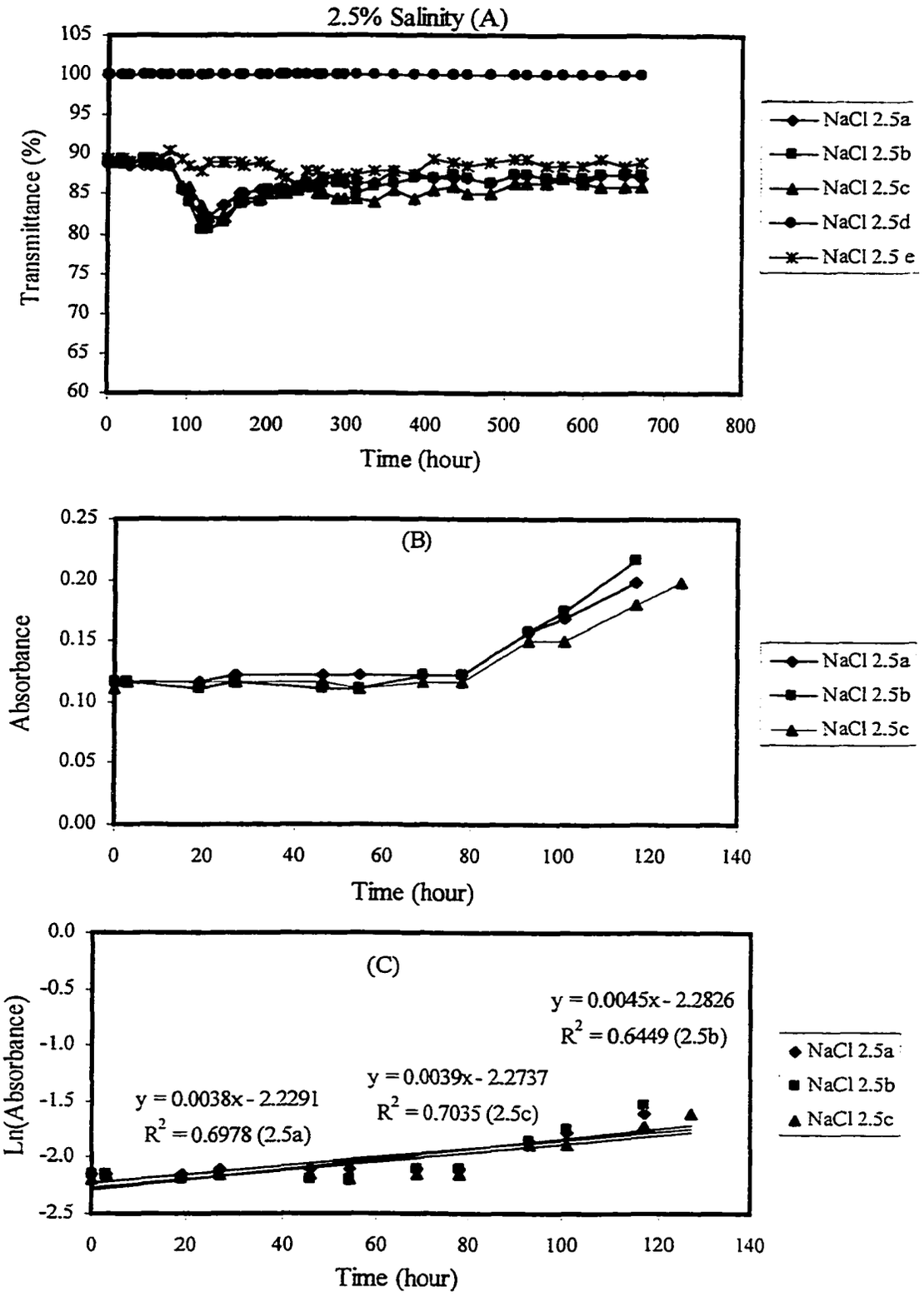


Figure 4.47 Interference of salinity (2.5%) on perchlorate biodegradation

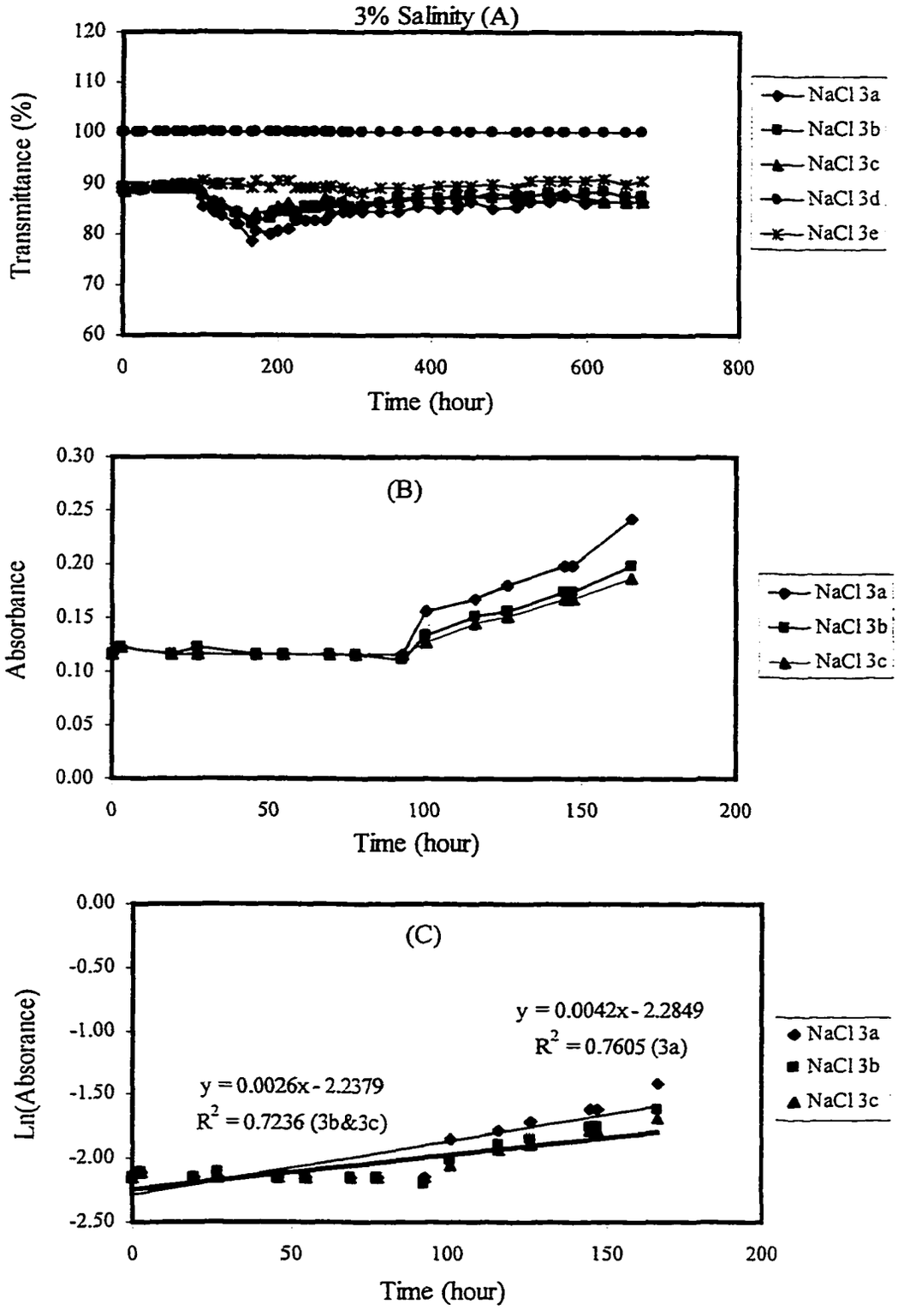


Figure 4.48 Interference of salinity (3.0%) on perchlorate biodegradation

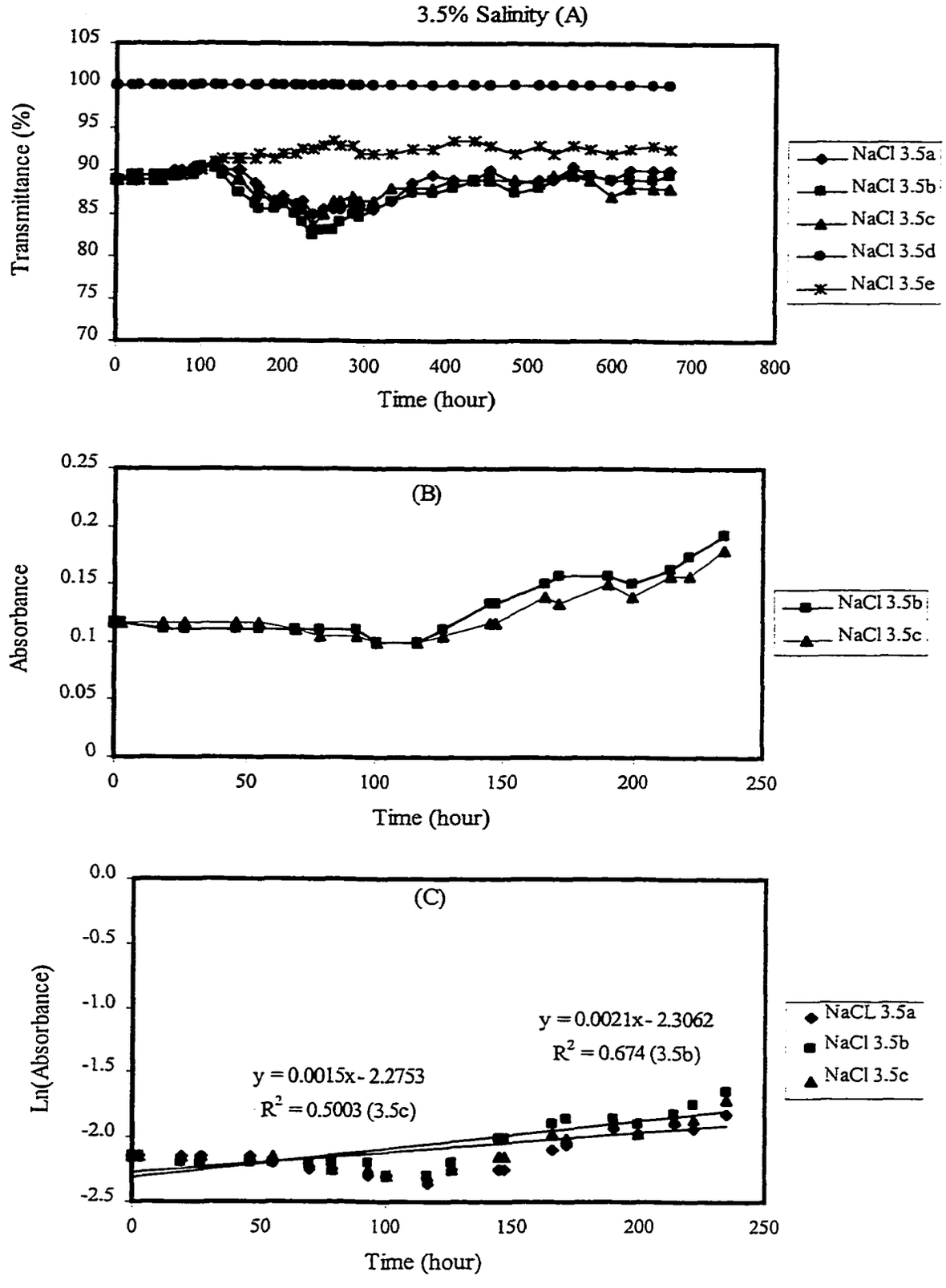


Figure 4.49 Interference of salinity (3.5%) on perchlorate biodegradation

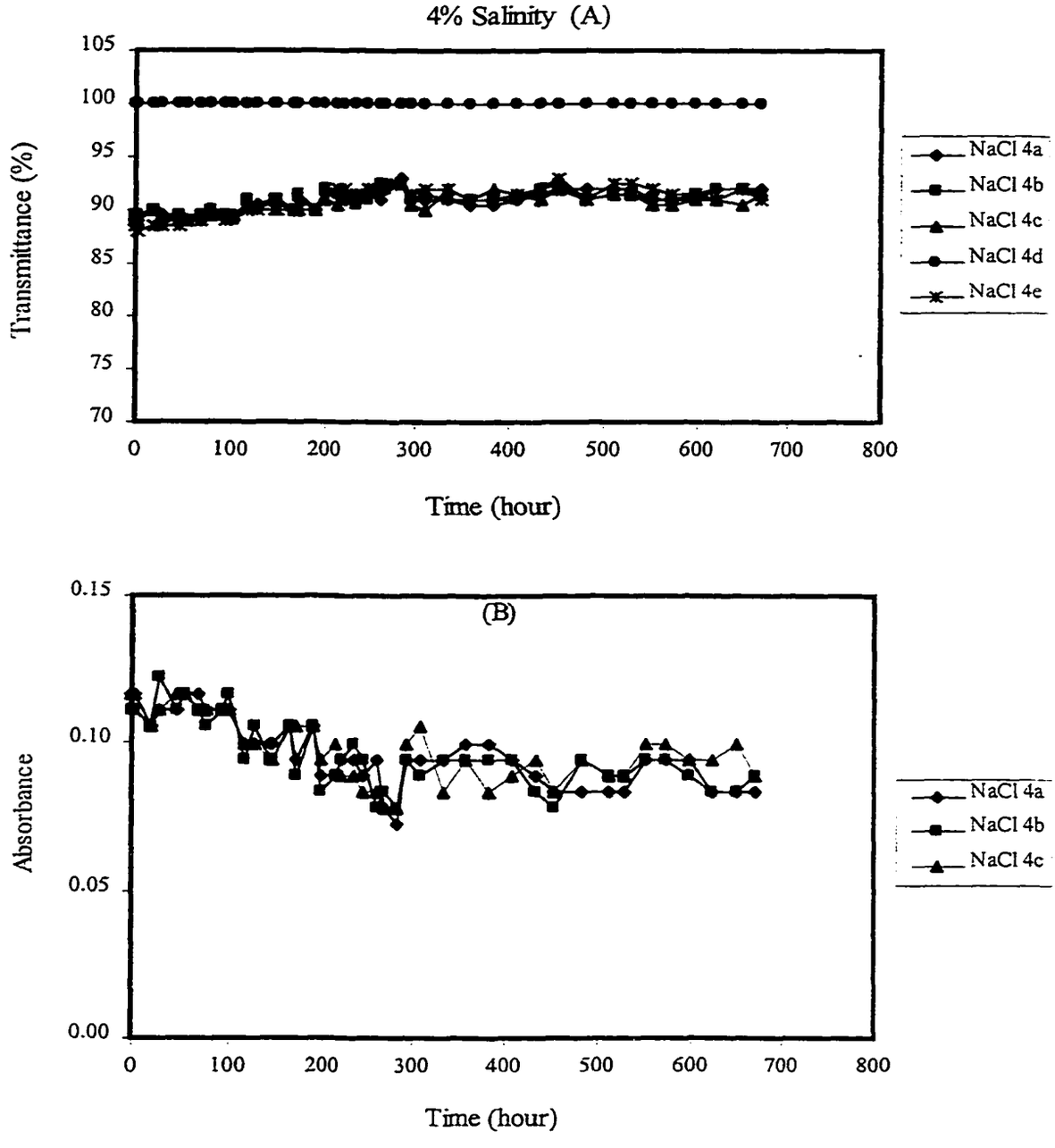


Figure 4.50 Interference of salinity (4.0%) on perchlorate biodegradation

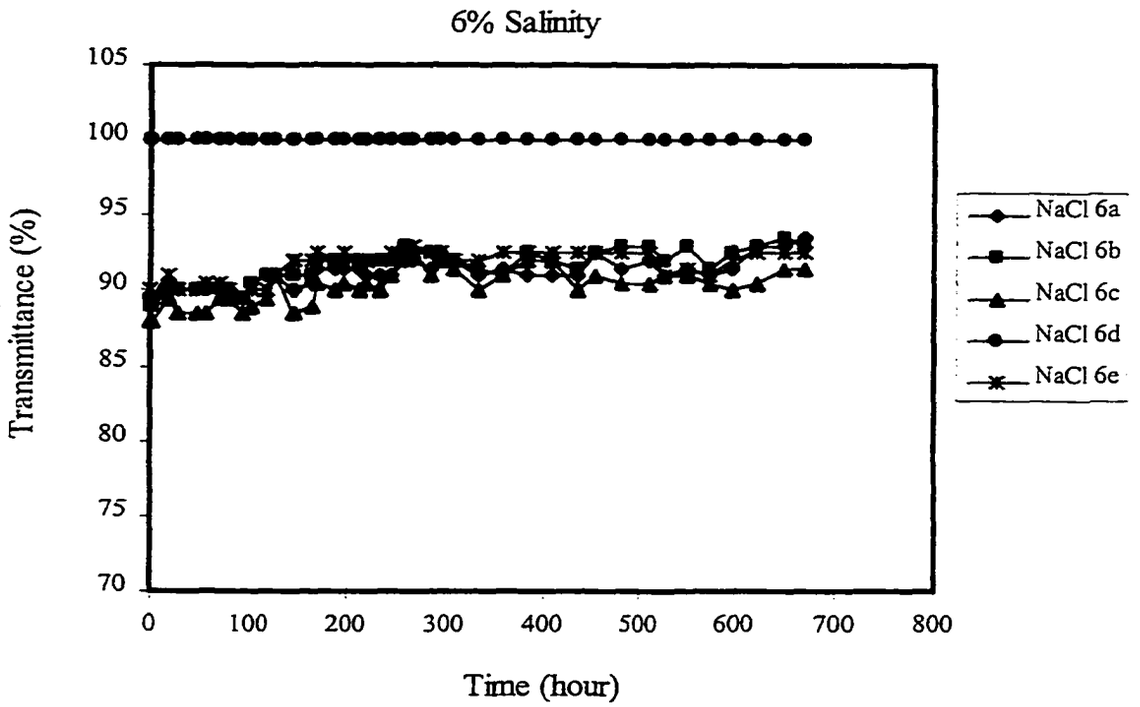


Figure 4.51 Interference of salinity (6.0%) on perchlorate biodegradation

Table 4.53 shows the calculated specific growth rate at different salinity levels as well as the R^2 obtained in fitting the data to a linear model. In the last column of Table 4.53, the fractions of the specific growth rate, for different salinity levels, as compared to the growth rate at 0% salinity are shown.

Notice that the growth rate in the absence of salinity is 0.26 day^{-1}. This value is well within the average growth rate for anaerobes (Rittmann and McCarty, 2001). As the salinity increases, the growth rate decreases. Even at 0.5% salinity (5,000 mg/L as NaCl) 27% reduction in the growth rate occurred. At 3.5 % salt, the growth rate decreases 82 % and at 4% salinity no growth is observed.

Most potable waters have total dissolved solids (TDS) values between 25 and 1000 mg/L (conductivity between 50 and 2000 μmhos/cm), thus for most drinking water

contaminated with perchlorate, the effects of salinity on perchlorate biodegradation may not be of great concern. However, some groundwaters contaminated with perchlorate (e.g. Kerr McGee site in Las Vegas and the Aeroject site in California) have very high TDS and microbial growth would be affected by the salinity. The Kerr McGee groundwater contaminated with perchlorate, for example, has TDS values between 5,000 - 12,000 mg/L (NDEP, 2000).

The major implication of salinity on perchlorate biodegradation, however, relates to the biodegradation of regenerant brines from ion-exchange systems. These brines have salinity varying from 6 to 12% and biodegradation under such conditions would require dilution or isolation of salt tolerant microbes capable of perchlorate biodegradation.

Table 4.53

Specific growth rate in different salinity condition

Salinity Concentration (%)	μ (day^{-1})	R^2	fraction of μ as compared to 0 % salinity
0 %	0.26	0.93	100 %
0.5 %	0.19	0.78	73 %
1.0 %	0.096	0.61	37 %
1.5 %	0.072	0.544	28 %
2.0 %	0.096	0.69	37 %
