NITRATE REMOVAL FROM DRINKING WATER USING COMBINED ION-EXCHANGE/ RESIN BIOREGENERATION

by

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Abstract

Nitrate contamination is a drinking water concern in many areas in the world, especially rural communities. Excess nitrate can cause several environmental and health problems. The maximum acceptable concentration of nitrate in drinking water is set to be 45 mg/L as nitrate by USEPA. One of the most common methods for nitrate removal from water is ion exchange using nitrate selective resins. Although these resins have a great capacity for nitrate removal, they are considered non-regenerable. The ability to regenerate these resins multiple times will increase the environmental and economic sustainability of nitrate-contaminated water treatment processes. This dissertation tests the hypothesis that multi-cycle exhaustion/bioregeneration of resin enclosed in a membrane is feasible and temperature and salt concentration in the medium will have an influence on the processes involved.

The results showed that this method is an effective and innovative resin regeneration method. Applying 6 cycles of resin exhaustion/regeneration showed that resin could be used for 4 cycles without a loss of capacity, after 6 cycles only 6% of the capacity was lost. A pseudo-second order kinetic equation described the nitrate desorption analysis well. The initial desorption rates of nitrate from the resin in a membrane were significantly different at different salt concentrations and temperatures; maximum of 306±21 mg of nitrate/g of resin/h for 6% at 35 °C and minimum of 42±3 mg of nitrate/g of resin/h at 2% salt and 12 °C. The effect of different salt concentrations on the biological nitrate removal rate was dependent on the temperature; within each group of temperatures, the nitrate degradation rate decreased with increasing the salt concentration. \( \text{rate} \left( \frac{mg \ nitrate}{mg \ VSS. \ hr} \right) = 0.0102 + (0.00114 \times temperature) - (0.000291 \times salt) \) fit the data for the effect of salt concentration and temperature
on nitrate degradation rate with the best correlation ($R^2 = 0.93$). The results from the kinetic studies were used to develop a mathematical model which incorporated physical desorption process with biological removal kinetics. The model fit well to experimental data ($R^2 = 0.94 \pm 0.06$ for 6 bioregeneration cycles) and was applied in the design of a bioreactor for a local nitrate contaminated drinking water source.
Preface

Parts of chapter 2 have been published as a book chapter. Ebrahimi, S., Xiao, Y., Roberts, D. J. 2012, Interactions of perchlorate and nitrate in biological treatment systems. “Nitrate: Occurrence, Characteristics and Health Considerations”. Nova science publishers INC. This book chapter is based on the analysis of the background literature related to my research, working with previous PhD student to understand the background of the microbial culture that I was planning to work with. I wrote the book chapter, Dr. Xiao, revised he chapter and Dr. Roberts did the final edition.

Chapter 3 is based on the published journal paper. Ebrahimi, S., Roberts, D. J. 2013, Sustainable nitrate-contaminated water treatment using multi cycle ion-exchange/bioregeneration of nitrate selective resin. Journal of Hazardous Materials, 262, 539-544. This paper published in a great journal (impact factor of 5.277) was the proof of concept regarding my proposed innovative method for nitrate removal from drinking water. For this publication, I did the all of the lab work as well as the paper preparation. Dr. Roberts took care of the final edition and submission.

The results of chapter 4 are published in Journal of Separation and Purification Technology which is another top journal in our filed (Impact factor: 3.494). Ebrahimi, S., Roberts, D. J. 2015, Bioregeneration of single use nitrate selective ion-exchange resin enclosed in a membrane: kinetics of desorption. Separation & Purification Technology, 146, 268-275. This journal paper is based on part of my experimental work on nitrate desorption rate in which all of the lab work was done by myself as well as data analysis, paper preparation and submission.
A version of chapter 5 is published as another journal paper. Ebrahimi, S., Nguyen, T. H., Roberts, D. J. 2015, Effect of temperature & salt concentration on salt tolerant nitrate-perchlorate reducing bacteria: nitrate degradation kinetics. Water Research, 83, 345-353. Water Research is the best journal in our filed (impact factor: 6.279). The lab work of this part of experiment was done by myself and an undergrad students (Hau), I was responsible for further data analysis, writing the paper and submitting it. Dr. Roberts helped with data analysis as well as final edition of the paper.

A version of chapter 6 has been published in Water Research. Ebrahimi, S., Roberts, D. J. 2016. Mathematical modelling & reactor design for multi-cycle bioregeneration of nitrate exhausted ion exchange resin. Water Research, 88c. 766-776. I have developed the mathematical model, validated the model, and analyzed the results as well as preparing the journal paper for publication. Dr. Roberts has done the final revision and submitting the paper.
Publications arising from the work presented in this dissertation are listed as follows:

**Book chapters**


**Journal articles**


**Conferences & Presentations**

- Ebrahimi, S., Roberts, D. J., 2015, Optimization of resin bioregeneration process by adapting a salt tolerant nitrate-perchlorate reducing bacteria to different salt
concentrations and temperatures. 115th General Meeting of American Society for Microbiology (ASM 2015), New Orleans, US.

- Ebrahimi, S., Roberts, D. J., 2013, Multi cycle ion exchange/ biodegradation removal of nitrate from drinking water by using salt tolerant nitrate-perchlorate reducing bacteria. 113th General Meeting of American Society for Microbiology (ASM 2013), Denver, Colorado, US.


- Ebrahimi, S., Roberts, D. J., 2013, Nitrate and perchlorate removal from drinking water by multi cycle ion-exchange/bioregeneration of tributylamine strong base anion exchange resin. AWWA 2013 Biological Treatment Symposium, Denver, Colorado, US.

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List of abbreviations

USEPA  United States Environmental Protection Agency
WHO   World Health Organization
MAC   Maximum Acceptable Concentration
ppb   part per billion
RO    Reverse Osmosis
IX    Ion Exchange
DO    Dissolved Oxygen
EBCT  Empty Bed Contact Time
VSS   Volatile Suspended Solids
ANOVA Analysis of Variance
S     substrate concentration
T     time
K     maximum specific nitrate removal rate
K_s   half saturation constant for nitrate
X     microbial concentration
C_t   nitrate concentration in the aqueous phase at time t
C_e   equilibrium concentration of nitrate in aqueous phase
K_{des} kinetic rate constant
K_{\text{des}C_e^2} initial desorption rate
K_0   temperature-independent factor
E_a   activation energy of desorption
R     universal gas constant
T     solution temperature
\( X_{\text{res}} \) concentration of fixed ionic group
\( C_{\text{aq}} \) concentration of the free ion in solution
\( D \) film diffusion coefficient
\( r_0 \) mean resin bead radius
\( \Delta \) liquid film thickness
\( M_t \) Thiele modulus
\( D_e \) diffusivity coefficient
\( k_{\text{rxn}} \) reaction kinetic coefficient
\( r_{d,t} \) desorption rate
\( C_{\text{NO}_3, a, t} \) nitrate concentration in the aqueous phase at time \( t \)
\( C_{\text{e,NO}_3,t} \) nitrate equilibrium concentration of nitrate in aqueous phase
\( r_{b,t} \) nitrate biodegradation rate
\( \alpha \) binary separation factor
\( X_{\text{Cl}} \) equivalent fraction or mole fraction of chloride in aqueous phase
\( X_{\text{NO}_3} \) equivalent fraction or mole fraction of nitrate in aqueous phase
\( Y_{\text{Cl}} \) resin-phase equivalent fraction or mole fraction of the chloride
\( Y_{\text{NO}_3} \) resin-phase equivalent fraction or mole fraction of the nitrate
\( C^r \) total ionic concentration
\( C_{\text{x-NO}_3} \) equivalent of nitrate on the resin
\( C_{\text{x-Cl}} \) equivalent of chloride on the resin
\( C^r \) total ionic concentration
\( C_R \) the capacity of resin
\( V \) volume of the aqueous phase
\( g_R \) mass of resin
\( M_{\text{NO}_3,t} \) the equivalent of NO\(_3\) on resin and in the aqueous phase at any time
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{e,Cl}$</td>
<td>equilibrium concentration of chloride in the aqueous phase</td>
</tr>
<tr>
<td>$V_T$</td>
<td>total volume</td>
</tr>
<tr>
<td>MLSS</td>
<td>Mixed Liquor Suspended Solid</td>
</tr>
<tr>
<td>$X_T$</td>
<td>MLSS concentration at full volume</td>
</tr>
<tr>
<td>$V_S$</td>
<td>Settled volume after decant</td>
</tr>
<tr>
<td>$X_S$</td>
<td>MLSS concentration in settled volume</td>
</tr>
<tr>
<td>SVI</td>
<td>Sludge Volume Index</td>
</tr>
<tr>
<td>$V_F$</td>
<td>fill volume</td>
</tr>
</tbody>
</table>
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Haruki Murakami, Japanese writer and marathon runner, suggests a great way to keep up doing important things in our life. He says: “... the point being to let the exhilaration I feel at the end of each run carry over to the next day. This is the same sort of tack I find necessary when writing a novel. I stop every day right at the point where I feel I can write more. Do that and the next day’s work goes surprisingly smoothly. To keep on going, you have to keep up the rhythm. This is the important thing for long-term projects. Once you set the pace, the rest will follow. The problem is getting the flywheel to spin at a set speed—and to get to that point takes as much concentration and effort as you can manage.” It is not easy to follow up with what he says. During my PhD I tried to do so and now that everything is done, I would like to thank each of everyone who played a role in this journey.

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To all of those who have taught me.
Chapter 1: Introduction

This chapter provides a general introduction to the research project, including the background, issues and objectives, which are necessary to understand the significance of this research. The structure of the thesis is described as well to provide a roadmap for readers.

1.1 General background

Nitrate contamination of groundwater is a growing concern worldwide. Excess nitrate can cause several environmental and health problems (Campbell et al., 2006; Islam et al., 2009). The major sources of nitrate are agricultural activities and discharges of municipal and industrial wastewaters, processed food, dairy and meat products, and the decomposition of decaying buried organic matter (Hall et al., 2001; Hudak, 2000; Schilling & Wolter, 2001). To limit the risk to human health from nitrate in drinking water, the maximum acceptable concentration (MAC) is set to be 10 mg-N/L (45 mg NO$_3^-$/L) in Canada and the United States, while the World Health Organization (WHO) and the European Community have set the MAC at 11.3 mg-N/L (50 mg NO$_3^-$/L) (USEPA, 2013; W. H. O., 2010).

One of the most commonly used methods for the removal of nitrate from drinking water is ion exchange (Bae et al., 2002; Samatya et al., 2006). Industry is trending toward the use of nitrate selective single use resins. These resins have a great capacity for nitrate but except resin regeneration with a high concentration of salt which results in high volume of brine production, there are no economically viable methods for regenerating them. They are replaced after exhaustion and incinerated or disposed of in a landfill. The production of greenhouse gases and re-contamination of the environment are very important disadvantages of incineration and landfill, respectively, not to mention the environmental cost of resin
production, the cost of the resin itself, and the environmental costs of shipping the resin to each treatment site.

The wide use of ion-exchange processes to remove nitrate from drinking water creates an urgency for the development of a process that can be used to regenerate of these resins. Since these resins cost in the thousands of dollars per cubic foot, the ability to reuse the resin for even a few times, will increase the economic viability and sustainability of nitrate-contaminated water treatment processes.

Most of studies on nitrate selective resins focus on adsorption characteristics of the resin (Bae et al., 2002; Clifford & Liu, 1993; Samatya et al., 2006) rather than understanding the desorption which is really important since the productivity of the process depends on the total life cycle of resin. There are a few of studies discussing the regeneration performance of nitrate selective resin (Cyplik et al., 2008; Van der Hoek et al., 1988a; Van der Hoek et al., 1988b). The main issue with regeneration for both regenerable and non-regenerable resin is disposal or treatment of spent regenerant brine. Biological treatment of the waste stream of the resin regeneration process with high concentration of salt and nitrate is quite a challenge. Denitrification needs a source of carbon as electron donor (such as methanol, acetate, ethanol) as well as nutrients that should be provided by mixing the brine stream with municipal wastewater stream or be added separately to the brine treatment plant (Bae et al., 2002; Clifford & Liu, 1993; Cyplik et al., 2008; Samatya et al., 2006; Van der Hoek et al., 1988). These issues lead to the idea to develop a new method to combine the resin regeneration process with brine treatment, so both processes can be done at the same time. This is even more important when considering the application to nitrate selective or non-regenerable resins. At this time these resins are mainly incinerated not regenerated.
Researchers have studied the direct bioregeneration of perchlorate-selective resin (Venkatesan et al., 2010; Y. Xiao, Roberts et al., 2010). In both of these studies, the resin was in direct contact with the culture so at the end of each cycle, bio-fouling removal and disinfection of resin was performed before the resin was reused in another cycle. Recently, Meng et al., 2014 studied the bioregeneration of exhausted resin for nitrate treatment from water, looking at the effect of different environmental parameters on bioregeneration. They do not mention the effect of the bioregeneration on reuse of the resin in another ion exchange cycle. In all of these studies, because of the direct contact of biomass with resin there is a possibility that the biomass will affect the desorption of perchlorate/nitrate from the resin. These factors result in increasing the cost and time required for each regeneration cycle (Venkatesan et al., 2010).

How to improve the bioregeneration process in order to have a sustainable method for nitrate removal from drinking water is still a question. In this research I hypothesize that I can develop a new method using resin enclosed in a membrane to prevent direct contact of the resin with the bacterial culture, so post-treatment processes will not be needed. Once this was proven feasible I conducted a comprehensive study of the effects of temperature and salt on the process.

1.2 Research objectives

Performing detailed study of the performance of multi-cycle bioregeneration of exhausted resin was the main goal of this research. The objectives of my research are as follows:
1. Investigate the potential of sustainable treatment of nitrate-contaminated water by multi-cycle ion exchange/ bioregeneration of selective resin as proof of concept with the following specific sub-objectives:

1.1. to determine the feasibility of multi cycle regeneration of nitrate-selective resin;

1.2. to identify the capacity loss of the resin after several ion exchange/bioregeneration cycles;

1.3. to investigate the kinetics observed during bioregeneration process;

1.4. to evaluate the results in the context of the literature to date.

2. Identify the effect of the temperature and salt concentration on desorption of nitrate from resin enclosed in the membrane including the following specific sub-objectives:

2.1. to investigate the effect of salt concentration and temperature on the desorption rate of nitrate from resin;

2.2. to determine the effect of temperature and salt concentration on the kinetics parameters;

2.3. to recommend operational parameters for the most rapid desorption kinetics.

3. Investigate the culture behavior under different salt concentrations and temperature including the following specific sub-objectives:

3.1. to understand the effect of salt concentration and temperature on the nitrate removal rate;

3.2. to identify the interaction of temperature and salt on the nitrate removal rate;
3.3. to create an equation describing the effect of temperature and salt concentration on nitrate removal rate that can be used in a bioregeneration model.

4. Optimize the bioregeneration process by developing a general mathematical model for multi-cycle bioregeneration of resin which includes the following sub-objectives:

4.1. to assess the process controlling mechanism;

4.2. to develop mathematical model for bioregeneration of a nitrate selective resin;

4.3. to validate the developed model with experimental results.

5. Design the bioreactor for multi-cycle bioregeneration of the resin.

1.3 Thesis structure

After this general introduction, Chapter 2 provides the background information from a literature review of nitrate contamination (effects and sources), treatment technologies (physical-chemical as well as biological methods), and advantages and disadvantages of different technologies as well as method comparison and the literature gap regarding the objectives of this research. Chapters 3 to 6 document the detailed methods and results addressing the research objectives mentioned above and chapter 7 presents the final conclusions of the research as well as suggestions for possible future studies.
Chapter 2: Literature review

2.1 Nitrate, sources of contamination, and regulation

In many parts of the world, groundwater serves as the sole source of drinking water in rural communities and urban areas. However, in recent years, increased industrial and agricultural activities have resulted in the generation of toxic pollutants such as inorganic anions, metal ions, synthetic organic chemicals which have increased public concern about the quality of ground waters. Inorganic anions are of great importance since these are toxic and harmful to humans and animals at very low concentrations (ppb). As there are usually no organoleptic changes in drinking water due to the presence of trace levels of toxic inorganic anions, it is therefore possible that some of them may remain undetected, thereby increasing the possible health risks (DeZuane, 1997; Shrimali & Singh, 2001). A number of inorganic anions have been found in potentially harmful concentrations in numerous drinking water sources (DeZuane, 1997; Petrovi et al., 2003; Smith et al., 2002). Of these, nitrate (NO$_3^-$) is of prime concern on a global scale. Nitrate is a naturally occurring ion in the nitrogen cycle and is the stable form of N for oxygenated systems. It can be reduced by microbial action into nitrite (NO$_2^-$) or other forms. The nitrite ion contains N in a relatively unstable oxidation state. Chemical and biological processes can further reduce nitrite to various compounds or oxidize it to nitrate. Due to high water solubility of nitrate (Thomson, 2001), it is possibly the most widespread groundwater contaminant in the world, imposing a serious threat to drinking water supplies (Majumdar & Gupta, 2002).

Excess of nitrate can cause several environmental problems. The effect of nitrate itself is described as primary toxicity. Intake of high nitrate concentrations (more than 60 mg/L) causes abdominal pains, diarrhea, vomiting, hypertension, increased infant mortality, central
nervous system birth defects, diabetes, spontaneous abortions, respiratory tract infections, and changes to the immune system (Fewtrell, 2004; Lohumi et al., 2004; Tate et al., 1990).

Secondary toxicity of nitrate is from microbial reduction to the reactive nitrite ion by intestinal bacteria. Nitrite has been implicated in methemoglobinemia, especially to infants under six months of age (Brunning-Fann & Kaneene, 1993; Kross et al., 1992).

Methemoglobin (MetHb) is formed when nitrite (for our purposes, formed from the endogenous bacterial conversion of nitrate from drinking water) oxidizes the ferrous iron in hemoglobin (Hb) to the ferric form. MetHb cannot bind oxygen, and the condition of methemoglobinemia is characterized by cyanosis, stupor, and cerebral anoxia (Brunning-Fann & Kaneene, 1993; Kross et al., 1992).

Many studies have shown that agricultural activities are the main source of elevated nitrate concentrations in ground water (Ator & Ferrari, 1997; Hudak, 2000; Keeney, 1986; Spalding & Exner, 1993; Wylie et al., 1995). Nitrogen is an essential nutrient required by all crops. Legumes fix N\textsubscript{2} from the atmosphere, but non-leguminous crops often require applied N to optimize crop growth and yield (Campbell et al., 2006; Drury & Tan, 1995). Nitrogen is added to non-leguminous crops primarily via fertilizer and manure and to a lesser extent via atmospheric deposition. Other sources of N for crop uptake include the decomposition and mineralization products of crop residues and soil organic matter. However, the addition of N to agricultural farmland is not without environmental risks. Unfavorable weather conditions (e.g., drought, excess rain, and frost), various soil physical and chemical factors and plant diseases can limit crop growth and thus N uptake, which may then result in increased amounts N being left in the soil at the end of the growing season. Environmental risks may occur when large surpluses of mineral N are present in the soil, especially between cropping
seasons in humid regions. Most of the residual inorganic N, especially which in the form of
nitrates, is water soluble. It is therefore susceptible to leaching through the soil into
groundwater, to loss through tile drains into ditches, streams and lakes, and to loss via
surface runoff and erosion. Eutrophication which is explosive growth of plants and algae in
water bodies is a common environmental problem caused by high concentration of nitrate (25
to 40 mg/l as nitrogen (Romano & Zeng, 2009; Hall et al., 2001).

