Preliminary Investigation of Ferrate for Drinking Water Treatment: Mussel Control and Synergy with Powdered Activated Carbon

by

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A thesis submitted in conformity with the requirements for the degree of Masters of Applied Science

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Synergy with Powdered Activated Carbon

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ABSTRACT

Ferrate has been observed under experimental conditions to be a potentially effective drinking water treatment chemical, serving as an oxidant, coagulant, and disinfectant. Although studies have ascertained ferrate’s potential to remove substances like bacteria, viruses, and organic matter, there is limited research for its use in mussel control and its synergy with other treatment methods.

This research first explored ferrate’s ability to control dreissenids. Preliminary results from acute toxicity testing suggested that ferrate could be an effective alternative to chlorine in the inactivation of quagga and zebra mussel veligers. Additionally, veliger settlement tests showed similar performance between ferrate and chlorine. This work, however, was limited in scope, and would require future investigation.

The research also explored the hypothesis that ferrate could enhance or inhibit the adsorption of geosmin by powdered activated carbon, through a series of jar tests. Overall, there was little observed impact of ferrate.
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Karlye Wong
# TABLE OF CONTENTS

**Abstract** ................................................................................................................................... ii

**Acknowledgments** ........................................................................................................... iii

**Table of Contents** ............................................................................................................ iv

**List of Tables** ...................................................................................................................... xii

**List of Figures** ................................................................................................................... xiv

1 **Introduction and Research Objectives** ......................................................................... 1

1.1 Motivation ........................................................................................................................ 1

1.2 Research Objectives ......................................................................................................... 2

1.3 Description of Chapters .................................................................................................... 3

1.4 References ........................................................................................................................ 4

2 **Literature Review** ............................................................................................................ 5

2.1 Fundamentals of Ferrate (FeO$_4^{2-}$) .............................................................................. 5

2.1.1 Chemical and Physical Properties ............................................................................. 6

2.1.2 Reaction and Kinetics ............................................................................................... 7

2.1.2.1 Potassium Ferrate (K$_2$FeO$_4$) ............................................................................ 10

2.2 Ferrate Suppliers ............................................................................................................ 12

2.3 Ferrate as a Coagulant Aid ............................................................................................ 13

2.3.1 Removal of NOM (Natural Organic Matter) .......................................................... 13

2.3.2 Removal of Algae ................................................................................................... 15

2.3.3 Effect on Turbidity and Suspended Solids .............................................................. 15

2.3.4 Removal of Metals .................................................................................................. 16

2.4 Ferrate as an Oxidant ...................................................................................................... 17

2.4.1 NOM (Natural Organic Matter) ................................................................................ 18
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4.2 Organic Compounds</td>
<td>19</td>
</tr>
<tr>
<td>2.4.3 Inorganic Compounds</td>
<td>19</td>
</tr>
<tr>
<td>2.4.4 Formation of Disinfection By-Products (DBPs)</td>
<td>19</td>
</tr>
<tr>
<td>2.4.5 Bacteria and viruses</td>
<td>20</td>
</tr>
<tr>
<td>2.4.6 Emerging Micropollutants</td>
<td>21</td>
</tr>
<tr>
<td>2.5 Production of Ferrate</td>
<td>22</td>
</tr>
<tr>
<td>2.5.1 Electrochemical Production</td>
<td>22</td>
</tr>
<tr>
<td>2.5.2 In-Situ Production</td>
<td>22</td>
</tr>
<tr>
<td>2.6 Pilot Scale Tests</td>
<td>23</td>
</tr>
<tr>
<td>2.7 Zebra and quagga mussels</td>
<td>24</td>
</tr>
<tr>
<td>2.7.1 History of Zebra and Quagga Mussels</td>
<td>24</td>
</tr>
<tr>
<td>2.7.2 Preferred Water Characteristics</td>
<td>27</td>
</tr>
<tr>
<td>2.7.3 Ecology</td>
<td>29</td>
</tr>
<tr>
<td>2.7.4 Life Cycle</td>
<td>29</td>
</tr>
<tr>
<td>2.7.4.1 Reproduction and Veliger Stage</td>
<td>32</td>
</tr>
<tr>
<td>2.7.4.2 Adult Mussels</td>
<td>33</td>
</tr>
<tr>
<td>2.7.5 Characteristics of Zebra and Quagga Mussels</td>
<td>34</td>
</tr>
<tr>
<td>2.7.5.1 Zebra Mussels (Dreissena polymorpha)</td>
<td>34</td>
</tr>
<tr>
<td>2.7.5.2 Quagga Mussels (Dreissena rostriformis bugensis)</td>
<td>35</td>
</tr>
<tr>
<td>2.8 Lake Simcoe Mussel Distribution</td>
<td>36</td>
</tr>
<tr>
<td>2.8.1 Treating Dreissenids</td>
<td>40</td>
</tr>
<tr>
<td>2.8.2 Dreissena Control and Removal</td>
<td>40</td>
</tr>
<tr>
<td>2.8.3 Response of Dreissena Veligers to Oxidants</td>
<td>42</td>
</tr>
<tr>
<td>2.8.1.1 Contaminants and Concerns</td>
<td>48</td>
</tr>
<tr>
<td>2.9 References</td>
<td>49</td>
</tr>
</tbody>
</table>
4.3.1 Water Source ........................................................................................................... 93
4.3.2 Biobox Apparatus Assembly .................................................................................. 94
  4.3.2.1 Prepping Settling Plates ................................................................................... 95
4.3.3 Initial Set Up ........................................................................................................... 96
4.3.4 Reagents .................................................................................................................. 97
  4.3.4.1 ENVIFER ........................................................................................................ 97
  4.3.4.2 Buffer Solution ................................................................................................ 98
4.4 Methodology .................................................................................................................. 99
  4.4.1 Original Experimental Design ................................................................................ 99
  4.4.2 Modified Experimental Design ............................................................................. 100
  4.4.3 Decay of Oxidants ................................................................................................. 100
  4.4.4 Stock solutions ...................................................................................................... 100
    4.4.4.1 Dosing of Stock Solutions ............................................................................. 101
  4.4.5 Setting Peristaltic Pumps ...................................................................................... 101
  4.4.6 Daily Tests ............................................................................................................ 102
  4.4.7 Settling Analysis ................................................................................................... 102
4.5 Results and Discussion ................................................................................................. 103
  4.5.1 Settling Plate Analysis .......................................................................................... 103
4.6 Summary and Conclusions ........................................................................................... 105
  4.6.1 Improvements and Suggestions ............................................................................ 105
  4.6.2 Conclusions ........................................................................................................... 106
4.7 References .................................................................................................................... 108
4.8 Acknowledgements ...................................................................................................... 109

5 ENHANCEMENT OF GEOSMIN AND DOC REMOVAL ........................................ 110
5.1 Introduction .................................................................................................................. 111
5.2 Literature Review ......................................................................................................... 112
  5.2.1 Powdered Activated Carbon ................................................................................. 112
    5.2.1.1 PAC and Adsorption Sites ............................................................................. 113
    5.2.1.2 Interaction with PAC and Coagulants ........................................................... 114
    5.2.1.3 Interaction of PAC with Oxidants ................................................................. 116
    5.2.1.4 PAC Stirring Protocol .................................................................................... 117
    5.2.1.5 Types of PAC ................................................................................................ 118
  5.2.2 Model Taste and Odour compound: Geosmin ...................................................... 121

5.3 Objective ...................................................................................................................... 122

5.4 Materials and Methods ................................................................................................. 123
  5.4.1 Water source ......................................................................................................... 123
  5.4.2 Chemicals and reagents......................................................................................... 123
  5.4.3 Bench-scale protocol ............................................................................................. 124
  5.4.4 Analysis ................................................................................................................. 125

5.5 Results and Discussion ................................................................................................. 126
  5.5.1 PAC only ............................................................................................................... 126
  5.5.2 Ferrate only ........................................................................................................... 126
  5.5.3 Ferrate and PAC added simultaneously................................................................. 128
  5.5.4 PAC and ferrate mixing order ............................................................................... 129
  5.5.5 Removal of Geosmin with PAC and Ferrate in Milli-Q Water ............................ 131

5.6 Summary and Conclusions ........................................................................................... 132
  5.6.1 DOC Removal ....................................................................................................... 132
  5.6.2 Geosmin Removal ................................................................................................. 133

5.7 References .................................................................................................................... 136

6 RECOMMENDATIONS FOR FUTURE WORK................................................................. 140
6.1 References........................................................................................................................................... 142

7 APPENDICES ........................................................................................................................................... 143

A. APPENDIX Ferrate Properties ............................................................................................................ 144

A.1 Material Properties ........................................................................................................................... 145

A.1.1 Envifer Properties (from NanoIron, CZ) ...................................................................................... 145

A.1.2 Material Properties ........................................................................................................................ 146

A.2 Ferrate Analysis .................................................................................................................................... 148

A.2.3 Ferrate Purity Determination using Chromite Method ............................................................... 148

A.2.4 Ferrate (K₂FeO₄) Residual Determination: Indirect Method with ABTS ................................. 152

A.2.5 Ferrate (FeO₄²⁻) Residual Determination: Direct Method .......................................................... 156

A.3 Ferrate Decay Throughout Experiment ......................................................................................... 160

B. APPENDIX B Veliger Acute Toxicity Test ......................................................................................... 164

B.1 Sourcing Dreissena Veligers ............................................................................................................... 165

B.1.1 Finding Sampling Locations ....................................................................................................... 165

B.1.2 Collection ....................................................................................................................................... 165

B.1.3 Storage of Dreissena veligers ..................................................................................................... 166

B.1.4 Concentration Determination of Samples .................................................................................. 167

B.1.5 Alive Dead Analysis and Practice ............................................................................................... 168

B.1.6 Veliger Microscopy ....................................................................................................................... 168

B.1.7 Preliminary Test: quenching agent on veligers to observe toxicity ............................................ 169

B.2 Veliger Behavior ............................................................................................................................... 170

B.2.1 Veliger Stress ................................................................................................................................. 170

B.3 Ferrate Concentration in Solution .................................................................................................. 172

B.3.1 Direct Method .................................................................................................................................. 173

B.3.2 Indirect Method ............................................................................................................................ 174
LIST OF TABLES

Table 2-1: Variation of kinetic constant (k) of Fe(VI) decomposition with pH ............................ 9
Table 2-2: Oxidation-reduction protential of various disinfectants/oxidants ............................... 17
Table 2-3: Summary of data for zebra and quagga mussel study in Lake Simcoe in 2009/2015 by LSRCA................................................................. 37
Table 2-4: Literature comparison of zebra mussel experiments.................................................. 44
Table 3-1: Characteristics and appearance of active and inactive veligers ................................. 71
Table 4-1: Oxidation states of Fe in ENVIFER compound using Mossbauer spectroscopy, analysis performed by NanoIron (CZ)......................................................... 98
Table 4-2: Percentage weight proportion of compounds in ENVIFER compound ................... 98
Table 4-3: Experimental design of initial experiment ............................................................... 99
Table 4-4: Experimental design of modified experiment performed October 2015............... 100
Table 5-1: Literature review of various stirring protocols and addition sequences of PAC and oxidants/coagulants ............................................................. 118
Table 5-2: Literature Review of Types of PAC used for removal of various microcontaminants .......................................................... 119
Table 5-3: Experimental design for initial simultaneous addition of ferrate and PAC jar tests .............................................................................................................. 124
Table 7-1: The decomposition rate of Envifer in various concentrations as pseudo-first order constant (Nano Iron s.r.o., 2016)................................................................ 146
Table 7-2: Mössbauer performed by NanoIron CZ (batch 19).................................................. 147
Table 7-3: AAS analysis of K and Fe in Envifer ........................................................................ 147
Table 7-4: Active components of Envifer by weight distribution .............................................. 147
Table 7-5 Potassium Ferrate Purity Determination - Summary of Reagents ......................... 148
Table 7-6: UV-vis spectrophotometer conditions ..................................................................... 154
Table 7-7: Potassium Ferrate Residual Determination – Summary of Reagents ................. 154
Table 7-8: UV-vis spectrophotometer conditions ..................................................................... 158
Table 7-9: Ferrate Residual Determination – Summary of Reagents ........................................ 159

Table 7-10: ABTS indirect absorbance of Fe(VI) to sample potassium ferrate concentration ratio results for dates of measurements ................................................................. 163

Table 7-11: Determination of estimated Fe(VI) percentage purity of sample potassium ferrate for experiments ............................................................................................ 163

Table 7-12: Locations of veliger sampling in Ontario ......................................................... 183

Table 7-13: Volume of stock solutions dispersed per time period in one day .................... 192

Table 7-14: Veliger viability in different environments ....................................................... 197

Table 7-15: Powdered activated carbon specifications (WP260-90, Calgon Carbon) ........ 199

Table 7-16: Experimental design for ferrate only jar test ............................................... 200

Table 7-17: pH data for ferrate only jar test ...................................................................... 201

Table 7-18: Experimental design for ferrate, then PAC jar test ....................................... 202

Table 7-19: pH data for ferrate, then PAC, jar test ............................................................. 203

Table 7-20: Experimental design for PAC, then ferrate jar test ...................................... 204

Table 7-21: pH data for PAC, then ferrate jar test ............................................................. 205

Table 7-22: Experimental design for PAC and ferrate added simultaneously jar test ...... 206

Table 7-23: pH data for PAC and ferrate simultaneous jar test ....................................... 207

Table 7-24: Reagents for geosmin analytical procedure .................................................. 208

Table 7-25: GC/MS operation conditions ........................................................................ 210

Table 7-26: Parameters of the MS scan for determining taste and odour compounds ...... 211

Table 7-27: Calculation of method detection limit for geosmin ...................................... 213
# LIST OF FIGURES

| Figure 2-1: Structure of ferrate ion | 6 |
| Figure 2-2: Ferrate VI in aqueous solution with three resonance hybrid structures | 7 |
| Figure 2-3: Species distribution of Fe(VI) in aqueous solution | 8 |
| Figure 2-4: Potassium ferrate in solid state | 11 |
| Figure 2-5: The self decay of potassium ferrate in aqueous solution at 0.4, 1.0, and 2.0 gL⁻¹: (a) 7 minutes after preparations, (b) after 67 minutes at room temperatures | 11 |
| Figure 2-6: Spread of *Dreissena polymorpha* through North America between 1986 and 2013 (USGS 2016b) | 25 |
| Figure 2-7: Zebra and quagga mussel distribution in North America as of March 2016 | 26 |
| Figure 2-8: Geographical risk areas of zebra and quagga mussel invasion based on calcium concentration levels in surface water (Whittier et al., 2008) | 28 |
| Figure 2-9: Growth stages of a *Dreissena polymorpha* veliger. (a) egg, (b) straight-hinged veliger 97-112 microns, (c) umbonal 112-347 microns, (d) pediveliger 231-462 microns | 30 |
| Figure 2-10: Life stages of *Dreissena polymorpha* showing relative depth in water table. Double-headed arrows indicate the benthic stages where the mussels can translocate in water column (Ackerman et al., 1994). | 31 |
| Figure 2-11: Growth stages of *Dreissena bugensis* from (a) pediveliger to (b) early plantigrade to (c) late plantigrade compared to *Dreissena polymorpha* as (d) pediveliger, (e) early plantigrade, and (f) late plantigrade | 32 |
| Figure 2-12: Comparison of adult zebra and quagga mussel | 35 |
| Figure 2-13: Zebra and quagga mussel density map of Lake Simcoe in 2009 | 38 |
| Figure 2-14: Zebra and quagga mussel density map of Lake Simcoe in 2015 | 39 |
| Figure 3-1. Location of veliger sampling for acute toxicity experiments | 68 |
| Figure 3-2: Pediveligers in a Petri dish | 70 |
| Figure 3-3: Veliger in an "Alive with Protrusion" state | 72 |
| Figure 3-4: A live veliger without outer protrusions, but intact organs | 73 |
Figure 3-5: Recent veliger killed by oxidant addition, organs emptied outside of shell ............ 75

Figure 3-9: Comparison of dead and open veliger under 100x microscopy with Leica M125 microscope ........................................................................................................................ 77

Figure 3-10: Veliger and Pediveligers in sample under 100x using Leica M125 stereo microscope ..................................................................................................................... 78

Figure 3-11: Initial behaviors of veligers before chlorine (mgL$^{-1}$ as Cl$_2$) (a) average distribution of number of veligers in each sample, (b) percentage (%) distribution of veligers and types in each sample .................................................................................. 79

Figure 3-12: Initial behaviors of veligers before ferrate addition (mgL$^{-1}$ as FeO$_4$) (a) average distribution of number of veligers in each sample, (b) percentage (%) distribution of veligers and types in each sample .................................................................................. 80

Figure 3-13: Percentage of veligers that adopt ideal behavior after an acute exposure of 7.1, 14.1, 28.2, 42.3, 70.5, and 141.0 µM as Cl$_2$ at 0 minutes and observed over 30 minutes .................................................................................................................. 82

Figure 3-14: Percentage of veligers that adopt ideal behavior after an acute exposure of 7.1, 14.1, 28.2, 42.3, 70.5, and 141.0 µM as FeO$_4$$^{2-}$ at 0 minutes and observed over 30 minutes .................................................................................................................. 84

Figure 4-1: Inside black polypropylene lining of coolers with silicone for attaching exposed .... 95

Figure 4-2: Hanging polypropylene plates for coolers .................................................................. 96

Figure 4-3: Experimental set up with peristaltic pumps, magnetic stirrers and stock solution .... 97

Figure 4-4: Juvenile mussel from control tank #1, under 400x Nikon E600 microscopy .......... 103

Figure 4-5: Juvenile mussel #2 (slightly larger) from control tank #1, under 400x Nikon E600 microscopy .................................................................................................................. 104

Figure 5-1: Effect of PAC and ferric chloride dosages on % removal of UV$_{254}$ .......................... 115

Figure 5-2: Molecular structure of geosmin (Juttner and Watson 2007) .................................... 122

Figure 5-3: Percentage DOC and geosmin removal in spiked Georgina WTP raw intake water (spiked with 100 ngL$^{-1}$ geosmin) using varying ferrate concentrations of 1, 5, 10, and 20 mgL$^{-1}$ in jar tests ........................................................................................................ 127

Figure 5-4: Percentage removal of DOC and geosmin after simultaneous addition of PAC and ferrate in jar tests with raw Georgina WTP intake water, spiked with 100 ngL$^{-1}$ geosmin .................................................................................................................. 128
Figure 5-5: DOC removal from Georgina WTP raw water (spiked with 100 ngL\(^{-1}\) geosmin) after the addition of (1) PAC added first with 1 hr contact time before ferrate, (2) ferrate added first with 1 hr contact time before ferrate, (3) PAC and ferrate added simultaneously. .......................................................................................................................... 130

Figure 5-6: Geosmin removal from Georgina WTP raw intake water (spiked with 100 ngL\(^{-1}\) geosmin) after the addition of (1) PAC added first with 1 hr contact time before ferrate, (2) ferrate added first with 1 hr contact time before ferrate, (3) PAC and ferrate added simultaneously. .................................................................................. 130

Figure 5-7: Geosmin concentration (ngL\(^{-1}\)) after treatment with various combinations of ferrate and PAC added simultaneously in Georgina WTP raw intake water and Milli-Q® water. Initial geosmin concentration ~ 67.3 ngL\(^{-1}\). .................................................................................................................. 132

Figure 7-2: Detection of ferrate using the direct method and the ABTS method. Ferrate at an initial concentration of 4.5 µM or approximately 0.29 mg/L (a) phosphate buffered solution, (b) borate buffered solution (Lee et al. 2005) ................................................................. 158

Figure 7-3: Purity determination of Fe(VI) in sample potassium ferrate and Envifer by Mössbauer spectrometry .......................................................................................................................... 161

Figure 7-4: Absorbance of Fe(VI) to sample potassium ferrate concentration ratio of ABTS indirect Fe(VI) measurements on dates following June 18, 2015 ............ 162

Figure 7-5: Direct visible spectra of Fe(VI) in aqueous solution, peak at 510 nm..................... 172

Figure 7-6: Detection of ferrate using the direct method and the ABTS method. Ferrate at an initial concentration of 4.5 µM or approximately 0.29 mgL\(^{-1}\) in a phosphate buffered solution (Lee et al. 2005) .................................................................................................................. 173

Figure 7-7: Standard curves obtained by the ABTS method prepared in a buffer solution of pH 9.1 (5 mM Na\(_2\)HPO\(_4\)/1 mM borate) (Lee et al., 2005) .............. 175

Figure 7-8: ABTS solution of 1 mgL\(^{-1}\) of ferrate over time ....................................................... 176

Figure 7-9: Calibration curve for direct method ferrate determination using the Hach D2800 Spectrophotometer ....................................................................................... 177

Figure 7-10: Comparison of dead and open veliger under 100x microscopy with Leica M125 microscope .................................................................................................................. 179

Figure 7-11: Zebra mussel veligers in a closed, but living state using 100x compound microscopy .......................................................................................................................... 181
Figure 7-12: Lake Ontario average temperature ranges since 1984 (surf-forecast.com) .......... 182

Figure 7-13: Decay of free chlorine over 24 h in filtered Lake Ontario water. \( C_0 = 1.2 \, \text{mgL}^{-1} \)  
(a) concentration over time (b) modeled as a first-order reaction ......................... 186

Figure 7-14: Decay of ferrate over 24 h in filtered Lake Ontario water. \( C_0 = 1.05 \, \text{mgL}^{-1} \)  
(a) concentration over time (b) modeled as a first-order reaction ....................... 187

Figure 7-15: Decay of Envifer as ferrate over 24 h in Milli-Q water \( C_0 = 1.05 \, \text{mgL}^{-1} \)  
(a) concentration over time (b) modeled as a first-order reaction ....................... 188

Figure 7-16: Ferrate as Envifer decay in Lake Ontario water over 24 h: (a) concentration over time, (b) second order reaction decay rate .............................................. 189

Figure 7-17: Ferrate as Envifer decay in Lake Ontario water with veligers over 24 h, \( C_0 = 1.46 \, \text{mgL}^{-1} \), modelled as a second-order reaction decay rate ....................... 190

Figure 7-18: Decay of Envifer as ferrate in pH = 9.1 phosphate buffer solution prepared with Milli-Q water over 24 h, \( C_0 = 34 \, \text{mgL}^{-1} \) .......................................................... 191

Figure 7-19: Second order decay of Envifer as ferrate in pH = 9.1 phosphate buffer solution prepared with Milli-Q water over 24 h, \( C_0 = 34 \, \text{mgL}^{-1} \) ...................................................... 191

Figure 7-20: Decay in pH 9.1 Borate buffer over 24 h, \( C_0 = 21.8 \, \text{mgL}^{-1} \) ........................................ 192

Figure 7-21: Dosing rates to maintain a 1 mgL\(^{-1}\) Envifer as ferrate residual in a 30 L tank given a 75 mgL\(^{-1}\) stock solution dosed at programmed intervals .............................. 193

Figure 7-22: Dosing rates to maintain a 1 mgL\(^{-1}\) free chlorine residual in a 30 L tank given a 24 mgL\(^{-1}\) stock solution dosed at programmed intervals ................................. 194

Figure 7-23: Dosing rates to maintain a 1 mgL\(^{-1}\) free chlorine residual in a 30 L tank given a 30 mgL\(^{-1}\) stock solution dosed at programmed intervals ................................. 195

Figure 7-24: Quality control chart for geomsin .......................................................................... 212

Figure 7-25: Calibration curve for geosmin analysis GC-MS Scarborough Campus Ferrate added first + PAC ................................................................. 212
1 INTRODUCTION AND RESEARCH OBJECTIVES

1.1 MOTIVATION

Chlorine is a common disinfectant for zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena bugensis rotiformis*) at the intakes of drinking water treatment plants. However, its use in the pre-treatment stages may contribute to the increase of disinfection-by-products in tap water (Rajagopal et al., 2002a).

Chlorine is currently dosed at low concentrations at intake pipes to provide a small residual of oxidant to prevent settlement, but little is known about how veligers of different stages in their life behave after exposure to oxidants (Claudi and Mackie, 1993). Once mussels are settled on a surface, they are typically removed by more costly mechanical methods (Nalepa and Schloesser, 2013). In order to emphasize the prevention of mussel settlement, focus must be changed from treating adult dreissenids to mussels in the veliger larval stage.

While studies have ascertained ferrate’s potential to act as a coagulant, co-precipitate heavy metals, and remove a variety of substances like bacteria, viruses, and tastes and odours, there has been limited research observing its efficacy in the control of mussels (Ma and Liu, 2002b; Yates et al., 2014). This work is meant to explore ferrate’s effect on veligers. There is a misconception that in order for a veliger to not attach to a surface, it must be dead. On the contrary, veligers exhibit a variety of behaviors in response to different situations, many of which are unable to attach to surfaces. In fact, veligers require a significant disinfectant concentration, administered for a long period of time, in order to be killed (Van Benschoten et al., 1993). As a result, it is more effective and efficient to focus on the concentrations of oxidant that will illicit the minimal response that will prevent them from adhering to surfaces. As a result, this work explores ferrate’s impact on preventing veliger attachment to surfaces, in addition to outright acute
inactivation effects. For comparison, the ferrate study is carried out in parallel to chlorine under identical conditions.

If ferrate were to be added at a plant intake for mussel control, it may also have effects on downstream treatment processes. One potential impact that has not been explored to date is ferrate’s effect on powdered activated carbon (PAC) adsorption. PAC’s interaction with other treatment chemicals, such as chlorine, have an effect on adsorption (Gillogly et al., 1998). More specifically, the effect of ferrate on the adsorption of geosmin and dissolve organic carbon (DOC) by PAC has not yet been investigated. The purpose of this additional experiment is to observe the removal of these compounds using varying combinations of concentration and mixing order of PAC and ferrate and to investigate their corresponding interactions with each other.

1.2 Research Objectives

The overall goals of this study were to (1) explore Dreissena control at water intake sites using ferrate, and (2) observe the synergy of PAC and ferrate in the removal of geosmin compounds and DOC. The detailed objectives of this research were as follows:

1. To develop a protocol for testing and observing acute toxicity of ferrate and chlorine on Dreissena veligers;
2. To determine the effectiveness of ferrate in inactivating Dreissena veligers in a static system in relation to chlorine;
3. To observe the ability of ferrate in the prevention of mussel settlement in relation to chlorine;
4. To develop a protocol for testing the dosing of varying oxidants on the settlement of Dreissena veligers;
5. To develop a jar test method for observing PAC and the coagulation enhancement properties of ferrate in the removal of geosmin and DOC
6. To investigate the synergy and varying mixing conditions whereby ferrate and PAC are able to remove of geosmin and DOC in Lake Simcoe water

1.3 Description of Chapters

Chapter 2 is a literature review on previous research on the kinetics, reactions, and potential applications of ferrate. It is also an overview of zebra and quagga mussels – their life cycle, characteristics, current status in the environment, and current control techniques.

Chapter 3 compares the behavioural differences and inactivation of Dreissena veligers throughout 30 minutes of acute exposure to ferrate and chlorine.

Chapter 4 provides a methodology and preliminary results into the investigation of the effect of continuous ferrate and chlorine dosage on the settlement of Dreissena veligers.

Chapter 5 investigates the interaction of powdered activated carbon (PAC) and ferrate in the removal of geosmin in the presence of natural organic matter (NOM)

Chapter 6 is an overview of the significant findings from this research and provides recommendations for future research
1.4 References


2 LITERATURE REVIEW

2.1 FUNDAMENTALS OF FERRATE (FeO$_4^{2-}$)

The ferrate ion (FeO$_4^{2-}$), also known as Fe(VI), is an ion in the +6 oxidation state of iron. Normally, iron occurs as a free metal or can be found in the oxidation states of Fe(II) or Fe(III). However, there are higher oxidation states of iron that can be formed: Fe(IV), Fe(V), or Fe(VI). Out of these higher oxidation species, Fe(IV) and Fe(V) are most unstable and often decompose to Fe(III) or Fe(II). Fe(VI) is also considered to be a more stable oxidation state in its solid earth salt form, $M_2$FeO$_4$ (Li, Na, K, Rb, Cs, and Ag) and $M_1$FeO$_4$ (Ca, Ba, and Sr), such as potassium ferrate or sodium ferrate (Sharma, 2013a).

Despite its high oxidizing power, selectivity, stability as a salt, non-toxic by-products, and coagulating properties, ferrate is remains largely unused in the water treatment industry. This can be attributed to the fact that it can be difficult to store and is unstable when in a liquid solution (Sharma et al., 2003). However, advancements in the in-situ manufacturing of potassium ferrate (K$_2$FeO$_4$) has encouraged increased research into its applications, observing its diverse properties. Lee et al. (2004) have described ferrate as a green oxidant, coagulant, disinfectant, and an antifouling agent. Ferrate has applicable multi-purpose characteristics that make it suitable for a variety of water applications. It has an oxidation-reduction potential greater than that of chlorine at 2.20 V (Wood, 1957), is able to disinfect microorganisms, can degrade or oxidize organics and inorganics, and has the potential to treat emerging micropollutants. In fact, it is more powerful as an oxidant than hypochlorite, ozone, hydrogen peroxide, chlorine, chlorine dioxide, dissolved oxygen, or permanganate (Jiang and Lloyd, 2002a; Lee et al., 2004). The possibility of ferrate to supplement and improve existing water treatment plants to tackle emerging issues has made it an attractive chemical. However, further research into the chemistry and properties of ferrate in drinking water treatment would be required to integrate its use into widespread, large-scale applications.
Ferrate has been considered to have advantages as an oxidant for water treatment and remediation because unlike chlorine, ferrate does not react with natural organic matter (NOM) to produce halogenated disinfectant by-products (DBPs) (Yates et al., 2014). Upon decomposition, it reduces to a non-toxic Fe(III) or an insoluble end-product ferric (III) hydroxide (Fe(OH)$_3$). Ferric hydroxide is a conventional coagulant, which means that ferrate has the possibility of being a dual-function chemical reagent that could possibly perform coagulation and oxidation in one step (DeLuca et al., 1983). This could result in a lower costs of operations and capital costs and could reduce the amount of other chemical additives.

### 2.1.1 Chemical and Physical Properties

Ferrate has a tetrahedral molecular structure and is characterized by its violet colour as a solid or when in aqueous solution. The tetrahedral structure of ferrate was confirmed by Goff and Murmann (1971). Each of the four oxygen atoms were found in this study to be kinetically equivalent (Figure 2-1)

![Structure of ferrate ion](image)

Figure 2-1: Structure of ferrate ion

It is a reactive substance with its enthalpy being $-481$ kJ/mole (Wood, 1957). When ferrate decomposes to ferric hydroxide and molecular oxygen, the violet colour disappears. The decomposition of ferrate is complicated and it has been proposed that multiple intermediate ions
may be generated (Perfiliev and Sharma, 2008). Ferrate is also proposed to have three resonance hybrid structures in aqueous solution as seen in Figure 2-2. It is suggested that the structure of 1’ and 2’ are the most prevalent resonance structures that exist in aqueous solution (Lee et al., 2004).

