The effect of pH and alkalinity on drinking water biofiltration performance

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by

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Abstract

Two bench-scale biofiltration columns were monitored to examine the influence of water-quality parameters, including pH and alkalinity, as a cost-effective approach to enhance drinking water biofiltration efficiency in terms of organic carbon removal, ammonia removal, and headloss build-up reduction. The biofilters were operated at pH values 6.0, 7.5, 9.0, and 10.0 with low and high alkalinity levels (25–50) and (180–220) mg $CaCO_3$/L. Applying a higher pH level of 7.5 compared to 6.0 led to similar total organic carbon (TOC) removal efficiency (65% and 67%). Raising the pH to 10.0 resulted in a significantly lower TOC removal efficiency (31%). Increasing pH was also observed to influence ammonia removal significantly such that ammonia removal efficiency improved from 13% at pH 6.0 to 93% at pH 10.0; however, the higher pH was no longer attributed to biological removal but ammonia stripping. The assessment of theoretical oxygen demand revealed that dissolved oxygen (DO) availability was an influential factor in nitrification efficiency. The higher alkalinity levels at each pH level resulted in higher adenosine triphosphate (ATP) concentrations, but no direct correlation was observed between ATP and TOC removal. Overall, pH 7.5 demonstrated optimal biofilter conditions in terms of water quality and operational considerations with average TOC and ammonia removal at 68% and 48% efficiency, respectively, with the lowest headloss development.

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Table of Contents

List of Tables

List of Figures

List of Acronyms

ATP	Adenosine triphosphate
BOM	Biological Organic Matter
C: N: P	Carbon: Nitrogen: Phosphorous
DBP	Disinfection Byproduct
DI	Deionized
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
EBCT	Empty Bed Contact Time
EPA	Environmental Protection Agency
EPS	Extracellular polymeric substances
FDA	Food and Drug Administration
GAC	Granular Activated Carbon
H_2O_2	Hydrogen Peroxide
HL	Headloss
HLR	Hydraulic Loading Rate
MAC	Maximum Allowable Concentration
NOM	Natural Organic Matter
pH	-log (hydrogen ions concentration)
PR	Protein
PS	Polysaccharide
TOC	Total Organic Carbon
WHO	World Health Organization

1. Introduction

1.1. Background

Over the past decades, water treatment plants have faced various concerns owing to the presence of untreated natural organic matter (NOM) in drinking water sources. Although untreated NOM itself does not have any direct effect on human health, it has the potential to cause water-quality problems such as disinfection by-product (DBP) formation and biological regrowth in the distribution system (Emelko et al., 2006; McKie et al., 2015). The untreated NOM in water treatment plants reacts with chlorine and other disinfectants to form DBPs (Azzeh et al., 2015; McKie et al., 2015), which are suspected carcinogens. Water treatment processes are also affected by NOM presence regarding chlorine demand increase (McKie et al., 2015; Dhawan et al., 2017) and membrane fouling (Azzeh et al., 2015). Biofiltration technology has proven a sustainable and profitable treatment process to remove organic matter through biodegradation (Dhawan et al., 2017; Keithley and Kiristis, 2018). Also, by increasing NOM removal efficiency, the problem of microbial regrowth in water distribution networks can be mitigated, and resultantly, the concerns of water odor and color can be reduced. Therefore, over the past decades, biofiltration systems, owing to their efficiency to remove organic matters, have been used vastly in drinking water systems. However, the research on drinking water treatment mainly focuses on dissolved organic carbon (DOC) or total organic carbon (TOC) removal, and a few studies have investigated simultaneous organic carbon and ammonia removal in the surface water system to reduce the common issues resulting from the presence of ammonium in water. Although a threshold odor concentration of 1.5 mg/L and taste concentration of 35 mg/L have been proposed for the ammonium cation (WHO, 2003), Health Canada (2017) has established a limit of 0.1 mg/L for free ammonia concentration in drinking water entering the distribution system. Likewise, Lájer, (2012) has indicated that the ammonia entering the distribution system should be controlled to

reduce the chlorination demand, DBPs production, and nitrite formation caused by uncontrolled nitrification. Therefore, an investigation into the parameters that improve biofiltration performance will be beneficial for increasing the efficacy of water treatment plants. The optimization of biofiltration has a long and well-established history regarding "operational design parameters" such as filter media, empty bed contact time (EBCT), and backwashing. For example, granular activated carbon (GAC) has been found to be superior to anthracite in biodegradable organic matter (BOM) removal, especially under unfavorable conditions, such as low temperature or presence of chlorine (Liu et al., 2001; Emelko et al., 2006). Also, EBCT has received significant attention with results generally demonstrating the benefits of increasing EBCT up to 20 minutes in DOC/BOM removal (Peldszus et al., 2012; Granger et al., 2014; Basu et al., 2016). Moreover, Ikhlef and Basu, (2017) have observed that collapse pulsing and bed expansion up to 30% during backwashing improve DOC removal in biofilters. They have also noted that increasing bed expansion over 30% results in wasting more backwash water and hindering DOC removal.

More recently, researchers have looked at controlling water-quality parameters to improve biofiltration performance concerning NOM removal, specifically by controlling nitrogen and phosphorus supplementation. The most relevant studies in this regard are summarized in Table 1.1. The review of the literature (Table 1.1) evaluating the influence of nutrient enhancement on drinking water biofiltration performance does not indicate a strong relationship between nutrient addition and biofilter efficiency improvement in terms of TOC/DOC removal as well as adenosine triphosphate (ATP) and extracellular polymeric substance (EPS) concentration. While some authors have reported an increase in TOC/DOC removal with nitrogen and phosphorus supplementation (Lauderdale et al., 2012; Granger et al., 2014; Dhawan et al., 2017), others have observed no to little impacts on organic carbon removal (Azzeh et al., 2015; Fu et al., 2017; Nemani et al., 2018). On the one hand, nutrient addition has been observed to provide the specific

Table 1. 1: Literature Review Comparison

Reference	Nutrients	pH	Media	DOC / TOC removal		Microbial	
				Control	Test	Control	Test
Nemani et al., 2018 (Pilot-scale)	(C: N: P) $_{Control}$: 400:1:2 400: 1: 30	7.2~8	GAC	6.8%	9.1%	ATP (ng/g): 429	ATP (ng/g): 453
	200: 40: 1				~6.8%		521
Fu et al., 2017 (Pilot-scale)	1 g N/L + 0.23 g P/L	N.S.	Anthracite + GAC	22%	30%	-	-
	1.0 g NH_4Cl/L			23%	20%	-	-
	2.0 g NH_4Cl/L			24%	25%	-	-
Ikhlef and Basu, 2017 (Bench-scale)	(C: N: P) $_{Control}$: 546:24:1 25: 5: 1	7	GAC/sand	21%	34%	-	-
Dhawan et al., 2017 (Bench-scale)	(C: N: P) $_{Control}$: 546:24:1 25: 5: 1		GAC/sand	24%	32%	-	-
	100: 10: 1				28%	-	-
Peleato et al., 2016 (Pilot-scale)	0.5 mg/L NH_4Cl + 0.5 mg/L H_3PO_4	7.3 ~ 8.6	Anth. /sand GAC/sand	No significant changes		-	-
Rahman et al., 2016 (Pilot-scale)	(C: N: P) $_{Control}$: 100:10:0.01 100: 10: 1 (0.01 mg/L K_2HPO_4)	7.8 ~ 8.4	Anth. /sand	1.2%	7.2% initially, then 1.2%	-	Small initial decrease (1st month)
	(0.05 mg/L K_2HPO_4)				No significant changes	-	No significant changes
Azzeh et al., 2015 (Pilot-scale)	(C: N: P) $_{Control}$: 100:30:00 100: 30: 02	7~9	Anth. /sand GAC/sand	5%	4%	-	-
	100: 40: 02			7%	6%	-	-
	100: 40: 20			2%	4%	-	-
McKie et al., 2015 (Pilot-scale)	0.5 mg N/L + 0.5 mg P/L (Otonabee River)	7.3 ~ 8.3	Anth. /sand GAC/sand	Anth: 6%, GAC: 11%	no significant changes	ATP (ng/g): 1080~3083 EPS (µg/g): 695~1010	ATP (ng/g): 1000~3500 EPS (µg/g): 675~1210
	0.5 mg N/L + 0.5 mg P/L (Lake Ontario)	7.3 ~ 8.2	Anth. /sand GAC/sand	~ 0%	~ 0%	ATP (ng/g): 92 ~ 290	No significant changes
Granger et al., 2014 (Bench-scale)	(C: P) $_{Control}$: 100: 8 100: 15	6	GAC/sand	5%	19%	ATP (ng/g): 234	ATP (ng/g): 390
	100: 3				11%		864
	100: 15	6	Anth. /sand	1%	19%	938	1090
	100: 3				9%		1250
	100: 15	9	GAC/sand	5%	21%	234	349
	100: 3				15%		287
	100: 15	9	Anth. /sand	1%	19%	938	324
	100: 3				9%		68
Lauderdale et al., 2012 (Pilot-scale)	(C: N: P) $_{Control}$: 100:6:00 100: 10: 02	N.S.	GAC/sand	11%	20%	ATP (ng/ml): 1120 EPS (mg/L): 8.5	ATP (ng/ml): 1500 EPS (mg/L): 3.9
Sang et al., 2003 (Pilot-scale)	(C: P) $_{Control}$: 100: 0.9 100: 1.6	7.3 ~ 7.8	bio ceramic	18.7%	24.3%	-	-
Yu et al., 2003 (Pilot-scale)	(C: P) $_{Control}$: 100: 0.61 100: 1.05	7.9	GAC	14.8%**	20.7%**	-	-
Vahala et al., 1998 (Pilot-scale)	27 µg P/L	7.4	GAC	12%~14%	no significant changes	ATP (nmol/g): 4.6	ATP (nmol/g): 3.5
	Inorganic nutrients				no significant changes		2.9

* N.S. stands for Not Specified

**indicated as COD_{Mn} to report Organic Matter concentration

conditions to improve biological activity significantly (Lauderdale et al., 2012; Granger et al., 2014), as well as reduce the EPS level (Lauderdale et al., 2012). On the other hand, others have found no meaningful changes in ATP and EPS values through nutrient enhancement (Vahala et al., 1998; McKie et al., 2015; Rahman et al., 2016; Nemani et al., 2018). Different doses of supplemented nutrients, as well as organic carbon to ammonium-nitrogen to phosphorus (C: N: P) ratios, have been illustrated in Table 1.1. It appears nutrient addition has not proven to be as effective as expected, and organic carbon removals have improved only marginally. It appears that the effect of nutrient enhancement may be site-specific (McKie et al., 2015; Rahman et al., 2016) and may depend on water-quality parameters. Only one study has examined the complementary influence of pH with respect to nutrient ratio on organic carbon removal (Granger et al., 2014).

As different microorganisms have individual pH conditions for growth and activity (Rittmann and McCarty, 2001) as well as utilization of organic matters (Villaverde et al., 1997), pH may have an essential influence on biofiltration performance. Overall, nutrient studies have shown only marginal impacts on TOC or DOC removal; meanwhile, pH has received very little attention and is yet to be modified as a water-quality parameter. Moreover, so far, most studies on nitrification pertain to wastewater and groundwater treatment. Therefore, investigating ammonia removal in surface water treatment system becomes crucial.

1.2. Research Objectives

The primary goal of this study is to investigate the influence of pH and alkalinity adjustment on the performance of drinking water biological filters.

The specific objectives of this research study are as follows:

- Assessing the influence of water pH and alkalinity adjustment on TOC and ammonia removal

- Evaluating TOC and ammonia removal mechanisms
- Investigating the impact of water pH and alkalinity on EPS and ATP concentration and the potential correlations with headloss build-up

1.3. Article Summary

- **Simultaneous TOC and ammonia removal in drinking water biofilters: influence of pH and alkalinity** *(Planned submission to Journal of Water Supply: Research and Technology)*

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Author contribution:

- ***Hamideh Pirouz Hamidi:*** Conducted a series of biofiltration laboratory experiments and monitored several parameters including TOC, ammonia, EPS, ATP with assistance from undergraduate students Chamathka Varsushawithana and Umar Hafeez and collect results. Compiled data of headloss and analyzed all the data. Wrote the complete initial draft, implemented changes and edits as directed through Professor Onita D. Basu and Postdoctoral Fellow Seyedeh Laleh Dashtban Kenari.
- ***Seyedeh Laleh Dashtban Kenari:*** Provided edits and suggestion throughout the writing stage.
- ***Onita D. Basu:*** Thesis supervisor. Drafted the experimental design. Provided guidance and feedback throughout the process of thesis completion. Provided edits and suggestion throughout the writing stage.

1.4. Organization of Thesis Document

The thesis document contains four chapters. The literature review that is presented in chapter 2 provides background information for this research. Chapter 3 provides a description of the experimental setup that was used for this study along with a detailed description of the experimental and the analytical methods. The results are presented in chapter 4 in the form of a journal article. Chapter 5 concludes the main findings from this study and provides recommendation for future work. Chapter 6 contains a list of the references. Finally, chapter 7 contains appendices.

2. Literature Review

This chapter reviews the literature on the application of biological active filters in organic carbon and ammonia removal and the functional parameters influencing biofilter performances. The review of the literature on biological characterization and headloss build-up is also undertaken.

2.1. Organic Carbon Removal with Drinking Water Biofiltration

The regrowth of the microorganisms in water distribution networks, as well as the formation of disinfectant by-products, have encouraged water treatment plants to employ sustainable treatment processes to remove untreated NOM. The use of biological filtration, owing to its efficacy in reducing significant fractions of organic matter and producing stable water, is regarded as an efficient method and environmentally viable solution to mitigate the formation of chlorinated by-products and microbial regrowth in distribution networks (Emelko et al., 2006; Yapsakli et al., 2010; Liu et al., 2017).

Microorganisms, especially bacteria, play a fundamental role in biofiltration systems regarding producing high-quality drinking water by the biodegradation of pollutants attached to the filter media (Keithly and Kirisits, 2018). Components responsible for microorganism regrowth in water distribution pipelines, including organic matter, ammonia, nitrate, iron, and manganese, can be found across a broad area of North America (Rittmann and Huck, 1989).

Since the 1970s, disinfection by-products, recognized a health hazard, are known to be formed when the organic matter in water reacts with chlorine or other disinfectants. Other issues have also been attributed to untreated NOM, including microbial regrowth in the water distribution network and membrane fouling (Azzeh et al., 2015; McKie et al., 2015; Emelko et al., 2006). In water and

wastewater studies, TOC represents NOM and is the most comprehensive measurement to quantify NOM (Leenheer et al., 2003).

2.2. Factors Affecting TOC Removal in Drinking Water Biofilters

Over past decades, biological filtration as a sustainable technology has come to play an essential role in removing untreated NOM (Urfer et al., 1997; Huang et al., 2011) and producing biologically stable water with low turbidity (Urfer et al., 1997). Therefore, many studies have investigated the parameters increasing untreated NOM removal to improve biofiltration performance. Operational design parameters, including filter media, EBCT, and backwashing procedures, have been well studied by former researchers.

2.2.1. Operational Design Parameters

As the quality of biomass growth and its protection against shear stress depend on filter media characteristics, selecting the proper media is essential for improving biofiltration performance. Chaudhary et al., (2003) have indicated that GAC provides better biomass attachment sites compared to sand. Although Granger et al., (2014) observed no superiority of GAC biofilters over anthracite ones in terms of DOC removal, Azzeh et al., (2015) reported a 0.1 mg/L higher DOC removal in GAC filters than anthracite ($p = 0.05$). However, in earlier studies, more notable differences among the mentioned media in terms of organic matter removal were observed (Liu et al., 2001; Emelko et al., 2006). Liu et al., (2001) obtained higher BOM removal in GAC filters than anthracite filters under unfavorable conditions such as low temperature, especially in the presence of chlorine. Likewise, Emelko et al., (2006) reported significantly higher TOC removal in a temperature range of 1–3°C in GAC biofilters than anthracite biofilters, 23% and 14%,

respectively. However, they indicated that these filters were comparable in TOC removal at 21–24°C. Overall, the majority of the findings to date support the fact that the GAC media could be used in both high and low temperatures, while cold temperature and chlorine presence impair the performance of the anthracite filters more than the GAC ones.

Urfer et al., (1997) explained that since BOM removal was significantly affected by contact time, EBCT would be an essential factor in designing a biofilter. In this respect, Hammes et al., (2011) indicated that although EBCT has a vast range from a few minutes to around an hour, most studies adjusted the EBCT values between 4 and 20 minutes. While any meaningful changes in organic carbon removal in the filters with EBCT ranging between 4 to 20 minutes were not observed by some authors (Hozalski et al., 1995; Halle et al., 2009), higher DOC removal by increasing EBCT from 4 to 20 minutes were reported by others (Peldszus et al., 2012; Basu et al., 2016). Similarly, Granger et al., (2014) demonstrated that with a 10–20 minutes EBCT, significant portions of BOM could be removed. The different responses to EBCT likely depended on the carbon source characteristics, which was indicated by Yavich et al., (2004). They found that increasing ozone dose decreased the required EBCT of filters fed by Lansing Lake while increased the required EBCT of filters fed by Huron River. Overall, it seems that TOC/DOC removal gains benefit from increasing EBCT up to 20 minutes.

Backwashing has been found to be required for controlling biofilm thickness, resulting in the minimizing of filter clogging and improving the biodegradation of pollutants (Zhu et al., 2010). Several studies have examined the effects of different backwashing procedures, including chlorinated backwash water or air scouring in backwashing, on biofiltration performance. For example, a substantial adverse impact of the presence of free chlorine in backwash water on organic matter removal has been reported (Urfer et al., 1997; Liu et al., 2001). While Liu et al.,

(2001) suggested using air scour during backwashing provided proper media cleaning, Emelko et al., (2006) did not observe any notable influence of applying air-scour or air-scour at collapse pulsing condition on BOM removal compared to water-only backwash. On the other hand, Ikhlef and Basu, (2017) assessed the effect of backwash regime on biofiltration performance and concluded that organic carbon removal was clearly influenced by the chosen backwash protocol. Applying collapse pulsing to the backwashing method modified DOC removal compared to the water-only backwash under nutrient-limited condition. Moreover, they reported that undertaking collapse pulsing and bed expansion up to 30% during backwashing positively affected DOC removal in biofilters; however, higher bed expansions adversely influenced DOC removal and increased backwash requirements.

2.2.2. Nutrient Supplementation

Recent studies aimed at improving biofiltration performance have examined the influence of nutrient enhancement on organics removal. The biodegradation of organic matters is believed to depend on the availability of essential nutrients, and the ratios of C: N: P play a vital role on microbial growth (Sang et al., 2003; Lauderdale et al., 2012; Dhawan et al., 2017). The impact of phosphorus addition was previously evaluated on organic matter removal, and a 6% improvement was found (Sang et al., 2003; Yu et al., 2003). However, in an earlier study, no significant changes were observed by Vahala et al., (1998) in either filter supplemented by phosphorus or a combination of inorganic nutrients. Later, to promote biological activity, Lauderdale et al., (2012) investigated the impact of nutrient enhancement on biofiltration performance and observed significant improvements in removed organic carbon values from 0.4 mg/L to 0.7 mg/L. Likewise, Dhawan et al., (2017) and Ikhlef and Basu, (2017) indicated an 8% and 14% increase respectively

in DOC removal in nutrient-enhanced biofilters (25: 5: 1) compared to nutrient-limited conditions (546: 24: 1). However, the same outcomes were not obtained when several nutrient ratios were assessed by other researchers (Azzeh et al., 2015; Nemani et al., 2018). Similarly, other studies found no striking effects of adding 0.5 mg/L phosphoric acid and 0.5 mg/L of ammonium chloride as nutrient sources (McKie et al., 2015; Peleato et al., 2016).

The ineffectiveness of nutrient supplementation was probably due to the limitation of the biodegradable fractions of organic carbon (Azzeh et al., 2015; Balsher, 2016). To examine this hypothesis, Balsher, (2016) explored the pre-treatment of biofilter influent through peroxidation to promote the biodegradability of organic compounds. Although he observed a 17% improvement in DOC removal after the addition of ozone and hydrogen peroxide, they did not find any further increase in DOC removal by combining the peroxidation strategy with nutrient enhancement.

In one study, Fu et al., (2017) found a modest improvement in DOC removal by phosphorus and nitrogen addition at an enhanced dose of 0.23 g P/L and 1.0 g N/L, respectively; then, they attempted to evaluate the effect of only nitrogen supplementation on biofiltration efficiency. Interestingly, they did not observe any advantages of enhancing only nitrogen at either dose of 1.0 or 2.0 g/L and concluded that phosphorus had an essential effect on improving microbial activity and DOC removal. While in another study by Rahman et al., (2016), long-term effects were not observed after phosphorus supplementation and positive effect on DOC removal diminished after one month.

Overall, except in a few studies, nutrient enhancement was not found to be beneficial in improving biofiltration performance significantly. Thus, nutrient enhancement influence may depend on water characteristics (McKie et al., 2015; Rahman et al., 2016) and other factors. Nutrient enhancement may also be responsible for modifying biofiltration performance.

2.2.3. Operating Water Temperature

Temperature effect as a water-quality parameter has also been evaluated in past studies. Some authors found that fewer organic matters were removed at low temperatures (≤ 5°C) than high temperatures (20°C) in anthracite or sand filters (Moll and Summers, 1999; Liu et al., 2001; Emelko et al., 2006). In some studies, low temperature (≤ 5°C) was reported to reduce TOC removal by 9% (Moll and Summers, 1999) and 6% (Emelko et al., 2006). Also, Liu et al., (2001) indicated that the removal of various fractions of organic carbons in the range of 70% to 90% at 20°C reduced to 10% to 50% at 5°C. However, this adverse temperature impact was not observed in GAC filters (Liu et al., 2001; Emelko et al., 2006).

On the other hand, DOC removal was not affected by temperature changes at values higher than 10°C (Moll and Summers, 1999; Lauderdale et al., 2012; Rahman et al., 2016). As the studies reviewed in the previous section were mainly indoor bench-scale or pilot-scale studies, the seasonal temperature variation could not be accounted to be a compelling factor for the different results obtained in different studies. Therefore, other water-quality parameters need to be assessed.

2.2.4. pH and Alkalinity

Although most microorganisms require a narrow pH range between 6.0 and 8.0 for growth and activity, some bacteria prosper better under highly acidic condition. Therefore, the optimum pH condition for the growth and activity of bacteria of interest must be recognized and considered to design a proficient treatment system (Rittmann and McCarty, 2001). In a study conducted by Moll and Summers, (1999), they observed that the overall DOC removal did not change in biofilters fed by different water sources with different operating pH values in the range of 5.5 to 9.5. However, a BIOLOG analysis, conducted by Moll and Summers, (1999), suggested that pH levels change

the utilization degree of different carbon types. For example, carboxylic and amino acids were better utilized at neutral or acidic pH, while carbohydrates were better utilized at pH 7.5.

Although Jeong et al., (2017) observed a slight improvement in DOC removal at pH 7.5 compared to 6.5 (22% versus 20%), Granger et al., (2014) found different responses to pH adjustment in nutrient-enhanced biofilters. An average 6% higher DOC removal were observed in filters operated at pH 6.0 rather than pH 9.0 with C: P ratio of 100: 15, while DOC removal was an average 14% higher at pH 9.0 rather than pH 6.0 in filters operated with C: P ratio of 100:3 (Granger et al., 2014).

Zanacic et al., (2016) indicated that high alkalinity impeded the performance of ozone-assisted biofiltration plants. They compared two water treatment plants with the alkalinity level of 127~204 mg $CaCO_3$/L and 300~450 mg $CaCO_3$/L and observed lower DOC removal in the second plant due to a higher alkalinity level, which hindered the influence of ozonation by reducing the oxidation rate. However, they mentioned that a direct comparison between these plants was not possible due to the differences in design and sources of water. Similarly, Camel and Bermond, (1998) recommended reducing the carbonate level to improve TOC removal.

