COMPARISON OF MICROC™, METHANOL AND ACETATE AS EXTERNAL CARBON SOURCES IN REMOVAL OF NITRATE AND PERCHLORATE FROM WASTWATER BY DENITRIFYING ENRICHMENTS

A Thesis Presented

by

Ibrahim Nimer EL-Shawabkeh

to

The Department of Civil and Environmental Engineering

in partial fulfillment of the requirements for the degree of

Master of Science

in

Civil Engineering

In the field of

Environmental Engineering

Northeastern University

Boston, Massachusetts

August 2008
Abstract

In this study, we used Sequencing Batch Reactors (SBR) to enrich for three denitrifying cultures that were fed with three carbon sources: Methanol, Acetate and MicroC™ with the aims to 1) evaluate and compare the denitrification rates and kinetics among different carbon sources, and 2) evaluate the ability of these enrichments to remove perchlorate from wastewater. Three SBR systems, using Methanol, Acetate and MicroC™ as carbon sources, were developed and monitored for nitrogen removal under two temperature (10 and 20 °C) conditions. Results obtained from denitrification batch tests during the monitoring process showed that the Methanol acclimated sludge had the highest denitrification rate followed by MicroC™ acclimated sludge then Acetate acclimated sludges. Results obtained from denitrification batch test at 10°C showed that MicroC™ acclimated sludge denitrification rate was better than Methanol acclimated sludge. Acetate acclimated sludge affected by the temperature change from 20°C to 10°C, this sensitivity caused to the accumulation of nitrite due to partial denitrification. Results showed that culture acclimated with different carbon sources (electron donors) had different denitrification rates and kinetics, therefore requires for different design considerations for denitrification processes. All the denitrifying enrichments were able to reduce perchlorate at different rates even with the presence of nitrate.
# Table Of Content

Abstract ........................................................................................................................................... I

Table Of Content .......................................................................................................................... II

List Of Figures ................................................................................................................................ IV

List Of Tables ............................................................................................................................... VI

1 Introduction .................................................................................................................................. 1

1.1 Nitrogen in Wastewater .................................................................................................... 1

1.2 Perchlorate in the Environment .................................................................................... 2

1.3 Objective and Goals .......................................................................................................... 3

2 Background: Nitrogen and Perchlorate Removal Processes ............................................ 4

2.1 Introduction ....................................................................................................................... 4

2.2 Nitrification ...................................................................................................................... 5

2.2.1 Nitrification Stoichiometry ....................................................................................... 5

2.2.2 Microbiology of Nitrification ..................................................................................... 6

2.2.3 Factors Effecting Nitrification .................................................................................... 7

2.3 Denitrification .................................................................................................................... 8

2.3.1 Microbiology of Denitrification ................................................................................... 8

2.3.2 Denitrification Stoichiometry .................................................................................... 9

2.3.3 Factors Effecting Denitrification ............................................................................... 10

2.3.4 Carbon Source in Denitrification ............................................................................. 11

2.4 Perchlorate .......................................................................................................................... 12

2.4.1 Chemistry of Perchlorate ............................................................................................ 12

2.4.2 Perchlorate Removal .................................................................................................... 14

3 Material and Methods .............................................................................................................. 16

3.1 Carbon Sources .................................................................................................................. 16

3.1.1 MicroC™ .................................................................................................................. 16

3.1.2 Methanol .................................................................................................................. 17

3.1.3 Acetate .................................................................................................................... 18

3.2 Sequencing Batch Reactor (SBR) System Design ......................................................... 19

3.2.1 Overview .................................................................................................................. 19

3.2.2 System Design .......................................................................................................... 20
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.3</td>
<td>Influent Properties</td>
<td>22</td>
</tr>
<tr>
<td>3.3</td>
<td>Denitrification Evaluation</td>
<td>28</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Denitrification Kinetics Batch Testing</td>
<td>28</td>
</tr>
<tr>
<td>3.3.2</td>
<td>Measurement of Denitrification Kinetics at 10°C</td>
<td>30</td>
</tr>
<tr>
<td>3.3.3</td>
<td>Measurement of Denitrification Kinetics 20°C</td>
<td>31</td>
</tr>
<tr>
<td>3.3.4</td>
<td>Measurement of Denitrification Kinetics Influence of Carbon Source</td>
<td>31</td>
</tr>
<tr>
<td>3.4</td>
<td>Perchlorate Removal</td>
<td>31</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Experiment 1</td>
<td>32</td>
</tr>
<tr>
<td>3.4.2</td>
<td>Experiment 2</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>Results and Discussion</td>
<td>35</td>
</tr>
<tr>
<td>4.1</td>
<td>Monitoring of the SBR Systems</td>
<td>35</td>
</tr>
<tr>
<td>4.1.1</td>
<td>MicroC™ SBR Monitoring</td>
<td>35</td>
</tr>
<tr>
<td>4.1.2</td>
<td>Methanol SBR Monitoring</td>
<td>37</td>
</tr>
<tr>
<td>4.1.3</td>
<td>Acetate SBR Monitoring</td>
<td>38</td>
</tr>
<tr>
<td>4.2</td>
<td>Denitrification Kinetics</td>
<td>40</td>
</tr>
<tr>
<td>4.2.1</td>
<td>Measurement Denitrification Kinetics at 10°C</td>
<td>40</td>
</tr>
<tr>
<td>4.2.2</td>
<td>Measurement Denitrification Kinetics at 20°C</td>
<td>47</td>
</tr>
<tr>
<td>4.2.3</td>
<td>Cross Response</td>
<td>54</td>
</tr>
<tr>
<td>4.3</td>
<td>Perchlorate Removal</td>
<td>59</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Experiment 1</td>
<td>60</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Experiment 2</td>
<td>62</td>
</tr>
<tr>
<td>4.4</td>
<td>Discussion</td>
<td>67</td>
</tr>
<tr>
<td>4.4.1</td>
<td>SBR Monitoring</td>
<td>67</td>
</tr>
<tr>
<td>4.4.2</td>
<td>Denitrification Kinetics</td>
<td>69</td>
</tr>
<tr>
<td>4.4.3</td>
<td>Perchlorate Removal</td>
<td>71</td>
</tr>
<tr>
<td>5</td>
<td>Conclusion</td>
<td>74</td>
</tr>
<tr>
<td>References</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Acknowledgement</td>
<td>81</td>
<td></td>
</tr>
</tbody>
</table>
List Of Figures

Figure 1. Biological nitrogen removal ................................................................. 5
Figure 2. SBR reactor scheme ........................................................................... 20
Figure 3. Three SBR systems, each using a different carbon source ............... 21
Figure 4. A schematic of the SBR system components ..................................... 22
Figure 5. Denitrification batch testing set up ..................................................... 29
Figure 6. Measurement of K_D ........................................................................ 30
Figure 7. Example of chemical parameters of MicroC™ monitoring .............. 36
Figure 8. Example of chemical parameters of Methanol monitoring ............... 38
Figure 9. Example of chemical parameters of Acetate monitoring ................. 39
Figure 10. Nitrogen compounds consumption in MicroC™ sludge 10°C .......... 41
Figure 11. COD consumption in MicroC™ sludge 10°C .................................... 42
Figure 12. Nitrogen compounds consumption in Methanol sludge 10°C .......... 43
Figure 13. COD consumption in Methanol sludge 10°C ................................... 44
Figure 14. Nitrogen compounds consumption in Acetate sludge 10°C .......... 45
Figure 15. COD consumption in Acetate sludge 10°C ..................................... 46
Figure 16. Nitrogen compounds consumption in MicroC™ sludge 20°C ......... 47
Figure 17 COD consumption in MicroC™ sludge 20°C .................................... 48
Figure 18. Nitrogen compounds consumption in Methanol sludge 20°C ......... 49
Figure 19. COD consumption in Methanol sludge 20°C ................................... 50
Figure 20. Nitrogen compounds consumption in Acetate sludge 20°C .......... 52
Figure 21. COD consumption in Acetate sludge at 20°C ................................ 53
Figure 22. Measurement of K_D for MicroC™ sludge using different carbon sources .... 56
Figure 23. Measurement of K_D for Methanol sludge using different carbon sources .... 57
Figure 24. Measurement of K_D for Acetate sludge using different carbon sources ...... 58
Figure 25. ClO_4^- removal in MicroC™ sludge .............................................. 60
Figure 26. ClO_4^- removal in Methanol sludge ............................................... 61
Figure 27. ClO_4^- removal in Acetate sludge ................................................ 62
Figure 28. NOx and ClO_4^- removal in MicroC™ sludge ............................... 64
Figure 29. NOx and ClO_4^- removal in methanol sludge ............................... 65
Figure 30. NOx and ClO₄⁻ removal in Acetate sludge.......................................................... 66
List Of Tables

Table 1. Feeding of the SBR systems ................................................................. 23
Table 2. Microelements used in the feeding ......................................................... 24
Table 3. Starting and ending time of each cycle .................................................. 26
Table 4 Phases and duration in every single phase .............................................. 27
Table 5. Concentrations of COD & ClO$_4^-$ used in experiment 1 of ClO$_4^-$ removal........ 33
Table 6. Concentration of NO$_3^-$, ClO$_4^-$, and COD used in experiment 2 of ClO$_4^-$, removal. ................................................................. 34
Table 7. Summary of all $K_D$ rates obtained from the three acclimated sludge. ....... 54
Table 8. Summary of the cross respond of the three acclimated sludges. ............... 59
Table 9. Summary of two experiments for measuring ClO$_4^-$ degradation ............... 67
Table 10. Measurement of ClO$_4^-$ degradation rates ........................................... 72
1 Introduction

1.1 Nitrogen in Wastewater

Nitrogen probably comes in the second place after carbon in the complexity of its cycling (Vaccari et al., 2006). The major accessible source of Nitrogen in the biosphere is the element nitrogen gas (N\(_2\)) that makes up of 78% (by volume) of the atmosphere (Vaccari et al., 2006). Nitrogen chemistry is complex due to the several oxidation states that nitrogen can consume (Metcalf and Eddy, 2003), as shown in the following equation:

\[
\begin{align*}
\text{NO}_3^- & \rightarrow \text{NO}_2^+ \rightarrow \text{HNO}_2 \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2 \rightarrow \text{NH}_4^+ \\
+5 & \rightarrow +4 \rightarrow +3 \rightarrow +2 \rightarrow +1 \rightarrow 0 \rightarrow -3
\end{align*}
\]

Discharged effluents from wastewater treatment plants usually contain nutrients such as nitrogen and phosphorus. Nitrogen together with phosphors cause eutrophication, the excessive growth of algae blooms in aquatic surfaces. Typical concentration of nitrogen in domestic wastewater is 40 mg/l as total nitrogen, which can be divided into organic compounds and ammonia (NH\(_4\)) with typical concentrations of 15 and 25 mg/l, respectively (Metcalf and Eddy, 2003). Nitrogen compounds can consume dissolved oxygen in aquatic surfaces, produce toxicity to stimulate aquatic growth life, threat to public health, and affect the suitability of wastewater effluents for reuse purposes. The United State Environmental Protection Agency (USEPA) listed nitrate (NO\(_3^-\)) as a

\[1\] http://chem-faculty.ucsd.edu/trogler/CurrentNitroWeb/Section1/section2.shtm
contaminant in drinking water since it can cause Methemoglobinemia (blue babies) (Walter et al., 2005).

Biological treatment is considered a flexible approach for wastewater treatment. It is usually designed to remove carbonaceous and nitrogenous pollutant compounds found in the wastewater. Since the majority of nitrogen compounds found in wastewater are in the form ammonium (NH$_4$), biological nitrification and denitrification are the most accessible processes for its removal from wastewater. In general, nitrification requires an aerobic environment for oxidation of ammonium to nitrite (phase I) and then to nitrate (phase II), whereas, denitrification occurs by utilizing electron donors under an anoxic condition (Gao et al., 2003).