![Figure 2-2: Ferrate VI in aqueous solution with three resonance hybrid structures (adapted from Ghermaout and Naceur, 2011)](image)

### 2.1.2 Reaction and Kinetics

The chemistry of ferrate changes in aqueous solution and requires different pHs for different applications. There are four Fe (VI) species in aqueous solutions through acid-base equilibria. In neutral and alkaline solution, HFeO$_4$ and FeO$_4^{2-}$ are the predominant species (Figure 2-3).
In aqueous media, ferrate can be a powerful oxidant over a broad pH range. However, Fe(VI) is most effective at a slightly acidic pH of around 6.4. At lower pH levels, ferrate produces a redox potential of 2.20 V, while higher pH levels will yield lower redox potentials of around 0.72 V. This can be seen by the redox reactions of ferrate below:

\[
FeO_4^{2-} + 8H^+ + 3e^- \rightarrow Fe^{3+} + 4H_2O \quad E^0 = 2.20 \text{ V}
\]

\[
FeO_4^{2-} + 4H_2O + 3e^- \rightarrow Fe(OH)_3 + OH^- \quad E^0 = 0.72 \text{ V}
\]

The reactivity of ferrate with various organic compounds has been described as showing a second-order behavior (Sharma, 2013a) (Eq 1):

\[
-d[Fe(\text{VI})]/dt = k[Fe(\text{VI})_{\text{total}}][X]_{\text{total}}
\]

where k is the second order rate constant that is usually determined as a function of pH.
Most of the literature k-constants are at basic pH levels, but Fe (VI) exists in multiple dissociated states. The Fe (VI) in the four different pronation states below (Eq 2-4), have different reactivity states (Lee et al., 2004).

\[ H_3Fe_4^+ \leftrightarrow H_2FeO_4 + H^+ \quad pK_1 = 1.5^{21} \]  
\[ H_3FeO_4 \leftrightarrow HFeO_4^- + H^+ \quad pK_2 = 3.5^{22} \]  
\[ HFeO_4^- \leftrightarrow FeO_4^{2-} + H^+ \quad pK_3 = 7.2^{22} \]

At higher pH levels from 9 to 10, ferrate observes maximum stability (Luo et al., 2014). Increased stability in aqueous solution is also achieved at lower concentrations (Li et al., 2005). When ferrate is dissolved in water, oxygen is evolved and ferric hydroxide is precipitated. On its own, Fe (VI) as self-decay produces Fe (IV) and H\(_2\)O\(_2\) through a two-electron transfer with the initial step being rate limiting (Lee et al., 2014). Fe(IV) then reacts with H\(_2\)O\(_2\) generating Fe(II) and O\(_2\). Finally Fe(VI) further oxidizes Fe(II), producing Fe(III) and Fe(V) (k = \(\sim 10^7 \text{ M}^{-1}\text{s}^{-1}\)) (Lee et al., 2014). Since there are a variety of pathways in which Fe(VI) self-decays, the observed oxidation capacity of Fe(VI) is typically lower than expected theoretical values. Table 2-1 below shows the variation of kinetic constant (k) of Fe(VI) decomposition in accordance with pH.

<table>
<thead>
<tr>
<th>pH</th>
<th>K(s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1</td>
<td>39 x 10(^{-4})</td>
</tr>
<tr>
<td>8.0</td>
<td>16 x 10(^{-4})</td>
</tr>
<tr>
<td>9.0</td>
<td>6 x 10(^{-4})</td>
</tr>
<tr>
<td>9.2</td>
<td>1 x 10(^{-4})</td>
</tr>
<tr>
<td>9.4</td>
<td>1 x 10(^{-4})</td>
</tr>
<tr>
<td>10.3</td>
<td>4 x 10(^{-4})</td>
</tr>
<tr>
<td>11.9</td>
<td>8 x 10(^{-4})</td>
</tr>
</tbody>
</table>
As mentioned, the decomposition depends on pH, but also initial ferrate concentration, pre-existing ions, and the temperature of the solution. The higher the initial concentration of ferrate, the poorer the stability. Jiang and Lloyd (2001) found that if initial ferrate ion concentration was less than 0.025 M, 89% will remain after 60 min. If the initial ferrate concentration was greater than 0.03 M, almost all the ferrate ions were found to be decomposed for the same period of time.

Similar to other oxidants in use for drinking water treatment, ferrate is able to be quenched by reducing agents. The use of sodium sulfite (Li et al., 2008) and sodium thiosulfate (Lee et al., 2008) are common compounds used to quench ferrate.

**2.1.2.1 Potassium Ferrate (K$_2$FeO$_4$)**

Potassium ferrate (K$_2$FeO$_4$) is the most stable and easily prepared salt form that includes ferrate (Thompson et al., 1951). It is a black-purple compound in its solid state that can be stable for long periods of time in air without moisture (Figure 2-4). Its stability as a salt also is highly dependent on its purity (Li et al., 2005). Since it has a high stability it is used in the preparation of other ferrate salts such as SrFeO$_4$ and BaFeO$_4$ and AgFeO$_4$. 
The decomposition rate has been reported to be in the order of hours at higher relative humidity of 65-95%, while decomposition occurred over days at lower relative humidity of 55-60% (Machala et al., 2009). Storing potassium ferrate in a dry environment is essential for preventing self-decay. Nowik et al. (2005) found through Mossbauer studies of $\text{K}_2\text{FeO}_4$ that after a period of 14 months in a “not extremely well sealed” sample holder at room temperature, the product had 17% of the original Fe (VI), with the remaining 83% as $\text{FeO}_3$ nanoparticles.

Figure 2-5: The self decay of potassium ferrate in aqueous solution at 0.4, 1.0, and 2.0 gL$^{-1}$: (a) 7 minutes after preparations, (b) after 67 minutes at room temperatures (NanolIron, s.r.o.)
Figure 2-5 shows the self-decay of potassium ferrate over time in aqueous solution. The deep purple colour is more intense at a greater concentration and after decay, the solution takes the orange colour of the iron hydroxides formed. There has been research in the amalgamation of potassium ferrate with diatomite. Since diatomite has a highly porous structure with high surface area, it would allow a slow release of potassium ferrate into water to prolong the reaction time without adjusting for pH or compounds that may react with it quickly (Xu et al., 2014).

### 2.2 Ferrate Suppliers

While many benefits of ferrate have been investigated and proven, challenges remain in the implementation of the technology at full scale, in particular due to its instability in solution and its high production cost. There is little information on the use of ferrate in large-scale and real world applications. Further research would be required to understand the feasibility of ferrate in terms of operating costs and treatment performance.

NanoIron (Czech Republic) manufactures iron-based compounds with a variety of applications. Envifer is an iron product blend that contains various states of ferrate, including Fe(V) and Fe(VI), in a potassium blend; the main components being K₂FeO₄, K₃FeO₄, and KFeO₂. NanoIron claims the product is suitable for removal of arsenic, selenium, organic compounds, and microorganisms, such as Cryptosporidium, coliforms, and salmonella.

Ferrate Treatment Technologies, LLC, is one of the first companies to have commercialized ferrate manufacturing by providing an on-site synthesis option. Their patented reactor, the Ferrator®, is a small synthesis reactor that the company claims can produce large quantities of ferrate inexpensively through the use of caustic bleach and ferric chloride. The company reports that it therefore removes the difficulty of transportation, storage, and the high expense for bulk industrial use by producing a liquid product that can be pumped and injected directly (Ferrate Treatment Technologies 2016).
2.3 Ferrate as a Coagulant Aid

When ferrate is in aqueous solution, it is reduced to $\text{Fe}^{3+}$ or an insoluble precipitate end product, ferric hydroxide $\text{Fe(OH)}_3$ (Jiang and Lloyd, 2002a). As a result, ferrate is able to be a dual-function chemical reagent in water applications that may be able perform oxidation and coagulation in one step (Li et al., 2005). Graham et al. (2010) found that ferrate achieved comparable or better floc formation to ferric chloride over a broader dose at pH 5 and 7. However, it was found that there was a lower degree of organics removal than ferric chloride.

The coagulation properties of ferrate have demonstrated successful removal of a variety of compounds like arsenic (Lee et al., 2003), phosphate (Lee et al., 2009), plutonium (Potts and Churchwell, 1994), and NOM (Jiang and Wang, 2003). Studies have also shown that it is effective in the removal of colour (White and Franklin, 1998) and coagulating colloidal particles (Jiang et al., 2001). In many cases, ferrate has been effectively added in the preoxidation step and the coagulation step. Ma and Liu (2002) proposed that pre-oxidation improves the coagulation process by destroying the organic coating on the surface of particles and destabilizing them. Ferrate is more successful as a coagulant aid in the presence of particles (Ma and Liu, 2001).

2.3.1 Removal of NOM (Natural Organic Matter)

Ferrate can effectively coagulate NOM, humic and fulvic acids. Similar to the removal of most compounds, ferrate is more effective at coagulating dissolved organic carbon (DOC) in an acidic solution than in alkaline solutions. Jiang and Wang (2003) found that ferrate was able to perform better than ferric sulphate at lower doses in treating water containing humic and fulvic acids in the removal of DOC and THMFP. Ferrate was more effective than ferric sulphate at concentrations between 2 - 8 mgL$^{-1}$ Fe for DOC removal and for UV$_{254}$ absorbance removal. At these
concentrations, it was found that the coagulation pH level had no significant effect on the ferrate performance. On the other hand, at study by Jiang et al. (2006) found that the removal efficiency of DOC decreased when the ferrate dose was greater than 3 mgL\(^{-1}\).

Jiang et al. (2006) explored the use of potassium ferrate as a coagulant in water treatment and found that pH has a significant effect on the removal of NOM, with superior performance in removing fulvic acid between a pH of 6 and 8. It was found that the removal efficiency by ferrate was similar to that of typical drinking water coagulants, like alum, ferric sulphate, and ferric hydroxide. For a Fe(VI) dose of 2 to 46 mgL\(^{-1}\) as Fe, the removal was 21 to 74\% for mgL\(^{-1}\) humic acid. For fulvic acid, the removal was 48 to 78\% for 10 mgL\(^{-1}\) (Lim and Kim, 2010).

Ferric chloride and ferrate differ in the coagulation properties due to the oxidation effects of ferrate. As an oxidant, ferrate tends to increase the carboxylic functions, decreases the high molecular weight fractions, and increases the smaller ones (Graham et al., 2010). Furthermore, through ferrate oxidation, the humic macromolecular structures are cleaved into more hydrophilic, electronegative fractions, thus affecting the charge interactions.

When placed in a phosphate buffer, ferrate was observed to be unable to form insoluble coagulation products with humic acids, through Fe-phosphate complexation. Furthermore, the removal of humic acid increased at higher pH levels and higher doses of ferrate. Graham et al. (2010) observed a DOC reduction of almost 20\% with a concentration of 100 – 200 µM and a pH of 5. They also observed that ferrate and ferric chloride had similar optimal Fe doses to produce a maximum floc index, but ferrate was able to achieve better floc formation at doses above and below the optimal dose. Overall, the inter-related processes of ferrate oxidation, reduction, formation of Fe(OH)\(_3\), and adsorption are complicated and require further research.
2.3.2 Removal of Algae

Microcystins are a significant concern to public health as they are toxins released during cyanobacterial blooms in water. Yuan et al. (2002) found that there was successful oxidation of microcystin-LR through oxidation with Fe(VI). At a pH of 7 – 7.5, a ferrate dose of 20 mgL\(^{-1}\) and a contact time of 30 min was able to remove 93% of ferrate and 98% at 40 mgL\(^{-1}\). Below a pH of 6, the removal of microcystin-LR increased, however coagulation was not as evident. 200 mg of freeze-dried cyanobacteria was able to be treated with 100% removal of cyanotoxins with a dosage of 40 mgL\(^{-1}\).

Pre-treatment of water containing algae using potassium ferrate resulted in the reduction of the coagulant dosage required to cause efficient algae coagulation. Ferrate preoxidation was also able to inactivate the algae and act as a coagulant aid causing agglomeration of algal cellular particles without the addition of any coagulant (Ma and Liu, 2002b).

Jiang et al. (2014) discovered that the removal of microcystin-LR in buffer solution was much greater than in lake water due to the interference of dissolved organic matter in the water matrix. Fulvic acid up to 0.2 mgL\(^{-1}\) had no effect on the removal of microcystin-LR, however the removal efficiency decreased at greater concentrations. Water with a concentration of 6 mgL\(^{-1}\) of fulvic acid resulted in a 40% removal of microcystin-LR.

Yuan et al. (2002) determined that ferrate is able to treat various cyanotoxins because it is able to destroy the peptide toxin which is produced by various species of cyanobacteria. This destruction of the peptide toxin is pH dependent and effective at pH 6 – 10.

2.3.3 Effect on Turbidity and Suspended Solids

Studies have shown that ferrate is effective for removing colour (White and Franklin, 1998) and coagulating colloidal particles (Jiang et al., 2001). Preoxidation with ferrate produces an
enhancement in the coagulation of surface waters. Ferrate has been shown to remove turbidity. A study by Graham et al. (2010) observed that the maximum floc index at the optimal ferrate dose was 20 to 50% greater than ferric chloride over a much broader range of doses.

When water had preoxidation with 0.5 to 1.0 mgL$^{-1}$ of ferrate, there was a substantial reduction in residual turbidity after sedimentation. An addition of 0.5 mgL$^{-1}$ of K$_2$FeO$_4$ reduced residual turbidity after sedimentation by about a third. Turbidity and suspended solids were reduced by an even greater percentage by ferrate in waters with higher concentrations of organic content (Ma and Liu, 2002). A study by Jiang et al. (2006) determined that ferrate can remove 10 to 20% more UV$_{254}$ absorbance and DOC than ferric sulphate for the same dose in a pH range of 6 – 8. At a 50 mgL$^{-1}$ concentration, ferrate is able to remove 99% of the settled solids and 94% of turbidity in surface water. In fact, any concentrations of ferrate over 6 mgL$^{-1}$ results in over 80% removal of suspended solids (Qu et al., 2003).

### 2.3.4 Removal of Metals

Since ferrate is able to act both as an oxidant and a coagulant, it can remove certain metals. Ferrate encourages enhanced coagulation caused by iron colloids co-precipitating with metals. For example, in the removal of arsenite, ferrate is able to oxidize it to become arsenate, which is subsequently removed by adsorption through ferric hydroxide (Jain et al., 2009). Ferrate is effective in the removal of some metals and can destabilize colloidal particles, but it has no effects on the removal of Cr$^{6+}$ and Zn$^{2+}$ (Jiang and Lloyd, 2002b).

There are some significant risks due to arsenic in drinking water sources. Lee et al. (2003) added ferrate in concentrations of 2 mgL$^{-1}$ into river water with approximately 517 ppb arsenic and was able to reduce it to 50 ppb. Other studies showed that Fe(VI) was able to combine with As(III) to produce the highly insoluble ferric arsenate, which immediately precipitated and settled to the bottom of the solution (Vogels and Johnson, 1998).
In general, the removal efficiency of heavy metals (Cu, Mn, and Zn) increased with increasing pH at doses between 0.03 – 0.7 mM (as Fe). Not all metals are removed in a 1:1 ratio by Fe(VI). In the absence of NOM, Cu or Mn:Fe(VI) was a 1:1 ratio, while Zn:Fe(VI) was a 1:3 ratio. The presence of NOM requires larger concentrations of Fe(VI) to remove the same concentrations of heavy metals (Lim and Kim, 2010). Ferrate preoxidation also increased the removal efficiency of other heavy metals like lead and chromium. Removal of heavy metals increase with increasing pH (Ma et al., 2008).

2.4 Ferrate as an Oxidant

The oxidation potential of ferrate in an acidic solution is the strongest of all oxidants that are currently used in water treatment, such as chlorine, ozone, hydrogen peroxide, chlorine dioxide, dissolved oxygen, and permanganate (Jiang and Lloyd, 2002b). The half-cell reduction potential of ferrate has been estimated to be approximately +2.20 V in acidic solution and +0.72 V in basic solution (Wood, 1957).

<p>| Table 2-2: Oxidation-reduction potential of various disinfectants/oxidants |
|---------------------------------------------------|-----------------|-----------|</p>
<table>
<thead>
<tr>
<th>Disinfectant/oxidant</th>
<th>Reaction</th>
<th>( E^o ), V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine</td>
<td>( \text{Cl}_2(g) + 2e^- \rightleftharpoons 2\text{Cl}^- )</td>
<td>1.358</td>
</tr>
<tr>
<td></td>
<td>( \text{ClO}^- + \text{H}_2\text{O} + 2e^- \rightleftharpoons \text{Cl}^- + 2\text{OH}^- )</td>
<td>0.841</td>
</tr>
<tr>
<td>Hypochlorite</td>
<td>( \text{HClO} + \text{H}^+ + 2e^- \rightleftharpoons \text{Cl}^- + \text{H}_2\text{O} )</td>
<td>1.482</td>
</tr>
<tr>
<td>Chlorine dioxide</td>
<td>( \text{ClO}_2(aq) + e^- \rightleftharpoons \text{ClO}_2^- )</td>
<td>0.954</td>
</tr>
<tr>
<td>Perchlorate</td>
<td>( \text{ClO}_4^- + 8\text{H}^+ + 8e^- \rightleftharpoons \text{Cl}^- + 4\text{H}_2\text{O} )</td>
<td>1.389</td>
</tr>
<tr>
<td>Ozone</td>
<td>( \text{O}_3 + 2\text{H}^+ + 2e^- \rightleftharpoons \text{O}_2 + \text{H}_2\text{O} )</td>
<td>2.076</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>( \text{H}_2\text{O}_2 + 2\text{H}^+ + 2e^- \rightleftharpoons 2\text{H}_2\text{O} )</td>
<td>1.776</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>( \text{O}_2 + 4\text{H}^+ + 4e^- \rightleftharpoons 2\text{H}_2\text{O} )</td>
<td>1.229</td>
</tr>
<tr>
<td>Permanganate</td>
<td>( \text{MnO}_4^- + 4\text{H}^+ + 3e^- \rightleftharpoons \text{MnO}_2 + 2\text{H}_2\text{O} )</td>
<td>1.679</td>
</tr>
<tr>
<td></td>
<td>( \text{MnO}_4^- + 8\text{H}^+ + 5e^- \rightleftharpoons \text{Mn}^{2+} + 4\text{H}_2\text{O} )</td>
<td>1.507</td>
</tr>
<tr>
<td>Ferrate(VI)</td>
<td>( \text{FeO}_4^{2-} + 8\text{H}^+ + 3e^- \rightleftharpoons \text{Fe}^{3+} + 4\text{H}_2\text{O}^- )</td>
<td>2.20</td>
</tr>
</tbody>
</table>
2.4.1 NOM (Natural Organic Matter)

Ferrate is effective in reducing fulvic acid in water. A weight ratio of ferrate to fulvic acid of 12:1 is able to reduce 90% of fulvic acid. Furthermore, the adsorption and co-precipitation of fulvic acid to Fe(OH)_3, the precipitate produced by ferrate decomposition, can contribute to additional removal of fulvic acid (Qu et al., 2003).

Ferric chloride and ferrate were found to react differently with NOM and produce different resultant particles. In raw water, the addition of ferrate created more nanoparticles than with ferric addition. These nanoparticles have a negative surface charge, improving stable colloidal suspension, but are much smaller, which may indicate the need for additional coagulation (Goodwill, 2015). On the other hand, the nanoparticles also have a much higher surface area, which could result in higher adsorption capacities in the removal of arsenic or reduced manganese (Knocke et al., 1988).

However, NOM hydrophobicity and pH have an effect on the final particle characterization and need to be studied further with ferrate. One benefit of ferrate is that there is a higher external surface area to the same mass of ferric iron. When ferrate was added to a sample of water, it was found that ferrate attacked the electron-rich moieties in NOM molecules, but had limited capability to mineralize NOMs. Ferrate degraded the hydrophobic and transphilic fractions of NOM, but had difficulty decomposing the hydrophilic fraction. FEEM and FRI showed that ferrate was able to react with fulvic and humic-like substances and aromatic proteins, but was not able to decompose soluble microbial by-products and rarely oxidized aromatic proteins (Song et al., 2015).

Ferrate has been observed by Jiang and Wang, (2003) to have significant trihalomethane formation potential (THMFP) reduction. A 1 mgL⁻¹ dose as Fe of ferrate can reduce THMFP by about 80%. The same level of reduction was achieved with a 3 -4 mgL⁻¹ dose of ferric sulphate.
This study also found that ferrate was successful in removing humic and fulvic acids at low doses (< 2 mgL$^{-1}$ as Fe). Jiang et al., (2006) found that the greatest removal of humic substances also achieved the maximum DOC and THMFP removal.

### 2.4.2 Organic Compounds

Ferrate is able to oxidize organic compounds like organosulfur compounds, amines, phenols, alcohols, hydrocarbons, ascorbate, and pharmaceuticals. Similar to other compounds, the rate constant of these reactions decrease with an increase in pH. Generally, Fe(VI) oxidizes organic compounds with a 2-e$^-$ transfer step: the first electron transfer occurring with a step from Fe(VI) to Fe(V) and a second electron transfer step to Fe(III) as the final reduced product (Sharma, 2013b).

### 2.4.3 Inorganic Compounds

The oxidation of inorganic pollutants also takes place through a one-electron or two-electron transfer process. Sharma et al., (1997) describe the oxidation of hydrogen sulfide by ferrate through a one-electron transfer step ($\text{HFe}^{\text{VI}}\text{O}_4^- + \text{H}_2\text{S} \rightarrow \text{H}_2\text{Fe}^{\text{VI}}\text{O}_4^- + \text{HS}^-$). On the other hand the conversion of arsenite to arsenate by ferrate occurs with a two-electron transfer process ($\text{HFe}^{\text{VI}}\text{O}_4^+ + \text{As}^{\text{III}}\text{O}_3^{2-} + 2\text{OH}^- \rightarrow \text{HFe}^{\text{IV}}\text{O}_4^{3-} + \text{As}^{\text{V}}\text{O}_4^{2-} + \text{H}_2\text{O}$).

### 2.4.4 Formation of Disinfection By-Products (DBPs)

A study by Yang et al. (2013) observed the interaction of ferrate with source water and the production of disinfection by-products (DBPs). Using source water, the presence of DBPs was observed with and without ferrate for pre-oxidation followed by chlorination. The formations of
DBPs was also observed with varying pH levels. It was found that while increasing the pH from 5.0 to 9.0, THM formation decreased using ferrate, except at a pH of 9.0. Ferrate successfully decreased formation of DBPs at acidic pH levels, but this lessened with a more alkaline pH. Yang et al. (2013) also found that the formation of NDMA and THMs was reduced after ferrate was added following a ClO₂ treatment.

### 2.4.5 Bacteria and viruses

K₂FeO₄⁻² is a very strong oxidant in aqueous media. Following dissolution in water, ferrate can release large amount of atomic oxygen which is able to disinfect microorganisms, bacteria, and viruses effectively (Jiang et al., 2006). The effectiveness of ferrate has been observed on microorganisms, particularly coliforms (Murmann and Robinson, 1974), resistant bacteria and spore formers (Sharma et al., 2005), and parasites (Ling et al., 2010). From these studies, it was determined the ferrate was successful in producing a 3-log reduction of microorganisms in laboratory and pilot-scale experiments with a low dosage in wastewater.

A study on the treatment of the bacterial community in municipal secondary effluent compared ferrate to chlorine (Gombos et al., 2012). It was found that a low ferrate dose of 5 mgL⁻¹ Fe(VI) was effective to produce a 99.9% reduction in indigenous bacteria. Furthermore, a similar dose was able to inactivate chlorine-resistant bacteria.

Jiang et al. (2006) compared the performance of potassium ferrate to sodium hypochlorite in terms of inactivation of *Eschericha coli*, an indicator organism of fecal contamination in water. It was found that ferrate at a low dose was superior to sodium hypochlorite in the inactivation of *E.coli* in terms of less contact time required and dosage. Furthermore, the disinfection performance was less affected by solution pH and the rate was faster. Since ferrate seems to have higher oxidant strength at lower pH levels, its effectiveness in reducing microorganisms at higher pH is less effective. However, the other species at higher pH levels are effective in inactivating
E. coli; \( \text{HFeO}_4^+ \) and \( \text{H}_2\text{FeO}_4 \) were found to be 3 and 265 times as effective as \( \text{FeO}_4^{2-} \) in \textit{E. coli} inactivation (Cho et al., 2006).

Overall, ferrate was found to have performed better as an oxidant and coagulant, reducing 30% more COD and 3-log more bacteria compared to aluminum sulphate and ferric sulphate at the same or a smaller dose (Jiang et al., 2007).

Ferrate was able to inactivate bacteriophage coliphage Qβ effectively from pH levels of 6 to 8, but the inactivation rate depended on pH (Kazama, 1995). Ferrate has also been shown to rapidly inactivate \textit{Enterobacteriaphage f2} at low concentrations at pH 6 to 8 in water (Schink and Waite 1980). In the reduction of coliforms, a study by Kwon et al., (2013) found that Fe(VI) was faster in disinfecting coliform than chlorine of the same concentration.

Furthermore, ferrate has a steric resemblance to the phosphate anion and is therefore able to bind to enzyme phosphoryl groups. It is also able to act similar to phosphatase, dehydrogenase, phosphorylase, and phosphoglucomutase enzymes (Basu et al., 1987).

### 2.4.6 Emerging Micropollutants

There is increasing concern with pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) in water supplies. These are both considered emerging micropollutants because they can have significant effects on environmental and human health in low concentrations in the parts per billion to parts per trillion levels.

Since ferrate has not been investigated as widely as other oxidants in use, there has not been as much research into the abilities of ferrate in oxidizing emerging compounds. However, recent research has found that the aqueous reaction of ferrate with bisphenol A (BPA) was successful in removing approximately 90% of BPA after 60 seconds at a Fe(VI):BPA molar ratio of 5:1 (Zhang et al., 2012).
Furthermore, ferrate effectively removed 68 selected EDCs and PPCPs in a waste water matrix (Jiang et al., 2013). Ferrate treatment resulted in selective oxidation of the electron rich organic moieties of these compounds. Removal of pharmaceuticals like ciprofloxin and naproxen were 70% and 50% respectively. Acidic pharmaceuticals, like ibuprofen, showed less reactivity with ferrate (Jiang et al., 2013).

### 2.5 Production of Ferrate

#### 2.5.1 Electrochemical Production

Producing and storing ferrate is difficult and has resulted in no full-scale water treatment operations as of yet. Due to ferrate’s high reactivity, a more economical and feasible method of integrating it into a system would be through using on-site production.

There are currently three methods for synthesizing ferrate (Bielski and Thomas, 1987). The first is an electrochemical method using anodic oxidation with an iron or alloy as the anode and NaOH/KOH as the electrolyte. Efficient production using this method is dependent on the electrolyte temperature and current density. The second method, the dry method, requires various iron oxide-containing minerals. These are heated under high pH and oxygen. While this method is simple, it is dangerous and could cause detonation at elevated temperatures. The third method, the wet method, is where Fe(III) salt is oxidized under strong alkaline conditions and is also considered the most practical method. In this case, hypochlorite or chlorine is used as an oxidant.

#### 2.5.2 In-Situ Production

Jiang et al. (2015) investigated the efficiency of ferrate that was produced on site and compared it with FeClSO\(_4\) and poly-aluminum chloride (PACl) based on microbial, trace organic micro-
pollutant, turbidity, and DOC removal. The study was performed using raw water from the Danube River. Ferrate production was done by using an electrochemical reactor system with four iron electrodes made of steel iron plates for anodes and cathodes and concentrated sodium hydroxide solution as an electrolyte. The ferrate produced was then delivered at a dose of 0.5 mgL$^{-1}$ as Fe and was found to be sufficient to remove the majority of pollutants and achieved disinfection targets for the study condition. By comparison, FeClSO$_4$ and PACl were not able to remove any organic micropollutants. As a result, this study found that ferrate may have the potential to replace conventional coagulants and disinfection reagents when prepared in-situ.

### 2.6 Pilot Scale Tests

There have been several pilot-scale tests in recent years that have tested ferrate addition. The number of these studies in the literature have been increasing year by year. In a study by Jiang et al. (2015), a ferrate pilot-scale plant was observed to see whether it could be used as a replacement to an existing FeCl$_3$ drinking water treatment plant in the Zweckverband Landeswasserversorgung Water Board in Germany. Results from this experiment showed that the dose of 0.1 mgL$^{-1}$ ferrate can achieve an average particle removal percentage of 93 % for raw water and 97% for ozonated water. This experiment was compared to treatment with ozone oxidation and ferric chloride coagulation and it was found to have additional benefits in that it did not result in the production of N-nitrosodimethyl-amine (NDMA) after treatment. Furthermore, it was found that ferrate could replace both ferric chloride and hydrogen peroxide in meeting the performance needs.
2.7 ZEBRA AND QUAGGA MUSSELS

Zebra and quagga mussels (*Dreissena polymorpha, Dreissena rostiformis*) are invasive species that have spread throughout North America after being brought over by shipping vessels from Europe. They are now common in enormous numbers in freshwater systems and have been connected to rapid ecological changes and fouling on existing infrastructure (Brown and Stepien, 2010). They affect natural wildlife and the composition of the water, while also clogging up intake pipes of drinking water treatment and power plants, which could be causing about $5 billion per year worth of damages to these facilities (Khalanski, 1997). It is estimated that the United States spends about $1 billion per year in damages and control costs (Pimentel et al., 2005). Aside from the economic impact, the introduction of mussels have had a lasting impact on the dynamic of ecosystems, changing both inshore and offshore communities (Hecky et al., 2004)

The instances of invasion in North America have been prolific and have changed ecological systems significantly. With *Dreissena* being an already prevalent issue in freshwater systems, the addition of eutrophication events, additional invasive species, and climate change could be additional stressors that may create further ecological and critical issues in the future.

2.7.1 History of Zebra and Quagga Mussels

The zebra mussel species originated from the Ponto-Caspian region of central Europe (Smirnova et al., 1992) and made its way via ballast water transport to Lake St. Clair in 1986. They spread substantially through all of the Great Lakes, the inland waters of Ontario and Quebec, and 19 states in the USA by 1994 (Schloesser et al., 1996). Since then, the spread has been continued to the west and south of the continent.
The quagga mussel (*Dreissena rostriformis bugensis*) was the second mussel to invade and colonize North America since the arrival of the zebra mussel. The quagga mussel first settled in the eastern basin of Lake Erie in August 1989, but had spread through the Niagara River to Lake Ontario by 1990 (Dermott and Munawar, 1993).

Models have shown that dreissenids have spread by both natural processes and human transport. In North America, the spread was exacerbated by transport through the Mississippi River basin and through transport of improperly cleaned recreational boats. Spread of mussels can also occur through turtles, crayfish, and birds, but is not well-documented (Carlton, 1993). Figure 2-6 and Figure 2-7 show the extent of the spread of zebra mussels between 1986, 2013, and 2016 (Whittier et al., 2008) (USGS 2016a). Due to the rapid spread of the species, there has been an abundance of information of zebra mussels in North America. Intensive studies, recording, and observations have been able to curb the spread and mitigate the effects over time.

**Figure 2-6:** Spread of *Dreissena polymorpha* through North America between 1986 and 2013 (USGS 2016b)
As the most vulnerable bodies of water have already been colonized, the spread of the zebra and quagga mussels have slowed down (Johnston et al., 2006). Journals have said that the spread of mussels will eventually be limited by extreme temperatures, nutrient abundance, and inadequate calcium concentrations (Whittier et al., 2008). However, recent observations have shown that quagga mussels have possibly been able to adjust to both colder and warmer conditions outside their norm, which have significant implications for further spread (Thompson et al., 1997; Ginn et al., 2015).

**Figure 2-7:** Zebra and quagga mussel distribution in North America as of March 2016 (USGS 2016b)
2.7.2 Preferred Water Characteristics

*Dreissena* veligers and adults can populate an area and have an effect on an ecosystem rapidly. This can be attributed to the fact that a small proportion of zebra mussels—only two settled juveniles out of 10,000 produced—need to survive in order to maintain their population in a body of water (Lucy 2006). Once they have begun to settle in an area, they are able to reproduce and can become impractical and expensive to remove.

Dreissenids prefer higher temperatures (18–20 °C), but have an upper tolerance of about 26-32 °C (Strayer 1991). Veligers also require a pH level greater than about 7.3, so that it that their shell structure and reproduction is not affected. In fact, it was found that there was a 90% reduction in maximum settlement at a pH of 7.0 (Claudi et al., 2012a). Calcium is required for the growth of their shells and without its presence in a water matrix, veligers are unable to develop (Hincks and Mackie, 1997). The threshold of calcium for survival is approximately 12 mg/L, with an ideal concentration of 28.3 mgL$^{-1}$ (Ramcharan 1992). While these values may be at the threshold, infestation levels of abundance likely do not occur until the pH exceeds about 7.5–8.0 and calcium levels exceed at least 15–20 mgL$^{-1}$ (Mackie and Schloesser 1996). The geographical risk areas for *Dreissena* invasion associated with calcium concentration can be seen in Figure 2-8.
Figure 2-8: Geographical risk areas of zebra and quagga mussel invasion based on calcium concentration levels in surface water (Whittier et al., 2008)

In terms of depth, zebra mussel veligers are situated in the water column anywhere from 2 to 20 m below the surface, but this can change with wind velocities (Mackie and Schloesser, 1996). Veligers can be found deeper in the water column when there is no mixture from the wind (Fraleigh et al., 1993). This effect is more profound in lakes with large surface areas like in the Great Lakes. Conversely, quagga mussel have been observed to be less selective with depths, occupying the profundal zone in Lake Ontario (45 -110 m) and also seen at extreme depths of 130 m (Reid et al., 2010).
The main limiting factors that determine where *Dreissena* can inhabit is determined by their tolerance at the larval stages of their life. Research has shown that zebra mussels are able to live in slightly brackish conditions where the salinity does not exceed 8 to 12 mgL\(^{-1}\) (Kilgour et al., 1994). Overall, the North American mussel varieties have adapted more to temperature extremes than the European varieties. For example, Lake St. Clair populations are able to display shell growth at temperatures of 6 to 8°C. Shell growth has been also been reported to occur at temperatures as low as 3°C (USGS 2016a).