Elevated nitrate concentrations in ground water are also common around dairy and
poultry operations, barnyards, and feedlots due to seepage from manure piles (Hii et al.,
1999; Wang et al., 2007). As an illustration, reports of elevated nitrate concentrations in the
Abbotsford Aquifer in southwestern British Columbia have increased public concern
regarding water quality (Hii et al., 1999; Keeney, 1986; Wylie et al., 1995). Agricultural
production, and particularly intensive poultry production, has been suggested as a major
contributor to the elevated nitrate concentrations. In addition to agricultural practices, the
major point sources include septic tanks and dairy lagoons. Many studies have shown high
concentrations of nitrate in areas with septic tanks (Keeney, 1986; MacQuarrie et al., 2001).
This is of particular concern to rural homeowners who use shallow drinking water wells.
Seepage from dairy lagoons has been found to be a source of elevated nitrate in shallow
ground water (Erickson, 1997).

To limit the risk to human health from nitrate in drinking water, the Maximum
Acceptable Concentration (MAC) and Maximum Contaminant Level (MCL) are set to be 10
mg nitrate, N/L (45 mg NO₃/L) in Canada and the United States respectively, while the
World Health Organization (WHO) and the European Community have set the MCL at 11.3
mg NO₃ N/L (50 mg NO₃/L) (USEPA, 2013; W. H. O., 2010).
2.2 Nitrate treatment technologies

The conventional water treatment methods including coagulation, filtration, and disinfection, are not efficient for the elimination of the nitrate ion from the water. To remove the nitrate ion, a supplementary method is necessary. Nitrate removal can be achieved using two main groups of treatment processes; physicochemical and biological treatment methods. Each of these methods has some advantages and disadvantages which are discussed below.

2.2.1 Physical-chemical process

The most common physical-chemical processes to remove nitrate are adsorption, reverse osmosis, ion exchange, electrodialysis, and chemical denitrification including zero-valent metals (iron and magnesium). Also, a wide interest towards some new methods such as metallic iron-aided abiotic nitrate reduction is reported by several researchers (Ahn et al., 2008; Chen, et al., 2005; Cheng et al., 1997; Choe et al., 2004; Devlin et al., 2000; Ginner, et al., 2004; Huang & Zhang, 2004; Kumar & Chakraborty, 2006; Liou et al., 2005). The most common physical-chemical processes that can be used for removal of nitrate are discussed in this chapter.

2.2.1.1 Adsorption processes

Adsorption has been explored widely for removal of nitrate from water, and offers satisfactory results especially with mineral-based and/or surface modified adsorbents (Bhatnagar & Sillanpaa, 2011). There are different materials such as activated carbon, resins, hydrotalcites, that can be used as the adsorbent for removal of nitrate. Table 2.1 presents the absorption characteristics of various adsorbents found in the literature.
Although, activated carbon is considered as a universal absorbent to remove different contamination from water, it is not highly beneficial for the removal of anionic compounds including nitrate. Only a few studies available report the sorption of nitrate by activated carbon. Afkhami et al. (2007), reported the use of processed carbon cloth in acidic form to remove nitrate from water which resulted in removal rate of 2 mmol nitrate per g of cloth. Powdered activated carbon and commercial granular activated carbon (produced from coconut shells) are reported to remove nitrate from aqueous solution with the removal rate of 10 and 0.03-0.16 mmol/g, respectively. Increasing temperature and treating the granular activated carbon with ZnCl₂ during the synthesis process affect the nitrate removal rate (Bhatnagar et al., 2008; Khani et al., 2008). Among all of the studies on nitrate removal using carbon based adsorbent it has been shown nitrate is competitive with perchlorate since both prefer the same surface sites in the adsorption process (Kaneko et al., 1991; Mahmudov & huang, 2011; Mizuta et al., 2004; Ohe et al., 2003).

Clays play an important role in the environment as a natural scavenger of cations and anions. Untreated clays cannot remove nitrate or show very poor nitrate adsorption (Xi et al., 2010). In order to remove nitrate, clay should be modified and treated. Modification of calcium bentonite by HCl or KCl helps nitrate removal due to the interaction between chlorine and nitrate (Bhattacharyya & Gupta, 2008; Mena-Duran et al., 2007). The main disadvantage of using clay as a treatment process is the low porosity of clay which makes the flow of water through the absorbent challenging.

As another adsorbent for nitrate removal, the synthesis, characterization and investigation of nitrate adsorption on Ca-Al-Cl hydrotalcite-like compound have been done by Islam (2011). Hydrotalcites, also known as layered double hydroxides or ionic clays are
based upon the brucite [Mg(OH)$_2$] structure. In the synthesis of this compound, some of the divalent cations are replaced by trivalent cations (e.g. Al$_3^+$, Fe$_3^+$, Cr$_3^+$, etc.) resulting in a layer charge. This layer charge is counter balanced by anions such as carbonate, nitrate, sulfate or chloride in the interlayer spaces resulting in removing them from the environment. Although this specific type of hydrotalcite selectively removes nitrate, it cannot be easily regenerated and reused.

There are many other materials and compounds that can be sued as absorbent for removal of nitrate. Table 2.1 summarizes some of the reported results by other researchers. The table is categorized based on the different adsorbent types. Carbon based adsorbents and chitosan have relatively high nitrate uptake compare to other natural absorbents. Agricultural wastes show an appreciable potential for nitrate removal too. More studies need to be done to optimize the adsorption process to consider the cost of the process as well as efficiency of the absorbent. More than economic factor and sorption efficiency, the range of nitrate concentration, the effect of other competing ions, and the effect of pH should be considered to select the best adsorbent option for removal of nitrate.

<table>
<thead>
<tr>
<th>Category</th>
<th>Adsorbent</th>
<th>Amount of nitrate adsorbed</th>
<th>Concentration range (mg/l)</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon-based sorbent</td>
<td>Powdered activated carbon</td>
<td>10 mmol/g</td>
<td>NA</td>
<td>25</td>
<td>&lt;5</td>
<td>Khani &amp; Mirzaei, 2008</td>
</tr>
<tr>
<td></td>
<td>ZnCl$_2$ treated coconut granular activated carbon</td>
<td>10.2 mg/g</td>
<td>5-200</td>
<td>25</td>
<td>5.5</td>
<td>Bhatnagar et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Commercial activated carbon</td>
<td>1.22 mg/g</td>
<td>0-25</td>
<td>15</td>
<td>NA</td>
<td>Mishra &amp; Patel, 2009</td>
</tr>
<tr>
<td></td>
<td>H$_2$SO$_4$ treated carbon</td>
<td>2.03 mmol/g</td>
<td>115</td>
<td>25</td>
<td>7</td>
<td>Afkhami et al.,</td>
</tr>
<tr>
<td>Category</td>
<td>Adsorbent</td>
<td>Amount of nitrate adsorbed</td>
<td>Concentration range (mg/l)</td>
<td>Temperature (°C)</td>
<td>pH</td>
<td>Reference</td>
</tr>
<tr>
<td>----------</td>
<td>-----------</td>
<td>-----------------------------</td>
<td>-----------------------------</td>
<td>------------------</td>
<td>---</td>
<td>-----------</td>
</tr>
<tr>
<td>cloth</td>
<td>Carbon nanotubes</td>
<td>25mmol/g</td>
<td>NA</td>
<td>25</td>
<td>&lt;5</td>
<td>Khani &amp; Mirzaei, 2008</td>
</tr>
<tr>
<td></td>
<td>Untreated coconut granular activated carbon</td>
<td>1.7mg/g</td>
<td>5-200</td>
<td>25</td>
<td>5.5</td>
<td>Bhatnagar et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Coconut shell activated carbon</td>
<td>0.27 mmol/g</td>
<td>NA</td>
<td>30</td>
<td>2-4</td>
<td>Ohe et al., 2003</td>
</tr>
<tr>
<td></td>
<td>Rice hull</td>
<td>1.32 mmol/g</td>
<td>1-30</td>
<td>30</td>
<td>NA</td>
<td>Orlando, et al., 2002</td>
</tr>
<tr>
<td></td>
<td>Wheat straw charcoal</td>
<td>1.10 mg/g</td>
<td>0-25</td>
<td>15</td>
<td>NA</td>
<td>Mishra &amp; Patel, 2009</td>
</tr>
<tr>
<td></td>
<td>Mustard straw charcoal</td>
<td>1.3 mg/g</td>
<td>0-25</td>
<td>15</td>
<td>NA</td>
<td>Mishra &amp; Patel, 2009</td>
</tr>
<tr>
<td></td>
<td>Raw wheat residue</td>
<td>0.02 mmol/g</td>
<td>50-500</td>
<td>21-25</td>
<td>6.8</td>
<td>Wang et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Modified wheat residue</td>
<td>2.08 mmol/g</td>
<td>50-500</td>
<td>21-25</td>
<td>6.8</td>
<td>Wang et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Bamboo charcoal</td>
<td>0.104 mmol/g</td>
<td>NA</td>
<td>32</td>
<td>2-4</td>
<td>Ohe, et al., 2003</td>
</tr>
<tr>
<td></td>
<td>Bamboo powder charcoal</td>
<td>1.25 mg/g</td>
<td>00-10</td>
<td>10</td>
<td>NA</td>
<td>Mizuta, et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Chitosan beads</td>
<td>90.7 mg/g</td>
<td>25-100</td>
<td>30</td>
<td>5</td>
<td>Chatterjee, et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Cross linked Chinese reed</td>
<td>7.55 mg/g</td>
<td>1000-4000</td>
<td>25</td>
<td>5.8</td>
<td>Namasivayam &amp; Holl, 2005</td>
</tr>
<tr>
<td></td>
<td>Surface modified sepiolite</td>
<td>0.453 mmol/g</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Ozcan, et al., 2005</td>
</tr>
<tr>
<td></td>
<td>Coated zeolite</td>
<td>0.6-0.74 mmol/g</td>
<td>10-3100</td>
<td>4-20</td>
<td>NA</td>
<td>Xi, et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Modified bentonite</td>
<td>12.83-14.76 mg/g</td>
<td>100</td>
<td>23</td>
<td>5.4</td>
<td>Xi, et al., 2010</td>
</tr>
</tbody>
</table>
2.2.1.2 Reverse osmosis

In the reverse osmosis (RO) process, contaminated water passes through a semipermeable membrane due to the pressure difference between both sides of the membrane resulting in leaving the contamination at one side of the membrane and providing clean water on the other side. Although the main use of RO systems is desalination of brackish water, it has been one of the best technologies for removing nitrate and other inorganic contamination from water (USEPA, 2004). Since RO is an expensive process, it is more feasible to be used for nitrate removal when other organic and inorganic contamination is present in water as well. Nitrate removal from ground water, full-scale RO plants are in use in the cities of Riverside and Tusain in southern California as well as Chino Basin Desalter (Bergman, 2007).

RO processes usually need pre-treatment systems to prevent fouling of the membrane, to maintain the performance of the system, and to extend the lifetime of the membranes. The general pre-treatment processes for RO systems include conventional methods (flocculation/coagulation, filtration, disinfection) and non-conventional methods (microfiltration, ultrafiltration and beach-well) (Ebrahimi et al., 2001; Bergman, 2007). Although blending feed and product water is a strategy to stabilize the product water and adjust pH and alkalinity after water treatment, RO systems may need a proper post-treatment method as well to readjust the quality of water after treatment (Bergman, 2007; Kapoor & Viraraghavan, 1997; Metcalf & Eddy, 2004).
2.2.1.3 Chemical denitrification

Iron and aluminum powder can be used as zero-valent metals for nitrate removal from water. Their tendency to donate electrons can be used to reduce contamination such as nitrate. Nitrate removal from water using zero-valent metals is called chemical denitrification. There are different pathways through which nitrate is reduced by the zero-valent iron process but in all of them iron is oxidized to ferrous iron and nitrate is reduced to ammonia on experimental conditions, rxn 2.1 (Kumar et al., 2006; Luk et al., 2002; Shrimali, & Singh, 2001; Yang et al., 2005).

\[ 4\text{Fe}^+ + \text{NO}_3^- + 7\text{H}_2\text{O} \rightarrow 4\text{Fe}^{+2} + \text{NH}_4^+ + 10\text{OH}^- \quad \text{rxn 2.1} \]

2.2.1.4 Electrodialysis

Electrodialysis is another physical-chemical technology that can be used for removal of nitrate from water. In this process, ions are transferred by electric current flow through cation and anion membranes, depending on ion charge, from a less concentrated solution to a more concentrated one, leaving a demineralized stream (Symons et al., 2001). Due to membrane scaling problems, this process requires pre-treatment. Fouling in electrodialysis can be controlled by modification of method as electrodialysis reversal (EDR) in which the electrical polarity of the electrodes is reversed on a set time cycle (Symons et al., 2001).

2.2.2 Biological processes

Reduction of nitrate can happen through two main processes, denitrification and assimilatory/dissimilatory nitrate reduction to ammonia. Dissimilatory nitrate reduction to ammonia (DNRA) and assimilatory nitrate reduction to ammonia retain the nitrogen in a fixed form, ammonia, keeping it available for further biological processes (rxn 2.2).
\[ C_6H_{12}O_6 + 3NO_3^- + 3H^+ \rightarrow 6CO_2 + 3NH_3 + 3H_2O \quad \text{rxn 2.2} \]

Dissimilatory nitrate reduction to nitrogen gas (denitrification) is an important pathway in nitrogen cycle. Four different enzymatic reactions are responsible for denitrification; nitrate to nitrite (rxn 2.3), nitrite to nitric oxide (rxn 2.4), nitric oxide to nitrous oxide (rxn 2.5), and the last step nitrous oxide to nitrogen gas (rxn 2.6) (Giblin et al., 2013). Each step is performed by a specific enzyme. Nitrate to nitrite is performed by nitrate reductase which is responsible for following reaction in which two electrons are needed to reduce nitrate to nitrite (Metcalf & Eddy, 2004):

\[ \text{NO}_3^- + 2e^- + 2H^+ \rightarrow \text{NO}_2^- + H_2O \quad \text{rxn 2.3} \]

Nitrite reductase is an enzyme which is responsible for reducing nitrite to nitric oxide through the following reaction:

\[ \text{NO}_2^- + e^- + 2H^+ \rightarrow \text{NO} + H_2O \quad \text{rxn 2.4} \]

Nitric oxide reductase reduces nitric oxide to nitrous oxide immediately after it is produced.

\[ 2\text{NO} + 2e^- + 2H^+ \rightarrow \text{N}_2\text{O} + H_2O \quad \text{rxn 2.5} \]

The last step of denitrification process is reduction of nitrous oxide (\( \text{N}_2\text{O} \)) to nitrogen gas based on the following reaction which is catalyzed by nitrous oxide reductase:

\[ \text{N}_2\text{O} + 2e^- + 2H^+ \rightarrow \text{N}_2 + H_2O \quad \text{rxn 2.6} \]

Biological systems prefer to go through the DNRA pathway when nitrate is limiting compared to the source of electrons because DNRA needs more organic compound as an electron source compared to denitrification (Megonigal et al., 2004). In this research, acetate was used as the electron and carbon source with 1.5 of the stoichiometric ratio of acetate to
nitrate in order to provide just enough carbon and electron source for denitrification (Metcalf & Eddy, 2004).

The concentration of dissolved oxygen (DO) can control the denitrification process. High concentrations of DO can be a repressor for genes that are responsible for nitrate reduction as well as an inhibitor for enzyme activity. Both autotrophic and heterotrophic bacteria possess the enzymes which result in nitrate reduction to nitrogen gas. Most of the denitrifiers can use other sources, such as oxygen and perchlorate as electron acceptors. Most of the denitrifiers are facultative heterotrophs that use organic carbon as an electron donor and nitrate as an electron acceptor. Since in the reduction of oxygen more energy will be available, in presence of oxygen the facultative aerobes prefer aerobic respiration. So, nitrate will be used as an electron acceptor only when DO concentration is depleted in solution (Giblin et al., 2013; Madigan et al., 2011; Metcalf & Eddy, 2004).

Although biological denitrification is commonly used for wastewater treatment, it is not commonly applied to drinking water treatment processes in North America because of direct contact of water with biomass. Application for drinking and groundwater treatment has mostly been studied in the lab scale and only occasionally developed in full-scale plants. There are many studies looking at biological removal of nitrate using different carbon sources and focusing on nitrate removal rate under different environmental conditions. Table 2.2 summarizes some of these studies. The exact values of biological nitrate denitrification rate from different studies are difficult to compare since the environmental conditions including temperature, salt concentration, pH, as well as types of the reactors, source of carbon, retention time, etc. are different. Although the denitrification rates are different, in all temperature-dependent studies the denitrification rate increased with
increasing temperature; following the Arrhenius’s law (Dawson & Murphy, 1972) or linear equation (Elefsiniotis & Li, 2006; Lewandoswki, 1982). Also, all salt-dependent studies have come to the same conclusion; nitrate removal rate is decreased by increasing the salt concentration (Glass & Silverstein, 1998; Ucisik & Henze, 2004; Van der Hoek et al., 1987).

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Nitrate degradation rate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium citrate</td>
<td>0.057 mg nitrate/mg VSS.h</td>
<td>Dawson &amp; Murphy, 1972</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>0.3 mg nitrate/mg VSS.h</td>
<td>Dawson &amp; Murphy, 1972</td>
</tr>
<tr>
<td>methanol</td>
<td>0.09 mg nitrate/mg VSS.h</td>
<td>Folger et al., 2005</td>
</tr>
<tr>
<td>acetate</td>
<td>0.03 mg nitrate/mg VSS.h</td>
<td>Glass &amp; Silverstein, 1998</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.01 mg nitrate/mg VSS.h</td>
<td>Pansward &amp; Anan, 1999</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.053 mg nitrate/mg VSS.h</td>
<td>Pansward &amp; Anan, 1999</td>
</tr>
<tr>
<td>VFA</td>
<td>0.0005 mg nitrate/mg VSS.h</td>
<td>Elefsiniotis &amp; Li, 2006</td>
</tr>
<tr>
<td>VFA</td>
<td>0.004 mg nitrate/mg VSS.h</td>
<td>Elefsiniotis &amp; Li, 2006</td>
</tr>
<tr>
<td>VFA</td>
<td>0.005 mg nitrate/mg VSS.h</td>
<td>Elefsiniotis &amp; Li, 2006</td>
</tr>
<tr>
<td>Ethanol</td>
<td>95% reduction (200-1000 nmol/L/h)</td>
<td>Salminen, et al., 2014</td>
</tr>
<tr>
<td>Methanol</td>
<td>12 kg-N/m3 .d</td>
<td>Rabah &amp; Dahab, 2004</td>
</tr>
</tbody>
</table>

Since the direct contact of the biomass with water in traditional biological denitrification causes difficulties regrading water treatment, membrane bioreactors/membrane contactors have been developed to overcome this problem. In these processes an ion exchange membrane is used to separate the biomass from water while denitrification takes place. Nitrate from contaminated water transfers through the membrane to the biomass side in which denitrification happens using a source of carbon. Separating the
biomass and water eliminates the further use of disinfection process after denitrification (Fabbricino & Petta, 2007; Matos et al., 2006; Ricardo et al., 2012; Upadhaya et al., 2010).