2.5 %	0.096	0.70	37 %
3.0 %	0.072	0.72	28 %
3.5%	0.048	0.67	18 %
4.0 %	No growth		
> 5 %	No growth		

Microbial Study

Gram-stain photomicrographs of the "BALI' culture show that BALI is a mixed culture with gram-positive and gram-negative microorganisms (Figures 4.52 & 4.53). The gram-negative microorganisms are dominant with the majority being motile rods. Also present are gram-negative microorganisms with a helical morphology. The figures below (Figure 4.52 & Figure 4.53) show Gram-stain slides of samples taken from the batch reactors used in the kinetics study and from the membrane-immobilized biofilm growth reactor. "BALI". The investigation of the characteristics of these microbes (e.g. preferred carbon sources, sensitivity to environmental components, etc) is very important to the performance of bioreactor to remove perchlorate from waters. There have been at least two reports (Coates, 1999 and Malmqvist, 1991) of perchlorate-degrading microorganisms similar to "BALI" isolated from the environment.

Figure 4.52 Gram-stain of "BALI" taken from growth reactor (TK1) showing the predominance of rod and helical gram-negative microorganisms. (magnification: x1000)

Figure 4.53 Gram-stain of "BALI" taken from kinetics study (KC5d) showing helical morphology growing microorganisms (magnification: x1000)

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

From this research, the following conclusions are drawn:

1. The diffusion coefficient for perchlorate through the three membranes tested, Memcor BTS-55, Memcor PVDF and Millipore FGLP, were found to be 6.64x 10^{-6}, 3.75x 10^{-6}, 6.67x 10^{-6} cm^2/sec, respectively. These diffusion coefficients are much smaller than that of perchlorate in water in the absence of a membrane (1.53 x 10^{-5} cm^2/sec) as calculated by the Wilke-Chang method. These results confirm the initial hypothesis of this study that perchlorate would migrate through semi-permeable membrane solely by diffusion.
2. The diffusion coefficient for nitrate through the Memcor BTS-55 membrane was determined to be 4.74 x 10^{-6} cm^2/sec. This data is very close to the value previously reported in the literature for a similar membrane type. However, this diffusion coefficient is much smaller than that of nitrate in water (2.12 x 10^{-5} cm^2/sec).
3. The diffusion coefficient for sulfate through the Memcor BTS-55 membrane was found to be 3.79 x 10^{-6} cm^2/sec. This coefficient is much smaller than that of sulfate in water (1.47 x 10^{-5} cm^2/sec).

