Integrating Ozonation to Lagoon Treatment: lab-scale bioreactor methodologies and pilot-scale preliminary costs

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Abstract

Lagoon systems constitute an important part of wastewater treatment infrastructure in Canada and they are common in small and remote communities, especially in First Nations (FN) communities. In fact, more than 50% of wastewater treatment systems in FN communities are based on facultative and aerobic lagoons. Although simple and low cost, these passive treatment systems depend almost entirely on the natural biodegradation power of microorganisms that develop inside, which are dictated by the surrounding climate conditions. Therefore, in regions subject to seasonal variability such as Canada, a lagoon's performance is challenged, and its treatment period is limited. Indeed, a national assessment of FN wastewater infrastructure revealed that a fraction of lagoon stations did not meet federal effluent discharge limits and approximately half of the facultative lagoons were not reporting any effluent discharge data. Consequently, the new wastewater systems effluent regulations that were implemented in June 2012 provided an incentive for FNs to develop long-term strategies that focus on enforcing standards and protocols, operator training and infrastructure investments.

A solution that has been proposed by the 3Cs laboratory to improve the performance of lagoon systems is the integration of low dose ozonation to increase the biodegradability of wastewater, while preserving microbial populations responsible for its treatment. In recent previous work, two pilot tests were initiated during which a 15% volume fraction of a lagoon was ozonated at a low dose in order to accelerate organic matter degradation. Preliminary results were promising, but due to the difficulty in controlling and predicting lagoon conditions, further investigation was required to confirm the role of ozone in the pilot tests. Therefore, the objective of this thesis was to study the role of ozone in the pilot tests by developing a testing strategy at laboratory scale to investigate and optimize the integration of ozonation to lagoon systems for enhancement of wastewater quality. Additionally, a preliminary economic analysis was performed to assess the costs involved in integrating ozonation to lagoon systems at pilot-scale.

The experimental design focused on simulating the aerobic conditions in a lagoon system by performing a biological treatment in a bench-scale bioreactor. This setup was operated batch-wise to treat synthetic wastewater using pre-incubated raw sludge collected at a local lagoon station. Each experiment was carried out for a duration of 12 hours and involved ozonating 25% of the bioreactor contents at various ozone doses during the different growth phases exhibited by bacteria (i.e. lag, exponential and stationary). The ozonated portion was then returned to the bioreactor and the biological treatment was resumed. The impact on COD removal rates, bacterial growth and biodegradability of several emerging contaminants of concern (carbamazepine, atrazine, ibuprofen, naproxen and gemfibrozil) was evaluated. During the method development phase, it was found that performing ozonation towards the end of the lag phase (t = 5.5 hours) generated a response in the biomass growth and removal of organic matter. In fact, in experiments conducted in 100% synthetic wastewater, ozonating 25% of the volume at a dose of 10 mg O_3/L increased the biomass specific growth rate to 0.671 hr^{-1} from 0.603hr^{-1}, which was recorded during the control run. Similarly, a higher removal of COD was observed, as indicated by a final COD/COD_o ratio of 0.68 as opposed to 0.73 in the control run. When using a 50% mixture of raw and synthetic wastewater to consider a more complex matrix, the specific growth rate was again increased to 0.439 hr^- compared to 0.306 hr^{-1} in the control run. The final COD/COD_o ratio followed a similar

trend with values of 0.88 and 0.80 in the control and ozonation experiments, respectively. These results suggest that early ozonation might enhance biomass growth, which eventually facilitates organic matter uptake. For the contaminants of emerging concern, although partial ozonation led to significant removal of certain compounds, it did not promote further biological degradation. However, in order to capture the full potential of low dose ozonation integration to a lagoon system, it would be beneficial to conduct the experiments presented in this thesis at a wider range of ozone doses and environmental conditions, and preferably in raw wastewater.

Finally, the preliminary economic analysis indicated that the use of a shared ozonation unit between lagoon stations is a low-cost option compared to other conventional alternatives. However, for lagoons that require significant improvement in treatment efficiency or that are severely undersized, it seems that this strategy, although very economical, may not be sufficient. If the results obtained are supported by more experiments, low dose ozonation could potentially be used as a short-term solution to help lagoon system managers to reach compliance.

Résumé

Les lagunages constituent une partie importante de l'infrastructure de traitement des eaux usées au Canada et ils sont courants dans les petites collectivités éloignées, en particulier dans les collectivités des Premières Nations (PN). En fait, plus de 50% des systèmes de traitement des eaux usées dans les communautés des PN sont basés sur des lagunes facultatives et aérobies. Bien que simples et peu coûteux, ces systèmes de traitement passifs dépendent presque entièrement du pouvoir de biodégradation naturelle des micro-organismes qui s'y développent, qui sont influencés par les conditions climatiques environnantes. Par conséquent, dans les régions sujettes à de grandes variations saisonnières comme le Canada, la performance d'une lagune est mise à l'épreuve et sa période de traitement efficace est limitée. En effet, une évaluation nationale de l'infrastructure des eaux usées des PN a révélé qu'une fraction des stations lagunaires ne respectaient pas les limites fédérales de rejet d'effluent et qu'environ la moitié des lagunes facultatives ne rapportaient aucune donnée sur les rejets d'effluents. Par conséquent, le nouveau règlement sur les effluents des systèmes de traitement des eaux usées qui a été mis en œuvre en juin 2012 a incité les PN à élaborer des stratégies à long terme axées sur l'application des normes et des protocoles, la formation des opérateurs et les investissements dans les infrastructures.

Une solution qui a été proposée par notre laboratoire pour améliorer les performances des systèmes lagunaires est l'intégration d'une ozonation à faible dose aux lagunes pour augmenter la biodégradabilité des eaux usées, tout en préservant les populations microbiennes responsables de leur traitement. Lors de récents travaux antérieurs, deux essais pilotes ont été effectués au cours desquels une fraction volumique de 15% d'une lagune a été ozonée à faible dose afin d'accélérer la dégradation de la matière organique. Les résultats préliminaires étaient prometteurs, mais en raison de la difficulté à contrôler et à prévoir les conditions de la lagune, une étude plus approfondie était nécessaire pour confirmer le rôle de l'ozone dans les essais pilotes. Par conséquent, l'objectif de cette thèse était d'étudier le rôle de l'ozone en développant une stratégie d'essais à l'échelle de laboratoire pour étudier et optimiser l'intégration de l'ozonation aux systèmes lagunaires pour l'amélioration de la qualité des eaux traitées. De plus, une analyse économique préliminaire a été réalisée pour évaluer les coûts liés à l'intégration de l'ozonation aux systèmes lagunaires.

La conception expérimentale a été centrée sur la simulation des conditions aérobies dans un système lagunaire en effectuant un traitement biologique dans un bioréacteur de laboratoire. Les expériences ont été menées de manière discontinue pour traiter les eaux usées synthétiques à l'aide de boues brutes pré-incubées collectées dans une station lagunaire locale. Chaque expérience a été réalisée pendant une durée de 12 heures et impliquait l'ozonation de 25% du contenu du bioréacteur à différentes doses d'ozone au cours des différentes phases de croissance des bactéries (c'est-à-dire latence, exponentielle et stationnaire). La partie ozonée était ensuite remise dans le bioréacteur pour poursuivre le traitement biologique. L'impact sur les taux d'élimination de la DCO, la croissance bactérienne et la biodégradabilité de contaminants préoccupants (carbamazépine, atrazine, ibuprofène, naproxène et gemfibrozil) a été évalué. Au cours de la phase de développement de la méthode, il a été constaté que l'exécution de l'ozonation vers la fin de la phase de latence (t = 5,5 heures) génère une réponse dans la croissance de la biomasse et l'élimination de la matière organique. En fait, dans des expériences menées dans des eaux usées 100% synthétiques,

l'ozonation de 25% du volume à une dose de 10 mg O_3 / L a augmenté le taux de croissance spécifique de la biomasse à 0,671 h^{-1} comparativement à 0,603 h^{-1} observé pour le contrôle. De même, une élimination plus élevée de la DCO a été observée, comme indiqué par un rapport final DCO/DCO_o de 0,68 au lieu de 0,73 pour le contrôle. Lors de l'utilisation d'un mélange à 50% d'eaux usées brutes et synthétiques pour considérer une matrice plus complexe, le taux de croissance spécifique a de nouveau été augmenté à 0,439 h^{-1} comparativement à 0,306 h^{-1} pour le contrôle. Le rapport final DCO/DCO_o a suivi une tendance similaire avec des valeurs de 0,88 et 0,80 dans les expériences de contrôle et d'ozonation, respectivement. Ces résultats suggèrent que l'ozonation précoce pourrait améliorer la croissance de la biomasse, ce qui facilite finalement l'absorption de matière organique. Pour les contaminants d'intérêt émergent, bien que l'ozonation partielle ait conduit à une élimination significative de certains composés, elle n'a pas favorisé une dégradation biologique supplémentaire. Cependant, afin de capter tout le potentiel de l'intégration d'ozonation à faible dose dans un système lagunaire, il serait bénéfique de mener les expériences présentées dans cette thèse en utilisant une plus large gamme de doses d'ozone et de conditions environnementales, et de préférence dans les eaux usées brutes.

Enfin, l'analyse économique préliminaire a indiqué que l'utilisation d'une unité d'ozonation partagée entre les stations lagunaires est une option peu coûteuse par rapport aux autres alternatives conventionnelles. Cependant, pour les lagunes qui nécessitent une amélioration significative de l'efficacité du traitement ou qui sont gravement sous-dimensionnées, il semble que cette stratégie, bien que très économique, puisse ne pas être suffisante. Si les résultats obtenus sont étayés par davantage d'expériences, l'ozonation à faible dose pourrait potentiellement être utilisée comme solution à court terme pour aider les gestionnaires du système lagunaire à atteindre la conformité.

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Abbreviations

Abbreviation	Definition
BOD	Biodegradable Oxygen Demand (mg/L)
cBOD	Carbonaceous Biodegradable Oxygen Demand (mg/L)
cBOD-5	cBOD measured over 5 days (mg/L)
COD	Chemical Oxygen Demand (mg/L)
DO	Dissolved Oxygen (mg/L)
DO_{sat}	Dissolved Oxygen at saturation(mg/L)
TSS	Total Suspended Solids (mg/L)
TDS	Total Dissolved Solids (mg/L)
FN	First Nations
WW	Wastewater
SWW	Synthetic Wastewater
WSERs	Wastewater Systems Effluent Regulations
TAN	Total Ammonia Nitrogen
CECs	Contaminants of Emerging Concern
WWTP	Wastewater Treatment Plant
TKN	Total Kjeldahl Nitrogen (mg/L)
MLVSS	Mixed Liquor Volatile Suspended Solids (mg/L)
WWTP	Wastewater Treatment Plant
OD_{600}	Optical density at 600 nm
DOM	Dissolved organic matter
CAPEX	Capital expenditure
OPEX	Operational expenditure

1 Introduction

1.1 Background

The ever-growing environmental pressures of managing domestic wastewater is a pressing matter for smaller and remote communities in Canada, especially for First Nations (FN) communities. Due to financial restrictions and lack of human resources, many of them opt to treat their wastewater using lagoons as opposed to other conventional wastewater treatment such as activated sludge. Lagoon systems are comprised of one or more pond-like bodies of water that receive, hold and treat wastewater through natural processes (Metcalf & Eddy et al., 2013).

As a response to the pervasive and longstanding issues of water infrastructure quality and maintenance, the Government of Canada introduced the First Nations Water and Wastewater Action Plan (Islam & Yuan, 2018). This was based on a 2011 extensive study done by Neegan Burnside in which 97% of First Nations participated. A total of 532 wastewater systems serving 418 First Nations were examined. It was found that lagoons are the most common type of treatment, of which 41% are facultative lagoons and 11% are aerated lagoons (Neegan Burnside, 2011). This national assessment revealed that 18% of lagoon-based treatments did not meet federal effluent discharge limits and that 24% of the aerated lagoons and 47% of the facultative lagoons were not reporting any effluent discharge data (Neegan Burnside, 2011). Consequently, the new wastewater systems effluent regulations (WSER) that were implemented in June 2012 provided an incentive for FNs to develop long-term strategies that focus on enforcing standards and protocols, operator training and infrastructure investments (Minister of Justice, 2012). These regulations, shown in ***Table 1***, were put in place for wastewater treatment systems that are designed to discharge wastewater effluent to natural receiving waters at a daily influent volume of 100 m^3 per day or more, which is the case for many FN lagoons (Minister of Justice, 2012).

The WSER limits came into force on January 1, 2015, but the monitoring provisions came into effect on January 1, 2013. According to a latest study led by Neegan Burnside in 2014-2015, 6% of wastewater systems were identified as high risk and 41% as medium risk as opposed to 14 and 51% in 2011, respectively (Islam & Yuan, 2018). This risk analysis used was based on five elemental weighing risks of effluent discharge (20%), system design (25%), operations (25%), reporting (10%), and operators (20%) of the respective wastewater treatment facility (INAC, 2011). Overall, that assessment indicated that many municipalities were still struggling to adapt their wastewater systems in order to follow the newly established regulations. However, the timeline to for these municipalities to upgrade their wastewater treatment infrastructure is until 2040.

Table 1. Wastewater Effluent Quality Objectives (Minister of Justice, 2012)

Parameter	Objective	Source
cBOD-5	25 mg/L	Provincial
TSS	25 mg/L	Provincial
Unionized ammonia expressed as nitrogen	1.25 mg/L	Federal

Therefore, considering the widespread use of sewage lagoons across First Nations in Canada coupled with a growing demand for safer and cleaner water, there is a need to investigate new affordable and easily scalable processes to improve the effluent quality of municipal wastewater from lagoon-based treatments.

1.2 Previous work

A solution that has been proposed by our laboratory was the integration of low dose ozonation to lagoons as an integrated system to increase the biodegradability of wastewater (Larcher & Yargeau, 2013; Schlageter, 2018). Ozone is an extremely reactive oxidant and is typically used as a disinfection step after biological treatment (Metcalf & Eddy et al., 2013) but it can also be used to speed up biological processes by partially oxidizing compounds found in wastewater, with the goal of increasing their biodegradability in the subsequent biological treatment (Gottschalk, Libra, & Saupe, 2008). Therefore, it was hypothesized that integrating ozonation to lagoons might facilitate the assimilation of contaminants by microorganisms and ultimately assist municipalities in reaching the required effluent discharge limits.

This project is a continuation of Beauregard Schlageter's Master's thesis (Schlageter, 2018) supervised by Prof. Yargeau, which consisted primarily of conducting two pilot tests of integrating ozone to lagoon treatment. The pilot tests were carried out at two FN communities in Ontario where 15% of the volume of the secondary lagoons was ozonated prior to seasonal discharge. These were conducted in partnership with Aclarus Ozone Water Systems and Trent University. At both locations, results confirmed that statistically significant decreases in BOD and total ammonia were observed. In addition, some preliminary ozonation laboratory experiments were performed on synthetic wastewater (SWW) at various ozone doses with a mix of three non-pathogenic bacterial species often found in wastewater effluent. The aim was to attempt to emulate lagoon conditions. However, the results obtained demonstrated that no significant changes were observed for the parameters that actually decreased during the pilot tests and revealed issues with the methodology used to mimic the biological treatment at lab-scale.

1.3 Objectives

Based on the previous work presented above, further investigation was required to confirm the role of ozone in the pilot tests and obtain a suitable methodology for lab-scale investigation of strategies to integrate ozone to lagoon treatment. Therefore, the specific objectives of this research were to:

1. **Develop a testing strategy at laboratory scale to investigate and optimize the integration of ozonation to lagoon systems for enhancement of wastewater quality.**

2. **Perform a preliminary financial analysis to assess the economic feasibility of integrating ozonation to lagoon systems.**

The main challenges that were addressed throughout the development of the methodology involved obtaining a consortium of microorganisms that would be representative of the biomass present in a lagoon and optimizing the use of ozone so that it enhances biodegradability without preventing biodegradation from occurring.

2 Literature Review

2.1 Lagoon Treatment Systems

2.1.1 Fate of suspended solids, organic matter and ammonia in lagoons

Lagoons are commonly used as passive treatment systems for municipal wastewater in remote communities due to their low cost where land is available, simple design and operation, and minimal operator expertise required. The complex natural ecosystem that develops in a facultative lagoon is fundamental to its use for wastewater treatment. The incoming wastewater is treated naturally through a combination of physical, biological and chemical processes. This type of treatment mainly favours the partial removal of suspended solids (TSS) through settling and the removal of biodegradable material (BOD) through degradation by various bacteria and microorganisms present in the lagoon. Suspended solids are particles present water that can be classified into various groups depending of their source; living or nonliving, size, mineral or organic, dispersed as individual entities or associated in flocs (Zucker et al., 2015).

The importance of removing these two contaminants (i.e. TSS and BOD) is derived from the negative impacts they have on the aquatic environment. For instance, a discharge of waste containing high levels of BOD into water leads to an increased oxygen uptake by bacteria necessary for the degradation of the incoming organic waste. This leads to a depletion of oxygen in the water creating anoxic conditions and killing other aquatic species. As for the suspended solids, they can increase the turbidity of water, which affects light penetration and can potentially reduce photosynthesis leading to lower daytime release of oxygen. As well, fine particles can damage sensitive gill structures of fish.

Although lagoons were not initially designed for ammonia removal, they are now required by federal regulations to decrease the level of unionized ammonia to 1.25 mg-N/L or less. Nitrogen is introduced into a lagoon via the influent water which usually contains organic nitrogen N from fecal matter and other organic material. Through microbial activity, this organic nitrogen gets converted into unionized ammonia NH_3 and ammonium ion NH_4 +, which can be measured as the total ammonia nitrogen (TAN). This process is termed ammonification. Unionized ammonia can react with water to form the ammonium ion as presented in the chemical equilibrium in equation 1. This equilibrium is pH and temperature dependent, with higher pH value (i.e. pH levels above 9) and temperature favouring the formation of unionized ammonia (Rezagama, Hibbaan, & Arief Budihardjo, 2017). This latter form is toxic to aquatic organisms because it is uncharged and lipid soluble which allows it to permeate through biological membranes more easily (Körner, Das, Veenstra, & Vermaat, 2001).

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^- \quad (1)$$

2.1.2 Natural processes occurring in a lagoon

Since a facultative lagoon is not artificially aerated, both aerobic and anaerobic conditions coexist, and three distinct layers form naturally as seen in **Figure 1**. The top layer is the aerobic zone where most of the dissolved oxygen is introduced through algae photosynthesis, and the mixing of the water surface by wind or rain. An equilibrium is established between algae that utilize sunlight, carbon dioxide along with nutrients and ammonia to release oxygen, and bacteria that utilize this oxygen to metabolize various biodegradable matter present in the wastewater into nutrients, releasing carbon dioxide. The dead algae and bacteria cells re-enter the food chain as organic matter that is to be degraded by various microorganisms. Therefore, aerobic treatment of the wastewater in this layer provides odor control, nutrient and BOD removal (EPA, 2011). The depth of this layer is dependent on the climate, the amount of sunlight and wind, and the growth of algae (Casey, Knott, Hause, Favley, & Gloyd, 1997).

The bottom layer is the anaerobic zone where no oxygen is present. This zone also includes a layer of sludge which forms due to the settling of suspended solids present in the wastewater. In this layer, anaerobic bacteria utilize incoming organic material and metabolize it to various organic acids and emit carbon dioxide, methane, ammonia and hydrogen sulfide. The anaerobic treatment of the wastewater involves processes such as sludge digestion, denitrification and some BOD removal (EPA, 2011).

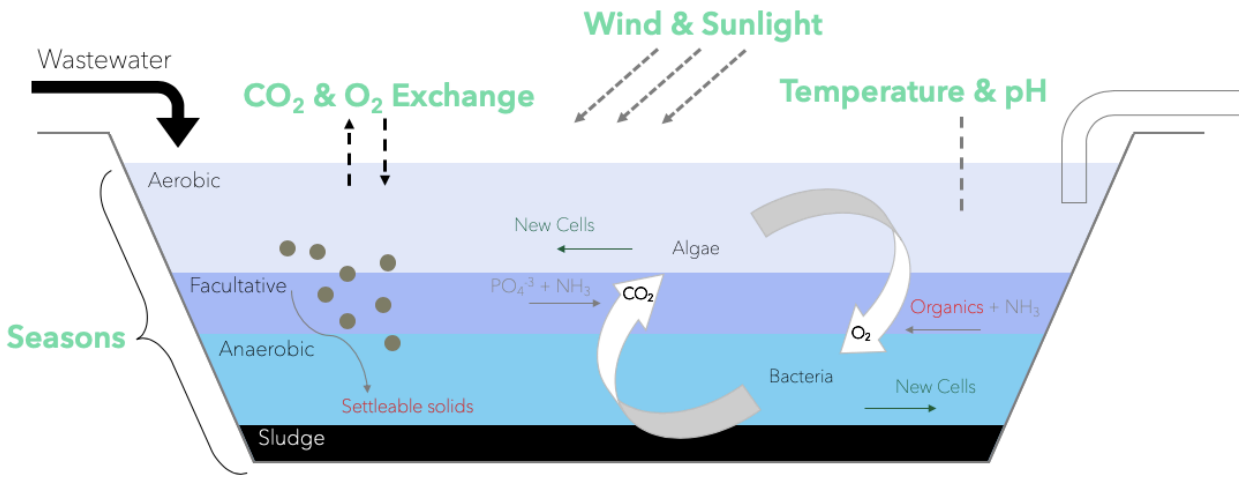

Figure 1. Biochemistry of a wastewater lagoon system, adapted from (Smith Engineering, 1995)

The middle zone is the transition between the aerobic and the anaerobic layers where both of these conditions exist. To avoid leaks to the groundwater below, lagoons are usually lined with material such as clay or an artificial liner.