1.2 Perchlorate in the Environment

Recent studies suggest that perchlorate (ClO$_4^-$) may need to be removed to less than 1 mg/L in drinking water to protect human health (USEPA, 2002). High concentrations of perchlorate prevent the iodide uptake by thyroid gland in humans, which will cause a lack in hormones production (Wolff, 1998). There is an increased concern regarding perchlorate due to the high-level concentrations that are reported in the environment, especially in groundwater. Major uses of perchlorate are as an oxidation accelerator in munitions, rocket fuel, and fireworks. The difficulty of perchlorate degradation arises from its nonvolatile, highly soluble, and kinetic characteristics (Gu et al., 2001). Studies have shown that perchlorate can be degraded by chemical and microbial processes. Chemical techniques, such as ion exchange, are capable in reducing perchlorate concentrations to part per billion levels (Logan, 1998). Microbial degradation of perchlorate can occur under certain conditions by a wild range of microorganisms
(Herman and Frankenberger, 1998). Recent studies on perchlorate pathway indicate that the most widely accepted pathway for bacterial respiration using ClO$_4^-$ and ClO$_3^-$ is:

\[
\begin{align*}
\text{ClO}_4^- & \rightarrow \text{ClO}_3^- \rightarrow \text{ClO}_2^- \rightarrow \text{O}_2 + \text{Cl}_2
\end{align*}
\]

(2)

1.3 Objective and Goals

The overall objective of this study is to develop SBR systems acclimated to MicroC™, Methanol and Acetate as carbon sources and evaluate their ability for biological denitrification and ClO$_4^-$ removal from wastewater. The specific goals are:

1. To study denitrification kinetics using different carbon sources in Sequencing Batch Reactors (SBR) including MicroC™, Methanol and Acetate.

2. Evaluate the performance of the acclimated sludge (developed for Goal 1) for removal of perchlorate.

The reason behind studying perchlorate removal by denitrification process is due the hypothesis and previous observation that the enzyme nitrate reductase which is an enzyme involved in the denitrification process is also responsible of reduce chlorate (Zumft, 1997). Two types of tests were conducted to assess the effect of perchlorate concentration and the effect of NO$_3^-$ on the performance.
2 Background: Nitrogen and Perchlorate Removal Processes

2.1 Introduction

Nitrogen exists in all ecological systems that are found on Earth. Nitrogen enters these ecosystems in several forms including nitrate nitrogen (NO$_3^-$-N) (e.g. fertilizers), particulate nitrogen (e.g. litter fall from trees), ammonium (e.g. sewage and animal waste), and nitrous oxides (N$_2$O) (e.g. fossil fuel combustion). This means that the type of nitrogen compound determines the pathway entry (Mayer et al, 2005). For example, N$_2$O enters aquatic bodies through atmospheric deposition, while NO$_3^-$ often enters through groundwater, and particulate nitrogen follows terrestrial routes. N$_2$ may be transformed by biological processes such as uptake by plants and microbial denitrification. Denitrification can remove nitrogen compounds from a system, whereas, uptake by plants allows the return of nitrogen to the system through microbial decay. Some of Nitrogen forms are considered toxic due to their negative effects on the environment. For example, NO$_3^-$ is considered an environmental stressor because of its biological reactivity that poses a human health risk (Mayer et al, 2005). On the other hand, N$_2$O is known as greenhouse gas.

Nitrogen is a major concern in wastewater process due to its harmful effects on the environment (Halling-Sørensen and Jorgensen 1993). Biological nutrient removal (BNR) processes are the most common approach to achieve nitrogen removal from wastewater treatment. Biological Nitrogen processes are an important technique in wastewater treatment. It is a combination of an aerobic process (nitrification) followed by an anoxic process (denitrification). A typical nitrification-denitrification process is shown in Figure...
1. The process involves the internal recirculation of the treated effluent (effluent from nitrification) to the anoxic zone for denitrification.

![Diagram of nitrogen removal](image)

**Figure 1. Biological nitrogen removal (Jeyanayagam, 2005).**

### 2.2 Nitrification

Nitrification is the oxidation of ammonia (NH₃) to nitrate (NO₃⁻) via nitrite (NO₂⁻). It is a process that involves two distinct activities and carried out by two categories of autotrophic microorganisms (Bitton, 1999).

#### 2.2.1 Nitrification Stoichiometry

The first phase is the oxidation of \((\text{NH}_4^+)\) to \((\text{NO}_2^-)\) with hydroxyl as an intermediate:

\[
2 \text{NH}_4^+ + 3 \text{O}_2 \rightarrow 2\text{NO}_2^- + 4 \text{H}^+ + 2 \text{H}_2\text{O} \quad (3)
\]
This step is done mainly by *Nitrosomonas* species (e.g., *N. europaea*, *N. oligocarbogenes*). *Nitrosomonas* species, also known as ammonia-oxidizer bacteria (AOB), are autotrophic bacteria.

The second phase is the oxidation of NO$_2^-$ to NO$_3^-$:

$$2 \text{NO}_2^- + \text{O}_2 \rightarrow 2 \text{NO}_3^- \quad (4)$$

This step is carried out mainly by *Nitrobacter* species (e.g., *N. agilis*, *N. winogradski* and *Nitrobacter*) which are also autotrophic bacteria and are known as nitrite-oxidizer bacteria (NOB) (Bitton, 1999). The growth of *Nitrobacter* species is higher than *Nitrosomonas* species thus, the rate-limiting step in nitrification is the conversion of (NH$_3$) to (NO$_2^-$) by *Nitrosomonas* (Bitton, 1999).

### 2.2.2 Microbiology of Nitrification

The oxidation of (NH$_3$) to (NO$_3^-$) by microorganisms is essential for cultivation of field plants and for nitrogen cycle (Barton, 2005). The process of nitrification is ecologically relevant because of its role in determining the fate of nitrogen in any environment (Staley et al., 2002). Most nitrifying bacteria belong to *Proteobacteria* which is a vast phylum including many of gram-negative species. Nitrifying bacteria are chemoautotrophs, which are divided to AOB and NOB. The AOB include *Nitrosomanas*, *Nitrosospia*, and *Nitrosocoxus* the first two genera belong to β-*Proteobacteria* and the third is belonging to γ-*Proteobacteria*. On other hand, the NOB includes *Nitrobacter*, which belongs to α-*Proteobacteria*, and *Nitrococcus*, which belongs to γ-*Proteobacteria*. Most studies on nitrifying bacteria were conducted mainly on *Nitrosomanas* species as AOB bacteria and *Nitrobacter* species as NOB bacteria. In addition, there is another NBO species known as *Nitrospira*, which belongs to *Xenobacteria*. These groups were discovered over the past
few years as new species that can perform nitrification in the environment (Barton, 2005).

Several methods are used in investigating nitrifying bacterial populations in situ. The most popular method is cultivation analysis. Cultivation sometimes leads to unreadable results since nitrifying bacteria are slow grower and large numbers of them are uncultivable. Measurements of potential nitrifying activities by the $^{14}$C method are used to estimate the biomass but do not identify the bacterial populations. However, over the past few years, molecular techniques have proved to be best techniques in studying these bacteria. The advantage of molecular techniques is the ability of assessing the population of interests without requiring culturing (Cebron et al., 2003).

2.2.3 Factors Effecting Nitrification

There are several factors that control nitrification:

1- Ammonia / nitrite concentration, which affect the growth of bacteria (Barton, 2005). In practice, ammonia oxidation is the limiting factor, this means that nitrite can appear in high amounts when the process is non-stationary (Henze et al., 1997).

2- Dissolved Oxygen (DO): Oxygen serves as electron acceptor in the nitrification process and nitrification consumes large amounts of oxygen also, oxygen should be sufficient and well distributed in the environment (Barton, 2005).

3- pH / Alkalinity: optimum pH for nitrification is between 8-9. It is well know that free ammonia, NH$_3$ and HNO$_2$ can inhibit both Nitrosomonas and Nitrobacter (Henze et al., 1997).
4- Temperature: The rate of nitrification is greatly influenced by temperature. As the temperature increases, the nitrification rate increases. Temperatures between 20°C and 35°C enhance nitrification. Nitrification slows down dramatically or may stop altogether at around 5°C.

5- Presence of Toxins: Nitrifying bacteria are more sensitive to inhibitory compounds such as heavy metals than the BOD reducing bacteria (Barton, 2005). It had been found that in some treatment plants, nitrification could be stopped completely due to the presence of inhibiting substance (Henze et al., 1997).

2.3 Denitrification

In Denitrification, nitrate reducing bacteria serves as an electron acceptor in anoxic respiration due to the absence of O₂ in the system as shown in the following equation:

\[ 2\text{NO}_3^- + 10\text{e}^- + 12\text{H}^+ \rightarrow \text{N}_2 + 6\text{H}_2\text{O} \]  

(5)

In denitrification, organic compounds are used as electron and carbon as sources by the microorganisms, which are known as denitrifying microorganisms. Most of the identified denitrifying bacteria belong to the *Proteobacteria* (Staley et al., 2002).

2.3.1 Microbiology of Denitrification

The denitrification process is carried out by a diversity of bacteria belonging taxonomically to the various subclasses of the *Proteobacteria*. Denitrification also extends beyond the bacteria to the archaea, where it is found among the halophilic and hyper thermophilic branches of this kingdom and may have evolutionary significance. Intriguingly, the NO and N₂O utilizing enzymes share structural elements with certain terminal oxidases of the aerobic respiratory chain. An entirely new development is the
recognition of the core enzymes of denitrification in the mitochondria of certain fungi (Kobayashi et al. 1996). Bacteria capable of denitrification are both heterotrophic and autotrophic. The heterotrophic include the following genera: Achromobacter, Acinetobacter, Agrobacterium, Alcaligenes, Arthrobacter, Bacillus, Chromobacterium, Corynebacterium, Flavobacterium, hypomicrobium, Moraxella, Neisseria, Paracoccus, Propionibacterium, Pseudomonas: -Rhizobium, Rhodospseudomonas, Spirillum, and Vibrio. Autotrophic denitrifying bacteria includes Thiobacillus denitrificans and Thiomicropira denitrificans (Brettar et al., 2006) Most of the denitrification bacteria are facultative aerobic organisms, which allow them to consume oxygen in absence of nitrate or nitrite as electron acceptor (Metcalf and Eddy, 2003).