4. When comparing the diffusion coefficients of perchlorate, nitrate, and sulfate, as expected from the values of their molar volumes, the diffusivity in water is from the fastest to the slowest: nitrate > perchlorate > sulfate. However, the diffusivity through the BTS-55 membrane follows: perchlorate > nitrate > sulfate. Thus, through the BTS-55 membrane, the diffusivity of nitrate is higher than that of sulfate, as expected from their diffusivities in water. However, perchlorate diffusivity is larger than that of nitrate and sulfate. Thus, sulfate and nitrate contained in perchlorate-contaminated waters may not reach the biofilm located on the BR side of the reactor) as fast as perchlorate and their effects on perchlorate biodegradation may be minimized.
5. Enrichment of a perchlorate-reducing microbial culture, from returned activated sludge taken from the Clark County Wastewater Treatment Plant, was achieved quickly and easily by acclimating the culture anaerobically to perchlorate.
6. With time and after the enrichment culture has been used in several bioreactor tests, the culture developed a red coloration. This culture containing red perchlorate-reducing microorganisms was named "BALI" (for Batista and Liu). Microscopic examination and Gram staining testing revealed that BALI is a mixed culture containing gram-positive and gram-negative microorganisms. The gram-negative microorganisms are dominant with the majority being motile rods. Also present are gram-negative microorganisms with a helical morphology.
7. The development of biofilms on the surface of the membranes was accomplished in a short period by feeding the culture with a high carbon (lactate) to perchlorate ratio (approximately 5:1).

8. Biodegradation of perchlorate through a FGLP membrane- immobilized biofilm was found very slow because of the poor perchlorate diffusion through the thick biofilm developed on the surface of this membrane. This happened because this membrane has a plastic backing that makes microbial attachment easier. It was concluded from the results that the FGLP membrane is not suitable for immobilizing biofilm for perchlorate biodegradation.
9. Biodegradation of perchlorate through a BTS-55 membrane-immobilized biofilm was found very fast. The concentration of perchlorate in the BR reactor, at all times, was very small. The lactate in the BR reactor decreased proportionally to perchlorate in the DR reactor. The molar ratios of perchlorate biodegraded to that of chloride formed were 0.82, 1.03 and 0.86 for three testing cycles. Theoretically, each mole of perchlorate would generate one mole of chloride, thus these ratios are approximately stoichiometric considering the larger size of the reactor used.
10. For the BTS-55 membrane, the thickness of the biofilm during the first testing cycle was found to be 24.7 μm. Perchlorate biodegradation rates were found to be 1.95, 0.9 and 1.75 moles ClO4- biodegraded /day for the three testing cycles. The difference in biodegradation rates in the three cycles may be explained by the change in biofilm thickness during the three testing cycles.
11. During the BTS-55 testing, chloride concentrations changed significantly from one reactor to another. This is attributed to diffusion of chloride through the membrane due to its small size and to movement of water from the DR reactor (smaller concentration of ions) to the BR reactor (higher concentration of ions) by osmotic pressure. Thus, in all the tests performed with this membrane, migration of water

from the DR to the BR reactor was observed. That means, with time, the volume of water in BR increased. The movement of water from the DR to the BR reactor by osmotic pressure, during perchlorate biodegradation, has implications in the design of full-scale membrane-immobilized biofilm systems. Product water (perchlorate free) could migrate to the BR reactor decreasing the total volume of treated water produced. In this study, DI water was used in the DR reactor. However, natural waters contaminated with perchlorate contain several other ions and the difference of ionic gradient, between the DR and BR reactors, may not be as great as that of this test.

12. Biodegradation of perchlorate through a PVDF membrane-immobilized biofilm was found faster than that of the FGLP membrane, but slower than that of the BTS-55 membrane. The biodegradation rate (0.1 moles of perchlorate per day) was much smaller than the values found for the BTS-55 membrane (0.9 to 1.95 moles of perchlorate per day). The PVDF membrane has the smallest diffusion coefficient from all three membranes tested. Therefore, the lower biodegradation rate is the result of low diffusion of perchlorate through the membrane. The perchlorate to chloride ratio for this test was found to be 0.99.
13. The results of the kinetic studies on perchlorate biodegradation showed that the "BALI" culture requires a minimum organic carbon to perchlorate ratio for perchlorate biodegradation to occur at acceptable rates. Under lactate-limited conditions, no microbial growth was observed, except in bottles in which the lactate to perchlorate ratio was equal to three. For all perchlorate-limited bottles, microbial growth was observed. The microbial growth was coupled with a decrease in

perchlorate and lactate concentrations and a stoichiometric increase in chloride concentration. The results further indicated that a lactate to perchlorate ratio of at least three is needed for perchlorate biodegradation to occur at acceptable rates.

14. The results of batch tests on the influence of nitrate on perchlorate biodegradation showed that the rate of nitrate biodegradation was faster than that of perchlorate. These results show that the enrichment mixed culture ("BALI") prefers nitrate to perchlorate as an electron acceptor. Thus, nitrate negatively affects perchlorate biodegradation.
15. The results of nitrate interference on perchlorate biodegradation in the membrane-immobilized biofilm reactor showed: (a) for high perchlorate levels and moderate nitrate levels, the nitrate biodegradation rates are much faster than those of perchlorate. It also showed that at lower nitrate concentration and for the same concentration of lactate (carbon source), perchlorate biodegradation occurred at greater extent; (b) for low perchlorate levels and high nitrate levels, the presence of nitrate will significantly affect perchlorate biodegradation, even when the concentration of perchlorate is very low (1mg/L). The results seem to strongly suggest that the negative effect of nitrate on perchlorate biodegradation is more significant when the perchlorate concentrations are lower. Thus, for drinking waters moderately contaminated with perchlorate, the presence of nitrate will significantly hinder perchlorate biodegradation. This findings imply higher cost for the design of perchlorate removal systems because of increased carbon source (e.g.: lactate) usage and larger reactor volume needed for biodegradation.

16. The results of sulfate interference on perchlorate biodegradation in batch testing showed that perchlorate biodegradation occurred before sulfate reduction. Sulfate biodegradation, after almost all the perchlorate had been reduced, was very slow.
17. The results of the interference of sulfate on perchlorate biodegradation in BTS-55 membrane-immobilized biofilm reactors showed that the higher the initial perchlorate concentration, the higher the perchlorate biodegradation rate, independent of the initial sulfate concentration. The sulfate biodegradation rates, in the presence of perchlorate, were much smaller (or null) than those of perchlorate. In the absence of perchlorate, however, the sulfate biodegradation rates were larger than those in the presence of perchlorate, except for the case in which the perchlorate and sulfate concentrations were about 50 mg/L. The reason for that is not clear from the data available. However, it can be concluded that sulfate does not affect perchlorate biodegradation indicating that the"BALI" mixed culture prefers perchlorate to sulfate as an electron acceptor.
18. The studies on the interference of salinity on perchlorate biodegradation showed that as the salinity increases, the growth rate decreases. Even at 0.5% salinity (5,000 mg/L as NaCl) 27% reduction in the growth rate occurred. At 3.5 % salt, the growth rate decreases 82 % and at 4% salinity no growth was observed. However, most potable waters have total dissolved solids (TDS) values between 25 and 1000 mg/L (conductivity between 50 and 2000 μmhos/cm), thus for most drinking water contaminated with perchlorate, the effects of salinity on perchlorate biodegradation may not be of great concern. Some groundwaters contaminated with perchlorate have very high TDS and microbial growth would be affected by the salinity. The

major implication of salinity on perchlorate biodegradation, however, relates to the biodegradation of regenerant brines from ion-exchange systems. These brines have salinity varying from 6 to 12% and biodegradation under such conditions would require dilution or isolation of salt tolerant microbes capable of perchlorate biodegradation.

The following topics deserve further research:

Perchlorate-reducing enrichment culture:

1. To determine the maximum growth rate and the half-saturation constant for the mixed "BALI" culture growing on perchlorate.
2. To fully characterize the "BALI" culture by isolating and identifying the major bacteria strains that compose the mixed culture.
3. To determine perchlorate biodegradation rates for every isolated microorganism from the "BALI" culture.
4. To determine the biodegradation kinetics for each isolate using different electron donors (e.g. lactate, acetate and hydrogen).
5. To determine, for every microbial isolate, the preferred electron donors and electron acceptors.

Membrane-immobilized biofilm reactor:

6. To improve the design of the biofilm growth chamber to ensure the growth of biofilms with homogenous thickness on the membrane.

7. To determine optimum perchlorate to electron donor ratios, so that the presence of organic carbon in the finished water is minimized.
8. To test the reactor using hydrogen as an electron donor.
9. To determine the optimum alkalinity concentration needed, when hydrogen is used as the electron donor, to promote microbial growth.
10. To determine the ionic concentration in the BR reactor needed to balance the DR reactor's ionic concentration, so that water movement by osmotic pressure from one reactor to another will be minimized.
11. To determine the interference of other factors, such as temperature, pH, and other organic contaminants, on perchlorate biodegradation by the biofilm.