### 2.7.3 Ecology

Dreissenids are filter feeders that have an inhalant and an exhalant siphon. They can consume about one liter of water per day and select algae to consume. *Dreissena* in their veliger stage are most sensitive to temperature, oxygen, and pH variations. However, once they are attached to a surface in their adult stage, the lifespan can vary from 3 to 9 years. In terms of growth rate, dreissenids can see growth of up to 0.5 mm/day and 1.5 to 2.0 cm/year (USGS 2016a).

### 2.7.4 Life Cycle

There are several stages of *Dreissena* development. They begin in egg form and once they are fertilized, the veligers are formed within 3 to 5 days. Through the veliger stages (Figure 2-9) there is development of organs, siphon, foot, and blood. There is a subcategorization of veliger, postveliger, and juvenile stages: (veliger) preshell, straight-hinged, umbonal, (postveliger) pediveliger, plantigrade, and juvenile settling stage (Ackerman et al., 1994) (Figure 2-10). At the final postveliger stage, as seen in Figure 2-9d, byssal threads are released and it can then settle on a suitable substrate to become an adult mussel.
Figure 2-9: Growth stages of a *Dreissena polymorpha* veliger. (a) egg, (b) straight-hinged veliger 97-112 microns, (c) umbonal 112-347 microns, (d) pediveliger 231-462 microns (Oliva et al., 2014)

Under the microscope, veligers of *Dreissena bugenis* and *Dreissena polymorpha* take on a unique shape, distinct from other zooplankton that may appear in solution. Figure 2-11 shows the recognizable shapes of veligers in later stages of growth. These forms of veligers are ideal for use in settlement experiments, as they are ready to settle within days after these crucial stages of development.
Figure 2-10: Life stages of *Dreissena polymorpha* showing relative depth in water table. Double-headed arrows indicate the benthic stages where the mussels can translocate in water column (Ackerman et al., 1994).
2.7.4.1 Reproduction and Veliger Stage

Dreissenids have distinct sexes and any adult mussel over 8 mm in shell length will begin to develop ovaries and testes during the winter months. The reason for the prolific success of the dreissenids can be attributed to their characteristic of external reproduction. Large sheets of dreissenids are able to release eggs and sperms in great volumes, thereby exacerbating fouling issues. They reproduce by releasing eggs and sperm into the water as soon as the temperature reaches about 12°C for zebra mussels and as low as 4°C for quagga mussels (Mills et al., 1996). While eggs and sperm are released at this stage of their life, reproductive stages peak near the end of May and June, while the numbers of veligers does not peak until mid-summer. The gametogenesis stages of a mussel typically end in early fall, from late September to October, but this is different per season depending on temperatures (Ackerman et al., 1994).
Dreissena veligers hatch from egg capsules and become free-floating and free-swimming. At this stage, they are at their most vulnerable state. In the interest of water treatment plants, eradicating Dreissenids at this point are the most effective because they have not yet settled on a hard surface. Dreissenids feed in the veliger stage on phytoplankton for weeks in order to develop to a point where they develop organ systems. Straight-hinged larva have transparent shells and are quite sensitive to changes in the environment at this point. When they grow to their next stage, the umbonal veligers are rounder, have lines visible on their shell, and are a little bit more resilient. Their distinct organs are more visible and they are able to swim around in a circular motion (Sprung, 1993).

Finally, pediveligers are the final veliger stage that are on the verge of settling. The shell at this stage is much thicker and more opaque than in the earlier stages. A foot is now visible as an extendible muscular organ located in the mid-ventral region and it is used to crawl. A velum also becomes present which helps the pediveliger propel in swimming (Mackie and Schloesser, 1996).

While veliger and adult mussel activity is elevated above 12°C, the viability of eggs and sperm have different characteristics. Eggs and sperm viability appear to have an inverse relationship to water temperature. Eggs are viable for 5 h at 12°C and sperm 22 h. On the other hand, at 24°C, eggs and sperm are only viable for about 2 h (Ackerman et al., 1994). In areas where the temperature of water becomes warmer sooner, like in shallow bays and shorelines, veligers may become more concentrated (Ackerman et al., 1994).

2.7.4.2 Adult Mussels

Most zebra mussels are sexually mature at a shell length of 8 to 10 mm. However, some mussels can mature much earlier at 5 mm of length (Claxton and Mackie, 1998). Claudi and Mackie (1993) found that the mussels that were able to settle in June and July were able to reproduce by
August. Warmer temperatures allowed development to increase in speed, thereby allowing veligers to develop into their adult reproductive stages much more quickly.

Adult mussels are able to live for four to seven years depending on the species of the mussel. They are also able to grow up to 50 mm in length with the largest ones exhibiting the greatest filtration rates. The depth of maximum density is variable among lakes and varies by season (Mackie and Schloesser, 1996). A power plant in Michigan observed a population density on its pipes as high as 700,000/m$^2$ (Kovalak et al., 1993). They are able to attach to many substrates in the water column: quagga mussels can settle on sand and soft surfaces, while zebra mussels prefer rock, macrophytes, cement, steel, rope, or other animals, forming colonies called druses. Furthermore, adult mussels have been reported to migrate from shallow water in the summer, to deep water in the winter. Smaller mussels are able to translocate at higher rates than the larger mussels which have numerous, already established byssal threads (Martel, 1993).

### 2.7.5 Characteristics of Zebra and Quagga Mussels

#### 2.7.5.1 Zebra Mussels (*Dreissena polymorpha*)

Zebra mussels thrive exceptionally well in water where there is a high source of phytoplankton and food levels and high temperature. They can grow up to 27 times faster than quagga mussels at higher temperatures (Baldwin et al., 2002). As seen in Figure 2-12, there are physical differences between zebra and quagga mussels. Zebra mussels have a clear angle between then ventral and dorsal surface, while the quagga has a rounded one. They are also the smaller of the two and have the distinct black and white stripes along their shell.

In terms of habitat, zebra mussels prefer settling on harder surfaces and are able to withstand a greater flow rate and more turbulence than quagga mussels (Wilson et al., 2006). It is for this reason that it is possible to see zebra mussels on the shoreline of lakes, as opposed to lakebeds.
Quagga mussels were the secondary mussel that arrived in North America and were confined to the deepwater habitats of the lower Great Lakes. However, recently these mussels have been able to dominate many *Dreissena* habitats throughout many profundal and littoral zones of lakes (Baldwin et al., 2002). It had been noticed that in European lakes in places like Ukraine, the quagga mussels have already been observed to outcompete zebra mussels. There are concerns that based on these previous species shifts there could be an intensification of the effects of dreissenids in North America (Mills et al., 1996). It was found that quagga mussels survived as well as zebra mussels while having an equal or greater growth rate. Quagga mussels have been able to outperform zebra mussels by growing significantly during critical low food levels. In terms of morphology or behavior, no significant differences were found (Wong et al., 2011).

Work by Ginn et al. (2015) has shown that there has been a quick speciation change from zebra mussels to quagga mussels in Lake Simcoe. This could possibly be attributed to the behavioural characteristics exhibited by quagga mussels. Quagga mussels inhabit and proliferate in the
profoundal and littoral zones of a lake, indicating that they can withstand different temperature and food regimes than zebra mussels (Baldwin et al., 2002). Further exacerbating their spread is their ability to settle, grow and spawn over a larger depth and temperature range. This has been specifically shown in Lake Simcoe where quagga mussel have been found settling at depths greater than 20 m and showing significant activity around 4°C (Spidle et al., 1995). In fact, a female quagga mussel with mature gonads was found in Lake Erie at a temperature of 4.8 °C (Claxton and Mackie 1998). Other studies have shown that quagga mussels can be present due to the decrease of phytoplankton in the Great Lakes, since quagga mussels can survive on lower levels of food (Roditi et al., 2000). As lakes across North American begin to decline in phytoplankton population, quaggas have become the dominant species (Ginn 2015).

Overall, quagga mussels have a longer shell length, which may mean that they have a greater longevity or growth rate compared to zebra mussels (Mackie, 1991). Baldwin et al. (2002) found that quagga mussels grow as well or better than zebra mussels with a higher instantaneous growth rate. In fact, under all conditions of food type, level, and temperature, quagga mussel growth was unaffected and was as much as 19 times greater than zebra mussels. As a result, the transition of zebra mussels to quagga mussels is thought to possibly intensify the ecological impacts that dreissenids have as an invasive species in North America.

2.8 Lake Simcoe Mussel Distribution

Zebra mussels were first detected at a density of 12 veligers/m³ in one site in August 1992 and then at greater densities at three other sites in August 1994. During mid-June to August 1995, it was estimated that there were about 11,249 – 31, 477 settles/m²/day in certain sites in Lake Simcoe’s main basin (Evans et al., 2011). Since then the densities of zebra mussels have risen and fallen and have now been replaced by the more resilient quagga mussel.
The rapid growth of quagga mussels has occurred in the profundal and littoral zones where phytoplankton biomass has declined (Idrisi et al., 2001). Mussels have not been as present in the profundal zones, which may mean that there will be declines in native macroinvertebrates that may in turn affect fish production and the rest of the food chain (Nalepa and Schloesser, 2013). There is also concern that since the quagga mussels operate at temperatures as low as 6°C, they will feed more actively during the spring and consume a large portion of spring phytoplankton blooms that are essential for ecological stability.

A study was performed in Lake Simcoe in 2009 and again in 2015 by the Lake Simcoe Region Conservation Authority (LSRCA) in order to map the species distribution of zebra and quagga mussels over six years. Ponar grab samples were taken at 747 sites in 2009 and 715 sites in 2015. The mussels were evaluated to determine their species, length, wet weight, and dry weight (Table 2-3) below.

Table 2-3: Summary of data for zebra and quagga mussel study in Lake Simcoe in 2009/2015 by LSRCA (Ginn et al., 2015)

<table>
<thead>
<tr>
<th></th>
<th>2009</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean Abundance (# / m²)</strong></td>
<td>2300</td>
<td>1463</td>
</tr>
<tr>
<td><strong>Mean Biomass (g / m²)</strong></td>
<td>11.5</td>
<td>15.7</td>
</tr>
<tr>
<td>% zebra mussels</td>
<td>84</td>
<td>12</td>
</tr>
<tr>
<td>% quagga mussels</td>
<td>16</td>
<td>88</td>
</tr>
<tr>
<td>% profundal sites with mussels</td>
<td>12</td>
<td>78</td>
</tr>
</tbody>
</table>

The results of this intensive *Dreissena* sampling showed that in the past six years there have been some significant changes in the species distribution of mussels in Lake Simcoe. Figure 2-13 shows that there is an abundance of zebra mussels mostly in the shallower areas of Lake Simcoe.
and that they are the predominant species. Meanwhile, quagga mussels had been reserved to the northeastern area of the lake and the deeper regions.

Figure 2-13: Zebra and quagga mussel density map of Lake Simcoe in 2009 (Ginn et al., 2015)

In 2015, when the study was performed again, sampled at 715 sites, it was found that quagga mussels had become the dominant species out of the two. Quagga mussels had outcompeted the zebra mussels and flourished within a small time frame. Figure 2-14 shows that zebra mussels are now confined to the Cook’s Bay area and the western arm in Kempenfelt Bay. Conversely, the quagga mussels have significant densities all throughout the lake.
It is assumed that the reason for this can be attributed to the food sources in Lake Simcoe. Since the lake was in such abundance of zebra mussels, the phytoplankton that they fed on began to become short in supply. Quaggas are able to survive on less of a diet and may have outcompeted the zebra mussels.

This creates some concerns since quagga mussels are the larger of the two species and may be able to foul surfaces more easily. Moreover, they are able to withstand greater temperature extremes, reproducing even under cold temperatures. Since a lot of pipe infrastructure is located at greater depths, the existence of quaggas on the lakebed could cause more significant fouling issues (Ginn et al., 2015).

Figure 2-14: Zebra and quagga mussel density map of Lake Simcoe in 2015 (Ginn et al., 2015)
2.8.1 Treating Dreissenids

The identification of veliger appearance in a treatment plant’s source water may be critical for the implementation of a control program. Continuous monitoring identifying reproduction and settling periods may optimize success. The most important sources of veligers are ones upstream of the facility’s uptake system. According to Mackie (1991), the adult mussels that settle in or around the intake pipe do not contribute directly to the increased settlement that may occur there later.

While pre-oxidation through a low concentration of chlorine or ozone is conventionally added near the intake pipe of a system, this is only really effective in preventing settlement around the intake pipe system. In order to address veliger and mussel development inside a water treatment plant, the plant processes may need to be adjusted. Typically, the addition of a coagulant and settling in the plant will help any infiltration further down the treatment train. Creating an environment that is unfavourable to mussel inhabitation is essential in preventing infiltration and settlement (Claudi and Mackie, 1993).

2.8.2 Dreissena Control and Removal

Research by Flemming et al. (2008) has indicated that a low level of chlorination (0.5 mgL\(^{-1}\)) can provide an adequate maintenance dose to prevent the establishment of dreissenids as a preventative measure. However, low doses do not seem to have a significant effect on dreissenids that have already reached adulthood. Two consecutive shock doses of 200 mgL\(^{-1}\), once every 24 hours was able to produce 100% mortality in 9 days (Khalanski, 1993), however lower shock concentrations may not actually kill established adult mussels, but may encourage translocation. While exposure to chlorine may not kill veligers or adult mussels, it can shorten their lives. Despite the fact that 99% of veligers do not survive the post-veliger stage to settle and
become adults, under favourable conditions, very few surviving settled veligers are needed to reproduce to start a successful colony (Stanczykowska and Lewandowski, 1993).

Aside from oxidation, there are other options for removal in a plant where dreissenids are already established. Oxygen removal and lowering the pH of the system can kill *Dreissena* veligers (Claudi et al., 2012b; Hincks and Mackie, 1997). As a result, acid washes of a system can be effective in eradicating existing populations. Alkalinity and hardness are both factors required for survival, as they need both to produce their shells. Finally, aggressive and energy intensive measures such as a hot water wash of 60°C for 5 s can kill 100% of dreissenids (Comeau et al., 2011).

Distilled white vinegar with a concentration of 5% acetic acid has been suggested by some United States government agencies to decontaminate adult mussels. In fact, a study by Davis et al., (2015) observed that concentrations of 25, 50, 75, and 100% of 5% acetic acid caused 100% mortality in adult mussels within four hours.

Other proprietary treatments, such as EarthTec®, are also effective in treating dreissenids. EarthTec® is a copper sulfate pentahydrate with a proprietary base acid, cupric acid, as its active ingredient, and has applications as an algaecide and a bactericide. Using EarthTec®, a 100% veliger mortality was found within 30 min at 3 ppm (Watters et al., 2013). Although much higher doses were required to kill adults depending on their maturity, a concentration of 1 ppm was able to deter colonization.

Several additional experiments on dreissenids in the literature have been compared in Table 2-4 below.
2.8.3 Response of *Dreissena* Veligers to Oxidants

A study was performed by Van Benschoten et al., (1993) to observe the use of sodium hypochlorite, potassium permanganate and hydrogen peroxide on veligers in the control of zebra mussels at water plant intakes. The study used static measurements to observe general trends in the reaction of veligers to exposure. Veligers were collected using an 80 µm net with a 250 µm sieve. The veligers were exposed in separate beakers to 0.5, 1.0, and 2.5 mgL\(^{-1}\) of each of the oxidants. Veligers were observed under 40x stereo magnification and determined whether they were alive or dead. Confirmation of mortality was determined by observing ciliary movement inside the translucent shell or extended velum. The results showed that sodium hypochlorite was the most effective oxidant in comparison to potassium permanganate and hydrogen peroxide. There was an increase in mortality with increasing exposure time to each of the oxidants.

The study also observed an experiment with more practical applications in a flow-through experiment. Unlike a static experiment, a flow through experiment observes hydraulic conditions closer to those that would be seen near an intake pipe. The flow rates of raw water entering the boxes were controlled by in-line valves at 1 Lmin\(^{-1}\). Stock solutions were added at a rate to maintain residual oxidant concentrations of 0.5, 1.0, and 2.5 mgL\(^{-1}\). The experiment was run for several days and instead of observing veliger response to the addition of an oxidant, the ability for mussels to adhere to the surface of plastic, glass and metal plates were observed.

In this study, it was found that static exposures to chlorine showed that intermittent treatments were much less effective than continuous treatment. The mussels that were exposed to oxidants spent less time filtering and displaying active behavior than those that were unexposed. Under intermittent exposure, the mussels were able to not be killed by simply closing their shells and avoiding the treatment. However, with continuous exposure, the mussels cannot live by remaining closed.
In the flow-through experiment, the authors came across many problems which prevented them from determining a conclusion. The main reason was because veligers settle during a specific time period in their growth period. While the flow-through system always had a fresh supply of veligers passing through, there may not have been an adequate density and they also may not have been in the right stage of their lives.
Table 2-4: Literature comparison of zebra mussel experiments

<table>
<thead>
<tr>
<th>Journal Article and Year</th>
<th>Experiment</th>
<th>Results</th>
<th>Apparatus</th>
<th>Methods</th>
<th>Possible Limitations and Considerations</th>
</tr>
</thead>
</table>
| Van Benschoten et al., (1993): Response of Zebra Mussel Veligers to Chemical Oxidants | Response of zebra mussel veligers to chlorine, ozone, hydrogen peroxide Niagara River | • prolonged exposure resulted in veliger mortality  
• 90% removal of veligers with 20 h exposure  
• ozone and chlorine produced similar results - H2O2 required greater concentrations | • Baffled flow-through tanks  
• HRT = 30min  
• Flow rate 1 L/min  
• Settling plates to observe veliger mortality  
• Oxidants were introduced to the water via pump at the bottom of chamber | • 500 to 1000 veligers per replicate  
• Chlorine - stock solution continuously pumped  
• H2O2 - continuously fed in pulse mod for 30 min every 12 h  
• single ozone contact chamber between constant head tank and baffled tanks  
• conducted from 1 to 18hours continuous exposure at 0.5 and 1.0 mg/L | • Non-attachment or mortality depends on hydraulic conditions and exposure time  
• Pulse of oxidants vs. continuous stream |
| Klerks and Fraleigh (1991): Controlling Adult Zebra Mussels with Oxidants | Effects of continuous and intermittent hypochlorite, permanganate, and peroxide with iron were compared for effectiveness against adult zebra mussels - Lake Erie | • Hypochlorite > permanganate > peroxide iron  
• intermittent treatments were ineffective (mussels closed up during treatment)  
• Oxidants less toxic at lower temperatures | • water pumped continuously into headboxes, which provided gravity flow into clear acrylic bioboxes  
• flow rate 1 L/min solutions pumped by peristaltic pumps at 2mL/min  
• Static mixers built into line between injection point and | • only 10-15mm mussels used  
• Applied continuously for 28 or 56 days in flow through systems | • Temperature  
• Size |
<table>
<thead>
<tr>
<th>Study</th>
<th>Methodology</th>
<th>Findings</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angarano et al., (2009):</td>
<td><strong>Cannabinoids inhibit zebra mussel byssal attachment: a potentially green antifouling technology</strong> 9 cannabinoid compounds were tested for their ability to inhibit zebra mussel byssal attachment, six efficacious compounds, however some exhibited toxicity towards non-target organism. Beakers each with 8 mussels were used with methanol as another control. Compounds were diluted to 1:1000 v/v and added to each beaker. Observed mortality and byssal attachment during 48 h exposure and 48h post exposure. Attached mussels were cut from the byssus and placed in new container with distilled water to observe reattachment.</td>
<td>• six efficacious compounds, however some exhibited toxicity towards non-target organism. • beakers each with 8 mussels. • used methanol as another control. • compounds were diluted to 1:1000 v/v and added to each beaker. • observed mortality and byssal attachment during 48 h exposure and 48h post exposure. • attached mussels were cut from the byssus and placed in new container with distilled water to observe reattachment.</td>
<td>• non-target organisms • observed post-exposure period • static water • 8 mussels is not a large sample • observes reattachment</td>
</tr>
<tr>
<td>Mackie and Kilgour, (1995):</td>
<td><strong>Efficacy and role of alum in removal of zebra mussel veliger larvae from raw water supplies</strong> Determine if alum kills veligers - lake Erie Alum is not sufficient to kill veligers, prechlorination removed veligers best. Additions of alum which acidified water killed veligers. A 100 mL of solution of lake water placed in 100 mm dia x 50 mm high culture dish. Observations made by stereo microscope, recorded as living or dead. Many replicates because observations were hard to record depended on numbers of veligers or definition of &quot;dead&quot;.</td>
<td>• Alum is not sufficient to kill veligers, prechlorination removed veligers best. • additions of alum which acidified water killed veligers.</td>
<td>• many replicates because observations were hard to record depended on numbers of veligers or definition of &quot;dead&quot;.</td>
</tr>
<tr>
<td>Rajagopal et al., (2002b):</td>
<td><strong>Does status of attachment influence survival time of zebra mussel</strong> Observing how the status of attachment could influence the survival of zebra mussels. Attached ZM are more resistant to oxidation than unattached ZM (27% increase of survival for attached ZM). Continuous once through flow-system (20 L tanks having an outlet at 17L mark) via peristaltic pump. Mussels used from same acclimatized stock for experiment.</td>
<td>• Attached ZM are more resistant to oxidation than unattached ZM (27% increase of survival for attached ZM). • peristaltic pump</td>
<td>• attached vs. unattached • are they naturally unattached or scraped off? • stress due to removal</td>
</tr>
<tr>
<td>Exposure to Chlorination?</td>
<td>Toxicity Response of Mussels</td>
<td>Mixing Pumps for Chlorine</td>
<td>About 100 Mussels Per Tank</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------------------</td>
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</tr>
<tr>
<td>Meehan (2014): Assessment and Utilization of Zequanox for Zebra Mussel Control in Irish Waters</td>
<td>Observing the effects of Zequanox on dreissenids in field tests</td>
<td>Mortality rates in Ireland were less than in North America, possibly due to higher metabolism due to temperature</td>
<td>Various solutions added to the jars</td>
</tr>
<tr>
<td>Meehan et al., (2014): Zebra Mussel Control using Zequanox in an Irish Waterway</td>
<td>Observing the effects of Zequanox on veligers and juvenile ZM</td>
<td>Mortality of ZM was attributed to Zequanox treatment</td>
<td>Settlement plates used to gather natural mussel settlement and collected 7 weeks after deployment</td>
</tr>
<tr>
<td>Cottrell, (2000): Zebra Mussel Adhesion and Aspects of Its Prevention Using Copper</td>
<td>Observe adhesion strength of zebra mussels</td>
<td>Wall-jet apparatus used to observe shear stress required to disrupt ZM adhesion</td>
<td>Adhesion only, no testing on ZM mortality</td>
</tr>
<tr>
<td>Wildridge et al., (1998): Acute Toxicity of Potassium to the Adult Zebra Mussel</td>
<td>Toxicity of potassium was tested on Lake Ontario ZM</td>
<td>K⁺ was not found to be useful in methods to control mussel infestations due to recovery after 96h</td>
<td>Mussel removed from Lake Ontario in May through August and held for 2 weeks in 19L aquarium with Lake Ontario water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 mussels placed in 250 mL beaker with lake water</td>
<td>Static concentrations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beakers were placed in a water bath that maintained temperature</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mussel removed from Lake Ontario in May through August and held for 2 weeks in 19L aquarium with Lake Ontario water</td>
<td>Mussel may be nonresponsive after treatment and may not respond to probing. However, after a dormancy period, they</td>
</tr>
</tbody>
</table>
beaker 48 h before test
- after exposure to substance for a period of 6, 12, and 24 hours, beakers were replaced with fresh lake water
- mussels were tested for responsiveness every 24 h
may become active again
- K+ was supposed to inhibit valve closure

| Waller et al., (1993): Toxicity of Candidate Molluscicides to Zebra Mussels and Selected Nontarget Organisms | • 18 chemicals were selected for two sizes of zebra mussels and two nontarget fish - Lake Erie | • two to three times more toxic to ZM than the nontarget species | • Mussels were held in 2000L aquaria with municipal water
- ZM exposed in 3.8L glass jars with 2.5 L test water without aeration | • (5-8mm) small and (20-25) large ZM were examined and placed in petri dishes and allowed to attach•those attached were used for testing10 mussels per jar with an exposure of 48 h
- mortality recorded at 48h and placed in untreated water for 48h to observe delayed mortality | • ensure candidate mussels are able to form byssus threads• observe delayed mortality and delayed reattachment |


2.8.1 Contaminants and Concerns

The arrival of dreissenids in North America has contributed to substantial economic and ecological ramifications. The economic effects, destruction of sport fisheries, and the decline of native shellfish contributed to a $4 billion loss in the first decade (Roberts, 1990). Zebra mussels greatly affected the planktonic food webs through the mass consumption of phytoplankton and small zooplankton. In fact, in some lakes, the population of phytoplankton and zooplankton reduced by more than 50%, leaving inedible or toxic phytoplankton, such as microcystins, behind (Vanderploeg et al., 2001). With a reduction in the planktonic concentration in the water, the clarity of the water in bodies of water greatly improve, along with concentrations of soluble nitrogen and phosphorus. These nutrients contribute to eutrophication and the growth of rooted plants and attached algae (Zhu et al., 2006). This creates an increase of population in the littoral zones, but affects the fish in the benthic areas and large zooplankton. The excessive fouling and competition for food caused by the presence of dreissenids has caused many native populations of mussels to decline substantially (Strayer and Malcom, 2007). Furthermore, the dense *Dreissena* populations have reduced dissolved oxygen in the water column and have reduced concentrations of calcium carbonate in the summer, further improving the clarity of the water at a greater extent than the reduction in phytoplankton (Barbiero et al., 2006).

An additional issue of the presence of dreissenids is their contribution to the biomagnification of heavy metals and polychlorinated biphenyls (PCBs). This has also led to avian botulism due to the consumption of dreissenids by birds (Bruner et al., 1994).
2.9 REFERENCES


Khalanski, M. (1993). Testing of five methods for the control of zebra mussels in cooling circuits of power plants located on the Moselle River (Electricite de France (EDF)).


Lim, M., and Kim, M.-J. (2010). Effectiveness of Potassium Ferrate (K\textsubscript{2}FeO\textsubscript{4}) for Simultaneous Removal of Heavy Metals and Natural Organic Matters from River Water. Water, Air, Soil and0 Pollution 211, 313–322.


and Habitat Evaluation of the Upper Great Lakes Connecting Channels, (Springer), pp. 251–268.


Wood, R.H. (1957). The heat, free energy, and entropy of the ferrate (VI) and selenide ions (University of California Radiation Laboratory).


3 ACUTE VELIGER TOXICITY: COMPARISON OF FERRATE AND CHLORINE

ABSTRACT

Zebra and quagga mussels (Dreissena polymorpha and Dreissena rostriformis bugenis) are common, non-native freshwater mollusks found in abundance in major waterways in eastern North America. The spread of these invasive organisms has resulted in the disruption of ecosystems and biofouling of critical water infrastructure, such as water intake pipes. Currently, chlorine has been used as the principal disinfectant for Dreissena control, but its use in the pre-treatment stages contributes to the increase of disinfection-by-products (DBPs), which can possibly be harmful to human and environmental health (Rajagopal et al., 2002a).

In this research, acute toxicity tests were performed to observe ferrate (FeO4^2-) as a possible alternative in Dreissena control instead of chlorine. Clusters of Dreissena veligers in the pediveliger and veliger life stages were exposed to varying one-time doses of ferrate and chlorine (7.0, 14.1, 28.2, 42.3, 70.5, 141.0 µM). The resulting behaviors over a 30 minute period of exposure were observed.

Overall, results showed that Dreissena veligers are inactive (dead or dormant) for longer periods of time when exposed to the same molar concentration of ferrate over chlorine. After 30 minutes, ferrate had a 30% increased effectiveness in the
inactivation of veligers over chlorine. For concentrations of ferrate over 14.1 µM, 100% of veligers remained in an inactive state for the duration of the experiment, while chlorine led to 72.3% of veligers inactive under the same molar concentration. The percentage of veligers that were dead, dying, or inactive as a result of varying molar concentration over time was overall more effective with ferrate. Furthermore, the veligers were able to recover with a reduction in oxidant residual, which indicates that a one-time dose of oxidant may not be effective to prevent future veliger settlement.

3.1 INTRODUCTION

Chlorine is the primary method of treatment used by treatment operators to prevent the fouling of intake pipes by zebra and quagga mussels. It is economical, effective, and the most regularly used technique in North America, Asia, and Europe (Rajagopal et al., 2002b). While chlorine is an oxidizing biocide that can treat all sorts of fouling organisms, there is a concern that raw water treated by a chlorine pre-oxidation could have environmental and health-related ramifications. Chlorine addition can produce toxicity to non-target organisms and when combined with natural organic matter (NOM), could produce potentially harmful disinfection by-products (DBPs) (Jenner and Janssen-Mommen, 1993). As a result, many forms of potential mussel treatment have been examined, such as potassium permanganate, ozone, chlorine dioxide, EarthTec®, and heat treatment (Claudi and Mackie, 1993; Harrington et al., 1997; Van Benschoten et al., 1993; Watters et al., 2013). Chemical control is often dosed during summer and autumn seasons at low-concentrations at intake pipes to provide a constant, small residual of oxidant to prevent settlement (Jenner and Janssen-Mommen, 1993).
In the interest of water treatment plants, eradicating dreissenids in the veliger stage of their life cycle is effective because they have not yet settled on a hard surface and become more resistant to disinfectants. While mussels become problematic in their adult stage, veligers are the larval state of bivalve mollusks and also when they are the most vulnerable. They hatch from egg capsules and become free-floating and free-swimming. These veligers float and swim around until they reach a certain age and then settle where there is ample food and a suitable surface to attach to (Marsden, 1992).

This experiment observes how veligers react after different concentrations of chlorine or ferrate are added under static conditions. There is a misconception that in order for a veliger to not attach to a surface, it must be dead. On the contrary, veligers exhibit a variety behaviors in response to exposures to different environments, many of which are unable to attach to surfaces. In fact, veligers require a significant concentration of chlorine in order to be killed. In fact, only 40% mortality is achieved in concentrations of 10 mgL\(^{-1}\) (Claudi 1997). As a result, it is more effective and efficient to focus on the concentrations that will illicit the minimal response that will prevent them from simply adhering to surfaces.

This experiment examines the behaviors of veligers after being exposed to chlorine and ferrate of different concentrations. Ferrate was compared to chlorine in order to observe the effects of a potential novel treatment to a currently widely-used one.

### 3.2 Objective

The purpose of this experiment is to observe the behavioral response of zebra and quagga mussel veligers after being exposed to a one-time dose of ferrate or chlorine at dosages of 7.0, 14.1, 28.2, 42.3, 70.5, and 141.0 µM. The observed veliger behavioral responses include: active (alive swimming, filtering), inactive (closed with no observable movement), or dead. These states were determined using a stereo microscope regularly over an oxidant-exposure period of 30 minutes in
order to simulate the span of time of a one-time dose at the head of an intake pipe at a water treatment plant. Since a plug of water travelling into a water treatment plant typically only is pre-treated once and does not undergo any further dosing, it is important to determine if the veligers contained in this volume of water have the ability to settle as they travel along the pipe and into the plant. Inactive or dead veligers are unable to release their byssal threads and attach to a surface and as a result, are the preferred states in the prevention of veliger settlement.

3.3 MATERIALS AND METHODS

3.3.1 Sample Source and Site Description

Experiments were performed at the Glenora Fisheries Station (Ontario Ministry of Natural Resources, Lake Ontario Management Unit) in Glenora, Ontario. The source water for this experiment was collected from the Bay of Quinte in Lake Ontario. Veligers were retrieved Sept 2, 2015 from Station 81, a Department of Fisheries and Oceans (DFO) buoy located in the Kingston Basin of Lake Ontario (44 01.01N, -76 40.13W) (Figure 3-1).