Notwithstanding the importance of pH in the utilization degree and microbial activity of organic matters, limited studies have examined the impacts of pH or alkalinity on biofiltration performance in terms of DOC/TOC removal, and the reported results are inconsistent.

2.3. Nitrogen/Ammonia Removal via Biological Mechanisms in Drinking Water

The presence of ammonium (NH_4^+) or its conjugate base ammonia (NH_3) in water sources does not exert a direct health concern, while their removal due to their adverse impacts on water quality is advisable (Lájer, 2012). Ammonia is a form of ammonium salt; for example, ammonium

chloride at a dose more than 33.7 mg per kg of body weight per day may interfere with human metabolism and have toxic effects (WHO, 2003). Besides, Environmental Protection Agency (EPA), Food and Drug Administration (FDA), and Health Canada have stipulated the maximum allowable concentration (MAC) of 10 mg/L nitrate and 1 mg/L nitrite as nitrogen in drinking water. Thus, they do not pose a direct health concern; however, their presence may impair the quality of drinking water, such as taste and odor, and interfere with satisfactory quality. The presence of ammonium can affect the odor and taste of water at a threshold of 1.5 and 35 mg/L respectively (WHO, 2003), while 0.1 mg/L has been determined by Health Canada (2017) as the free ammonium limitation in drinking water. In addition to the water-quality problems caused by ammonium, including dissolved oxygen consumption in water treatment plants and the reduction of disinfection efficiency, it can also increase the corrosion of metal pipes in distribution systems. Nitrification is regarded as the most efficient and cost-effective way to remove ammonia compared to conventional processes such as break-point chlorination or ion exchange (Yu et al., 2007; Yapasakli et al., 2010; Lájer, 2012). Moreover, the volatilization of ammonia at high pH is another potential method for ammonia transformation (Lájer, 2012; Delgadillo-mirquea et al., 2016). Assimilation is a possible way where ammonia can be utilized if adequate C: N: P ratio is available for metabolism (Yu et al., 2007).

2.3.1. Critical Parameters Affecting Ammonia Removal

According to Eq. (2.1), complete nitrification causes 4.57 mg O_2/mg NH_4^+-N dissolved oxygen demand as well as 7.14 mg $CaCO_3$/mg NH_4^+-N alkalinity consumption (Metcalf and Eddy, 2013).

$$\mathbf{NH_4^+ + 2\ O_2 + 2\ HCO_3^- \rightarrow NO_3^- + 3\ H_2O + 2\ CO_2} \qquad \mathbf{(2.1)}$$

Alkalinity may be a limiting factor in the nitrification process, such that in wastewater studies 40–80 mg $CaCO_3$/L was required as the minimum alkalinity to complete nitrification (Colt, 2006; Summerfelt et al., 2015). In addition to the researchers who indicated that particular concentrations of alkalinity were required to complete nitrification (Chen et al., 2006; Rusten et al., 2006; Metcalf and Eddy, 2013), others illustrated that alkalinity was needed to maintain pH and prevent significant pH decline (Villaverde et al., 1997; Summerfelt et al., 2015). As nitrifiers with prolonged growth rates are sensitive to low pH, avoiding low pH is beneficial for ammonia removal. A study by Villaverde et al., (1997) on wastewater evaluated the effect of the pH variation between 5 and 8.5 on nitrification efficiency in a filter with influent NH_4^+-N concentration of 80-100 mg/L and reported that per each unit pH increase, nitrification improved by 13%. Improvement in ammonia removal by increasing pH was also likely due to the higher relative proportion of NH_3 to NH_4^+, which led to more NH_3 availability at higher pH (Allison and Prosser, 1993; Loyless and Malone, 1997; Colt, 2006). Furthermore, Sajuni et al., (2010) examined the impact of temperature, DO, pH, and alkalinity on the ammonia removal efficiency of wastewater biofilters and indicated the pH range of 7.5 to 9.0 to be the optimum range for nitrification.

Eq. 2.1 shows that the removal of ammonia through nitrification also results in a DO consumption, and oxygen is perhaps the other limiting factor in nitrification.

In addition to oxygen limitation in biofiltration system, DO paradox was also reported in a few studies. Nitrogen–oxygen balance carried out by Yu et al., (2007) illustrated that the removed ammonia was 30% higher than it could be removed through nitrification and thus other mechanisms except for nitrification removed about 30% of ammonia. In a later study, Cai et al., (2014) also found significant nitrogen loss compared to oxygen consumption. Anaerobic ammonia

oxidation (Anammox) is a well-known ammonia removal method where oxygen is not required. As DO presence is a detrimental parameter for the anammox process, it is unsure whether the anammox bacteria will grow in aerobic conditions such as drinking water biofilters. However, the resistance of microorganisms in the outer layer of the biofilm may prevent the diffusion of DO into the inner layer and provide a suitable anoxic environment in the inner layer for the anammox bacteria to grow (Yu et al., 2007; Cai et al., 2014). Based on Yu et al.'s (2007) study, aerobic de-ammonification, the nitrification followed by anammox, could be a possible method for extra ammonia removal than nitrification.

The nitrification process has the simultaneous impact of reducing oxygen and alkalinity and increasing nitrate level (Chen et al., 2006; Yu et al., 2007; Fu et al., 2017). However, in the case of the following nitrification by denitrification, increasing nitrate formation may be limited by the denitrification (Delatollah et al., 2015). Furthermore, Van Rijn et al., (2006) suggested that a portion of the consumed alkalinity in the nitrification phase would be regained in the denitrification stage and resulted in the alkalinity increase. Expected alkalinity increases would be approximately 3.57 mg $CaCO_3$ per each mg of nitrate-N that reduced to N_2 according to the following simplified stoichiometry (Eq. 2.2).

$$\mathbf{2\ NO_3^- + 12\ H^+ + 10\ e^- \rightarrow N_2 + 6\ H_2O} \qquad \mathbf{(2.2)}$$

2.3.2. Other Influential Parameters in Nitrification

In addition to oxygen and alkalinity limitation, nitrification can also be limited by low contact time, low temperature, as well as toxic material availability (Lopez-Ponnade et al., 2017). A few studies were conducted to review these parameters. A study by Andersson et al., (2001) on drinking

water illustrated the importance of temperature impact on nitrification activity. Ammonia removal efficiency decreased from 90% at a temperature higher than 10°C to less than 30% at 4°C. The severe impact of low temperature was furthered by decreasing the temperature to 3°C with 10% ammonia removal. Andersson et al., (2001) also found that in warm temperatures, an EBCT of five minutes would be enough to secure complete nitrification, while longer EBCTs would be required in cold temperatures.

Similarly, Wert et al., (2008) indicated that nitrification would improve at a lower filtration rate and higher EBCT. They observed that by increasing the filtration rate from 4.8 m/h to 14.6 m/h to reduce EBCT from 9.7 to 3.2 minutes, the conversion of ammonia to nitrate reduced by 60%. They also suggested that dechlorinated backwash water was required to improve nitrification efficiency in the system.

2.4. Biological Characterization

In biological filtration systems, biofilms, composed of microbial cells and EPS, form on filter media to facilitate microbial activity. Several processes such as adsorption, desorption, attachment, detachment, and microbial activity take place in biofilters to form biofilms (Liu et al., 2001). Although too little biomass slows down pollutant degradation, excessive biofilm would increase the headloss development rate (Zhu et al., 2010). Regarding this fact, other researchers investigated the beneficial and detrimental effect of EPS in biofilters. For instance, EPS encases and assists bacteria in attaching to the surface of media (Keithly and Kirisits, 2018). EPS also protects bacteria from unexpected adverse conditions such as swings in pH or exposure to oxidants (Lauderdale et al., 2012).

On the other hand, EPS may be the primary contributor to biofilter clogging and headloss development, which result in increasing backwash frequency and energy costs (Lauderdale et al., 2012; Xia et al., 2016; Keithly and Kirisits, 2018). Similarly, Hammes et al., (2010) indicated that increasing biological activity without the overproduction of EPS would be beneficial to have an efficient biofilter.

Nutrient content was found to be one of the possible contributive factors in EPS production. Sheng et al., (2010) found that EPS concentration and composition were significantly affected by nutrient levels. They indicated that nutrient deficiency promoted EPS formation to compensate for substrate shortage; likewise, C: N ratio impacted EPS characteristics such as PR/PS ratio. The importance of nutrient availability was also noticed by other authors when they attempted to correlate the microbial activity and biomass development in biofilters to the biodegradable dissolved organic carbon availability in source water and raw water quality (Pharand et al., 2014; McKie et al., 2015; Rahman et al., 2016).

McKie et al., (2015) observed that nutrient addition did not affect biological activity, while 10 times higher ATP concentration was measured in biofilters fed by Otonabee River with 2.5 times higher DOC level compared to Lake Ontario. Similarly, three times higher nitrogen concentration in Otonabee River resulted in up to 10 times higher protein formation in relevant biofilters compared to that of Lake Ontario. Although they did not find any contribution of nutrient enhancement to microbial activity and headloss formation, Lauderdale et al., (2012) reported a 30% improvement in biological activity in biofilters operated at the C: N: P ratio of 100: 10: 2. Additionally, they observed a 30% lesser EPS production, which reduced the headloss development rate by 15%. Furthermore, a 60% increase in the terminal headloss in biofilters operated under N-limited condition, reported by Lauderdale et al., (2012), illustrated that nutrient

supplementation improved biofiltration performance in terms of decreased EPS production and hydraulic performance. This hypothesis was also supported by Fang et al., (2008) who examined phosphorus supplementation at a dose of 3, 30, and 300 μg/L on EPS formation. Their results illustrated that EPS concentration decreased by 33% at the initial stage of phosphorus addition, further increased to 30 and 300 μg/L, and decreased EPS concentration by 81% and 77%, respectively. In contrast with these findings, lower ATP levels were observed in biofilters enhanced by phosphorus and inorganic nutrients (Vahala et al., 1998; Lee, 2014). Lee, (2014) compared biofilters operated under nutrient-rich and nutrient-limited conditions with the C: N: P ratios100: 25: 1, 100:00:1, 100: 10: 5, and 100: 10: 0 and observed higher EPS and lower ATP concentration under all nutrient ratios compared to the balanced condition (100: 10: 1).

While McKie et al., (2015) did not report any signs of hydrogen peroxide addition on ATP concentration and headloss development, a 66% and 40% reduction in headloss development were observed by others (Lauderdale et al., 2012; Azzeh et al., 2015). Meanwhile, Azzeh et al., (2015) found a 52% reduction in EPS concentration, while Lauderdale et al., (2012) did not observe any remarkable effect on biological activity in terms of ATP concentration. Thus, it seems the headloss was reduced due to the oxidation of inactive biomass and EPS rather than active microorganisms. The review of the literature indicates that not all researchers could observe the effectiveness of nutrient enhancement on biological features and headloss build-up improvement. Although, nutrient addition provided a more favorable biological condition with respect to ATP and/or EPS concentrations (Fang et al., 2008; Sheng et al., 2010; Lauderdale et al., 2012), other researchers did not find any direct contribution of nutrient supplementation to improve biological activity or reduce EPS formation (Vahala et al., 1998; Lee, 2014; Pharand et al., 2014; McKie et al., 2015). Thus, other factors may affect EPS and ATP concentrations as well as headloss development.

Granger et al., (2014) investigated the effect of the combination of phosphorus enhancement along with pH adjustment on biological activity. While similar ATP concentration was observed in control biofilters operated at pH 6.0 and 9.0 with a C: P ratio of 100:8, higher ATP concentration was found in biofilters operated at pH 6.0 compared to pH 9.0 at both C: P ratios of 100:3 and 100:15. However, the higher ATP concentration at pH 6.0 did not correlate to higher DOC removal, and an average of 6% improvement in DOC removal was found at pH 9.0 compared to pH 6.0. Additionally, minimal bacteria and no biofilms were observed in the filters operated at pH 9.0, while the biofilms were better developed at pH 6.0. They suggested that this observation, along with a higher ATP level at pH 6.0, demonstrated better bacterial growth at this pH rather than pH 9.0.

3. Material and Methods

This chapter first describes the experimental set-up used for biofiltration performance optimization by pH and alkalinity adjustment. Then, it explains the methods used for water-quality and biological characteristics measurements.

3.1. Biofiltration System

3.1.1. Experimental Setup Description

Two laboratory-scale dual media biofiltration columns containing 520 mm of GAC on top of 180 mm of sand as the filter media were used in parallel. 15 mm of synthetic underdrain was considered in each column to support the media as well as prevent media loss. The columns were constructed of a 1250-mm plexiglass pipe with a 50-mm internal diameter. Each column had 5 sampling ports at different media depths and an overflow with an attached tube to drain backwash water at the height of 1060 mm. These columns were mounted on a steel frame located on top of a steel drain tray. The hydraulic loading rate of 3 m/hr (flow rate: 100 mL/min) was adjusted for each column to provide an empty bed contact time of 14 minutes.

The bottom of each column had four openings, which were used for required attachments. One of them was used to direct the continuous effluent to the rotameters (Cole Parmer), then to turbidimeters, to measure the effluent turbidity. Another opening attached to a pressure transmitter (Wika-10) was used to transfer the pressure data continuously. The remained openings were employed for introducing backwash water and air scour.

The design parameters for each column are provided in Table 3.1.

Table 3. 1: Filter and Media Design Parameters	
Parameter	**Value**
Column Height (mm)	1250
Inside Diameter (mm)	50
Flow Rate through Each Column (ml/min)	100
EBCT (min)	15
GAC Bed Depth (mm)	520
GAC Effective Size (mm)	0.70
Sand Bed Depth (mm)	180
Sand Effective Size (mm)	0.50
Number of Sampling Port	5
Synthetic Underdrain Depth (mm)	15

3.1.2. Process Configuration

The biofiltration set-up in the current study is illustrated in Figure 3.1. Two 50 L Nalgene cylindrical containers were used as the nutrient dosing tanks. One of the tanks contained a mixture of carbon solutions (acetic acid, formic acid, and glyoxal), and the other one contained a mixture of inorganic nutrients such as nitrogen and phosphorous. Each solution tank was equipped with a mixer to ensure that the solution was homogenized. In addition, one water tank was installed to collect dechlorinated tap water to facilitate providing the columns with synthetic water at a C: N: P ratio of 25: 5: 1 continuously. The carbon, nitrogen, and phosphorus concentrations remained constant throughout the whole study. The water tank contained a toilet flush system to maintain water at a certain level. Using a Masterflex peristaltic pump (model 07528-30) with two easy load pump heads and Masterflex tubing (LS-14), the carbon and nutrients solutions were dosed into a line where they mixed with dechlorinated tap water pumped continuously from the water tank using a Masterflex peristaltic pump (model 07528-10) with an easy load pump head (model 77200-

60) and 16 inch Masterflex tubing (06440-16 Tygon E-LFL). An inline static mixer was used (McMaster Carr) to provide adequate mixing for the dosing solutions with water. Then the mixed flow was passed through a tee to split it equally into the rotameters (Cole Palmer) to control each biofilter influent flowrate. The flowrate of each biofilter effluent was controlled by the same type of rotameters (Cole Palmer). Then the effluent was directed toward two inline low range turbidimeters (HACH, Filter Track 660) to measure the effluent turbidity continuously.

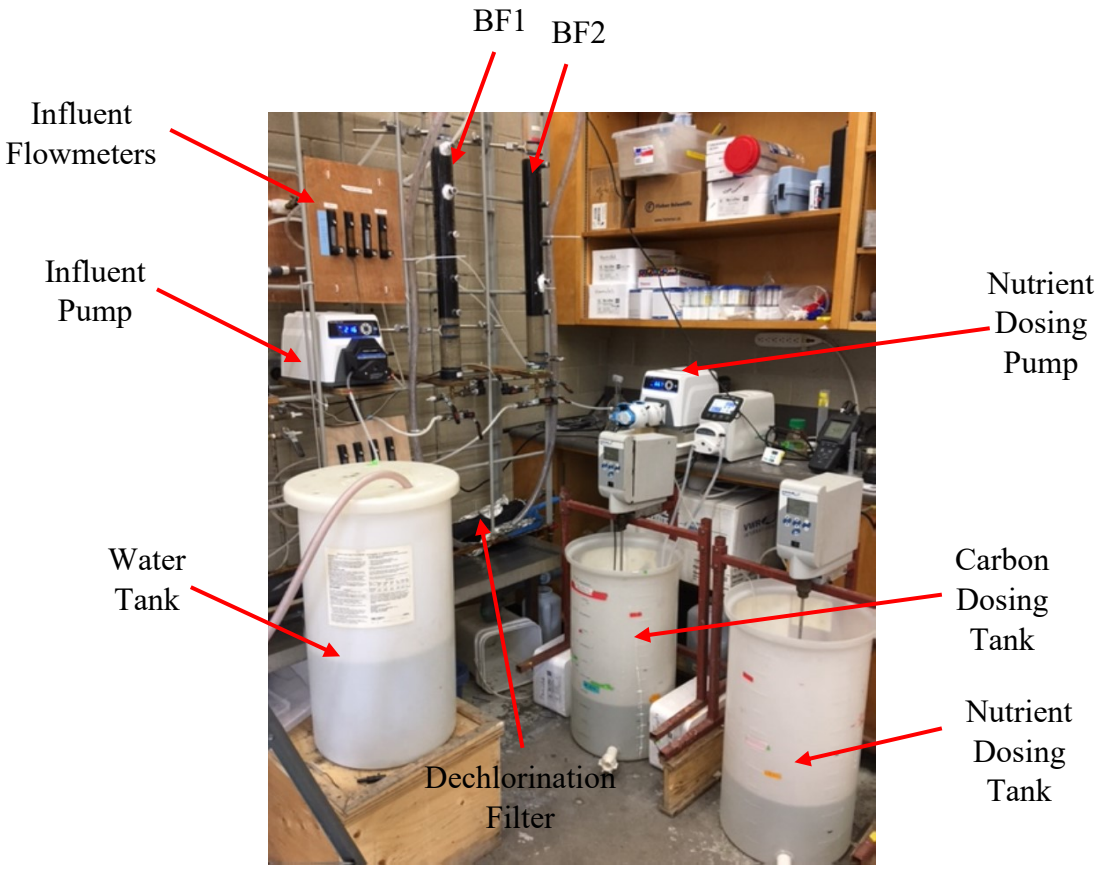

Figure 3. 1: Biofiltration setup

Throughout the conditioning phase, the biofilters were inoculated several times with water from Ottawa River and backwash water from the Ottawa Britannia water treatment plant full-scale filters to enhance the bacterial growth in the biofilters.

3.1.3. Backwash Procedure

The effluent from the biofilters was collected to be used as the unchlorinated backwash water. Before starting the backwash, the biofilter columns were drained to 5 cm above the filter media, as well as air and water supplies were attached to the relevant connectors at the bottom of the columns. In the conditioning phase and the first experiment of the study phase, one biofilter was backwashed once a week, and the other biofilter was backwashed twice a week. The results showed that these filters were required to be backwashed more frequent than once a week; therefore, in the following experiments, both columns were backwashed twice a week. The backwash procedure consisted of a 6-minute slow wash (35 m/hr) with air scour followed by a 4-minute high-rate water wash (30 m/hr) to achieve the 30% bed expansion, and finally a 2-minute low-rate water wash (velocity=10 m/hr) at 10% bed expansion.

3.1.4. Phases of Research

A summary of the different phases of the current research study is provided in Table 3.2. The experiments consisted of two phases, namely conditioning and experimental phases.

The conditioning phase lasted around six months, and both columns reached the same TOC removal efficiency. During the conditioning period, the biofilters received organic carbon, nitrogen, and phosphorus at a ratio of 25: 5: 1 (Ikhlef and Basu, 2017).

The experimental phase had an identical nutrient ratio as that of the conditioning phase (C: N: P = 25: 5: 1) along with pH and alkalinity adjustment. The identical nutrient conditions were provided in the columns during the experiments to avoid nutrient-limited conditions and ensure reproducibility of results.

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Table 3. 2: Phases of biofiltration research		
Phases	**Objective**	**Comments**
I	Conditioning Phase C: N: P ratio of 25: 5: 1 without any NaOH and $NaHCO_3$ addition	pH 6.0 and low alkalinity level
II	Experimental Phase C: N: P ratio of 25: 5: 1 NaOH and $NaHCO_3$ addition to adjusting pH and alkalinity	pH 6.0 and low alkalinity level pH 6.0 and high alkalinity level pH 7.5 and low alkalinity level pH 7.5 and high alkalinity level pH 9.0 and low alkalinity level pH 9.0 and high alkalinity level pH 10.0 and low alkalinity level pH 10.0 and high alkalinity level
low alkalinity: 25 - 50 mg $CaCO_3$/L high alkalinity: 180 - 220 mg $CaCO_3$/L		

3.2. Analytical Methods

Table 3.3 illustrates a summary of the parameters examined in this study and their sampling frequency.

Table 3. 3: Parameter sampling frequency		
Parameter	**Sampling Frequency**	**Comment**
Total Organic Carbon	5 times per week	
Nitrogen, ammonia	3 times per week	
Turbidity	Continuously	
Headloss	Continuously	
EPS	2 times per week	before backwash and 24 hours after backwash
Adenosine Tri-Phosphate Method	2 times per week	before backwash and 24 hours after backwash
Nitrate	3 times per week	At high alkalinity level of pH 6.0
pH	Daily	
Alkalinity	3 times per week	
Dissolved Oxygen	5 times per week	At the same time of TOC sampling
Temperature	Daily	
SDI test	Once a week	

3.2.1. Dosing Solutions

The nutrient (nitrogen and phosphorus) solutions were prepared using distilled water, and the organic carbon solutions were prepared using tap water. Each stock solution was prepared in a 1-L volumetric flask rinsed with DI water and allowed to mix overnight on a stir plate with a magnetic stirrer. Then each solution was placed in its relevant dosing tank and filled to the mark. Each dosing tank was equipped with a stand mixer to mix the solution thoroughly at 350 rpm for about 2–3 hours. Dosing solutions calculations and associated concentrations can be found in Appendix A. The synthetic raw water make-up of the lab-scale biofilter setup is provided in Table 3.4 and shows the chemicals used to prepare the dosing solutions. A summary of the different chemicals used to prepare the dosing solutions is provided in Table 3.4.

Table 3. 4: Chemicals used for dosing solutions preparation

Purpose	Chemical Used	Phase
Carbon Source	Acetic acid, formic acid, glyoxal	All phases
Phosphorous Source	Potassium dihydrogen phosphate	
Nitrogen Source	Ammonium sulfate	
pH adjustment	Sodium hydroxide	pH 7.5 and low/high alk. pH 9.0 and low/high alk. pH 10.0 and low/high alk.
pH adjustment	Hydrochloric acid	pH 6.0 and high alk.
Alkalinity adjustment	Sodium bicarbonate	pH 7.5 and low/high alk. pH 9.0 and low/high alk. pH 10.0 and low/high alk.

3.2.2. Total Organic Carbon (TOC)

Water samples were collected and stored in clean amber glass bottles placed in 10% HCl solution overnight. Following this, all bottles were rinsed with DI water, wrapped in aluminum foil, and baked at 400°C for a minimum of 40 minutes to ensure that they were free of any organic contaminants. All the samples were preserved with 3 drops of phosphoric acid to keep the pH less than 2.0 and were stored at 4°C up to a week if not analyzed immediately.

TOC and DOC were measured under the Persulfate Ultraviolet Oxidation Method in Standard Methods 5310C (APHA, 2012). Before DOC analysis, the samples were filtered through a 0.45-μm filter (Millipore). As dissolved organic carbon was used in this study to prepare the dosing solution, only TOC was measured.