2.2.3 Ion exchange

Ion exchange can be considered as the exchange of ions between two electrolytes or between an electrolyte solution and a complex. Basically, ion exchange technology as a water treatment process is based on exchange of contaminant ion existing in water with an innocuous ion attached to the ion exchange materials (resin). The ion, attached to the functional groups of the resin in its original form, is called counter-ion (Helfferich, 1962).

There are three different types of ion exchange processes; cation exchangers are used for exchanging positively charged ions, anion exchangers for negatively charged ions, and amphoteric exchangers that are used for both cations and anions at the same time (Samatya et al., 2006). There are different types of developed synthetic resins that can be used in ion exchange processes. Synthetic resins are either acrylic or styrenic. Acrylic resins have an aliphatic (open carbon chain) matrix but styrenic resins have an aromatic matrix. The styrenic resins are more common in the market compared to acrylic ones. The most important resin classification is the characteristic of the functional group attached to the main matrix of the resin. Resins can be classified in four different groups; strong-acid, weak-acid, strong-base, weak-base. This classification is based on the pKₐ (dissociation constant) of the functional group to the matrix of resin. Resins with pKₐ higher than 13 are strong-base and those ones with very low value of pKₐ are strong-acid. The resin with moderate value of pK are weak-acid and weak-base (Helfferich, 1962; Sharbatmaleki, 2010).

Since nitrate removal is the main goal of this research, this discussion will focus on the structure of anion exchange resin. Table 2.3 shows the types of resin based on their
functional group. Strong-base anion exchange resins are categorized as type I and type II. The functional group in strong base resins is a positively charged quaternary amine which is attached to the main polymeric matrix. The counter ion is ionically bonded to the functional group and will be exchanged with available anions in water (Helfferich, 1962; Sharbatmaleki, 2010). In type II resin, one of the methyl groups of the amine will be replaced by ethanol. This gives the type II resins higher capacity and regeneration efficiency compared to type I. On the other hand, type I has a stronger matrix and does not react with the chemicals in the environment (Sharbatmaleki, 2010).

Table 2.3: Common types of anion exchange resin

<table>
<thead>
<tr>
<th>Type of resin</th>
<th>Functional group</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak-base anionic</td>
<td>Secondary amine</td>
<td>([R(CH_3)_2N]HOH)</td>
</tr>
<tr>
<td>Strong-base anionic type I</td>
<td>Trimethyl-amine</td>
<td>([R(CH_3)_3N^+]Cl^-)</td>
</tr>
<tr>
<td>Strong-base anionic type II</td>
<td>Dimethyl ethanol-amine</td>
<td>([R(CH_3)_2(CH_2CH_2OH)N^+]Cl^-)</td>
</tr>
</tbody>
</table>

Figure 2.1 shows the chemical structure of the resin that is used in our study, macroporous styrene strong base anion (SBA) resin with N-tributylamine functional groups which is nitrate selective resin.
2.3 Methods comparison

Darbi et al., (2003) compared three different methods for nitrate removal from ground water in Winnipeg, Manitoba; reverse osmosis, biological denitrification and ion exchange. Using a WGR-600 membrane, the RO system achieved 85% nitrate removal while ion exchange and biological denitrification resulted in 90% and 96% removal of nitrate, respectively. Biological denitrification is a promising method for removal of nitrate. But a long start up time as well as the water contamination due to the direct contact of the biomass with water makes this process not practical for removal of nitrate from drinking water. Chemical denitrification is still in the research phase and does not have very high removal efficiency. Also, the high cost of iron, long reaction time, pH constraints are disadvantages which limit the use of zero-valent technology. Water treatment by electrodialysis is limited to soft waters due to membrane scaling problems unless a pre-treatment process is included which is not cost effective. Electrodialysis also demands high energy. Since time and efficiency are important factors, among all of the technologies discussed previously, ion
exchange and reverse osmosis are the most commonly used technologies because of short start-up time, minimal temperature and pH constraints, high efficiency, etc, especially for small communities. The RO process requires pre-treatment and sometimes post treatment processes which make it an expensive option and more practical for desalination compared to a nitrate removal from water. Therefore, among these methods, nitrate removal by ion exchange has been favored over other technologies in the last decade and it is been suggested by United States Environmental Protection Agency (USEPA) as an accepted treatment method for removal of nitrate form water (USEPA, 2013).

2.4 Ion exchange challenges

Based on the selectivity of the resin, for nitrate removal from water, there are generally two kinds of resins as the ion exchanger; one is a nitrate selective and the other one is low-selective (or non-selective) anion exchange resin.

Non-selective resins have lower affinity for nitrate and other anions can compete with nitrate in ion exchange process. The biggest issue associated with using low/non-selective resins is the presence of other anions such as perchlorate, sulfate, and bicarbonate that affect the available sites on the resin and the adsorption capacity for nitrate. In this type of resin breakthrough points occur more frequently and although the resin is easily regenerable, frequent regeneration of the resin produces brine that must be disposed of properly before discharge (Faccini, 2011; Venkatesan et al., 2010). Although this type of resin is regenerable, because of the presence of other anions in contaminated water, mostly it is not economical to use non-selective resins in treatment processes.

Water treatment utilities prefer to use highly nitrate selective resins because of their great capacity; they can be used for 100,000 to 200,000 bed volumes depending on the water
contaminant. Selective resins have high efficiency for nitrate removal from water and can remove nitrate for many bed volumes without any leakage. However, the regeneration of this type of resin using the conventional methods is not practical since the regeneration of these highly selective resins needs a high concentration of salt (≈12%) and huge amount of regeneration solution which results in large quantity of brine solution with high concentration of salt which needs to be discharged to the water bodies (ocean, lake, river, …) or sent out to the wastewater treatment plants, on-site septic system, dry well, etc. Regeneration by normal concentration of brine is ineffective for most selective resins such as Purolite, bi-functional and some other strong-base anion exchange resins and should be done by using of different chemicals such as; ferric chloride, hydrochloric acid, etc which is not economically feasible (Aldex Chemical Company Ltd., 2015; Aldridge et al., 2005; Brown et al., 2003). Because of this, most of the highly selective resins are considered as non-regenerable especially for small communities and they will be replaced by fresh resin and the exhausted resin will be disposed of as a hazardous waste, usually through incineration or sending it to a landfill (Clifford & Liu, 1993; Samatya et al., 2006; Xiao et al., 2010) neither of which is cost-effective. So, because of the wide use of ion exchange processes as a promising method for nitrate removal from contaminated water, developing methods for regeneration of exhausted non-regenerable selective resins is urgent.

There are a few studies looking at regeneration of nitrate selective resins. (Cyplik et al., 2008) investigated the adsorption capacity and conventional regeneration of a selective resin (IONAC SR-7). The main goal of this study was to investigate the effect of complete and partial regeneration process on brine production. In their research, it was found that as a result of resin partial regeneration using 12% salt the resin’s working sorption capacity
decreased 70% of theoretical resin ion-exchange capacity. Also, complete regeneration of resin using 12% salt for 9 cycles was performed. As a result, they suggest that using partial regeneration results in a 50% reduction of the volume of environmentally harmful waste after regeneration. As far as the resin capacity loss is not a concern, this is a good solution to decrease brine production. However, still brine treatment is an issue. Biological treatment of the waste stream of resin regeneration process with high concentration of salt and nitrate is quite a challenge. Denitrification needs a source of carbon as electron donor (such as methanol, acetate, ethanol, …) as well as nutrients that should be provided by mixing the brine stream with municipal wastewater stream or be added separately to the brine treatment plant (Clifford & Liu, 1993; Cyplik et al., 2008; Seidel et al., 2011; Van der Hoek et al., 1988). If the brine stream is not diluted the microbial community must be salt tolerant.

This issue leads to the idea to develop a new method to combine the resin regeneration process with brine treatment, so both processes can be done at the same time. Resin bioregeneration is a new concept which is even more important when considering the application to nitrate selective or non-regenerable resins which are mainly incinerated not regenerated. Van der Hoek et al., (1988a), has studied biological regeneration of a nitrate selective resin (IRA996). In this study, exhausted resin was regenerated in an up-flow sludge blanket denitrification reactor (using methanol as a carbon source) for 6 h of regeneration and 1 h rinsing following by resin disinfection system. Although they do not mention anything specific about the multi-cycle use of resin in the process, they achieved a noticeable reduction of brine production. In another similar study, Van der Hoek et al., (1988b), performed studies using denitrifying bacteria in the resin to avoid high volume production of brine. In this study after 24 h of regeneration 18% of the nitrate was still on the resin which
resulted in reduction in the capacity of the resin when it was used in another ion exchange cycle (72% nitrate capacity compared with conventional regenerated ion exchange column). Although this method helps to reduce salt consumption and waste brine production, it is not practical for every water treatment system (especially small communities) because of the cost of adding carbon source and nutrients as well as having the required expertise to maintain the biological system (Clifford & Liu, 1993; McAdam & Judd, 2008; Orlando et al., 2002; Seidel et al., 2011; Van der Hoek et al., 1987; Van der Hoek et al., 1988a; Van der Hoek et al., 1988b).

In other studies, researchers have studied the direct bioregeneration of perchlorate-selective resin (Venkatesan et al., 2010; Xiao et al., 2010) and bioregeneration of exhausted resin for nitrate treatment from water (Meng et al., 2014), looking at the effect of different environmental parameters on bioregeneration. Since in these studies resin was in direct contact with the culture, at the end of each cycle, bio-fouling removal and disinfection of resin was performed before the resin was reused in another cycle. Also, there is a possibility of losing the capacity of the resin as resin is in direct contact of biomass. These factors result in increasing the cost and time required for each regeneration cycle (Venkatesan et al., 2010).

To improve this sustainable method for nitrate removal from water, we have enclosed the resin in a membrane to prevent direct contact of the resin with the bacterial culture during the bioregeneration stage. For the resin bioregeneration method, the biomass should still be salt tolerant. In the last decade, biological reduction of perchlorate and nitrate using salt-tolerant microorganisms was developed in Dr. Roberts’ research laboratory. A nitrate-perchlorate reducing mixed culture (NP30) was isolated from marine sediment in 2001 and has been fed perchlorate and nitrate in 3% salt since then. The microbial composition of
NP30 was analyzed using DGGE (denaturing gradient gel electrophoresis) and FISH (fluorescence in-situ hybridization). The results showed that *Halomonas* was the dominant (>18%) nitrate-reducing organism and *Azoarcus/Denitromonas* was the dominant (>22%) perchlorate-reducing organism (Xiao et al., 2010). In a later study a pure culture of a *Marinobacter* (P4B1) strain that could use both perchlorate and nitrate was isolated (Xiao et al., 2010).

By using the same mixed salt tolerant culture (NP30), in the first step of our research, as a proof of concept, the use of multi-cycle exhaustion/bioregeneration of resin enclosed in membrane was shown to be quite effective; in the next steps of the research the effect of salt concentration and temperature as the main environmental factors on important processes (desorption & degradation) involved in resin bioregeneration was studied to allow the mathematical modelling and optimization of the process as the final step of the project. Chapters 3 to 6 present the methodology and the results of this research.
Chapter 3: Multi-cycle bioregeneration of resin; proof of concept

As a proof of concept and the first step of the project, multi cycle loading and bioregeneration of tributylamine strong base anion (SBA) exchange resin was studied. In this study, resin was enclosed in a membrane to eliminate direct contact of the biological system with the resin. The main objectives of first part of the research was to determine: the feasibility of multi cycle regeneration of nitrate-selective resin; the capacity loss of the resin after several ion exchange/ bioregeneration cycles; the kinetics observed during each of the processes; and to evaluate the results in the context of the literature to date.

3.1 Materials & methods

3.1.1 Resin

This research was performed using ion exchange resin commercially available as highly nitrate selective product (CalRez 2190). This resin is classified as a macroporous styrene strong base anion (SBA) resin with N-tributylamine functional groups. The resin beads are 0.77±0.06 mm. Based on previous studies the nitrate-chloride separation factor and capacity of the resin was identified as 780 and 1.48 meq/g, respectively (Faccini, 2011). The resin was conditioned and converted to the chloride form using HCl (1N) and NaOH (1N) using 3 cycles of acid- base cycling. One gram of the washed resin was enclosed in a biological inert, ultra-pure cellulose ester membrane (Spectrum Spectra/por 6RC Dialysis membrane tubing 50,000 Dalton molecular weight cut-off: ≈0.04 μm) to avoid direct contact of the resin with the culture.

Figure 3.1 shows resin enclosed in the membrane (using clamps) in the reactor (1 litre flask). The mass of resin was chosen to present as close to a monolayer of resin as possible to
maximize the transfer of nitrate out of the resin and into the culture. The resin was placed in the membrane in as close to monolayer as possible to remove the effect of inter-particle diffusion (in a packed bed) from the mass transfer system. This was done by clamping the middle of the membrane and adding the same amount of resin on both sides of the clamp while the membrane was sitting horizontally. Also, the length of the membrane was selected to make sure that resin is monolayer in the membrane (Figure 3.1).

![Figure 3.1: Schematic of set-up; resin beads, resin enclosed in membrane in the reactor (1 liter flask), and column exhaustion set-up for first and last cycle of exhaustion](image)

### 3.1.2 Culture

A mixed perchlorate- and nitrate-reducing bacterial culture (NP30) was enriched from marine sediment in previous research (Cang et al., 2004). In this research the perchlorate was
not needed so the culture was adapted to 6% salt and 500 mg/L nitrate using synthetic medium based on general marine medium composition with the addition of a group of trace metals as reported previously (Cang et al., 2004). The medium contained 23 g/L 

\[
\begin{align*}
\text{MgCl}_2 \cdot 6\text{H}_2\text{O}, & \quad 1.4 \text{ g/L CaCl}_2 \cdot 2\text{H}_2\text{O}, \\
0.72 \text{ g/L KCl}, & \quad 60 \text{ g/L NaCl}, \\
3.9 \text{ g/L CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}, & \quad 0.3 \text{ g/L NaHCO}_3, \\
0.48 \text{ g/L NH}_4\text{Cl}, & \quad 1 \text{ mL/L 67 mM Na}_2\text{S} \cdot 9\text{H}_2\text{O}, \\
1 \text{ mL/L phosphate solution}, & \quad \end{align*}
\]

(50 g/L KH$_2$PO$_4$), 1 mL/L mineral solution (which contained 50 g/L (NH$_4$)$_6$Mo$_7$O$_{24}\cdot4\text{H}_2\text{O}$, 0.05 g/L ZnCl$_2$, 0.3 g/L H$_3$BO$_3$, 1.5 g/L FeCl$_2 \cdot 4\text{H}_2\text{O}$, 10 g/L CoCl$_2 \cdot 6\text{H}_2\text{O}$, 0.03 g/L MnCl$_2 \cdot 6\text{H}_2\text{O}$, and 0.03 g/L NiCl$_2 \cdot 6\text{H}_2\text{O}$). Acetate was applied to achieve 1.5 acetate to nitrate ratio as the carbon and electron source. Strict anaerobic technique was used in medium preparation.

Monitoring the nitrite in the system showed that there was no nitrite accumulation in the system. A nitrogen mass balance and gas production of the system showed that complete denitrification of nitrate to the nitrogen gas had occurred in all cultures.

### 3.1.3 Exhaustion/ regeneration

In this research, 3 test (biological regeneration) and 3 control (salt only regeneration) samples were studied using 6 cycles of exhaustion/ regeneration. Tests and controls were treated identically during exhaustion. The resin was exhausted in columns in the first and last cycles to reveal the breakthrough curve and capacity for this resin using glass columns with 5 cm diameter and 50 cm length (Figure 3.1). To do this, nitrate solution (500 mg/L) was added to the influent with a flow rate of 2.5 ml/min to obtain an empty bed contact time (EBCT) of 10 min. Samples of the effluent were collected periodically and analysed for nitrate concentration. The resin was considered as exhausted when nitrate appeared in the
effluent. The resin operating capacity was estimated from the breakthrough curve by calculating the area above the curve and the mass of resin loaded.

Exhaustion for cycles 2-5 were done as a batch where both control and test samples were enclosed in membranes and placed in 1 litre flasks with 300 ml of 700 mg/L nitrate solution and mixed on a rotary shaker (110 RPM & 23°C) for 3 days to ensure complete exhaustion. A sample of the aqueous phase was analysed for nitrate before and after exhaustion.

During regeneration studies the test membranes containing resin were placed in 1 liter flasks with 250 ml of mixed synthetic medium and culture (2500 mg/L VSS) and mixed by shaking on a rotary shaker (110 RPM & 23°C) for 48 hours. In addition to the 3 replicate of test conditions, 3 replicate of control conditions were placed in the same medium without the culture to show nitrate desorption from resin due to the salt. For the controls the medium was replaced with new medium at the beginning of each regeneration cycle. For the tests the culture was settled and about 90% of the medium above the biomass was replaced with fresh medium.

Biomass concentrations were measured based on the volatile suspended solids (VSS) before the first cycle and after the last cycle. Aqueous phase samples were taken in intervals during regeneration for nitrate analysis. After each complete cycle, 0.1 g of resin was taken for analysis of the nitrate attached to the resin.

3.1.4 Analytical methods

Nitrate in the aqueous phase was measured using the nitrate test kit and an Orion 9512BNWP ammonia ion selective electrode (Thermo Fisher Scientific) when the
concentration of nitrate was higher than 1 mg/L. Lower concentrations of nitrate and chloride concentrations were measured using a Dionex ICS-2100 with KOH eluent using a multi-step gradient of 1, 1, 30, 60, 1, and 1 mM respectively at time of 0, 8, 28, 38, 38, and 43 minutes and flow rate of 1.5 ml/min. Calibration curves were established using nitrate standard solutions of 250 µg/L, 500 µg/L, 750 µg/L and 1 mg/L and chloride standard solutions of 750 µg/L, 1 mg/L, 10 mg/L, 100 mg/L, and 500 mg/L.

The measurement of nitrate attached to the resin was carried out by resin combustion in a calorimeter chamber (Bomb calorimeter). After combustion of the sample, the combustion chamber was rinsed thoroughly with Type 1 (18.2 MΩm/cm²) water. The chloride concentration in the rinsate was analyzed using ion chromatography. With each set of samples, three replicates of the resin sample in complete chloride form was combusted. The chloride concentration from the resin sample in complete chloride form was compared to the chloride concentration of the exhausted resin (in triplicate). The difference of the chloride concentration in exhausted resin samples and samples from the resin in complete chloride form was used to calculate the nitrate attached to the resin. The subtractive method was applied because the nitrogen on the functional groups of the resin formed nitrate on combustion and prevented the direct measurement of nitrate on the resin. The method was verified by combustion of resin with known nitrogen loading prior to the use of the technique for samples with 96±2% recovery.

Volatile suspended solids (VSS, biomass) was measured using Standard Methods 254-E (Eaton et al., 2005). A sample volume of two ml was filtered through a glass fiber filter. The filter was dried and then combusted. For each sample VSS was measured in triplicate and the average of all three measurements was used as the culture VSS.
3.1.5 Statistical analysis

Statistical analysis was performed to determine whether there is a significant difference in nitrate concentration between the different treatments. The statistical significance of the differences was determined using one way ANOVA test (p=0.05) and a Student Neuman Keuls test when the ANOVA showed significant differences using SPSS Statistic 20 software.

