

Effects of Prolonged Shutdown on the Performance and Microbial Activity of Intermittently Operated Biofilters for Manganese Removal from Groundwater: Pilot Scale Study

by

Ujwal Kishor Zore

A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Applied Science

in

Civil Engineering - Water

Waterloo, Ontario, Canada, 2024

© Ujwal Kishor Zore 2024

Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Statement of Contributions

The contents of Chapter 4, Chapter 5, and Chapter 6, were presented at the following conferences:

- Zore, U.K., Arora, H., Peldszus, S., Huck, P.M. (2023), Performance of intermittently operated biofilters for manganese removal from groundwater, AWWA ACE 2023, Toronto, Canada.
- Zore, U.K., Lamba-Rautapuro, N., Slawson, R., Peldszus, S., Huck, P.M. (2023), Effects of a prolonged shutdown on intermittently operated pilot-scale biofilters for manganese removal from groundwater, AWWA WQTC 2023, Dallas, Texas, USA.
- Zore, U.K., Peldszus, S., Huck, P.M. (2024), Performance of intermittently operated biofilters for manganese removal from groundwater after a prolonged shutdown, OWWA/WEAO Ontario Water For The Future Conference And Trade Show 2024, Niagara Falls, Ontario, Canada. [ACCEPTED]

Abstract

Apart from aesthetic concerns, manganese (Mn) in drinking water has been associated with cognitive and neurobehavioral effects in children. Recently Health Canada has changed its drinking water guidelines for Mn from an aesthetic parameter to a regulated parameter with a maximum acceptable concentration (MAC) of 120 $\mu\text{g/L}$, an aesthetic objective (AO) of 20 $\mu\text{g/L}$, and a treatment objective limit of 15 $\mu\text{g/L}$. While biofiltration has proved to be an effective treatment option for Mn and iron (Fe), it is not widely used in North America. Also, in small-scale communities, treatment plants often adopt intermittent operation schemes, wherein, water treatment occurs for a few hours per day, based on demand. Among the various operational aspects that could potentially affect the performance of intermittently operated biofilters, prolonged filter shutdown remains a critical concern. However, limited insights exist regarding both the intermittent operation for Mn and Fe removal from groundwater and the impact of a prolonged shutdown. This study aimed to fill these knowledge gaps by comparing the performance of intermittently and continuously operated biofilters for Mn and Fe removal from groundwater, and by investigating the impact of a prolonged shutdown of these filters. To achieve these objectives, three pilot-scale biofilters were located at a drinking water treatment facility in Southern Ontario, Canada, out of which two biofilters were operated continuously and one intermittently (6 h per day). The biofilters were initially operated for a couple of months, then underwent a six-month shutdown period, and then resumed active operation for three months. Sand was used as the biofilter media providing an empty bed contact time (EBCT) of 15.6 minutes. Raw groundwater containing Mn (31 $\mu\text{g/L}$) and Fe (96 $\mu\text{g/L}$) was fed to the biofilters and the effluent flow rate was maintained at 5 m/h. Mn and Fe removal was assessed before and after the extended shutdown in order to gauge its impact on biofilter performance. In addition, turbidity, pH, DO, oxidation-reduction potential (ORP) and total organic carbon (TOC), were periodically monitored. Microbiological changes were analyzed using AxP (adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP)) and other microbiological analyses. Lastly, the storage strategy (SS) utilized during an extended shutdown period might play a crucial role, therefore four different storage strategies were investigated. To briefly summarize the results, during the start-up phase, after 56 days, all biofilters achieved Mn removal to below 15 $\mu\text{g/L}$, with the intermittently operated biofilter exhibiting lower removal rates and not reaching a steady state, while Fe removal reached a steady state within a week for all biofilters. Next, during the six-month extended shutdown,

a notable reduction in ATP occurred, but all biofilters seemed to have remained viable for 4 months, potentially due to the SS utilized. Also, it was found that manganese oxidizing bacteria (MnOB) were able to persist and actively oxidize Mn even after the six-month-long shutdown. Lastly, post-extended shutdown all biofilters approached the previous Mn removal rates within 20 days of restart and then reached a steady state within a month of operation. Notably, intermittently operated biofilter performed equally well as continuously operated biofilters. Also, Fe removal was not found to be affected by the extended shutdown. This suggests that the biofilters were able to quickly regain their performance even after a six-month extended shutdown highlighting the resilient nature of these biofilter systems. Overall, this study enhances the understanding of Mn removal in intermittently operated biofilters, and suggests ways to mitigate the effects of prolonged shutdowns, thereby expanding the applicability of these biofilters, especially to smaller communities.

Acknowledgements

First, I would like to express my gratitude to my supervisors, Dr. Peter M. Huck and Dr. Sigrid Peldszus for their invaluable advice and continuous support throughout. I am thankful to be a part of such an interesting project and I am grateful for your immense knowledge, insightful feedback, and time. Also, my sincere thanks goes to Dr. Robin Slawson for her valuable counsel and professional expertise with the microbiological aspects of this project. Further, I would like to extend my thanks to the readers of this thesis, Dr. William B. Anderson and Dr. Mahesh Pandey for their feedback.

I profusely thank Hemant Arora for the initial design and assembly of the pilot scale setup that was remodified by me and then used for the experimental study; to Nicholas Lamba-Rautapuro, an MSc student from Wilfrid Laurier University for his contribution to the microbiological analysis, as part of the collaboration between the NSERC Chair in Water treatment research group and Dr. Slawson's microbiology research group. Thanks to Dr. Brian Kendall for performing the manganese and iron analysis, and to Steven Ngo for performing the TOC analysis. Also, thanks to technicians/technologists including but not limited to Mark Sobon, Mark Hummel, Mark Merlau, and Fred Bakker for helping me along the way. I would also like to acknowledge NSERC Chair in Water Treatment students, Sina Golchi, Mahmoud Badawy, and fellow water enthusiasts/colleagues for their support and their companionship in the lab and Lorena Baku for her assistance with numerous administrative tasks.

This project was conducted in partnership with the industrial research chair partner. I am deeply grateful to the friendly staff including but not limited to Thomas Mulrooney, Mark Vandergeest, Shane Steele, Chris Prince, Greg Sealey, Rebecca Hornick, Deanna Davies, Kayla Vandenburg and Motiul Islam for their continuous support at every stage of the project. Huge thanks to Thomas for assisting me with sample collection and analysis as well as with the regular maintenance of the experimental setup.

Finally, I would like to thank my parents, my sisters, my twin brother and my relatives for their unconditional love and unwavering encouragement. Also, thanks to M. Zaeem Khalid, Abubakr Shafique, Nilanti Sen Gupta, Abbey Sayles, Noman Sajjad, Navya Nair, Xuanye Bai, Khader Khan, Sripadh Guptha, TSV Mahesh, Sajal Gupta and my other friends whose support has brightened this journey. I am also grateful to everyone I crossed paths with during badminton matches, music jam sessions, outdoor adventures, gym sessions, and runs as your presence made this challenging master's phase a smooth journey.

This project was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) in the form of an Industrial Research Chair in Water Treatment at the University of Waterloo. The current Chair partners are listed at <https://uwaterloo.ca/nserc-chair-water-treatment/partners>.

Dedication

This work is wholeheartedly dedicated to the almighty Lord Krishna and my beloved parents Mrs. Usha Zore and Dr. Kishor Zore, who first taught me the value of education and helped me to come to where I am today.

I also dedicate this work to Dr. Nisha Kothari (a mentor every student dreams of) and all my former teachers who showed me the right path at the right time and ignited an enduring flame within me.

Thank you!

Table of Contents

Author's Declaration	ii
Statement of Contributions	iii
Abstract	iv
Acknowledgements	vi
Dedication	viii
List of Figures	xiii
List of Tables.....	xviii
List of Acronyms.....	xx
Disclaimer	xxi
Chapter 1 General Introduction.....	1
1.1 Problem Statement.....	1
1.2 Research Objectives.....	3
1.3 Research Approach.....	3
1.4 Thesis Structure	5
Chapter 2 Background and Literature Review	8
2.1 Mn in Drinking Water Sources.....	8
2.1.1 Properties and Occurrence	8
2.1.2 Issues and Regulations.....	11
2.2 Mn Removal from Drinking Water	13
2.2.1 Physical/Chemical Treatment Processes for Mn Removal.....	15
2.2.2 Biological Methods.....	18
2.2.3 Treatment for Mn Removal in Canada	18

2.3 Mn Removal from Drinking Water	19
2.3.1 General Principles of Biofiltration.....	20
2.3.2 Biofiltration for Mn Removal.....	21
2.3.3 Factors Influencing Biofilter Performance	23
2.4 Intermittent Operation	26
2.4.1 Why Operate Intermittently?	26
2.4.2 Problems Associated with Intermittent Operation.....	26
2.4.3 Pertinent Research Investigations on Intermittent Biofilter Operation	27
2.5 Prolonged Shutdown.....	33
2.6 Conclusions and Research Gaps.....	34
Chapter 3 Materials and Research Methodology	37
3.1 Overview.....	37
3.2 Pilot Plant Location	37
3.3 Pilot Plant.....	42
3.3.1 Pilot Plant Set-Up	42
3.3.2 Filter Media Selection and Properties.....	45
3.3.3 Pilot Operations	46
3.4 Sample Collection and Handling.....	48
3.4.1 Water Samples.....	49
3.4.2 Media Samples.....	50
3.5 Analysis Methods	51
3.5.1 Water Quality Parameters.....	51
3.5.2 Microbial Parameters.....	52
3.5.3 Statistical Analysis.....	54

Chapter 4 Effect of Intermittent Operation on Manganese Removal During Biofilter Start-Up.....	56
4.1 Overview.....	56
4.2 Results and Discussion	57
4.2.1 Feed Water Quality	57
4.2.2 Biofilter Performance During the Start-Up Period	59
4.2.3 Mn and Fe Removal at Different Biofilter Depths	65
4.2.4 Effect of Short-Term Shutdown on Biofilter Performance	69
4.2.5 Effect of Backwash on Microbial Activity	73
4.2.6 Turbidity Removal by the Biofilters.....	76
4.3 Conclusions.....	78
Chapter 5 Effects of an Extended Shutdown on the Microbial Activity of Biofilters Previously Operated Intermittently and Continuously	80
5.1 Overview.....	80
5.2 Results and Discussion	80
5.2.1 Impact of the Extended Shutdown and the Different Storage Strategies on Basic Water Quality Parameters	80
5.2.2 Impact of the Extended Shutdown on Microbial Activity in the Biofilters.....	85
5.2.3 Culture-Based Microbiological Analysis Results.....	95
5.3 Conclusions.....	100
Chapter 6 Performance of Intermittently and Continuously Operated Biofilters for Manganese Removal from Groundwater after a Prolonged Shutdown	103
6.1 Overview.....	103
6.2 Results and Discussion	103
6.2.1 Feed Water Quality	103
6.2.2 Biofilter Performance After Restart Following the Extended Shutdown	106

6.2.3 Mn And Fe Removal in Depth-Wise Zones of the Biofilter	111
6.2.4 Microbial Activity in the Biofilters in Phase 3 (Post Extended Shutdown)	115
6.2.5 Effect of 18-h Regular Intermittent Shutdown on Biofilter Performance	121
6.2.6 Turbidity Removal by the Biofilters	125
6.3 Conclusions.....	127
Chapter 7 Conclusion and Recommendations for Future Work	129
7.1 Conclusions.....	129
7.2 Recommendations for Future Work	132
References	133
Appendices.....	149
Appendix A - Supporting Document for Chapter 3: Materials and Research Methodology.....	149
Appendix B - Supporting Data and Figures for Chapter 4: Effect of Intermittent Operation on Manganese Removal During Biofilter Start-Up.....	174
Appendix C - Supporting Data and Figures for Chapter 5: Effects of an Extended Shutdown on the Microbial Activity of Biofilters Previously Operated Intermittently and Continuously	188
Appendix D - Supporting Data for Chapter 6: Effect of Intermittent Operation on Manganese Removal Post Extended Shutdown.....	195

List of Figures

Figure 1.1 Timeline of the experimental work.....	4
Figure 2.1 Negative logarithm of electron activity (pε) and redox potential (EH) as a function of pH for the Mn species in an aqueous medium (Stumm & Morgan, 1996).....	9
Figure 2.2 Negative logarithm of electron activity (pε) and redox potential (EH) as a function of pH for the Fe species in aqueous medium (Snoeyink & David Jenkins, 1980).	9
Figure 2.3 Negative logarithm of electron activity (pε) and redox potential as a function of pH for the nitrogen species in aqueous medium (Russell & Hall, 2010).	10
Figure 2.4 Pathways for the Mn transformation (MnOB: Manganese oxidizing bacteria).....	14
Figure 2.5 Treatment alternatives for Mn removal from water (Tobiason et al., 2016)	15
Figure 2.6 Over time evolution of biofilter media from acclimation period up until biofilters reach maturity and are in pseudo steady state (Cheng et al., 2017; Keithley et al., 2023; Yang et al., 2014).	23
Figure 3.1 Water quality of 5 out of 14 groundwater wells in the city having the highest Mn composition along with the guideline limits. “Maximum” represents the highest recorded value within the dataset spanning 2019-2021 for the respective wells.....	41
Figure 3.2 Current groundwater treatment process at groundwater well 13 (site chosen for pilot operation)	42
Figure 3.3 Schematic diagram of the pilot set-up assembled at the experimental site	43
Figure 3.4 Pilot set up on-site	44
Figure 3.5 Influent and effluent flow rate data for all biofilters over a week	47

Figure 4.1 Mn concentrations in pilot biofilter influent and effluents over time. Data from Jan. 18, 2023 = Day 7 to Mar. 8, 2023 = Day 56.	60
Figure 4.2 Fe concentrations in pilot biofilter influent and effluents over time. Data from Jan. 18, 2023 = Day 7 to Mar. 8, 2023 = Day 56.	61
Figure 4.3 Variations in key water quality parameters over time: (a) pH, (b) ORP, and (c) DO	63
Figure 4.4 Field of activity of Mn and Fe oxidizing bacteria: pH- Eh diagram (Mouchet, 1992).....	64
Figure 4.5 (a) Mn and (b) Fe concentrations across various biofilter bed depths on day 49	67
Figure 4.6 ATP measured for the media at different biofilter bed depths. Sample collection was done before backwashing after intermittently operated biofilter had been operating for 2-3 h after 18 h shutdown	68
Figure 4.7 Impact of 18 h shutdown on (a) Mn and (b) Fe concentrations during biofilter C start up on day 56. Sample collection began 5 minutes (0.1 h) after the biofilter restart and then continued at hourly intervals. To have a reference point a sample was collected on the previous day just before the shutdown.	70
Figure 4.8 ATP of biofilter media before and after restart of biofilter C after an 18 h shut down period, while column A and B were running continuously on day 7, 42 and 56. BR indicates that the sampling was done before restart when the continuously operated biofilters were still operating while the intermittently operated biofilter was shut down, and AR indicates the sampling which was done after restart, when all the biofilters were operating. Media samples were collected from the top-most layer i.e. from 10 cm before backwashing the biofilter columns. ATP measurements were done in duplicate. The error bars here represent the standard deviation from the mean value.	72
Figure 4.9 DO levels before and after restart of biofilter C after a 18 h shut down period, while column A and B were running continuously. BR indicates that the sampling was done before restart when the continuously operated biofilters were still operating while the intermittently operated biofilter was shut down, and AR indicates the sampling which was done after restart, when all the biofilters were operating.....	73

Figure 4.10 Backwash flow rate data for all biofilters.....	74
Figure 4.11 ATP on biofilter media before and after backwash. The media samples were collected from the top-most layer i.e. from 10 cm. BB indicates that the sampling was done before backwash and AB representing the sampling which was done after backwash. After backwash the sampling was performed after 30 minutes.....	75
Figure 4.12 Turbidity data in pilot biofilter influent and effluents. (a) Data from Jan 18, 2023 = Day 7 to Mar 8, 23 = Day 56, (b) Hourly after 18 h shutdown during biofilter C start up on day 27 and 54, (c) before and after backwash on day 42 and day 54. Note that different y-axes are used to highlight specific trends, patterns and for clarity of the graphs.....	77
Figure 5.1 Trends in temperature at different biofilter bed depths during the extended shutdown: Top layer (10 cm) and bottom layer (110 cm) [SS: Storage strategy (Refer to Table 3.4)].....	81
Figure 5.2 Trends in DO at different biofilter bed depths during the extended shutdown: Top layer (10 cm) and bottom layer (110 cm).....	83
Figure 5.3 Trends in pH at different biofilter bed depths during the extended shutdown: Top layer (10 cm) and bottom layer (110 cm).....	84
Figure 5.4 Trends in oxidation-reduction potential (ORP) at different biofilter bed depths during the extended shutdown: Top layer (10 cm) and bottom layer (110 cm).....	85
Figure 5.5 ATP data for three biofilter bed depths during the extended biofilter shutdown (a) Top layer (10 cm), (b) Middle layer (50 cm) and (c) Bottom layer (110 cm). Duplicate sand media samples were taken for all samples and the error bars represent the standard deviation from the mean value.....	87
Figure 5.6 AMP, ADP and ATP values for sand media samples from biofilters A, B and C during the extended biofilter shutdown (a) on Day 0 (i.e. the day when the extended shutdown began), (b) Day 35 (c) Day 63, (d) Day 99, (e) Day 134 and (f) Day 203 (last day of extended shutdown).....	90
Figure 5.7 EC values for sand media samples from biofilters A, B and C in (a) top layer (10 cm), (b) middle layer (50 cm) and (c) bottom layer (110 cm). Dashed lines are to interpret the status of the biofilters (Refer Table 5.1).....	94

Figure 5.8 Culturable cell counts of bacteria isolated from sand media samples from three different biofilters after plating on R2A + Mn media, at three different depths (10, 50 and 110 cm), over the course of two sampling events during the extended shutdown (4.5 (day 134) and 6 months (day 203)). Prior to the shutdown, biofilters A and B: operated continuously for 24 h per day, while biofilter C operated intermittently for 6 h a day.	96
Figure 5.9 LBB-confirmed plate, illustrating LBB stained blue colony and phenotypic diversity	98
Figure 5.10 Average Well Colour Development (AWCD) derived after 96 h of incubation on Biolog Ecoplates.	100
Figure 6.1 Mn concentrations in pilot biofilter influent and effluents over time (Data from Sept. 28, 2023 = Day 0 to Dec. 13, 2023 = Day 75)	106
Figure 6.2 Mn concentrations in feedwater and pilot biofilter effluents from phase 1-3 (Jan.-Dec. '23) (a) actual timeline and data from Jan. 11, 2023 = Week 0 to Dec. 13, 2023 = Week 48 (b) 6-month shutdown timeframe shrunk and data from Jan. 11, 2023 to Dec. 13, 2023. In phase 1, day 7= Jan. 18, 2023 and day 56 = Mar. 8, 2023 and in phase 3, day 0 = Sep. 28, 2023 and Day 75 = Dec. 13, 2023	108
Figure 6.3 Fe concentrations in pilot biofilter influent and effluents over time (Data from Sep. 28, 2023 = Day 0 to Dec. 13, 2023 = Day 75)	109
Figure 6.4 Fe concentrations in biofilter influent and effluent for all 3 phases (Jan.-Dec. '23) with 6-month shutdown timeframe shrunk.....	109
Figure 6.5 Mn concentrations across various biofilter bed depths on day 27 and 55 after the extended shut-down. Note that the y-axis in (a) is different from (b) to see the trends more clearly	112
Figure 6.6 Fe concentrations across various biofilter bed depths on day 27 and 55 after the extended shut-down. Note that the y-axis in (a) is different from (b) to see the trends more clearly	114
Figure 6.7 ATP measured for the biofilm attached to the filter media at different biofilter bed depths. Samples were collected before backwashing and after the intermittently operated biofilter C had been	

operating for 2-3 h post 18 h shutdown. Measurements were done in duplicates and the error bars represent the standard deviation..... 116

Figure 6.8 AxP (AMP + ADP + ATP) values for the biofilters A, B and C at (a) last day of phase 1 (day 56 during phase 1), (b) on Day 0 (i.e. the day when extended shutdown ended), (c) day 13, (d) day 28, (e) day 56 and (f) day 85 of phase 3. Note that all the media samples were collected after backwashing 118

Figure 6.9 EC values for the biofilters A, B and C in the (a) top layer (10 cm), (b) middle layer (50 cm) and (c) bottom layer (110 cm) in phase 3. Dashed lines are to interpret the status of the biofilters (Refer Table 5.1) 120

Figure 6.10 Impact of 18 h shutdown on Mn concentrations during biofilter C restart on (a) day 28 and (b) day 56. Sample collection began 5 minutes after the biofilter C was restarted and then continued at regular intervals. To have a reference point a sample was collected on the previous day just before the shutdown (in this case biofilters A and B continued running without a shut-down)..... 122

Figure 6.11 Impact of 18 h shutdown on Fe concentrations during biofilter C restart on (a) day 28 and (b) day 56. Sample collection began 5 minutes after the biofilter C was restarted and then continued at regular intervals. To have a reference point a sample was collected on the previous day just before the shutdown (in this case biofilters A and B continued running without a shut-down)..... 124

Figure 6.12 Turbidity removal a) over time in phase 3 (post extended shutdown) and b) on day 56 immediately after resuming operations following an 18 h shut down of the intermittent biofilter C (in this case biofilters A and B continued running without a shut-down)..... 126

List of Tables

Table 1.1 Sub-objectives for each phase of the pilot study	5
Table 2.1 Major causes of Mn contamination in water source	11
Table 2.2 Issues associated with excess Mn in finished drinking water (Health Canada, 2022).....	12
Table 2.3 Summary of aesthetic, health-based and operational guidelines for Mn in µg/L	13
Table 2.4 List of oxidizing bacteria for different contaminants in water.....	22
Table 2.5 Studies focusing on intermittently operated biofilters for organics removal.....	28
Table 2.6 Studies focusing on intermittently operated biofilters for Mn removal.....	31
Table 3.1 Information on groundwater wells in the city (2019-2021) along with the respective guideline values (Health Canada, 2019) (Health Canada, 2022)	39
Table 3.2 Sand media characteristics (Arora, 2021).....	46
Table 3.3 EBCTs for each sampling port depth.....	46
Table 3.4 Different storage strategies (SS) studied during extended shutdown	48
Table 3.5 Water parameters studied and their respective sampling frequencies	50
Table 3.6 Microbiological parameters studied and their respective frequencies	51
Table 4.1 Feedwater* quality data monitored at well 13 during the start-up phase (parameter set 1) .	58
Table 4.2 Feedwater* quality data monitored at well 13 during the start-up phase (parameter set 2) .	58
Table 4.3 t-test to compare the effluent Mn concentration of continuously and intermittently operated biofilters	62

Table 4.4 t-test to compare the effluent Fe concentration of continuously and intermittently operated biofilters	62
Table 5.1 Interpretation of EC values	93
Table 5.2 LBB assay results for R2A + Mn media plates from 5.2.3.1 confirming presence of MnOB	99
Table 6.1 Feedwater* quality data measured in phase 3.....	105
Table 6.2 t-test to compare the effluent Mn concentration of continuously and intermittently operated biofilters	110
Table 6.3 t-test to compare the effluent Fe concentration of continuously and intermittently operated biofilters	110

List of Acronyms

ADP	Adenosine Diphosphate
AMP	Adenosine Monophosphate
AO	Aesthetic Objective
ATP	Adenosine Triphosphate
AxP	AMP+ADP+ATP
CFU	Colony-forming unit
CLPP	Community level physiological profiling
CONT	Continuous
DO	Dissolved oxygen
DWTP	Drinking water treatment plants
EBCT	Empty Bed Contact Time
EC	Energy charge
Fe	Iron
ICP-MS	Inductively Coupled Plasma-Mass-Spectrometry
INT	Intermittent
IOB	Iron oxidizing bacteria
LBB	Leucoberbelin blue
MAC	Maximum Acceptable Concentration
Mn	Manganese
MnOB	Manganese Oxidizing Bacteria
NTU	Nephelometric Turbidity unit
OG	Operational guidelines
ORP	Oxidation Reduction Potential
SD	Standard deviation
SOP	Standard operating procedure
SS	Storage strategy
TOC	Total Organic Carbon

Disclaimer

In several instances in this study, commercial products and/or trade names were mentioned. The use of commercial names does not constitute endorsement or recommendation of these products and/or instruments by the author.

Chapter 1

General Introduction

1.1 Problem Statement

NASA satellites have confirmed that 21 out of the world's 37 largest aquifers are running out too fast to be replenished and according to data from various surveys done by UNICEF and WHO, worldwide one out of three people do not have basic access to clean water (Keenan, 2019; World Health Organisation, 2019). Also, water requires treatment before distribution and consumption in order to provide safe and aesthetically pleasing water to the consumers. One concern is the presence of manganese (Mn) in drinking water, which has been gaining attention due to the cognitive and neurobehavioral effects associated with it, especially in children. Previously, Mn in drinking water was mainly viewed as an aesthetic problem (Bouchard et al., 2007; Oulhote et al., 2014). Amongst the water sources, Mn contamination has been known to be more prevalent and occurs at higher concentrations in groundwater as compared to surface water as its major source is the dissolution of minerals and rocks (Health Canada, 2019). In general, the concentration of Mn is below 0.1 mg/L in the freshwater sources in Canada, but they can fluctuate depending on the sources of Mn (World Health Organisation, 2017). With respect to the toxicological findings, in 2019, Health Canada revised the federal guidelines for Mn in finished drinking water from a mere aesthetic objective (AO), introduced a maximum acceptable concentration (MAC) value of 120 µg/L, and lowered the AO value to 20 µg/L. Furthermore, Health Canada has recommended treatment utilities to maintain the treated water goal of 15 µg/L (Health Canada, 2019; Trueman et al., 2019). Increased concerns about Mn toxicity and the change in regulatory importance of Mn is the driver of utilities to revisit their treatment processes for Mn reduction and to look at innovative, economically feasible and environmentally friendly technologies, particularly for regions where such interventions were not previously warranted (Teodosiu et al., 2018).

The treatment methodology is generally based on source water characteristics, i.e. the form in which Mn exists. In natural water, Mn is generally found in the oxidation states of Mn^{+2} (dissolved form) or Mn^{+4} (particulate form) (Benefield et al., 1982; Therdkiattikul et al., 2020). Several physical, chemical and biological treatment methods have been successful in reducing the Mn concentration by oxidizing the soluble Mn^{2+} form to the precipitate form Mn^{+4} , followed by physical removal usually using filtration (Burger et al., 2008; Patil et al., 2016). Mn^{2+} can be removed via ion exchange, a combination of sorption and catalytic oxidation, biological oxidation or a combination of direct

oxidation and precipitation (Tobiason et al., 2016). However, physical and chemical processes have some drawbacks, including iron complexation issues, dependency on pH, and total organic carbon (TOC), and issues with reagent dosage location (Mouchet, 1992).

Due to the benign nature and other benefits like less sludge production, lower operation & maintenance costs, and no disinfection by-product generation, biofiltration technology has been gaining profound attention for drinking water treatment including for Mn (Hasan et al., 2020; Chaudhary et al., 2003; Kim et al., 1997). Mn removal can happen either biologically (via Mn oxidizing bacteria (MnOB)) or physiochemically (via Mn oxides (MnOx)). In general, Mn biofilters transition from biological Mn removal initially to physico-chemical Mn removal as the biofilters mature (Breda et al., 2019; Bruins, 2017). Regarding optimal biofilter performance and operation, water quality parameters, design and operational parameters and microbiological parameters have all been reported to significantly affect Mn removal (Tobiason et al., 2016). While biofiltration has numerous benefits, it has some drawbacks for example a long acclimation time. This period (ranging from a few weeks to several months) can be quite long and alternative treatment may have to be in place when implementing biofiltration for Mn removal.

With the implementation of rapid filtration technologies and the rise in water conservation policies, treatment plants often do not need to run at their full capacities and it is anticipated that the number of such plants will increase in the near future (McKie & Andrews, 2022). Also, in small-scale communities, treatment plants often adopt intermittent operation schemes (shutting down water production for up to 20 h/day), where water is treated for several hours each day, based on demand. However intermittent operations might potentially result in periods of substrate and oxygen deprivation in biofilters depending on local conditions and thereby may affect their efficiencies (Brown et al., 2020). Biofilters may experience anoxic conditions i.e. dissolved oxygen (DO) <1 mg/L during intermittent shutdown. This could potentially affect biofilm formation due to reduced microbial activity, reduce MnO_x formation and therefore affect biofilter performance. Furthermore, prolonged biofilter shutdown (two or more months of shutdown) is amongst the various operational aspects that could potentially affect the performance of biofilters. These shutdowns may occur due to hardware/software component concerns, raw water quality issues or other external factors. These events can occur unexpectedly and may surpass the initially expected timeframes and therefore have the potential to severely affect the biofilm on the filter media. However, limited insights exist regarding

both the intermittent operation for Mn removal from groundwater and the impact of a prolonged shutdown on biofilter performance and microbial activity.

1.2 Research Objectives

This research focused on a comparative investigation of pilot-scale intermittently and continuously operated biofilters for efficient Mn removal from groundwater. Various operational parameters that could potentially affect the performance of the biofilters were studied. The objectives are listed below. Objectives 1 to 5 were applied to the biofilter startup period and following restart after the six-month biofilter shutdown:

1. Assess the time required for intermittently and continuously operated biofilters to reach a pseudo-steady state with respect to Mn and iron (Fe) removal.
2. Evaluate biofilter performance at different empty bed contact times (EBCT) by monitoring Mn and Fe removal at various depths of the continuously and intermittently operated biofilters.
3. Investigate the impact of the 18-h shutdown periods of the intermittent biofilter on its performance and microbial activity.
4. Study the effect of backwashing on the microbial activity in the biofilter biofilms.
5. Ascertain the ability of biofilters to mitigate turbidity in response to turbidity spikes.
6. Document the impact of a six-month shutdown of all biofilters on microbial activity in the biofilter biofilms and basic water quality parameters
7. Monitor the effect of different storage strategies on biofilter biofilms and water quality parameters during the six-month shutdown

1.3 Research Approach

A pilot-scale set-up was assembled at a drinking water treatment facility in Southern Ontario, Canada. Three rapid conventional biofilters were operated side by side, for the comparative investigation between intermittently and continuously operated biofilters. Sand was used as the media and raw non-chlorinated water from a groundwater well was pumped as feed to the biofilter columns. The overall study was designed to run for 9-10 months (starting January 11th, 2023), but after 56 days of normal operation, an unprecedented groundwater well shutdown took place, which impacted the research

operations and data collection as there was no access to the raw groundwater. The operations were halted on March 8th, 2023, and resumed on September 28th, 2023. Figure 1.1 shows the timeline of the experimental work performed for this thesis. The unexpected and prolonged shutdown of the well provided a unique opportunity to conduct thorough microbiological studies and delve deeply into a situation that could potentially occur at any well. This experience also offered valuable insights into how wells that use biofiltration technology for water purification can navigate such challenges in the future.

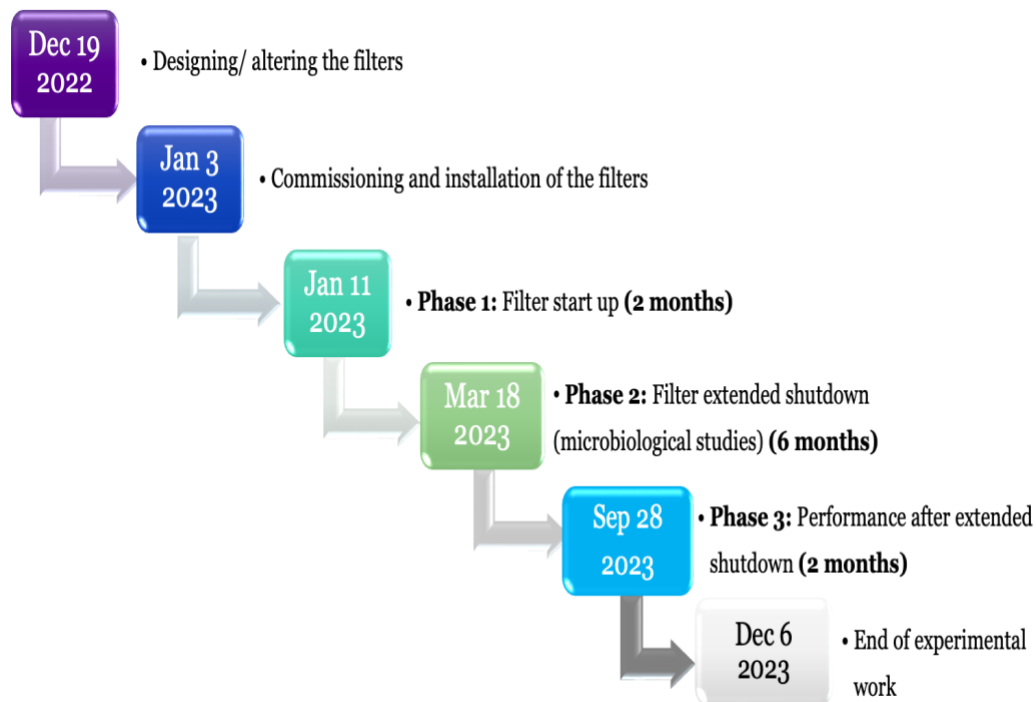


Figure 1.1 Timeline of the experimental work

Phase 1, which began in January 2023 and spanned two months, saw the filter start-up, during which the water quality parameters and the microbial activity were periodically assessed for the two continuously and one intermittently operated biofilters. The subsequent phase, which commenced in March 2023, lasted for six months and involved the study of biofilter media during an extended shutdown period. Originally unplanned, this phase had to be integrated in response to the unforeseen shutdown of the groundwater well. In September 2023 the third and last phase of the research study commenced, lasting two months, during which the filters were evaluated to assess their performance in

the wake of the extended shutdown. All experimental work was concluded in December 2023. The research sub-objectives for each phase are tabulated in Table 1.1.

Table 1.1 Sub-objectives for each phase of the pilot study

Phase 1 Filter start-up (Chapter 4)	Phase 2 Extended shutdown (Chapter 5)	Phase 3 Post extended shutdown (Chapter 6)
✓ Time required to reach steady state during start-up period ✓ Mn and Fe removal in depth-wise zones of the biofilter ✓ Impact of short-term shutdown time on biofilter performance and microbial activity ✓ Effect of backwashing ✓ Turbidity mitigation in response to turbidity spikes	✓ Impact of extended shutdown on microbial activity in the biofilters ✓ Effect of different storage strategies on DO levels	✓ Time required to reach steady state post extended shutdown ✓ Impact of extended shutdown on biofilter performance and microbial activity after bringing the biofilters back online ✓ Mn and Fe removal in depth-wise zones of the biofilter

1.4 Thesis Structure

This thesis contains seven chapters in total, with chapters 4, 5, and 6 each dedicated to one of the three research phases undertaken throughout this project.

Chapter 1 outlines the problem statement and offers a concise overview of research dedicated to intermittently operated biofilters for Mn removal from drinking water, emphasizing the existing research gaps. This is followed by the research objectives, the thesis structure and the research approach undertaken to address the defined research objectives.

Chapter 2 is a comprehensive literature review, which provides an overview of the current understanding of the occurrence, challenges and regulatory frameworks concerning Mn in drinking water. Various treatment methods are also introduced, with particular emphasis on biofiltration. The mechanisms and principles behind biofiltration technology, along with its applications and benefits are listed to demonstrate how biofiltration is used in water treatment processes. Furthermore, intermittent operation in water treatment is discussed and the studies published to date that have assessed intermittently operated biofilters from Mn removal are summarized. Lastly, the research gaps related to the application of intermittently operated biofilters for Mn removal in the drinking water system have been identified.

Chapter 3 covers the detailed methodology and approaches used in the experimental work. The details of the pilot set-up, its operations, the location and the feed water utilized, are also included. Lastly, the sampling methods, analysis methods and statistical tools utilized have been described. Appendix A provides supportive information related to this chapter.

Chapter 4 includes the experimental results and discussion of the startup phase, wherein the performance of continuously and intermittently operated biofilters was compared. Mn and Fe removal were investigated along with the microbial activity in terms of adenosine triphosphate (ATP) to discern the efficacy of each biofilter and understand the biological processes underlying biofiltration. Appendix B shows the supportive information related to this chapter.

Chapter 5 includes the experimental results and discussion of the six-month shutdown phase, wherein its effects on the microbial activity of the biofilters were investigated. For this in-depth microbiological analysis, AxP and ATP-only analysis were performed by the author, while, MnOB analysis, leucoberbelin blue (LBB) analysis and community level physiological profiles (CLPP) analysis were performed by Nicholas Lamba-Rautapuro, a masters student at Wilfrid Laurier University, as part of a collaboration with Prof. Slawson's research group. Further, to mitigate the effects of the prolonged shutdowns, different storage strategies were studied. Appendix C specifies the supportive information related to this chapter.

Chapter 6 includes the experimental results and discussion of the biofilter restart following the six-month shutdown, wherein the performance of continuously and intermittently operated biofilters was compared to each other and also with the results from the startup phase (chapter 4). For

this phase, the methods and approaches described in chapters 4 and 5 were utilized. Appendix D includes the supportive information related to this chapter.

Chapter 7 summarizes the experimental results and draws conclusions based on those results. Recommendations are also made for follow-up research and water treatment practice.

Chapter 2

Background and Literature Review

2.1 Mn in Drinking Water Sources

2.1.1 Properties and Occurrence

Mn is a transition metal with an atomic weight of 54.938 atomic mass unit (AMU). It is present in abundance in the earth's crust in the form of minerals and is generally found together with Fe ores (Chiswell & Huang, 2006). It can have oxidation states in the range of -3 to +7 (-1, 0, +2, +3, +4, +6, +7 being the most common), but in water, it is generally found in the oxidation states of +2 or +4 (Therdkiattikul et al., 2020). While in the oxidation state +2, the metal exists in dissolved form, in +4 oxidation state, it is found in precipitate form (Benefield et al., 1982). The dominance of a particular Mn species depends on the conditions in its milieu (i.e., in the water source), especially oxidation-reduction potential and pH (Mouchet, 1992). Figure 2.1 shows the graphical representation of the complex Mn redox equilibria or the Pourbaix diagram for Mn, and it shows which Mn species dominate in natural waters for existing pH and oxidation-reduction potential (ORP) conditions. Further, in context of Mn removal, it is known that Fe and ammonia have significant influence as competitors, thus, their redox equilibrium diagrams are displayed in Figure 2.2 and Figure 2.3 respectively. From the figures it can be clearly noticed that Mn exists in Mn^{+2} state and Fe exists in Fe^{2+} and Fe^{3+} states, dissolved in water at low pH values (below 8) and in anoxic conditions, whereas both metals exist in the precipitate form at higher pH values.

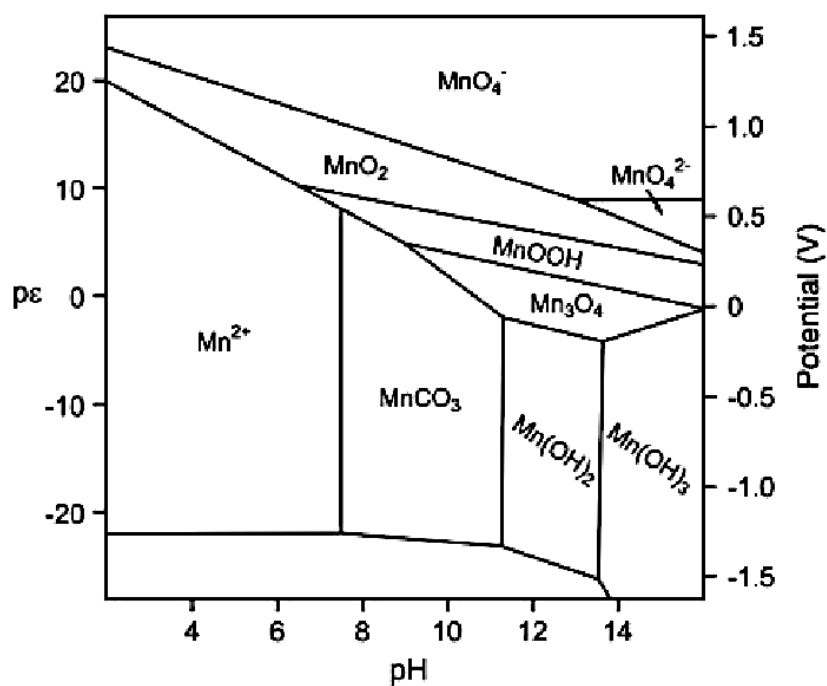


Figure 2.1 Negative logarithm of electron activity ($p\varepsilon$) and redox potential (EH) as a function of pH for the Mn species in an aqueous medium (Stumm & Morgan, 1996).

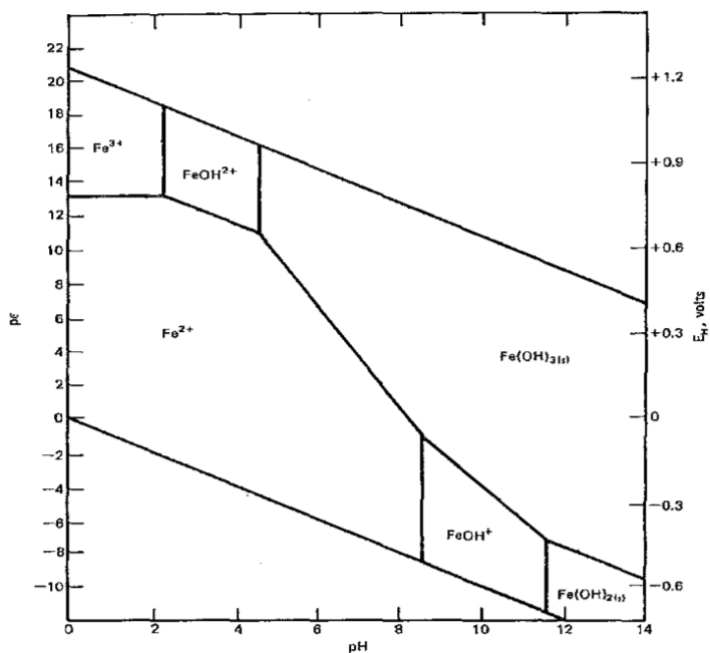


Figure 2.2 Negative logarithm of electron activity ($p\varepsilon$) and redox potential (EH) as a function of pH for the Fe species in aqueous medium (Snoeyink & Jenkins, 1980).

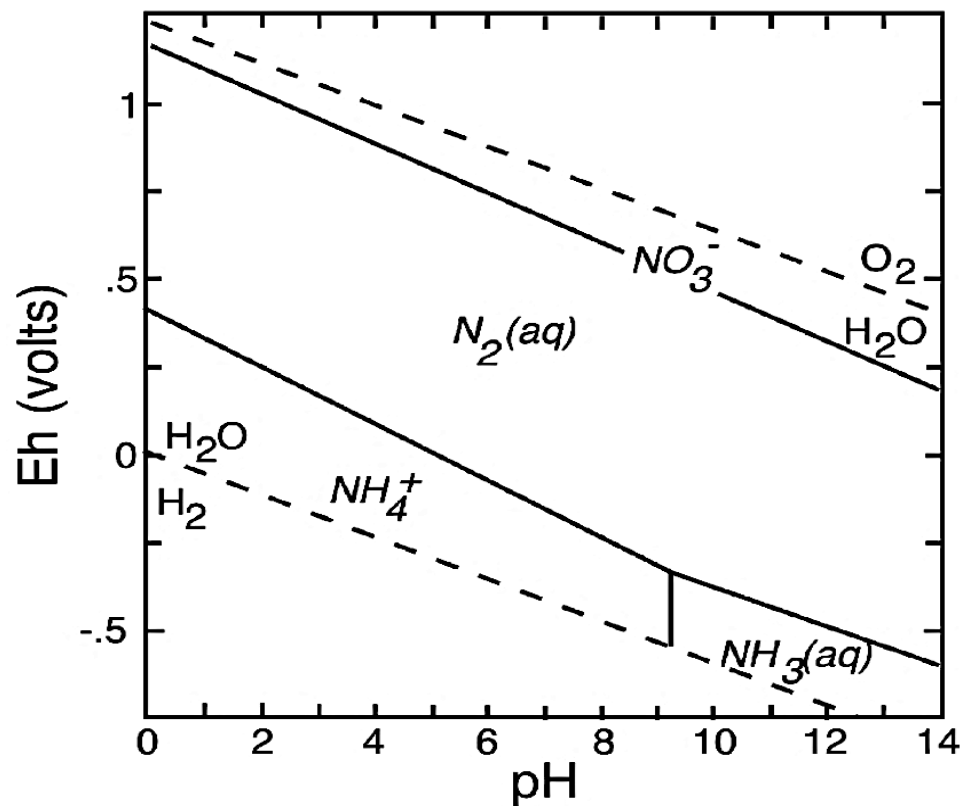


Figure 2.3 Negative logarithm of electron activity ($p\varepsilon$) and redox potential as a function of pH for the nitrogen species in aqueous medium (Russell & Hall, 2010).

Canada has more groundwater than surface water and an estimated 30.3% of population of the Canada depends on groundwater as its main source of water because of its reliability and relatively lower treatment/distribution-related expenses (Government of Canada, 2013). Compared to surface water, groundwater is less likely to be influenced by pathogenic contaminants, but the mineral content is typically higher in groundwater. In general, the concentration of Mn is below 0.1 mg/L in the freshwater sources in Canada, but natural reasons including acidic and reducing conditions in sources and anthropogenic sources can cause surges in Mn levels (World Health Organisation, 2017). In general, Mn is a geogenic pollutant, that limits the use of groundwater polluted by it for domestic use. The major causes of Mn occurrence in groundwater and surface water are tabulated in Table 2.1. Apart from those highlighted, Mn contamination can also happen due to in-situ generation during treatment at water treatment plants, like KMnO_4 addition as an oxidant or ferric chloride addition as a coagulant (Health Canada, 2019; Pei et al., 2022). It is important to note here that Mn contamination in

groundwater is more common and occurs at higher concentrations compared to surface water. Also, groundwater Mn concentrations are more stable over time, while surface water concentrations can vary seasonally.

Table 2.1 Major causes of Mn contamination in water source

Groundwater sources	Surface water sources
1. Dissolution of Mn bearing minerals and rocks. 2. Sewage and landfill leachate 3. Acid-mine drainage *(2) and (3) are for groundwater under direct influence of surface water (GUDI)	1. Weathering or dissolution of Mn bearing minerals and rocks. 2. Sewage and landfill leachate 3. Acid-mine drainage 4. Industrial discharge 5. Deposition from the air

2.1.2 Issues and Regulations

Mn plays a vital role in the human diet. As a macronutrient, it is required for the normal functioning of the brain, the nervous system, and many of the body's enzyme systems. Mn in the human body contributes to bone health, facilitates the formation of the antioxidant superoxide dismutase (SOD) for free radical protection, regulates blood sugar, aids nutrient metabolism, and enhances brain function and the adequate intake of Mn ranges from values as low as 0.003 mg for infants (up to 6 months of age) to as high as 2.6 mg for lactating mothers (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes et al., 2002). Although food is a significant source of Mn exposure for humans, Mn is more bioavailable when taken up in water compared to food (Pei et al., 2022).

Mn is crucial for humans, but in excess, it can be harmful to human health. Drinking water with elevated Mn concentrations has been associated with cognitive and neurobehavioral effects (Bouchard et al., 2007; Oulhote et al., 2014). Infants, the elderly and those with liver disease are particularly susceptible to the adverse effects of excess Mn (O'Neal & Zheng, 2015). Also, chronic Mn exposure has been linked to manganism, a condition resembling Parkinson's disease (Pfalzer & Bowman, 2017).

In addition to the adverse health effects, its problems related to the synergistic adverse impacts with co-constituents like Fe, arsenic and lead have been known (Bruins, 2017). An example of deleterious synergy is the lead release that gets exacerbated in the distribution system as a result of MnO₂ coating formation, which acts as a good adsorber (Friedman et al., 2010). Further, at drinking water treatment

utilities, it can give rise to operational and distribution supply issues (Refer to Table 2.2). The presence of Mn in water can lead to visual aesthetic issues, including stains and discoloration of plumbing fixtures, coloured water at the tap, while also contributing to the formation of scales in waterpipes, affecting both the appearance and functionality of drinking water distribution systems. Furthermore, operational problems like energy losses caused by scaling/fouling in the water treatment/distribution equipments, corrosion in the distribution lines and black or brown-coloured water at the tap makes one think about the solution to this problem. The considerably relevant disadvantages of excess Mn are tabulated in **Table 2.2**.

Table 2.2 Issues associated with excess Mn in finished drinking water (Health Canada, 2022)

Health effects (long-term exposure)	Aesthetics and others
1. Cognitive and neurobehavioral effects in young children 2. Affect memory, attention, and motor skills (movement) in adults 3. Manganism	1. Visual aesthetics- Coloured tap water 2. Unusual taste 3. Staining of plumbing fixtures or laundry 4. Reduced flow in distribution lines due to clogging with Mn precipitates 5. Increase in growth of unwanted bacteria 6. Can promote lead issues in the distribution systems

Although WHO has established guidelines for Mn, countries have been regulating the levels of Mn in drinking water individually. Health-based standard guidelines have been set for Mn by USEPA to 300 µg/L, along with a secondary maximum contamination level of 50 µg/L, which is also the aesthetic objective (USEPA, 2004). The European Union has also set 50 µg/L as the guidance value for Mn level (European Commission, 2020). Keeping track of the newer toxicological findings, in 2019, Health Canada revised the guidelines for Mn concentrations in finished drinking water, introduced a MAC value of 120 µg/L and lowered the AO value to 20 µg/L (Health Canada, 2019). The evolving understanding of the health impacts necessitated this shift in regulatory focus, prompting Health Canada to set an enforceable MAC for Mn. The change in regulatory importance of Mn from a mere aesthetic objective to an enforceable MAC value emphasizes that the change would ensure a proactive approach to managing Mn levels thereby safeguarding water quality and addressing both visual aesthetic issues

and potential health risks associated with elevated Mn concentrations. The primary concern associated with Mn is human health and not aesthetics, but drinking water providers aim for a more stringent value and since the aesthetic objective value is lower than the MAC value, treatment technologies should be devised for that threshold. In addition to that, Health Canada has recommended treatment utilities to maintain the treated water goal of 15 µg/L (Health Canada, 2019). Furthermore, in Japan, the water treatment plants aim to maintain the Mn concentration below 10 µg/L (Kim, 2015). Refer to Table 2.3 for a summary of Mn regulations (Marsidi et al., 2018; Pei et al., 2022).

Table 2.3 Summary of aesthetic, health-based and operational guidelines for Mn in µg/L

Guidelines	Health-based	Aesthetic	Treatment	Reference
Australia	500 µg/L	100 µg/L	NA	(NHMRC & NRMMC, 2011)
Canada	120 µg/L	20 µg/L	15 µg/L	(Health Canada, 2019)
China	NA	100 µg/L	NA	(SAMC & SAC, 2022)
Europe	NA	50 µg/L	NA	(European Commission, 2020)
India	NA	100 µg/L	NA	(Bureau of Indian Standards, 2012)
Japan	NA	50 µg/L	10 µg/L	(Kim, 2015; MHLW, 2003)
USEPA	300 µg/L	50 µg/L	NA	(USEPA, 2004)
WHO	80 µg/L	50 µg/L	NA	(World Health Organization, 2021)

***NA- Not Available**

2.2 Mn Removal from Drinking Water

Mn and Fe removal facilities were first established in Germany (1874) and then in the United States (1893) (Connor & Iron, 1971). Since then, significant advancements in the methodologies for the effective removal of the two metals have been reported. Water treatment processes are mainly classified into chemical, physical and biological processes, and based on the treatment objective and source water quality the choice of an appropriate treatment pathway is made. In source water, Mn exists in various forms, predominantly as dissolved Mn in the Mn²⁺ and particulate Mn which are usually Mn oxides (MnOx) where Mn has an oxidation state of +4. These distinct chemical forms present in the water source necessitate different treatment approaches, as their physical and chemical properties vary

significantly. Dissolved Mn (i.e. the Mn^{2+} cation) can be oxidized both physiochemically and biologically to particulate Mn oxides (i.e. MnO_x with an Mn oxidation state of +4), which can then easily be removed physically using processes including, but not limited to, filtration (Tatari et al., 2016). Note that that MnO_x in turn acts as a catalyst promoting the oxidation of dissolved Mn^{2+} to solid MnO_x . Figure 2.4 shows the different pathways through which the Mn transformation from Mn^{2+} to MnO_x happens on the filter media (Cheng et al., 2019).

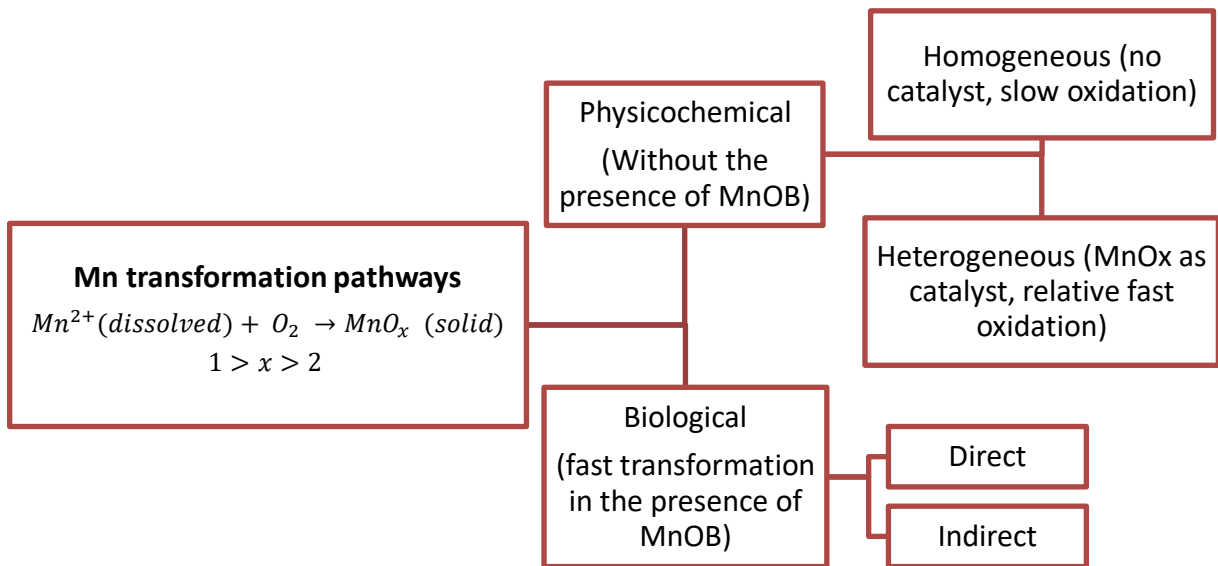


Figure 2.4 Pathways for the Mn transformation (MnOB: Manganese oxidizing bacteria)

Particulate Mn requires different treatment strategies. Filtration processes, such as multimedia filtration or membrane filtration, are typically employed to capture and remove solid Mn particles. Additionally, chemical additives may be used to facilitate precipitation and subsequent filtration. The treatment methodology is based to a large extent on source water characteristics, i.e. the form in which Mn exists. Understanding the specific forms of Mn present in source water is crucial for designing effective treatment processes that address the challenges posed by both dissolved and particulate Mn, ensuring the production of water that meets quality standards. Figure 2.5 shows the range of alternatives available for Mn removal from water.

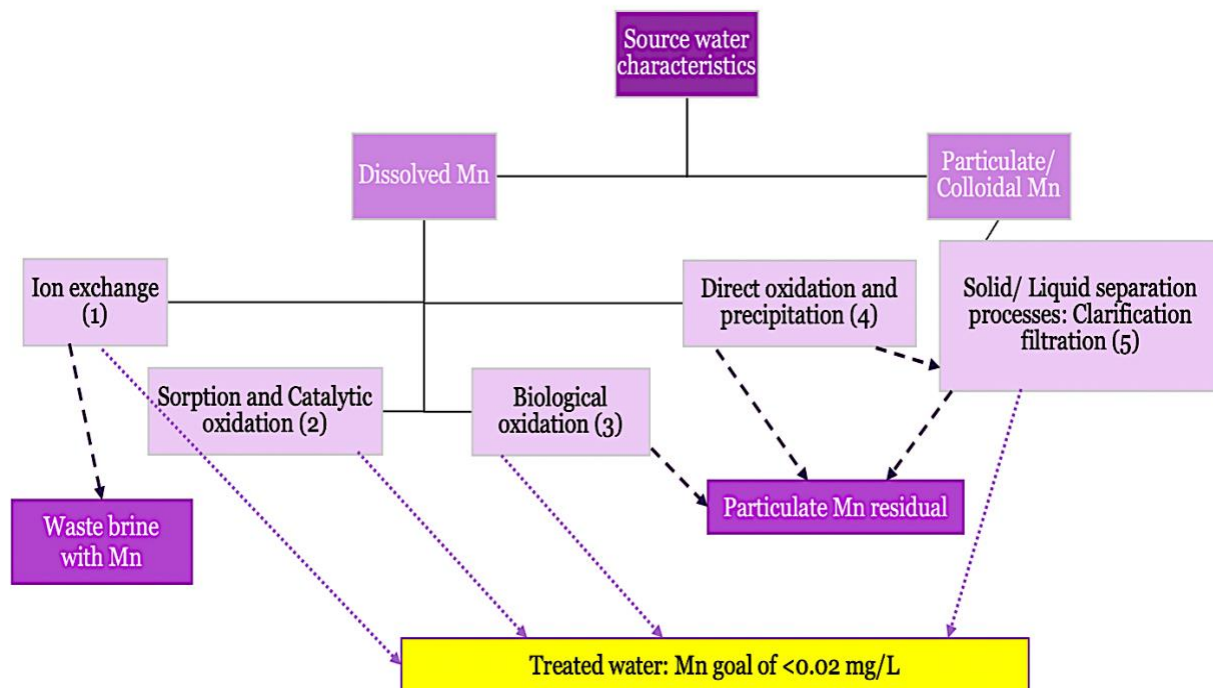


Figure 2.5 Treatment alternatives for Mn removal from water (Tobiason et al., 2016)

If the source water contains Mn in the dissolved form, i.e. the Mn^{2+} cation, then it can be removed via ion-exchange, a combination of sorption and catalytic oxidation, biological oxidation or a combination of direct oxidation and precipitation (Refer to Figure 2.5, processes 1-4). For particulate/colloidal type Mn, a solid/liquid separation process can be utilized. The treatment process is selected with the objective of meeting the regulated Mn threshold, which varies by jurisdiction and for Canada it is 20 $\mu\text{g/L}$. It is important to note that in Canada, treatment plants adhere to a more stringent target, aiming to reduce Mn levels below 15 $\mu\text{g/L}$ (Health Canada, 2019). Also, Mn is often present together with Fe in source water, necessitating the treatment of both metals. But reviewing Fe removal processes in addition to, or in combination with Mn removal processes goes beyond the scope of this review and is not discussed here.

2.2.1 Physical/Chemical Treatment Processes for Mn Removal

Physical conventional processes for water treatment have been profoundly known for their simplicity and flexibility with respect to individual needs. Using natural forces, physical processes including sedimentation, filtration, and flotation, have been found efficient, but they also faced some

disadvantages like high initial investment, high maintenance cost, clogging issues and requirement for post-treatment (Crini & Lichtfouse, 2019). On the other hand, chemical processes for water treatment like oxidation, adsorption, chemical precipitation, electro-coagulation and flocculation have been found effective in treating water to the desired extent, by either converting the contaminations into stabilized end products or by degrading them (Yargeau, 2012). But chemical processes also have been known to face challenges like consumption of hazardous chemicals, production of hazardous by-products, high sludge generation, high cost of chemicals and expensive regeneration. In the context of Mn removal, Tobiasson et al., (2016) have summarized some published research works and provided inferences. The underlying principles, advantages and disadvantages of three prominent methods: ion exchange, sorption and catalytic oxidation, and direct oxidation and precipitation (Refer to Figure 2.5, processes 1, 2 and 4) are discussed in more detail below.

Ion exchange involves the substitution of cations such as Na^+ or H^+ by soluble Mn cations (Mn^{2+}) within a cationic ion exchange resin or media. The resin selectively attracts and retains Mn ions, releasing other monovalent ions in exchange (Tobiasson et al., 2016). It offers selective removal of soluble Mn cations, which is operated continuously until the resin saturates. This leads to a periodic regeneration of the resin and therefore waste generation.

Direct oxidation and precipitation, a commonly practised Mn removal method, involves the addition of oxidizing agents to convert soluble Mn into insoluble Mn dioxide, followed by physical removal through sedimentation or filtration. Some of the oxidants found effective for Mn removal include chlorine dioxide, permanganate and ozone (Tobiasson et al., 2016). Direct oxidation is suitable for high Mn concentrations and varying water qualities, but it requires careful management of the residual oxidizing chemicals, potentially impacting water quality. Additionally, it leads to sludge generation, necessitating proper disposal measures. Further, the kinetics of oxidants like oxygen and hypochlorite (HOCl) have been reported to be very slow, and hydrogen peroxide (H_2O_2) has been found ineffective for Mn^{2+} oxidation, making these oxidants unapplicable (Knocke et al., 1987; Pankow & Morgan, 1981). The dosage of oxidants like ClO_2 and O_3 were also reported to be influenced to a great extent by oxidant consumption through water constituents other than Mn^{2+} (Knocke et al., 1987). Also, while using permanganate, chlorine dioxide and ozone as oxidants, the possibility of generating colloidal Mn particles was reported (Ministry of Health British Columbia, 2022).

Sorption and catalytic oxidation involve the use of a catalyst attached to filter media to adsorb soluble Mn^{2+} and facilitate its oxidation to solid MnO_x . Excess MnO_x is then removed from backwashing the filter media. Preferably a metal oxide, like Mn-oxide, which is referred to as MnO_x (x between 1.5 to 2) is used as a catalyst and is coated on different filter media including greensand, pyrolusite, ceramic material, anthracite coal or silica. Additionally, chlorine is frequently applied to the filters to support the oxidation of the Mn^{2+} , which is adsorbed onto the catalyst-coated filter media, to solid MnO_x . Catalytic oxidation has been known to effectively and quickly convert soluble Mn to its particulate form, with the extent of adsorption being dependent on the MnO_x coating level of the filter media (Dutta et al., 2021; Tobiasson et al., 2016). Also, compared to direct oxidation and precipitation, this process has lower sludge production, although over time, the sorptive filter media may need replacement should regeneration with free chlorine be insufficient. Further, not all of the media-associated MnO_x was reported available for Mn uptake (Islam et al., 2010). Activated carbon without catalyst coating has been utilized as an effective benign adsorbent (developed using biomass) for both Fe and Mn, but it was also associated with high regeneration costs and thereby faced difficulties with large-scale applications (Marsidi et al., 2018). Catalytic oxidation technology using oxidants like chlorine, and an oxide-coated surface has been known to be effective for Mn removal but has also been associated with halogenated disinfection by-product (DBP) generation which are carcinogenic and genotoxic (Richardson et al., 2007).

Furthermore, some of the reported effective physical and chemical routes for Mn removal include, using ferrate (Fe(VI)) as an oxidant, elevating pH by adding lime, membrane filtration, sorption using Mn-coated adsorbents, employing second-stage adsorptive contractors and using zeolites for ion exchange (Ellis et al., 2000; Goodwill et al., 2016; Hu et al., 2004; Knocke et al., 2010; Taffarel & Rubio, 2009; Teng et al., 2001; Tobiasson et al., 2016). However, physical and chemical processes have some drawbacks, including iron complexation issues, interference by nitrification, TOC, and issues with reagent dosage location (Mouchet, 1992). In the context of ultrafiltration, fouling issue was reported due to Mn oxide particulates, when prechlorinating was not performed, thereby suggesting prechlorinating as a necessary step when dealing with considerate Mn levels in the water (Choo et al., 2005). Additionally, it has been reported that for the removal of Mn below the regulated standards, chemical oxidation processes are not adequate (Therdkiattikul et al., 2020).