3.2.3. Nitrogen-Ammonia

Influent and effluent water samples were collected in clean glass bottles. The pH of the samples was adjusted to less than 2 with concentrated hydrochloric acid if the samples were not tested immediately. HACH DR 2800 was used to test the nitrogen–ammonia concentration based on the salicylate method (#10023). A calibration curve was prepared and saved in the HACH machine to read the results in mg NH_3-N/L.

3.2.4. Dissolved Oxygen and Temperature

Dissolved oxygen and temperature measurements were taken using the same probe (Thermo Scientific ORION).

3.2.5. pH

pH was monitored using a pH probe (Thermo Scientific ORION 087003). The device was calibrated using standard reagent at pH 4, 7, and 10.

3.2.6. Alkalinity

Influent and effluent water samples were collected in clean glass bottles. HACH DR 2800 and TNT 870 kits were used to measure the total alkalinity in the alkalinity range of 25–400 mg $CaCO_3$/L based on the HACH method (#10239). In this method, the pH changed due to the reaction between carbonates and the reagent in the vial. This variation is shown by an indicator and is photometrically evaluated.

3.2.7. Nitrate

Influent and effluent water samples were collected in clean glass bottles. HACH DR 2800 and TNT plus 835 kits were used to measure the total nitrate content based on the HACH dimethylphenol method (#10206). The samples needed to be analyzed immediately for best results; otherwise, they had to be filtered and stored at or below 6°C for a maximum of 48 hours.

3.2.8. Adenosine Tri-Phosphate (ATP)

ATP tests were performed on top of the media before the backwash and 24 hours after the backwash. The tests were conducted according to the LuminUltra Technologies test kit instruction. The test steps involve calibration, sample preparation, and total ATP analysis. The samples were collected directly after the filters were taken offline. 1 g of media samples were collected in sterile containers, and the microbial activity was measured using a deposit and surface analysis test kit (DSA^{TM}), which was tailored to attached growth measurement such as biological filter media.

3.2.9. EPS Analysis

The EPS analysis was conducted on the top 5 cm of the biofilter media. The polysaccharide analysis method was adapted from Azzeh et al., (2015), and protein analysis was carried out based on Pierce^{TM} BCA Protein Assay Kit's instructions (Thermo Scientific). New calibration curves were prepared for each test; serial dilution curves of glucose and Bovine Serum Albumin (BSA)

solution were used for polysaccharides and proteins, respectively. Hach spectrophotometer (Hach DR/2500 Scanning Spectrophotometer) and CE 3055 Single Beam Cecil UV/visible spectrophotometer (Cambridge, UK) were used for polysaccharide and protein analysis, respectively.

2 g of filter media were collected in 10-mL falcon tubes. The samples could be stored in the fridge at 4°C up to 48 hours before analysis. They were brought to room temperature before beginning the test.

3.2.9.1. Extraction Method

For sample extraction, Tris-EDTA buffer (10 mM Tris, 10 mM EDTA) was prepared from 1 M Tris at pH 8.0 and 0.5 M EDTA solution. The stock solution was placed on a magnetic stirrer until the reagents were fully dissolved, then sterilized in the autoclave at 121 °C for 20 minutes and maintained at room temperature. 10 mL of prepared Tris-EDTA buffer was added to the filter media. The samples were incubated at 4 °C on a shaker at 300 rpm for 4 hours then were centrifuged at 12000 g for 15 minutes. After that, the supernatant was pipet and filtered using a 0.45-µm filter into sterile glass containers. The samples were stored in a freezer at -20 °C if they were not analyzed immediately.

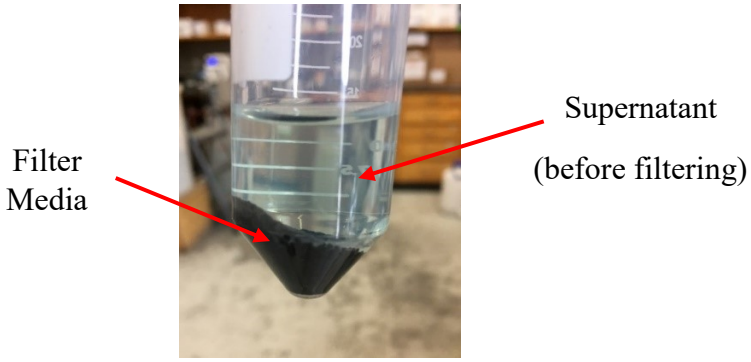

Figure 3. 2: Extraction result

3.2.9.2. Polysaccharide Analysis

The water bath was placed on a heater and heated to 100 °C. Then, standard solutions were prepared to provide new calibration curves. At first, 50 mg/mL standard solution was prepared out of 2.5 g of glucose anhydrides; then, it was diluted to 1 mg/mL using MilliQ water.

200 µL of 1 mg/mL glucose solution and 1800 µL of MilliQ water were added to a sterile glass vial to prepare 2 mL of 100 µg/mL standard. 1 mL of each standard concentration was added to 1 mL of MilliQ water in another glass vial to make the next lower concentration in the serial dilution standards (100, 50, 25, 12.5, 6.25, 3.125, and 0 mg/L). The glass vials were then capped with acid-resistant lids. 1 mL of the sample (in triplicates), including a blank (Milli-Q water), was added to the glass vials, and 1 mL of 5 % phenol solution was added to each vial. Then, in a fume hood, 5 mL of 98 % sulfuric acid was added to each vial. Then the samples were mixed by a vortex mixer at a low speed and placed in a 100° C water bath for 5 minutes. After removing the samples from the water bath, they were mixed again. The samples were covered in aluminum foil and incubated in the dark, at room temperature, for 10 minutes. Then each sample was gently mixed by the vortex, covered again in foil, incubated in the dark at room temperature for an additional 30 minutes, and afterward were mixed gently. Finally, the absorbance was read at 492 nm using the Hach spectrophotometer (Hach Odyssey DR 2500 Scanning Spectrophotometer).

3.2.9.3. Protein Analysis

The water bath was placed on a heater and heated to 60° C. Then standard solutions were prepared by adding 50 parts reagent A to 1-part reagent B of the Pierce™ BCA working reagent. The

required reagent volume was calculated by multiplying the number of samples, including blanks, standard curve, and duplicates by 2.

500 µL of bovine serum albumin (BSA) was added to 1500 µL of MilliQ water at 2 mg/mL to prepare a standard solution at 500 µg/mL. Then 100 µL of each concentration was added to 100 µL of MilliQ water to make the serial dilution.

100 µL of the sample (in triplicates), including a blank (Milli-Q water), and standard solutions were added to a sterile glass vial. 2 mL of BCA reagent mixture were added to each glass vial and mixed well. The vials were covered and incubated in the water bath at 60° C for 30 minutes. Then all the samples were cooled to room temperature.

CE 3055 Single Beam Cecil UV/visible spectrophotometer was set to 562 nm. The absorbance of all the samples was measured within 10 minutes.

Using the calibration curve, which was prepared based on each BSA standard versus its concentration in µg/m, the protein concentration of each sample was determined.

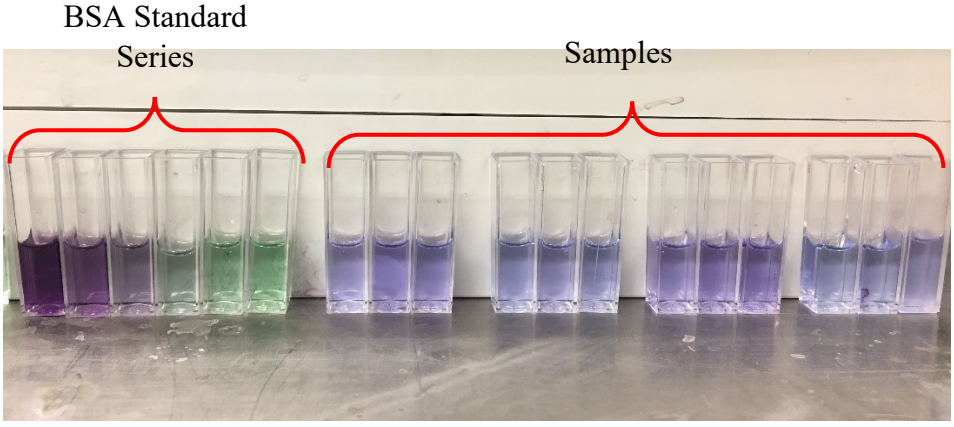

Figure 3. 3: Protein test

3.2.10. Turbidity

The effluent turbidity was monitored continuously by two inline low range turbidimeters (HACH SC 200). The data were recorded in 15-minute intervals and reduced to 30 seconds after each backwash to record ripening time accurately. Influent turbidity was also measured using a portable HACK turbidimeter.

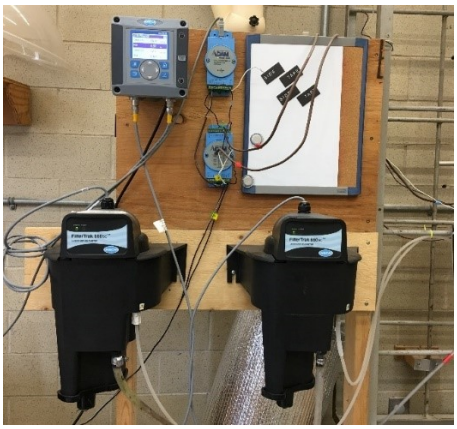

Figure 3. 4: Inline turbidimeters

3.2.11. Pressure

A pressure transmitter (Wika A-10) was installed at the bottom of each biofilter to transfer data through inline ADAM-4561 to collect the pressure data of each column continuously.

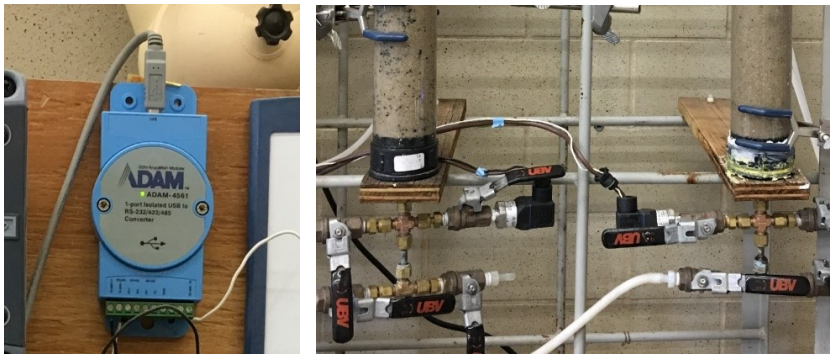

Figure 3. 5: Pressure transmitter and inline convertor

3.2.12. SDI

The silt density index (SDI) of water was measured following ASTM D4189-07 to quantify the particle matter in water. This standard applies to relatively low turbid water (<1.0 NTU). The method is based on measuring the flow rate of the water passed through a 0.45-µm membrane filter at a constantly applied gauge pressure of 30 psi. The calculation of the SDI is as follows:

$$SDI_T = \frac{\left[1 - \frac{t_i}{t_f}\right] 100}{T}$$

Where: T = total elapsed flow time, usually 15 minutes

t_i = initial time required to collect 500 mL of sample, (s)

t_f = time required to collect 500 mL of the sample after test time T, (s)

3.2.13. Statistical and Data Analysis

Statistical analysis was performed in the form of a paired t-test. The average, maximum, minimum, and standard deviations of the sample range were determined. The error bars in the figures represents the standard deviation.

4. Simultaneous TOC and Ammonia Removal in Drinking Water Biofilters: Influence of pH and Alkalinity

This chapter is written in the form of a journal article for submission to the journal Water Supply: Research and Technology. This paper will be published by co-authors Hamideh Pirouz Hamidi (student), Seyedeh Laleh Dashtban Kenari (Postdoctoral Fellow) and Dr. Onita Basu (supervisor).

Abstract

A bench-scale biofiltration study was conducted to investigate the potential benefits of adjusting water pH and alkalinity as a simple water quality control mechanism. Biofilter efficacy in terms of organic carbon removal, ammonia removal, and headloss development was assessed. Two lab-scale biofilters were tested at pH values between 6.0 and 10.0 with low and high alkalinity levels (25–50) and (180–220) mg $CaCO_3$/L. At the pH values 6.0 and 7.5, no difference was found in the total organic carbon (TOC) removal at approximately 67%. However, as the pH increased the TOC removal decreased to 31% removal at pH 10. Conversely, as the pH increased, ammonia removal increased from 13% at pH 6.0 to 93% at pH 10.0. An assessment of the available dissolved oxygen (DO) with organic carbon and ammonia concentration indicated that the available DO was a limiting factor in complete TOC and ammonia removal. Changes in alkalinity demonstrated a modest impact on biofilter activity with slightly higher ATP values observed at the higher alkalinity level but no associated impact on TOC or ammonia removal.

Overall, pH 7.5 demonstrated an optimum condition for water quality and headloss control with 68% and 48% removal in terms of TOC and ammonia removal, respectively, and with the lowest headloss development.

Key word: drinking water biofilter, pH, alkalinity, TOC removal, ammonia removal, headloss

Abbreviation: ATP, Adenosine Triphosphate, DO, Dissolved Oxygen, EPS, Extracellular polymeric substances, HL, Headloss, HLR, Hydraulic Loading Rate, NOM, Natural Organic Matter, PR, Protein, PS, Polysaccharide, TOC, Total Organic Carbon

4.1. Introduction

Natural organic matter (NOM) is a complex mixture of organic compounds found in surface water sources. It needs to be removed from surface water to reduce the production of disinfection by-products (DBPs) due to reactions with disinfectants (Azzeh et al., 2015; McKie et al., 2015). NOM can also hinder water treatment processes by increasing chlorine demand (McKie et al., 2015; Dhawan et al., 2017). Also, NOM removal can reduce the microbial regrowth in water distribution networks, thus minimize the chances of water with odor and color issues. Biofiltration is a method that can be employed to decrease NOM and is considered a sustainable treatment process that transforms and removes organic matter through biodegradation (Dhawan et al., 2017; Keithley and Kiristis, 2018). Surface water treatment has generally focused on dissolved organic carbon (DOC) removal with biofiltration performance, while ammonia removal has received little attention. However, the World Health Organization (WHO), has set 1.5 and 35 mg NH_4^+/L as the odor and taste thresholds, respectively, Health Canada (2017) recommends controlling the ammonia entering distribution networks to 0.1 mg/L ammonia–nitrogen. This will serve to minimize the

side-effects of uncontrolled nitrification in the distribution system, such as nitrite formation, which is dangerous for humans (Lájer, 2012).

Several operational design parameters have been examined for the optimization of biofiltration behavior related to organic carbon removal. In particular, operational design parameters, including filter media, empty bed contact time (EBCT), and backwashing procedures, have received attention. The most prominent outcomes indicate that granular activated carbon (GAC) outperforms anthracite under low-temperature conditions or in the presence of chlorine (Liu et al., 2001; Emelko et al., 2006). Increasing EBCT up to 20 minutes has been found to have positive impacts concerning DOC removal efficiency (Granger et al., 2014; Basu et al., 2016). Ikhlef and Basu, (2017) demonstrated that collapse pulsing improved DOC removal versus water-only backwash, but bed expansion more than 30% had harmful impacts on DOC removal. Despite the comprehensive research on organic carbon removal, scant attention has been paid to the examination of the influence of operational design parameters on ammonia removal in drinking water treatment.

Beyond the operational parameters noted, more recent studies have aimed to increase biofiltration performance by enhancing biofilter with nutrients, including nitrogen and phosphorus. The nutrient enhancement for organic matter removal has received mixed results with some researchers indicating positive findings (Lauderdale et al., 2012; Granger et al., 2014; Dhawan et al., 2017), while others reporting negligible to no changes (Azzeh et al., 2015; Fu et al., 2017; Nemani et al., 2018). Furthermore, nutrient supplementation has been found to increase microbial activity, which can be a symptom of improving the biofiltration ability to remove contaminants (Lauderdale et al., 2012; Granger et al., 2014), while no significant improvement in biological condition was observed under nutrient enhancement in other researches (McKie et al., 2015; Rahman et al., 2016;

Nemani et al., 2018). The hydraulic performance of biofiltration following nutrient supplementation has also received little attention. While Lauderdale et al., (2012) observed a 15% reduction in head loss in nutrient-enhanced biofilters, McKie et al., (2015) found no improvement in the filters' run time. The review of prior research results indicates that nutrient enhancement strategies on biofiltration efficiency have not resulted in drastically positive outcomes and that the remarkable differences in the obtained results may be due to the different water-quality parameters that were not adjusted or only reported. Among previous studies, only one study evaluated the effect of pH along with nutrient enhancement on organic matter removal (Granger et al., 2014).

This study investigated the impact of pH and alkalinity on the concurrent total organic carbon (TOC) and ammonia removal performance as well as the headloss development rate. Additional parameters were also analyzed to assess any supplementary trends on head loss or TOC removal, including EPS, adenosine triphosphate (ATP), and dissolved oxygen (DO) concentrations.

4.2. Materials and Methods

4.2.1. Synthetic Water

Two biofilters were fed with synthetic water to mimic a surface water source (Ikhlef and Basu, 2017). Stock solutions were prepared weekly in two separate tanks for carbon and nutrient enhancement and mixed with dechlorinated tap water using an inline static mixer to feed the biofilters. The carbon dosing tank consisted of glyoxal (Sigma-Aldrich, Canada, Ontario), acetic acid (Sigma-Aldrich, Canada) and formic acid (Sigma-Aldrich, Canada, Ontario) at equivalent carbon levels mixed with dechlorinated tap water to provide 10 mg TOC/L level at the filter's inlet. Ammonium sulfate (Fisher Scientific, Canada, Ontario) and potassium dihydrogen phosphate (Sigma-Aldrich, Canada, Ontario) served as the nitrogen and phosphorus source,

respectively. These nutrients were mixed with deionized (DI) water in the second tank to provide a C: N: P ratio of 25:5:1 (Ikhlef and Basu, 2017) on a w/w basis. The characteristics of the synthetic water used are presented in Table 4.1. Also, a schematic of the lab-scale experimental set-up is shown in Figure 4.1.

Table 4. 1: Synthetic water quality parameters

	Synthetic water (filter influent)	
Parameters	**Range**	**Average**
TOC (mg/L)	8.16 - 11.09	9.96
Ammonia (mg NH_3-N/L)	1.79 - 2.21	1.99
Nitrate (mg NO_3-N/L)	1.66 - 1.90	1.78
Hardness (mg $CaCO_3$/L)	48.30 - 51.30	50.21
DO (mg/L)	5.31 - 10.38	-
Temp (°C)	15 -23	18
pH	5.90 - 6.22	6.0
	7.25 – 7.70	7.5
	8.78 – 9.15	9.0
	9.80 – 10.10	10.0
Alkalinity (mg $CaCO_3$/L)	25 - 50	49
	180 - 220	200

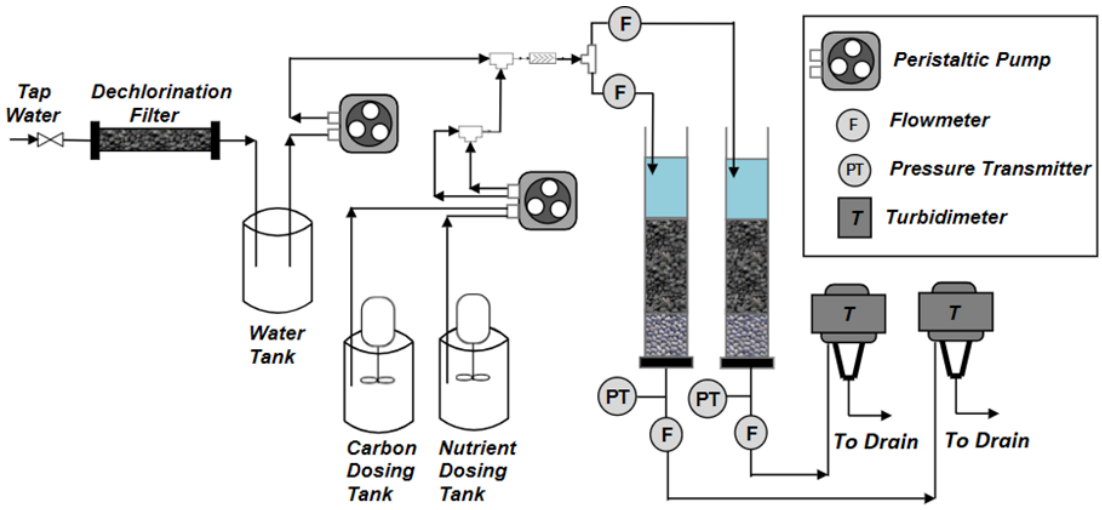

Figure 4. 1: Schematic of the experimental set-up

4.2.2. Biofilter Setup

The lab-scale biofiltration setup consisted of two identical biofilter columns, operated at a hydraulic loading rate of 3 m/h (flow rate: 100 mL/min), providing an EBCT of 14 minutes. The biofilters were run under identical conditions to ensure the reproducibility of results. The biofilter columns were constructed of plexiglass pipes with of 1250 mm length and 50 mm internal diameter. Both columns were filled up to 520 mm with exhausted GAC (effective size d = 0.7 mm) over 180 mm of sand (effective size d = 0.5 mm) and consisted of an overflow located at the height of 1060 mm, followed by a synthetic underdrain material.

4.2.3. Experimental Design

The two dual media biological filters were operated parallelly for several months before initiating the experiments. The phase I of the study (acclimation phase) lasted for 6 months until steady-state conditions of consistent TOC removal were reached. At the beginning of phase I, both biofilters were inoculated with water from Ottawa River and backwash water from the Ottawa Britannia water treatment plant full-scale filters to promote bacterial attachment and growth on the media. TOC removal was measured during this period to determine when steady state biological acclimation had occurred. Once a paired t-test analysis of the TOC removal showed no statistical differences ($p > 0.05$), phase II was started. In phase II (experimental phase), the filters were operated for 12–13 days following the pH or alkalinity adjustments before sampling. In this phase, the performance of biofilters was tested at different pH values of 6.0, 7.5, 9.0, and 10.0 and two alkalinity levels, low alkalinity level ranging between 25 and 50 mg $CaCO_3$/L and high alkalinity between 180 and 220 mg $CaCO_3$/L. In the study phase, each experiment lasted for 17–21 days, during which TOC and DO were measured 5 times per week. In addition, ammonium and alkalinity

were measured 3 times per week. Also, before each backwash and 24 hours after each backwash, the GAC media were collected for ATP and EPS tests. At a high alkalinity level of pH 6.0, nitrate removal was also measured 3 times per week. Sodium hydroxide (Fisher Scientific, Canada, Ontario), hydrochloric acid (Fisher Scientific, Canada, Ontario), and sodium bicarbonate (Arm and Hammer) solutions were added to adjust the pH and alkalinity of the synthetic water, as needed. Both filters were backwashed twice a week with their unchlorinated effluent. The backwash procedure consisted of a slow wash with air scour for 6 minutes (water velocity = 12 m/hr and air velocity = 35 m/hr), followed by 4 minutes of high-rate water wash, which ensured 30% bed expansion (water velocity = 30 m/hr), and finally a low-rate water wash at 10% bed expansion for 2 minutes (velocity=10 m/hr) (Dhawan et al., 2017).

4.2.4. Analytical Methods

TOC was measured using the Persulfate Ultraviolet Oxidation method following Standard Methods 5310D (APHA, 2012) with Shimadzu TOC-VCPH/CPN total organic carbon analyzer (Shimadzu, Canada). Filter influent and effluent samples were collected and tested daily. The samples were preserved with 3 drops of phosphoric acid (purity: 85–88%) (Sigma-Aldrich, Canada, Ontario) to maintain the pH at less than 2.0. The nitrogen–ammonia concentration was measured using the salicylate method (#10023) with HACH DR 2800 spectrophotometer. In addition, Hach method #10206 (TNT 835) was used for measuring nitrate concentration. The alkalinity of water was estimated with Hach method #10239 (TNT 870), following the manufacturer's instruction.