All samples were collected using a plankton net with a 63 µm mesh size (= size 25:200 mesh openings per inch). The concentrated solution was strained through a coarse sieve of 250 µm in order to remove large plankton and algae that may confound the microscopic analysis, as well as possible predators. For this analysis, the desired veliger sizes are between 80 to 230 µm. Since veligers have no real protection in their early stages of life, they are vulnerable to all predators that consume zooplankton at the microscopic scale: small fish, copepods, freshwater Cnidaria (hydras), and freshwater sponges (Liebig and Vanderploeg, 1995).
3.3.2 Chemicals and Reagents

Potassium ferrate was purchased in May 2012 from Ferratec, a ferrate manufacturer. All remaining chemicals including potassium ferrate, sodium hypochlorite, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), sodium phosphate (Na$_2$HPO$_4$), sodium borate (Na$_2$B$_4$O$_7$·10H$_2$O), and acetic acid were purchased from Sigma Aldrich. Stock solutions and buffers were prepared using ultrapure water from a Milli-Q® system. Ferrate analysis was performed using the direct and indirect UV-spectrometry method (Appendix B).

3.3.3 Veliger Sample Processing

Following collection from the lake, the veliger solution was sieved at 250 µM to remove larger predators. The veligers were transported in Nalgene bottles and placed on ice in a cooler to slow down their physiological functions.

In the lab, the veligers were diluted with coarse-sieved lake water and stored in 4°C. All samples of veligers were brought to room temperature prior to testing to ensure that they had acclimated and had similar rates of activity.
Typically, veligers were used for experiments before four days had passed in order to reduce the effects of limited food, oxygen, and waste concentration. Veligers kept in cool storage for longer than four days had a greater number of initial mortality and were found to die in experiments more quickly. While veliger collections were able to remain alive for a maximum of about 10 days, different exposures to stress, concentration of veligers, food levels, water quality, and oxygen fluctuated during their life span in the container. Bacteria was also found to be more active in older veliger solutions, which made counting difficult and mortality higher. Details about the methodology for veliger analysis and processing can be found in Appendix B.

3.3.4 Veliger Analysis

The beaker containing the solution from the plankton net was set aside to allow veligers to settle to the bottom and warm it up to room temperature. A 5 mL pipette was used to extract a sub-sample from the bottom of the beaker and transfer it to a small Petri plate. Both stereo and compound microscopes were used to identify veligers. The compound microscope was a Nikon E600 Eclipse 100x. Using a glass 1 mL Sedgewick-Rafter cell, 1 mL samples of solution were observed at a time. Depending on the clarity of the matrix of the solution, small beige dots near the bottom of the dish are veligers visible to the eye. Figure 3-2 below shows veligers in a 5 mL Petri dish. Each white-beige particle is an individual veliger at about 2-5 weeks of age.

Pasteur pipettes were used to transfer the solution to Sedgewick-Rafter cells and Petri dishes. The turbidity, matrix, and plankton content of each solution was different and veligers tended to stick together in clumps and together with any algae that may be present (Claudi 2015a).
3.3.5 Veliger Behavior

Veligers were observed through stereo and compound microscopy to determine their present activity. Since veliger behaviours can be difficult to conclude definitively, veligers were categorized into two distinct categories: active or inactive. These categories of veliger status were further subdivided into four different behaviors: alive with protrusion, alive, closed, or dead (Table 3-1). Details of the protocol are in Appendix B.
Table 3-1: Characteristics and appearance of active and inactive veligers

<table>
<thead>
<tr>
<th>Category</th>
<th>Sub-category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active (able to settle on surfaces)</td>
<td>Swimming</td>
<td>Highest activity level and occurs at room temperature or around 20 – 24 °C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Movement in circular pattern using its velum as propulsion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Able to release byssal threads to settle</td>
</tr>
<tr>
<td></td>
<td>Open</td>
<td>Shell open</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Velum protruding or foot outside of shell</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rolling, rotating, pulsing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Able to release byssal threads to settle</td>
</tr>
<tr>
<td>Inactive (unable to settle on surfaces)</td>
<td>Dead</td>
<td>No movement or pulsing inside shell</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Organs sometimes excreted outside of body, resembling a cloud and is sometimes “shimmery” under a microscope</td>
</tr>
<tr>
<td></td>
<td>Dying</td>
<td>Shell is damaged (broken or cracked)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Organs outside of shell, but still moving uncharacteristically</td>
</tr>
<tr>
<td></td>
<td>Closed</td>
<td>Sometimes indistinguishable between dead and alive.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May be organ movement inside shell</td>
</tr>
</tbody>
</table>

It is difficult to determine whether a veliger is clearly dead unless their inner organs are on the outside of their shell and they are immobile. Veligers that have died prior to the experiment due to turbulence and/or agitation that has disarranged their physiological structure or ones with shells that have been damaged or “eaten” away by an oxidant were ignored.

The moment an oxidant is added, veligers will close up their shells immediately. They will still be alive, but will protect themselves by shutting down their feeding and filtering mechanisms. The only way that the oxidant will kill the veliger is if their shell is improperly shut and allows oxidant infiltration to harm their organs. Otherwise, the oxidant must be strong enough to break
down their calcium carbonate shell to enter and destroy their organs. It is possible to see the breakdown of their shell as holes over time when exposed to an oxidant (Claudi 2015b).

3.3.5.1 Alive – with protrusion

A veliger that is alive with protrusion has clearly observable movements outside of its shell and is visibly moving and alive (Figure 3-3). A veliger in this state exhibits a high level of activity through swimming, active filtering, and motoring around the water solution. In this state, the veliger’s shell is open with its foot or velum outside of the shell and may be propelling itself around. For the purposes of this experiment, this state is not preferable. In this state, a mature veliger will be able to actively release byssal threads, settle, and foul a surface.

![Figure 3-3: Veliger in an "Alive with Protrusion" state.](image)

3.3.5.2 Alive – closed with internal movements

In this state, a veliger is alive, but its shell is not open. This behaviour is commonly exhibited following the addition of an oxidant or an unfavorable condition, causing the veliger to close its
shell, but remain alive. While the veliger may still be alive, it remains in a condition where it will not actively attach to any surface because it is unable to release its byssal threads. Under a microscope, it is often difficult to determine whether the veliger is alive. This may be confirmed with an examination of its moving internal organs seen through the shell.

As seen in Figure 3-4, there are no cilia or organs protruding out of the shell, but its organs are distinctly present and undamaged. However, upon closer observation, internal movements inside the shell such as pulsing or cilia movement may indicate that the veliger is indeed alive.

![Figure 3-4: A live veliger without outer protrusions, but intact organs](image)

### 3.3.5.3 Indeterminable State

A veliger in an indeterminable state may look similar to the veliger in Figure 3-4. Due to temperature or environmental conditions, a veliger may not exhibit characteristics that indicate it is alive. If there is no outer or inner movement visible, the veligers were classified to be in an indeterminable state. While ambiguous, the veliger remains in an inactive state where it will not actively attach to a surface.
3.3.5.4 Dead

When a veliger is killed by the addition of oxidant, it means that the oxidant has penetrated the shell of the veliger and no longer has a protective covering (Claudi 2015b). The oxidant can then enter and damage its vulnerable organs. As a result, veligers exhibit death when the internal organs are oozing out of the shell, as seen in Figure 3-5.

A veliger that has been damaged long before sampling or in the environment may be seen as simply an empty veliger shell with no organs inside or around it. In this experiment, these were ignored and not included in the counting. Since veligers exhibit numerous behaviors, simplifications were made for veligers who were observed as “dying”. Typically, when first affected by the addition of an oxidant, a veliger will not die immediately; its cilia and organs may continue moving although its organs are clearly outside of its body. For simplification, these veligers were considered as dead.

Finally, a veliger that has been dead for a period of time but not long enough for its organs to be distributed or decomposed may have its shell occupied by other microorganisms. This is misleading to the untrained eye, as it can look like the veliger is alive and that there is organ movement. A veliger in this state will have no organs visible, but will have very small circular microorganisms and bacteria moving around individually, digesting the contents of the shell. In this experiment, veligers in this state were ignored.

From observations, it was found that solutions with bacterial activity and active veliger decomposition did not last much longer than a few days. After this time, nearly all of the veligers were in a dead state and consumed by bacteria and microorganisms.
3.3.6 Purity of Stock Ferrate

Potassium ferrate used in this experiment was stored in a desiccator, but obtained in 2012. Since ferrate observes self-decay when humidity is present, it is necessary to ensure that the purity of the ferrate is known. In this solid state, it can be stable in air without moisture. The decomposition rate of ferrate is known to be in the order of hours at higher relative humidity of 65-95%, while decomposition occurred over days at lower relative humidity of 55-60% (Machala et al., 2009). As a result, the purity of this potassium ferrate was determined using the chromite method (Schreyer et al., 1950). This method measures the concentration of ferrate (VI) through observing the oxidation of chromium (III) in a strongly alkaline solution with the ferrate (VI) ion. This oxidation is described in the next equation:

$$\text{Cr(OH)}_4^{4-} + \text{FeO}_4^{2-} + 3\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_3(\text{H}_2\text{O})_3 + \text{CrO}_4^{2-} + \text{OH}^-$$

A weighed sample of potassium ferrate is added to an excess of alkaline chromite solution. The chromate (VI) solution produced by the oxidation is acidified and the resulting dichromate is titrated with a standard solution of ferrous ions. This method allows the measurement of low
concentrations of ferrate (VI). This method can report ferrate concentrations as low as $5.46 \times 10^{-4}$ M as reported by Golovko et al. (2011).

In order to verify the results from the chromite method, results were also obtained from Mossbauer spectrometry from the University of Rochester (Appendix A). The potassium ferrate used in this research had an estimated purity of approximately 82% based on an estimate of decay from these results.

### 3.3.7 Methodology

This experiment observes molar concentrations of oxidants equal to 0.5, 1, 2, 3, 5, and 10 mgL$^{-1}$ as Cl$^-$ (7.1, 14.1, 28.2, 42.3, 70.5, and 141.0 µM). Both ferrate and free chlorine were tested under these concentrations, as well as three controls with no oxidant added.

A concentrated solution of veligers was extracted from a volume of solution obtained from plankton net pulls. The solution was allowed to settle for several minutes before removing 50 mL from the bottom to collect veligers. A 5 mL volume of this solution was transferred into a Petri dish where approximately 20 – 40 veligers were counted and separated for observation. Three of these Petri dishes of 5 mL were prepared for replicates, each with at least 20-40 veligers. The remaining 35 mL of solution was distributed into seven remaining Petri dishes in volumes of 5 mL in order to calculate the residual concentrations of oxidant and pH of the solution over 30 minutes.

The veligers that were allocated into each Petri dish were counted and evaluated to determine their initial life cycle stage prior to the addition of the oxidant. Veliger life span can be defined into multiple subcategories, but the time restriction of the experiment made it difficult to accurately determine exact life stages. As a result, specimens were categorized into (1) veligers (D-shaped larvae) and (2) pediveligers, similar to protocol used by Thompson et al. (1997).
Furthermore, in order to quantify the effects of oxidants on the veligers, their behaviors were categorized into clear and differentiable behaviors. The following categories were used when describing the behavior of veligers: active (swimming, open), inactive (dead, not moving, dying), or indeterminable (Figure 3-6). The initial behavior of the veligers was recorded and the microscopic light was turned off to ensure that the temperature of the sample did not rise and thus, encourage increased veliger activity.

Once the veligers were counted, categorized by life cycle stage and behaviour, and labelled, the first petri dish was placed under the microscope. Chlorine and ferrate were dosed into the designated Petri dishes and resulting behaviors were counted and recorded every 5 to 10 minutes. The Petri dishes for the residuals were measured using the Hach spectrophotometer DR 2800 every 10 minutes.

Figure 3-6: Comparison of dead and open veliger under 100x microscopy with Leica M125 microscope
3.4 RESULTS AND DISCUSSION

3.4.1 Veliger Behaviors

The effects of seven concentrations of chlorine and ferrate were observed with three replicates each. The initial behavior and life stages exhibited by the veligers could have a direct influence in the outcome of the results. It is for this reason, that prior to addition of the oxidant, the veligers allocated to each Petri dish were counted and evaluated to determine their initial life cycle stage (veliger or pediveliger).

![Veliger (~90 µm) and Pediveliger (~200 µm)](image)

Figure 3-7: Veliger and Pediveligers in sample under 100x using Leica M125 stereo microscope

The initial life cycle is important to note in order to ensure that resulting behaviors are not due to the veligers being in different life stages. A possible difference in the reaction of an early stage veliger to a pediveliger could be significant since early stage veligers may have less of a
tolerance to oxidants because of their lesser developed shells and organs than later stage veligers. Figure 3-8 below shows the average of the initial count of total specimens, pediveligers, and veligers in the three replicates before the addition of chlorine.

**Figure 3-8:** Initial behaviors of veligers before chlorine (mgL$^{-1}$ as Cl$_2$) (a) average distribution of number of veligers in each sample, (b) percentage (%) distribution of veligers and types in each sample.
Figure 3-9 shows the distribution of veligers in different life stages for the samples preceding the acute dose of ferrate. In these samples, there was much more consistency in the distribution of veligers for each sample. Outside of the 14.0 µM ferrate sample, the distribution of veligers different life stages in each of the samples were not significantly different.

**Figure 3-9:** Initial behaviors of veligers before ferrate addition (mgL$^{-1}$ as FeO$_4$) (a) average distribution of number of veligers in each sample, (b) percentage (%) distribution of veligers and types in each sample.
3.4.2 Veliger Reaction to Oxidants

A dose of chlorine was added to produce a concentration in the veliger solution of 0.5 mgL\(^{-1}\) or 7.1 µM. Since the oxidant was added as a one-time dose at the beginning of the experiment, there was decay of free chlorine or ferrate over the 30 minutes. The dotted lines in Figure 3-10 show the residual concentration of chlorine and ferrate, respectively, of the samples over the 30 minutes of the experiment. Overall, Fe(VI) observed a much slower rate of decay over free chlorine.

When first added, the chlorine led to about 92% inactivation of veligers, but it started decline immediately after such that 30 minutes after the dose, only 72% of veligers were inactive. While not as dramatic, all of the veligers which were dosed with chlorine up to 10 mgL\(^{-1}\) or 141.0 µM experienced a slight resurgence of active veligers again. It appeared as though the veligers were starting to become active again after the chlorine residual had dissipated. The percentage of veligers in the inactive state and concurrent chlorine residual concentration over 30 minutes can be seen in (Figure 3-10).
Figure 3-10: Percentage of veligers that adopt ideal behavior after an acute exposure of 7.1, 14.1, 28.2, 42.3, 70.5, and 141.0 µM as Cl₂ at 0 minutes and observed over 30 minutes.

It is possible that the slower rate of decay seen by Fe(VI) (Figure 3-11) could be a reason as to why veligers remained in a closed and inactive state for a longer period of time than those in
tested in the chlorine samples. The kinetics and reaction mechanism with the Fe(VI) sample seem to exhibit different characteristics than the chlorine samples. Further research into the reaction and mechanisms of veligers and these two oxidants would need to be conducted in order to gather any conclusions from this study.

Ferrate exhibited a much longer lasting effect on the veligers. The dose of 1.4 mgL\(^{-1}\) of potassium ferrate or 7.1 µM encouraged 94.8% of veligers to remain inactive. As time progressed, longer exposures resulted in higher percentages of inactive veligers, reaching 97.7% after 30 minutes. It was observed that all veliger solutions that were dosed with a ferrate concentration of at least 1.0 mgL\(^{-1}\) resulted in almost 100% inactivation for up to 30 minutes. In all samples and replicates, none of the veligers that were classified as inactive made a resurgence 30 minutes after acute exposure. The percentage of inactive veligers and the concurrent concentrations of ferrate over 30 minutes can be seen in Figure 3-11 below.
Figure 3-11: Percentage of veligers that adopt ideal behavior after an acute exposure of 7.1, 14.1, 28.2, 42.3, 70.5, and 141.0 µM as FeO$_4^{2-}$ at 0 minutes and observed over 30 minutes.
3.4.3 Considerations

Due to the complications of working with live organisms, this experiment observed several possible difficulties. One of the most significant issues was ensuring that all the veligers were in a consistent, live, and healthy state where it would be possible to easily identify the reaction to added oxidants. It is imperative to retrieve veliger samples and preserve the sample by removing predators and storing at a low temperature. Immediate use is highly recommended as veligers will be exposed to less stress and therefore, will possibly elicit a more genuine response to the oxidant.

The acute toxicity experiment was time-based over the course of 30 minutes. The samples were observed approximately every five to 10 minutes. With three replicates, the sample observations were staggered, but each time observation required a veliger count and a test for oxidant residual. Despite filming the reaction of each group of veligers to the oxidant, the determination of the reaction of veligers as alive or dead may not have been as accurate due to the time restriction.

The samples of veligers for each Petri dish may have had a discrepancy between the amount of lesser developed veligers and ones that were almost of pediveliger stage. This poses a possible issue because more developed veligers may be more resistant to oxidant doses due to a thicker shell. Not all veligers in the sample were in the same stage of life. While an effort was made to ensure an equal distribution of pediveligers and D-shaped veligers, the final counts yielded varying maturities. It is a double-edged sword to over-handle the sample in order to produce similar percentages of veligers with differing maturities. While the make-up of the sample may be more equal, further prodding may add to the increased stressed state of the veligers.
3.5 Summary and Conclusions

Based on the results and observations of this experiment, both ferrate and chlorine are effective in immediately eliciting an inactive response in the targeted veligers. While the state of a veliger may be affected initially, the purpose of this experiment is observe what happens to a veliger over time after being exposed to an oxidant. Overall, it was observed that chlorine dosages simply inactivated the veligers for the first few minutes, but after the residual was used up, the veligers began to re-activate. For all concentrations of chlorine dosage, veligers began to rebound in activity after 30 minutes. This may indicate that any dosing at an intake pipe might make no difference once inside the plant. Veligers are still active and able to settle after 30 minutes of being exposed.

On the other hand, ferrate proved to have greater longevity in keeping the veligers in an ideal state. All doses of ferrate were effective for the entire 30 minutes. No veligers were able to rebound from their ideal state after 30 minutes of acute treatment. This might mean that there is a less likely chance of veligers settling throughout the intake pipe or settling on surfaces during the 30 minute travel time. While ferrate may produce some precipitate and has an unfavourable colouring, ferrate proved to be significantly more effective than chlorine in maintaining the population of veligers in an inactive state.

Furthermore, it requires a high concentration of oxidant to produce mortality in veligers. Even at high levels of chlorine at 141.0 µM, only around 16-18% of veligers actually died after 30 minutes of exposure. Focus should now be placed on the inactivation of veligers as death may not be a feasible end-goal for water treatment plant operators.

This experiment revealed some novel findings in regards to the effectiveness of ferrate in comparison to chlorine in encouraging inactive veliger behavior. Similar molar concentrations of ferrate to chlorine were successful in prolonging an ideal response in the veligers. For all concentrations greater than 1 mgL⁻¹, the veligers were inactive for the entire 30 minutes. Since
not all intake pipes are within the span of 30 minutes, it would be useful to observe the duration for which veligers are able to stay inactive and whether it correlates to a certain residual of the oxidant. Furthermore, observing whether veligers respond to a reduction in concentration by adding lake water to the Petri dish might also be insightful.

Further experiments should segregate pediveligers from the umbonal and hinged early-stage veligers. Pediveligers and earlier-stage veligers may react differently to the oxidants. Pediveligers are much more resilient to oxidants and due to their more developed shells they are able to close up while the oxidant is present and open up to resume feeding and filtering once the oxidant has decayed. More importantly, pediveligers are the critical stage in a dreissenid’s life and behaviors after applying oxidants at this point should be observed.
3.6 REFERENCES


4 DREISSENID SETTLEMENT IN RESPONSE TO OXIDANTS

ABSTRACT

Zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena bugensis rostiformis*) are both invasive *Dreissena* species that have spread rampantly throughout North America after being brought over by shipping vessels from Europe. They are now common in large numbers across North America, impacting ecosystems and fouling existing infrastructure such as drinking water intake pipes.

Targeting dreissenids at the larval, veliger stage before they have gained the ability to adhere firmly to surfaces is essential (Waller et al., 1993). Neutralizing free swimming veligers will mitigate blockages that may occur when they reach adulthood and settle in the insides of pipes. In order to prevent fouling and blockage, chlorine pre-treatment has typically been used to prevent settlement of mussels. However, chlorine use in pre-treatment with raw water could be related to the increase of disinfection-by-products (DBPs) that could be consumed by the public through tap water (Rajagopal et al., 2002a).

In this research, an alternative oxidant to chlorine, ferrate (FeO$_4^{2-}$), was investigated as a possible disinfectant to inhibit *Dreissena* settlement and attachment on a polypropylene surface. In order to observe the ability to prevent veliger settlement, ferrate and chlorine were compared by dosing solutions at low quantities to
continuously maintain a residual of 1 mgL\(^{-1}\) in lab-scale settlement tanks with untreated Lake Ontario water. After a period of 10 days, there was no observed settlement in the tanks continuously dosed with chlorine or ferrate, while the control with no oxidant experienced settlement of two juvenile dreissenids. This may indicate that ferrate could potentially be another option in the prevention of *Dreissena* settlement. The addition of oxidant to a greater population of veligers under environmental conditions would need to be conducted in order to verify conclusions drawn from these experiments.

### 4.1 INTRODUCTION

Quagga and zebra mussels have become two of the most significant nonindigenous biofouling organisms that have been introduced into North America (La Bounty and Roefer, 2007). Since arriving in Lake St. Clair in 1986, these mussels have spread throughout the Great Lakes to Mississippi, the Colorado River, lakes and reservoirs in Arizona, California, Colorado, and Utah (LaBounty and Burns, 2005).

Dreissenids exist in three main forms: larval veligers, juvenile, and adult mussels (Ackerman et al., 1994). Veligers are free-swimming and able to transport themselves in water. The juvenile and adult stages are mostly motile by releasing their byssal threads and attaching to favourable substrates. Within the veliger state, they are divided into four different stages: trochophore, straight-hinged veliger, umbonal veliger, and pediveliger. The pediveliger is the most significant, as they are able to swim with their velum and crawl using their foot, and are at the stage where they secrete byssal threads to settle and develop on a substrate. This period of development to a
juvenile mussel can last anywhere from 18-90 days (Ackerman et al., 1994; Crosier and Molloy, 2001).

The previous acute toxicity experiment focused on chlorine and ferrate being employed in an acute setting. However, this is ineffective in the real world. While the input of a high concentration of oxidants can be effective in reducing *Dreissena* veliger populations, the application near an intake pipe can potentially be detrimental to water quality. Furthermore, this method is impractical in real-world situations since settlement rates can vary with different environmental conditions. Previous research has shown that small concentrations of oxidants can effectively produce inactivity in veligers, making them unlikely to settle (Claudi et al., 2012b; Rajagopal et al., 2002a; Van Benschoten et al., 1993). It may only take a small non-lethal dosage to prevent mussels from releasing byssal threads and settling. By addressing veligers at this stage in their life, they are prevented from settling on a surface and can be swept away alive or dead. Focusing on mussels at their adult stage would require more oxidants and could potentially cost more in clean up and disposal (Klerks and Fraleigh, 1991). Adjusting treatment strategies to focus on addressing the veliger stage is preventative and possibly more cost effective.

Chlorine has been proven to be an effective solution for treating both mussels and veligers (Rajagopal et al., 2002b; Van Benschoten et al., 1993). However, chlorine can combine with natural organic matter (NOM), producing disinfection by-products (DBPs) which may be linked to health effects, such as bladder and rectum cancer (Bharadwaj, 2006). The purpose of this research is to investigate the possibility of using ferrate as an alternative. Unlike chlorine, ferrate reaction with NOM produces iron-based by-products which are less toxic than the DBPs produced using chlorine (Yang et al., 2013).

Larval settlement can be the turning point in the development of the community development and structure of mussels (Underwood and Denley, 1984). It has been found that monitoring control measures in the early stages of settlement are much more effective than when the population has already achieved settlement on surface (Barton, 1993). In fact, settlement rates
can vary significantly from weeks, to days, to a matter of hours (Claudi and Mackie, 1993). The purpose of this settlement experiment is to prevent the mussels from settling initially, thereby preventing the need for any biofouling clean-up.

4.2 OBJECTIVE

The primary objective of this study was to develop a method of observing the ability of *Dreissena* veligers to settle on a substrate under different various conditions. This method requires the concentration of mussel pediveligers from Lake Ontario and counting the settlement after an exposure period of 10 days. The detailed objectives were as follows:

1. To observe the ability of ferrate in the prevention of mussel settlement in relation to chlorine,
2. To observe the effect of ferrate dosing on final water quality,
3. To investigate the feasibility of using bioboxes to simulate the prevention of *Dreissena* settlement on intake pipes,
4. To develop a protocol for testing the dosing of varying oxidants on the settlement of *Dreissena* veligers.

4.3 MATERIALS

4.3.1 Water Source

All lake water used for the experiment was collected from Humber Bay Park West from the boat launch area and filtered accordingly. Before the experiment, each cooler was filled with filtered Lake Ontario water. Filtered lake water was achieved by filtering the water through a 180 µm Nitex bolt cloth to remove any unwanted predators from the tank water.
Veligers for this experiment were collected in early October 2015 from Lake Ontario near the King St. Water treatment plant pier in Kingston, Ontario. Veligers were collected using a 63 µm plankton net submerged off-shore and pulled vertically from the lake bed (10 m). The samples were then filtered through 250 µm coarse sieve and stored in carboys.

4.3.2 Biobox Apparatus Assembly

Identical Coleman 40 L coolers were modified to be used in this experiment as tanks for lake water and veligers to be stored in and observed for settling. The cooler was lined with a favourable substrate for veligers to release byssal threads and settle on, black polypropylene sheets (Claudi and Mackie, 1993) (Johnston Plastics Ltd., Toronto). The polypropylene sheets were cut to the dimensions of the bottom and sides of each of the coolers and cut with a table saw. GE Waterproof Weatherproof 100% silicone sealant was tested to observe any possible toxic effects on veligers and used to adhere the sheets to the sides and bottom of the cooler.

The feed lines from the two aquarium air pumps were connected to a T-junction and each tubing was capped with an aquarium air stone. The tubing used was black Masterflex 14 gauge and was tested to ensure that there was no reactivity with the oxidants used. The airstones were evenly distributed and adhered to the bottom of the bioboxes (Figure 4-1).
4.3.2.1 Prepping Settling Plates

Before the experiment, each tank was filled with filtered Lake Ontario water. Using Trilene fish wire attached to 1.5” pine hobby wood, dark coloured polypropylene plates were hung into the tank. Porcelain weights were also hung from the bottom of each plate to ensure that they would not float up due to the aeration. The dark plates were hung and side panels were submerged in lake water for five days to form a biofilm prior to the experiment (Figure 4-2).
4.3.3 Initial Set Up

Initially the lake water should be the same for each of the tanks and the parameters should be identical. The lake water in each of the tanks was fresh with enough algae and consumables for the veligers. Water was filtered through a 250 µm sieve to ensure that there were no copepods or predators. Since only three coolers were used in this portion of the experiment, 90 L was collected for the coolers themselves, while an additional 4-6 carboys were collected each time for cleaning and redundancy. Every three days, half the volume of each cooler was replaced with fresh, filtered, raw lake water. Once collected and distributed into their individual coolers, the coolers were stored in the lab at a temperature that did not exceed 22°C.

Based on previous experiments, there were several factors that are essential in maintaining the viability of veligers in the tanks. Identical air pumps with air stones were placed in each cooler to maintain a supply of oxygen to the water. Once the water had been distributed into each cooler,
preliminary parameters were tested to ensure that all the coolers had identical and appropriate characteristics.

Figure 4-3: Experimental set up with peristaltic pumps, magnetic stirrers and stock solution

4.3.4 Reagents

All reagents except for Envifer, a ferrate composite product, were obtained from Sigma Aldrich. This includes sodium phosphate dibasic, acetic acid, monosodium phosphate, and sodium tetraborate decahydrate. The potassium ferrate was stored in a desiccator since 2012.

4.3.4.1 ENVIFER

In place of pure ferrate, an inexpensive potassium ferrate composite product, Envifer (supplied by NanoIron Inc., Czech Republic), was used. Envifer contains approximately 50% ferrate (VI) and costs €80 per kilogram, while pure ferrate can cost approximately $100 per gram (Sigma-Aldrich Inc., United States). Hereafter, any stated quantities or concentrations of ferrate will correspond to measurements in pure ferrate.
Envifer comprises several potassium iron compounds, including K$_2$FeO$_4$, K$_3$FeO$_4$, KFeO$_2$, and K$_2$O. It is a dark purple colour, but has larger agglomerates than pure ferrate. A sample breakdown of the constituents of Envifer is found in Table 4-1 below. This analysis was performed by NanoIron on their batch produced in July 2015. The sum of the percentages may not equal 100% due to impurities, measurement errors, and possible unidentified components. In regards to impurities, there may be some SiO$_2$ present, as well as some traces of metal elements.

Table 4-1: Oxidation states of Fe in ENVIFER compound using Mossbauer spectroscopy, analysis performed by NanoIron (CZ)

<table>
<thead>
<tr>
<th>Oxidation State</th>
<th>Mole Fraction of the total Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(III)</td>
<td>49 ± 3 %</td>
</tr>
<tr>
<td>Fe(IV)</td>
<td>&lt; 3 %</td>
</tr>
<tr>
<td>Fe(V) + Fe(VI)</td>
<td>51 ± 3 %</td>
</tr>
</tbody>
</table>

Table 4-2: Percentage weight proportion of compounds in ENVIFER compound

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Weight proportion of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>K$_3$Fe(V)O$_4$</td>
<td>56 ± 3 %</td>
</tr>
<tr>
<td>KFe(III)O$_2$</td>
<td>29 ± 3 %</td>
</tr>
<tr>
<td>K$_2$O</td>
<td>&lt; 3 %</td>
</tr>
</tbody>
</table>

4.3.4.2 Buffer Solution

A buffer solution was required for the ferrate solution, as it is most stable around pH 9.1 (Lan et al., 2013). Since a ferrate solution had to be continuously dosed into the coolers to maintain a stable concentration, a more stable stock solution had to be created. Kim et al., (2014) observed a stable buffer solution for ferrate with a ratio of 5mM Na$_2$HPO$_4$/1mM Na$_2$B$_4$O$_7$ in Milli-Q water.
This solution was prepared in advance of the experiment in large quantities for all ferrate solutions.

4.4 Methodology

4.4.1 Original Experimental Design

The original experimental design was to observe two low concentrations of oxidants against two high concentrations of oxidants and a control. Table 4-3 below outlines the intended experimental design for this research.

Table 4-3: Experimental design of initial experiment

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Condition</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control 1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Control 2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Control 3</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Ferrate Low 1</td>
<td>0.1 mgL⁻¹</td>
</tr>
<tr>
<td>5</td>
<td>Ferrate Low 2</td>
<td>0.1 mgL⁻¹</td>
</tr>
<tr>
<td>6</td>
<td>Ferrate High 1</td>
<td>1.0 mgL⁻¹</td>
</tr>
<tr>
<td>7</td>
<td>Ferrate High 2</td>
<td>1.0 mgL⁻¹</td>
</tr>
<tr>
<td>8</td>
<td>Chlorine Low 1</td>
<td>0.1 mgL⁻¹</td>
</tr>
<tr>
<td>9</td>
<td>Chlorine Low 2</td>
<td>0.1 mgL⁻¹</td>
</tr>
<tr>
<td>10</td>
<td>Chlorine High 1</td>
<td>1.0 mgL⁻¹</td>
</tr>
<tr>
<td>11</td>
<td>Chlorine High 2</td>
<td>1.0 mgL⁻¹</td>
</tr>
</tbody>
</table>
4.4.2 Modified Experimental Design

Due to a low environmental veliger concentration leading up to the experiment during autumn 2015, all 11 of the conditions were unable to be tested. Instead, an abbreviated test was performed that consisted of three conditions (Table 4-4).