3.2 Results & discussion

3.2.1 Resin exhaustion

Before and after each exhaustion cycle nitrate was measured in the aqueous phase of each test and control reactor. Based on the difference of nitrate concentration before and after exhaustion and the mass of applied resin, the amount of nitrate adsorbed on the resin was calculated. Figure 3.2 presents the nitrate adsorbed in test and control reactors for each cycle compared to theoretical capacity. Since a sample was taken from resin at the end of each cycle, adsorbed nitrate has been normalized based on mass of resin at each cycle.
Figure 3.2: Nitrate adsorbed onto the resin during exhaustion for each cycle for tests and controls. The data are calculated from the difference between nitrate concentrations in the aqueous phase before and after exhaustion. The bar heights indicate the average and the error bars indicate one standard deviation of triplicate test or controls.

Statistical analysis revealed there was no significant difference in the adsorbed nitrate over the first 4 cycles. For cycle 5, the difference is significant, a 12% reduction in adsorbed nitrate was observed in cycle 5 compare to other cycles. There was also no significant difference in adsorbed nitrate in the controls in first 3 cycles, the amount of adsorbed nitrate decreased significantly in cycles 4 and 5. The statistical analysis also showed that there was significantly more nitrate adsorbed in the tests than in the controls for the 4th and 5th cycle.

3.2.2 Resin bio-regeneration

During each bioregeneration cycle, the nitrate concentration in the aqueous phase was measured periodically during the 48 hours of bioregeneration. Figure 3.3 shows the
normalized nitrate concentration (mg of nitrate per gram of dry resin) for test and control reactors in each cycle. The concentrations of nitrate in the test reactors reached a peak in first 0.5 to 1 hour after placing the resin in medium because of desorption of nitrate from resin. After this time the microorganisms began to degrade the nitrate in the aqueous phase and so the nitrate concentration decreased rapidly over the next 9 hours. The nitrate concentration was generally non-detectable after 10 hours. Although nitrate was no longer measurable in the aqueous phase, the resin was left in the flask for 48 hours to ensure that the nitrate attached to the resin had time to completely desorb.
Figure 3.3: Nitrate concentration in the aqueous phase during regeneration a) test b) control; nitrate concentrations in aqueous phase are normalized by dividing to the mass of resin in each sample. The data points represent the average of three replicate of each test and control samples. The error bars are omitted to avoid crowding in the figure.
In the control reactors, the nitrate concentration in the aqueous phase reached equilibrium by about 10 hours. A statistical analysis of the nitrate concentration in controls showed that the equilibrium concentration in control samples in cycle 1 and 2 are not significantly different but they are different from cycle 4 and 5.

The peak concentrations shown in Figure 3.3 decrease in each cycle because the biodegradation rate is increasing. To further demonstrate this, the cultures were incubated with a direct nitrate spike with no resin. Biomass was spiked with nitrate to measure the direct nitrate degradation rate of the biomass. Figure 3.4 presents the concentrations of nitrate in the aqueous phase. Initially the culture was able to remove 500 mg/L of nitrate from the aqueous phase experiment in 12 hours and after using the culture for 6 bioregeneration cycles it was able to remove the same amount of nitrate in <8 hours. This can be explained by an increase in biomass (VSS increased from 2500 mg/L to 5400±600 mg/L). As the number of cycles increased the biomass also increased the biodegradation of nitrate in the aqueous phase occurred before the first sampling point.

\[
\begin{align*}
\text{y} &= -42.13x + 494.15 \\
R^2 &= 0.9965 \\
\text{y} &= -63.299x + 470.49 \\
R^2 &= 0.9895
\end{align*}
\]

Figure 3.4: Nitrate degradation in aqueous phase cultures used during the initial and final cycle
At the end of each regeneration cycle, samples of resin were taken to determine the mass of nitrate attached to the resin using the bomb calorimeter to combust the resin. Figure 3.5 presents the results of these analyses. There was insignificant nitrate left on the resin in test samples for the 1\textsuperscript{st} three cycles. After cycle 4 some nitrate residual remained attached to the resin. The culture achieved more than 94% and 78% nitrate removal from resin in cycle 4 and 5, respectively. There was always some residual nitrate attached to the resin in control reactors. This increased with each cycle. Through cycle 1-5, for control samples, removal of nitrate attached to the resin was 99%, 96%, 89%, 79% & 59%, respectively.

![Figure 3.5: Residual nitrate on resin after each cycle as determined from the rinsate from ignition of the resin. The bar height presents the average and the error bars present one standard deviation of triplicate results.](image_url)
3.2.3 Time series resin analysis

During regeneration, nitrate was removed from the aqueous phase within 10 hours, but the samples were incubated for 48 hours to regenerate the resin as much as possible. In order to understand the exact time when nitrate was completely removed from the resin (i.e. no new nitrate desorbed from the resin) in cycle 6, samples of resin were taken at different times and analysed for nitrate attached to the resin using the bomb calorimeter. The results (Figure 3.6) show that for fresh resin the nitrate in the resin was removed to non-detectable levels within 10 hours, but after six cycles of exhaustion/bioregeneration the process was much slower. A projection of the slope of line suggests that it would have taken 80 hours to completely regenerate the resin.

Figure 3.6: Residual nitrate (as determined from the rinsate from ignition of the resin) on resin at different intervals during the first and last regeneration cycle
3.2.4 Resin capacity & analysis of the process

Column experiments were conducted as the first and last exhaustion cycle in order to observe resin performance and determine resin capacity and obtain a breakthrough curve. The result of the column exhaustion test is shown in Figure 3.7. The bed volume of the exhaustion solution at which the nitrate concentration in the effluent was half of the influent, was considered as the 50% breakthrough point of the resin.
Figure 3.7: Column exhaustion breakthrough curve. a) first cycle of all samples, b) last cycle of control samples, c) last cycle of test samples. The data points represent the normalized ($C/C_0$) nitrate concentrations in the effluent of columns fed with 500 mg/L nitrate using an EBCT of 10 minutes.
Table 3.1 summarizes the change in bed volume to the 50% breakthrough point and the calculated capacity of resin. Initially the 50% breakthrough point was at 68 bed volumes (BV) of resin. After using resin in 6 exhaustion/bioregeneration cycles, less than 6% of the resin capacity was lost while in the controls without bioregeneration, the resin lost 20% of its capacity.

Table 3.1. Bed volumes required to 50% breakthrough point and resin capacity, initially and after 6 cycles

<table>
<thead>
<tr>
<th>Sample</th>
<th>Breakthrough point (BV)</th>
<th>BV reduction (%)</th>
<th>Capacity (meq/g of dry resin)</th>
<th>Capacity reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>61±2</td>
<td>13</td>
<td>1.34±0.06</td>
<td>5.9</td>
</tr>
<tr>
<td>Control</td>
<td>48±2</td>
<td>33</td>
<td>1.15±0.08</td>
<td>19.7</td>
</tr>
<tr>
<td>Initial</td>
<td>68</td>
<td>-</td>
<td>1.43</td>
<td>-</td>
</tr>
</tbody>
</table>

One major concern of multi-cycle use of resin in ion-exchange processes is resin capacity loss. In this research the capacity of resin during 6 exhaustion/bioregeneration cycles (test samples) was compared to the capacity of resin in control samples (no biological reduction). During regeneration cycles in control samples the desorption of nitrate from the resin continued to the point when the nitrate concentration in aqueous phase reached equilibrium with the nitrate concentration on the resin.

The difference between the final equilibrium concentration and the aqueous phase concentration of nitrate at any time can be considered as the driving force, which allows the incorporation of the concepts of kinetic limitations and end point equilibrium. In control samples, based on the equilibrium concentration of nitrate at each cycle an average desorption rate of 0.099±0.003 h⁻¹ was calculated. In test samples the equilibrium
concentration is changing continually as the desorption rate and the degradation rate change due to the concentration in the aqueous phase. At low concentrations, and early in the process, desorption is favored, as higher concentrations develop in the aqueous phase degradation becomes favored. So the test samples never reach the equilibrium concentration of control samples at each cycle.

Additionally, nitrate was removed faster from the test samples with each test cycle. This is reflected in the reduction in the peak concentration of nitrate in each cycle. The substrate utilization rate in the biological systems can be modeled with the following expression for soluble substrates such as nitrate.

\[
\frac{dS}{dt} = -\frac{kXS}{K_S + S}
\]  

(eq 3.1)

Where S is the nitrate concentration (mg/L); t is the time (h); k is the maximum specific nitrate utilization rate (mg of nitrate/mg of VSS•h); K_s is the half saturation constant for nitrate (mg/L); and X is the microbial concentration (mg VSS/L). A calculated specific degradation rate of - 0.017 and - 0.012 mg NO_3-/mg VSS•h respectively for initial and final tests shows that since the rates were very comparable, the increasing biomass must be responsible for the increased removal from the system.

Based on these results, the resin did not lose significant capacity until after 4 cycles. The capacity in cycle 5 and 6 was still much better in biologically regenerated resin than for resin that had not undergone biological regeneration. There appears to be an increase in the non-regenerable sites in the resin in each cycle. Because of the degradation of nitrate in the aqueous phase, the maximum concentration of nitrate in test samples never reaches the equilibrium concentration of nitrate in controls. So, the nitrate accumulation in the less
Regenerable sites is decreased and the capacity is maintained. Increasing the non-regenerable sites of the resin in each cycle can also be explained based by the shrinking core model which was developed for ion uptake by resins (Cang et al., 2004; Faccini, 2011; Sharbatmaleki & Batista, 2012; Sharbatmaleki et al., 2015; Venkatesan et al., 2010; Y. Xiao et al., 2010; Y. Xiao et al., 2010). The model shows that ions first occupy the outer region of the resin, and progressively move to the inner unused resin core with time. Based on this model, it is reasonable to assume that nitrate ions in the outer region of the resin become available to biomass more easily when compared to nitrate ions present in the inner core of the resin. So, in each cycle, as more and more nitrate is loaded in to the core, more time is required for the nitrate to dissolve out of the core. The calorimeter results suggest that leaving the resin in the bioreactor for a longer time would allow complete regeneration of resin.

Sharbatmaleki & Batista (2012), reported that the capacity loss was more significant through the first cycles and stabilized after a few cycles which differs significantly from the results reported here. However, the results are difficult to compare directly since their experiment was done using perchlorate and the resin was in direct contact with the culture.

The ultimate application of the process would see resin sold in membrane cartridges that could be plugged in to the ion exchange column housing for exhaustion, then removed and put in a bioregeneration tank for regeneration. This would probably be done in regional facilities where the culture can be maintained, rather than at each site. This would require shipping the resin to and from the regional facility and the treatment sites but would still result in a net savings due to the ability to reuse the resin several times. A full cost benefit analysis would be performed for each site to account for the cost of using fresh resin for each
use, as well as incineration or transport of the exhausted resin to the landfill, as compared to the costs associated with housing the resin in a membrane and applying bioregeneration process along with losing some capacity of resin after 6 cycles.

Although the proof of concept study for the extension of the use of the non-generable resins by use of multi-cycle exhaustion/ bioregeneration demonstrated that nitrate selective resin can be bioregenerated and reloaded for at least 6 cycles, to develop a process for the commercialization of this technology it is important to fully understand the bioregeneration process and determine the effect of important environmental factors on resin bioregeneration. The next two chapters focus on the effect of temperature and salt concentration on the main processes involved in resin bioregeneration (nitrate desorption and degradation).
Chapter 4: Effect of temperature & salt concentration on nitrate desorption from resin

Based on the proof of concept study (chapter 3), multi-cycle bioregeneration of exhausted resin is possible, but it is important to fully understand the process in order to develop a bioregeneration process for the commercialization of this technology.

Two main processes are involved in resin bioregeneration; desorption of nitrate from resin and biological degradation of nitrate. Environmental conditions affect these two processes especially temperature and salt concentration. Therefore, the effect of environmental parameters should be investigated on these two processes separately to provide valuable information for bioregeneration of the resin. Higher concentrations of salt and higher temperature are beneficial for regeneration of the resin since initial desorption rate of nitrate from resin is increasing by increasing the temperature and salt concentration. On the other hand, high temperature may improve nitrate degradation by the culture, but high concentrations of salt can have a negative impact on biomass performance. Since in direct resin bioregeneration the desorption of nitrate from resin and degradation of nitrate is happening at the same time, it is important to find the best environmental condition for both desorption and degradation processes.

Most studies on nitrate selective resins focus on adsorption characteristics rather than desorption but desorption investigation is really important since the productivity of the process depends on the total cycle life of resin (Bae et al., 2002; Lin et al., 2007; Orlando et al., 2002; Samatya et al., 2006). To the best of my knowledge there are just a few studies discussing the regeneration performance of nitrate selective resin (Cyplik et al., 2008; Van
der Hoek et al., 1988a; Van der Hoek et al., 1988b). Even in these studies, only the possibility of regeneration of the resin has been studied not the effect of environmental parameters on the regeneration process.

In this chapter the effect of temperature and salt concentration on the desorption of nitrate from resin rate has been investigated. The results of this part of research provide much needed data of the kinetics as well as the typical equilibrium as such is a significant contribution to the overall design of systems for the reuse of highly selective resin in multi-cycle ion exchange processes without any further disinfection or cleaning procedures. The specific objectives of this part of research are to present the effect of salt concentration and temperature on the desorption rate of nitrate from resin, to use this data to present the kinetics observed during each of the processes, and to determine the effect of temperature and salt on the kinetics parameters, finally recommending operational parameters for the most rapid desorption kinetics.

4.1 Materials & methods

4.1.1 Water sample

Water analysis of 27 wells in Osoyoos, BC, Canada, shows that 15 wells have nitrate contamination problems. A challenge water for this experiment was selected based on the maximum concentrations of nitrate, sulfate, and chloride in well samples; 216 mg/L, 186 mg/L, and 200mg/L, respectively for nitrate, sulfate, and chloride.

4.1.2 Resin & reactor

For this part of experiment, resin was prepared the same as the first exhaustion cycle as it was explained in section 3.1.1.
The desorption tests were performed with ~0.1 g of resin enclosed in dialysis membrane in 250 ml Erlenmeyer as the desorption reactor (Figure 4.1).

![Figure 4.1: Reactor set-up; resin enclosed in membrane using closures in the Erlenmeyer flask as a reactor with regeneration solution](image)

The mass of resin was chosen to present as close to a monolayer of resin as possible to maximize the transfer of nitrate out of the resin and into the culture. The regeneration solution was made based on the chemical composition of the culture medium mentioned in section 3.1.2 for 6% salt concentration. The magnesium chloride and sodium chloride concentrations were changed to 6.33 g/L and 20 mg/L, respectively for 2% salt, and were changed to 12.7 g/L and 40 g/L, respectively for 4% salt to provide desired salt concentration and keep the molar ratio of the Mg/Na at 0.11 as it is required for the stable activity of the culture in high salt-concentrated solutions (Lin et al., 2007).

### 4.1.3 Analytical methods

Nitrate, chloride, and sulfate concentrations were measured using a Dionex ICS-2100 with KOH eluent using a multi-step gradient of 1, 10, 22, 1, and 1 mM respectively at time of 0, 10, 23, 26, and 27 minutes and flow rate of 1.5 ml/min. Calibration curves were
established using standard solutions of 1 mg/L, 10 mg/L, 100 mg/L, and 250 mg/L of each salt. The detection limit of ICS-2100 for all of the desired anions was 1 mg/L.

4.1.4 Statistical analysis

Statistical analysis was performed to determine whether there is a significant difference in kinetics parameters at different processes. Normal distribution and test of homogeneity were performed on the data to make sure that two way ANOVA test is valid for the set of experimental data. The statistical significance of the differences was determined by Univariate analysis of variance (two-way ANOVA) at 95% confidence interval for differences using SPSS Statistic 20 software.

4.1.5 Kinetics model selection

A comprehensive study was performed regarding adsorption and desorption characteristics of the same type of resin (Faccini, 2011). In that research, kinetic equations including pseudo-first and pseudo-second order equations, the Elovich's equation, as well as intra-particle diffusion models including the equations for the film diffusion mass transfer rate, Weber-Morris model, Dumwald-Wagner model and Boyd kinetic model were tested. From all the models tested only the pseudo-second order kinetic model was able to reproduce the experimental results. The work was done using perchlorate as the main contaminant to calculate the adsorption and desorption rate. To make sure that the same model can be used using nitrate as the contaminant, the same study was done on the experimental results of our research, resulting in the same outcome. Therefore, for this research pseudo-second order kinetic model (eq 4.1) was used to interpret the data and analysis the effect of temperature and salt concentration on desorption process.
\[
\frac{dC_t}{dt} = K_{des} (C_e - C_t)^2 \quad eq \ 4.1
\]

Where, \(C_t\) (mg/g of resin) is the nitrate concentration in the aqueous phase at time \(t\), \(C_e\) (mg/g of resin) is the equilibrium concentration of nitrate in aqueous phase and \(K_{des}\) \([(mg/ g \ of \ resin) \cdot h^{-1}]\) is kinetic rate constant.

4.2 Results & discussion

4.2.1 Effect of temperature & salt concentration on desorption kinetic parameters

Figure 4.2 shows the nitrate concentration in the aqueous phase during regeneration of resin at different temperatures (constant salt concentration for each graph).

The samples were left for 24 hours at 23°C and 35°C and 3 days at 12°C to make sure equilibrium concentration was achieved. The nitrate concentration was at equilibrium after 3 hours so the graphs show only first 3 hours of the experiment (just before the equilibrium) to see the changes in nitrate desorption rate in a better scale.
Figure 4.2: Effect of temperature on nitrate desorption at different salt concentrations; (a): 2% salt, (b): 4% salt, and (c): 6% salt (The data inside the ovals were used in defining kinetic parameters and the rest of the data were used to determine the equilibrium concentration). The data points represent the average of three replicate tests and the error bars are the standard deviation.
At each salt concentration, the initial rate of nitrate desorption from resin was higher at 35°C. Based on Figure 4.2, the equilibrium concentration of nitrate in the aqueous phase at 23°C & 35°C is higher than 12 but it is hard to differentiate between 23°C and 35°C just based on the graphs.

Regarding the effect of salt concentration, Figure 4.3 shows the nitrate concentrations at different salt concentrations for each temperature. Although at each temperature, the initial desorption and equilibrium concentration of nitrate in the liquid phase is higher with 6% salt concentration, the exact effect of salt concentration on each kinetic parameter cannot be seen only based on the graph.
Figure 4.3: Effect of salt concentration on nitrate desorption at different temperatures; (a): 12°C, (b): 23°C, and (c): 35°C (The data inside the ovals were used in defining kinetic parameters and the rest of the data were used to determine the equilibrium concentration). The data points represent the average of three replicate and the error bars are the standard deviation.
In order to understand the exact effect of temperature and salt concentration on the desorption process statistical analysis of the kinetics parameters was performed. As explained in section 4.1.5, pseudo second order kinetics were used to interpret the data of nitrate desorption from resin. \( \frac{t}{C_t} \) was plotted vs \( t \) for each set of data in order to calculate the kinetic parameters (Figure 4.4). \( C_e \) (mg of nitrate/ g of resin), \( K_{des} ([\text{mg/ g of resin} \cdot h])^{-1} \), and \( K_{des} C_e^2 \) (mg of nitrate/ g of resin\( \cdot h \)) were calculated and considered as equilibrium concentration, kinetic rate constant, and initial desorption rate, respectively. It should be mentioned that initial data before experimental equilibrium (first 3 hours of experiment) was considered to predict the equation constants and statistical analysis.