The microbial populations in a lagoon are loosely grouped as heterotrophs as they metabolize mainly organic carbon, as well as a portion of nitrogen and other nutrients required for cell growth. Heterotrophs have a very fast growth rate which renders them very resilient to environmental stresses. The bacteria responsible for uptaking most of the nitrogen are autotrophs and are classified as nitrifying bacteria. They have a much slower growth rate compared to heterotrophs

which makes them more sensitive to stresses such as seasonal change in temperature (i.e. winter and spring snowmelt). In fact, efficiency of nitrification drops significantly at low temperatures (Water Pollution Control Federation, 1998).

The mechanisms through which NH_3 is removed from lagoons are:

1. **Volatilization**

   Unionized ammonia is gaseous and will volatilize. The degree of volatilization is dependent on the fraction of unionized ammonia in the lagoon which is pH and temperature dependent as mentioned above. Mixing conditions will affect the magnitude of the mass transfer. Therefore, at lower temperatures when biological activity is decreased and the lagoon water is well mixed due to wind, the main process of ammonia removal is volatilization (EPA, 2011).

2. **Biological nitrification**

   This process involves the biological sequential oxidation of ammonia into nitrite NO_2^- and nitrate NO_3^- as presented in equation 2 and 3:

$$NH_3 + O_2 \rightleftharpoons NO_2^- + 3H^+ + e^- \quad (2)$$

$$NO_2^- + H_2O \rightleftharpoons NO_3^- + 2H^+ + e^- \quad (3)$$

   The bacteria associated with the first step are *Nitrosomonas* and those associated with the second step are *Nitrobacter* (EPA, 2002).

   It should be noted that nitrification will only occur once cBOD levels are low as nitrifying bacteria do not compete well against BOD-removing bacteria (Smith Engineering, 1995).

   A portion of the nitrate produced can undergo denitrification which reduces it into nitrogen gas (N_2) as depicted in equation 4. Some of the species of bacteria involved in this process include *Pseudomonas*, *Micrococcus*, *Achromobacter* and *Bacillus* (EPA, 2011).

$$6NO_3^- + 5CH_3OH \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \quad (4)$$

   These reactions are taking place within the bottom sediments under anoxic conditions in facultative lagoons and are affected by temperature, redox potential and sediment characteristics. In lagoons equipped with adequate mixing and aeration, denitrification is negligible (EPA, 2011).

   As well, the low concentrations of nitrates/nitrites measured in lagoon effluents indicate that nitrification is generally not a significant process in terms of ammonia removal from lagoons (Pano & Middlebrooks, 1982). However, the presence of nitrates in a natural body

of water can be detrimental to its ecosystem as it is assimilated by algae and stimulates eutrophication.

3. **Assimilation into biomass**

   The NO_3^- that is produced during the nitrification process as well as a portion of NH_4^+ can be taken up by organisms to produce N-containing compounds such as proteins. Ammonia assimilation in algal biomass could also account for a significant portion of its removal given adequate conditions. It should be noted that this type of removal may result in apparent reductions in ammonia, but unless algae is removed from the water, once the algae starts to decay, whatever it had assimilated will get released back into the water body.

All of these processes are affected by temperature, DO concentration, pH levels, retention times and wastewater characteristics. It is important to note that seasonal variations have a direct impact on microbial and algae growth which subsequently affects the conversion of organic nitrogen into its other forms.

Finally, the wastewater is kept inside the lagoon until the quality parameters meet the required criteria. The wastewater is then discharged to natural water bodies.

2.1.3 Factors affecting lagoon treatment performance

As the use of a lagoon is mainly dictated by natural biological processes, its performance is highly dependent on factors such as temperature, sunlight, dissolved oxygen and pH. These factors are in turn affected by seasonal changes, which consequently leads to limitations in cold- climate lagoons. In regions subject to colder weather, lagoons may spend several months per year covered by an ice sheet blocking the diffusion of oxygen subsequently inhibiting aerobic processes. Below freezing temperatures along with the lack of sunlight also slow down microbial activity. Therefore, the performance of a cold-climate lagoon is tightly related to seasonal changes.

One of the immediate consequences of operating a lagoon in a cold-climate region such as Canada is the discharge schedule, which is intermittent, and typically occurring once a year. The discharge may occur in spring to take advantage of the higher flow rates from the receiving waters, or during summer/early fall as this period provides a higher quality effluent in terms of BOD removal, but may have a higher TSS concentration due to algae (Tilsworth & Smith, 1984). However, discharging the lagoon once per year means that the wastewater must be retained in the lagoon for one year which requires the use of a large land area.

The fundamental parameter that enables a lagoon to be used as a wastewater treatment system is algal growth as this is simultaneously a nutrient sink and an oxygen provider to heterotrophic/aerobic bacteria that enhance biodegradation of BOD. Once again, in cold-climate regions, algal growth is limited to several months during summer during which efficient BOD removal is provided. **Figure 2** shows how the seasons affect the different layers found in a lagoon.

In spring, the increased sunlight melts the ice sheet covering the lagoon and warms up the top layer of the lagoon which creates convection currents disturbing the thermal stratification that settled in

during the winter months. The increased turbulence results in benthal feedback which is when the sludge accumulated at the bottom containing nutrients and other organic and inorganic material gets released back into the lagoon (Hill, 2015b). This may result in temporarily higher ammonia and BOD levels.

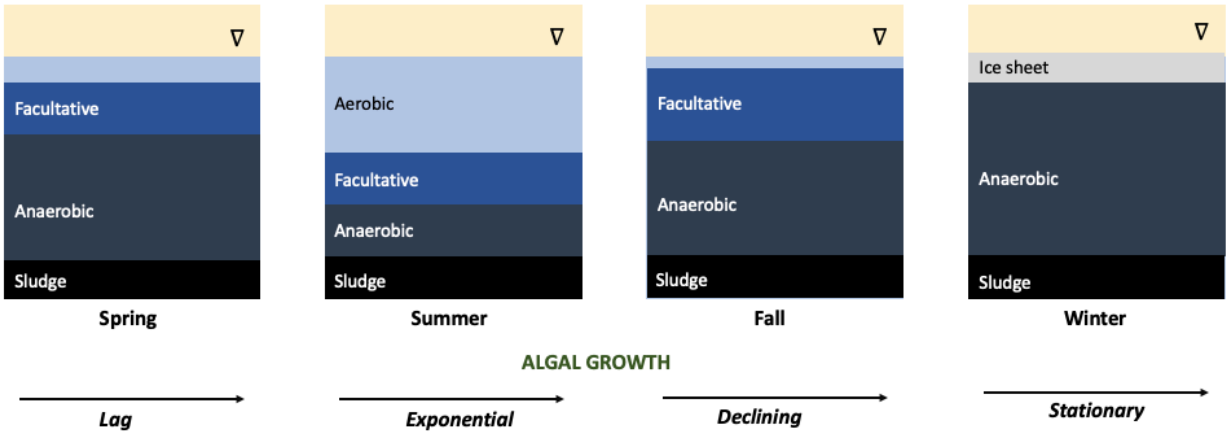

Figure 2. Lagoon layer differentiation due to seasonal variation

During summer, as the amount of sunlight increases and temperature rises, algae proliferates and utilizes the nutrients released from the sludge, releasing oxygen in the lagoon. Most of the biodegradable matter is metabolized by bacteria during this period.

As fall and winter approach, the decrease in temperature can have a considerable effect on lagoon performance (Hill, 2015a):

- **Causes destratification**: as the temperature begins to drop, the top layer of the lagoon gets colder and sinks which displaces the warmer water layer at the bottom to the top;
- **Builds up BOD**: metabolism of bacteria and algae slows down with decreased temperature;
- **Reduces DO**: this is caused by ice covering the lagoon preventing intake of oxygen; and
- **Creates sludge**: BOD degradation is almost halted as the level of DO decreases, therefore allowing easily degradable BOD to settle at the bottom increasing the sludge volume of the lagoon.

2.2 Ozonation

Ozone (O_3) is a highly unstable gas that is generated from oxygen molecules. It is typically produced at the point of use and the most common method used is a corona discharge. Ozone is formed as the electrical discharge ionizes oxygen molecules which then combine with molecular oxygen.

Ozone is much more soluble in water than oxygen. When dissolved in water, it can undergo reactions with some water matrix components. Ozone is an electrophile with high selectivity that reacts mainly with double bonds, activated aromatic rings and non-protonated amines. However,

its applicability in wastewater treatment is enhanced by the decomposition of ozone which leads to the formation of hydroxyl radicals (·OH), which are the strongest oxidants in water (Gottschalk, Libra, & Saupe, 2010). They are non-selective and react fast with many dissolved compounds in the water matrix.

The stability of ozone is pH-dependant because hydroxide ions (OH^-) initiate ozone decomposition as shown in equations 5 and 6 (Lee & von Gunten, 2016):

$$O_3 + OH^- \rightarrow HO_2^- + O_2 \quad (5)$$

$$O_3 + HO_2^- \rightarrow \ ^{\cdot}OH + O_2^{\cdot -} + O_2 \quad (6)$$

Therefore, ozonation of wastewater will undergo two different reaction mechanisms depending on the water matrix and its conditions (Gottschalk et al., 2010):

1. **Direction reaction**: direct and selective oxidation of organic matter by ozone.

2. **Indirection reaction**: decay of ozone accelerated by initiators such as OH^-, followed by formation of secondary oxidants such as ·OH which react non-selectively and immediately with target molecules.

2.3 Integration of ozonation to biological treatment

The application of ozonation in wastewater treatment has mainly been for disinfection purposes and to some extent tertiary treatment. However, given the ability of ozone to partially or completely degrade organic compounds (Scott & Ollis, 1995), its use prior a biological process has been identified as a valid approach to facilitate the removal of contaminants present in water (Ried, Mielcke, Wieland, Schaefer, & Sievers, 2007a).

According to Scott & Ollis (1995), the combination of these two processes can benefit four types of wastewater contaminants: recalcitrant compounds, biodegradable wastes with small amounts of recalcitrant compounds, inhibitory compounds and intermediate dead-end products.

In the case of recalcitrant compounds, chemical oxidation with ozone can be applied prior to biological treatment in order for significant biodegradation to occur. Compounds are recalcitrant either due to their large size or lack of reactive sites (Scott & Ollis, 1995). Chemical oxidation leads to smaller chain lengths, increased biological activity and eventually greater degradation of compounds (Scott & Ollis, 1995). The same sequence is applied to inhibitory compounds which may be toxic to the microorganisms used in the biological treatment. The use of a chemical oxidant may potentially degrade these compounds into less toxic or biodegradable intermediates. This facilitates biotreatment and leads to a more robust biological process.

In the case of biodegradable wastes with small amounts of recalcitrant compounds, which is often the case for domestic wastewaters, the use of biological treatment followed by chemical oxidation was initially considered more suitable. The large biodegradable portion is mineralized during the biological process and the remaining persistent compounds are degraded with subsequent chemical

oxidation (Scott & Ollis, 1995). The initial biological step also reduces the concentration of compounds that may compete for the chemical oxidant. However, the use of ozone after biological treatment may lead to the accumulation of some partially oxidized compounds which may be more toxic than the parent contaminants. It is possible that in this case, the cost of ozone rises in order to attempt to achieve full mineralization of biologically recalcitrant compounds.

More recently, our labs and others have explored other strategies for combined treatment processes due to the high diversity of pollutants found in wastewater, including the integration of ozonation prior to biological treatment of wastewater (Beltrán, García-Araya, & Álvarez, 1999; Larcher & Yargeau, 2013; Ordoño & Rollon, 2012; Schlageter, 2018). The integration of a chemical oxidation process such as oxidation to biological treatment may have synergistic effects leading to improved global contaminant removal efficiency and cost reductions. For an efficient and economic integrated process, the individual and combined effect of each process on the pollutants of concern must be studied. In the present case, the pollutants of main concern are organic matter, ammonia and suspended solids. Although a brief discussion of their removal in a lagoon (i.e. biological treatment) has been covered in sub-section 2.1.2, the following sub-sections will cover the fate of these contaminants during ozonation and the potential interactions when combined with biological treatment.

2.2.1 Effects of ozone on organic matter

Ozonation of organic matter leads to compounds of lower molecular weight that are more easily degradable and thus, its application prior to a biological oxidation (i.e. activated sludge system) process has been considered as a valid approach to promote the removal of organic contaminants present in water (Gottschalk et al., 2008). In other words, pre-ozonation of wastewater can increase its biodegradability, therefore facilitating the subsequent biological oxidation.

A measure of biodegradability as the BOD/COD ratio has surfaced as an indicator for the ability of ozone to increase the biodegradable portion in wastewater therefore enhancing the performance of biological processes (Beltrán, García-Araya, & Álvarez, 1997). The chemical oxygen demand (COD) is a measure of the amount of organic matter present in the water and it is reported as the amount of oxygen that it takes to fully oxidize it. BOD is the biodegradable portion of COD. The recalcitrant portion of COD is often of synthetic origin and may persist and bioaccumulate in the environment having potentially harmful effects on downstream aquatic environments and on the water quality (van Leeuwen, Sridhar, Kamel Harrata, Esplugas, & Onuki, 2009). Common recalcitrant substances are phenol compounds, pesticides and textile dyes (Achisa C. Mecha, Maurice S. Onyango, Aoyi Ochieng, & Maggy N. B. Momba, 2016).

In pre-ozonated wastewater, the portion of recalcitrant compounds will thus be lower than in non-ozonated wastewater due to the increase in the biodegradable fraction provided by ozone. Consequently, this will decrease the amount of energy microorganisms will spend on biodegradation. This energy saving is likely used to increase the oxidation rate of readily degradable material and the biomass formation (Mao & Smith, 1995).

Table 2 shows an overview of studies that have been conducted on the use of ozonation and its effect on biodegradability. Overall, according to these studies, this process has increased biodegradability of different types of wastewater at various operating conditions.

Table 2. Ozone effects on the biodegradability of various types of wastewater

Study	Ozone dose (mg/L)	BOD_i/COD_i	BOD_f/COD_f	Conditions
Ozonation of primary municipal water (Beltrán, García-Araya, & Alvarez, 1997)	100	0.54	0.71	pH=7.7 T=20°C
Pre-ozonation of refractory landfill leachate *(Imai, Onuma, Inamori, & Sudo, 1998)*	37	0.06	0.35	pH=8.1
Ozonation of domestic wastewater (Beltrán, García-Araya, & Álvarez, 1999)	40	0.57	0.69	pH=7.3-7.8 T=20°C
Ozonation of tannery effluent *(Preethi et al., 2009)*	2000	0.18	0.49	pH=11
Ozonation of cork processing water (Gomes, Silva, Simões, Canto, & Albuquerque, 2013)	507-4939	0.27	0.63	pH=10 T=20°C
Ozonation of primary municipal wastewater (Achisa C. Mecha et al., 2016)	21	0.22	0.53	pH=11
Integrated ozonation and biotreatment of pulping wastewater *(Zhang, Lei, Li, & Chen, 2017)*	25	0.15	0.36	pH=7.5 T=25°C
Ozonation of textile wastewater *(Ulucan-Altuntas & Ilhan, 2018)*	26	0.18	0.32	pH=9
The effect of ozone on the biodegradation of 17α-ethinylestradiol and sulfamethoxazole by mixed bacterial cultures *(Larcher & Yargeau, 2013)*	5	0.2	5.1	-

Although pre-ozonation of wastewater is reported to be a viable solution in conjunction with biological oxidation, several parameters can influence its performance:

- **Ozone dose applied**

Figure 3 shows the BOD/COD ratio as a function of the ozone dose during continuous ozonation of domestic wastewater from an experiment done by Beltrán, García-Araya, & Álvarez (1999) which seems to reach a maximum at approximately 42 mg/L of ozone. In order to understand these results, it is of interest to investigate the mechanisms of ozone attack on the organic matter. In fact, at low ozone consumption BOD increases while COD decreases which leads to an overall higher biodegradability index suggesting that the ozone reacts mainly with refractory compounds. This is the desired effect of pre-ozonation as it increases the efficiency the subsequent biological treatment. However, at higher ozone doses, it seems that ozone also reacts with the biodegradable portion of wastewater, including the one generated by oxidation of recalcitrant compounds, resulting in a decrease of final BOD and ultimately yielding a smaller BOD/COD ratio. Therefore, an ozone dose above the optimum level actually decrease the efficiency of the system since it results in highly oxidized products with little metabolic value for microorganisms and it leads to large amounts of ozone wasted on easily degradable compounds.

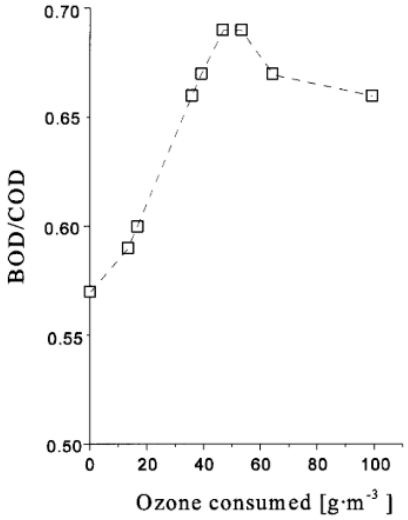

Figure 3. BOD/COD as a function of ozone dosage
(Beltrán, García-Araya, et al., 1999)

In a related study, another interesting aspect investigated by Beltrán, García-Araya, & Alvarez (1997) was the effect of the ozone dosage on the relative portions of biodegradable and non-biodegradable material in wastewater attacked by ozone. For instance, for an applied dose of 47 mg- O_3/L, both biodegradable and non-biodegradable fractions were reduced by 7 and 10%, respectively. However, at 100 mg-O_3/L, it was only the non-biodegradable fraction that was further reduced by as much as 20%. This explains that the latter dose was optimal because it contributed to a net increase in biodegradability.

- **Temperature**

This parameter is important during both the ozonation and the biological oxidation process. During ozonation, this parameter has opposing effects on ozone solubility and reaction rates. Increasing the temperature will decrease ozone solubility but will increase reactions rates. During biological oxidation, an increase in temperature (until a certain optimal point e.g. 35-40°C) will speed up biochemical reactions rates and will also allow for adequate growth of aerobic microorganisms in wastewater until a certain optimal temperature range is reached.

Beltrán, García-Araya, & Álvarez (1997) found that increasing the temperature of wastewater from 15 to 20°C led to an increase in COD removal during the ozonation experiments. Past that temperature, no significant changes were observed (i.e. between 20 and 30°C). In a subsequent experiment, ozonation followed by biological oxidation were tested at different temperatures. It was found that temperatures higher than 30-35°C inhibit degradation. However, for temperatures of 5 and 20°C, higher COD reductions rates during biological degradation were observed for the pre-ozonated samples as opposed to the non-ozonated sample.

- **pH**

The pH of the wastewater is extremely important as it directly affects the reaction mechanism of ozone as previously mentioned. Also, in terms of the biological process, microorganisms require a specific pH range in order to grow properly (i.e. around 7).

Once again, Beltrán, García-Araya, & Álvarez (1997) tested the effect of pH on COD reduction and no effect was observed at short ozonation contact times (i.e. less than 10 min), while for longer times (i.e. 30 min) a slight increase of COD reduction is observed with higher pH. It was deducted that since pH has no influence on the oxidation rate, ozone direct reactions presumably develop at the start of ozonation. However, the increased COD reduction rates due to increased pH observed at higher contact times could either be explained by the pH-dependant reactivities of the reacting compounds or by the fact that ozone may be decomposing into free radicals that would increase the oxidation rate of the compounds present. Therefore, after the initial period of direct ozone reactions, ozone may start to accumulate and if the pH is high, it likely to be converted into hydroxyl free radicals.

If the wastewater studied has high alkalinity, therefore containing high levels of bicarbonates and carbonates, hydroxyl free radical reactions may not be apparent as carbonate species are strong inhibitors of the reactions between hydroxyl free radicals and organic matter (Buxton, Greenstock, Helman, & Ross, 1988). Experiments by Beltrán, García-Araya, & Álvarez (1997) on wastewater whose carbonate ions were removed yielded significant COD removal rates for a contact time of 30 min at higher pH (i.e. 9).

On the other hand, the oxidation of organic compounds with ozone typically leads to a decrease in pH throughout the reaction due to the formation of organic acids of lower molecular weight (Achisa C. Mecha et al., 2016; Gomes et al., 2013; Shin & Lim, 1996).

- **Water matrix**

The study done by Achisa C. Mecha et al. (2016) included a section on the effect of water matrix on COD reduction by ozonation. Experiments were done on a primary effluent (i.e. after sedimentation), a secondary effluent (i.e. after biological treatment) and synthetic wastewater (i.e. SWW). The COD reductions obtained were 58%, 72% and 93% for the primary effluent, the secondary effluent and the SWW, respectively. These results showcase the overestimation of COD reduction when performing ozonation on SWW. This is due to the lack of competing substances in SWW which limit the ozone demand as opposed to the primary and secondary effluent, which have a more complex water matrix resulting in a higher ozone demand. Similar results were obtained by Shin & Lim (1996) while ozonating naphthalene refinery wastewater and naphthalene-spiked synthetic wastewater. Higher reductions of COD were obtained for the synthetic wastewater (i.e. approximately 80%) as opposed to the real wastewater (i.e. approximately 60%).