2.2.2 Biological Methods

Biological treatment supporting manganese oxidizing bacteria (MnOB) growth, has been reported an effective process for manganese removal from groundwater (Burger et al., 2008). Biological filters have been known to have effective manganese removal because of the high surface area for microbial attachment and high Mn oxidizing capabilities (Abu Hasan et al., 2020; Chaudhary et al., 2003; Liu et al., 2017; Kim et al., 1997).

Biofilters are filters having biomass attached to the filter media and rapid sand filters (flow rate: 2-10 m/h) are most commonly utilized (Logsdon et al., 2002), while slow sand filters (flow rate: 0.1-0.3 m/h) with their large footprint are less often utilized usually due to their space requirements (Marsidi et al., 2018). Anyways, both rapid and slow sand filtration can successfully treat Mn and Fe. Pacini et al., (2005) reported in their research work that removal up to 97% of Fe and Mn can be achieved using slow sand filtration. In another research investigation performed on a small community, (Manav Demir, 2016) reported 90-95% removal of Mn and Fe using slow sand biofilters. Similarly, researchers have also reported high efficiency of Mn and Fe removal using rapid sand filtration (Olanczuk-Neyman & Bray, 2000; Sodamade & Pearse, 2013).

Physical and chemical treatment methods have reported successful results in reducing the Mn concentration below MAC and AO limits (Patil et al., 2016). However, due to its benign nature and other benefits like less sludge production, lower operation & maintenance cost, and no by-product generation, biofiltration technology has been gaining profound attention for drinking water treatment. Over the last couple of decades, biofilm-based biological treatment technology has been gaining popularity over the conventional physiochemical processes (Marsidi et al., 2018). Biofiltration has demonstrated significant potential in small to medium-sized communities due to low operating expenditure, no chemical requirement, simple operation and adequate treatment performance. These advantages make biofiltration particularly well-suited for settings with lower water demand, contributing to its growing popularity in such communities.

2.2.3 Treatment for Mn Removal in Canada

In Quebec, it has been reported that Mn is one of the major inorganic contaminants that exceeds the Canadian regulated value in finished drinking water (Bondu et al., 2020). In British Columbia, the Mn concentration was found to be greater than 10 mg/L in some source water samples (Ministry of Health British Columbia, 2022). Apart from this, Mn related concerns are substantial in New Brunswick (33%

of treatment facilities are non-compliant with the MAC), Nova Scotia (22% of treatment facilities are non-compliant with the MAC), Saskatchewan and Manitoba (Health Canada, 2016). The introduction of MAC values for Mn in finished drinking water by Health Canada has posed challenges for utilities, as they navigate the complexities of adhering to the updated standard. The utilities were asked to submit plans to meet the regulations set on Mn in the distribution supply (Rural Municipalities of Alberta, 2019). While designing suitable treatment methods, it is important to note that the recommendation is to maintain a treated water goal of 15 µg/L for Mn (Health Canada, 2019).

In Canada, physical-chemical treatment processes for Mn removal have been widely used in the past, but the problems associated including the use of strong oxidants, high expenses, etc. have prompted the search for a more economically efficient and resilient treatment technology. In Saskatchewan, most of the treatment facilities currently use Mn greensand filters in combination with oxidation using permanganate (Health Canada, 2016). Further, the application of greensand filters has been reported at a water treatment plant in Hamilton, Ontario (Walkerton Clean Water Centre, 2018). It was reported that, after comparing the performance of different treatment technologies for Mn removal at pilot scale, cities/municipalities like Woodstock in New Brunswick and Waterloo in Quebec have installed the biological filters at their wells (Williams & Ondeo, 2002). Biofiltration of drinking water for Mn removal has been successfully utilized and documented in Europe, and Asia. The regulations on disinfectant by-products and growing environmental awareness has led to increased interest in biofiltration technology in North America as well, including Canada (Burger et al., 2008; Pontius, 1993; Rittmann et al., 1989; Rittmann & Snoeyink, 1984).

2.3 Mn Removal from Drinking Water

Amongst the various treatment options, there has been a surge in interest in research related to biofiltration due to its broad application and benign nature. From the beginning of its utilization in Scotland to its widespread application in Europe, North America and some other countries, biofiltration has been successfully implemented at numerous water treatment facilities and has been researched ever since with the aim of understanding its science and capabilities (Halle, 2010; Thuptimchang et al., 2021; Yu et al., 2003, 2007).

2.3.1 General Principles of Biofiltration

Biofiltration involves the use of native microorganisms to break down toxins or to convert/oxidize dissolved Mn to its oxidized, particulate form that can easily be removed physically (Sharma et al., 2005; Urfer et al., 1997). In a biofilter, bacteria present in the source water attach to the filter media while flowing through the filter, when attached, excrete extracellular polymeric substances (EPS) and start building up a biofilm. On this biofilm, as the effluent flows along, pollutants either degrade or get converted. Mass transport within the biofilm occurs based on the concentration gradient of contaminants and following the attachment of microbes, detachment may take place due to factors such as abrasion, pressure fluctuations, or self-oxidation of contaminants. Aeration is also provided, in order to maintain the dissolved oxygen levels required for metabolic activities. (Rittmann & McCarty, 1980, 2001; Rittmann & Stilwell, 2002; Williamson & McCarty, 1976).

Due to the non-requirement of chemicals for treatment, biofiltration has been utilized as a benign technology for the removal of dissolved organic carbon, heavy metals and fine particulates from drinking water. It has also been found to be economical with a significant reduction in the investment, operational and maintenance costs, thereby making it a preferred technique (Li et al., 2005). Also, biofiltration allows treatment to happen at high filtration rates, while ensuring flexibility and better sludge treatability (Mouchet, 1992). Biofiltration has been found advantageous over conventional processes for water treatment, as it may more efficiently treat organic matter which would eventually get converted into DBP in the disinfection treatment step (Kim et al., 1997).

While biofiltration has numerous benefits, there are some drawbacks associated with it as well. Biofiltration is associated with an acclimation period at the commissioning/start-up phase, during which the formation of biofilm coating happens on the filter media until a pseudo-steady state performance is achieved (Ramsay et al., 2018). This period (ranging from a few weeks to several months) is generally time-consuming and might also cause some unwanted disruptions at the treatment facility if other treatment processes are not in place to guarantee the water supply (McCormick et al., 2021; Morales Medina et al., 2023). To minimize this acclimation phase, treatment facilities have adopted “proactive inoculation” and “inherent inoculation” methods like utilizing matured biofilter media and backwash sludge from established biofilters at other treatment plants or using microorganisms inoculated/cultured in laboratories or exposing the filter media to the water utilized earlier for backwashing (Breda et al., 2017; Cai et al., 2015; Gülay et al., 2016; Qin et al., 2009; Ramsay et al., 2018). Apart from this, the mechanism behind contaminant degradation using biofiltration is complex.

2.3.2 Biofiltration for Mn Removal

Mn removal can happen either biologically or physiochemically (Refer to Figure 2.4) and in general for Mn, initially the removal primarily happens biologically and when the filters mature, it happens mostly via physical-chemical processes (Breda et al., 2019; Bruins, 2017). For Mn removal, it is possible that various processes occur in parallel. During the start-up period, Mn^{2+} is transported to the biofilm, where it interacts with the microbial community and other constituents of the biofilm matrix. The biological process involved in converting soluble Mn to its particulate form can be either intracellular oxidation via enzymatic action or adsorption of dissolved Mn on cell membrane surfaces, followed by oxidation by enzymatic action or simple biocatalysis (Mouchet, 1992). Cheng et al., (2019) suggested two biological reaction pathways, namely direct enzymatic oxidation and an indirect chemical pathway for Mn^{+2} degradation by MnOB. It is known that Mn oxidation (homogeneous pathway) using oxygen is not possible when the pH is below 9, so for most of the drinking water sources that have a pH in the range of 6-8, direct enzymatic oxidation by MnOB is the dominant Mn removal pathway (Yang et al., 2014). In the direct enzymatic pathway, microorganisms, particularly certain bacteria and fungi, play a crucial role in mediating the transformation of Mn ions. There are many MnOB species including *Lepothrix*, *Crenothrix*, *Hyphomicrobium*, *Siderocapsa*, *Siderocystis*, *Metallogenium* and *Pseudomonas manganoxidans*, wherein except the last one, all species can also oxidize Fe. A detailed list of known MnOB, iron-oxidizing bacteria (IOB) and ammonia-oxidizing bacteria is shown in Table 2.4 (Abu Hasan et al., 2012; Granger et al., 2014; Li et al., 2013; McKee et al., 2016; Tang et al., 2016; Yang et al., 2014).

Over time, from the acclimation period to reaching maturity and a pseudo-steady state, the biofilter media evolves, developing distinct sections capable of effectively removing both Fe and Mn, as shown in Figure 2.6 (Abu Hasan et al., 2012; Cheng et al., 2017; Han et al., 2013; Yang et al., 2014). Note that ammonia, Fe and Mn need to be present in the feedwater to observe the development of these zones. Some of the prominent microorganisms detected in biofilters include *Nitrobacter* for nitrates, *Pseudomonas* for Mn, *Gallionella* for Fe, etc. (Bruins et al., 2017; Cai et al., 2015; Chung et al., 1996; Myung Cha et al., 1999). Some researchers also examined the biofilter depth-wise to enumerate the relative abundance of different oxidizing species for Fe^{+2} , Mn^{+2} and NH_4^+-N removal including *Pseudomonas*, *Nitrospira*, *Gallionella*, *Leptothrix*, *Hyphomicrobium* (Li et al., 2013). Notably, the physical-chemical conditions necessary for bacterial species to oxidize Mn play an important role. For Mn removal, a minimum DO of 5 mg/L, pH in the range of 7.2-8.9, and ORP in the range of 200-550

mV is considered favourable (Kohl & Dixon, 2012; Mouchet, 1992). And it is important to note that the conditions favourable for Mn removal are different than those favorable for Fe removal. Some researchers mentioned that unless the biofilters are running at low flow rates, it is not possible to remove Mn and Fe simultaneously in the same reactor, mainly due to the difference in the operational conditions required for the removal of the two metals (Frischherz, 1985). Studies were conducted where biofilters were in series with the first one removing predominantly Fe and the second one Mn after doing the required pH adjustments (Bourgine et al., 1994).

Table 2.4 List of oxidizing bacteria for different contaminants in water

MnOB	IOB	Others
<i>Leptothrix, Pseudomonas, Arthrobacter, Hyphomicrobium, Siderocapsa, Crenothrix, and Metalloaenium</i>	<i>Gallionella, Leptothrix, Bacillus, Crenothrix, Pseudomonas</i>	Ammonia oxidizing bacteria: <i>Nitrosomonas europaea, Nitrosomonas halophila, Nitrosomonas mobilis, Nitrospira, Comamonas, Nitrosovibrio, Nitrosococcus, and Acinetobacter</i> Arsenic oxidizing bacteria: <i>Alcaligenes, Pseudomonas</i> Methane oxidizing bacteria: <i>Methylomonas, Methylothermobacter</i> Organic oxidizing bacteria: <i>Paludibacter, Novosphingobium, Flavobacterium, Burkholderia</i> Sulfur oxidizing bacteria: <i>Sulfuritalea</i>

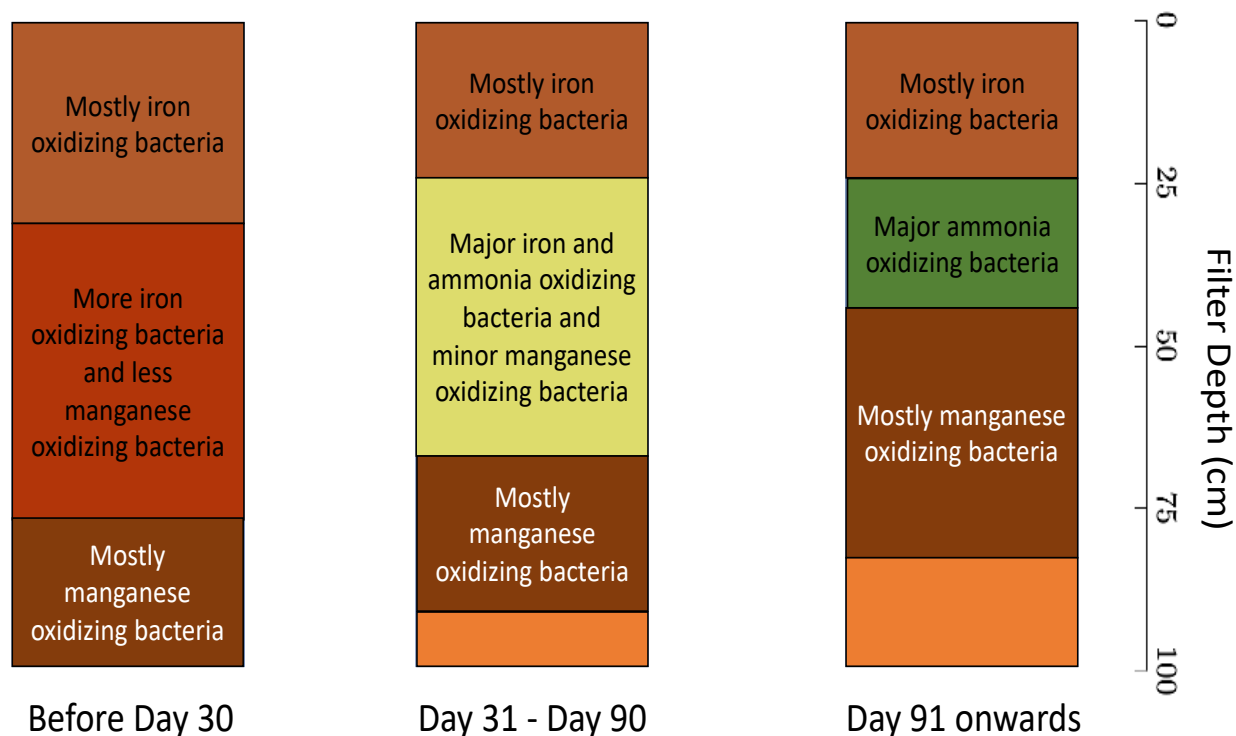


Figure 2.6 Over time evolution of biofilter media from acclimation period up until biofilters reach maturity and are in pseudo steady state (Cheng et al., 2017; Keithley et al., 2023; Yang et al., 2014).

2.3.3 Factors Influencing Biofilter Performance

2.3.3.1 Water Quality Parameters

A thorough understanding of influent water quality parameters can ensure optimal biofilter performance and operation. The concentration and form of Mn, Fe, pH, alkalinity, hardness, temperature, DO and ORP were reported as significant factors affecting Mn removal (Tobiason et al., 2016). Alkalinity affects the buffering capacity of the water and can influence pH stability, thus it is essential for maintaining favourable pH conditions for Mn removal. pH and ORP especially have been reported to influence the biofilter performance for Mn removal significantly. The field of activity for MnOB, as reported by (Mouchet, 1992), was found to be within a pH range of 7.2 to 8.9 and an ORP range of 200 to 550 mV. Optimal pH conditions are also critical for the formation of solid MnOx via physical-chemical oxidation processes, thus adjusting pH can ensure effective removal by promoting the conversion of soluble Mn²⁺ to solid MnOx. Apart from

this, an appropriate temperature and DO can also support biofilter operations. For biofilm growth and Mn oxidation, an aerobic environment is critical and DO has been known to play an important role in the crystal structure of MnOx filter media samples (Cheng et al., 2019). In the indirect chemical pathway for Mn²⁺ degradation, Mn oxidation can occur through chemical reactions in the presence of DO or other oxidizing agents, and is often catalyzed by MnOx. In aerobic conditions, DO promotes the enzymatic oxidation of Mn²⁺ by MnOB, which is a key step in the formation of MnOx and subsequent removal. After surveying 100 water treatment plants, which used biofiltration, it was reported that NH₄⁺ removal efficiencies above 85%, pH above 7.1 and DO above 1 mg/L would result in effective Mn removal (Bruins, 2017). Apart from pH and DO, DOC was reported as important for the transformation of Mn and influencing the levels of dissolved Mn in the source water and a high DOC (>7 mg/L) was reported unfavourable for Mn removal (Li et al., 2021). Possible reasons were oxidizing conditions, where chemical mineralization of DOC depletes dissolved oxygen (DO), or the presence of humic acid with Mn(II)-complexing capabilities, leading to the reductive dissolution of Mn oxides and a return to the Mn(II) state. Also, DOC along with the metal constituents (Mn, Fe) and other nutrients are important for biomass development (McKie et al., 2019). Temperature can influence the rate of chemical reactions and microbial activity and therefore impact the efficiency of biological and chemical processes involved in Mn removal. Although for groundwater, temperature remains mostly stable, temperature variations can have some impact on the performance of biofilters, especially on biological activity (Morales Medina et al., 2023b).

2.3.3.2 Biofilter Design and Operational Parameters

Flow rates, EBCT, filter run time, and backwashing requirements are considered to be pertinent design and operational parameters of a biofilter related to efficacy of the treatment, production capacity and cost. Bruins, (2017) reported that the Fe loading rate per filter run played the most significant role in Mn removal, while filtration rate and EBCT played a secondary role. From their survey of 100 water treatment plants, which used biofiltration, it was reported that a Fe loading rate per filter run of less than 2.7 kg/m² in the raw water, a filtration rate less than 10.5 m/h and an EBCT greater than 11.5 min was favourable for effective Mn removal. Further, in terms of operation modes, when gravity and pressure filters were compared, it was found that gravity filters performed better than pressure filters, even when the filtration rates of the pressure filters were in the acceptable range (i.e. <10.5 m/h) (Bruins, 2017). Also, backwashing is necessary in biofiltration to remove accumulated solids, debris, and microbial biomass from the filter media and thereby avoid clogging, reduced hydraulic conductivity, and decreased treatment efficiency. In this context, it was reported that the backwashing frequency is important, especially during the filter ripening period and excess backwashing was reported unfavourable (Bruins, 2017).

The choice of media, its characteristics and filter bed depth also plays an important role in Mn removal (Bruins et al., 2015b). Various filter bed materials have been examined by researchers, out of which sand, anthracite and granular activated carbon (GAC) were found most preferable (Cescon & Jiang, 2020). Amongst the various characteristics of filter media, specific surface area has been known to play a key role in biological oxidation (Tekerlekopoulou et al., 2008). Apart from this, it was also reported that inoculated media could accelerate the start-up and show higher Mn removal rates than non-inoculated ones (Breda et al., 2019).

2.3.3.3 Microbiological Parameters

Basu et al., (2016) in their review pointed out research gaps related to bacterial growth within biofilters for DOC removal. Lautenschlager et al., (2014) investigated the bacterial community composition within the filter biomass and effluent and documented its impact on the degradation of organic carbon during drinking water treatment. Further, although the underlying reason was not clear, it was reported that the biomass density was higher in cold-water conditions compared to that in warm-water conditions (McKie & Andrews, 2022). In the context of Mn biofiltration, understanding the type of MnOB and MnO_x during different time points, i.e. during the acclimation period and after filter maturation, is essential for unravelling the mechanisms underlying Mn removal processes in biofilters. The presence, abundance, and activity of MnOB influence the rate of Mn oxidation, the formation of MnO_x, and the overall success of biological Mn removal processes. A lot of different types of Mn oxides have been identified, and among them, birnesite has been reported most effective for Mn removal (Bruins et al., 2015a). Apart from this, the overall composition of the microbial community in the filter media affects the diversity of microorganisms involved in Mn oxidation and removal. ATP measurements can help provide an overall picture of the microbial activity in the filter, although no direct correlation between ATP and biofilter performance has been reported (Pharand et al., 2014). Although Earle et al. (2023) reported that Mn removal increased as the biomass accumulated over time. Also, adequate nutrient availability, such as carbon and nitrogen sources, that support the growth and activity of MnOB is a key factor controlling the health of the biofilm. Lastly, EPS serves as a crucial microbiological parameter for Mn removal by serving as a nutrient and energy source for microorganisms, contributing to biofilm formation and stability, facilitating extracellular Mn oxidation, etc. (Tobiason et al., 2016). McCormick et al., (2023) reported that the composition of EPS can be affected by the source water along with the microbial community.

2.3.3.4 Biofilter Operation

There are two modes in which a biofilter can be operated, continuously or intermittently (a few hours per day). Due to the way a biofilter functions, it is likely that the operational mode adopted might result in

considerable variations in hydraulics, microbiological characteristics and filter operation. Thus, factors including, but not limited to operational elements like backwash frequency, water quality parameters like DO, and biofilter storage options for intermittent biofilters, need to be given consideration so that the biofilter functions optimally.

2.4 Intermittent Operation

2.4.1 Why Operate Intermittently?

Recently, with the advent of implementing rapid filtration technologies and with the rise in water conservation policies, it has been found that treatment plants do not have the need to run at full capacity, which might lead to intermittent operation of filters. And it is anticipated that the number of such plants will increase in the near future (McKie & Andrews, 2022). Further, based on water demand, currently, many groundwater wells are operated intermittently. Apart from the demand point of view, intermittent operation can also be associated with savings of operational costs, resulting in economic benefits. By optimizing the operating times of a biofilter, while maintaining the effluent water quality according to the regulatory considerations, significant energy savings can be achieved. For Mn removal from groundwater sources, if biofiltration is to be implemented, then it is logical that intermittent operation should be investigated for Mn biofilters, which has only been done to a limited extent to date (Bruins, 2017; Burger et al., 2008; Evans et al., 2021; Zeng et al., 2019).

2.4.2 Problems Associated with Intermittent Operation

Intermittent operations might result in periods of oxygen deprivation depending on the local conditions and thereby affect the biofilter efficiencies (Zeng et al., 2019). Biofilters can experience anoxic conditions i.e. $DO < 1$ mg/L during shutdown and it can be hypothesized that this would affect the biofilm formation due to reduced microbial activity and also reduce the Mn-oxide formation. The shutdown can also lead to decreased nutrient loading, which can cause a nutrient-stressed condition in the biofilters for the microorganisms, which can lead to a decline in active biomass contents, and enzyme activity and therefore affect bioactivity McKie et al., (2016). In the context of turbidity, it was hypothesized by McKie & Andrews, (2022) that frequent shutdowns can cause an increase in turbidity spikes. Also, Niquette et al., (1998) highlighted the hazards of releasing endotoxins that have been known to be generated during biofilter shutdown due to bacterial cell lysis.

2.4.3 Pertinent Research Investigations on Intermittent Biofilter Operation

The earliest works related to intermittently operated biofilters for drinking water application date back to 1998 when Niquette et al. investigated the effects of a biofilter shutdown on water quality. The biofilter focused on biodegradation of DOC and nitrification of ammonia. It was reported that the DO levels dropped significantly and quickly during the first two hours of filter shutdown, promoting anaerobic conditions, and the biomass levels declined in the filter at several depths after a 24-hour shutdown. They also reported that performing filter backwash before restarting the biofilter, favoured biofilter performance post-shutdown and brought the filters to pre-shutdown standards in terms of performance (Niquette et al., 1998). Recently, McKie & Andrews, (2022) reported that a shutdown does not have any detrimental effect on the biofilter in terms of biological activity, production rate and DOC removal performance, rather enhanced results were achieved. In their study, backwash was done before biofilter shutdown and the reasons behind the enhanced results were unclear. Few researchers have been investigating the intermittent operation for organics removal (Refer to Table 2.5), but even fewer have investigated it with Mn being of prime focus (Refer to Table 2.6).

Table 2.5 Studies focusing on intermittently operated biofilters for organics removal

Sr. no.	Biofilter set-up details	Major inferences, knowledge gaps	Reference
1.	1. Pilot scale study at a surface water treatment plant 2. Biofilter media: matured GAC 3. Operations (for 7 months)- Continuously and intermittently (12 h/d) 4. Water quality parameters- Temperature: 4 - 22°C, pH: 6.6 -7.0 5. Water quality parameters- TOC: 3.7-4.7 mg-C/L; Temp.: 5-17 °C; pH: 6.7-8.11; DO: 8.8-14.1 mg/L	1. Initially virgin GAC exhibited higher DOC and DBP precursor removal rates as compared to biologically activated carbon due to the absorptive capacity, but with time the rates became statistically similar as biological removal became predominant. 2. Nutrient enhancement (nitrogen and phosphorus) did not seem to have a significant impact on the performance 3. Intermittently operated biofilters showed improved performance compared to continuous biofilters with respect to DOC removal but not for DBP precursors	(Nemani et al., 2018)
2.	1. Pilot scale study at a surface water treatment plant 2. Biofilter media: matured GAC 3. Operations (for 10 month): Continuously and intermittently (4-12 h/day) 4. Water quality parameters- Temp.: 17-18 °C; pH: 7.6-7.63; DO: 8.9-9.8 mg/L; DOC: 3.5- 3.9 mg-C/L	1. Anoxic conditions created during stagnation periods 2. ATP and DO were higher in biofilters operated continuously compared to those operated intermittently 3. DBP removal rates per biomass density were higher for intermittently operated biofilters than the continuously operated biofilters but the removal rates per DO consumption was higher for continuously operated biofilters 4. Removals were not significantly different with respect to EBCT and DOC values	(Mian et al., 2019)

3.	1. Pilot and full-scale study at a surface water treatment plant 2. Biofilter media: matured GAC 3. Operations (for 6 weeks): Only Intermittently (12 h/d) 4. Water quality parameters- Temp.: 3.1-4.1 °C; pH: 7.9-8.3; DO: 13.1-14.3 mg/L; DOC: 3.9-4.5 mg-C/L	1. The intermittently operated biofilters maintained N-nitrosodimethylamine (NDMA) precursors concentrations in the effluent post shutdown of 6 h or more 2. After the stagnation period and after start-up, NDMA precursors were not released 3. Biofilters consistently removed DBP precursors 30 mins after restart and the effluent concentrations remained unaffected post-shutdown, upon restarting the biofilters 4. Microbial activity was reported to remain stable during cold water conditions and the extracted biofilm contained high amounts of humic substances 5. ATP increased for 20 min post restart and so did the DO and both stabilized in 30 min after restart	(Di Tommaso et al., 2019)
4.	1. Pilot scale study at a surface water treatment plant 2. Biofilter media: matured GAC 3. Water quality parameters- Temperature: 4 - 22°C, pH: 6.6 -7.0 4. Operations: Continuously and intermittently (8h/day) 5. EBCT- 15 and 30 mins 6. Pre-treatment: Ultrafiltration, Post treatment, UV disinfection 7. Backwash: Every two months	1. Effective activity (enzyme activity multiplied by EBCT) measurement helped to better predict the biofilters performance 2. Cyclical operations did not affect DOC removal 3. Biomass density and cellular activity declined by more than 50% after shutdown and ATP increased until 4 h after the start-up. 4. Biomass levels for EBCT = 15 min were higher than that of EBCT = 30 mins, but organics and DBP removal was higher for EBCT = 30 mins.	(McKie et al., 2019)

		5. Biomass levels were higher in the upper levels than the lower depths of the biofilter bed 6. Higher esterase activity reported for cyclical operation, thereby resulting in higher DOC removal 7. Unique bacterial communities might be present in the filters operated cyclically 8. Suggested to increase surface area and reduce filter depth for optimization 9. Cyclical operation might minimize the costs associated with production costs especially if operated during low energy cost periods	
--	--	--	--

Table 2.6 Studies focusing on intermittently operated biofilters for Mn removal

Sr. no.	Biofilter details	Results, major inferences and knowledge gaps	Reference
1.	1. 4 groundwater wells in New Brunswick (full-scale test) 2. Operation type: continuous/intermittent (4 h/day) 3. Filter media- Uniform sand 4. Water quality parameters- Mn: 0.86-1.39 mg/L, Fe: <0.26 mg/L, pH: 6.46-7.5 5. Backwash frequency- about every 300-336 h (continuous), 75 - 300 h (intermittent) 6. Biofilter ripening time- 5 min	1. 100% Mn removal (detection limit 0.03 mg/L) achieved in all filters 2. Predominance of biological oxidation for Mn removal 3. The concentration of Mn in raw water has minimal impact on filter operation once they reach a steady state 4. Presence of MnOB observed in 3 of 4 municipalities, with Leptothrix bacteria recorded in 1 of 4.	(Burger et al., 2008)
2.	1. Full scale and pilot scale test at a groundwater treatment plant 2. Filter media: Sand 3. Iron and Mn oxidizing bacteria (IMOB) was captured from a local groundwater treatment plant, cultured and then inoculated in the filter 4. Operation type: intermittent (6-7 h/day)/ continuous 5. Raw water characteristics: T: 12-14°C; pH: 6.8; Turbidity: 32.3-39 NTU; Mn conc.: 0.8-2 mg/L; Fe conc.: 0.15-0.2 mg/L	1. The maximum Mn removal rates attained by intermittently operated biofilters was 50%, while the continuously operated biofilters reached 96% removal rates. 2. Transition from continuous to intermittent operation took 40 days to reach a steady state 3. Intermittent operation showed no impact on the microbial activity of biofilter media 4. Intermittent operation delayed the biofilters from reaching a steady state 5. During shutdowns, emptying is recommended rather than keeping the filter media filled with water 6. MnOB and IOB exhibit low metabolic and growth rates, indicating that microorganisms responsible for	(Zeng et al., 2019)

		Fe and Mn removal differ significantly from those involved in carbon and nitrogen removal	
3.	1. Full scale test at a surface water treatment plant 2. Filter media: GAC and anthracite 3. Raw water characteristics: pH- 6.7, Turbidity- 1.85 NTU 4. Note: Extended shutdown for 2 months	1. Acclimation time for Mn removal was higher in winter than in summer 2. Total Mn removal was not affected by the extended shutdown in some cases, while it was affected in others. 3. Leaving the filters submerged and backwashing before bringing the filters online may have contributed to maintaining the biomass 4. Suggestions: • Bringing biofilters into service during warmer water seasons • Using GAC media to provide an additional barrier for organic carbon removal during acclimation • Potentially increasing the EBCT of filters during acclimation	(Evans et al., 2021)

2.5 Prolonged Shutdown

Amongst the various operational aspects that could potentially affect the performance of biofilters, one is prolonged filter shutdowns (two or more months of shutdown). These shutdowns may occur due to hardware/software component concerns, raw water quality issues or other external factors. These events can occur unexpectedly and may surpass the initially expected timeframes and therefore have the potential to result in microbial mortality or trigger a state of dormancy in the biofilm on the filter media. A prolonged shutdown might cause a depletion in the DO levels in the filter bed, which can potentially lead to an anaerobic state and therefore affect the microbial activity and metabolism (Zeng et al., 2019). Based on the existing literature and to the best of the researcher's knowledge, only one study has explored an extended shutdown of biofilters to date. (Evans et al., 2021) investigated the dynamics of the startup phase of new biofilters and the acclimation process of existing biofilters following an extended shutdown. This study was done at full-scale water treatment plants, which treated surface water from lakes. One of the water treatment plants was under an extended shutdown for about two months during the winter due to maintenance work at the raw water facilities. During this shutdown, the biofilters were kept submerged in water and before the operations were restarted, the biofilters were backwashed. The latter is consistent with recommendations by Niquette et al., (1998) to backwash biofilters just prior to restarting them after a shutdown. Regarding biofilter performance after the extended shutdown, findings indicated relatively low TOC removal rates initially, which gradually increased over time. In terms of Mn and Fe, varied results were reported. Some utilities experienced lower removals post-shutdown, while others showed minimal impact of the two-month shutdown on Mn and Fe removals. Furthermore, confounding outcomes were observed concerning microbial activity. Certain utilities documented a decline in microbial community levels during the shutdown, followed by a gradual increase after restart. In contrast, other utilities maintained high microbial activity, possibly due to specific storage practices and backwashing procedures applied to the biofilters during the shutdown and before restart (Brown et al., 2020; Evans et al., 2020). This highlights the significance of adequate biofilter storage during shutdown periods. Other suggestions to better handle the extended shutdown situation, include restarting operations in warmer conditions and implementing thorough monitoring of the different water quality parameters as different parameters might take different time periods to acclimate. Being relatively unexplored, a comprehensive understanding of these operational disruptions on biofilter performance and microbial ecology is needed. Furthermore, the potential risk of releasing the hazardous endotoxins during a shutdown, as emphasized by Niquette

et al., (1998) in their research, underscores the necessity for further investigation into this operational challenge.

2.6 Conclusions and Research Gaps

An estimated 30.3% of the Canadian population depends on groundwater as its main source of water and although it is less likely to be influenced by pathogenic contaminants, the mineral content is typically higher in groundwater (Government of Canada, 2013). Mn is a geogenic pollutant, which limits the domestic use of groundwater for aesthetic concerns. Mn in drinking water has been associated with cognitive and neurobehavioral effects in children (Bouchard et al., 2007; Oulhote et al., 2014). Keeping track of the newer toxicological findings, in 2019, Health Canada revised the guidelines for Mn concentrations in finished drinking water, introduced a MAC value of 120 µg/L and lowered the AO value to 20 µg/L (Health Canada, 2019). The introduction of a MAC value for Mn in finished drinking water by Health Canada has posed challenges for treatment utilities, as they navigate the complexities of adhering to the updated standard.

In source water, Mn exists in various forms, predominantly as dissolved Mn (Mn^{2+}) and sometimes as particulate Mn (mostly $MnOx$). This necessitates different treatment approaches, as their physical and chemical properties vary significantly. Physical and chemical processes that have reported successful results in reducing the Mn concentration include ion exchange, direct oxidation and precipitation, and sorption in combination with catalytic oxidation (Tobiason et al., 2016). However, biofiltration technology has been gaining profound attention for drinking water treatment due to its benign nature and other benefits like less sludge production, lower operation & maintenance cost, and no disinfection by-product generation. Biofiltration involves the use of native microorganisms to convert/oxidize dissolved Mn either biologically or physiochemically to its oxidized, particulate form that can easily be removed physically (Sharma et al., 2005; Urfer et al., 1997). Biofiltration technology has achieved commercial availability in Europe and Asia, and the regulations on disinfectant by-products and growing environmental awareness has led to increased interest in this treatment method in North America as well. Also, sand has been used as the media of choice for Mn removal by biofilters in Europe, but limited exploration has been conducted regarding its utilization in North America.

Notably, knowledge gaps persist in the scientific understanding, of biofiltration for Mn removal. The knowledge gaps include details about Mn removal pathway, microorganisms responsible for the biological oxidation and the oxides catalyzing physico-chemical oxidation. Further, depending on the plant capacity and drinking water requirements, biofilters for groundwater treatment might need to be operated intermittently, but little is known about its benefits, effects, and potential, and currently, not much evidence is available to comment on the impact of the intermittently operated biofilters on Mn removal. A key question is whether intermittent biofilters achieve the same Mn removals as continuously operated biofilters, during the startup phase i.e. the acclimation phase, and more importantly once biofilters are at steady state. This underlying knowledge gap possibly relates to the period of non-steady state conditions that might arise just after the intermittent shutdown, when the biofilters restart. The Mn removal rates may differ from those observed prior to the shutdown, and it may take some time for the biofilters to return to those levels. Nothing could be found in the literature, indicating the biofilter performance once the intermittently operated biofilters restart. Also, the time required to reach steady state i.e. the acclimation period may vary, possibly be slower for the intermittently operated biofilters in comparison to the continuously operated biofilters. The acclimation phase of the biofilter is crucial in decision making, and none of the research studies have specifically addressed this aspect. In the context of the microbiology, intermittent shutdown may affect biological activity measured as ATP. Due to possible nutrient limited and DO stressed conditions, the microbial composition might decline during intermittent shutdown. Also, limited literature exists with respect to the microbial activity before and after the restart of intermittently operated biofilters and there is a need for further investigation. Additionally, most of the studies that performed microbial analyses during shutdown were focused on surface water sources, whereas Mn is primarily encountered in groundwater sources.

Furthermore, a major operational challenge that utilities might have to deal with is an extended shutdown (more than a week or two up to several months), which might last for more than the anticipated time and can potentially cause the biofilm on the filter media to be affected. It is important to know how to overcome this challenge and find ways, in which the effect of a prolonged shutdown can be minimized. It is important to conduct an in-depth investigation into the optimal storage conditions for biofilters during an extended shutdown situation to preserve their operational status. To the best of the author's knowledge, limited research has been conducted on this aspect, despite the fact that this problem can unexpectedly arise and significantly impact water treatment utilities. The extended

shutdown can potentially adversely impact the microbial activity during the shutdown, which in turn may impact the performance of the biofilters when they are restarted. After restart any potential difference in performance of intermittently and continuously operated biofilters needs to be investigated. Also, understanding the duration required for these biofilters to reach a steady state after restart is very important to understand the impact of such operational interruptions.

Chapter 3

Materials and Research Methodology

3.1 Overview

A pilot-scale set-up consisting of three rapid, conventional biofilters was used for this research study, which was assembled at a drinking water treatment facility in Southern Ontario, Canada, while sample analysis was primarily performed at the University of Waterloo. Sand was used as the media and raw non-chlorinated water from a groundwater well was utilized and pumped as feed to the biofilter columns. The pilot biofilters were installed in December 2022, and operations commenced in January 2023. The details of the pilot set-up and its operation are covered in section 3.3, while the pilot location is described in section 3.2. Based on the sub-objectives defined for each phase, the sampling and analysis plan was designed.

The sampling frequency for each parameter was determined based on factors including significance level, time availability and budget. Sampling details are shown in section 3.4, and analytical methods, including water quality parameters and microbial analysis of the biofilm on filter media samples, are described in section 3.5. For microbiological studies including MnOB, LBB and CLPP analysis the samples were analyzed by Nicholas Lamba-Rautapuro, a MSc student at Wilfrid Laurier University.

3.2 Pilot Plant Location

For this research work the partner facility was at a drinking water treatment facility in Southern Ontario, Canada, where the pilot scale set-up was assembled and operated. The city has access to two types of drinking water sources: surface water and groundwater. The Kempenfelt Bay connected to Lake Simcoe is a surface water source, that supplies drinking water to the north and central parts of the city and the water demand from the south end of the city is fulfilled by the groundwater reserves from well fields. The choice to utilize groundwater as our feed water source was driven by the specific challenge posed by Mn contamination, which is predominantly prevalent in groundwater. The city has 14 existing groundwater wells and because the groundwater is naturally filtered through layers of rocky sediments, it requires less treatment than surface water. The groundwater wells in the city are known to be supplied by four aquifers and most of the wells get the water from Aquifer 3 and 4, which are extensively sand

and gravel aquifers. The groundwater treatment facilities currently run intermittently from two/three hours per day up to nine hours per day.

Amongst the 14 wells, the site selection for pilot scale set-up and experimental work was done based on:

1. Water quality requirements: Concentration of Mn and other important co-constituents like Fe, turbidity, TOC, etc. were of paramount significance and wells with higher concentrations of Mn and Fe were given precedence due to their alignment with the project's focus on mitigating these contaminants.
2. Space requirements: The availability of adequate space and height clearance at each well location was assessed to ensure sufficient room available to accommodate the pilot set-up and ancillary equipment.

To gauge all these constraints a site visit was conducted on September 23rd, 2022, well diagrams were referred to, and past water quality data (2019-2021) was analyzed. Refer to Appendix A.1 for the original biofilter design and the space and raw water flow rate requirements based on that design.

The water quality parameters of 14 groundwater wells within the city were examined using historical data made available from 2019-2021 (Refer to Table 3.1). Some of the significant insights regarding these groundwater wells are:

- Mn Concentration: All wells located within the city exhibited Mn concentrations below the MAC threshold of 120 µg/L. However, it is noteworthy that 13 out of the 14 wells surpassed the AO value of 20 µg/L for Mn concentration.
- Fe Concentration: Among the five wells with the highest Mn concentrations, Fe concentrations remained below the AO value of 0.3 mg/L, except for well 3A, where the Fe concentration exceeded this threshold.
- pH Variation: The pH levels across the wells exhibited a range of 7.78 to 8.12, falling within the desirable range for the effective removal of Mn.
- Alkalinity Range: Alkalinity levels spanned from 186 to 272 mg/L, with well 13 displaying the highest alkalinity among all wells.
- Turbidity Levels: Turbidity measurements ranged from 1 to 8 NTU (Nephelometric Turbidity Units). Notably, well 3A displayed the highest turbidity value among all the wells studied.

Table 3.1 Information on groundwater wells in the city (2019-2021) along with the respective guideline values (Health Canada, 2019)

(Health Canada, 2022)

Well	Mn (mg/L)	Fe (mg/L)	NO₃²⁻ (mg/L)	NH₄⁺ (mg/L)	TOC (mg/L)	Cl⁻ (mg/L)	TDS (mg/L)	pH	Conductivity (µS/cm)	Alkalinity (mg/L)	Hardness (mg/L)	Turbidity (NTU)
5	0.041	1.26	NA	0.06	2.7	33.9	285	NA	513	189	248	2.7
7	0.026	0.619	NA	0.08	3	33.9	286	NA	533	192	261	5.3
9	0.004	0.006	4.2	0.03	1.3	99.9	468	NA	851	252	338	0.3
11	0.074	0.117	0.6	0.07	1.1	213	601	7.78	1130	231	362	1.9
12	0.04	0.309	NA	0.13	1.2	309	731	NA	1410	218	352	5.3
13	0.07	0.151	4.1	0.16	2.9	90.4	500	7.86	925	272	424	1.3
14	0.088	0.3	0.1	0.11	1.3	149	462	7.83	895	200	301	4.6
15	0.028	0.51	NA	0.12	2.2	73.1	326	7.83	623	186	266	6.1
16	0.025	0.01	1.2	0.12	1.2	28.2	281	NA	525	211	265	0.3
17	0.024	0.484	NA	0.09	3	29.9	281	NA	528	195	248	6.1
18	0.023	0.327	NA	0.09	2.7	9.1	251	NA	462	186	231	3.1
19	0.022	0.53	NA	0.11	1.1	6.8	202	8.12	391	188	203	6
3A	0.038	1.33	0.1	0.74	5.4	122	442	NA	808	214	311	8.1
4A	0.022	0.552	NA	0.17	1.1	31.2	243	8.1	467	171	218	5.6
Guideline value	0.02 (AO)	0.3 (AO)	-	-	2 (OG)	250 (AO)	500 (AO)	-	-	30-500 (OG)	80-100 (OG)	1 (OG)

NO₃²⁻ : Nitrate; NH₄⁺ : Ammonia; Cl⁻ : Chloride; TDS: Total dissolved solids

As evident from Table 3.1, the maximum reported Mn concentrations in 13 out of 14 wells exceeded the established AO limit of 20 µg/L set by Health Canada. Notably, among these wells, numbers 14, 11, 13, 5, 12, and 3A exhibited substantially elevated Mn levels, listed in descending order of concentration, which underwent additional scrutiny. Wells 5 and 3A were not considered further as they had very high amounts of Fe, which might have caused hindrance in studying the removal of the contaminant of our interest, Mn. Also, well 12 was excluded due to its high turbidity. Figure 3.1. shows the plots for different water quality parameters of five wells which have reported the highest Mn contamination. Also, for reference, the guideline limits for the parameters are shown on the plots. There was an abundance of calcium, magnesium, sodium, aluminum, and silicon found in the selected five groundwater wells. Also, high fluoride and bicarbonate was reported in the wells. Site visits were conducted for wells 11, 14, and 13 and based on this visit, well 13 was selected for the pilot study. It was only well 13 that had adequate water chemistry including a high enough Mn concentration, and sufficient space to accommodate the pilot plant. The feed water utilized for the experiment was procured before undergoing both chlorination and iron sequestering using sodium silicate processes.

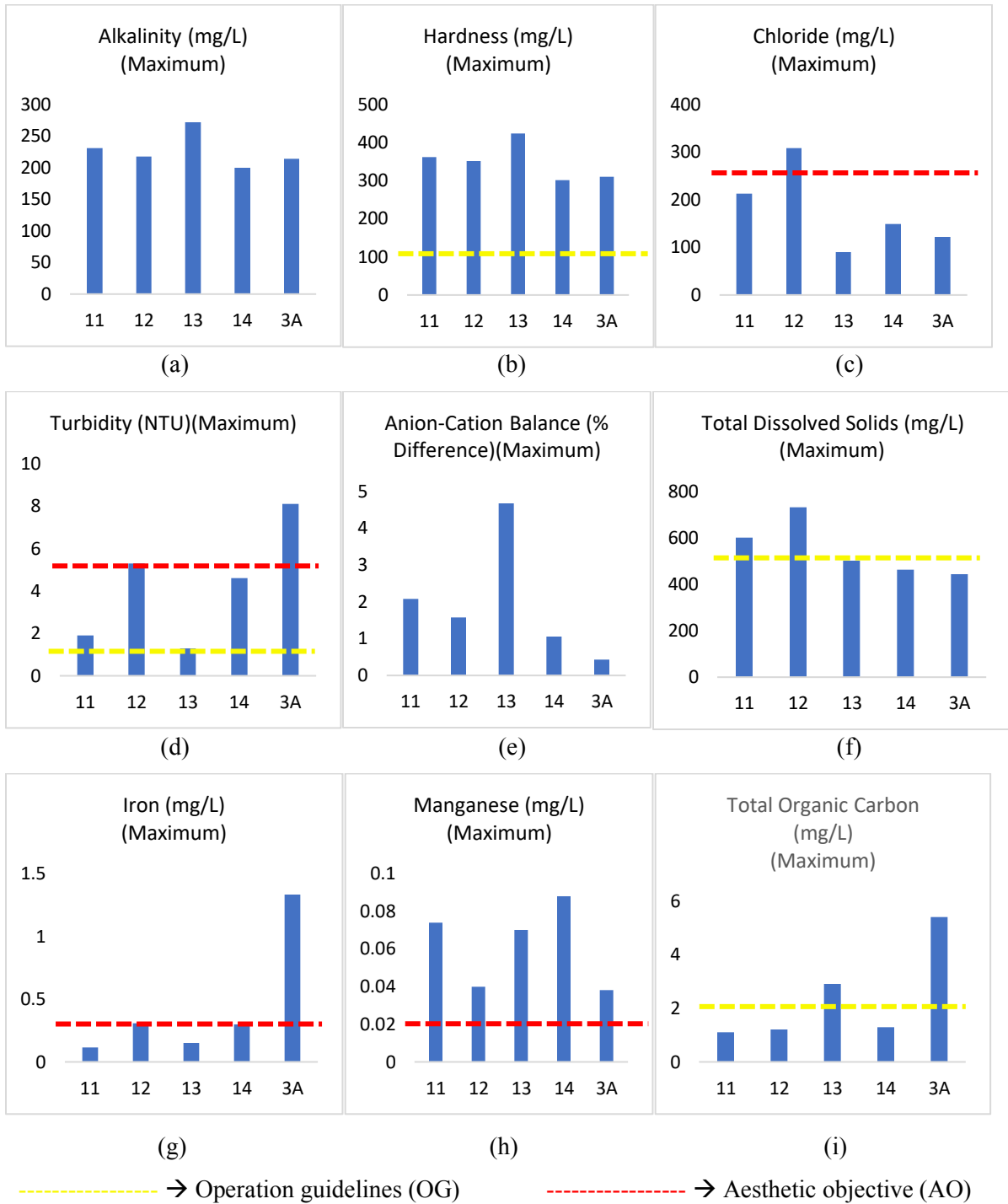


Figure 3.1 Water quality of 5 out of 14 groundwater wells in the city having the highest Mn composition along with the guideline limits. “Maximum” represents the highest recorded value within the dataset spanning 2019-2021 for the respective wells

Groundwater well 13 (chosen for pilot operation), comprises screens, pumps, motors, and casings. For this well, the treatment process begins as groundwater is drawn from depths of 50 to 100 meters below the earth's surface. A protective screen prevents particles from entering and damaging the equipment as the water is pumped to the surface, often using vertical turbine pumps. The raw water first goes through the sand separation step and before distribution, chlorine is added to disinfect the water, ensuring the inactivation of potentially harmful contaminants. The groundwater naturally contains substances like Fe and Mn and to mitigate this, sodium silicate is introduced at well 13. The current groundwater treatment process train followed by the city at well 13 is described in Figure 3.2.

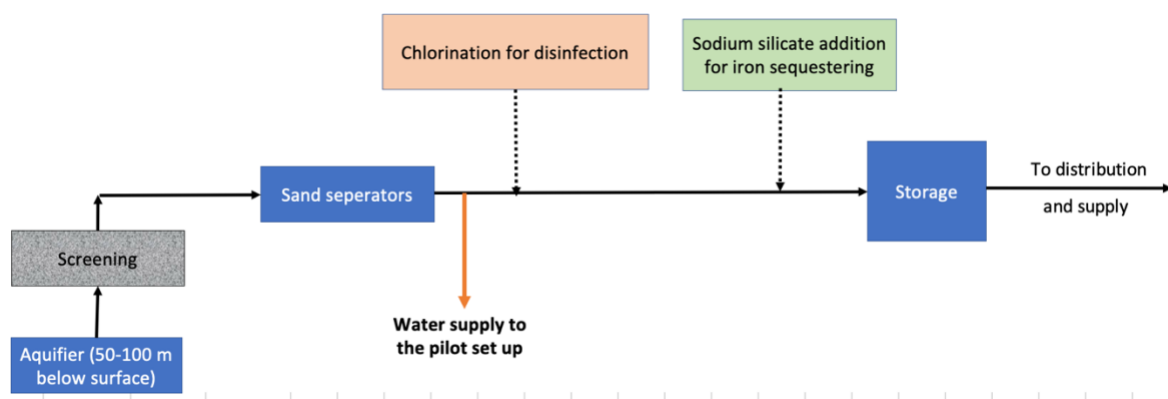


Figure 3.2 Current groundwater treatment process at groundwater well 13 (site chosen for pilot operation)

3.3 Pilot Plant

3.3.1 Pilot Plant Set-Up

The pilot-plant setup was comprised of three identical biofilters as shown in Figure 3.3. The setup of these biofilters was based on an earlier design (Arora, 2021). Appendix A.4 contains the well 13 utility building drawing which illustrates that the utility had just about 3.2 m height clearance, whereas the biofilters designed earlier stood 3.5 m high. The biofilter columns were shortened to ensure that the pilot set-up would fit properly inside the utility building. Following the adjustments, each biofilter exhibited dimensions of 2.8 meters in overall height and a diameter of 12.5 centimetres. Further, to ensure robust structural support for the biofilter columns, sturdy metal beams were employed to connect the biofilter columns to the building wall. Additionally, to exclude light and therefore prevent algae

growth, the biofilter columns were wrapped in silver insulation (cool shield thermal bubble roll) as depicted in Figure 3.4.

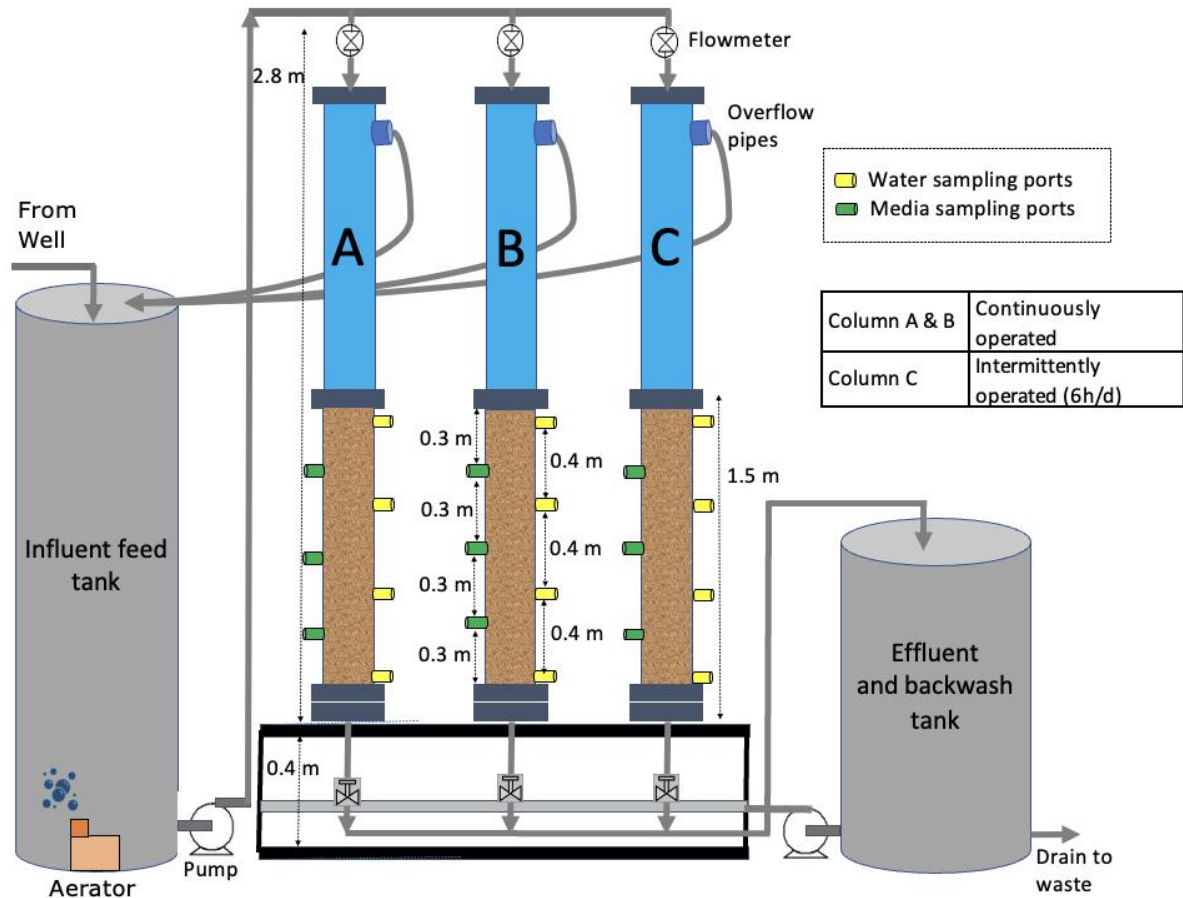


Figure 3.3 Schematic diagram of the pilot set-up assembled at the experimental site

The filter media was introduced to fill the biofilter halfway to its capacity (1.3 m total depth). Sand was used as the filter media in the columns, and it was layered above a bed of gravel (0.2 m total depth). Using gravel as the bed of a filter column ensured support and stability to the sand media above and facilitate water good flow distribution during backwashing. The hydraulic loading was maintained at 5 m/h (~ 40 L/h). For all three biofilters, an overflow port was located 0.9 m from the surface of the media (Refer to Figure 3.3), thereby maintaining a stable water level and uniform hydraulic conditions across the biofilters. The excess water from the overflow was directed back into the influent feed tank.

Two separate tanks (overall capacity 2500 L) were interconnected and used to store and provide feed water to the biofilter columns. Typically, groundwater contains a low level of DO, which might fall short of the conditions necessary for effective Mn removal. To ensure the essential DO levels in the influent, aerators were positioned within the influent storage tank. This measure was taken to guarantee an adequate supply of DO, which might play a crucial role in promoting efficient Mn removal processes. Water was drawn from the tanks and directed into the biofilter columns. To achieve this, a well-calibrated pump (IWAKI (W)MD-30RZ(T) with a low flow rate (maximum: 17 LPM) and high head (maximum: 11 m) was employed, ensuring a suitable and controlled water flow for the intended processes. An additional tank with a capacity of 1000 litres was utilized to collect the treated effluent from all the columns and the overflow from this tank was directed to the drainage system. The effluent tank water was also used for backwashing the biofilters. For backwashing the water was pumped from the effluent tank and IWAKI (W)MD-70RZ(T) pump with a low flow rate (maximum: 43 LPM) and high head (maximum: 20.3 m) capacity was employed. The water generated during the backwashing process was directly channelled into the drainage system.



Figure 3.4 Pilot set up on-site

In terms of operation, two biofilters were run continuously and one biofilter was run intermittently. To enable intermittent operation, solenoid valves were programmed for automatic shutoff/start of the

flow to column C. The precise timing of these switch events was recorded and stored on a PC. LabVIEW software was employed both for creating the user interface on the PC and for data acquisition. The PC utilized a multifunctional input/output device, the PCI-6034E, to facilitate communication with the experimental setup. This device featured six analog inputs for collecting flow readings and two digital outputs for controlling the solenoid valves.

A compact laboratory workspace (Refer to Figure 3.4) was established adjacent to the desktop area. This designated workspace was used for carrying out crucial tasks related to sampling procedures. Specifically, it facilitated activities such as treating water samples with nitric acid for preservation purposes, preparing diluted samples from the collected media samples in preparation for ATP/AxP analysis, and storing the necessary calibration fluids for calibrating the pH and oxidation ORP measurements of the multi-probe meter. This measure was taken to minimize the impact of time delays that arise during the transfer of samples from the field site to the university laboratory for subsequent analysis.

3.3.2 Filter Media Selection and Properties

The possible filter media material options were granular activated carbon (GAC), anthracite or sand. The factors considered in choosing the filter media were industrial suitability, cost-effectiveness, media characteristics, and backwashing feasibility. GAC has been known to be advantageous due to its high surface area property, while sand has been widely used in the water industry as a non-expensive, effective biofilter media material. Sand was reported as the media of choice for Mn biofiltration in Europe. Consequently, considering these factors, suitability for the study's objectives, and to make the experimental conditions comparable to the experimental work of Arora, (2021), sand was chosen as the filter media for this research. The sand media used in the biofilter columns was obtained from the Continental Carbon Group, Canada. The effective size or D_{10} value of the material obtained was between 0.85 to 1.00 mm. This material was sieved further using ASTM E-11 test sieves to obtain sand media in the size range of 0.85 -1.7 mm, in order to be consistent with the media in the pilot setup of (Arora, 2021). The filter media characteristics have been tabulated in Table 3.2. The detailed sieve analysis results can be found in Appendix A.7.

Table 3.2 Sand media characteristics Arora, 2021)

Porosity	0.57	Surface area (BET) (m²/g)	0.19
Media size (mm)	0.85-1.7	Bulk density (g/cm³)	2.16
Grain density (g/cm³)	2.18	Pore volume (mL/g)	0.0025

3.3.3 Pilot Operations

Three rapid conventional filters pilot-scale biofilters were operated side by side, for the comparative investigation between intermittently and continuously operated biofilters. Out of these three biofilters, biofilter A and B were operated continuously for 24 h a day and biofilter C was operated intermittently for 6 h a day and remained offline for 18 h per day and the flow rates in the biofilters were continuously monitored and regulated (on weekly basis). For Phase 1 and Phase 3, the biofilters were operating continuously/intermittently, while during Phase 2, all three biofilters were under shutdown. The detailed standard operating procedures (SOP) for the pilot set-up are in Appendix A.5.

The total media depth (excluding gravel depth) was 1.3 m and with a loading of 5 m/h the corresponding EBCT was 15.6 min. In Table 3.3, the EBCTs for each sampling bed depth are compiled. Figure 3.5 shows the recorded influent and effluent flow rates during a typical week of operation. To confirm the flow meter reading, flow rates were checked manually by collecting effluent in a measuring cylinder and using a stopwatch. As can be observed in Figure 3.5, the effluent flow rate stayed within the 10% margin of the desired flow rate of 0.65 L/min, suggesting that during a week, the increase in head loss was not substantial. Although some fluctuations were observed, the possible reasons behind this can be the change in the influent flow rate or the effect of the head loss over time.

Table 3.3 EBCTs for each sampling port depth

Sampling ports (from top)	Distance from the top of the filter bed (m)	EBCT (min)
1	0.1	1.2
2	0.4	4.8
3	0.5	6.0
4	0.7	8.4
5	0.9	10.8
6	1	12.0
7	1.3	15.6

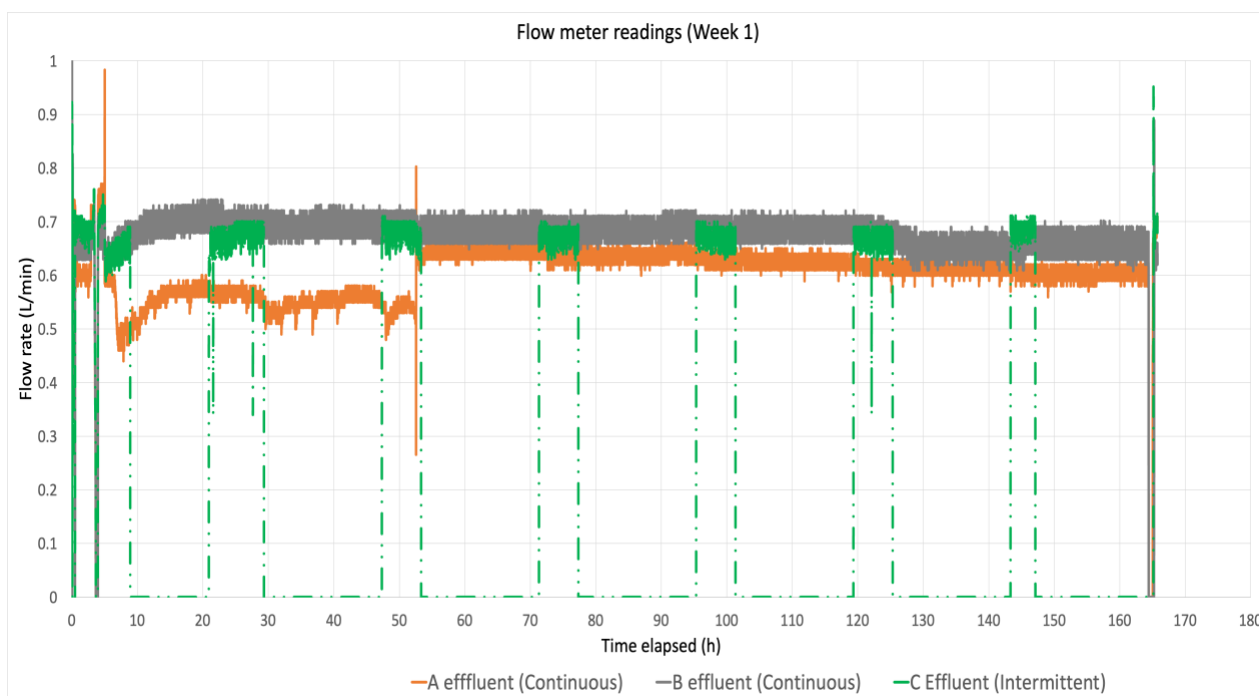


Figure 3.5 Influent and effluent flow rate data for all biofilters over a week

A regular backwashing process was implemented at a rate of 20 to 30 m/h regularly to dislodge accumulated particles and debris and it was performed for each biofilter one at a time. Typically, the backwashing process was triggered either when the biofilters experience a certain level of headloss or when there was a sudden increase in treated water turbidity. However, despite a week of operation, neither of these conditions were observed. The turbidity levels remained consistently below 1 NTU, and the increase in head loss was not substantial. But, for the well-being of the biofilters, the biofilters operated continuously for 24 h a day were backwashed weekly, and for the biofilter operated intermittently, the backwashing was performed monthly. This approach was employed to ensure that an equivalent volume of water was treated by all the biofilters between successive backwash cycles. The backwash cycle started with an initial low water flow rate backwash (1.5-2 L/min) and air scour for 3 minutes and was followed by a high water flow rate backwash (10-12 L/min) for at least 5 minutes or until discharge water clears. For air scour an oil-free diaphragm-based air compressor was used. The reason behind choosing this was to avoid any type of contamination and an oil-free air compressor served the purpose.

The high water flow rate was carefully selected, considering the optimal expansion of the filter bed. A regular backwashing process was implemented at a rate of 20 to 30 m/h. The selected backwash rate resulted in a filter bed expansion of 25 to 30 percent, which aligns with recognized industry standards for biofiltration systems (Laurent et al., 2003). During the operation of the biofilter, several challenges were encountered. These included issues such as improper mixing of sand media during backwashing in columns B and C, system disruptions caused by on-site power switching, software errors in the computer system, well lockout due to elevated turbidity, and inaccuracies in flow meter readings. Further comprehensive insights into these observed anomalies and disruptions throughout the experimental process can be found in Appendix A.6.