![Graph showing desorption kinetics](image)

**Figure 4.4: Pseudo-second-order desorption kinetics.** The data points represent the average of three replicate tests and the error bars are the standard deviation.

As a summary, Table 4.1 shows the average values of Pseudo-second order kinetic parameters at different temperatures and salt concentrations.
A statistical analysis was done to analyze the effect of both temperature and salt concentration on kinetic parameters including equilibrium concentration, initial desorption rate, as well as kinetic rate constant. Univariate analysis of variance (two-way ANOVA) was done at 95% confidence interval to interpret the results.

The statistical analysis showed that the difference in the mean values (of final equilibrium concentration) among the different levels of salt concentration is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in temperature (there is not a statistically significant difference, P = 0.204). So, the salt concentration does not have significant effect on the final equilibrium concentration. After allowing the exhausted resin and regeneration solution to come to the equilibrium, even the lowest concentration of salt (2%) is high enough to exchange the maximum available adsorbed nitrate on the resin with chloride in the regeneration solution.
However, regarding the kinetic rate constant and initial desorption rate, for both parameters, the difference in the mean values among the different levels of salt concentration is greater than would be expected by chance after allowing for effects of differences in temperature ($P \leq 0.001$). To isolate which group(s) differ from the others, all pairwise multiple comparison procedure using Holm-Sidak method with overall significance level of 0.05 was used. Pairwise comparison shows that both initial desorption rate and kinetic rate constant at 2% salt is lower than 4 and 6% but there is no significant difference between mean values of 4 and 6% salt concentration.

The effect of salt concentration on different kinetics parameters can be explained based on the chemical structure of the resin and Le Chatelier's principle. According to the functional group of the resin, the general ion exchange equation regarding regeneration of resin (desorption of nitrate from resin) can be expressed as:

$$[\text{R(C}_4\text{H}_9\text{)}_3\text{N}^+]\text{NO}_3^- + \text{Cl}^- \leftrightarrow [\text{R(C}_4\text{H}_9\text{)}_3\text{N}^+]\text{Cl}^- + \text{NO}_3^- \quad \text{rxn 4.1}$$

The concentration of salt in the regeneration solution is orders of magnitude higher than the ion concentrations on the resin phase even at 2% salt. Therefore, increasing the salt concentration is not affecting the final equilibrium concentration of nitrate since at this regeneration solution concentration the amount of nitrate that can be desorbed from resin based on the separation factor of the nitrate to chloride is limited. However, increasing the salt concentration will increase the chloride concentration on the left side of the equation which based on the Le Chatelier's principle forwards the desorption reaction to the right side, the original portion of adsorbed nitrate on the resin diffuses out rapidly and results in increasing the initial desorption rate of nitrate from resin before reaching equilibrium. As a matter of fact, as the salt concentration is increasing, salt diffuses rapidly within the resin
particles and is creating a large driving force for desorption of nitrate from the resin. However, the effective diffusivity is limited and after specific concentration of salt, increasing the concentration of salt doesn’t have any effect on the diffusivity and the kinetic rate constant and initial desorption rate are not changing anymore. On the other hand, as the salt concentration is decreasing the importance of equilibrium reaction is more significant. There is a possibility that a portion of desorbed nitrate is adsorbed again on the resin and decreases the desorption rate. Although at lower concentration of salt desorbed nitrate becomes unbound slowly, the total concentration of chloride is still high enough to free all of the available nitrate on the resin sites and to achieve the same final equilibrium nitrate concentration as higher salt concentration.

Other studies on analysis of the effect of salt concentration on desorption kinetics (looking at different contamination, different types of resin, …) validate the concept of our research. Although most of the researchers have been working on the effect of the salt concentration on ion exchange process mainly on the adsorption side, it is reported that desorption rates are faster at higher concentration of salt to the specific level of salt concentration which matched the experimental data of our research (Conder & Hayek, 2000; Hunter & Garta, 2001).

Regarding the effect of temperature, the Arrhenius equation was used to calculate desorption activation energy (Gokmen & Serpen, 2002; Zacharia et al., 2004).

\[ K_{des} = K_0 \exp \left(- \frac{E_a}{RT} \right) \]  \hspace{1cm} (eq 4.2)

Where \( K_0 \) is the temperature-independent factor (g of resin/mg of nitrate•h), \( E_a \) is the activation energy of desorption (kJ/mol), \( R \) is the universal gas constant (8.314 J/mol•K) and
T the solution temperature (K). Rearranging Arrhenius equation and plotting ln(K_{des}) vs 1/T, K_0 and E can be calculated from intercept and slope, respectively. ln(K_{des}) vs 1/T was plotted for each salt concentration (Figure 4.5). Based on the results the activation energy and K_0 value was calculated as 15±4 Kj/mol and 17±6 g of resin/mg of nitrate•h, respectively. Therefore, temperature dependency of the desorption process can be shown as:

\[
K_{des} = 17 \exp \left( - \frac{15 \times 10^3}{RT} \right)
\]

(eq 4.3)

The desorption process is endothermic which means applying more energy to the process will shift the reaction forward resulting in more removal of nitrate from the resin sites.

Statistical analysis shows that for all of the kinetic parameters, including equilibrium concentration, kinetic rate constant, and initial desorption rate, the effect of different temperatures does not depend on what level of salt is present and there is no correlation between salt and temperature. Therefore, the effect of each independent factor (temperature and salt) can be investigated separately.
Figure 4.5: Plot of $\ln(k_{des})$ against reciprocal temperature to calculate Arrhenius equation constant. The data points are the average of triplicates and error bars represent the standard deviation.

Statistical analysis shows that for all kinetic parameters with $P<0.001$ there is statistically significant difference among the different temperatures. To understand the exact different between these values, all pairwise multiple comparison procedure was done (Holm-Sidak method).

Mean values of both kinetic rate constant, and initial desorption rate increased with increasing temperature which proves the results of modified Arrhenius equation for this specific desorption reaction.

Although the reaction rate constant is increasing with temperature, statistical analysis of final equilibrium concentration shows that there is no significant difference between final equilibrium concentration of nitrate in the solution at 23 and 35 °C and both of them are significantly higher than the mean value of equilibrium concentration at 12°C. These results show that although the reaction rate at 23°C is slower than 35°C, leaving the resin in contact
with regeneration solution for a longer time results in the same regeneration capacity since the final equilibrium concentration is the same. On the other hand, slow desorption rate of nitrate as 12°C provides the opportunity for desorbed nitrate molecule to become readsoberbed on the resin which results in the lower final equilibrium concentration of nitrate in the solution compared to the other temperatures. Although in our research the effect of temperature on the final equilibrium concentration of nitrate has been studied, it is not the main concern of our research which is bioregeneration of the exhausted resin. These results are important when a complete cycle of resin regeneration is the main aspect of the process. In bioregeneration system, the process never gets to the final equilibrium concentration since nitrate leaving the resin is removed by the biomass and the process reaches a new equilibrium concentration. In this type of process, the initial desorption rate plays a much more important role rather than equilibrium concentration.

4.2.2 Modeling the desorption process

Figure 4.6 depict the lab and proposed full scale application of the bioregeneration process for resin enclosed in a membrane. In the lab scale the resin was enclosed in a membrane as close to a monolayer as could be obtained. For the actual treatment process the eluent would flow into the reactor through the resin where the desorption would occur and carry the nitrate out through the membrane to the culture in the bulk liquid flow. Mass transfer processes involved inside the resin and outside the resin to the bulk of the liquid include; 1: transfer Cl\(^-\) ion of high concentrated regeneration solution to the resin beads, 2: transfer the Cl\(^-\) ion into the pores to get to the exchangeable sites, 3: desorption of nitrate from functional group 4: diffusion of the desorbed nitrate from the inside the resin to the
surface of resin, and finally, 5: diffusion of nitrate ion from the surface of the resin through the liquid film to the outside of the membrane.

**Figure 4.6: Different mass transfer processes involved in bioregeneration (both lab and large scale)**

Based on the literature, it is accepted that the chemical reaction of functional group is not a controlling process (Sharbatmaleki, 2010; Sharbatmaleki & Batista, 2012; Sharbatmaleki et al., 2015; Venkatesan et al., 2010). Then, step 3 is negligible. Also, because of the high concentration of chloride in the regeneration solution, step 1 and 2 do not have a controlling effect on the desorption process. The diffusion of ions inside the resin and through the pores can be explained by a shrinking core model in which the ions in the outer region of the resin bead are exchanged and diffuse to the bulk of liquid before those ones that are in the deeper exchangeable sites of the resin (Pritzker, 2005; Sharbatmaleki & Batista, 2012). Pore diffusion is controlled by different parameters, such as, viscosity, concentration
gradient, resin bead size, structure of resin (number of crosslinking bonds in the resin), and resin pore size (macro-porous or gel-type resin).

In terms of bioregeneration of resin enclosed in a membrane, the goal of this research, it is assumed that regeneration is limited by the rate of diffusion of the ions in the external solution (through the resin beads to get to the biomass solution) and in the pores of resin particles. In this case predicting the concentration changes with time would require the solution of unsteady-state diffusion equations (including shrinking core model, film diffusion, and mass-transfer through membrane at the same time). For engineering applications, the mass transfer rate can be approximated using an overall coefficient and a driving force (McCabe et al., 2005).

In the lab scale experiment, the total diffusion coefficient which is the desorption rate can be calculated experimentally and the total desorption kinetics can be considered based on desorption rate and concentration gradient. Based on the result of this experiment, Pseudo-second order equation was the best model to explain the desorption of nitrate from resin (eq 4.1) which matched with the previous study of the same resin (Faccini, 2011).

During bioregeneration of the resin, since desorbed nitrate will be degraded by biomass and will be removed from the aqueous phase, the desorption of nitrate from resin will never reach a constant equilibrium. Therefore, the initial desorption rate plays the most important role in the process. Based on the results, 6% salt and 35°C are the best experimental condition for controlling the desorption rate of nitrate from resin. Selecting the best operational condition also depends on the behavior of the biomass at 6% salt and temperature of 35°C which must be determined. Chapter 5 presents the results of the effect of
the same factors on biological removal of nitrate to provide enough information to model and optimize the bioregeneration process.
Chapter 5: Interaction of salt concentration & temperature on the biological nitrate removal rate

Discussed previously, in order to model and design a commercial scale bioregeneration process, an understanding of the major environmental operational parameters for resin regeneration is necessary which provides a need to study the desorption of nitrate from resin and degradation of nitrate in the aqueous phase. Desorption of nitrate from resin was presented in Chapter 4. This chapter focuses on the second process, biological removal of nitrate in the aqueous phase. In this part of the research, the effect of two independent factors (temperature and salt concentration) on denitrification rate (dependent parameter) were studied to allow the optimization of the bioregeneration process along with the previous study of the effect of the same factors on the nitrate desorption rate from the resin which was presented in Chapter 4.

Removal of nitrate from the aqueous phase can occur through different pathways including, denitrification which is converting nitrate to nitrogen gas, and dissimilatory nitrate reduction to ammonia (DNRA). Complete denitrification and prevention of DNRA can be controlled by providing excess carbon source for the biomass.

There are several studies looking at the effect of temperature and salt concentration on the biological denitrification rate as separate factors (Cang et al., 2004; Ebrahimi et al., 2012; Ucisik, A. S., Henze, 2004; Xiao & Roberts, 2013) but to the best of my knowledge there is no study on the interaction of salt concentration and temperature.

The main objectives of this part of research were to understand and investigate 1) the effect of salt concentration on nitrate removal rate considering 4 different levels of salt, 2) the
effect of temperature on denitrification including 3 different temperatures, 3) the interaction of temperature and salt on the nitrate removal rate, and, 4) to mathematically describe the effect of temperature and salt concentration on nitrate removal rate for future use in a bioregeneration model.

5.1 Materials & methods

5.1.1 Culture, medium & bioreactor

The same culture and medium that were described in Section 3.1.2 was used for this part of the experiment. Nitrate perchlorate reducing bacteria (NP30) was fed just with nitrate for 3 month. After this adaptation period, nitrate was the only electron acceptor (N30: nitrate reducing bacteria at 3% salt). Salt concentration was controlled using NaCl at different concentrations; 20, 30, 40, 50, and 60 g/L for 2, 3, 4, 5, and 6% salt, respectively. Also, the molar ratio of the Mg/Na was kept at 0.11 (using MgCl$_2$•6H$_2$O) as it is required for the stable activity of the culture in high concentration solutions (Lin et al., 2007; Xiao & Roberts, 2013). Also, nitrate and acetate concentrations were controlled using NaNO$_3$ and CH$_3$COONa•3H$_2$O to get 100 and 500 mg/L nitrate wherever was desired and to achieve 1.5 acetate to nitrate ratio as the carbon and electron source.

During the adaptation period three replications of each reactor were performed at the same time to show the reproducibility of the experiment. Biomass concentration of each bioreactor was kept at $\approx$2000±50 mg/L VSS by calculating the exact volume of the fresh medium that should be added to the bioreactor to keep the biomass concentration constant. If it was needed excess biomass was discarded as well. Bioreactors were spiked by nitrate and acetate every other day. To provide the nutrients for the culture medium was changed weekly
by settling and replacing the 80% of the medium with fresh medium. The whole process was done under anoxic/anaerobic condition by boiling the medium and flushing nitrogen gas.

### 5.1.2 Culture adaptation in batch bioreactors

Salt concentration and temperature were considered as two independent variables and the nitrate degradation rate as the dependent variable. Using factorial design of experiment method, a series of batch reactors were designed to reveal the effect of temperature and salt concentration on the nitrate degradation rate. Five levels of salt concentration (2, 3, 4, 5, and 6% salt) and three temperatures (10, 23 and 35°C) were examined. Early studies showed the culture was sensitive to rapid changes in salt or temperature and so a two phase adaptation method was used; salt-temperature and temperature-salt adaptation methods. In the salt-temperature method, N30 was adapted to different levels of salt first and then the temperature was changed and in the temperature-salt method, N30 was adapted to different temperatures first and then the salt concentration was changed. Figure 5.1 shows the different steps of the acclimation procedure.
Figure 5.1: Adaptation protocol: N shows that culture was fed with just nitrate as electron acceptor, the number exactly after N shows the salt concentration, and the other number shows the temperature of the bioreactor. For example, N40-10 is a bioreactor in which the culture was fed with nitrate and 40 g/L of NaCl and was adapted to 10°C. The order of temperature and salt concentration in each box represents the different adaptation method; N20-10 means that the original culture (3% salt at 23°C) was adapted to 2% salt (at the same temperature; 23°C) and then temperature was changed to 10°C, and 10-N20 represents that the original culture was adapted to 10°C first (at the same salt concentration; 3%) and then salt concentration was changed to 2%. Each biomass adaptation method was done in triplicate (three separate bioreactors) to show the reproducibility of the results.
Each box represents a complete cycle of adaptation. Each adaptation cycle was carried out for a month in 1L Erlenmeyer flasks as the anaerobic bioreactor. Each adaptation cycle included weekly changes of the medium (to provide nutrients) and spikes with nitrate-acetate spike solution every other day to keep the nitrate concentration at desired levels. Bioreactors were kept in the shaker (110 RPM) with adjustable temperature. At lower temperature the shaker could provide 10°C below the room temperature. In this research the temperature of samples labelled as 10°C varied between 10 & 12°C, depending on the room temperature.

5.1.3 Analytical methods

Nitrate concentration in the samples and biomass concentration was measured using the same method explained in Section 4.1.3. Sample was filtered through 0.2 µm syringe filter (25mm diameter with polypropylene housing) just after sampling to remove the biomass and to make sure no nitrate degradation occurred while the sample was running on the IC.

The biomass concentration was measured based on the volatile suspended solids (VSS) using Standard Methods 254-E (Eaton et al., 2005). A sample volume of 1-2 ml (depending on the concentration of sample) was filtered through a glass microfiber filter (934-AH™ with 42.5 mm diameter). The filter was dried at 98°C overnight and then combusted for an hour at 550°C. VSS measurement of the samples was done in triplicate and the average of all three measurements was used in data analysis.

5.1.4 Statistical analysis

Statistical analysis was performed to determine whether there was a significant difference in nitrate degradation rate at different experimental conditions using Sigma Stat
3.0. More details about each statistical method used for each specific analysis is explained in the results and discussion section.

5.2 Results & discussion

5.2.1 Degradation rate analysis

After each cycle of adaptation (30 days) the degradation rate of nitrate was studied. Each nitrate degradation rate analysis was performed as triplicate for each bioreactor. Figure 5.2 shows a sample of results at 6% salt concentration and different temperatures.
Figure 5.2: Nitrate removal at different temperature for culture adaptation to 6% salt; a) 10°C, b) 23°C, and c) 35°C this graph is a sample of nitrate removal data used to calculate the degradation rate based on the slope of the lines on the graph. The lines are the best linear fit to the data points which present the zero-order kinetics for the nitrate degradation rate. Each adaptation set had three bioreactors as triplicate and for each bioreactor nitrate degradation rate analysis was done in triplicate. The average and standard deviation of all 9 normalized nitrate degradation rates were used to further analysis of the results.
Initially the nitrate degradation rate study was done starting with 500 mg/L of nitrate, taking samples at different intervals, for 12 hours or until nitrate was depleted from the aqueous phase. The nitrate degradation was linear from ~500 mg/L to ~100 mg/L (zero order kinetics). To determine if the degradation rate would become dependent on the concentration (1<sup>st</sup> order) at lower concentrations, additional degradation rate studies were performed starting with 100 mg/L of nitrate. Understanding the kinetics at lower nitrate concentrations is also important with respect to the final goal of the project (bio-regeneration of resin) since at each desorption equilibrium nitrate concentration in the aqueous phase is usually less than 100 mg/L.

The biological denitrification study at nitrate concentrations lower than 100 mg/L also showed that the nitrate removal rate still followed zero order kinetics. At 35°C nitrate removal was so fast, it was hard to distinguish between first & zero order kinetics even by taking samples every 10 minutes. Therefore, for this study, zero order kinetics (nitrate removal rate is not dependent on initial nitrate concentration) was considered for all of the experiments, the nitrate degradation rate was calculated based on the slope of the lines for each sample. The average and standard deviation of all three replicate was used for further analysis of the results.

Other similar studies support our experimental results. In these studies different sources of carbon such as; acetate, methanol, ethanol, etc. have been used. Despite different electron donor for denitrification, in all of these studies it has been reported that when the carbon source and other nutrients were in high enough concentration not to limit the growth, denitrification was a zero-order reaction with respect to nitrate (Dawson & Murphy, 1972;

5.2.2 Effect of the adaptation method on nitrate degradation rate

The effect of adaptation method on nitrate degradation rate was statistically analyzed using t-test and 95 percent confidence interval for the difference of means. Table 5.1 shows the statistical results of comparing salt-temperature & temperature –salt adaptation methods. The difference in the mean values of each two groups was not great enough to reject the possibility that the difference is due to random sampling variability. There was not a statistically significant difference between groups since the p-values for all the tests are greater than 0.005. This shows that by providing enough adaptation time at each specific environmental condition (at least one month for our culture), culture behavior was stable and the nitrate degradation rate was consistent.