2.2.2 Effects of ozone on nitrogenous compounds

The oxidation of ammonia in wastewater into nitrate can be achieved through ozonation as shown in equation 7 (Haag, Hoigné, & Bader, 1984) below:

$$4O_3 + NH_3 \rightarrow H^+ + NO_3^- + H_2O + 4O_2 \quad (7)$$

According to Haag et al., ozone cannot oxidize NH_4^+, but it can oxidize NH_3 either directly or indirectly (i.e. with molecular ozone or free hydroxyl radicals). This makes ammonia ozonation highly dependent on the pH of the solution as the equilibrium of ammonia and ammonium in water is dictated by the pH (see equation 1). In alkaline conditions, the NH_3 form dominates and in acidic conditions, NH_4^+ takes over. Therefore, oxidation of ammonia occurs faster and more significantly at higher pH since its availability is increased. Singer & Zilli (1975) reported that the oxidation of ammonia is about 10-20 times faster at a pH of 9 than 7. Similar results were found by other studies (Lin & Wu, 1996; Luo, Yan, Wang, Luo, & Zhou, 2015) reporting that the higher the initial pH value, the greater the ammonia efficiency removal through ozonation. Though, the conversion of ammonia into nitrate forms H+ ions (see equation 7) which lowers the pH and the amount of ammonia available, therefore lowering the oxidation rates as the reaction proceeds (Khuntia, Majumder, & Ghosh, 2013). This may problematic for pre-ozonated wastewater that undergoes subsequent biological oxidation as the microorganisms might be affected by pH values that drop below 7. **Table 3** shows an overview of studies that have been conducted on the use of ozonation and its effect on nitrogenous compounds at various conditions.

These studies also monitored the increase of nitrate as it is a result of ammonia oxidation. The concentration of nitrite usually remains very low, less than 1 mg/L (Beltrán, García-Araya, et al., 1999; Luo et al., 2015), as it is readily oxidized to nitrate.

However, the studies done solely on solutions of ammonium chloride do not account for the other compounds found in typical domestic wastewater. In fact, ammonia oxidation in wastewater containing a significant amount of COD is slower than in simple ammonium chloride solutions as ammonia competes for ozone with dissolved organic compounds (Singer & Zilli, 1975). Indeed, (Beltrán, García-Araya, et al., 1999) stated that ozonation alone is not appropriate to remove

nitrogenous compounds from wastewater, especially at low ozone doses and neutral pH which is typical in domestic sewage. At these conditions, ozone reacts primarily with organics. However, when combined with a subsequent biological treatment, it was found that ozonation actually increases its efficiency at removing nitrogenous compounds. Two possible explanations were provided: (1) a higher amount of nitrogen was metabolized when pre-ozonation occurred since an increase in biomass growth was measured, (2) a higher percentage of nitrifiers was observed in pre-ozonated wastewater which suggested that ozonation may favour the growth of *Nitrosomas* species.

Table 3. Ozone effects on ammonia in various types of wastewater

Study	Ozone dose (mg/L)	Initial (mg/L)	Final (mg/L)	Conditions
Ozonation of an ammonium chloride solution *(Singer & Zilli, 1975)*	71	$[NH_3] = 45$	$[NH_3] = 8$	pH=9 T=20°C
Ozonation of an ammonium chloride solution *(Lin & Wu, 1996)*	161	$[NH_3\text{-}N] = 50$	$[NH_3\text{-}N] = 37$	pH_i= 9.2 pH_f =7 T=25°C
Ozonation of nitrogenous compounds in primary municipal wastewater (Beltrán, García-Araya, et al., 1999)	40	[TKN] = 35	[TKN] = 32	pH=7.3-7.8 T=20°C
Ozonation followed by biological oxidation of primary municipal wastewater (Beltrán, García-Araya, et al., 1999)	40	[TKN] = 35	[TKN] = 17	Ozonation: T=20°C pH=7.3-7.8 Biological oxidation: T=20°C pH=7.2-7.6
Ozonation of ammonia-containing wastewater (Luo et al., 2015)	100	$[NH_3] = 100$	$[NH_3] = 59$	pH_i =11 pH_f =6.3 T=25°C

2.2.3 Effects of ozone on suspended solids

Table 4 shows an overview of studies that have been conducted on the use of ozonation and its effect on suspended solids in various types of wastewater.

Ozone can affect particles present in wastewater by impacting their surface and changing their properties. For instance, it was found that ozone can desorb organic matter from particle surfaces, reducing the electrostatic-stabilizing effect of organic matter and improving particle aggregation (Jekel, 1994). In fact, the destabilization of suspended particles is necessary for particle aggregation and floc formation, which is in turn needed prior to removal by clarification and filtration. This phenomenon often causes a net reduction in the negative charge and increases the

chances for particle collision and coagulation as opposed to the adsorption of organic matter onto particles which enhances their colloidal stability and inhibit aggregation (Chandrakanth & Amy, 1996).

Some of the mechanisms through which ozone contributes to particle destabilization include polymerization of organic matter leading to particle aggregation, lysis of algae which liberates biopolymers that may act as coagulants, reduction in molecular weight of the adsorbed organics causing desorption, and the possible rupture of iron and manganese complexes resulting in a production of coagulant (Reckhow, Singer, & Trusell, 1986).

On the other hand, suspended particles can also affect the interaction between ozone and other compounds. For example, microbes or other compounds such as pharmaceuticals embedded in the pores of porous particles may be protected from ozonation because ozone penetration into particles is limited (Zucker et al., 2015).

Table 4. Ozone effects on suspended solids in various types of wastewater

Study	Ozone dose (mg/L)	Initial (mg/L)	Final (mg/L)
Ozonation of hatchery influent water *(Rueter & Johnson, 1995)*	0.8-1.1	TSS: 28	TSS: 13
Ozonation of a commercial aquaculture effluent *(Sandu, Brazil, & Hallerman, 2008)*	55	TSS: 553 Turbidity: 132	TSS: 129 Turbidity: 21
Integrated biological system and ozonation of wastewater *(Di Iaconi, 2012)*	Landfill leachate: 420 Tannery WW: 180 Textile WW: 60	Landfill leachate TSS: 220 Tannery WW TSS: 230 Textile WW TSS:106	Landfill leachate TSS: 28 Tannery WW TSS: 6 Textile WW TSS: 15
Combined process of ozone and bio-filtration in the treatment of secondary effluent *(Tripathi & Tripathi, 2011)*	10	Turbidity: 40	Turbidity: 6

2.2.4 Full-scale applications

The studies presented in the sub-sections above showcase the well-known potential and performance of combined biological and ozonation processes for wastewater treatment. These studies are limited to bench-scale experiments conducted with either synthetic wastewater or wastewater obtained from municipal WWTPs. Yet, there are more than 40 full-scale applications of combined integrated ozonation and biological processes that exist (Ried et al., 2007a). Although these were designed primarily to address recalcitrant compounds in landfill leachate and industrial wastewater, there is now a need for such approaches for the treatment of wastewaters containing small amounts of recalcitrant compounds such as domestic wastewater.

Some examples of full-scale combined ozonation and biological treatment technologies are shown in **Table 5**. It can be seen that the combined ozonation and biological treatment has been adapted in various form throughout the years. The post-ozonation of biologically pre-treated wastewater (Bio-O_3) was one of the first iterations. It was intended for treating remaining recalcitrant compounds of biologically treated wastewater. In the Bioquint process, the ozonation is placed in a cycle loop around the second biological treatment step. The effluent from the biological process is ozonated and recycled back several times into the bioreactor. This combination reduces the specific ozone consumption due to a higher number of compounds being biodegraded, instead of mineralized by extended ozonation (Ried, Mielcke, Wieland, Schaefer, & Sievers, 2007b). In order to reduce costs, a similar process of biological pre-treatment followed by ozonation and then by a biological post-treatment (Bio-O_3-Bio) was implemented. This was dictated by the high costs of ozone production and the much lower costs of biological treatment (Ried et al., 2007b).

Table 5. Full-scale combined ozonation and biological treatment technologies

Operator	Wastewater type	Process	Ozone dose [kg/m^3]	Source
WWTP Prato, Italy	Textile + sewage	Bio-O_3	0.032	(Kaulbach, 1993)
LLTP Hellsiek, Germany	Leachate	Bioquint	0.533	(Ried & Melke, 1999)
LLTP Bornum, Germany	Leachate	Bioquint	1.280	(Ried & Melke, 1999)
Lang Papier	Paper industry	Bio-O_3-Bio	0.172	(Ried, Mielcke, & Kampmann, 2000)
SCA-Laarkirchen	Paper industry	Bio-O_3-Bio	0.068	(Liechtu & Baig, 2005)
WWTP Kalundborg, Denmark	Industry + sewage	Bio-O_3-Bio	0.180	(Ried, Mielcke, & Kampmann, 2006)

LLTP – landfill leachate treatment plant

Although the full-scale applications discussed here are not primarily focused on the treatment of domestic wastewater, the availability of large-scale technologies implementing the combination of ozonation and biological treatment demonstrate the viability of such combined treatment at large scale. More specifically, the Bioquint and Bio-O_3-Bio processes are analogous to the integration of ozonation to lagoon treatment since the ozone is applied during the ongoing biological degradation occurring in a lagoon. Therefore, the wastewater is pre-treated biologically to some extent before ozonation, followed by biological post-treatment until the lagoon is discharged.

These full-scale technologies combined the use of ozonation to biological treatment having performances that can be controlled through various operating parameters (eg. return rate of activated sludge) and based on the use of high doses of ozone. However, the integration of low

doses of ozone to minimally control biological treatments such as a facultative lagoon has not been explored.

The work presented in this thesis attempts to fill this gap by providing an experimental methodology to investigate at lab-scale the integration of ozonation to lagoon treatment and to estimate the cost associated with such an approach.

3 Methodology

As mentioned in the Introduction, the first objective presented in this thesis is to develop a strategy at bench-scale that optimizes the integration of ozonation to lagoon systems for the enhancement of wastewater quality. The initial stage consisted of setting the foundation of a working biological treatment system through the use of a lab-scale batch bioreactor which emulates as closely as possible the conditions in lagoon systems. This ensured that the bacterial populations grown in the bioreactor are capable of providing a minimal wastewater treatment which can then be used as a base for comparison when applying partial ozonation, as it would be done during the integration of an ozonation system in a lagoon treatment. Once the biological treatment was established, ozonation experiments were conducted at different bacterial growth phase and ozone doses, as well as in different wastewater matrices.

This section covers the approach utilized to achieve this objective and lays out a detailed description of selection of bacterial inoculum and wastewater, the bioreactor and ozonation system, the experimental protocol for each test, as well as the methods used for the characterization and analysis of the samples.

3.1 Bacterial inoculum

In order to emulate the diverse bacterial populations found in a lagoon, several options were considered to replace the use of pure strains of bacteria. We considered isolating and growing bacteria from the lagoon wastewater, but this option was quickly eliminated as it would diminish the variety of microorganisms in the inoculum and extend the duration of the experiment preparation. Another alternative that was considered was using activated sludge from a municipal wastewater treatment plant to directly seed the bioreactor, in case there was no access to a lagoon station. Ultimately, a lagoon station in Terrebonne/Mascouche was selected as the sampling site for the bacterial inoculum used in all experiments. This allowed for the inoculum to be more representative of the microorganism consortium present in a lagoon.

This lagoon station comprises four aerated ponds totaling at a volume of 350 000 m^3. The average flowrate is 28 000 m^3/day. There is an attached growth biological reactor between the first and the second pond. There is also ferric sulphate addition to the fourth pond to improve phosphorus removal. The system is continuous, and the average retention time is 17-20 days. The average depth of all four ponds is approximately 3-4 meters, but there is a significant layer of sludge at the bottom. The wastewater that is treated is mostly municipal (i.e. Terrebonne and Mascouche municipalities), and a small portion (exact value unknown) of industrial wastewater coming from Terrebonne.

Depending on the number of lagoon cells at the station, it is preferable to collect sludge in the lagoons closest to the effluent. This is due to the fact that the lagoons that treat the raw influent often contain higher concentrations of toxic compounds and have higher levels of organic matter that may take much longer to degrade during the conditioning period that must be conducted in the lab before the sludge can be used as inoculum in experiments. Through various trials, it was established that the first pond had very high levels of organics and the second pond had high levels

of ammonia. The fourth pond could not be used as a source of inoculum due to the chemical addition. The collection of sludge was therefore done in the third pond for the majority of the experiments. Sampling in the second pond was only done once due to technical problems (i.e. the boat required used during sampling could not be moved from the second pond).

Since these lagoons are aerated, the sample was collected close to the location where air is sparged to ensure higher populations of aerobic bacteria in the sample. Since the lagoons were not equipped with sampling locations for the settled sludge, it was necessary to use the sediment sampler AMS 445.11.

Compared to activated sludge from a wastewater treatment plant, sludge from a lagoon needs to be conditioned or pre-incubated in order to be used as inoculum in aerobic experiments. The main reasons being the high concentrations of organics present in the sludge as well as the high levels of suspended solids, as can be seen in **Figure 4.**

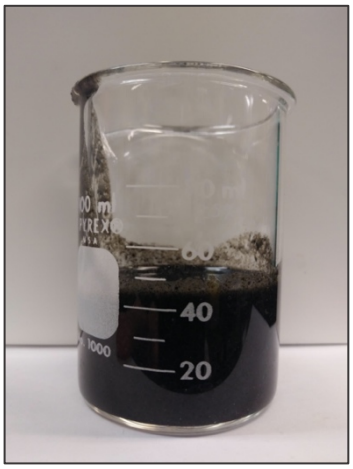

Figure 4. Settled lagoon sludge

3.2 Synthetic wastewater and Wastewater source

Ideally, all experiments would be conducted using real wastewater to get as close as possible to the conditions encountered during large scale treatment and take into account the possible effects of the wastewater matrix on the treatment efficiency. However, using synthetic wastewater as a media offers certain advantages: (i) ensures better control of the experimental conditions and reproducible conditions, (ii) creates the desired redox conditions, (iii) useful for studying and assessing the effects of different wastewater composition, or (iv) evaluating the inhibitory or toxic effects of certain solutions or compounds (van Loosdrecht, Nielsen, Lopez-Vazquez, & Brdjanovic, 2016).

To be used as a substrate for microorganisms, the synthetic wastewater should contain a mixture of carbon sources, composed of a readily biodegradable COD source like volatile fatty acids (such as acetate or proprionate) or glucose and macro- (ammonium, phosphorous, magnesium, sulphate, calcium, potassium) and micro-nutrients (iron, boron, copper, manganese, molybdate, zinc, iodine

cobalt) to ensure that cells are not limited by their absence. Generally, they can all be mixed together in the same media, as long as precipitation is not observed. As previously used in our group (Kolosov, Peyot, & Yargeau, 2018) and based on Klamerth et al. (2010), the synthetic wastewater recipe shown in **Table 6** was used.

Table 6. Synthetic wastewater recipe

Component	Concentration (mg/L)
Peptone	32
Meat extract	22
Urea	6
K_2HPO_4	28
$CaCl_2 \cdot 2H_2O$	4
$MgSO_4 \cdot 7H_2O$	2
NaCl	7
Na_2SO_4	96
$CaSO_4 \cdot 2H_2O$	60
KCl	4
Trace solution	300 µl

An additional trace element solution (van Loosdrecht et al., 2016) was incorporated in the recipe containing the following micro-nutrients (per liter): 10 g EDTA, 1.5 g $FeCl_3 \cdot 6H_2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4 \cdot 5H_2O$, 0.12 g $MnCl_2 \cdot 4H_2O$, 0.06 g $Na_2MoO_4 \cdot 2H_2O$, 0.12 g $ZnSO_4 \cdot 7H_2O$, 0.18 g KI and 0.15 g $CoCl \cdot 6H_2O$.

Considering the importance of validating results using real wastewater, the optimal conditions determined using synthetic wastewater were used to conduct an experiment with real wastewater. **Table 7** shows the initial characteristics of the raw and synthetic wastewater used in all the experiments.

Table 7. Wastewater characteristics

Characteristics	SWW	Raw WW
pH	6.90 – 7.20	8.20 – 8.40
COD (mg/L)	200 – 300	100 – 150
Ammonia (mg NH_3-N/L)	2 – 5	8 – 15

Preliminary testing of the wastewater was conducted in 500 ml Erlenmeyer flask filled with 200 ml of media and inoculated with 100 µl of conditioned sludge. These were kept at 25°C in an incubator shaker set at 120 rpm. As shown in **Table 8**, raw WW alone or wastewater spiked with micro-nutrients was not sustaining growth of the bacteria and could not be used for this validation experiment. The use of a mixture of raw WW mixed with 15% SWW showed an increase in biomass growth, although not as significant as when using SWW alone. It was thus decided to run the validation experiment using a mixture of 50:50 SWW : raw WW to ensure both bacterial growth and the additional effect of water matrix. To avoid having suspended solids in the bioreactor during experiment, the raw WW was filtered through a 0.1 µm pore size glass filter.

Table 8. Biomass growth in various media

Time (hours)	SWW	Raw WW	Raw + trace solution	Raw WW + 15% SWW
0	0.001	0.008	0.010	0.011
10	0.038	0.009	0.010	0.015
26	0.137	0.012	0.013	0.053

Finally, given the expertise of Prof. Yargeau's laboratory on the detection of contaminants of emerging concern (CECs), it was decided to include several of them in the synthetic/raw wastewater in order to assess their fate during partial ozonation. The CECs that were analyzed are presented in **Table 9** and they were selected from a larger list of 15 CECs that was used by Beauregard Schlageter when analyzing the wastewater samples collected from the lagoons during the pilot tests. The ones that were retained included some compounds that were detected in the lagoon samples, among which only a few were actually affected by ozonation. The wastewater in the bioreactor, raw or synthetic, was spiked with 550 µl of a 320 ppm mixture of the CECs mentioned. This was done to achieve a concentration in the bioreactor of approximately 100 ppb, which would be detectable by liquid chromatography mass chromatography without complex and costly sample preparation.

Table 9. List of selected CECs, adapted from (Schlageter, 2018)

Compound	Description	Effect of ozonation
Caffeine	Stimulant	None
Carbamazepine	Antiepileptic	Decrease during ozonation
Atrazine	Herbicide	None
Ibuprofen	Nonsteroidal anti-inflammatory	Decrease during ozonation
Naproxen	Nonsteroidal anti-inflammatory	None
Gemfibrozil	Blood lipid regulator	Decrease during and after ozonation

3.3 Bioreactor and ozonation system

The bioreactor setup that was used to simulate a biological treatment of wastewater is a 3.6 L Labfors 4 bioreactor. **Figure 5** shows the main parameters that were controlled and/or monitored throughout the experiments. Temperature and DO levels were controlled to values specific to each experiment. However, the pH of the wastewater was not adjusted as in practice, at the lagoon scale, the pH of the wastewater would not be adjusted. Data monitoring was accomplished through the use of the IRIS software. This program captured data from sensors (temperature, pH, DO), pumps (acid, base, feed, antifoam), gas flows and stirring speed.