The biological activity of the biofilters, indicated by ATP, was assessed by collecting 1 g of filter media and using a Deposit Surface Analysis kit (LuminUltra Technologies Ltd, NB). The test was

conducted based on the manufacturer's instruction that includes calibration, sample preparation, and total ATP analysis. Extracellular polymeric substances (EPS) concentration was determined by the quantification of proteins and polysaccharides. For EPS extraction, 10 mL of Tris-EDTA buffer (10 mM Tris, 10 mM EDTA) was added to 2 g of filter media, shaken at 300 rpm at 4° C for 4 hr, and centrifuged at 12000 g for 15 minutes (Azzeh et al., 2015). The supernatant was pipetted and filtered using a 0.45-µm syringe filter and stored at -20° C if the analysis was not conducted immediately. Protein was quantified according to Pierce™ BCA (Thermo Scientific) instruction using a CE3055 single Beam Cecil UV/visible spectrophotometer (Cambridge, England). Polysaccharide quantification was conducted based on the method described by Azzeh et al., (2015), employing a Hach Odyssey DR 2500 Scanning spectrophotometer.

For each biofilter, the effluent turbidity and pressure change were monitored and recorded continuously by two inline low range turbidimeters (Hach SC 200) and pressure transmitters (Wika A-10), respectively. Temperature and DO were recorded by Thermo Scientific ORION 087003 probe.

4.3. Results and Discussion

4.3.1. Impacts of Water pH and Alkalinity on TOC Removal in Biofilters

Table 4.2 shows the TOC removal performance under different water pH levels at two low and high alkalinity values. Overall, the TOC removal secured in this study was significantly higher than that of previous studies (Lauderdale et al., 2012; Granger et al., 2014; Azzeh et al., 2015; Nemani et al., 2018). This difference is attributed to the presence of readily biodegradable organic carbon in the synthetic water with an ideal nutrient (N and P) ratio. As shown in Table 4.2, pH is an influential factor in TOC removal in biofilters (Rittman and McCarty, 2001).

Table 4. 2: TOC removal efficiency (ave ± std dev) over study phase, average influent TOC: 9.96 mg/L		
Experimental condition	**TOC removal efficiency %**	**Number of samples**
pH=6.0, low alk.	67.1 ± 4.9	42
pH=6.0, high alk.	62.5 ± 5.2	24
pH=7.5, low alk.	69.2 ± 3.2	32
pH=7.5, high alk.	66.7 ± 2.0	28
pH=9.0, low alk.	63.4 ± 5.3	24
pH=9.0, high alk.	53.2 ± 5.8	24
pH=10.0, low alk.	55.9 ± 5.3	24
pH=10.0, high alk.	31.4 ± 10.0	22
low alk.: 25 - 50 mg $CaCO_3$/L high alk.: 180 - 220 mg $CaCO_3$/L		

In the current study, between pH of 6.0 and 7.5, the TOC removal was found to be similar and ranged between 63 and 69% at both alkalinity levels. Likewise, Jeong et al., (2017) observed only 2% improvement in TOC removal by increasing the pH from 6.5 to 7.5. However, a further increment in pH to 9.0 and 10.0 significantly reduced the TOC removal to 53% and 31%, respectively. Moll and Summers, (1999) reported that carboxylic and amino acids were better utilized in a neutral or acidic pH environment, while microorganisms used carbohydrates better in filters operated at a pH value of 7.5. In this study, the carbon mixture was primarily composed of carboxylic functional groups; thus, the decrease in performance is partially attributed to this factor.

Interestingly, higher alkalinity levels resulted in lower TOC removal in all cases, and the differences between TOC removal efficiencies at two alkalinity level increased as pH increased, with a maximum change of 25% observed at a pH of 10.0. The adverse impact of high alkalinity on TOC removal was likely due to the oxygen-scavenging effect of carbonate ions that remove oxygen or prevent oxidation (Zanacic et al., 2016).

4.3.2. Impacts of Water pH and Alkalinity on Ammonia Removal in Biofilters

Table 4.3 shows the ammonia removal performance under different pH levels and the two test alkalinity levels. As can be observed in the table, increasing pH from 6.0 to 10.0 increased ammonia removal from 13% to 93%.

Table 4. 3: Ammonia removal efficiency (ave ± std dev) over study phase, average influent ammonia: 1.99 mg NH_3-N/L

Experimental condition	Ammonia removal efficiency %	Number of samples
pH=6.0, low alk.	13.3 ± 5.6	16
pH=6.0, high alk.	13.4 ± 4.5	18
pH=7.5, low alk.	44.5 ± 4.8	10
pH=7.5, high alk.	53.0 ± 5.2	10
pH=9.0, low alk.	75.6 ± 4.2	12
pH=9.0, high alk.	91.1 ± 7.9	12
pH=10.0, high alk.	92.5 ± 6.2	12
low alk.: 25 - 50 mg $CaCO_3$/L high alk.: 180 - 220 mg $CaCO_3$/L		

The results show that ammonia removal at pH 6.0 was significantly lower than the other experiments. At pH 6.0 (pH < 9.25), the shifting of the ammonia acid-base equilibrium toward NH_4^+ (Eq. 4.1) (O'Farrell, 1975; Lájer, 2012) suggests that nitrification is most probably the main pathway for ammonia removal.

$$\mathbf{NH_4^+ + OH^- \leftrightarrow NH_4OH \leftrightarrow NH_3 + H_2O} \qquad \mathbf{(4.1)}$$

In the nitrification process attributed to H^+ production, the pH value was expected to reduce. However, as experiments were conducted at pH 6.0 in this study, the effluent pH increase indicated inadequate or incomplete nitrification at this pH value.

The overall reaction for the complete nitrification process (Eq. 4.2) represents that the nitrification process consumes 4.57 mg O_2/mg NH_4^+-N and 7.14 mg $CaCO_3$/mg NH_4^+-N (Metcalf and Eddy, 2013).

$$\mathbf{NH_4^+ + 2\ O_2 + 2\ HCO_3^- \rightarrow NO_3^- + 3\ H_2O + 2\ CO_2} \qquad \mathbf{(4.2)}$$

In the current study, the required alkalinity for complete nitrification (14.21 mg $CaCO_3$/L) was lower than the alkalinity available in all conditions tested, and thus alkalinity was not the limiting factor in ammonia removal. According to Eq. 4.2, available oxygen can also be a limiting factor in securing complete ammonia removal through the nitrification. Despite the existing aerobic conditions in drinking water biofilters, the growth rate of nitrifying bacteria would be affected by the reduction of oxygen concentration to below 2 mg/L (Knowles et al., 1965). Moreover, the microorganism activity in the outer layer of the biofilm and inhibition of DO diffusion into the inner layer would result in creating anaerobic conditions in drinking water biofilters (Yu et al., 2007; Cai et al., 2014). Therefore, non-nitrifying mechanisms such as anaerobic ammonia oxidation (Anammox) may also be responsible for ammonia removal (Yu et al., 2007; Cai et al., 2014). However, inadequate ammonia removal at pH 6.0 shows that a low pH value has a higher detrimental effect on ammonia removal (Villaverde et al., 1997) than alkalinity and oxygen limitation.

By increasing the pH to 7.5 and 9.0 at both low and high alkalinity levels, high ammonia removal was observed. In addition, at these influent pH levels, the effluent pH was observed to decrease (upward of 1.5 pH units), which emphasized that nitrification was the main ammonia removal pathway in this pH range. However, by increasing the pH to 10.0, no noticeable change in effluent pH occurred (a minimal decrease of 0.2 pH units), indicating a shift in the ammonia removal process from nitrification to ammonia volatilization and air stripping. In particular, it is well established that shifting the equilibrium in favor of NH_3 (Eq. 4.1) (at pH > 9.25) results in ammonia stripping at such high pH conditions (Lájer, 2012).

A detailed analysis of the alkalinity levels in relation to ammonia removal was carried out at a high alkalinity level and pH 6.0 to demonstrate how nitrification consumed alkalinity and how a change in alkalinity was observed in this study (Table 4.4). The theoretical reaction of nitrification (Eq. 4.2) shows that approximately 7.14 mg of alkalinity is consumed for every mg of ammonia converted to nitrate; thus, a decrease in alkalinity is expected if nitrification occurs. However, as indicated in Table 4.4, an increase in alkalinity occurred by a maximum change of 23 mg/L in the effluent of biofilters. Van Rijn et al., (2006) have demonstrated that the alkalinity consumed in the nitrification stage is regained in the denitrification stage (i.e., conversion to carbon dioxide) at a rate of 3.57 mg $CaCO_3$ per mg of nitrate. Moreover, the minor differences ($p > 0.05$) between the actual and calculated alkalinity changes observed, as shown in Table 4.4, indicate that denitrification had occurred in the system. Denitrification also consumes organic carbon through a biochemical reaction process (Eq. 4.3, 4.4). Thus, in the current study, a portion of organic carbon was consumed to complete the denitrification process.

$$\mathbf{CH_2O_2 + NO_3^- \rightarrow NO_2^- + CO_2 + H_2O} \qquad \mathbf{(4.3)}$$

$$3\ CH_2O_2 + 2\ NO_2^- + ½\ O_2 \rightarrow 3\ CO_2 + N_2 + H_2O + 4\ OH^- \quad (4.4)$$

Table 4. 4: Analysis of alkalinity levels with nitrogen removal at pH 6.0 and high alkalinity level. Average influent TOC: 9.96 ± 0.39 mg/L and ammonia: 1.99 ± 0.05 mg NH_3-N/L.

Time	Experimental			Calculated		
	Removed ammonia (mg NH_3-N/L)	Removed nitrate (mg NO_3-N/L)	Alkalinity changes (mg $CaCO_3$/L)	Alk. decrease (mg $CaCO_3$/L) (nitrification)	Alk. increase (mg $CaCO_3$/L) (denitrification)	Alkalinity changes (mg $CaCO_3$/L)
Day#1	0.35	5.36	+ 23	2.50	19.14	+ 16.64
Day#3	0.32	2.93	+ 2	2.28	10.46	+ 8.18
Day#5	0.16	5.58	+ 19	1.14	19.93	+ 18.79
Day#8	0.17	4.76	+ 16	1.21	17.00	+ 15.79
Day#12	0.18	2.35	+ 15	1.29	8.38	+ 7.09
Day#15	0.16	1.73	+ 9	1.14	6.17	+ 5.03
Day#17	0.20	1.28	+ 8	1.43	4.59	+ 3.16

4.3.3. Impact of Dissolved Oxygen on Carbon and Ammonia Removal

To determine the consumed oxygen for substrate removal in theoretical terms, during aerobic heterotrophic biodegradation, the relationship between the consumed oxygen and removed organic carbon was examined. In the current study, organic carbon is represented as CH_2O_2 (formic acid), CH_3COOH (acetic acid), and $C_2H_2O_2$ (glyoxal), and new cell material is represented as $C_5H_7NO_2$ (Metcalf and Eddy, 2013). Thus, biological oxidation equations can be written as follows:

$$6\ CH_2O_2 + 2\ O_2 + 0.2\ NH_3 \rightarrow 0.2\ C_5H_7NO_2 + 5\ CO_2 + 5.6\ H_2O \quad (4.5)$$

$$3\ CH_3COOH + O_2 + NH_3 \rightarrow C_5H_7NO_2 + CO_2 + 4\ H_2O \quad (4.6)$$

$$4\ C_2H_2O_2 + O_2 + NH_3 \rightarrow C_5H_7NO_2 + 3\ CO_2 + 2\ H_2O \quad (4.7)$$

Furthermore, the balanced stoichiometric reaction for the oxidation of each carbon source used in this examination can be written as follows:

$$\mathbf{CH_2O_2 + 1/2\ O_2 \rightarrow CO_2 + H_2O} \quad \textbf{(4.8)}$$

$$\mathbf{CH_3COOH + 2\ O_2 \rightarrow 2\ CO_2 + 2\ H_2O} \quad \textbf{(4.9)}$$

$$\mathbf{C_2H_2O_2 + 3/2\ O_2 \rightarrow 2\ CO_2 + H_2O} \quad \textbf{(4.10)}$$

Using these generalized chemical formulas for organic carbon, the theoretical oxygen demand for complete organic carbon removal is estimated at 3.31 mg O_2 (0.332 mg O_2/mg C) (Metcalf and Eddy, 2013). Furthermore, as discussed in section 4.3.2, based on Eq. 4.2, each mg of NH_4^+-N consumes 4.57 mg O_2 for complete oxidation to nitrate through nitrification. Thus, in this study, 12.40 mg O_2/L is required to secure complete TOC and ammonia removal, 9.96 mg C/L and 1.99 mg NH_3-N/L, respectively. Therefore, the available oxygen in all experiments illustrates that it is impossible to reach 100% removal. The calculated oxygen demand for removed TOC and ammonia at each pH level, as well as experimentally removed oxygen, is demonstrated in Table 4.5. At pH 6.0 and 7.5, the calculated required oxygen for TOC and ammonia removal was always less than the actual oxygen removed during the experiments (range of 30 to approx. 50%). This observation shows that other competing reactions occurred that simultaneously required dissolved oxygen. On the other hand, at pH 9.0 and 10.0, experimentally removed oxygen was always less than the calculated amount for the biological oxidation of the observed removed organic carbon and ammonia. This finding helps to understand that at pH $\geq$ 9.0, the removal mechanism of pollutants, especially ammonia, likely changes from nitrification to ammonia volatilization. Figure 4.2 also demonstrates that effluent DO in most cases tested at pH 6.0 and 7.5 are near zero. The impact of dissolved oxygen in surface water biofiltration is somewhat understudied, and the results reported here suggest that more research should be undertaken in this area of the field.

Table 4. 5: Calculated oxygen demand for carbon removal and nitrification versus experimental DO consumption						
Experimental condition	**TOC removal (mg/L)**	**Calculated O_2 demand for carbon removal (mg/L)**	**NH_3 removal (mg NH_3-N/L)**	**Calculated O_2 demand for nitrification (mg/L)**	**Total calculated O_2 demand (mg/L)**	**Experimental DO removal (mg/L)**
pH=6.0, LA	6.35	2.11	0.25	1.14	3.25	6.55
pH=6.0, HA	5.85	1.94	0.25	1.14	3.08	6.78
pH=7.5, LA	6.48	2.15	0.84	3.84	5.99	7.48
pH=7.5, HA	6.28	2.08	1.04	4.75	6.83	9.13
pH=9.0, LA	6.27	2.08	1.61	7.36	9.44	7.74
pH=9.0, HA	4.92	1.63	1.74	7.95	9.58	7.70
pH=10.0, LA	4.83	1.60	N.A.	N.A.	N.A.	4.50
pH=10.0, HA	3.10	1.03	1.81	8.27	9.30	6.65
LA: low alk.: 25 - 50 mg $CaCO_3$/L HA: high alk.: 180 - 220 mg $CaCO_3$/L						

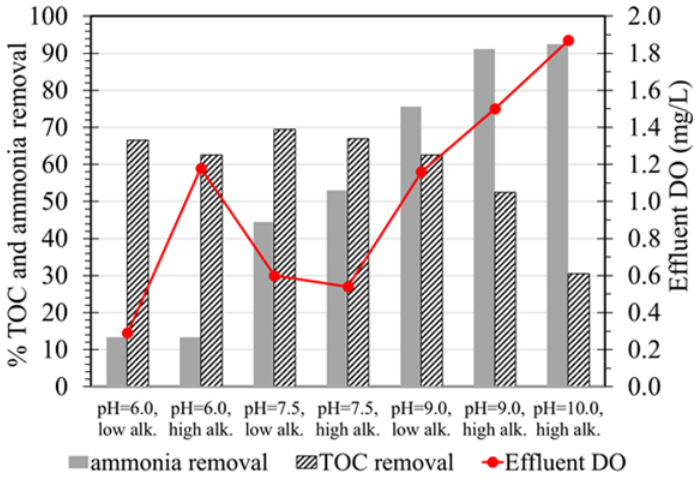

Figure 4. 2: Average TOC and ammonia removal efficiency over study phase versus effluent DO, average influent TOC: 9.96 mg/L, ammonia: 1.99 mg NH_3-N/L, HLR: 3 m/h, low and high alkalinity level: 25 - 50 and 180 - 220 mg $CaCO_3$/L, respectively

4.3.4. Biological Characterization and Headloss Development

EPS, which is primarily formed by polysaccharides and proteins along with microbial cells, composes the biofilm that supports the microbial species that grow and develop on the filter media surface. EPS has both beneficial and detrimental effects on the operation of a biofilter. For instance, the EPS protects bacteria from unexpected adverse conditions such as swings in pH or exposure to oxidants. On the other hand, EPS has been postulated as the primary contributor to biofilter clogging and headloss development (Xia et al., 2016). As EPS has been proposed to play an essential role in biofilter headloss development, the investigation into the role of pH and alkalinity on EPS formation may help to improve the hydraulic performance of biofilters. The hydraulic performance of filters can be enhanced by reducing the headloss development rate, which impacts filter run time and backwash requirements.

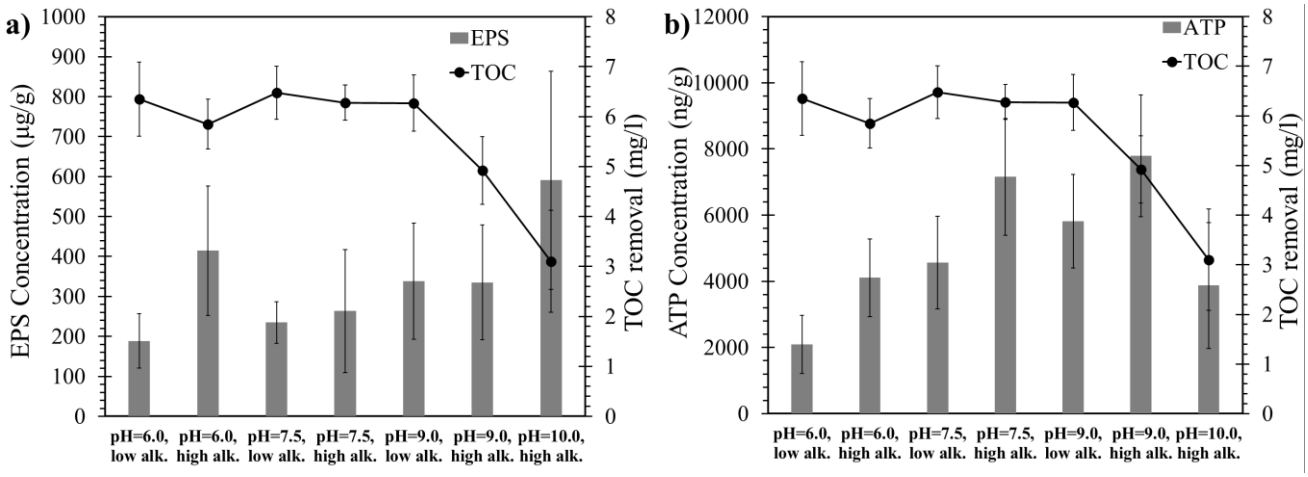

Figure 4. 3: Correlation between removed TOC and a) EPS concentration b) ATP concentration, under different conditions. Average influent TOC: 9.96 mg/L, HLR: 3 m/h

The EPS results presented in Table 4.6 and Figure 4.3 show that by increasing pH, EPS production increases and reaches the highest value at pH 10.0 after a relatively consistent period of measured EPS values (235–338 μg/g) over pH 7.5 to 9.0. Also, it is observed that at pH 7.5 and 9.0, EPS production is not significantly affected by alkalinity change. The observed differences in EPS values at pH 6.0 and low/high alkalinity can be attributed to other parameters such as oxygen availability or temperature.

ATP, which is often described as the "energy currency" of living cells, is considered a useful indicator of microbial activity (Hammes et al., 2010). Having an efficient biofilter will be beneficial to promote biological activity without the overproduction of EPS. Figure 4.3b illustrates that at each constant pH, higher alkalinity is observed to correlate to a higher microbial activity indicated by ATP. Also, microbial activity increased at pH ranging from 6.0 to 9.0 with no correlation with TOC removal. However, the observed decline in ATP at pH 10.0 (Figure 4.3b) may be due to carbon and other nutrient deficiency caused by chemical sequestrations (McKie et al., 2015) or ammonia volatilization. Meanwhile, nutrient inadequacy can also increase the EPS concentration at pH 10.0 (Lauderdale et al., 2012), as observed in Figure 4.3a. On the other hand, during EPS extraction, as cell lysis may occur at pH 10.0 (McSwain et al., 2005), EPS concentration increases at this pH value and misleads the attempts to find the relationship between EPS and ATP.

Overall, Figure 4.4 shows that headloss development does not have any consistent trend over pH and alkalinity changes. However, the highest and the lowest headloss rate at pH 6.0 and 10.0, respectively, correspond to the highest and lowest TOC removal (Figure 4.4b), no direct correlation is found between headloss development and microbial activity. Although headloss

development rate is increased by increasing EPS concentration at pH ranging from 7.5 to 9.0, opposing trend between them at pH 6.0 and 10.0 indicates that headloss improvement would not

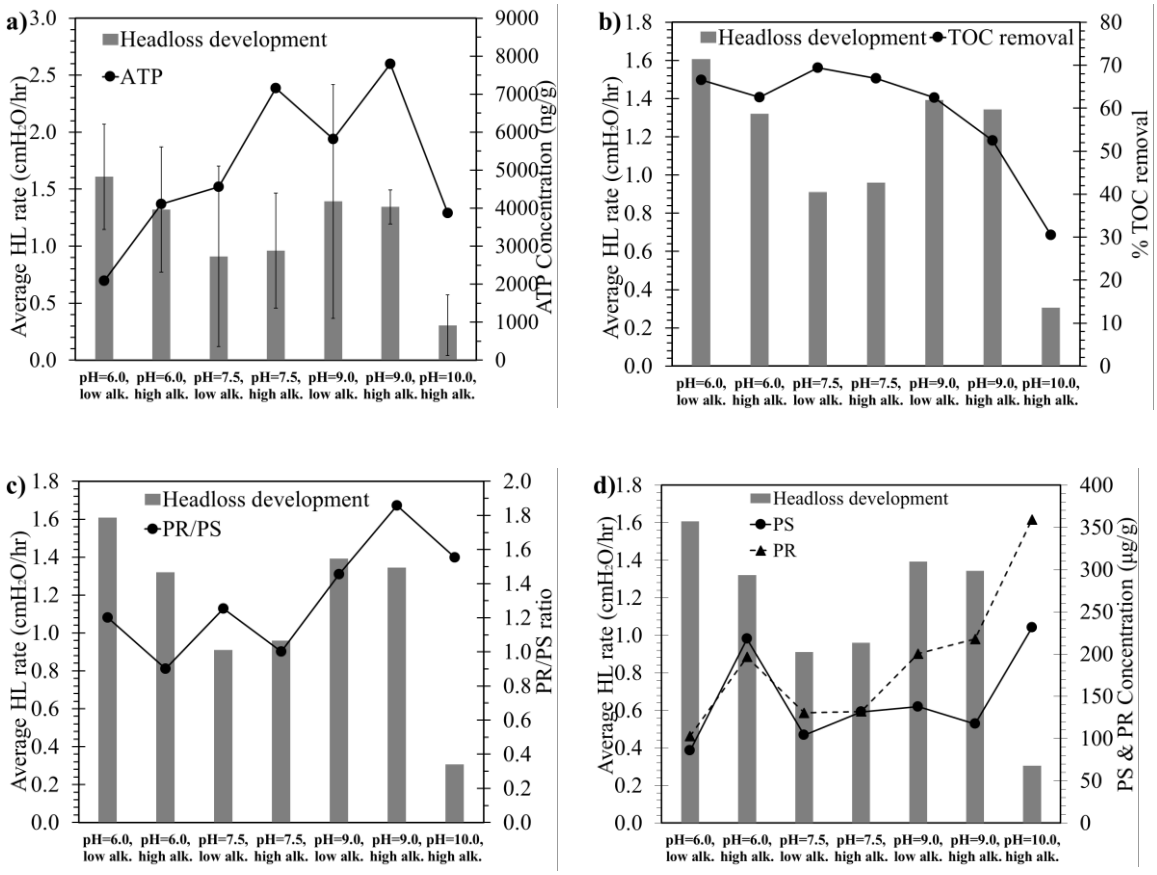

Figure 4. 4: Headloss development rate correlation with a) ATP b) TOC, c) PR/PS ratio and d) PR and PS concentration, HL: headloss, PS: polysaccharide, and PR: protein, average influent TOC: 9.96 mg/L, and low and high alkalinity level: 25 - 50 and 180 - 220 mg $CaCO_3$/L, respectively

be necessarily achieved by controlling EPS production (Xia et al., 2016). Meanwhile, Wang et al., (2012) suggested that due to existing a complex interaction between pH and EPS traits with regards to cell size, density and EPS flocculation ability a high pH does not necessarily exert higher headloss. Moreover, as discussed earlier, high pH and heat treatment prompts critical cell lysis and produce more protein and polysaccharide (McSwain et al., 2005). As cell lysis was not measured

in this study, overestimated EPS values at high pH may cause no to the weak correlation between EPS with ATP and headloss.