Table 5.1: Comparison of the mean nitrate degradation rates using different adaptation methods

<table>
<thead>
<tr>
<th>Sample group</th>
<th>Mean rate (mg nitrate/mg VSS•h)</th>
<th>P-values</th>
<th>Adaptation method</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-N20</td>
<td>0.0104</td>
<td>0.094</td>
<td>temperature-salt</td>
</tr>
<tr>
<td>N20-10</td>
<td>0.0123</td>
<td></td>
<td>salt-temperature</td>
</tr>
<tr>
<td>10-N40</td>
<td>0.00740</td>
<td>0.333</td>
<td>temperature-salt</td>
</tr>
<tr>
<td>N40-10</td>
<td>0.115</td>
<td></td>
<td>salt-temperature</td>
</tr>
<tr>
<td>10-N50</td>
<td>0.00754</td>
<td>0.667</td>
<td>temperature-salt</td>
</tr>
<tr>
<td>N50-10</td>
<td>0.00571</td>
<td></td>
<td>salt-temperature</td>
</tr>
<tr>
<td>10-N60</td>
<td>0.00534</td>
<td>0.333</td>
<td>temperature-salt</td>
</tr>
<tr>
<td>N60-10</td>
<td>0.00504</td>
<td></td>
<td>salt-temperature</td>
</tr>
<tr>
<td>Sample group</td>
<td>Mean rate (mg nitrate/mg VSS•h)</td>
<td>P-values</td>
<td>Adaptation method</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------------------</td>
<td>----------</td>
<td>-------------------</td>
</tr>
<tr>
<td>35-N20</td>
<td>0.0446</td>
<td>0.352</td>
<td>temperature-salt</td>
</tr>
<tr>
<td>N20-35</td>
<td>0.0473</td>
<td></td>
<td>salt-temperature</td>
</tr>
<tr>
<td>35-N40</td>
<td>0.0381</td>
<td>0.611</td>
<td>temperature-salt</td>
</tr>
<tr>
<td>N40-35</td>
<td>0.0373</td>
<td></td>
<td>salt-temperature</td>
</tr>
<tr>
<td>35-N50</td>
<td>0.0352</td>
<td>0.772</td>
<td>temperature-salt</td>
</tr>
<tr>
<td>N50-35</td>
<td>0.0341</td>
<td></td>
<td>salt-temperature</td>
</tr>
<tr>
<td>35-N60</td>
<td>0.0308</td>
<td>0.657</td>
<td>temperature-salt</td>
</tr>
<tr>
<td>N60-35</td>
<td>0.0320</td>
<td></td>
<td>salt-temperature</td>
</tr>
</tbody>
</table>

Based on these results, the nitrate removal rates from the different adaptation methods were combined to calculate the average nitrate removal rate for each specific temperature and salt concentration. For example, nitrate degradation rate at 10°C and 2% salt was reported as the average and STD of all degradation rates for 10-N20 and N20-10 sample groups. Based on this analysis, Table 5.2 shows the nitrate degradation rates for each sample group.

**Table 5.2: Average and standard deviation of replicate for nitrate degradation rate at different salt concentrations and different temperatures**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Salt concentration (g/L of NaCl)</th>
<th>Nitrate degradation rate (mg nitrate/mg VSS•h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>20</td>
<td>0.0118±0.0008</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.017±0.002</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.009±0.002</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.007±0.001</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>Salt concentration (g/L of NaCl)</td>
<td>Nitrate degradation rate (mg nitrate/mg VSS•h)</td>
</tr>
<tr>
<td>------------------</td>
<td>----------------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>23</td>
<td>60</td>
<td>0.0052±0.0002</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.0286±0.004</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.031±0.002</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.027±0.003</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.020±0.003</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.020±0.002</td>
</tr>
<tr>
<td>35</td>
<td>20</td>
<td>0.039±0.003</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.046±0.001</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.038±0.001</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.035±0.004</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.031±0.003</td>
</tr>
</tbody>
</table>

5.2.3 **Effect of temperature on nitrate degradation rate**

The effect of temperature on the nitrate degradation rate is shown in Figure 5.3. As can be expected, at each specific salt concentration, the rate of nitrate removal increased with increasing temperature.
Figure 5.3: The effect of temperature on nitrate degradation rate at different salt concentrations. The data points are the average of replicate and error bars represent the standard deviation.

Table 5.3 shows the values from the linear regression of data for each salt concentration with the lower and higher intercept values for 6 and 3% salt.

Table 5.3: Linear regression analysis of the effect of temperature on nitrate degradation rate at different salt concentrations

<table>
<thead>
<tr>
<th>Salt concentration (g/L of NaCl)</th>
<th>Slope (mg nitrate/mg VSS.h.°C)</th>
<th>R² values</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.0011</td>
<td>0.9899</td>
<td>Rate = 0.0011T + 0.0015</td>
</tr>
<tr>
<td>30</td>
<td>0.0012</td>
<td>0.9974</td>
<td>Rate = 0.0012T + 0.0049</td>
</tr>
<tr>
<td>40</td>
<td>0.0011</td>
<td>0.9813</td>
<td>Rate = 0.0011T - 0.0008</td>
</tr>
<tr>
<td>50</td>
<td>0.0011</td>
<td>0.9988</td>
<td>Rate = 0.0011T - 0.0048</td>
</tr>
<tr>
<td>60</td>
<td>0.001</td>
<td>0.9967</td>
<td>Rate = 0.001T - 0.0049</td>
</tr>
</tbody>
</table>
A statistical analysis on the results shows the slope of the lines are not statistically different (p<0.001). This slope shows that the rate-increase with temperature is the same for all the salt concentrations with the average value of 0.00111±0.00006 mg nitrate/mg VSS•h•°C. Although the intercepts are statistically significantly different using 95% confidence interval (P=0.763), with $R^2$ values of 0.82, an overall linear regression can be used to show the effect of temperature on the nitrate degradation rate (equation 5.1). Since this equation is derived based on the experimental data in the range of 10-35 °C, this equation can be used for predicting the nitrate degradation range only in this range of temperature. If the nitrate degradation rate at lower temperature than 10°C or higher than 35°C is needed, the experimental analysis should be done to cover broader temperature range.

$$\text{rate} \left( \frac{\text{mg nitrate}}{\text{mg VSS} \cdot \text{hr}} \right) = -0.0005 + (0.00111 \times \text{temperature})$$ \hspace{1cm} \text{eq 5.1}$$

The statistical analysis of the effect of temperature was done using a two-way analysis of variance with the nitrate degradation rate as the dependent variable. The difference in the mean values among the different levels of temperature was greater than would be expected by chance after allowing for effects of differences in salt concentration; using 95% confidence interval there was a statistically significant difference (P <0.001). Also, the power of the performed test with alpha = 0.0500, for temperature was 1.000. Table 5.4 shows the statistical results of comparison considering temperature as an independent factor.

<table>
<thead>
<tr>
<th>Group</th>
<th>Difference of means</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 vs 10</td>
<td>0.0292</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group</td>
<td>Difference of means</td>
<td>P-value</td>
</tr>
<tr>
<td>---------</td>
<td>---------------------</td>
<td>---------</td>
</tr>
<tr>
<td>35 vs 23</td>
<td>0.0137</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>23 vs 10</td>
<td>0.0155</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

To isolate which group(s) differ from the others, an all pairwise multiple comparison procedure (Holm-Sidak method) among each group of salt concentrations was performed. Comparisons for the temperature factor within each group of salt also indicates the same results; although the difference of means is changing within each group, nitrate degradation rates at each temperature are significantly different with the adjusted mean values of 0.0100, 0.0255, and 0.0392 (mg nitrate/mg VSS⦁h) for 10, 23, and 35°C, respectively.

Similar studies looking at only the effect of temperature on nitrate degradation rate, report the same concept. Elefsiniotis & Li, (2006) explored the effect of temperature and carbon source on the denitrification rate. Based on their results, in the range of 10-30°C, nitrate degradation rate is greater at higher temperature and the denitrification rate in linearly related to the temperature. In another study (Lewandoswki, 1982) using different sources of carbon such as methanol and acetic acid the same results have been shown; a linear increase in the nitrate degradation rate with increasing temperature.

### 5.2.4 Effect of salt concentration on nitrate degradation rate

The effect of salt concentration on the nitrate degradation rate at each specific temperature is shown in Figure 5.4. The curves show the general trend of the change in nitrate removal rate with salt concentration. The ovals in the figure show the groups of equality based on the statistical analysis with 95% confidence.
Figure 5.4: The effect of salt concentration on nitrate degradation rate at different temperatures. The data is categorized to show the effect of salt concentration on nitrate degradation rate within each group of temperature. The data points are the average of replicate and error bars represent the standard deviation.

As shown in Figure 5.4 at all of the studied temperatures, the best nitrate removal was obtained at 3% salt concentration. Before starting this experiment, the mixed culture (NP30) had been fed using 3% salt medium for more than 10 years. Although the salt concentration was decreased to 2% in the experiment, because of the original medium of the culture, the highest degradation rate at each temperature occurred at 3% salt. Excluding 3% salt, general trend of graph shows that increasing the salt concentration in the medium results in reduction of the nitrate removal rate. To clarify this effect, a statistical analysis, using two-way ANOVA was done using nitrate degradation rate as a dependent variable and salt concentration as an independent factor. The results clarify that the difference in the mean
values among the different levels of salt concentration is greater than would be expected by chance after allowing for effects of differences in temperature (P<0.001). Moreover, to understand the effect of salt concentration within each group (different temperature) all pairwise multiple comparison procedure was performed. Based on Table 5.5, overall nitrate degradation rate of 50 & 60 g/L of NaCl and 20 & 30 g/L of NaCl are not significantly different.

Table 5.5: All pairwise multiple comparisons for factor of salt concentration on nitrate degradation rate

<table>
<thead>
<tr>
<th>Group</th>
<th>Difference of means</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 vs 60</td>
<td>0.0125</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30 vs 50</td>
<td>0.109</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30 vs 40</td>
<td>0.001654</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30 vs 20</td>
<td>0.00266</td>
<td>0.061</td>
</tr>
<tr>
<td>20 vs 60</td>
<td>0.00988</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20 vs 50</td>
<td>0.00823</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20 vs 40</td>
<td>0.00388</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>40 vs 60</td>
<td>0.00599</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>40 vs 50</td>
<td>0.00435</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>50 vs 60</td>
<td>0.00164</td>
<td>0.154</td>
</tr>
</tbody>
</table>

Based on the Figure 5.4 and the statistical analysis, the effect of salt on the nitrate degradation rate does not follow a specific pattern at all temperatures and it really depends on which temperature was used. Figure 5.4 also shows the categorized data based on the statistical analysis. At each specific temperature, the mean values of the data that are grouped (each oval) are not significantly different.
Although the effect of salt concentration on the denitrification rate depends on the temperature, a similar pattern can be observed at each specific temperature 3% and 6% salt concentration has the maximum and minimum nitrate degradation rate, respectively. The difference between minimum and maximum nitrate degradation rate is greater at lower temperature. At 35°C, a 32% reduction in the nitrate degradation rate between 3 and 6% salt was observed while at 12°C and 23°C this reduction was 69% and 34%, respectively. Although the culture is adapted to room temperature it performed better at the higher temperature. The culture was more sensitive to the environmental changes (such as change in the salt concentration of the medium) at lower temperatures. In other words, temperature affects the level of salinity tolerance and the culture is less tolerant to high concentration of salt at low temperature. A higher sensitivity of different cultures at severe environmental condition has been shown by other researchers as well (McArthur, 2006; Srivastava & Srivastava, 2003).

5.2.5 Interaction of temperature & salt concentration on degradation rate

The final goal of this research is to develop a model for bioregeneration of exhausted resin with nitrate. This work isolated the biodegradation phase of the process. Based on the results of our study, a general linear relation between temperature and denitrification rate was found regardless of salt concentration (eq 5.1), the effect of different salt concentrations was dependent on the temperature that was used. Considering both salt concentration and temperature at the same time as independent factors a statistical analysis shows that there is a significant interaction between salt concentration and temperature.

A regression analysis was done to determine the best model for the correlation of salt concentration and temperature with the observed degradation rates. Equation 5.2 fit the data
for the effect of salt concentration and temperature on nitrate degradation rate with the best correlation ($R^2 = 0.93$). This equation is only valid for the range of temperature and salt concentration in our experimental study (2-6% salt and 10-35°C).

\[
\text{rate} \left( \frac{mg \text{nitrate}}{mg \text{VSS.hr}} \right) = 0.0102 + (0.00114 \times \text{temperature}) - (0.000291 \times \text{salt}) \quad \text{eq 5.2}
\]

Comparing equation 5.2 with 5.1 shows the addition of the salt effect to the equation alters the intercept but not the temperature correction factor. If the effect of salt concentration is independent from the temperature of the experiment, by ignoring the salt concentration term on eq 5.2 (0.000291*salt) equation 5.2 should be equal to equation 5.1. The fact that the temperature correction factor is not changing shows that the nitrate degradation rate increase due to temperature is not dependent on salt concentration. However, the intercept of the two equations are different enough to show that there is some combined effect of salt on the overall nitrate degradation rate.

In figure 5, the raw data has been plotted as well as the best surface area fitting the interaction of salt concentration and temperature on nitrate removal rate (eq 5.2). Figure 5.5a, is a 3-D graph to show the position of the experimental data to the surface area of the linear regression of the experimental data (eq 5.2). Figure 5.5b and Figure 5.5c are looking at the same results from salt concentration side and temperature side, respectively to show the change of the nitrate degradation rate with each of these independent factors in a 2-D graph. The lines in 2-D graphs are the side view of the same surface (eq 5.2).
Figure 5.5: Interaction of salt concentration and temperature; a) scattered raw data with the best surface fits the results (eq 2, 3D), b) raw data compared to the best fitted surface showing the change with salt concentration, c) raw data compared to the best fitted surface showing the change with temperature
Table 5.6 presents the results from other research reporting biological nitrate removal rates. The exact values of biological nitrate denitrification rate from different studies are difficult to compare since the environmental conditions including temperature, salt concentration, pH, as well as types of the reactors, source of carbon, retention time, etc. are different. Although the denitrification rates are different, in all temperature-dependent studies denitrification rate increased with increasing temperature; following the Arrhenius’s law (Dawson, R. N., Murphy, K. L., 1972) or linear equation (Elefsiniotis & Li, D., 2006; Lewandoswki, 1982). Also, all salt-dependent studies have come to the same conclusion; nitrate removal rate is decreased by increasing the salt concentration (Glass & Silverstein, 1998; Ucisik & Henze, 2004; Van der Hoek et al., 1987).

Our experimental results are in the range of reported values for nitrate degradation rate at different environmental conditions. Among different studies, Dawson and Murphy have reported high degradation values even at low temperature using *Pseudomonas denitrificans* (Glass & Silverstein, 1998). Although they have reported the results as nitrate removal rate, in the experiment the mechanism of nitrate removal is dissimilatory nitrate reduction to ammonia (DNRA) and not nitrate reduction to nitrogen eliminating a direct comparison with the other reported denitrification rates. Excluding their results, the lowest reported rate (0.0005 mg nitrate/mg VSS•h at 10°C ) (Elefsiniotis & Li, 2006) is one order of magnitude below our lowest rate value (0.0052 mg nitrate/mg VSS•h at 10°C & 6% salt) and the highest reported value by (Pansward, T., Anan, C., 1999) (0.053 mg nitrate/mg VSS•h at 23°C & 3% salt) is close to our highest removal rate (0.046 mg nitrate/mg VSS•h at 35°C & 3% salt).
Table 5.6: Biological nitrate removal rate at different environmental conditions

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Salt concentration (g/L of NaCl)</th>
<th>Carbon source</th>
<th>Nitrate degradation rate (mg nitrate/mg VSS•h)*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Not specified</td>
<td>Sodium citrate</td>
<td>0.057</td>
<td>Dawson &amp; Murphy, 1972</td>
</tr>
<tr>
<td>20</td>
<td>Not specified</td>
<td>Sodium citrate</td>
<td>0.3</td>
<td>Dawson &amp; Murphy, 1972</td>
</tr>
<tr>
<td>25</td>
<td>5</td>
<td>methanol</td>
<td>0.09</td>
<td>Folger et al., 2005</td>
</tr>
<tr>
<td>23</td>
<td>60</td>
<td>acetate</td>
<td>0.03</td>
<td>Glass &amp; Silverstein, 1998</td>
</tr>
<tr>
<td>23</td>
<td>5</td>
<td>Acetic acid</td>
<td>0.01</td>
<td>Pansward &amp; Anan, 1999</td>
</tr>
<tr>
<td>23</td>
<td>30</td>
<td>Acetic acid</td>
<td>0.053</td>
<td>Pansward &amp; Anan, 1999</td>
</tr>
<tr>
<td>10</td>
<td>Not specified</td>
<td>VFA</td>
<td>0.005</td>
<td>Elefsiniotis &amp; Li, 2006</td>
</tr>
<tr>
<td>20</td>
<td>Not specified</td>
<td>VFA</td>
<td>0.004</td>
<td>Elefsiniotis &amp; Li, 2006</td>
</tr>
<tr>
<td>30</td>
<td>Not specified</td>
<td>VFA</td>
<td>0.005</td>
<td>Elefsiniotis &amp; Li, 2006</td>
</tr>
</tbody>
</table>

*the reported unit for nitrate removal was different in some of the studies but we have recalculated it from information in the paper so it can be compared with the other results.

The understandings of the kinetics of desorption of nitrate from the resin (chapter 4) and the nitrate biodegradation of the culture (chapter 5) can be used to develop a complete resin bioregeneration model which is presented in the next chapter.
Chapter 6: Model development and reactor design for bioregeneration of resin enclosed in a dialysis membrane

Looking at the effect of temperature and salt concentration on nitrate desorption rate and nitrate biological degradation rate, provided enough information to develop a mathematical model for bioregeneration of resin exhausted with nitrate. Developing a general mathematical model as well as a design of bioregeneration reactor is presented in this chapter. The main objectives of this chapter are to assess the process controlling mechanism; to develop mathematical model for bioregeneration of a nitrate selective resin; to validate the developed model with experimental results; and to design the bioregeneration reactor.

6.1 Materials & methods

6.1.1 Experimental design and data collection

This chapter presents the development of a model based on the experimental results presented in Chapters 3, 4, and 5.

6.1.2 Model reactor description

The envisioned treatment reactor would be able to receive the exhausted ion exchange column directly. The medium would be encased in a membrane within the exhaustion column. This would be released from the casing and placed in the center of an annular reactor. An influent line would flush through the ion exchange media and out into the surrounding space that contains the biological system. Figure 6.1 presents a schematic of the system.
Figure 6.1: Overall set up for bioregeneration of exhausted resin

Since the ion exchange process is a batch process, the bioregeneration process should be considered as a batch process too. A basic reactor sizing was done based on the concepts of sequencing batch reactor (SBR) and experimental results of first cycle of bioregeneration which is presented in Table 6.1 (Ebrahimi & Roberts, 2013; Ebrahimi & Roberts, 2015).