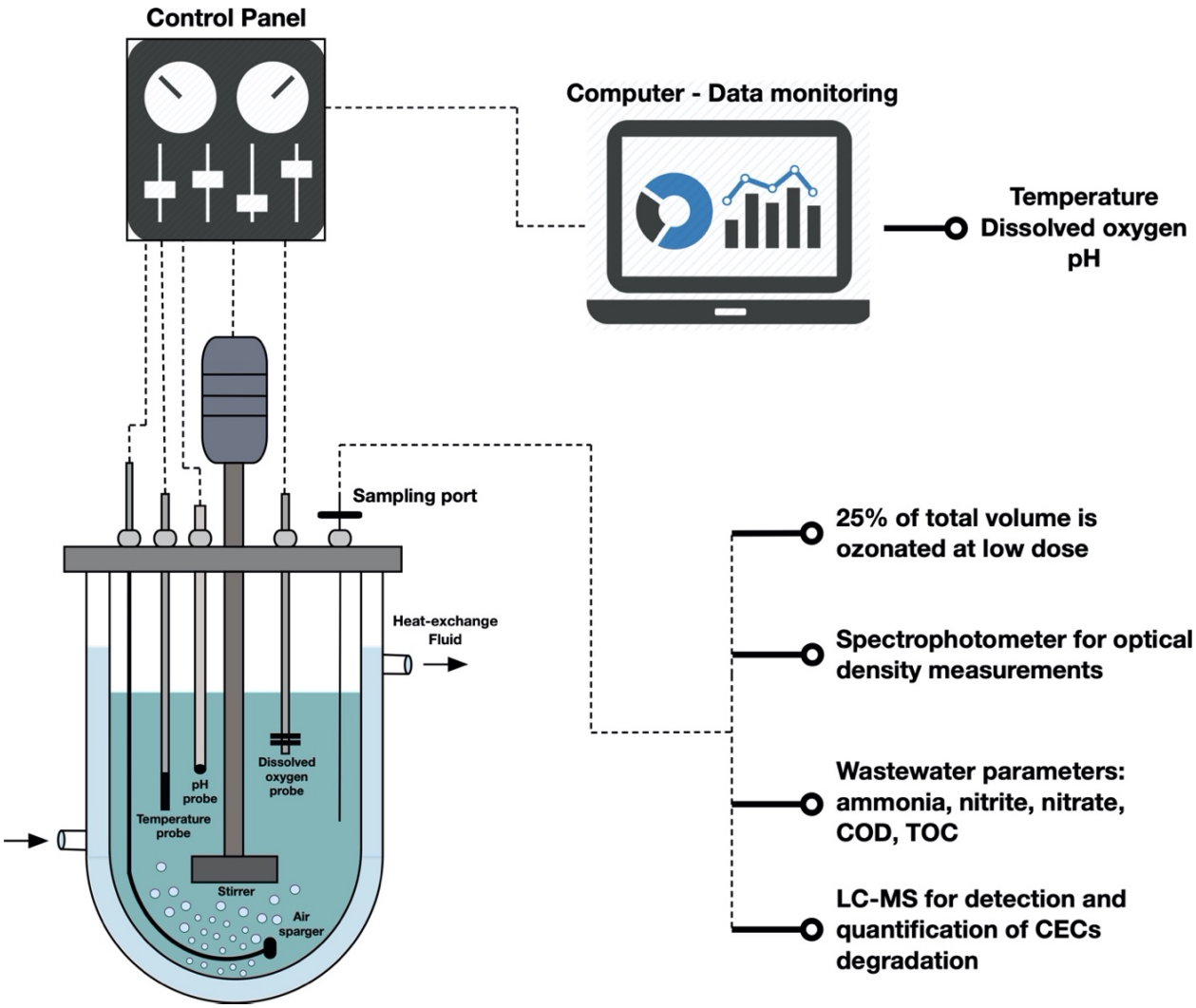

Figure 5. Bioreactor setup

For the experiments that require ozonation (all experiments except the controls), a determined portion of the total volume (i.e. 25% of the volume) of wastewater was removed from the bioreactor and placed into the reaction vessel of the ozonation unit. **Figure 6** shows an overview of the ozonation system schematic. The ozone generator was a Triogen TOGC2B ozone generator from Ozonia. Ozonation of the wastewater was conducted by sparging the desired dose of ozone into the wastewater. The ozone dose transferred to the wastewater was calculated based on the inlet and outlet ozone concentrations in the gas and the flow rate of the gas through the system. **Figure 33** in Appendix III shows the ozone generator as well as the inlet and outlet ozone concentration monitors, Wedeco HC-400plus and Wedeco MC-400plus, respectively.

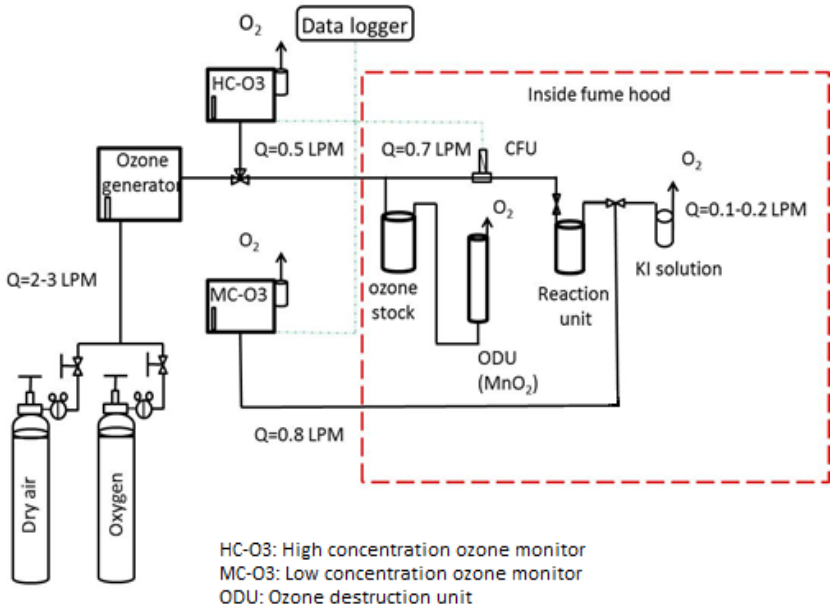

Figure 6. Ozonation system schematic used in the 3Cs laboratory (Chassaing, 2018). LPM = L/min, KI = Potassium Iodide

3.4 Experimental protocol for each test

The experimental protocol for each test can be broken down into three main phases, described below in further details.

3.4.1 PHASE I – Sludge conditioning

The initial phase is used to prepare the sludge collected from the lagoon station by ‘’activating’’ the bacteria. The process of pre-incubating the sludge will also allow for the dilution of organics, solids and other compounds. This step is performed in the same conditions (e.g. temperature, pH, DO, stirring speed) as the experiment using a 9:1 ratio of (raw or synthetic) wastewater to sludge. **Figure 7** shows the bioreactor contents during the sludge conditioning phase. After one or two days in the bioreactor, in order to verify if the inoculum contains heterotrophic aerobic bacteria, the H-BART test tube can be used to easily confirm that bacteria are present, active and aerobic, as shown in **Figure 8**. The conditioned sludge can then be used as inoculum for experiments.

Ideally, a new sample of sludge should be conditioned prior to each experiment to ensure that the inoculum remains consistent, at least in terms of pre-incubation conditions. However, in order to avoid long delays between each experiment, when it was possible to perform two or, at most, three experiments back-to-back (i.e. over 4-6 days), the same conditioned sludge was used. Preliminary experiments indicated higher variability in growth if the conditioned sludge was used over a longer period of time.

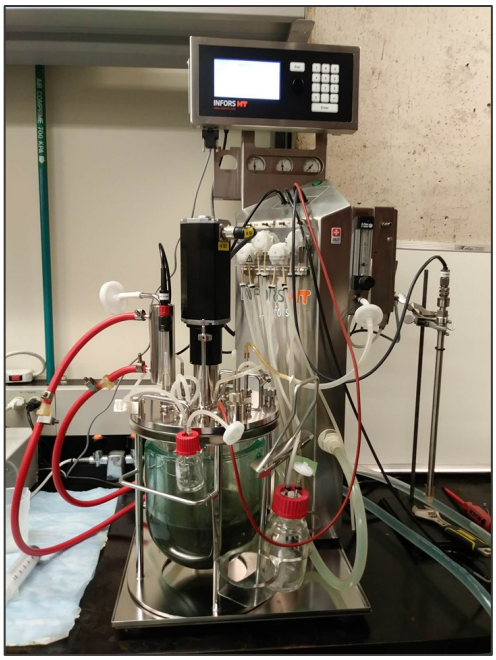

Figure 7. Bioreactor setup for sludge conditioning

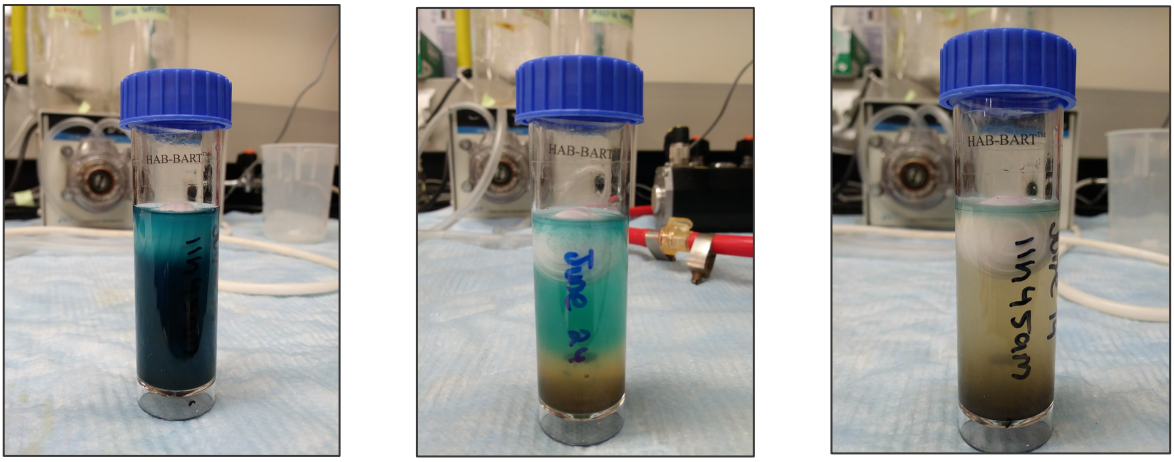

Figure 8. H-BART test tubes. The wastewater sample is placed in a test tube where it is colored with methylene blue (left). Bleaching of the methylene blue solution from bottom to top indicates the presence of aerobic heterotrophic bacteria (center). The number of days after which the entire tube is bleached (right) indicates the abundance of bacteria present (less days, higher biomass).

Although in-depth characterization of bacterial populations was not conducted, additional information was obtained by using the N-BART tubes which confirmed the presence of nitrifying bacteria in the pre-incubated sludge. The pink coloration developed after 5 days indicates the presence of nitrifying bacteria, as shown in **Figure 9**.

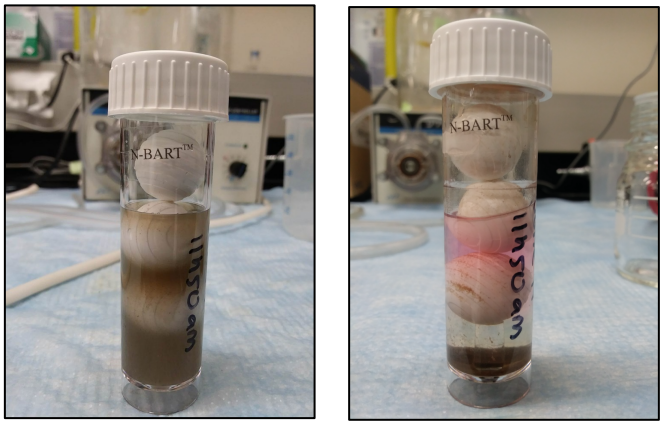

Figure 9. N-BART tubes. The wastewater sample is placed in a test tube for five days (left) to allow for nitrification to occur. After the incubation period, a chemical reagent is added to detect the product of nitrification (nitrite). The pink coloration (right) indicates presence nitrite, and therefore of nitrifying bacteria. The stronger the coloration, the higher the concentration of nitrifying bacteria.

3.4.2 PHASE II – Control run

Once the presence of heterotrophic aerobic bacteria was confirmed, a control run was conducted (i.e. biological treatment only) to assess if the bacteria were able to grow adequately inside the bioreactor and treat the lagoon wastewater. Additionally, even though a lagoon has different layers and levels of treatment, it is important to keep in mind that the bioreactor is used to simulate the aerobic portion of the lagoon as it is that portion that is responsible for the majority of the undergoing organic matter reduction.

Initially, several control runs were carried out to optimize the parameters presented in **Table 10** as well as monitor the evolution of some of the water quality parameters of interest such as COD and ammonia. Furthermore, trials of various sampling intervals were carried out which allowed to obtain a timeline for the cell growth and provide a good indication on the level of treatment that the bacteria can achieve without ozonation. The bacterial growth was monitored via optical density (i.e. 600 nm) using a spectrophotometer and the data collected was used to generate the cell growth curve.

Table 10. Bioreactor operation parameters

Conditions	Description
Operation mode	Batch
Temperature	25 or 15°C
Wastewater	Synthetic or 50:50 synthetic : raw
Air flowrate	3 L/min
pH (not adjusted)	6.9 – 8.5
Stirring speed	150
Duration	12 hours
CECs spiking volume	550 µl
Initial dissolved oxygen level (not maintained)	100% (saturated)

Figure 10 shows the various stages involved in the control run. First, the conditioned sludge that has been transferred in a 250 ml Erlenmeyer flask is allowed to settle in order to avoid transferring any solids into the bioreactor that could interfere with the optical density readings.

Next, 1700 ml of wastewater is placed in bioreactor and allowed to reach the desired temperature and dissolved oxygen saturation. The bioreactor contents are then spiked with 550 µl of a spiking solution containing the list of CECs shown in **Table 9**. Assuming, the concentration of these CECs is negligible in the conditioned sludge and/or raw wastewater, the initial desired concentration in the bioreactor was 100 ppb.

Prior to the inoculation of the bioreactor, the OD_{600} of the wastewater was measured and subtracted from the biomass OD_{600} measurements. This is because a sample of Milli Q water is used as a blank. In order to determine the amount of conditioned sludge to be added to the wastewater in the bioreactor, an approximation defined by the equation below was used.

$$V_{cond.\ sludge} = \frac{V_{WW}}{OD_{600,\ cond.\ sludge} \Big/ OD_{600,initial\ in\ bioreactor}} \tag{8}$$

Therefore, for a wastewater volume of 1700 ml and an average OD_{600} of 0.255 nm for the conditioned sludge, an approximate volume of 100 ml of conditioned sludge is required to obtain an initial OD_{600} of 0.015 nm for the inoculated bioreactor. **Figure 10** also shows how the optical density of the biomass increases from the beginning to the end of the experiment. This emphasizes the importance of avoiding the addition of suspended solids from the conditioned sludge to avoid having overestimated values of optical density.

Figure 10. Conditioned sludge after settling (left). Bioreactor contents at the start of the experiment (center). Bioreactor contents after biomass growth (right).

Once the bioreactor is inoculated with the conditioned sludge, a sample of 10-15 ml is taken every hour. Three OD_{600} measurements are taken right away and the remainder of the samples are filtered through 0.45 µm pore size Nylon syringe filter. They are then stored in the fridge for 1-2 days or in the freezer to preserve them for the remaining analyses.

3.4.3 PHASE III – Ozonation

The ozonation experiments involved ozonating of a fraction of the total volume of the wastewater inside the bioreactor. The ozonation reactor is shown in **Figure 11**. As previously mentioned, a sludge pre-incubation and control run are performed prior to each ozonation experiment. The sludge pre-incubation as well as the preparation for the experiment are identical to Phase I and II. In terms of the ozonation procedure, the parameters described below were considered crucial to the efficiency of the process.

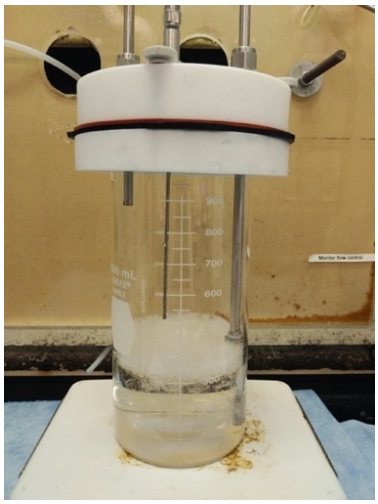

Figure 11. Ozonation reactor

1. **Ozone dosage**: 10, 15 and 20 mg-O_3/L (applied to the ozonated volume fraction)

These ozone doses were selected based on Beauregard Schlageter's experiments (Schlageter, 2018). All of these doses were tested in order to determine the range at which bacteria were not harmed by the ozone, but its effect is significant enough to observes changes in biomass growth or removal of contaminants.

2. **Volume of WW to be ozonated**: 25%

The fraction selected as to be within the range of values in Beau Schlageter's experiments, which were 20% and 50% (Schlageter, 2018). Although other volumes could have been tested, due to time constraints, a volume fraction of 25% was selected as it seems more realistically applicable for a large-scale lagoon as opposed to a fraction closer to 50%, which would result in large volumes of water to be treated.

3. **Time of ozonation**: end of lag phase, exponential and stationary

All stages of the growth curve (except for decline), as seen in **Figure 12**, were used as a potential time to ozonate. Initially, in the research proposal, it was hypothesized that ozonating during the stationary phase would be the most beneficial. It was considered that ozonating during the lag phase might put too much stress on the bacterial populations inside the bioreactor, especially since they will not be acclimated to ozonated water. Doing so might inhibit further growth of bacteria. On the other hand, ozonating during the exponential phase may have a lesser impact since the bacterial population is growing exponentially and using much of the organic content present in the wastewater. Therefore, ozonating during this phase might lead to a loss of ozone since bacterial activity will presumably be maximal. The stationary phase might be the most adequate for conducting partial ozonation since bacteria would supposedly be more stable and acclimatized to the conditions of the reactor. But since their growth rate is constant, the wastewater treatment will have also slowed down which might allow for the effect of partial ozonation to be more obvious.

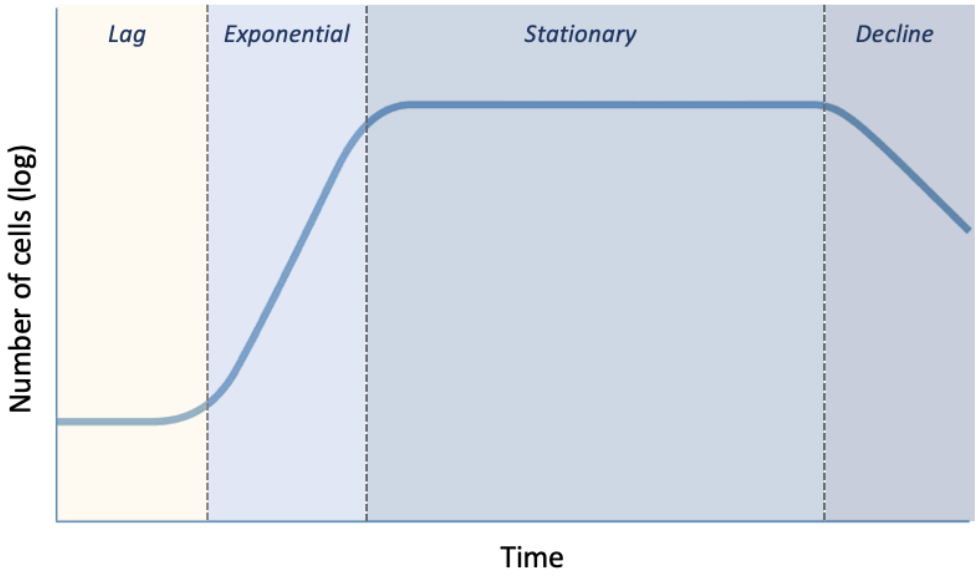

Figure 12. Cell growth curve

Moreover, a large portion of the readily biodegradable material may have already been consumed which means ozone will be more prone to oxidizing refractory compounds therefore increasing biodegradability. In order to validate the hypotheses mentioned above, ozonation trials were conducted during various growth phases in to determine which one showed a significant impact on the biomass growth.

4. **Wastewater matrix**: 100% synthetic and 50% mixture of raw and synthetic

The synthetic wastewater was first used to ensure better control of the experimental conditions and reproducibility. Once the protocol and optimal conditions were established, a mixture of 50% raw and synthetic wastewater was used in order to mimic more closely the conditions of a lagoon. A 100% raw wastewater was not used because preliminary work indicated that bacterial growth was not sustainable in this matrix using the current system.

5. **Temperature**: 25 and 15°C

A temperature of 25°C was selected as an "optimal" temperature at which the biomass growth and treatment levels would be maximal. Although, bacteria grow optimally at a temperature of 35-37°C, this range is not representative of lagoon temperatures. A lower temperature of 15°C was added to verify the extent of biological treatment and integrated ozonation. This was implemented to mimic the environmental conditions of a lagoon during colder months of the year.

Overall, the balance between these parameters was especially important for this type of experiment where ozonation is performed during biological treatment. In many studies presented in ***Table 2***, the wastewater contained a large amount of recalcitrant organic matter (i.e. tannery effluents, textile dyes, landfill leachate) and the main purpose of ozonation was to convert it into biodegradable organic matter so that subsequent biological treatment is facilitated. In the case of this project, since ozonation and biological treatment occur simultaneously, the objective leans more towards "helping" bacteria to biodegrade the present organic matter.

3.5 Characterization of samples

3.5.1 Determination of chemical oxygen demand

Chemical oxygen demand (COD) refers to the amount of oxygen that would be required to fully oxidize the compounds containing carbon to carbon dioxide. The value obtained is then used as an indicator of the amount of organic material, biodegradable and non-biodegradable, that is present in a sample of wastewater.

The Hach Method 8000 (digestive method) was used for the determination of COD. The full method is presented in Appendix I. Wastewater samples from each experiment were filtered through 0.45 µm pore sized Nylon filters and then diluted such that their COD values falls within the range of detection (0-150 mg/L) of the COD kit used. In a fume hood, 2 mL of the diluted sample was transferred to a COD vial. The vial contents were then mixed and placed in the digester at 150°C for 120 minutes. After the digestion period, the samples were cooled to room temperature. The samples were then analyzed for COD content using the DR/2500 Spectrophotometer (Hach).