Lastly, during an extended shutdown, it is crucial to properly store the biofilter to preserve its integrity, prevent excessive loss of beneficial microbial populations, degradation of biofilm structure and ensure its continued effectiveness upon resumption of operations. Thus, four different storage strategies were investigated, and their details can be found in Table 3.4.

Table 3.4 Different storage strategies (SS) studied during extended shutdown

SS 1	Fresh water (collected and stored in influent tank on the last day of phase 1) circulated between column heads and influent tank continuously
SS 2	The water stored in the biofilters was drained and fresh water was added into the biofilters every week
SS 3	Biofilters were kept submerged in the same water, but, on a weekly basis, this water was drained from the columns and pumped back into the columns
SS 4	Biofilters were left submerged in the same water without recirculation (no draining water and pumping it back into columns) for four weeks

3.4 Sample Collection and Handling

The sampling ports featuring a 125 μm mesh at one end to restrict the passage of sand media were designed to facilitate the collection of water and media samples (Refer to Figure 3.3).

3.4.1 Water Samples

When collecting samples from the sampling ports, water was allowed to run from the port for approximately 1-2 minutes before the actual sample collection. Additionally, when collecting water samples from various depths within the biofilter, sampling followed a bottom-to-top approach, i.e. samples were initially drawn from the lowest port, progressing upward to the top. Depending on the specific analysis requirements, samples were collected into designated containers, and duplicates were taken for influents and effluents as needed.

Effluent samples, totalling 50 mL each, were collected from all three biofilters on a weekly basis to assess the biofilter's performance in terms of Mn and Fe removal. Additionally, on a monthly schedule, samples were gathered from sampling ports at different biofilter bed depths to investigate the effectiveness of Mn and Fe removal within the filter beds. Furthermore, a monthly sampling routine included the collection of samples at one-hour intervals immediately after intermittently operated biofilters resumed operation following a brief shutdown. This investigative measure aimed to determine the duration required for the intermittently operated biofilters to attain a steady state performance.

For samples related to TOC, clear glass vials capped with septa were employed for collection, and they were stored in a cooler with ice packs during transit from the experimental site to the laboratory at the University of Waterloo. On the other hand, samples for ammonia, nitrate, nitrite, and phosphate were collected on-site using 50 mL sterilized polystyrene centrifuge tubes. These samples were also stored in a cooler with ice packs during transportation to the laboratory for subsequent analysis. TOC, nitrate, nitrite, ammonia, and phosphorus were assessed at the outset, midpoint, and conclusion of each experimental phase. Further water quality parameters including DO levels, pH, temperature, conductivity and ORP were measured on-site. The frequency of these measurements varied from once to three times per week depending on the operating conditions and study requirements. Table 3.5 summarizes the various water parameters studied during different research phases and their respective sampling frequencies.

Table 3.5 Water parameters studied and their respective sampling frequencies

Water parameters	Experimental phase	Frequency
DO, ORP, temperature, conductivity, pH	1, 3	1-2x per week
DO, ORP, temperature, conductivity, pH	2	2-3x per week
Turbidity	1, 3	1-2x per week
Mn, Fe (over time)	1, 3	1x per week
Mn, Fe (post 18-h intermittent shutdown)	1, 3	1x per month
Mn, Fe (depth-wise profile)	1, 3	1x per month
TOC, nitrate, nitrite, ammonia, phosphate	1, 3	1x per month

3.4.2 Media Samples

Media samples were collected periodically. To understand the effect of the shutdown on the microbial activity media samples were collected before and after shutdown and to understand the effect of backwashing on the microbial activity media samples were also collected before and after backwashing. For ATP, AxP and other microbiological analyses, biofilter columns were drained entirely and sampling ports were removed before collecting media samples. In terms of media sampling for ATP analysis, it was strategically scheduled just before filter backwash. Sterile spatulas were used for media retrieval. For MnOB, CLPP, and LBB analysis, filter media for each of the samples was collected from the biofilters in sterile centrifuge tubes, transported to Wilfrid Laurier University and stored in a storage cooler before analysis. Note that, during Phase 2 of the study, a change in the sampling depth for the bottom layer became necessary due to a threading issue in the sampling port at 120 cm. This issue prevented the repetitive opening of the port at 120 cm, prompting the adjustment in the sampling depth from 120 cm to 110 cm to ensure smooth and consistent data collection. Table 3.6 summarizes the various microbiological parameters studied during various experimental phases and their respective frequencies.

Table 3.6 Microbiological parameters studied and their respective frequencies

Microbiological parameters	Experimental phase	Frequency
ATP	1	1x in two weeks
AxP	2	1x per week
AxP	3	1x in two weeks
MnOB, CLPP, LBB	2	End of phase
MnOB, CLPP, LBB	3	Start and end of phase

3.5 Analysis Methods

3.5.1 Water Quality Parameters

3.5.1.1 Metal Analysis

For metal analysis, effluent water samples were collected in 50 mL acid-rinsed centrifuge tubes at the site, acidified with HNO₃ (35%) onsite to a pH below 2 and transferred to UW in a cooler. 10 mL of the acidified sample was transferred to a 15 mL centrifuge tube and stored at 4°C before analysis. The analysis was done in the Metal Isotope Geochemistry laboratory, University of Waterloo using Agilent 8800 triple quadrupole inductively coupled plasma mass spectrometer (ICP-MS) following Method 6020B which had a detection limit of 0.012 µg/mL for Mn and 0.231 µg/mL for Fe (US Environmental Protection Agency, 2014). To validate the results, influent samples were analyzed in duplicate. It is important to note that the total Mn and Fe concentration encompasses the particulate form, dissolved form, and colloidal form.

3.5.1.2 TOC

To measure the TOC, effluent samples were collected in glass vials covered with Teflon septa and were acidified using HCl (pH lowered to 2) after bringing them to the University of Waterloo lab from the pilot plant. Between collection and analysis, the samples were stored at 4 °C and analyzed by the Water STP group at the University of Waterloo. The analysis of this parameter was conducted using the Standard Method 5310B, which specifically utilizes the high-temperature combustion method (American Public Health Association, 2017). The analysis process was facilitated using a Shimadzu

TOC-L CSH total organic carbon analyzer and the limit of quantification for this method was 0.100 mg C/L (or 100 µg C/L).

3.5.1.3 DO, Temperature, Conductivity, ORP, pH

The professional plus YSI multiprobe meter was used to measure DO, temperature, conductivity, oxidation-reduction potential (ORP), and pH on-site. To ensure data accuracy and consistency, all samples underwent duplicate analyses. The multiprobe meter was calibrated for pH, ORP and DO weekly or when deemed necessary.

3.5.1.4 Turbidity

A Hach 2100Q portable turbidity meter was utilized for turbidity analysis. This analysis was conducted on-site, with water samples collected into centrifuge tubes for assessment. It was measured weekly, at regular intervals after biofilter restart and pre/post-backwash to understand the efficiency of biofilters in mitigating turbidity. To ensure data reliability, the samples were analyzed in triplicate.

3.5.1.5 Nitrate, Nitrite, Ammonia and Phosphate

Effluent and influent water samples were collected into centrifuge tubes and subsequently transported to the laboratory at the University of Waterloo for analysis. The analysis was conducted using a Hach DR 1900 Spectrophotometer. For nitrate analysis, the cadmium reduction method (HACH method number 8171) was followed, with a detection limit of 0.1 mg/L NO_3^- -N. Nitrite analysis followed the USEPA diazotization method (HACH method number 8507), which had a detection limit of 0.002 mg/L NO_2^- -N, ammonia levels were determined using the salicylate Method (HACH method number 8155) with detection limit of 0.01 mg/L NH_3 -N and phosphate levels were determined using the USEPA Ascorbic acid method (HACH method number 8048) with detection limit of 0.02 mg/L PO_4^{3-} .

3.5.2 Microbial Parameters

3.5.2.1 ATP and AxP

The MnOB analysis was performed at Wilfrid Laurier University by Nicholas Lamba-Rautapuro, an MSc student. This procedure is following Burger et al., (2008). 1.5 g of filter media was placed inside a stomaching sample bag and suspended in 10 mL of 0.9% NaCl solution. The sample was stomached in a Seward Circulator 400 Stomacher for 5 minutes for biofilm detachment. 1 mL of the detached biofilm supernatant was inoculated into 9 mL of a 0.9% NaCl solution, creating a dilution of 10^{-1} .

Dilutions were prepared for ranges of 10^{-1} to 10^{-4} and 100 μ L (0.1 mL) from each dilution level was spread plated onto culture media. Three different culture media's were used:

- Minimal media: 17 mg/L MnSO_4 , 15g/L agar.
- Supplemented media: 17 mg/L MnSO_4 , 1g/L Beef extract, 75 mg/L yeast extract, 150 mg/L Ferrous ammonium sulphate, 150 mg/L sodium citrate, 12g/L agar.
- R2A+Mn media: 0.5 g/L Yeast Extract, 0.5 g/L Protease Peptone No. 3, 0.5 g/L Casamino Acids, 0.5 g/L Dextrose 0.5 g/L Soluble Starch, 0.3 g/L, Sodium Pyruvate, 0.3 g/L Dipotassium Phosphate, 0.05 g/L, Magnesium Sulfate, 5g/L Agar, 17 mg/L MnSO_4 .

Plates were left in the dark at room temperature for approximately 4 weeks and plate counts were performed to determine CFU/mL.

3.5.2.2 Manganese Oxidizing Bacteria (MnOB)

3.5.2.3 Leucoberberlin Blue (LBB) Analysis

The LBB analysis was performed at Wilfrid Laurier University by Nicholas Lamba-Rautapuro, an MSc student. This procedure is following Breda et al., (2017). LBB reacts with oxidized Mn to form a blue colour on the surface of bacterial colonies. LBB reagent (0.04% LBB in 45 mM acetic acid) was created by adding 0.04 g Leucoberberlin blue in 100 mL 45 mM acetic acid. 45 mM acetic acid was created from 17.4 M glacial acetic acid and sterile Milli-Q water. Starting at 10^{-4} , 1-2 drops of LBB reagent were applied to 5-10 colonies on each plate. If any colonies turned blue, the dilution factor was recorded, a positive reaction confirming the presence of MnOB. This procedure was repeated for subsequent lower dilution levels until a positive reaction was observed, or no reaction was observed at all. The dilution factor provides an estimation of the degree or magnitude of MnOB found on each respective plate. For example, a plate with MnOB only on 10^{-2} plates may have 10x less MnOB than a plate with MnOB on 10^{-3} plates. Degree of Mn oxidation was also documented, with “+++” being strong, recorded if colonies immediately changed to a strong blue colour, or an existing brown or black colouration was present before LBB was added; “++” being medium, recorded if colonies took a bit longer to change to an average blue colour; “+” being weak, recorded if colonies barely or weakly changed to a blue colour.

3.5.2.4 Community Level Physiological Profile (CLPP) Analysis

The CLPP analysis was performed at Wilfrid Laurier University by Nicholas Lamba-Rautapuro, an MSc student. This procedure is following Németh et al., (2021). The CLPP procedure is as follows: 0.5 g of filter media was placed inside a stomaching sample bag and suspended in 10 mL of 0.9% NaCl solution. The sample was stomached in a Seward Circulator 400 Stomacher for 5 minutes for biofilm detachment. 150 μ L of the sample was inoculated into each of the wells of a Biolog Ecoplate. Biolog Ecoplates contained 31 unique carbon sources in triplicate, allowing for the analysis of changes in microbial communities based on the metabolic profile of the community. Within each well, a tetrazolium dye is found, that when reduced via cellular respiration, yields a purple colour that can be measured using a spectrophotometer. Biolog Ecoplates were measured at 590 nm using an xMark spectrophotometer every 24 h, with 96 h being the optimal time point for further analysis. Average well colour development (AWCD) was used to estimate the average metabolic rate of the microbial community, by calculating the sum of absorbance readings and dividing that value by the total number of carbon sources available, i.e., 31. Substrate AWCD was calculated using the sum of absorbance readings for a specific group of substrates and dividing it by the number of substrates found in that group. Six categories were used: polymers, carbohydrates, carboxylic acids, amino acids, amines, and phenolic compounds. Shannon Diversity Index (H') was calculated to determine substrate utilization diversity, where the maximum value possible is 3.434, the natural logarithm for the total number of substrates available, which is 31, occurring when all substrates are metabolized on the plate. Values greater than or equal to 3 are considered high diversity, while lower values such as 1 and 2 are considered low diversity.

3.5.3 Statistical Analysis

Statistical significance was assessed at the 5% significance level across the various response variables studied. To minimize potential biases stemming from fluctuations in influent water quality, paired student t-tests were used to determine the significance of the difference in performance between the continuously and intermittently operated biofilters regarding the effluent concentrations of Mn and Fe. Calculations of t and P values were employed within a 95% confidence interval to assess the statistical significance of these differences. The resulting p-values indicated the degree to which a relationship exhibited a significant difference, with higher p-values suggesting a greater likelihood of statistical

similarity. Further, an F-test was performed to confirm whether the assumption of equal variance is valid while performing the t-test.

Chapter 4

Effect of Intermittent Operation on Manganese Removal During Biofilter Start-Up

4.1 Overview

This chapter includes the results and discussion of the research phase wherein the performance of continuously and intermittently operated biofilters during the start-up phase was investigated. This phase commenced in January 2023 and extended over a duration of two months, for the reason explained below. The sub-objectives of this phase were:

- ✓ Assess the time required for intermittently and continuously operated biofilters to reach a pseudo-steady state during the start-up period
- ✓ Evaluate biofilter performance at different EBCTs by monitoring Mn and Fe removal in depth-wise zones of the continuously and intermittently operated biofilters
- ✓ Investigate the impact of 18-h intermittent shutdown on biofilter performance and microbial activity
- ✓ Study the effect of backwashing
- ✓ Ascertain the ability of biofilters to mitigate turbidity in response to turbidity spikes

These evaluations were conducted for two continuously operated biofilters and one intermittently operated biofilter (6h/d on; 18h/d shut down) which commenced their operation at the same time. It is important to note that, based on previous literature, a specific SS was applied to the intermittently operated biofilter. To ensure their integrity, these biofilters were deliberately kept submerged during the 18-hour shutdown periods (Evans et al., 2021).

Regrettably, after a continuous operational period of 56 days, the well, which fed the pilot biofilters, was shut down unexpectedly by the utility due to water quality issues. This meant that pilot biofilter operations had to be shut down as well. Hence, March 8th, the shutdown date, marked the conclusion of this research phase. Therefore, the dataset for this phase includes observations and data collected from January 11th to March 8th, 2023. This unexpected interruption posed challenges, but the available data within this timeframe still provides valuable insights into pilot biofilter performance and the impacts of operational factors assessed during this start-up phase.

4.2 Results and Discussion

4.2.1 Feed Water Quality

Feed water quality parameters are presented in Tables 4.1 and 4.2. The temperature of the raw water ranged from 10-12 °C, suggesting that the operation was happening in the low-temperature range, which is typical for groundwater in cold regions. The average DO in the raw groundwater was 3.9 mg/L, which was considerably low, and thus, aerators were submerged within the influent feed tank to enhance the DO levels. Note that this practice was not followed at the treatment utility as they utilized chemical treatment. Aeration increased the DO levels in the feed water to 9-10 mg/L, which was more than adequate for biofiltration operation and efficient functioning. Further, the ORP was found to be in the range of 175-210 mV, which was close to the lower range of the field of activity for Mn- and Fe-oxidising bacteria (Mouchet, 1992). The nitrate concentration suggested that there was a good amount of nutrients present in the feed water for biofilm formation and the low amount of ammonia should not be harmful to biofilms. The alkalinity level (272 mg CaCO₃/L) demonstrates the water's good buffering capacity against changes in pH. The pH was found to be in the range of 7.5-7.9 which was appropriate within the field of activity for Mn and Fe oxidizing bacteria (Mouchet, 1992). Turbidity exhibited a notable range, essentially from a low of 0.72 NTU to a higher value of 2.89 NTU. Similarly, the Mn and Fe concentrations were variable and had a relatively high standard deviation. The recorded minimum concentrations for Mn and Fe were 21.8 µg/L and 57.0µg/L, respectively, while the maximum concentrations reached 55.8 µg/L for Mn and 235.4 µg/L for Fe. Although Mn concentrations were relatively low, they still exceeded the aesthetic value (20 µg/L). Lastly, the TOC was found to be in the range of 0.8 mg/L, which was lower than that reported historically for well 13 by the utility.

During the experimental duration, noteworthy fluctuations were noticed in the concentrations of Mn, Fe, and turbidity levels. These fluctuations were particularly pronounced during the latter weeks of the experiment, which coincided with the repeated short-term well intermittent shutdowns due to turbidity spikes experienced in the treated effluent stream. During this time the operational status of the well was at risk and was therefore suspended on March 8, 2023, which marked the end of this experimental phase.

Table 4.1 Feedwater* quality data monitored at well 13 during the start-up phase (parameter set 1)

Parameter	DO (mg/L)	Nitrate (mg -N/L)	Ammonia (mg-N/L)	Alkalinity (mg/L CaCO₃)	Hardness (mg/L CaCO₃)	ORP (mV)	Temperature (°C)
Average value	3.9	1.25	0.06	272	424	181	11.1
S.D	0.3	0.09	0.01	-	-	12	0.4
No. of observations	23	3	3	1	1	23	23

Table 4.2 Feedwater* quality data monitored at well 13 during the start-up phase (parameter set 2)

Parameter	Mn (µg/L)	Fe (µg/L)	TOC (mg/L)	Turbidity (NTU)	pH	Conductivity (µS/cm)
Minimum value	21.8	57.0	0.75	0.7	7.5	676
Maximum value	55.8	235.4	0.83	2.9	7.9	741
Average value	30.5	96.7	0.80	1.6	7.7	703
S.D	10.0	44.5	0.05	1.0	0.1	16
No. of observations	37	37	3	15	23	23

*Feedwater was sourced directly from the groundwater well

4.2.2 Biofilter Performance During the Start-Up Period

The aim was to achieve effective Mn removal and meet both aesthetic and treatment objectives set by Health Canada for Mn. The AO for Mn removal was set at 20 µg/L, while the treatment objective is 15 µg/L (Health Canada, 2022). Figure 4.1 visually depicts the progress of Mn removal over time. During the initial 28 days, limited Mn removal was observed in all three biofilters. However, after this period, a distinct trend emerged. Biofilters A and B, which were operated continuously, showed an increase in Mn removal, although it appeared that column B was performing slightly slower than column A until day 42. On the other hand, biofilter C operated intermittently, did not show substantial improvement in Mn removal, although it was able to meet the treatment objective. The reason behind the unidentical behaviour of the intermittently operated biofilter in comparison to the continuously operated biofilters was understood but the variation in the performance of biofilters A and B, which were operated identically, is still unclear, although the microbial activity results (discussed later in the chapter) are in line with these findings. It can be hypothesized that even if the operating conditions are the same, it is still possible that the biofilters can behave differently during the start-up phase. Detailed microbiological analysis and understanding of the removal mechanism might help understand this better. Overall, all three biofilters were able to remove Mn to below the treatment objective limit of 15 µg/L. It can be seen in Figure 4.1 that continuously operated biofilter A was able to remove Mn consistently, with the effluent concentrations below 1 µg/L for two consecutive weeks, indicating biofilters reached a pseudo-steady state. (Pseudo-steady state refers to the phase where the system exhibits stable conditions, resembling a steady state, but with certain transient effects. In this thesis, we will refer to this state simply as 'steady state' for clarity). However, that was not the case for continuously operated biofilter B, which although able to remove Mn consistently, did not report similar Mn concentrations in the effluent for at least two consecutive weeks. The effluent Mn concentrations in biofilters A and B were almost identical towards the end and thus it can be hypothesized that column B was also close to a steady state. This could potentially have been confirmed if the biofilters had been able to run for a couple more weeks, which unfortunately was not possible due to the unprecedented well shutdown. Furthermore, intermittently operated biofilter C clearly did not reach a steady state. Overall, after 49-56 days of operation biofilters A and B recorded >90% Mn removal rates (max. 98% reached by column A on day 56), while C just showed around 64% Mn removal. This variation in the performance could potentially be attributed to a reduction in DO levels in the biofilter column during shutdown (Refer to Figure 4.9), which might have subsequently constrained the growth and maturation of Mn-oxidizing

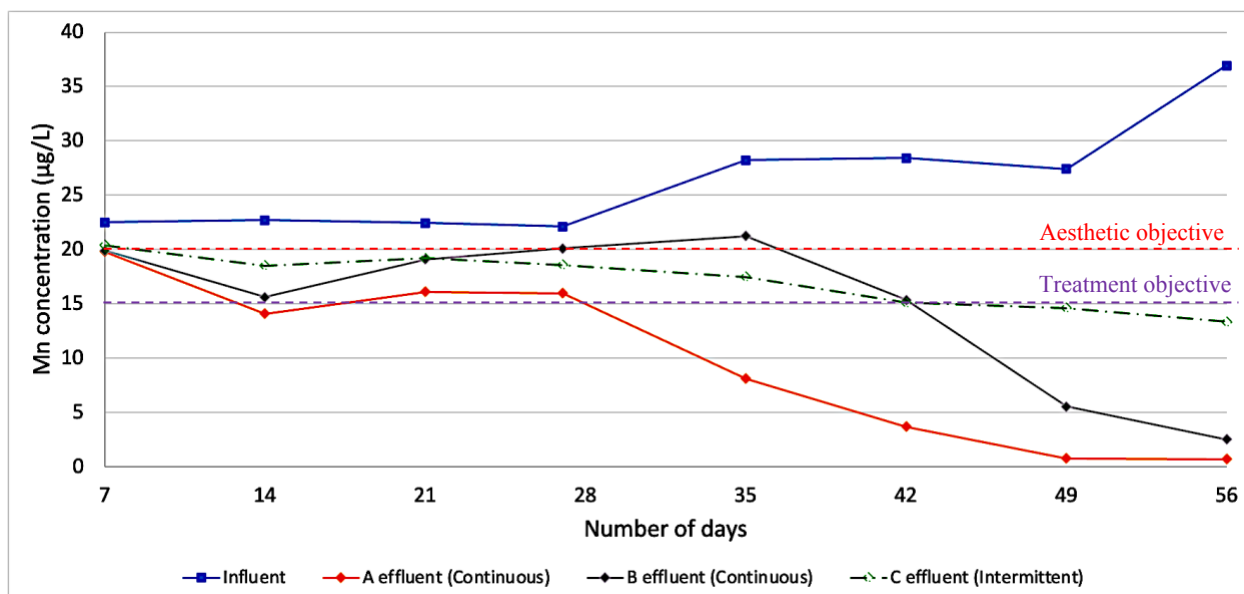


Figure 4.1 Mn concentrations in pilot biofilter influent and effluents over time. Data from Jan. 18, 2023 = Day 7 to Mar. 8, 2023 = Day 56.

bacteria on the filter media, which, in turn, could have contributed to a deceleration in the filtration improvement process. These findings align with the outcomes reported in a previous research study. Notably, Zeng et al., (2019), in their research work, emphasized a key expectation, that intermittently operated filters might initially require more time to reach their optimal performance condition compared to the continuously operated biofilters, but once the filter matures, the biofilter under intermittent mode would likely perform like the one operating in continuous mode. Therefore, although it can not be concluded that the intermittently operated biofilter reached a steady state in 56 days of operation, had it been the case that the biofilter could have been operated longer, it could have been postulated based on the trends observed that the intermittently operated biofilter could have performed similarly to the continuously operated biofilters.

Apart from Mn, the Fe removal performance of the biofilters was also investigated. Figure 4.2 visually depicts the progress of Fe removal over time. The results revealed that the continuously operated biofilters demonstrated excellent Fe removal capabilities, achieving a removal rate of over 90%. On the other hand, the intermittently operated biofilter exhibited a Fe removal rate of 84%.

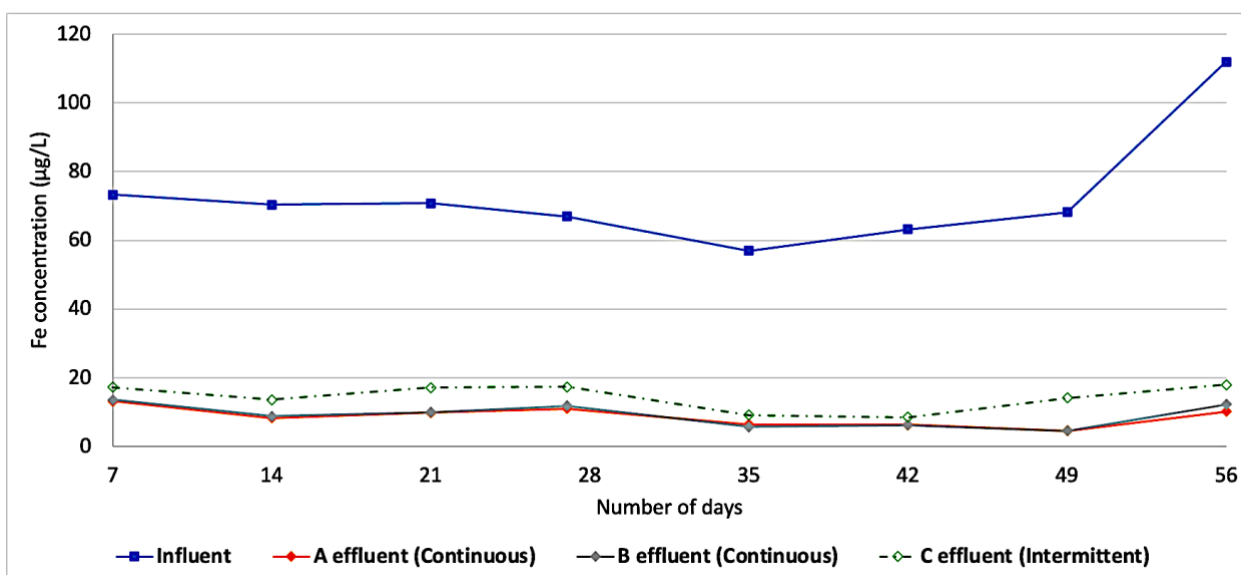


Figure 4.2 Fe concentrations in pilot biofilter influent and effluents over time. Data from Jan. 18, 2023 = Day 7 to Mar. 8, 2023 = Day 56.

Interestingly, all biofilters reached a steady state within a week of operation, which indicates that the biofilters adapted to the treatment conditions and established consistent Fe removal, regardless of the operation mode. The possible reason behind this performance can be attributed to the predominant removal mechanism for Fe. Based on how quickly the biofilters showed substantial removal, the combination of chemical oxidation and filtration processes might be considered as the dominant mechanism. Aeration, along with conditions in the influent tank, may have triggered the chemical precipitation of Fe, contributing to its removal in the biofilters.

A notable observation from Figure 4.1 and Figure 4.2 is the significant increase in Mn (~68% increase) and Fe (~53% increase) concentration in the influent on Day 56 (compared to Day 49) when all three biofilters demonstrated remarkable resilience. All the biofilters were able to maintain effluent Mn concentrations below the treatment objective of 15 µg/L and also maintain excellent Fe removal rates. It should be noted that the operations were halted on Day 56 due to a well shutdown. Had the biofilters been able to continue operating, the observed trends suggest that intermittently operated biofilter, under the conditions tested, might have performed comparably to continuously operated biofilters.

A t-test was conducted to determine the significance of the difference in performance between the continuously and intermittently operated biofilters regarding the effluent concentrations of Mn and Fe. F-test was performed to confirm whether the assumption of equal variance is valid while performing the t-test. The statistical analysis results can be found in Appendix B.2. For Mn, as seen in Table 4.3, columns A and B, which were operated continuously, did not exhibit a statistically significant difference in their

removal rates, however, column C which was operated intermittently showed significant difference in comparison with column A. Further, column C did not show a significant variation in removal rates when compared to column B, which was also operated continuously. The statistical results support the difference in the performance of column B as compared to identically operated column A. For about 42 days of operation, column B displayed removal rates similar to those of column C. However, it transitioned to exhibit removal rates similar to those of column A by day 56. Further for Fe, as seen in Table 4.4, columns A and B which were operated continuously did not exhibit a statistically significant difference in their removal rates, however, column C which was operated intermittently showed significant variation in comparison with both biofilters A and B.

Table 4.3 t-test to compare the effluent Mn concentration of continuously and intermittently operated biofilters

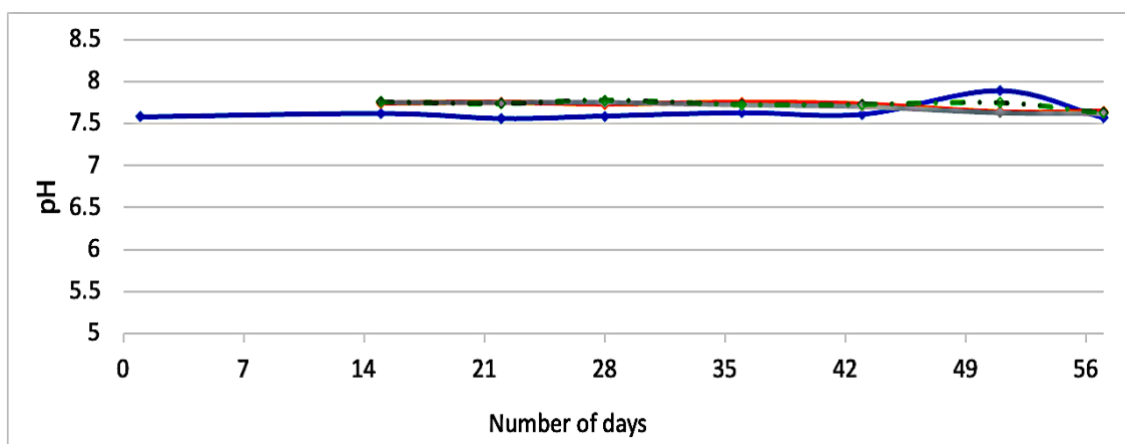
Effluent concentration compared	F value	t value	P value	Result
A (continuous) and B (continuous)	1.187	1.114	0.142	Not significant
A (continuous) and C (intermittent)	3.081	1.814	0.046	Significant
B (continuous) and C (intermittent)	2.596	0.532	0.302	Not significant

Table 4.4 t-test to compare the effluent Fe concentration of continuously and intermittently operated biofilters

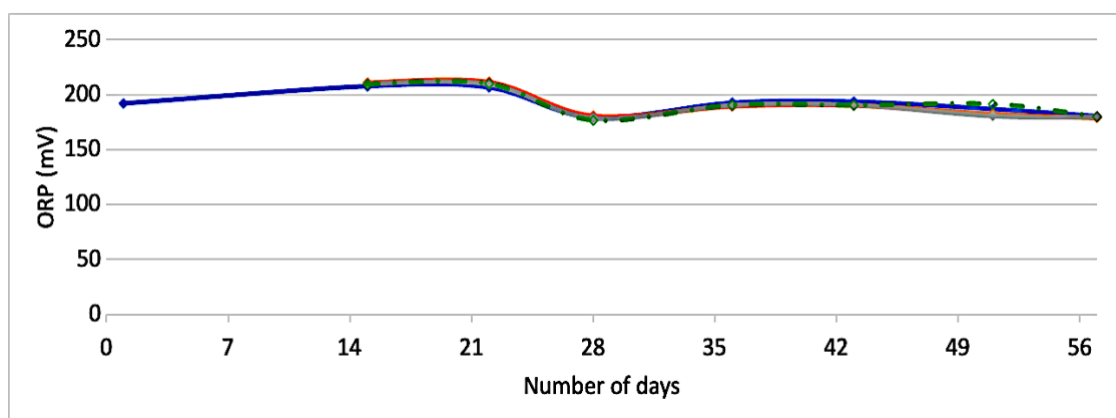
Effluent concentration compared	F value	t value	P value	Result
A (continuous) and B (continuous)	1.042	0.202	0.421	Not significant
A (continuous) and C (intermittent)	1.171	3.753	0.001	Significant
B (continuous) and C (intermittent)	1.124	3.437	0.002	Significant

In addition to monitoring Mn and Fe removal, the removal of TOC was also tracked. On day 49 of operation, it was observed that column A achieved a noteworthy TOC removal rate, effectively reducing 10.4% of the influent TOC concentration (0.83 mg/L). In comparison, column B exhibited an 8.3% TOC removal rate, while column C, which operated intermittently, had a 6.6% reduction in TOC levels. It was assumed that the remaining organic matter could have been non-biodegradable organic matter which could not be degraded due to the nature of the biofiltration process, which primarily relies on biological degradation mechanisms.

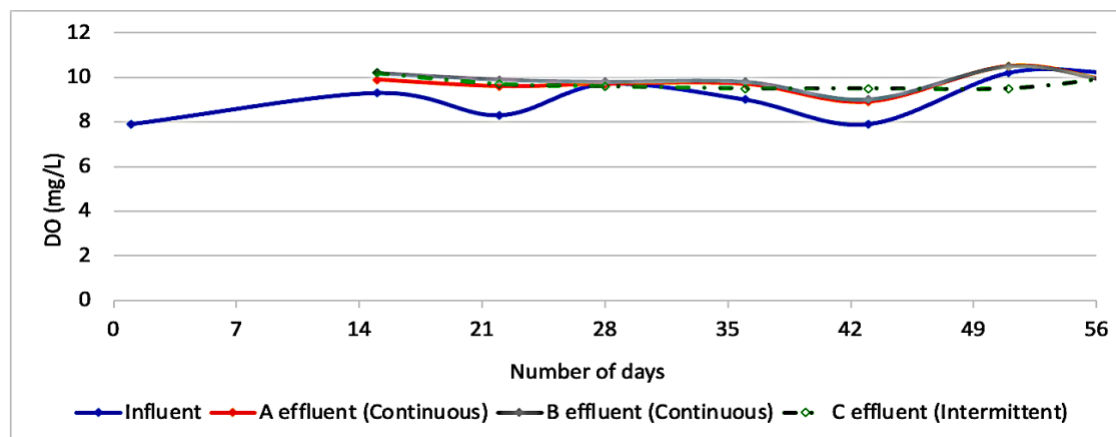
Apart from Mn, Fe and TOC a few other pertinent water quality parameters were analysed regularly. As is evident in Figure 4.3 (a), throughout the entire duration of this experimental phase, the pH levels in both influent and effluent water samples from all biofilters consistently fell within the range of 7.5 to 8.



(a)



(b)



(c)

Figure 4.3 Variations in key water quality parameters over time: (a) pH, (b) ORP, and (c) DO

These pH values were within the documented range for the activity of Mn-oxidizing bacteria, but they were on the outer edge of the documented range for Fe-oxidizing bacteria (Mouchet, 1992) (Refer to Figure 4.4). The oxidation-reduction potential (ORP) values displayed minimal variations across differently

operated biofilters as is evident in Figure 4.3 (b). The values were close to the lower end of the documented range for Mn-oxidizing bacteria activity, and they were within the range for the activity of Fe-oxidising bacteria (Mouchet, 1992) (Refer to Figure 4.4). Furthermore, DO levels exhibited remarkable stability throughout the 56-day operation period, consistently ranging between 9.5 and 10.5 mg/L as shown in Figure 4.3 (c). In some instances, it can be seen in Figure 4.3 (c) that the DO values in the influent were lower than those in the effluents of the biofilter columns. Although the reason behind this is not completely clear, the possible reasons include the inadequacy in sample collection and DO measurement from the biofilter effluents. The water samples were collected by opening the valves, which might have resulted in diffusion during transfer, while collecting the samples in the sample collector, thereby potentially increasing the DO levels in the effluents. In addition, instrument error could be another potential factor, despite conducting calibration immediately before utilizing the DO probe meter.

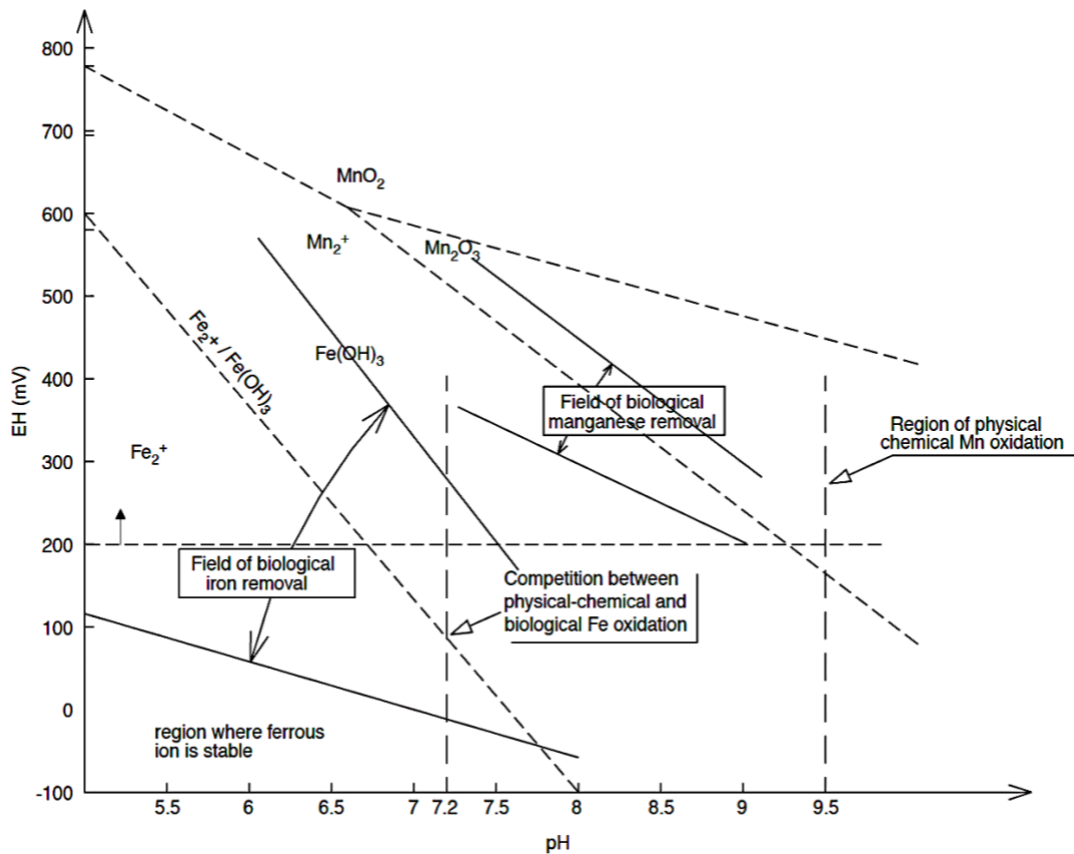


Figure 4.4 Field of activity of Mn and Fe oxidizing bacteria: pH- Eh diagram (Mouchet, 1992)

These water quality parameter trends underline the consistent conditions within the biofilters and their consistent operation throughout the study duration, providing valuable context for interpreting the observed Mn and Fe removal trends. In addition, the nitrite and ammonia concentrations in influent and effluent

water samples were found to approach the detection limits (0.002 mg/L NO_2^- -N for nitrite and 0.01 mg/L NH_3 -N for ammonia) of the analytical instrument employed for analysis. Furthermore, nitrate concentrations showed notable similarity between influent and effluent samples, suggesting that nitrate removal did not happen in any of the biofilters during the start-up phase. Additional supporting plots for the water quality parameters can be found in Appendix B.1, providing a comprehensive visual representation of the data.

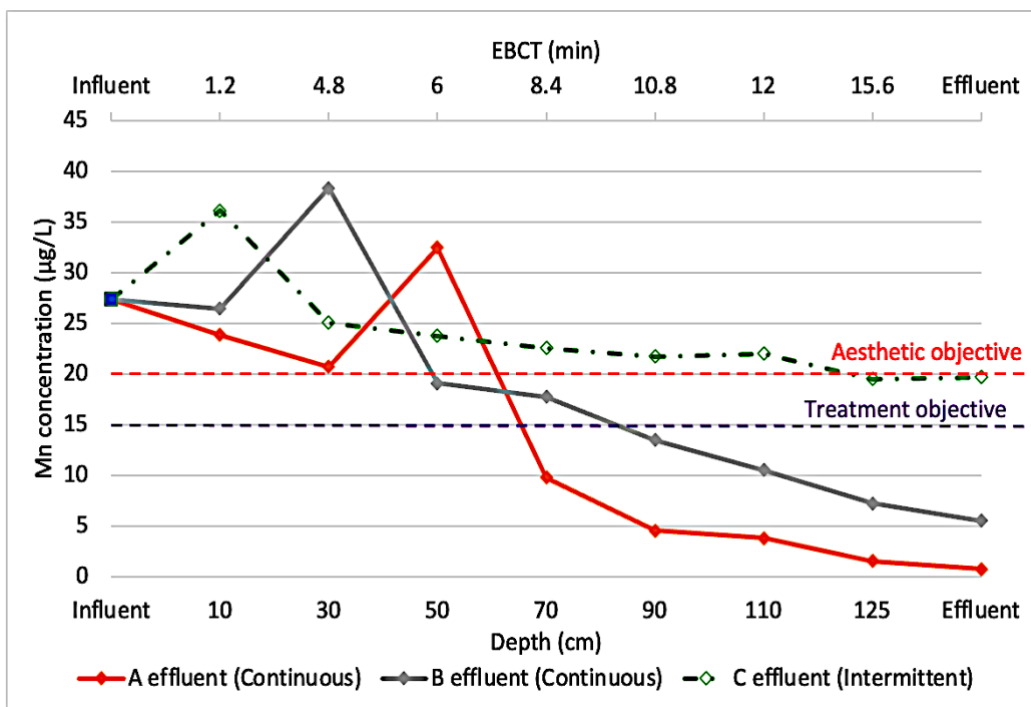
4.2.3 Mn and Fe Removal at Different Biofilter Depths

Literature has emphasized the importance of having sufficient EBCT to achieve effective TOC removal. However, when it comes to Mn removal, it was found in the literature that as long as EBCT falls within an acceptable range, ideal Mn removal can be achieved under pseudo-steady state conditions. Specifically, research indicated that an EBCT exceeding 11.5 minutes was conducive to favourable Mn removal from surface water sources (Basu et al., 2016; Evans et al., 2020; Evans et al., 2021). However, precise figures for groundwater sources were not identified in the literature. This study considered the impact of EBCT on both Mn and Fe removal, aiming to discern the regions where Mn and Fe removal predominated during the initial start-up phase. This approach allowed for a more comprehensive understanding of the factors influencing Mn removal within the biofilters. Figure 4.5 (a) illustrates the Mn concentrations at different depths of the biofilter bed recorded on the 49th day of operation. Samples from different biofilter depths were also collected on the 21st day of operation when none of the biofilters showed substantial Mn removal and the corresponding plots can be found in Appendix B.3. On the 49th day the biofilters A, B and C were performing at 97%, 80% and 47% Mn removal rates respectively, with biofilter A seemingly have reached the steady state, biofilter B seemingly approaching the steady state and lastly column C showing improved performance and moving toward the steady state. It is important to note that the total Mn concentration encompasses the particulate form, dissolved form, and colloidal form and the concentrations depicted here are the total Mn concentrations.

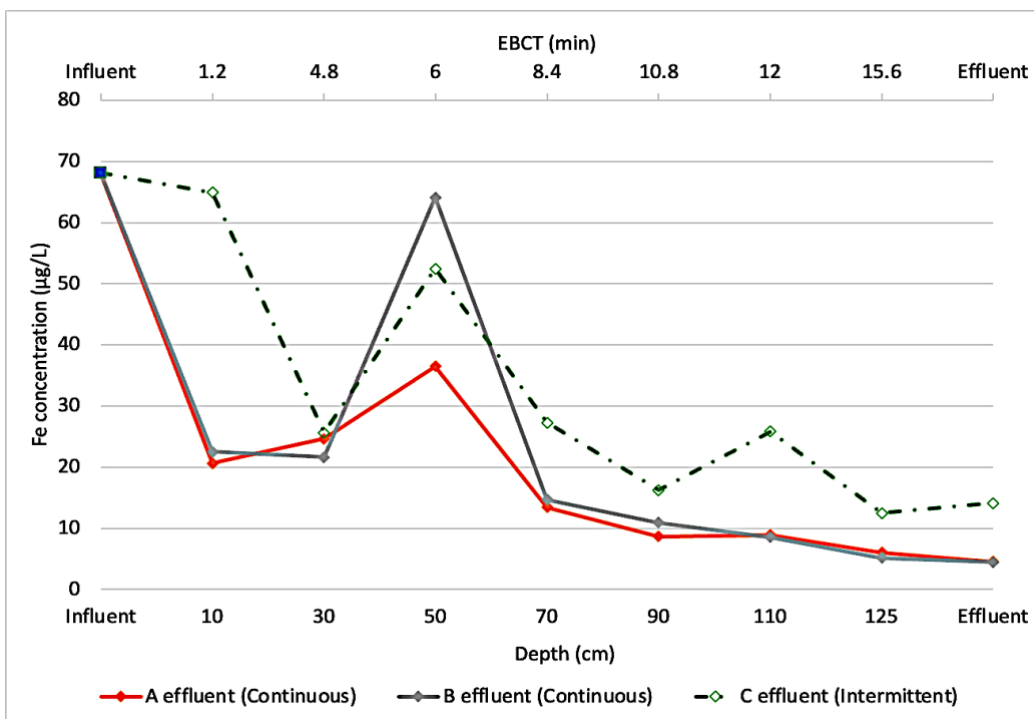
In Figure 4.5 (a) a common trend can be observed: the majority of Mn removal occurred within the middle layer of the biofilter, specifically between depths of 30-70 cm, which aligns with the existing literature on Mn removal mechanisms within biofiltration systems. Previous studies mentioned that during the start-up phase, Mn-oxidising bacteria were more prominent in the middle layer of the biofilter column (Bruins, 2017; Burger et al., 2008; Burger et al., 2008(a)). The reasoning behind Mn removal in the middle layer might be the favourable conditions for the growth and activity of Mn-oxidizing bacteria, which are responsible for the biological oxidation of Mn. Also, substantial variation can be observed between the biofilters operated continuously and the biofilter operated intermittently. The intermittently operated biofilter had comparatively lower removal rates at all the filter depths. Lastly, some unusual peaks were

observed at specific filter bed depths (i.e. C-10 cm, B-30 cm, A-50 cm). Although the exact reason behind the unusual peaks is unclear, they may be attributed to the accumulation of slogged Mn oxide within those particular layers or possible leaching from the upper layers of the biofilter. However, this reasoning contradicts the interpretation that the majority of Mn removal might have happened in the middle layer. If the majority of removal had happened in the middle layer, then the accumulation should have also happened in that layer but it can be seen in Figure 4.5 (a) that for biofilters B and C, the peaks were observed either at the top layer (biofilter C) or somewhere closer to the top layer (biofilter B). Leaching from the upper layer might explain this, but a better understanding of the removal mechanism might help understand this further and thus further research is required in this area.

The results from the investigation for Fe removal on the 49th day of operation are shown in Figure 4.5 (b). On this day the biofilters A, B and C were performing at 93%, 93% and 79% Fe removal respectively. For Fe, it can be observed that the majority of the removal occurred within the top layer of the biofilter, specifically between depths of 0-30 cm, which aligns with the existing literature on Fe removal mechanisms within biofiltration systems. Previous studies mentioned that during the start-up phase, Fe-oxidizing bacteria had higher dominance in the upper layers of the biofilter column (Bruins, 2017; Burger 2008; Burger et al., 2008 (a)). Interestingly, unlike Mn, a substantial difference was not observed between intermittently and continuously operated biofilters, although, intermittently operated biofilter had slightly lower removal rates at most filter depths. Unusual peaks were observed at a specific filter bed depth (50 cm) in this case as well. Although the reason behind this is unclear, one possibility might be leaching from the upper layer. Based on the literature and our findings, most of the Fe removal occurred in the top layer. So, while collecting samples, it is likely that the accumulation of Fe in the top layer might have influenced the concentration peaks observed during analysis in the samples collected from the middle layer.



(a)



(b)

Figure 4.5 (a) Mn and (b) Fe concentrations across various biofilter bed depths on day 49

To further understand the microbial activity associated with Mn and Fe removal, biomass on the biofilter media was analyzed by performing ATP analysis. This analysis was performed at two distinct time

points, day 28 and day 56, to facilitate a comparison. From Figure 4.6, four major observations can be drawn. Firstly, the ATP levels were consistently highest in the uppermost layers of the biofilter across most cases (4 out of 6) and there was a decrease in the ATP levels as the bed depth increased. This observation highlights the important role of the top layer in microbial activity, potentially related to biofilter performance. Also, other studies, especially those based on TOC removal have supported the trend of decreasing ATP levels with increasing bed depth. For example, (ElHadidy, 2016) reported ATP values in the middle of the filter to be approximately half of those found at the top (550 ng/cm³ compared to 1,000 ng/cm³). Secondly, it is evident that the ATP values for intermittently operated biofilters are substantially lower compared to the continuously operated biofilter A, at all the depths. Further, on day 28 the ATP levels were higher in continuously operated biofilter B compared to intermittently operated biofilter C, but not much variation was seen between the two biofilters on day 56, and the reason behind this is unclear. Thirdly, over the period from day 28 to day 56 a noticeable increase in ATP levels was generally observed across various depths, especially at the 70 cm depth i.e. the middle layer, which hints at an incremental enhancement in microbial activity as the operation of the biofilter system advanced. Lastly, some variation was seen in the ATP values of the continuously operated biofilters A and B on day 56. The ATP values at all the depths of biofilter B were substantially lower as compared to that of biofilter A and were similar to those of intermittently operated biofilter C. This observation aligns with the differences in Mn removal observed in the continuously operated biofilters A and B.

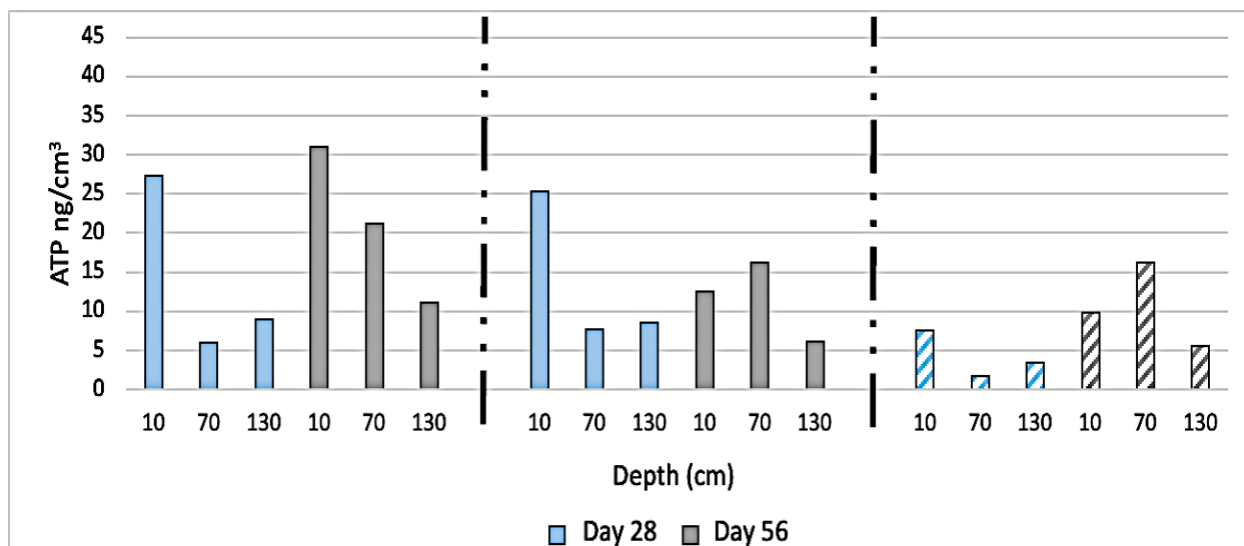


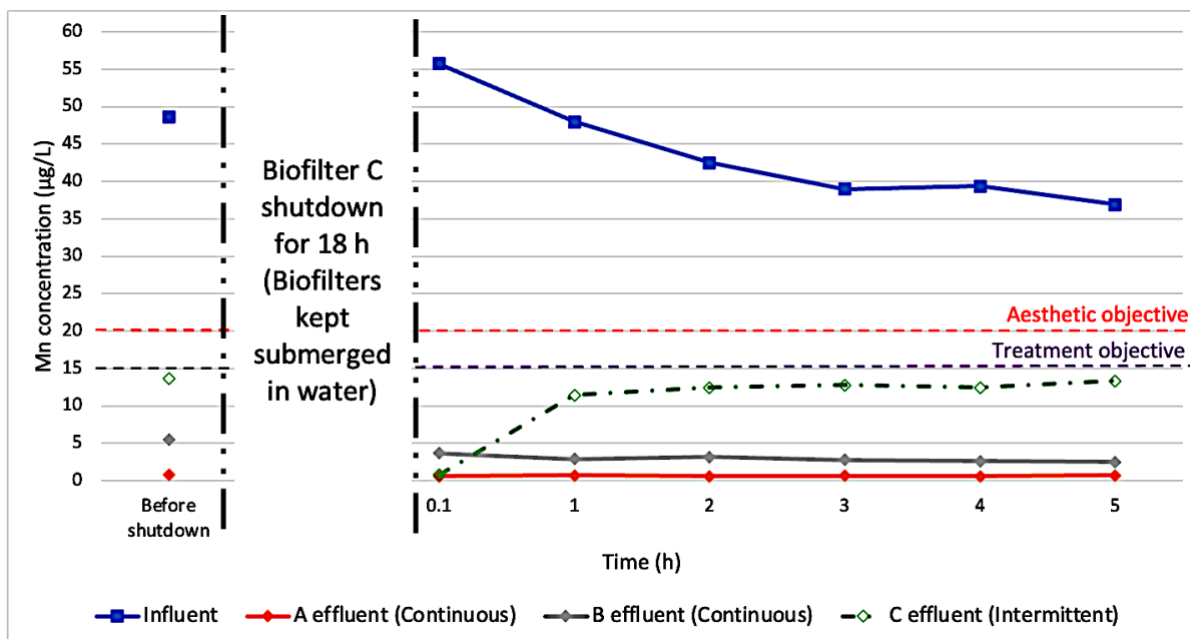
Figure 4.6 ATP measured for the media at different biofilter bed depths. Sample collection was done before backwashing after intermittently operated biofilter had been operating for 2-3 h after 18 h shutdown

Based on these observations, several inferences could be drawn. Firstly, on day 28 of the study, the ATP values indicated higher microbial activity in the topmost layer (10 cm) of the biofilters, while the middle layer (70 cm) exhibited relatively low ATP levels in all the biofilters. However, by day 56, a substantial increase in ATP levels could be observed in the middle layer. This variation suggests that microbial communities in the middle layer became more active over time, aligning with the period when substantial Mn removal was also observed. This correlation suggests a potential link between microbial activity and Mn removal in the middle layer. Secondly, the ATP data might also support the findings regarding depth-wise Fe removal. The higher ATP levels in the top layer of the biofilters on both day 28 and day 56 imply consistent microbial activity in this region. This observation corresponds with the previous findings that Fe removal was consistent since the beginning of the operation. Now, although based on the biofilter performance for Fe removal, the potential predominant removal mechanism for Fe was thought to be chemical oxidation and precipitation, the rationale provided above may support the biological contribution to the Fe removal process. Overall, the distribution of ATP levels aligns with the observations of Mn and Fe removal patterns at different filter depths and thereby enhance the comprehension of the biofiltration processes involved in Mn and Fe removal.

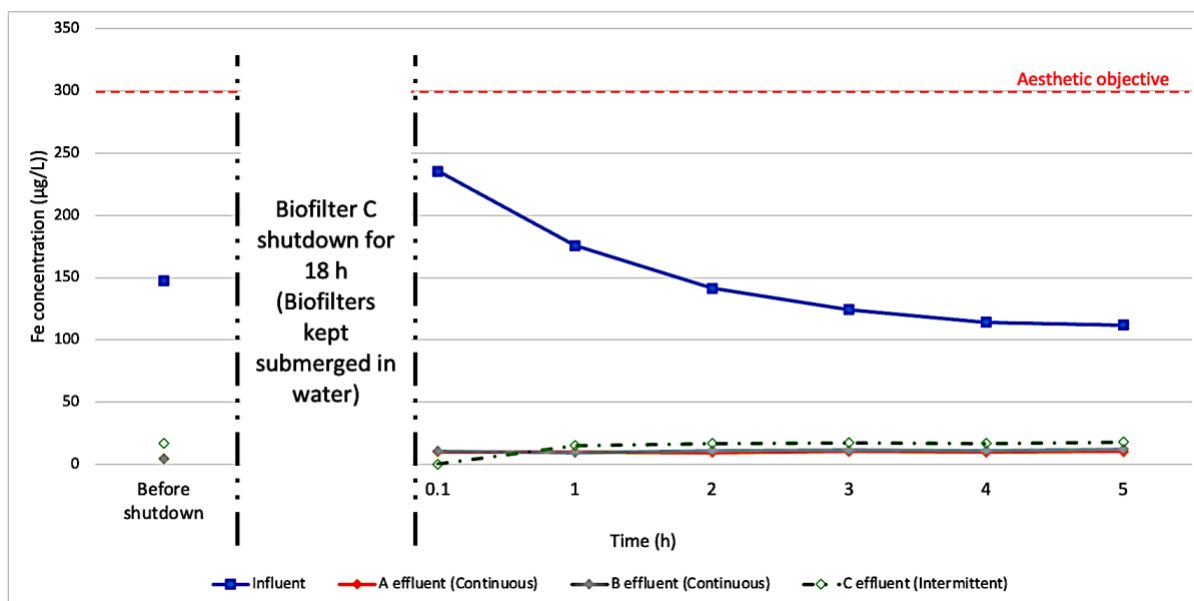
4.2.4 Effect of Short-Term Shutdown on Biofilter Performance

To assess the impact of an 18-hour shutdown on biofilter performance, effluent samples were collected at regular intervals of one hour following the restart of the intermittently operated biofilter C. The ICP-MS results for Mn are shown in Figure 4.7(a). This assessment was performed on Day 56 of pilot operation, when biofilter C had not reached pseudo-steady state yet, but a prolonged emergency shut down of the well feeding the pilot biofilters was imminent. It was observed that following the restart, the biofilter operated intermittently demonstrated a rapid recovery within just 1 hour. A dip was observed at 0.1 h i.e. 5 mins post restart, which is discussed later in this section. The Mn concentration in the effluent remained consistently below the treatment objective of 15 µg/L throughout the sampling period. Further, on comparing the Mn concentrations before the shutdown and post-recovery, it was observed that there was no notable difference in biofilter performance. This indicates that the 18 h shutdown did not have a substantial impact on the ability of the biofilter to remove Mn. Although, the removal rates for the intermittently operated biofilter C, before shutdown and post restart weren't substantially different, but overall, they were lower (64%) as compared to the removal rates of the continuously operated biofilters (>90%). Also, despite facing challenges in the form of a fluctuating Mn concentration in the influent on Day 56 (Refer to Figure 4.7(a)), all three biofilters showcased remarkable resilience and maintained their performance. Mn concentrations as high as 55 µg/L were recorded on this day while the average Mn concentration was 31 µg/L. Samples

were also collected at regular intervals on the 27th day of operation, when none of the biofilters showed substantial Mn removal, and the corresponding plots can be found in Appendix B.4.



(a)



(b)

Figure 4.7 Impact of 18 h shutdown on (a) Mn and (b) Fe concentrations during biofilter C start up on day 56. Sample collection began 5 minutes (0.1 h) after the biofilter restart and then continued at hourly intervals. To have a reference point a sample was collected on the previous day just before the shutdown.

Furthermore, post-restart, Fe removal was also investigated, and the results are shown in Figure 4.7(b). Similar trends were observed for Fe as the biofilter operated intermittently demonstrated a rapid recovery within just 1 hour post restart. A dip was observed at 0.1 h i.e. 5 mins post restart for Fe as well, which is discussed later in this section. Further, unlike Mn removal, Fe removal showed lesser difference between the intermittently (84%) and continuously operated biofilters (~90%). Also, despite facing challenges in the form of a substantial increase in Fe concentration in the influent on Day 56, all three biofilters showcased remarkable resilience and maintained their performance. Fe concentrations as high as 235 µg/L was recorded on this day while the average Mn concentration was 57 µg/L.

On a side note, it was observed that, at the 0.1-hour or 5-minute time point following the restart, the Mn and Fe concentrations in the effluent samples were nearly zero for column C. During the 18-hour shutdown period, the biofilter did not receive any new influent water and was kept submerged in water. As a result, the sample collected at the 5-minute time point after the restart may have been stagnant water that remained within the biofilter during the shutdown. This stagnant water could possibly have undergone extensive Mn and Fe removal processes during the shutdown period, leading to substantially reduced concentrations.

Apart from biofilter performance before and after restart, the microbial activity was also investigated during this transition. This assessment was performed on Day 7, 42 and 56 of pilot operation. One notable finding (Refer to Figure 4.8) was that the overall microbial activity was substantially lower in the media of the intermittently operated biofilter as compared to continuously operated biofilter A. The variation was also observed between continuously operated biofilter B and intermittently operated biofilter C on day 7 but then surprisingly, ATP values for continuously operated biofilter B dropped on day 42 and day 56 and didn't show substantial differences. The reason behind this decline in the ATP value is unclear. Given the confounding results observed in relation to the impact of intermittent operation on microbial activity within the biofilter media, it becomes apparent that ATP alone cannot serve as an exclusive indicator to comprehensively comprehend the impact of 18 h shutdown on the microbial community residing within the biofilters. The low ATP values do support the lower Mn removal rates observed for the intermittently operated biofilter, and also the differences in the performance of the continuously operated biofilters A and B. These findings align with prior research conducted by Lauderdale et al., which reported a direct relationship between elevated ATP levels and increased Mn removal rates in biofiltration systems operating under nutrient-supplemented conditions (Lauderdale et al., 2012). However, from the before restart (BR) and after restart (AR) plots of the intermittently operated biofilter, it was observed that an 18-hour shutdown had a variable effect on the ATP values. On day 7 the ATP values were almost similar before and after restart, on day 42, the ATP was higher after restart, while on day 56, the ATP values were similar. Overall,

Figure 4.8 indicates that the temporary shutdown did not cause much disruption to the microbial activity within the biofilter media.

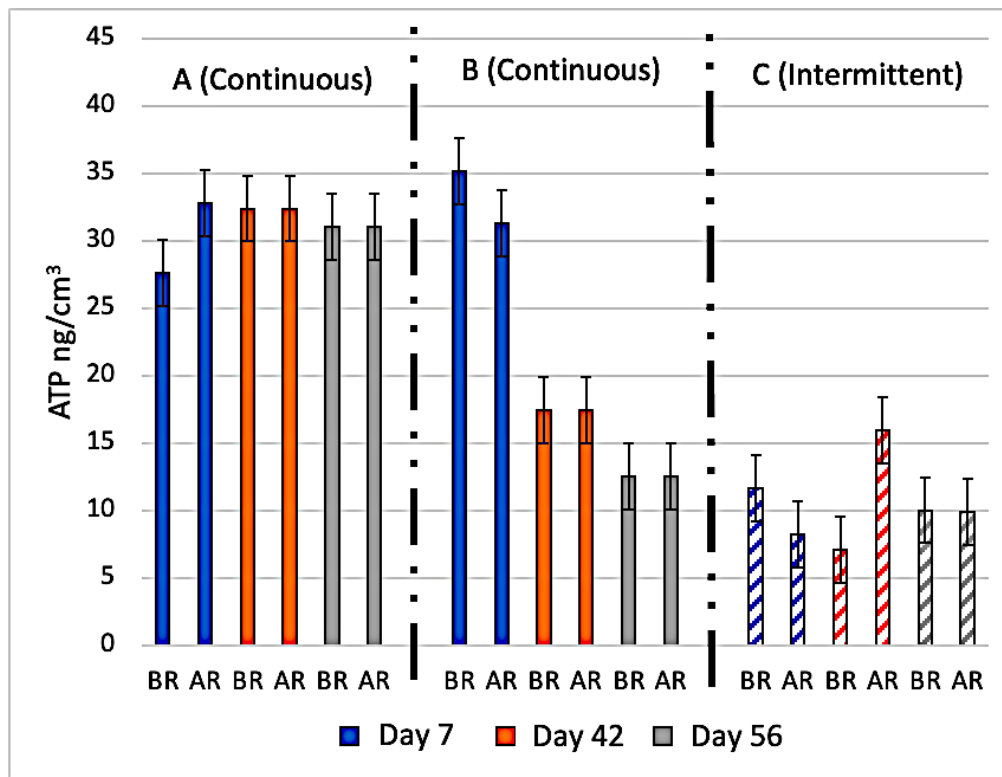


Figure 4.8 ATP of biofilter media before and after restart of biofilter C after an 18 h shut down period, while column A and B were running continuously on day 7, 42 and 56. BR indicates that the sampling was done before restart when the continuously operated biofilters were still operating while the intermittently operated biofilter was shut down, and AR indicates the sampling which was done after restart, when all the biofilters were operating. Media samples were collected from the top-most layer i.e. from 10 cm before backwashing the biofilter columns. ATP measurements were done in duplicate. The error bars here represent the standard deviation from the mean value.