Table 4-4: Experimental design of modified experiment performed October 2015

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Condition</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0 mgL(^{-1})</td>
</tr>
<tr>
<td>2</td>
<td>Ferrate</td>
<td>1 mgL(^{-1})</td>
</tr>
<tr>
<td>3</td>
<td>Chlorine</td>
<td>1 mgL(^{-1})</td>
</tr>
</tbody>
</table>

4.4.3 Decay of Oxidants

Overall, the purpose of this experiment was to observe the settlement of pediveligers on plates placed in an environment where they would receive a constant exposure of 1 mgL\(^{-1}\) of oxidant in Lake Ontario water. Tests were performed to observe the stability of ferrate and chlorine in Lake Ontario water. Based on the data found from the decay of chlorine and ferrate over time, it was then possible to determine how much ferrate and chlorine must be replenished to maintain a constant level of 1 mgL\(^{-1}\) ferrate and free chlorine. The decay of chlorine and ferrate in lake water and buffer is found in Appendix C.

4.4.4 Stock solutions

Chlorine is able to remain stable in Milli-Q® water in dark, cool conditions. On the other hand, ferrate or Envifer is known to be quite unstable in solution. Envifer’s decay rate in solution
increases with higher concentrations and in this case, the stock solution had a concentration of around 60 mg\textsuperscript{L}\textsuperscript{-1}.

As mentioned earlier, Envifer is most stable in Milli-Q® water buffered to pH 9.1 (Luo et al., 2014). The characteristics of ferrate reaction in Milli-Q® water with pH 9.1 buffer was also observed so that these conditions could be employed for stock solutions. The stability of ferrate in Lake Ontario water (pH 7.9) was also observed in order to understand the speed of oxidant decay inside the tanks where the mussel veligers were being held. By determining both the decay rates of ferrate in Lake Ontario water, as well as in a stable buffer, a suitable pumping rate of ferrate stock solution was determined in order to maintain an adequate residual for the experiment (Appendix C).

### 4.4.4.1 Dosing of Stock Solutions

The dosing of stock solutions was conducted to maintain a residual of approximately 1 mg\textsuperscript{L}\textsuperscript{-1} of chlorine or ferrate in the tanks at all times. Solutions were pumped from 1 L beakers, covered in aluminum foil to reduce light-induced reaction and set on magnetic stirrers to ensure the solution was homogeneous. Based on several earlier experiments for both chlorine and ferrate in Lake Ontario water, an average k-constant was determined. Using this, a model taking into consideration the decay in the tank and the decay of the stock solution was developed using excel. Using this model, an initial concentration, as well as any necessary additions over the course of the experiment were determined (Appendix C).

### 4.4.5 Setting Peristaltic Pumps

All of the peristaltic pumps for all of the tanks were identical and are set as 125 ml/min for a set amount of time every 3 hours. The volume of stock solution in this experiment was dispersed as
outlined in Appendix C and was repeated for 10 days. This dosing scheme was the starting point for the experiment, since the residual was quite variable over the 10 days. This scheme also required maintenance every 12 hours, which was manageable for one person to supervise. The flow rates were adjusted if there was any odd fluctuation in residual concentrations. The same Masterflex 16-gauge, black tubing was used for all pumps with the same pumping rates.

4.4.6 Daily Tests

Tests were performed daily in order to ensure that the most favourable conditions were present to facilitate health and growth in the veligers.

- pH
- Temperature
- ORP (oxidation reduction potential)
- EC (electrical conductivity)
- DOC
- Ferrate or chlorine residual
- UV-spec: absorbance at 415 and 510

4.4.7 Settling Analysis

After 10 days, at the end of the experiment, the settling plates from the bioboxes were removed carefully and examined to assess colonization of veligers. Settled mussels at this stage should be in their juvenile state. The following guidelines were administered when analyzing the plates:

1. Colonization must be observed in the control sample for experiment to be valid
2. A magnifying glass or stereo microscope should be used to observe settled veligers
3. Once settling is determined on the surface of the control, remove settling plates from remaining tanks and count
4. Calculate settlement /m² by calculating settlement per designated area
5. Record final water quality properties of the water
4.5 RESULTS AND DISCUSSION

4.5.1 Settling Plate Analysis

Unfortunately, the number of viable pediveligers at the start of the settlement experiment was relatively small, leading to a small number of settled mussels. Nevertheless, no settlement at all was observed in the coolers with a constant residual of 1 mgL$^{-1}$ chlorine or 1 mgL$^{-1}$ ferrate, but two settled juvenile mussels were observed in the control (Figure 4-4; Figure 4-5). Likely quagga mussels, both had settled on the bottom sheet of the cooler and had grown to 2 and 4 mm. It would have been implausible for larger veligers to have been present prior to the experiment as the water was filtered through a mesh of 250 μm. Samples of the water near the bottom of the oxidant-containing tanks had shown that 100% of the veligers had died. Approximately 97% of veligers had died at the end of the 10 day period in the control.

Figure 4-4: Juvenile mussel from control tank #1, under 400x Nikon E600 microscopy
Although the numbers obtained from the settlement experiment were not significant, there was absolutely no settlement in the tanks containing oxidant. This may indicate that veligers are not likely to settle in an environment where there is a residual oxidant concentration available. Since the veligers had settled and grown to a juvenile stage of 2 and 4 mm, we can assume that the control had a suitable environment for veligers to settle and develop. It would have been impossible for larger veligers to have been present prior to the experiment as the water was filtered through a mesh of 250 µm.

While chlorine remained between 0.8 and 1.2 mgL\(^{-1}\) throughout the 10 days, the ferrate dosing had to be tweaked constantly and observed a greater fluctuation between 0.5 – 2 mgL\(^{-1}\).

The use of Envifer provided the most uncertainty and unpredictability throughout the experiment. The Envifer produced Fe(III) precipitate which coated the bottom of the tank. This also resulted in interference with the UV-spectrometer readings. While ferrate ions should have been homogeneously mixed and in solution, stirring the tank made a significant difference in
absorbance readings. The addition of ABTS to the solution to detect residual ferrate was also slow reacting, which could mean that ABTS was reacting with another compound in the impure Envifer product; reaction with ABTS should have been instantaneous. Measurement of oxidation-reduction potential assisted with understanding the concentration of ferrate ions in the cooler, but more research and contact with the manufacturer will be necessary for future research.

4.6 SUMMARY AND CONCLUSIONS

4.6.1 Improvements and Suggestions

- For this experiment to run at full capacity with 11 coolers, enough veligers needed to be collected. Throughout this experiment, Station 81 was accessed with the help of the Glenora Ministry of Natural Resources and Fisheries. Located near the outlet of the Bay of Quinte, it seemed that all of the veligers were washed out from that area and concentrated where the station was located. It is highly recommended that veligers be collected from this location in August to early September. 20 pulls from 20 m below the water surface would be sufficient for the experiment.

- There was a significant amount of difficulty in the final observation of the plates to determine settlement. Since the plates were secured using silicone to the sides and bottom of the cooler it was difficult to remove the plates and observe them. In order to make this process easier, it is recommended that the plates be fastened temporarily in a way that is easy to remove.

- One of the major issues was tracking the ferrate residual provided by the Envifer. There are still some questions that remain about the product in regards to its homogeneity in solution and the method of determining the concentration. It is best that Envifer be used
at high flow rates and that the stock and the cooler be changed often to prevent too much precipitate from accumulating.

### 4.6.2 Conclusions

While Envifer is an affordable alternative, it had some drawbacks witnessed in this experiment. One of the most significant hurdles was the dosing of Envifer so that a residual could remain at 1 mgL$^{-1}$. A solids dispenser would not be feasible for this product because the sizing of the ground Envifer substance ranges from 0.1 to 5 mm in diameter – it is very heterogeneous. When this ground substance is dissolved in a liquid to produce a ferrate solution, Envifer requires a lot more agitation in order for a homogeneous solution to be produced. There are some larger granules that are unable to be dissolved and remain at the bottom of the beaker. It was found that even after vigorous mixing and leaving the solution to rest, the ferrate solution would begin to stratify.

Since using a solids doser was impossible, peristaltic pumps were used to dose ferrate from an aqueous state. While this was the preferred alternative, there were still difficulties given the stratifying state of the ferrate mixture. At low flow rates, precipitate from the ferrate would settle out into the tubes. The ferrate concentration of the liquid going in to the tube was compared to the ferrate concentration of the liquid leaving the tube. Where the stock entering the tube at about 2 mL/min was 75 mgL$^{-1}$, it was found that the concentration of ferrate was nearly 0 mgL$^{-1}$. As a result, ferrate was settling or reacting with the tube. A much faster flow rate over a shorter period of time is recommended.

Finally, Envifer was tricky to determine using the direct method or ABTS. The water in the tank and in the samples had to be agitated to create a homogeneous mixture. Solution taken from the top of a tank yielded nearly 0 mgL$^{-1}$ concentration, while the same solution mixed would yield a measurable concentration. According to NanoIron, this should not be the case, ferrate should be
in solution and a similar concentration should be taken from all stratus of the solution. In addition, the ABTS would not react immediately with the Envifer solution, it would develop over time. In a typical pure ferrate solution, ABTS would react immediately. This characteristic of Envifer requires further research.

Based on the results from this experiment, it was found that the tanks with a chlorine and ferrate residual of 1 mgL⁻¹ did not observe any veliger growth or attachment. However, there were not enough replicates to form a conclusion on the effects of a residual oxidant on the settlement of zebra mussels. Given that there was a low concentration of veligers and unfavorable circumstances, the settlement and development of two mussels in the control cooler may be a good indicator for future experiments. There is a possibility that ferrate at a residual of about 1 mgL⁻¹ may be an effective deterrent in the settlement of mussels.

While there were a significant number of uncertainties in the experiment, this experiment served as a trial which will leave room for experimental improvements, greater efficiency, and success in the future.
4.7 References


4.8 ACKNOWLEDGEMENTS

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5 ENHANCEMENT OF GEOSMIN AND DOC REMOVAL

ABSTRACT

The interaction effects of ferrate and powdered activated carbon (PAC) for geosmin and dissolved organic carbon (DOC) removal were monitored. The experiment used Lake Simcoe water, which has a DOC concentration of approximately 4.0 mgL$^{-1}$.

Ferrate and PAC were added in varying sequences in jar tests to observe the potential interaction: with the two compounds added simultaneously, with PAC first, or with ferrate first. The maximum DOC removal was achieved when PAC was added first (20%), while adding ferrate first or together with PAC led to only about 11% DOC removal. The greater DOC removal when PAC is added before ferrate suggests that pretreatment with ferrate either leads to compounds that foul the PAC and block DOC adsorption, or else oxidize the DOC to render it less adsorbing. Similarly, geosmin removal also appeared to be slightly greater when PAC was applied before ferrate, with up to approximately 80% removal compared to about 60% removal when ferrate was added first or together with PAC.
5.1 INTRODUCTION

Taste and odour (T&O) compounds have a significant influence on the public’s perception of drinking water quality. While T&O is not often associated with health effects, it is often the most frequent customer complaint for drinking water. For many customers, it is the main way of determining the quality and safety of the tap water. The presence of these compounds may trigger less consumer trust and may induce a consumer switch to alternate sources. One frequently monitored T&O compound that will be focused on in this study is geosmin (trans-1,10-dimethyl-trans-9 decalol, C\textsubscript{12}H\textsubscript{22}O). The lowest detectable concentration for humans is about 4 to 20 ngL\textsuperscript{-1} (Young et al., 1996). Geosmin can be difficult to remove through conventional treatment and plants must often add additional technologies to manage T&O issues, since they can be a seasonal occurrence.

Common methods for removing T&O compounds are granular activated carbon (GAC), powdered activated carbon (PAC), biofiltration, and advanced oxidation processes (AOP). Of these options, PAC is an effective technology that can easily and economically be adopted into an existing system. A study by Kim et al. (2014) showed that it is hard to control geosmin through PAC only; PAC typically can provide the additional removal that will complement the existing system. It was also found that the combined treatment of oxidants and PAC was more effective than solely one treatment on its own.

Since typical NOM and T&O compound concentrations are about 2 – 10 mgL\textsuperscript{-1} and 10-100 ngL\textsuperscript{-1}, respectively, the general problem of the adsorption process of geosmin is that the adsorption capacity of PAC is often reduced by NOM. Larger NOM molecules are able to outcompete micropollutants such as T&O compounds for removal (Newcombe et al., 1997). Sidney Seckler et al. (2013) observed the interference of iron as a coagulant on T&O removal by PAC adsorption. It was also found that greater coagulant doses and subsequent accumulation of PAC particles in the metal hydroxide reduced the liquid phase removal of T&O compounds (Ho and Newcombe, 2005).
PAC is often added to an existing treatment system. However, its interaction with other treatment chemicals may have an effect on adsorption. The effect of ferrate on the adsorption of geosmin and dissolved organic compounds (DOC) has not yet been explored. As a result, this study is being performed to observe the removal of these compounds using PAC and ferrate and their corresponding interactions with each other based on the sequence of addition, pH, presence of DOC, and ferrate concentration.

5.2 Literature Review

5.2.1 Powdered Activated Carbon

PAC can be selective in removing NOM in terms of the types and sizes that it can adsorb. The adsorption of NOM by PAC is described by the Freundlich isotherm. Geosmin and 2-MIB (2-methylisoborneol) are two of the most frequently observed T&O compounds. After 30 minutes, the geosmin and 2-MIB follow the Freundlich isotherm. However geosmin has a greater adsorbance than 2-MIB in water containing NOM (Zoschke et al., 2011). PAC is a versatile compound to use because most of the time it can easily be added to an existing treatment system without requiring any significant upgrades. The most common application point for PAC to be added into an existing treatment system is at the rapid mix stage of the coagulation process. This makes it much easier to settle out and dispose PAC with the rest of the sludge waste. This may pose some issues as the flocs that are developed during this stage may interfere with the PAC adsorption of the target compound (Ho and Newcombe, 2005).

Furthermore, the use of PAC can reduce coagulation and pH modification requirements (Álvarez-Uriarte et al., 2010). A concerning factor for the use of PAC with other treatment chemicals is that it is strongly affected by surface chemistry. The surface chemistry of PAC can be altered through pH, the addition of oxidants, or the water matrix.
Under correct doses and conditions of PAC, it can be effective during T&O events and in the removal of specific contaminants. A low dose of PAC can reduce the coagulant dose required and also can increase the DBP formation potential (Kristiana, 2011). In particular, PAC is most effective in the removal of small, fast-acting DBP precursors and aiding in the formation of flocs (Álvarez-Uriarte et al., 2010). PAC has been employed in many treatment plants because it can offer a more cost-effective treatment process than enhanced coagulation only (Najm et al., 1998).

5.2.1.1 PAC and Adsorption Sites

Competition is an important aspect of PAC to observe since interfering compounds will have a direct impact on the ability of PAC to adsorb targeted compounds. Zoschke et al. (2011) have found that pretreated water that has been flocculated shows no competition with taste and odour compounds. However, NOM tends to be a major factor for PAC adsorbance and of the NOM compounds, it has been found that the small and highly adsorbable ones are the most competitive with T&O compounds. It has been understood that the general problem with the adsorption of targeted compounds using PAC is the competition from water containing high levels of NOM. This can be attributed to the discrepancies in concentrations of the targeted substance and the interfering substance. For example, typical NOM concentrations are in the range of 2 - 10 mgL\(^{-1}\) of DOC while the concentration of micropollutants is in the range of 10 – 100 ngL\(^{-1}\). Newcombe et al. (1997, 2002a) describe two mechanisms that may illustrate the interference of NOM in PAC adsorption. Firstly, large NOM particles may block the pores of the activated carbon and prevent the targeted pollutants from accessing the sites. Secondly, smaller NOM particles may directly compete with the micropollutant for adsorption sites. These mechanisms are not always the case, as the effect is correlated to the concentration and character of the NOM (Newcombe et al., 1997). Other factors that may affect the adsorption of PAC include the alkalinity of the water matrix. A greater removal for PAC was found with lower alkalinity in higher DOC containing waters (Watson et al., 2015).
In a study performed by Kilduff et al. (1996), it was found that the presence of DOC sufficiently changed the mechanism of adsorption by PAC for different organic molecules. For example, trichloroethylene and atrazine adsorption by PAC was reduced, possibly by the blocking of sorption sites by organic molecules (Kilduff, 2002; Li et al., 2003).

5.2.1.2 Interaction with PAC and Coagulants

The US EPA has identified that enhanced coagulation (EC) combined with granular activated carbon (GAC) is the best technology in the reduction of DBP precursors (USEPA). However, removal through EC depends greatly on factors such as pH, alkalinity, coagulant type, dosage, and type and concentration of NOM. The interaction of coagulants and PAC have been observed to have both positive and negative interactive effects. It is thought that a specific dose of coagulant can remove a finite limit of NOM. As a result, PAC may be a means of supplementing existing coagulation to improve NOM removal and to control the production of DBPs. Najm et al. (1998) found that PAC was able to remove the low molecular weight and uncharged NOM substances unaffected by coagulation.

Ho and Newcombe (2005) observed that the interaction of PAC with greater coagulant dosages, especially those with metal hydroxide, reduced the removal of 2-MIB from the liquid phase. On the contrary, Uyak et al. (2007) found that the use of ferric chloride with PAC increased removal of DOC. Figure 5-1 below shows the effect of PAC with varying ferric chloride dosages on % removal of UV$_{254}$ (Uyak et al., 2006). Reduced dose of coagulant and PAC led to large fractions of NOM being removed effectively at higher and lower ranges of PAC concentrations. Alvarez-Uriarte et al. (2010) observed that coagulants did not hinder adsorption performance by PAC. In fact, in the removal of micropollutants, the removal achieved through the combination of PAC and coagulants was equal to the sum of the individual removals. In pilot-scale applications of PAC with enhanced coagulation, it was found that there was a 70% improvement in the removal
of NOM and an 80-95% reduction in DBPs (Kristiana et al., 2011). This may mean that the addition of PAC may reduce treatment costs and possibly sludge production as well.

![Figure 5-1: Effect of PAC and ferric chloride dosages on % removal of UV$_{254}$ (Uyak et al., 2007)](image)

The correlation of PAC to micropollutants removal can be heavily influenced by water composition. The study by Uyak et al., (2007) further emphasizes that the dosing of PAC and coagulants should focus on the optimization of mixing conditions and contact time over all other factors. Further emphasizing this point, Gillogly et al. (1999) and Graham et al. (2000) reported that the removal of T&O compounds is independent of initial concentrations, but is dependent on carbon dose.

Furthermore, the acidity of a solution greatly dictates the efficiency of PAC to adsorb by affecting its surface chemistry. Where there is a low pH of the water matrix, the electrostatic interactions favour the adsorption of the deprotonated NOM molecules onto the positive charged surface of PAC, thereby creating an interference for the targeted molecule (Alvarte-Uriarte et al., 2011). For example, Tennant and Mazyck (2007) observed that DI water observes a positive correlation with increased PAC and acidity. On the other hand, in raw Florida water, surface
chemistry had no effects on adsorption of geosmin and MIB. As a result, it is necessary to observe all types of surface water matrices and the effects of surface acidity on pore adsorption. In real-world scenarios however, the amount of coagulation and the need for acidification will increase operational costs.

### 5.2.1.3 Interaction of PAC with Oxidants

It is important to understand the interaction of PAC with current chemical treatments in a system prior to application since some have adverse effects. Gillogly et al. (1998) found that upon the application of chlorine and PAC, they were most effective in removing T&O compounds when their contact time was minimized or eliminated. The reason for this is because the MIB adsorption sites were oxidized by the chlorine. Chlorine readily oxidized sites containing MIB as well, releasing it back into the aqueous phase.

The oxidation of the surface of activated carbon produced a decrease in the ability of the PAC to adsorb organic substances. Activated carbon also can act as a catalyst in reactions with aqueous chlorine (McGuire and Suffet, 1984). MIB is difficult to oxidize and directly oxidized by chlorine. It is also possible that absorption sites were built up with surface oxides, thus interfering with the adsorptive capacity of MIB (Faust and Aly, 1998). Previous studies have shown that the addition of chlorine has resulted in the reduction in the ability for activated carbon to adsorb organics like phenol and p-nitrophenol (McGuire and Suffet, 1984).

In the study by Gillogly et al. (1998), MIB removal in Lake Michigan water was 68% in 4 h using 11.5 mgL\(^{-1}\) of PAC. The simultaneous addition of 3 mgL\(^{-1}\) chlorine as Cl\(_2\) resulted in only 26% of MIB removed in 4 h. A delay in the addition by 12 min resulted in only 54% of MIB adsorbed in 4 h (Ho et al., 2008).
On the other hand, there are oxidant and PAC combinations that have complementary effects. In a study on ozone pretreatment and PAC addition, it was found that baseline coagulation with 200 mg L\(^{-1}\) of FeCl\(_3\) achieved 39% DOC and 54% UV\(_{254}\) removal. The addition of PAC helped remove and additional 9% and 25% of DOC and UV\(_{254}\), respectively (Agbaba et al., 2015). Furthermore, a study performed by Agbaba et al. (2015) also investigated the removal of NOM using ozone pretreatment and PAC addition. Pre-ozonation changed the structure of the NOM in water, which enhanced the efficiency of coagulation in its removal. PAC added to the water slightly improved the coagulation efficacy in NOM removal. Pre-ozonation followed by PAC and FeCl\(_3\) (0.65 mg O\(_3\)/mg DOC, 5 mg L\(^{-1}\) PAC, and 200 mg FeCl\(_3\)/L\(^{-1}\)) resulted in a more effective coagulation treatment, removing 58% DOC and 72% UV\(_{254}\). Investigations into the surface chemistry and adsorptive properties of activated carbon following interaction with an oxidant must be determined individually.

### 5.2.1.4 PAC Stirring Protocol

A literature study was performed to compare the mixing regimes for the jar test in the experiment. Since it is uncertain what the optimal stirring regime is for PAC and ferrate, the stirring regimes of various combinations of PAC and other oxidants and/or coagulants were compared. In a study by Altmann et al. (2015), the sequence of addition was not critical for effective adsorption of micropollutants. In treatment plants, PAC is typically added in the rapid mix stage along with coagulants and then settles in the sedimentation stage (Tomaszewska et al., 2004). In real world applications, selecting the point of addition includes the provision of good mixing, good contact between PAC and water being treated, sufficient time of contact for adsorption of contaminant, little interference with treatment chemical and the adsorption mechanism of PAC, and no reduction of the finished water quality. It is necessary to determine the optimum time of contact since it will depend on the concentration and characteristic of the contaminant and water matrix.
Studies of PAC investigated the efficacy of the additional coagulant or oxidant added both before (Agbaba et al., 2015), simultaneously (Tomaszewksa et al., 2004; Uyak et al., 2006) and after PAC addition (Alvarez-Uriarte et al., 2010; Uyak et al., 2006).

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Journal Article</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agbaba et al. (2015)</td>
<td>Ozone pretreatment and PAC addition in removal of NOM by coagulation</td>
<td>Coagulant addition: 120 rpm for 2 min Flocculate with anionic flocculent: 30rpm for 30 min Settling: 60 min</td>
</tr>
<tr>
<td>Ma and Liu 2001</td>
<td>Effectiveness of ferrate pre-oxidation in enhancing the coagulant of surface water</td>
<td>Ferrate addition: 300 rpm for 1 min Flocculate with coagulant: 60 rpm for 10 min Settling: 30 min</td>
</tr>
<tr>
<td>Tomaszeska et al. (2004)</td>
<td>Removal of organic matter by coagulant enhanced with adsorption on PAC</td>
<td>PAC/Coagulant addition: 100 rpm for 1 min Flocculate: 10 rpm for 15 min with pH adjustment Settling: 60 min</td>
</tr>
<tr>
<td>Alvaraz-Uriarte et al. (2010)</td>
<td>The effect of mixed oxidants and Pac on the removal of NOM</td>
<td>PAC addition: 100 rpm for 5 min Coagulant addition: 80 rpm for 2 min Anionic flocculation: 30 min Settling: 60 min</td>
</tr>
</tbody>
</table>

5.2.1.5 Types of PAC

Activated carbon is classified based on its mesh size, shape, and the material that it is derived from. The effectiveness of different PACs in the removal of different contaminants varies.
significantly between types of carbon. In a study by Donati et al. (1994), it was found that wood-based carbons were the most effective in adsorbing microcystin-LR, followed by coal-based carbons. On the other hand, coconut and peat moss-based carbons were seen as the poorest adsorbents of microcystin-LR. Based on the contaminant being observed, different types of PAC can be more effective. Yu et al. (2007) examined five types of PACs in the removal of geosmin and 2-MIB from raw water where coconut-shell based carbon had the highest adsorption capacity compared to wood and coal-based carbons. This can be attributed to the large micropore volumes of the shell-based PAC. In the case of geosmin and MIB, a correlation was found between the size of the micropore volumes of activated carbons and the ability of geosmin and MIB to be adsorbed.

While the iodine number and methylene blue number are two parameters that are used to define the adsorbance properties of PAC, they were found to be inconsequential in correlation with the adsorption capacities of MIB and geosmin. The study also found that other defining characteristics of PAC, such as meso and total pore volumes, surface area, O and C-O contents, were statistically insignificant. Overall, micropore volume was found to be the most effective indicator for PAC selection in the removal of MIB and geosmin. Table 5-2 is an overview of the different types of PAC that are used in the removal of various microcontaminants in raw water.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Article</th>
<th>PAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meinel et al. (2014)</td>
<td>Pilot Scale Investigation of Micropollutants Removal with Granular and PAC</td>
<td>AquaSorb 5000 P grainsize (d50) 15-35 µm</td>
</tr>
<tr>
<td>Kim et al. (2014)</td>
<td>Removal of Geosmin and 2MIB by membrane system combined with PAC for drinking water treatment</td>
<td>C-PAC 90-180 µm Norit (Darco KB-B, USA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 27 µm coconut-based</td>
</tr>
</tbody>
</table>
Zoshcke et al. (2011)  
Adsorption of geosmin and 2MIB onto PAC at non-equil conditions: influence of NOM and process modeling  

PAC SA Norit from Norit  
- Pore volume distribution 0.41 cm³/g  
- Micropores <2 nm diameter

Sidney Seckler et al. (2012)  
Interference of iron as a coag on MIB removal by PAC adsorption for low turbidity waters  

PAC - Brascarbo Agroindustrial, Brazil (CarboActiv K)  
- Iodine number of 600 mg/g

Cook et al. (2001)  
The Application of PAC for MIB and geosmin removal  

Picaef 1100 PAC (PICA)  
- Coconut based steam activated carbon  
- 23 µm  
- Microporous  
- Very little meso or macroporosity

Wang et al. (2014)  
Removal of geosmin by PAC as an Emergency Method  

Coal based PAC  
Bamboo based PAC

Tennant and Mazyck (2006)  
The role of surface acidity and pore size distribution in the adsorption of 2-MIB via PAC  

Two PACs were observed  
- Passing a 325 mesh but retained by 400 mesh  
- Similar surface chemistry and different pore structure  
- Different surface chemistry and similar pore structure

Graham et al. (2000)  
Modeling Equilibrium Adsorption of 2MIB and Geosmin in Natural Waters  

PAC: Hydrodarco B, American Norit Co.
Dixon et al. (2011) | A coagulation-powdered activated carbon UF, Australian cyanobacterial blooms | Acticarb PS1000 (Activated Carbon Technologies, Australia)  
| Coal based, steam activated carbon |

Carriere et al., (2009) | Supplementing coagulation with PAC as control strategy for THMs: application to an existing utility | Hydrodarco B by Norit  
| Calgon WPLCoal-based PAC |

### 5.2.2 Model Taste and Odour compound: Geosmin

T&O events are caused by secondary metabolites of cyanobacteria (blue-green algae), including geosmin and 2-MIB (Figure 5-2). Cyanobacteria bloom in surface water where there are high levels of nutrients at warm temperatures. There are several harmful cyanotoxins that are derived from cyanobacteria, however, geosmin is non-toxic. During the growth stages, cyanobacteria synthesizes geosmin. When there is biodegradation of these cells or death, the geosmin is released from the cyanobacteria. In these specific surface water locations where this occurs, especially during warmer temperatures and eutrophic conditions, T&O events ensue. Consumers of water can detect T&O compounds with a musky and earthy odour and/or taste at levels as low as 10 ngL⁻¹ (Zoschke et al., 2011).

PAC has been identified as an effective method in the removal of geosmin since it can be applied into existing treatment systems and applied at the early stages of conventional treatment trains seasonally or whenever there are T&O events. In this research, geosmin will be analyzed as the model T&O compound (Figure 5-2).
5.3 OBJECTIVE

In this experiment, geosmin removal was tested specifically in order to represent T&O compounds. The purpose of this study was develop a method to investigate the interaction of powdered activated carbon (PAC) and ferrate for the removal of geosmin in a closed system reactor. The detailed objectives of this study are as follows:

1. To investigate the effects of mixing scheme for PAC and ferrate in the removal of geosmin and DOC using a jar test protocol;
2. To investigate the effects of NOM in the removal of geosmin using PAC and ferrate;
3. To develop a protocol for observing the interaction of PAC and an oxidant/coagulant;
4. To observe the effects of pH on GAC and ferrate interactions in the removal of DOC and geosmin.
5.4 MATERIALS AND METHODS

5.4.1 Water source

Water source for this experiment was collected from the Georgina Water Treatment Plant in Georgina, Ontario. The water used for this experiment was collected from the raw water line that fed into the plant. This water was untreated without pre-chlorination and is sourced from southern Lake Simcoe. Lake Simcoe water has a DOC of about 3.6 – 4.2 mgL$^{-1}$ and a pH of 8.2 – 8.5.

5.4.2 Chemicals and reagents

Geosmin, geosmin-D3, NaCl, methanol, sodium thiosulfate, potassium ferrate, and the ingredients for acetate and sodium carbonate buffers were all purchased from Sigma Aldrich. Geosmin-D3 was used as the internal standard for the geosmin analysis. For the extraction of the samples, reagent grade sodium chloride was used. Stock and buffer solutions were prepared using ultrapure Milli-Q® water, while calibration stock and check standards used high-purity GC grade methanol.

The PAC used was Pulsorb 260-90 by Calgon Carbon Corporation, which is part of the company’s series of virgin coal based PACs, ideal for removal of taste and odour compounds, endocrine disrupting compounds, and organics (Appendix D).

A stock solution of 10 mgL$^{-1}$ of geosmin was used to spike approximately 100 ngL$^{-1}$ into each sample. Sodium azide was used to preserve the water samples from each of the jar tests (166 µL of 23 µgL$^{-1}$ sodium azide into 23 mL samples).
5.4.3 Bench-scale protocol

Two jar tests with two Phipps & Bird apparatus jar testers were run in parallel for experimental replicates, at room temperature (22°C). 2 L of Lake Simcoe water is poured into each jar prior to the test. Eight jar test samples were run with two replicates each. A carbonate buffer was also added in order to maintain pH levels.

Geosmin was spiked at 100 ngL\(^{-1}\) into each jar and stirred to ensure a homogeneous mixture. All the volumes were adjusted with Milli-Q® water to ensure that the volumes were consistent. The experimental design for the initial trials with ferrate (0 – 20 mgL\(^{-1}\)) and PAC (20 mgL\(^{-1}\)) are listed in Table 5-3 below. For each jar test run, a constant concentration of PAC was added along with varying amount of ferrate.

**Table 5-3: Experimental design for initial simultaneous addition of ferrate and PAC jar tests**

<table>
<thead>
<tr>
<th>Test #</th>
<th>[Ferrate] (mgL(^{-1}))</th>
<th>[PAC] (mgL(^{-1}))</th>
<th>PAC mass (mg)</th>
<th>Ferrate mass (mg)</th>
<th>Geosmin (ngL(^{-1}))</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>100</td>
<td>10 mgL(^{-1}) Ferrate only</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>20</td>
<td>40</td>
<td>0</td>
<td>100</td>
<td>20 mgL(^{-1}) PAC only</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>20</td>
<td>40</td>
<td>0</td>
<td>100</td>
<td>1 mgL(^{-1}) Ferrate + 20 mgL(^{-1}) PAC</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>20</td>
<td>40</td>
<td>2</td>
<td>100</td>
<td>5 mgL(^{-1}) Ferrate + 20 mgL(^{-1}) PAC</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>20</td>
<td>100</td>
<td>10 mgL(^{-1}) Ferrate + 20 mgL(^{-1}) PAC</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>40</td>
<td>100</td>
<td>20 mgL(^{-1}) Ferrate + mgL(^{-1}) PAC</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>Raw Lake Simcoe water with geosmin spike</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Raw Lake Simcoe water only</td>
</tr>
</tbody>
</table>

Combinations of PAC and ferrate were added to each jar using the same stirring protocol. PAC was initially added in a rapid mix stage at 200 rpm for two minutes. Immediately after, ferrate
was added followed by another two minutes of rapid mixing. Observations were taken and then the paddle speed was reduced to 30 rpm to initiate 30 minutes of flocculation. Finally, the mixing of the jar tests was ceased to allow 60 minutes of settling. Once this was completed, sodium thiosulfate was added in excess to each jar in order to quench any available ferrate that had not reacted. This was done in order to prevent any available unused oxidant from interfering with the gas chromatography required to detect geosmin.