3.5.2 Determination of ammonia concentration

The Hach Method 10023 (salicylate method) was used to determine the ammonia concentration in wastewater samples. The full method is presented in Appendix II. Wastewater samples from each experiment were filtered through 0.45 µm pore sized Nylon filters and the diluted such that their ammonia concentration falls within the range of detection (0-2.50 mg NH_3-N/L) of the kit used. In a fume hood, 2 mL of sample was transferred to a Hach NH_3 vial followed by the addition of the salicylate and cyanurate reagents (in that order). The vials were then mixed until the reagents were dissolved and left to react for 20 minutes. The samples were then analyzed for ammonia content using the DR/2500 Spectrophotometer from Hach. This test measures the total ammonia present in the water sample and the results is a sum of both the unionized ammonia and ammonium ions. Using the pH values of each sample recorded by the pH electrode in the bioreactor, the Henderson-Hasselbalch equation (Po & Senozan, 2001) was used to determine the portion of

unionized ammonia. [A^-] is the concentration of the acid (i.e. unionized ammonia) and [HA] is the concentration of conjugate base (i.e. ammonium).

$$pH = pK_a + log\frac{[A^-]}{[HA]} = pK_a + log\frac{[NH_3]}{[NH_4^+]} \quad (9)$$

Using the sum of both ammonia forms obtained from this test, the equation can be rearranged as follows to solve for the concentration of unionized ammonia:

$$[NH_3] = \frac{[NH_3] + [NH_4^+]}{10^{pKa-pH} + 1} \quad (10)$$

3.5.3 Quantification of contaminants of emerging concern

The separation and detection of each CEC was conducted using a method previously developed and published by our group (Kolosov et al., 2018). Briefly, an Accela LC system coupled to a LTQ Orbitrap XL was used. The separation of compounds was done using an optimized gradient achieved by two solvents, water with 2 mM ammonium formate and 0.1 % formic acid (solvent A) and methanol with 0.1% formic acid (solvent B). The column temperature was maintained at 30 °C and the flow of solvents was maintained at 0.250 ml/min, The injection volume was 25 µL and chromatographic separation of the compounds was achieved using a Hypersil Gold column (50 Å~ 2.1 mm, 1.9 µm) with an in-line Direct-Connection UHPLC 0.2 µm filter. Mass spectrum data acquisition was conducted in full scan mode (50 – 700 m/z) with the high-resolution detector. Additional work was conducted to determine the interaction of the wastewater matrix with the CECs, as shown in Appendix IV. Due to potential adsorption effects exhibited by ibuprofen, atrazine and carbamazepine onto dissolved organic matter, it was decided to prepare the calibration curves in the same medium as the experiment.

4 Results and Discussion

This section outlines the results obtained during the method development which were focused mainly on establishing a working biological process in the bioreactor and optimizing the ozonation parameters, as well as ozonation experiments at various conditions (i.e. wastewater matrix, temperature). The order onto which they are presented demonstrates not only the different conditions at which biological treatment and ozonation are operated but as well the progression of the method development. For this reason, the number of samples and the duration of experiments evolve from experiment to experiment as the optimal timing of the biomass growth phases is identified.

It should be noted that all control runs (i.e. biological treatment only) and experiments (i.e. biological treatment + ozonation) will be referred to by their respective number, as outlined in Appendix V. **Table 19** presented in Appendix V shows a detailed description of each experiment in chronological order.

4.1 Characterization of bacterial inoculum and optimization of biological treatment

Prior to testing the integration of ozonation to biological treatment, the first phase of experimentation was used to perform biological treatment control runs. These experiments were crucial for assessing the treatment efficiency of the bacteria in the conditioned sludge and their effect on the wastewater quality. Additionally, it was equally critical to determine the reproducibility of the biological growth in the bioreactor when using different raw sludge samples. By achieving reproducible control runs, it ensured that the eventual comparison between control and ozonation experiments is adequate and reliable.

4.1.1 Reproducibility and biological growth in the bioreactor

The initial stage of experimentation consisted of making sure that the bacteria contained in the conditioned lagoon sludge are able to grow in synthetic wastewater under given conditions. This also allowed for the monitoring of wastewater quality parameters. For this reason, the initial experiments were often run for longer periods of time (i.e. 4-9 days) in order to assess the extent of biological treatment provided by the lagoon bacteria. The results presented in **Figure 13** show optical density measurements of six different experiments conducted with six different samples of sludge, collected at the lagoon on different days over a period of six months. For experiments five and six (i.e. EXP 5 and EXP 6) and control runs three and four (i.e. CTL 3 and CTL 4), only the portion of the results relevant to the analysis performed in this section are included on the graph. These results were analyzed in order to evaluate reproducibility of the experiments and establish a suitable timeline for the experimental protocol based on the growth rate of the lagoon bacteria, which was unknown considering that the inoculum used is made up of many diverse natural microorganisms.

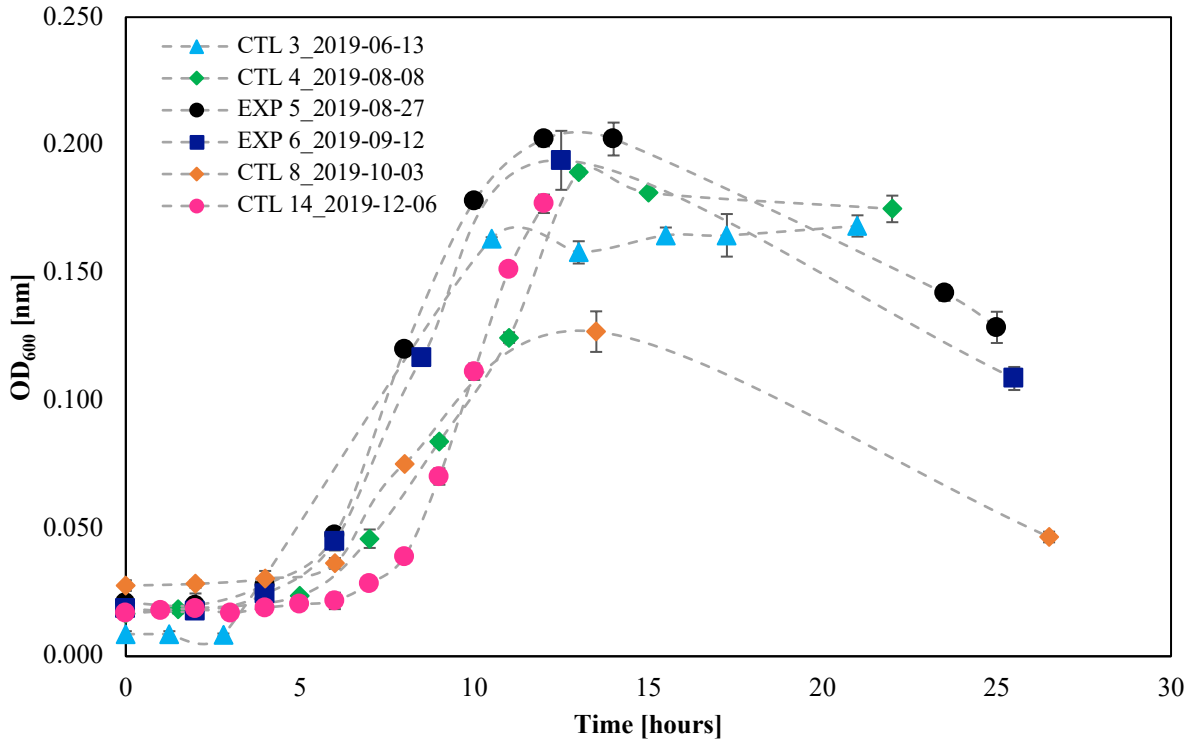

Figure 13. Biomass growth using sludge samples collected on different days. Total experiment duration ranging from 12 to 25 hours at 25°C, 150 rpm mixing and initial DO_{sat} levels. The error bars indicate that for each time point, three separate samples were collected to measure the OD_{600}.

Although not all runs reach the same maximal optical density value, the phases of biomass growth are apparent and demonstrate the experiments were sufficiently reproducible. From **Figure 13**, the lag phase is approximately four to six hours and the exponential phase approximately six to eight hours. The stationary phase was difficult to estimate due to the sampling schedule. Experiments were either started early in the morning which allowed for the detection of the lag and exponential phase, or they were started late in the evening which allowed for the detection early lag phase and the stationary phase in the next morning. The transition between lag and exponential along with exponential and stationary were then sometimes missed as they were occurring at night. The maximal optical density values ranged from 0.202 nm in EXP 5 to 0.127 nm in CTL 8.

It can be noted in **Figure 13** that after a duration of 12 hours, there is no optical density decline observed in CTL 3 and CTL 4 as opposed to the other control runs. Although it was not possible to determine the specific cause of this decline in bacterial growth, a few observations might help explain it. For instance, the sludge that was used for each experiment was sampled on different days over a period of six months. These sludge samples may have contained slightly different bacterial populations or leftover toxic compounds from the wastewater at the lagoon station. Additionally, it was observed during all experiments that after approximately 24 to 48 hours, the bacteria tend to flocculate and aggregate in small lumps which may affect optical density measurement. Finally, it should also be noted that the data collection frequency in CTL 3 and 4 is slightly different which may give the impression that the stationary phase is more pronounced in these experiments.

Similar studies were conducted by Dahiya & Venkata Mohan (2016) where various consortia of indigenous wastewater bacteria were grown in synthetic wastewater and achieved a maximal optical density ranging from 1.200 nm to 0.6 nm over a period of 48 hours. The reported transition between the exponential and stationary phases was approximately at six hours which is comparable to that shown in **Figure 13**. However, each consortium only contained three to four strains of bacteria, all of them belonging to either the *Pseudomonas* or *Bacillus* genus. These bacteria are found to prevail in the wastewater microbiome; *Bacillus* species have been identified to degrade proteins, starch and lipids, and *Pseudomonas* can easily break down carbon-containing compounds (Dahiya & Venkata Mohan, 2016). In addition, they used a temperature of 29°C, which may partially explain the greater biomass growth.

Another important factor to consider is that growth of individual organisms is different than the growth of a consortium containing many different organisms. In fact, bacteria can form complex associations with other organisms within the wastewater medium (Dahiya & Venkata Mohan, 2016). These association can either be neutral, positive or negative. In a neutral interaction, different bacterial species co-exist without affecting one another. Positive interactions occur when these bacterial species develop a synergetic relationship that help them grow, such as co-metabolism (Modestra & Mohan, 2014). Finally, negative associations can inhibit biomass growth. Since the inoculum used for the experiments conducted in this thesis contained a mixture of natural lagoon microorganisms, it is possible that their growth inside the bioreactor in SWW might have been inhibited by some negative interactions resulting in lower optical densities than those reported in other studies.

COD reduction reached levels near 100% when the experiment was allowed to run more than 3-4 days, as shown in **Table 11**. These experiments indicated that the bacteria in the conditioned sludge were capable of oxidizing the organic matter present in the SWW.

Table 11. COD removal capability of the lagoon bacteria

Experiment	COD removal (%)	Duration of COD removal (hours)
CTL 2	96.4	117 (4 days, 21 hours)
CTL 3	97.6	107 (4 days, 11 hours)
CTL 4	94.3	72 (3 days)
EXP 5	96.3	76 (3 days, 4 hours)
EXP 6	96.1	98 (4 days, 2 hours)

The studies conducted by Dahiya & Venkata Mohan (2016) reported an COD reductions between 86% and 50% in 48 hours, which are similar to the COD reductions observed in the experiments listed in **Table 11** : within 48 hours the levels of removal were between 57% and 74%.

4.1.2 Synthetic wastewater volume change

Considering that the integration of the ozonation step during the biological treatment requires that a volume of water be extracted and returned to the bioreactor, experiments were conducted to assess the bacteria's ability to recover from a volume exchange. **Figure 14** shows that CTL 3 and CTL 4 both had a volume exchange, at 45 hours and 22 hours, respectively. This was performed

much earlier for CTL 4 because the intention was to capture the stationary phase, during which the integration of ozonation would eventually be tested. This volume exchange involved changing 25% of the bioreactor contents with fresh SSW. This fresh volume of SWW would not contain any biomass, which would initially cause a dilution when added to the bioreactor. This dilution effect can be seen more clearly for CTL 4 as the optical density decreases from a value of 0.175 nm at 22 hours to 0.119 nm at 23 hours. A similar trend is observed for CTL 3, where the optical density decreases from 0.088 to 0.071 nm. However, since this was in the decline phase, this decrease can also be partially attributed to decaying biomass.

However, **Figure 14** shows that for both controls, the addition of fresh SWW did not disturb the biomass growth. In fact, when the volume change was performed sooner, like in CTL 4 at 22 hours, the optical density measurements returned to the value before the volume change in less than an hour. In CTL 3, the volume change was performed much later when the optical density measurements were already decreasing and there was only a small increase in biomass growth observed several hours later. Therefore, these experiments, particularly CTL 4, indicate a volume exchange does not affect the bacteria's growth.

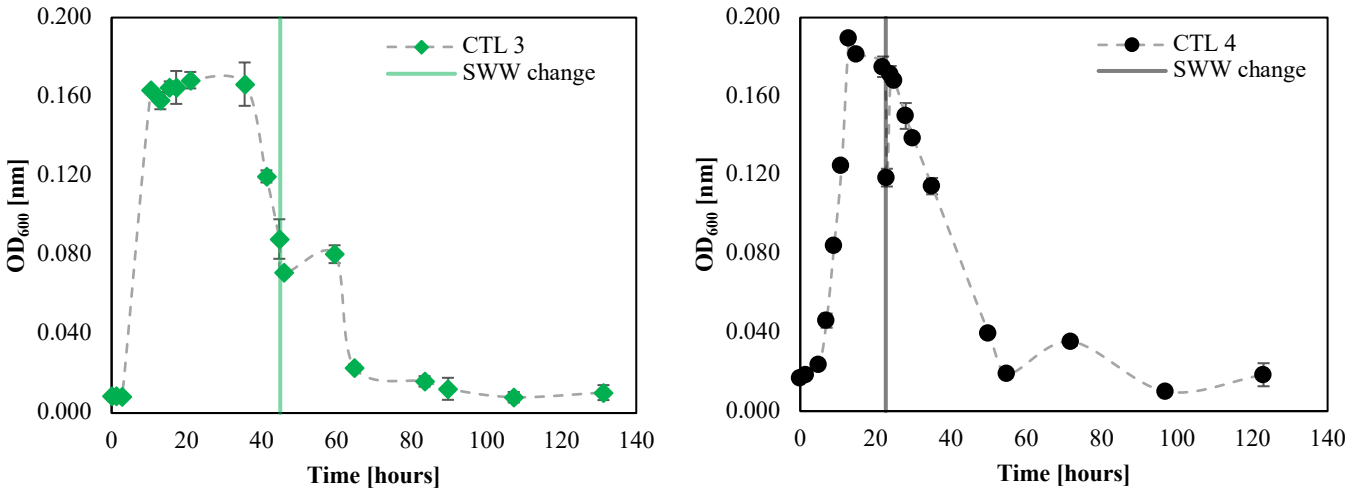

Figure 14. Biomass growth in control runs with SWW volume change at 45 hours (CTL 3, left) and 22 hours (CTL 4, right). Experiment conducted at 25°C, 150 rpm mixing and initial DO_{sat} levels. The error bars indicate that for each time point, three separate samples were collected to measure the OD_{600}.

4.1.3 Ammonia removal during biological treatment and presence of nitrifying bacteria

For all the experiments conducted, as the COD levels decrease, the levels of ammonia increase. An example of these trends is shown in **Figure 15** from control run three (CTL 3). The synthetic wastewater recipe (shown in **Table 6**), suggests that the main source of organics, the peptone and meat extract, which contain high protein levels, are most probably the source of this nitrogen. As these compounds are biodegraded, the nitrogen contained in their peptide bonds get released into solution and ammonia is formed.

Given the high levels of ammonia that develop throughout each experiment caused by the biodegradation of organic matter, an additional test was performed to verify if nitrifying bacteria

would eventually develop and use the accumulated ammonia as a source of nutrients. Unlike carbonaceous organic material that is readily used by bacteria, there is a lag time in the growth of nitrifying bacteria which delays the decomposition of nitrogenous organic matter. Additionally, all samples of conditioned sludge contained at the very least traces of nitrifying bacteria confirmed by the N-BART test tubes as mentioned in the Methodology section.

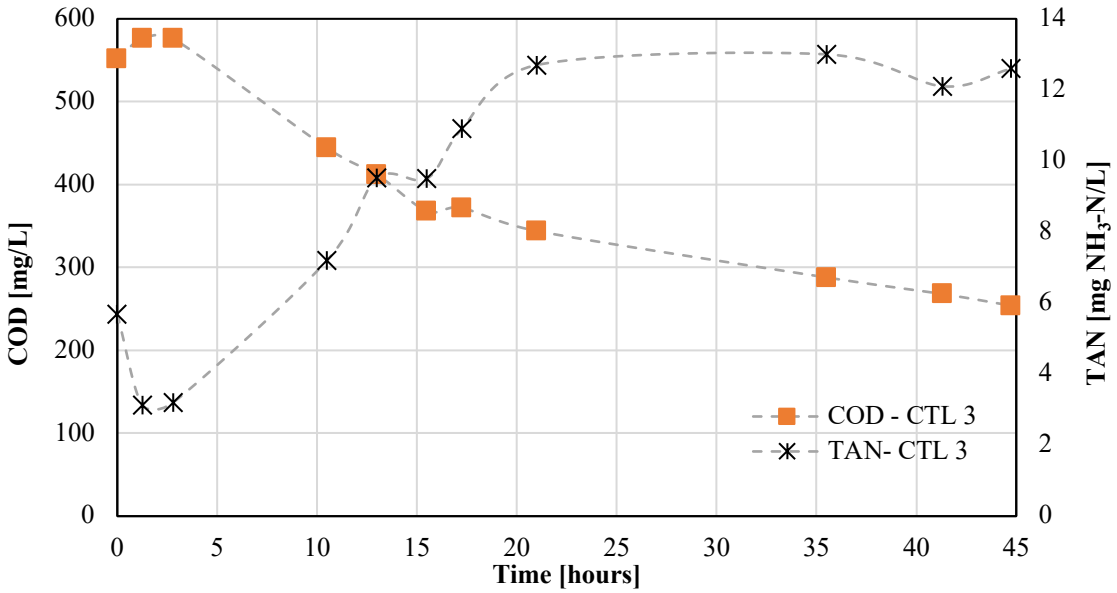

Figure 15. Relationship between COD and TAN evolutions throughout biological treatment. Total experiment duration of 45 hours at 25°C, 150 rpm mixing and initial DO_{sat} levels.

Figure 16 displays CTL 3 which was run for a longer total duration of 205 hours. A SWW volume change occurred at 45 hours and it was confirmed that 97.6% of COD was removed within 107 hours. The ammonia levels were rising until around 84 hours, after which they seem to remain constant and did not start declining once the COD were low. This suggests that nitrifying bacteria did not grow upon depletion of the organic material.

There are several environmental factors that can have an effect on the rate of nitrification in wastewater such as substrate concentration (i.e. NH_4^+), pH, temperature and oxygen availability (Princic, Mahne I, Megusar, Paul, & Tiedje, 1998). The pH, temperature and oxygen availability can be removed as possible causes for failed growth since the pH range of ammonia oxidizers is 6.0 – 9.0 (Bioscience, 2020), the optimal temperature range is 20-35°C (Bioscience, 2020) and the bioreactor was continuously aerated. Depletion of the trace elements by heterotrophic bacteria but required by nitrifying bacteria could have contributed to their lack of growth. This was not verified, but future experiments could include the addition of trace elements after the organic matter is depleted. Furthermore, it is possible that the time duration of experiment was not long enough since autotrophs grow more slowly than heterotrophs. In fact, ammonia oxidizers such as *Nitrosomas* reproduce once every eight hours (Princic et al., 1998), as opposed to common heterotrophic bacteria which have an average doubling time of 30-60 minutes (Rumbaugh, 2016). During the COD removal phase, spatial and nutrients competition between heterotrophic and nitrifying bacteria may have played a role. High levels of organic matter also have an inhibiting

effect on the growth of nitrifying bacteria by entering their cells and inactivating their enzyme systems (ECOS, 2013).

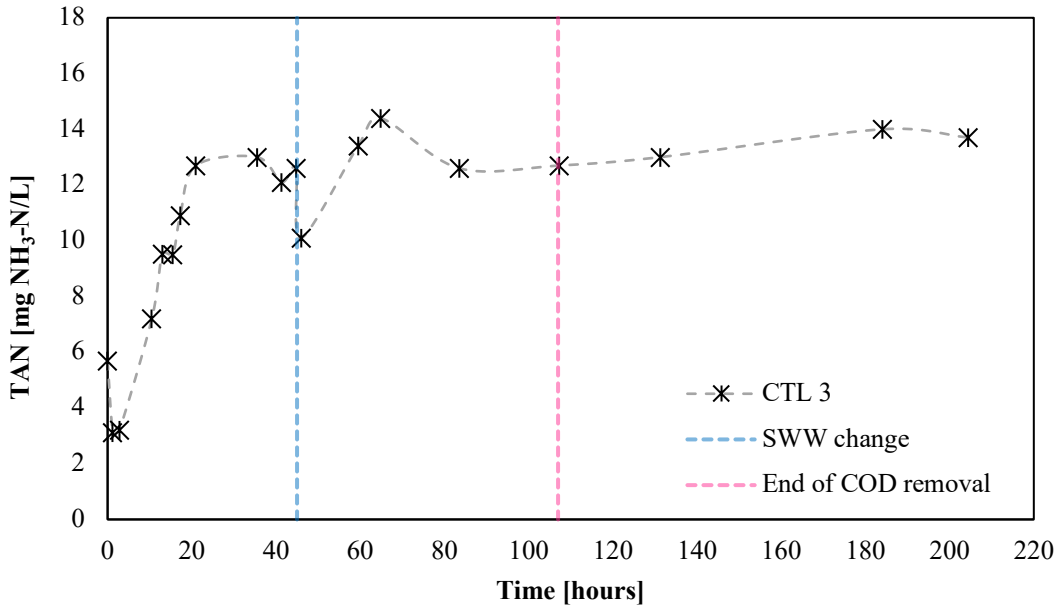

Figure 16. Biological treatment conducted over extended period of time to verify presence of nitrifying bacteria. Total experiment duration of 205 hours at 25°C, 150 rpm mixing and initial DO_{sat} levels.