APPENDIX A

RAW DATA FOR SALINITY TESTS

Table A-1

The interference of different salinity levels on perchlorate biodegradation

(Raw data -- Figure 4.41)

Time (hour)	Transmittance (%)									
	NaCl 0a	NaCl 0.5b	NaCl 1a	NaCl 1.5b	NaCl 2b	NaCl 2.5b	NaCl 3b	NaCl 3.5c	NaCl 4b	NaCl 6a
0	89.5	89.5	89.5	89	89	89	89	89	89.5	89.5
3	89	89.5	89.5	89	88.5	89	88.5	89	89.5	89.5
19	87.5	89	89.5	89	89	89.5	89	89	90	90
27	87.5	88.5	89	89	89	89	88.5	89	88.5	90
46	78.5	82	86	88.5	89	89.5	89	89	89.5	90
54.5	76	79	80.5	86.5	89	89.5	89	89	89	90
69	76.5	79	80.5	81	86.5	88.5	89	89.5	89.5	90
78	76.5	79	80.5	81.5	86	88.5	89	90	90	90
93	76.5	78.5	80.5	81.5	83	85.5	89.5	90	89.5	89.5
101	76.5	79	80.5	81.5	83	84	87.5	90.5	89	90.5
117	76.5	79	81.5	82	83	80.5	86	90.5	91	91
127	76	79.5	81.5	82.5	84	80.5	85.5	90	90	91
145	50.5	78	82	82.5	84	81.5	84	89	91	90
147	50.5	78	82	82.5	84	81.5	84	89	91	90
166	55	58	82	83.5	85.5	84	82	87	90	90.5
171	54.5	53	82	84.5	85.5	84	82.5	87.5	91.5	91.5
190	42.5	57.5	82	84.5	86	84	83	86	90	91.5
199	40.5	61	81.5	84.5	87	85.5	84.5	87	92	91.5
214.5	28	70.5	57.5	84.5	87	85.5	84.5	85.5	91.5	91.5
222	24.5	71.5	58	85	87	85	83.5	85.5	91	91
235	28.5	71.5	62	83.5	85.5	85.5	85.5	83.5	90.5	91
247	28.5	71.5	63.5	76.5	86.5	86.5	85.5	85	91	91
261	29	71.5	73.5	65.5	87	86	85.5	86.5	92.5	92
268.5	30	71.5	75	68	87	86.5	86	86.5	92	92
285	30.5	72	74.5	71	86.5	86.5	86	87	92.5	91.5
295	30.5	71	75	72	85	86.5	85.5	86.5	91	92.5
310	31.5	72	75	73.5	86	85	85.5	86.5	91.5	92
334	32	72	75.5	77.5	86.5	86	86	88	91	91
359	32.5	72.5	75.5	78.5	86	86.5	87	88	91	91.5
385	34	72.5	76	80	86	87	87	88	91	91
410	34	73	78	81.5	86.5	87	87	89	91	91
435	35	73.5	78	82	87	87.5	87.5	89	92	91
454	36.5	74	78.5	83	87.5	87	87.5	89	92.5	92.5
482	37	74	78	82.5	87.5	86.5	87	89	91	91.5
512	38.5	74.5	80	82	88	87.5	87.5	88.5	91.5	92
529	39	74.5	78	81.5	86.5	87.5	87.5	89.5	91.5	91
552	40	75.5	79.5	82	87	87	88	89.5	91	91.5
573	41	75.5	79.5	82	87.5	87	87.5	89	91	91
598	41.5	75	79	81	87	87	88	87	91.5	91.5
622	42.5	76	80	81	87.5	87.5	88.5	88	92	93
650	42.5	76	80	80.5	88	87.5	87.5	88	92	93
671.5	43.5	76	79	80	86.5	87.5	87.5	88	91.5	93.5

Table A-2

The interference of salinity on perchlorate biodegradation (0 %)

(Raw data -- Figure 4.42 A, B & C)

Time	NaCl 0a			NaCl 0b			NaCl 0c			NaCl 0d	NaCl 0e
(hr)	Trans	Abs.	Ln(Abs.)	Trans.	Abs.	Ln(Abs)	Trans	Abs.	Ln(Abs.)		
0	89.5	**0.1109**	**-2.1988**	88	**0.1278**	**-2.057**	89	0.11653	-2.15	100	89.5
3	89	**0.1165**	**-2.1496**	88	**0.1278**	**-2.057**	89	0.11653	-2.15	100	89
19	87.5	**0.1335**	**-2.0134**	88	**0.1278**	**-2.057**	89	0.11653	-2.15	100	89
27	87.5	**0.1335**	**-2.0134**	88	**0.1278**	**-2.057**	89	0.11653	-2.15	100	89
46	78.5	**0.2421**	**-1.4185**	76.5	**0.2679**	**-1.3172**	79	0.23572	-1.445	100	89
54.5	76	**0.2744**	**-1.293**	76	**0.2744**	**-1.293**	79.5	0.22941	-1.472	100	89
69	76.5	**0.2679**	**-1.3172**	76	**0.2744**	**-1.293**	79.5	0.22941	-1.472	100	89.5
78	76.5	**0.2679**	**-1.3172**	76	**0.2744**	**-1.293**	79	0.23572	-1.445	100	89.5
93	76.5	**0.2679**	**-1.3172**	76	**0.2744**	**-1.293**	79	0.23572	-1.445	100	89.5
101	76.5	**0.2679**	**-1.3172**	76.5	**0.2679**	**-1.3172**	79	0.23572	-1.445	100	89.5
117	76.5	**0.2679**	**-1.3172**	75	**0.2877**	**-1.2459**	78.5	0.24207	-1.419	100	89
127	76	**0.2744**	**-1.293**	75	**0.2877**	**-1.2459**	78	0.24846	-1.392	100	90.5
145	50.5	**0.6832**	**-0.381**	52	**0.6539**	**-0.4248**	72	0.3285	-1.113	100	90
147	50.5	**0.6832**	**-0.381**	52	**0.6539**	**-0.4248**	72	0.3285	-1.113	100	90
166	55	**0.5978**	**-0.5144**	55	**0.5978**	**-0.5144**	55	0.59784	-0.514	100	89.5
171	54.5	**0.607**	**-0.4993**	52	**0.6539**	**-0.4248**	55	0.59784	-0.514	100	90
190	42.5	**0.8557**	**-0.1559**	41.5	**0.8795**	**-0.1284**	55.5	0.58879	-0.53	100	89
199	40.5	**0.9039**	**-0.1011**	35	**1.0498**	**0.0486**	57	0.56212	-0.576	100	89.5
214.5	28	**1.273**	**0.2413**	25.5	**1.3665**	**0.3122**	55.5	0.58879	-0.53	100	89
222	24.5	**1.4065**	**0.3411**	27.5	1.291	0.2554	55.5	0.58879	-0.53	100	89
235	28.5	1.2553	0.2273	28	1.273	0.2413	58	0.54473	-0.607	100	89
247	28.5	1.2553	0.2273	28	1.273	0.2413	60	0.51083	-0.672	100	89
261	29	1.2379	0.2134	29	1.2379	0.2134	62	0.47804	-0.738	100	89
268.5	30	1.204	0.1856	29.5	1.2208	0.1995	63	0.46204	-0.772	100	89
285	30.5	1.1874	0.1718	29.5	1.2208	0.1995	64	0.44629	-0.807	100	89
295	30.5	1.1874	0.1718	29.5	1.2208	0.1995	63	0.46204	-0.772	100	89
310	31.5	1.1552	0.1443	30	1.204	0.1856	59.5	0.51919	-0.655	100	88.5
334	32	1.1394	0.1305	31	1.1712	0.158	59	0.52763	-0.639	100	88
359	32.5	1.1239	0.1168	32	1.1394	0.1305	60.5	0.50253	-0.688	100	88
385	34	1.0788	0.0759	33	1.1087	0.1032	62.5	0.47	-0.755	100	88
410	34	1.0788	0.0759	33.5	1.0936	0.0895	62.5	0.47	-0.755	100	87.5
435	35	1.0498	0.0486	35.5	1.0356	0.035	40.5	0.90387	-0.101	100	88
454	36.5	1.0079	0.0078	35.5	1.0356	0.035	34	1.07881	0.0759	100	88
482	37	0.9943	-0.0058	36	1.0217	0.0214	35	1.04982	0.0486	100	88
512	38.5	0.9545	-0.0466	37.5	0.9808	-0.0194	36	1.02165	0.0214	100	89
529	39	0.9416	-0.0602	38	0.9676	-0.033	36	1.02165	0.0214	100	88
552	40	0.9163	-0.0874	38.5	0.9545	-0.0466	37	0.99425	-0.006	100	88
573	41	0.8916	-0.1147	40	0.9163	-0.0874	37.5	0.98083	-0.019	100	88
598	41.5	0.8795	-0.1284	40.5	0.9039	-0.1011	37.5	0.98083	-0.019	100	88
622	42.5	0.8557	-0.1559	42	0.8675	-0.1421	38.5	0.95451	-0.047	100	88
650	42.5	0.8557	-0.1559	42	0.8675	-0.1421	39	0.94161	-0.06	100	88
671.5	43.5	0.8324	-0.1834	42.5	0.8557	-0.1559	39.5	0.92887	-0.074	100	88

Table A-3

The interference of salinity on perchlorate biodegradation (0.5 %)

(Raw data -- Figure 4.43 A, B & C)

Time (hr)	NaCl 0.5a			NaCl 0.5b			NaCl 0.5c			NaCl 0.5d	NaCl 0.5e
	Trans.	Abs.	Ln(Abs.)	Trans.	Abs.	Ln(Abs.)	Trans.	Abs.	Ln(Abs.)	Trans.	Tran.
0	90	0.11	-2.199	90	**0.111**	**-2.2**	89	**0.117**	**-2.15**	100	89.5
3	90	0.11	-2.199	90	**0.111**	**-2.2**	88	**0.128**	**-2.057**	100	89.5
19	89	0.12	-2.15	89	**0.117**	**-2.15**	89	**0.122**	**-2.102**	100	89.5
27	89	0.12	-2.15	89	**0.122**	**-2.1**	89	**0.122**	**-2.102**	100	89.5
46	81	0.21	-1.557	82	**0.198**	**-1.62**	82	**0.198**	**-1.617**	100	88.5
54.5	80	0.23	-1.472	79	**0.236**	**-1.45**	82	**0.205**	**-1.587**	100	88.5
69	80	0.23	-1.472	79	**0.236**	**-1.45**	82	**0.205**	**-1.587**	100	88.5
78	80	0.22	-1.5	79	**0.236**	**-1.45**	82	**0.198**	**-1.617**	100	88.5
93	80	0.23	-1.472	79	**0.242**	**-1.42**	82	**0.205**	**-1.587**	100	88
101	79	0.24	-1.445	79	**0.236**	**-1.45**	82	**0.205**	**-1.587**	100	88
117	80	0.23	-1.472	79	**0.236**	**-1.45**	81	**0.211**	**-1.557**	100	88
127	80	0.23	-1.472	80	**0.229**	**-1.47**	81	**0.211**	**-1.557**	100	88
145	78	0.25	-1.392	78	**0.248**	**-1.39**	80	**0.223**	**-1.5**	100	87.5
147	78	0.25	-1.392	78	**0.248**	**-1.39**	80	**0.223**	**-1.5**	100	87.5
166	52	0.66	-0.41	58	**0.545**	**-0.61**	64	**0.454**	**-0.789**	100	87.5
171	52	0.65	-0.425	53	**0.635**	**-0.45**	53	**0.635**	**-0.454**	100	87.5
190	58	0.54	-0.607	58	0.553	-0.59	58	0.545	-0.607	100	88
199	61	0.5	-0.688	61	0.494	-0.7	62	0.486	-0.721	100	88
214.5	71	0.35	-1.051	71	0.35	-1.05	72	0.335	-1.092	100	88
222	72	0.34	-1.092	72	0.335	-1.09	73	0.315	-1.156	100	88.5
235	71	0.34	-1.072	72	0.335	-1.09	73	0.315	-1.156	100	88
247	72	0.34	-1.092	72	0.335	-1.09	73	0.315	-1.156	100	88
261	71	0.34	-1.072	72	0.335	-1.09	73	0.315	-1.156	100	88
268.5	71	0.35	-1.051	72	0.335	-1.09	73	0.315	-1.156	100	89
285	71	0.35	-1.051	72	0.329	-1.11	74	0.308	-1.178	100	88.5
295	71	0.35	-1.051	71	0.342	-1.07	72	0.329	-1.113	100	87
310	72	0.33	-1.113	72	0.329	-1.11	73	0.322	-1.134	100	87.5
334	66	0.42	-0.878	72	0.329	-1.11	73	0.322	-1.134	100	88
359	64	0.45	-0.807	73	0.322	-1.13	74	0.308	-1.178	100	89
385	65	0.44	-0.824	73	0.322	-1.13	75	0.294	-1.223	100	88
410	67	0.41	-0.897	73	0.315	-1.16	75	0.294	-1.223	100	88
435	69	0.38	-0.972	74	0.308	-1.18	75	0.294	-1.223	100	88
454	70	0.36	-1.011	74	0.301	-1.2	76	0.281	-1.269	100	88
482	70	0.36	-1.011	74	0.301	-1.2	75	0.294	-1.223	100	88
512	73	0.31	-1.156	75	0.294	-1.22	76	0.281	-1.269	100	88.5
529	72	0.34	-1.092	75	0.294	-1.22	75	0.288	-1.246	100	88
552	66	0.42	-0.878	76	0.281	-1.27	76	0.281	-1.269	100	88
573	33	1.11	0.103	76	0.281	-1.27	76	0.274	-1.293	100	88
598	33	1.11	0.103	75	0.288	-1.25	76	0.281	-1.269	100	88
622	35	1.05	0.049	76	0.274	-1.29	76	0.281	-1.269	100	88
650	35	1.05	0.049	76	0.274	-1.29	76	0.274	-1.293	100	87
671.5	35	1.05.	0.049	76	0.274	-1.29	77	0.268	-1.317	100	87