Table 4. 6: EPS and ATP concentration and headloss development rate (ave ± std dev) over the different experimental condition

Experimental condition	EPS concentration (µg/g)	ATP concentration (ng/g)	Headloss development cmH_2O/hr
pH=6.0, low alk.	188.7 ± 67.9	2089 ± 877	1.61 ± 0.46
pH=6.0, high alk.	414.6 ± 162.2	4106 ± 1179	1.32 ± 0.55
pH=7.5, low alk.	234.6 ± 52.3	4565 ± 1398	0.91 ± 0.79
pH=7.5, high alk.	263.6 ± 154.4	7159 ± 1763	0.96 ± 0.50
pH=9.0, low alk.	338.2 ± 145.9	5816 ± 1418	1.39 ± 1.02
pH=9.0, high alk.	335.2 ± 143.8	7793 ± 1838	1.34 ± 0.15
pH=10.0, high alk.	590.5 ± 273.4	3873 ± 1904	0.31 ± 0.27
low alk.: 25 - 50 mg $CaCO_3$/L high alk.: 180 - 220 mg $CaCO_3$/L			

4.4. Conclusion

The influence of pH and alkalinity adjustment on water quality, headloss build-up, and biological activity in drinking water biofilters has been studied.

- A pH of 7.5 at both alkalinity levels is the optimal condition for biofiltration systems in terms of TOC and ammonia removal with the lowest headloss development rate.
- The changes in alkalinity demonstrate a modest impact on biofilter activity with slightly higher ATP values observed at the higher alkalinity level but no associated impact on TOC or ammonia removal.
- TOC removal efficiency decreases significantly by increasing pH from 67% removal at pH 6.0 and 7.5 to 31% at pH 10.

- Ammonia removal efficiency is most impacted by changing pH levels; with removal levels ranging from 13% to 93%; however, with respect to microbially mediated removal the preferred pH was approximately pH 7.5 at approximately 45% removal.
- No direct correlation between ATP, EPS, or DO and TOC removal is observed.

Acknowledgments

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4.5. References

1. Azzeh, J., Taylor-Edmonds, L. and Andrew, R.C. 2015 Engineered biofiltration for ultrafiltration fouling mitigation and disinfection by-product precursor control. Water Science & Technology. 15 (1): 124-133.
2. Basu. O.D., Dhawan, S., and Black. K. 2016 Application of biofiltration in drinking water treatment – a review, Journal of Chemical Technology & Biotechnology, 91 (3): 585-595
3. Cai, Y., Li, D., Liang, Y., Zeng, H. and Zhang, J. 2014 Autotrophic nitrogen removal process in a potable water treatment biofilter that simultaneously removes Mn and NH_4^+-N, Bioresource Technology (172) 226–231
4. Dhawan, S., Basu. O.D. and Banihashemi, B. 2017 Influence of nutrient supplementation on DOC removal in drinking water biofilters, Water Science and Technology-Water Supply, 17(2):422-432

5. Emelko, M. B., Huck, P. M., Coffey, B. M., and Smith, E. F. 2006 Effects of media, backwash, and temperature on full-scale biological filtration. Journal of American Water Works Association, 98 (12): 61-73
6. Fu, J., Lee, W., Coleman, C., Meyer, M., Carter, J., Nowack, K. and Huang, C. 2017 Pilot investigation of two-stage biofiltration for removal of natural organic matter in drinking water treatment, Chemosphere (166) 311-322
7. Granger H. C., Stoddart A. K. and Gagnon G.A. 2014 Direct Biofiltration for Manganese removal from Surface Water, J. Environ. Eng., 140(4): 04014006
8. Hammes, F., Goldschmidt, F., Vital, M. and Wang, Y. 2010 Measurement and interpretation of microbial adenosine tri-phosphate (ATP) in aquatic environments, Water Research 44: 3915-3923
9. Health Canada. 2017 Guidelines for Canadian Drinking Water Quality - Summary Table. Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario
10. Ikhlef, S., and Basu, O.D. 2017 Influence of backwash regime on biofilter performance in drinking water treatment, Journal of Chemical Technology and Biotechnology, 92(7): 1777+
11. Jeong, S., Cho,K., Jeong, D., Lee, S., Leiknes, T., Vigneswaran, S. and Bae, H. 2017 Effect of engineered environment on microbial community structure in biofilter and biofilm on reverse osmosis membrane. Water Research 124: 227-237.
12. Keithley, S.E. and Kirisits, M. 2018 An improved protocol for extracting extracellular polymeric substances from granular filter media, Water Research (129): 419-427
13. Knowles, G., Downing, A.L. and Barrett, M.J. 1965 Determination of kinetic constants for nitrifying bacteria in mixed culture, with the aid of an electronic computer, Journal of general Microbiology (38) 268-278

14. Lájer, K. 2012 Ammonium removal by nitrification in drinking water treatment. Journal American Water Works Association 10: 47-53
15. Lauderdale, C., Chadik, P., Kirisits, M. and Brown, J. 2012 Engineered biofiltration: enhanced biofilter performance through nutrient and peroxide addition. Journal American Water Works Association, 104(73): 289-309
16. Liu, X., Huck, P. M., and Slawson R. M. 2001 Factors affecting drinking water biofiltration. Journal American Water Works Association, 93(12): 90-101
17. McKie, M.J., Taylor-Edmonds, L., Andrew, S.A., Andrew, R.C. 2015 Engineered biofiltration for the removal of disinfection byproduct precursors and genotoxicity, Water Research 81: 196-207
18. McSwain, B. S., Irvine, R. L., Hausner, M. and Wilderer, P. A. 2005 Composition and Distribution of Extracellular Polymeric Substances in Aerobic Flocs and Granular Sludge, Applied and Environmental Microbiology, 1051–1057
19. Metcalf, and Eddy, Fifth Edition, 2013 Wastewater engineering: Treatment and resource recovery
20. Moll, D.M., Summers, R.S. 1999 Assessment of drinking water filter microbial communities using taxonomic and metabolic profiles, Water Sci. Technol. 39, 8349
21. Nemani, V.A., McKie, M.J., Taylor-Edmonds, L. and Andrews R.C. 2018 Impact of biofilter operation on microbial community structure and performance, Journal of Water Process Engineering (24) 35–41
22. O'Farrell, T.P., Frauson, F.P., Cassel A.F. and Bishop D.F. 1972 Nitrogen Removal by Ammonia Stripping, Water Pollution Control Federation, 44 (8): 1527-1535

23. Rahman, I., Van Dyke, M.I., Anderson, W.B., Jin, X., Ndiongue, S. and Huck, P.M. 2016 Effect of phosphorus addition on biofiltration pre-treatment to reduce ultrafiltration membrane fouling, Desalination and Water Treatment 57: 25057–25069
24. Rittmann, B.E. and McCarty, P.L. 2001 Environmental biotechnology: Principles and applications, McGraw-Hill Series in Water Resources and Environmental Engineering
25. Van Rijn, J., Tal, Y., Schreier, H.J. 2006 Denitrification in recirculating systems: theory and applications. Aquacultural Engineering 34 (3): 364-376
26. Villaverde, S., Garcia-encina, P.A., and Fdz-Polanco F. 1997 Influence of pH over nitrifying biofilm activity in submerged biofilters. Water Research 31(5):1180-1186
27. Wang, L., Wang, L.F., Ren, X., Ye, X., Li, W., Yuan, S., Sun, M., Sheng, G., Yu, H., and Wang, X. 2012 pH Dependence of Structure and Surface Properties of Microbial EPS, Environmental Science and Technology, 46: 737−744
28. World Health Organization, 2003 Ammonia in drinking water, Background document for development of WHO Guidelines for Drinking-water Quality, WHO/SDE/WSH/03.04/01
29. Xia, L., Zheng, X., Shao, H., Xin, J., Sun, Z. and Wang, L. 2016 Effects of bacterial cells and two types of extracellular polymers on bioclogging of sand columns, Journal of Hydrology 535: 293–300
30. Yu, X., Qi, Z., Zhang, X., Yu, P., Liu, B., Zhang, L. and Fu, L. 2007 Nitrogen loss and oxygen paradox in full-scale biofiltration for drinking water treatment, water research (41) 1455 – 1464
31. Zanacic, E., Stavrinides, J., and McMartin. D. 2016 Field-analysis of potable water quality and ozone efficiency in ozone assisted biological filtration systems for surface water treatment, Water Research 104: 397- 407

5. Conclusion

A bench-scale biofiltration study was conducted to evaluate the potential benefits and impacts of adjusting water pH and alkalinity to improve biofiltration efficiency in terms of organic carbon, ammonia removal, and headloss build-up. Additional parameters, such as EPS, ATP, and DO concentrations, were also examined to identify any possible correlation between these parameters and TOC removal or headloss development.

The study was conducted using two parallel dual media plexiglass columns with 50 mm internal diameter operated at a C: N: P ratio of 25: 5: 1 with an EBCT of 14 minutes. The two biofilters were tested at 4 pH conditions ranging between 6.0 and 10.0 with low and high alkalinity levels, 25–50 and 180–220 mg $CaCO_3$/L, respectively.

The primary outcomes of this research study are as follows:

- Increasing pH in the range of 6.0 to 10.0 has an adverse effect on TOC removal in biological filters. TOC removal efficiency decreases significantly from 67% and 63% at low and high alkalinity levels of pH 6.0 to 55% and 31% at respective alkalinity levels at pH 10.0.
- Increasing pH from 6.0 to 10.0 improves ammonia removal significantly from 13% at pH 6.0. to 93% at pH 10.0. The possible method for ammonia removal at tested pH less than 9.0 is nitrification. By increasing pH and shifting the ammonium–ammonia equilibrium in favor of NH_3, the ammonia removal mechanism changes from nitrification to ammonia volatilization.
- The increase of alkalinity in biofilter effluent is the symptom of denitrification, which also causes some extra organic carbon removal.

- Increasing pH from 6.0 to 10.0 results in the highest EPS concentrations at pH 10.0. On the other hand, the highest and the lowest headloss rates were observed at pH 6.0 and pH 10.0, respectively. Therefore, according to this study, EPS is not the principal constituent of headloss development, and other parameters are also responsible for the clogging in biofilters.
- At each respective pH, the higher alkalinity appears to correlate to higher microbial activity (ATP) but not headloss development.
- A direct correlation between ATP, EPS, or DO and TOC removal is not observed.

In conclusion, for the investigation into the influence of pH and alkalinity adjustment on the efficiency of biofilters tested at pH 6.0, 7.5, 9.0, and 10.0, pH 7.5 showed an optimum condition in terms of TOC and ammonia removal with 68% and 48% efficiency, respectively, and with the lowest headloss development..

5.1 Future Work

The results of this study warrant further investigation as follows:

- As the focus of this work is investigating the effect of water-quality parameters on biofiltration performance, further investigation to combine pH adjustment along with the pre-oxidation of organic matter via ozone or hydrogen peroxide is suggested.
- In this study, the biofilters are fed by synthetic water. To investigate the coincident effect of water quality and carbon source, using feeding columns with enhanced raw water or different carbon sources is suggested.

- Due to low influent turbidity, the evaluation of the effect of influent pH on turbidity removal is not practical. Further studies are required to assess the overall performance of pH and alkalinity adjustment on biofiltration performance in high turbid water for turbidity removal.
- Although any correlation between TOC removal and DO has not been observed in this study, it is helpful to provide the constant DO condition in biofilter influent to assess the pH influence on biofiltration performance regardless of initial DO concentration.
- Cell lysis measurement is recommended to secure a comprehensive assessment of the influence of pH and alkalinity adjustment on biological characteristics and find the possible correlation between EPS concentration and ATP values or headloss development rate.

6. Reference

1. Allison, S.M. and Prosser, J.I. 1993 Ammonia oxidation at low pH by attached populations of nitrifying bacteria. Soil Biology and Biochemistry 25: 935-941
2. Andersson, A., Laurent, P., Kihn, A., Prévost, M. and Servais, P. 2001 Impact of temperature on nitrification in biological activated carbon (BAC) filters used for drinking water treatment, Water research 35 (12) 2923-2934
3. Azzeh, J., Taylor-Edmonds, L. and Andrew, R.C. 2015 Engineered biofiltration for ultrafiltration fouling mitigation and disinfection by-product precursor control. Water Science & Technology. 15 (1): 124-133
4. Balsher, S. S. 2016 Pre-Oxidation Strategies for Improvement of Biofiltration Performance, Master's thesis submitted to the Faculty of the Graduate School of the University of Toronto
5. Basu. O.D., Dhawan, S., and Black. K. 2016 Application of biofiltration in drinking water treatment – a review, Journal of Chemical Technology & Biotechnology, 91 (3): 585-595
6. Cai, Y., Li, D., Liang, Y., Zeng, H. and Zhang, J. 2014 Autotrophic nitrogen removal process in a potable water treatment biofilter that simultaneously removes Mn and NH_4^+-N, Bioresource Technology (172) 226–231
7. Camel. V. and Bermond, M.A. 1998 The use of ozone and associated oxidation processes in drinking water treatment, Water Research 32 (11): 3208-3222
8. Chaudhary, D. S., Vigneswaran, S., Ngo, H. H., Shim, W. G., and Moon, H. 2003 Biofilter in water and wastewater treatment. Korean Journal of Chemical Engineering, 20 (6): 1054-1065

9. Chen, S., Ling, J., Blancheton, J.-P., 2006. Nitrification kinetics of biofilm as affected by water quality factors. Aquacultural Engineering 34, 179–197.
10. Colt, J. 2006 Water quality requirements for reuse systems. Aquacultural Eng. 34: 143–156
11. Delgadillo-Mirquez, L. Lopes, F., Taidi, B., and Pareau. D. 2016 Nitrogen and phosphate removal from wastewater with a mixed microalgae and bacteria culture, Biotechnology Reports 11: 18–26
12. Delatolla, R., Séguin, C., Springthorpe, S., Gorman, E., Campbell, A. and Douglas, I. 2015 Disinfection byproduct formation during biofiltration cycle: Implications for drinking water production, Chemosphere (136) 190–197
13. Dhawan, S., Basu. O.D. and Banihashemi, B. 2017 Influence of nutrient supplementation on DOC removal in drinking water biofilters, Water Science and Technology-Water Supply, 17(2):422-432
14. Emelko, M. B., Huck, P. M., Coffey, B. M., and Smith, E. F., 2006. Effects of media, backwash, and temperature on full-scale biological filtration. Journal of American Water Works Association, 98 (12): 61-73
15. Fang, W., Hu, J.Y., and Ong, S.L. 2008 Influence of phosphorus on biofilm formation in model drinking water distribution systems, Journal of Applied Microbiology ISSN 1364-5072
16. Fu, J., Lee, W., Coleman, C., Meyer, M., Carter, J., Nowack, K. and Huang, C. 2017 Pilot investigation of two-stage biofiltration for removal of natural organic matter in drinking water treatment, Chemosphere (166) 311-322

17. Granger H. C., Stoddart A. K. and Gagnon G.A. 2014 Direct Biofiltration for Manganese removal from Surface Water, J. Environ. Eng., 140(4): 04014006
18. Hallé, C., P. M. Huck, S. Peldszus, J. Haberkamp, and M. Jekel. 2009 Assessing the performance of biological filtration as pretreatment to low pressure membranes for drinking water. Environmental Science and Technology, 43(10): 3878-3884
19. Hammes, F., Goldschmidt, F., Vital, M. and Wang, Y. 2010 Measurement and interpretation of microbial adenosine tri-phosphate (ATP) in aquatic environments, Water Research 44: 3915-3923
20. Hammes, F., Velten, S., Egli, T., and Juhna, T. 2011 Biotreatment of drinking water, Elsevier, 6.41: 517-530
21. Health Canada. 2017 Guidelines for Canadian Drinking Water Quality - Summary Table. Water and Air Quality Bureau, Healthy Enviroments and Consumer Safety Branch, Health Canada, Ottawa, Ontario
22. Hozalski, R.M., Goel, S., and Bouwer, E.J. 1995 TOC removal in biologically active sand filters: effect of NOM source and EBCT. Journal of American Water Work Association, 87:12-40
23. Huang, G., Meng, F., Zheng, X., Wang, Y., Wang, Z., Liu, H., and Jekel, M. 2011 Biodegradation behavior of natural organic matter (NOM) in a biological aerated filter (BAF) as a pretreatment for ultrafiltration (UF) of river water, Appl Microbiol Biotechnol 90:1795–1803
24. Ikhlef, S., and Basu, O.D. 2017 Influence of backwash regime on biofilter performance in drinking water treatment, Journal of Chemical Technology and Biotechnology, 92(7): 1777+

25. Jeong, S., Cho,K., Jeong, D., Lee, S., Leiknes, T., Vigneswaran, S. and Bae, H. 2017 Effect of engineered environment on microbial community structure in biofilter and biofilm on reverse osmosis membrane. Water Research 124: 227-237.
26. Keithley, S.E. and Kirisits, M. 2018 An improved protocol for extracting extracellular polymeric substances from granular filter media, Water Research (129): 419-427
27. Knowles, G., Downing, A.L. and Barrett, M.J. 1965 Determination of kinetic constants for nitrifying bacteria in mixed culture, with the aid of an electronic computer, Journal of general Microbiology (38) 268-278
28. Lájer, K. 2012 Ammonium removal by nitrification in drinking water treatment. Journal American Water Works Association 10: 47-53
29. Lauderdale, C., Chadik, P., Kirisits, M. and Brown, J. 2012 Engineered biofiltration: enhanced biofilter performance through nutrient and peroxide addition. Journal American Water Works Association, 104 (73): 289-309
30. Lee, H., 2014 The effect of influent nutrient conditions and biofiltration pretreatment on membrane biofouling, Master's thesis submitted to the Faculty of the Graduate School of the University of Colorado
31. Leenheer, J. A. and J. P. Croué. 2003 Characterizing aquatic dissolved organic matter. Environmental Science and Technology, 37(1): 18A-26A
32. Liu, C., Olivares, C.I., Pinto, A.J., Lauderdale, C.V., Brown, J., Selbes, M. and Karanfil, T. 2017 The control of disinfection byproducts and their precursors in biologically active filtration processes, Water Research (124) 630-653
33. Liu, X., Huck, P. M., and Slawson R. M. 2001 Factors affecting drinking water biofiltration. Journal American Water Works Association, 93(12): 90-101

34. Lopez-Ponnada, E. V., Lynn, T. J., Peterson, M., Ergas, S. J. and Mihelcic, J. R. 2017 Application of denitrifying wood chip bioreactors for management of residential non-point sources of nitrogen, Journal of Biological Engineering 11:16
35. Loyless, J.C., and Malone, R.F. 1997 A sodium bicarbonate dosing methodology for pH management in fresh water-recirculating aquaculture systems, The progressive fish-culturist 59:198-205
36. McKie, M.J., Taylor-Edmonds, L., Andrew, S.A., Andrew, R.C. 2015 Engineered biofiltration for the removal of disinfection byproduct precursors and genotoxicity, Water Research 81: 196-207
37. McSwain, B. S., Irvine, R. L., Hausner, M. and Wilderer, P. A. 2005 Composition and Distribution of Extracellular Polymeric Substances in Aerobic Flocs and Granular Sludge, Applied and Environmental Microbiology, 1051–1057
38. Metcalf, and Eddy, Fifth Edition, 2013 Wastewater engineering: Treatment and resource recovery
39. Moll, D.M., Summers, R.S. 1999 Assessment of drinking water filter microbial communities using taxonomic and metabolic profiles, Water Sci. Technol. 39, 8349
40. Nemani, V.A., McKie, M.J., Taylor-Edmonds, L. and Andrews R.C. 2018 Impact of biofilter operation on microbial community structure and performance, Journal of Water Process Engineering (24) 35–41
41. O'Farrell, T.P., Frauson, F.P., Cassel A.F. and Bishop D.F. 1972 Nitrogen Removal by Ammonia Stripping, Water Pollution Control Federation, 44 (8): 1527-1535

42. Peldszus, S., Benecke, J., Jekel, M. & Huck, P. M. 2012 Direct biofiltration pretreatment for fouling control of ultrafiltration membranes. Journal American Water Works Association, 104 (7): 430–445

43. Peleato, N.M., McKie, M., Taylor-Edmonds, L., Andrews, S.A., Legge, R.L. and Andrews, R.C. 2016 Fluorescence spectroscopy for monitoring reduction of natural organic matter and halogenated furanone precursors by biofiltration, Chemosphere (153) 155-161

44. Pharand, L., Van Dyke, M.I., Anderson, W.B. and Huck, P.M. 2014 Assessment of biomass in drinking water biofilters by adenosine triphosphate, Journal American Water Works Association, 106 (10) 433–444

45. Rahman, I., Van Dyke, M.I., Anderson, W.B., Jin, X., Ndiongue, S. and Huck, P.M. 2016 Effect of phosphorus addition on biofiltration pre-treatment to reduce ultrafiltration membrane fouling, Desalination and Water Treatment 57: 25057–25069

46. Rittmann, B.E. and McCarty, P.L. 2001 Environmental biotechnology: Principles and applications, McGraw-Hill Series in Water Resources and Environmental Engineering

47. Rittmann, B. E. and Huck, P. M. 1989 Biological treatment of public water supplies, CRC Critical Reviews in Environmental Control 19: 119

48. Rusten, B., Eikebrokk, B., Ulgenes, Y., Lygren, E. 2006 Design and operations of the Kaldnes moving bed biofilm reactors. Aquacultural Engineering 34: 322–331.

49. Sajuni, N.R., Ahmad, A.L. and Vadivelu, V.M. 2010 Effect of filter media characteristics, pH and temperature on the ammonia removal in the wastewater, Journal of Applied Sciences 10 (12): 1146-1150

50. Sang, J., Zhang, X., Li, L. and Wang, Z. 2003 Improvement of organics removal by bio-ceramic filtration of raw water with addition of phosphorus, Water Research (37) 4711–4718

51. Sheng, G., Yu, H., and Li, X. 2010 Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review, Biotechnology Advances 28: 882-894

52. Summerfelt, S.T., Zühlke,A., Kolarevi, J., Reiten, B.K.M., Selset, R., Gutierrez, X. and Terjesen, B.F. 2015 Effect of alkalinity on ammonia removal, carbon dioxide stripping, and system pH in semi-commercial scale water recirculating aquaculture systems operated with moving bed bioreactors, Aquacultural Engineering 65: 46–54

53. Urfer, D., Huck, P. M., Booth, S. D. J., & Coffey, B. M. 1997 Biological filtration for BOM and particle removal: a critical review. Journal of American Water Works Association, 89 (12): 83–98

54. Vahala, R., Moramarco, V., Niemi, R. M., Rintala, J. and Laukkanen, R. 1998 The effects of nutrients on natural organic matter (NOM) removal in biological activated carbon (BAC) filtration, Acta hydrochim. hydrobiol. (26) (3) 196-199

55. Van Rijn, J., Tal, Y., Schreier, H.J. 2006 Denitrification in recirculating systems: theory and applications. Aquacultural Engineering 34 (3): 364-376.