One possible reason for this could be attributed to the operational approach employed during the 18-hour shutdown, wherein the biofilters were kept submerged in stagnant water. Figure 4.9 displays the recorded DO levels both just before the intermittently operated biofilter restarted and shortly after its restart. Note that the DO concentration in the influent was recorded as 11.2 mg/L during this period. As observed from the Figure 4.9, while DO levels did experience a decline in the intermittently operated biofilters during the shutdown period, they did not drop to substantially low levels. The lowest DO during recorded shutdown was just about 20% lower than what was maintained by the biofilters after restart. Another noteworthy observation is the relatively high DO levels detected in the topmost layer. This can likely be

attributed to the water recirculation occurring at the upper part of the biofilter column during the intermittent shutdown period. It's important to mention that this recirculation was a default feature of the pilot-scale biofilter's design, details of which can be found in section 3.3.1. Furthermore, DO values surged immediately after the intermittently operated biofilter resumed operation following an 18-hour shutdown, mostly due to the reintroduction of fresh water into the biofilter.

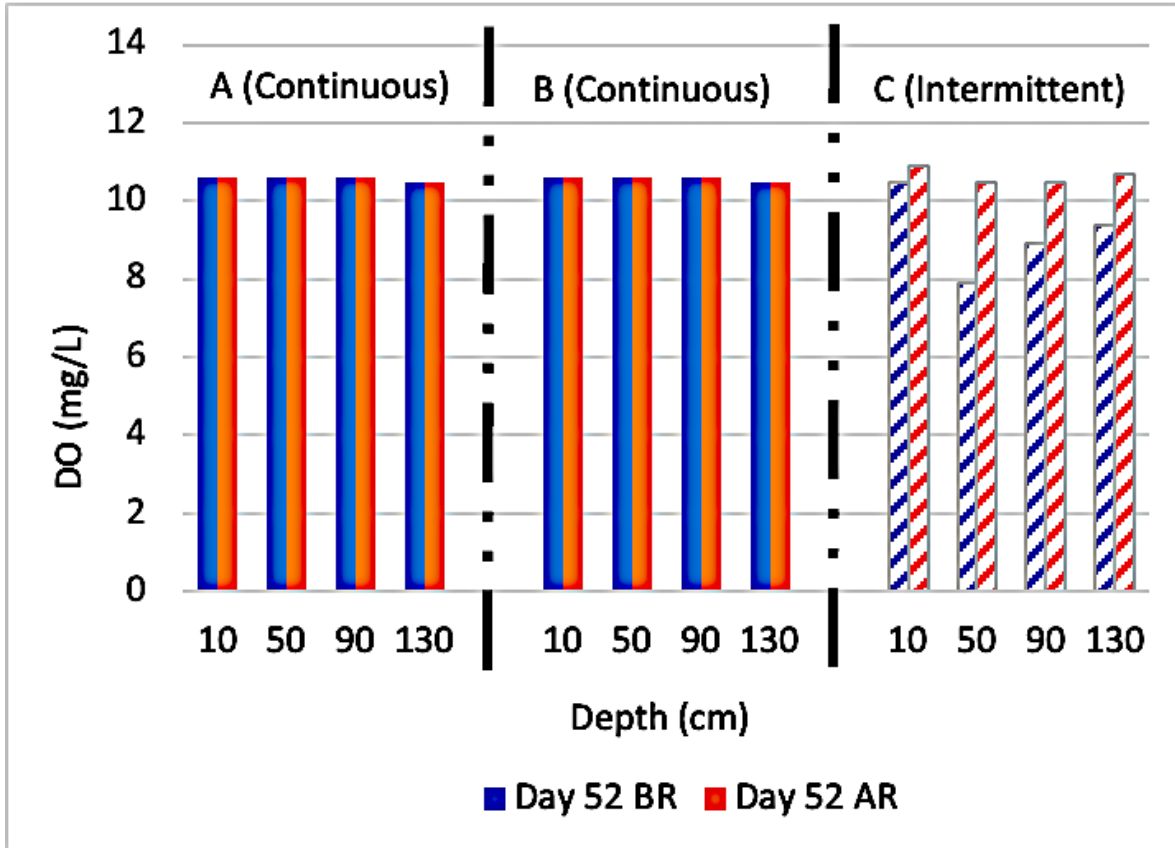


Figure 4.9 DO levels before and after restart of biofilter C after a 18 h shut down period, while column A and B were running continuously. BR indicates that the sampling was done before restart when the continuously operated biofilters were still operating while the intermittently operated biofilter was shut down, and AR indicates the sampling which was done after restart, when all the biofilters were operating.

4.2.5 Effect of Backwash on Microbial Activity

The effect of backwashing on the microbial activity in terms of ATP concentrations is very limited (Pharand et al., 2014) and limited research has explored this aspect, particularly with Mn as the concerning contaminant to be removed. In the study by Burger et al., (2008), backwashing was conducted at a rate of 20 m/hr for a duration of 5 minutes. The backwashing frequency was set at intervals of 300-336 h for

continuously operated biofilters and 75-300 h (operating time) for intermittently operated biofilters. In a separate study conducted by Niquette et al., (1998), backwashing was performed without air scour at a 20-35 m/h rate for 2 mins and with air scour at same rate but for 30 seconds. In one research, it was suggested that backwashing could be carried out either immediately after the shutdown or just before the system's restart, although performing just before restart was reported as favourable (Bruins, 2017).

In the present study a weekly backwashing schedule was implemented for the continuously operated biofilters, while the intermittently operated biofilters underwent backwashing on a monthly basis, to ensure uniformity in the treated volume per backwash cycle for each biofilter column. Backwashing was always performed before the intermittently operated biofilter would restart after the 18 h shutdown. Figure 4.10 shows the backwash flow rate recorded in the PC during a backwashing cycle when all the biofilters had to be backwashed. As seen in Figure 4.10, for each biofilter there was a period of initial low water flow rate backwash (1.5-2 L/min) and air scour for 3 minutes, which was then followed by high water flow rate backwash (10-12 L/min) for at least 5 minutes. An important observation from Figure 4.10 is the disturbance in the flow that was observed for the continuously operated biofilter B and intermittently operated biofilter C, but not in continuously operated biofilter A during the high waterflow backwash period. This problem was always faced and the possible reason behind this was found to be the lump formation in the biofilter media, which didn't allow a smooth backwashing process for these two biofilters. This observation was also in line with the variation in the performance between the two continuously operated biofilters A and B and the similarity in the performance of biofilter B and C initially during the start-up phase.

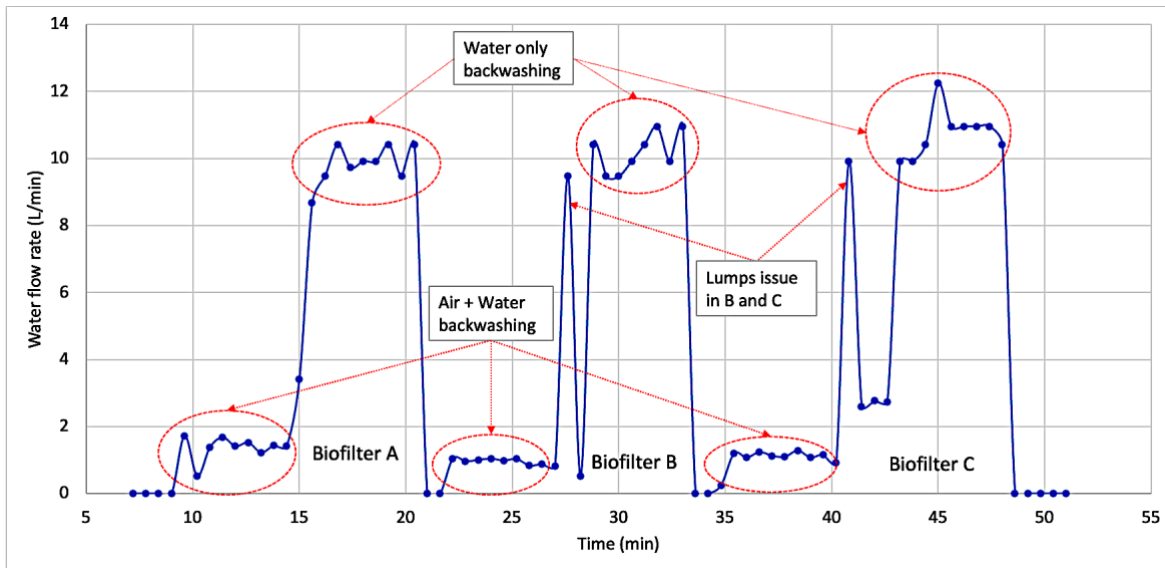
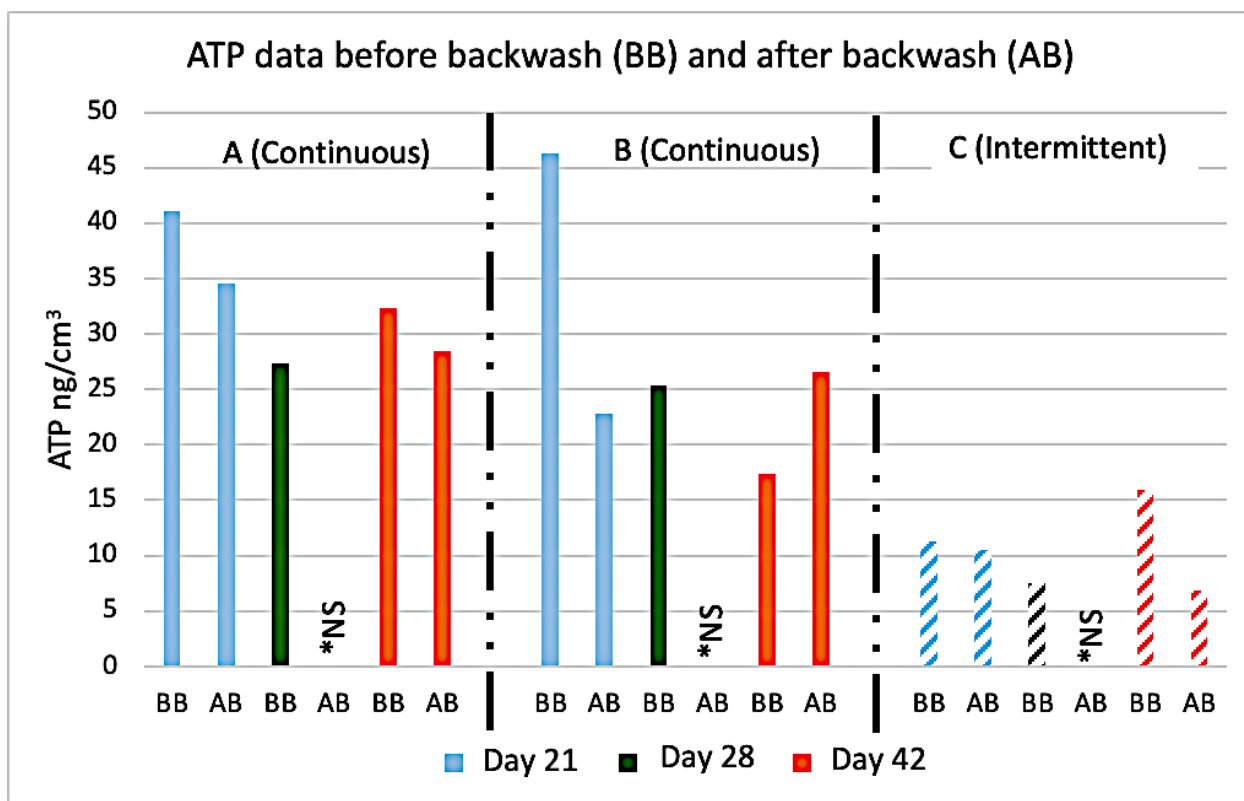


Figure 4.10 Backwash flow rate data for all biofilters.

To understand the effect of backwashing, the microbial activity was investigated before and after backwash. As seen in Figure 4.11, although ATP values decreased in most of the cases after backwashing (increase in just one case), the effect was not much, which indicates that backwashing did not cause a substantial disruption to the microbial activity within the biofilter media, indicating that the biofilters were intact after the backwash. The interpretation of these finding is consistent with previous research outcomes (Ikhlef & Basu, 2017). In the literature focused on organic carbon removal, it was found that media collisions and abrasions due to backwashing with air scour did not lead to substantial biofilm detachment, which also aligns with the findings of this research (Emelko et al., 2006; Huck et al., 2000).



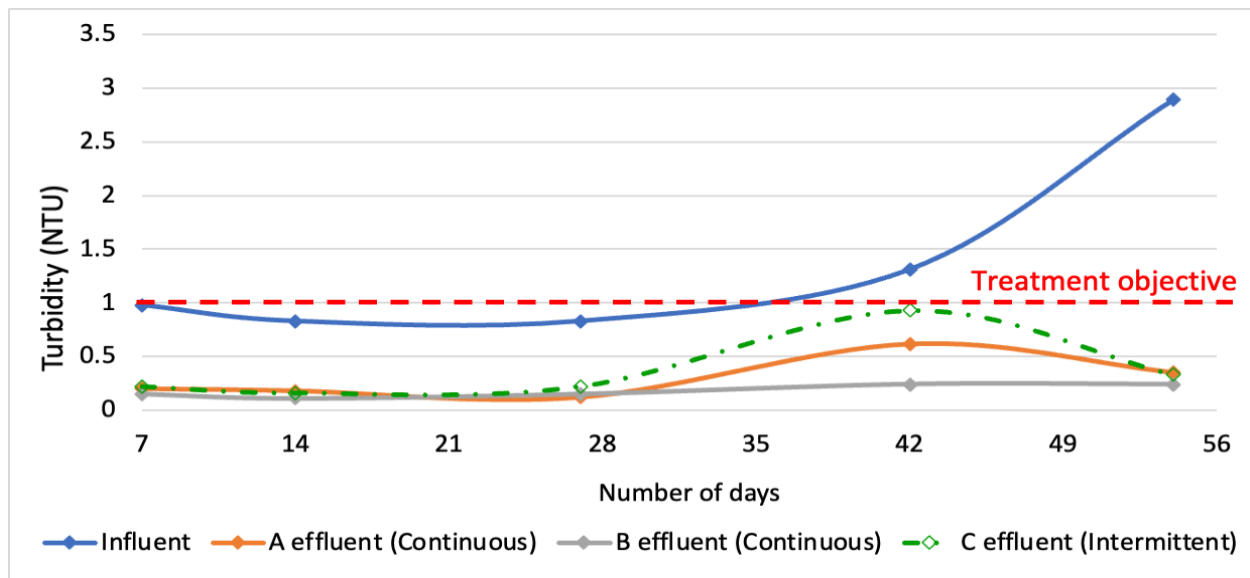
*NS- Not sampled

Figure 4.11 ATP on biofilter media before and after backwash. The media samples were collected from the top-most layer i.e. from 10 cm. BB indicates that the sampling was done before backwash and AB representing the sampling which was done after backwash. After backwash the sampling was performed after 30 minutes.

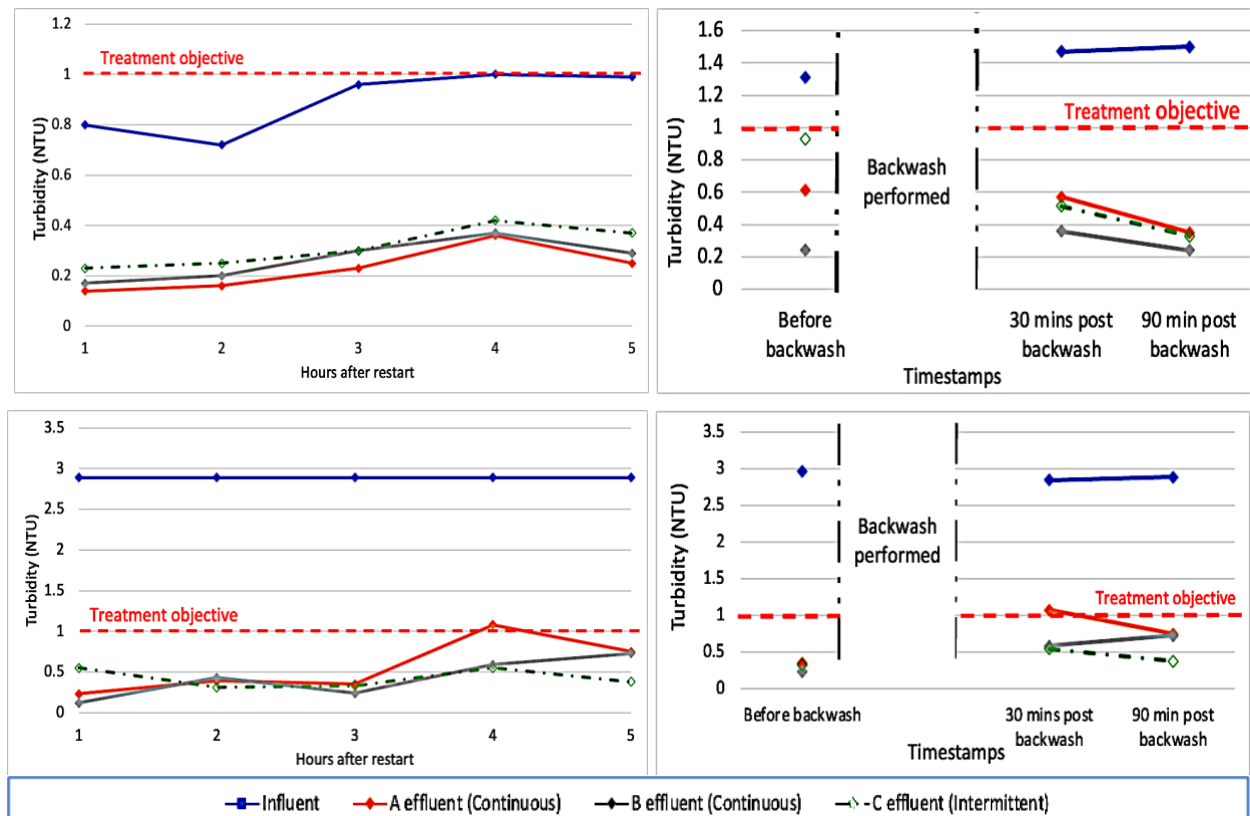
4.2.6 Turbidity Removal by the Biofilters

Turbidity is an important parameter that indicates the quality of water. One important observation from the study was the ability of all the biofilters to consistently maintain turbidity values below 1 NTU, even while facing increased influent turbidity, as shown in Figure 4.12 (a). The utility's treatment objective for the groundwater well was 0.1 NTU, which the biofilters were not able to meet but except for day 42, it can be seen that the biofilters were able to produce turbidity levels below 0.5 NTU. The minor rise in effluent turbidity observed on day 42 is not substantially noteworthy. This increase in Biofilter C appears to be consistent with the trend observed in continuously operated biofilter A, suggesting a potential influent-related cause for the elevated turbidity levels, as depicted in Figure 4.12 (a). On day 54, it can be seen that the turbidity in the influent is close to 3 NTU and still the intermittently operated biofilter was able to maintain the turbidity levels in the effluent below 1 NTU post restart.

Further, to see the effect of 18-h shutdown on the intermittently operated biofilter, the trends for turbidity observed post-restart are shown in Figure 4.12 (b) for day 27 and day 54. It can be observed that no substantial variation was found in between the effluent turbidity levels in intermittently and continuously operated biofilters. Surprisingly, there was one instance on day 54 for column A (operated continuously), when the turbidity level was recorded above 1 NTU. Turbidity levels were also monitored before and after backwash and the results are illustrated in Figure 4.12 (c). As seen on day 42, the turbidity values post backwash were lower than those before backwash for 2 out of 3 biofilters and the turbidity values reduced as the time passed after backwash. On day 54 as well, it can be seen that as the time passed the turbidity values came down, although for column A (operated continuously), 30 mins after backwash the turbidity level was recorded above 1 NTU. It's noteworthy to point out here that the pilot scale set up used for the experimental work was not coagulant-assisted rapid granular media filtration, which is generally applied for turbidity removal. Although the biofilters employed were able to perform well, if better turbidity removal had been the goal, then coagulation addition prior to biofiltration would have been done.



(a)



(b)

(c)

Figure 4.12 Turbidity data in pilot biofilter influent and effluents. (a) Data from Jan 18, 2023 = Day 7 to Mar 8, 23 = Day 56, (b) Hourly after 18 h shutdown during biofilter C start up on day 27 and 54, (c) before and after backwash on day 42 and day 54. Note that different y-axes are used to highlight specific trends, patterns and for clarity of the graphs

4.3 Conclusions

The main objective of this phase of the research was to compare the performance of continuously and intermittently operated biofilters during the start-up phase. To achieve this goal, a pilot scale setup consisting of three sand biofilters was assembled at a groundwater treatment utility and operated for 56 days. After 56 days of operation, an groundwater well shutdown took place, which impacted the research operations and data collection as there was no access to the raw groundwater. The major findings from this start-up phase based on the results obtained are:

- During the 56 days of operation, all the biofilters were able to remove Mn below a treatment objective value of 15 µg/L after day 42. While the continuously operated biofilter A appeared to have reached steady state, continuously operated biofilter B seemed to be reaching steady state and the intermittently operated biofilter C did not reach steady state, but based on the Mn removal trends observed, it can be concluded that the performance of the intermittently operated biofilter had started to improve.
- When comparing intermittently and continuously operated biofilters, notable differences were observed during the start-up period. Mn removal rates in the intermittently operated biofilter were approximately 64%, which were lower compared to the consistently high removal rates of over 90% observed in the continuously operated biofilters. Also, a variation in the performance of the two continuously operated biofilters was observed. Although column B eventually exhibited almost similar removal rates as column A, its performance diverged significantly until day 49 of operation.
- High Fe removal rates with minimal variation (~8%) between intermittently and continuously operated biofilters was recorded, indicating that both operational modes could effectively remove Fe from the water. With substantial removal observed from week one itself, it can be hypothesised that chemical oxidation and precipitation might be the prominent removal mechanism for Fe.
- Mn removal was found to predominantly occur in the middle layer of the biofilter, specifically between depths of 30-70 cm. This middle layer accounted for approximately 55-80% of the overall Mn removal. This finding highlights the importance of the middle section of the biofilter bed for efficient Mn removal during the start-up period.
- Further, in terms of depth-wise removal, it was observed that the removal of Fe predominantly occurred in the top 10 cm of the biofilter bed, accounting for approximately 65-70% of the overall Fe removal. This suggests that Fe removal is more prominent in the upper layers of the biofilter.
- In the context of microbial activity, ATP values were observed to be lower in the intermittently operated biofilters compared to continuously operated biofilter A. A possible reason behind lower ATP values might be the nutrient and dissolved oxygen situation during the shutdown period, which might have led to restricted growth and maturation of MnOB, and therefore affected the microbial activity. The ATP

values of continuously operated biofilter B were similar to those of intermittently operated biofilter C rather than the continuously operated biofilter A and the reason behind this is unclear, although it aligns with the Mn removal results.

- The data interpretations potentially indicate that there is no direct relationship between the removal of Mn and the biomass concentration as quantified through the ATP concentration. While ATP measurements provide valuable insights into active microbial biomass, they may not provide a straightforward correlation with Mn removal.
- Neither backwash nor an 18-hour shut down substantially affected the performance of the biofilters. The biofilters were able to recover their performance within just one hour after restart.
- In the context of effect of backwash and 18 h shutdown on the microbial activity of the biofilters, it was found that backwash didn't affect the ATP values substantially. However, confounding results were obtained while investigating the impact of the 18 h shutdown, indicating that ATP alone cannot serve as an exclusive indicator to comprehensively comprehend the impact of 18 h shutdown on the microbial community residing within the biofilters.
- Lastly, all the biofilters were able to keep the turbidity value below 1 NTU consistently.

These findings underscore the complexity of the interactions within biofilter systems. Additionally, the study highlights the need for a multifaceted approach when assessing the effects of operational changes, such as backwashing or short-term shutdowns, on the microbial communities within biofilters. Further research and exploration are warranted to gain a more comprehensive understanding of the interplay between microbial communities, Mn removal, and operational changes in biofilter systems.

Chapter 5

Effects of an Extended Shutdown on the Microbial Activity of Biofilters Previously Operated Intermittently and Continuously

5.1 Overview

This chapter includes the results and discussion of the research phase 2 wherein the effects of an extended shutdown on the microbial activity of biofilters was investigated. It is important to highlight that this phase was initially not part of the research plan; instead, it was incorporated due to an unexpected shutdown at the groundwater utility. The shutdown resulted from concerns related to the raw water and led to a halt in the supply of raw water to the pilot biofilter setup. This phase commenced on 8th March 2023 and lasted six months until 27th September 2023. During this phase a uniform approach was applied to all biofilters, i.e. both previously operated intermittent and continuous biofilters were subjected to identical measures. The sub-objectives of this phase were:

- ✓ Assess the impact of the extended shutdown on microbial activity in the biofilter biofilms and on basic water quality parameters
- ✓ Evaluate the effect of different storage strategies (Refer to Table 3.4) during prolonged shutdown on biofilters

To investigate the effects of an extended shutdown on the microbial activity of biofilters, apart from ATP, (Adenosine Diphosphate) ADP and (Adenosine Monophosphate) AMP (all together called AxP) were also measured. To the authors knowledge, this has not been investigated previously for this application and the overall literature on the utilization of ATP, ADP, and AMP to investigate microbial activity is limited.

5.2 Results and Discussion

5.2.1 Impact of the Extended Shutdown and the Different Storage Strategies on Basic Water Quality Parameters

Figure 5.1 illustrates the trends in temperature at two biofilter depths, the top layer (10 cm) and the bottom layer (110 cm). The temperature in the biofilters ranged from around 15^oC at the beginning of the shutdown to nearly 25 ^oC by the end of the shutdown, which is an increment of 10^oC. It can be inferred from the figure that the temperature was the same within each biofilter and among all the biofilters and they consistently

exhibited an upward temperature trend over time. The rise in temperature can be attributed to two main factors:

1. Stagnation of the water within the biofilters resulted in the water temperature in the biofilters being equivalent to the room temperature (represented by the blue line in the plots).
2. The shutdown coincided with the summer season when room temperatures were increasing, as evident in the plotted data (Refer to Figure 5.1). Hence, the water temperatures in the biofilters were also increasing.

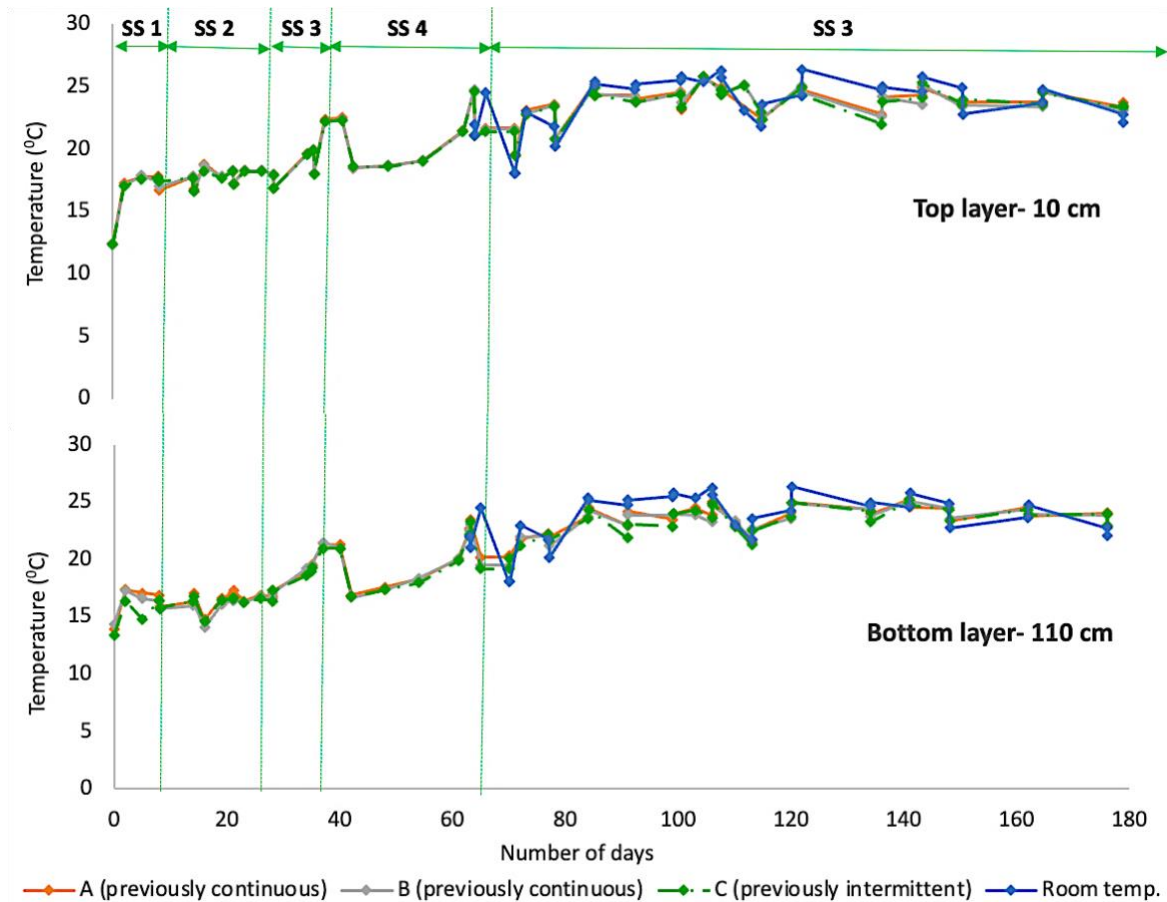


**Figure 5.1 Trends in temperature at different biofilter bed depths during the extended shutdown:
Top layer (10 cm) and bottom layer (110 cm) [SS: Storage strategy (Refer to Table 3.4)]**

An increase in temperature can lead to higher evaporation rates, which can potentially affect the moisture content in biofilter media if biofilters are drained during shutdown. Thus, during shutdown periods, keeping biofilters submerged in water can be recommended. Furthermore, temperature might affect the metabolic activity of microorganisms within the biofilter. With an increasing temperature observed during the extended shutdown, it is possible that the environment might have created favourable conditions

for portions of the microbiological community to thrive, while for a different segment of the microbes, this created a stressed situation. Furthermore, as the temperature rise was fairly gradual, there is a possibility of a shift in the microbial community towards organisms which have their optimum growth at higher temperatures. The details of the effect of temperature on the microbial activity in terms of ATP levels have been discussed in section 5.2.2.

To verify the occurrence of a stressed situation, i.e. ascertain whether the biofilters turned anoxic during the extended shutdown, measurement of DO levels was found relevant. Figure 5.2 illustrates the trends in DO at two biofilter depths, the top layer (10 cm) and the bottom layer (110 cm). Until day 63, the DO in all biofilters declined at the lower level, but this decline was not that pronounced in the upper layer. However, after day 63, the observed effect was significantly reduced when SS 3 was being used during shutdown. From the plots, it can be inferred that DO values exhibited a steady decrease from day 35 to 63 when SS 4 was being used where the biofilters were left submerged under the same water for 4 weeks. However, after day 63, when the biofilters were being drained weekly to take media samples and were refilled with the same water, these DO values quickly rebounded to the same elevated levels, which explains the zig-zag trend in Figure 5.2. This process aerated the water back to the same DO level before being filled back into the biofilter, thus maintaining an almost consistent DO level over time. This suggests that the periodic water circulation process likely played a crucial role in mitigating the risk of steady DO depletion and, consequently, helped prevent the biofilters from entering an oxygen-stressed state. In contrast, during the period between day 35 and day 63 when the biofilters were left submerged in the same water for about four weeks, the DO values visibly decreased, reaching levels as low as 4 mg/L. Such a decline may not be suitable to maintain bacterial activity and it might potentially cause microbial dormancy.

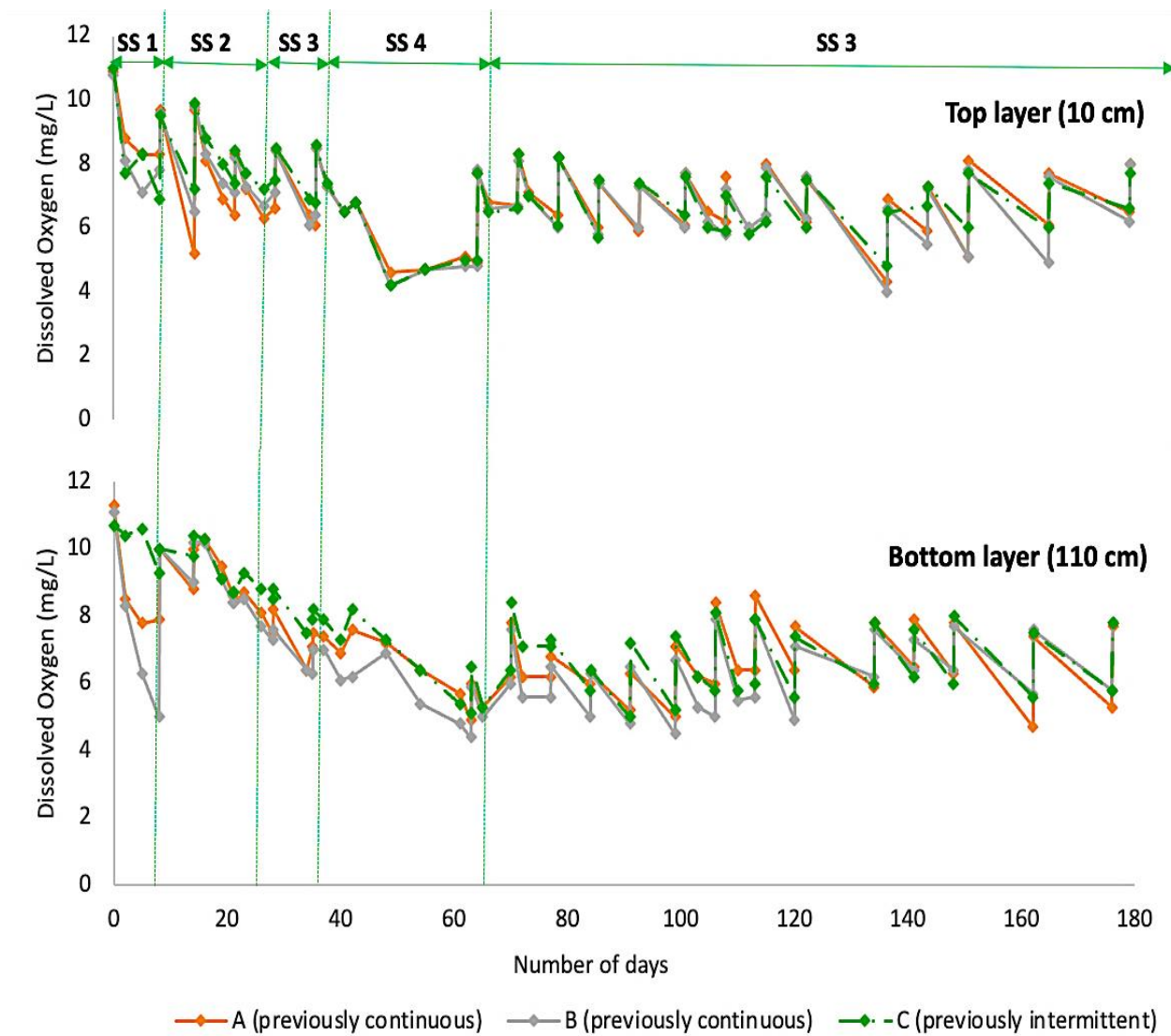


Figure 5.2 Trends in DO at different biofilter bed depths during the extended shutdown: Top layer (10 cm) and bottom layer (110 cm)

Trends similar to DO were observed for pH as well as shown in Figure 5.3. Overall, there were no notable variations in pH observed across all biofilters as it consistently remained within the range of 7.3 to 8 throughout the entire extended shutdown period. The most significant depletion occurred when the water circulation process was suspended between day 35 and 63 of the extended shutdown. However, it is essential to emphasize that despite this decline being notable, it remained within the acceptable range for biological Mn removal processes.

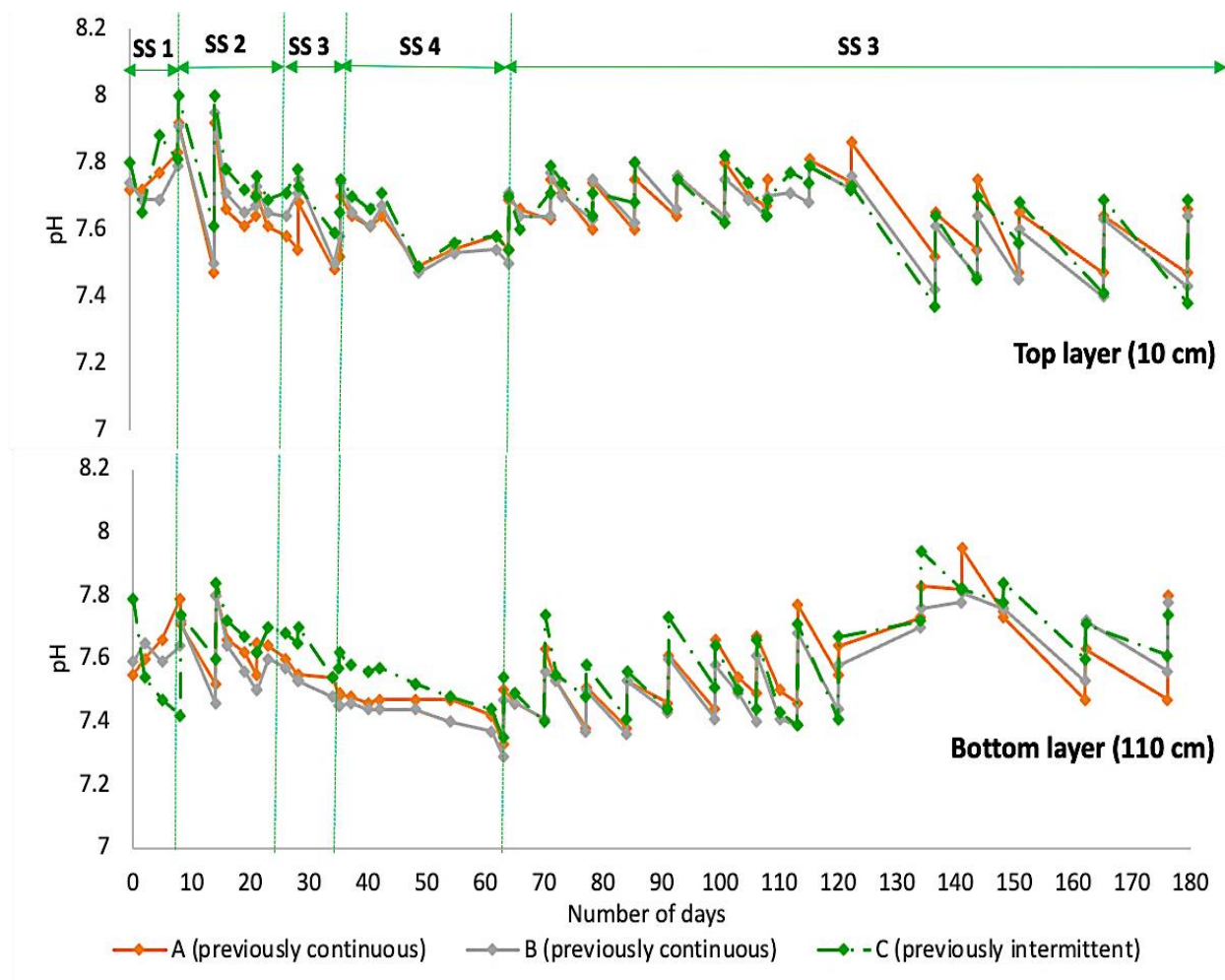


Figure 5.3 Trends in pH at different biofilter bed depths during the extended shutdown: Top layer (10 cm) and bottom layer (110 cm)

Additionally, apart from its importance for redox processes, ORP has been known for its role in assessing the favourability of environmental conditions for the metabolic activities of microorganisms, thereby influencing microbial community composition (Reichart et al., 2007). Therefore, ORP was monitored and the trends for ORP can be found in Figure 5.4. The ORP was stable up until day 100 for all the biofilters, and it was somewhat decreased in both the top and bottom layers, especially after 120 days into the extended shutdown. Biofilter C, in particular, experienced more pronounced reductions in ORP during this period. In the top layer of biofilter C, ORP levels reached a minimum of approximately 130 mV and in the bottom layer, the ORP minimum was below 100 mV, indicating a significant decline in the redox potential. Interestingly, ORP did not exhibit the degree of fluctuations (zig-zag pattern) that was observed in the trends of DO and pH. This suggests that the various storage strategies employed did not have a distinct impact on the ORP.

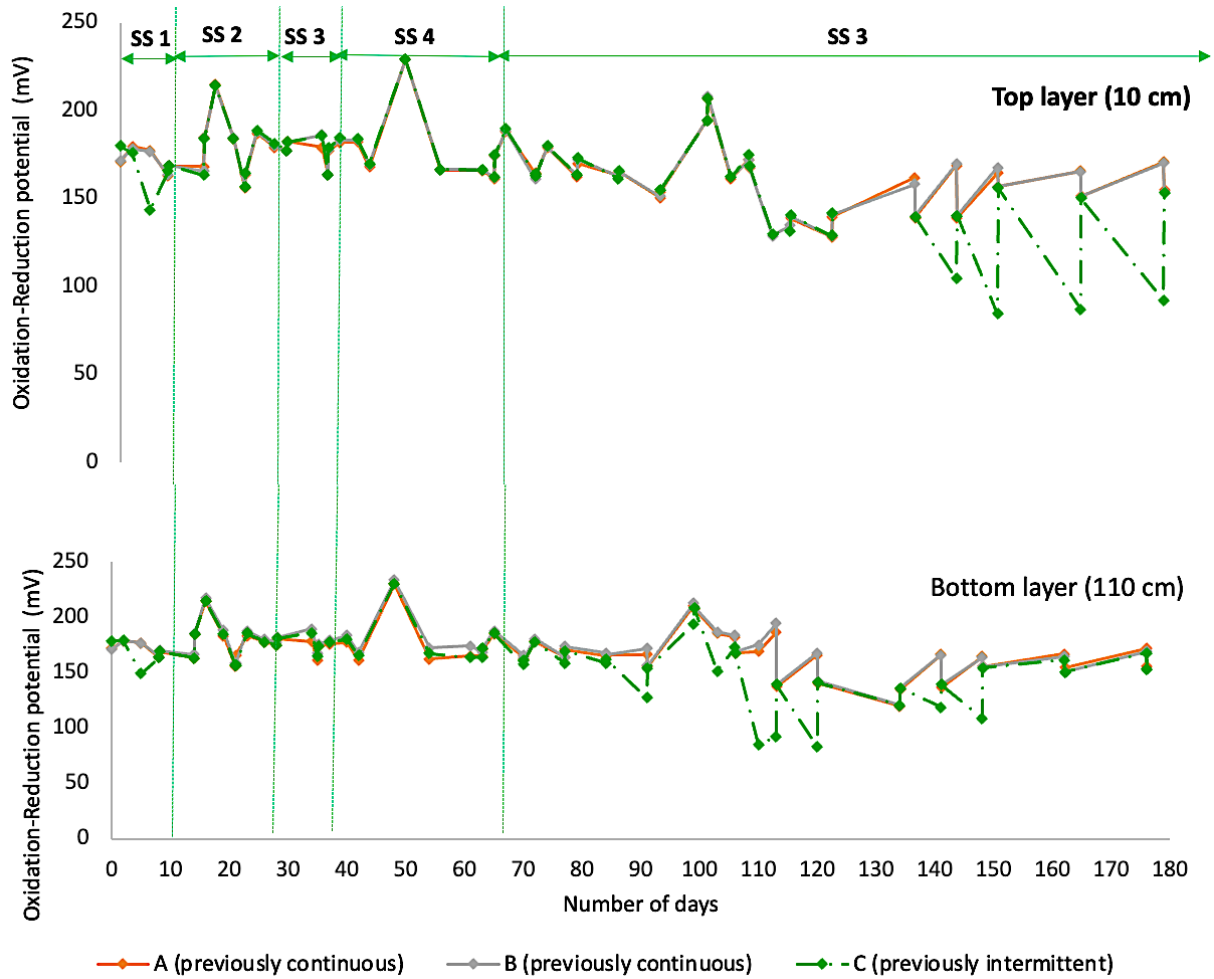


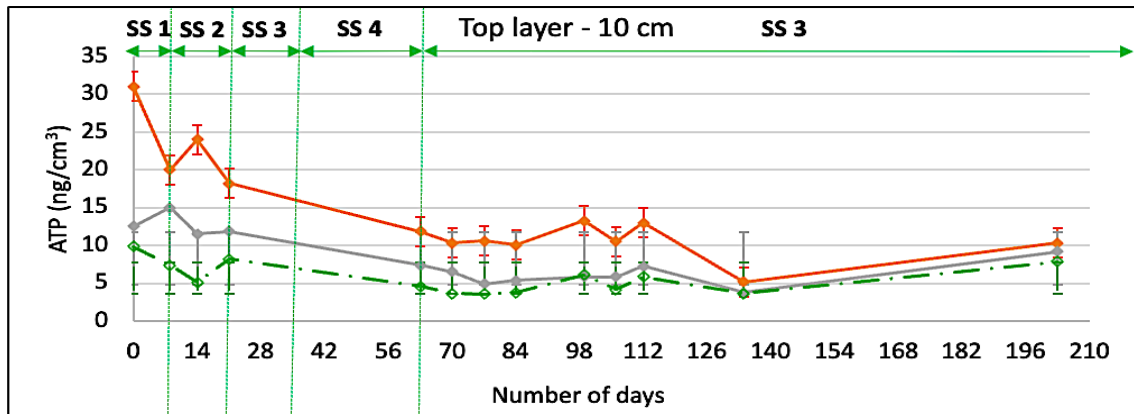
Figure 5.4 Trends in oxidation-reduction potential (ORP) at different biofilter bed depths during the extended shutdown: Top layer (10 cm) and bottom layer (110 cm)

Lastly, in addition to monitoring the fundamental water quality parameters, conductivity was also measured, and the corresponding plots can be found in Appendix C, Figure C.1. Also, apart from the top and bottom layers, water quality parameter values were recorded from the middle layer of the biofilters, and these measurements are also available in Appendix C, Figure C.2.

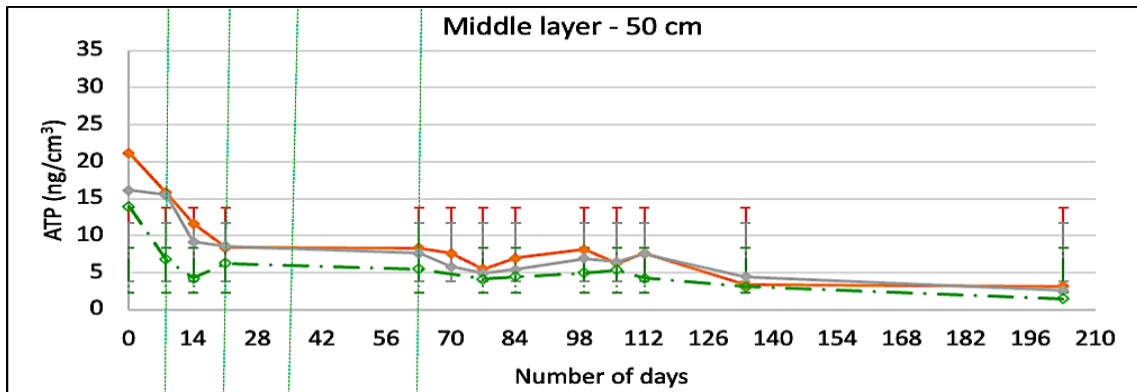
5.2.2 Impact of the Extended Shutdown on Microbial Activity in the Biofilters

Apart from monitoring water quality parameters during the extended shutdown, a critical dimension of the study involved delving into the changes observed in the microbial activity within the biofilm attached to the biofilter media. To understand the effects of an extended shutdown on microbial activity, biomass on the biofilter media was analyzed by performing various microbial analyses.

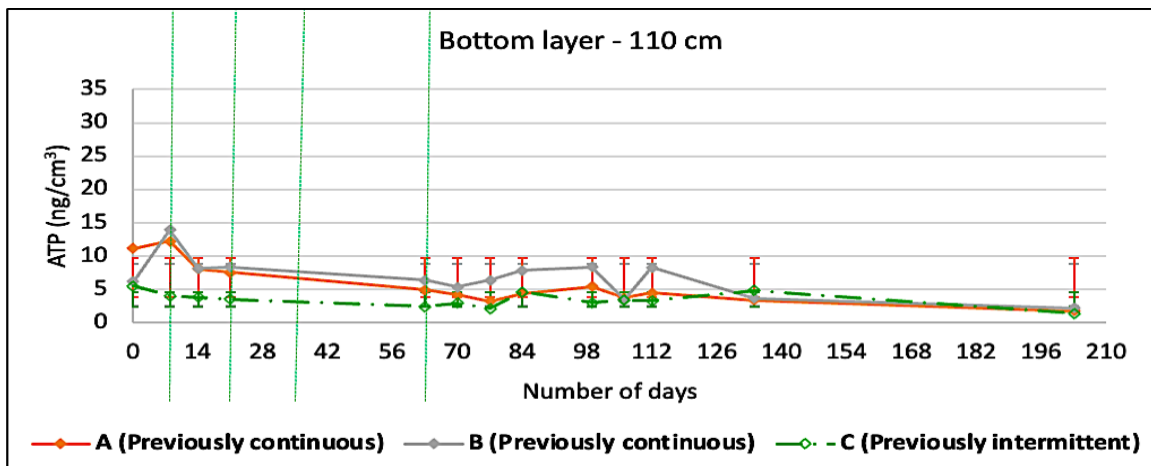
ATP quantification has been known to provide a direct measure of cellular energy status and a quick insight into the metabolic activity (Pharand et al., 2014). Figure 5.5 displays the recorded ATP values during the extended shutdown for three different biofilter depths and several intriguing patterns can be seen. Notably, the previously intermittently operated biofilter C consistently exhibited lower ATP values compared to the previously continuously operated biofilters, particularly biofilter A until day 112 (about 4 months into shutdown). Following day 112, the ATP values for all the biofilters seemed to become quite similar. Furthermore, although biofilters A and B were both operated continuously before the shutdown, they showcased differences in ATP levels, especially noticeable in the top layer. This variation was also in line with their difference in performance and microbial activity observed in the continuously operated biofilters during the start-up phase, the reason for which was not completely clear. Further, in the top layer, a substantial decline in ATP levels was seen in all the biofilters for about 70 days, with biofilter A having the steepest decline amongst the three biofilters and biofilter C having the least decline. On day 0, i.e. the onset of extended shutdown, biofilter A also had the highest and biofilter C had the lowest ATP level in the top layer as seen in Figure 5.5 (a). After 70 days, the decline became less pronounced, and the ATP levels seemed to stabilize. A steep decline in the ATP values was also observed for all the biofilters in the middle layer in the first 14 days, after which the ATP levels seemed to stabilize. Overall, a notable reduction in ATP levels, up to approximately 84%, was evident in the top and middle layers during the extended shutdown period. ATP levels in the bottom layer remained consistently low, with a minimal decline throughout the extended shutdown. Overall, the most substantial ATP loss occurred in the top layer within the initial 28 days, tapering off notably after 84 days into the shutdown period.



(a)



(b)



(c)

Figure 5.5 ATP data for three biofilter bed depths during the extended biofilter shutdown (a) Top layer (10 cm), (b) Middle layer (50 cm) and (c) Bottom layer (110 cm). Duplicate sand media samples were taken for all samples and the error bars represent the standard deviation from the mean value

While limited literature exists regarding the impact of an extended shutdown on ATP levels, the sole research study found on this subject aligns with the results obtained in this study. Evans et al., 2021 reported in their work that the ATP was reduced during the 2.5-month extended shutdown and mentioned that keeping biofilters submerged during the shutdown might have helped maintain the biomass. Despite the different ATP levels at the onset of the extended shutdown in the present study, a convergence occurred in ATP levels across all three biofilters approximately 4 months into the extended shutdown, after which they recorded similar ATP levels consistently, with almost no decline. This seems to indicate that all the biofilters potentially harboured a resilient microbial community probably adapting to the extended shutdown conditions. The results from the temperature analysis support this hypothesis. Although researchers have previously reported that the microbial activity in terms of ATP is not affected by temperature and no direct relationship was found between temperature and ATP (Pharand et al., 2013, 2014; Rahman, 2013), other researchers have reported that the microbial activity is reduced at lower temperatures and conversely increases at higher temperatures until it reaches an optimum, after which increases in temperature cause a loss of the ability of microbes to degrade contaminants (Lautenschlager et al., 2014; Robinson, 2015). In the present study, as the temperature rise was fairly gradual, there is the possibility of a shift in the microbial community towards organisms, which have their optimum growth at higher temperatures. Further, the differences in the ATP levels between the previously continuously operated biofilters A and B might be attributed to differences in the composition of their microbial communities. Overall, the ATP level trends suggest complex dynamics of factors influencing microbial activity during the extended shutdown, with distinct responses in different biofilter layers and previous operation modes.

The use of ATP as a sole metric provides a measure of viable biomass within the biofilm on the biofilter media. However, to gain a more nuanced understanding of the microbial activity and energy status, the inclusion of ADP and AMP in the analysis was reported useful (Zhao et al., 2022). ATP represents the total cellular energy, but when accompanied by its degradation products, ADP and AMP, it allows for the differentiation between active and dormant microbial cells. AMP, which is the lowest energy state of the adenosine phosphate molecule can be correlated to the mass of dormant cells in a biological sample and high AMP levels might indicate a cellular stress situation. ATP on the other hand can be used to characterize active biomass and high ATP levels can indicate an energy-rich state. Lastly, high ADP can indicate a state wherein there is demand for energy because ATP has been utilized by microbes for cellular activities (Atkinson & Walton, 1967; Chapman et al., 1971). To delve deeper into

the complexity and gain a better understanding of the effects of the extended shutdown, trends in ATP, ADP, and AMP concentrations (all together termed AxP) are discussed below. To the best of the authors knowledge, AxP analysis has not been employed previously during an extended biofilter shutdown.

In Figure 5.6, the plots of AMP, ADP, and ATP values for biofilters A, B, and C provide insights into the biofilters cellular energy components at various stages of the extended shutdown. Note that the ATP data in Figure 5.6 were measured separately from the ones reported in Figure 5.5 and reagents from the AxP kit were used. The ATP, ADP and AMP values were also measured on additional days during the shutdown, the plots for which can be found in Appendix C, Figure C.3.

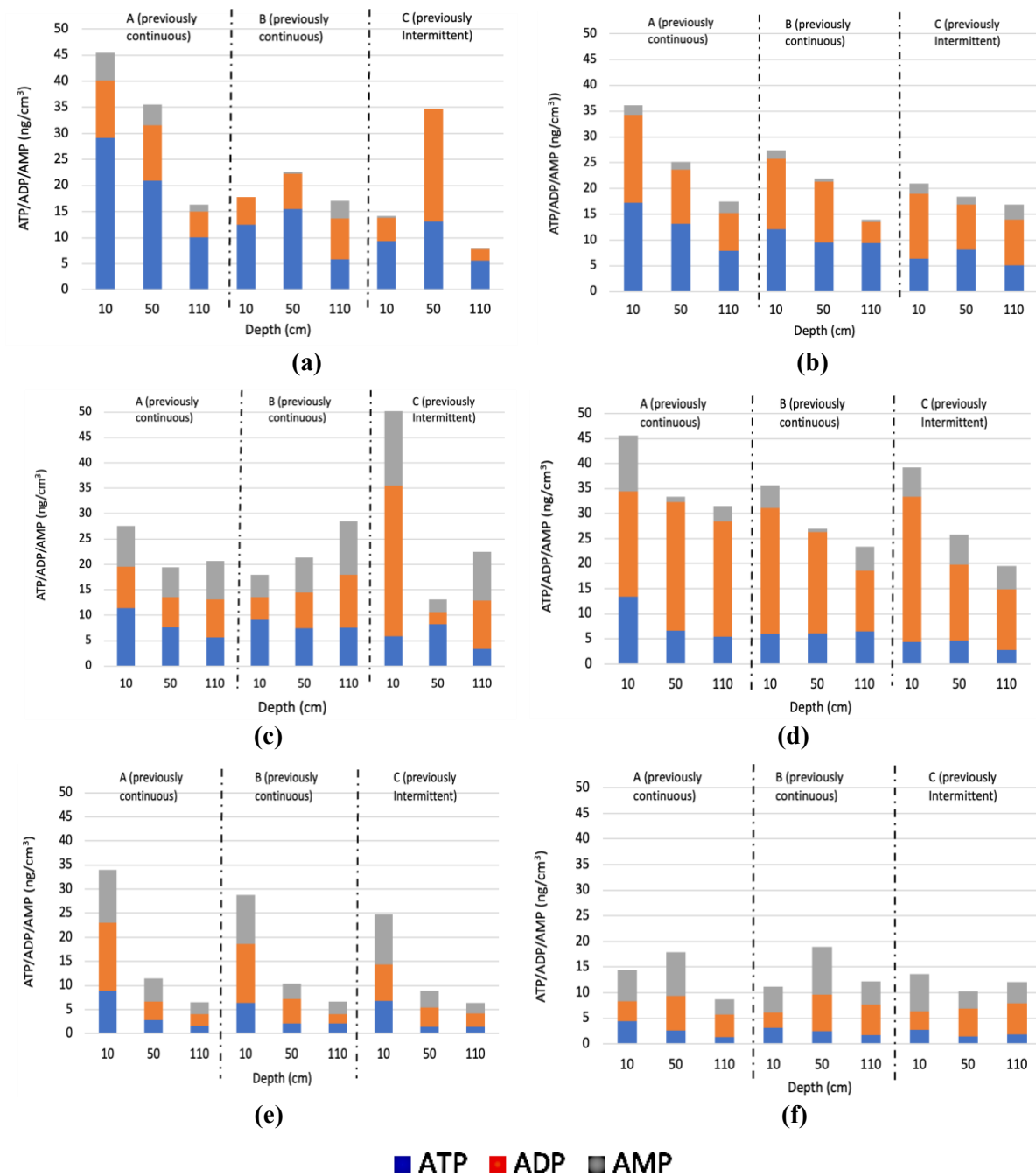


Figure 5.6 AMP, ADP and ATP values for sand media samples from biofilters A, B and C during the extended biofilter shutdown (a) on Day 0 (i.e. the day when the extended shutdown began), (b) Day 35 (c) Day 63, (d) Day 99, (e) Day 134 and (f) Day 203 (last day of extended shutdown)

On Day 0 (Refer to Figure 5.6 (a)), all biofilters at almost all depths exhibited the anticipated pattern with elevated ATP levels, lower ADP and minimal AMP levels. By day 35 (Refer to Figure 5.6 (b)), a shift was observed, as ATP and ADP appeared in almost equal amounts, although AMP levels still remained relatively low. Also, the total amounts of ATP, ADP, and AMP were slightly reduced compared to day 0, with ATP levels depletion ranging from 3% to 41% across different filter bed depths of the three biofilters. Moving on to day 63 (Refer to Figure 5.6 (c)), there was a notable increase in AMP levels, accompanied by substantial reductions in both ADP and ATP. By day 99 (Refer to Figure 5.6 (d)), all biofilters experienced a reduction of over 50% (with respect to Day 0) in their ATP levels, but an unusual spike in the overall quantity was observed, primarily attributed to an exceptionally high ADP amount. Day 134 (Refer to Figure 5.6 (e)), which marked four months into the extended shutdown, witnessed a considerable decrease in the total ATP, ADP, and AMP, with the decrease in the ATP surpassing 67% (with respect to Day 0) for all biofilters, with the highest reduction reaching 89% (with respect to Day 0). Also, ATP and ADP levels were notably low, and the AMP levels were observed to be higher than both ATP and ADP levels. Further, the AMP/ATP ratio was found to be in between 1-10, which might be a signal that the energy status of the cell is compromised and potentially indicating a greater metabolic stress and possible dormancy of the bacteria (Pepper & Brooks, 2021). Further observation from day 134 strongly suggests that, after 4 months into the extended shutdown, inactive microbes have become more predominant than their active counterparts within the biofilters. Finally, on Day 203 (Refer to Figure 5.6 (f)), which marked the conclusion of the extended shutdown, a further reduction in ATP, ADP, and AMP was observed, particularly in the top layer of the biofilter.

Based on the trends observed, a few important inferences can be made. Firstly, the ATP/ADP/AMP levels observed on day 35 suggest that the microbes might have been adjusting their metabolic activities to cope with the changing environmental conditions caused by the shutdown. Further, on day 63, the increased AMP levels indicate a transition towards reduced metabolic activity, which could potentially indicate that ATP was not completely replenished and was being transformed into AMP and ADP. Following on to day 99, when ADP levels rose and the AMP levels went down, the fluctuation from day 63 to day 99 may be explained by changes in the biofilter storage strategies. The transition from SS 4 (biofilters being left submerged in the same water for four weeks without draining and pumping it back into the biofilters – days 35-62) on day 63 to weekly oxygenation of water (as a result of draining biofilters for media sampling and pumping the same water back into the biofilters, SS 3 – days 63-203) could have influenced these changes. Due to a reduction in physiological stress i.e. higher DO levels,

the change in ATP/ADP/AMP concentrations might have occurred (Karl & Holm-Hansen, 1978). Moreover, throughout the extended shutdown period, while ATP values displayed a steady decline, ADP and AMP levels showed fluctuations. On certain occasions, the AMP/ATP ratio was surprisingly low, possibly because of the rise in temperature. The extended shutdown coincided with the summer season, leading to an observed increase in the water temperature of the biofilters (Refer to Figure 5.1). A study by Hooper et al., (2019) reported a decrease in the AMP/ATP ratio with an increase in temperature, providing a potential explanation for the observed variations. Lastly, the substantial decrease in ATP on day 134, followed by continued reduction on day 203 and the comparative rise in the AMP, implies a sustained period of reduced microbial activity. Overall, the levels of ATP, ADP and AMP have declined over the shutdown period. Regarding ADP and AMP levels, no clear trends were evident across filter bed depths, but overall, the distribution of ATP, ADP and AMP seemed to change, with ADP and AMP levels increasing relative to ATP. This observation strongly suggests that with time, inactive microbes had become more predominant than their active ones within the biofilters or microbes were a lot less active overall, indicating a potential nutrient-stressed condition.