2.3.2 Denitrification Stoichiometry

Biological denitrification involves the biological oxidation of many organic substrates in wastewater treatment using nitrate or nitrite as the electron acceptor instead of oxygen. In the absence or under limited DO concentrations, the nitrate reductase enzyme in the electron transport respiratory chain is induced, and helps to transfer hydrogen and electrons to nitrate as the terminal electron acceptor. The nitrate reduction reactions involve the following reduction steps from nitrate to nitrite, to nitric oxide, to nitrous oxide, and to nitrogen gas shown in the following equation (Metcalf & eddy, 2003)

\[
\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2
\]  

Barth et al. (1968) found that 4 g of BOD is needed to reduce 1 gram of \(\text{NO}_3^-\). In general, the real value of BOD need to degrade \(\text{NO}_3^-\) varies depending on: the system operating conditions, the type of electron donor used for denitrification and the denitrifying
microorganisms involved. At the steady state, the reaction stoichiometry using external carbon source as electron donor is as follows:

\[
C_{10}H_{19}O_3N + 10\text{NO}_3^- \rightarrow 5\text{N}_2 + 10\text{CO}_2 + 3\text{H}_2\text{O} + \text{NH}_3 + 10\text{OH} \quad (7)
\]

This compound is usually used as the biodegradable organic matter in wastewater. Most of wastewater treatment plants use Methanol as external carbon source due to economic reasons in denitrification process as shown in the following equation

\[
5\text{CH}_3\text{OH} + 6\text{NO}_3^- \rightarrow 3\text{N}_2 + 5\text{CO}_2 + 7\text{H}_2\text{O} + 6\text{OH} \quad (8)
\]

Other compounds can be used in denitrification process includes Acetate, ethanol, and methane. The stoichiometry of Acetate in denitrification process is represented by:

\[
5\text{CH}_3\text{COOH} + 8\text{NO}_3^- \rightarrow 4\text{N}_2 + 10\text{CO}_2 + 6\text{H}_2\text{O} + 8\text{OH} \quad (9)
\]

For cell synthesis, the biodegradable soluble Chemical Oxygen Demand (bsCOD \text{syn}) is calculated from the net biomass yield and the ratio of 1.42 g O2/g YSS. The oxygen equivalent of the biomass is equal to the bsCOD incorporated into biomass (Metcalf & eddy, 2003).

### 2.3.3 Factors Effecting Denitrification

There are several factors that control denitrification process:

1- pH / Alkalinity: optimum pH for denitrification is around 7-9 with a variation of the local conditions (Henze et al., 1997).

2- Dissolved Oxygen (DO): it is well known that oxygen inhibits denitrification since it became the electron acceptor in the system. The effect of oxygen can be evaluated using the following expression:

\[
K_d = \frac{K_{s,O_2(NO_3^-)}}{K_{s,O_2(NO_3^-)} + S_{O_2}} \quad (10)
\]

where

- \(K_{s,O_2(NO_3^-)}\) The saturation constant of oxygen inhibition
The oxygen concentration in the liquid phase (Henze et al., 1997).

3- Temperature: the change of temperature effect microbial growth, which will lead to the change in the rate of denitrification process; considering the effect of temperature can be calculated using the following equation

\[ K_d \text{ at } 20^\circ C = K_d \text{ at } T \theta \] (20-T) \hspace{1cm} (11)

where: \( K_d \) denitrification rate

\( T \) temperature

\( \theta \) temperature coefficient (Metcalf and eddy, 2003)

4- Carbon source: wide range of chemical includes natural, synthetic compound and even inorganic compounds. It is well known that organic compounds such as Methanol and Acetate are wildly used as carbon source (Henze et al., 1997).

2.3.4 Carbon Source in Denitrification

The addition of external carbon source helps in increasing denitrification rates as well enhances the nitrogen removal. This addition of external carbon source to the denitrification process is required if the influent COD/N ratio is not sufficient or if effluent TN requirement is really less than 3mg/L. The most common carbon source is Methanol due to its availability and its economic efficiency (Zayed and Winter, 1998) and for to the low yield and low COD/N needed, however the use of Methanol applies on risks since it is inflammable and neurotoxic solvent. Other studies have proposed the use of other carbon sources such as glucose, ethanol, Acetate and methane (Sage et al., 2006). Sage el al. in (2006) designed a predictive model to study the denitrification potential and rates performance using different carbon sources based on compositional data. The experiment varied from using single carbon source like components (lactose, lactate,
proteins, fat) in some test to a complex mixtures (modeled “process water”) in other tests. The experiment showed high potential data in the case of using the mixture when it compared to the single carbon source. Results of denitrification rate showed similarity in both cases (Sage et al., 2006).

Dos Santos et al., (2004) used three electron donor sources: Methanol, ethanol, and methane to measure denitrification rate. The denitrification rates estimated from the three carbon sources indicated that a complete removal of NO$_2^-$ and NO$_3^-$ was accomplished. Measurement of the denitrification rates were estimated in two phases, the first phase was after 2 months while the second phase was after 4 months. The variation was in the time of complete denitrification process. Biased on this research Methane was the fastest compound to complete the process within 50 minutes with denitrification rates of 0.06±0.002 and 0.09±0.0001 mgN/gVSS-h for the first and the second phase, respectively. On the other hand, Methanol and Methane achieved the process within 120 and 315 minutes with rates of (0.07±0.009 and 0.10±0.004), (0.23±0.04 and 0.47±0.03) mgN/gVSS-h, respectively (Dos Santos et al., 2004).

### 2.4 Perchlorate

#### 2.4.1 Chemistry of Perchlorate

Perchlorate (ClO$_4^-$) is a highly oxidized (+7) chlorine oxy-anion (Logan, 2001). The ClO$_4^-$ molecular structure consisted of the single chlorine atom in the center of a tetrahedral banded to four atoms of oxygen. This structure allows the negative charge to appear anywhere of the molecular as well to spread between the four oxygen atoms in even way. This dispersion gives ClO$_4^-$ the ability to bind to positive charge metallic centers. ClO$_4^-$ could be found in the forms of perchloric acid (HClO$_4$) and salts such as
ammonium perchlorate (NH₄ClO₄), potassium perchlorate (KClO₄), and sodium perchlorate (NaClO₄) (McCarty and Meyer, 2005). Chemical characteristics of ClO₄⁻ include high solubility and mobility and stability in the environment. Due to these characteristics, ClO₄⁻ is capable to form long contaminants in aquatic surfaces. On the other hand, ClO₄⁻ dose not have any activity of interest in soil surface chemistry. Harmful effect of ClO₄⁻ includes the un normal production of hormones by the thyroid gland, having potential for carcinogenic, neurodevelopment, reproductive, and immune toxic effects (Urban Sky and Schlock 1999). Major uses includes as rocket fuels, flares, fireworks, automotive air bags, matches, batteries, and analytical chemistry (McCarty and Meyer, 2005). ClO₄⁻ in surface and groundwater has become an ever-increasing water quality concern (Tan et al., 2003). On 1998, ClO₄⁻ was added to the drinking water contaminated list by the United States environmental protection agency (Urban sky, 2000). ClO₄⁻ can be traced in the environment using many methods including ion-selective electrodes, ion chromatography, capillary electrophoresis, High-performance liquid chromatography (HPLC), and spectrophotometry (Urban Sky, 2000). Degradation can occur chemically or naturally. Bacteria capable of perchlorate degradation are widely distributed in natural systems, and it is possible to develop acclimated cultures for ClO₄⁻ reduction using a variety of inocula (Coates et al., 1999).

Early studies on bacterial degradation of ClO₄⁻ were resulted from chlorate reduction. Most of these studies indicated that chlorate (ClO₃⁻) was degraded to chlorite (ClO₂⁻) by one strain of Escherichia coli without further reduction of ClO₂⁻. The failure of complete ClO₃⁻ respiration were due to the toxic effects of c ClO₂⁻ and an absence of the enzyme chlorite dismutase. In the following years, studies found the ability of some bacteria to
convert $\text{ClO}_3^-$ to chloride ($\text{Cl}^-$). One of the earliest studies on reduction of $\text{ClO}_4^-$ to $\text{Cl}^-$ by heterotrophic bacteria was first identified by Hackenthal in (1964) by using a $\text{Cl}^-$ labeled. In his study, Hackenthal proposed the first pathway equation (12) of $\text{ClO}_4^-$ degradation obtained from nitrate-adapted cells of *Bacillus cereus* (Rikken et al., 1996), as follows:

$$\text{ClO}_4^- \rightarrow \text{ClO}_3^- \rightarrow \text{ClO}_2^- \rightarrow \text{Cl}_2 \text{O}_2 \rightarrow \text{Cl}_2$$  \hspace{1cm} (12)

The reduction of $\text{ClO}_4^-$ or $\text{ClO}_3^-$ to $\text{Cl}^-$ by bacteria was subsequently confirmed by other researchers. Results of these hypothesized pathways did not make any further progress until the purification of a new enzyme called chlorite dismutase from the Perchlorate Respiring Bacteria (PRB) strain GR-1 by (Rikken et al., 1996). This study found that oxygen was produced from $\text{ClO}_2^-$ (Rikken et al., 1996). The study proposed a three-step mechanism of perchlorate reduction in which $\text{ClO}_3^-$, $\text{ClO}_2^-$, and dissolved oxygen were sequentially produced:

$$\text{ClO}_4^- \rightarrow \text{ClO}_3^- \rightarrow \text{ClO}_2^- \rightarrow \text{O}_2 + \text{Cl}_2$$  \hspace{1cm} (13)  \hspace{1cm} (Rikken et al., 1996).

### 2.4.2 Perchlorate Removal

Most of $\text{ClO}_4^-$ studies focused on its removal from groundwater since it concentrated in high levels. Xu et al. in (2004) observed the ability of *Dechlorosoma* sp. KJ, which is known as denitrifies bacteria, to stimulate $\text{ClO}_4^-$ respiratory in the presence of $\text{ClO}_3^-$ or $\text{ClO}_4^-$ in the media. This bacterium were able to grow in an aerobic medium contained only $\text{ClO}_3^-$ or $\text{ClO}_4^-$ or $\text{NO}_3^-$ despite a lag time of 0.3 to 2 days while there were no lag time in the case of using of identical media. Result of this study showed that 10% of $\text{NO}_3^-$ reduction happened when bacteria were grown using $\text{ClO}_3^-$ or $\text{ClO}_4^-$. As well, a small degradation of $\text{ClO}_3^-$ or $\text{ClO}_4^-$ was gained from grown the bacteria on medium contained
NO$_3^-$; Same results obtained when cell extract with methyl violgen used as electron carrier. The main result obtained from this study was that ClO$_4^-$ degradation enzyme was expressed in a separated way from NO$_3^-$ degradation enzyme. This assumption came from the ability of strain KJ to degrade ClO$_4^-$ in a better way when it was pre grown on NO$_3^-$ and ClO$_4^-$ rather than growing it only of ClO$_4^-$; This lead to hypothesize that NO$_3^-$ in groundwater would increase ClO$_4^-$ degradation rate. On the other hand, the study found that there were no sign of ClO$_4^-$ degradation in the case of using Pseudomonas sp. PDA (Xu, 2004).

Min et al., (2004) designed two side-by-side pilot-scale fixed-bed bioreactors packed with sand or plastic media and perchlorate-degrading bacterium Dechlorosoma sp. KJ. The system was used to treat groundwater contained ClO$_4^-$ (77 µg/L), NO$_3^-$ (4 mg/L), and O$_2$ (7.5 mg/L). Acetic acid as external and nutrients were also added to the system. ClO$_4^-$ was removed to levels less than 4 µg/L in the sand and plastic medium bioreactors at flow rates of 0.063–0.126 L/s and 0.063 L/s, respectively. Acetate in the sand reactor was decreased from 43±8 to 13±8 mg/L after day 100, and ClO$_4^-$ was completely removed from the system. Detention times to complete ClO$_4^-$ removal were more efficient in mixed cultures (10–18 min) than those of the pure culture (1 min) reported in previous studies. Analysis of intra-column ClO$_4^-$ indicated that removal of dissolved O$_2$ and NO$_3^-$, were completed prior to complete ClO$_4^-$ degradation. This study demonstrated for the first time in a pilot-scale system, that with regular backwashing cycles, fixed-bed bioreactors could be used to remove perchlorate in groundwater to a suitable level for drinking water (Min et al., 2004).
3 Material and Methods

3.1 Carbon Sources

Three SBR systems were designed to acclimate sludge using three different carbon sources: Methanol, Acetate and MicroC™. Methanol is selected because most of the wastewater plants that operate denitrification process use Methanol and has been recommended as the most efficient carbon source in wastewater treatment, primarily because it is the economic solution. On the other hand, the use of Methanol is associated with risk because it is flammable. Acetate is selected because it is a good chemical for this process and can serve for comparisons. MicroC™ is selected because it is a new chemical compound with an unidentified chemical formula designed by Environmental Operation Solution Inc. to enhance the denitrification process. A brief description of the three carbon sources is provided below.