The absence of nitrifying bacteria in the bioreactor eliminates the possibility of testing the impact of ozonation on ammonia removal. It would have been interesting to test the findings of Beltrán, García-Araya, & Álvarez (1999) mentioned in the literature review. They reported that a higher amount of nitrogen was metabolized when pre-ozonation of domestic sewage from a WWTP occurred since a higher percentage of nitrifiers was observed in pre-ozonated wastewater which suggested that ozonation may favour the growth of *Nitrosomas* species.

4.2 Integration of ozonation to biological treatment experiments

Upon completing the biological treatment validation phase, the ozonation experiments were initiated. The experiments conducted covered the parameters and conditions outlined in the methodology section: time of ozonation, ozone dose, ozonated volume portion, wastewater matrix and temperature.

4.2.1 Time of ozonation

Ozonation during the stationary phase

Identifying the stationary growth phase was particularly difficult due to scheduling difficulties. However, since it was hypothesized that ozonating in the stationary phase would prove to be most beneficial, it was selected as the first time point to integrate the ozonation. The specific time selected as part of the stationary phase was about 24-26 hours because this allowed for a whole day of sampling on the first day of the experiment and the ozonation could then be performed right

away on the second day. The ozone dose selected was 15-20 mg O_3/L, which was on the higher end of the range discussed in the Methodology section. The choice of starting with a higher ozone dose was implemented in order to determine early on if it may cause stress to the biomass present in the bioreactor.

Figure 17 shows that in both ozonation experiments, growth subsequent to ozonation was not observed suggesting that the biomass might have been too affected by the ozone dose or already not able to thrive again. Although a slight increase in optical density for EXP 5 was observed approximately 50 hours later, a similar small increase in OD_{600} can also be noticed in CTL 4. These slight increases might be caused by cells being detached from various parts of the bioreactor (e.g. sensors, impellers, baffles) or flocculation, which was observed after longer periods of time. Moreover, when comparing the organic matter removal reported in **Table 11**, it is observed that COD removal was slowed down for EXP 6: COD removal was completed in 72 hours in CTL 4 and 76 hours in EXP 5 as opposed to 98 hours in EXP 6. This suggest that the higher ozone dose in EXP 6 (20 mg O_3/L compare to 15 mg O_3/L in EXP 5) might have provided additional stress for bacteria. These results indicate that ozonating in the stationary phase does not improve treatment efficiency at an ozone dose of 15 mg O_3/L and may even slow down biodegradation at a higher dose of 20 mg O_3/L.

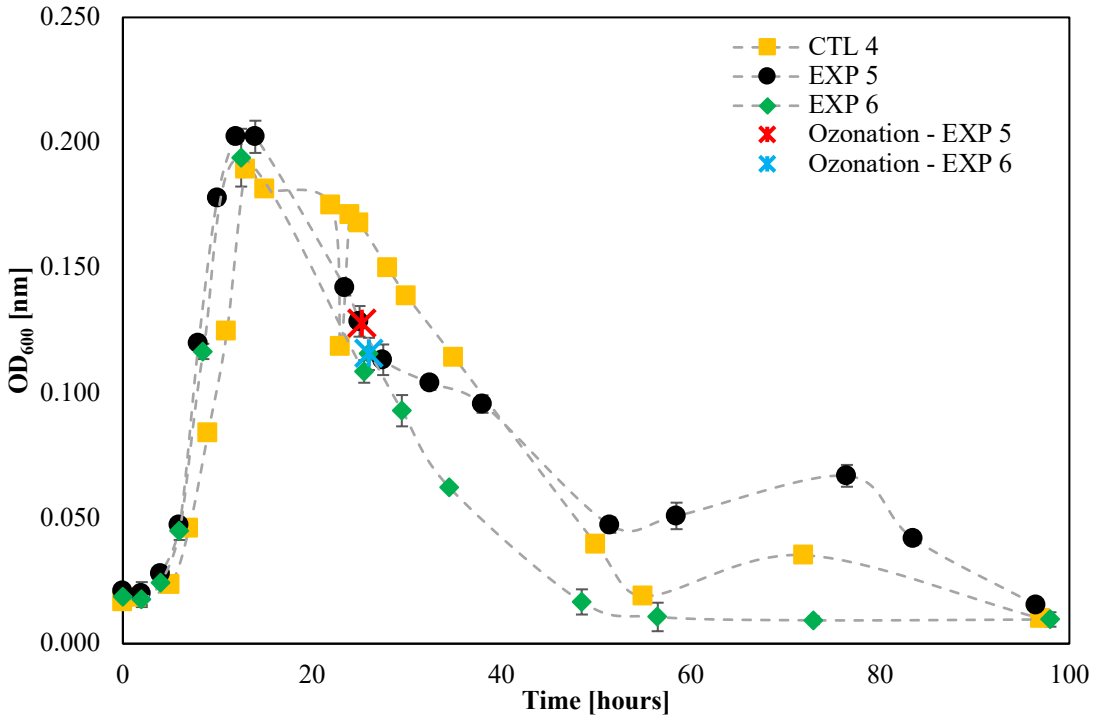

Figure 17. Biomass growth. Total experiment duration ranging from 96 to 98 hours at 25°C, 150 rpm mixing and initial DO_{sat} levels. Ozonation conditions: ozonated fraction = 25% of total volume (approx. 400 ml), ozone dose= 15 mg O_3/L for EXP 5 and 20 mg O_3/L for EXP 6. CTL: control run – only biological treatment; EXP: biological treatment + ozonation (at t=25-26 hrs). The error bars indicate that for each time point, three separate samples were collected to measure the OD_{600}.

Ozonation during the exponential phase

While ozonating in this growth phase was initially not considered, the results obtained by ozonating during the stationary phase suggested that other options should be considered. Additionally, it was decided to keep the ozone dose at 20 mg O_3/L due to the high biomass concentration during the exponential phase which could potentially use up the ozone before it reacts with contaminants present in wastewater.

Figure 18 shows that EXP 7 exhibited a very similar biomass growth when ozonating half-way through the exponential phase to the one observed in the CTL 8. Similarly, the COD removal trends were also similar in both the control run and the ozonation experiment, which confirmed that ozonating in this phase did not improve the treatment efficiency. As previously mentioned, as the bacteria are growing exponentially, the high biomass density that is generated most likely depleted the ozone at such a rate that the partial oxidation of organic matter was minimal. Therefore, ozonating during this phase might lead to a loss of ozone since bacterial activity will presumably be maximal.

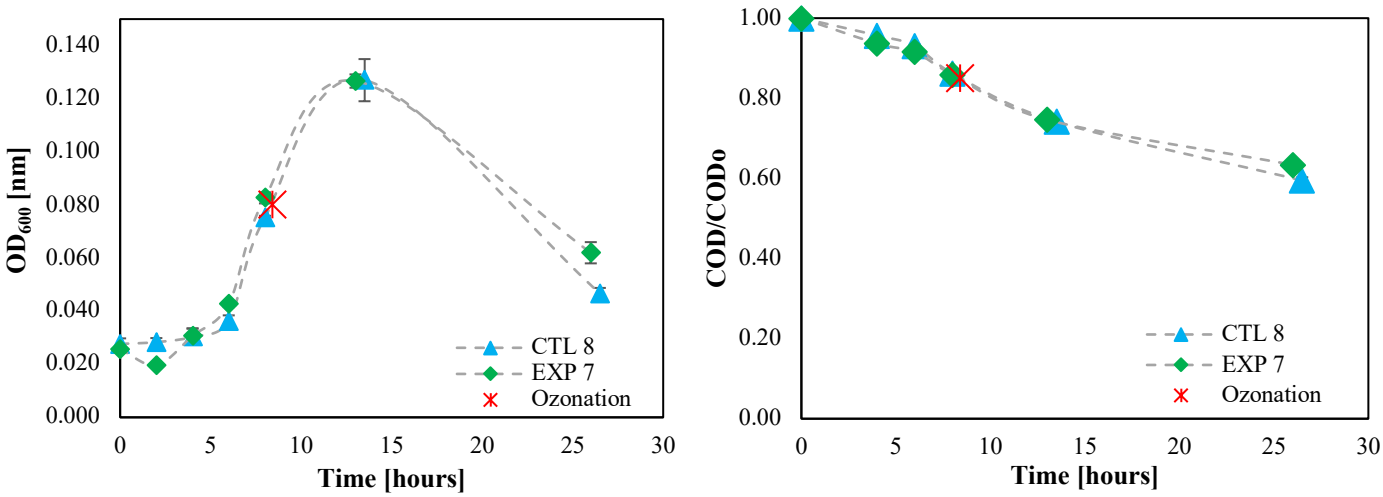

Figure 18. Biomass growth (left) and COD removal (right). Total experiment duration 26 hours at 25°C, 150 rpm mixing and initial DO_{sat} levels. Ozonation conditions: ozonated fraction = 25% of total volume (approx. 400 ml), ozone dose= 20 mg O_3/L. CTL: control run – only biological treatment; EXP: biological treatment + ozonation (at t=8.4 hrs). The error bars indicate that for each time point, three separate samples were collected to measure the OD_{600}.

Ozonation at the end of the lag phase

Contrary to what was initially speculated, ozonating towards the end of the lag phase seemed to have a more significant effect on the biomass growth. However, due to the biomass being more fragile during this growth phase, it was decided to start testing at a lower dose of ozone of 10 mg O_3/L. It can be seen in **Figure 19** that the optical density measurements in the ozonation experiment show a rapid increase following the ozonation at 5.5 hours. During the exponential growth, most data points in the ozonation experiment are two hours ahead of those in the control run. In fact,

after 12 hours of treatment, the optical density in the ozonation experiment is slightly greater than the one in the control run. Similarly, the organic matter levels also reached a lower level in the ozonation experiment (i.e. 0.68 COD/COD_o) as opposed to the control run (i.e. 0.73 COD/COD_o). Since these experiments were the first ones showing promising results, they were done in triplicates in order to confirm that they are statistically significant.

The results shown in **Figure 19** suggest that early ozonation might enhance biomass growth. One possible explanation is that towards the end of the lag phase, the biomass inside the bioreactor is still minimal but has had the time to acclimate to its new environment. Therefore, when ozonating only a portion of the bioreactor contents, some of the organic matter is likely to react with the ozone and get degraded into lower molecular weight molecules (Scott & Ollis, 1995). Since, the biomass is minimal, the ozone demand is small and can react more with the organic matter. In turn, these smaller organic molecules will be more easily taken up by the biomass in the bioreactor which could explain the accelerated growth shown in the results. Therefore, these preliminary results suggest that a lower ozone dose coupled with the change in time of ozonation may lead to a more effective integration of ozonation into biological treatment.

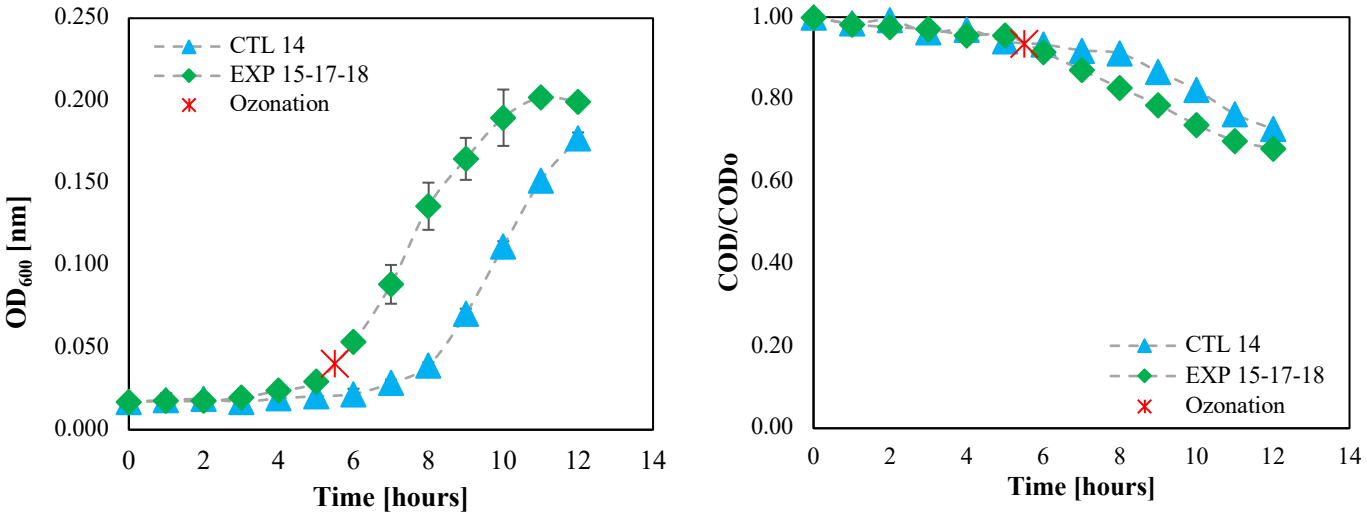

Figure 19. Biomass growth (left) and COD removal (right). Total experiment duration 12 hours at 25°C, 150 rpm mixing and initial DO_{sat} levels. Ozonation conditions: ozonated fraction = 25% of total volume (approx. 400 ml), ozone dose= 10 mg O_3/L. CTL: control run – only biological treatment; EXP: biological treatment + ozonation (at t=5.5 hrs). The error bars indicate that for each time point, three separate samples were collected to measure the OD_{600}. The ozonation experiments were performed in triplicates (n=3).

These results align with the findings of Larcher & Yargeau (2013) where a low ozone dose of 5 mg O_3/L was used to increase the biodegradability of sulfamethoxazole and 17α-ethinylestradiol. Their results show that the application of ozone increased the biodegradability index (BOD_5/COD) from 0.2 to 5.1. On the other hand, Schlageter (2018) also studied the effect of ozone dosing strategies on wastewater quality parameters in laboratory experiments. The ozone doses ranged from 5 to 20 mg O_3/L applied to 20% or 50% of the bioreactor volume. However, it was reported that ozone was ineffective in improving the wastewater quality parameters in the laboratory experiments but was effective in the removal of two caffeine and sulfamethoxazole.

So far the benefits of pre-ozonation have been focused on its ability to increase the biodegradability of contaminants, but an interesting mechanism reported by Fu et al. (2019) described the interaction of ozone with microorganisms and their ability to degrade organic matter. In their study, a low ozone dose of 5 mg O_3/L was reported to have increased COD removal by 24% in the biological process compared to the control. On the other hand, a high ozone dose of 50 mg O_3/L showed the opposite effect. The argument put forward was that low dose ozonation alters the EPS (i.e. extracellular polymeric substance) fraction surrounding bacteria and increases the contact between cells and DOM (i.e. dissolved organic matter). In fact, bacterial cells in activated sludge have a double layer consisting of loosely-bound (LB)-EPS and tightly-bound (TB)-EPS (Poxon & Darby, 1997). In order for DOM to be assimilated by microbial cells, it has to first pass through the EPS layers (Comte, Guibaud, & Baudu, 2006). Therefore, if the ozone dose is low enough to not damage the cell, but high enough to alter the EPS, this would eventually enhance the mass transfer of DOM to microorganisms without comprising their resistance to ozone toxicity.

Overall, these results seem indicate that there is an optimal range of biomass density and ozone dose which may stimulate growth and organic matter uptake. However, the experiments here conducted were able to pinpoint to only one set of conditions which achieved that goal: ozonating 25% of the volume at an ozone dose of 10 mg/L at the end of the lag phase (i.e. t = 5.5 hours or OD_{600} of approximately 0.029 nm). Nevertheless, it should be noted that since the ozone dose of 10 mg/L was not tested in the other growth phases (i.e. exponential and stationary), it is difficult to conclude which one of the two factors (ozone dose or timing) contributed to the increase in biomass growth and organic matter uptake.

4.2.2 Wastewater matrix

Following the promising results obtained in experiments 15-18 and the time constraints due to the closure of the lab during the Covid-19 pandemic, it was decided that rather than testing for other ozone doses or ozonated volume portions, it was important to validate the results obtained in synthetic wastewater with a real matrix. However, as described in the methodology section, using 100% raw wastewater led to low biomass growth and would have required further modifications of the bioreactor operation, which again was not possible due to time constraints. Therefore, a 50% mixture of raw and synthetic wastewater was used, and these experiments were performed in three replicates.

The maximal optical density in this water matrix (approx. 0.090 nm) shown in **Figure 20** was half the value of that reached in 100% synthetic wastewater (approx. 0.200 nm) shown in **Figure 19**. Also, the lag phase seems to be slightly longer as the exponential phase seems to start at around 8 hours. However, it can be seen in **Figure 20** that in the ozonation experiments, the optical density reached almost twice the value of that in the control run. This trend is therefore similar to that obtained in the experiments 14-18.

Moreover, it can be noticed that during the exponential growth, most data points in the ozonation experiment are three hours ahead of those in the control run. For instance, in the ozonation experiments, an optical density of 0.042 nm is reached after nine hours as opposed to the control run where this optical density value is only reached after 12 hours. The lower biomass growth observed in the mixture of raw and synthetic wastewater can be explained by the possible presence

of recalcitrant, toxic or complex compounds which are not as easily assimilated by bacteria (Achisa C. Mecha et al., 2016).

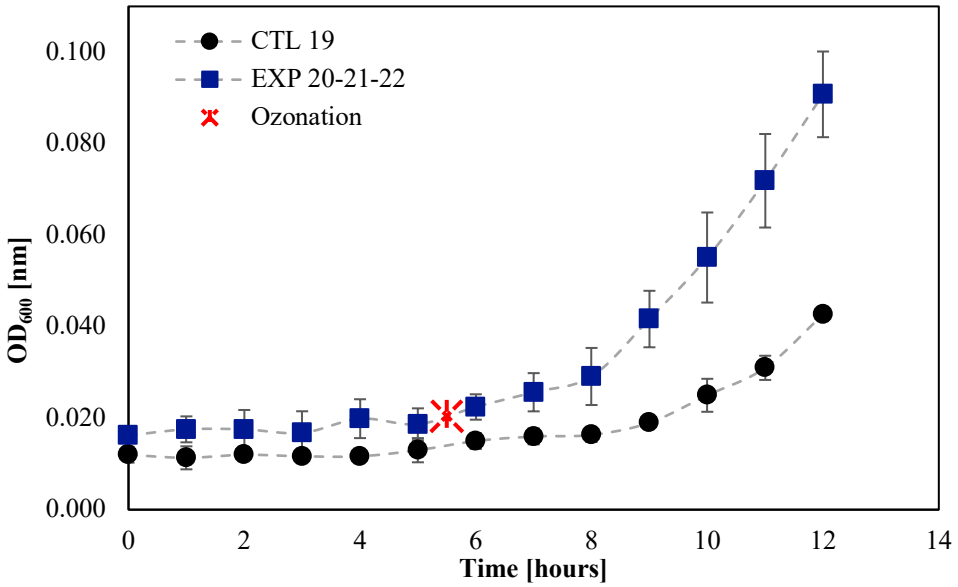

Figure 20. Biomass growth in 50:50 synthetic:raw wastewater. Total experiment duration of 12 hours at 25°C, 150 rpm mixing and initial DO_{sat} levels. Ozonation conditions: ozonated fraction = 25% of total volume (approx. 400 ml), ozone dose= 10 mg O_3/L. CTL: control run – only biological treatment; EXP: biological treatment + ozonation (at t=5.5 hrs). The error bars indicate that for each time point, three separate samples were collected to measure the OD_{600}. The ozonation experiments were performed in triplicates (n=3).

For the organic matter removal, **Figure 21** shows how the removal efficiency in a mixture of 50% of raw and synthetic wastewater compares to that achieved using 100% synthetic wastewater. The removal efficiencies in the latter water matrix are lower with COD_f/COD_o ratio of 0.88 and 0.80 ± 0.01 for the control run and the ozonation experiment, respectively, compare to 0.73 and 0.68 ± 0.02 in SSW. A similar trend in terms of efficiency of biological treatment based on the water matrix was reported by Achisa C. Mecha et al. (2016) where COD removal was much more efficient in synthetic wastewater (i.e. 93%) than in raw wastewater obtained from a biological treatment unit (i.e. 72%).

However, in both water matrices, the difference in COD removal between the control and the ozonation is similar. Therefore, although the water matrix seems to interfere with the efficiency of biological treatment, the additional removal provided by ozonation is similar in both water matrices and was maintained in the more complex matrix.

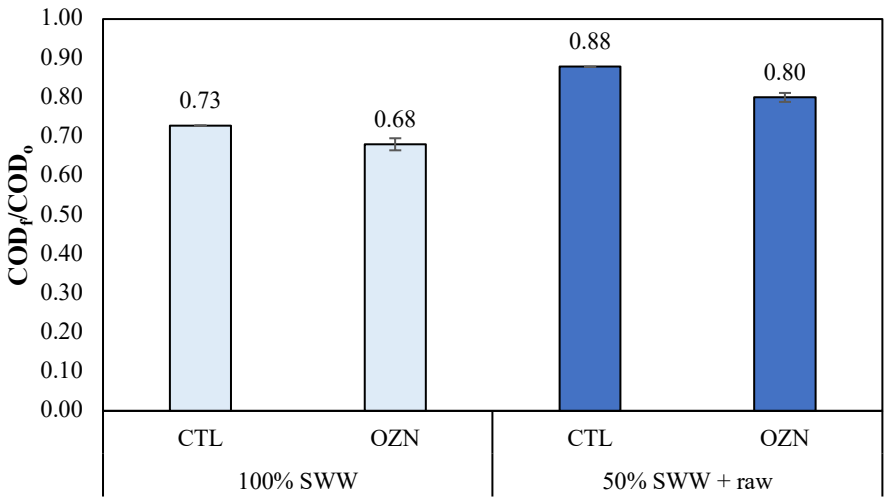

Figure 21. Comparison of COD removal in different water matrices (100 % SWW and 50% mixture of raw and synthetic wastewater). Only the ozonation experiments were performed in triplicates (n=3).