Table A-4

The interference of salinity on perchlorate biodegradation (1 %)

(Raw data -- Figure 4.44 A, B & C)

Time	NaCl 1a			NaCl 1b			NaCl 1c			NaCl 1d	NaCl 1e
(hr)	Trans.	Abs.	Ln(Abs.)	Trans.	Abs.	Ln(Abs.)	Trans.	Abs.	Ln(Abs.)	Trans.	Trans.
0	90	**0.111**	**-2.199**	89	**0.117**	**-2.15**	89	**0.117**	**-2.15**	100	89
3	90	**0.111**	**-2.199**	89	**0.117**	**-2.15**	89	**0.117**	**-2.15**	100	89
19	90	**0.111**	**-2.199**	89	**0.117**	**-2.15**	89	**0.117**	**-2.15**	100	89.5
27	89	**0.117**	**-2.15**	89	**0.117**	**-2.15**	88.5	**0.122**	**-2.1**	100	89
46	86	**0.151**	**-1.892**	85	**0.163**	**-1.817**	86	**0.151**	**-1.89**	100	89
54.5	81	**0.217**	**-1.528**	80.5	**0.217**	**-1.528**	81.5	**0.205**	**-1.59**	100	89
69	81	**0.217**	**-1.528**	80.5	**0.217**	**-1.528**	81.5	**0.205**	**-1.59**	100	90
78	81	**0.217**	**-1.528**	80.5	**0.217**	**-1.528**	81	**0.211**	**-1.56**	100	89.5
93	81	**0.217**	**-1.528**	80.5	**0.217**	**-1.528**	81	**0.211**	**-1.56**	100	88.5
101	81	**0.217**	**-1.528**	80	**0.223**	**-1.5**	80.5	**0.217**	**-1.53**	100	88
117	82	**0.205**	**-1.587**	81	**0.211**	**-1.557**	81.5	**0.205**	**-1.59**	100	89
127	82	**0.205**	**-1.587**	81.5	**0.205**	**-1.587**	81.5	**0.205**	**-1.59**	100	88
145	82	**0.198**	**-1.617**	81.5	**0.205**	**-1.587**	82	**0.198**	**-1.62**	100	88.5
147	82	**0.198**	**-1.617**	81.5	**0.205**	**-1.587**	82	**0.198**	**-1.62**	100	88.5
166	82	**0.198**	**-1.617**	82.5	**0.192**	**-1.648**	82.5	**0.192**	**-1.65**	100	88
171	82	**0.198**	**-1.617**	82.5	**0.192**	**-1.648**	82.5	**0.192**	**-1.65**	100	88
190	82	**0.198**	**-1.617**	81.5	**0.205**	**-1.587**	82.5	**0.192**	**-1.65**	100	88
199	82	**0.205**	**-1.587**	79	**0.236**	**-1.445**	82	**0.198**	**-1.62**	100	88
214.5	58	**0.553**	**-0.592**	55.5	**0.589**	**-0.53**	79.5	**0.229**	**-1.47**	100	88.5
222	58	0.545	-0.607	59.5	0.519	-0.655	73	**0.315**	**-1.16**	100	89
235	62	0.478	-0.738	62	0.478	-0.738	57.5	**0.553**	**-0.59**	100	88.5
247	64	0.454	-0.789	65.5	0.423	-0.86	60.5	0.503	-0.69	100	88.5
261	74	0.308	-1.178	75.5	0.281	-1.269	63.5	0.454	-0.79	100	88.5
268.5	75	0.288	-1.246	75.5	0.281	-1.269	68	0.386	-0.95	100	89
285	75	0.294	-1.223	75	0.288	-1.246	75	0.288	-1.25	100	87.5
295	75	0.288	-1.246	75	0.288	-1.246	75	0.288	-1.25	100	86.5
310	75	0.288	-1.246	75.5	0.281	-1.269	75.5	0.281	-1.27	100	86.5
334	76	0.281	-1.269	76	0.274	-1.293	75	0.288	-1.25	100	87.5
359	76	0.281	-1.269	75.5	0.281	-1.269	75.5	0.281	-1.27	100	86.5
385	76	0.274	-1.293	76.5	0.268	-1.317	75.5	0.281	-1.27	100	86.5
410	78	0.248	-1.392	78	0.248	-1.392	76	0.274	-1.29	100	88
435	78	0.248	-1.392	78.5	0.242	-1.419	77	0.261	-1.34	100	87.5
454	79	0.242	-1.419	78.5	0.242	-1.419	77.5	0.255	-1.37	100	88
482	78	0.248	-1.392	78.5	0.242	-1.419	76	0.274	-1.29	100	88.5
512	80	0.223	-1.5	80	0.223	-1.5	78	0.248	-1.39	100	88.5
529	78	0.248	-1.392	78.5	0.242	-1.419	77	0.261	-1.34	100	87.5
552	80	0.229	-1.472	80	0.223	-1.5	79	0.236	-1.45	100	88
573	80	0.229	-1.472	80	0.223	-1.5	78.5	0.242	-1.42	100	88
598	79	0.236	-1.445	80	0.223	-1.5	78	0.248	-1.39	100	87.5
622	80	0.223	-1.5	80.5	0.217	-1.528	78.5	0.242	-1.42	100	88
650	80	0.223	-1.5	80	0.223	-1.5	78	0.248	-1.39	100	88
671.5	79	0.236	-1.445	80.5	0.217	-1.528	79	0.236	-1.45	100	87.5

Table A-5

The interference of salinity on perchlorate biodegradation (1.5 %)

(Raw data -- Figure 4.45 A, B & C)

Time	NaCl 1.5a			NaCl 1.5b			NaCl 1.5c			NaCl 1.5d	NaCl 1.5e
(hr)	Trans.	Abs.	Ln(Abs.)	Trans	Abs.	Ln(Abs.)	Trans.	Abs.	Ln(Abs.)	Trans.	Trans.
0	89	0.12	-2.15	89	**0.1165**	**-2.15**	89	**0.12**	**-2.14957**	100	89
3	89	0.12	-2.15	89	**0.1165**	**-2.15**	89	**0.12**	**-2.14957**	100	89
19	89	0.12	-2.15	89	**0.1165**	**-2.15**	89	**0.12**	**-2.14957**	100	89.5
27	89	0.12	-2.15	89	**0.1165**	**-2.15**	89	**0.12**	**-2.14957**	100	89
46	88.5	0.12	-2.102	88.5	**0.1222**	**-2.102**	88.5	**0.12**	**-2.10236**	100	89
54.5	86.5	0.15	-1.931	86.5	**0.145**	**-1.931**	88	**0.13**	**-2.05703**	100	89.5
69	82	0.2	-1.617	81	**0.2107**	**-1.557**	81	**0.21**	**-1.55722**	100	89.5
78	82	0.2	-1.617	81.5	**0.2046**	**-1.587**	81.5	**0.2**	**-1.58686**	100	89.5
93	82	0.2	-1.617	81.5	**0.2046**	**-1.587**	81.5	**0.2**	**-1.58686**	100	89
101	82	0.2	-1.617	81.5	**0.2046**	**-1.587**	81.5	**0.2**	**-1.58686**	100	88
117	82	0.2	-1.617	82	**0.1985**	**-1.617**	82	**0.2**	**-1.61721**	100	88.5
127	82	0.2	-1.617	82.5	**0.1924**	**-1.648**	83	**0.19**	**-1.68024**	100	88
145	81.5	0.2	-1.587	82.5	**0.1924**	**-1.648**	83	**0.19**	**-1.68024**	100	88
147	81.5	0.2	-1.587	82.5	**0.1924**	**-1.648**	83	**0.19**	**-1.68024**	100	88
166	83	0.19	-1.68	83.5	**0.1803**	**-1.713**	83.5	**0.18**	**-1.713**	100	88
171	83.5	0.18	-1.713	84.5	**0.1684**	**-1.781**	84	**0.17**	**-1.74667**	100	88
190	83.5	0.18	-1.713	84.5	**0.1684**	**-1.781**	84	**0.17**	**-1.74667**	100	88
199	84	0.17	-1.747	84.5	**0.1684**	**-1.781**	84.5	**0.17**	**-1.7813**	100	88.5
214.5	84.5	0.17	-1.781	84.5	**0.1684**	**-1.781**	84	**0.17**	**-1.74667**	100	88
222	85	0.16	-1.817	85	**0.1625**	**-1.817**	84.5	**0.17**	**-1.7813**	100	88.5
235	84.5	0.17	-1.781	83.5	**0.1803**	**-1.713**	81	**0.21**	**-1.55722**	100	88
247	84	0.17	-1.747	76.5	**0.2679**	**-1.317**	72	**0.33**	**-1.11321**	100	88
261	84.5	0.17	-1.781	65.5	**0.4231**	**-0.86**	65	**0.43**	**-0.84215**	100	88
268.5	86	0.15	-1.892	68	0.3857	-0.953	68	0.39	-0.95279	100	88.5
285	85.5	0.16	-1.854	71	0.3425	-1.072	72	0.33	-1.11321	100	88.5
295	84.5	0.17	-1.781	72	0.3285	-1.113	72	0.33	-1.11321	100	88
310	85	0.16	-1.817	73.5	0.3079	-1.178	73.5	0.31	-1.17803	100	88
334	84.5	0.17	-1.781	77.5	0.2549	-1.367	77	0.26	-1.34184	100	89
359	84.5	0.17	-1.781	78.5	0.2421	-1.419	78	0.25	-1.39247	100	88.5
385	85	0.16	-1.817	80	0.2231	-1.5	79	0.24	-1.4451	100	88
410	85	0.16	-1.817	81.5	0.2046	-1.587	80	0.22	-1.49994	100	88
435	85.5	0.16	-1.854	82	0.1985	-1.617	81.5	0.2	-1.58686	100	89
454	86	0.15	-1.892	83	0.1863	-1.68	81.5	0.2	-1.58686	100	89
482	83.5	0.18	-1.713	82.5	0.1924	-1.648	81	0.21	-1.55722	100	88
512	84	0.17	-1.747	82	0.1985	-1.617	81	0.21	-1.55722	100	88.5
529	82	0.2	-1.617	81.5	0.2046	-1.587	81	0.21	-1.55722	100	88
552	82	0.2	-1.617	82	0.1985	-1.617	80.5	0.22	-1.52826	100	88
573	80	0.22	-1.5	82	0.1985	-1.617	81	0.21	-1.55722	100	88
598	76	0.27	-1.293	81	0.2107	-1.557	80.5	0.22	-1.52826	100	88
622	71	0.34	-1.072	81	0.2107	-1.557	80.5	0.22	-1.52826	100	88
650	66.5	0.41	-0.897	80.5	0.2169	-1.528	79.5	0.23	-1.47223	100	88
671.5	64.5	0.44	-0.824	80	0.2231	-1.5	79.5	0.23	-1.47223	100	88.5