3.1.1 MicroC™

MicroC™ is a new liquid chemical that can be used in wastewater treatment and was developed by Environmental Operating Solutions, Inc.² as electron/carbon source in wastewater plants that uses denitrification process. MicroC™ is produced from renewable agricultural products that are abundant in the United States. MicroC™ is a light blue/green color with a mild alcohol odor. It has a specific gravity of 1.18 at 25°C, a density of 9.84 lbs/gal, pH value between 5.3-5.8 and has viscosity of 16.4 centipoises. MicroC™ is 100% soluble in water and has a COD value of 670,000 mg/L.³

² http://www.eosenvironmental.com/product/index.htm
³ http://www.eosenvironmental.com/docs/MicroC-technicalspecifications.pdf
The development of MicroCTM was driven by the following (a) the shortcomings of existing carbon sources in terms of safety, cost and environmental sustainability; and (b) the trend among communities and regulators to limit nitrogen pollution by placing increasingly stringent nitrogen limits on wastewater treatment plants. MicroCTM is becoming a valid carbon source through the largest technology manufacturers in the industry and commercial use at a large number of wastewater treatment facilities due to:

- MicroCTM derived from renewable agricultural products, which gives him, has environmental advantages when compared with other electron donor/carbon sources.
- MicroCTM is far less energy-intensive than Methanol and results in an overall energy savings of 72,000 BTU for each gallon of Methanol replaced by MicroCTM.
- MicroCTM allows manufacturers to extend denitrification capabilities to smaller systems, reducing the overall nitrogen pollution contributed by these small wastewater treatment systems.

### 3.1.2 Methanol

Methanol is the simplest alcohol, also known as methyl alcohol, carbinol, and wood alcohol. Methanol was originally produced by the destructive distillation of wood chips in the absence of air. Today, Methanol is synthesized by a catalytic reaction equation (14) of carbon monoxide with hydrogen in a high temperatures and pressure environment (Wade, 1999),

\[
\begin{align*}
300-400 \, ^\circ C, 200-300 \, atm \, H_2 \\
CO + 2H_2 & \longrightarrow \text{CH}_3\text{OH} \\
\text{ZnO-Cr}_2\text{O}_3
\end{align*}
\]

---

Methanol has a melting point of -97 °C, boiling point of 65 °C and relative density of 0.8. Methanol is a colorless volatile liquid with a faintly sweet pungent odor similar to ethyl alcohol. It is soluble in water. Methanol is listed as a “Poison-Class B”, and is harmful if swallowed or absorbed through the skin. Ingestion to human body can cause irreversible injury to the nervous system, blindness, or death. It causes eye and respiratory system irritation and may cause skin irritation. Methanol is also a good fuel for internal combustion, is an important industrial material, is used in the manufacturing of formaldehyde, as a solvent in the paint and varnish industry, and as an anti-Freeze in car radiators (Wade, 1999).

Methanol is the common chemical that is used as carbon source due its cost effectiveness, because of its low cost and availability on large quantities and for its favorable yield less (the sludge production). On the other hand, using Methanol in denitrification process can be dangerous because it is volatile, inflammable and neurotoxic chemical.

### 3.1.3 Acetate

Acetate is the most common oxidized fermentation product that links between fermentative organic and syntrophs and sink organisms (Lewandowski and DeFilippi, 1998). The Acetate anion (CH$_3$COO$^-$) is a carboxylate and is the conjugate base of acetic acid. Acetate ion is formed by deportation of acetic acid:

\[
\text{CH}_3\text{COOH} \rightarrow \text{CH}_3\text{COO}^- + \text{H}^+ \quad (15)
\]

Acetate is found in many forms such as sodium Acetate, ethyl Acetate, trenbolone Acetate, megestrol Acetate, calcium Acetate. Sodium Acetate (CH$_3$COONa) is the salt of acetic acid and usually found as sodium Acetate trihydrate or Sodium Acetate Anhydrous. Sodium Acetate is a white crystal, odorless or faint acetous odor. Acetate has
been used as external carbon source for wastewater treatment due its availability and comparable cost.

### 3.2 Sequencing Batch Reactor (SBR) System Design

#### 3.2.1 Overview

As part of the continuous processes for the biological treatment of wastewater, organic matter oxidation, as well as liquid and solid phase separation reactions are usually carried out in two separate tanks: the aeration tank and the clarifier. However, these two processes can also be carried out efficiently in a single reactor such as sequencing batch reactors (SBR) (Dagot, 2001). The SBR is a fill-and-draw activated sludge technology with a timed sequence of processes (phases) (Figure 2) that take place in the same tank. Its advantage over other techniques includes the operational flexibility, which allows the adjustment of the duration of one single phase (aeration, settling, etc.), depending on the influent characteristics (flow and concentration) (Spagni, 2007). The treatment cycle can be adjusted to undergo aerobic, anaerobic, and anoxic conditions in order to achieve BNR, including nitrification, denitrification, and some phosphorus removal (Al-Rekabi, 2007).

1. **Fill**: during the fill operation, volume and substrate are added to the reactor. During this phase, the reactor may be only mixed or mixed and aerated to activate the biological reactions. The feeding time for the three reactors was 27 minutes.

2. **React**: in this phase, the biomass consumes the substrate under controlled environmental conditions. The react time consisted of anoxic phase, which lasted for two hours, and aerobic phase, which lasted for four hours.
3. Settle: this phase allows the solids to be separated from the liquid under quiescent conditions, resulting into an effluent supernatant (clarified). Settling time lasted for one hour and ten minutes.

4. Draw (Decant): the clarified effluent is removed in this phase. The draw phase lasted for 10 minutes.

5. Idle: this phase used when a multitank system is used which provide time to the reactor to complete one cycle before starting a second one. The idle time in this experiment lasted for 10 minutes.

![Figure 2. SBR reactor scheme](image)

**3.2.2 System Design**

The system in this study consists of three SBR setups as shown in Figure 3. Each setup is developed to use MicroC™, Methanol or Acetate as a carbon source.
The Details of the operation of three SBR systems is described Figure 4. Each setup in the system consists of a six litters container with a mixer fixed at the top rotating at 34 rpm. The setup is connected to an aeration system, which is activated by a pump, tubing and perusing air by air stone located at the end of the rigid tubing, used to enhance the air bubbling diffusion. Two separated peristaltic pumps were used as a feeding influent and supernatant discharge effluent.
Figure 4. A schematic of the SBR system components

All three systems were connected to a timer, with the previously described devices that control and activate the influent, effluent, mixing and aeration system using different circuits. The influent pump was in circuit one, the effluent pump was in circuit two, the aeration pump was in circuit three and the mixing pump was in circuit four.

### 3.2.3 Influent Properties

The SBR Influent for the three reactors was synthetic wastewater that contained all the necessary macro and microelements (summarized in Table 1) to support the microbial activity. Macro elements were weighed then added directly to the feeding. The microelements (summarized in Table 2) were prepared in a solution and were added to the feeding. As described earlier, the difference between the three SBR systems was the carbon sources, which are MicroC™, Methanol, and Acetate. All the three solutions were
prepared daily and stored in three different ten liters containers, one for each carbon sources used, and connected with a small diameter tube to the pumps and to the reactors. Three different bottles were be also used to store the effluent after drawing.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon source</td>
<td>300 as COD</td>
</tr>
<tr>
<td>MgSO₄ * 7H₂O</td>
<td>40</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>7.5</td>
</tr>
<tr>
<td>Fe(SO₄) * 7H₂O</td>
<td>1</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>220</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>560</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>40</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>30</td>
</tr>
<tr>
<td>MnSO₄ * 4H₂O</td>
<td>0.2</td>
</tr>
<tr>
<td>Trace element addition</td>
<td>8ml / 10 l</td>
</tr>
</tbody>
</table>
Table 2. Microelements used in the feeding

<table>
<thead>
<tr>
<th>Trace element solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>453 mg/l</td>
</tr>
<tr>
<td>62.5 mg/l</td>
</tr>
<tr>
<td>710 mg/l</td>
</tr>
<tr>
<td>550 mg/l</td>
</tr>
<tr>
<td>20 mg/l</td>
</tr>
<tr>
<td>26 mg/l</td>
</tr>
</tbody>
</table>

The Hydraulic Retention Time (HRT) for both of the anoxic and aerobic phases were found as shown in the following equations:

\[
HRT_{(\text{ox})} = \frac{V_{\text{ox}}}{Q} = \frac{4200 \text{ m}^3}{6600 \text{ m}^3 \text{ day}} = 15.27 \text{ h}
\]  \hspace{1cm} (16)

\[
HRT_{(\text{anox})} = \frac{V_{\text{anox}}}{Q} = \frac{1300 \text{ m}^3}{6600 \text{ m}^3 \text{ day}} = 4.73 \text{ h}
\]  \hspace{1cm} (17)

Based on this data, a 15 h HRT for the aerobic phase is assumed for the SBR systems, and considering the volume is six liters, the daily influent flow was:

\[
Q_{SBR} = \frac{V_{SBR}}{HRT_{\text{ox}}} = \frac{6}{15} = 0.4 \text{ l day} \approx 9 \text{ l day}
\]  \hspace{1cm} (18)

Considering three daily cycles:

\[
Q_{SBR} \approx 9 \text{ l day} = 3 \frac{\text{l}}{\text{cycle}}
\]  \hspace{1cm} (19)

The initial volume of the SBR is consequently calculated:
Feeding phase was done in 56 minutes with a speed of 60 rpm in order to avoid sudden variations in the reactors conditions. Usually in wastewater treatment plants it is opportune to have the sludge waste phase after the reaction step to easily calculate the Sludge Retention Time (SRT), the following assumptions should be used to calculate SRT in SBR design:

- \( x = 2200 \text{ mgSS/l} \) is the biomass concentration into the reactor;
- \( x_r = 3300 \text{ mgSS/l} \) is the biomass concentration in the return activated sludge line;
- \( Q_s = 8 \text{ mc/h} \) is the flow of the wasted sludge.

The SRT can be calculated as follow:

\[
SRT = \frac{x \cdot V_s}{Q_s \cdot x_r} = \frac{2200 \cdot 4200}{8 \cdot 3300} = 357h = 14.8d \approx 15d
\]

(22)

In addition, the sludge wasting was operated manually only once a day, with the following flow:

\[
Q_s = \frac{V}{SRT} = \frac{6}{15} = 0.4 \text{ l/day}
\]

(23)

The time used for wasting was two minutes and 200 ml of sludge were wasted for each minute.

The duration of anoxic and aerobic phases was calculated based on the volumetric ratio between anoxic and aerobic reactors.

\[
\frac{V_{anox}}{V_{ox}} = \frac{1300m^3}{4200m^3} = 0.31
\]

(24)
To establish for the duration for the aerobic and the anoxic phases the following assumptions should be used $V_{\text{anox}} \approx 31\% V_{\text{ox}}$ in previous conclusions. The aerobic phase was run for four hours and thirty minutes and consequently the duration of the anoxic phase was:

$$t_{\text{anox}} \approx 31\% t_{\text{ox}} \Rightarrow t_{\text{anox}} = 0.31 \times 270 \text{ min} = 83.7 \text{ min} = 1\text{h}24' \quad (25)$$

This time was increased to two hours so that all the six liters of sludge were in anoxic conditions at least one hour. The settling phase was fixed to one hour and ten minutes for the three liters while the effluent discharge was for thirteen minutes each cycle. Ten minutes of idle phase were provided to prepare the systems for the next cycle. The partition of the five different phases and their duration are summarized in Table 3 and Table 4.