Table 6.1: Available data from experimental process

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVI</td>
<td>130 ml/g</td>
</tr>
<tr>
<td>VSS</td>
<td>2500 mg/L</td>
</tr>
<tr>
<td>K_s</td>
<td>15 mgNO₃/L</td>
</tr>
<tr>
<td>k</td>
<td>0.02 mgNO₃/mgVSS.h</td>
</tr>
<tr>
<td>Resin capacity</td>
<td>0.5 meq/ml (1.62 meq/g)</td>
</tr>
<tr>
<td>Y_{obs}</td>
<td>0.45 mg VSS/mg acetate removed</td>
</tr>
</tbody>
</table>
6.1.3 Mathematical approach

6.1.3.1 Bioregeneration mechanism

Bioregeneration of resin enclosed in a membrane includes different processes; a) transfer of the chloride ion of high concentrated regeneration solution to the resin beads, b) transfer of the chloride ion into the pores to the exchangeable sites (inter-diffusion), c) exchange of nitrate with chloride on the resin sites d) diffusion of the desorbed nitrate from the inside the resin to the surface of resin (inter-diffusion), e) diffusion of nitrate from the surface of the resin through the liquid film around the resin beads (film diffusion) and through to outside the membrane, d) biodegradation of nitrate. In the bulk solution outside the membrane any concentration differences are constantly leveled out in the well-mixed solution, therefore there is no bulk diffusion outside of the membrane and the only kinetics involved would be nitrate biodegradation.

Inside the membrane, based on the literature, it is accepted that chemical reaction of functional group is not a controlling process (Helfferich, 1962; Lahav & Green, 2000; Parkhurst, 1990; Sharbatmaleki, 2010; Sharbatmaleki et al., 2015; Xiong et al., 2007), thus, step c is negligible. The diffusion of ions inside the resin and through the pores (inter-diffusion) can be explained by shrinking core model based on which the ions in the outer region of the resin bead are exchanged and diffused to the bulk of liquid before those ones that are in the deeper exchangeable sites of the resin (Pritzker, 2005; Sharbatmaleki et al., 2015; Venkatesan et al., 2010). Pore diffusion is controlled by different parameters, such as, viscosity, concentration gradient, resin bead size, structure of resin (number of crosslinking bond in the resin), and resin pore size (macro-porous or gel-type resin). On the other hand, film diffusion (diffusion of ions from surface of resin to the bulk of liquid) can be affected by
flow rate, turbulence, and viscosity which affect the thickness of diffusion boundary layer (Helfferich, 1962; Sharbatmaleki et al., 2015). Comparing film and pore diffusion in order to understand which process is controlling the total diffusion process can be explained mathematically as (Helfferich, 1962):

\[
\frac{X_{res} \delta \bar{D}}{C_{aq} D r_0} (5 + 2\alpha) \ll 1 \rightarrow \text{pore diffusion control} \quad \text{eq 6.1a}
\]

\[
\frac{X_{res} \delta \bar{D}}{C_{aq} D r_0} (5 + 2\alpha) \approx 1 \rightarrow \text{pore/film diffusion control} \quad \text{eq 6.1b}
\]

\[
\frac{X_{res} \delta \bar{D}}{C_{aq} D r_0} (5 + 2\alpha) \gg 1 \rightarrow \text{film diffusion control} \quad \text{eq 6.1c}
\]

Where, \(X_{res}\) is concentration of fixed ionic group (eq/L), \(C_{aq}\) is the concentration of the free ion in solution (eq/L), \(\bar{D}\) is the pore diffusion coefficient (cm\(^2\)/s), \(D\) is the film diffusion coefficient (cm\(^2\)/s), \(r_0\) is the mean resin bead radius in cm, \(\delta\) is the liquid film thickness (cm), and \(\alpha\) is selectivity coefficient for the involved ions. \(\bar{D}, D,\) and \(\delta\) can be identified experimentally for different resin and ions (Helfferich, 1962; Sharbatmaleki et al., 2015).

To understand the overall bioregeneration process, whether it is diffusion-limited process (including both inter-diffusion and film diffusion) or kinetic-limited process, the Thiele modulus, can be used which is derived for reactant penetration into the pores of beads considering spherical geometry.

\[
M_t = r_0 \sqrt{\frac{k_{rxn}}{D_e}} \quad \text{eq 6.2}
\]
Where $M_t$ is Thiele modulus, $r_0$ is the mean resin bead radius (cm), $D_e$ is the diffusivity coefficient in cm$^2$/s, and $k_{rxn}$ is the reaction kinetic coefficient (1/sec). The factors which determine the extent of diffusion limitation including particle size, rate of chemical reaction, and reaction mobility are all reflected in the Thiele modulus (Helfferich, 1962; Sharbatmaleki et al., 2015). Low Thiele modulus ($M_t < 3$) indicates that reaction kinetics are not a controlling step.

6.1.3.2 Bioregeneration model development

A numerical model was developed to predict the bioregeneration process. In this model the major assumptions were:

1. Biological denitrification happens immediately and there is no lag time. Experimental results support this assumption since after as little as 10 minutes the nitrate concentration has decreased.

2. Within a 24 hour time period, biomass concentration remains constant. This assumption is valid since the high biomass concentration that is normally used in these systems does not show a significant change with each allotment of nitrate.

3. Sulfate is desorbed from resin immediately and it does not affect biological denitrification.

4. Agitation is sufficient to eliminate bulk diffusion in the solution and keeps the concentration of nitrate and chloride in the solution uniform.

In the lab scale experiments the total diffusion coefficient (desorption rate) can be calculated experimentally and the total desorption kinetics can be considered based on desorption rate and the concentration gradient. A comprehensive study was performed to
determine the desorption kinetics and the effect of temperature and salt concentration on kinetics parameters in Chapter 4. Based on the result of this experiment, a pseudo-second order equation was the best model to explain the desorption of nitrate from resin which matched with the previous study of the same resin (Faccini, 2011). Therefore, for model development, pseudo-second order kinetic model (eq 6.3) was used. The difference between the final equilibrium concentration and aqueous phase concentration is considered as driving force at each specific time which is proportional to the available fraction of active sites (Faccini, 2011). This model incorporates both the concept of kinetic limitation and the final point of equilibrium and describes the desorption of nitrate from the resin very well.

\[ r_{d,t} = K_{des}(C_{e,NO3,t} - C_{NO3,a,t})^2 \]  
\[ eq. \ 6.3 \]

Where, \( r_{d,t} \) is the desorption rate (eq/g of resin h), \( C_{NO3,a,t} \) (eq/g of resin) is the nitrate concentration in the aqueous phase at time \( t \), \( C_{e,NO3,t} \) (eq/g of resin) is the nitrate equilibrium concentration of nitrate in aqueous phase and \( K_{des} \) \(((eq/ g of resin)\cdot h)^{-1})\) is kinetic rate constant.

Chapter 5 presented the detailed study of biological denitrification of nitrate using nitrate reducing bacteria shows that the biodegradation rate of nitrate in the range of 30-500 mg/L of nitrate at different temperature and salt concentration is independent of the initial nitrate concentration and at each specific salt concentration, the nitrate removal rate increased with increasing temperature with the average value of 0.00111±0.00006 mg-nitrate/mgVSS•h•°C \( (r_{bt}) \). In order to show that the substrate utilization rate is switching from zero-order kinetics to first-order kinetics, the substrate utilization rate in the biological systems is modeled using the following expression:
\[ r_{b,t} = \frac{kX_{NO3,a,t}}{K_s + C_{NO3,a,t}} \quad eq \, 6.4 \]

In which, \( r_{b,t} \) is the nitrate biodegradation rate (eq/mg VSS•h), \( k \) is maximum specific nitrate degradation rate (eq/mg VSS•h) and \( K_s \) is half saturation constant (eq/L). Based on the results it is predictable that at nitrate concentration lower than 30 mg/L, nitrate removal follows first order kinetics. Since in our experimental results, biological removal of nitrate at lower concentration was really fast and it was not possible to calculate Monod constant directly, the zero order nitrate removal rate was considered as \( \mu_{\text{max}} \) and 15 mg/L (0.000242eq/L) as \( K_s \).

In bioregeneration of ion exchange resin, desorption and degradation occurs at the same time and as a result, the equilibrium concentration in the aqueous phase will be changing with time. So the change of concentration in the aqueous phase (\( C_{a,t} \)) can be calculated based on the difference of desorption and degradation.

\[ \frac{dC_{NO3,a,t}}{dt} = r_{d,t} - r_{b,t} \quad eq \, 6.5 \]

The equilibrium of the ion exchange reaction can be expressed in terms of equivalent fractions which presents the separation factor.

\[ \alpha_{Cl}^{NO3} = \frac{Y_{NO3}X_{Cl}}{Y_{Cl}X_{NO3}} \quad eq \, 6.6 \]

Where \( \alpha_{Cl}^{NO3} \) is the binary separation factor, \( X \) are equivalent fraction or mole fraction of ions in aqueous phase, \( Y \) are the resin-phase equivalent fraction or mole fraction of the ions (for monovalent ion exchange mole fraction and equivalent fraction are the same). Also, each term of equation 1 can be expressed as:
\[ X_{NO3} = \frac{C_{NO3}}{C} \quad eq \ 6.7 \]
\[ X_{Cl} = \frac{C_{Cl}}{C} \quad eq \ 6.8 \]

In which \( X \) is equivalent fraction of nitrate and chloride \( C_{NO3} \) and \( C_{Cl} \) are equivalent concentrations of nitrate and chloride (eq/L), respectively, and \( C \) is the total anionic concentration in solution (eq/L). The same definition can be used on resin phase:

\[ Y_{NO3} = \frac{C_{x-NO3}}{C^r} \quad eq \ 6.9 \]
\[ Y_{NO3} = \frac{C_{x-Cl}}{C^r} \quad eq \ 6.10 \]

In which \( Y \) is equivalent fraction of nitrate, \( C_{x-NO3} \) and \( C_{x-Cl} \) are equivalent of nitrate and chloride on the resin (eq/L), respectively, and chloride on the resin and \( C^r \) is the total ionic concentration (eq/L) in the resin.

\[ X_{NO3} + X_{Cl} = 1, \quad Y_{NO3} + Y_{Cl} = 1 \quad eq \ 6.11 \]

The concentration on the resin can be calculated using following equations (\( C_e \) is equilibrium concentration).

\[ X_{Cl} = \frac{C_{e,Cl}}{C_T} \quad eq \ 6.12 \]
\[ X_{NO3} = \frac{C_{e,NO3}}{C_T} = 1 - \frac{C_{e,Cl}}{C_T} \quad eq \ 6.13 \]
\[ Y_{Cl} = 1 - Y_{NO3} \quad eq \ 6.14 \]

At equilibrium the fraction of nitrate on the resin can be calculated as:
\[ Y_{NO3,t} = \frac{M_{NO3,t} - C_{e,NO3,t}V}{C_R \cdot g_R} \]  

\textit{eq 6.15}

Where, \(C_R\) is the capacity of resin (eq/g of resin), \(V\) is the volume of the aqueous phase, \(g_R\) is mass of resin (g), and \(M_{NO3,t}\) is the equivalent of NO\(_3\) on resin and in the aqueous phase at any time which can be defined as

\[ \frac{dM_{NO3,t}}{dt} = -r_{b,t} V \]  

\textit{eq 6.16}

Substituting equations 6.12 to 6.15 into equation 6.6 results in a definition for nitrate equilibrium nitrate concentration at each specific time \((C_{e,NO3,t})\), \textit{eq 6.17}.

\[ C_{e,NO3,t} = \sqrt{\left( -VC_{e,Cl} - C_R g_R \alpha + \alpha M_{NO3,t} \right)^2 + 4VC_{e,Ci}C_{e,Cl}C_R g_R + V C_{e,Ci} C_{e,Cl} - C_R g_R \alpha + \alpha M_{NO3,t}} \]  

\textit{eq 6.17}

The mathematical model can be solved using developed algorithm (Figure 6.2). During the regeneration process, the chloride concentration is high (2-6%), so changes in the chloride concentration in the aqueous phase would be negligible and can be calculated based on the salt concentration of medium (for example 6% salt=1.034 eq/L). At time zero, \(C_{NO3,a}\) would be zero and \(M_{NO3}\) can be measured by analyzing completely exhausted resin using oxygen calorimeter. Initial biomass concentration \((X)\), \(\alpha\), \(V\), \(C_R\), \(K_{des}\), and \(g_R\) can be assumed based on the experimental results. In the algorithm the cycle is done when 99% of resin capacity is recovered.
6.2 Results & discussion

6.2.1 Process-controlling mechanism

The importance of pore diffusion or film diffusion processes was mathematically identified using \( \frac{X_{res} \delta D}{caq \delta r_0} (5 + 2\alpha) \) term and parameters reported in Table 6.2 to evaluate the control processing in bioregeneration.
Table 6.2: Ion exchange mass transfer parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta$, liquid film thickness</td>
<td>$10^{-3}$</td>
<td>cm</td>
<td>Helfferich, 1962; Sharbatmaleki et al., 2015</td>
</tr>
<tr>
<td>$D$, film diffusion</td>
<td>$10^{-5}$</td>
<td>cm$^2$/s</td>
<td>Helfferich, 1962; Sharbatmaleki et al., 2015</td>
</tr>
<tr>
<td>$\bar{D}$, pore diffusion</td>
<td>$4.67 \times 10^{-9}$</td>
<td>cm$^2$/s</td>
<td>Helfferich, 1962; Sharbatmaleki et al., 2015</td>
</tr>
<tr>
<td>$\alpha$, selectivity (Cl to NO$_3$)</td>
<td>$\approx 800$</td>
<td>NA</td>
<td>Faccini, 2011</td>
</tr>
<tr>
<td>$r_0$, mean resin bean radius</td>
<td>0.39</td>
<td>mm</td>
<td>Cal-Res Coatings Ltd and this research</td>
</tr>
</tbody>
</table>

The concentration of fixed ionic group was considered based on the full exhaustion of the resin with nitrate (1.51 eq/L). The concentration of the free ion (chloride) in the solution was calculated based on the salt concentration of regeneration solution as minimum 2% salt (0.34 eq/L) and maximum 6% salt (1.03 eq/L). Based on the results the value of $\frac{x_{\text{res}} \delta D}{C_{aq} D r_0}$ $(5 + 2\alpha)$ varies between $9.7 \times 10^{-7}$ - $0.3 \times 10^{-4}$, depending on the salt concentration of the solution. Regardless of the salt concentration of the solution, this specific resin is a pore-diffusion controlled process ($\frac{x_{\text{res}} \delta D'}{C_{aq} D r_0}$ $(5 + 2\alpha)$ $\ll 1$) which means the mass-transfer through the pores of the resin plays an important role in the process (Helfferich, 1962; Sharbatmaleki et al., 2015).

Since the pore-diffusion mass transfer was the controlling diffusion process, in the next step, the Thiele modulus was employed, using the pore-diffusion coefficient as the diffusivity coefficient, to identify whether the overall bioregeneration process is controlled by diffusion or biological removal of nitrate. Using these kinetic values the Thiele modulus
was calculated to be between 4 and 12 depending on the temperature and salt concentration. High Thiele ($M_T > 3$) modulus shows that the bioregeneration process is controlled by reaction kinetics. In other words, biological nitrate removal plays an important role in the resin bioregeneration process.

### 6.2.2 Model performance

To validate the developed model, the results from the model were compared to the experimental results of multi-cycle bioregeneration of resin, using parameters explained in the initial proof of concept study, Chapter 3. The model explained in section 6.1.3.2 is for one cycle of bioregeneration of exhausted resin. The experimental results for control samples (samples without biological removal of nitrate in the aqueous phase) showed that after using resin in each cycle, the desorption rate decreased (Ebrahimi & Roberts, 2013). The change of desorption rate after each cycle can be explained based on shrinking core model and the fact that in our studied resin pore diffusion is the controlling process. Based on the shrinking core model about 10% of the nitrate will be adsorbed in the core of the resin where radius of the core ($r$) is half of the average radius of the resin beads ($r_0$) (Pritzker, 2005; Sharbatmaleki et al., 2015). In the desorption process, nitrate on the resin sited outside of this core will be desorbed easily first and then nitrate in the inner resin sites will be desorbed with slower rate since pore diffusion is controlling. There is a possibility that after each regeneration cycle which is basically swelling and shrinking of the resin beads, $r$ is increasing (Sharbatmaleki et al., 2015), which results in decreasing the desorption rate at each cycle. The model was used to predict the reduction rate of the nitrate desorption rate after each cycle. Model prediction shows about 30% reduction of desorption rate after each cycle. Figure 6.3 presents the experimental results (Ebrahimi & Roberts, 2013) as well as the model-predicted data for test
and control samples. Test samples are resin enclosed in membrane, bio-regenerated in presence of the biomass and control samples are resin enclosed in membrane in regeneration solution without biomass (Ebrahimi & Roberts, 2013). The R-squared value was calculated to show how close the experimental data are to the fitted regression curves from the developed model. The average of R-squared values for cycle 1 to 5 for test and control samples are 0.94±0.06 and 0.86±0.04, respectively which shows that the developed model predicted the experimental results very well.
Figure 6.3: Comparison of experimental and model predicted results; a) regeneration of control samples and b) bioregeneration of test samples. During regeneration studies samples were placed in 1 litre flasks with 250 ml of mixed synthetic medium and culture (2500 mg/L VSS) and mixed well for 48 hours. In addition to the 3 replicate of test conditions (resin in the membrane in contact with culture in anaerobic flasks) 3 replicate of control conditions were placed in the same medium without the culture to show nitrate desorption from resin (Ebrahimi & Roberts, 2013)
The main goal of developing a mathematical model for multi-cycle bioregeneration is for use in reactor design and predicting the time required for regeneration of the resin at each specific capacity. That the model can be used to predict 1) how long resin should be left in the bioreactor to be regenerated completely? (if it is possible based on the resin structure), 2) how many cycles can the resin be regenerated?, 3) what is the required time for partial resin regeneration if the time of regeneration is much more important than loss of capacity of the resin. Since the feasibility of resin regeneration is one of the most important items that can help with resin selection for an ion exchange process, this model’s outcome can be used by an ion exchange specialist to choose the best resin option for an ion exchange process to remove specific contamination from water.

Table 6.3 shows the predicted residual nitrate on the resin after 24 hours of bioregeneration/regeneration of the resin for both test and control samples. A regeneration time of 24 hours was considered to compare the capacity-loss of the resin with experimental data. Experimental results revealed that after using the resin in 5 regeneration cycles, test samples lost about 6% capacity and control samples for which normal regeneration process was carried out lost 19% capacity. The model predicted capacity loss for the resin of 7 and 26% for test and control samples, respectively.

Table 6.3: Model-predicted residual nitrate and resin capacity loss for both test and control samples

<table>
<thead>
<tr>
<th># of regeneration cycles</th>
<th>Test samples</th>
<th>Control samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Residual nitrate (eq/g of resin)</td>
<td>Capacity reduction after (%)</td>
</tr>
<tr>
<td>1</td>
<td>2.0E-05</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>3.09E-05</td>
<td>2.3</td>
</tr>
<tr>
<td>3</td>
<td>4.3E-05</td>
<td>3.4</td>
</tr>
<tr>
<td># of regeneration cycles</td>
<td>Test samples</td>
<td>Control samples</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------</td>
<td>----------------</td>
</tr>
<tr>
<td></td>
<td>Residual nitrate (eq/g of resin)</td>
<td>Capacity reduction after (%)</td>
</tr>
<tr>
<td>4</td>
<td>6.3E-05</td>
<td>4.9</td>
</tr>
<tr>
<td>5</td>
<td>9.2E-05</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Figure 6.4 shows the nitrate remaining on the resin at different times during the regeneration process for different cycles. Based on this graph, the capacity loss of resin at each specific time can be predicted and based on that the time the resin will need to be regenerated to recover specific capacity of the exhausted resin can be determined.