56. Villaverde, S., Garcia-encina, P.A., and Fdz-Polanco F. 1997 Influence of pH over nitrifying biofilm activity in submerged biofilters. Water Research 31(5):1180-1186

57. Wang, L., Wang, L.F., Ren, X., Ye, X., Li, W., Yuan, S., Sun, M., Sheng, G., Yu, H., and Wang, X. 2012 pH Dependence of Structure and Surface Properties of Microbial EPS, Environmental Science and Technology, 46: 737−744

58. Wert, E. C., J. J. Neemann, D. J. Rexing, and Zegers. R.E. 2008 Biofiltration for removal of BOM and residual ammonia following control of bromate formation. Water Research, 42(12): 372-378

59. World Health Organization, 2003 Ammonia in drinking water, Background document for development of WHO Guidelines for Drinking-water Quality, WHO/SDE/WSH/03.04/01

60. Xia, L., Zheng, X., Shao, H., Xin, J., Sun, Z. and Wang, L. 2016 Effects of bacterial cells and two types of extracellular polymers on bioclogging of sand columns, Journal of Hydrology 535: 293–300

61. Yapsakli, K., Mertoglu, B. and Cecen, F. 2010 Identification of nitrifiers and nitrification performance in drinking water biological activated carbon (BAC) filtration, Process Biochemistry (45) 1543–1549

62. Yavich, A. A., Lee, K., Chen, K., Pape, L. and Masten, S. J. 2004 Evaluation of biodegradability of NOM after ozonation. Water Research, 38(12): 2839-2846

63. Yu, X., Qi, Z., Zhang, X., Yu, P., Liu, B., Zhang, L. and Fu, L. 2007 Nitrogen loss and oxygen paradox in full-scale biofiltration for drinking water treatment, Water research 41: 1455 – 1464

64. Yu, X., Zhang, X. and Wang, Z.S. 2003 Improving removal efficiency of organic matters by adding phosphorus in drinking water biofiltration treatment, Biomedical and environmental sciences 16, 29-39

65. Zanacic, E., Stavrinides, J., and McMartin. D. 2016 Field-analysis of potable water quality and ozone efficiency in ozone assisted biological filtration systems for surface water treatment, Water Research 104: 397- 407

66. Zhu, I. X., Getting, T. & Bruce, D. 2010 Review of biologically active filters in drinking water applications. Journal – American Water Works Association 102 (12): 67–77

7. Appendices

7.1. Appendix A- Dosing Solution Calculation

- ***Carbon Dosing Solution***

10 mg/L of carbon is needed in the biofilters' influent

Average TOC level of tap water: 3.30 mg/L

6.70 mg/L should be supplied from 3 different carbon sources.

4 ml/min × C_C = 200 ml/min × 6.70 mg/L

C_C = 335 mg/L ×50 L = 16.75 gr

16.75 / 3 = 5.58 gr from each carbon sources

Acetic Acid:

$$5.58\ gr\ C \times \frac{1\ molC}{12\ gr\ C} \times \frac{1\ mol\ CH3COOH}{2\ mol\ C} \times \frac{60.05\ gr\ CH3COOH}{1\ mol\ CH3COOH} = 13.96\ gr\ CH3COOH$$

$$\frac{13.96\ gr\ CH3COOH}{1.049\ g/ml} \times \frac{1}{0.997} = 13.36\ ml\ of\ Acetic\ Acid$$

Glyoxal:

40 wt% in water, density: 1.265 g/ml

$$5.58\ grC \times \frac{1\ molC}{12\ gr\ C} \times \frac{1\ mol\ C2H2O2}{2\ mol\ C} \times \frac{58.04\ gr\ C2H2O2}{1\ mol\ C2H2O2} = \frac{13.50\ grC2H2O2}{0.4} = 33.75\ gr$$

$$\frac{33.75\ gr\ C2H2O2}{1.265\frac{g}{ml}} = 26.68\ ml\ of\ Glyoxal$$

Formic Acid:

$$5.58\ grC \times \frac{1\ molC}{12\ gr\ C} \times \frac{1\ mol\ CH2O2}{1\ mol\ C} \times \frac{46.03\ grCH2O2}{1\ mol\ CH2O2} = 21.42\ grCH2O2$$

$$21.42\ \frac{gr\ CH2O2}{1.22\ \frac{g}{ml}} \times \frac{1}{0.99} = 17.73\ ml\ of\ Formic\ Acid$$

- ***Nutrient Dosing Solution***

2 mg/L of nitrogen is needed in the biofilters' influent

4 ml/min × C_N = 200 ml/min × 2 mg/L

C_N = 100 mg/L ×50 L = 5 gr

$$5.0\ gr\ N \times \frac{1\ mol\ N}{14.01\ gr\ N} \times \frac{1\ mol\ (NH4)2SO4}{2\ mol\ N} \times \frac{132.13\ gr\ (NH4)2SO4}{1\ mol\ (NH4)2SO4} = 23.58\ gr\ (NH4)2SO4$$

0.4 mg/L of phosphorus is needed in the biofilters' influent

4 ml/min × C_P = 200 ml/min × 0.4 mg/L

C_P = 20 mg/L ×50 L = 1.0 gr

$$1.0\ gr\ P \times \frac{1\ mol\ P}{30.97\ gr\ P} \times \frac{1\ mol\ KH2PO4}{1\ mol\ P} \times \frac{136.09\ gr\ KH2PO4}{1\ mol\ KH2PO4} = 4.39\ gr\ KH2PO4$$

7.2. Appendix B- Conditioning Phase Result

Table 7. 1: Conditioning phase result (TOC removal)

Experiment	BF1			BF2		
	Inf. (mgTOC/L)	Eff. (mgTOC/L)	% Removal efficiency	Inf. (mgTOC/L)	Eff. (mgTOC/L)	% Removal efficiency
1	8.64	2.68	68.95	8.79	2.65	69.89
2	9.49	3.31	65.12	9.81	3.15	67.90
3	9.96	3.29	66.96	9.37	3.09	67.03
4	10.37	2.96	71.49	10.23	2.88	71.82
5	10.72	3.27	69.51	10.60	2.89	72.75
6	10.24	3.25	68.26	10.17	2.88	71.64
7	10.01	3.20	68.08	9.88	2.95	70.11
8	8.66	3.31	61.84	8.81	3.17	64.02
9	9.56	5.18	45.79	9.35	3.45	63.11
10	9.32	2.94	68.45	9.12	2.86	68.60
11	9.34	3.03	67.56	8.80	5.82	33.83
12	10.51	3.54	66.32	10.56	3.08	70.80
13	10.60	3.52	66.78	10.70	3.28	69.36
14	9.67	3.34	65.43	10.34	3.23	68.78
15	10.53	3.25	69.12	9.63	3.23	66.47
16	9.53	3.44	63.91	9.47	3.15	66.75
17	9.28	3.18	65.76	9.55	3.01	68.50
18	10.70	3.98	62.80	10.56	3.45	67.33
19	8.96	3.18	64.53	8.84	3.22	63.57
20	8.50	3.06	64.00	8.57	3.05	64.39
21	9.79	3.47	64.59	9.36	3.67	60.78
22	10.54	3.69	64.99	10.56	3.71	64.87
23	8.29	2.27	72.60	8.45	2.25	73.41
24	10.06	3.78	62.39	9.32	4.32	53.64
25	8.77	2.78	68.28	8.74	3.01	65.56
26	10.95	3.65	66.67	10.73	3.45	67.85

7.3. Appendix C- TOC Removal Graphs

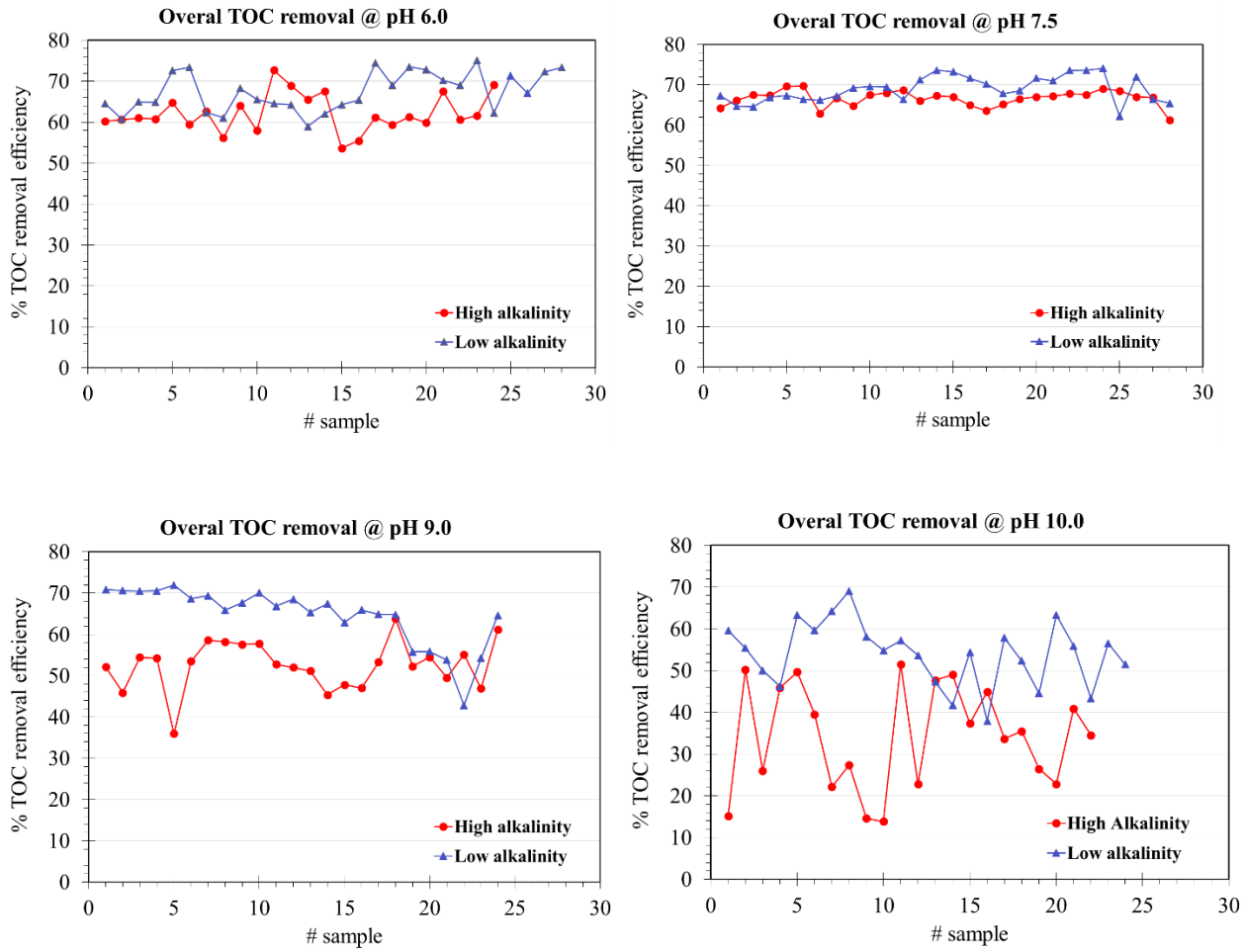

Figure 7. 1: TOC removal efficiency at pH 6.0, 7.5, 9.0, and 10.0

7.4. Appendix D- Additional TOC Results

Table 7. 2: TOC removal efficiency

Exp.	IN 1 (mg/L)	EFF 1 (mg/L)	% Removal	IN 2 (mg/L)	EFF 2 (mg/L)	% Removal
pH 6 and low alkalinity level	9.79	3.47	64.59	9.36	3.67	60.78
	10.54	3.69	64.99	10.56	3.71	64.87
	8.29	2.27	72.60	8.45	2.25	73.41
	10.06	3.78	62.39	10.86	4.20	61.33
	8.77	2.78	68.28	8.74	3.01	65.56
	9.09	3.22	64.56	9.16	3.27	64.29
	8.69	3.57	58.96	8.68	3.30	62.04
	8.40	3.00	64.27	8.32	2.88	65.41
	9.62	2.46	74.46	9.85	3.05	69.03
	8.52	2.25	73.54	8.52	2.31	72.83
	9.35	2.78	70.25	9.25	2.87	69.03
	9.38	2.34	75.05	9.31	3.52	62.19
	9.21	2.64	71.36	9.16	3.02	67.03
	9.71	2.69	72.32	9.65	2.57	73.35
	9.96	3.01	69.74	10.05	2.49	75.27
	10.01	2.75	72.57	10.01	2.37	76.35
	9.48	3.33	64.90	9.40	3.61	61.57
	10.72	3.27	69.51	10.44	3.69	64.66
	11.09	4.03	63.67	9.62	2.97	69.08
	10.01	3.16	68.48	9.81	3.69	62.36
	9.70	3.05	68.51	9.63	3.06	68.25
pH 6 and high alkalinity level	10.01	3.98	60.24	10.21	4.02	60.63
	9.98	3.89	61.02	9.87	3.87	60.79
	9.87	3.48	64.74	9.82	3.98	59.47
	9.06	3.38	62.63	8.92	3.91	56.17

	IN 1 (mg/L)	EFF 1 (mg/L)	% Removal	IN 2 (mg/L)	EFF 2 (mg/L)	% Removal
	8.97	3.22	64.07	9.26	3.89	57.97
	9.40	2.56	72.76	9.53	2.96	68.97
	8.89	3.06	65.56	8.88	2.88	67.55
	9.37	4.34	53.68	9.50	4.23	55.47
	9.90	3.84	61.21	9.64	3.92	59.34
	9.80	3.79	61.33	9.49	3.81	59.85
	9.57	3.11	67.50	9.63	3.79	60.64
	9.35	3.59	61.60	9.20	2.84	69.13
pH 7.5 and low alkalinity level	8.83	2.88	67.36	8.76	3.09	64.69
	9.25	3.27	64.61	9.24	3.06	66.95
	9.62	3.14	67.35	9.77	3.28	66.40
	8.41	2.84	66.26	8.51	2.79	67.23
	9.62	2.96	69.29	9.58	2.91	69.61
	9.46	2.89	69.47	9.35	3.15	66.38
	9.71	2.79	71.27	9.75	2.57	73.63
	9.58	2.56	73.25	9.55	2.71	71.65
	8.49	2.53	70.21	8.37	2.69	67.85
	8.67	2.72	68.58	8.79	2.49	71.69
	9.20	2.66	71.04	9.24	2.44	73.58
	9.75	2.57	73.61	9.73	2.52	74.11
	9.58	3.61	62.27	9.52	2.66	72.03
	9.26	3.11	66.46	9.38	3.24	65.46
	9.98	2.70	72.92	9.90	2.76	72.11
	10.42	3.43	67.06	10.21	3.01	70.57
pH 7.5 and high alkalinity level	8.62	3.08	64.30	8.38	2.83	66.19
	8.85	2.88	67.51	8.88	2.89	67.41
	9.85	2.99	69.66	9.60	2.90	69.78
	10.77	4.00	62.88	10.16	3.38	66.73

	IN 1 (mg/L)	EFF 1 (mg/L)	% Removal	IN 2 (mg/L)	EFF 2 (mg/L)	% Removal
	9.11	3.21	64.74	9.22	2.99	67.51
	9.56	3.06	68.00	9.54	2.98	68.76
	9.60	3.26	66.05	9.57	3.13	67.34
	9.41	3.10	67.03	9.10	3.19	64.98
	9.62	3.50	63.61	9.71	3.38	65.16
	9.01	3.02	66.49	9.01	2.97	67.02
	9.19	3.01	67.19	9.10	2.93	67.82
	9.46	3.07	67.55	9.49	2.94	69.05
	9.60	3.02	68.51	9.85	3.25	67.02
	9.80	3.25	66.87	9.88	3.83	61.28
pH 9 and low alkalinity level	10.07	2.93	70.86	9.79	2.88	70.60
	9.90	2.92	70.48	9.69	2.85	70.56
	9.14	2.57	71.95	9.37	2.94	68.66
	9.50	2.91	69.40	9.12	3.11	65.90
	9.43	3.05	67.69	9.62	2.88	70.07
	9.68	3.21	66.86	9.69	3.05	68.54
	9.72	3.37	65.35	9.52	3.10	67.42
	10.16	3.77	62.92	9.80	3.34	65.94
	10.06	3.53	64.89	9.94	3.50	64.80
	9.99	4.42	55.76	9.85	4.35	55.82
	9.40	4.34	53.84	9.25	5.29	42.85
	10.12	4.63	54.23	10.13	3.59	64.60
pH 9 and high alkalinity level	10.92	5.23	52.11	10.48	5.67	45.93
	10.02	4.57	54.43	10.28	4.70	54.25
	10.26	6.57	35.96	9.79	4.55	53.49
	10.22	4.23	58.61	10.03	4.19	58.23
	10.56	4.48	57.60	10.42	4.40	57.75
	10.48	4.95	52.73	10.25	4.92	52.02

	IN 1 (mg/L)	EFF 1 (mg/L)	% Removal	IN 2 (mg/L)	EFF 2 (mg/L)	% Removal
	10.45	5.10	51.19	10.27	5.61	45.35
	9.08	4.74	47.81	9.21	4.88	47.01
	10.71	5.01	53.27	10.42	3.77	63.78
	10.98	5.24	52.28	11.09	5.05	54.50
	10.60	5.35	49.53	10.48	4.70	55.12
	9.94	5.28	46.91	9.77	3.79	61.17
pH 10 and low alkalinity level	9.28	3.75	59.59	8.16	3.64	55.39
	9.31	4.63	50.27	8.84	4.75	46.27
	9.27	3.40	63.32	8.31	3.35	59.69
	9.56	3.42	64.23	9.79	3.03	69.05
	9.50	3.98	58.11	9.28	4.19	54.85
	8.95	3.83	57.21	8.80	4.08	53.64
	8.53	4.49	47.36	8.80	5.13	41.70
	8.66	3.95	54.39	8.99	5.58	37.93
	9.14	3.85	57.88	9.17	4.37	52.34
	9.69	5.37	44.58	9.79	3.59	63.33
	8.42	3.78	55.11	8.43	4.75	43.65
	9.41	4.09	56.54	8.72	4.23	51.49
pH 10 and high alkalinity level	10.30	8.74	15.18	10.41	5.18	50.24
	8.95	6.59	26.37	8.99	4.85	46.05
	10.35	5.21	49.66	10.92	6.61	39.51
	10.18	7.93	22.13	10.34	7.50	27.44
	10.55	9.01	14.59	10.73	9.24	13.86
	10.35	5.02	51.52	10.78	8.32	22.80
	10.20	5.33	47.71	10.35	5.28	49.02
	10.94	6.85	37.37	10.76	5.92	44.95
	9.44	6.26	33.66	9.38	6.05	35.49
	8.87	6.52	26.48	8.82	6.80	22.90

	IN 1 (mg/L)	EFF 1 (mg/L)	% Removal	IN 2 (mg/L)	EFF 2 (mg/L)	% Removal
	8.65	5.11	40.92	8.82	5.78	34.52

7.5. Appendix E- Ammonia Removal Graphs

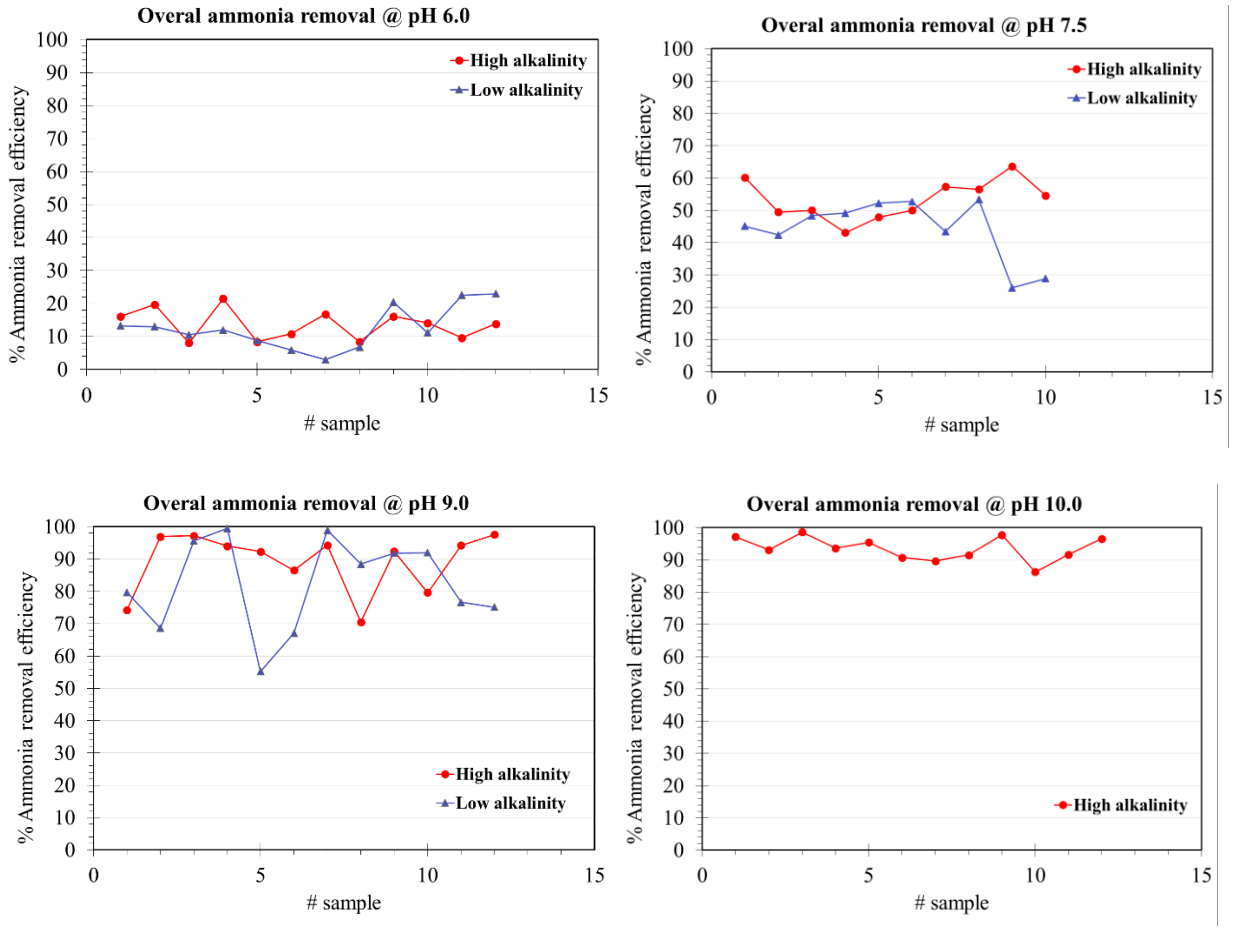

Figure 7. 2: Ammonia removal efficiency

7.6. Appendix F- Additional Ammonia Results

Table 7. 3: Ammonia removal efficiency

Exp.	IN 1 (mg NH_3-N/L)	EFF 1 (mg NH_3-N/L)	% Removal	IN 2 (mg NH_3-N/L)	EFF 2 (mg NH_3-N/L)	% Removal
pH 6, LA	1.87	1.62	13.37	1.88	1.64	12.77
	1.92	1.72	10.42	1.86	1.64	11.83
	1.91	1.74	8.90	1.92	1.81	5.73
	1.88	1.83	2.66	1.86	1.74	6.45
	2.21	1.76	20.36	1.98	1.76	11.11
	2.09	1.62	22.49	2.05	1.58	22.93
	2.11	1.83	13.27	2.12	1.96	7.55
	1.81	1.56	13.81	1.89	1.63	13.76
pH 6, HA	1.89	1.54	18.52	1.81	1.55	14.36
	1.96	1.64	16.22	1.95	1.57	19.49
	1.94	1.78	8.25	1.97	1.55	21.32
	1.99	1.83	8.29	1.97	1.76	10.66
	1.85	1.54	16.76	1.89	1.73	8.47
	1.88	1.58	16.22	1.85	1.59	14.05
	1.88	1.70	9.57	1.89	1.63	13.76
	1.90	1.74	8.42	1.88	1.64	12.77
	1.91	1.71	10.47	1.90	1.69	11.05
pH 7.5, LA	1.83	1.005	45.08	1.84	1.06	42.39
	1.88	0.97	48.40	1.85	0.94	49.19
	2.05	0.98	52.20	2.01	0.95	2.05
	1.79	1.012	43.46	1.81	0.845	53.31
	1.96	1.45	26.02	1.94	1.38	28.87