### Table 3. Starting and ending time of each cycle

<table>
<thead>
<tr>
<th>Cycle</th>
<th>START</th>
<th>END</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st cycle</td>
<td>9:30 AM</td>
<td>5:30 PM</td>
</tr>
<tr>
<td>2nd cycle</td>
<td>5:30 PM</td>
<td>1:30 AM</td>
</tr>
<tr>
<td>3rd cycle</td>
<td>1:30 AM</td>
<td>9:30 AM</td>
</tr>
</tbody>
</table>

The SBR systems using the different carbon sources were run for 1 year. During that time microbiological studies on the three SBR system populations were successfully conducted. Samples of the daily wasted sludge were used to measure the amount of Unbiodegradable Soluble COD (USCOD) in order to confirm the Rapidly Biodegradable COD (RBCOD) value.
In the last 10 minutes of the aerobic phase, the oxygen aeration was completely stopped to allow the nitrifying bacteria to consume all of the remaining oxygen, ensuring an anoxic condition for next cycle. During the monitoring stage, there were some difficulties controlling the pH in the MicroC™ reactor due to the appearance of filamentous bacteria. This problem was solved by adding some drops of NaOH (2N) to increase the pH to approximately 7.5 and increase the amount of sodium bicarbonate.

**Table 4 Phases and duration in every single phase.**

<table>
<thead>
<tr>
<th>FROM</th>
<th>TO</th>
<th>CYCLE</th>
<th>PHASE</th>
<th>CIRCUIT ON</th>
<th>CIRCUIT ON</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:30 AM</td>
<td>10:26 AM</td>
<td>1st</td>
<td>Anoxic + Feeding</td>
<td>Mixer + Influent Pump</td>
<td>1</td>
</tr>
<tr>
<td>10:26 AM</td>
<td>11:30 AM</td>
<td>1st</td>
<td>Anoxic</td>
<td>Mixer</td>
<td>4</td>
</tr>
<tr>
<td>11:30 AM</td>
<td>3:58 PM</td>
<td>1st</td>
<td>Aerobic</td>
<td>Mixer + Aeration</td>
<td>3</td>
</tr>
<tr>
<td>3:58 PM</td>
<td>4:00 PM</td>
<td>1st</td>
<td>Aerobic + Sludge wasting</td>
<td>Mixer + Aeration</td>
<td>3</td>
</tr>
<tr>
<td>4:00 PM</td>
<td>5:07 PM</td>
<td>1st</td>
<td>Settling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5:07 PM</td>
<td>5:20 PM</td>
<td>1st</td>
<td>Supernatant drawing</td>
<td>Effluent pump</td>
<td>2</td>
</tr>
<tr>
<td>5:20 PM</td>
<td>5:30 PM</td>
<td>1st</td>
<td>Pause</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5:30 PM</td>
<td>6:26 PM</td>
<td>2nd</td>
<td>Anoxic + Feeding</td>
<td>Mixer + Influent Pump</td>
<td>1</td>
</tr>
<tr>
<td>6:26 PM</td>
<td>7:30 PM</td>
<td>2nd</td>
<td>Anoxic</td>
<td>Mixer</td>
<td>4</td>
</tr>
<tr>
<td>7:30 PM</td>
<td>12:00 AM</td>
<td>2nd</td>
<td>Aerobic</td>
<td>Mixer + Aeration</td>
<td>3</td>
</tr>
<tr>
<td>12:00 AM</td>
<td>1:07 AM</td>
<td>2nd</td>
<td>Settling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:07 AM</td>
<td>1:20 AM</td>
<td>2nd</td>
<td>Supernatant drawing</td>
<td>Effluent pump</td>
<td>2</td>
</tr>
<tr>
<td>1:20 AM</td>
<td>1:30 AM</td>
<td>2nd</td>
<td>Pause</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:30 AM</td>
<td>2:26 AM</td>
<td>3rd</td>
<td>Anoxic + Feeding</td>
<td>Mixer + Influent Pump</td>
<td>1</td>
</tr>
<tr>
<td>2:26 AM</td>
<td>3:30 AM</td>
<td>3rd</td>
<td>Anoxic</td>
<td>Mixer</td>
<td>4</td>
</tr>
<tr>
<td>3:30 AM</td>
<td>8:00 AM</td>
<td>3rd</td>
<td>Aerobic</td>
<td>Mixer + Aeration</td>
<td>3</td>
</tr>
<tr>
<td>8:00 AM</td>
<td>9:07 AM</td>
<td>3rd</td>
<td>Settling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:07 AM</td>
<td>9:20 AM</td>
<td>3rd</td>
<td>Supernatant drawing</td>
<td>Effluent pump</td>
<td>2</td>
</tr>
<tr>
<td>9:20 AM</td>
<td>9:30 AM</td>
<td>3rd</td>
<td>Pause</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.3 Denitrification Evaluation

Once the acclimated sludges were developed, they were then used in batch tests to evaluate denitrification kinetics under different environmental conditions. A series of denitrification batch tests were used to measure the denitrification rates using the sludges from three SBR systems, using MicroC™, Methanol and Acetate as carbon sources. The denitrification rates and kinetics of the denitrification process with different carbon sources was studied at two different temperatures at (10 and 20 °C).

3.3.1 Denitrification Kinetics Batch Testing

All denitrification kinetic tests were run using the same batch system as shown in Figure 5. The setting of the batch consisted of 1.5 L of sludge with a mixing device to ensure the homogeneous conditions. The carbon source was added into the reactor in order to provide different sCOD concentration for denitrification process and a known amount of Potassium Nitrate solution (5gN/l) was added to obtain an adequate concentration of NO₃⁻-N. Samples were taken over time with a 15 ml syringe and pre-filtered through a 100 µm filter paper, and after that filtered with 0.45 µm syringe filters. The concentrations of NO₂⁻, NO₃⁻ and COD have been analyzed over the time of testing. Other than the above constituents, pH, temperature and biomass concentration (TSS, VSS) were also monitored at the end of the test. Nitrogen gas and parafilm were used to cover the top of the reactor to prevent oxygen transfer through the surface and to reach anoxic conditions. The pH was checked continually and regulated at 7.5 throughout the test by adding NaOH (1M) or HCl (1M).
Figure 5. Denitrification batch testing set up

Figure 6 shows the profile of NO$_3^-$ and NO$_2^-$ obtained during the test. The analysis of the NO$_3^-$ and NO$_2^-$ has obtained using Ion Chromatography (IC). When NO$_2^-$ was present, NOx calculation was calculated as:

$$\text{NOx} - N = \text{NO}_3^- + 0.6 \text{ NO}_2$$ (26)

The time profile of the NOx was used to determine the denitrification rate using the following equation:

$$\kappa d = \frac{(M \times 60)}{V_{ss}}$$

$$\frac{1000}{(1000)}$$ (27)

Where

$M_1$ the slope of NOx time profile (in the example: -0.0.0639).
Temperature is considered one of the environmental factors that affect denitrification. We evaluated the denitrification rates and kinetics at both 10°C and 20°C using the three acclimated sludges.

### 3.3.2 Measurement of Denitrification Kinetics at 10°C

The batch test, which was described in 3.3.1, was used to measure denitrification kinetics at 10°C, consisted of 1.5 L of sludge from the three SBR systems was used for each test.
A known amounts of carbon source and Potassium Nitrate (KNO₃) (5g/l) were added into the reactor to provide initial concentrations of 220 mg/l and 20 mg/l as COD and NO₃⁻-N, respectively, for the denitrification process.

3.3.3 Measurement of Denitrification Kinetics 20°C

The batch test, which was described in 3.3.1, was used to measure denitrification kinetics at 20°C, consisted of 1.5 L of sludge from the three SBR systems was used for the tests. A known amount of carbon source and KNO₃ (5g/l) were added into the reactor to provide initial concentrations of 220 mg/l and 20 mg/l as COD and NO₃⁻-N, respectively, for the denitrification process.

3.3.4 Measurement of Denitrification Kinetics Influence of Carbon Source

The batch test, which was described in 3.2.2, was used to measure the denitrification kinetics. The types of carbon sources include Micro C, Methanol, Acetate, glucose and ethanol. Addition of the carbon source and KNO₃ (5g/l) to the reactors provided initial concentrations of 220 mg/l and 20 mg/l as COD and NO₃⁻-N, respectively, for the denitrification process.

3.4 Perchlorate Removal

Due to the increasing concern in ClO₄⁻ removal from wastewater, the efficiency of the biousers grown on the SBR reactors described in section (3.2.2) in removing ClO₄⁻ were studied by two test experiments. The targets of these trials were to determine the reduction rate of ClO₄⁻ using different concentration of ClO₄⁻ with the three sludges and to determine the reduction rate in the presence of NO₃⁻.
3.4.1 Experiment 1

In this experiment, the acclimated sludge from the three SBR systems were used to remove a known concentration of $\mathrm{ClO}_4^-$ as described in Table 5. The protocol was as follows: a 1 L of mixed liquor sludge was collected from each setup and then centrifuged at 5000 rpm for five minutes. The sludge was then re-suspended in 150 ml of growth media, and poured in a serum bottle for testing so that the final concentration of VSS in the bottles was approximately 500 mg/l. To ensure the proper mixing, all serum bottles were placed on a shaking plate at speed of 320 rpm throughout the duration of the test. The COD equivalent concentrations were injected into the bottles. After one hour, the $\mathrm{ClO}_4^-$ equivalent concentration was injected into the bottles. A 5 ml sample was taken with a syringe and immediately replaced with 5 ml of media and nitrogen gas, through pumping, to prevent an aerobic environment. Then the samples were filtered through 0.45 $\mu$m membrane filter. Samples were taken twice a day for the first 2 days, and then once a day for the next 2-3 weeks. All samples were stored in pre-labeled containers and kept at 4 °C until analysis (stable for about 1 month). At the end of the test, a 50 ml sample was taken from each bottle for TSS and VSS analysis, and another 100 ml was frozen and later use for DNA extraction. The controls were also used with autoclaved sludge to verify any observation of the $\mathrm{ClO}_4^-$ on the sludge.
### Table 5. Concentrations of COD & ClO$_4^-$ used in experiment 1 of ClO$_4^-$ removal

<table>
<thead>
<tr>
<th>TEST ID</th>
<th>INOCULUM</th>
<th>Electron donor (mg/l as COD)</th>
<th>Perchlorate (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>AS_SBR_Acetate</td>
<td>Acetate_50</td>
<td>10</td>
</tr>
<tr>
<td>B1</td>
<td>AS_SBR_Methanol</td>
<td>Methanol_50</td>
<td>10</td>
</tr>
<tr>
<td>C1</td>
<td>AS_SBR_MicroC™</td>
<td>MicroC™_50</td>
<td>10</td>
</tr>
<tr>
<td>Control_A</td>
<td>Autoclaved seed SBR Acetate</td>
<td>Acetate_50</td>
<td>10</td>
</tr>
<tr>
<td>Control_B</td>
<td>Autoclaved seed SBR Methanol</td>
<td>Methanol_50</td>
<td>10</td>
</tr>
<tr>
<td>Control_C</td>
<td>Autoclaved seed SBR MicroC™</td>
<td>MicroC™_50</td>
<td>10</td>
</tr>
<tr>
<td>Control_D</td>
<td>Medium</td>
<td>-</td>
<td>10</td>
</tr>
</tbody>
</table>

#### 3.4.2 Experiment 2

In this experiment, the acclimated sludge from the SBR systems was used to remove a known concentration of ClO$_4^-$ from wastewater in the presence of NO$_3^-$ as described in Table 6. The protocol was as follows: a 1 L of mixed liquor sludge was collected from each setup and then centrifuged at 5000 rpm for five minutes. The sludge was then re-suspended in 150 ml of growth media, and poured in a serum bottle for testing so that the final concentration of VSS in the bottles was approximately 500 mg/l. To ensure the proper mixing, all serum bottles were placed on a shaking plate at speed of 320 rpm throughout the duration of the test. The COD equivalent concentrations were injected into the bottles. After one hour, the ClO$_4^-$ and NO$_3^-$ equivalent concentrations were injected into the bottles. A 5 ml sample was taken with a syringe and immediately replaced nitrogen gas, through pumping, to prevent an aerobic environment. Then the samples were filtered through 0.45 µm membrane filter. Samples were taken four times a day.
for the first 2 days, then twice a day for the next two and once a day in days five and six. At the end of day, six a second dose of ClO$_4^-$ was spiked to the bottles and samples were taken for often days. All samples were stored in pre-labeled containers and kept at 4 °C until analysis (stable for about 1 month). At the end of the test, a 50 ml sample was taken each bottle for TSS and VSS analysis, and another 100 ml was frozen and later use for DNA extraction. The controls were also used with autoclaved sludge to verify any observation of the ClO$_4^-$ on the sludge.