4.2.3 Temperature

In order to investigate if the results obtained in the experiments 14-22 would also apply in colder environments, a lower temperature of 15°C was used in the experiments described below. This was important to the research as the majority of the lagoons in Canada are subject to colder temperature for several months during the year. The water matrix used in these experiments at lower temperature was also a 50% mixture of raw and synthetic wastewater.

Figure 22 shows that no biomass growth was observed during the 12 hours of each experiment, control or ozonation. To check if these conditions could actually sustain biomass growth, EXP 24 was allowed to run overnight. The lag phase was longer at this lower temperature and biomass growth was only observed the next day, with an optical density of 0.064 nm recorded after 26 hours. This longer lag phase can be explained by the fact that temperature affects biomass composition, nutrient requirement and most importantly, metabolic reaction rates (Mayo & Noike, 1996).

Considering the long lag-phase, the experiment was repeated using a different timeline than in the previous experiments. The experiment was started later in the evening to allow the lag phase to run through the night and the ozonation was performed the next morning. **Figure 23** shows a comparison between EXP 24 (using the usual timeline) and EXP 26 (performing the ozonation 15.5 hours later). For EXP 26 with a delayed ozonation, an immediate increase in biomass optical density was observed right after ozonation. However, the value attained of 0.039 nm attained in 22 hours, which is still much lower than the optical density values obtained at 25°C in 12 hours (i.e. 0.091 nm in 50% raw and synthetic wastewater). This lower cell density at lower temperature is in line with a decrease in heterotrophic bacterial density measurements at lower temperatures reported by Mayo & Noike (1996). However, additional experiments are required to assess the reproducibility of EXP 26.

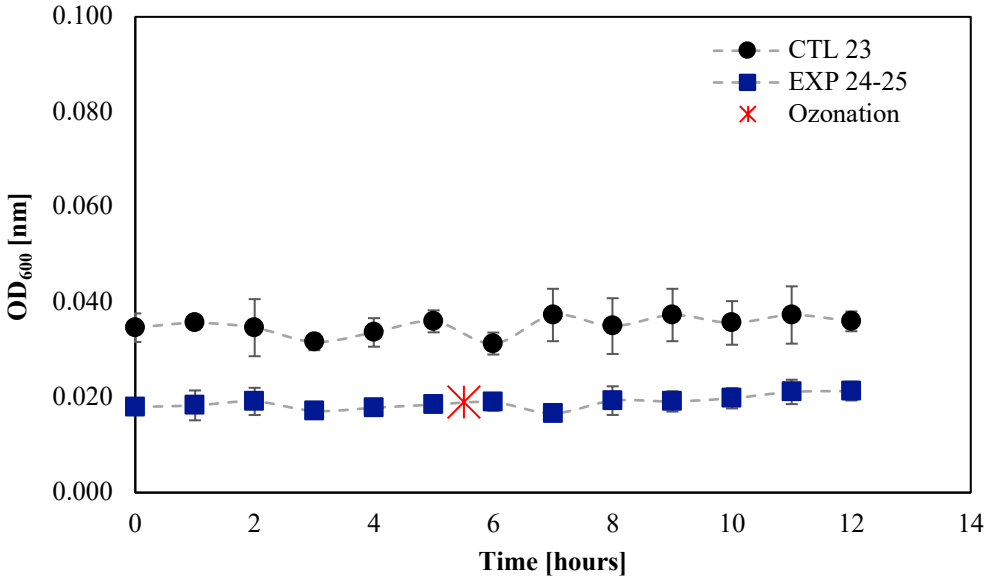

Figure 22. Biomass growth in 50:50 synthetic:raw wastewater. Total experiment duration of 12 hours at 15°C, 150 rpm mixing and initial DO_{sat} levels. Ozonation conditions: ozonated fraction = 25% of total volume (approx. 400 ml), ozone dose= 10 mg O_3/L. CTL: control run – only biological treatment; EXP: biological treatment + ozonation (at t=5.5 hrs).

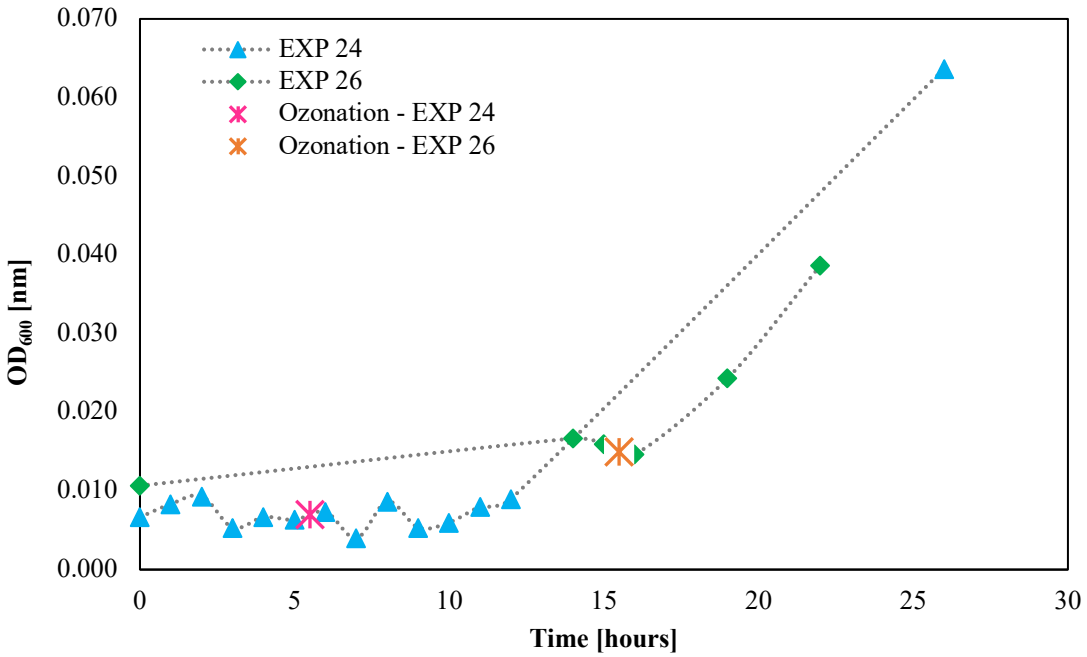

Figure 23. Biomass growth in 50:50 synthetic:raw wastewater. Total experiment duration of 12 hours at 15°C, 150 rpm mixing and initial DO_{sat} levels. Ozonation conditions: ozonated fraction = 25% of total volume (approx. 400 ml), ozone dose= 10 mg O_3/L. CTL: control run – only biological treatment; EXP: biological treatment + ozonation (at t=5.5 hrs for EXP 24 and t=15.5 hrs for EXP 26).

4.3 Assessment of the integration of ozonation to biological treatment

The integration of ozonation to biological treatment was assessed based on the experiments where ozonation was performed at the end of the lag phase at a dose of 10 mg O_3/L in 100% synthetic wastewater at 25°C, in 50% raw wastewater at 25°C and 15°C (i.e. EXP 14-26). All prior experiments were used for method development purposes and were not included in this analysis. As discussed in the previous section, increased biomass optical density was observed when ozonating. However, in order to translate those observations into comparable metrics, the growth rate of the biomass was calculated. Additional consideration was given to the levels of dissolved oxygen and pH in the bioreactor during experiments to assess the integration of these two processes.

4.3.1 Quantification of the impact on the growth rate of integrating ozone

In order to determine the growth rate, the optical density data points in the exponential phase can be modelled using the following equations, where μ is the specific growth rate (hr^{-1}) and X is the biomass (mg/L, or in this case nm):

$$\mu = \frac{dX/dt}{X} \tag{11.1}$$

$$X = X_o e^{\mu(t-t_{lag})} \tag{11.2}$$

$$\ln\left(\frac{X}{X_o}\right) = \mu(t - t_{lag}) \tag{11.3}$$

The linear model (i.e. equation 11.3) is fitted to the exponential portion of the biomass growth curves obtained in the experiments conducted in 100% synthetic wastewater at 25°C (i.e. CTL 14 and EXPs 15-17-18). As shown in **Figure 24**, the exponential portion was approximated to occur from 5 to 8 hours in EXP 15-17-18, and from 6 to 10 hours in the CTL 14. The calculated specific growth rate for the control run was 0.603 hr^{-1}, and that of the ozonation experiment was slightly higher at a value of 0.671 hr^{-1}. This represents an increase of approximately 11.3%.

The same procedure was followed for the experiments conducted in 50% raw wastewater at 25°C (i.e. CTL 19 and EXP 20-23). The linear portions were fitted as seen in **Figure 25** and the exponential phases were approximated to occur from 8 to 12 hours for both the ozonation and control experiments. The specific growth rate for the control run was 0.306 hr^{-1}, and that of the ozonation experiment was 0.439 hr^{-1}. This represents an increase of approximately 43.5%. Similar to the optical density measurements, the specific growth rates of the biomass in the 50% mixture of raw and SWW were also approximately half the rates observed in the experiments in 100% SWW.

Although there is some variability in the literature values for the specific growth rates of heterotrophic biomass, the values shown in **Figure 24** and **Figure 25** fall within several reported general ranges. Kappeler & Gujer (1992) reported a range of 0.042 to 0.333 hr^{-1}, and Chen et al.

(2009) reported specific growth rates for various strains of wastewater bacteria ranging from 0.385 to 0.772 hr^{-1}. However, it should be noted that the growth rates calculated above are not net growth rates as the biomass decay rate remains unknown. As well, using optical density as measurement of biomass growth can lead to overestimations as this method does not differentiate between live and dead cells.

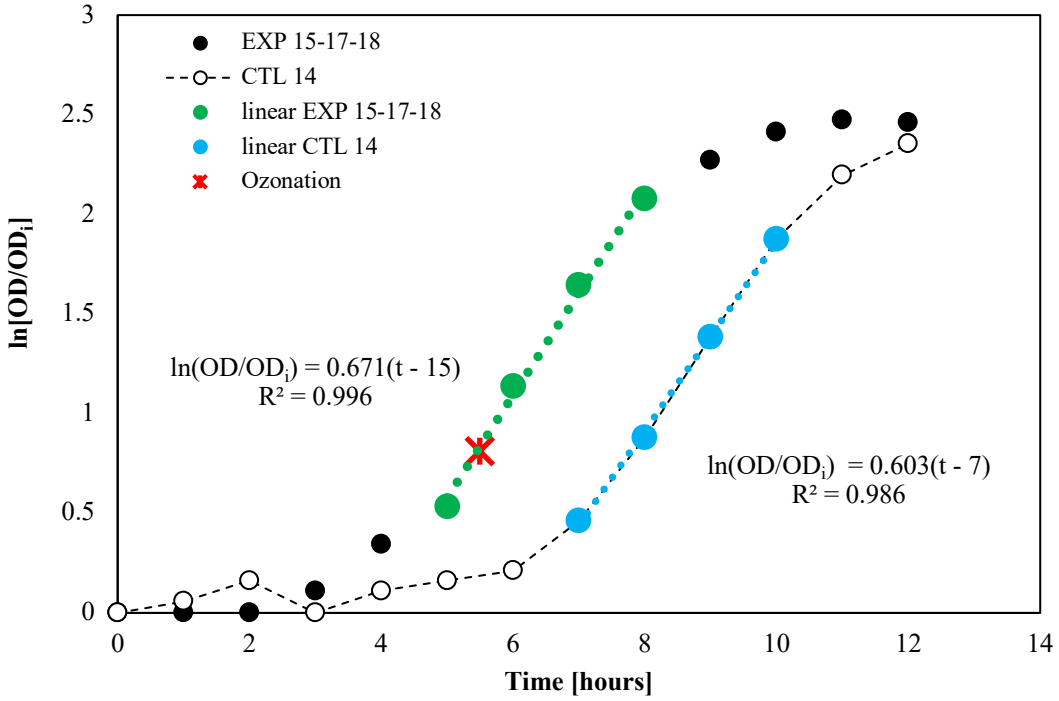

Figure 24. Specific growth rate. Total experiment duration 12 hours at 25°C, 150 rpm mixing and initial DO_{sat} levels. Ozonation conditions: ozonated fraction = 25% of total volume (approx. 400 ml), ozone dose= 10 mg O_3/L. CTL: control run – only biological treatment; EXP: biological treatment + ozonation (at t=5.5 hrs).

Although the exponential phase was not captured in the experiments conducted at 15°C due to the prolonged lag phase (EXP 26), the supposed exponential portion (i.e. t=16, 19 and 22 hours) was fitted. The specific growth rate was found to be 0.216 hr^{-1}. No additional control was performed with the modified ozonation time frame due to time restrictions, but the value of the specific growth rate indicates that biomass growth is restrained at lower temperatures even with ozonation.

These results suggest that the ozonation experiments conducted at the end of the lag phase (t=5.5 hours) at an ozone dose of 10 mg O_3/L had an impact on the specific growth rate of the biomass inside the bioreactor. This impact is maximal when the biomass is grown in 100% synthetic wastewater at 25°C and decreases when the medium is switched to 50% raw wastewater at 25°C.

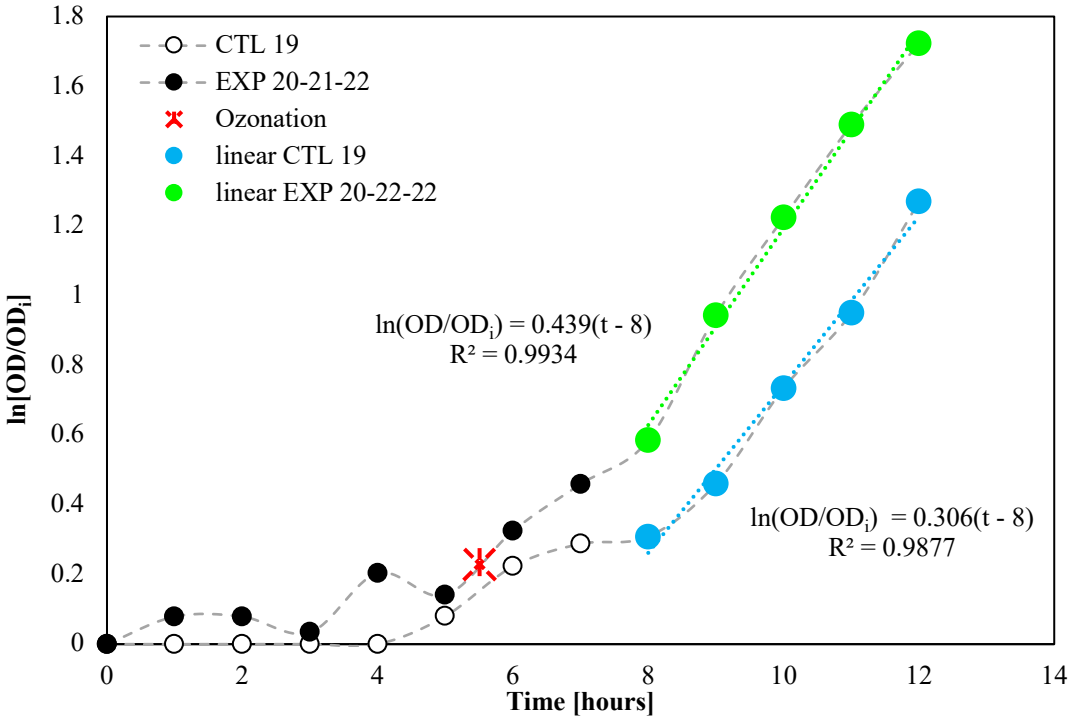

Figure 25. Specific growth rate in 50:50 synthetic:raw wastewater. Total experiment duration of 12 hours at 25°C, 150 rpm mixing and initial DO_{sat} levels. Ozonation conditions: ozonated fraction = 25% of total volume (approx. 400 ml), ozone dose= 10 mg O3/L. CTL: control run

4.3.2 pH levels

The pH levels in the experiments using 100% synthetic wastewater follow a trend very similar to the optical density measurements, as displayed in **Figure 26**. The pH levels mimic the biomass growth even in terms of the time frame as they start increasing in the ozonation experiment two hours earlier than those in the control run. The pH for both the control and the ozonation experiments remains in the range of 7.0 to 7.8. However, the fact that the pH is increasing is an interesting pattern as with the oxidation of organic matter, the pH is expected to decrease due to the formation of organic acids of lower molecular weight (Dahiya & Venkata Mohan, 2016). In this case, the increase in pH is most likely due to the increase in ammonia levels.

A different pattern is observed in the experiments conducted using a 50% mixture of raw and synthetic wastewater. As shown in **Figure 27**, the pH rises slightly in the first three hours of the experiment remaining constant until the last four hours when it starts to decline. It should be noted that the starting pH of 8.4 is higher than the one observed when using 100% synthetic wastewater. Due to technical issues, the pH data for CTL 19 is not available. The levels and fluctuations in pH can also be tied to the initial levels of ammonia present in the wastewater. Another aspect to be noticed is that the initial ammonia content of the 50% wastewater mixture is much higher due to the presence of raw wastewater. For instance, the initial ammonia concentration in CTL 14, composed of SWW, is 1.38 mg NH_3-N/L and increases to 7.92 mg NH_3-N/L in 12 hours. On the other hand, the ammonia concentration in CTL 19, composed of 50% raw wastewater, remains at approximately 13 mg NH_3-N/L throughout the entire experiment. Since the raw wastewater is mainly from municipal sources, the source of the high levels of ammonia is most likely urea.

The pH provided by the two wastewater matrices could be one of the factors that impacted the biomass growth, since pH is known to influence biomass regulation, ion transport and metabolic rate (Mayo & Noike, 1996). In fact, the high optical density of biomass observed when using 100% synthetic wastewater was favoured by its pH of approximately 7, which is optimal for microorganism. The more alkaline environment provided by the mixture of raw and synthetic wastewater (pH 8.4) could have been less favourable for biomass growth.

Ultimately, the high levels of ammonia in the raw wastewater might have contributed to the high pH levels and limited the biomass growth in the experiments using 50% raw wastewater. Lower biomass density may have limited the organic matter degradation, which consequently limited the pH fluctuations. On the other hand, using 100% synthetic wastewater ensures a neutral pH which promotes biomass growth. This in turn promotes biodegradation which leads to higher ammonia levels during treatment.

It should be noted however that the pH levels in the lagoon stations where the sludge was collected and in the lagoons where the pilot tests were conducted by Schlageter (2018) also had alkaline conditions with pH levels fluctuating between 8.0 and 9.0.

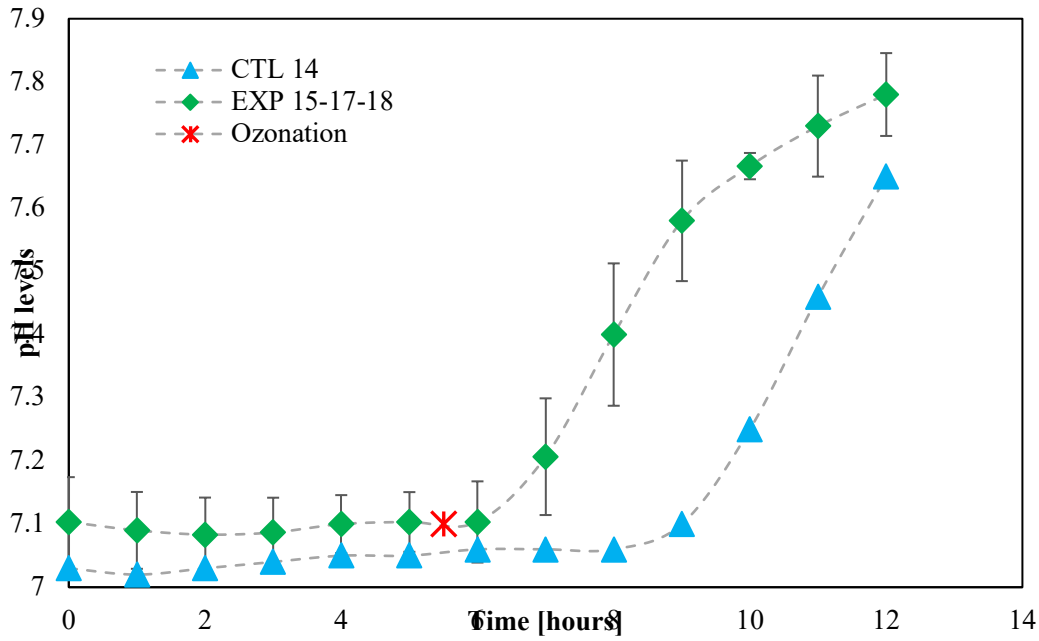

Figure 26. pH levels. Total experiment duration 12 hours at 25°C, 150 rpm mixing and initial DO_{sat} levels. Ozonation conditions: ozonated fraction = 25% of total volume (approx. 400 ml), ozone dose= 10 mg O_3/L. CTL: control run – only biological treatment; EXP: biological treatment + ozonation (at t=5.5 hrs). The ozonation experiments were performed in triplicates (n=3).