The following experimental designs were tested in subsequent jar tests to investigate various concentrations and PAC and ferrate separately. For all procedures, the same stirring protocols were followed:

1) Varying concentrations of only ferrate
2) Varying concentrations of only PAC
3) PAC (20 mgL\(^{-1}\)) and ferrate added simultaneously
4) Ferrate added first, 1 hr contact time, PAC (20 mgL\(^{-1}\)) added second
5) PAC (20 mgL\(^{-1}\)) added first, 1 hr contact time, ferrate added second
6) Ferrate and PAC added simultaneously in Milli-Q®

### 5.4.4 Analysis

Samples of approximately 250 mL were taken from each of the jar tests in order to account for the volume required to perform DOC analysis and GC-MS. The volumes were brought to the vacuum apparatus and filtered using a 45 µM filter. Since ferrate increases the pH of a sample, pH after the experiment was recorded to ensure that the buffer prevented any significant pH fluctuations.

The geosmin analysis was run at the TRACES lab at University of Toronto, Scarborough Campus using an Agilent 7890A gas chromatographer and 5975 mass spectrometer. The column was an HP-5MSx 0.25 mm. Secondary geosmin analysis was performed at the DWRG lab at the
University of Toronto, St. George campus using a solid phase micro extraction (SPME) fiber and analyzed on an Agilent 3800 gas chromatography system with a 4200 mass spectrometer. The method detection limit for geosmin was 7 ngL⁻¹.

5.5 RESULTS AND DISCUSSION

5.5.1 PAC only

PAC is already used as a method of removal for T&O compounds. In this experiment Pulsorb 260-90 by Calgon Carbon Corporation was used, which is a sub-bituminous based compound. PAC at concentrations of 10 mgL⁻¹ and 20 mgL⁻¹ were observed separately from ferrate to investigate geosmin and DOC removal on its own. Over several experiments, 20 mgL⁻¹ of PAC was able to remove DOC by approximately 16% from an initial DOC concentration of about 3.7 mgL⁻¹. This was consistent throughout several separate jar tests with different Lake Simcoe raw water. In the geosmin removal analysis, it was observed that PAC was effective at a concentration of 10 mgL⁻¹. In this sample, the geosmin was removed beyond the MDL.

Furthermore, geosmin removal using PAC was examined with varying pH levels (5.5, 6.5, 7.5, 8.5, and 9.5). It was found that following jar test and treatment with PAC at varying pH levels, the remaining geosmin concentration was below the MDL. As a result, this experiment shows that PAC is effective at removing geosmin at all pH levels.

5.5.2 Ferrate only

The removal of DOC was observed solely using ferrate. Ferrate was dosed in jars with concentrations of 0, 1, 5, 10, and 20 mgL⁻¹. The initial DOC concentration in Lake Simcoe water was approximately 3.96 mgL⁻¹. As the dose of ferrate increased to 20 mgL⁻¹, it was observed that
the concentration of DOC was reduced to approximately 3.5 mgL$^{-1}$. Figure 5-3 below shows the observed percentage removal of DOC and geosmin given varying concentrations of only ferrate (1, 5, 10, and 20 mgL$^{-1}$).

While the DOC concentration decreased as ferrate concentration increased, the removal was not significant. A 5 mgL$^{-1}$ dose of ferrate had a DOC removal of approximately 6%, while a 20 mgL$^{-1}$ dose of ferrate had a DOC removal of approximately 12.5%. It can be concluded that based on this data, ferrate would not be an adequate primary method of removing DOC.

In terms of geosmin removal, the results have no discernable correlation with increase of ferrate concentration and geosmin removed. The highest removal occurs with 1 mgL$^{-1}$ of ferrate. It is possible that there may be a reaction with the NOM in the water matrix that may be contributing to the changes in geosmin removal. While the results were reproducible, these experiments should be repeated with varying concentrations of NOM.

![Figure 5-3: Percentage DOC and geosmin removal in spiked Georgina WTP raw intake water (spiked with 100 ngL$^{-1}$ geosmin) using varying ferrate concentrations of 1, 5, 10, and 20 mgL$^{-1}$ in jar tests](image-url)
5.5.3 Ferrate and PAC added simultaneously

Ferrate and PAC were added simultaneously in order to observe any possible synergistic or interfering effects in the removal of DOC. This experiment used various concentrations of ferrate (1, 5, 10, and 20 mgL\(^{-1}\)) with a constant concentration of PAC (20 mgL\(^{-1}\)).

The results between seven trials of the jar tests were reproducible and similar results were yielded each time (Figure 5-4). In this jar test, the initial DOC concentration was approximately 3.77 mgL\(^{-1}\). While there is a correlation between concentration of ferrate and removal of DOC, overall, it can be seen that there is no significant removal of DOC. The addition of ferrate and PAC makes DOC removal less efficient by a very small amount (about 0.5 mgL\(^{-1}\) removed instead of 0.7 mgL\(^{-1}\) for PAC alone).

Figure 5-4: Percentage removal of DOC and geosmin after simultaneous addition of PAC and ferrate in jar tests with raw Georgina WTP intake water, spiked with 100 ngL\(^{-1}\) geosmin
When PAC and ferrate were added simultaneously, there was an inverse effect in the removal of geosmin as ferrate was increased in concentration. Figure 5-4 shows the percentage geosmin removal in a solution with about 100 ngL$^{-1}$ of geosmin in each jar test. 20 mgL$^{-1}$ of only PAC and PAC with 1 mgL$^{-1}$ of ferrate had similar removals, but as ferrate concentration increased, geosmin removal decreased.

### 5.5.4 PAC and ferrate mixing order

In research performed by Gillogly et al. (1998), it was observed that T&O compounds were most effectively removed when the interaction time between chlorine and PAC was minimized or eliminated. This experiment showed similar patterns in that ferrate reacted similarly when in interaction with PAC. Overall, the addition of ferrate to PAC simultaneously had a negative effect on DOC and geosmin adsorption.

The analysis of DOC removal indicated that the addition of PAC preceding ferrate was the sequence that produced the greatest removal. PAC and 20 mgL$^{-1}$ of ferrate removed the greatest percentage of DOC at about 21.1 %. This order of treatment also had the most significant ability to remove geosmin from water. However, the removal of geosmin diminished with increased concentrations of ferrate. In fact, almost all sequences gave diminishing success in geosmin removal as concentration of ferrate increased. For both DOC and geosmin removal, 5 mgL$^{-1}$ of only ferrate was not sufficient in removing DOC (2 – 21%) (Figure 5-5) or geosmin (0 – 25%) (Figure 5-6). Additional replicate experiments will need to be performed in order to determine that there may be a significant difference in geosmin removal.
Figure 5-5: DOC removal from Georgina WTP raw water (spiked with 100 ngL\(^{-1}\) geosmin) after the addition of (1) PAC added first with 1 hr contact time before ferrate, (2) ferrate added first with 1 hr contact time before ferrate, (3) PAC and ferrate added simultaneously.

Figure 5-6: Geosmin removal from Georgina WTP raw intake water (spiked with 100 ngL\(^{-1}\) geosmin) after the addition of (1) PAC added first with 1 hr contact time before ferrate, (2) ferrate added first with 1 hr contact time before ferrate, (3) PAC and ferrate added simultaneously.
5.5.5 Removal of Geosmin with PAC and Ferrate in Milli-Q Water

In order to observe the adsorbance and oxidation effects of PAC and ferrate in the absence of NOM, the same jar test was performed using Milli-Q® water instead of the raw Georgina water. Newcombe et al. (1997, 2002) observed that large NOM particles are capable of blocking the pores of activated carbon and can prevent pollutants from accessing the sites. The study also found that smaller NOM particles may compete with micropollutants for adsorption sites.

Figure 5-7 compares the simultaneous addition of ferrate and PAC in Milli-Q® and in Georgina WTP water, showing the percentage removal of geosmin after treatment with 20 mgL⁻¹ of PAC and varying concentrations of ferrate (0, 1, 5, 10, 20 mgL⁻¹) added simultaneously. The initial concentration of the geosmin in the spiked Milli-Q® sample was about 67.3 ngL⁻¹. Ferrate applied on its own at a concentration of 10 mgL⁻¹ had the least removal with a remaining concentration of about 43.3 ngL⁻¹ compared to 0% removal in Georgina water. However in Milli-Q®, the remaining combinations removed geosmin below the MDL for all of the concentrations of ferrate with PAC. This may mean that the interference caused by NOM in the adsorption of geosmin may be greater than the interference or change in surface chemistry caused by the addition of ferrate. There could be a reaction between ferrate and NOM causing increasing interference with adsorption onto PAC with increased concentrations of ferrate. This experiment shows that PAC removes the majority of geosmin through adsorption.
5.6 SUMMARY AND CONCLUSIONS

5.6.1 DOC Removal

DOC removal by PAC in the presence of ferrate was observed in a variety of sequences: simultaneously, PAC prior to ferrate, and ferrate prior to PAC. When added simultaneously, the removal of DOC by adding ferrate and PAC simultaneously decreased in comparison to removal with solely PAC. When added simultaneously, a larger concentration of ferrate (20 mgL\(^{-1}\)) had the greatest removal of DOC (11.9%).

When ferrate was added prior to PAC addition and given 1 hr of contact time, the concentration of DOC removed followed no predictable pattern with increase in ferrate concentration. This
may be attributed to the interactions between NOM and ferrate, causing possible interferences with by-products. In only the jar tests where ferrate was added first, there was difficulty in replicating the removal percentages of DOC. This could have been attributed to the coagulation and oxidant properties of ferrate occurring between NOM, PAC, and geosmin, as well as self-decay due to ferrate concentration in a jar test.

The combination with the most effective DOC removal occurred when PAC was added first and followed by the addition of increasing concentrations of ferrate. The 20 mgL⁻¹ ferrate and PAC combination was able to remove a greater concentration of DOC than solely PAC, 20.5% compared to 17.8% It is possible that the adsorption of NOM to PAC sites was uninhibited by the ferrate, which is applied an hour after. In the removal of DOC, it was found the ferrate only at 5 mgL⁻¹ removed approximately 5% of DOC in every test.

Overall, the combination of ferrate and PAC treatment simultaneously reduces DOC adsorption by PAC. However, when one hour of contact with PAC is then followed by ferrate addition, there seems to be greater DOC removal than with using PAC alone. It can be inferred that adsorption of DOC may be increased with the longer contact time with PAC and then some remaining DOC may be oxidized through the addition of ferrate. The treatment with 20 mgL⁻¹ PAC followed an hour later by 20 mgL⁻¹ ferrate has a marginal improvement over treatment with only PAC, at about 3%.

### 5.6.2 Geosmin Removal

When ferrate was added before PAC, the amount of geosmin removed decreased as the concentration of ferrate added increased. The samples using only PAC and PAC with 1 mgL⁻¹ ferrate had similar removals, but as the concentration of ferrate increased, the removal of geosmin decreased. This could indicate that either ferrate or by-products formed with NOM and ferrate were interfering with adsorption.
When ferrate was added after PAC, the removal of geosmin remained the same for varying concentrations of ferrate. Ferrate and PAC added simultaneously was least able to remove geosmin. PAC was maintained at a constant concentration of 20 mgL\(^{-1}\) and had ferrate concentrations of 1 to 20 mgL\(^{-1}\) added. 20 mgL\(^{-1}\) of only PAC was able to remove approximately 91.5% of the geosmin in the sample. On the other hand, ferrate only was found to be able to remove any geosmin at 5 mgL\(^{-1}\). When ferrate and PAC were combined, a negative correlation was observed between the increased additions of ferrate to a constant concentration of PAC. At 20 mgL\(^{-1}\) of ferrate and 20 mgL\(^{-1}\) of PAC, geosmin removal reduced to about 63.1%, indicating that it is possible that ferrate interferes with PAC’s ability to adsorb geosmin. The removal of geosmin for the treatment sequence where ferrate was added first or simultaneous addition dipped to the lowest for the highest concentration of ferrate added (20 mgL\(^{-1}\)). In these combinations, it was observed that ferrate had no additional benefit in the removal of geosmin after PAC addition.

In a study by Park et al. (2007), it was found that ferrate alone could not oxidise more than 25% of geosmin and MIB. Furthermore, oxidants like ozone caused substantial structural changes to NOM by removing any aromatic rings, reducing adsorption of NOM by hydrophobic interaction, and increasing the surface carboxylic groups (Wang et al., 2016). These changes to the structure of NOM created an interference for the adsorbance of geosmin by the PAC. Another issue may be that the oxidant is altering the surface of the PAC itself.

For enhanced uptake, PAC should have a net positive charge. PAC with acidic characteristics had reduced uptake. Park et al., (2007) observed that the removal of geosmin decreases with increasing pH. In this experiment, the final pH for all the jar tests varied from 8 to 9, but all had increased by about a unit individually. This may be a contributing factor for the reduced uptake of geosmin in this experiment. At pH levels below 6, ferrate is very effective (Lee et al., 2004), however, the current experiment was run at a pH of 8 to 9. In future experiments, altering pH levels to suit the preferred ranges of the removal substances may help improve results.
Overall, it was found that this combination of ferrate and PAC was inefficient in removing DOC and particularly geosmin. Since ferrate and PAC had no difficulty in adsorbing geosmin without the presence of NOM, it can be assumed that the natural water matrix combined with ferrate may have had an adverse effect on the adsorption of geosmin. This may be attributed to the oxidization of the surface of PAC (Pakuła et al., 1998) or competition for adsorption sites (Newcombe et al., 2002b). Since the make-up of the NOM is still unknown in Lake Simcoe water, it is unclear what mechanism was occurring between NOM and the surface of the PAC.
5.7 References


6 RECOMMENDATIONS FOR FUTURE WORK

The main objective of this research was to perform a preliminary investigation into the potential for integrating the use of ferrate into current water treatment. The overall goals of this study were to (1) explore *Dreissena* control at water intake sites using ferrate, and (2) observe the synergy of PAC and ferrate in the removal of geosmin compounds and DOC. Results for *Dreissena* control showed that ferrate could possibly illicit a greater inactivity of dreissenids under acute toxicity test settings and a continuously low-dose of ferrate may prevent settlement. Additionally, the results from the geosmin removal experiment showed that interactions between NOM and ferrate may be interfering with PAC adsorption of geosmin.

The results from both of these experiments must be explored further and optimized to create significant conclusions. As a result, several recommendations derived from this study for future work with ferrate are listed below:

1. The results presented here reveal that ferrate could be a possible alternative to chlorine for the control of veliger settlement. However, the effects of an oxidant can depend on different variables. An increase in the concentration of veligers used and taking into greater consideration the life stage and initial characteristics could provide more accurate results. Additionally, a more definite alive/dead protocol would reduce any false positives.

2. The effect of ferrate on settled adult dreissenids could be explored in acute and flow-through settings to determine concentrations or dosing time required to achieve mortality.

3. In regards to the removal of geosmin, the effect of NOM and PAC and ferrate should be investigated further. A characterization of the by-products between ferrate and NOM could help provide some insight into the interference with PAC.

4. In future experiments, it may be beneficial to observe differences through adjusting the addition of PAC. Flipping the addition of PAC and ferrate or giving more time for PAC and/or ferrate to react with the NOM or geosmin may alter results as well. Zheng et al.
(2013) found that PAC binds to the flocs produced by ferrate, the ferric hydroxide, and interferes with the pores on the surface, thereby preventing the adsorption of geosmin and/or NOM. It is for this reason that it may be worthwhile to spread out the additions or changing the sequence of addition. Different types of PAC will have surface properties that may interact differently with ferrate and its by-products.
6.1 REFERENCES


7 APPENDICES
A. APPENDIX Ferrate Properties
A.1 MATERIAL PROPERTIES

A.2.1 Envifer Properties (from NanoIron, CZ)

Product composition:

- \( \text{K}_2\text{FeO}_4 \)
- \( \text{K}_3\text{FeO}_4 \)
- \( \text{KFeO}_2 \)
- \( \text{K}_2\text{O} \)

Characteristics of the product:

- Colour: dark purple
- Form: solid/agglomerates of powder
- Agglomerates are easy soluble in water
- The material is hygroscopic and decomposes already at normal air moisture
- Solid ferrate can be stored in dry condition in original packaging for few months

Chemical-physical properties of solid ferrate

Main components of product are \( \text{K}_2\text{FeO}_4 \) and \( \text{K}_3\text{FeO}_4 \), trivalent iron in form of \( \text{KFeO}_2 \) and K in form of \( \text{K}_2\text{O} \). The material is hygroscopic and decomposes already at normal air moisture \( \text{Fe(V)} \) and \( \text{Fe(VI)} \) are changed into \( \text{Fe(III)} \).

Chemical-physical properties of liquid ferrate

\( \text{K}_3\text{FeO}_4 \) is decomposed into \( \text{Fe(III)} \) and \( \text{K}_2\text{FeO}_4 \) in water environment, the ratio is probably pH dependant. After that \( \text{K}_2\text{FeO}_4 \) is decomposing to iron oxides/hydroxides. The rate of decomposition of \( \text{K}_2\text{FeO}_4 \) and \( \text{K}_3\text{FeO}_4 \) is faster in the acidic range, or in strongly alkaline pH. The most stable solution of ferrate was
made in water solution at pH 9-10.5, at temperature of 7°C, in concentration 0.4 g/L of solid ferrate. It seems that the ferrate is more stable in earthy water comparing to the demineralized water.

**Solid ferrate**

Solid ferrate is necessary to store under dry atmosphere (ideally under nitrogen). Under these conditions, the product can be stored theoretically for unlimited time. Material is not homogenous so there might be differences when analyzing small weights of the product.

**Liquid ferrate**

Dry ferrate is easy soluble in water. Liquid ferrate must be processed very quickly (within half an hour) and the solution is not intended for storage.

### A.2.2 Material Properties

**Table 7-1:** The decomposition rate of Envifer in various concentrations as pseudo-first order constant (Nano Iron s.r.o., 2016)

<table>
<thead>
<tr>
<th>Demineralized water</th>
<th>$\text{pH}_{\text{theoretic}}$</th>
<th>$\text{pH}_{\text{measured}}$</th>
<th>$k / \text{s}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 g.L$^{-1}$Fe(VI)/Fe(V)</td>
<td>11.2</td>
<td>10.9</td>
<td>$-1.3 \pm 0.36 \times 10^{-7}$</td>
</tr>
<tr>
<td>1.0 g.L$^{-1}$Fe(VI)/Fe(V)</td>
<td>11.6</td>
<td>11.5</td>
<td>$-7.8 \pm 0.06 \times 10^{-6}$</td>
</tr>
<tr>
<td>2.0 g.L$^{-1}$Fe(VI)/Fe(V)</td>
<td>11.9</td>
<td>11.9</td>
<td>$-1.9 \pm 0.07 \times 10^{-5}$</td>
</tr>
<tr>
<td>0.4 g.L$^{-1}$Fe(VI)/Fe(V)</td>
<td>11.2</td>
<td>10.9</td>
<td>$-1.3 \pm 0.36 \times 10^{-7}$</td>
</tr>
</tbody>
</table>
Table 7-2: Mössbauer performed by Nanolron CZ (batch 19) (Nano Iron s.r.o., 2016)

<table>
<thead>
<tr>
<th>Oxidation state</th>
<th>Molar weight ratio / total Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(III)</td>
<td>49 ± 3 %</td>
</tr>
<tr>
<td>Fe(IV)</td>
<td>&lt; 3 %</td>
</tr>
<tr>
<td>Fe(V)</td>
<td>45 ± 3 %</td>
</tr>
<tr>
<td>Fe(VI)</td>
<td>6 ± 3 %</td>
</tr>
</tbody>
</table>

Table 7-3: AAS analysis of K and Fe in Envifer (Nano Iron s.r.o., 2016)

<table>
<thead>
<tr>
<th>Element</th>
<th>Weight distribution [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>21,0</td>
</tr>
<tr>
<td>Fe</td>
<td>26,1</td>
</tr>
</tbody>
</table>

Table 7-4: Active components of Envifer by weight distribution (sum is 76 % due to presence of oxide impurities (cca 5 – 10%), accumulation of measurement errors and unidentified components with different stoichiometry (Nano Iron s.r.o., 2016)

<table>
<thead>
<tr>
<th>Species</th>
<th>Weight distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(III)</td>
<td>12,9 ± 3 %</td>
</tr>
<tr>
<td>Fe(IV)</td>
<td>–</td>
</tr>
<tr>
<td>Fe(V)</td>
<td>11,7 ± 3 %</td>
</tr>
<tr>
<td>Fe(VI)</td>
<td>1,6 ± 3 %</td>
</tr>
<tr>
<td>KFe(III)O₂</td>
<td>29 ± 3 %</td>
</tr>
<tr>
<td>K₃Fe(V)O₄</td>
<td>50 ± 5 %</td>
</tr>
<tr>
<td>K₂Fe(VI)O₄</td>
<td>6 ± 2 %</td>
</tr>
<tr>
<td>K₂O</td>
<td>&lt; 3 %</td>
</tr>
</tbody>
</table>
A.2 Ferrate Analysis

A.2.3 Ferrate Purity Determination using Chromite Method

Schreyer et al. (1950) published a method to determine the concentration of ferrate in a given sample through a method they called “the chromite method”, which is based in the oxidation of chromite in a strongly alkaline solution with the ferrate (VI) ion. This oxidation is described in the next equation:

\[
\text{Cr(OH)}_4^- + \text{FeO}_4^{2-} + 3\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_3(\text{H}_2\text{O})_3 + \text{CrO}_4^{2-} + \text{OH}^-
\]

A weighed sample of potassium ferrate, or an aliquot containing the ferrate (VI) ion is added to an excess of alkaline chromite solution. The chromate (VI) solution produced by the oxidation is acidified and the resulting dichromate is titrated with a standard solution of ferrous ions. This method allows the measurement of low concentrations of ferrate (VI).

This method has good results with ferrate concentrations as low as 5.46x10^{-4} M as reported by Golovko et al. (2011)

<table>
<thead>
<tr>
<th>Reagent Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromic chloride hexahydrate</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>Potassium ferrate</td>
</tr>
<tr>
<td>Sulfuric acid</td>
</tr>
<tr>
<td>Phosphoric acid</td>
</tr>
<tr>
<td>Standard dichromate solution</td>
</tr>
<tr>
<td>Ferrous ammonium sulfate</td>
</tr>
<tr>
<td>Sodium diphenylamine sulfonate</td>
</tr>
<tr>
<td>Sodium sulfate</td>
</tr>
</tbody>
</table>
The chromite method, as described by Licht et al. (2001)

Solution Preparation:

For the chromic chloride stock solution:

1. Add 16.67 g of chromic chloride hexahydrate to 100 mL of Milli-Q water

For the saturated sodium hydroxide solution, free of reducing agents:

1. Dissolve 720 g of sodium hydroxide in 1 L of Milli-Q water
2. Add 0.1 g of potassium ferrate
3. Destroy the excess of potassium ferrate by boiling

*This step removes potential interferences of compounds that could compete with the ferrate present in the sample to be analyzed

For the sulfuric acid solution:

1. Prepare a 1 to 5 sulfuric acid solution (from a 95-97% sulfuric acid)

For the sulfuric-phosphoric acid mixture

1. Put 240 mL of Milli-Q water in a beaker
2. Add 60 mL of 95-97% sulfuric acid
3. Add 150 mL of 85% phosphoric acid

For the standard potassium dichromate solution:

1. Add 1.042 g of potassium dichromate to 250 mL of Milli-Q water

For the ferrous ammonium sulfate solution (0.085 N = 33.332 g/L)

Equivalent Weight (EW) of ferrous ammonium sulfate = 392.14 g/eq

\[ m = N \times EW \times V = 0.085 \text{eq L} \times 392.14 \text{g eq} \times 1 \text{ L} = 33.332 \text{ g} \]
1. Add 8.34 g of ferrous ammonium sulfate to 250 mL of Milli-Q water

For the sodium diphenylamine sulfonate solution (≈0.01 M)

1. Dissolve 0.32 g of barium diphenylamine sulfonate in 100 mL of Milli-Q water
2. Add 0.5 g of sodium sulfate.

Or

1. Dissolve 0.068 g of sodium diphenylamine-4-sulfonate in 25 mL of Milli-Q water

Standardization of the ferrous ammonium sulfate solution:

1. Put 25 mL of the standard potassium dichromate solution in an Erlenmeyer flask
2. Add 150 mL of Milli-Q water
3. Add 65 mL of 1 to 5 sulfuric acid dilution
4. Add 15 mL of sulfuric-phosphoric acid mixture
5. Add 7-8 drops of sodium diphenylamine sulfonate
6. Titrate with the ferrous ammonium sulfate solution

The end point of the titration is marked by a change to a green color

Procedure:

1. Prepare an alkaline chromite solution. A fresh solution should be prepared prior to every analysis performed:
   a. Put 5 mL of chromic chloride solution in an Erlenmeyer flask
   b. Add 20 mL of saturated sodium hydroxide solution, free from reducing agents
   c. Add 5 mL of Milli-Q water
2. Cool the solution in an ice bath until it is at room temperature

3. Weigh a sample that contains 0.15 – 0.20 g of potassium ferrate

4. Add the potassium ferrate to the flask containing the alkaline chromite solution (do not allow the potassium ferrate to stick to the walls of the flask, it is recommended to take around 5 mL of the chromite solution with a micropipette in order to wash all of the potassium ferrate from the weighing dish and the walls of the flask if that is the case)

5. Swirl the flask until the potassium ferrate is completely dissolved

6. Add 150 mL of Milli-Q water

7. Add 65 mL of 1 to 5 sulfuric acid

8. Add 15 mL of sulfuric-phosphoric acid mixture

9. Add 7 – 8 drops of sodium diphenylamine sulfonate indicator

10. Titrate immediately with standard ferrous ammonium sulfate solution

The ferrous ammonium sulfate solution should be standardized against the standard dichromate solution immediately prior to use.

The end point is marked by a change from purple to light green.

The calculation of the percentage of potassium ferrate is:

\[ \% K_2FeO_4 = \frac{V(NH_4)_2Fe(SO_4)_2 \times 6H_2O \times N(NH_4)_2Fe(SO_4)_2 \times 6H_2O \times K_2FeO_4 \times 100}{3000 \times \text{Weight of Sample}} \]

- \( V(NH_4)_2Fe(SO_4)_2 \times 6H_2O \) is the volume of the ferrous ammonium sulfate solution used until the end point was reached (in mL)
- \( N(NH_4)_2Fe(SO_4)_2 \times 6H_2O \) is the normal concentration of the ferrous ammonium sulfate solution, obtained from the standardization against the standard dichromate solution (in eq/L)
- \( K_2FeO_4 \) is the molecular weight of potassium ferrate (in gmol⁻¹)
• Weight of sample should be in g
• 3000 is a conversion factor to change mL to L and g mol\(^{-1}\) to g/eq
• 100 is to obtain a percentage

References


A.2.4 Ferrate (K\(_2\)FeO\(_4\)) Residual Determination: Indirect Method with ABTS

The measurement of ferrate (VI) in water can be done with a colorimetric procedure based on the oxidation of ABTS. Ferrate oxidizes ABTS and forms a positively charged radical ABTS\(^{++}\) that has an absorbance peak at 415 nm (Lee et al. 2005). This method is more effective for measuring low concentrations of ferrate and is suitable for a tap water matrix (Luo et al. 2011).

There is a 1:1 stoichiometric ratio between ferrate (VI) and ABTS\(^{++}\), so the absorbance at 415 nm can be directly correlated to the concentration of ferrate in a solution. This positively charged radical has a molar absorptivity of 34,000 ±500 M\(^{-1}\) cm\(^{-1}\). This high molar absorptivity is an order of magnitude greater than the direct method. Luo et al. (2011), determined that absorbance was linear with Fe(VI) concentrations in the range of 0.03 µM to 35 µM (about 0.02 to 2.23 mg/L).
The stoichiometric ratio of ferrate (VI) and ABTS in the overall reaction is 1:2. Ferrate (VI) reacts with two ABTS molecules but only forms one green-coloured ABTS$^{•+}$ radical. There is also production of ferrate (V), which reacts faster with ABTS than ferrate (VI) does and also on different sites in the molecule so ABTS$^{•+}$ is not produced. Because of this, the method requires ABTS to be present in a ratio greater than 2:1 (ABTS:Ferrate (VI)) molar ratio so there are no interferences.

However, ABTS is used to measure other oxidants such as bromine and chlorine (Pinkernell et al. 2000) and there might be positive bias if these oxidants are also present during the experimentation.

To make stock solutions

1. Stock solutions of ABTS reagent are prepared by dissolving 1 g diammonium-ABTS in 1 L Milli-Q® water. If the ABTS stock solution is high (0.02 at 415 nm in a 10 cm path length), it should be replaced.

2. A 5mM Na$_2$HPO$_4$/1 mM borate buffer (pH ≈ 9.1) should be prepared for ferrate standards.

3. Primary stock solutions were prepared by dissolving K$_2$FeO$_4$ in 5mM Na$_2$HPO$_4$/0.5mM borate buffer (pH ≈ 9.1). Mass measurements should be performed in the fume hood.

4. Working stock solutions must not exceed 40 μM, so a maximum of 2.3 mg/L mg of potassium ferrate. Primary solutions are added to 5 mM phosphate / 1 mM borate buffer.

5. Stock solutions should be used immediately.

6. For standardization of stock solutions, take measurements of the absorbance of each dilution in the UV-vis spectrophotometer at 510 nm.
To make standards:

1. A buffer solution of 34.3 mL of CH$_3$CO$_2$H, 6.9 g of NaH$_2$PO$_4$·H$_2$O, and 26.7 g of Na$_2$HPO$_4$·2H$_2$O in 1 L distilled water to give 0.6 M acetate and 0.2 M phosphate (pH ≈ 4.1). This buffer stabilizes iron (III), which otherwise precipitates.

2. Add 5 mL of buffer (pH = 4.1, 0.6 M acetate/0.2 M phosphate) solution to the 25 mL volumetric flask

3. Add 1 mL of ABTS solution to the volumetric flask

4. Dilute the sample if necessary and fill the remainder of the 25 mL volumetric flask with the sample.

5. Formation of green colour should occur in less than 1 sec.

6. Measure absorbance at 415 nm

Table 7-6: UV-vis spectrophotometer conditions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>System</td>
<td></td>
</tr>
<tr>
<td>Wavelength</td>
<td>415 nm</td>
</tr>
</tbody>
</table>

Table 7-7: Potassium Ferrate Residual Determination – Summary of Reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS)</td>
<td></td>
</tr>
<tr>
<td>Phosphate Buffer Solution</td>
<td></td>
</tr>
</tbody>
</table>
Equipment and Materials:

- 25 mL Erlenmeyer flask
- 25 mL graduated cylinder or volumetric flask
- 5 mL of phosphate buffer solution
- 1 mL of ABTS solution (250 mg of diammonium-ABTS salt should be dissolved in 250 mL of Milli-Q® water)
- 19 mL of sample (dilute if necessary)
- UV-vis spectrophotometer

Sample Preparation:

7. A buffer solution of 34.3 mL of CH$_3$CO$_2$H, 6.9 g of NaH$_2$PO$_4$·H$_2$O, and 26.7 g of Na$_2$HPO$_4$·2H$_2$O in 1 L distilled water to give 0.6 M acetate and 0.2 M phosphate (pH ≈ 4.1). This buffer stabilizes iron (III), which otherwise precipitates.