**Table 6. Concentration of NO$_3^-$, ClO$_4^-$, and COD used in experiment 2 of ClO$_4^-$ removal.**

<table>
<thead>
<tr>
<th>TEST ID</th>
<th>INOCULUM</th>
<th>$e^-$donor (mg/l COD)</th>
<th>ClO$_4^-$ (mg/l)</th>
<th>NO$_3^-$ (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Acetate</td>
<td>Acetate_100</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>B1</td>
<td>Methanol</td>
<td>Methanol_100</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>C1</td>
<td>MicroC™</td>
<td>MicroC™_100</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Control_A</td>
<td>Autoclaved SBR Acetate</td>
<td>Acetate_100</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Control_B</td>
<td>Autoclaved seed SBR Methanol</td>
<td>Methanol_100</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Control_C</td>
<td>Autoclaved seed SBR MicroC™</td>
<td>MicroC™_100</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Control_D</td>
<td>Medium</td>
<td>-</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
4 Results and Discussion

4.1 Monitoring of the SBR Systems

In the first part of this study, MicroC™, Methanol and Acetate were used as external carbon sources in a denitrification process to remove nitrogen compounds from wastewater using the SBR system.

Monitoring of the three SBR systems was done before beginning any other experiment. The monitoring process took about 2 to 3 months to ensure that denitrification cultures are enriched with three carbon sources and the denitrification activities are stable.

4.1.1 MicroC™ SBR Monitoring

A series of monitoring tests were run, using MicroC™ as a carbon source, to measure the physical and the chemical parameters to evaluate the nitrogen removal. Since the nitrogen removal was operated in the SBR system using nitrification / denitrification process, the monitoring was to evaluate nitrogen consumption during one cycle. The chart in Figure 7 is an example of result of the nitrification/ denitrification process that was obtained from the monitoring of the MicroC™ SBR system. The chart can be divided into two separate phases: anoxic and aerobic.
During the anoxic phase, denitrified bacteria were activated to consume most of NO$_3^-$ in this phase, NO$_3^-$ became the electron acceptor with the absence of O$_2$ and reduced to N$_2$. Measurement of NO$_3^-$ in this example indicated that it was dropped from 4.58 mg/l to 0.35 mg/l within the first 120 minutes of the cycle. The denitrification rate ($K_D$) during this phase was 1.41 mgN/gVSS-h. In the aerobic phase, O$_2$ was pumped to the SBR through aeration allowing for the nitrification process to take place in the MicroC™ SBR.
system. Figure 4.1 showed the NH$_4^+$, which was added through the feeding, was dropped from 7.8 mg/l to about 0.015 mg/l. On the other hand, due to decrease of NH$_4^+$, NO$_3^-$ was increased from 0.35 mg/l to about 5.94 mg/l. This increase indicated that an efficient nitrification process took place during the aerobic phase with a nitrification rate of 0.93 mgN/gVSS-h. The chart also shows a low increase in NO$_2^-$ which can be indicating that a partial incomplete nitrification process took place.

4.1.2 Methanol SBR Monitoring

A series of monitoring tests were run to measure the physical and the chemical parameters to evaluate the use of Methanol as a carbon source. Figure 8 shows an example of the chemical parameters measured during one cycle.

The results of the chart of Methanol SBR monitoring shown in Figure 8, can be described in the same way as the MicroC™ system. During the anoxic phase, denitrified bacteria consumed most of NO$_3^-$ . In this phase, NO$_3^-$ became the electron acceptor with the absence of O$_2$ and reduced to N$_2$. Measurement of NO$_3^-$ in this example indicated that it dropped from 4.09 mg/l to zero within the anoxic time of the cycle, while NO$_2^-$ concentration in this phase was constant. The calculated value of denitrification rate (K_D) during this phase was 3.16 mgN/gVSS-h. In the aerobic phase, O$_2$ was pumped to the SBR through aeration allowing for the nitrification process to take place in the Methanol SBR system. In this phase, as Figure 8 shows, there is a decrease of NH$_4^+$, from 10.22 mg/l to about 0.19 mg/l. On the other hand, due to this decrease NO$_3^-$ was increased about 7.72 mg/l at the end of the aerobic phase. This increase indicated that an efficient nitrification process was taken place during the aerobic phase with a nitrification rate of 2.70 mgN/gVSS-h.
4.1.3 Acetate SBR Monitoring

A series of monitoring tests were run to measure the physical and the chemical parameters to evaluate the use of Acetate as a carbon source. Figure 9 shows an example of the chemical parameters measured during one cycle.
Results of the chart of Acetate SBR monitoring shown in Figure 9, can be described in the same way to MicroC™ and Methanol SBR systems. During the anoxic phase, denitrified bacteria consumed most of NO$_3^-$ . In this phase, NO$_3^-$ became the electron acceptor with the absence of O$_2$ and reduced to N$_2$. Measurement of NO$_3^-$ in this example indicated that it dropped from 4.77 mg/l to 0.05 mg/l within the anoxic time of the cycle, while NO$_2^-$ concentration in this phase was constant and equal too. The calculated value
of $K_D$ during this phase was 3.65 mgN/gVSS-h. In the aerobic phase, $O_2$ was pumped to the SBR through aeration allowing for the nitrification process to take place in the Acetate SBR system. In this phase, as Figure 9 showed, there was a decrease in NH$_4$ from 10.6 mg/l to about 0.02 mg/l. On the other hand, due to this decrease NO$_3^-$ was increased to about 7.67 mg/l at the end of the aerobic phase. This increase indicated that an efficient nitrification process was taken place during the aerobic phase with a rate of 2.66 mgN/gVSS-h.

4.2 Denitrification Kinetics

4.2.1 Measurement Denitrification Kinetics at 10°C

The effect of temperature on the denitrification kinetic using MicroC™, Methanol and Acetate sludge was evaluated using batch tests at 10°C and 20°C.

Measurement of MicroC™ Denitrification Kinetics at 10°C

Figure 10 shows the consumption of the nitrogen within the test time using MicroC™ sludge. From this chart, the results of nitrogen consumption, allowed the calculation of the denitrification rate using MicroC™ sludge. The denitrification rate ($K_D$) of MicroC™ sludge at 10°C was 2.51 mgN/gVSS-h.
Figure 10. Nitrogen compounds consumption in MicroC™ sludge 10°C

Figure 11 shows the change on COD concentration within the test time for MicroC™.

The initial COD was found to be 1018 mg/l, while the concentration at the end after four days was 289 mg/l.
Figure 11. COD consumption in MicroC™ sludge 10°C

**Measurement of Methanol Denitrification Kinetics at 10°C**

Figure 12 shows the consumption of the nitrogen, which was used to calculate the denitrification rate using Methanol sludge. The denitrification rate ($K_D$) of Methanol sludge at 10°C was 2.2 mgN/gVSS-h.
Figure 12. Nitrogen compounds consumption in Methanol sludge 10°C

Figure 13 shows the change on COD concentration within the test time for Methanol. The initial COD was found to be 648 mg/l, while the concentration at the end after four days was 65 mg/l.
Figure 13. COD consumption in Methanol sludge 10°C

Measurement of Acetate Denitrification Kinetics at 10°C

The results of nitrogen consumption, Figure 14, allowed the calculation of the denitrification rate using Acetate-acclimated sludge.
In this case, two values for denitrification rate ($K_D$) were used. The first value was $1.39 \text{ mgN/gVSS-h}$, which represents $K_D$ of NOx since it represents $\text{NO}_2^-$ and $\text{NO}_3^-$. The second $K_D$ was $9.94 \text{ mgN/gVSS-h}$, which represented the consumption of $\text{NO}_3^-$. The reason for using two measured values of $K_D$ is due to the increase of $\text{NO}_2^-$ concentration as shown in Figure 14.
Figure 15 shows the consumption of the COD within the test time using Acetate sludge. From this chart, the initial COD was 148.58 mg/l, while the concentration at the end of the test after four hours, and was 67.36 mg/l.
4.2.2 Measurement Denitrification Kinetics at 20°C

Measurement of MicroC™ Denitrification Kinetics at 20°C

Figure 16 shows the consumption of the nitrogen within the test time using MicroC™ sludge. The $K_D$ of MicroC™ sludge at 20°C was 4.58 mgN/gVSS-h.
Figure 17 shows the consumption of the COD within the test time using MicroC™ sludge. From this chart, the initial COD was found to be 196 mg/l, while the concentration at the end after four days, was 87 mg/l.

Figure 17 COD consumption in MicroC™ sludge 20°C
Measurement of Methanol Denitrification Kinetics at 20°C

Figure 18 shows the consumption of the nitrogen within the test time using Methanol sludge. Figure 18 allowed the calculation of the denitrification rate using Methanol sludge. The $K_D$ of Methanol sludge at 20°C was 5.56 mgN/gVSS-h.

![Graph showing nitrogen compounds consumption in Methanol sludge 20°C](image)

**Figure 18. Nitrogen compounds consumption in Methanol sludge 20°C**
Figure 19 shows the consumption of the COD within the test time using Methanol sludge.

From this chart, the initial COD was found to be 209 mg/l, while the concentration at the end after four days, was 79 mg/l.

![Figure 19. COD consumption in Methanol sludge 20°C](image-url)
**Measurement of Acetate Denitrification Kinetics at 20°C**

The results of nitrogen consumption, Figure 20, allowed calculation the denitrification rate using Acetate sludge. In this case, two values $K_D$ were used. The first value was 13.47 mgN/gVSS-h, which represents $K_D$ of NOx since it represents $\text{NO}_2^-$ and $\text{NO}_3^-$. The second $K_D$ was 35.68 mgN/gVSS-h, which represented the consumption of $\text{NO}_3^-$. The reason for using two measured values of $K_D$ is due to the increase of $\text{NO}_2^-$ concentration as shown in Figure 20.

Figure 21 shows the consumption of the COD within the test time using Acetate sludge. From this chart, the initial COD was found to be 198 mg/l, while the concentration at the end after four days, was 59 mg/l.
Figure 20. Nitrogen compounds consumption in Acetate sludge 20°C
Observation from the effect of temperature indicated that Methanol sludge had $K_\text{D}$ at 10°C followed by MicroC™ then by Acetate. On the other hand, Acetate sludge had a better $K_\text{D}$ over Methanol and MicroC™, respectively. In the addition, this experiment showed how temperature could be affecting the denitrification process the result from the Acetate.
sludge showed. Table 7 summarizes all rates obtained from the three acclimated sludge at the two temperatures used in this experiment.