Table A-6

The interference of salinity on perchlorate biodegradation (2 %)

(Raw data -- Figure 4.46 A, B & C)

Time	NaCl 2a			NaCl 2b			NaCl 2c			NaCl 2d	NaCl 2e
(hr)	Trans.	Abs.	Ln(Abs.)	Trans.	Abs.	Ln(Abs.)	Trans.	Abs.	Ln(Abs.)	Trans.	Trans
0	88	**0.1278**	**-2.05703**	89	**0.1165**	**-2.14957**	89	**0.117**	**-2.15**	100	89
3	88.5	**0.1222**	**-2.10236**	89	**0.1222**	**-2.10236**	89	**0.117**	**-2.15**	100	89
19	88.5	**0.1222**	**-2.10236**	89	**0.1165**	**-2.14957**	89	**0.117**	**-2.15**	100	89
27	88.5	**0.1222**	**-2.10236**	89	**0.1165**	**-2.14957**	89	**0.117**	**-2.15**	100	89
46	88	**0.1278**	**-2.05703**	89	**0.1165**	**-2.14957**	89	**0.117**	**-2.15**	100	89.5
54.5	88	**0.1278**	**-2.05703**	89	**0.1165**	**-2.14957**	89	**0.117**	**-2.15**	100	89.5
69	85	**0.1625**	**-1.81696**	87	**0.145**	**-1.93084**	86.5	**0.145**	**-1.931**	100	89
78	84	**0.1744**	**-1.74667**	86	**0.1508**	**-1.89165**	86	**0.151**	**-1.892**	100	89.5
93	81.5	**0.2046**	**-1.58686**	83	**0.1863**	**-1.68024**	83	**0.186**	**-1.68**	100	89
101	79	**0.2357**	**-1.4451**	83	0.1863	-1.68024	83	0.186	-1.68	100	89
117	81.5	0.2046	-1.58686	83	0.1863	-1.68024	84	0.174	-1.747	100	88
127	81.5	0.2046	-1.58686	84	0.1744	-1.74667	84	0.174	-1.747	100	89
145	81.5	0.2046	-1.58686	84	0.1744	-1.74667	82	0.198	-1.617	100	88
147	81.5	0.2046	-1.58686	84	0.1744	-1.74667	82	0.198	-1.617	100	88
166	83.5	0.1803	-1.713	86	0.1567	-1.85372	83.5	0.18	-1.713	100	88
171	83.5	0.1803	-1.713	86	0.1567	-1.85372	84.5	0.168	-1.781	100	88.5
190	84	0.1744	-1.74667	86	0.1508	-1.89165	84.5	0.168	-1.781	100	88
199	84.5	0.1684	-1.7813	87	0.1393	-1.9714	86	0.151	-1.892	100	88
214.5	85	0.1625	-1.81696	87	0.1393	-1.9714	86	0.151	-1.892	100	88
222	84.5	0.1684	-1.7813	87	0.1393	-1.9714	85	0.163	-1.817	100	88
235	84.5	0.1684	-1.7813	86	0.1567	-1.85372	85.5	0.157	-1.854	100	88
247	86.5	0.145	-1.93084	87	0.145	-1.93084	86.5	0.145	-1.931	100	88
261	85.5	0.1567	-1.85372	87	0.1393	-1.9714	87	0.139	-1.971	100	88
268.5	87	0.1393	-1.9714	87	0.1393	-1.9714	87	0.139	-1.971	100	88
285	86.5	0.145	-1.93084	87	0.145	-1.93084	87	0.139	-1.971	100	88
295	85.5	0.1567	-1.85372	85	0.1625	-1.81696	86.5	0.145	-1.931	100	87.5
310	85.5	0.1567	-1.85372	86	0.1508	-1.89165	85.5	0.157	-1.854	100	88
334	86.5	0.145	-1.93084	87	0.145	-1.93084	86.5	0.145	-1.931	100	89
359	86.5	0.145	-1.93084	86	0.1508	-1.89165	87	0.139	-1.971	100	88
385	86	0.1508	-1.89165	86	0.1508	-1.89165	87	0.139	-1.971	100	88
410	87	0.1393	-1.9714	87	0.145	-1.93084	88	0.128	-2.057	100	88.5
435	88	0.1278	-2.05703	87	0.1393	-1.9714	88	0.128	-2.057	100	88.5
454	88.5	0.1222	-2.10236	88	0.1335	-2.01342	87.5	0.134	-2.013	100	89
482	87	0.1393	-1.9714	88	0.1335	-2.01342	87.5	0.134	-2.013	100	88.5
512	87.5	0.1335	-2.01342	88	0.1278	-2.05703	88	0.128	-2.057	100	88.5
529	86.5	0.145	-1.93084	87	0.145	-1.93084	87	0.139	-1.971	100	88
552	86.5	0.145	-1.93084	87	0.1393	-1.9714	87.5	0.134	-2.013	100	88
573	86	0.1508	-1.89165	88	0.1335	-2.01342	86.5	0.145	-1.931	100	89
598	85.5	0.1567	-1.85372	87	0.1393	-1.9714	86.5	0.145	-1.931	100	88
622	85.5	0.1567	-1.85372	88	0.1335	-2.01342	86.5	0.145	-1.931	100	88.5
650	84.5	0.1684	-1.7813	88	0.1278	-2.05703	87	0.139	-1.971	100	88.5
671.5	84.5	0.1684	-1.7813	87	0.145	-1.93084	86.5	0.145	-1.931	100	88

Table A-7

The interference of salinity on perchlorate biodegradation (2.5 %)

(Raw data -- Figure 4.47 A, B & C)

Time	NaCl 2.5a			NaCl 2.5b			NaCl 2.5c			NaCl 2.5d	NaCl 2.5e
(hr)	Trans.	Abs.	Ln(Abs.)	Trans	Abs.	Ln(Abs.)	Trans	Abs.	Ln(Abs.)	Trans.	Trans.
0	89	**0.1165**	**-2.1496**	89	**0.11653**	**-2.14957**	89.5	**0.111**	**-2.1988**	100	89.5
3	89	**0.1165**	**-2.1496**	89	**0.11653**	**-2.14957**	89	**0.117**	**-2.1496**	100	89
19	89	**0.1165**	**-2.1496**	89.5	**0.11093**	**-2.19884**	89	**0.117**	**-2.1496**	100	89.5
27	88.5	**0.1222**	**-2.1024**	89	**0.11653**	**-2.14957**	89	**0.117**	**-2.1496**	100	89
46	88.5	**0.1222**	**-2.1024**	89.5	**0.11093**	**-2.19884**	89	**0.117**	**-2.1496**	100	89
54.5	88.5	**0.1222**	**-2.1024**	89.5	**0.11093**	**-2.19884**	89.5	**0.111**	**-2.1988**	100	89.5
69	88.5	**0.1222**	**-2.1024**	88.5	**0.12217**	**-2.10236**	89	**0.117**	**-2.1496**	100	89.5
78	88.5	**0.1222**	**-2.1024**	88.5	**0.12217**	**-2.10236**	89	**0.117**	**-2.1496**	100	90.5
93	85.5	**0.1567**	**-1.8537**	85.5	**0.15665**	**-1.85372**	86	**0.151**	**-1.8916**	100	89.5
101	84.5	**0.1684**	**-1.7813**	84	**0.17435**	**-1.74667**	86	**0.151**	**-1.8916**	100	88.5
117	82	**0.1985**	**-1.6172**	80.5	**0.21691**	**-1.52826**	83.5	**0.18**	**-1.713**	100	88
127	82	0.1985	-1.6172	80.5	**0.21691**	**-1.52826**	82	**0.198**	**-1.6172**	100	89
145	83.5	0.1803	-1.713	81.5	0.20457	-1.58686	82	0.198	-1.6172	100	89
147	83.5	0.1803	-1.713	81.5	0.20457	-1.58686	82	0.198	-1.6172	100	89
166	85	0.1625	-1.817	84	0.17435	-1.74667	84	0.174	-1.7467	100	89
171	85	0.1625	-1.817	84	0.17435	-1.74667	84.5	0.168	-1.7813	100	88.5
190	85.5	0.1567	-1.8537	84	0.17435	-1.74667	84.5	0.168	-1.7813	100	89
199	85.5	0.1567	-1.8537	85.5	0.15665	-1.85372	85	0.163	-1.817	100	88.5
214.5	85.5	0.1567	-1.8537	85.5	0.15665	-1.85372	85	0.163	-1.817	100	87.5
222	85.5	0.1567	-1.8537	85	0.16252	-1.81696	85	0.163	-1.817	100	87
235	86	0.1508	-1.8916	85.5	0.15665	-1.85372	85.5	0.157	-1.8537	100	86
247	86.5	0.145	-1.9308	86.5	0.14503	-1.93084	86	0.151	-1.8916	100	88
261	86.5	0.145	-1.9308	86	0.15082	-1.89165	85	0.163	-1.817	100	88
268.5	87	0.1393	-1.9714	86.5	0.14503	-1.93084	85	0.163	-1.817	100	87
285	87	0.1393	-1.9714	86.5	0.14503	-1.93084	84.5	0.168	-1.7813	100	87.5
295	86.5	0.145	-1.9308	86.5	0.14503	-1.93084	84.5	0.168	-1.7813	100	87
310	86.5	0.145	-1.9308	85	0.16252	-1.81696	84.5	0.168	-1.7813	100	87.5
334	86.5	0.145	-1.9308	86	0.15082	-1.89165	84	0.174	-1.7467	100	88
359	88	0.1278	-2.057	86.5	0.14503	-1.93084	85.5	0.157	-1.8537	100	88
385	87.5	0.1335	-2.0134	87	0.13926	-1.9714	84.5	0.168	-1.7813	100	87.5
410	87	0.1393	-1.9714	87	0.13926	-1.9714	85.5	0.157	-1.8537	100	89.5
435	87	0.1393	-1.9714	87.5	0.13353	-2.01342	86	0.151	-1.8916	100	89
454	87	0.1393	-1.9714	87	0.13926	-1.9714	85	0.163	-1.817	100	88.5
482	86.5	0.145	-1.9308	86.5	0.14503	-1.93084	85	0.163	-1.817	100	89
512	87.5	0.1335	-2.0134	87.5	0.13353	-2.01342	86.5	0.145	-1.9308	100	89.5
529	87.5	0.1335	-2.0134	87.5	0.13353	-2.01342	86.5	0.145	-1.9308	100	89.5
552	87	0.1393	-1.9714	87	0.13926	-1.9714	86.5	0.145	-1.9308	100	88.5
573	87	0.1393	-1.9714	87	0.13926	-1.9714	87	0.139	-1.9714	100	88.5
598	86.5	0.145	-1.9308	87	0.13926	-1.9714	86.5	0.145	-1.9308	100	88.5
622	87.5	0.1335	-2.0134	87.5	0.13353	-2.01342	86	0.151	-1.8916	100	89.5
650	87.5	0.1335	-2.0134	87.5	0.13353	-2.01342	86	0.151	-1.8916	100	88.5
671.5	87	0.1393	-1.9714	87.5	0.13353	-2.01342	86	0.151	-1.8916	100	89