	IN 1 (mg/L)	EFF 1 (mg/L)	% Removal	IN 2 (mg/L)	EFF 2 (mg/L)	% Removal
pH 7.5, HA	1.93	0.77	60.10	1.92	0.97	49.48
	1.88	0.94	43.10	1.84	1.05	43.10
	1.86	0.9	51.61	1.86	0.93	50.00
	2.12	0.905	57.31	2.07	0.9	56.52
	1.98	0.72	63.64	1.96	0.89	54.59
pH 9, LA	2.03	0.412	79.70	2.07	0.65	68.60
	1.94	0.085	95.62	1.98	0.01	99.49
	1.88	0.843	55.16	1.91	0.629	67.07
	1.93	0.021	98.96	1.89	0.218	88.47
	2.08	0.171	91.83	1.99	0.16	91.96
	1.84	0.431	76.63	1.89	0.472	75.13
pH 9, HA	1.93	0.497	74.25	1.97	0.06	96.95
	1.89	0.053	97.20	1.92	0.115	94.01
	1.86	0.143	92.31	1.89	0.255	86.51
	1.99	0.113	94.32	2.02	0.595	70.54
	1.97	0.15	92.39	1.94	0.395	79.64
	2.07	0.12	94.20	2.02	0.05	97.52
pH 10, HA	1.89	0.0535	97.17	1.88	0.129	93.14
	1.88	0.03	98.62	1.86	0.12	93.66
	1.97	0.09	95.43	1.89	0.175	90.74
	1.95	0.202	89.64	1.97	0.167	91.52
	2.02	0.045	97.77	2.04	0.28	86.27
	1.94	0.162	91.65	1.91	0.066	96.54

7.7. Appendix G- Additional DO Results

Table 7. 4: DO removal efficiency

Exp.	IN 1 (mg/L)	EFF 1 (mg/L)	% Removal	IN 2 (mg/L)	EFF 2 (mg/L)	% Removal
pH 6 and low alkalinity level	6.60	0.36	94.55	6.40	0.68	89.38
	6.74	0.65	90.36	6.70	0.30	95.52
	6.63	0.20	96.98	6.71	1.08	83.90
	6.71	0.20	97.02	6.72	0.10	98.51
	6.84	0.08	98.83	6.76	0.07	98.96
	6.93	0.05	99.28	6.86	0.08	98.83
	6.85	0.09	98.69	6.84	0.07	98.98
	6.31	0.07	98.89	6.24	0.05	99.20
	7.27	0.11	98.49	7.11	0.11	98.45
	7.44	0.29	96.10	7.41	0.12	98.38
	6.99	0.35	94.99	6.95	0.24	96.55
	6.99	0.38	94.56	7.12	0.78	89.04
	7.13	0.28	96.07	7.20	0.72	90.00
	6.43	0.18	97.20	6.39	0.19	97.03
	7.25	0.31	95.72	7.31	0.42	94.25
	7.40	0.38	94.86	7.21	0.12	98.34
	6.81	0.22	96.77	7.09	0.16	97.74
	6.80	0.25	96.32	7.00	0.23	96.71
	6.76	0.19	97.19	6.55	0.16	97.56
	6.56	0.17	97.41	6.62	0.19	97.13
	6.00	0.24	96.00	6.43	0.29	95.49
pH 6 and high alkalinity level	8.93	0.87	90.26	8.95	0.48	94.64
	8.86	1.18	86.68	9.11	1.17	87.16
	8.80	0.56	93.64	8.91	0.71	92.03
	8.21	0.27	96.71	8.21	1.11	86.48
	8.51	2.16	74.62	8.51	2.23	73.80

	IN 1 (mg/L)	EFF 1 (mg/L)	% Removal	IN 2 (mg/L)	EFF 2 (mg/L)	% Removal
	8.09	0.80	90.11	8.09	0.19	97.65
	7.43	0.50	93.27	7.43	0.57	92.33
	8.46	2.12	74.94	8.28	2.22	73.19
	7.99	1.85	76.85	7.99	1.66	79.22
	7.33	1.71	76.67	7.58	1.14	84.96
	7.47	0.90	87.95	7.86	0.88	88.80
	7.82	0.50	93.61	8.01	0.51	93.63
pH 7.5 and low alkalinity level	7.38	0.34	95.39	7.48	0.44	94.12
	7.55	0.35	95.36	7.55	0.47	93.77
	5.31	0.23	95.67	4.92	0.17	96.54
	6.54	0.38	94.19	6.44	0.44	93.17
	8.08	0.35	95.67	8.10	0.28	96.54
	7.81	1.96	74.90	8.02	0.39	95.14
	8.17	0.32	96.08	8.35	0.29	96.53
	8.01	0.92	88.51	8.11	0.41	94.94
	7.40	0.28	96.22	7.52	0.22	97.07
	7.46	0.33	95.58	7.52	0.25	96.68
	8.39	0.31	96.31	8.37	0.32	96.18
	9.14	0.89	90.26	9.12	0.92	89.91
	9.40	0.35	96.28	9.35	0.44	95.29
	10.38	3.20	69.17	10.33	0.92	91.09
	9.90	1.09	88.99	9.96	1.36	86.35
	8.23	0.25	96.96	8.27	0.45	94.56
pH 7.5 and high alkalinity level	9.77	0.76	92.22	9.50	1.60	83.16
	9.20	0.40	95.65	9.26	0.80	91.36
	10.17	1.29	87.32	10.20	0.49	95.20
	9.06	0.34	96.25	9.06	0.46	94.92
	9.94	0.68	93.16	10.02	0.43	95.71

	IN 1 (mg/L)	EFF 1 (mg/L)	% Removal	IN 2 (mg/L)	EFF 2 (mg/L)	% Removal
	9.16	0.27	97.05	9.75	0.30	96.92
	9.70	0.65	93.30	9.73	0.43	95.58
	9.98	0.39	96.09	10.05	0.32	96.82
	10.01	0.37	96.30	9.94	0.46	95.37
	9.46	0.56	94.08	9.50	0.55	94.21
	9.75	0.23	97.64	9.70	0.29	97.01
	10.03	0.45	95.51	10.14	0.57	94.38
	9.58	0.69	92.80	9.68	0.58	94.01
	9.17	0.40	95.64	9.21	0.38	95.87
pH 9 and low alkalinity level	8.21	0.77	90.62	8.31	0.76	90.85
	8.29	1.15	86.13	8.58	1.01	88.23
	9.69	1.22	87.41	9.65	1.16	87.98
	9.07	1.30	85.67	9.15	0.94	89.73
	8.67	1.06	87.77	8.69	1.13	87.00
	9.35	1.20	87.17	9.39	1.24	86.79
	9.45	1.18	87.51	9.49	1.22	87.14
	10.06	1.42	85.88	9.66	1.04	89.23
	8.79	1.34	84.76	9.17	1.26	86.26
	8.30	2.20	73.49	8.06	1.03	87.22
	8.01	0.93	88.39	8.06	1.25	84.49
	8.68	1.12	87.10	8.70	0.83	90.46
pH 9 and high alkalinity level	9.67	2.18	77.46	9.81	1.02	89.60
	8.62	1.72	80.05	8.73	1.16	86.71
	9.17	2.52	72.52	9.18	1.51	83.55
	8.51	2.26	73.44	9.25	1.50	83.78
	9.19	1.20	86.94	9.48	1.16	87.76
	8.09	1.64	79.73	8.30	0.99	88.07
	9.34	1.24	86.72	9.35	1.04	88.88
	9.30	1.40	84.95	9.06	1.02	88.74

	IN 1 (mg/L)	EFF 1 (mg/L)	% Removal	IN 2 (mg/L)	EFF 2 (mg/L)	% Removal
	9.49	2.38	74.92	9.22	1.07	88.39
	9.51	1.31	86.23	9.46	1.00	89.43
	9.88	2.28	76.92	10.15	1.25	87.68
	9.23	1.76	80.93	9.22	1.26	86.33
pH 10 and low alkalinity level	5.40	0.33	93.89	6.05	0.24	96.03
	4.54	0.47	89.65	4.89	0.30	93.87
	5.42	0.34	93.73	5.50	0.40	92.73
	5.63	0.29	94.85	5.36	0.25	95.34
	5.77	0.38	93.41	5.53	0.60	89.15
	5.36	0.26	95.15	5.13	0.40	92.20
	4.75	0.28	94.11	4.30	0.31	92.79
	5.04	0.30	94.05	4.29	0.26	93.94
	4.73	0.28	94.08	4.58	0.32	93.01
	5.95	2.10	64.71	5.89	1.20	79.63
	4.20	0.25	94.05	3.10	0.20	93.55
	3.78	0.38	89.95	3.39	0.33	90.27
pH 10 and high alkalinity level	8.37	1.33	84.11	8.47	1.98	76.62
	9.11	4.35	52.25	9.26	4.29	53.67
	9.85	4.35	55.84	9.93	2.25	77.34
	9.58	1.69	82.36	9.65	2.28	76.37
	8.99	5.40	39.93	9.12	2.80	69.30
	9.05	1.17	87.07	9.34	0.97	89.61
	8.56	1.93	77.45	8.81	1.03	88.31
	8.88	0.53	94.03	8.87	1.20	86.47
	6.55	0.60	90.84	6.58	0.68	89.67
	7.50	2.88	61.60	7.28	1.33	81.73
	7.31	1.32	81.94	7.39	0.20	97.29

7.8. Appendix H- Additional Turbidity Results

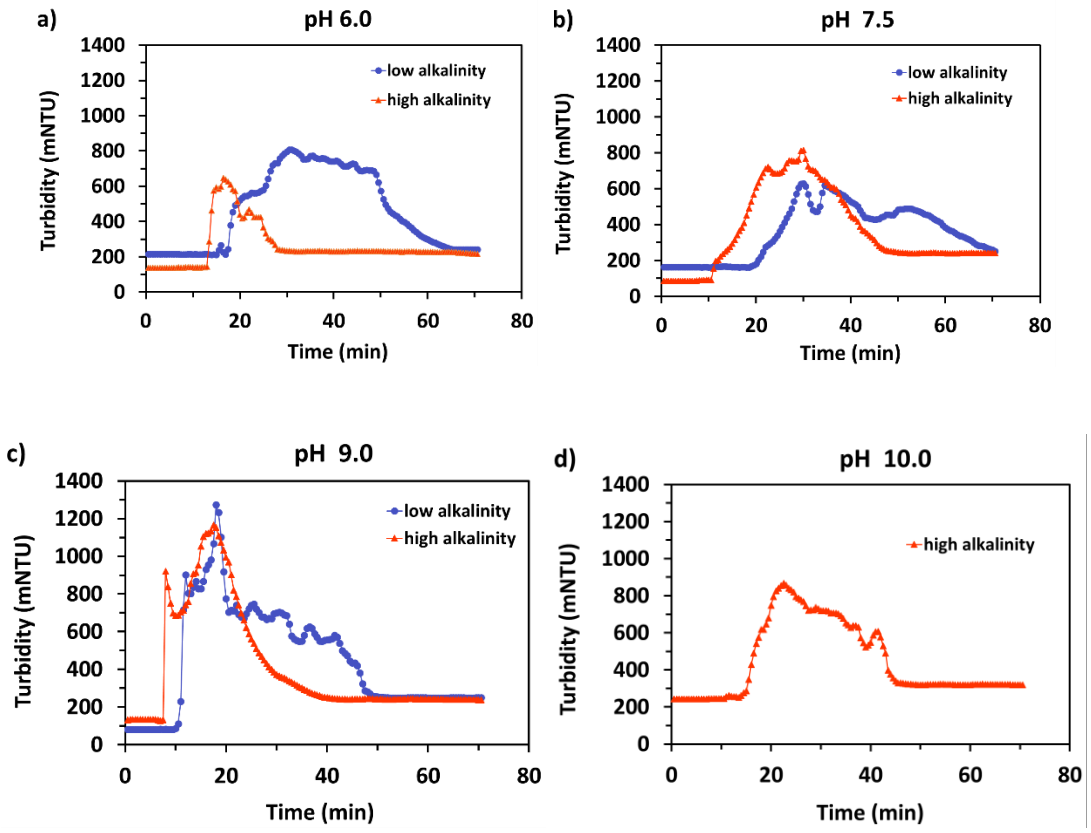

Figure 7. 3: Ripening curve development

7.9. Appendix I- Additional EPS and ATP Results

Table 7. 5: EPS and ATP concentrations

Exp.	Before backwash				24 hours after backwash			
	PS Conc. (µg/g)	PR Conc. (µg/g)	EPS conc. (µg/g)	ATP conc. (ng/g)	PS Conc. (µg/g)	PR Conc. (µg/g)	EPS conc. (µg/g)	ATP conc. (ng/g)
pH 6, low alk.	116.8	119.4	236.2	1047.7	52.8	78.0	130.9	1191.0
	78.9	85.4	164.3	2196.2	127.0	173.3	300.3	1245.3
	60.5	76.8	137.3	2754.9	86.1	86.5	172.6	1744.8
	NA	NA	NA	NA	153.4	153.9	307.4	2315.1
	NA	NA	NA	NA	45.0	71.6	116.6	3610.0
	NA	NA	NA	NA	64.7	73.6	138.3	2784.8
pH 6, high alk.	165.9	240.7	406.5	4828.8	178.5	215.1	393.5	3650.1
	242.0	212.0	454.0	2680.7	247.0	172.1	419.1	3772.5
	241.5	176.6	418.2	4688.5	245.3	188.2	433.5	5098.9
pH 7.5, low alk.	157.5	85.3	242.8	3704.6	144.9	92.7	237.6	4315.2
	65.3	121.1	186.4	4186.2	104.2	106.3	210.5	3960.3
	66.2	140.8	206.9	5630.9	76.4	162.4	238.8	5320.7
	89.9	145.6	235.5	4550.7	119.9	190.3	310.2	4903.9
pH 7.5, high alk.	89.0	87.5	176.5	6646.8	101.3	91.1	192.4	6785.6
	162.6	149.5	312.1	8197.8	110.2	155.5	265.7	5649.3
	99.8	164.7	264.5	8126.7	230.9	158.3	389.1	7574.8
pH 9.0, low alk.	108.7	296.7	405.4	4558.5	121.6	253.8	375.4	5257.9
	56.9	152.9	209.9	6375.3	66.6	169.1	235.7	6261.5
	200.1	157.5	357.6	5537.8	272.4	173.1	445.5	6907.3
pH 9.0, high alk.	59.6	160.5	220.1	6864.7	87.5	228.7	316.1	7830.5
	73.6	131.4	205.0	6043.6	91.6	269.2	360.7	9062.6
	181.0	224.7	405.7	8086.2	215.7	283.4	499.1	8870.4

Exp.	Before backwash				24 hours after backwash			
	PS Conc. (µg/g)	PR Conc. (µg/g)	EPS conc. (µg/g)	ATP conc. (ng/g)	PS Conc. (µg/g)	PR Conc. (µg/g)	EPS conc. (µg/g)	ATP conc. (ng/g)
pH 10, high alk.	124.9	287.2	412.1	2899.7	118.0	261.5	379.6	2489.9
	290.8	480.1	770.9	6142.7	207.8	359.4	567.2	3770.3
	402.7	479.1	881.8	4823.2	252.2	282.3	534.5	3110.3

7.10. Appendix J- Effect of backwash on EPS and ATP concentration

Table 7. 6: Average EPS and ATP results, before backwash and 24 hours after backwash

Experiment	EPS concentration (µg/g)		ATP concentration (ng/g)	
	before backwash	24 hours after backwash	before backwash	24 hours after backwash
pH 6.0, low alkalinity	93.86	194.34	1999.59	2148.49
pH 6.0, high alkalinity	426.23	415.38	4065.98	4173.81
pH 7.5, low alkalinity	217.91	249.25	4518.06	4625.02
pH 7.5, high alkalinity	251.01	282.41	7657.10	6669.88
pH 9.0, low alkalinity	324.30	352.18	5490.54	6142.25
pH 9.0, high alkalinity	276.91	391.99	6998.14	8587.82
pH 10.0, high alkalinity	688.25	493.76	4621.86	3123.50

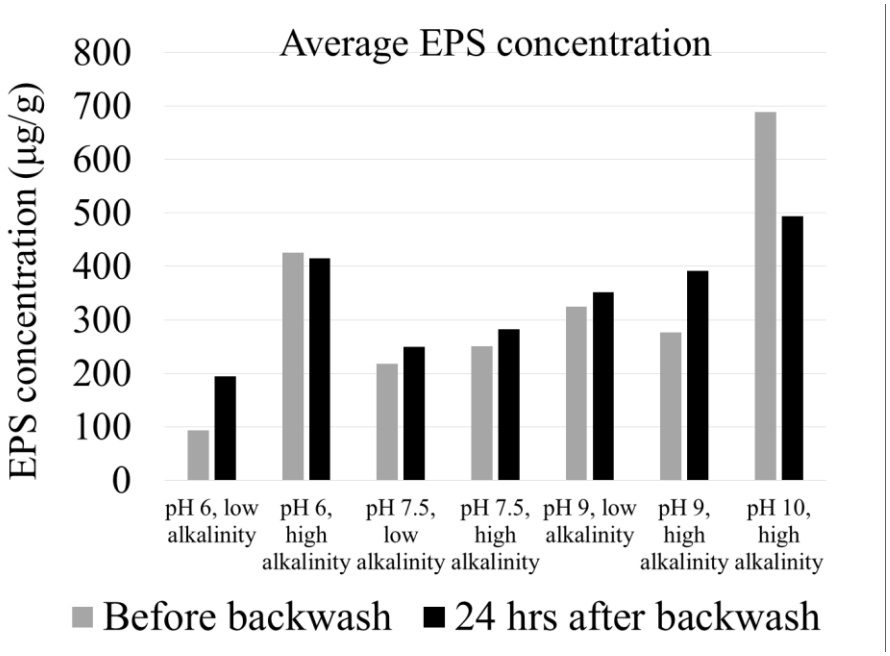

Figure 7. 4: Effect of backwash on EPS concentration

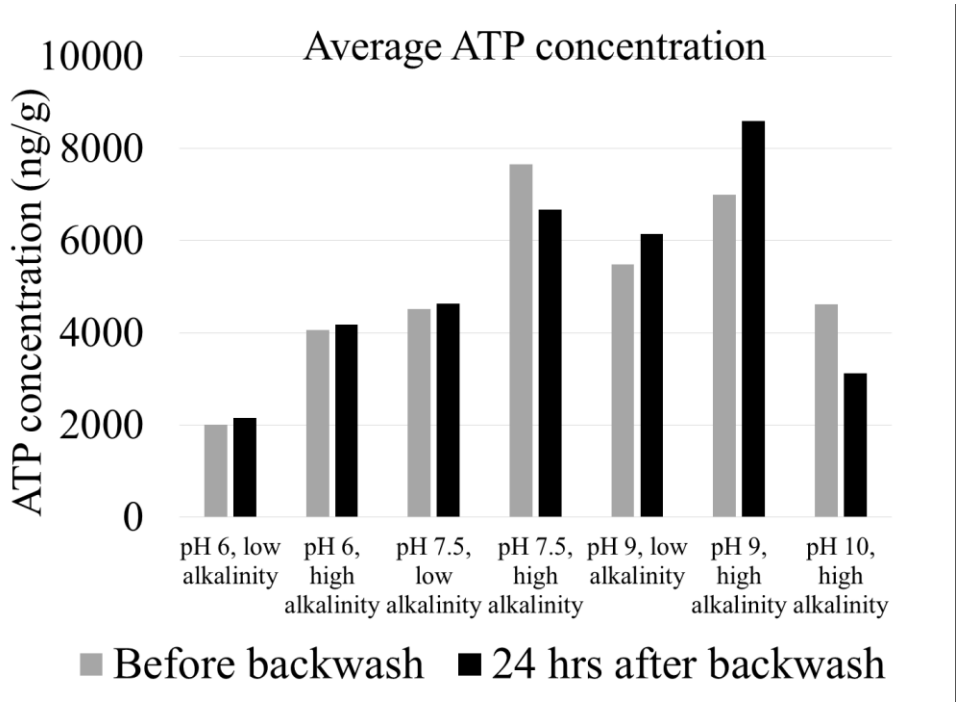

Figure 7. 5: Effect of backwash on ATP concentration

7.11. Appendix K- SDI Results

Table 7. 7: Effect of pH and alkalinity adjustment on SDI value

Exp.	BF1				BF2			
	t_i (sec)	t_f(sec)	T (min)	SDI	t_i (sec)	t_f(sec)	T (min)	SDI
pH 6, high alkalinity	11.28	40.00	5.00	**14.36**	11.04	37.67	5.00	**14.14**
	11.10	38.67		**14.26**	10.80	41.33		**14.77**
	10.89	40.00		**14.56**	11.12	39.00		**14.30**
	11.28	59.67	10.00	**8.11**	11.04	70.67	10.00	**8.44**
	11.10	60.67		**8.17**	10.80	67.67		**8.40**
	10.89	79.00		**8.62**	11.12	65.00		**8.29**
	11.28	97.67	15.00	**5.90**	11.04	94.67	15.00	**5.89**
	11.10	79.33		**5.73**	10.80	93.00		**5.89**
	10.89	100.33		**5.94**	11.12	89.67		**5.84**
pH 7.5, low alkalinity	9.70	39.00	5.00	**15.03**	6.04	41.00	5.00	**17.05**
	9.16	52.00		**16.48**	6.04	42.00		**17.12**
	9.76	54.00		**16.39**	9.36	43.00		**15.65**
	9.70	69.00	10.00	**8.59**	6.04	127.00	10.00	**9.52**
	9.16	69.00		**8.67**	6.04	125.00		**9.52**
	9.76	72.00		**8.64**	9.36	69.00		**8.64**
	9.70	91.00	15.00	**5.96**	6.04	202.00	15.00	**6.47**
	9.16	74.00		**5.84**	6.04	134.00		**6.37**
	9.76	81.00		**5.86**	9.36	87.00		**5.95**
pH 7.5, high alkalinity	10.66	61.07	5.00	**16.51**	12.57	54.31	5.00	**15.37**
	10.33	60.33		**16.58**	13.04	44.67		**14.16**
	12.26	61.67		**16.02**	11.82	55.43		**15.74**
	13.17	74.67		**16.47**	13.16	73.67		**16.43**
	10.66	84.67	10.00	**8.74**	12.57	67.00	10.00	**8.12**
	10.33	84.33		**8.78**	13.04	72.00		**8.19**
	12.26	83.13		**8.53**	11.82	90.03		**8.69**