Table 7. Summary of all $K_D$ rates obtained from the three acclimated sludge.

<table>
<thead>
<tr>
<th>Sludge</th>
<th>$K_D$ (mgN/gVSS-h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature</td>
</tr>
<tr>
<td></td>
<td>10°C</td>
</tr>
<tr>
<td>MicroC™</td>
<td>2.51</td>
</tr>
<tr>
<td>Methanol</td>
<td>2.2</td>
</tr>
<tr>
<td>Acetate</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td>9.94 (NO$_3^-$)</td>
</tr>
</tbody>
</table>

4.2.3 Cross Response

The cross response studies were conducted to assess the ability of different carbon-acclimated culture to immediately utilize other carbon sources. For all three sludges, the tests were run with various common carbon sources including MicroC™, Methanol, Acetate, Ethanol, and Glucose. All tests were run under the same conditions using 220 mg/l and 20mg/l as initial COD and NO$_3^-$ to ensure the evaluation and comparison of the maximum specific denitrification rate between the different carbon sources. All tests were stopped after 75 minutes.

MicroC™

Figure 22, shows the different responses of the MicroC™ acclimated sludge to the different carbon sources. $K_D$, obtained from the cross of using MicroC™ sludge, was 0.4 mgN/gVSS-h for Methanol, 3.61 mgN/gVSS-h for Acetate (in the case of NOx) and 9.14
mgN/gVSS-h (in the case of NO₃⁻), 3.96 mgN/gVSS-h for Ethanol and 0.15 mgN/gVSS-h for Glucose (in the case of NOx) and 12.69 (in the case NO₃⁻).

**Methanol**

Five batch tests using the Methanol acclimated sludge were conducted using MicroC™, Methanol, Acetate, Ethanol, and Glucose as the carbon source to determine the NO₂⁻, NO₃⁻, NOx and COD uptake rates. Figure 23, shows the different responses of the Methanol acclimated sludge to the different carbon sources. K_D, obtained from the cross response of using Methanol sludge, was 10.38 mgN/gVSS-h for MicroC™, 11.29 mgN/gVSS-h for Acetate, 6.4 mgN/gVSS-h for Ethanol and 0.05 for Glucose (in the case of NOx) and 12.69 mgN/gVSS-h (in the case NO₃⁻).

**Acetate**

Five batch tests using the Acetate acclimated sludge, were conducted using MicroC™, Methanol, Acetate, Ethanol, and Glucose as the carbon source to determine the NO₂⁻, NO₃⁻, NOx and COD uptake rates. Figure 24, shows the different respond of the Acetate acclimated sludge to the different carbon sources. K_D, obtained from the cross respond of using Acetate sludge, was 0.33 mgN/gVSS-h for MicroC™, 1.3 mgN/gVSS-h for Methanol, 3.21 mgN/gVSS-h for Ethanol and 4.23 mgN/gVSS-h for Glucose (in the case of NOx) and 12.64 mgN/gVSS-h (in the case of NO₃⁻).
Figure 22. Measurement of $K_D$ for MicroC™ sludge using different carbon sources
Figure 23. Measurement of $K_D$ for Methanol sludge using different carbon sources
Figure 24. Measurement of $K_D$ for Acetate sludge using different carbon sources
The overall results obtained from the cross response inducted that MicroC™-acclimated sludge was able use Acetate and Ethanol as carbon sources in the denitrification process, while in the case of Methanol and Glucose there was low response. Methanol-acclimated sludge had a higher denitrification rates in using MicroC™, Acetate and Ethanol, while Glucose yield low respond. Finally, Acetate-acclimated sludge showed the ability of using all the carbon sources with high denitrification rates with the exception of MicroC™. Table 8 summarizes the $K_D$ rates of all used carbon sources with the three acclimated sludges.

Table 8. Summary of the cross respond of the three acclimated sludges.

<table>
<thead>
<tr>
<th>Carbon Source</th>
<th>Sludge</th>
<th>MicroC™ $K_D$</th>
<th>Methanol $K_D$</th>
<th>Acetate $K_D$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MicroC™</td>
<td>5.47</td>
<td>10.33</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.4</td>
<td>5.56</td>
<td>1.23</td>
</tr>
<tr>
<td>Mehtanol</td>
<td>MicroC™</td>
<td>3.58 ± 1.40</td>
<td>6.26 ± 0.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.4</td>
<td>5.56</td>
<td>1.23</td>
</tr>
<tr>
<td>Acetate</td>
<td>MicroC™</td>
<td>3.61 (NOx)</td>
<td>11.29</td>
<td>17.58</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.4</td>
<td>5.56</td>
<td>1.23</td>
</tr>
<tr>
<td>Ethanol</td>
<td>MicroC™</td>
<td>3.61 (NOx)</td>
<td>11.29</td>
<td>17.58</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.4</td>
<td>5.56</td>
<td>1.23</td>
</tr>
<tr>
<td>Glucose</td>
<td>MicroC™</td>
<td>12.64 (NO₃⁻)</td>
<td>0.05</td>
<td>4.23</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.15 (NOx)</td>
<td>0.05</td>
<td>4.23</td>
</tr>
</tbody>
</table>

4.3 Perchlorate Removal

This section shows the results of two trials of ClO$_4^-$ removal. The targets of these trials were to determine the reduction rate of ClO$_4^-$ using different concentrations in the first trial while the second one was to determine the reduction rate in the presence of NO$_3^-$.
4.3.1 Experiment 1

In this trial the acclimated sludge from the SBR were used to remove ClO$_4^-$.
Figure 25, presents the degradation ClO$_4^-$ using MicroC™ sludge with degradation rate of 4.33 mg/gVSS-d.

![Graph showing ClO$_4^-$ removal in MicroC™ sludge](image)

Figure 25. ClO$_4^-$ removal in MicroC™ sludge

Figure 26, presents the degradation ClO$_4^-$ using Methanol sludge with degradation rate of 3.96 mg/gVSS-d.
Figure 26. ClO$_4^-$ removal in Methanol sludge

Figure 27, presents the degradation ClO$_4^-$ using Acetate sludge with degradation rate of 5.24 mg/gVSS-d.
4.3.2 Experiment 2

This trial was to study effect of NO$_3^-$ on ClO$_4^-$ degradation using two spick of ClO$_4^-$ one at the start of the experiment and the second was at day 6. Results of this trial indicated that ClO$_4^-$ was accomplished after removing NOx from the system. Result obtained from using MicroC™ sludge Figure 28 indicted that $K_D$ for NO$_3^-$ was 2.86 mgN/gVSS-h.
While ClO$_4^-$ degradation rate of the first spike was 2.51 mg/gVSS-d while on the second spike, degradation rate was 3.41 mg/gVSS-d.

Results of this trial indicate that ClO$_4^-$ was accomplished after removing NOx from the system. Result obtained from using methanol sludge Figure 29 indicted that $K_D$ for NO$_3^-$ was 12.6 mgN/gVSS-h. While ClO$_4^-$ degradation rate of the first spike was 0.45 mg/gVSS-d, while on the second spike, degradation rate was 5.55 mg/gVSS-d.

Results from this trial show that ClO$_4^-$ removal was accomplished after removing NO$_3^-$ from the system. Result obtained from using acetate sludge in Figure 30, indicted that $K_D$ for NO$_x$ was 4.70 mgN/gVSS-h, while ClO$_4^-$ degradation rate of the first spike was 3.23 mg/gVSS-d, while on the second spike, degradation rate was 18.69 mg/gVSS-d.
Figure 28. NOx and ClO⁴⁻ removal in MicroC™ sludge.
Figure 29. NOx and ClO_4^- removal in Methanol sludge.
All results obtained from two experiments for measuring ClO$_4^-$ degradation are summarized in Table 9. These results showed that using the Acetate sludge yield the higher ClO$_4^-$ degradation in both experiments followed by MicroC™ then by Methanol.
Table 9. Summary of two experiments for measuring ClO$_4^-$ degradation

<table>
<thead>
<tr>
<th>Unit</th>
<th>ClO$_4^-$</th>
<th>NO$_3^-$</th>
<th>ClO$_4^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MicroC™</td>
<td></td>
<td>1st spike</td>
<td>2nd spike</td>
</tr>
<tr>
<td>mg/gVSS-h</td>
<td>4.33</td>
<td>2.86</td>
<td>2.51</td>
</tr>
<tr>
<td>Methanol</td>
<td>3.96</td>
<td>12.96</td>
<td>0.45</td>
</tr>
<tr>
<td>Acetate</td>
<td>5.24</td>
<td>5.70</td>
<td>3.23</td>
</tr>
</tbody>
</table>

4.4 Discussion

4.4.1 SBR Monitoring

Results obtained from the monitoring process section (4.1) are aimed to evaluate the ability and capability of three SBR systems using of MicroC™, Methanol and Acetate as external carbon sources in the denitrification process to achieve biological nitrogen removal using SBR systems. All charts in section (4.1) summarize changes in the concentrations of N-NOx and N-NH$_4^+$ during one cycle of the operation time. In addition to these parameters, COD concentration was studied within the same cycle.

Results obtained from tracking COD show that it was completely consumed during the anoxic phase allowing the nitrification process to occur without any inhibiting effects. The N-NOx trends over time for one cycle show the ability to use Methanol, Acetate and MicroC™ as external carbon sources in the denitrification process to achieve complete nitrate removal in the SBR systems without the appearance of nitrite at the end of the anoxic phase. Moreover, the profiles of the nitrogen species during the aerobic phase
showed a complete and efficient nitrification. To determine the nitrification rate a complete nitrification test was conducted taking samples at regular time interval from the SBR during the aerobic phase. Results showed that the Methanol acclimate sludge had the highest nitrification rate followed by MicroC™ acclimated sludge then Acetate acclimated sludge. The measured rates were 7.25, 4.72 and 4.02 mgN/gVSS-h, for Methanol, MicroC™, and Acetate, respectively.

During the monitoring process, physical parameters such as temperature, dissolved oxygen (DO), pH and color were also monitored. Temperature of the SBR systems were between 20-23˚C. Measurement of DO varied depending on the phase. In the anoxic phase, DO was zero to ensure the use of NO$_3^-$ as electron acceptor in the denitrification process. In the aerobic phase, DO increased from zero to 2 mg/l at the first 20 minutes then up to 7-8 mg/l until the last 10 minutes of this phase. The aeration was shut down of in the last 10 minutes to ensure the complete consumption of O$_2$ by nitrification microorganisms so the system would be ready for the next anoxic phase. With this operating condition nitrogen purging was not needed, and denitrification could take place in the SBR from the beginning of the start of the anoxic phase.

At the beginning of the monitoring process, it was difficult to control and keep the pH within the optimum range during the anoxic phase. This problem affected mostly the MicroC™ acclimated sludge followed by Methanol. Measured pH in the Acetate reactor was always within the optimum range. The low pH in the MicroC™ reactor was probably due to the low pH of the MicroC™ stock solution, which is between 5.3 and 5.8. This problem was solved by increasing the amount of sodium bicarbonate in the feeding and by adding some drops of NaOH (2N) to increase the pH to greater than 7.5.
Another problem that was observed only in MicroC™ reactor was the appearance of filamentous bacteria, which could have been a result from the low pH. The filamentous growth, which happened two times during the monitoring, did not however affect the biological removal of nitrogen. The filamentous appearance caused unstable biomass settling in the MicroC™ SBR during the settling phase leading to a loss of some of the biomass through the draw phase. To solve this problem sodium hypochlorite 5% was added for couple of times into MicroC™ reactor at the end of the aerobic phase as 5g Cl/Kg TSS-day.