Table A-8

The interference of salinity on perchlorate biodegradation (3 %)

(Raw data -- Figure 4.48 A, B & C)

Time	NaCl 3a			NaCl 3b			NaCl 3c			NaCl 3d	NaCl 3e
(hr)	Trans	Abs.	Ln(Abs)	Trans	Abs.	Ln(Abs.)	Trans.	Abs.	Ln(Abs.)	Trans	Trans
0	89	**0.1165**	**-2.14957**	89	**0.1165**	**-2.14957**	89	**0.1165**	**-2.1496**	100	89
3	88.5	**0.1221**	**-2.10236**	88.5	**0.1221**	**-2.10236**	88.5	**0.1222**	**-2.1024**	100	89
19	89	**0.1165**	**-2.14957**	89	**0.1165**	**-2.14957**	89	**0.1165**	**-2.1496**	100	89
27	89	**0.1165**	**-2.14957**	88.5	**0.1221**	**-2.10236**	89	**0.1165**	**-2.1496**	100	89
46	89	**0.1165**	**-2.14957**	89	**0.1165**	**-2.14957**	89	**0.1165**	**-2.1496**	100	89.5
54.5	89	**0.1165**	**-2.14957**	89	**0.1165**	**-2.14957**	89	**0.1165**	**-2.1496**	100	89.5
69	89	**0.1165**	**-2.14957**	89	**0.1165**	**-2.14957**	89	**0.1165**	**-2.1496**	100	90
78	89	**0.1165**	**-2.14957**	89	**0.1165**	**-2.14957**	89	**0.1165**	**-2.1496**	100	90
93	89	**0.1165**	**-2.14957**	89.5	**0.1109**	**-2.19884**	89	**0.1165**	**-2.1496**	100	90
101	85.5	**0.1566**	**-1.85372**	87.5	**0.1335**	**-2.01342**	88	**0.1278**	**-2.057**	100	90.5
117	84.5	**0.1684**	**-1.7813**	86	**0.1508**	**-1.89165**	86.5	**0.145**	**-1.9308**	100	90
127	83.5	**0.1803**	**-1.713**	85.5	**0.1566**	**-1.85372**	86	**0.1508**	**-1.8916**	100	90
145	82	**0.1984**	**-1.61721**	84	**0.1743**	**-1.74667**	84.5	**0.1684**	**-1.7813**	100	90
147	82	**0.1984**	**-1.61721**	84	**0.1743**	**-1.74667**	84.5	**0.1684**	**-1.7813**	100	90
166	78.5	**0.2420**	**-1.41852**	82	**0.1984**	**-1.61721**	83	**0.1863**	**-1.6802**	100	89
171	80.5	0.2169	-1.52826	82.5	0.1923	-1.64832	84	0.1744	-1.7467	100	90.5
190	80	0.2231	-1.49994	83	0.1863	-1.68024	84.5	0.1684	-1.7813	100	89
199	80.5	0.2169	-1.52826	84.5	0.1684	-1.7813	85.5	0.1567	-1.8537	100	90.5
214.5	81	0.2107	-1.55722	84.5	0.1684	-1.7813	86	0.1508	-1.8916	100	90.5
222	82.5	0.1923	-1.64832	83.5	0.1803	-1.713	84.5	0.1684	-1.7813	100	89
235	82.5	0.1923	-1.64832	85.5	0.1566	-1.85372	85.5	0.1567	-1.8537	100	89
247	82.5	0.1923	-1.64832	85.5	0.1566	-1.85372	85.5	0.1567	-1.8537	100	89
261	82.5	0.1923	-1.64832	85.5	0.1566	-1.85372	87	0.1393	-1.9714	100	89
268.5	84	0.1743	-1.74667	86	0.1508	-1.89165	86.5	0.145	-1.9308	100	89.5
285	84.5	0.1684	-1.7813	86	0.1508	-1.89165	86.5	0.145	-1.9308	100	89
295	84.5	0.1684	-1.7813	85.5	0.1566	-1.85372	85.5	0.1567	-1.8537	100	88.5
310	84.5	0.1684	-1.7813	85.5	0.1566	-1.85372	86	0.1508	-1.8916	100	88
334	84.5	0.1684	-1.7813	86	0.1508	-1.89165	86.5	0.145	-1.9308	100	89
359	84.5	0.1684	-1.7813	87	0.1392	-1.9714	86.5	0.145	-1.9308	100	89
385	85.5	0.1566	-1.85372	87	0.1392	-1.9714	87	0.1393	-1.9714	100	88.8
410	85	0.1625	-1.81696	87	0.1392	-1.9714	87	0.1393	-1.9714	100	89.5
435	85	0.1625	-1.81696	87.5	0.1335	-2.01342	87	0.1393	-1.9714	100	89.5
454	86.5	0.1450	-1.93084	87.5	0.1335	-2.01342	87	0.1393	-1.9714	100	89.5
482	85	0.1625	-1.81696	87	0.1392	-1.9714	88	0.1278	-2.057	100	90
512	85.5	0.1566	-1.85372	87.5	0.1335	-2.01342	87.5	0.1335	-2.0134	100	89
529	86.5	0.1450	-1.93084	87.5	0.1335	-2.01342	87.5	0.1335	-2.0134	100	90.5
552	86.5	0.1450	-1.93084	88	0.1278	-2.05703	88	0.1278	-2.057	100	90.5
573	87.5	0.1335	-2.01342	87.5	0.1335	-2.01342	88	0.1278	-2.057	100	90.5
598	86	0.1508	-1.89165	88	0.1278	-2.05703	87	0.1393	-1.9714	100	90.5
622	86.5	0.1450	-1.93084	88.5	0.1221	-2.10236	86.5	0.145	-1.9308	100	91
650	86.5	0.1450	-1.93084	87.5	0.1335	-2.01342	86.5	0.145	-1.9308	100	90
671.5	86.5	0.1450	-1.93084	87.5	0.1335	-2.01342	86.5	0.145	-1.9308	100	90.5

Table A-9

The interference of salinity on perchlorate biodegradation (3.5 %)

(Raw data -- Figure 4.49 A, B & C)

Time	NaCl 3.5a			NaCl 3.5b			NaCl 3.5c			NaCl 3.5d	NaCl 3.5e
(hr)	Trans.	Abs.	Ln(Abs.)	Trans.	Abs.	Ln(Abs.)	Trans.	Abs.	Ln(Abs)	Trans.	Trans
0	89	0.12	-2.15	89	**0.12**	**-2.1496**	89	**0.117**	**-2.15**	100	89
3	89	0.12	-2.15	89	**0.12**	**-2.1496**	89	**0.117**	**-2.15**	100	89
19	89	0.12	-2.15	89.5	**0.11**	**-2.1988**	89	**0.117**	**-2.15**	100	89.5
27	89	0.12	-2.15	89.5	**0.11**	**-2.1988**	89	**0.117**	**-2.15**	100	89
46	89	0.12	-2.15	89.5	**0.11**	**-2.1988**	89	**0.117**	**-2.15**	100	89
54.5	89.5	0.11	-2.199	89.5	**0.11**	**-2.1988**	89	**0.117**	**-2.15**	100	89.5
69	90	0.11	-2.25	89.5	**0.11**	**-2.1988**	89.5	**0.111**	**-2.199**	100	89.5
78	90	0.11	-2.25	89.5	**0.11**	**-2.1988**	90	**0.105**	**-2.25**	100	89.5
93	90.5	0.1	-2.304	89.5	**0.11**	**-2.1988**	90	**0.105**	**-2.25**	100	90
101	90.5	0.1	-2.304	90.5	**0.1**	**-2.3044**	90.5	**0.1**	**-2.304**	100	90
117	91	0.09	-2.361	90.5	**0.1**	**-2.3044**	90.5	**0.1**	**-2.304**	100	91
127	90	0.11	-2.25	89.5	**0.11**	**-2.1988**	90	**0.105**	**-2.25**	100	91.5
145	90	0.11	-2.25	87.5	**0.13**	**-2.0134**	89	**0.117**	**-2.15**	100	91.5
147	90	0.11	-2.25	87.5	**0.13**	**-2.0134**	89	**0.117**	**-2.15**	100	91.5
166	88.5	0.12	-2.102	86	**0.15**	**-1.8916**	87	**0.139**	**-1.971**	100	91.5
171	88	0.13	-2.057	85.5	**0.16**	**-1.8537**	87.5	**0.134**	**-2.013**	100	92
190	86.5	0.15	-1.931	85.5	**0.16**	**-1.8537**	86	**0.151**	**-1.892**	100	91.5
199	87	0.14	-1.971	86	**0.15**	**-1.8916**	87	**0.139**	**-1.971**	100	92
214.5	86	0.15	-1.892	85	**0.16**	**-1.817**	85.5	**0.157**	**-1.854**	100	92
222	86.5	0.15	-1.931	84	**0.17**	**-1.7467**	85.5	**0.157**	**-1.854**	100	92.5
235	85	0.16	-1.817	82.5	**0.19**	**-1.6483**	83.5	**0.18**	**-1.713**	100	92.5
247	85.5	0.16	-1.854	83	0.19	-1.6802	85	0.163	-1.817	100	93
261	85.5	0.16	-1.854	83	0.19	-1.6802	86.5	0.145	-1.931	100	93.5
268.5	85.5	0.16	-1.854	84	0.17	-1.7467	86.5	0.145	-1.931	100	93
285	86	0.15	-1.892	85	0.16	-1.817	87	0.139	-1.971	100	93
295	85.5	0.16	-1.854	84.5	0.17	-1.7813	86.5	0.145	-1.931	100	92
310	85.5	0.16	-1.854	85.5	0.16	-1.8537	86.5	0.145	-1.931	100	92
334	86.5	0.15	-1.931	86.5	0.15	-1.9308	88	0.128	-2.057	100	92
359	88.5	0.12	-2.102	87.5	0.13	-2.0134	88	0.128	-2.057	100	92.5
385	89.5	0.11	-2.199	87.5	0.13	-2.0134	88	0.128	-2.057	100	92.5
410	89	0.12	-2.15	88	0.13	-2.057	89	0.117	-2.15	100	93.5
435	89	0.12	-2.15	89	0.12	-2.1496	89	0.117	-2.15	100	93.5
454	90	0.11	-2.25	89	0.12	-2.1496	89	0.117	-2.15	100	93
482	88.5	0.12	-2.102	87.5	0.13	-2.0134	89	0.117	-2.15	100	92
512	89	0.12	-2.15	88	0.13	-2.057	88.5	0.122	-2.102	100	93
529	89	0.12	-2.15	89	0.12	-2.1496	89.5	0.111	-2.199	100	92
552	90.5	0.1	-2.304	89.5	0.11	-2.1988	89.5	0.111	-2.199	100	93
573	89.5	0.11	-2.199	89.5	0.11	-2.1988	89	0.117	-2.15	100	92.5
598	89	0.12	-2.15	89	0.12	-2.1496	87	0.139	-1.971	100	92
622	90	0.11	-2.25	89	0.12	-2.1496	88	0.128	-2.057	100	92.5
650	90	0.11	-2.25	89	0.12	-2.1496	88	0.128	-2.057	100	93
671.5	90	0.11	-2.25	89.5	0.11	-2.1988	88	0.128	-2.057	100	92.5