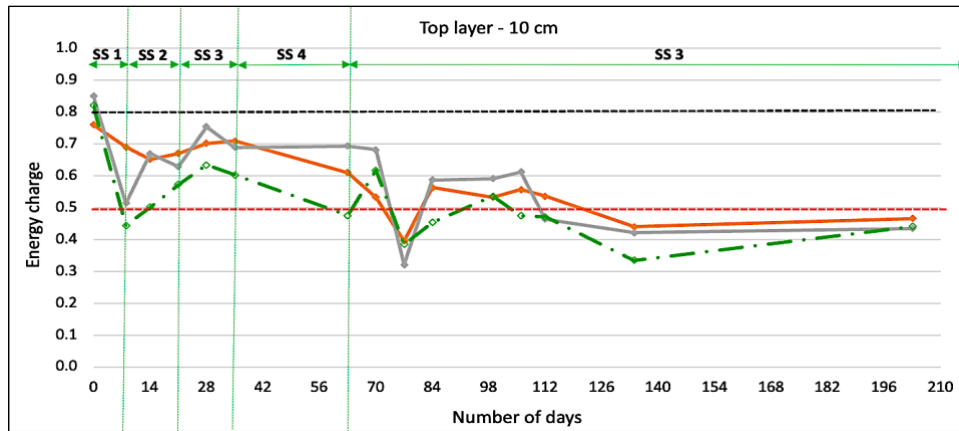
The ratio of ATP, ADP and AMP has been reported to provide a comprehensive view of a biological system's energy status, distinguishing between active, dormant, and stressed microbial populations (Zhao et al., 2022). Further, it has been reported that the ratios of $(AMP + ADP)/ATP$ and AMP/ATP could be a useful indicator of bioactivity with a high value of AMP/ATP signalling that the energy status of the cell is compromised (Hooper et al., 2019). But the most commonly used ratio, the total adenylate energy charge (EC) has been proposed to reflect the energy status of a cell, which enumerates the amount of metabolic energy momentarily stored in the adenine nucleotide pool (Atkinson & Walton, 1967). Its theoretical range is from 0.0 (all AMP) to 1.0 (all ATP) with a low EC quotient (<0.5) suggesting that growth is no longer possible and that cells may be nutrient stressed (Jewson & Dokulil, 1982; Vang, 2013). The interpretations corresponding to the specific range of EC values are elucidated in Table 5.1 (Chapman et al., 1971). Using the ATP, ADP and AMP data, EC was calculated (Refer to section 3.6.2.1), and the corresponding plots can be found in Figure 5.7.

Table 5.1 Interpretation of EC values

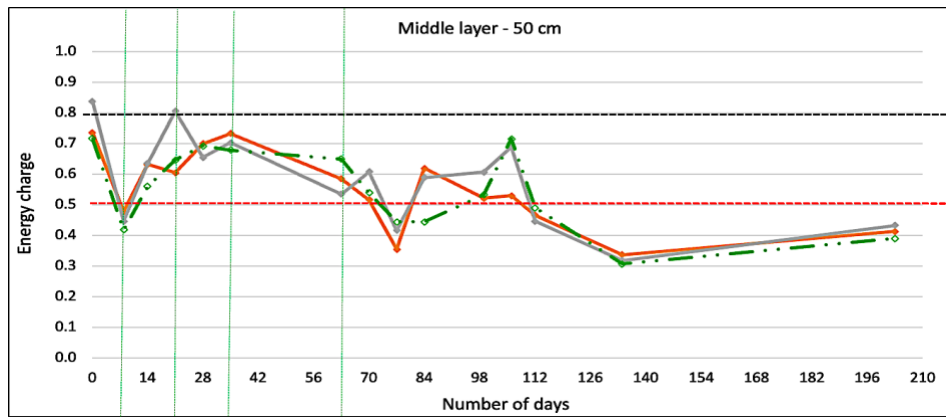
EC	Interpretation
> 0.8	Microorganisms are actively growing
0.5 – 0.8	Microorganisms are viable but not actively growing
< 0.5	Microorganisms are dying or have gone dormant

From the plots (Refer to Figure 5.7(a)), it can be observed that, in the previously intermittently operated biofilter C, the EC values for the top depth were consistently lower than those in the previously continuously operated biofilters up to day 63. However, no substantial difference was seen in the middle and the bottom layers (Refer to Figure 5.7(b) and (c)). Further, after 63 days, no substantial difference was observed among the biofilters in the top layer as well. Within just 8 days of shutdown, the EC fell below 0.8, marking a transition from active to potentially viable but not actively growing microbial status. Also, immediately following backwashing on day 8, EC values were notably low, registering a value below 0.5. In subsequent weeks, a gradual increase in the EC value was observed, bringing the biofilters back into the viable range, potentially influenced by the change in biofilter SS. Note that a backwash was only performed on the 8th day of extended shutdown.

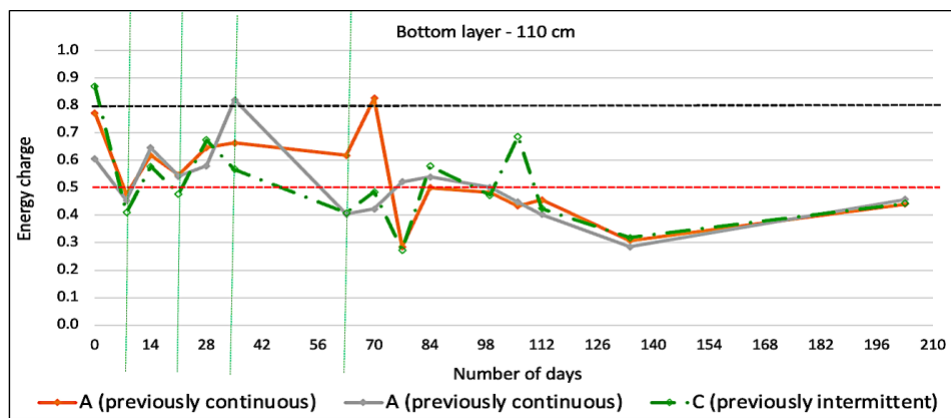
While occasional declines were noted, the majority of layers within all three biofilters remained potentially viable for approximately 112 days, translating to around 4 months, with the EC value recorded between 0.5 and 0.8. Some dips and spikes occurred, but after day 112, EC values for almost all biofilters potentially entered a state of dormancy at all depths (EC below 0.5). The EC values in the bottom layer were observed to be comparatively lower than the top and the middle layers from the beginning of the shutdown, but the state of dormancy seemed to have been reached for all the layers at almost the same time, i.e. after around 112 days of shutdown.



(a)



(b)



(c)

Figure 5.7 EC values for sand media samples from biofilters A, B and C in (a) top layer (10 cm), (b) middle layer (50 cm) and (c) bottom layer (110 cm). Dashed lines are to interpret the status of the biofilters (Refer Table 5.1).

The choice of storage strategies might have played a crucial role in sustaining the biofilters during the extended shutdown, maintaining microbial activity, and influencing EC values. It was observed that, between day 8 and day 28, the weekly renewal of biofilters with fresh raw water (employing storage strategies 1 and 2) resulted in an increase in EC values, effectively maintaining them within the viable range. However, from day 35 to day 63, a month-long storage in the same water without replenishment (implementing SS 4) led to a significant decline in EC values across most biofilter depths (ranging from 5% to 42%). This decline was expected, as the depleting DO levels might have led to an oxygen-stressed situation in the biofilters, which might have impacted the microorganisms in the biofilm. Under such conditions, microorganisms might have prioritized existence over growth, potentially leading to a decrease in the EC value, with ADP and AMP levels surpassing ATP levels. A substantial drop in EC was observed on day 77. It is unclear why it happened, though it might hypothetically be a delayed impact linked to the change in SS. Post day 63, reintroducing the same water weekly (employing SS 3) resulted in a gradual but sustained decrease in EC values within the viable range, highlighting the effectiveness of this strategy in maintaining a slow rate of microbial activity decline. In summary, while confirmation remains elusive, the implementation of SS 3 demonstrated potential benefits, likely contributing to the biofilter's ability to maintain viability during the extended shutdown. Although storage strategies 1 and 2 also showed benefits, their scalability to full operation is uncertain. Therefore, among the various storage strategies, SS 3 emerges as the most effective option, wherein the biofilters were kept submerged in the same water, but, on a weekly basis, this water was drained from the columns and pumped back into the columns.

5.2.3 Culture-Based Microbiological Analysis Results

The culture-based microbiological analysis was performed at Wilfrid Laurier University by Nicholas Lamba-Rautapuro, an MSc student.

5.2.3.1 Occurrence of Manganese Oxidizing Bacteria (MnOB)

Three different culture medium types were used to measure culturable cell counts of bacteria isolated from the sand media from the biofilters, but only the data from the R2A + Mn culture media are discussed in detail. The results from the other two media types can be found in Appendix C, Figure C.4. As observed in Figure 5.8, from 4.5 months to 6 months, no substantial variation in overall microbial counts was observed during the extended shutdown for biofilters A and B, while biofilter C showed some decrease in microbial cell counts. All biofilters at all depths yielded viable, culturable

bacteria with similar cell counts. Also, it was found that bacteria were able to grow on the Mn min culture medium, which consisted only of Mn, indicating the presence of MnOB (Refer to Figure C.3). Furthermore, no substantial differences in cell counts were observed with biofilter depth for Mn min culture media results as well (Refer to Figure 5.8, Figure C.4). No correlation between former operational conditions (from phase 1, when biofilters A and B were operated continuously and biofilter C was operated intermittently for 6 h/day) and cell count was observed as all three biofilters had culturable cell counts in the same range. However, the decrease in culturable cell counts in biofilter C from 4.5 to 6 months was an exception. Note that the CFU levels recorded in another study, examining intermittently operated biofilters that were acclimated in similar operating conditions, fell within a similar range as those observed in this study (Arora, 2024). This confirmed the viability of the microorganisms.

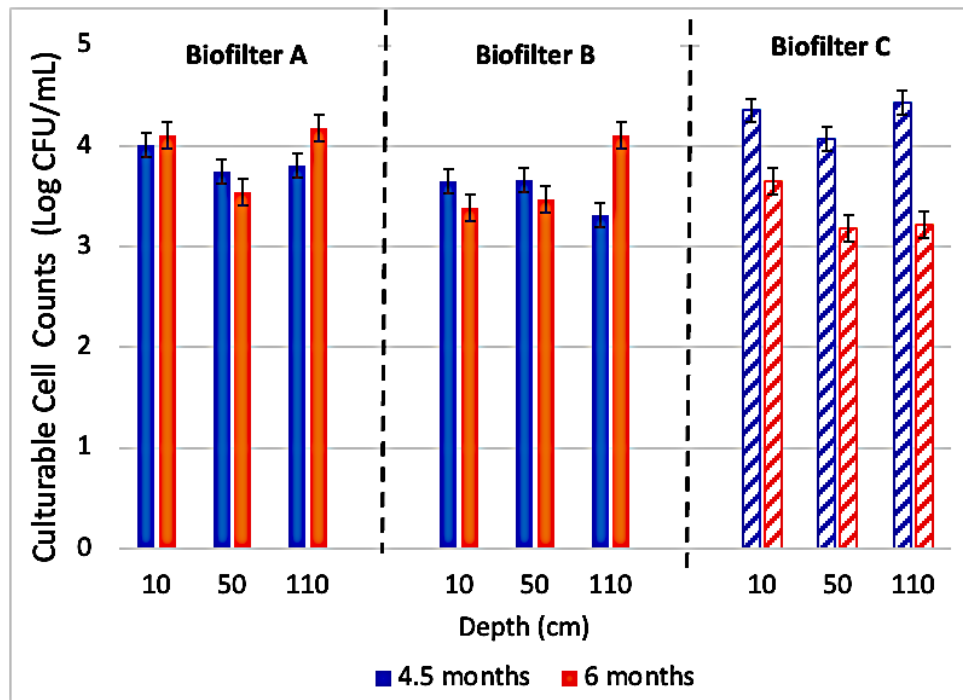


Figure 5.8 Culturable cell counts of bacteria isolated from sand media samples from three different biofilters after plating on R2A + Mn media, at three different depths (10, 50 and 110 cm), over the course of two sampling events during the extended shutdown (4.5 (day 134) and 6 months (day 203)). Prior to the shutdown, biofilters A and B: operated continuously for 24 h per day, while biofilter C operated intermittently for 6 h a day.

No definitive trends regarding log CFU/mL and filter age, filter depth or former operational conditions were observed. This may be attributed to either microbial diversity or insufficient data since sampling and analysis were done just twice during the extended shutdown. Microbial complexity may be attributed to the possibility that while some microorganisms may have struggled to survive in the low nutrient conditions present during the shutdown certain microorganisms might have thrived (Hirsch, 1986). Further, additional data points, particularly when EC values were above 0.5 during the extended shutdown period, would have provided valuable insights. Overall, viable, culturable cell counts at all depths suggest the possibility that despite the extended shutdown, the bacteria present on the biofilter media remained viable even after 6 months of shutdown.

5.2.3.2 Leucoberbelin Blue (LBB) Results Confirming the Presence Of MnOB

While culturable cell counts provided initial insights into the potential viability of microbes on the biofilter media during an extended shutdown, the LBB analysis was deemed necessary to ascertain whether the isolated bacteria were oxidizing Mn. Adding manganous sulphate to culture media, as was done for all media in 5.2.3.1, did not always result in plated microbial colonies turning black as a result of Mn oxide production, which was expected and was reported in the study done by (Burger et al., 2008). As a result, colonies on the culture plates could not be discerned as MnOB or non-MnOB solely through phenotypical observation. Hence, LBB solution was applied to individual colonies on each plate as a confirmatory step. After the addition of LBB, colonies that were not already black in appearance, but rather beige, turned blue (after reacting with oxidized Mn). Positive blue colourations observed after the application of LBB solution (Refer to Figure 5.9) suggest the possibility that MnOB were present within the biofilters despite the extended shutdown, confirming the persistence of MnOB. It is important to note that, positive blue colouration did not necessarily mean that these microorganisms were oxidizing Mn in the biofilters as they are usually facultative Mn oxidizers. Figure 5.9 shows the results from just one culture plate, more photos (confirming the presence of MnOB at different depths in all biofilters) can be seen in Appendix C, Figure C.5.

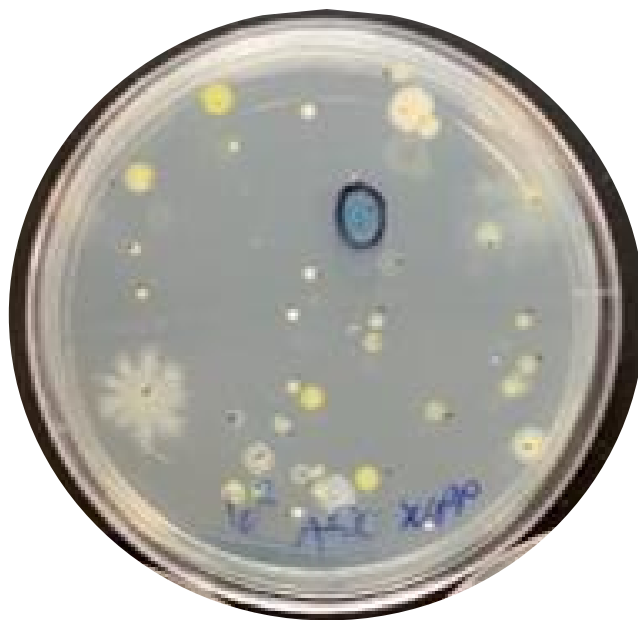


Figure 5.9 LBB-confirmed plate, illustrating LBB stained blue colony and phenotypic diversity

In Table 5.2, the results from LBB assay for R2A + Mn culture media plates are tabulated. The results from the other two media types can be found in Appendix C, Table C.1 and C.2. A slight decrease in the presence of LBB-confirmed MnOB over time can be seen. LBB confirmation of MnOB was observed in 4 out of 9 samples after 4.5 months of shutdown, with all three biofilters indicating some Mn oxidation. But fewer samples (3/9) were confirmed after 6 months of shutdown, with biofilter B media plates not indicating Mn oxidation at any depth. Overall, despite the extended shutdown, the microbial counts showed no substantial variation and LBB confirmed that MnOB might be able to persist and actively oxidize Mn in the biofilters.

Table 5.2 LBB assay results for R2A + Mn media plates from 5.2.3.1 confirming presence of MnOB

Samples collection	Biofilter	Depth		
		10 cm	50 cm	110 cm
4.5 months since shutdown commenced	A	(-)	(+)	(+)
	B	(-)	(-)	(+)
	C	(-)	(-)	(++)
6 months since shutdown commenced	A	(-)	(-)	(++)
	B	(-)	(-)	(-)
	C	(++)	(-)	(+++)

* The symbol “(-)” indicates a negative test result, while “+” represents weak blue colouration, “++” represents moderate blue colouration and “+++” represents strong blue colouration.

5.2.3.3 Community Level Physiological Profile (CLPP) Analysis Results

LBB helped in confirming cultured MnOB, but MnOB constitutes simply a fraction of the total microbial population in the biofilters. CLPP facilitates the generation of a functional and metabolic profile of the entire microbial community, thereby providing an in-depth examination of the health and dynamics of the microbial system as a whole. The significance of CLPP lies in the fact that bacteria do not exist in isolation. By assessing how microorganisms utilize different carbon sources and gauging their overall metabolic activity, CLPP has been known to offer insights into the functional diversity and vitality of the microbial community. In CLPP analysis, the overall metabolic activity of the microbial community is quantified by measuring the colour development in media samples containing different carbon sources. While average well colour development (AWCD) values represent the average colour change, specific average well colour development (SAWCD) values normalize this value based on the number of carbon sources used, providing a more specific measure of the metabolic efficiency per substrate (Garland, 2006; Weber & Legge, 2010, 2011). Only the AWCD results are discussed here.

As observed in Figure 5.10, AWCD decreased from 4.5 months to 6 months of extended shutdown, within all biofilters at all depths, except for biofilter C at 10 cm depth, where it remained almost similar. Since AWCD is used to estimate the overall metabolic rate, decreases in AWCD are related to lower metabolic activity within the system (Morrison, 2014). During the extended shutdown, bacteria may have been subjected to lower nutrient conditions. Under low-nutrient conditions, bacteria might have prioritized survival over quick reproduction, thereby resulting in lowering metabolic activity. The

AWCD results also support the reduction in ATP values seen during the extended shutdown. Overall, despite low nutrient conditions during the extended shutdown, diverse microbial metabolism took place, indicating that the biofilter media biofilms contained bacteria that can metabolize a breadth of substrates from varying carbon guilds.

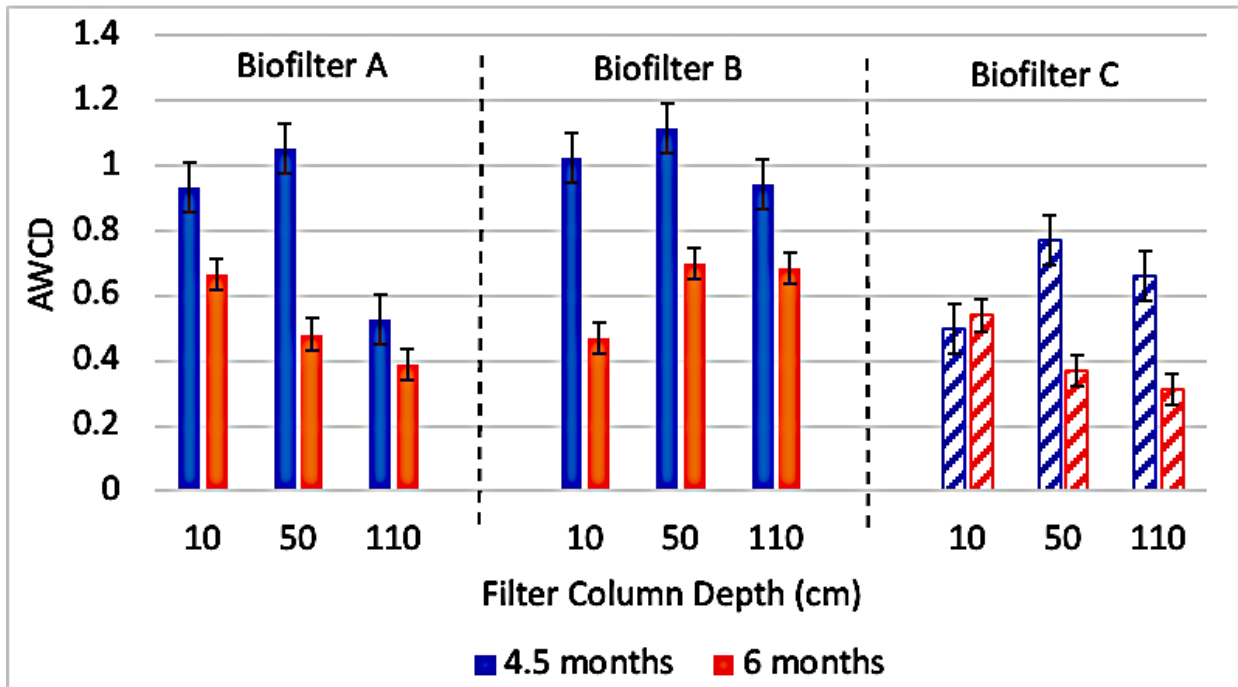


Figure 5.10 Average Well Colour Development (AWCD) derived after 96 h of incubation on Biolog Ecoplates.

5.3 Conclusions

The main objective of this phase of the research project was to study the effects of an extended shutdown on the microbial activity of biofilters that were operated continuously and intermittently prior to the extended shutdown. This phase was incorporated into the research project in response to an unexpected shutdown of the groundwater well feeding the pilot plant. Extended shutdowns, such as the one encountered, can happen unexpectedly and can extend for prolonged, undefined timeframes. Thus, while this area was found to be relatively unexplored, it holds significant importance for treatment utilities relying on biofiltration for water treatment. During this extended shutdown, the basic water quality parameters were closely monitored along with some microbiological parameters and four

different storage strategies were investigated. The major findings from this phase based on the results obtained are:

1. Overall, the DO in all the biofilters decreased over time up until SS 3 was employed, where the weekly water circulation process likely played a crucial role in reoxygenating the water, reducing the risk of steady DO depletion and, consequently, helped prevent the biofilters from entering an oxygen-stressed state.
2. ORP remained stable in all biofilters until day 100 of the extended shutdown, and slightly declined thereafter in both the top and bottom layers, with somewhat more pronounced reductions observed in the top layer. Amongst all the biofilters, biofilter C exhibited more pronounced reduction. Also, unlike DO, the storage strategies did not appear to have a substantial impact on ORP.
3. A notable reduction in ATP levels in the biofilm attached to the biofilter media, reaching up to approximately 84%, was evident in the top and middle layers of the biofilters during the extended shutdown period, while the ATP levels in the bottom layer remained consistently low, with minimal decline throughout the extended shutdown.
4. The previously intermittently operated biofilter C exhibited lower ATP values compared to the previously continuously operated biofilters, particularly biofilter A until day 112 (about 4 months into shutdown), but after that the ATP values for all the became similar. Differences in ATP values were also observed between biofilters A and B, although they were operated identically before the extended shutdown.
5. Overall, the distribution of ATP, ADP and AMP changed during the extended shutdown with ADP and AMP levels increasing relative to ATP. The nucleotide levels indicated that the microbes might have been adjusting their metabolic activities to cope with the changing environmental conditions caused by the extended shutdown.
6. The biofilm on the majority of the biofilter media layers within all three biofilters remained potentially viable for approximately 112 days, translating to around 4 months, with EC values recorded between 0.5 and 0.8. Reintroducing the same water weekly (employing SS 3) might have possibly resulted in a less pronounced decrease in EC values, highlighting the effectiveness of this strategy in maintaining a slow rate of microbial activity decline. After day 112, EC values for almost all biofilters might have entered a state of dormancy at all depths (EC below 0.5).
7. The presence of MnOB was confirmed by using culture-based analysis methods combined with the LBB assay. No substantial variation in overall microbial counts was observed during the extended

shutdown and viable, culturable cell counts at all depths suggested that despite the extended shutdown, the bacteria present on the filter media remained viable.

These findings underscore the fact that the choice of storage strategies might have played a crucial role in sustaining the biofilters during the extended shutdown, maintaining microbial activity, and influencing EC values. Additionally, the study highlights that microbes stayed viable in environments with low nutrient levels, by adapting to these conditions.

Chapter 6

Performance of Intermittently and Continuously Operated Biofilters for Manganese Removal from Groundwater after a Prolonged Shutdown

6.1 Overview

This chapter includes the results and discussion of the research phase, wherein the performance of continuously and intermittently operated biofilters was investigated after a six-month extended shutdown. This phase commenced in the last week of September 2023 and extended over a duration of almost three months. The sub-objectives of this phase were:

- ✓ Assess time required for intermittently and continuously operated biofilters to reach a pseudo-steady state after an extended shutdown
- ✓ Evaluate biofilter performance at different EBCTs by monitoring Mn and Fe removal in depth-wise zones of the continuously and intermittently operated biofilters
- ✓ Investigate the impact of intermittent shutdowns on biofilter performance and microbial activity
- ✓ Ascertain the ability of biofilters to mitigate turbidity

In phase 3 (post-extended shutdown) the biofilters were operated using the same approach as in phase 1, i.e. the biofilter start-up phase. Specifically, two biofilters (A and B) were continuously operated around the clock (24 h/day), while one biofilter (C) followed an intermittent operation schedule (6 h/day). Furthermore, during the 18-hour intermittent shutdown period, to ensure its integrity, the intermittently operated biofilter C was kept submerged in water.

6.2 Results and Discussion

6.2.1 Feed Water Quality

Feed water quality parameters measured during phase 3 (post-extended shutdown) are presented in Table 6.1. The recorded minimum concentrations for Mn and Fe were 18.6 µg/L and 24.2 µg/L,

respectively, while the maximum concentrations reached 26.7 µg/L for Mn and 76.4 µg/L for Fe. The average Mn and Fe concentrations in the influent were 23.5 µg/L and 50.4 µg/L, respectively. Although Mn concentrations were relatively low, they still exceeded the AO (20 µg/L), creating a need for treatment. During phase 1 i.e. the biofilter start-up phase (Refer to table 4.1 and 4.2), the average Mn and Fe concentrations in the influent were 30.5 µg/L and 96.7 µg/L respectively, which in comparison to phase 3 (post extended shutdown), were higher. The possible reason for these differences might be the maintenance work that was conducted at the well during the shutdown period.

The TOC was found to be in the range of 0.7 mg C/L, which was similar to concentrations recorded during phase 1 (average 0.8 mg-C/L). Turbidity ranged from 0.46 NTU to 2.58 NTU, with an average value of 1.28 NTU. This was similar to the trends observed during phase 1, the start-up phase. The pH was found to be in the range of 7.43-7.67, which was appropriate for Mn and Fe oxidizing bacteria (Mouchet, 1992). The temperature of the raw water ranged from 10-13 °C. Further, the conductivity was recorded in the range of 583-708 µS/cm. The nitrate concentration suggested that there was a good amount of nutrients present in the feed water for biofilm formation and the low amount of ammonia should not be harmful to biofilms.

Table 6.1 Feedwater* quality data measured in phase 3

Parameter	Mn (µg/L)	Fe (µg/L)	TOC (mg C/L)	Turbidity (NTU)	pH	Conductivity (µS/cm)	Temp. (°C)	Ammonia (mg-N/L)	Nitrate (mg-N/L)
Minimum value	18.6	24.2	0.69	0.46	7.43	583	10.1	0.04	2.1
Maximum value	26.7	76.4	0.71	2.85	7.67	708	13.4	0.29	2.7
Average value	23.5	50.4	0.70	1.28	7.54	656	11.3	0.17	2.5
S.D	1.9	15.5	0.01	0.96	0.08	39	0.8	0.11	0.3
# sampling	28	28	4	9	20	20	20	4	4

*Feedwater was sourced directly from the groundwater well

6.2.2 Biofilter Performance After Restart Following the Extended Shutdown

The aim was to achieve effective Mn removal and meet both, aesthetic, and treatment objectives set by Health Canada for Mn. The AO for Mn removal was set at 20 µg/L, while the treatment objective is 15 µg/L (Health Canada, 2022). Figure 6.1 depicts the progress of Mn removal over time in phase 3 i.e. after the prolonged shutdown and the data can be found in appendix table D1. Noteworthy trends were observed in the Mn removal performance of the biofilters during the phase 3. Initially, just after restart, none of the biofilters demonstrated immediate effectiveness in removing Mn and the removals were minimal. However, within 20 days, all biofilters successfully achieved Mn removals below the treatment objective limit, although the intermittently operated biofilter C exhibited slightly lower removals compared to the continuously operated biofilters A and B. Further, a month after the restart (precisely day 34), the intermittently operated biofilter C consistently removed an equivalent amount of Mn compared to the continuously operated biofilters A and B. Pseudo-steady state conditions were reached within 34 days after the restart for all three biofilters, and the removals by that time were >95%. Notably, in contrast to phase 1, the startup phase where biofilter B and biofilter A, despite similar operation, exhibited differences in removals, their behaviors aligned closely after the restart.

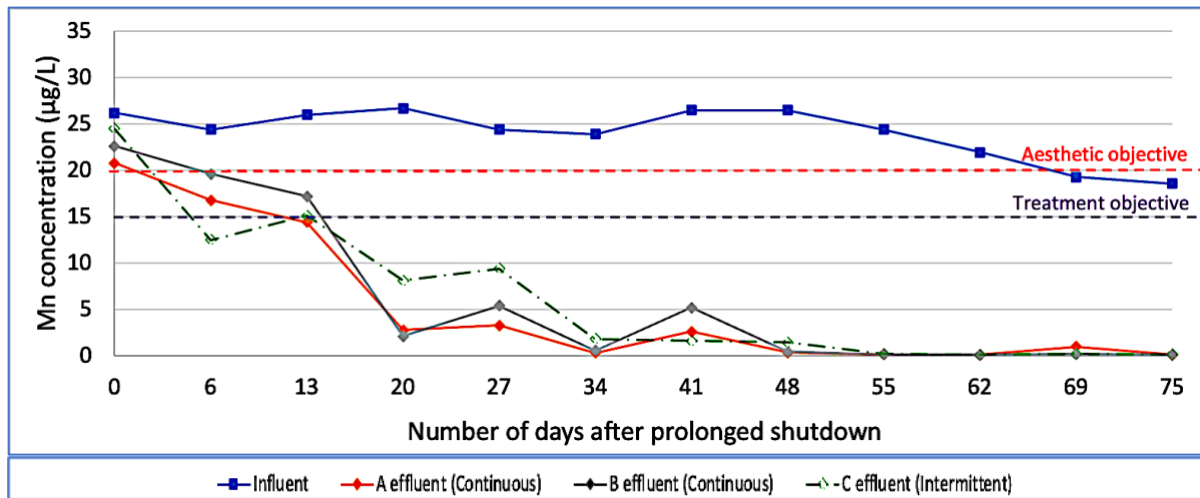
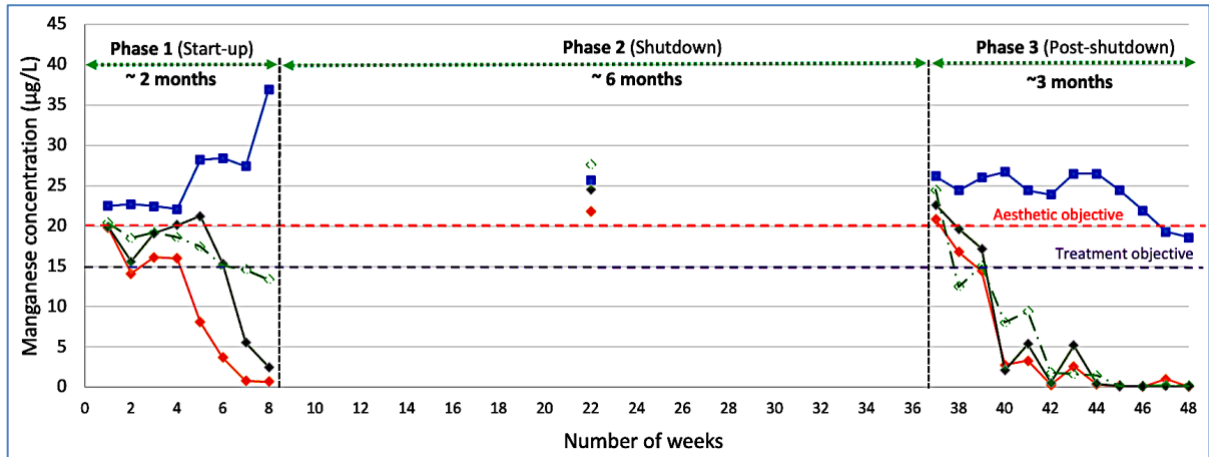


Figure 6.1 Mn concentrations in pilot biofilter influent and effluents over time (Data from Sept. 28, 2023 = Day 0 to Dec. 13, 2023 = Day 75)

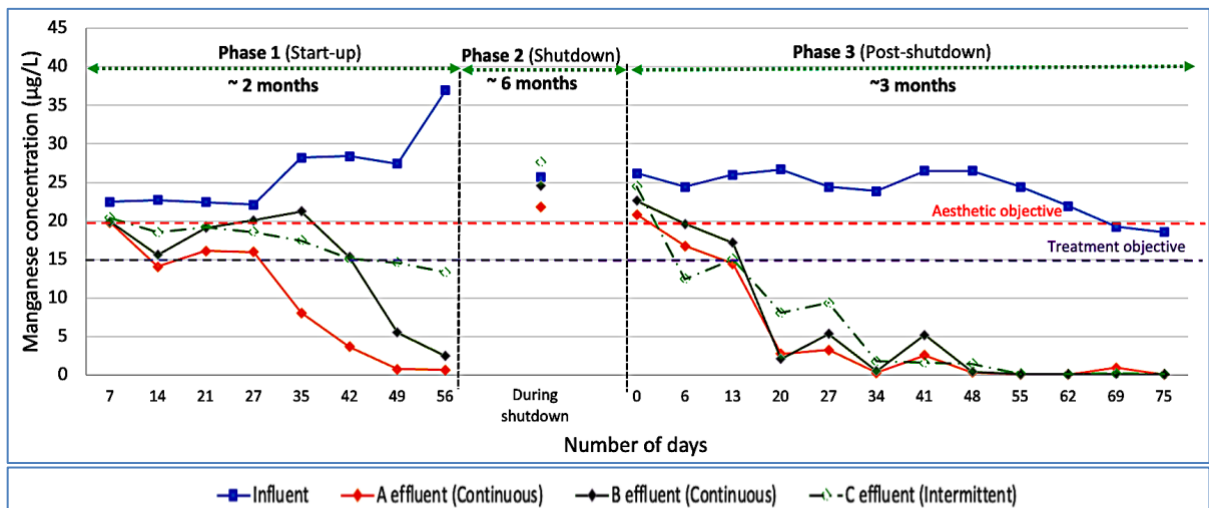
As seen in Figure 6.2, compared to Phase 1, notable improvements in Mn removal efficiency were observed for all three biofilters during the phase 3. All three biofilters showed quicker and higher Mn removals. Specifically, the continuously operated biofilters A and B exhibited a quick increase in Mn removals. While during the phase 1, over 90% Mn removal was achieved by day 56 (week 8) for the

continuous biofilters A and B, in phase 3, this level of removal was attained from as early as 20 days onwards (Refer to Figure 6.2(b)). Further, when comparing the performance of the intermittently operated biofilter C, substantial enhancements in Mn removal performance were observed in phase 3 compared to phase 1. It was found that the intermittently operated biofilter C was able to consistently remove equivalent amounts of Mn compared to the continuously operated biofilters A and B within a month after restart, whereas during phase 1, even after 56 days (week 8) of operation, the removals recorded by the intermittently operated biofilter C was significantly lower than those for the continuously operated biofilter A. While about 65% of Mn was removed after 56 days of operation during Phase 1 by the intermittent biofilter C, within just 20 days of operation in phase 3 (post-shutdown), approximately 69% Mn removal was achieved. The Mn removals exceeded 95% from 34 days onwards for all biofilters, indicating a substantial improvement in efficiency compared to phase 1.

Several factors likely contributed to the observed trends in Mn removal in phase 3. During phase 1, biofilters were undergoing acclimation and were establishing microbial communities likely including MnOBs. This phase was followed by the extended shutdown period, when the microbial activity was affected, but it was observed that the microorganisms remained viable during the shutdown (Refer to sections 5.2.2 and 5.2.3), although growth was restricted. In phase 3, the presence of a mature and active microbial community might have facilitated the rapid oxidation of soluble Mn^{2+} to insoluble MnOx , enhancing overall Mn removal. The swift response observed in Mn removals suggests that the microbial community was not only sustained during the extended shutdown but also quickly became active after restart, highlighting its resilience and adaptability to the famine situation caused by the extended shutdown. Also, the stability of the biofilm on the media surface within the biofilters may have improved over time. This might have provided a favourable environment for MnOB colonization and activity and thereby would have promoted consistent Mn removal efficiency.



(a)



(b)

Figure 6.2 Mn concentrations in feedwater and pilot biofilter effluents from phase 1-3 (Jan.- Dec. '23) (a) actual timeline and data from Jan. 11, 2023 = Week 0 to Dec. 13, 2023 = Week 48 (b) 6-month shutdown timeframe shrunk and data from Jan. 11, 2023 to Dec. 13, 2023. In phase 1, day 7= Jan. 18, 2023 and day 56 = Mar. 8, 2023 and in phase 3, day 0 = Sep. 28, 2023 and Day 75 = Dec. 13, 2023

Apart from Mn, Fe removal performance of the biofilters was also investigated. Figure 6.3 depicts the progress of Fe removal over time and the data can be found in Appendix Table D2. The results revealed that all biofilters removed Fe immediately after restart and reached a steady state. The continuously operated biofilters A and B demonstrated excellent Fe removal capabilities, achieving an

average removal rate of around 84%, with an unusual, reduced removal on day 41, when the removal for biofilter A was 69% and that for B was just 50%. On the other hand, the intermittently operated biofilter C exhibited consistent Fe removals, with an average of approximately 77%. The unusual variation in the removals was not observed on day 41 for the intermittently operated biofilter. Moreover, as seen in Figure 6.4, Fe removal efficiency was similar before and after the shutdown phase for all three biofilters. During phase 1, the intermittent biofilter C removals were significantly less than the continuous biofilters A and B removals. This has improved after the shutdown in that this difference has become even smaller.

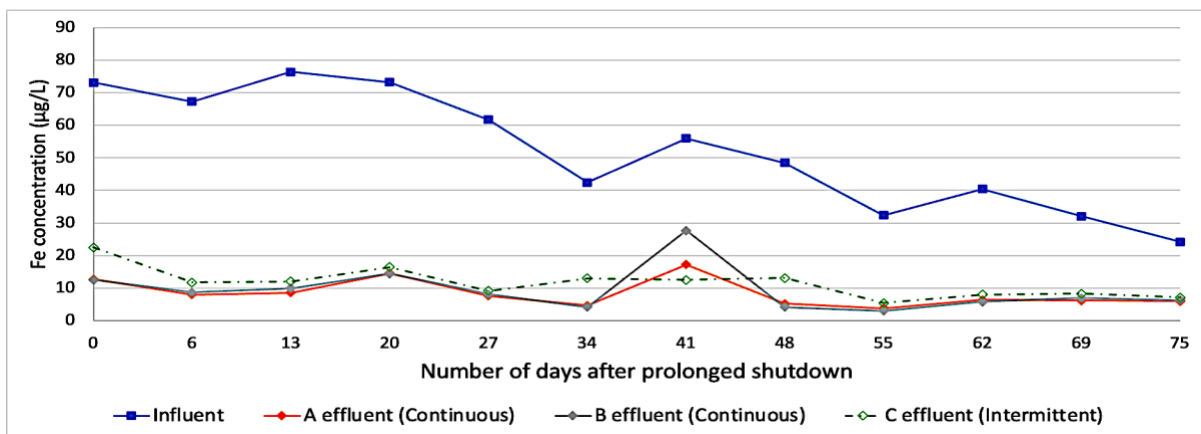


Figure 6.3 Fe concentrations in pilot biofilter influent and effluents over time (Data from Sep. 28, 2023 = Day 0 to Dec. 13, 2023 = Day 75)

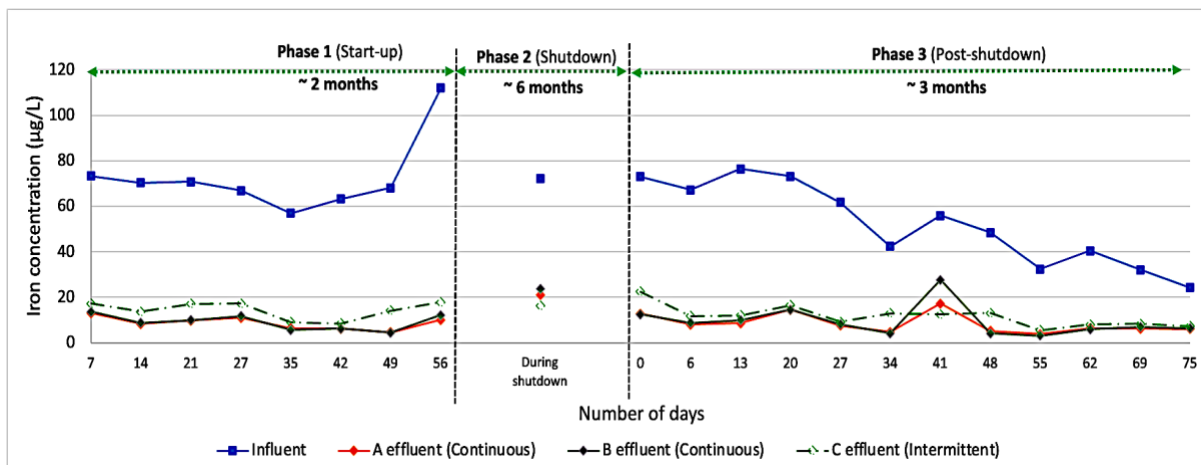


Figure 6.4 Fe concentrations in biofilter influent and effluent for all 3 phases (Jan.-Dec. '23) with 6-month shutdown timeframe shrunk

Interestingly, all biofilters reached a steady state quickly after restart (i.e. day 0), which indicates that the biofilters adapted to the treatment conditions and established consistent Fe removal, regardless of the operation mode and the extended shutdown. Further, even during the shutdown, substantial Fe removal was observed (Refer to Figure 6.4). The possible reason behind this performance may be attributed to the predominant removal mechanism for Fe. Based on how quickly the biofilters showed substantial removal, the combination of chemical oxidation and filtration processes might be considered as the dominant mechanism. Aeration, along with conditions in the influent tank, may have triggered the chemical precipitation of Fe, contributing to its removal in the biofilters.

A t-test was conducted to determine the significance of the difference in performance between the continuously and intermittently operated biofilters regarding the effluent concentrations of Mn and Fe. F-test was performed to confirm whether the assumption of equal variance is valid before performing the t-test. The statistical analysis results can be found in Appendix Table D3-D7. For Mn, as seen in Table 6.2, biofilter C, which was operated intermittently, did not exhibit a statistically significant difference in its removals in comparison with biofilters A and B which were operated continuously. Further, for Fe, as seen in Table 6.3, biofilters A and B, which were operated continuously, did not exhibit a statistically significant difference in their removal rates. Biofilter C, which was operated intermittently, showed a statistically significant difference in comparison with biofilter A but not B.

Table 6.2 t-test to compare the effluent Mn concentration of continuously and intermittently operated biofilters

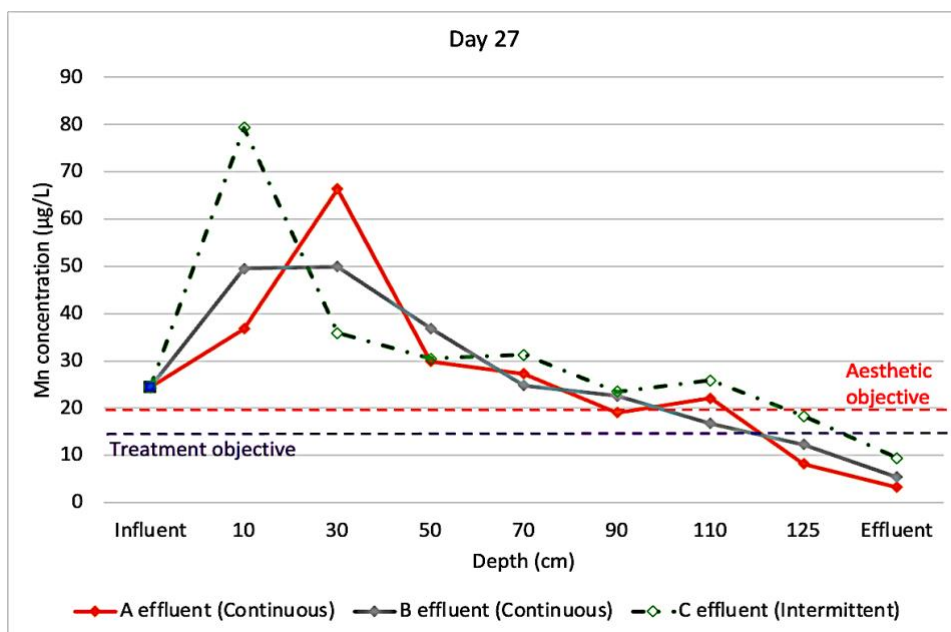
Effluent concentration compared	F value	t value	P value	Result
A (continuous) and B (continuous)	0.771	0.274	0.393	Not significant
A (continuous) and C (intermittent)	0.939	0.324	0.375	Not significant
B (continuous) and C (intermittent)	1.217	0.032	0.487	Not significant

Table 6.3 t-test to compare the effluent Fe concentration of continuously and intermittently operated biofilters

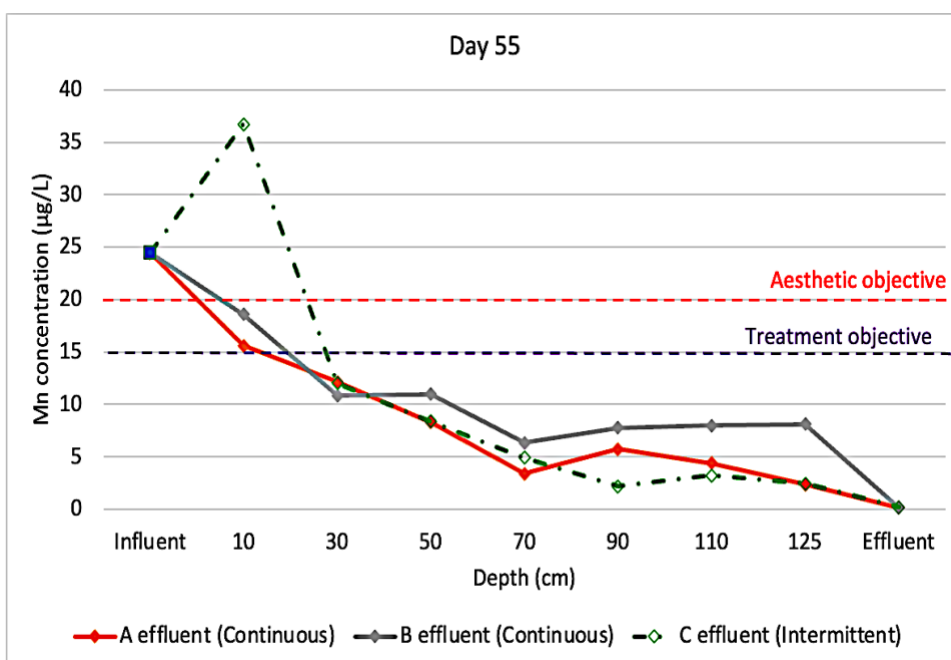
Effluent concentration compared	F value	t value	P value	Result
A (continuous) and B (continuous)	0.317	0.411	0.343	Not significant
A (continuous) and C (intermittent)	1.155	2.559	0.009	Significant
B (continuous) and C (intermittent)	3.642	1.326	0.099	Not significant

6.2.3 Mn And Fe Removal in Depth-Wise Zones of the Biofilter

To discern the regions where Mn and Fe removal predominated during phase 3, this study measured Mn concentrations along the biofilter bed. This investigation allowed for a more comprehensive understanding of the factors influencing Mn removal within the biofilters. Figure 6.5(a) illustrates the Mn concentrations at different depths of the biofilter bed recorded on the 27th day of operation after prolonged shutdown and the data can be found in Appendix Table D8. Samples from different biofilter depths were also collected on the 55th day of operation after prolonged shutdown, and the corresponding plots can be found in Figure 6.5(b) and the data can be found in Appendix Table D9. On the 27th day, the biofilters A, B and C were performing at 87%, 78% and 62% Mn removals respectively, with biofilters A, B and C showing improved performance and moving toward a steady state (Refer to Figure 6.1). On the 55th day, all biofilters were performing at more than 99% Mn removal rates, and all of them had reached a steady state. In Figure 6.5, on day 27, the intermittently operated biofilter C had slightly higher Mn concentrations from about 70 cm onwards compared to the continuous biofilters A and B, whereas on day 55, Mn concentrations for the intermittent biofilter C were similar to those for the continuous biofilters A and B from about 30 cm onwards. Further, on day 27, it can be seen that all biofilters showed an increase in Mn concentrations at 10 and 30 cm bed depth and the concentrations declined with depth thereafter with the majority of the Mn removal appearing to be happening in the bottom layers. Accumulation of manganese oxide within the top layer might explain this, but further research is required to confirm this hypothesis. Similar observations were also made during phase 1, indicating a phenomenon, which is not completely understood. Interestingly, this phenomenon was only observed for intermittent biofilter C on day 55 and not the continuous biofilters A and B. On day 55, more than half of the Mn removal occurred within the top 30 cm of the media in all biofilters and the remainder of Mn removal happened in the middle, within 70 cm. When compared to phase 1, wherein the majority of the removal was observed in the middle layer (30-70 cm) and less removal was observed in the top layer (0-30 cm), it can be inferred that there was a shift in the Mn removal zones from the middle to the top after the prolonged shut-down, which is in-line with what was found in the literature (Abu Hasan et al., 2012; Cheng et al., 2017; Han et al., 2013; Yang et al., 2014).



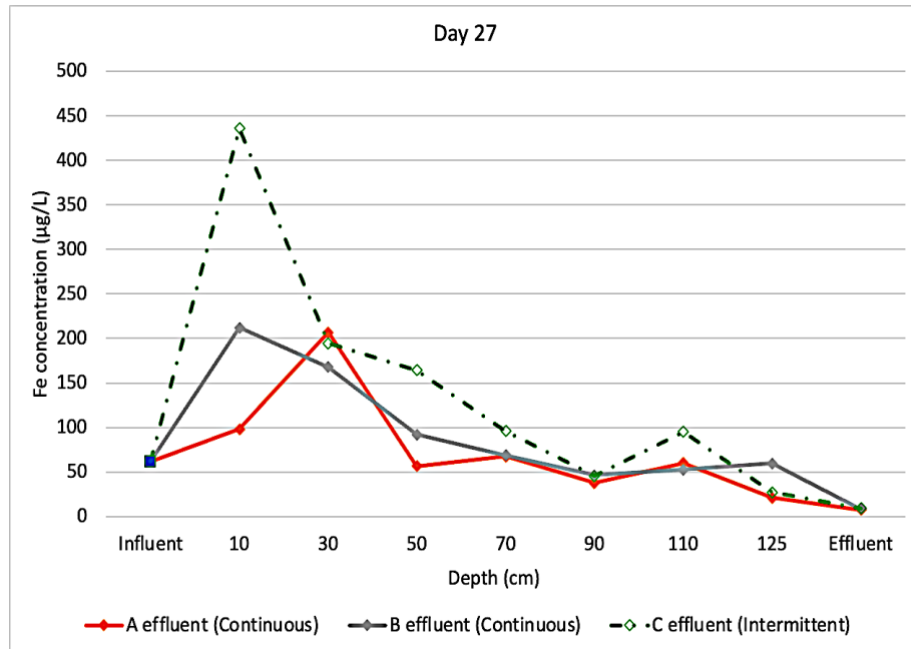
(a)



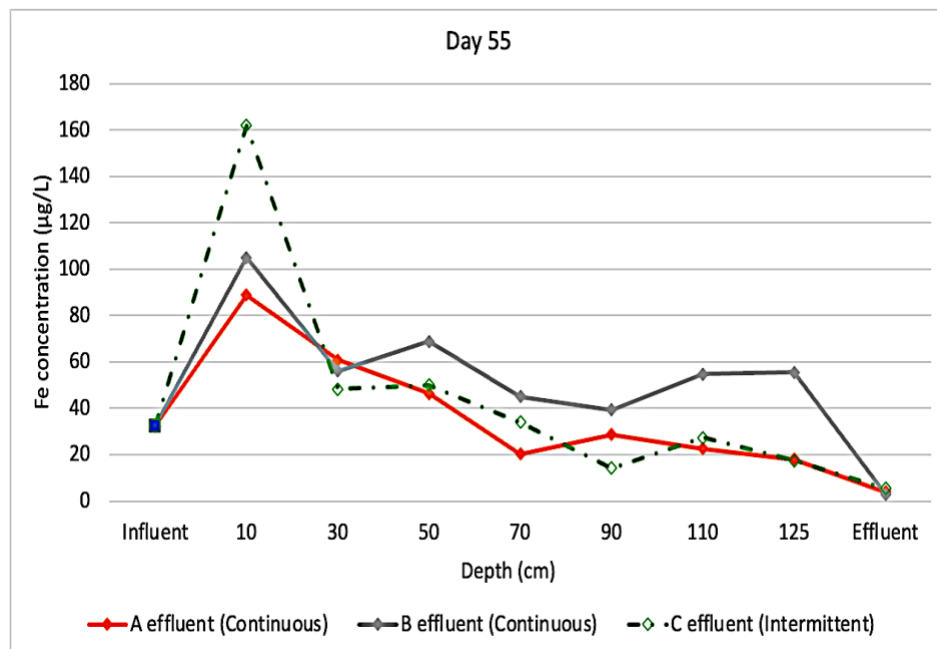
(b)

Figure 6.5 Mn concentrations across various biofilter bed depths on day 27 and 55 after the extended shut-down. Note that the y-axis in (a) is different from (b) to see the trends more clearly

Apart from Mn, Fe removal at different biofilter depths was also investigated. Figure 6.6 illustrates the Fe concentrations at different depths of the biofilter bed recorded on the 27th day and 55th days of operation after the prolonged shutdown. On the 27th day, biofilters A, B and C were performing at 88%, 87% and 85% Fe removal rates respectively, and all of them had reached a steady state (Refer to Figure 6.3). On the 55th day, the biofilters A, B and C were performing at 88%, 91% and 83% Fe removal rates respectively, and all of them had reached a steady state. In Figure 6.6 (a), it can be seen that on day 27, all biofilters initially showed an increase in Fe concentrations. Notably, the initial increase in concentrations at 10 cm bed depth was more pronounced for the intermittently operated biofilter C compared to the continuously operated biofilters A and B, and thereafter the Fe concentrations appeared to decline continuously in all biofilters. An increase in Fe concentrations in the top layers was also observed during phase 1 and it can potentially be due to accumulation of Fe in that region. The intermittently operated biofilter C also had higher Fe concentrations at 50, 70 and 110 cm bed depth. On day 55 (Refer to Figure 6.6 (b)), all biofilters still showed an increase in Fe concentrations at 10 cm depth, although this initial increase in Fe concentrations was much lower compared to day 27. Also, Fe concentrations in all the biofilters appeared to decrease similarly from 30 cm onwards. Further, majority of the Fe removal appeared to have occurred in the top and the middle layer i.e. 0-70 cm. Note that, during phase 1, it was found that most of the removal occurred in the top layer (0-30 cm). Further, it can be observed that on day 27, compared to the continuously operated biofilters A and B, the intermittently operated biofilter C had lower removals until a biofilter depth of 110 cm. But on the 55th day of sampling, except for the increase at 10 cm bed depth similar removals were observed at all biofilter depths of the intermittently operated biofilter C and continuously operated biofilter A.



(a)



(b)

Figure 6.6 Fe concentrations across various biofilter bed depths on day 27 and 55 after the extended shut-down. Note that the y-axis in (a) is different from (b) to see the trends more clearly

6.2.4 Microbial Activity in the Biofilters in Phase 3 (Post Extended Shutdown)

To understand the microbial activity associated with Mn and Fe removal, active biomass on the biofilter media was quantified by performing ATP analysis. This analysis was performed at five distinct time points post-shutdown to facilitate a comprehensive comparison. One set of samples was collected before the biofilters were restarted (referred to as “day 0 before backwash” in Figure 6.7) and another set was collected after the biofilters were backwashed (referred to as “Day 0 (after backwash)” in Figure 6.7). This was followed by sampling on day 13, 28, 56 and 85 before backwash (referred to as “Day 13, 28, 56 and 85” in Figure 6.7). The ATP values obtained are plotted in Figure 6.7, wherein for comparison, the ATP values from the last day (56th day) of phase 1 were also included. Corresponding ATP data can be found in Appendix Table D10. From Figure 6.7, it can be observed that, in the top layer there was a significant decrease in ATP levels across all biofilters from the last day (56th day) of phase 1 to the final day of the extended shutdown i.e. phase 2, with a further drop observed once a backwash was performed. However, in the middle and bottom layers, the ATP values were similar or slightly increased after backwash for all the biofilters. Upon resuming operation in phase 3, an increase in ATP levels was observed for all biofilters at all depths with time. Particularly, 56 days into phase 3, ATP values in the top layer surpassed those recorded on the last day (56th day) of phase 1. On the 85th day of operation in phase 3 (post extended shutdown), the ATP values appeared to increase slightly for biofilter A, while for biofilter B and C, they remained similar. Further, ATP values exhibited a decreasing trend with biofilter depth for all biofilters, although this was more pronounced for the continuously operated biofilters A and B compared to the intermittent one. Also, unlike phase 1, where variations were observed in ATP values between the continuously operated biofilters A and B (Refer to Figure 4.6), in phase 3, ATP values for the continuous biofilters were similar at all depths most of the time. Interestingly, although the performance of the intermittently operated biofilter C in terms of Mn and Fe removal matched that of the continuously operated biofilters A and B, its ATP values were significantly lower across all biofilter depths and times. This suggests that while ATP provides an indication of microbial activity on the media, it may not directly correlate with biofilter performance.

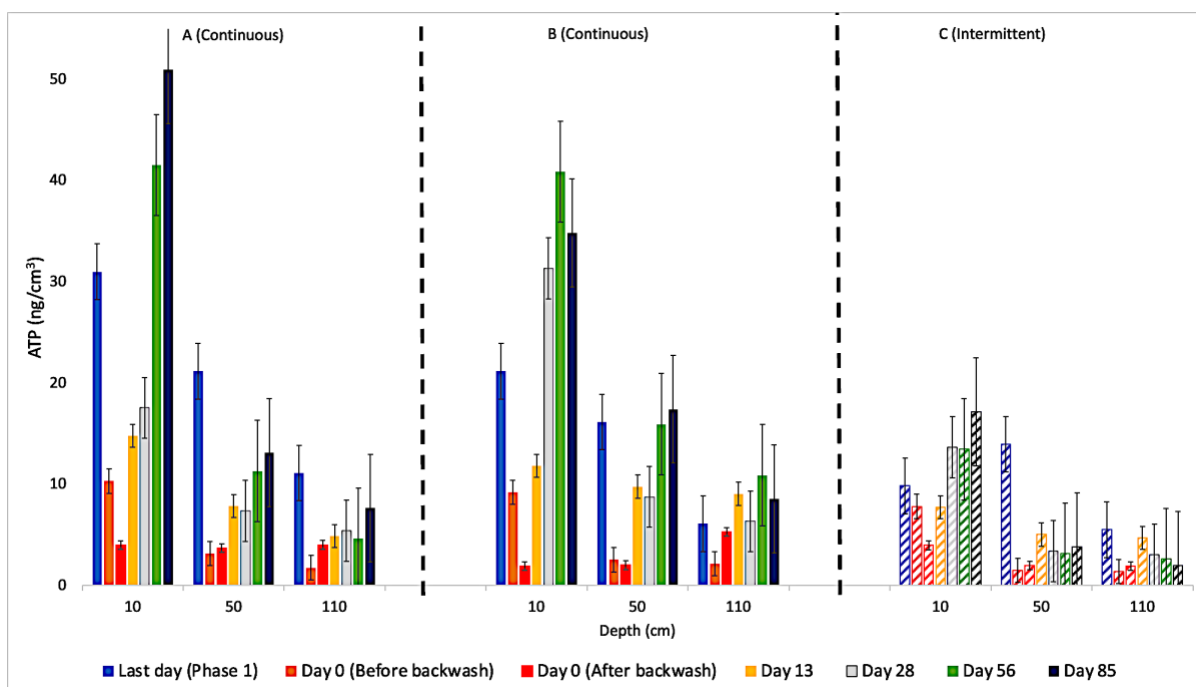


Figure 6.7 ATP measured for the biofilm attached to the filter media at different biofilter bed depths. Samples were collected before backwashing and after the intermittently operated biofilter C had been operating for 2-3 h post 18 h shutdown. Measurements were done in duplicates and the error bars represent the standard deviation

Based on these observations, several inferences can be drawn. The prolonged shutdown might have led to a decline in the microbial activity within the biofilters and the further drop in ATP levels observed after backwash might be linked to the partial dislodging of biofilm accumulated on the media during the cleaning process. The decrease in ATP levels in the top layer post-backwash and a slight increase in the levels in the bottom layer at 110 cm was in line with research work done by Morales Medina et al., 2023. In their work on Mn and TOC removal, they reported that after backwash the microbial composition and active biomass (ATP) increased in the bottom layer, while it decreased in the top layer. It was further mentioned that 30 h after backwash the microbial diversity became more uniform, and it was back to higher levels at the top level. Upon resuming operation in phase 3, the increase in ATP levels suggests a shift in the activity level of the microorganisms and a rebound in microbial growth and metabolic activity as the biofilters were reintroduced to the fresh raw water as the treatment proceeded. The similarities in ATP values between the continuously operated biofilters A and B in phase 3 suggest a more consistent microbial response compared to phase 1, possibly due to the stabilization of microbial communities over time. Lastly, the lower ATP values observed in the

intermittently operated biofilter C despite comparable Mn and Fe removal performance may indicate differences in microbial composition, metabolic pathways, or activity levels. The possible reason behind this might be the lesser nutrient loading over time compared to the continuously operated biofilters A and B. This also highlights the complexity of the microbial community within the biofilm on the biofilter media.

To further gain a more nuanced understanding of the microbial community dynamics, AxP (i.e. ATP+ADP+AMP) was measured and the plots of ATP, ADP and AMP values can be found in Figure 6.8 and the corresponding AxP data can be found in Appendix Table D11. This measurement was done on the 1st day of biofilter operation after the six-month extended shutdown after the biofilters were backwashed. Following this, the analysis was done every two weeks, after the biofilters were backwashed. For comparison, the AxP levels recorded on the last day of phase 1 are also shown in Figure 6.8(a). Over time, an increase in AxP was observed in all biofilters and at all depths, indicating increasing microbial activity. However, ADP, and to a lesser extent AMP, constituted a larger portion of the adenylate pool just after biofilter restart in all biofilters and at all depths. Notably, ATP, ADP, and AMP values were generally lower for the intermittently operated biofilter C compared to the continuously operated ones (A and B), once started following the extended shutdown.