4.4.2 Denitrification Kinetics

The effect of temperature on denitrification rates using the MicroC™, Methanol and Acetate acclimated sludges, was studied to evaluate the efficiency of denitrification process at 10 and 20 °C. Results obtained from these tests are summarized in sections (4.2.1 and 4.1.2) and indicate the ability of all three acclimated sludges to accomplish the denitrification process with some variation in the obtained the denitrification rate ($K_D$).

Results obtained from using MicroC™ as a single carbon source in denitrification process indicate that MicroC™ sludge a decrease in the rates from 4.28 to 2.51 mgN/gVSS-h when the temperature dropped from 20 to 10 °C. On the other hand, denitrification rates using Methanol sludge decreased from 5.56 to 2.2 mgN/gVSS-h when the temperature dropped from 20 to 10 °C. The results indicate that MicroC™-acclimated sludge is comparable to Methanol-acclimated sludge under normal temperature (20 °C); the results also show that the Methanol-utilizing bacteria are more sensitive to low temperatures than the bacteria that use MicroC™, showing a 60% decrease of the denitrification rate at 10 °C versus a 40% drop with MicroC™.
When using Acetate-acclimated sludge, the results show that Acetate yielded the highest denitrification rate at 20°C with rate of 13.47 (mgN/gVSS-h), while it had the lowest denitrification rate at 10°C with 1.39 (mgN/gVSS-h). This variation indicated that the use of Acetate as carbon source was very sensitive to the change of temperature. This sensitivity appeared in the form of partial denitrification occurring which allowed for converting of NO₃⁻ into NO₂⁻ by the nitrate-reducing microorganism only but not for the converting of NO₂⁻ into N₂.

The ability to use other carbon sources with the three acclimated sludges was evaluated. General results obtained from these tests Section (4.2.3) show that using different carbon sources caused variation in the response. This is clearly demonstrated in the denitrification rates obtained from the three acclimated sludges Table 10.

The calculated denitrification rates using Methanol, Acetate, Ethanol and glucose with MicroCTM acclimated sludge showed that the converting of NO₃⁻ to N₂ via NO₂⁻ occurred using Ethanol and Methanol without any problems. When Acetate and Glucose were used, MicroCTM acclimated sludge managed to complete the first step of denitrification by converting NO₃⁻ into NO₂⁻, however the second step, which was the limiting step of the reduction of NO₂⁻ into N₂, did not occur.

Observations on Methanol acclimated sludge in the cross response tests showed higher denitrification rates when MicroCTM, Acetate and Ethanol were used. On the other hand, when Glucose was used the nitrate-reducing microorganisms were not able to reduce NO₃⁻ due to the lack of ability of using this compound as carbon source.

Results obtained from using Acetate sludge in the cross response tests showed the ability of microorganisms acclimated to Acetate to use all the compounds used in this
experiment without any exception as external carbon sources to complete the converting of NO$_3^-$ to N$_2$ via NO$_2^-$.

Table 10. Summary of the cross response effect.

<table>
<thead>
<tr>
<th>Carbon Source</th>
<th>MicroC™</th>
<th>Methanol</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>MicroC™</td>
<td>5.47</td>
<td>10.33</td>
<td>0.33</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.4</td>
<td>5.56</td>
<td>1.23</td>
</tr>
<tr>
<td>Acetate</td>
<td>3.61 (NOx)</td>
<td>11.29</td>
<td>17.58</td>
</tr>
<tr>
<td>Ethanol</td>
<td>3.96</td>
<td>6.41</td>
<td>3.21</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.15 (NOx)</td>
<td>0.05</td>
<td>4.23</td>
</tr>
</tbody>
</table>

4.4.3 Perchlorate Removal

Further studies on the denitrification microorganisms acclimated to MicroC™, Methanol and Acetate sludges were conducted to investigate of the ability of these sludges to degrade perchlorate. Two separate experiments were performed in the lab using different concentrations of perchlorate and to study the influences of nitrate (in experiment 2). Table 11 summarizes the degradation rates of perchlorate in the two experiments as well as the denitrification rates for experiment 2.
Table 11. Measurement of ClO$_4^-$ degradation rates.

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ClO$_4^-$</td>
<td>NO$_3^-$</td>
</tr>
<tr>
<td>MicroCTM</td>
<td>4.33 mg/gVSS-h</td>
<td>2.86 mg/gVSS-h</td>
</tr>
<tr>
<td>Methanol</td>
<td>3.96 mg/gVSS-h</td>
<td>12.96 mg/gVSS-h</td>
</tr>
<tr>
<td>Acetate</td>
<td>5.24 mg/gVSS-h</td>
<td>5.70 mg/gVSS-h</td>
</tr>
</tbody>
</table>

Observations from experiment (1) indicate the ability of denitrifying microorganisms acclimated to three different carbon sources to use ClO$_4^-$ as electron acceptor under anaerobic conditions. The results from this experiment support this observation and the ability of enzyme nitrate reductase to reduce chlorate.

The complete degradation, using the three sludges, was accomplished within the first 10 days. The difference in performance between the sludges was in the time requirements to complete the degradation. Acetate sludge was the fastest to degrade perchlorate within 8 days with rate of 5.24 mg/gVSS-d. Methanol and MicroCTM were able to degrade ClO$_4^-$ within the same time with degradation rates of 4.33 and 3.96 mg/gVSS-d, respectively. Observations on the control samples show the persistence of ClO$_4^-$ concentrations in all samples.

Experiment 2, was conducted to study the effect on NO$_3^-$ on ClO$_4^-$ degradation using the same sludges under anoxic conditions. NO$_3^-$ was completely removed within the first two day of the experiment. This allows the denitrifying microorganisms acclimated to the three carbon sources to use ClO$_4^-$ as electron acceptor instead of NO$_3^-$.

Major observations were that Acetate and MicroCTM were able to degrade ClO$_4^-$ with the two
spikes without any problem, while Methanol started to degrade ClO$_4^-$ only after the second spike.

Results obtained from this experiment do not provide full characterization of the relationship between on NO$_3^-$ removal and ClO$_4^-$ degradation, since the amount of NO$_3^-$ was small and there were two spikes in ClO$_4^-$ serum bottles; however they show that perchlorate removal was reduced when nitrate was present, and started only when nitrate level was low.
5 Conclusion

This study was designed in the lab scale to enrich for three denitrifying cultures that were fed with three carbon sources: Methanol, Acetate and MicroC™ to evaluate the denitrification process reactors under different conditions and to study the ability of acclimated sludge to remove nitrogen compounds and perchlorate from wastewater.

The study show the potential of using MicroC™ as a carbon source as well as Methanol and Acetate, to support the denitrification process. Also no inhibition on nitrification was observed for any of the compound tested.

pH and DO are very important parameters to control for the optimization of the SBR systems performance. Inefficient pH control caused growth of filamentous bacteria, which determined settling problems; after this problem the pH was adjusted and the filamentous bacteria were successfully removed from the system.

Once the SBR systems were ready, the effects of temperature at (10 and 20)°C as well as the influence of different carbon sources were used as key factors in comparing the denitrification rates and kinetics among the three acclimated sludges. Results obtained from testing the effect of temperature indicate that the denitrification rate using MicroC™-acclimated sludge was comparable to the Methanol-acclimated sludge at 20°C. However the results also show that the Methanol-utilizing bacteria are more sensitive to low temperatures than the bacteria that use MicroC™, showing a 60% decrease of the denitrification rate at 10 °C versus a 40% drop with MicroC™.

When Acetate-acclimated sludge, used at 10°C only partial denitrification occurred, causing the accumulation of NO₂⁻. This result implies to that the nitrite-reducing
denitrification microorganisms that uses acetate as carbon source are more sensitive to temperature than the nitrate-reducing microorganisms. These values measured at 10°C are “transitional’ kinetics since the sludge was not acclimated at 10°C and these tests only simulate instant population response to temperature decrease. Further studies are needed to determine if acclimatization to lower temperature will yield different results.

The effect of using of different external carbon sources with MicroC™-acclimated sludge, varied depending on the type of the compound used. Denitrifying bacteria were able to use Ethanol and Methanol as carbon sources to convert NO$_3^-$ to N$_2$ via NO$_2^-$. Acetate and Glucose were able to accomplish partial denitrification by activating the nitrite-reducing microorganisms but not nitrate-reducing microorganisms.

All types carbon compounds were able to activate both nitrite and nitrate-reducing microorganisms yielding high denitrification rates when Methanol-acclimated sludge was used, with the exception of Glucose, which yielded no response.

Acetate-acclimated sludge microorganisms showed the ability to use all types of carbon compounds to achieve complete denitrification with similar values for the denitrification rates of using the carbon compounds.

In general, cross response tests were conducted in a short period. Further work should be done using these same tests or by changing the initial amounts of chemical used (carbon source and potassium nitrate) to observe the effect of the proposed conditions over a long period or by using other carbon compounds.

This study showed that MicroC™, Methanol and Acetate acclimated sludges microorganisms were able to use perchlorate as the only electron acceptor in the absence
of O₂ and NO₃⁻ allowing them to convert ClO₄⁻ to Cl⁻. Further work should be conducted to find the maximum biomass yield for these microorganisms using different concentrations of perchlorate, and to determine which microorganisms are responsible of the ClO₄⁻ degradation

Observations from the experiment indicate that when both nitrate and perchlorate were fed into the serum bottle, NO₃⁻ removal was accomplished in the first hours of the experiment, and ClO₄⁻ degradation started only after the nitrate was completely removed from the system. This seems to indicate that NO₃⁻ presence can inhibit or reduce the ClO₄⁻ degradation; however, the bacteria responsible of the denitrification were able to easily use ClO₄⁻ when nitrate was absent.

Further work on perchlorate can be conducted to evaluate which are the bacteria responsible for the degradation of perchlorate in the activated sludge. Another interesting investigation of the relationship between NO₃⁻ removal and ClO₄⁻ degradation could be designing system for ClO₄⁻ degradation then test the ability of NO₃⁻ removal since most of the studies indicate that perchlorate degradation microorganisms are unable to remove NO₃⁻ from wastewater.
References


Xu Jianlin, Trimble John J., Steinberg Lisa and Logan Bruce E., 2004, *Chlorate and nitrate reduction pathways are separately induced in the perchlorate-respiring bacterium Dechlorosoma sp. KJ and the chlorate-respiring bacterium Pseudomonas sp. PDA*, Water Research, **38**:673–68.


Acknowledgement

Firstly, GOD blessing combined with the blessing of my parents Nimer and Hind, my beautiful wife Dema, and my whole family and friends were the light giving me the strength and well to finish this work.

Thought this research, there were numerous whom gave there supports to complete this work. First, I wish to expresses my sincere gratitude to Professor Gu, for supervision, encouragement and mostly, giving me a chance to learn new experience in this research combined with the knowledge she passed to me. Also I would like also to gratefully thank Dr. Annalisa Onnis Hayden for her technical support and help. Also I would like to thank my lab mates Carla Cherchi and Nehreen Majed for their help during the time working in the lab. Finally, I would like to thank the department of Civil and environmental engineering for giving the chance to be one of their graduate students.

Most grateful thanks to my brothers and sisters, the rest of my family and my friends, for their full support knowing that even am far away from them but they always remember me in their prayers.

My special thanks to Professor Akarm Alshawabkeh for giving me the chance to follow my dream, his wife Reham, their kids: Sammy, Rania and Leena for their great generous hospitality and full support.

Last but not least I dedicate my work to my beloved parents and to my best friend and my soul mate my wife, for their care and understanding and full support.