	13.17	100.67		**8.69**	13.16	138.33		**9.05**
	10.66	121.67	15.00	**6.08**	12.57	88.00	15.00	**5.71**
	10.33	109.00		**6.03**	13.04	93.33		**5.74**
	12.26	113.33		**5.95**	11.82	132.33		**6.07**
	13.17	164.67		**6.13**	13.16	275.00		**6.35**
pH 9, low alkalinity	14.55	63.33	5.00	**15.41**	13.60	60.33	5.00	**15.49**
	13.10	42.86		**13.89**	13.96	57.33		**15.13**
	14.42	71.33		**15.96**	16.12	60.33		**14.66**
	14.55	85.33	10.00	**8.29**	13.60	85.67	10.00	**8.41**
	13.10	68.00		**8.07**	13.96	81.33		**8.28**
	14.42	105.67		**8.64**	16.12	96.67		**8.33**
	14.55	114.33	15.00	**5.82**	13.60	117.33	15.00	**5.89**
	13.10	87.67		**5.67**	13.96	103.80		**5.77**
	14.42	156.33		**6.05**	16.12	150.67		**5.95**
pH 9, high alkalinity	15.72	58.33	5.00	**13.36**	13.36	49.67	5.00	**13.36**
	19.64	65.93		**10.80**	10.80	38.40		**10.80**
	12.84	51.91		**9.37**	9.54	35.21		**9.37**
	15.72	85.33	10.00	**13.36**	13.36	86.00	10.00	**13.36**
	19.64	81.13		**10.80**	10.80	48.30		**10.80**
	12.84	90.33		**9.37**	9.54	55.70		**9.37**
	15.72	122.67	15.00	**13.36**	13.36	124.33	15.00	**13.36**
	19.64	110.00		**10.80**	10.80	74.00		**10.80**
	12.84	154.33		**9.37**	9.54	72.00		**9.37**
pH 10, high alkalinity	18.55	66.00	5.00	**14.38**	13.19	51.33	5.00	**14.86**
	12.00	50.00		**15.20**	11.50	33.00		**13.03**
	14.08	51.80		**14.56**	11.85	43.21		**14.52**
	18.55	102.00	10.00	**8.18**	13.19	78.87	10.00	**8.33**
	12.00	69.67		**8.28**	11.50	58.50		**8.03**
	14.08	75.10		**8.13**	11.85	64.20		**8.15**
	18.55	157.00	15.00	**5.88**	13.19	108.33	15.00	**5.85**

	12.00	87.33		**5.75**	11.50	77.50		**5.68**
	14.08	124.67		**5.91**	11.85	77.47		**5.65**

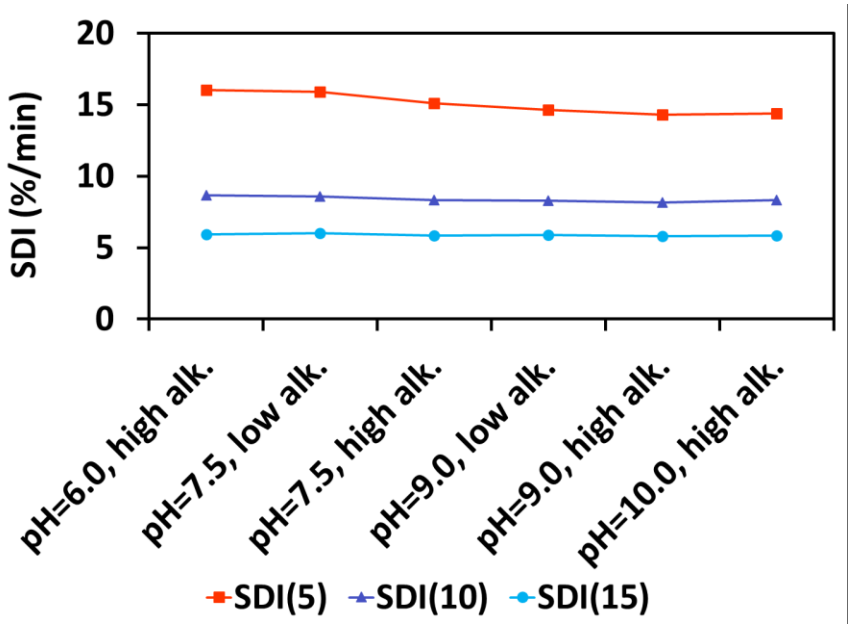

Figure 7. 6: Effect of pH and alkalinity adjustment on SDI value

7.12. Appendix L- Biological Oxidation

Organic matters were represented as CH_3COOH, $C_2H_2O_2$ and CH_2O_2 (carbon source in this study); and new cell was represented as $C_5H_7NO_2$ (Metcalf and Eddy, 2013).

Conversion of organic matter (COD) to simple product (Eq. 7-3, Metcalf and Eddy)

$6\ CH_2O_2 + 2\ O_2 + 0.2\ NH_3 \rightarrow 0.2\ C_5H_7NO_2 + 5\ CO_2 + 5.6\ H_2O$

$3\ CH_3COOH + O_2 + NH_3 \rightarrow C_5H_7NO_2 + CO_2 + 4\ H_2O$

$4\ C_2H_2O_2 + O_2 + NH_3 \rightarrow C_5H_7NO_2 + 3\ CO_2 + 2\ H_2O$

Yield on COD basis:

Balanced stochiometric reaction for oxidation of organic carbon to CO_2:

a) Formic acid

$CH_2O_2 + 1/2\ O_2 \rightarrow CO_2 + H_2O$ *(from Eq. 7-4, Metcalf and Eddy)*

The COD of substrate (acetic acid) is:

$$COD = \frac{\Delta(O2)}{\Delta\ (CH2O2)} = \frac{1/2 \times (32)\ gr\ O2}{46.03\ gr\ CH2O2} = 0.35\ \frac{gr\ O2}{gr\ CH2O2} = 0.35\ \frac{gr\ COD}{gr\ CH2O2}$$

$C_5H_7NO_2 + 5\ O_2 \rightarrow 5\ CO_2 + NH_3 + 2\ H_2O$ *(Eq. 7-5, Metcalf and Eddy)*

The COD of cell tissue is:

$$COD = \frac{\Delta(O2)}{\Delta\ (C5H7NO2\ )} = \frac{5 \times (32)\ gr\ O2}{1\ \times (113)\ gr\ C5H7NO2} = 1.42\ \frac{gr\ O2}{gr\ C5H7NO2}$$

$COD_r = COD_{cell} + COD_{ox}$

COD_{ox}: oxygen consumption

COD_r: $COD_{utilized}$

Oxygen consumed: $COD_{utilized} - COD_{cell}$

$$\left(\frac{0.35\ gr\ O2}{gr\ CH2O2} \times 6\ mole \times \frac{46.03\ gr\ CH2O2}{1\ mole}\right) - \left(\frac{1.42\ gr\ O2}{gr\ \text{cell}} \times 0.2\ mole \times \frac{113\ gr\ cell}{1\ mole}\right)$$

$$= 64.57\ gr\ O2$$

$$\frac{oxygen\ consumed}{substrate\ as\ COD} = \frac{64.57\ gr\ O2}{\frac{0.35\ gr\ COD}{gr\ CH2O2} \times \frac{46.03\ gr\ CH2O2}{1\ mole} \times 6\ mole} = 0.668\ \frac{gr\ O2}{COD\ used}$$

b) Acetic acid

$CH_3COOH + 2\ O_2 \rightarrow 2\ CO_2 + 2\ H_2O$ *(from Eq. 7-4, Metcalf and Eddy)*

The COD of substrate (acetic acid) is:

$$COD = \frac{\Delta(O2)}{\Delta\ (CH3COOH)} = \frac{2 \times (32)\ gr\ O2}{60.05\ gr\ CH3COOH} = 1.07\ \frac{gr\ O2}{gr\ CH3COOH} = 1.07\ \frac{gr\ COD}{gr\ CH3COOH}$$

$C_5H_7NO_2 + 5\ O_2 \rightarrow 5\ CO_2 + NH_3 + 2\ H_2O$ *(Eq. 7-5, Metcalf and Eddy)*

Similarly, the COD of cell tissue is $1.42\ \frac{gr\ O2}{gr\ C5H7NO2}$

Oxygen consumed: $COD_{utilized} - COD_{cell}$

$$\left(\frac{1.07\ gr\ O2}{gr\ CH3COOH} \times 3\ mole \times \frac{60.05\ gr\ CH3COOH}{1\ mole}\right) - \left(\frac{1.42\ gr\ O2}{gr\ \text{cell}} \times \frac{113\ gr\ cell}{1\ mole}\right) = 31.58\ gr\ O2$$

$$\frac{oxygen\ consumed}{substrate\ as\ COD} = \frac{31.58\ gr\ O2}{\frac{1.07\ gr\ COD}{gr\ CH3COOH} \times \frac{60.05\ gr\ CH3COOH}{1\ mole} \times 3\ mole}$$

$$= 0.164\ \frac{gr\ O2}{COD\ used}$$

c) Glyoxal

$C_2H_2O_2 + 3/2\ O_2 \rightarrow 2\ CO_2 + H_2O$

The COD of substrate (glyoxal) is:

$$COD = \frac{\Delta(O2)}{\Delta\ (\mathrm{C2H2O2})} = \frac{3/2 \times (32)\ gr\ \mathrm{O2}}{58.04\ gr\ \mathrm{C2H2O2}} = 0.83\ \frac{gr\ \mathrm{O2}}{gr\ \mathrm{C2H2O2}} = 0.83\ \frac{gr\ COD}{gr\ \mathrm{C2H2O2}}$$

$C_5H_7NO_2 + 5\ O_2 \rightarrow 5\ CO_2 + NH_3 + 2\ H_2O$

Similarly, the COD of cell tissue is $1.42\ \frac{gr\ \mathrm{O2}}{gr\ \mathrm{C5H7NO2}}$

Oxygen consumed: $\mathrm{COD}_{\text{utilized}} - \mathrm{COD}_{\text{cell}}$

$$\left(\frac{0.83\ gr\ \mathrm{O2}}{gr\ \mathrm{C2H2O2}} \times 4\ mole \times \frac{58.04\ gr\ \mathrm{C2H2O2}}{1\ mole}\right) - \left(\frac{1.42\ gr\ \mathrm{O2}}{gr\ \mathrm{cell}} \times \frac{113\ gr\ cell}{1\ mole}\right) = 31.77\ gr\ O2$$

$$\frac{oxygen\ consumed}{substrate\ as\ COD} = \frac{31.77\ gr\ O2}{\frac{0.83\ gr\ COD}{gr\ \mathrm{C2H2O2}} \times \frac{58.04\ gr\ \mathrm{C2H2O2}}{1\ mole} \times 4\ mole}$$

$$= 0.165\ \frac{gr\ O2}{gr\ COD\ used}$$

Finally, we can make an average between calculated oxygen requirement.

In our study carbon was supplied in equal portion from each carbon sources, thus required oxygen is 0.332 gr/ removed carbon (gr)

7.13. Appendix L- Biological Oxidation

Table 7. 8: Effluent pH variation under different experimental conditions

Experimental condition	average influent pH	average effluent pH
pH=6.0, low alk.	6.00	6.15
pH=6.0, high alk.	6.05	6.04
pH=7.5, low alk.	7.50	7.00
pH=7.5, high alk.	7.50	7.00
pH=9.0, low alk.	9.00	7.50
pH=9.0, high alk.	9.00	8.10
pH=10.0, high alk.	10.00	9.80

low alk.: 25 - 50 mg $CaCO_3$/L
high alk.: 180 - 220 mg $CaCO_3$/L

7.14. Appendix M- TOC removal versus DO removal

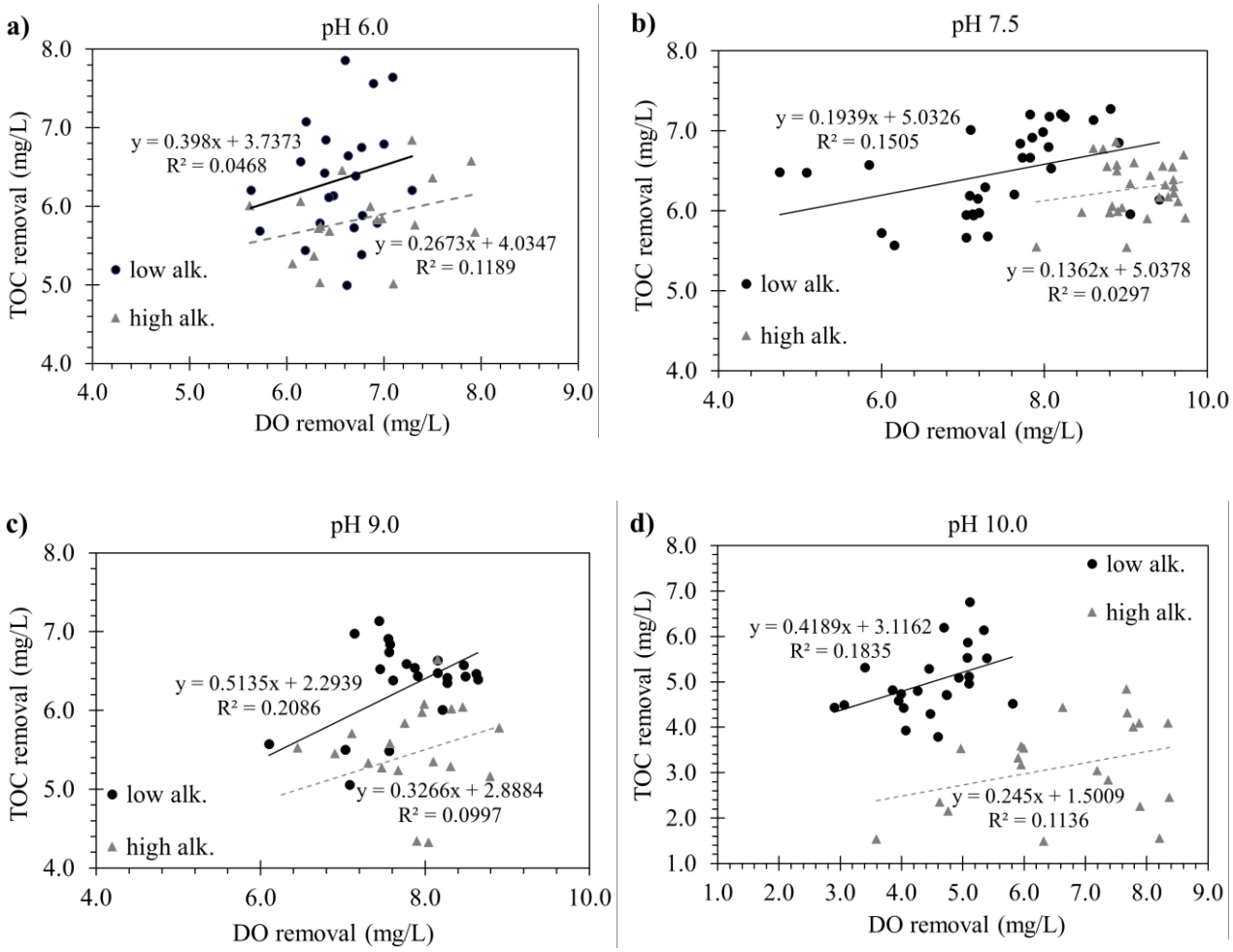

Figure 7. 7: Relation between TOC removal and DO removal, average influent TOC: 9.96 mg/L, influent DO: 5.31 – 10.38 mg/L, HLR: 3 m/h and experimental duration: 17 days

7.15. Appendix N- Calibration Curves

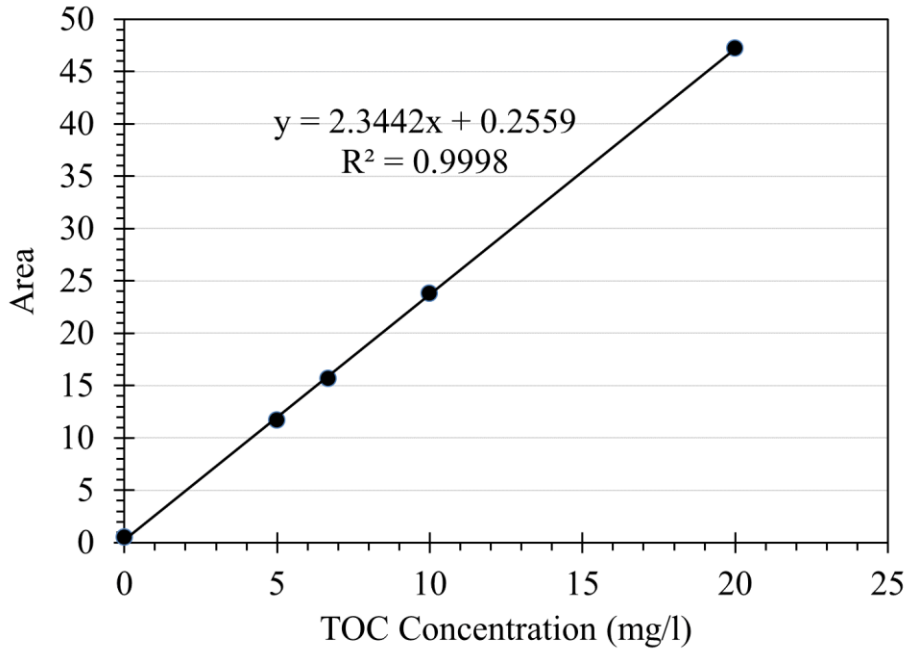

Figure 7. 8: Sample TOC calibration curve

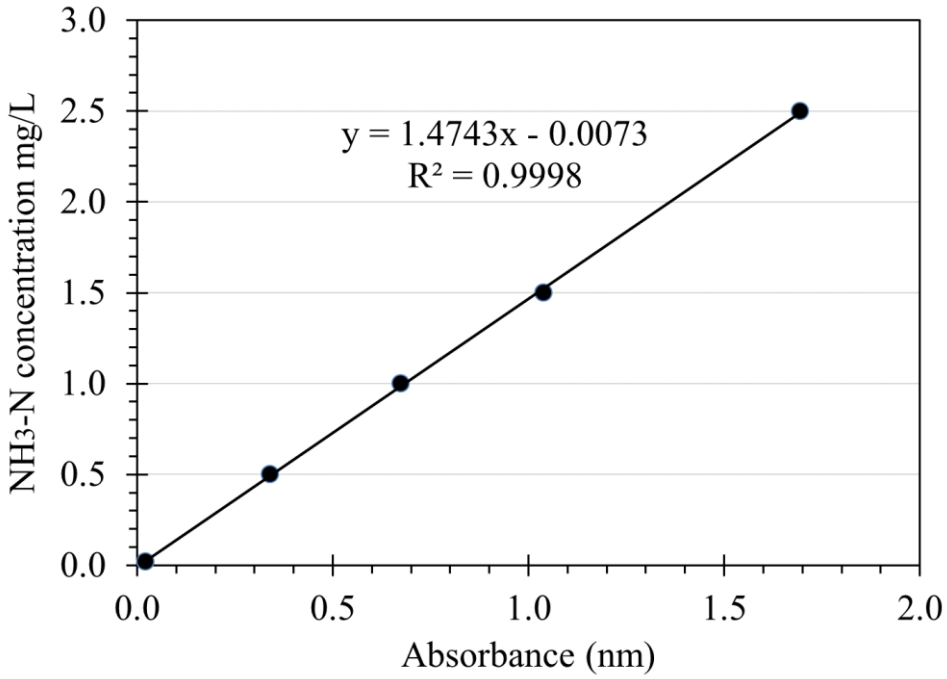

Figure 7. 9: Sample ammonia calibration curve

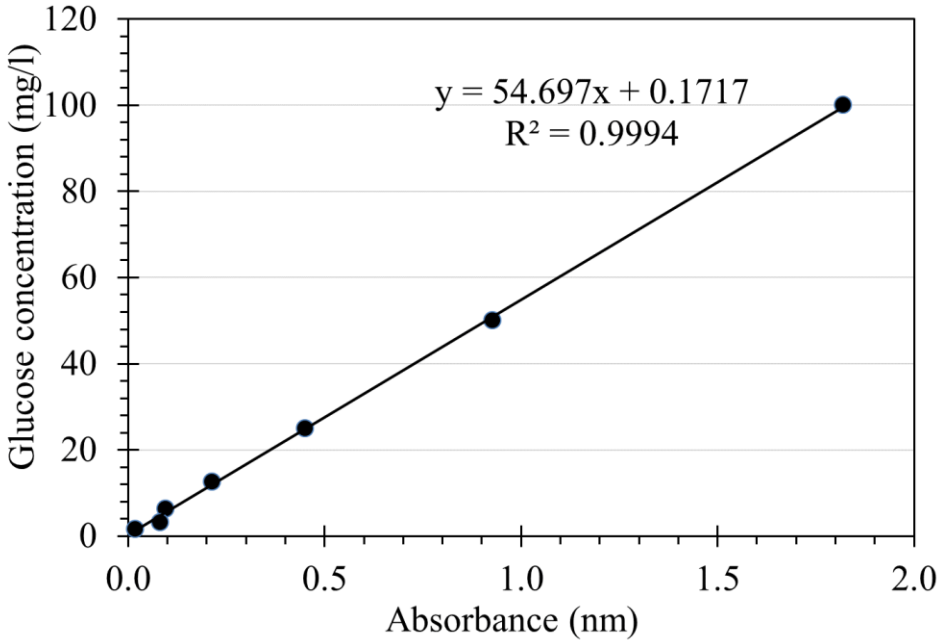

Figure 7. 10: Sample glucose calibration curve

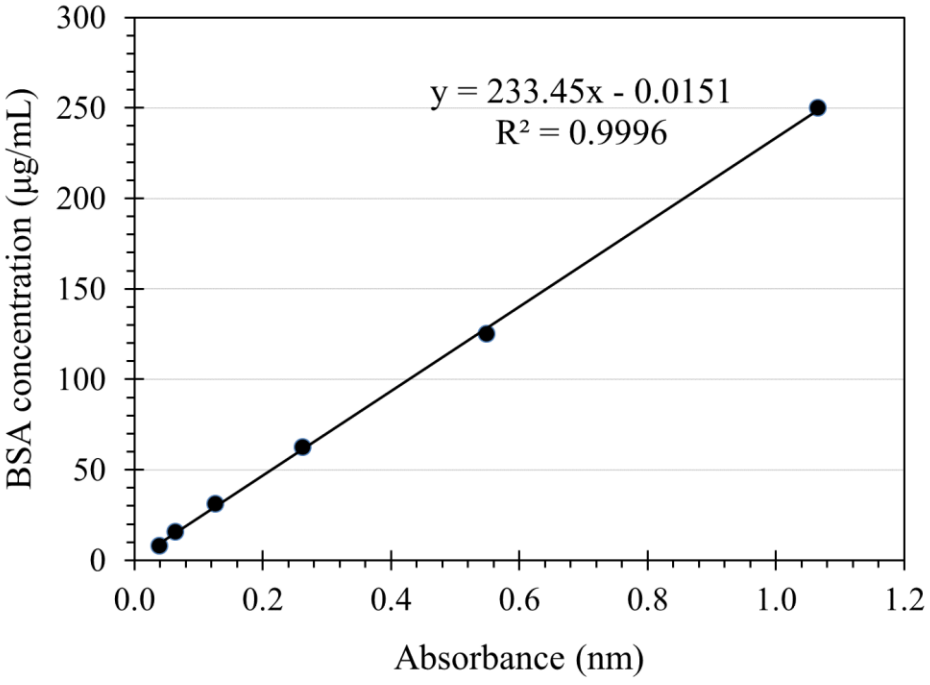

Figure 7. 11: Sample BSA calibration curve