On day 0, the AxP levels were found to be very low and were mostly comprised of AMP and ADP. On day 13, ADP comprised the majority of the AxP, followed by ATP, while AMP was found in a smaller proportion. By day 28, ATP was either predominant or equal to ADP. Moving on to day 56, the overall AxP was quite high, which was surprising (Refer to Figure 6.8(e)). ATP, ADP and AMP were either found to be in equal proportion, or ADP and AMP were found in a higher proportion. However, on day 85, there was a shift observed, as ATP made up the largest portion of the three compounds. ATP values were reportedly higher than the ADP values for all biofilters and at all depths, and the AMP values were quite low. Surprisingly on day 85, the AxP levels in the top layer of the continuously operated biofilter B were lower than those observed in the continuously operated biofilter A, although the AxP values were similar in the lower two depths. This aligned with the observations from the results obtained in phase 1 (Refer to Figure 6.8 (a)), wherein the AxP levels recorded at 10 and 50 cm depths of the continuously operated biofilter B were lower than those in A.

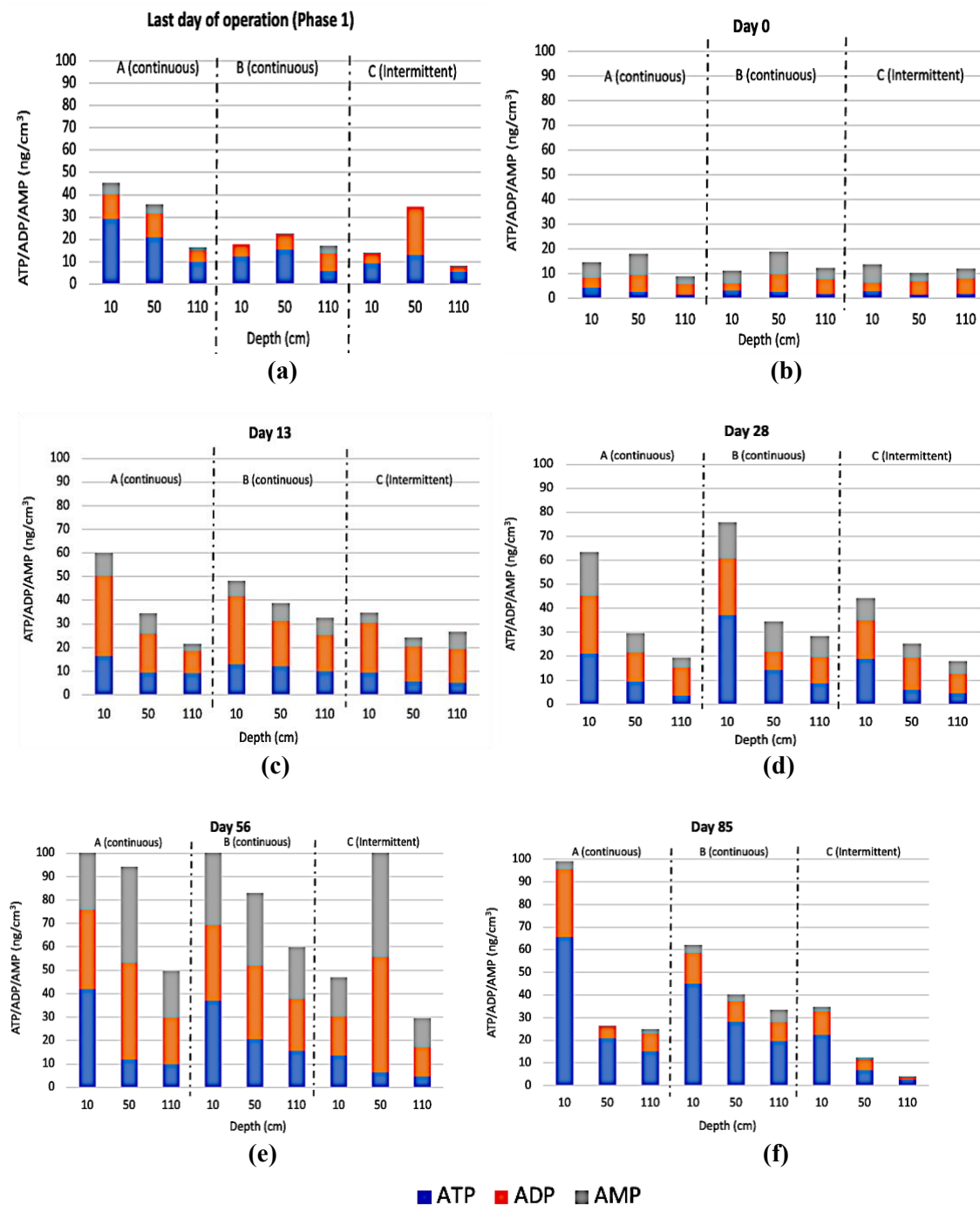
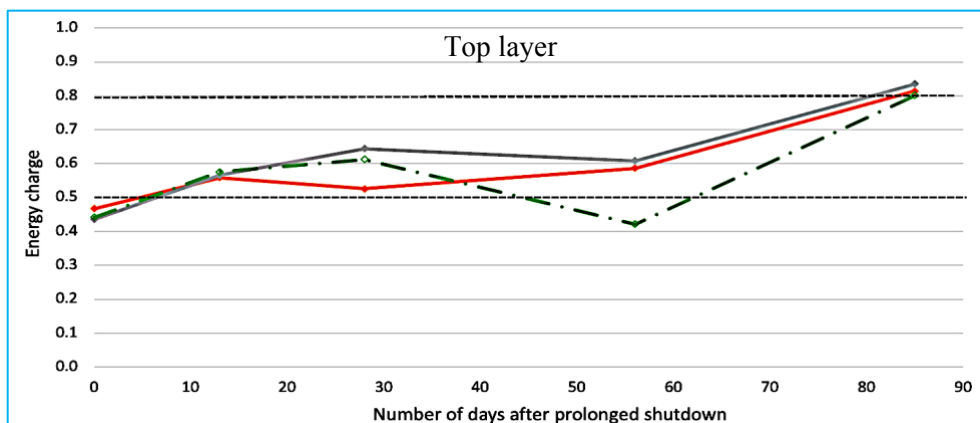


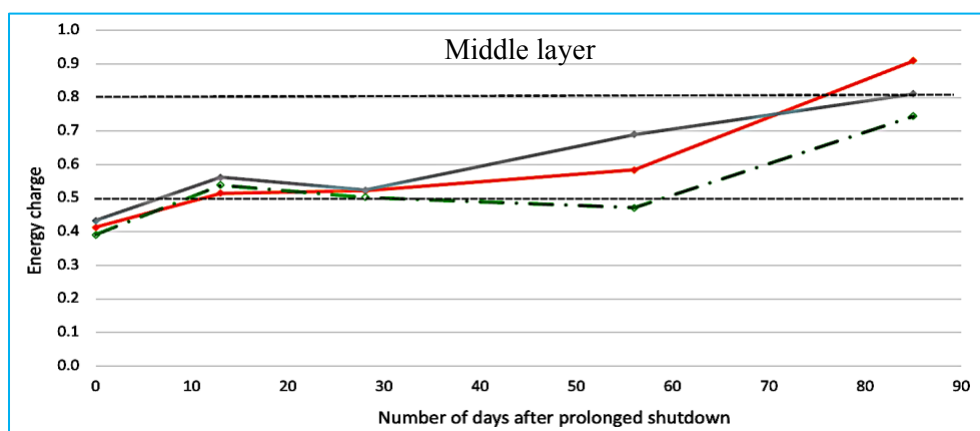
Figure 6.8 AxP (AMP + ADP + ATP) values for the biofilters A, B and C at (a) last day of phase 1 (day 56 during phase 1), (b) on Day 0 (i.e. the day when extended shutdown ended), (c) day 13, (d) day 28, (e) day 56 and (f) day 85 of phase 3. Note that all the media samples were collected after backwashing

Based on the trends observed, a few important inferences can be made. Firstly, the ATP/ADP/AMP levels observed suggest that the microbes might have been adjusting their metabolic activities after restart. Further, it can be interpreted from this data that 56 days after the restart (Refer to Figure 6.8 (e)), the biofilters were eventually able to attain the microbial activity levels that were recorded during the phase 1 after 56 days (Refer to Figure 6.8. (a)). Although in terms of biofilter performance, it was seen that the biofilters achieved improved results within a month of operation compared to performance during phase 1. This further supports the conclusion from the previous section (ATP only results), that the microbial activity might not be directly correlated with the Mn removal performance of the biofilters.

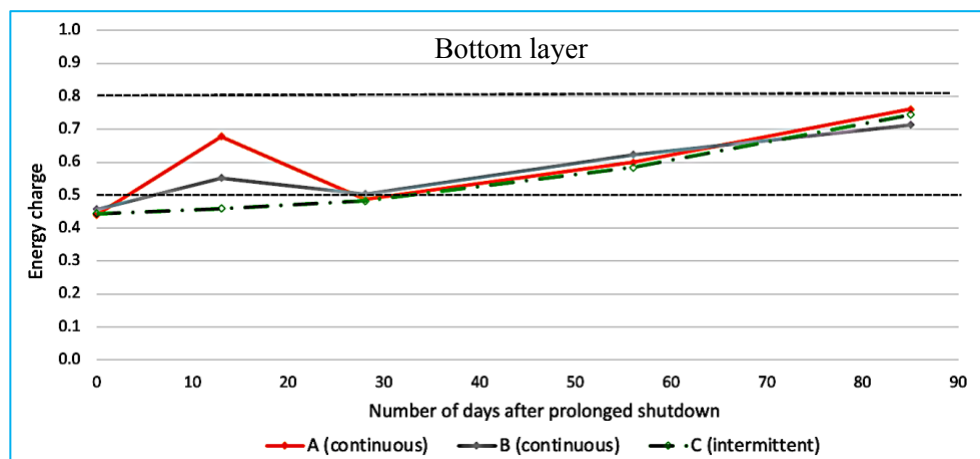
EC has been proposed to reflect the energy status of a cell and that it can enumerate the amount of metabolic energy momentarily stored in the adenine nucleotide pool (Atkinson & Walton, 1967). Using the ATP, ADP and AMP data, EC was calculated (Refer to section 3.6.2.1), and the corresponding plots can be found in Figure 6.9. It can be observed that EC values increased over time for all biofilters at all depths after restart though this increase was not linear. At the end of phase 1 i.e. after 56 days of operation (Refer to Figure 5.7- 0th day of phase 2), the EC values were either close to 0.8 or above 0.8, especially in the top layer. In phase 3 (Refer to Figure 6.9), the EC values stayed in the viable region i.e. between 0.5 to 0.8 until day 56. Over time, after the restart, an increasing trend was observed in the EC values for all the biofilters in almost all the depths and on day 85, the EC values were found to be either more than or close to 0.8, indicating that the biofilters were changing their status from viable microorganisms to active microorganism.



(a)



(b)

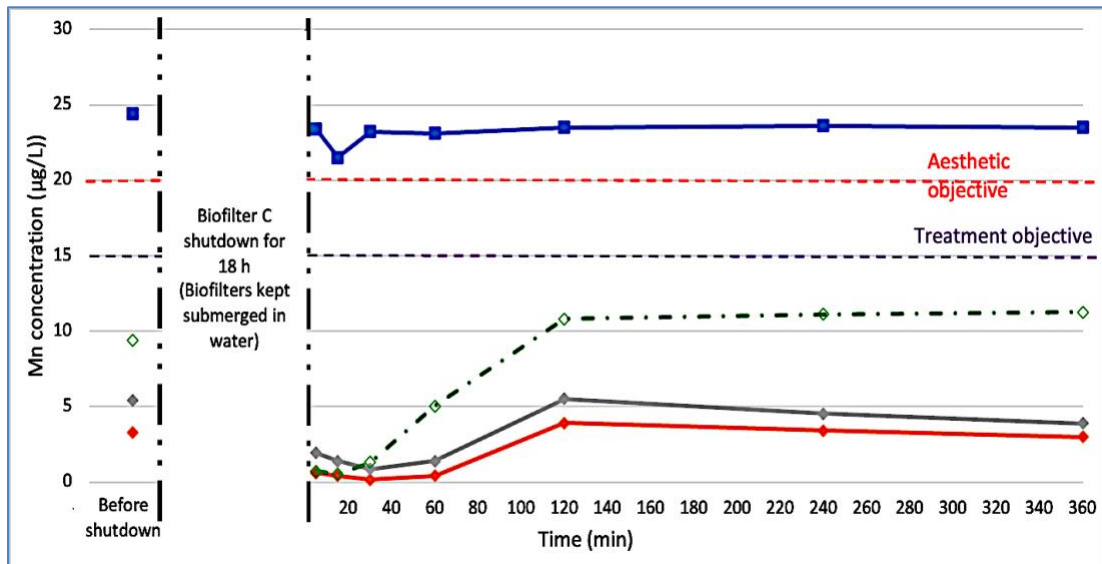


(c)

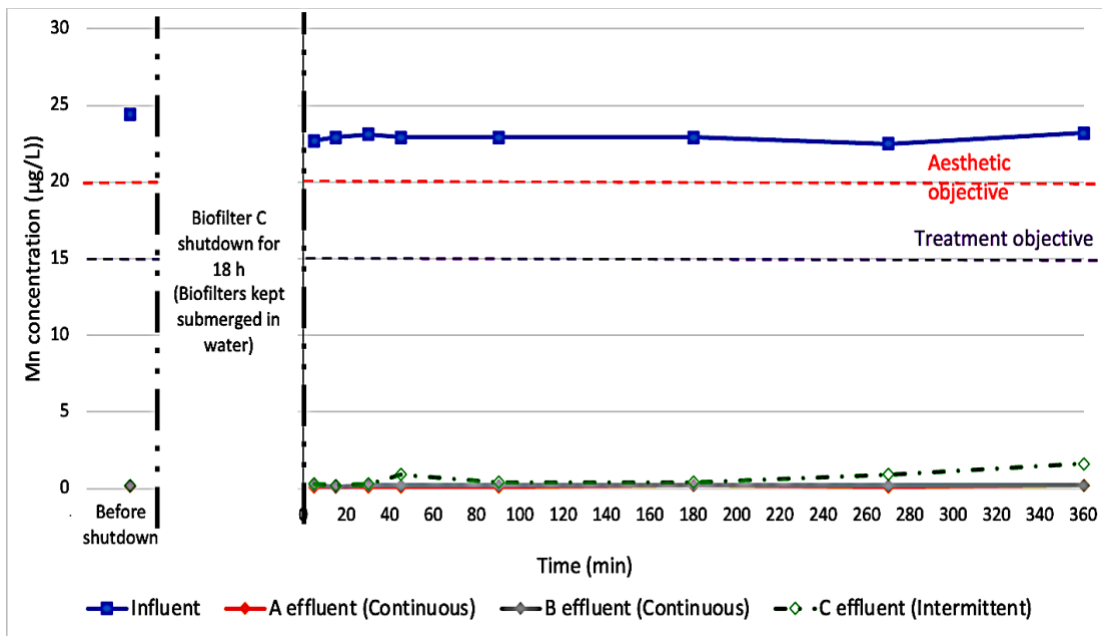
Figure 6.9 EC values for the biofilters A, B and C in the (a) top layer (10 cm), (b) middle layer (50 cm) and (c) bottom layer (110 cm) in phase 3. Dashed lines are to interpret the status of the biofilters (Refer Table 5.1)

6.2.5 Effect of 18-h Regular Intermittent Shutdown on Biofilter Performance

To assess the impact of an 18-hour intermittent shutdown on biofilter performance, effluent samples were collected at regular intervals following the restart of the intermittently operated biofilter C. During phase 1, it was found that the intermittently operated biofilter C recovered to its normal removal efficiency (comparable to those achieved prior to the 18-h shutdown) within an hour of restart. In this phase, the samples were taken more frequently in the first hour of operation, to know exactly how much time the intermittently operated biofilter C takes to recover (if less than an hour). Mn biofilter effluent concentrations are shown in Figure 6.10 and the corresponding ATP data can be found in Appendix Table D12. This assessment was performed on day 28 and 56 of pilot operation in phase 3 (post-extended shutdown). On the 28th day, the biofilters A, B and C were showing improved performance and moving toward a steady state, while on the 56th day, all biofilters had reached a steady state (Refer to Figure 6.1). In Figure 6.10 it was observed that the Mn concentration in the effluent remained consistently below the treatment objective of 15 µg/L throughout the sampling period on both days of the assessment. But a shift in the performance from day 28 to day 56 was observed immediately after restarting intermittent biofilter C. On day 28, when the intermittently operated biofilter C exhibited a removal rate of 62%, and the continuously operated biofilters A and B exhibited approximately 85% removal, for the intermittently operated biofilter, Mn effluent concentration decreased to 1-2 µg/L within the first hour after restarting the biofilter, before increasing back to 10 µg/L thereafter (Refer to Figure 6.10). This was unexpected, as an increase in Mn effluent concentration after restart was expected. In addition, both continuous biofilters A and B showed the same behaviour as the intermittent biofilter C, which was surprising as they were kept running during the time when biofilter C was shut down. Note that in phase 1 the decrease followed by an increase in Mn effluent concentrations was also observed for the intermittent biofilter C after restart, but not for the continuously running biofilters A and B (Refer to Figure 4.7), which was expected. The underlying reason for these observations remains incompletely understood and necessitates further investigation. The Mn concentration in the influent was notably low, and the observed behaviour may differ when dealing with feed water containing higher Mn concentrations. Moving on, by day 56, when removal rates for all biofilters, including the intermittently operated biofilter C, had reached 99%, it was noted that the intermittently operated biofilter performed almost similar to the continuously operated biofilters A and B within minutes post restart, and it demonstrated removals similar to those observed before the shutdown instantaneously.



(a)

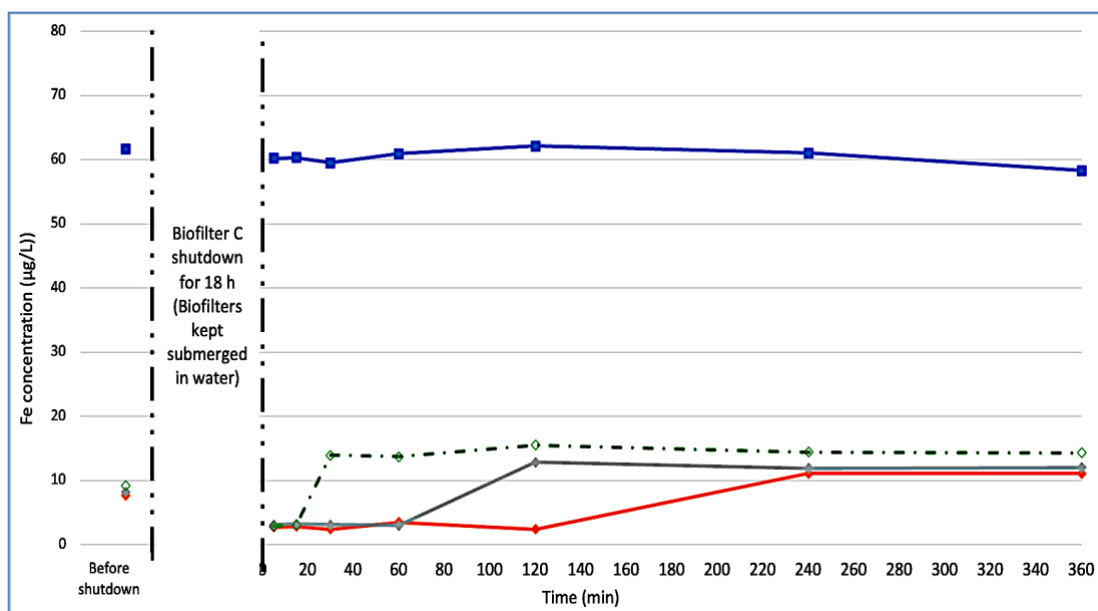


(b)

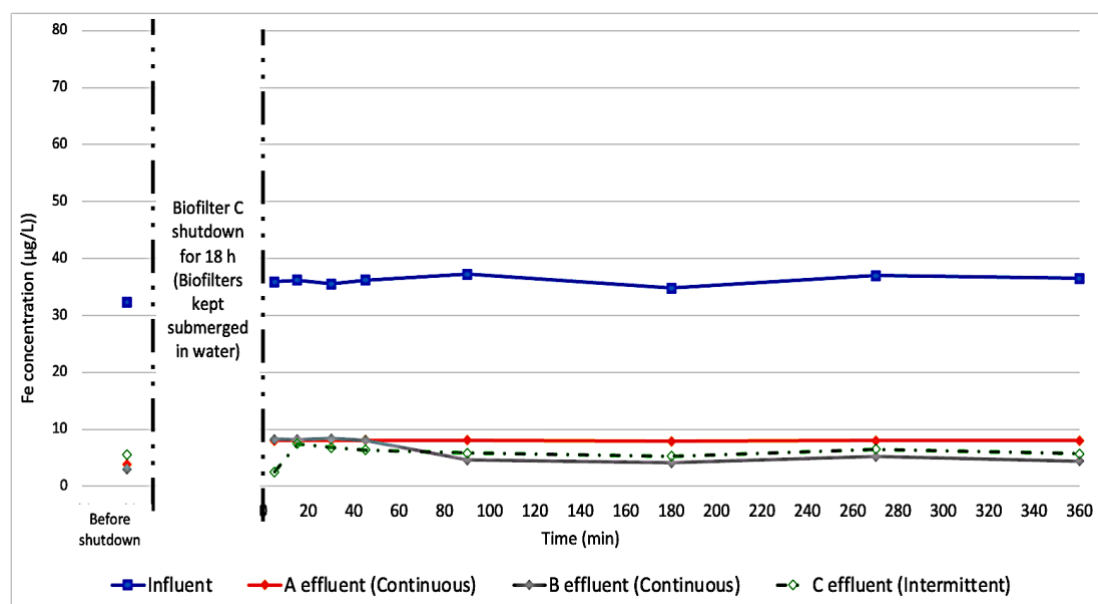
Figure 6.10 Impact of 18 h shutdown on Mn concentrations during biofilter C restart on (a) day 28 and (b) day 56. Sample collection began 5 minutes after the biofilter C was restarted and then continued at regular intervals. To have a reference point a sample was collected on the previous day just before the shutdown (in this case biofilters A and B continued running without a shut-down)

Furthermore, post 18-h shutdown, Fe removal was also investigated on day 28 and 56, and the results are shown in Figure 6.11 and the corresponding ATP data can be found in Appendix Table D13. Trends similar to those for Mn were observed for Fe as well including a shift in the performance from day 28 to day 56. On day 28, the time until the intermittently operated biofilter C reached its previous removals was around 30 mins, which was faster than for Mn (2 h). On day 56, it was observed that the Fe concentration was quite low (3 µg/L) for the intermittently operated biofilter C for the first sampling point (5 min), but from the next sampling point (15 mins onwards) the Fe concentration in the effluent were equivalent to the levels recorded for the continuously operated biofilters A and B. This phenomenon (low concentration initially followed by consistent levels) was also observed during phase 1.

Overall in the context of Mn and Fe, the quick recovery of the intermittently operated biofilter C after the 18-h shutdown suggests that the microbial communities may not have been affected by the 18-h shutdown conditions, allowing for rapid restoration of removal efficiencies. The consistent performance of all biofilters by day 56 might indicate the stabilization of microbial communities over time, resulting in uniform removals across all biofilters. Lastly, a possible reason behind the lower Mn and Fe concentrations for the intermittently operated biofilter C in the initial 5 to 15 minutes post-restart might be attributed to the 18-hour shutdown period. During this time the biofilter did not receive any new influent water and was kept submerged in water. As a result, the sample collected at 5 to 15 minutes after restart of biofilter C may have been the stagnant water that remained within the biofilter during the shutdown (theoretical EBCT of the biofilter was 15.6 mins). This stagnant water has possibly undergone Mn and Fe removal during the shutdown period, leading to substantially reduced Mn and Fe effluent concentrations for biofilter C in the initial 15 min after restart.



(a)

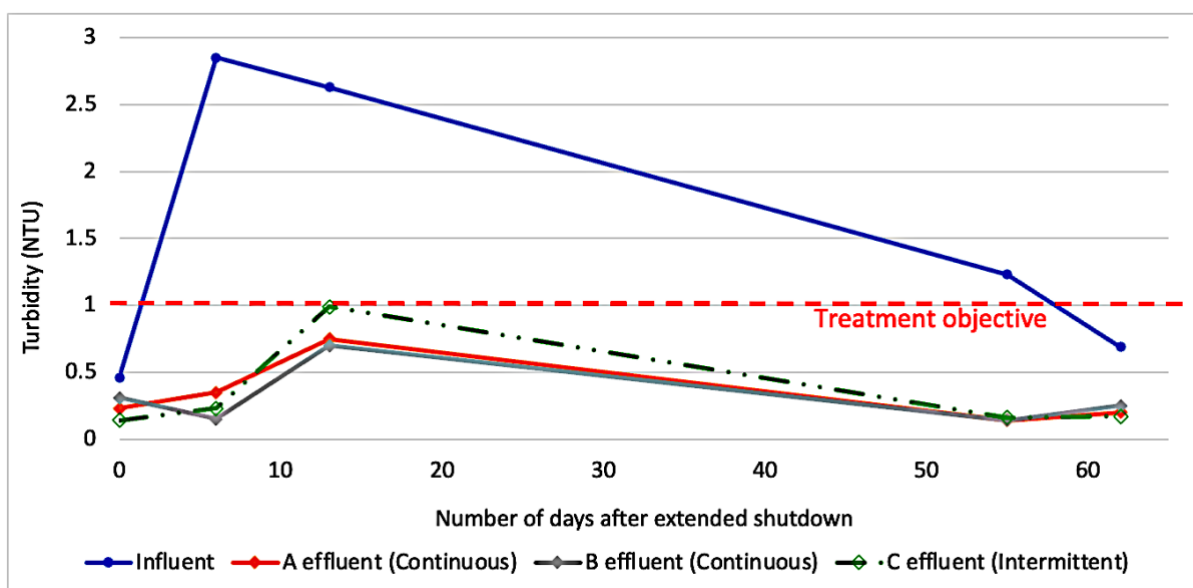


(b)

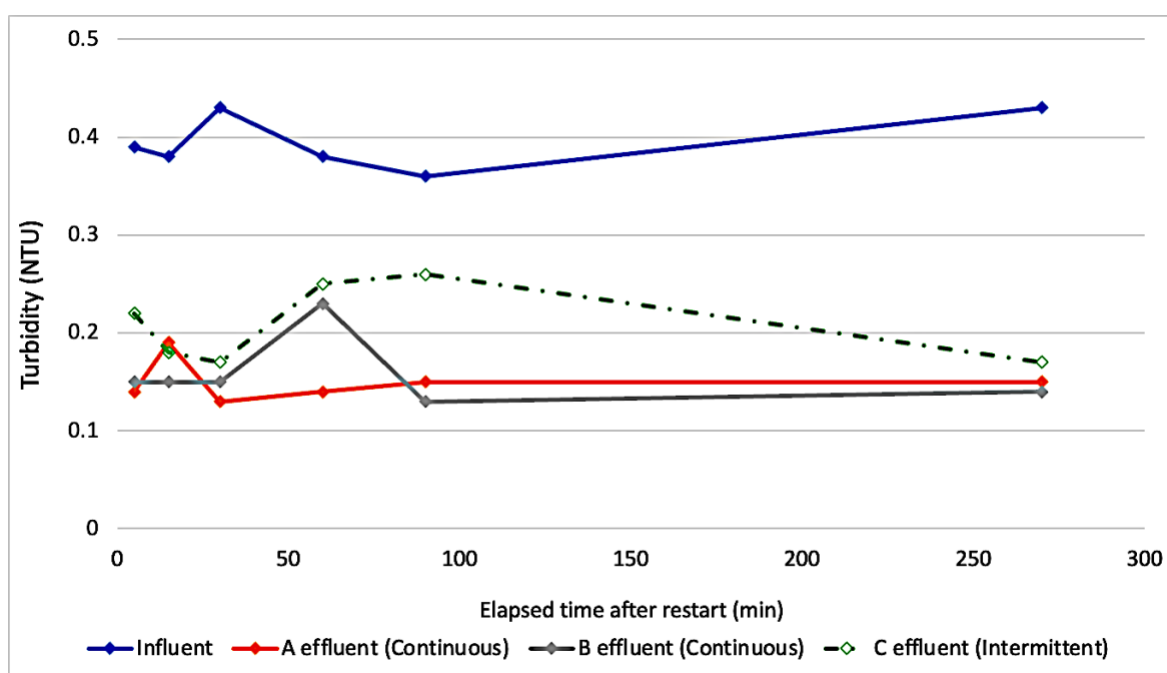
Figure 6.11 Impact of 18 h shutdown on Fe concentrations during biofilter C restart on (a) day 28 and (b) day 56. Sample collection began 5 minutes after the biofilter C was restarted and then continued at regular intervals. To have a reference point a sample was collected on the previous day just before the shutdown (in this case biofilters A and B continued running without a shut-down)

6.2.6 Turbidity Removal by the Biofilters

Figure 6.12(a) depicts the turbidity removal over time in phase 3. One important observation from the study was the ability of all biofilters to consistently maintain turbidity values below 1 NTU, even while facing varying influent turbidities. Also, all the biofilters appeared to perform similarly irrespective of the operational runtimes. On day 56 immediately after resuming operations following the 18 h shutdown of the intermittently operated biofilter C (while biofilter A and B continued running without a shutdown) turbidity was measured at regular intervals until the intermittently operated biofilter shut down again (Refer to Figure 6.12(b)). No significant change was noted in the effluent turbidity levels over time following the restart of the intermittent biofilter C after its 18-hour shutdown period, and the effluent turbidities of all biofilters remained consistent with those observed throughout phase 3. This indicates the resilience of the biofilter systems and their ability to quickly recover and maintain treatment efficiency following shutdown periods. It is noteworthy to point out here that, the pilot scale setup used for the experimental work was not coagulant assisted, which is generally the case when targeting turbidity removal. Although the biofilters employed were able to perform well, if better turbidity removal had been the goal, then coagulant addition prior to biofiltration would need to be done.



(a)



(b)

Figure 6.12 Turbidity removal a) over time in phase 3 (post extended shutdown) and b) on day 56 immediately after resuming operations following an 18 h shut down of the intermittent biofilter C (in this case biofilters A and B continued running without a shut-down)

6.3 Conclusions

The main objective of this phase of the research project was to compare the performance of continuously and intermittently operated biofilters after a 6 months extended shutdown. The major findings from this phase based on the results obtained are:

- In comparison to phase 1, it was observed post-extended shutdown i.e. in phase 3 that the raw feedwater had comparatively lower Mn and Fe concentrations, although they were still above the AO value (20 µg/L) and the treatment objective limit (15 µg/L).
- During the 85 days of operation in phase 3, all biofilters were able to remove Mn below the treatment objective limit (15 µg/L) within 20 days after restart. The intermittently operated biofilter C recorded the same Mn removals as the continuously operated biofilters A and B a month after the restart and a pseudo-steady state was reached for all the biofilters within 34 days with >95% removal.
- All biofilters removed Fe immediately after restart and a steady state was observed. The extended shutdown had no effect on Fe removal, from which it can be hypothesized that chemical oxidation and precipitation combined with physical removal in the biofilter might be the prominent removal mechanism for Fe.
- When compared to the end of phase 1, wherein the majority of the Mn removal was observed in the middle layers of the biofilters, at the end of phase 3 the majority of the Mn removal occurred in the top layers, suggesting a shift in the Mn removal region as the biofilter matured.
- Regarding the impact of an 18-hour shutdown on biofilter performance, no significant effect was observed over time. The intermittently operated biofilter C exhibited behaviour comparable to those of the continuously operated biofilters A and B.
- After restarting the biofilters, microbial activity, measured as ATP increased over time at almost all bed depths, in all biofilters, and 56 days after biofilter restart ATP levels were similar to those at the end of phase 1. ATP levels in the intermittently operated biofilter C were substantially lower compared to those in the continuously operated biofilters A and B. Overall, there does not seem to be a direct relationship between the removal of Mn and active biomass as quantified by ATP.
- After restart, ATP, ADP and AMP levels increased over time and the EC values, although increasing, were within the 0.5-0.8 range, which is reported to be the viable region for microbial activity. On day 85 the end of phase 3, there was a shift with ATP increasing while ADP and

AMP decreased resulting in levels similar to the ones observed at the end of phase 1 just before the extended shutdown began. Also, on day 85, the EC values were either higher or close to 0.8, suggesting the shift in the microbial activity status from viable to actively growing.

- Lastly, all the biofilters consistently maintained turbidity values below 1 NTU, even when facing varying influent turbidities.

These findings underscore the resilient nature of the biofilter systems irrespective of intermittent operations and relatively low water temperatures, as the biofilters were able to quickly regain their performance after a 6-month extended shutdown.

Chapter 7

Conclusion and Recommendations for Future Work

7.1 Conclusions

While significant progress has been made in understanding biofiltration systems for water treatment, knowledge is limited concerning their application for Mn removal from groundwater. Specifically, the dynamics of intermittently operated biofilter and the effects of prolonged shutdowns on biofilter performance have not been thoroughly investigated. The goal of this project was to compare the performance of intermittently and continuously operated biofilters, removing Mn from groundwater, during the start-up phase and after a six-month extended shutdown. A pilot scale setup consisting of three sand biofilters was assembled at a groundwater treatment utility. While treating raw groundwater, two biofilters were operated continuously (24 h/day), while one was operated intermittently (6 h/day).

To achieve this goal, the pilot study was divided into three research phases. In the first research phase, i.e. the start-up phase, spanning 56 days, the biofilter performance and the microbial activity on the biofilter media were investigated. Based on the results observed, the following conclusions can be made for Phase 1:

- In regards to performance, all three biofilters were able to remove Mn below the treatment objective value of 15 µg/L after day 42, but the removals of the intermittently operated biofilter were significantly lower than those of the continuously operated biofilters. By day 56, when the biofilters had to be shut down, the continuously operated biofilters approached a steady state, while the intermittently operated biofilter did not. This suggests that during the start-up period, intermittent operation delayed the Mn removal efficiency.
- Further, in terms of depth-wise removal, Mn removal was found to predominantly (55-80%) occur in the middle layer of the biofilter, highlighting the importance of the middle section of the biofilter bed for efficient Mn removal during the start-up period.
- In terms of microbial activity, over time ATP values were observed to be lower in the intermittently operated biofilter compared to the continuous ones, however, post intermittent 18-h shutdown the ATP levels appeared to remain relatively similar. This indicated that microbial activity developed slower on the intermittently operated biofilter.

- Although quite gradual, based on the trends observed, the intermittent biofilter would probably have achieved steady state performance eventually, were it not for the extended shutdown.

For phase 2, the six-month unprecedented well shutdown gave an opportunity to look into the impact of this shutdown on the biofilters. To the author's knowledge, only one study has examined the impact of an extended shutdown, but that was less than three months (Evans et al., 2021). A uniform approach was applied to all biofilters, i.e. both previously operated intermittently and continuously biofilters were subjected to the same storage strategies. To understand the effects, water quality parameters of the water used to store the biofilters were investigated in depth along with several microbial parameters for the biofilm on the biofilter media. For studying microbial activity, apart from ATP, ADP and AMP were also measured. Additional microbiological analysis included MnOB, LBB and CLPP, which were performed at Wilfrid Laurier University by Nicholas Lamba-Rautapuro, an MSc student. Based on the results observed, the following conclusions can be made for phase 2:

- DO levels were found to decrease over time, but the employment of SS 3 likely played a crucial role in re-oxygenating the water. Periodic water circulation (SS 3) seemed to have helped prevent the biofilters from entering an oxygen-stressed state and therefore can potentially be recommended for treatment practice.
- ATP levels were reduced substantially in all biofilters and at all filter bed depths. The distribution of ATP, ADP and AMP changed during the extended shutdown with ADP and AMP levels increasing relative to ATP over time. This indicated that the microbes might have been adjusting their metabolic activities to cope with the changing environmental conditions caused by the shutdown.
- All three biofilters remained potentially viable (EC between 0.5 and 0.8) for approximately 4 months and the presence of MnOB was confirmed by using culture-based analysis methods combined with the LBB assay. After 4 months, all biofilters potentially entered a state of dormancy (EC below 0.5), while the presence of MnOB was still confirmed. These findings underscore the fact that MnOB were able to persist within the biofilters despite the extended shutdown.
- The choice of SS 3 for the majority of the shutdown period might have played a crucial role in sustaining the biofilters during the extended shutdown, maintaining microbial activity, and influencing EC values.

For phase 3, after the prolonged shutdown, when the access to the raw water was restored, the performance of the continuously and intermittently operated biofilters was investigated. Biofilters were

operated the same way as during the start-up phase. Biofilter performance was assessed alongside all the same microbial analyses utilized during phase 2. Based on the results observed, the following conclusions can be made for phase 3:

- All biofilters were able to remove Mn below the treatment objective limit (15 µg/L) within 20 days after restart and the intermittently operated biofilter recorded similar Mn removals as the continuously operated biofilters within a month of operation. Also, the intermittently operated biofilter approached a steady state in a time frame comparable to that of the continuously operated biofilters. Compared to the start-up phase, the accelerated performance indicates that the biofilters swiftly regained their efficiency following the 6-month shutdown, which underscores the resilient nature of these biofilter systems.
- Compared to the start-up phase, wherein the majority of the Mn removal was observed in the middle layer, in this phase, the majority of the Mn removal occurred in the top layer, suggesting a shift in the Mn removal region as the biofilter matured.
- In terms of microbial activity, there was a shift observed in the nucleotide levels with ATP increasing and ADP and AMP decreasing in all the biofilters. Also, ATP levels in the intermittently operated biofilter continued to be substantially lower compared to the continuously operated biofilter, potentially indicating no correlation between Mn removal and microbial activity.

Overall, the findings from this comprehensive study provide a better understanding of the Mn removal from groundwater using biofilters operated intermittently in cold temperature regions. It also helped understand the effects of a prolonged shutdown on intermittently and continuously operated biofilters for Mn removal and suggested ways, by which these effects can be minimized. These results indicate that it is possible and feasible to use intermittently operated biofilters to remove Mn in full-scale drinking water treatment plants. Eventually, it is expected that this critical information will enhance the applicability of intermittently operated biofilters, especially in small-scale communities, where operations are periodic, and resources are limited. Finally, it is anticipated that findings from this study can be applied for Mn removal from groundwater wells located elsewhere in North America & Europe and help provide contaminant-free drinking water to its population sustainably.

7.2 Recommendations for Future Work

Based on the research performed herein recommendations for future work are:

- In this study, the effect of an 18-h intermittent shutdown was investigated. In the real world depending on water demands, treatment plants can have varying operating hours. So future work could focus on investigating the effects of varying intermittent shutdown durations.
- Differences in performance and microbial activity were noted between the two continuously operated biofilters in phase 1. Conducting duplicate experiments for intermittent operation would help to ascertain any possible differences and validate results.
- The Mn concentrations in the raw water were relatively low, although exceeding both the treatment objective and the AO. To comprehensively assess the efficacy of the pilot-scale biofilters in Mn removal, it would be beneficial to employ water spiked with Mn for further evaluation or perform tests at wells with higher Mn concentrations.
- No direct relationship was found between ATP and Mn removal. Additional microbial analyses going beyond ATP quantification are recommended to gain a more comprehensive understanding of the microbial communities and their relationship to Mn removal.
- Sand media, which has been known to lack adsorbing properties, was employed as the filter media for this study. Further investigations and comparisons with other commonly used biofilter media including granular activated carbon (GAC) and anthracite, that are capable of adsorbing Mn are warranted. The choice of media type may significantly influence biofilter performance in Mn removal. Additionally, exploring the impact of an extended shutdown on various biofilter media types is necessary for a comprehensive understanding of their behaviour in such conditions.

References

- Abu Hasan, H., Muhammad, M. H., & Ismail, N. 'Izzati. (2020). A review of biological drinking water treatment technologies for contaminants removal from polluted water resources. *Journal of Water Process Engineering*, 33, 101035. <https://doi.org/10.1016/j.jwpe.2019.101035>
- Abu Hasan, H., Sheikh Abdullah, S. R., Tan Kofli, N., & Kamarudin, S. K. (2012). Effective microbes for simultaneous bio-oxidation of ammonia and manganese in biological aerated filter system. *Bioresource Technology*, 124, 355–363. <https://doi.org/10.1016/j.biortech.2012.08.055>
- A.Keenan. (2019, June 18). *1 in 3 people globally do not have access to safe drinking water – UNICEF, WHO*. World Health Organisation.
- American Public Health Association. (2017). *Standard Methods for the Examination of Water and Wastewater, Total Organic Carbon, (5310B)*.
- Arora, H. (2021). *Manganese Removal from Groundwater using Biofilters*.
- Arora, H. (2024). *Biofiltration for Manganese Removal from Groundwater: Mechanistic Insights and Operational Strategies*. University of Waterloo.
- Atkinson, D. E., & Walton, G. M. (1967). Adenosine Triphosphate Conservation in Metabolic Regulation. *Journal of Biological Chemistry*, 242(13), 3239–3241. [https://doi.org/10.1016/S0021-9258\(18\)95956-9](https://doi.org/10.1016/S0021-9258(18)95956-9)
- Basu, O. D., Dhawan, S., & Black, K. (2016). Applications of biofiltration in drinking water treatment - a review. *Journal of Chemical Technology & Biotechnology*, 91(3). <https://doi.org/10.1002/jctb.4860>
- Benefield, L. D., Judkins, J. F., & Weand, B. L. (1982). *Process chemistry for water and wastewater treatment*.
- Bondu, R., Cloutier, V., Rosa, E., & Roy, M. (2020). An exploratory data analysis approach for assessing the sources and distribution of naturally occurring contaminants (F, Ba, Mn, As) in groundwater from southern Quebec (Canada). *Applied Geochemistry*, 114, 104500. <https://doi.org/10.1016/j.apgeochem.2019.104500>

- Bouchard, M., Laforest, F., Vandelac, L., Bellinger, D., & Mergler, D. (2007). Hair Manganese and Hyperactive Behaviors: Pilot Study of School-Age Children Exposed through Tap Water. *Environmental Health Perspectives*, 115(1), 122–127. <https://doi.org/10.1289/ehp.9504>
- Bourgine, F. P., Gennery, M., Chapman, J. I., Kerai, H., Green, J. G., Rap, R. J., Ellis, S., & Gaumard, C. (1994). Biological Processes at Saints Hill Water-Treatment Plant, Kent. *Water and Environment Journal*, 8(4), 379–391. <https://doi.org/10.1111/j.1747-6593.1994.tb01121.x>
- Breda, I. L., Ramsay, L., & Roslev, P. (2017). Manganese oxidation and bacterial diversity on different filter media coatings during the start-up of drinking water biofilters. *Journal of Water Supply: Research and Technology - Aqua*, 66(8), 641–650. <https://doi.org/10.2166/aqua.2017.084>
- Breda, I. L., Søborg, D. A., Ramsay, L., & Roslev, P. (2019). Manganese removal processes during start-up of inoculated and non-inoculated drinking water biofilters. *Water Quality Research Journal*, 54(1), 47–56. <https://doi.org/10.2166/wqrj.2018.016>
- Brown, J., Upadhyaya, G., Nyfennegger, J., Pope, G., Bassett, S., Evans, A., Carter, J., Nystrom, V., Black, S., Alito, C., & others. (2020). Biofiltration Guidance Manual for Drinking Water Facilities. *Project 4719*.
- Bruins, J. H. (2017). *Manganese removal from groundwater Role of biological and physico-chemical autocatalytic processes*. CRC Press / Balkema - Taylor & Francis Group.
- Bruins, J. H., Petrusevski, B., Slokar, Y. M., Huysman, K., Joris, K., Kruithof, J. C., & Kennedy, M. D. (2015a). Biological and physico-chemical formation of Birnessite during the ripening of manganese removal filters. *Water Research*, 69, 154–161. <https://doi.org/10.1016/j.watres.2014.11.019>
- Bruins, J. H., Petrusevski, B., Slokar, Y. M., Huysman, K., Joris, K., Kruithof, J. C., & Kennedy, M. D. (2015b). Reduction of ripening time of full-scale manganese removal filters with manganese oxide-coated media. *Journal of Water Supply: Research and Technology-Aqua*, 64(4), 434–441. <https://doi.org/10.2166/aqua.2015.117>
- Bruins, J. H., Petrusevski, B., Slokar, Y. M., Wübbels, G. H., Huysman, K., Wullings, B. A., Joris, K., Kruithof, J. C., & Kennedy, M. D. (2017). Identification of the bacterial population in manganese removal filters. *Water Science and Technology: Water Supply*, 17(3), 842–850. <https://doi.org/10.2166/ws.2016.184>

- Bureau of Indian Standards. (2012). *Indian standard drinking water—Specification*.
- Burger, M. S., Krentz, C. A., Mercer, S. S., & Gagnon, G. A. (2008). Manganese removal and occurrence of manganese oxidizing bacteria in full-scale biofilters. *Journal of Water Supply: Research and Technology - AQUA*, 57(5), 351–359. <https://doi.org/10.2166/aqua.2008.050>
- Burger, M. S., Mercer, S. S., Shupe, G. D., & Gagnon, G. A. (2008a). Manganese removal during bench-scale biofiltration. *Water Research*, 42(19), 4733–4742. <https://doi.org/10.1016/j.watres.2008.08.024>
- Cai, Y., Li, D., Liang, Y., Luo, Y., Zeng, H., & Zhang, J. (2015). Effective start-up biofiltration method for Fe, Mn, and ammonia removal and bacterial community analysis. *Bioresource Technology*, 176, 149–155. <https://doi.org/10.1016/j.biortech.2014.11.025>
- Cescon, A., & Jiang, J.-Q. (2020). Filtration Process and Alternative Filter Media Material in Water Treatment. *Water*, 12(12), 3377. <https://doi.org/10.3390/w12123377>
- Chapman, A. G., Fall, L., & Atkinson, D. E. (1971). Adenylate Energy Charge in *Escherichia coli* During Growth and Starvation. *Journal of Bacteriology*, 108(3), 1072–1086. <https://doi.org/10.1128/jb.108.3.1072-1086.1971>
- Chaudhary, D. S., Vigneswaran, S., Ngo, H. H., Shim, W. G., & Moon, H. (2003). Biofilter in Water and Wastewater Treatment. *Korean Journal of Chemical Engineering*, 20(6), 1054–1065. <https://doi.org/10.1007/BF02706936>
- Cheng, Q., Nengzi, L., Bao, L., Huang, Y., Liu, S., Cheng, X., Li, B., & Zhang, J. (2017). Distribution and genetic diversity of microbial populations in the pilot-scale biofilter for simultaneous removal of ammonia, iron and manganese from real groundwater. *Chemosphere*, 182, 450–457. <https://doi.org/10.1016/j.chemosphere.2017.05.075>
- Cheng, Y., Huang, T., Liu, C., & Zhang, S. (2019). Effects of dissolved oxygen on the start-up of manganese oxides filter for catalytic oxidative removal of manganese from groundwater. *Chemical Engineering Journal*, 371, 88–95. <https://doi.org/10.1016/j.cej.2019.03.252>
- Chiswell, B., & Huang, S.-H. D. (2006). *Manganese removal* (pp. 179–192). [https://doi.org/10.1016/S1573-4285\(06\)80079-7](https://doi.org/10.1016/S1573-4285(06)80079-7)

- Choo, K., Lee, H., & Choi, S. (2005). Iron and manganese removal and membrane fouling during UF in conjunction with prechlorination for drinking water treatment. *Journal of Membrane Science*, 267(1–2), 18–26. <https://doi.org/10.1016/j.memsci.2005.05.021>
- Chung, Y.-C., Huang, C., & Tseng, C.-P. (1996). Operation optimization of *Thiobacillus thioautotrophicus* CH11 biofilter for hydrogen sulfide removal. *Journal of Biotechnology*, 52(1), 31–38. [https://doi.org/10.1016/S0168-1656\(96\)01622-7](https://doi.org/10.1016/S0168-1656(96)01622-7)
- Connor, O., & Iron, J. (1971). *Manganese. Water Quality and Treatment*. McGraw-Hill.
- Crini, G., & Lichtfouse, E. (2019). Advantages and disadvantages of techniques used for wastewater treatment. *Environmental Chemistry Letters*, 17(1). <https://doi.org/10.1007/s10311-018-0785-9>
- Di Tommaso, C., Taylor-Edmonds, L., Andrews, S. A., & Andrews, R. C. (2019). The contribution of biofilm to nitrogenous disinfection by-product formation in full-scale cyclically-operated drinking water biofilters. *Water Research*, 155, 403–409. <https://doi.org/10.1016/j.watres.2019.02.025>
- Dutta, D., Borah, J. P., & Puzari, A. (2021). Adsorption of Mn²⁺ from Aqueous Solution Using Manganese Oxide-Coated Hollow Polymethylmethacrylate Microspheres (MHPM). *Adsorption Science & Technology*, 2021, 1–10. <https://doi.org/10.1155/2021/5597299>
- Earle, M. R., Stoddart, A. K., & Gagnon, G. A. (2023). Raw water biofiltration for surface water manganese control. *Scientific Reports*, 13(1), 9020. <https://doi.org/10.1038/s41598-023-36348-1>
- ElHadidy, A. (2016). *Performance of Biological Filters for Drinking Water Treatment and Their Use for High Pressure Membrane Biofouling Control*. University of Waterloo.
- Ellis, D., Bouchard, C., & Lantagne, G. (2000). Removal of iron and manganese from groundwater by oxidation and microfiltration. *Desalination*, 130(3), 255–264. [https://doi.org/10.1016/S0011-9164\(00\)00090-4](https://doi.org/10.1016/S0011-9164(00)00090-4)
- Emelko, M. B., Huck, P. M., Coffey, B. M., & Smith, E. F. (2006). Effects of Media, Backwash, and Temperature on Full-Scale Biological Filtration. *Journal AWWA*, 98(12), 61–73. <https://doi.org/10.1002/j.1551-8833.2006.tb07824.x>
- European Commission. (2020). *Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast)*.

- Evans, A. N., Carter, J. T., Kuhnel, B., & Nystrom, V. E. (2021). Seasonal impacts on biofiltration acclimation dynamics and performance. *AWWA Water Science*, 3(3). <https://doi.org/10.1002/aws2.1225>
- Evans, A., Onesios-Barry, K., Casteloos, K., Carter, J., Ha, C., Hakes, L., Stoddart, A., Earle, M., Anderson, L., Allward, N., Gagnon, G., & Knocke, W. (2020). *Optimizing Biofiltration for Improved Manganese Control under Cold-Water Conditions*. www.waterrf.org
- Friedman, M. J., Andrew S. Hill, Steve H. Reiber, Richard L. Valentine, G. Larsen, A. Young, G. V. Korshin, & C. Y. Peng. (2010). *Assessment of inorganics accumulation in drinking water system scales and sediments* (Water Research Foundation, Ed.).
- Frischherz, H. (1985). Biological elimination of iron and manganese. *Water Supply*, 3, 125–136.
- Garland, J. L. (2006). Analysis and interpretation of community-level physiological profiles in microbial ecology. *FEMS Microbiology Ecology*, 24(4), 289–300. <https://doi.org/10.1111/j.1574-6941.1997.tb00446.x>
- Goodwill, J. E., Jiang, Y., Reckhow, D. A., & Tobiason, J. E. (2016). Laboratory Assessment of Ferrate for Drinking Water Treatment. *Journal - American Water Works Association*, 108, E164–E174. <https://doi.org/10.5942/jawwa.2016.108.0029>
- Government of Canada. (2013). *Water sources: groundwater*.
- Granger, H. C., Stoddart, A. K., & Gagnon, G. A. (2014). Direct Biofiltration for Manganese Removal from Surface Water. *Journal of Environmental Engineering*, 140(4), 04014006. [https://doi.org/10.1061/\(asce\)ee.1943-7870.0000819](https://doi.org/10.1061/(asce)ee.1943-7870.0000819)
- Gülay, A., Musovic, S., Albrechtsen, H.-J., Al-Soud, W. A., Sørensen, S. J., & Smets, B. F. (2016). Ecological patterns, diversity and core taxa of microbial communities in groundwater-fed rapid gravity filters. *The ISME Journal*, 10(9), 2209–2222. <https://doi.org/10.1038/ismej.2016.16>
- Halle, C. (2010). *Biofiltration in Drinking Water Treatment: Reduction of Membrane Fouling and Biodegradation of Organic Trace Contaminants*. UWSpace. <http://hdl.handle.net/10012/5022> [Doctor of Philosophy]. University of Waterloo.

- Han, M., Zhao, Z., Gao, W., & Cui, F. (2013). Study on the factors affecting simultaneous removal of ammonia and manganese by pilot-scale biological aerated filter (BAF) for drinking water pre-treatment. *Bioresource Technology*, 145, 17–24. <https://doi.org/10.1016/j.biortech.2013.02.101>
- Health Canada. (2016). *Manganese in Drinking Water*.
- Health Canada. (2019). *Guidelines for Canadian Drinking Water Quality- Guideline Technical Document: Manganese*.
- Health Canada. (2022). *Guidelines for Canadian Drinking Water Quality*.
- Hirsch, P. (1986). Microbial life at extremely low nutrient levels. *Advances in Space Research*, 6(12), 287–298. [https://doi.org/10.1016/0273-1177\(86\)90097-9](https://doi.org/10.1016/0273-1177(86)90097-9)
- Hooper, J., Vickstrom, K., Evans, P., Smith, C., Alito, C. L., Black, S., & Lauderdale, C. (2019). *Guidance manual for monitoring biological filtration of drinking water. The Water Research Foundation*.
- Hu, P.-Y., Hsieh, Y.-H., Chen, J.-C., & Chang, C.-Y. (2004). Adsorption of divalent manganese ion on manganese-coated sand. *Journal of Water Supply: Research and Technology-Aqua*, 53(3), 151–158. <https://doi.org/10.2166/aqua.2004.0013>
- Huck, P. M., B.M. Coffey, A. Amirtharajah, & E.J. Bouwer. (2000). *Optimizing filtration in biological filters*.
- Ikhlef, S., & Basu, O. D. (2017). Influence of backwash regime on biofilter performance in drinking water treatment. *Journal of Chemical Technology & Biotechnology*, 92(7), 1777–1784. <https://doi.org/10.1002/jctb.5178>
- Islam, A. A., Goodwill, J. E., Bouchard (Deceased), R., Tobiasson, J. E., & Knocke, W. R. (2010). Characterization of filter media $\text{MnO}_{x(s)}$ surfaces and Mn removal capability. *Journal AWWA*, 102(9), 71–83. <https://doi.org/10.1002/j.1551-8833.2010.tb10188.x>
- Jewson, D. H., & Dokulil, M. (1982). Adenylate Energy Charge Measurements in Freshwater Microbial Studies. *The Journal of Ecology*, 70(2), 595. <https://doi.org/10.2307/2259925>
- Karl, D. M., & Holm-Hansen, O. (1978). Methodology and measurement of adenylate energy charge ratios in environmental samples. *Marine Biology*, 48(2), 185–197. <https://doi.org/10.1007/BF00395018>

- Keithley, A. E., Gomez-Alvarez, V., Williams, D., Ryu, H., & Lytle, D. A. (2023). Depth profiles of biological aerated contactors: Characterizing microbial activity treating reduced contaminants. *Journal of Water Process Engineering*, 56, 104360. <https://doi.org/10.1016/j.jwpe.2023.104360>
- Kim, J. (2015). Manganese treatment to reduce black water occurrence in the water supply. *Environmental Engineering Research*, 20(3), 230–236. <https://doi.org/10.4491/eer.2015.003>
- Kim, W. H., Wataru Nishijima, Eiji Shoto, & Mitsumasa Okada. (1997). Pilot plant study on ozonation and biological activated carbon process for drinking water treatment. *Water Science and Technology*, 35(8). [https://doi.org/10.1016/S0273-1223\(97\)00147-9](https://doi.org/10.1016/S0273-1223(97)00147-9)
- Knocke, W. R., Hoehn, R. C., & Sinsabaugh, R. L. (1987). Using Alternative Oxidants to Remove Dissolved Manganese From Waters Laden With Organics. *Journal - American Water Works Association*, 79(3), 75–79. <https://doi.org/10.1002/j.1551-8833.1987.tb02818.x>
- Knocke, W. R., Zuravnsky, L., Little, J. C., & Tobiason, J. E. (2010). Adsorptive contactors for removal of soluble manganese during drinking water treatment. *Journal - American Water Works Association*, 102(8), 64–75. <https://doi.org/10.1002/j.1551-8833.2010.tb10171.x>
- Kohl, P. M., & Dixon, D. (2012). Water Quality Occurrence, Impacts, and Removal of Manganese in Biofiltration Processes. *Water Research Foundation* . www.WaterRF.org.
- Lauderdale, C., Chadik, P., Kirisits, M. J., & Brown, J. (2012). Engineered biofiltration: Enhanced biofilter performance through nutrient and peroxide addition. *Journal AWWA*, 104(5). <https://doi.org/10.5942/jawwa.2012.104.0073>
- Laurent, P., Kihn, A., Andersson, A., & Servais, P. (2003). Impact of backwashing on nitrification in the biological activated carbon filters used in drinking water treatment. *Environmental Technology*, 24(3), 277–287. <https://doi.org/10.1080/09593330309385560>
- Lautenschlager, K., Hwang, C., Ling, F., Liu, W.-T., Boon, N., Köster, O., Egli, T., & Hammes, F. (2014b). Abundance and composition of indigenous bacterial communities in a multi-step biofiltration-based drinking water treatment plant. *Water Research*, 62, 40–52. <https://doi.org/10.1016/j.watres.2014.05.035>
- Li, D., Zhang, J., Wang, H., Yang, H., & Wang, B. (2005). Operational performance of biological treatment plant for iron and manganese removal. *Journal of Water Supply: Research and Technology-Aqua*, 54(1). <https://doi.org/10.2166/aqua.2005.0002>

- Li, G., Chen, R., Ma, X., & Shi, B. (2021). Critical Factors Affecting the Level of Dissolved Manganese in Different Raw Water and its Removal at Water Treatment Plants. *ACS ES&T Water*, 1(10), 2259–2268. <https://doi.org/10.1021/acsestwater.1c00222>
- Li, X. kun, Chu, Z. rui, Liu, Y. jun, Zhu, M. ting, Yang, L., & Zhang, J. (2013). Molecular characterization of microbial populations in full-scale biofilters treating iron, manganese and ammonia containing groundwater in Harbin, China. *Bioresource Technology*, 147, 234–239. <https://doi.org/10.1016/j.biortech.2013.08.008>
- Liu, Y., Engel, B. A., Flanagan, D. C., Gitau, M. W., McMillan, S. K., & Chaubey, I. (2017). A review on effectiveness of best management practices in improving hydrology and water quality: Needs and opportunities. *Science of The Total Environment*, 601–602. <https://doi.org/10.1016/j.scitotenv.2017.05.212>
- Logsdon, G. S., Kohne, R., Abel, S., & LaBonde, S. (2002). Slow sand filtration for small water systems. *Journal of Environmental Engineering and Science*, 1(5), 339–348. <https://doi.org/10.1139/s02-025>
- Manav Demir, N. (2016). Experimental Study of Factors that Affect Iron and Manganese Removal in Slow Sand Filters and Identification of Responsible Microbial Species. *Polish Journal of Environmental Studies*, 25(4), 1453–1465. <https://doi.org/10.15244/pjoes/62679>
- Marsidi, N., Abu Hasan, H., & Sheikh Abdullah, S. R. (2018). A review of biological aerated filters for iron and manganese ions removal in water treatment. *Journal of Water Process Engineering*, 23, 1–12. <https://doi.org/10.1016/j.jwpe.2018.01.010>
- McCormick, N. E., Earle, M., Ha, C., Hakes, L., Evans, A., Anderson, L., Stoddart, A. K., Langille, M. G. I., & Gagnon, G. A. (2021). Biological and physico-chemical mechanisms accelerating the acclimation of Mn-removing biofilters. *Water Research*, 207, 117793. <https://doi.org/10.1016/j.watres.2021.117793>
- McCormick, N. E., Earle, M., Stoddart, A. K., Langille, M. G. I., & Gagnon, G. A. (2023). Understanding the impact of different source water types on the biofilm characteristics and microbial communities of manganese removing biofilters. *Environmental Science: Water Research & Technology*, 9(1), 48–61. <https://doi.org/10.1039/D2EW00568A>

- McKee, K. P., Vance, C. C., & Karthikeyan, R. (2016). Biological manganese oxidation by *Pseudomonas putida* in trickling filters. *Journal of Environmental Science and Health, Part A*, 51(7), 523–535. <https://doi.org/10.1080/10934529.2016.1141618>
- McKie, & Andrews. (2022). *Impact of Intermittent Operation on Biofilter Performance*. www.waterrf.org
- McKie, M. J., Bertoia, C., Taylor-Edmonds, L., Andrews, S. A., & Andrews, R. C. (2019). Pilot-scale comparison of cyclically and continuously operated drinking water biofilters: Evaluation of biomass, biological activity and treated water quality. *Water Research*, 149, 488–495. <https://doi.org/10.1016/j.watres.2018.11.033>
- McKie, M. J., L. Taylor-Edmonds, S.A. Andrews, & R.C. Andrews. (2016). Alternative Control Strategies for Improved Biofilter Performance. *Ontario Water Conference & Trade Show*.
- MHLW. (2003). *Ministerial ordinance on water quality standards, ordinance no. 101 of 2003 (平成十五年厚生労働省令第百一号, 水質基準に関する省令)*. Ministry of Health, Labour and Welfare (厚生労働省).
- Ministry of Health British Columbia. (2022). *Guidance on Manganese in Drinking Water, Version 1.1*.
- Morales Medina, W. R., To, P., Taylor, M., Nguyen, C., & Fahrenfeld, N. L. (2023b). Acclimation, manganese removal, and backwash impact on full-scale drinking water biofilter microbiome. *AWWA Water Science*, 5(2). <https://doi.org/10.1002/aws2.1334>
- Morrison, R. L. (2014). *Investigating the Persistence and “Naturalization” Potential of Salmonella in Non-host Environments using Culture-based and Molecular-Based Fingerprinting Techniques*. Wilfrid Laurier University.
- Mouchet, P. (1992). From Conventional to Biological Removal of Iron and Manganese in France. *Journal - American Water Works Association*, 84(4), 158–167. <https://doi.org/10.1002/j.1551-8833.1992.tb07342.x>. (Copyrights licence number: 5753980456597; Retrieved on March 21, 2024)
- Myung Cha, J., Suk Cha, W., & Lee, J. (1999). Removal of organo-sulphur odour compounds by *Thiobacillus novellus* SRM, sulphur-oxidizing microorganisms. *Process Biochemistry*, 34(6–7), 659–665. [https://doi.org/10.1016/S0032-9592\(98\)00139-3](https://doi.org/10.1016/S0032-9592(98)00139-3)

- Nemani, V. A., McKie, M. J., Taylor-Edmonds, L., & Andrews, R. C. (2018). Impact of biofilter operation on microbial community structure and performance. *Journal of Water Process Engineering*, 24, 35–41. <https://doi.org/10.1016/j.jwpe.2018.05.009>
- Németh, I., Molnár, S., Vaszita, E., & Molnár, M. (2021). The Biolog EcoPlate™ Technique for Assessing the Effect of Metal Oxide Nanoparticles on Freshwater Microbial Communities. *Nanomaterials*, 11(7), 1777. <https://doi.org/10.3390/nano11071777>
- NHMRC & NRMCC. (2011). *Australian drinking water guidelines paper 6 national water quality management strategy*. .
- Niquette, P., Prévost, M., Servais, P., Beaudet, J.-F., Coallier, J., & Lafrance, P. (1998). Shutdown of BAC filters: effects on water quality. In *Journal (American Water Works Association)* (Vol. 90, Issue 12).
- Olanczuk-Neyman, K., & Bray, R. (2000). The role of physico-chemical and biological processes in manganese and ammonia nitrogen removal from groundwater. *Polish Journal of Environmental Studies*, 9(2), 91–96.
- O’Neal, S. L., & Zheng, W. (2015). Manganese Toxicity Upon Overexposure: a Decade in Review. *Current Environmental Health Reports*, 2(3), 315–328. <https://doi.org/10.1007/s40572-015-0056-x>
- Oulhote, Y., Mergler, D., Barbeau, B., Bellinger, D. C., Bouffard, T., Brodeur, M.-È., Saint-Amour, D., Legrand, M., Sauvé, S., & Bouchard, M. F. (2014). Neurobehavioral Function in School-Age Children Exposed to Manganese in Drinking Water. *Environmental Health Perspectives*, 122(12), 1343–1350. <https://doi.org/10.1289/ehp.1307918>
- Pacini, V. A., María Ingallinella, A., & Sanguinetti, G. (2005). Removal of iron and manganese using biological roughing up flow filtration technology. *Water Research*, 39(18), 4463–4475. <https://doi.org/10.1016/j.watres.2005.08.027>
- Pankow, J. F., & Morgan, J. J. (1981). Kinetics for the aquatic environment. *Environmental Science & Technology*, 15(10), 1155–1164. <https://doi.org/10.1021/es00092a004>
- Patil, D. S., Chavan, S. M., & Oubagaranadin, J. U. K. (2016). A review of technologies for manganese removal from wastewaters. *Journal of Environmental Chemical Engineering*, 4(1), 468–487. <https://doi.org/10.1016/j.jece.2015.11.028>

- Pei, Y., Prepas-Strobeck, N. S., Payne, S. J., & She, Z. (2022). Considerations for new manganese analytical techniques for drinking water quality management. *AWWA Water Science*, 4(3). <https://doi.org/10.1002/aws2.1285>
- Pepper, I. L., & Brooks, J. P. (2021). Soil microbial influences on “One Health.” In *Principles and Applications of Soil Microbiology* (pp. 681–700). Elsevier. <https://doi.org/10.1016/B978-0-12-820202-9.00025-3>
- Pfalzer, A. C., & Bowman, A. B. (2017). Relationships Between Essential Manganese Biology and Manganese Toxicity in Neurological Disease. *Current Environmental Health Reports*, 4(2), 223–228. <https://doi.org/10.1007/s40572-017-0136-1>
- Pharand, L., Van Dyke, M., Halevy, P., Anderson, W., & Huck, P. (2013). Effects of seasonal changes and nutrient availability on the performance of full-scale drinking water biofilters. *AWWA Water Quality Technology Conf., Long Beach, California*.
- Pharand, L., Van Dyke, M. I., Anderson, W. B., & Huck, P. M. (2014). Assessment of biomass in drinking water biofilters by adenosine triphosphate. *Journal AWWA*, 106(10). <https://doi.org/10.5942/jawwa.2014.106.0107>
- Pontius, F. W. (1993). Federal Drinking Water Regulation Update. *Journal - American Water Works Association*, 85(2), 42–51. <https://doi.org/10.1002/j.1551-8833.1993.tb05936.x>
- Qin, S., Ma, F., Huang, P., & Yang, J. (2009). Fe (II) and Mn (II) removal from drilled well water: A case study from a biological treatment unit in Harbin. *Desalination*, 245(1–3), 183–193. <https://doi.org/10.1016/j.desal.2008.04.048>
- Rahman, I. (2013). *Direct Biofiltration and Nutrient (Phosphorus) Enhancement for Polymeric Ultrafiltration Membrane Fouling Control*. University of Waterloo.
- Ramsay, L., Breda, I. L., & Søborg, D. A. (2018). Comprehensive analysis of the start-up period of a full-scale drinking water biofilter provides guidance for optimization. *Drinking Water Engineering and Science*, 11(2), 87–100. <https://doi.org/10.5194/dwes-11-87-2018>
- Reichart, O., Szakmár, K., Jozwiak, Á., Felföldi, J., & Baranyai, L. (2007). Redox potential measurement as a rapid method for microbiological testing and its validation for coliform determination. *International Journal of Food Microbiology*, 114(2), 143–148. <https://doi.org/10.1016/j.ijfoodmicro.2006.08.016>

- Richardson, S., Plewa, M., Wagner, E., Schoeny, R., & Demarini, D. (2007). Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. *Mutation Research/Reviews in Mutation Research*, 636(1–3), 178–242. <https://doi.org/10.1016/j.mrrev.2007.09.001>
- Rittmann, B. E., Huck, P. M., & Bouwer, E. J. (1989). Biological treatment of public water supplies. *Critical Reviews in Environmental Control*, 19(2), 119–184. <https://doi.org/10.1080/10643388909388362>
- Rittmann, B. E., & McCarty, P. L. (1980). Model of steady-state-biofilm kinetics. *Biotechnology and Bioengineering*, 22(11), 2343–2357. <https://doi.org/10.1002/bit.260221110>
- Rittmann, B. E., & McCarty, P. L. (2001). *Environmental biotechnology: principles and applications*. (McGraw-Hill Education, Ed.).
- Rittmann, B. E., & Snoeyink, V. L. (1984). Achieving Biologically Stable Drinking Water. *Journal - American Water Works Association*, 76(10), 106–114. <https://doi.org/10.1002/j.1551-8833.1984.tb05427.x>
- Rittmann, B. E., & Stilwell, D. (2002). Modelling biological processes in water treatment: the integrated biofiltration model. *Journal of Water Supply: Research and Technology-Aqua*, 51(1). <https://doi.org/10.2166/aqua.2002.0001>
- Robinson, P. K. (2015). Enzymes: principles and biotechnological applications. *Essays in Biochemistry*, 59, 1–41. <https://doi.org/10.1042/bse0590001>
- Rural Municipalities of Alberta. (2019). *Change to Lead and Manganese MAC Guidelines in Drinking Water*.
- Russell, M. J., & Hall, A. J. (2010). *Reprinted (adapted) with permission from The Hydrothermal Source of Energy and Materials at the Origin of Life*. Copyright 2010 American Chemical Society. (pp. 45–62). <https://doi.org/10.1021/bk-2009-1025.ch003>
- SAMC & SAC. (2022). *Standards for drinking water quality (生活 饮用水卫生标准)*. State administration for market regulation (国家市场监督管理总局), Standardization administration (国家标准化管理委员会). (Catalogue No GB57849-2022).