8. Add 5 mL of buffer (pH = 4.1, 0.6 M acetate/0.2 M phosphate) solution to the 25 mL volumetric flask

9. Add 1 mL of ABTS solution to the volumetric flask

10. Dilute the sample if necessary and fill the remainder of the 25 mL volumetric flask with the sample.

11. Formation of green colour should occur in less than 1 sec.

12. Measure absorbance at 415 nm

Blank:

A cuvette with Milli-Q and buffer should be measured at 415 nm
Data Analysis and Reporting

\[ [K_2FeO_4] = \frac{A^{415} \times V_{final}}{\varepsilon \times l \times V_{sample}} \]

Where

- \( A^{415} \) is the absorbance at 415 nm
- \( \varepsilon \) is the molar absorptivity of potassium ferrate \((\approx 34000 \text{ M}^{-1}\text{cm}^{-1})\)
- \( l \) is the path length of the optical cell
- \( V_{final} \) is the final volume after addition of all reagents
- \( V_{sample} \) is the volume of the original sample

References:


A.2.5 Ferrate (FeO_4^{2-}) Residual Determination: Direct Method

For the purpose of measuring potassium ferrate (K_2FeO_4) decay in aqueous solution.

Ferrate concentration can be determined using direct spectrophotometry, as described and published by Lee et al. 2005. This compound has a peak of absorbance at 510 nm (Sharma 2010) in the visible portion of the electromagnetic spectrum. The molar absorptivity of ferrate is highly dependent on pH (Lee et al. 2005) and at pH 9.1 it is 1150M^{-1}cm^{-1} (Lee et al. 2005). This method of determining ferrate is effective at pH levels where ferrate is stable.
Standard solutions can be prepared using commercial, purified ferrate. Reaction rates of ferrate are pH dependent, and that the compound is most stable at pH 9-10 (Li et al. 2005). After reacting, ferrate (VI) might produce iron (III) hydroxide (FeOH₃), which also affects reaction rates (Sharma 2010).

With the direct method, the molar absorptivity of Fe(VI) is an order of magnitude lower than the ABTS method and can change with pH. As a result, slight changes in optical properties of the solution can cause significant errors with the direct method. This method has a limit when Fe(VI) salts sometimes decompose to yield colloidal Fe(III) oxides, which interfere with spectral measurements. This is minimized by using a phosphate buffer. Figure 1 (Lee et al. 2005) below shows both the absorbance using the direct and indirect methods with and without using a phosphate buffer. The initial concentration is 4.5 µM or about 0.29 mg/L.
Figure 7-1: Detection of ferrate using the direct method and the ABTS method. Ferrate at an initial concentration of 4.5 µm or approximately 0.29 mg/L. (a) phosphate buffered solution, (b) borate buffered solution (Lee et al. 2005)

Table 7-8: UV-vis spectrophotometer conditions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>System</td>
<td></td>
</tr>
<tr>
<td>Wavelength</td>
<td>510 nm</td>
</tr>
</tbody>
</table>
Table 7-9: Ferrate Residual Determination – Summary of Reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borate-phosphate buffer</td>
<td>1:5 mM ratio</td>
</tr>
<tr>
<td>Potassium Ferrate</td>
<td>99%, Sigma-Aldrich</td>
</tr>
</tbody>
</table>

Equipment and Materials:

- Cell or cuvette for UV-vis spectrophotometer
- 100 mL graduated cylinder
- 10 mL graduated cylinder
- 5 mL of borate-phosphate buffer (pH 9.1 (5 mM Na₂HPO₄:1 mM borate)
- 100 mL of sample (dilute if necessary)
- UV-vis spectrophotometer
- Magnetic stirrer

Method:

Sample Preparation:

1. Measure 100 mL of sample using a 100 mL graduated cylinder or volumetric flask or dilute sample to 100 mL if necessary
2. Add 5 mL of buffer solution to the sample
3. Add magnetic stirrer
4. Fill a cuvette and measure absorbance at 510 nm
Blank:
A cuvette with Milli-Q and buffer should be measured at 510 nm

Data Analysis and Reporting

\[ [K_2FeO_4] = \frac{A^{510} \cdot V_{final}}{\varepsilon \cdot l \cdot V_{sample}} \]

Where

- \( A^{510} \) is the absorbance at 510 nm
- \( \varepsilon \) is the molar absorptivity of potassium ferrate (≈1150 M\(^{-1}\) cm\(^{-1}\))
- \( l \) is the path length of the optical cell
- \( V_{final} \) is the final volume after addition of all reagents
- \( V_{sample} \) is the volume of the original sample

**A.3 Ferrate Decay Throughout Experiment**

The ferrate used throughout the experiment was from the same source from 2012. It was also experiencing self-decay throughout the duration of this project. As a result, the mass of the potassium ferrate did not correspond to the Fe(VI) that existed in the compound. It is likely that there was degradation into Fe(V), Fe(IV), or Fe(III).

The ferrate masses and concentrations indicated throughout the thesis correspond to the measured weight of the solid potassium ferrate. However, Mössbauer spectrometry from the University of Rochester determined the Fe(VI) content of the potassium ferrate and Envifer, about 60% and 33% purity, respectively (Figure 7-2).
Using the UV-vis spectrometry with the indirect ABTS method, calculations over the course of the experiment were plotted with a linear trend line (Table 7-10). By interpolating the dates of the experiments with the Mössbauer spectrometry determination of 60% purity on September 7, 2016, the purities of the ferrates from each component of the research was estimated. The percentage purity of the potassium ferrate for the acute veliger toxicity, veliger settlement, and PAC experiments were estimated to be 82.0, 79.0, and 60.5%, respectively (Table 7-11).
Assumptions were made in regards to the decay being linear and using the first ABTS measurement as day one. In terms of experimental effect, it changes the concentrations of ferrate being used. However, the effect of lower-order ferrate also have oxidation effects that may not be taken into consideration.

![Graph showing absorbance over time](image)

**Figure 7-3:** Absorbance of Fe(VI) to sample potassium ferrate concentration ratio of ABTS indirect Fe(VI) measurements on dates following June 18, 2015
**Table 7-10**: ABTS indirect absorbance of Fe(VI) to sample potassium ferrate concentration ratio results for dates of measurements

<table>
<thead>
<tr>
<th>Date</th>
<th>Days after first experiment</th>
<th>Absorbance/Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015-06-18</td>
<td>0</td>
<td>0.11479</td>
</tr>
<tr>
<td>2015-06-22</td>
<td>4</td>
<td>0.093374</td>
</tr>
<tr>
<td>2015-07-30</td>
<td>42</td>
<td>0.093</td>
</tr>
<tr>
<td>2015-10-01</td>
<td>105</td>
<td>0.0889</td>
</tr>
<tr>
<td>2016-03-23</td>
<td>279</td>
<td>0.073755102</td>
</tr>
<tr>
<td>2016-09-18</td>
<td>458</td>
<td>0.0722</td>
</tr>
</tbody>
</table>

**Table 7-11**: Determination of estimated Fe(VI) percentage purity of sample potassium ferrate for experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Date</th>
<th>Days after first experiment</th>
<th>Interpolated UV Absorbance</th>
<th>Fe(VI) Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mossbauer</td>
<td>2016-09-08</td>
<td>448</td>
<td>0.06854</td>
<td>59.5%</td>
</tr>
<tr>
<td>PAC Tests</td>
<td>2016-08-22</td>
<td>431</td>
<td>0.06973</td>
<td>60.5%</td>
</tr>
<tr>
<td>Acute Veliger</td>
<td>2015-09-03</td>
<td>77</td>
<td>0.09451</td>
<td>82.0%</td>
</tr>
<tr>
<td>Veliger Settlement</td>
<td>2015-10-22</td>
<td>126</td>
<td>0.09108</td>
<td>79.0%</td>
</tr>
</tbody>
</table>
B. APPENDIX B Veliger Acute Toxicity Test
B.1 SOURCING *Dreissena* VELIGERS

B.1.1 Finding Sampling Locations

In order to perform the experiment, the concentration must consist of a high natural concentration of *Dreissena* veligers, including quagga and/or zebra mussels. These are fairly undistinguishable in the environment. Grab samples should be taken from locations to determine presence of veligers in water. Choose a deep locations where the plankton net can be dropped vertically at least 10 m (boat, pier, boardwalk etc.). Veligers should be present in water table after about 10 consecutive days of water warmer than 15°C.

B.1.2 Collection

Correct collection protocol is critical to ensure that an adequate amount of veligers are collected and that that they have underwent little stress. This will ensure a quality sample that will permit the veligers to live a long period of time.

- Use 63 um plankton net to collect vertical tows by sinking the net as far down as the rope goes or before hitting the lake bed and slowly pull up no faster than 0.5 m/s. This will allow the column of water to be pulled through the sieve without being forced out.
- Allow all water to have left the sleeve before unscrewing the canister, then swirl the plankton canister until the excess water has been drained
- Pour the canister of water through a 250 um sieve, followed by a 180 um sieve to remove all larger zooplankton, plankton, copepods, and algae from the sample. Clean the sieves frequently.
- Dilute samples if necessary because overly concentrated samples will cause stress on the veligers upon transport and storage. It puts a stress on food supply and oxygen.
- All interfering zooplankton and algae must be sieved out of the sample to retain dissolved oxygen, prevent interference and unnecessary movement, and for visibility
Ensure that all mesh and sieving equipment has been submerged in water and rinsed thoroughly. There should be no algae.

While the area of the net and the depth submerged is usually recorded to determine the density of veligers in the water, it was unnecessary in this case to record the accurate amount of volume through the net. The purpose of veliger collection in this case was to collect as many veligers as possible. This was performed by dropping the net into the water and letting it sink to about 10 m below the surface. Once fully submerged, the net was hoisted up to the surface in a smooth and continuous manner. All the water was allowed to drain through the net and following that, the canister was unscrewed and the contents were filtered again.

B.1.3 Storage of *Dreissena* veligers

1. Store the sample containers in a refrigerator until ready to use
2. Ensure that storage temperature does not exceed 20°C

Veligers are without protection from environmental changes, vulnerable to stress so transporting them to the laboratory and storing them for several days can be difficult without a high level of mortality. Once veligers have been coarse sieved into a jar after collection from a freshwater source, it was quickly placed on ice. Keeping the veligers cool will slow down their physiological functions. Since the solution is now a closed system, there is a limited amount of food, space for waste, and dissolved oxygen. Slowing down their metabolic rates through keeping the solution cool will prolong the life of the veligers in solution.

Veliger were separated into more diluted solutions were stored in 4°C when not in use in order to reduce activity, metabolism, and to increase longevity. All samples of veligers were brought to room temperature prior to use to ensure that they acclimated and had similar rates of activity. Use veligers as soon as possible after sampling to ensure that environmental stress has been kept to a minimum to prevent any influence on the experiment.
B.1.4 Concentration Determination of Samples

Each sample taken may have a different concentration. Gentle mixing to ensure that it is a homogeneous solution.

1. Mix preserved sample thoroughly
2. Remove 1 mL from middle of jar, avoid the bottom
3. Place a cover diagonally across the Sedgewick-Rafter cell
4. Fill cell slowly until water is evenly in contact with the cover slip
5. Count veligers in as many Whipple squares as need to obtain 60 veligers

Using the Sedgewick-Rafter Cell

1. Count veligers in as many Whipple squares as needed to obtain 60 veligers. If there are more than 60 veligers per Whipple square dilute the original sample by one half until adequate density is reached
2. Count veligers unbiasedly by listing order of squares before counting the slide
3. Record dilution and number of squares counted.
4. Record total number of veligers in five squares
5. For low veliger densities: count number of veligers in entire S-R cell by scanning back and forth over cell. Count each cell until same total is reached
6. Repeat procedure for a total of 5 samples from each sample jar.

The number of veligers per milliliter of sample is calculated for each S-R cell:

\[
\frac{veligers}{mL} = \frac{N \times 1000 \text{ mm}^3 \times df}{A \times D \times F}
\]

Where:
- \(N\) = total number of veligers counted
- \(df\) = dilution factor (\(df = 2\) if sample was diluted by \(\frac{1}{2}\) for counting)
- \(A\) = area of a field (Whipple grid image area in mm\(^2\)
D = depth of a field (S-R) cell depth = 1 mm
F = number of fields counted

Use the dilution factor and volume of water filtered to determine an estimate of the total density of veligers in the sampling location.

6. Repeat this 5 times for each sample

**B.1.5 Alive Dead Analysis and Practice**

Perform alive dead analysis to get a good handle on what a dead *Dreissena* veliger looks like in comparison to a live one. Take a fresh sample and observe all different types and size of veligers. Every veliger that counts should be larger than 1 whipple square, anything smaller can be often confused with other zooplankton, ostracods.

Take the same sample and apply a concentration of chlorine greater than 5 mg/L or boil the water. Re-examine the sample so that a dead veliger can be characterized visually.

**B.1.6 Veliger Microscopy**

Remove a 1 ml aliquot and dispense on the Sedgewick-Rafter counting cell. Ensure that the aliquot is bulging over the edges by surface tension slightly. Carefully place the glass cover slip over the well by placing it diagonally across and allowing it to swing into place to hold the volume. Cleaning using an acetic acid bath helps dissolve the calcium of a veliger shell. Methodologies followed the USACE Environmental Laboratory methods of analyzing plankton tows (USACE 2002).
While cells with grids were not used, it is highly recommended for use to help count and keep track of moving veligers. Since veligers tend to sink due to their density in the water and propensity to settle, the majority of veligers will be found in the bottom portion of the solution.

Ensure that the light used to illuminate the solution in the compound or stereo microscope is turned off when not in use because veliger activity will be affected by the increase of temperature contributed by the light.

**B.1.7 Preliminary Test: quenching agent on veligers to observe toxicity**

Need to find an adequate quenching agent that will have no effect on the viability of veligers: sodium thiosulfate (STS) or ABTS

1. Determine working concentration of veliger solution. Count dead and alive veligers and ensure that there are no organisms in the samples that are capable of disturbing the veligers or reducing DO substantially
2. Test STS first because of the lack of colour.
3. Test initial and final water properties including: temperature, DOC, TOC, UV 254, Turbidity, pH, Fe residual, Chlorine residual.
4. Experimental Design: 3 Replicates using one time dosages and measuring toxicity after 30 minutes.

(Using 10 minutes because although I want to determine immediate dosage and effects of quenching agent on mortality, it will probably take 30 minutes until I can fully examine the sample)
<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. Ensure same sample of water and same concentration for each replicate. Count by taking 5 samples from each watch glass and counting each Sedgewick-Rafter cell.

**B.2 VELIGER BEHAVIOR**

**B.2.1 Veliger Stress**

A less quantitative and predictable external factor that affects the life span and sensitivity of a veligers to oxidants is stress. When handling veligers, one must be very careful to not create any additional stress factors that may influence a veliger physically. The following is a list of factors that may affect the health of veligers (Claudi 2015):

- Extreme temperatures
  - particularly those exceeding 24°C, as well as prolonged periods on ice
- Turbulence
  - Any pouring or transferring of veliger solutions should be done carefully. High flow rates can dismantle the veliger shells. Veligers close their shells when they experience stress or the presence of an unfavourable substance. Once their shells
are affected, they will be vulnerable to environmental changes and may die prematurely.

- Any mechanical stirring of a veliger solution performed by a magnetic stirrer, for example, is unnecessary and causes immediate death of the veligers. Any stirring of the veliger solution should be performed with a stirring rod or gentle suction and release with a pipet
- Veligers require aeration if kept in a tank for a long period of time. Try to avoid direct aeration as it may also disturb the veliger shells.

- The presence of unknown substances
  - Zebra mussel and quagga mussel veligers have not been studied in depth. While they are an invasive species that thrive in the Great Lakes, there are many substances that they are sensitive to. Ensure that any new environment that they are to be exposed to is thoroughly rinsed with lake water and that no possibly harmful substances are present. This could include any metals, salts, adhesives, etc.

- Hardness
  - The presence of CaCO$_3$ is absolutely integral to the survival of the zebra mussels. It is essential for the mussels to build their shells. A lack of this substance will result in the degradation of their shells.

- Direct light
  - Try and avoid direct light. Veligers tend to move up and down in the water column depending on temperature and light. While direct light is unfavourable, light is still required for any algae present in the water that the veligers consume.

- pH
  - pH is another factor that must be monitored daily. Veligers are extremely sensitive to any pH outside of 7.6- 8.8. The pH of lake water, at around 8 is the most favourable pH for them to thrive in.

- Lack of food
In several papers that observe veligers, the prolonged survival of veligers is facilitated with the addition of algae. In these experiments, an aquarium food for shrimp was added to the coolers and was effective in extending their lifespan.

**B.3 Ferrate Concentration in Solution**

Aqueous ferrate concentration can be determined using direct spectrophotometry, as described and published by Lee et al. (2005). Aqueous ferrate has a peak of absorbance at 510 nm (Sharma 2010) in the visible portion of the electromagnetic spectrum. As seen in Error! Reference source not found., the visible absorption spectra shows a clear peak under UV-spectroscopy. This method of determining ferrate is effective at pH levels where ferrate is stable and as a result aqueous ferrate was prepared in phosphate buffer. The molar absorptivity of ferrate is highly dependent on pH (Lee et al. 2005) and at pH 9.1 it is 1150 M$^{-1}$ cm$^{-1}$ (Lee et al. 2005). Standard solutions were prepared using commercial, purified ferrate (Sigma Aldrich).

![Figure 7-4: Direct visible spectra of Fe(VI) in aqueous solution, peak at 510 nm (adapted from Li et al., 2005)](image-url)
B.3.1 Direct Method

With the direct method, the molar absorptivity of Fe(VI) is an order of magnitude lower than the ABTS method and can change with pH. As a result, slight changes in optical properties of the solution can cause significant errors with the direct method. This method has a limit when Fe(VI) salts sometimes decompose to yield colloidal Fe(III) oxides, which interfere with spectral measurements. This is minimized by using a phosphate buffer. Figure 7-6 (Lee et al. 2005) below shows both the absorbance using the direct and indirect methods with and without using a phosphate buffer. The initial concentration is 4.5 µM or about 0.29 mg/L.

Figure 7-5: Detection of ferrate using the direct method and the ABTS method. Ferrate at an initial concentration of 4.5 µM or approximately 0.29 mgL\(^{-1}\) in a phosphate buffered solution (Lee et al. 2005)
B.3.2 Indirect Method

Smaller concentrations of ferrate less than about 2.23 mg/L or 35 µm can be detected using the indirect method, which involves the use of 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). The measurement of ferrate (VI) in water can be done with a colorimetric procedure based on the oxidation of ABTS. Ferrate oxidizes ABTS and forms a positively charged radical ABTS$^{•+}$ that has an absorbance peak at 415 nm (Lee et al. 2005). This method is more effective for measuring low concentrations of ferrate and is suitable for a tap water matrix (Luo et al. 2011).

There is a 1:1 stoichiometric ratio between ferrate (VI) and ABTS$^{•+}$, so the absorbance at 415 nm can be directly correlated to the concentration of ferrate in a solution. This positively charged radical has a molar absorptivity of 34,000 ±500 M$^{-1}$cm$^{-1}$. This high molar absorptivity is an order of magnitude greater than the direct method. Luo et al. (2011), determined that absorbance was linear with Fe(VI) concentrations in the range of 0.03 µM to 35 µM (about 0.02 to 2.23 mg/L).

The stoichiometric ratio of ferrate (VI) and ABTS in the overall reaction is 1:2. Ferrate (VI) reacts with two ABTS molecules but only forms one green-coloured ABTS$^{•+}$ radical. There is also production of ferrate (V), which reacts faster with ABTS than ferrate (VI) does and also on different sites in the molecule so ABTS$^{•+}$ is not produced. Because of this, the method requires ABTS to be present in a ratio greater than 2:1 (ABTS:Ferrate (VI)) molar ratio so there are no interferences. However, ABTS is used to measure other oxidants such as bromine and chlorine (Pinkernell et al. 2000) and there might be positive bias if these oxidants are also present during the experimentation. Methodology followed techniques used by (Lee et al., 2005).
Figure 7-6: Standard curves obtained by the ABTS method prepared in a buffer solution of pH 9.1 (5 mM Na$_2$HPO$_4$/1mM borate) (Lee et al., 2005)

Figure 7-7 shows that the absorbance of the ABTS ferrate solution does not diminish or change over time. Solutions kept for longer before undergoing UV-spectrometry procedures will remain accurate.
B.3.3 Hach Spectrometer DR2800

Since the acute experiment was performed in the at the Glenora Fisheries Ministry of Natural Resources and Fisheries laboratory, the portable Hach DR2800 Spectrophotometer was used to determine the ferrate and chlorine decay over the 30 minutes after the acute addition of the oxidant. The direct method was employed for determining ferrate concentration in the acute experiment. On the other hand, the DPD test was employed for use to determine the chlorine concentration.

A calibration curve was developed for the DR2800 for ferrate, but lower concentrations were likely less exact given that the direct method is not as sensitive to low concentrations of ferrate.

Figure 7-7: ABTS solution of 1 mgL$^{-1}$ of ferrate over time
B.4 Veliger Collection and Testing

B.4.1 Veliger Testing

In order to define the procedure for the experiment, the steps for the preparation of the 1 mg/L chlorine petri plates will be examined. In this portion of the experiment, eight petri dishes were prepared: three of these were to examine three replicates of veliger samples, while the remaining six were to obtain oxidant residual data. A concentrated veliger solution was prepared and was homogeneously mixed. 1 mL of this homogeneous veliger solution was pipetted into each one of the eight petri dishes. Following this 4 mL of lake water was added to each one of the eight dishes. A solution of chlorine was made with a concentration that when added to each of the 5 mL petri dishes would produce a concentration of 1 mg/L. The final volume of all the dishes was made to be 7 mL for consistency.

Before the application of the chlorine solution to the petri dishes, the three veliger replicate plates were observed under the microscope to count the initial number of veligers present in the dish. A portion of the veligers were observed so that it was manageable. The count was separated
into pediveligers and lesser developed veligers (umbonal or hinged stage). Once the veligers were counted and labelled, the first petri dish was placed under the microscope. The chlorine was then pipetted to observe the immediate behavior of the veligers. This was then repeated for all of the veliger replicates. The chlorine was also dosed into the petri dishes to determine the residual. The timer was set at this point to observe 30 minutes of veliger behavior.

Veliger behavior was filmed and counted every 5 to 10 minutes, but it varied for each concentration of oxidant. Ideally, the same time frame should have been observed for each concentration, but there were delays with filming, counting, and sometimes complications with timing arose since all three replicates were observed simultaneously.

The petri dishes for the residuals were measured using the Hach spectrophotometer DR 2800 every 10 minutes. Since each dish had 7 mL of solution, two dishes had to be combined for each time period, 0, 10, 30 minutes.

In order to quantify the effects of oxidants on the veligers, the behavior of the veligers must be defined into clear and differentiable categories. It is very difficult to determine whether a veliger is dead or not unless their innards are on the outside of their shell and they are immobile.

Essentially, a veliger only really dies if they have experience significant turbulence and agitation or their shell has been damaged or “eaten” away. The moment an oxidant is added a liquid, veligers will close up immediately. They will still be alive, but will protect themselves by shutting down their feeding and filtering mechanisms. The only way that the oxidant will kill the veliger is if their shell is improperly shut and allows oxidant infiltration to harm their organs. Otherwise, the oxidant must be strong enough to break down the calcium carbonate shell to enter and destroy their organs. It is possible to see the breakdown of the shell as holes in the shell over time when exposed to an oxidant.

The following categories were used when describing the behavior of veligers:

**Dead**

- A dead veliger will be completely immobile. There will be no movement whatsoever inside or out of the shell. It is difficult to describe what a veliger looks
like when it is dead or not because some have their shells open and some have their shells closed. One that is dead by recent oxidant dosing will have its organs excreted outside of its body. The organs outside of its body look like a cloud and is sometimes “shimmery” under a compound microscope. See Figure 7-9 below.

![Image of dead and open veligers under 100x microscopy with Leica M125 microscope]

**Figure 7-9:** Comparison of dead and open veliger under 100x microscopy with Leica M125 microscope

**Open**
- It this experiment, an open veliger is described as a veliger with its shell open. It can either have its velum protruding, or its foot out. An open shell means that the veliger is feeding, filtering, and very active. Typically, in this case an open veliger will be rolling, rotating around and might sometimes pick up a crawl or a swim. In this case, they are filed under the category that they are most seen doing. Open veligers are most definitely alive and are an unfavourable behavior (Figure 7-9).

**Swimming**
- An open and swimming veliger is at its highest activity level. Veligers motor around in a circular pattern with its velum as its propulsion. Veligers will typically open up and swim when the temperature of the water is around room
temperature or 20 – 24 °C. Veligers will not swim if there is an oxidant present or if there are unfavourable conditions.

Not moving

- In this experiment, a veliger that is not moving is in an indeterminable state where it is uncertain whether the veliger is alive or dead. The shell is typically closed and there is no movement in organs, no pulsing, or swimming. Veligers are filed under this category when there might be movement in surrounding organisms or in the fluid and the veligers might only be moving due to external factors and might be dead. Since the time allotted for determining veliger states is short, this category was given to quickly categorize veligers.

Dying

- Dying veligers are in a state where death is eminent. These veligers are visibly injured and exhibit uncharacteristic movement. They are either moving because their velum has been closed in their shell, their velum is still moving after their innards have been ejected from their shell, or there may be other organisms which have hijacked the shell and are feeding on the remnants of the veliger.

Closed

- A closed veliger is still alive but is simply closed and not moving. In order to determine whether or not the veliger is still alive, one must look closely at the internal organs. There is often pulsing, digesting, or movement in the internal organs which indicate that the veliger is likely alive. The organs are visibly intact in this state (Figure 7-10)
Overall, the veligers must be finally categorized into an ideal or non-ideal state. Therefore, any mistakes in determining behaviors is not as critical. Ideal states are behaviors which prevent veligers from settling and allow them to wash away: dying, closed, dead, or not moving. The remaining behaviors are non-ideal and allow veligers to exist in a state where they may begin to release their byssal threads and settle on a surface.
**B.4.2 Veliger Collection**

Collections were performed during periods of the summer season where the Lake Ontario water temperature exceeded 12°C according to surf-forecast.com (Figure 7-12). Collections during the summer 2015 season began in late May and continued until late October.

The majority of veliger samples were acquired with the assistance of Carolina Taraborelli of the Glenora Department of the Ministry of Natural Resources and Fisheries of the Government of Ontario, located in Prince Edward County, Ontario. The Glenora MNRF was performing biweekly sampling of plankton and water quality.

![Figure 7-11: Lake Ontario average temperature ranges since 1984 (surf-forecast.com)](image)

Veligers were extracted from identified locations over the course of the experiment. The identified locations and collection dates are listed in Table 7-12 below.
The collection locations for the experiment were all in Ontario. All locations and samples had some presence of veligers, at least one veliger for 10 mL. It was difficult to find a consistent location where there was adequate depth since the height of water changes during the season.

The following locations were used for the experiments in 2015. Table 7-12 lists the locations, dates, methods of access, depth, number of tows, and the veliger concentration yielded.

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Method of Access</th>
<th>Depth</th>
<th>Tows</th>
<th>Veliger Concentration of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>All throughout the summer</td>
<td>Whitby Lighthouse Pier</td>
<td>Pier access</td>
<td>10 m</td>
<td>20</td>
<td>max 10000/L</td>
</tr>
<tr>
<td>October 1, 2015</td>
<td>Oshawa Pier</td>
<td>Pier access</td>
<td>8 m</td>
<td>10</td>
<td>4000/L</td>
</tr>
<tr>
<td>All throughout the summer</td>
<td>Sugar Beach at George Brown Waterfront Campus</td>
<td>Pier Access</td>
<td></td>
<td></td>
<td>max 16000/L</td>
</tr>
<tr>
<td>July 10, 2015</td>
<td>Port Dover, Lake Erie</td>
<td>MNRF Fishing Boat</td>
<td>~25 m</td>
<td>20</td>
<td>2000/L</td>
</tr>
<tr>
<td>August 18, 2015</td>
<td>Station 81, Glenora, Lake Ontario</td>
<td>MNRF Sampling Boat</td>
<td>28 m</td>
<td>6</td>
<td>max 100000/L</td>
</tr>
<tr>
<td>September 3, 2015</td>
<td>King St. Water Treatment Plant, Kingston, Lake Ontario</td>
<td>Treatment Plant Dock</td>
<td>8 m</td>
<td>50</td>
<td>8500/L</td>
</tr>
<tr>
<td>September 16, 2015</td>
<td>King St. Water Treatment Plant, Kingston, Lake Ontario</td>
<td>Treatment Plant Dock</td>
<td>8 m</td>
<td>50</td>
<td>8500/L</td>
</tr>
<tr>
<td>October 8, 2015</td>
<td>Cook’s Bay, Georgina, Lake Simcoe</td>
<td>Small 14 ft Boat</td>
<td></td>
<td></td>
<td>750/L</td>
</tr>
<tr>
<td>July 30, 2015</td>
<td>Cook’s Bay, Georgina, Lake Simcoe</td>
<td>Small 14 ft Boat</td>
<td></td>
<td></td>
<td>750/L</td>
</tr>
</tbody>
</table>
C. APPENDIX C Veliger Settlement Experiment
C.1 OXIDANT DECAY

The purpose of this experiment is to observe the settlement of pediveligers on plates placed in an environment where they would receive a constant exposure of 1 mgL$^{-1}$ of oxidant in Lake Ontario water. Tests were performed to observe the stability of ferrate and chlorine in Lake Ontario water. Based on the data found from the decay of chlorine and ferrate over time, it was then possible to determine how much ferrate and chlorine must be replenished to maintain a constant level. Observations of the reaction mechanism will help determine the dosing rate of the stock solution required to maintain the cooler units at consistent concentration of 1.0 and 0.1 mgL$^{-1}$ ferrate and free chlorine.

C.2 CHLORINE DECAY

The decay of free chlorine over time in Lake Ontario water was observed (Figure 7-12). Lake Ontario water was collected from the raw water feed at the R.C. Harris Water Treatment Plant (Toronto, ON) and 250 µm filtered lake water from the shore of Lake Ontario. Since in the latter sample the water was taken directly from the lake, it was observed that that the decay of oxidant would be faster due to a higher concentration of organics.

C.1.1 Decay in Lake Ontario water

A decay of chlorine in Lake Ontario water was observed with a k-constant of about -6e-04 and an initial demand of about 0.38 mgL$^{-1}$ (Figure 7-12). A solution with a higher concentration of chlorine was to be used for the stock solution in the dosing of the coolers. Since chlorine is able to remain stable in Milli-Q water in dark, cool conditions, this solution was not adjusted for.
Figure 7-12: Decay of free chlorine over 24 h in filtered Lake Ontario water. $C_0=1.2$ mgL$^{-1}$ (a) concentration over time (b) modeled as a first-order reaction
C.3 Ferrate Decay

C.2.1 Lake Ontario Water

Potassium ferrate was added to room temperature (22 °C) Lake Ontario water. Using the indirect method, the concentration of ferrate was observed over 24 hours. The reaction observed first-order reaction characteristics.

![Graph](image)

**Figure 7-13:** Decay of ferrate over 24 h in filtered Lake Ontario water. $C_0=1.05$ mgL$^{-1}$ (a) concentration over time (b) modeled as a first-order reaction

$y = 0.724e^{-0.003x}$

$R^2 = 0.9898$

$y = -0.0013x - 0.1402$

$R^2 = 0.9898$
C.4 Envifer Decay

C.3.1 Milli-Q Water

Envifer was added to Milli-Q water to observe its decay over 24 hours.

**Figure 7-14**: Decay of Envifer as ferrate over 24 h in Milli-Q water $C_0=1.05 \text{ mgL}^{-1}$ (a) concentration over time (b) modeled as a first-order reaction
C.3.2 Lake Water

Envifer was added to Lake Ontario water in Figure 7-15 below. Similar to chlorine, there is an initial demand of ferrate within the first couple minutes of ferrate addition. As seen from the R value on the second order reaction graph, the organic content in the water and the impurity of ferrate results in a poor second order relationship. This decay experiment was replicated several times and each attempt delivered a slightly different decay rate. Based on this information, an amount of ferrate was to be added to the cooler to remove the initial demand. Following this, an assumed k-constant of 0.0003 was used to model a required concentration and rate for dosing.

Any required adjustments would be made as the experiment proceeded.

Figure 7-15: Ferrate as Envifer decay in Lake Ontario water over 24 h: (a) concentration over time, (b) second order reaction decay rate
C.3.3 Envifer Decay in Lake Ontario water with Veligers

Envifer was added to Lake Ontario water with a similar veliger solution that was used in the experiments. This was to account for the oxidation that would be occurring with a greater number organisms in the solution.