Table A-10

The interference of salinity on perchlorate biodegradation (4 %)

(Raw data -- Figure 4.50 A & B)

Time	NaCl 4a			NaCl 4b			NaCl 4c			NaCl 4d	NaCl 4e
(hr)	Trans.	Abs.	Ln(Abs.)	Trans	Abs.	Ln(Abs.)	Trans	Abs.	Ln(Abs.)	Trans	Trans
0	89	0.1165	-2.1495	89.5	0.1109	-2.19884	89	0.11653	-2.14957	100	88.5
3	89	0.1165	-2.1495	89.5	0.1109	-2.19884	89	0.11653	-2.14957	100	88
19	90	0.1053	-2.2503	90	0.1053	-2.25037	90	0.10536	-2.25037	100	88.5
27	89.5	0.1109	-2.1988	88.5	0.1221	-2.10236	89.5	0.11093	-2.19884	100	88.5
46	89.5	0.1109	-2.1988	89.5	0.1109	-2.19884	89	0.11653	-2.14957	100	88.5
54.5	89	0.1165	-2.1495	89	0.1165	-2.14957	89	0.11653	-2.14957	100	89
69	89	0.1165	-2.1495	89.5	0.1109	-2.19884	89.5	0.11093	-2.19884	100	89
78	89.5	0.1109	-2.198	90	0.1053	-2.25037	89.5	0.11093	-2.19884	100	89.5
93	89.5	0.1109	-2.1988	89.5	0.1109	-2.19884	89.5	0.11093	-2.19884	100	89
101	89.5	0.1109	-2.1988	89	0.1165	-2.14957	89.5	0.11093	-2.19884	100	89.5
117	90.5	0.0998	-2.3043	91	0.0943	-2.36116	90.5	0.09982	-2.30438	100	90
127	90.5	0.0998	-2.3043	90	0.1053	-2.25037	90.5	0.09982	-2.30438	100	90
145	90.5	0.0998	-2.3043	91	0.0943	-2.36116	91	0.09431	-2.36116	100	90
147	90.5	0.0998	-2.3043	91	0.0943	-2.36116	91	0.09431	-2.36116	100	90
166	90	0.1053	-2.2503	90	0.1053	-2.25037	90	0.10536	-2.25037	100	90
171	91	0.0943	-2.3611	91.5	0.0888	-2.42102	90	0.10536	-2.25037	100	90
190	90	0.1053	-2.2503	90	0.1053	-2.25037	90	0.10536	-2.25037	100	90
199	91.5	0.0888	-2.4210	92	0.0833	-2.48433	91	0.09431	-2.36116	100	92
214.5	91.5	0.0888	-2.4210	91.5	0.0888	-2.42102	90.5	0.09982	-2.30438	100	92
222	91	0.0943	-2.3611	91	0.0943	-2.36116	91.5	0.08883	-2.42102	100	92
235	91	0.0943	-2.3611	90.5	0.0998	-2.30438	91.5	0.08883	-2.42102	100	91.5
247	91.5	0.0888	-2.4210	91	0.0943	-2.36116	92	0.08338	-2.48433	100	92
261	91	0.0943	-2.3611	92.5	0.0779	-2.55154	92	0.08338	-2.48433	100	92.5
268.5	92.5	0.0779	-2.5515	92	0.0833	-2.48433	92.5	0.07796	-2.55154	100	92.5
285	93	0.0725	-2.6231	92.5	0.0779	-2.55154	92.5	0.07796	-2.55154	100	92.5
295	91	0.0943	-2.3611	91	0.0943	-2.36116	90.5	0.09982	-2.30438	100	91.5
310	91	0.0943	-2.3611	91.5	0.0888	-2.42102	90	0.10536	-2.25037	100	92
334	91	0.0943	-2.3611	91	0.0943	-2.36116	92	0.08338	-2.48433	100	92
359	90.5	0.0998	-2.3043	91	0.0943	-2.36116	91	0.09431	-2.36116	100	91
385	90.5	0.0998	-2.3043	91	0.0943	-2.36116	92	0.08338	-2.48433	100	91
410	91	0.0943	-2.3611	91	0.0943	-2.36116	91.5	0.08883	-2.42102	100	91.5
435	91.5	0.0888	-2.4210	92	0.0833	-2.48433	91	0.09431	-2.36116	100	92
454	92	0.0833	-2.4843	92.5	0.0779	-2.55154	92	0.08338	-2.48433	100	93
482	92	0.0833	-2.4843	91	0.0943	-2.36116	91	0.09431	-2.36116	100	91
512	92	0.0833	-2.4843	91.5	0.0888	-2.42102	91.5	0.08883	-2.42102	100	92.5
529	92	0.0833	-2.4843	91.5	0.0888	-2.42102	91.5	0.08883	-2.42102	100	92.5
552	91	0.0943	-2.3611	91	0.0943	-2.36116	90.5	0.09982	-2.30438	100	92
573	91	0.0943	-2.3611	91	0.0943	-2.36116	90.5	0.09982	-2.30438	100	91.5
598	91	0.094	-2.3611	91.5	0.0888	-2.42102	91	0.09431	-2.36116	100	91.5
622	92	0.0833	-2.4843	92	0.0833	-2.48433	91	0.09431	-2.36116	100	91
650	92	0.0833	-2.4843	92	0.0833	-2.48433	90.5	0.09982	-2.30438	100	92
671.5	92	0.0833	-2.4843	91.5	0.0888	-2.42102	91.5	0.08883	-2.42102	100	91

Table A-11

The interference of salinity on perchlorate biodegradation (6 %)

(Raw data -- Figure 4.51)

Time (hour)	Transmittance (%)				
	NaCl 6a	NaCl 6b	NaCl 6c	NaCl 6d	NaCl 6e
0	89.5	89	88	100	90
3	89.5	89	88	100	89.5
19	90	90	89.5	100	91
27	90	90	88.5	100	90
46	90	90	88.5	100	90
54.5	90	90	88.5	100	90.5
69	90	90	89.5	100	90.5
78	90	90	89.5	100	90
93	89.5	89.5	88.5	100	89.5
101	90.5	90.5	89	100	90
117	91	91	89.5	100	90
127	91	91	91	100	91
145	90	91	88.5	100	92
147	90	91	88.5	100	92
166	90.5	91	89	100	92
171	91.5	92	90.5	100	92.5
190	91.5	92	90	100	92
199	91.5	92	90.5	100	92.5
214.5	91.5	92	90	100	92
222	91	92	90.5	100	92
235	91	92	90	100	92
247	91	92	91	100	92.5
261	92	93	92.5	100	92.5
268.5	92	92.5	92.5	100	93
285	91.5	92.5	91	100	92.5
295	92.5	92	92	100	92.5
310	92	92	91.5	100	92
334	91	91.5	90	100	92
359	91.5	91	91	100	92.5
385	91	92.5	92	100	92.5
410	91	92	92	100	92.5
435	91	91.5	90	100	92.5
454	92.5	92.5	91	100	92.5
482	91.5	93	90.5	100	92.5
512	92	93	90.5	100	92.5
529	91	92	91	100	91
552	91.5	93	91	100	91.5
573	91	91.5	90.5	100	91
598	91.5	92.5	90	100	92
622	93	93	90.5	100	92.5
650	93	93.5	91.5	100	92.5
671.5	93.5	93	91.5	100	92.5

APPENDIX B

EXAMPLE CALCULATIONS

EXAMPLE 1: Moles of perchlorate biodegraded per day per m^2 of a membrane through a BTS-55 membrane-immobilized biofilm (data from Tables 6.24 and 6.25, p 103-104)

At day 1, the initial amount of perchlorate in the DR and BR reactors was:
(231.12 + 0.194) mg/L * 5 L =1156.57 mg

At day 7, the perchlorate concentration in both reactors was zero. Therefore, the perchlorate biodegraded per day was:
1156.57 / 6= 192. 76 mg/day = 0.19276 g/day
since the molecular weight of perchlorate is: 99.5 g/mole, so
$0.19276 / 99.5 = 1.94 \times 10^{-3}$ mole/day

The diameter of the membrane = 10 cm = 0.1 m
Thus, the area of the membrane = $3.14 \times 0.1^2 / 4 = 7.85 \times 10^{-3}$ m^2

Then, the perchlorate biodegradation rate was:
$1.94 \times 10^{-3} / (7.85 \times 10^{-3}) = 0.25$ mole/m^2-day

EXAMPLE 2: Calculation for perchlorate removal after acclimation in KC3 to KC5 (data from Table 4.33 p126)

Perchlorate removal in KC3 (data from Table 4.33, p126)
At day 0, the initial concentration of perchlorate in KC3 = 63.65 mg/L
After acclimation, at day 3, the concentration of perchlorate = 17.54 mg/L
Thus, perchlorate removed = (63.65-17.54)/63.65 = 72.4 %

Perchlorate removal in KC4 (data from Table 4.33, p126)
At day 0, the initial concentration of perchlorate = 108.72 mg/L
After acclimation, at day 5, the concentration of perchlorate = 52.17 mg/L

Thus, perchlorate removed = (108.72-52.17)/108.72 = 52%

Perchlorate removal in KC5 (data from Table 4.33, p126)

At day 0, the initial concentration of perchlorate = 232.2 mg/L

After acclimation, at day 5, the concentration of perchlorate = 166.68 mg/L

Thus, perchlorate removed = (232.2-166.68)/232.2 = 28.2%

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