- Sharma, S. K., Petrusovski, B., & Schippers, J. C. (2005). Biological iron removal from groundwater: a review. *Journal of Water Supply: Research and Technology-Aqua*, 54(4), 239–247. <https://doi.org/10.2166/aqua.2005.0022>
- Smith, T. (2014). *Manual on Test Sieving Methods: Guidelines for Establishing Sieve Analysis Procedures*.
- Snoeyink, V. L., & David Jenkins. (1980). *Water chemistry*. JOHN WILEY & SONS.
- Sodamade, G., & Pearse, L. (2013). Removal of iron and manganese using rapid sand filtration. *NSE Technical Transaction*, 47(3), 41–51.
- Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Subcommittee of Interpretation, Uses of Dietary Reference Intakes, Subcommittee on Upper Reference Levels of Nutrients, & Panel on Micronutrient. (2002). *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. National Academies Press. <https://doi.org/10.17226/10026>
- Stumm, W., & Morgan, J. J. (1996). *Aquatic Chemistry, chemical equilibria and rates in Natural Waters* (Copyright Licence number: 5743170674163; Retrieved on March 06, 2024) (Wiley, Ed.; 3rd ed.).
- Taffarel, S. R., & Rubio, J. (2009). On the removal of Mn²⁺ ions by adsorption onto natural and activated Chilean zeolites. *Minerals Engineering*, 22(4), 336–343. <https://doi.org/10.1016/j.mineng.2008.09.007>
- Tang, W., Gong, J., Wu, L., Li, Y., Zhang, M., & Zeng, X. (2016). DGGE diversity of manganese mine samples and isolation of a *Lysinibacillus* sp. efficient in removal of high Mn (II) concentrations. *Chemosphere*, 165, 277–283. <https://doi.org/10.1016/j.chemosphere.2016.08.134>
- Tatari, K., Smets, B. F., & Albrechtsen, H.-J. (2016). Depth investigation of rapid sand filters for drinking water production reveals strong stratification in nitrification biokinetic behavior. *Water Research*, 101, 402–410. <https://doi.org/10.1016/j.watres.2016.04.073>
- Tekerlekopoulou, A. G., Vasiliadou, I. A., & Vayenas, D. V. (2008). Biological manganese removal from potable water using trickling filters. *Biochemical Engineering Journal*, 38(3), 292–301. <https://doi.org/10.1016/j.bej.2007.07.016>

- Teng, Z., Yuan Huang, J., Fujita, K., & Takizawa, S. (2001). Manganese removal by hollow fiber micro-filter. Membrane separation for drinking water. *Desalination*, 139(1–3), 411–418. [https://doi.org/10.1016/S0011-9164\(01\)00342-3](https://doi.org/10.1016/S0011-9164(01)00342-3)
- Teodosiu, C., Gilca, A.-F., Barjoveanu, G., & Fiore, S. (2018). Emerging pollutants removal through advanced drinking water treatment: A review on processes and environmental performances assessment. *Journal of Cleaner Production*, 197, 1210–1221. <https://doi.org/10.1016/j.jclepro.2018.06.247>
- Therdkiattikul, N., Ratpukdi, T., Kidkhunthod, P., Chanlek, N., & Siripattanakul-Ratpukdi, S. (2020). Manganese-contaminated groundwater treatment by novel bacterial isolates: kinetic study and mechanism analysis using synchrotron-based techniques. *Scientific Reports*, 10(1), 13391. <https://doi.org/10.1038/s41598-020-70355-w>
- Thuptimrang, P., Siripattanakul-Ratpukdi, S., Ratpukdi, T., Youngwilai, A., & Khan, E. (2021). Biofiltration for treatment of recent emerging contaminants in water: Current and future perspectives. *Water Environment Research*, 93(7). <https://doi.org/10.1002/wer.1493>
- Tobiason, J. E., Bazilio, A., Goodwill, J., Mai, X., & Nguyen, C. (2016). Manganese Removal from Drinking Water Sources. In *Current Pollution Reports* (Vol. 2, Issue 3, pp. 168–177). Springer. <https://doi.org/10.1007/s40726-016-0036-2>
- Trueman, B. F., Gregory, B. S., McCormick, N. E., Gao, Y., Gora, S., Anaviapik-Soucie, T., L'Hérault, V., & Gagnon, G. A. (2019). Manganese Increases Lead Release to Drinking Water. *Environmental Science & Technology*, 53(9), 4803–4812. <https://doi.org/10.1021/acs.est.9b00317>
- Urfer, D., Huck, P. M., Booth, S. D. J., & Coffey, B. M. (1997). Biological filtration for BOM and particle removal: a critical review. *Journal - American Water Works Association*, 89(12), 83–98. <https://doi.org/10.1002/j.1551-8833.1997.tb08342.x>
- US Environmental Protection Agency. (2014). *Method 6020B Inductively Coupled Plasma—Mass Spectrometry*.
- USEPA. (2004). *Drinking water health advisory for manganese*.
- Vang, Ó. K. (2013). *ATP measurements for monitoring microbial drinking water quality*. Technical University of Denmark.

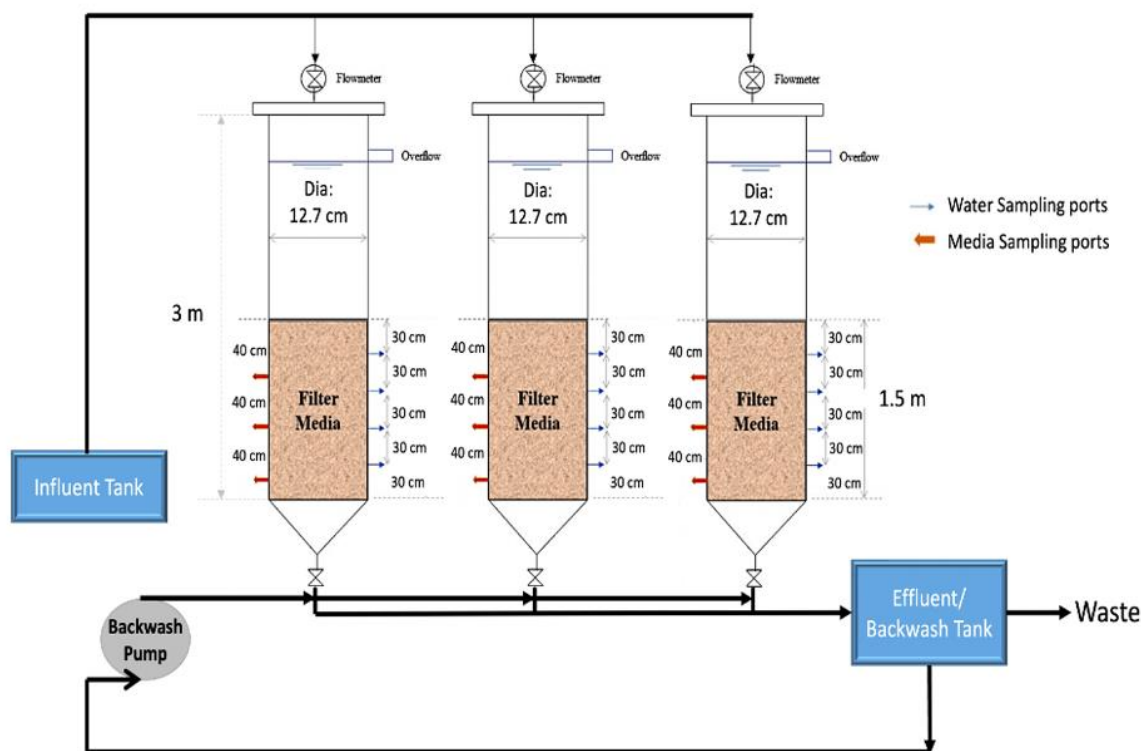
- Walkerton Clean Water Centre. (2018). *Evaluation of Greensand Filtration Operation for the Reduction of Manganese*.
- Weber, K. P., & Legge, R. L. (2010). *Community-Level Physiological Profiling* (pp. 263–281). https://doi.org/10.1007/978-1-60761-439-5_16
- Weber, K. P., & Legge, R. L. (2011). Dynamics in the bacterial community-level physiological profiles and hydrological characteristics of constructed wetland mesocosms during start-up. *Ecological Engineering*, 37(5), 666–677. <https://doi.org/10.1016/j.ecoleng.2010.03.016>
- Williams, P., & Ondeo, D. (2002, March 1). Biological iron and manganese removal as a viable alternative for groundwater treatment. *Environmental Science and Engineering*.
- Williamson, K., & Perry L. McCarty. (1976). A model of substrate utilization by bacterial films. *Water Pollution Control Federation*, 9–24.
- Woo Hang Kim, Wataru Nishijima, Eiji Shoto, & Mitsumasa Okada. (1997). Pilot plant study on ozonation and biological activated carbon process for drinking water treatment. *Water Science and Technology*, 35(8). [https://doi.org/10.1016/S0273-1223\(97\)00147-9](https://doi.org/10.1016/S0273-1223(97)00147-9)
- World Health Organisation. (2017). *Guidelines for drinking-water quality* (World Health Organization, Ed.; 4th Edition).
- World Health Organisation. (2019). *1 in 3 people globally do not have access to safe drinking water – UNICEF, WHO*.
- World Health Organization. (2021). *Manganese in drinking water: background document for development of WHO Guidelines for drinking-water quality*.
- Yang, L., Li, X., Chu, Z., Ren, Y., & Zhang, J. (2014). Distribution and genetic diversity of the microorganisms in the biofilter for the simultaneous removal of arsenic, iron and manganese from simulated groundwater. *Bioresource Technology*, 156, 384–388. <https://doi.org/10.1016/j.biortech.2014.01.067>
- Yargeau, V. (2012). Water and wastewater treatment: chemical processes. In *Metropolitan Sustainability*. Elsevier. <https://doi.org/10.1533/9780857096463.3.390>

- Yu, X., Qi, Z., Zhang, X., Yu, P., Liu, B., Zhang, L., & Fu, L. (2007). Nitrogen loss and oxygen paradox in full-scale biofiltration for drinking water treatment. *Water Research*, 41(7). <https://doi.org/10.1016/j.watres.2007.01.006>
- Yu, X., Zhang, X., Liu, X., Zhao, X., & Wang, Z. (2003). Phosphorus limitation in biofiltration for drinking water treatment. *Journal of Environmental Sciences*, 15(4), 494–499.
- Zeng, H., Yin, C., Zhang, J., & Li, D. (2019). Start-up of a biofilter in a full-scale groundwater treatment plant for iron and manganese removal. *International Journal of Environmental Research and Public Health*, 16(5). <https://doi.org/10.3390/ijerph16050698>
- Zhao, P., Zhao, S., Wang, H.-G., Lu, M., & Li, Z.-H. (2022). Encapsulation of bacteria in different stratified extracellular polymeric substances and its implications for performance enhancement and resource recovery. *Water Research*, 220, 118684. <https://doi.org/10.1016/j.watres.2022.118684>

Appendices

Appendix A - Supporting Document for Chapter 3: Materials and Research Methodology

Appendix A.1 Original biofilters design (*Not to scale) Arora, (2021) and space requirements based on the design



Based on the biofilter design above, the space and raw water flowrates requirements were as follows:

1. Height clearance: 3.7 - 4.2 m (ideal)
2. Footprint: Minimum 12-13 m² (130-140 ft²)
3. Feedwater to biofilter columns (5 m/h flow rate in columns) requirements:
 - Estimated minimum feed flow: 2,200 L/day (14,000 L/week) for 3 pilot biofilters (Two running continuously, one for 6 h/day)
 - Estimated maximum feed flow 4,600 L/day (32,200 L/week) for 3 pilot biofilters (all running continuously)
4. Required volume flow rates from the well (based on storage tank capacities):

- 2300 L/h if well operates 2 h/day
- 512 L/h if well operates 9 h/day

Appendix A.2 Photos taken during field visit of 4 utility wells that were considered for pilot set-up



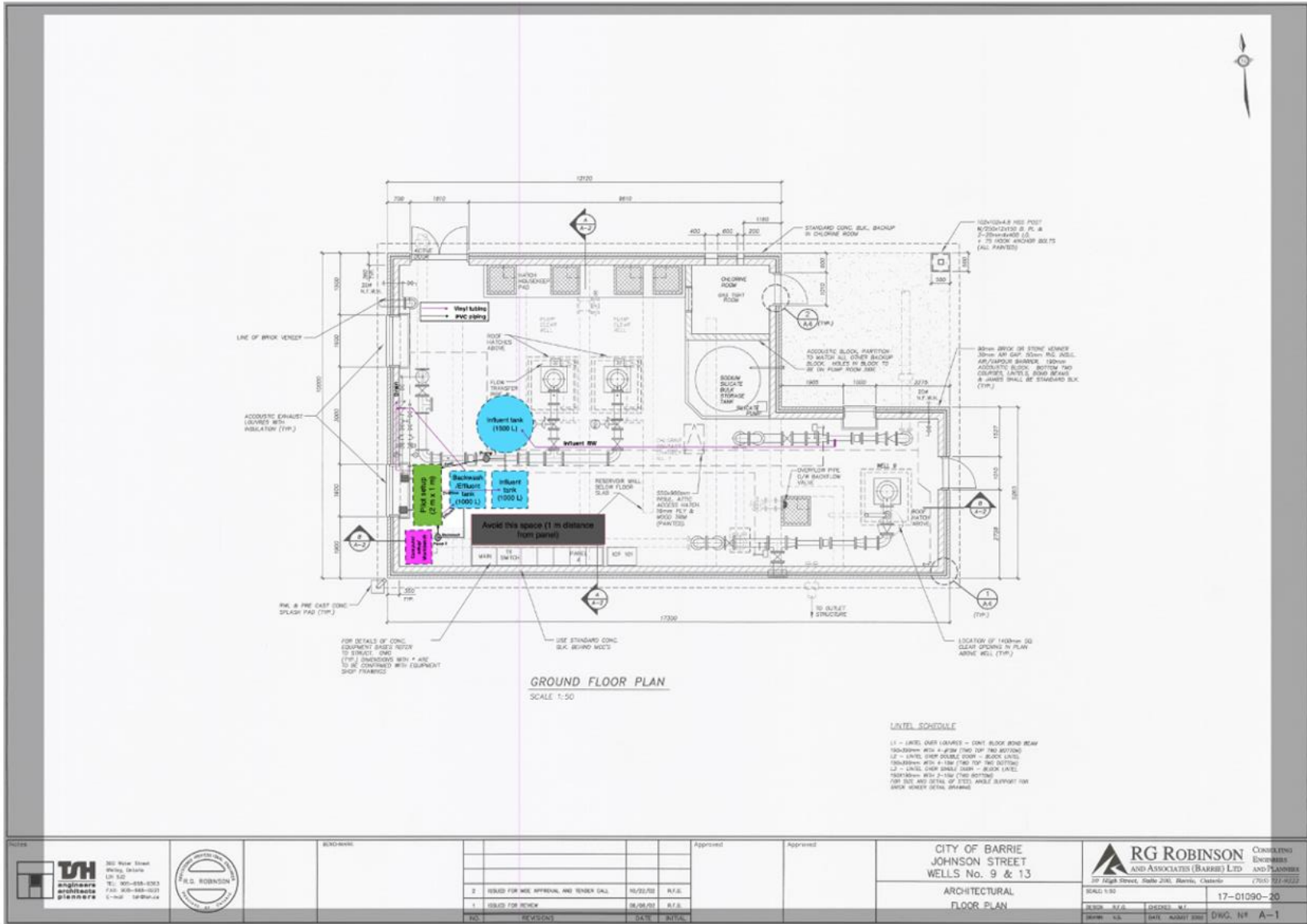
Appendix A.3 Data Compilation from Site Visits to Selected Wells on September 23, 2022

Table A.1 Important information about wells

	Well 13	Well 11/14	Well 3A
Operating hours	2.5 – 6 hrs (Depending on fill level in finished water tank) Can be increased if required	1 – 6 hrs (Depending on fill level in finished water tank). Can not be increased	0 to 24 hrs/ day
Space availability	Enough	Not adequate for two tables	Enough
Height clearance	A little bit more than 12 ft	11 ft	A little bit more than 12 ft
Flow regime ({Pilot work will require 0.274 L/sec (max)})	67 L/sec	80 L/sec	Any value possible
Non-chlorinated water access	Yes (influent line can be taken from a point which is before the chlorine gas addition segment)		
Onsite disposal of filter effluent and backwash water	Sanitary sewers available	Sanitary sewers available	Sanitary sewers available
Access to electricity for pumps etc.	Available to provide for a pump and a computer system.		
Internet access	No	No	No

Any important issue to be given consideration	Issues with iron bacteria and slime formation which is controlled through periodic maintenance (superchlorination)- Last time done in July or Aug 21'.	VOC plume issues. Therefore, can not increase the operation hours (one reason).	Pressure relief required. Very high TOC and turbidity. Not huge amount of Mn in water.
Analysis equipment	Available at the surface water treatment plant.		
Other comments	➤ 200 m³ storage underground. ➤ 30-35 years old system. ➤ Sand separator used (before influent line) ➤ No wall for clamping the filters.	➤ Sand separator used	➤ Shutdown. Last operated in 2010. Now just under maintenance and sampling done every 2 weeks. ➤ Turnover time not required. ➤ No sand issues here ➤ No influent feed tank required.

Appendix A.4 Well 13 drawings (Floor plan with the pilot plant layout)



Appendix A.5 Biofiltration Pilot Plant Standard Operation Procedure

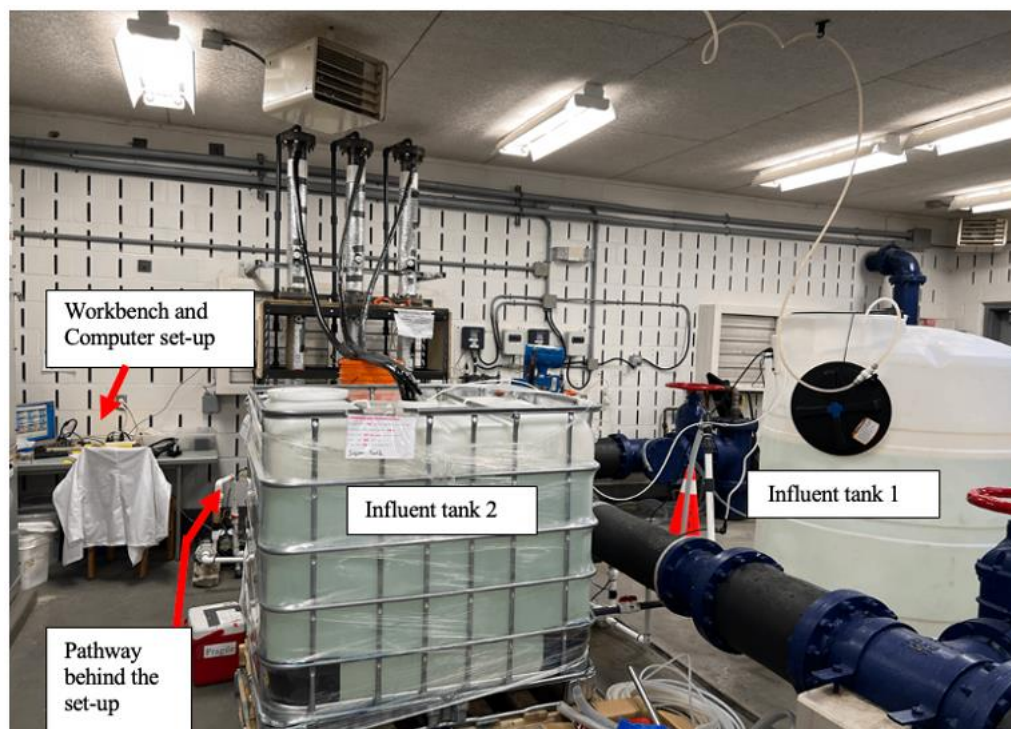


Figure A.1 Pilot scale set up on-site



Figure A.2 Image highlighting respective biofilter column runtimes

A.5.1 System start-up

This procedure describes the steps for start-up of the pilot set up.

A.5.1.1 Procedure

1. **OPEN** groundwater main influent feed valve completely (*to feed tank 1*). (V13, located on top of influent tank 1) (Refer to Figure A.3)
2. **OPEN** influent raw water line from feed tank to pump completely (V14) (Refer to Figure A.3)
3. **OPEN** influent raw water line from pump to set up completely (V15) (Refer to Figure A.3)
4. **OPEN** influent valves in the influent lines (located behind the biofilter columns) of the set up halfway (V18, V19, V20) (Refer to Figure A.4)

Note: Sometimes, there is a possibility that the valves must be opened a bit less or a bit more, just to reach the desired effluent flow rate.

5. **OPEN** the valves for effluent water line. (V21, V22, V23) (Refer to Figure A.4)

Note 1: They need to be very slightly opened, but the valves are very sensitive.

- Note 2:** Make sure the backwash valves (located below the biofilter columns) (V29, V30, V28) and biofilter column drain valves (V24, V35, V26) are completely **CLOSED** (Refer to Figure A.5)
6. **PLUG IN & START** the INFLUENT pump. (Refer to Figure A.3)
 7. **STEPS** for running the software. (Procedure is also posted on the wall behind the desktop on the workbench) (Refer to Figure A.6)
 - a. Switch on the computer and launch “Tubular Reactor” application.
 - b. Give a name to the file (Name will be the date in MM DD YYYY format of that day)
 - c. Click on the START option.
 8. **REGULATE** the effluent valves, so that the effluent flow rate on the desktop shows 5 m/h (OR ~0.65 L/min) for all the three biofilter columns. (V21, V22, V23) (Refer to Figure A.4)

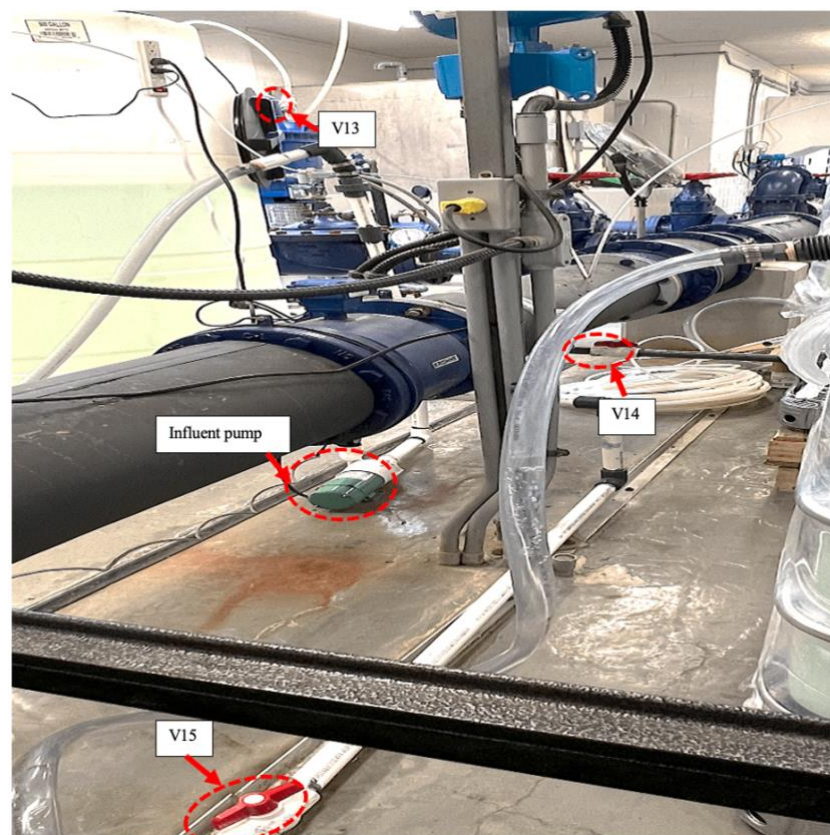


Figure A.3 Image highlighting influent valves common to all biofilter columns

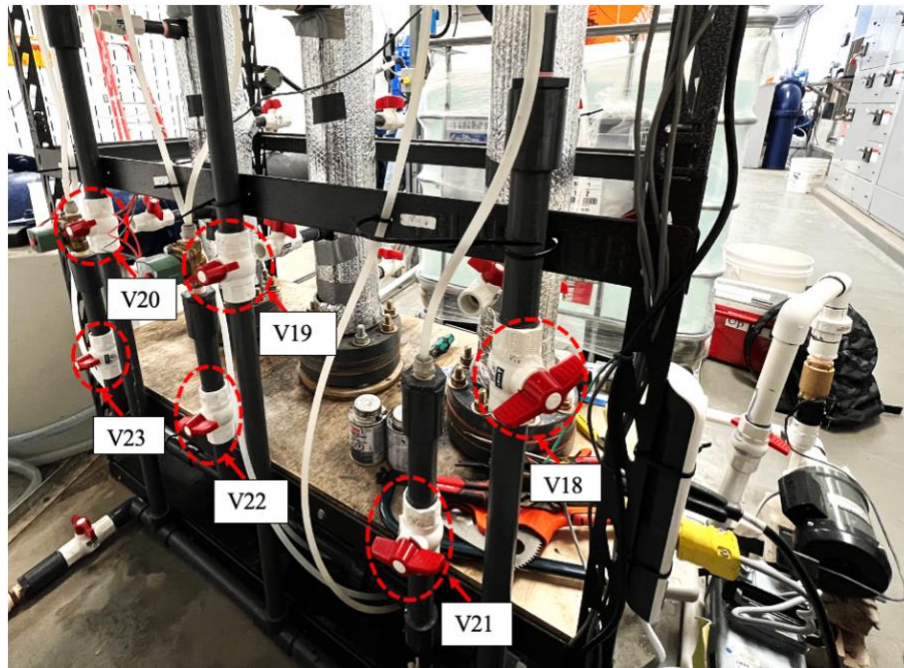


Figure A.4 Image highlighting influent (V18, V19, V20) and effluent (V21, V22, V23) flow valves for each biofilter column

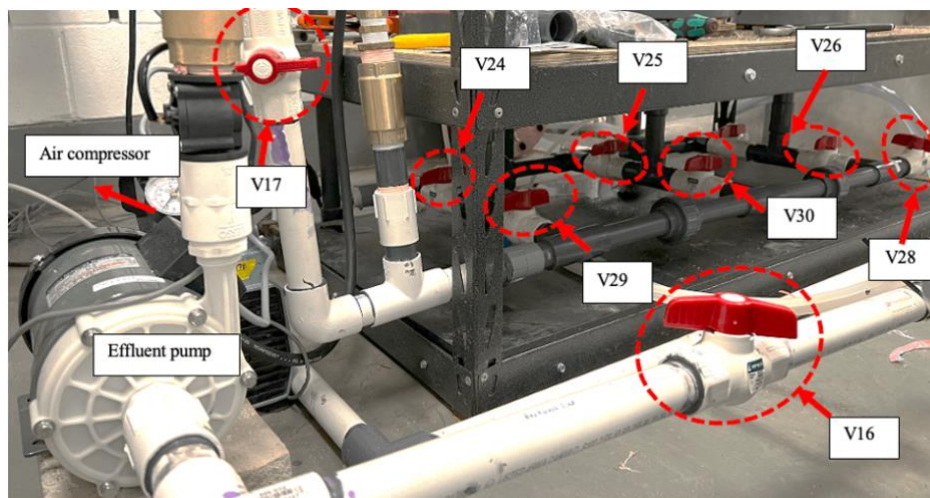


Figure A.5 Image highlighting backwashing valves (V16, V17, V28, V29, V30), biofilter column drain valves (V24, V25, V26)), effluent pump and air compressor

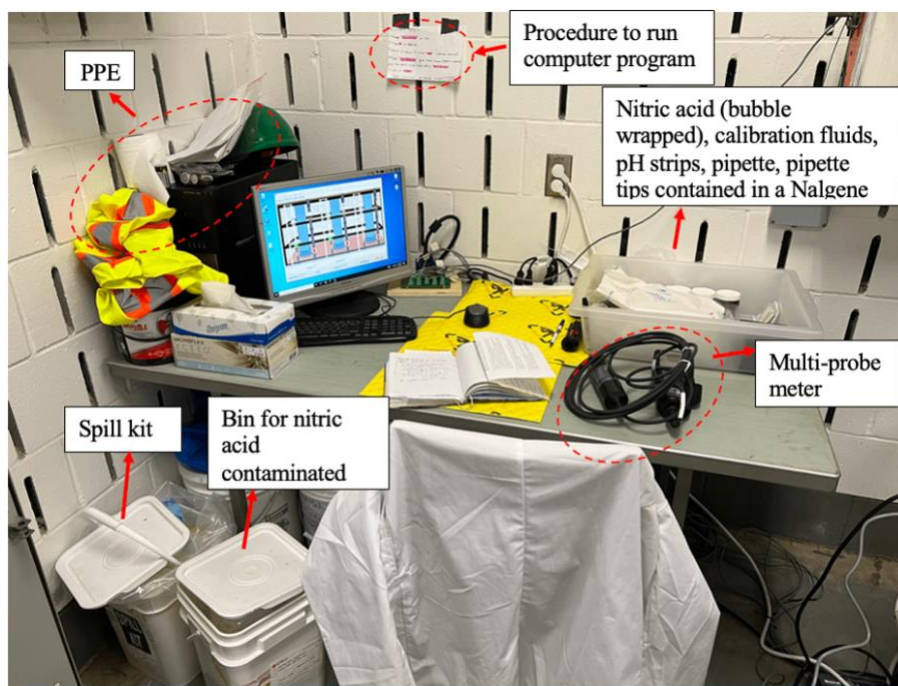


Figure A.6 Image describing the items on workbench besides biofilter set-up on-site

A.5.2 Backwash

This procedure describes the procedure for backwashing each biofilter of the pilot set up.

Note: The Procedure is general to all biofilter columnss. The valve numbers below are for biofilter column A for illustration. Refer to table 1 for biofilter specific valve numbers.

A.5.2.1 Procedure (for biofilter column 1)

*Biofilter column specific - see Table A.2 below for column-specific valves

1. **CLOSE** influent raw water line from pump to set up completely (V15) (Refer to Figure A.3)
2. **CLOSE** influent valves in the influent lines of the set up halfway (V18, V19, V20) (Refer to Figure A.4)
3. **CLOSE** the valves for effluent water line. (V21, V22, V23) (Refer to Figure A.4)

Table A.2 Valve IDs for each process per biofilter column

Valves common to all biofilter columns			
V13		Main line (Well 13 to influent tank 1)	
V14		Connecting Influent tank 2 and Influent tank 1	
V15		Feed line (Pump 1 to biofilter setup)	
V16		Backwash line (Tank to pump 2)	
V17		Backwash line (Pump 2 to biofilter setup)	
Valves specific to each biofilter columns			
Column A	Column B	Column C	Line description
V18	V19	V20	Influent
V21	V22	V23	Effluent
V29	V30	V28	Backwash
V24	V25	V26	Drain valves (used to drain the biofilters completely)

4. **ENSURE** enough water (at least 3/4th of the tank) is stored in the effluent/backwash tank for backwashing three columns and that water has stopped flowing to the backwash tank for this column.
5. Remove the overflow pipe of column A from the influent tank 2 and connect it to the spare drainpipe.
6. **OPEN** the **column drain valve V24**. (Refer to Figure A.5.)
7. **CLOSE** the **column drain valve V24** once the level has reached approximately 30 cm above the top of the media.
8. **OPEN** the **backwashing valve V29** connecting the column 1. (Refer to Figure A.5)
9. **OPEN** the **backwashing valve V16** connecting the backwash storage tank to the backwash pump. (Refer to Figure A.5)
10. **CLOSE** the **valve V17** connecting backwash pump to the set up. (Refer to Figure A.5)
11. **CLOSE** the **inlet knob** of the air compressor completely (**marked as Inlet knob on the**

compressor).

12. **OPEN** the **outlet knob** of the air compressor completely (**marked as Outlet knob on the compressor**).

***Note:** The inlet knob is to take in air from the atmosphere, whereas the outlet knob controls the amount of air that passes into the pipelines. The knobs have a hole on the top to purge out air. As we **CLOSE** the knobs, more air goes **INTO** the system than being purged out. A fully open outlet knob would lead to complete air purging outside, and as we close the outlet knob, the air will start passing into the pipeline from the compressor. Before starting the air compressor, the outlet knob should be kept completely **OPEN**. Once the compressor is started, slowly turn the knob clockwise to close it, which will lead some air to pass into the pipeline and therefore into the column. (Refer to Figure A.7)*



Figure A.7 Image showing the markings on the air compressor

13. **START** the **air compressor**. (Refer to Figure A.5)

14. **START** the BACKWASH pump. (Refer to Figure A.5)
15. **VERY SLOWLY OPEN** the **valve V17** connecting backwash pump to the set-up, just enough to let water go upwards in the column and NOT fluidise the media. (Markings on valve is made accordingly) (The backwash flowrate shown in the program interface on the desktop should be around **1.5-2 L/min**) (Refer to Figure A.7, Figure A.8)

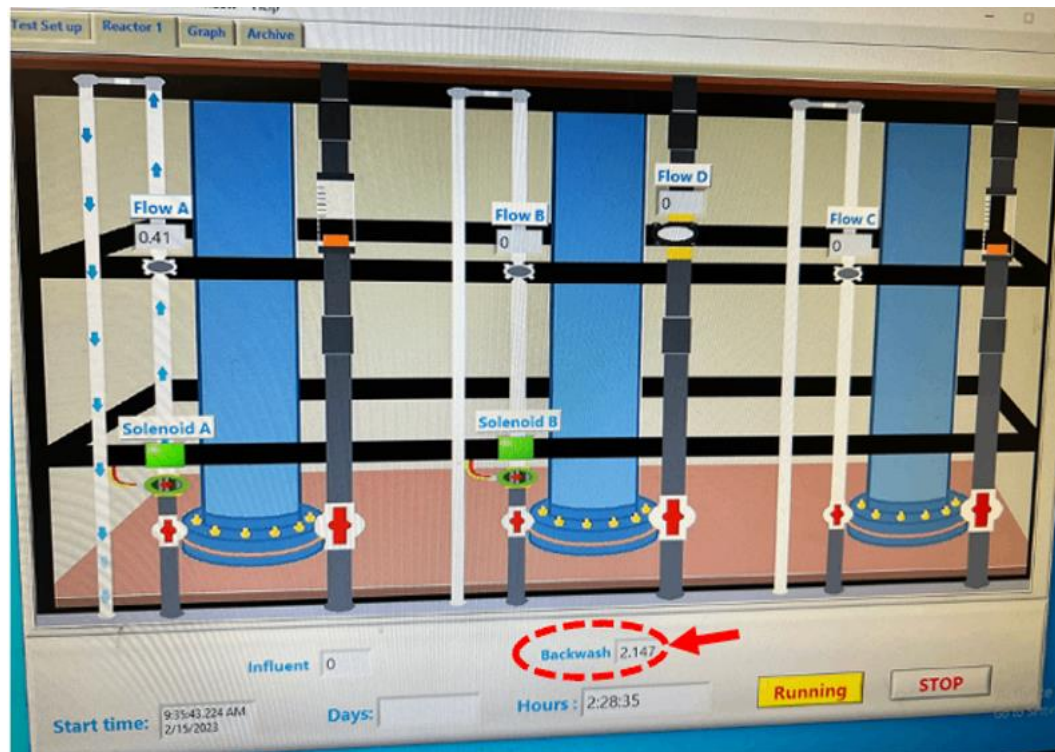


Figure A.8 Image highlighting the backwash flow rate while performing air scour + water backwash

16. **VERY SLOWLY CLOSE** (rotate clockwise) the outlet knob of the air compressor until the markings on knob and compressor body match. (*Note: Be very careful and keep a watch at the top of the column while opening this knob. Slightly higher air force can cause the media to overflow and therefore media loss*)
17. **Perform** this **air scour + water backwash** for **3 minutes**.
18. **OPEN** the **outlet knob** of the air compressor completely.
19. **STOP** the air compressor. (Make sure outlet knob is OPEN completely)
20. **SLOWLY OPEN** the **valve V17** connecting backwash pump to the set-up so that the bed

expansion happens. 35-40 % expansion of filter bed is required. (Markings on columns are made accordingly) (The backwash flowrate shown in the program interface on the desktop should be around 9.5-10.5 L/min) (Refer to Figure A.7, A.9, A.10)

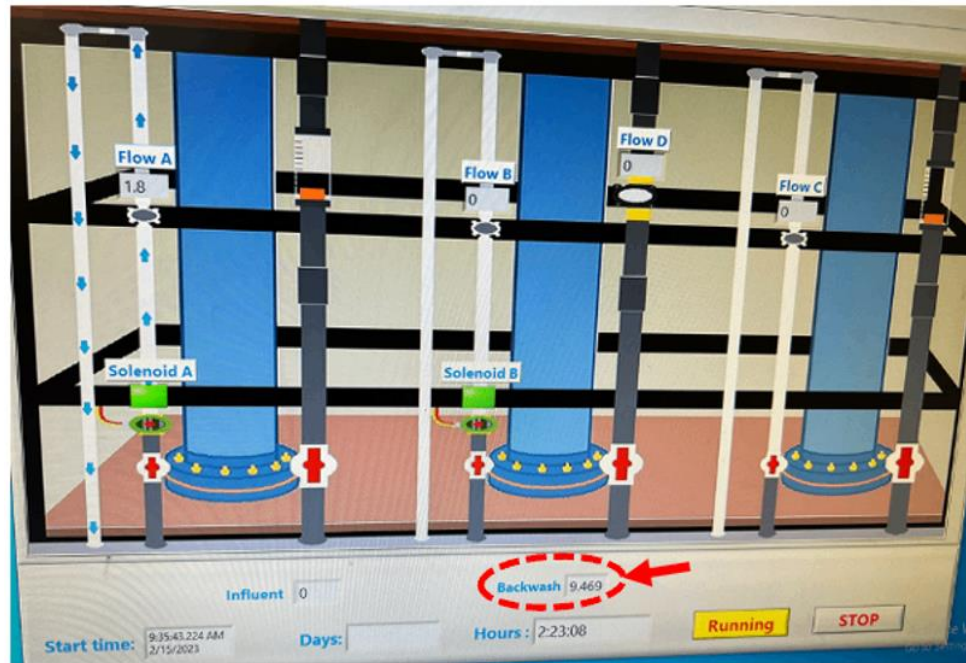


Figure A.9 Image highlighting the backwash flow rate while performing water only backwash



Figure A.10 Image highlighting the media expansion limit marking on the filter columns

Note: Initially valve might have to be opened a bit more to overcome the bed lifting resistance, but then as the bed starts to fluidise, reduce the valve opening accordingly.

21. **Perform this water only backwash for 5-7 minutes (or longer until the time that backwash water is clear).** (Clear water should come out from the overflow towards the end of backwash)
22. **STOP** the pump.
23. **CLOSE** the **valve V17** connecting backwash pump to the set up.
24. **CLOSE** the **backwashing valve V29** connecting the column 1.
25. **OPEN** the **column drain valve V24**.
26. **CLOSE** the **column drain valve V24** once the water is completely drained from the column.
27. Remove the connection of the overflow pipe of column A to the spare drainpipe and put the overflow pipe back in influent tank 2.
28. **REPEAT** STEP 7 to STEP 21 for column B and C.
29. **CLOSE** the **backwashing valve V16** connecting the backwash storage tank to the backwash pump.

30. **FOLLOW 1.0** to begin operating the biofilters again.

A.5.3 Emergency system shutdown

This procedure describes the procedure for shutting down operations of the pilot set up at a drinking water treatment facility in Southern Ontario, Canada.

A.5.3.1 Procedure

1. **CLOSE** influent raw water line from feed tank to pump (V14) (Refer to Figure A.3)
2. **UN-PLUG** the influent pump.
3. **CLOSE** influent raw water line from pump to set up (V15) (Refer to Figure A.3)
4. **CLOSE** the effluent line valves once the water level in the columns is about 10 cm above the filter media (V21, V22, V23). (Refer to Figure A.4)
5. **CLOSE** groundwater main influent feed valve (*to feed tank 1*). (V13, located on top of influent tank 1) (Refer to Figure A.3)

Also, emergency shutdown procedures have been posted (Refer to Figure A.11) on each of the tanks at the site.

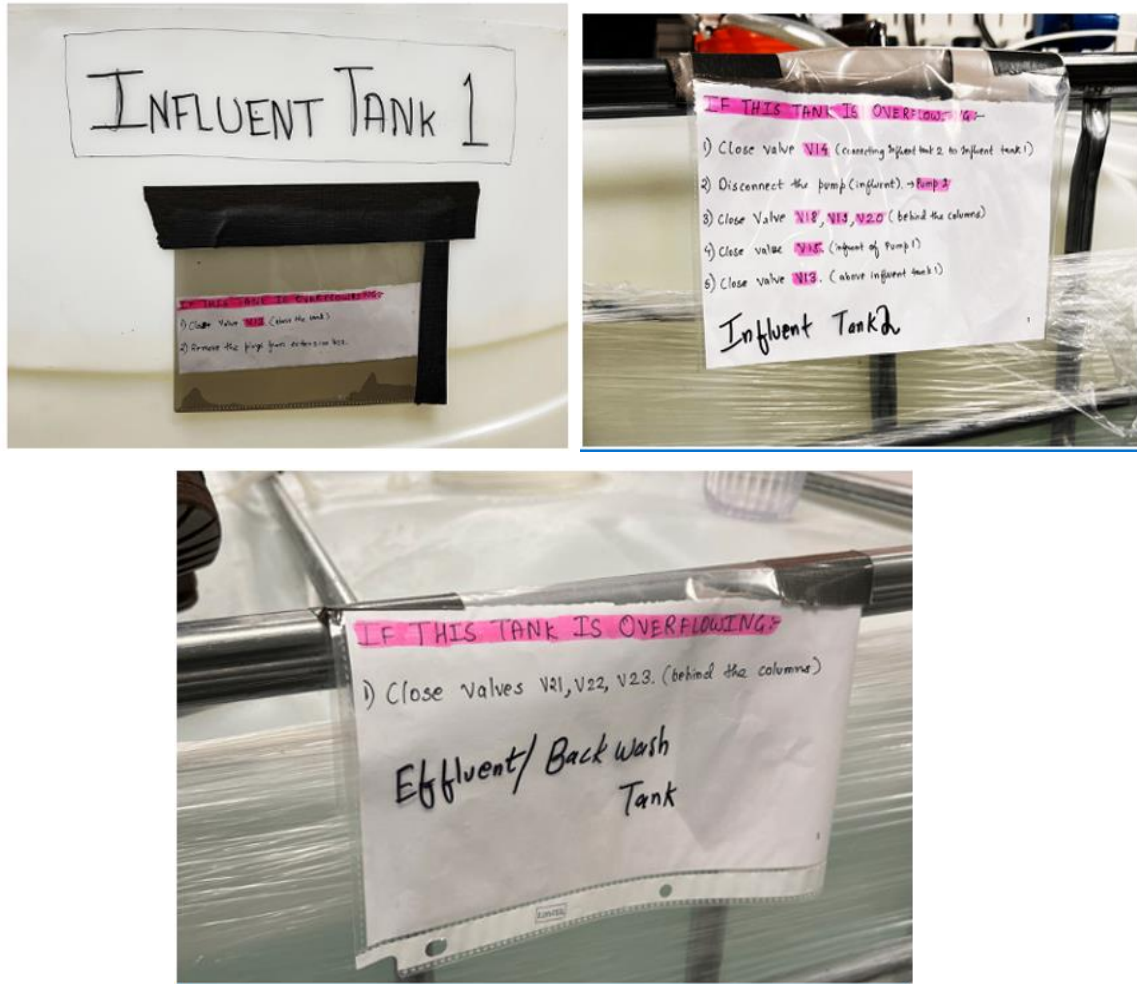


Figure A.11 Image showing the emergency shutdown procedures posted on-site on the tanks.

A.5.4 Mitigating blockages in biofilter columns

Particularly during summer operation, mud balls may form within the biofilter columns, affecting water flow and the backwashing process. If mud balls are present, the standard backwashing techniques described above will not adequately eliminate the formation of blockages in the media. The following procedure describes a method of removing blockages caused by mud balls.

A.5.4.1 Procedure

1. **CLOSE** the **influent water valve** and the **effluent water valve** of the affected column.
2. **OPEN** the **column drain valve** to lower the column water level.

3. **CLOSE** the **column drain valve** when the water level in the column is below the height of the sampling port nearest to the mud balls.
4. **CONNECT** a pump to the sampling port with tubing.
5. **OPEN** the **sampling port valve**.
6. **TURN ON** the pump and allow it to run until the mud balls have broken up.
7. **TURN OFF** the pump and **close** the **sampling port valve**.
8. **DISCONNECT** the tubing from the sampling port.
9. **OPEN** the **influent water valve** and the **effluent water valve**.
10. **BACKWASH** the column following the normal procedure. If the media level rises uniformly after the air scour, then the mud balls have successfully been removed.

A.5.5 Tasks performed by the water operator from groundwater treatment utility

This list describes the tasks performed by the operator:

1. Regular check-up of the following (at least once in three days):
 - a. Check for any overflows from tanks.
 - b. Check water level in the tanks. If the level is too low (less than 1/3rd of the tank level) in the influent tank 1, then manually fill the tank with a hose.
 - c. Check effluent flow rates (around 0.66 L/min) for the columns in the program running on the desktop. If too high (more than 0.72 L/min) or too low (less than 0.5 L/min), then regulate the valves V21, V22, V23. (Refer to Figure 3.5)
 - d. Check if the program on computer is running smoothly. If any error occurs, then stop the program, close the program, and restart the program. Instructions to do so are attached to the wall behind the desktop. Also refer SOP for “SYSTEM START-UP”, bullet point 7 for the steps.
 - e. Column C is shutting down after running for 6 h as set (operating times are posted on filter C for reference).
 - f. Samples will be taken weekly by Ujwal (graduate student, University of Waterloo), while sometimes the operator will help measure water quality parameters using the multi-probe meter.
 - g. Backwashing will be done by Ujwal and the operator will provide help.

2. System shutdown in case of emergency. This includes any malfunctioning in the pilot setup, water overflow from the tanks or columns, in case of plant shutdown or in situations which might potentially possess any risk to the plant or personal on-site.
3. Water quality analysis using multi probe meter and taking water sample, once a week (sometimes).

Appendix A.6 Important observations during operation and while performing biofilter backwash

Important observations during operations:

Backwashing was performed for the first time after 14 days of operation and it was observed that the flow rates were not affected significantly in between two backwash cycles. While performing backwashing, the complete process was quite smooth for columns A, but for columns B and C, some issues were faced. In column B and C, the sand media would not mix appropriately initially, but then by modulating the backwash flow rate, the sand media would separate and then continue to mix for the rest of the cycle. For columns A, B and C, during the initial period of filter backwash cycle wherein air scour was performed, the process was quite smooth and there was no need to regulate the water flow rate. The water flow rate was just enough to let water flow upwards in the columns and the air bubbles would create the desired disturbance in the media and potentially separating the sand media particles without causing much fluidization. One important observation during this part of the backwash cycle was that dark reddish-brown water was seen in the columns above the sand media, which could mainly be because of the light oxidised particulate Mn and iron oxides captured in the sand media coming out. After 2-3 minutes of air scour, the air compressor would be turned off and then slowly the water flow rate was increased so that the desired bed expansion could be achieved. The transition between the two parts of the backwash cycle was quite smooth for Column A, but in Columns B and C, there was a need to regulate the flow rate a little bit to create agitation inside the column and thereby separate the clogged sand media particles that did not get separated during the initial air scour process. Sometimes, punching the columns externally with fist also helped clear the lump issue in the columns. Once they got cleared, the rest of the backwashing process was quite smooth. Towards the end of the backwash cycle, clear water was observed flowing out from the columns from the overflow pipes.

System perturbations during operations:

In the first week, due to the varying run times of the groundwater well utility (4-8 h/day), sometimes the water level in the influent tank would reach a point when the pump did not have enough water to pump into the columns, so it would run dry. This problem was solved in the second week itself by changing the pipelines (larger diameter feed pipeline was used to meet the demands of the pilot biofilters) and then it never occurred again, until towards the end of Phase 1, when due to turbidity lockdowns at the treatment utility, the water supply to the influent tank used to get cut quite frequently. Apart from this, a couple of times due to maintenance at the treatment utility, the water supply used to

get cut and the biofilters would run dry. All this information was recorded by the computer and the operator at the plant used to mention this in the logbook that was kept on site.

The computer constantly captured the flow rate data from the flow sensors and periodically flow rate was measured manually to confirm the flow meter readings.

Table A.3 Details of the no. of times biofilters ran dry and the reasons behind

Sr. no.	Reason behind biofilter columns running dry	No. of times	Hours lost/event
1.	System interruption due to power switching on site (Jan 17, Feb 14)	2	~24 h
2.	Unprecedented error in computer software	1	~24-48 h
3.	Well lockout due to high turbidity	3	4-10 h
4.	Flow meter not recording correct values	4	2-3 h

Appendix A.7 Sieve analysis (Smith, 2014)

Fine series of ASTM E-11 test sieves, were utilised for the separation process. The sieves used include:

1. 0.85 mm (Mesh No.20)
2. 1.00 mm (Mesh no.18)
3. 1.19 mm (Mesh No.16)
4. 1.4 mm (Mesh No.14)
5. 1.7 mm (Mesh No.12)

Sieving was performed using the Oscillatap sieve shaker machine and was also done manually by shaking the sieves in hand. After sieving, to reduce the variability and to uniformly mix the media coning and quartering technique was used. The sieve analysis of the media was done using standard sieve analysis procedures. The effective size (D_{10}) came out to be 1.05 mm and the uniformity coefficient was 1.28 (typically for sand the uniformity coefficient should be <1.5).