![Figure 7-16: Ferrate as Envifer decay in Lake Ontario water with veligers over 24 h, $C_0 = 1.46 \text{ mgL}^{-1}$, modelled as a second-order reaction decay rate]

C.3.4 pH 9.1 Phosphate Buffer

Ferrate (VI) or Envifer is known to be unstable in an aqueous solution. Envifer’s decay rate in solution increases with higher concentrations. In this case, the stock solution had a concentration of around 30 mgL$^{-1}$. A borate buffer was prepared and the decay rate of a solution of 30 mg/L of Envifer was observed (Figure 7-17). When graphed as a second order reaction (Figure 7-18 Error! Reference source not found.), the R-value was significantly closer to 1 than the Lake Ontario water decay rates. This may be because Envifer was exhibiting self-decay and there were no organics for the Envifer to interact and interfere with.
Figure 7-17: Decay of Envifer as ferrate in pH = 9.1 phosphate buffer solution prepared with Milli-Q water over 24 h, $C_0 = 34\text{ mgL}^{-1}$

Figure 7-18: Second order decay of Envifer as ferrate in pH = 9.1 phosphate buffer solution prepared with Milli-Q water over 24 h, $C_0 = 34\text{ mgL}^{-1}$
Figure 7-19: Decay in pH 9.1 Borate buffer over 24 h, $C_o = 21.8 \text{ mgL}^{-1}$

C.5 DOING SCHEME

C.4.1 Pumping Scheme

Table 7-13: Volume of stock solutions dispersed per time period in one day

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Volume dispersed (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>180 (3 hours)</td>
<td>125</td>
</tr>
<tr>
<td>360</td>
<td>125</td>
</tr>
<tr>
<td>540</td>
<td>125</td>
</tr>
<tr>
<td>720 (12 hours)</td>
<td>250</td>
</tr>
<tr>
<td>900</td>
<td>250</td>
</tr>
<tr>
<td>1080</td>
<td>250</td>
</tr>
<tr>
<td>1260</td>
<td>250</td>
</tr>
<tr>
<td>1440 (before start of next day)</td>
<td>375</td>
</tr>
</tbody>
</table>
Figure 7-20 below is sample dosing scheme for Envifer as ferrate in a 30 L tank. The decay rates were determined by using the average k-constant, while an optimal dosing rate was determined by using the model. As indicated below, the orange points are the overall concentration in the tank over the 24 hour period. A new model was created for every following 24 hour time period.

It should be noted that these models were only used as guidelines for the entire experiment. Additional single doses were added where needed. The flow rates were also adjusted after reading daily residuals to ensure that the concentration did not exceed 1 mgL\(^{-1}\) by too much. The dosing of Envifer was much more complicated than for chlorine which does not decay in its Milli-Q stock (Figure 7-21).

![Figure 7-20: Dosing rates to maintain a 1 mgL\(^{-1}\) Envifer as ferrate residual in a 30 L tank given a 75 mgL\(^{-1}\) stock solution dosed at programmed intervals](image-url)
Figure 7-21: Dosing rates to maintain a 1 mgL$^{-1}$ free chlorine residual in a 30 L tank given a 24 mgL$^{-1}$ stock solution dosed at programmed intervals
C.4.2 Dosing Scheme

Figure 7-22 below is sample dosing scheme for Envifer as ferrate in a 30 L tank. The decay rates were determined by using the average k-constant, while an optimal dosing rate was determined by using the model. As indicated below, the orange points are the overall concentration in the tank over the 24 hour period. A new model was created for every following 24 hour time period.

It should be noted that these models were only used as guidelines for the entire experiment. Additional single doses were added where needed. The flow rates were also adjusted after reading daily residuals to ensure that the concentration did not exceed 1 mgL$^{-1}$ by too much. The dosing of Envifer was much more complicated than for chlorine which does not decay in its Milli-Q stock.

![Graph showing dosing rates](image-url)

**Figure 7-22:** Dosing rates to maintain a 1 mgL$^{-1}$ free chlorine residual in a 30 L tank given a 30 mgL$^{-1}$ stock solution dosed at programmed intervals.
C.4.3 Survival Rates in Cooler

Overall, it was found that veligers were uncomfortable in their environment in the lab. The veligers were unable to stay alive longer than 7-8 days. Lake water was used to simulate their living environments much more closely. Other parameters of the water that were observed were temperature, pH, hardness, dissolved oxygen, and oxidation-reduction potential. While many of these parameters stayed consistent and at a suitable level for the veligers, there was still premature veliger death. It is worth noting that each of the samples taken every three or so days had a mixture of veligers at different stages in their life cycle. As a result, approximately three samples were taken for each condition at each time slot.

As a result, tests were performed in more inert environments in order to determine if there was an additional substance that was causing death in the veligers. Based on these various tests, the cause of veliger death was indeterminable.

A factor that could not be determined through tests was the waste produced by the veligers themselves. This may have been a factor which could have affected the lifespan of the veligers. It is for this reason that half of the volume of water in the cooler was replaced with fresh, filtered lake water as well. While this extended the life longer by introducing new food and removing wastes, the veliger population observed a significant loss in number by day 10. Finally, fish food that was used for the fish tank in the lab was added as an experiment to see what would occur. Ocean Nutrition Flake Food, which is full of plankton and algae (consumables for the veligers), was added. The percentage of veliger death at the end of 10 days dropped a little less significantly.

Several tests were performed in order to ensure the optimal survival rate among the veligers after 10 day in a cooler (Table 7-14).
Table 7-14: Veliger viability in different environments

<table>
<thead>
<tr>
<th>Container</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooler with black panels with aeration (bottom)</td>
<td>85%</td>
<td>65%</td>
<td>30%</td>
<td>0%</td>
</tr>
<tr>
<td>Glass beaker with aeration</td>
<td>97.5%</td>
<td>70%</td>
<td>0%</td>
<td>5%</td>
</tr>
<tr>
<td>Glass beaker without aeration</td>
<td>95%</td>
<td>72%</td>
<td>5%</td>
<td>0%</td>
</tr>
<tr>
<td>Cooler without aeration</td>
<td>90%</td>
<td>66%</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>Cooler with black panels with aeration from top</td>
<td>85%</td>
<td>70%</td>
<td>60%</td>
<td>10%</td>
</tr>
<tr>
<td>Cooler with black panels without aeration</td>
<td>85%</td>
<td>75%</td>
<td>33%</td>
<td>5%</td>
</tr>
<tr>
<td>Cooler with black panels with aeration (from top) with pH buffer added</td>
<td>90%</td>
<td>66%</td>
<td>40%</td>
<td>5%</td>
</tr>
</tbody>
</table>

Due to the approaching winter season, the settlement experiment proceeded regardless of the less than favourable conditions for the veligers. The final tank, lined with black polypropylene, was set up with filtered lake water to be replaced every three days, with fish food and light aeration from the top of the tank instead of the bottom.
D. APPENDIX D Ferrate and PAC Interactions in the Removal of Geosmin
D.1 PAC PREPARATION

PAC used in this experiment was Pulsorb WP260-90 by Calgon Carbon. Specifications are listed below:

<table>
<thead>
<tr>
<th>Specifications</th>
<th>WP260-90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine Number</td>
<td>1000</td>
</tr>
<tr>
<td>Moisture by weight</td>
<td>10%</td>
</tr>
<tr>
<td>Screen size by weight, US Sieve Series</td>
<td>Through 325 mesh 90% (min)</td>
</tr>
</tbody>
</table>

D.2 EXPERIMENTAL DATA

D.3.1 Ferrate Only

Ferrate at different concentrations (1, 5, 10, and 20 mg/L) was added to Lake Simcoe Water. Each jar was spiked with 100 μL of 1 mg/L geosmin solution to achieve 100 ng/L. Ferrate was added to each jar using 5 mL of borate buffer (pH 8.2) and mixed at 200 rpm for two minutes and then 30 minutes at 30 rpm, then for the settlement phase the water was not mixed for 1 hour. 5 mL of 5 g/L sodium thiosulfate (STS) were added to the jars containing ferrate in order to quench any residuals.

5 drops (≈ 135 μL) of HCl 1M were added to each jar before adding anything else, these amount of HCl was added to keep conditions the same as in the previous experiments.
Table 7-16: Experimental design for ferrate only jar test

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ferrate (1 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>2</td>
<td>Ferrate (5 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>3</td>
<td>Ferrate (10 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>4</td>
<td>Ferrate (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>5</td>
<td>Lake water + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>6</td>
<td>Lake water</td>
</tr>
</tbody>
</table>

Observations

- 18.5 °C
- Filtration time was considerably longer than the one observed in previous experiments, even the higher concentrations of ferrate took longer to be filtered than in the experiments using the same concentration of ferrate and 20 mg/L of PAC
- pH increased throughout the experiment as ferrate concentrations increased
- There was no visible coloration attributable to ferrate at the end of the experiment, in contrast with the previous experiments, where jars containing 10 and 20 mg/L of ferrate still seem purple at the end of the settling period
Table 7-17: pH data for ferrate only jar test

<table>
<thead>
<tr>
<th>Jar</th>
<th>pH₀</th>
<th>pHᵣ</th>
<th>ΔpH</th>
<th>Jar</th>
<th>pH₀</th>
<th>pHᵣ</th>
<th>ΔpH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrate 1 mg/L</td>
<td></td>
<td></td>
<td></td>
<td>Ferrate 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1α</td>
<td>8.34</td>
<td>8.36</td>
<td>0.02</td>
<td>1β</td>
<td>8.34</td>
<td>8.39</td>
<td>0.05</td>
</tr>
<tr>
<td>Ferrate 5 mg/L</td>
<td></td>
<td></td>
<td></td>
<td>Ferrate 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2α</td>
<td>8.38</td>
<td>8.57</td>
<td>0.19</td>
<td>2β</td>
<td>8.39</td>
<td>8.57</td>
<td>0.18</td>
</tr>
<tr>
<td>Ferrate 10 mg/L</td>
<td></td>
<td></td>
<td></td>
<td>Ferrate 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3α</td>
<td>8.46</td>
<td>8.73</td>
<td>0.27</td>
<td>3β</td>
<td>8.51</td>
<td>8.77</td>
<td>0.26</td>
</tr>
<tr>
<td>Ferrate 20 mg/L</td>
<td></td>
<td></td>
<td></td>
<td>Ferrate 20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4α</td>
<td>8.67</td>
<td>8.97</td>
<td>0.30</td>
<td>4β</td>
<td>8.69</td>
<td>8.99</td>
<td>0.3</td>
</tr>
<tr>
<td>Raw water with geosmin</td>
<td></td>
<td></td>
<td></td>
<td>Raw water with geosmin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5α</td>
<td>8.36</td>
<td>8.44</td>
<td>0.08</td>
<td>5β</td>
<td>8.44</td>
<td>8.40</td>
<td>0.04</td>
</tr>
<tr>
<td>Raw water without geosmin</td>
<td></td>
<td></td>
<td></td>
<td>Raw water without geosmin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6α</td>
<td>8.40</td>
<td>8.39</td>
<td>0.01</td>
<td>6β</td>
<td>8.34</td>
<td>8.39</td>
<td>0.05</td>
</tr>
</tbody>
</table>

D.3.2 Ferrate, then PAC

Ferrate at different concentrations (1, 5, 10, and 20 mg/L) was added to Lake Simcoe Water. Each jar was spiked with 100 μL of 1 mg/L geosmin solution to achieve 100 ng/L. Initially, ferrate was added to each jar using 5 mL of borate buffer (pH 8.2) and mixed at 200 rpm for two minutes and then one hour at 30 rpm, then PAC was added using 5 mL of Milli-Q water and mixed at 200 rpm for two minutes, then 30 minutes at 30 rpm, then for the settlement phase the water was not mixed for 1 hour. 5 mL of 5 g/L sodium thiosulfate (STS) were added to the jars containing ferrate in order to quench any residuals.

The pH was tried to be set at 7 in every jar, so 5 drops (= 135 μL) of HCl 1M were added to each jar before adding anything else. Here it was observed that pH increased again after some minutes went by ("self-buffering water")
Table 7-18: Experimental design for ferrate, then PAC jar test

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ferrate only (5 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>2</td>
<td>PAC only (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>3</td>
<td>Ferrate (1 mg/L) + PAC (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>4</td>
<td>Ferrate (5 mg/L) + PAC (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>5</td>
<td>Ferrate (10 mg/L) + PAC (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>6</td>
<td>Ferrate (20 mg/L) + PAC (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>A</td>
<td>Lake water + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>B</td>
<td>Lake water</td>
</tr>
</tbody>
</table>

Observations

- 21.5 °C
- pH was not significantly modified through the experiment
- PAC formed considerably bigger particles than previously observed in any other jar tests. The particles also seemed to be bigger in size as ferrate concentration increased
- There was no visible coloration attributable to ferrate at the end of the experiment, in contrast with the previous experiments, where jars containing 10 and 20 mg/L of ferrate still seem purple at the end of the settling period
Table 7-19 : pH data for ferrate, then PAC, jar test

<table>
<thead>
<tr>
<th>Jar</th>
<th>pH$_o$</th>
<th>pH$_f$</th>
<th>ΔpH</th>
<th>Jar</th>
<th>pH$_o$</th>
<th>pH$_f$</th>
<th>ΔpH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrate only 5 mg/L</td>
<td>1α</td>
<td>8.35</td>
<td></td>
<td>1β</td>
<td>8.55</td>
<td>8.59</td>
<td>0.04</td>
</tr>
<tr>
<td>PAC only</td>
<td>2α</td>
<td>8.46</td>
<td></td>
<td>2β</td>
<td>8.48</td>
<td>8.42</td>
<td>0.06</td>
</tr>
<tr>
<td>PAC + Ferrate 1 mg/L</td>
<td>3α</td>
<td>8.49</td>
<td></td>
<td>3β</td>
<td>8.50</td>
<td>8.34</td>
<td>0.16</td>
</tr>
<tr>
<td>PAC + Ferrate 5 mg/L</td>
<td>4α</td>
<td>8.54</td>
<td></td>
<td>4β</td>
<td>8.55</td>
<td>8.56</td>
<td>0.01</td>
</tr>
<tr>
<td>PAC + Ferrate 10 mg/L</td>
<td>5α</td>
<td>8.59</td>
<td></td>
<td>5β</td>
<td>8.65</td>
<td>8.68</td>
<td>0.03</td>
</tr>
<tr>
<td>PAC + Ferrate 20 mg/L</td>
<td>6α</td>
<td>8.74</td>
<td></td>
<td>6β</td>
<td>8.80</td>
<td>8.88</td>
<td>0.08</td>
</tr>
<tr>
<td>Raw water with geosmin</td>
<td>Aα</td>
<td>8.31</td>
<td></td>
<td>Aβ</td>
<td>8.32</td>
<td>8.37</td>
<td>0.05</td>
</tr>
<tr>
<td>Raw water without geosmin</td>
<td>Bα</td>
<td>8.32</td>
<td></td>
<td>Bβ</td>
<td>8.32</td>
<td>8.38</td>
<td>0.06</td>
</tr>
</tbody>
</table>

D.3.3 PAC, then ferrate

Ferrate at different concentrations (1, 5, 10, and 20 mg/L) was added to Lake Simcoe Water. Each jar was spiked with 100 μL of 1 mg/L geosmin solution to achieve 100 ng/L. Initially, PAC was added to each jar using 5 mL of Milli-Q water and mixed at 200 rpm for two minutes and then one hour at 30 rpm, then ferrate was added using 5 mL of borate buffer (pH 8.2) and mixed at 200 rpm for two minutes, then 30 minutes at 30 rpm, then for the settlement phase the water was not mixed for 1 hour. 5 mL of 5 g/L sodium thiosulfate (STS) were added to the jars containing ferrate in order to quench any residuals.

5 drops (≈ 135 μL) of HCl 1M were added to each jar before adding anything else, these amount of HCl was added to keep conditions the same as in the previous experiment.
Table 7-20: Experimental design for PAC, then ferrate jar test

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ferrate only (5 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>2</td>
<td>PAC only (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>3</td>
<td>Ferrate (1 mg/L) + PAC (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>4</td>
<td>Ferrate (5 mg/L) + PAC (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>5</td>
<td>Ferrate (10 mg/L) + PAC (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>6</td>
<td>Ferrate (20 mg/L) + PAC (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>A</td>
<td>Lake water + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>B</td>
<td>Lake water</td>
</tr>
</tbody>
</table>

Observations

- 20 °C
- PAC particles that sedimented, seemed the same size as the experiments where PAC and ferrate were added simultaneously
- Jars containing larger concentration of ferrate had a significant increase of pH throughout the experiment (0.3 – 0.5)
- It was observed that the jars that were not continuously mixed (lake water with and without geosmin, jars A and B), had a much lower pH than the ones that were (around 0.7 points lower)
Table 7-21: pH data for PAC, then ferrate jar test

<table>
<thead>
<tr>
<th>Jar</th>
<th>pH₀</th>
<th>pHᵣ</th>
<th>ΔpH</th>
<th>Jar</th>
<th>pH₀</th>
<th>pHᵣ</th>
<th>ΔpH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrate only 5 mg/L</td>
<td>1α</td>
<td>8.56</td>
<td>8.64</td>
<td>1β</td>
<td>8.58</td>
<td>8.70</td>
<td>0.12</td>
</tr>
<tr>
<td>PAC only</td>
<td>2α</td>
<td>8.50</td>
<td>8.55</td>
<td>2β</td>
<td>8.53</td>
<td>8.55</td>
<td>0.02</td>
</tr>
<tr>
<td>PAC + Ferrate 1 mg/L</td>
<td>3α</td>
<td>8.53</td>
<td>8.58</td>
<td>3β</td>
<td>8.53</td>
<td>8.59</td>
<td>0.06</td>
</tr>
<tr>
<td>PAC + Ferrate 5 mg/L</td>
<td>4α</td>
<td>8.53</td>
<td>8.71</td>
<td>4β</td>
<td>8.52</td>
<td>8.71</td>
<td>0.19</td>
</tr>
<tr>
<td>PAC + Ferrate 10 mg/L</td>
<td>5α</td>
<td>8.53</td>
<td>8.83</td>
<td>5β</td>
<td>8.54</td>
<td>8.84</td>
<td>0.30</td>
</tr>
<tr>
<td>PAC + Ferrate 20 mg/L</td>
<td>6α</td>
<td>8.54</td>
<td>9.03</td>
<td>6β</td>
<td>8.54</td>
<td>9.03</td>
<td>0.49</td>
</tr>
<tr>
<td>Raw water with geosmin</td>
<td>Aα</td>
<td>7.84</td>
<td>7.99</td>
<td>Aβ</td>
<td>7.76</td>
<td>7.94</td>
<td>0.18</td>
</tr>
<tr>
<td>Raw water without geosmin</td>
<td>Bα</td>
<td>7.79</td>
<td>7.95</td>
<td>Bβ</td>
<td>7.76</td>
<td>7.92</td>
<td>0.16</td>
</tr>
</tbody>
</table>

D.3.4 PAC and Ferrate simultaneously

Ferrate at different concentrations (1, 5, 10, and 20 mg/L) was added to Lake Simcoe Water. Each jar was spiked with 100 μL of 1 mg/L geosmin solution to achieve 100 ng/L. Initially, PAC was added to each jar using 5 mL of Milli-Q water and mixed at 200 rpm for two minutes, then ferrate was added using 5 mL of borate buffer (pH 8.2) and mixed at 200 rpm for two minutes, then 30 minutes at 30 rpm, then for the settlement phase the water was not mixed for 1 hour. 5 mL of 5 g/L sodium thiosulfate (STS) were added to the jars containing ferrate in order to quench any residuals.

5 drops (≈ 135 μL) of HCl 1M were added to each jar before adding anything else, these amount of HCl was added to keep conditions the same as in the previous experiment.
Table 7-22: Experimental design for PAC and ferrate added simultaneously jar test

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ferrate only (5 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>2</td>
<td>PAC only (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>3</td>
<td>Ferrate (1 mg/L) + PAC (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>4</td>
<td>Ferrate (5 mg/L) + PAC (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>5</td>
<td>Ferrate (10 mg/L) + PAC (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>6</td>
<td>Ferrate (20 mg/L) + PAC (20 mg/L) + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>A</td>
<td>Lake water + geosmin (100 ng/L)</td>
</tr>
<tr>
<td>B</td>
<td>Lake water</td>
</tr>
</tbody>
</table>

Observations

- 21 °C
- pH remained fairly constant throughout the experiment
- pH of unmixed jars is considerably lower than those that are (around 0.7 points lower)
Table 7-23: pH data for PAC and ferrate simultaneous jar test

<table>
<thead>
<tr>
<th>Jar</th>
<th>pH&lt;sub&gt;o&lt;/sub&gt;</th>
<th>pH&lt;sub&gt;F&lt;/sub&gt;</th>
<th>ΔpH</th>
<th>Jar</th>
<th>pH&lt;sub&gt;o&lt;/sub&gt;</th>
<th>pH&lt;sub&gt;F&lt;/sub&gt;</th>
<th>ΔpH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrate only 5 mg/L</td>
<td></td>
<td></td>
<td></td>
<td>1β</td>
<td>8.74</td>
<td>8.71</td>
<td>0.03</td>
</tr>
<tr>
<td>PAC only</td>
<td>2α</td>
<td>8.63</td>
<td></td>
<td>2β</td>
<td>8.64</td>
<td>8.56</td>
<td>0.08</td>
</tr>
<tr>
<td>PAC + Ferrate 1 mg/L</td>
<td>3α</td>
<td>8.68</td>
<td>0.06</td>
<td>3β</td>
<td>8.73</td>
<td>8.65</td>
<td>0.08</td>
</tr>
<tr>
<td>PAC + Ferrate 5 mg/L</td>
<td>4α</td>
<td>8.78</td>
<td>0.06</td>
<td>4β</td>
<td>8.71</td>
<td>8.63</td>
<td>0.08</td>
</tr>
<tr>
<td>PAC + Ferrate 10 mg/L</td>
<td>5α</td>
<td>8.76</td>
<td>0.01</td>
<td>5β</td>
<td>8.82</td>
<td>8.82</td>
<td>0</td>
</tr>
<tr>
<td>PAC + Ferrate 20 mg/L</td>
<td>6α</td>
<td>8.95</td>
<td>0.05</td>
<td>6β</td>
<td>8.92</td>
<td>8.98</td>
<td>0.06</td>
</tr>
<tr>
<td>Raw water with geosmin</td>
<td>Aα</td>
<td>7.97</td>
<td>0.1</td>
<td>Aβ</td>
<td>7.92</td>
<td>7.89</td>
<td>0.03</td>
</tr>
<tr>
<td>Raw water without geosmin</td>
<td>Bα</td>
<td>7.97</td>
<td>0.08</td>
<td>Bβ</td>
<td>7.93</td>
<td>7.89</td>
<td>0.04</td>
</tr>
</tbody>
</table>

D.3 Geosmin Analytical Methods

D.3.5 Principle

Geosmin was extracted and concentrated from aqueous samples using headspace solid phase micro-extraction (HS-SPME) and analyzed using gas chromatography-mass spectrometry (GC-MS). The geosmin was quantified using an internal standard: d<sub>3</sub>-geosmin. The internal standard is used to observe retention time, relative response, and to more accurately quantify the analyte concentration in the sample. The analysis was performed with a Varian® 3800 gas chromatograph with a Varian® ion-trap mass spectrometer detector, using electron impact (EI) ionization and an autosampler.

Samples with a volume of 10 mL or 10 mL of Milli-Q® for calibration standards was added into a 20 mL clear vial (Supelco, Bellefonte, PA) that contained 3.5 g of reagent grade sodium chloride (NaCl). Internal standard was added to each sample and calibration standard by using a syringe to add 25 μL of a solution of 10 μg/L of d<sub>3</sub>-Geosmin to achieve a 25 ng/L concentration. The vial was capped with a Teflon®-lined septum magnetic crimp cap (Supelco, Bellefonte, PA). Vials were placed into a sample tray, where an autosampler delivered the sample vial to a spinning box that was
preset to 65°C ± 1 °C at a rotation speed of 500 rev/min (rpm). Samples were shaken for 5 minutes to dissolve the salt. The needle (23 gauge) containing a 1 cm long SPME fiber (Supelco, Bellefonte, PA) was inserted into through the septum, into the vial, and the fiber was extended into the vial’s headspace for 30 minutes. At the end of contact time, the fiber was retracted back into the needle, the needle injected into the GC/MS injection port, and the GC/MS run started. After five minutes of desorption, the sample was carried onto the GC column and analyzed for MIB and geosmin content.

### D.3.6 Reagents

#### Table 7-24: Reagents for geosmin analytical procedure

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Supplier and Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geosmin</td>
<td>Sigma Aldrich (47522-U), 100 μg/mL in methanol</td>
</tr>
<tr>
<td>d3-Geosmin</td>
<td>Sigma Aldrich (646377), ≥99.9%, Chromasolv® Plus</td>
</tr>
<tr>
<td>Methanol</td>
<td>Sigma Aldrich (323772-2L), ≥99.5%, Reagent Plus®</td>
</tr>
<tr>
<td>Acetone</td>
<td>EMD (DX0838), HPLC grade</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>Sigma Aldrich (S2002), ≥99.5%, Reagent Plus®</td>
</tr>
<tr>
<td>Sodium Azide</td>
<td>Sigma Aldrich (S2002), ≥99.5%, Reagent Plus®</td>
</tr>
</tbody>
</table>

### D.3.7 Method Outline

#### D.3.7.1 Sampling, Preservation, and Storage

1. Prepared micro-column sampling glassware by adding 166 μL of 25 g/L Reagent Plus®, ≥99.5% sodium azide solution (Sigma-Aldrich, Oakville, ON). Sodium azide acts as a preservative (Pei, 2003).
2. Collected samples in 23 mL amber vials with Teflon®-lined septa screw caps, ensuring that the sample was headspace free.
3. Stored samples at 4°C for up to two weeks.
D.3.7.2  Geosmin and MIB solution preparation for GC/MS analysis

**Calibration Standards Preparation**

1. Prepared calibration standards
2. Pipetted 10 mL of Milli-Q® water into a sample extraction vial that contained 3.5 g of NaCl.
3. Injected the appropriate volume of 10 μg/L combined geosmin and MIB stock solution.
4. Spiked 25 μL of 10 μg/L internal standard into the vial to achieve 25 ng/L.
   Note: The syringe was submerged into the water to avoid evaporation.
5. Repeated the above steps and prepare 5, 10, 30, 50, 100, 200 ng/L standard solutions.
6. Calibration standards were analyzed right away.
   Note: The calibration curve was prepared daily. When a new fiber was used, a new
   calibration curve was generated to demonstrate that the fiber could achieve linearity with a
   relative standard deviation (RSD) < 20% over the desired calibration range.

**Sample Preparation**

1. Added 10 mL of sample into a sample extraction vial (with 3.5 g NaCl).
2. Spiked 25 μL of 10 μg/L internal standard into the vial to achieve 25 ng/L.
3. Clamped vial cap and analyzed immediately.

**Blank Sample Preparation**

1. Poured 10 mL of Milli-Q® water into a sample extraction vial (with 3.5 g NaCl).
2. Spiked 25 μL of 10 μg/L internal standard into the vial to achieve 25 ng/L.
3. Analyzed a blank and check standard after every 10 samples.

**Running Standards Preparation (100 ng/L of Geosmin and MIB in Milli® Water)**

1. Pipetted 10 mL of Milli-Q® water into a sample extraction vial that contained 3.5 g NaCl.
2. Injected 100 μL of geosmin, MIB combined stock solution (10 μg/L) into the vial.
3. Spiked 25 μL of 10 μg/L internal standard into the vial to achieve 25 ng/L.
   Note: Analyzed a check standard after every 10 samples.
### D.3.7.3 GC/MS Operation

**Table 7-25: GC/MS operation conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>VF-5MS capillary column (30m × 0.25 mm, I.D., 0.25 μm film thickness)</td>
</tr>
<tr>
<td>Carrier Gas</td>
<td>Helium at 1 mL/min @ 25°C</td>
</tr>
</tbody>
</table>
| Injection Method          | Temperature: 250°C  
Desorbing time: 5min  
Mode: Splitless for first 2 minutes, split after 2 minutes with split ratio of 50  
Split Valve: Open after 2 min, Flow @ 50 mL/min  
Injection Volume: 1 μL @ normal speed |
| Auto Sampler method       | Syringe: SPME Fiber  
Supelco  
Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS), df 50/30 μm, needle size 24 ga  
Agitator Temperature: 65.0°C  
Pre-incubation time: 5min  
Extraction agitation speed: 400rpm  
Extraction time: 30min |
| GC Method                 | Initial: starts from 40°C, holds for 2min;  
Ramp: 1. increases to 250°C at 15°C/min;  
Equilibration: hold at 250°C for 7min |
| MS Conditions             | Scan Mode: SIS (Single Ion Selection)  
Ionization Type: EI  
Emission current: 30uAmps  
Scan average: 3microscans (0.89s/scan)  
Multiplier Offset: 150volts |
| Total Run Time            | 23.00 min/run                                                                                                                                |
Table 7-26: Parameters of the MS scan for determining taste and odour compounds

<table>
<thead>
<tr>
<th>T&amp;O Compounds</th>
<th>Quantitative ions (m/z)</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geosmin</td>
<td>112</td>
<td>11.2</td>
</tr>
<tr>
<td>2-MIB</td>
<td>95</td>
<td>9.0</td>
</tr>
<tr>
<td>D₃-Geosmin</td>
<td>115</td>
<td>11.2</td>
</tr>
</tbody>
</table>

D.3.8 Calculations

The taste and odour compound concentration was determined by correlation of the sample’s response ratio (ratio of sample’s response to that of the internal standard) with a calibration curve run daily. The calibration curve was determined using standards prepared with geosmin and MIB (d₃-geosmin as an internal standard) as an internal standard.

D.3.9 References


D.4 QA/QC

D.4.1 Check Standards

For every sample analysis run on the GC/MS, a check standard was run every 10 samples. A quality control chart was maintained for the analysis of geosmin with a concentration of 30 ngL⁻¹ of geosmin. Figure below shows the check standards plotted on a quality control chart. The performance of the GC/MS was likely varied since analysis was performed on two different instruments. Furthermore, the GC/MS located at the St. George campus was experiencing instrumental issues throughout the experiment and had multiple fibres and parts changed.
Figure 7-23: Quality control chart for geomsin

**D.4.2 Calibration Curves**

For every sample analysis with a new SPME fibre, a seven-point calibration curve was prepared for the beginning of the run. An example of a calibration curve from Scarborough campus is seen below (Figure 7-24).

Figure 7-24: Calibration curve for geosmin analysis GC-MS Scarborough Campus Ferrate added first + PAC
D.4.3 Method Detection Limit

The method detection limits (MDLs) for the analysis of geosmin was determined by taking the student t-value of 8 replicate samples of about 26 ng/L. Based on Table 7-27 below, the MDL of geosmin was 7.6 ng/L.

Table 7-27: Calculation of method detection limit for geosmin

<table>
<thead>
<tr>
<th></th>
<th>Peak Area Geosmin</th>
<th>Peak Area d3-geosmin</th>
<th>Area Ratio</th>
<th>Calculated concentration geosmin (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.75E06</td>
<td>2.42E07</td>
<td>0.04</td>
<td>26.6</td>
</tr>
<tr>
<td>2</td>
<td>4.46E06</td>
<td>3.70E07</td>
<td>0.12</td>
<td>27.8</td>
</tr>
<tr>
<td>3</td>
<td>4.06E06</td>
<td>3.31E07</td>
<td>0.12</td>
<td>28.7</td>
</tr>
<tr>
<td>4</td>
<td>4.36E06</td>
<td>3.27E07</td>
<td>0.13</td>
<td>25.2</td>
</tr>
<tr>
<td>5</td>
<td>4.08E06</td>
<td>2.91E07</td>
<td>0.14</td>
<td>27.1</td>
</tr>
<tr>
<td>6</td>
<td>4.37E06</td>
<td>3.20E07</td>
<td>0.14</td>
<td>25.9</td>
</tr>
<tr>
<td>7</td>
<td>4.36E06</td>
<td>3.04E07</td>
<td>0.14</td>
<td>26.8</td>
</tr>
<tr>
<td>8</td>
<td>4.06E06</td>
<td>3.53E07</td>
<td>0.11</td>
<td>20.4</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>Mean</td>
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<td>Standard Deviation</td>
</tr>
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<td></td>
<td></td>
<td>RSD</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MDL (ng/L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26.1</td>
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<td></td>
<td>2.5</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>9.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.6</td>
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</table>