Figure 6.4: Rate of residual nitrate rate on the resin at different bioregeneration cycle (model predicted results)
6.2.3 Effect of different parameters on bioregeneration of resin

In order to evaluate the effect of different parameters on the bioregeneration process, sensitivity analysis of the model was performed. The required time (h) for regeneration of 90% capacity of exhausted resin was considered as the desired output to compare the effect of different parameters. Resin mass and the biomass concentration were considered as independent factors. For sensitivity analysis of model regarding each of these parameters the other model parameters were considered as constant.

Since one of the main goals of this research was to evaluate the effect of temperature and salt concentrations as the main environmental factors on resin bioregeneration, the model sensitivity analysis was done for these two parameters as well. Previous research has revealed that salt concentration and temperature have a significant effect on nitrate desorption and biodegradation rates (Ebrahimi et al., 2015). Corresponding nitrate desorption rate and nitrate biological removal rate to each salt concentration and temperature obtaining from previous experimental analysis (Ebrahimi et al., 2015; Ebrahimi & Roberts, 2015) were used to perform the model sensitivity analysis for salt concentration and temperature. Table 6.4 presents the results of model sensitivity analysis for all dependent and independent parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Time to 90% capacity recovery (h)</th>
<th>Nitrate concentration in the aqueous phase at 90% resin recovery (mg/L)</th>
<th>Time to get lower than 30 mg/L nitrate in the aqueous phase (h)</th>
<th>Resin capacity recovery when nitrate in the aqueous phase is 30 mg/L (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin Mass (g)</td>
<td>1</td>
<td>4</td>
<td>252</td>
<td>30</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.5</td>
<td>20</td>
<td>7</td>
<td>88</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value</td>
<td>Time to 90% capacity recovery (h)</td>
<td>Nitrate concentration in the aqueous phase at 90% resin recovery (mg/L)</td>
<td>Time to get lower than 30 mg/L nitrate in the aqueous phase (h)</td>
<td>Resin capacity recovery when nitrate in the aqueous phase is 30 mg/L (%)</td>
</tr>
<tr>
<td>---------------------------------</td>
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<td>------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrient Bioavailability</td>
<td>4</td>
<td>15</td>
<td>0.5</td>
<td>6.5</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>44</td>
<td>0.2</td>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>Biomass concentration (mg VSS/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>3.8</td>
<td>252</td>
<td>31</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>3.7</td>
<td>217</td>
<td>15</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>3.6</td>
<td>151</td>
<td>7.5</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>2500</td>
<td>3.6</td>
<td>121</td>
<td>6</td>
<td>94</td>
</tr>
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<td>93</td>
</tr>
<tr>
<td>Temperature 12°C</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9</td>
<td>87</td>
<td>12</td>
<td>92.5</td>
</tr>
<tr>
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<td>6</td>
<td>96</td>
<td>9</td>
<td>93</td>
</tr>
<tr>
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</tr>
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<td>6</td>
<td>3.2</td>
<td>256</td>
<td>30</td>
<td>98</td>
</tr>
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<td>Temperature 23°C</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>2</td>
<td>5.3</td>
<td>20</td>
<td>5</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.3</td>
<td>44</td>
<td>4.6</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.8</td>
<td>146</td>
<td>5.5</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.8</td>
<td>183</td>
<td>7.5</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.6</td>
<td>190</td>
<td>7.6</td>
<td>96</td>
</tr>
<tr>
<td>Temperature 35°C</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>18</td>
<td>3.7</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>35</td>
<td>3</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.8</td>
<td>162</td>
<td>4</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.5</td>
<td>188</td>
<td>4.3</td>
<td>96</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value</td>
<td>Time to 90% capacity recovery (h)</td>
<td>Nitrate concentration in the aqueous phase at 90% resin recovery (mg/L)</td>
<td>Time to get lower than 30 mg/L nitrate in the aqueous phase (h)</td>
<td>Resin capacity recovery when nitrate in the aqueous phase is 30 mg/L (%)</td>
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<td>-------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.2</td>
<td>217</td>
<td>5</td>
<td>97</td>
</tr>
</tbody>
</table>

The first tested parameter was resin mass, as can be expected, by increasing the mass of resin the overall regeneration time increased. This is expected because the volume of the bioreactor was held constant and so the aqueous phase decreased as the resin mass increased. Having more mass of the resin in the same volume of reactor and the same regeneration solution affects the resin bioregeneration process. Because of higher mass of resin, there would be thick layer of resin on top of each other which decreases the desorption rate of nitrate from resin to the aqueous phase because of lower mass transfer rate in more packed area. On the other hand, Although the desorption rate decreases because of more mass of resin, nitrate biodegradation rate remain constant since it is happening outside of the membrane and it is not related to the mass of resin. When the mass of resin is increasing, the desorption rate of nitrate is decreasing, less nitrate defuses outside of the membrane and less nitrate will be available for the biomass with the same nitrate degradation rate. Therefore, whatever is coming to the aqueous phase will be degraded by the biomass immediately and the nitrate concentration in the aqueous phase will be low.

The second parameter examined was the biomass, increasing the biomass concentration did not significantly affect the time to achieve 90% regeneration or the resin capacity when nitrate was 30 mg/L but the nitrate concentration in the aqueous phase at 90% recovery and the time to lower than 30 mg/L in the aqueous phase were significantly
affected; as the biomass concentration increased these decreased. The resin regeneration depends on the concentration of salt in the bioreactor. Since the salt concentration has been considered constant while changing the biomass, the time required for 90% recovery of resin capacity happens quickly and stays almost the same at different biomass concentrations. But since biomass will affect the degradation of nitrate, the nitrate concentration in the aqueous phase at 90% recovery of the resin as well as required time for nitrate removal to lower concentrations are highly affected by biomass concentration.

Since previous results revealed an interaction between salt and temperature, the effects of these two parameters were examined in concert, i.e. the effects of salt concentration were determined at each temperature. At each specific temperature, increasing the salt concentration, decreased the time required to recover 90% of the resin. Increasing the salt concentration will increase the chloride concentration on the left side of the regeneration equation

$$[R(C_4H_9)_3N^+]NO_3^- + Cl^- \leftrightarrow [R(C_4H_9)_3N^+]Cl^- + NO_3^-$$

which based on the Le Chatelier's principle forwards the desorption reaction to the right side and results in faster desorption. Also, the overall time of resin regeneration is lower at higher temperature. The desorption process is endothermic which means applying more energy to the process will shift the reaction forward resulting in more removal of nitrate from the resin sites. But resin recovery is not the only goal of this process; nitrate in the aqueous phase should be removed to lower than maximum allowable concentration of nitrate. Since the nitrate desorption rate is faster than the biodegradation rate, resin regeneration to 90% recovery is achieved quickly but leaves a high concentration of nitrate in the aqueous phase because of the nitrate biological removal rate and prevents further regeneration of the resin. In order to achieve high resin regeneration recovery (more than 95%), nitrate in the aqueous phase should be
degraded to let more nitrate desorb from the resin sites. At each specific temperature, the
time required to decrease the nitrate concentration in the aqueous phase to lower than 30
mg/L is lowest at 3% salt and increases by increasing the salt concentration and decreases by
increasing the temperature. Before starting the experiment used to collect the biodegradation
data, the mixed culture (NP30) had been fed using 3% salt medium for years. This is why the
culture performs the best at 3% salt. When nitrate in the aqueous phase is lower than 30 mg/L
most of the adsorbed nitrate has been released and so there is very little effect of temperature
or salt on this value. The effect of temperature and salt concentration on the regeneration
time and nitrate removal support our conclusion that $M_t$ for this process is higher than 3 and
biodegradation of nitrate plays an important role in resin bioregeneration process.

### 6.2.4 Design of the bioregeneration reactor

Based on the experimental data presented in section 6.2.2 and the concept of SBR
design, a mass balance was developed based on solids in the reactor; mass of the solids at full
volume is equal to mass of settled solids (Droste, 1997; Metcalf & Eddy, 2004).

$$V_T X_T = V_S X_S \quad eq \ 6.18$$

Where, $V_T$ = total volume, m$^3$; $X_T$ = MLSS concentration at full volume, g/m$^3$; $V_S$ =
Settled volume after decant, m$^3$; $X_S$ = MLSS concentration in settled volume, g/m$^3$.

The mass balance was then solved to determine the fill fraction. First $X_S$ was
estimated based on the sludge volume index (SVI):

$$X_S = \frac{10^6}{SVI} = \frac{10^6}{130} = 7692 \frac{mg}{L} \quad eq \ 6.19$$

Then settled fraction was calculated:
\[
\frac{V_S}{V_T} = \frac{X}{X_S} = \frac{2500 \text{ mg/L}}{7692 \text{ mg/L}} = 0.33 \quad \text{eq 6.20}
\]

Usually 20% liquid should be provided above the sludge blanket so that solids are not removed by the decanting mechanism (Crittenden et al., 2012; Droste, 1997; Metcalf & Eddy, 2004).

\[
\frac{V_S}{V_T} = 1.2 \times 0.33 = 0.4 \quad \text{eq 6.21}
\]

The decant depth was calculated as: Decant depth = 0.4 full depth = 0.4*1.5 = 0.6m

The fill fraction was determined using (\(V_F\) is fill volume):

\[
V_S + V_F = V_T \rightarrow \frac{V_S}{V_T} + \frac{V_F}{V_T} = 1 \rightarrow \frac{V_F}{V_T} = 1 - 0.4 = 0.6 \quad \text{eq 6.22}
\]

Based on the selectivity of any specific resin regarding the anions in the water sample, the available capacity of resin for each anion can be calculated (Crittenden et al., 2012). Since our resin is highly nitrate selective, it is assumed that 20% of the resin capacity is occupied by the other anions. Therefore the available capacity of resin for removal of nitrate is 0.4 eq of NO\(_3\)/L of resin. Based on the chemical composition of challenge water (Ebrahimi & Roberts, 2013; Ebrahimi & Roberts, 2015) the volume of water that can be treated by each liter of resin can be calculated.

\[
\frac{216 \text{ mg nitrate}}{\text{L Water}} \times \frac{1 \text{ eq}}{62 \text{ g nitrate}} = 3.48 \frac{\text{meq of nitrate}}{\text{L Water}} \quad \text{eq 6.23}
\]

\[
\frac{0.4 \text{ meq/ml Resin}}{3.48 \text{ meq/L Water}} = 115 \frac{\text{L Water}}{\text{L Resin}} \quad \text{eq 6.24}
\]
Considering typical volume of IX process in small industrial drinking water treatment facilities as 2.5 m$^3$ of water that should be treated per each cycle of ion exchange, the mass of resin which is needed for complete removal of nitrate from drinking water was calculated.

\[
\frac{2.5 \times 10^3 \text{L}_{\text{Water}}}{115 \frac{\text{L}_{\text{Water}}}{\text{L}_{\text{Resin}}}} = 20 \text{ L}_{\text{Resin}} \quad eq 6.25
\]

\[
20 \times 10^3 \text{L}_R \times 0.5 \frac{\text{meq}}{\text{ml}_{\text{Resin}}} = 10000 \text{ meq Resin} \quad eq 6.26
\]

\[
\frac{10000 \text{ meq Resin}}{1.62 \frac{\text{meq}}{\text{g}_{\text{Resin}}}} = 6.2 \text{ Kg Resin} \quad eq 6.27
\]

Assuming the entire nitrate adsorbed during the IX process should be desorbed and biodegraded in bioregeneration reactor, the volume which is needed for full SBR reactor can be calculated.

\[
6.2 \times 10^3 \text{resin} \times 1.62 \frac{\text{meq}}{\text{g}_{\text{Resin}}} \times \frac{62 \text{ g nitrate}}{1\text{eq}} = 620 \times 10^3 \text{mg Nitrate} \quad eq 6.28
\]

Since the experimental analysis of bioregeneration of resin at a lab scale was done for nitrate concentration of not higher than 700 mg/L, in order to be in the same range the volume of the reactor should be 1 m$^3$ in order to provide 620 mg/L of nitrate during the bioregeneration process. Thus, the diameter of membrane part and whole reactor can be calculated:

\[
\text{Resin volume} = 0.02 \text{ m}^3
\]

\[
\text{Depth} = 1.5\text{m}
\]
\[
\frac{\pi D^2}{4} \times 1.5 = 0.02 \rightarrow D = 15 \text{ cm} \quad \text{eq 6.29}
\]

Considering 30% as safety factor for membrane cover: \( D = 20 \text{ cm} \)

Biodegradation volume = 1 m³

Depth = 1.5 m, typical bed depth used in industry range from 0.75 to 3 m (Crittenden et al., 2012; Droste, 1997).

\[
\frac{\pi D^2}{4} \times 1.5 = 1 \rightarrow D = 92 \text{ cm} \quad \text{eq 6.30}
\]

The total diameter would be 92+20 = 112 cm

Based on these calculations the size of the SBR was identified (Figure 6.5).

**Figure 6.5:** Size of bioregeneration reactor based on basic design

Based on the developed model using the calculated mass of resin and reactor volume to, nitrate will be completely removed from the aqueous phase in 15 hours.
According to the observed yield (0.45 mg VSS/mg acetate removed) the amount of VSS that will be produced at each cycle can be calculated. In order to have a constant biomass in the reactor, the produced biomass at each cycle should be wasted during the draw time. 620 g nitrate is removed at each bioregeneration cycle (assuming all of the adsorbed nitrate can be biodegraded). Based on the denitrification reaction considering acetate as a carbon source VSS produced at each cycle that should be removed was calculated as follow:

\[
\frac{4}{3} \text{NO}_3^- + \text{CH}_3\text{COO}^- \rightarrow \frac{2}{3} \text{N}_2 + \text{HCO}_3^- + \text{CO}_2 + \text{H}_2\text{O}
\]

\(\text{rxn 6.1}\)

\[
620 \text{ g nitrate} \times \frac{1 \text{ mol nitrate}}{62 \text{ g nitrate}} \times \frac{1 \text{ mol acetate}}{\frac{4}{3} \text{ mol nitrate}} \times \frac{59 \text{ g acetate}}{1 \text{ mol acetate}} = 445 \text{ g acetate}
\]

\(\text{eq 6.31}\)

\[
0.45 \frac{\text{mg VSS}}{\text{mg acetate}} \times 445 \times 10^3 \text{ mg acetate} = 200 \text{ g VSS produced at each cycle}
\]

\(\text{eq 6.32}\)

This is just biomass production based on the reduction of nitrate. Part of the acetate will be used for anabolism to make more biomass without using nitrate. For this reason in the experimental part of the research 1.5 molar ratio of acetate to nitrate was added to compensate for anabolism mechanism. Considering pyruvate as the biomass produced using acetate as the electron donor, 0.5 g cell COD is produced per g of COD as acetate (Metcalf & Eddy, 2004). Also, each gram of cell and each gram of acetate provides 1.42 g and 0.78 g COD, respectively. Based on this, if extra acetate is completely used for biomass production, about 80 gram of biomass is produced per each bioregeneration cycle which makes total 280 g VSS per each cycle for both nitrate reduction and cell production.

Regarding the regeneration solution, the minimum regeneration solution needed to complete regeneration of resin at 6% salt can be calculated as follow:
\[
\frac{1.62 \text{ meq of NaCl/mlResin} \times 20 \, L_{\text{resin}}}{1 \text{ meq of NaCL/LRegenration solution}} = 32.4 \, L_{\text{Regenration solution}} \quad eq \, 6.33
\]

Complete design of bioregeneration reactor including time required for each phase of the SBR, HRT, SRT, regeneration solution flow, etc. can be done when detailed information of a case study is available.
Chapter 7: Conclusion & future work

7.1 Conclusions

To improve resin bioregeneration process, in our research, we enclosed the resin in a membrane to prevent direct contact of the resin with the bacterial culture before the bioregeneration stage.

1. The proof of concept study showed that:
   1.1. the resin can be used for 4 cycles without a loss of capacity;
   1.2. after 6 cycles only 6% of the capacity was lost;
   1.3. direct regeneration of a resin enclosed in a membrane is possible without any disinfection or cleaning procedures.

2. Investigation the effect of temperature and salt concentration on nitrate desorption rate revealed that:
   2.1. the experimental data can be modeled using a pseudo-second order kinetic;
   2.2. the nitrate concentration is at the equilibrium after almost 3 hours;
   2.3. the salt concentration does not have significant effect on the final equilibrium concentration;
   2.4. the initial desorption rate at different concentrations are significantly different with higher and lower value for 6% and 2% salt, respectively;
   2.5. the difference in the mean value of equilibrium concentration at 12°C is significant than 23°C and 35°C but there is no significant difference between 23°C and 35°C mean values;
2.6. the initial desorption rate at different temperatures are significantly different with higher value at 35°C and lower value at 12°C.

3. The results of study on the interaction of salt concentration and temperature during biological denitrification showed:

3.1. the nitrate biodegradation rate at different temperature and salt concentration is independent of the initial nitrate concentration.

3.2. at each specific salt concentration, the nitrate removal rate increased with increasing temperature with the average value of 0.00111±0.00006 mg-nitrate/mg-VSS•h•°C.

3.3. the effect of different salt concentrations was dependent on the temperature;

3.4. there was a significant interaction between salt concentration and temperature;

3.5. within each group of temperatures, the nitrate degradation rate decreased with increasing the salt concentration;

3.6. the temperature affects the level of salinity tolerance and the culture was less tolerant to high concentration of salt at low temperature.

3.7. the difference between the minimum and maximum nitrate degradation rate is greater at lower temperature. At 35°C, a 32% reduction in the nitrate degradation rate was observed while at 12°C this reduction was 69%.

4. Optimization of the multi-cycle bioregeneration of the resin enclosed in membrane showed that:

4.1. pore diffusion is the controlling desorption process;
4.2. total resin bioregeneration process is governed by biological removal of nitrate;

4.3. developed mathematical model which incorporated physical desorption process with biological removal kinetics predicts the experimental results very well;

5. The sequencing batch bioreactor for the multi-cycle bioregeneration of selective resins was designed.

7.2 Future work

The results of this research can be used to develop new research topics to build on this project. Here are some recommendations:

- One of the obstacles through this research was the lack of a case study. All the water samples were made synthetically. Testing of the process on multiple natural water sources would allow a comprehensive evaluation of the performance of combined ion exchange/resin bioregeneration system under multiple loadings can further shed light on the performance (and limitations) of this process.

- Cost analysis of the large scale of this process and comparison of the capital cost of this method with the other developed ones can provide valuable information regarding the final decision on method selection.

- On the microbiological side, a comprehensive analysis of the changes in the microbial populations in the mixed culture based on the change of the environmental condition will provide important information about the stability of the culture.
• Since the mathematical model has been developed based on the basic characteristics of the studied resin, it would be beneficial to apply the developed model to a new selective resin to predict the bioregeneration results and validate the predicted results with multi-cycle bioregeneration of the resin.

• A comprehensive study of the performance of a large scale bioregeneration reactor is necessary to commercialize this project.
References


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