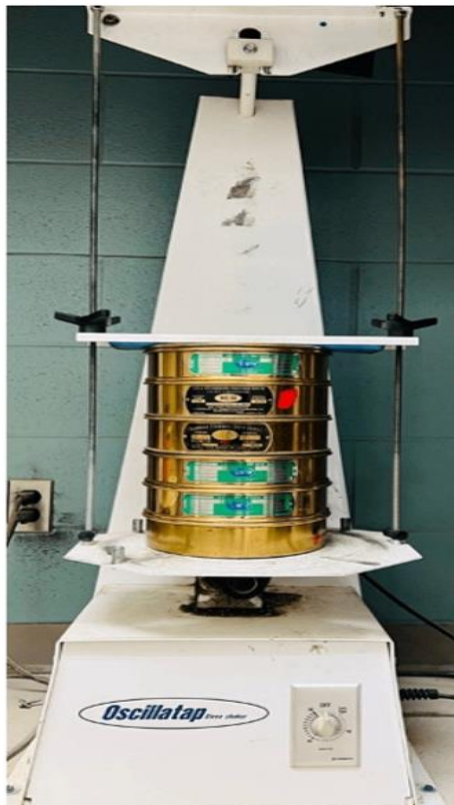


Figure A.12 Sieves staged on the Oscillatap sieve shaker

Table A.4 Sieve analysis data (before the pilot operation commenced)

Total mass used for sieve analysis: 302.3 g					
Sieve size (mm)	Mass retained (g)	Retained %	Cumulative weight (g)	Cumulative %	Pass %
1.7	0	0%	0	0%	100%
1.4	37.7	12%	37.7	12%	88%
1.19	153.4	51%	191.1	63%	37%
1	104	34%	295.1	98%	2%
0.85	7.2	2%	302.3	100%	0%

Sieve test results:

D ₁₀	1.05
d ₆₀	1.35
UC	1.28

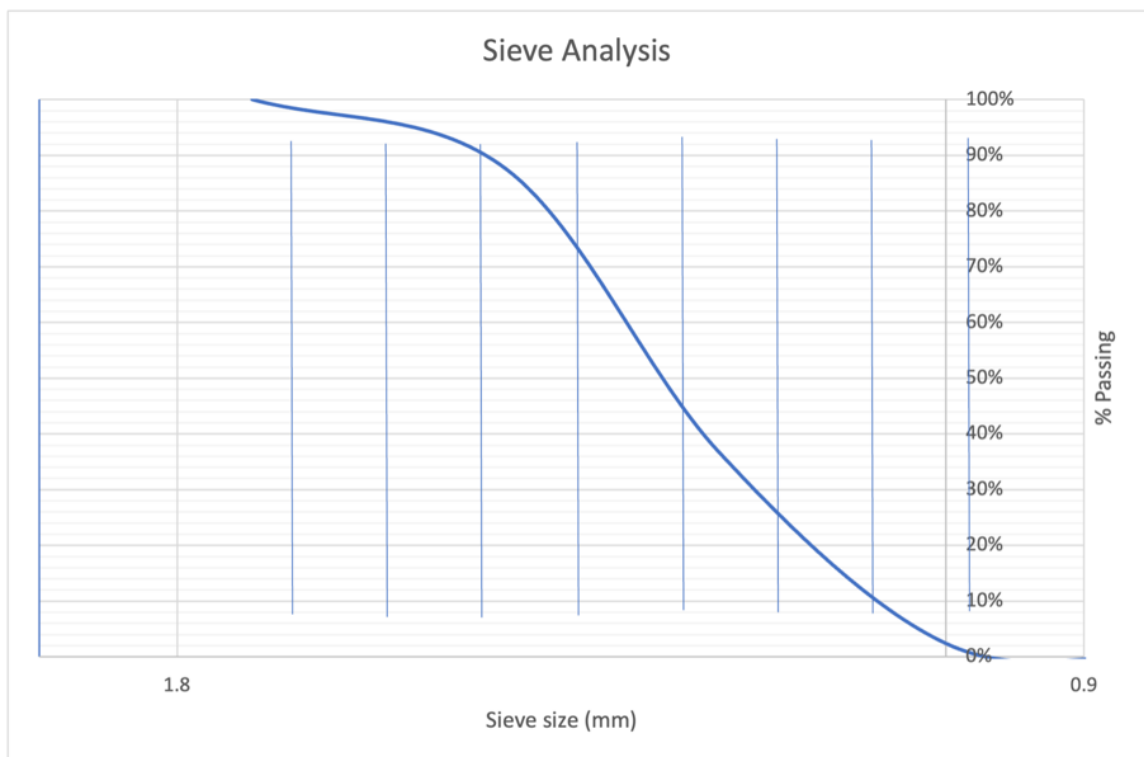


Figure A.13 Standard Particle Distribution Curve for sand media

The sieve analysis was also performed post biofilter dismantling and the results are below:

Table A.5 Sieve analysis data (after the pilot set up was dissassembled)

A					
Sieve size (mm)	Mass retained (g)	retained %	cumulative weight (g)	cumulative %	pass %
1.4	29.8	9%	29.8	9%	91%
1.19	161.9	49%	191.7	58%	42%
1	121.1	37%	312.8	95%	5%
0.85	16.2	5%	329	100%	0%
0.5	0	0%	329	100%	0%

B					
Sieve size (mm)	Mass retained (g)	retained %	cumulative weight (g)	cumulative %	pass %
1.4	16.4	6%	16.4	6%	94%
1.19	141.4	49%	157.8	55%	45%
1	115.2	40%	273	94%	6%
0.85	16	6%	289	100%	0%
0.5	0	0%	289	100%	0%

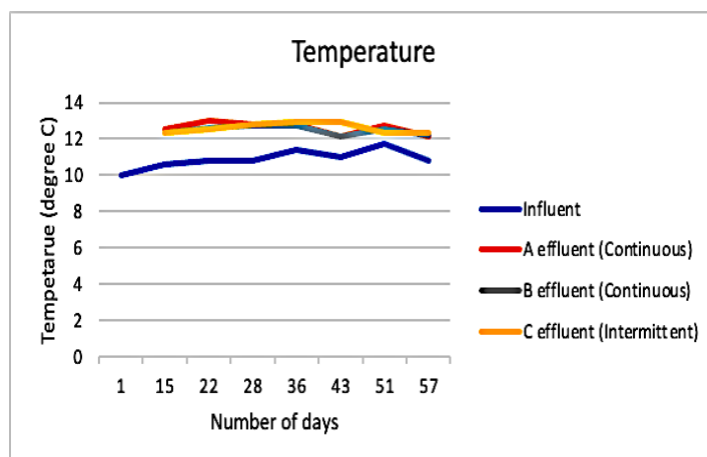
C					
Sieve size (mm)	Mass retained (g)	retained %	cumulative weight (g)	cumulative %	pass %
1.4	31.5	7%	31.5	7%	93%
1.19	276.4	60%	307.9	67%	33%
1	134.1	29%	442	97%	3%
0.85	14.5	3%	456.5	100%	0%
0.5	0.85	0%	457.35	100%	0%

Sieve test results:

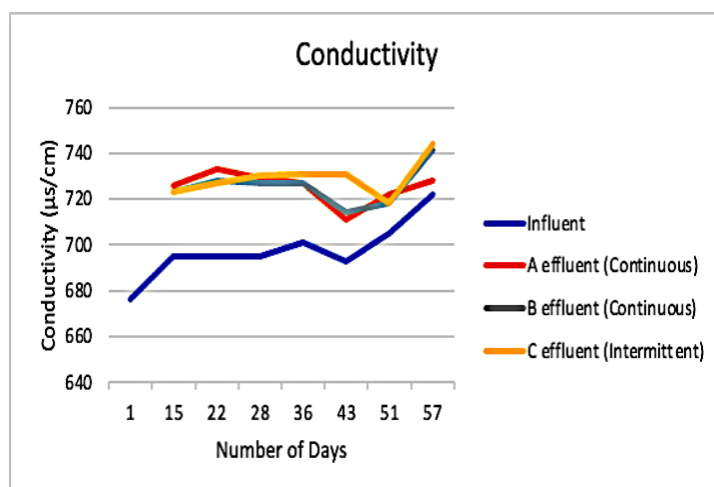
d10	d60	UC	
1.10	1.36	1.23	A
1.09	1.35	1.24	B
1.13	1.38	1.22	C
Acceptable range for UC:		1.3 to 1.7	

Appendix B - Supporting Data and Figures for Chapter 4: Effect of Intermittent Operation on Manganese Removal During Biofilter Start-Up

Appendix B.1: Additional supporting plots for the water quality parameters



(a)



(b)

Figure B.1 Variations in water quality parameters over time: (a) Temperature and (b) conductivity

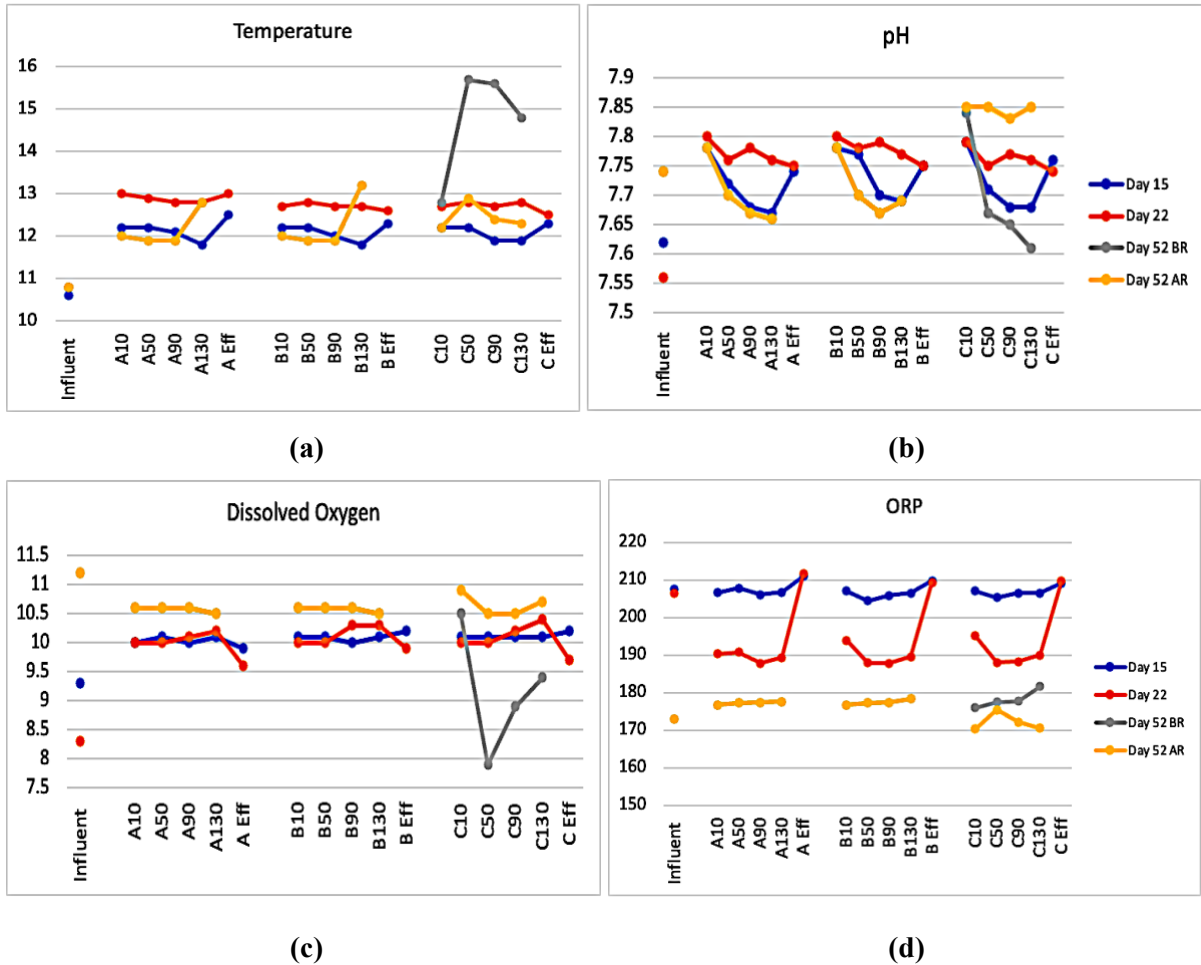


Figure B.2 Variations in water quality parameters recorded across various biofilter bed depths on day 15, 22 and 52 of operation (a) Temperature ($^{\circ}\text{C}$) (b) pH (c) DO (mg/L), (d) ORP (mV)

BR- Data collected before restart

AR- Data collected after restart

AX, BX, CX- Represents water samples collected from X cm depth of column A, B and C respectively, with X = 10 cm, 50 cm, 90 cm and 130 cm

A Eff, B Eff, C Eff- Represents water samples collected from effluents of column A, B and C respectively



Figure B.3 Impact of 18-h shutdown on water quality parameters during biofilter C start-up, on day 28 of operation (a) Temperature (°C) (b) pH (c) DO (mg/L), (d) ORP (mV), (e) conductivity (μS/cm)

AX, BX, CX- Represents water samples collected from X cm depth of column A, B and C respectively, with X = 10 cm, 50 cm, 90 cm and 130 cm

A Eff, B Eff, C Eff- Represents water samples collected from effluents of columns A, B and C respectively

Appendix B.2: Statistical analysis results

Table B.1 Day-wise Mn removal rates in the effluent water and the F values for each paired comparison to find whether we can assume equal variance while performing the student t-test

Day	Influent	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)	A-B	A-C	B-C
7	22.5	12%	12%	9%	0%	3%	2%
14	22.7	38%	31%	19%	7%	19%	13%
21	22.5	28%	15%	14%	13%	14%	0%
27	22.1	28%	9%	16%	19%	12%	7%
35	28.2	71%	25%	38%	47%	33%	13%
42	28.4	87%	46%	47%	41%	40%	1%
49	27.4	97%	80%	47%	17%	50%	33%
56	36.9	98%	93%	64%	5%	34%	29%
n	Avg	57%	39%	32%	19%	26%	12%
8	Std. dev	35%	32%	20%	17%	16%	13%
Null hypothesis	A_avg-B_avg = 0			F value	1.187	3.081	2.596
				p-value	0.41	0.08	0.12
				Variance	Equal	Equal	Equal

Table B.2 Day-wise Fe removal rates in the effluent water and the F values for each paired comparison to find whether we can assume equal variance while performing the student t-test

Day	Influent	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)	A-B	A-C	B-C
7	73.3	82%	81%	77%	1%	6%	5%
14	70.4	88%	88%	81%	1%	8%	7%
21	70.8	86%	86%	76%	0%	10%	10%
27	66.9	84%	82%	74%	1%	9%	8%
35	57.0	89%	90%	84%	-1%	5%	6%
42	63.2	90%	90%	87%	0%	3%	4%
49	68.2	93%	93%	79%	0%	14%	14%
56	112.0	91%	89%	84%	2%	7%	5%

Null hypothesis	$A_{avg} - B_{avg} = 0$
-----------------	-------------------------

F value	1.042	1.171	1.124
p-value	0.48	0.42	0.39
Variance	Equal	Equal	Equal

Table B.3 t-Test (A & B) results: Two-sample assuming equal variances

Manganese			Iron		
	<i>Variable 1</i>	<i>Variable 2</i>		<i>Variable 1</i>	<i>Variable 2</i>
Mean	0.57	0.38823	Mean	0.88	0.88
Variance	0.12	0.10211	Variance	0.00	0.00
Observations	8	8	Observations	8	8
Pooled Variance	0.11		Pooled Variance	0.00	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	14		df	14	
t Stat	1.11		t Stat	0.20	
P(T<=t) one-tail	0.14		P(T<=t) one-tail	0.42	
t Critical one-tail	1.76		t Critical one-tail	1.76	
P(T<=t) two-tail	0.28		P(T<=t) two-tail	0.84	
t Critical two-tail	2.15		t Critical two-tail	2.15	
Result	Not significant		Result	Not significant	

Table B.4 t-Test (A & C) results: Two-sample assuming equal variances

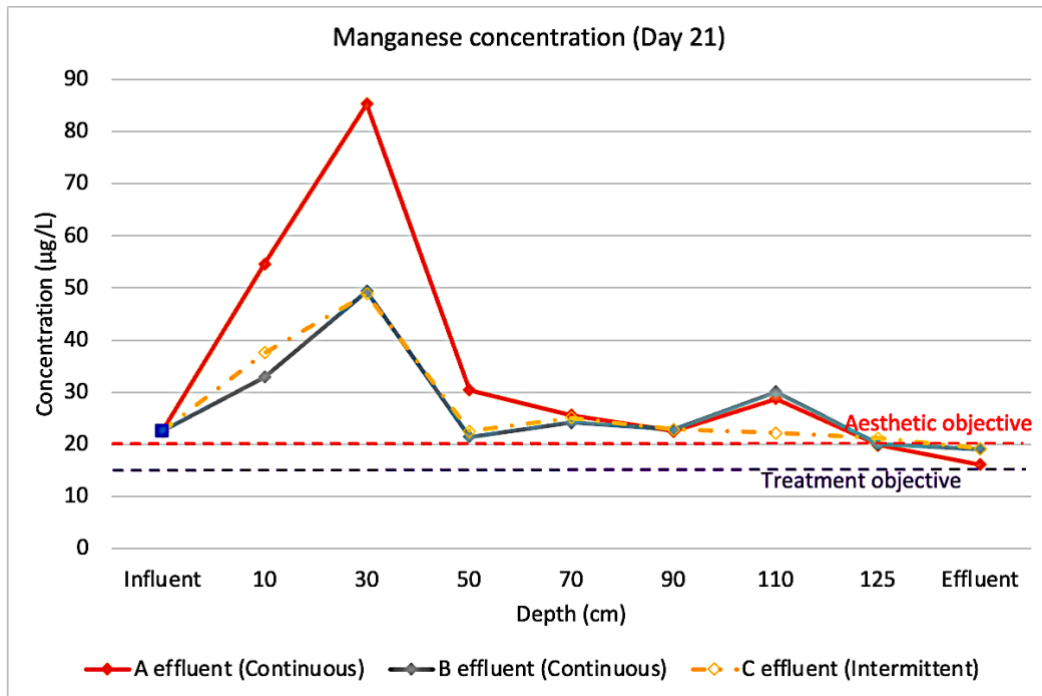
Manganese			Iron		
	<i>Variable 1</i>	<i>Variable 2</i>		<i>Variable 1</i>	<i>Variable 2</i>
Mean	0.57	0.31749	Mean	0.88	0.80
Variance	0.12	0.03933	Variance	0.00	0.00
Observations	8	8	Observations	8	8
Pooled Variance	0.08		Pooled Variance	0.00	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	14		df	14	

t Stat	1.8		t Stat	3.75	
P(T<=t) one-tail	0.05		P(T<=t) one-tail	0.00	
t Critical one-tail	1.76		t Critical one-tail	1.76	
P(T<=t) two-tail	0.09		P(T<=t) two-tail	0.00	
t Critical two-tail	2.15		t Critical two-tail	2.15	
Results	Significant		Results	Significant	

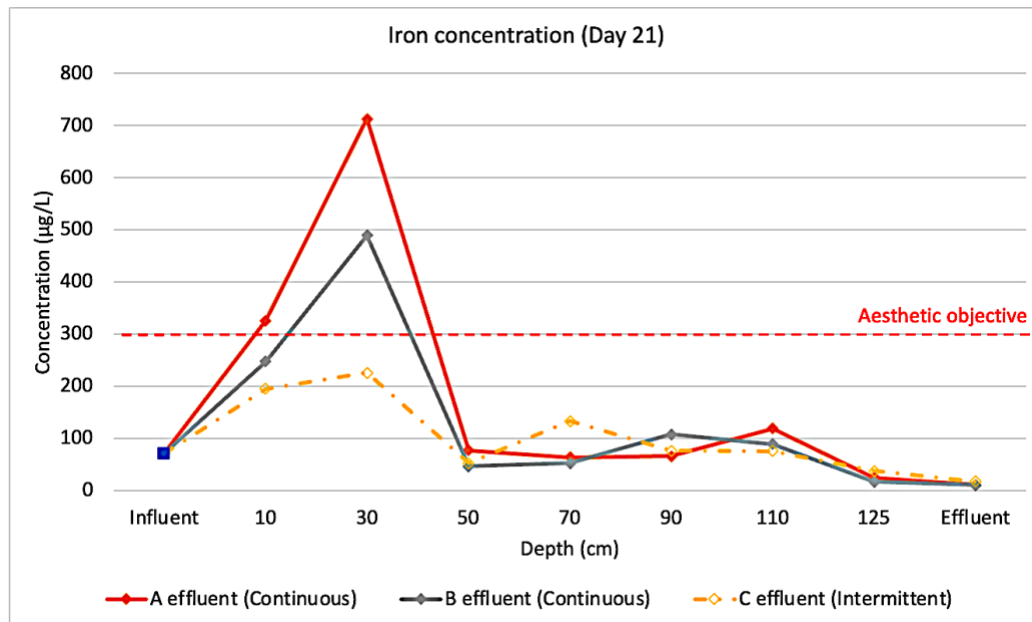
Table B.5 t-Test (B & C) results: Two-sample assuming equal variances

Manganese			Iron		
	<i>Variable 1</i>	<i>Variable 2</i>		<i>Variable 1</i>	<i>Variable 2</i>
Mean	0.39	0.31749	Mean	0.88	0.80
Variance	0.10	0.03933	Variance	0.00	0.00
Observations	8	8	Observations	8	8
Pooled Variance	0.07		Pooled Variance	0.00	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	14		df	14	
t Stat	0.53		t Stat	3.44	
P(T<=t) one-tail	0.30		P(T<=t) one-tail	0.00	
t Critical one-tail	1.76		t Critical one-tail	1.76	
P(T<=t) two-tail	0.60		P(T<=t) two-tail	0.00	
t Critical two-tail	2.15		t Critical two-tail	2.15	
Results	Not significant		Results	Significant	

Appendix B.3: Mn and Fe concentrations across various biofilter bed depths on day 21



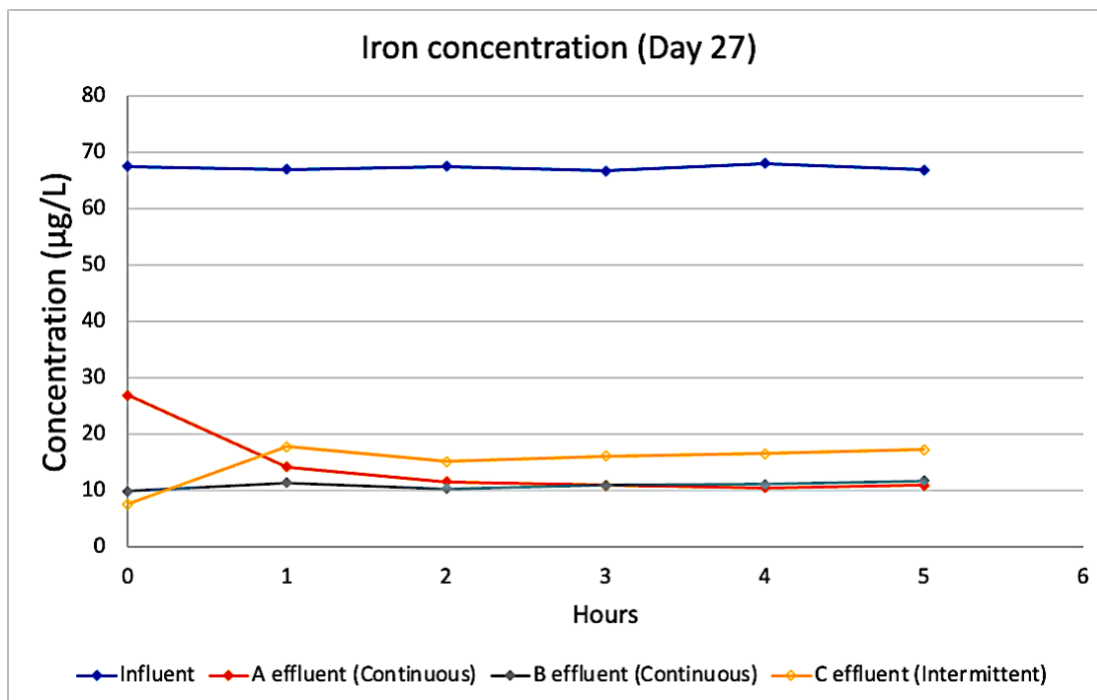
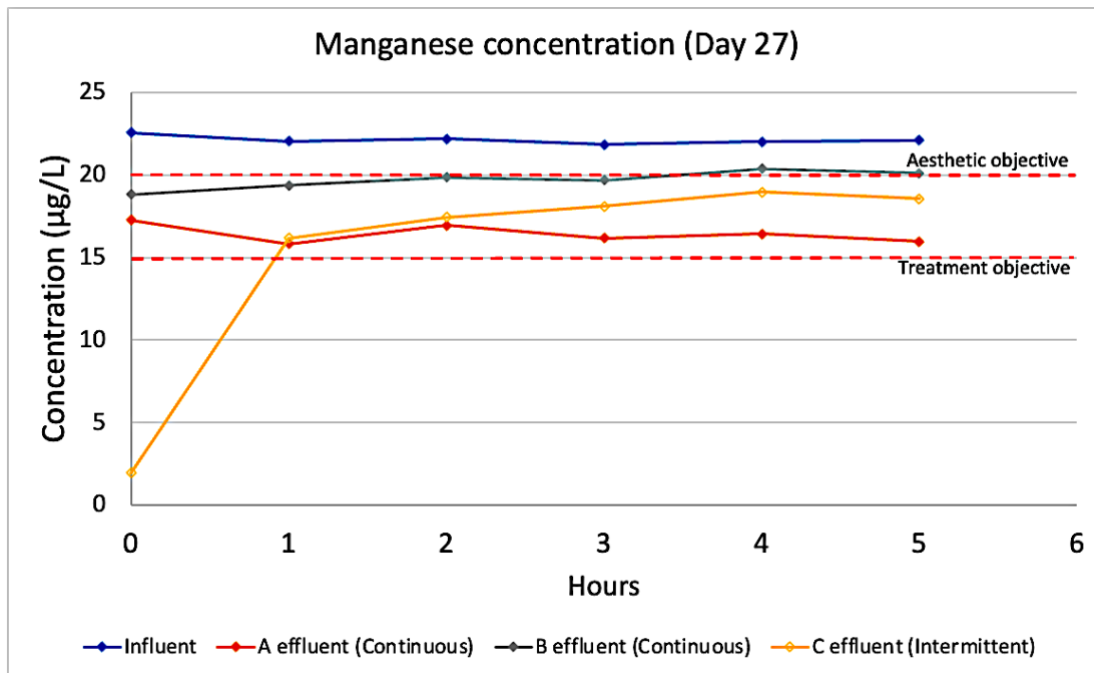
(a)



(b)

Note: Sample collection was done from the top to the bottom which is not conventional (bottom to top). Samples taken from top to bottom disturbs the flow through the column and therefore might affect the results.

Appendix B.4: Impact of 18 h shutdown on Mn and Fe concentrations over time



Appendix B.5: Data recorded to achieve the sub-objectives for phase 1 i.e. start-up phase

Table B.6 Mn concentration in the influent water and effluent water over time

Day	Influent	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
7	22.5	19.8	19.9	20.4
14	22.7	14.1	15.6	18.5
21	22.4	16.1	19.1	19.2
27	22.1	16.0	20.1	18.6
35	28.224	8.098	21.234	17.455
42	28.419	3.687	15.35	15.087
49	27.408	0.764	5.526	14.6
56	36.931	0.703	2.507	13.334

Table B.7 Fe concentration in the influent water and effluent water over time

Day	Influent	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
7	73.3	13.1	13.6	17.2
14	70.4	8.3	8.8	13.6
21	70.8	9.8	9.9	17.1
27	66.9	10.9	11.8	17.3
35	56.955	6.357	5.636	9.078
42	63.167	6.236	6.171	8.429
49	68.148	4.52	4.46	14.1
56	112.024	10.138	12.202	17.881

Table B.8 Mn concentration in the influent and effluents post intermittent 18 h shutdown during biofilter C start up on day 27. Sample collection began 5 minutes (0.1 h) after the biofilter restart and then continued at hourly intervals.

Hours post restart	Influent	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
0.1	22.6	17.3	18.8	1.9
1	22.0	15.8	19.4	16.2
2	22.2	16.9	19.9	17.4
3	21.8	16.2	19.7	18.1
4	22.0	16.4	20.4	19.0
5	22.1	16.0	20.1	18.6

Table B.9 Mn concentration in the influent and effluents post intermittent 18 h shutdown during biofilter C start up on day 56. Sample collection began 5 minutes (0.1 h) after the biofilter restart and then continued at hourly intervals. Here to have a reference point a sample was collected on the previous day just before the shutdown.

Hours post restart	Influent	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
Before shutdown	48.58	0.8	5.5	13.7
0.1	55.747	0.6	3.7	0.8
1	47.949	0.7	2.9	11.5
2	42.48	0.6	3.2	12.5
3	39.003	0.7	2.8	12.8
4	39.372	0.6	2.6	12.5
5	36.931	0.7	2.5	13.3

Table B.10 Fe concentration in the influent and effluents post intermittent 18 h shutdown during biofilter C start up on day 27. Sample collection began 5 minutes (0.1 h) after the biofilter restart and then continued at hourly intervals.

Hours post restart	Influent	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
0.1	67.5	26.9	9.9	7.6
1	67.0	14.2	11.4	17.8
2	67.5	11.5	10.3	15.2
3	66.7	11.0	10.9	16.2
4	68.0	10.5	11.1	16.5
5	66.9	10.9	11.8	17.3

Table B.11 Fe concentration in the influent and effluents post intermittent 18 h shutdown during biofilter C start up on day 56. Sample collection began 5 minutes (0.1 h) after the biofilter restart and then continued at hourly intervals. Here to have a reference point a sample was collected on the previous day just before the shutdown.

Hours post restart	Influent	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
Before shutdown	147.78	4.5	4.5	16.6
0.1	235.416	9.9	10.4	0
1	175.679	9.9	9.4	15.0
2	141.326	9.5	11.1	16.6
3	124.332	10.3	11.5	17.3
4	114.187	9.6	11.1	16.6
5	112.024	10.1	12.2	17.9

Table B.12 Mn concentration across various biofilter bed depths (Day 21)

Mn conc.	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
Influent	22.4	22.4	22.4
10	54.6	32.9	37.6
30	85.3	49.4	48.8
50	30.4	21.4	22.5
70	25.6	24.2	25.0
90	22.5	22.8	22.8
110	28.7	30.0	22.1
125	19.8	20.0	21.2
Effluent	16.1	19.1	19.2

Table B.13 Mn concentration across various biofilter bed depths (Day 49)

Mn conc.	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
Influent	27.4	27.4	27.4
10	23.9	26.5	36.2
30	20.7	38.4	25.1
50	32.5	19.1	23.8
70	9.8	17.8	22.6
90	4.6	13.5	21.7
110	3.8	10.6	22.0
125	1.6	7.3	19.5
Effluent	0.8	5.5	19.7

Table B.14 Fe concentration across various biofilter bed depths (Day 21)

Fe conc.	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
Influent	70.8	70.8	70.8
10	325.3	247.0	195.1
30	713	489.2	225.6
50	76.8	46.0	51.8
70	63.5	51.7	132.4
90	65.6	107.4	75.6
110	118.4	89.0	75.2
125	24.2	15.8	36.7
Effluent	9.8	9.9	17.1

Table B.15 Fe concentration across various biofilter bed depths (Day 49)

Fe conc.	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
Influent	68.2	68.2	68.2
10	20.6	22.5	64.9
30	24.7	21.6	25.6
50	36.5	64.1	52.5
70	13.4	14.7	27.3
90	8.6	10.9	16.2
110	8.9	8.5	25.8
125	6.0	5.1	12.5
Effluent	4.5	4.5	14.1

Table B.16 ATP measured for the media at different depths of each biofilter

Day	A0	B0	C0	A60	B60	C60	A120	B120	C120
28 days	26.62	26.69	8.60	6.97	9.58	1.87	9.06	8.79	3.24
28 days	28.14	23.98	6.38	5.07	5.74	1.53	8.93	8.23	3.48
56 days	34.71	15.05	10.31	17.16	15.29	11.53	11.46	5.62	5.74
56 days	27.28	9.95	9.42	25.22	17.03	16.39	10.78	6.61	5.29

Table B.17 ATP measured for the media before and after restart

	BR			AR		
Day	A(BR)	B(BR)	C(BR)	A(AR)	B(AR)	C(AR)
7	26.54	36.82	13.48	34.24	29.58	8.29
7	28.70	33.51	9.72	31.35	32.95	8.08
42	37.40	16.63	7.52	37.40	16.63	18.47
42	27.36	18.19	6.58	27.36	18.19	13.43
56	34.71	15.05	11.05	34.71	15.05	10.31
56	27.28	9.95	8.97	27.28	9.95	9.42

BR indicates that the sampling was done before restart when the continuously operated biofilters were still operating while the intermittently operated biofilter was shut down, and AR indicates the sampling which was done after restart, when all the biofilters were operating.

Table B.18 ATP measured for the media before and after backwash

	BB			AB		
Day	A(BB)	B(BB)	C(BB)	A(AB)	B(AB)	C(AB)
21	38.19	49.93	12.07	35.34	24.15	10.26
21	44.03	42.55	10.60	33.80	21.55	10.75
28	26.62	26.69	8.60	-	-	-
28	28.14	23.98	6.38	-	-	-
42	37.40	16.63	18.47	17.13	22.73	6.35
42	27.36	18.19	13.43	39.74	30.36	7.44

BB indicates that the sampling was done before backwash, and AB indicates the sampling which was done after backwash.

Appendix C - Supporting Data and Figures for Chapter 5: Effects of an Extended Shutdown on the Microbial Activity of Biofilters Previously Operated Intermittently and Continuously

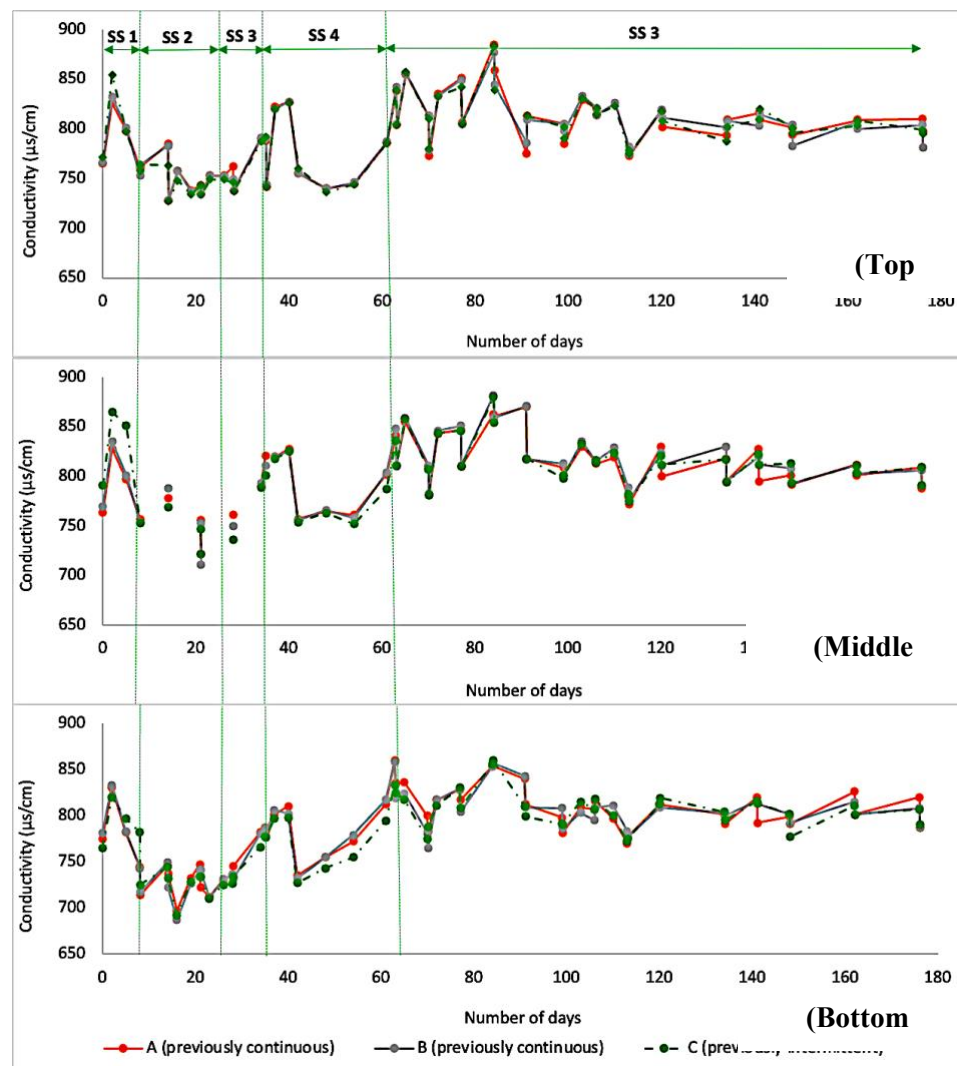


Figure C.1 Trends in conductivity at different biofilter depths during the extended shutdown in the (a) top layer (10 cm), (b) middle layer (50 cm) and (c) bottom layer (110 cm)
[SS: Storage strategy]

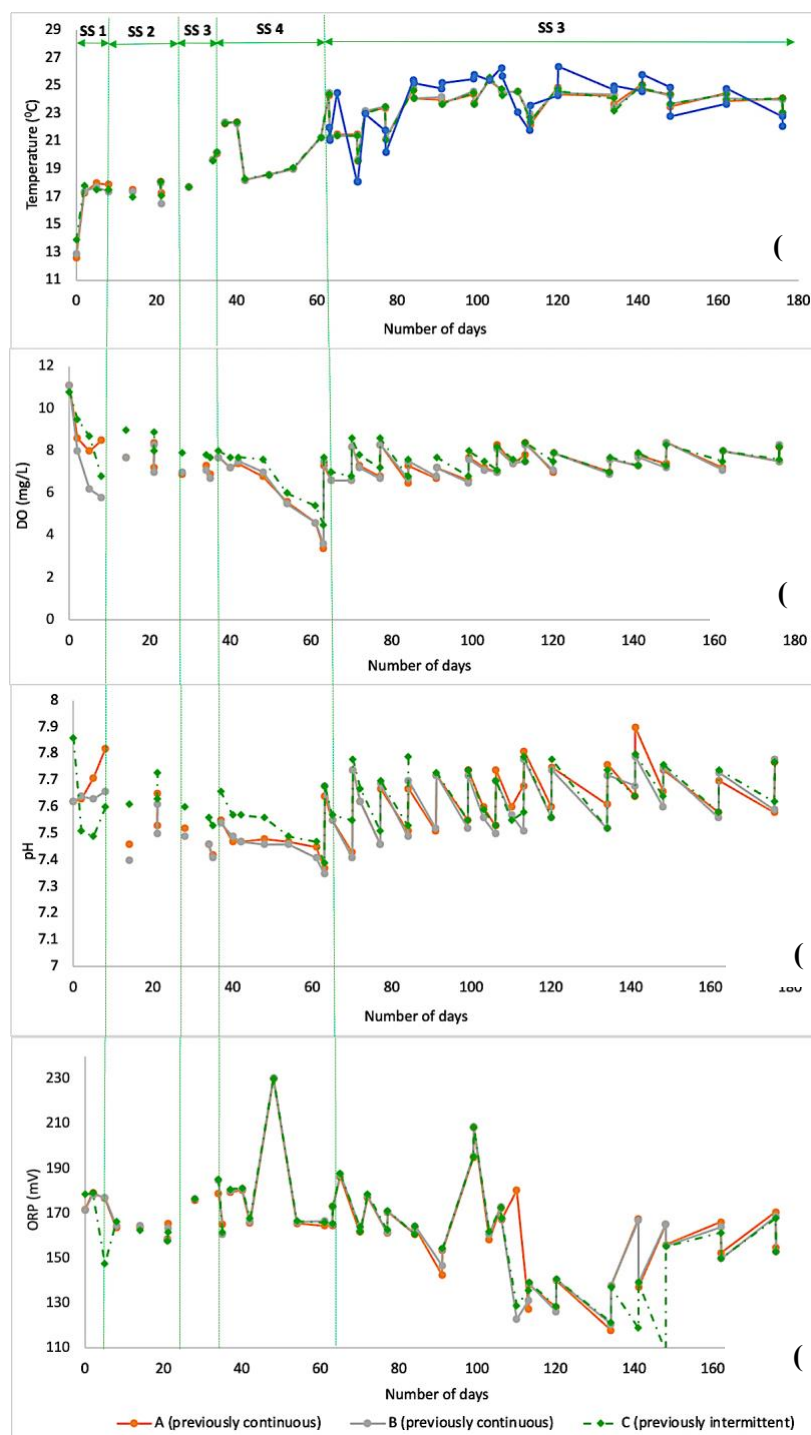
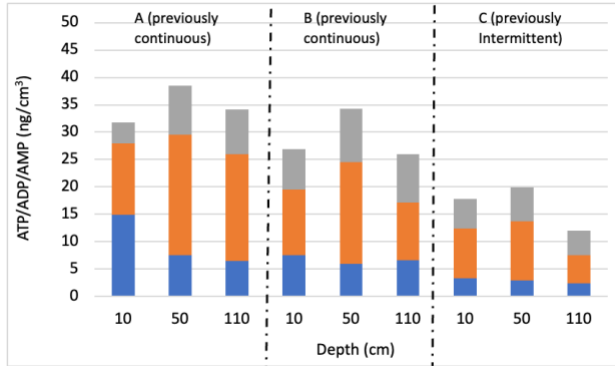
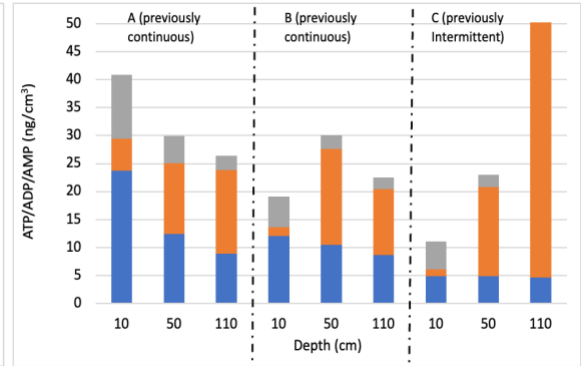


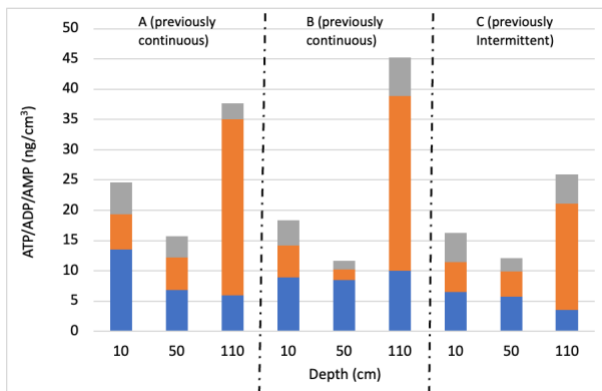
Figure C.2 Trends in (a) temperature, (b) DO, (c) pH and (d) ORP during the extended shutdown in the middle layer (50 cm) of the biofilters [SS: Storage strategy]



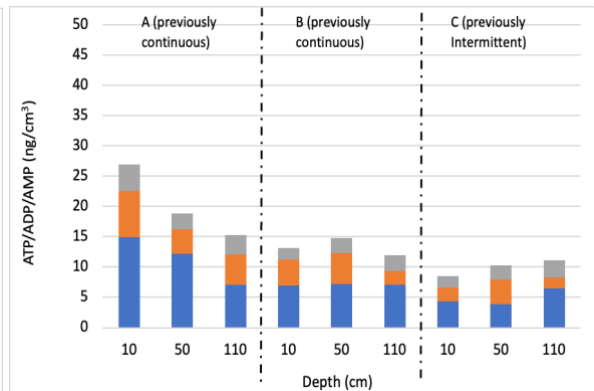
(a)



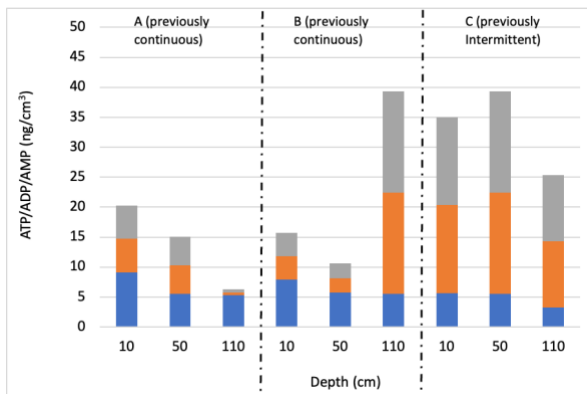
(b)



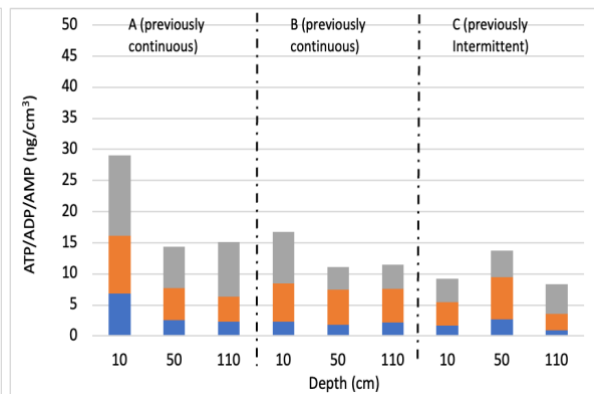
(c)



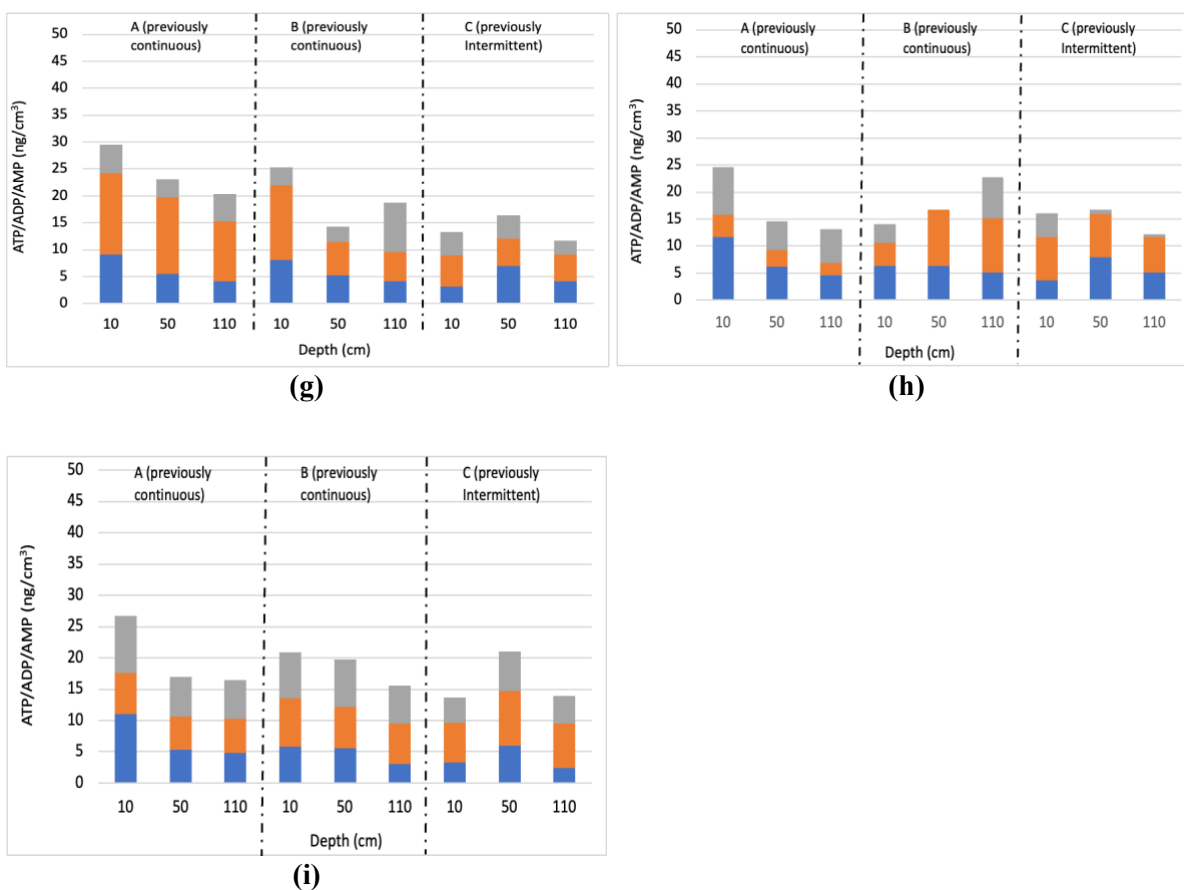
(d)



(e)

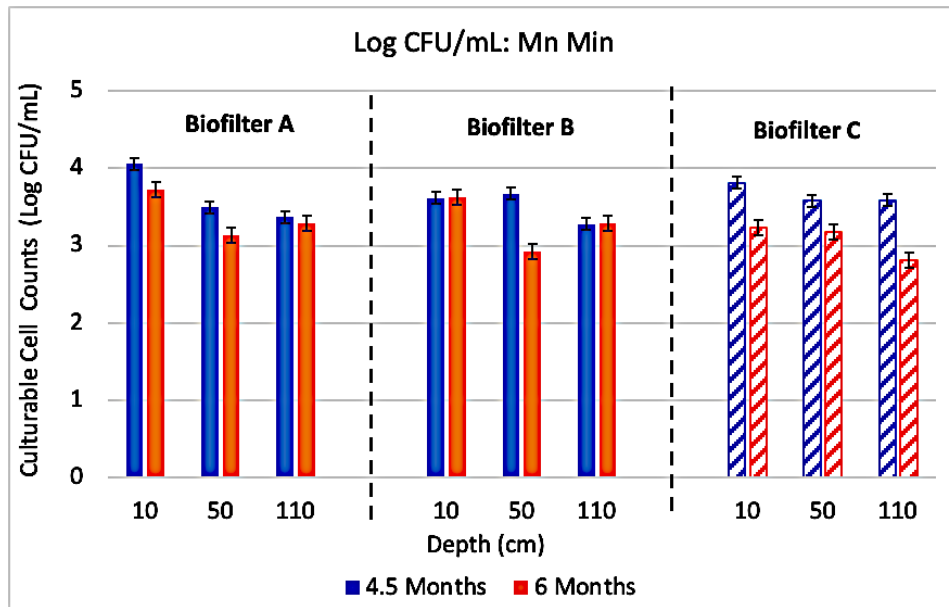


(f)

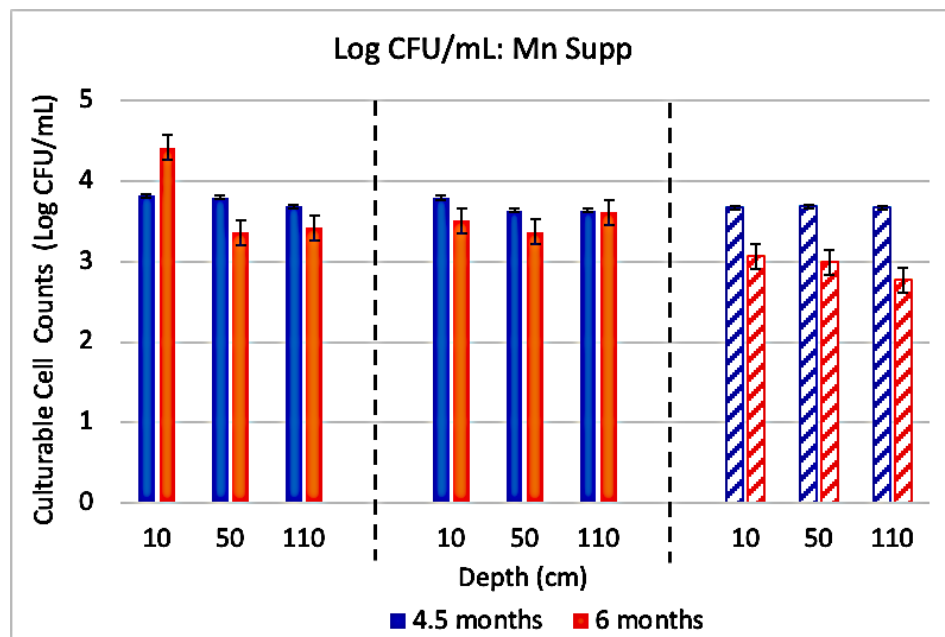


■ ATP ■ ADP ■ AMP

Figure C.3 AMP, ADP and ATP values for the biofilters A, B and C during the extended biofilter shutdown (a) on Day 8, (b) day 14, (c) day 21, (d) day 28, (e) day 70, (f) day 77, (g) day 84, (h) day 106, and (i) day 112



(a)



(b)

Figure C.4 Culturable cell counts of bacteria isolated from three different filter columns after plating on (a) Mn minimum and (b) Mn supplemental media, at three different depths (10, 50 and 110 cm), over the course of two sampling events during the extended shutdown (4.5 and 6 months). Prior to the shutdown, columns A and B: operated continuously for 24 h per day, while column C operated intermittently for 6 h a day.

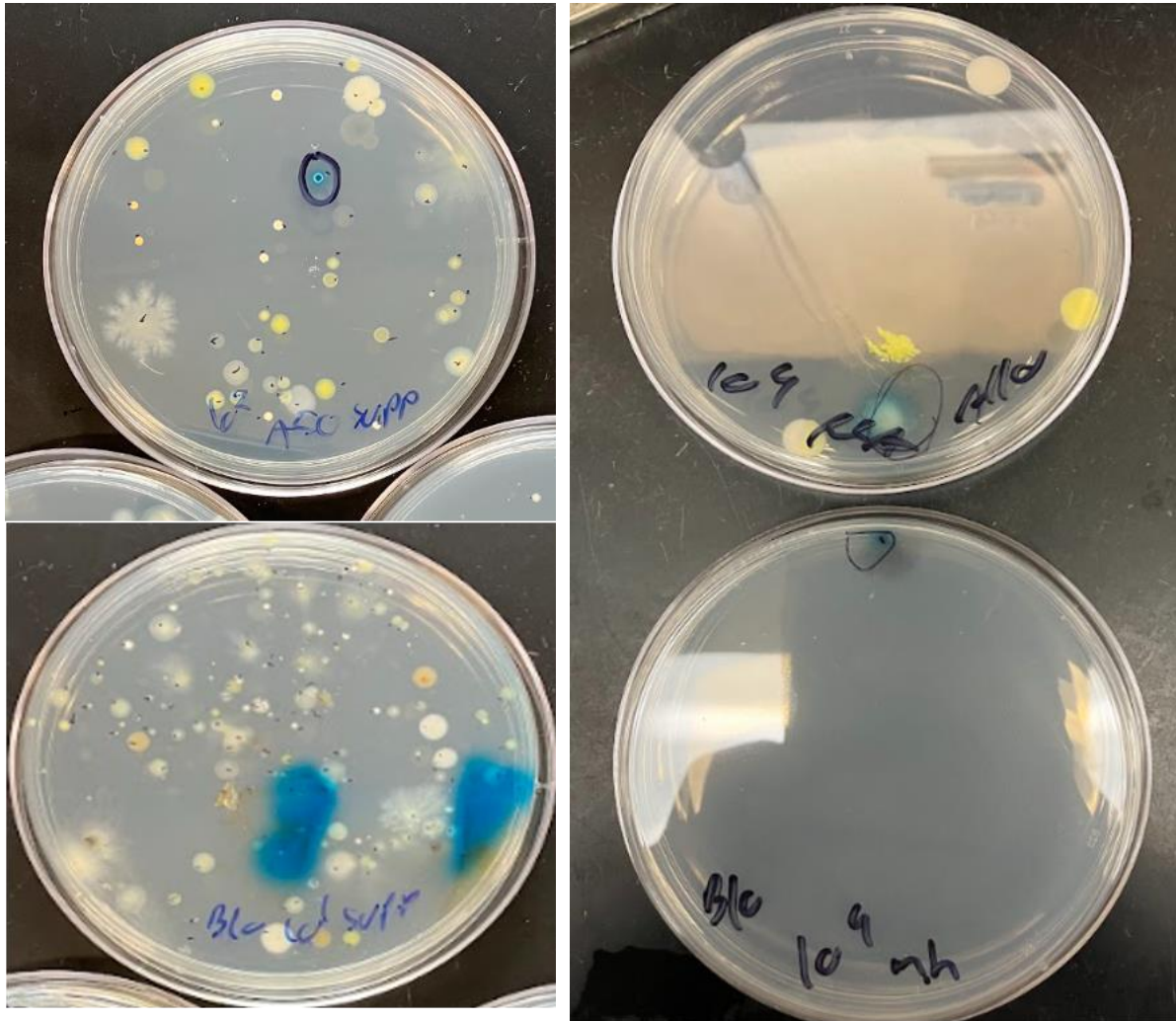


Figure C.5 LBB-confirmed plates, illustrating LBB stained colonies and phenotypic diversity.
Top left: Column A-50 cm after 4 months of shutdown on Mn Supp. Bottom Left: Column B-10 cm after 4 months of shutdown on Mn Supp. Top Right: Column A-110 cm after 6 months of shutdown on R2A + Mn. Bottom Right: Column B-10 cm after 6 months of shutdown on Mn Min.

Table C.1 Leucoberbelin Blue Assay on presumptive MnOB colonies after plating onto Mn supplemental culture medium. The symbol “-” indicates a negative test result, while “+” represents weak blue colouration, “++” represents moderate blue colouration and “+++” represents strong blue colouration.

Samples collection	Sample	Depth		
		10 cm	50 cm	110 cm
4.5 months since shutdown commenced	A	(+)	X	X
	B	(+++)	X	X
	C	X	X	X
6 months since shutdown commenced	A	X	X	X
	B	X	X	X
	C	X	X	X

Table C.2 Leucoberbelin Blue Assay on presumptive MnOB colonies after plating onto Mn min culture medium. The symbol “-” indicates a negative test result, while “+” represents weak blue colouration, “++” represents moderate blue colouration and “+++” represents strong blue colouration.

Samples collection	Sample	Depth		
		10 cm	50 cm	110 cm
4.5 months since shutdown commenced	A	X	X	X
	B	(++)	X	X
	C	X	X	X
6 months since shutdown commenced	A	X	X	X
	B	X	(++)	X
	C	X	X	X

Appendix D - Supporting Data for Chapter 6: Effect of Intermittent Operation on Manganese Removal Post Extended Shutdown

Table D.1 Mn concentration (µg/L) in the influent water and effluent water over time

Day	Influent	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
During shutdown	25.7	21.8	24.6	27.6
0	26.2	20.8	22.6	24.5
6	24.4	16.8	19.6	12.5
13	26.0	14.4	17.2	15.1
20	26.7	2.8	2.1	8.1
27	24.4	3.3	5.4	9.4
34	23.9	0.3	0.6	1.8
41	26.5	2.6	5.2	1.6
48	26.5	0.3	0.4	1.5
55	24.4	0.2	0.1	0.2
62	21.9	0.1	0.1	0.1
69	19.3	1.0	0.2	0.2
75	18.6	0.1	0.1	0.2

Table D.2 Fe concentration (µg/L) in the influent water and effluent water over time

Day	Influent	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
During shutdown	72.3	21.1	23.7	16.4
0	73.1	12.7	12.5	22.5
6	67.2	8.0	8.7	11.8
13	76.4	8.6	10.0	12.0
20	73.2	14.6	14.5	16.5
27	61.7	7.6	8.2	9.2
34	42.4	4.7	4.2	13.0
41	55.9	17.2	27.7	12.5
48	48.5	5.2	4.2	13.1
55	32.3	3.9	3.0	5.5
62	40.4	6.4	5.9	8.1
69	32.1	6.2	7.0	8.4
75	24.2	6.0	6.3	7.2

Table D.3 Mn removal over time in the effluent water and the F values for each paired comparison to find whether we can assume equal variance before performing the student t-test

Day	Mn influent concentration (µg/L)	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)	A-B	A-C	B-C
0	26.2	21%	14%	6%	7%	14%	7%
6	24.4	31%	20%	49%	11%	-18%	-29%
13	26.0	45%	34%	42%	11%	3%	-8%
20	26.7	90%	92%	70%	-3%	20%	22%
27	24.4	86%	78%	61%	9%	25%	16%
34	23.9	99%	98%	92%	1%	6%	5%
41	26.5	90%	80%	94%	10%	-4%	-14%
48	26.5	99%	98%	95%	0%	4%	4%
55	24.4	99%	99%	99%	0%	0%	0%
62	21.9	100%	99%	99%	0%	0%	0%
69	19.3	95%	99%	99%	-4%	-4%	0%
75	18.6	99%	99%	99%	0%	0%	0%
n	Avg	79%	76%	76%	4%	4%	0%
12	Std. dev	29%	33%	30%	6%	11%	13%
F value					0.77	0.94	1.22
p-value					0.663	0.541	0.375
Variance					Equal	Equal	Equal

Table D.4 Fe removals over time and the F values for each paired comparison to find whether we can assume equal variance before performing the student t-test

Day	Fe influent concentration (µg/L)	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)	A-B	A-C	B-C
0	73.1	83%	83%	69%	0%	13%	14%
6	67.2	88%	87%	82%	1%	6%	5%
13	76.4	89%	87%	84%	2%	4%	3%
20	73.2	80%	80%	77%	0%	3%	3%
27	61.7	88%	87%	85%	1%	3%	2%
34	42.4	89%	90%	69%	-1%	19%	21%
41	55.9	69%	51%	78%	19%	-8%	-27%
48	48.5	89%	91%	73%	-2%	16%	18%
55	32.3	88%	91%	83%	-3%	5%	8%
62	40.4	84%	85%	80%	-1%	4%	5%
69	32.1	81%	78%	74%	3%	7%	4%
75	24.2	75%	74%	70%	1%	5%	4%
n	Avg	84%	82%	77%	2%	6%	5%
12	Std. dev	6%	11%	6%	6%	7%	12%
				F value	0.32	1.16	3.64
				p-value	0.965	0.408	0.02
				Variance	Equal	Equal	Not equal

Table D.5 t-Test (A-B): Two-Sample Assuming Equal Variances

Manganese			Iron		
	<i>Variable 1</i>	<i>Variable 2</i>		<i>Variable 1</i>	<i>Variable 2</i>
Mean	0.794	0.759	Mean	0.835	0.820
Variance	0.086	0.111	Variance	0.004	0.013
Observations	12	12	Observations	12	12
Pooled Variance	0.098		Pooled Variance	0.008	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	22		df	22	
t Stat	0.274		t Stat	0.411	
P(T<=t) one-tail	0.393		P(T<=t) one-tail	0.343	
t Critical one-tail	1.717		t Critical one-tail	1.717	
P(T<=t) two-tail	0.787		P(T<=t) two-tail	0.685	
t Critical two-tail	2.074		t Critical two-tail	2.074	

Table D.6 t-Test (B-C): Two-Sample Assuming Equal Variances

Manganese			Iron		
	<i>Variable 1</i>	<i>Variable 2</i>		<i>Variable 1</i>	<i>Variable 2</i>
Mean	0.759	0.75500964	Mean	0.820	0.772
Variance	0.111	0.09154989	Variance	0.013	0.003
Observations	12	12	Observations	12	12
Pooled Variance	0.102		Pooled Variance	0.008	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	22		df	22	
t Stat	0.032		t Stat	1.326	
P(T<=t) one-tail	0.487		P(T<=t) one-tail	0.099	
t Critical one-tail	1.717		t Critical one-tail	1.717	
P(T<=t) two-tail	0.974		P(T<=t) two-tail	0.198	
t Critical two-tail	2.074		t Critical two-tail	2.074	

Table D.7 t-Test (A-C): Two-Sample Assuming Equal Variances

Manganese			Iron		
	<i>Variable 1</i>	<i>Variable 2</i>		<i>Variable 1</i>	<i>Variable 2</i>
Mean	0.794	0.755	Mean	0.835	0.772
Variance	0.086	0.092	Variance	0.004	0.003
Observations	12	12	Observations	12	12
Pooled Variance	0.089		Pooled Variance	0.004	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	22		df	22	
t Stat	0.323		t Stat	2.559	
P(T<=t) one-tail	0.375		P(T<=t) one-tail	0.009	
t Critical one-tail	1.717		t Critical one-tail	1.717	
P(T<=t) two-tail	0.749		P(T<=t) two-tail	0.018	
t Critical two-tail	2.074		t Critical two-tail	2.074	

Table D.8 Mn and Fe concentrations (µg/L) across various biofilter bed depths (Day 27)

Mn conc.	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
Influent	24.4	24.4	24.4
10	36.8	49.5	79.3
30	66.4	50.0	35.9
50	29.9	36.9	30.5
70	27.2	24.8	31.3
90	19.0	22.5	23.5
110	22.1	16.8	25.8
125	8.2	12.3	18.3
Effluent	3.3	5.4	9.4

Fe conc.	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
Influent	61.7	61.7	61.7
10	98.0	212.2	436.1
30	206.7	167.6	194.5
50	56.6	91.8	164.5
70	67.4	69.0	95.8

90	37.7	46.5	45.4
110	60.0	52.6	95.1
125	20.9	59.4	27.1
Effluent	7.6	8.2	9.2

Table D.9 Mn and Fe concentrations (µg/L) across various biofilter bed depths (Day 55)

Mn conc.	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
Influent	24.4	24.4	24.4
10	15.6	18.6	36.7
30	12.1	10.8	12.0
50	8.3	11.0	8.4
70	3.4	6.3	4.9
90	5.8	7.8	2.2
110	4.4	8.0	3.2
125	2.3	8.1	2.4
Effluent	0.2	0.1	0.2

Fe conc.	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
Influent	32.3	32.3	32.3
10	88.9	104.9	162.0
30	60.9	56.1	48.4
50	46.4	68.9	50.0
70	20.2	45.0	33.9
90	28.7	39.3	14.2
110	22.7	54.7	27.3
125	18.1	55.5	17.4
Effluent	3.9	3.0	5.5

Table D.10 ATP measured in ng/cm³ for the media at different depths of each biofilter. Media samples were collected before backwash on day 13, 28, 56 and 85.

	A (Continuous)			B (Continuous)			C (Intermittent)		
Depth (cm)	10	50	110	10	50	110	10	50	110
Last day (Phase 1)	31.00	21.19	11.12	21.19	16.16	6.12	9.86	13.96	5.51
Day 0 (Before backwash)	10.32	3.18	1.76	9.22	2.55	2.16	7.82	1.51	1.39
Day 0 (After backwash)	4.01	3.72	4.03	1.93	2.01	5.30	3.97	1.97	1.91
Day 13	14.77	7.85	4.90	11.81	9.78	9.06	7.71	5.04	4.71
Day 28	17.56	7.36	5.42	31.35	8.77	6.35	13.66	3.41	3.06
Day 56	41.54	11.32	4.64	40.88	15.94	10.91	13.47	3.17	2.63
Day 85	50.96	13.13	7.66	34.83	17.43	8.56	17.18	3.82	2.00

Table D.11 AxP measured in ng/cm³ for the media at different depths of each biofilter

	A (Continuous)			B (Continuous)			C (Intermittent)			
Average values	10	50	110	10	50	110	10	50	110	
During shutdown	0.47	0.41	0.44	0.44	0.43	0.46	0.44	0.39	0.44	EC
During shutdown	4.41	2.65	1.38	3.19	2.45	1.78	2.81	1.50	1.86	ATP
During shutdown	3.87	6.76	4.42	2.93	7.15	5.89	3.56	5.45	6.06	ADP
During shutdown	6.19	8.46	2.96	5.05	9.33	4.54	7.28	3.33	4.12	AMP
Day 0	0.48	0.44	0.63	0.34	0.57	0.75	0.20	0.51	0.38	EC
Day 0	4.58	4.68	7.89	2.19	12.10	5.29	1.59	5.97	1.69	ATP
Day 0	4.10	5.55	11.90	2.54	31.75	3.68	0.13	19.75	2.62	ADP
Day 0	5.24	7.23	2.62	5.49	4.86	0.55	6.54	5.29	3.56	AMP
Day 13	0.56	0.51	0.68	0.57	0.56	0.55	0.58	0.54	0.46	EC
Day 13	16.55	9.46	9.19	12.90	12.21	10.13	9.55	5.82	5.19	ATP
Day 13	33.80	16.50	9.54	28.75	18.99	15.10	20.97	14.64	14.33	ADP
Day 13	9.67	8.60	2.90	6.62	7.54	7.46	4.27	3.91	7.21	AMP
Day 28	0.53	0.52	0.49	0.64	0.52	0.50	0.61	0.50	0.48	EC
Day 28	21.19	9.51	3.54	37.25	14.22	8.75	19.00	5.98	4.47	ATP
Day 28	23.96	11.98	11.75	23.40	7.50	10.96	15.97	13.30	8.04	ADP
Day 28	18.24	8.20	3.99	15.28	12.70	8.57	9.16	6.04	5.50	AMP
Day 56	0.59	0.58	0.60	0.61	0.69	0.62	0.42	0.47	0.58	EC
Day 56	41.87	11.97	9.85	37.10	20.63	15.54	13.67	6.28	4.55	ATP
Day 56	33.92	41.08	19.95	32.29	31.16	22.12	16.60	49.27	12.50	ADP
Day 56	24.67	4.68	2.78	18.22	0.69	5.03	22.39	7.76	2.74	AMP
Day 85	0.81	0.91	0.76	0.84	0.81	0.71	0.80	0.74	0.74	EC
Day 85	65.77	20.83	15.16	45.00	28.09	19.56	22.45	6.93	2.63	ATP
Day 85	29.76	5.27	7.69	13.68	9.25	8.41	10.27	4.33	0.81	ADP
Day 85	3.47	0.34	2.12	3.40	2.98	5.39	1.92	1.14	0.64	AMP

Table D12. Mn and Fe concentrations (µg/L) post 18-h intermittent shutdown (Day 28) Sample collection began 5 minutes after biofilter C restart and then continued at regular intervals. To have a reference point a sample was collected on the previous day just before the shutdown.

Mn conc.	Influent	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
Before shutdown	24.4	3.3	5.4	9.4
5	23.4	0.6	1.9	0.7
15	21.5	0.4	1.4	0.5
30	23.2	0.2	0.8	1.3
60	23.1	0.4	1.4	5.0
120	23.5	3.9	5.5	10.8
240	23.6	3.4	4.5	11.1
360	23.5	3	3.9	11.3

Fe conc.	Influent	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
Before shutdown	61.7	7.6	8.2	9.2
5	60.2	2.7	3.1	2.9
15	60.3	2.8	3.2	3.0
30	59.5	2.4	3.1	13.9
60	60.9	3.5	3.0	13.7
120	62.1	2.4	12.8	15.5
240	61	11.1	11.9	14.4
360	58.3	11.1	12.0	14.3

Table D13. Mn and Fe concentrations (µg/L) post 18-h intermittent shutdown (Day 56) Sample collection began 5 minutes after biofilter C restart and then continued at regular intervals. To have a reference point a sample was collected on the previous day just before the shutdown.

Mn conc.	Influent	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
Before shutdown	24.4	0.2	0.1	0.2
5	22.7	0.1	0.2	0.3
15	22.9	0.1	0.1	0.2
30	23.1	0.1	0.2	0.3
45	22.9	0.1	0.2	0.9
90	22.9	0.1	0.2	0.4
180	22.9	0.2	0.2	0.4
270	22.5	0.1	0.2	0.9
360	23.2	0.2	0.2	1.6

Fe conc.	Influent	A effluent (Continuous)	B effluent (Continuous)	C effluent (Intermittent)
Before shutdown	32.3	3.9	3.0	5.5
5	35.9	8	8.3	2.5
15	36.2	8	8.2	7.5
30	35.5	8.1	8.4	6.8
45	36.2	8.1	8.1	6.4
90	37.2	8.1	4.6	5.8
180	34.8	7.9	4.1	5.3
270	37	8	5.2	6.5
360	36.5	8	4.4	5.7