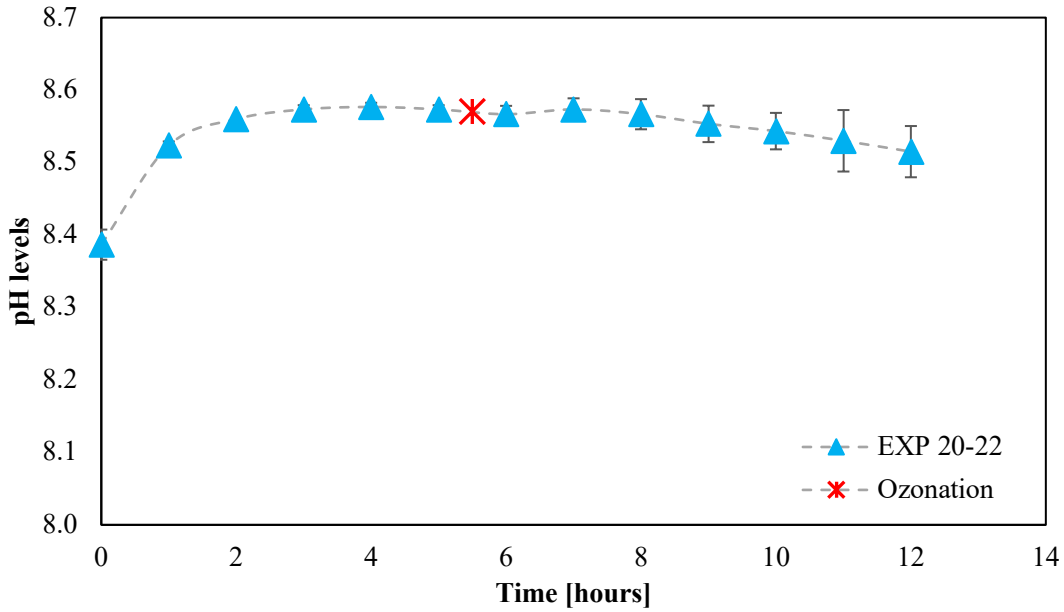

Figure 27. pH levels in 50:50 synthetic:raw wastewater. Total experiment duration of 12 hours at 25°C, 150 rpm mixing and initial DO_{sat} levels. Ozonation conditions: ozonated fraction = 25% of total volume (approx. 400 ml), ozone dose= 10 mg O_3/L. CTL: control run – only biological treatment; EXP: biological treatment + ozonation (at t=5.5 hrs). Ozonation experiments were performed in triplicates (n=3).

4.3.3 Dissolved oxygen levels

The dissolved oxygen levels were also monitored for EXP 14-18, and as seen in **Figure 28**, a similar trend was observed for both the control run and the ozonation experiment. Throughout the 12-hour experiments, the levels of dissolved oxygen dropped approximately 10-15% due to the on-going biodegradation activity. Another important aspect to observed is that the level of oxygen levels right after the addition of the ozonated portion into bioreactor, did not increase the overall amount of dissolved oxygen in the bioreactor. This indicates that the increase is growth is not due to an increase level of oxygen.

For the ozonation experiments, the drop in dissolved oxygen occurs earlier (around t = 9 hours) as opposed to the control run (around t = 11). Similarly, the dissolved oxygen levels reach a lower level in the ozonation experiments (86%), as opposed to the control run (88%). These results confirm the trends in results that have been presented so far, where the ozonation experiments exhibit a greater biomass activity earlier in the experiments. It should be noted that the levels of dissolved oxygen increase as the biological activity is transitioning from the exponential to the stationary growth phase. This change in oxygen requirement exhibited by bacteria can be observed since the air flowrate into the bioreactor is kept constant throughout the duration of every experiment (i.e. see **Table 10**) and it is set so that the bioreactor is initially saturated with oxygen prior to biological activity. Therefore, as the specific growth rate of bacteria decreases so does their oxygen requirement, therefore resulting in higher dissolved oxygen levels in the bioreactor.

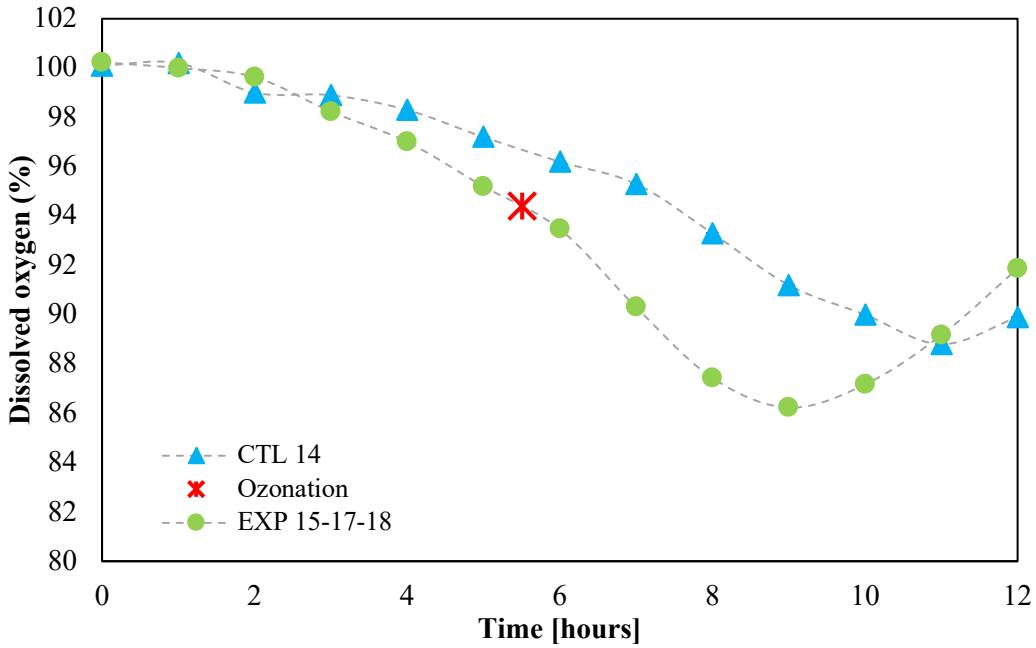

Figure 28. Dissolved oxygen levels during experiments 14-18.

4.4 Contaminants of emerging concern

Several contaminants of emerging concern were selected and added to the bioreactor in order to verify if the biological treatment with integrated ozonation increased their biodegradability. Caffeine was used as a positive control because it is known to biodegrade easily. This was confirmed in the several experiments conducted during the method development (i.e. CTL 3-4-8, EXP 5-7), during which caffeine, initially present at a concentration of 123 ppb, was completely biodegraded in 44 hours on average (results not shown). The removal of CECs is assessed both in the ozonated portion and in the biological treatment.

4.4.1 Removal of CECs in the ozonated portion

The removal of CECs in the ozonated portion (i.e. 25% of the total bioreactor volume) is presented in **Table 12**. These results show that the ozone itself did partially degrade some compounds in the ozonated portion. More specifically, a significant decrease was observed in both wastewater matrices (100% SWW and 50% mixture) for carbamazepine (56.9% and 33.5%), naproxen (52.1% and 37.0%) and gemfibrozil (47.7% and 47.2%). The low removal efficiencies observed for caffeine and atrazine are due to the fact that they are highly resistant to ozone (Acero, Stemmler, & von Gunten, 2000; Kim & Tanaka, 2010). On the other hand, carbamazepine, gemfibrozil and naproxen can be degraded at levels above 90% using ozone (Deng, 2020; Kim & Tanaka, 2010; Yao et al., 2018). This confirms the trend observed in **Table 12**, where higher removal efficiencies are observed for the compounds with high reactivity towards ozone.

Table 12. Direct effect of ozonation on CECs in the ozonated portion (25% of the total volume) in different wastewater matrices.

Compound	100% SWW (EXP 15-17-18) Removal (%)	50% raw + SWW (EXP 20-22) Removal (%)
Caffeine	5.6	3.3
Carbamazepine	56.9	33.5
Atrazine	13.1	8.7
Ibuprofen	8.3	8.0
Naproxen	52.1	37.0
Gemfibrozil	47.7	47.2

4.4.2 Removal of CECs in biological treatment with integrated ozonation

Although ozone alone can decrease the concentration of several CECs, the combined effect with biological treatment was assessed to verify is ozone can also increase their biodegradability and further enhance removal. **Figure 29** shows the final concentrations recorded for CTL 14 and EXP 15, experiments conducted in 100% synthetic wastewater. It can be noticed that in the control run, carbamazepine, atrazine and ibuprofen were not degraded at all and that naproxen and gemfibrozil only decreased by approximately 10%. In the ozonation experiment, similar results are observed suggesting that ozone did not contribute to the increase the biodegradability of these compounds.

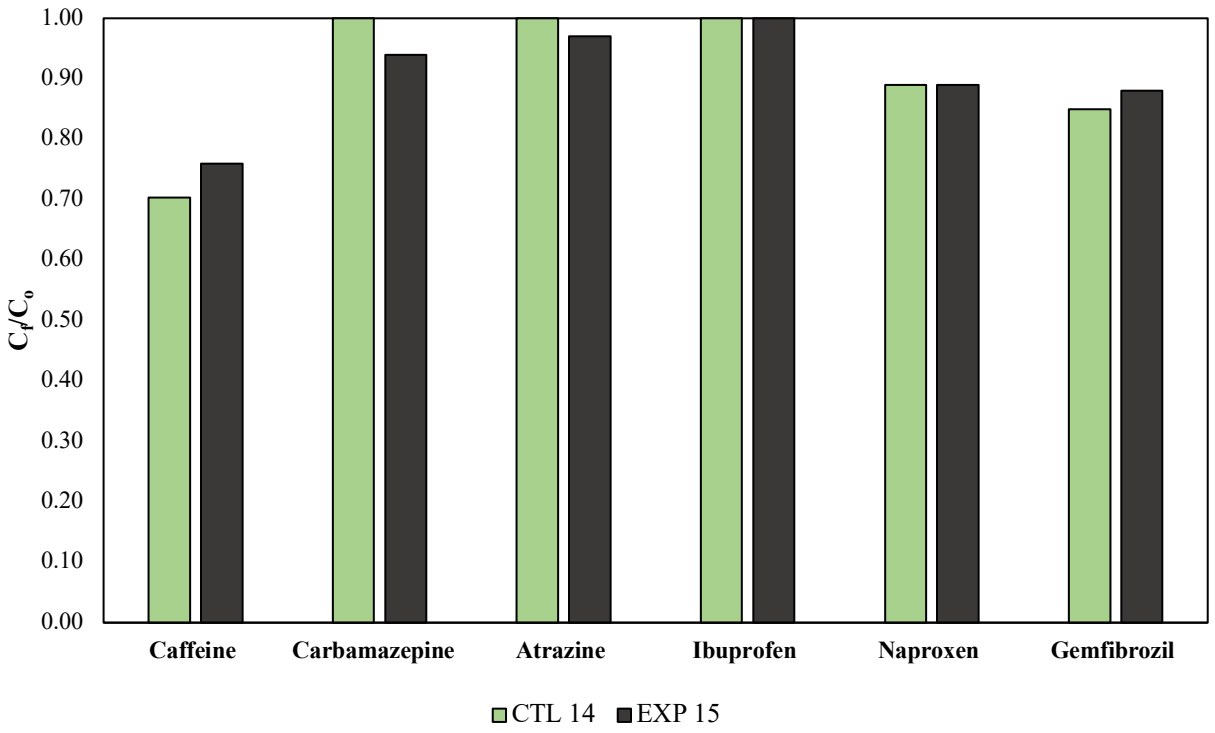

Figure 29. Removal of selected contaminants of emerging concern in 100% synthetic wastewater; final concentrations (t = 12 hours). Ozonation conditions: ozonated fraction = 25% of total volume (approx. 400 ml), ozone dose= 10 mg O_3/L. CTL: control run – only biological treatment; EXP: biological treatment + ozonation (at t=5.5 hrs).

Comparable results were obtained in the experiments conducted in the 50% mixture of raw and synthetic wastewater, as shown in **Figure 30**. Only gemfibrozil shows a decrease of 22% in the integrated biological-ozonation system as opposed to the control. Another aspect to consider in EXPs 19-22, is that the final concentrations of all compounds are greater than the initial ones. Jelic et al. (2011) states that some metabolites and derivates can recombine to form parents compounds and proposes this possible explanation for higher concentration of carbamazepine obtained in the effluent of a WWTP. Since EXPs 19-22 contained raw domestic wastewater, it is possible that it contained some metabolites and derivatives. Some desorption effects during treatment might also have contributed to this increase in concentration.

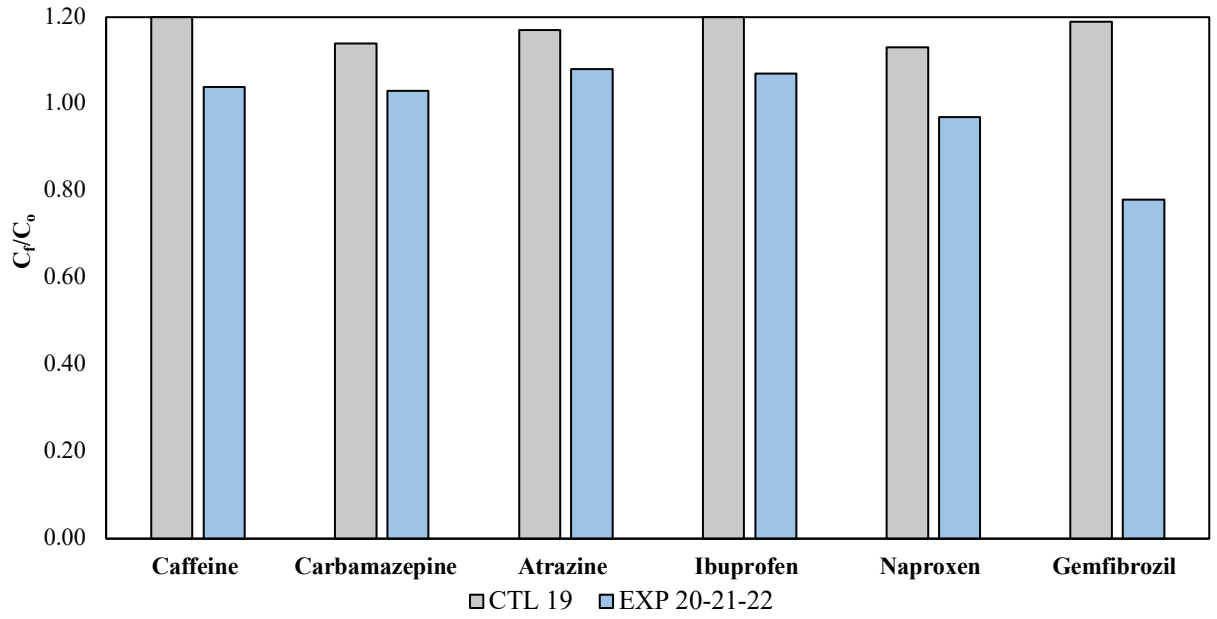

Figure 30. Removal of selected contaminants of emerging concern in a 50% mixture of raw and synthetic wastewater; final concentrations (t = 12 hours). Ozonation conditions: ozonated fraction = 25% of total volume (approx. 400 ml), ozone dose= 10 mg O_3/L. CTL: control run – only biological treatment; EXP: biological treatment + ozonation (at t=5.5 hrs).

The absence of CECs biodegradation is supported by results obtained during the control reproducibility runs where it was found that all the selected CECs had undergone minimal biodegradation by the bacteria in the conditioned sludge, with the exception of caffeine. This is not aligned with the reported literature which stipulates that most CECs listed above, especially ibuprofen and naproxen, are biodegradable to some extent (Tiwari et al., 2017). For instance, in conventional activated sludge systems, Samaras et al. (2013) reported removal of 100% for ibuprofen and 95% for naproxen, and Jelic et al. (2011) reported 90% removal for gemfibrozil. On the other hand, carbamazepine is considered to be an emerging recalcitrant organic pollutant and does not biodegrade well in conventional WWTP, with removals below 10% (Costa et al., 2019). Atrazine has a various removal efficiencies in biological treatment ranging from 40-90% (Liu, Huang, & Wang, 2008). In lagoons systems, higher CECs removal efficiencies were observed more regularly in lagoons located in arid and semi-arid regions where average annual temperature were higher than 25°C and were subject to minimal seasonal changes (Al Qarni, Collier, O'Keeffe,

& Akunna, 2016). Additionally, temperature stability and longer retention times favour greater microbial activity and adaptation (Al Qarni et al., 2016).

Aside from the reported biodegradation of these compounds, the increased biodegradability of pharmaceuticals has also been reported by Larcher & Yargeau (2013). They used a low ozone dose of 5 mg O_3/L to increase the biodegradability of sulfamethoxazole and 17α-ethinylestradiol. The results show that pre-ozonation removed 5 % to 40 % more sulfamethoxazole, but 2 % to 23 % less 17α-ethinylestradiol. This was attributed to the preferential degradation of a by-product of 17α-ethinylestradiol ozonation.

Ultimately, although partial ozonation led to significant removal of certain compounds, it did not seem to further promote their degradation by biological treatment. Additionally, since other studies in the literature report significant levels of biodegradation of most of the CECs in conventional activated sludge systems (Jelic et al., 2011; Samaras et al., 2013), one hypothesis is that the required microbial populations did not develop in the bioreactor for the biodegradation of these compounds to occur. It is also possible that the sludge conditioning might have reduced the diversity of the inoculum, or that that the lagoon itself did not contain the appropriate microorganisms for degrading these compounds. This is especially relevant for the experiments discussed here due to the times at which the sludge that was used was collected (i.e. December for EXPs 14-15, and May for EXPs 19-22).

5 Preliminary Economic Analysis

As part of the assessment of the feasibility of integrating ozonation to lagoons at a large scale, it is important to start tackling the financial aspects associated to this approach. The analysis conducted here is a preliminary estimation of the capital and operational costs involved in running and maintaining such a process. The concept of integrating ozonation to lagoons used in this analysis consists of implementing a network among several lagoon treatment locations within a certain perimeter that would allow the sharing of a mobile ozonation system in order to maximize its use, lower the requirements in terms of qualified personnel and decrease the overall cost of operation.

One lagoon at one of the two FN communities at which the pilot tests were conducted by Schlageter (2018) was used as a reference as it has already been the subject of a 20-year population and housing projection study. The results of the study, shared with us by the manager of the lagoon through personal communications, demonstrated that the existing lagoon system is considerably undersized. Studies were done to propose solutions and costs for upgrading the existing infrastructure, which can be used here as a baseline for comparison of the proposed integration of an ozonation system to the existing lagoon. For simplicity purposes, the lagoon will be addressed as *Lagoon A* in the remainder of this section.

5.1 Preliminary costs for the integration of ozonation to lagoons based on lab-scale results

The preliminary economic analysis was based on the results obtained in the experiments presented in the previous section as well as on the information provided by Aclarus, the company that collaborated on the pilot tests presented by Schlageter (2018). Given the limited use of the ozonation system in the context of partial ozonation, it was decided to perform the preliminary economic analysis assuming one portable ozonation can be shared between multiple lagoon stations. The number of lagoon stations that could share the ozonation unit would depend on their geographical proximity and most likely on a pre-defined period of time during which ozonation is performed (e.g. from ice-off to mid-summer) as well as the required duration of operation at each site.

The parameters that can be manipulated in terms of equipment are the ozone production rate of the ozone generator and the pumping rate of the pumps. These two parameters would eventually determine the ozone dose and the runtime of the operation. The information on the models available for each system (i.e. ozonation and pumping) were provided by Michael Doran (Aclarus, 2020), and are shown in **Table 13**. There are two ozone generator models available with an ozone production rate of 120 or 140 g/hr, and three pumps with pumping rates of 50, 90 and 150 gpm. Assuming 100% efficiency of the ozone system at dissolving and keeping the ozone in the water, the ozone dose can be calculated using the following equation:

$$\text{Ozone dose (mg/L)} = {}^{O_{3,\text{production rate}}\left(\frac{\text{g}}{\text{hr}}\right) \times 1000}\Big/_{\text{Pumping rate (gpm)} \times 3.78 \times 60} \quad (12)$$

Table 13. Summary of equipment information provided by Aclarus (Aclarus, 2020).

Unit		Information	Value	Units
Diesel generator		Unit cost	2,500	$
		Fuel consumption	1.8	gal/hr
		Power output	28	kW
		Diesel cost	1	$/L
Ozone* generator	**120 g/hr model**	Unit cost	32,000	$
		Energy requirement	1.1	kW
	140 g/hr model	Unit cost	34,000	$
		Energy requirement	1.3	kW
Oxygen system		Unit cost	10,000	$
		Energy requirement	1.2	kW
Pump	**50 gpm**	Unit cost	4,000	$
		Energy requirement	5	kW
	90 gpm	Unit cost	7,000	$
		Energy requirement	10	kW
	150 gpm	Unit cost	14,500	$
		Energy requirement	25	kW
Maintenance		Equipment	2,000	$/year
-		Electricity	0.073	$/kWh
-		Labour	2	people
-		System setup	2	days

*Price comprises control system and sensors.

Using equation 12, the ozone dose generated by each combination of ozone generator and pump were obtained, as shown in the table below.

Table 14. Ozone doses based on various ozone production rates and water pumping rates.

	Pump - 50 gpm	Pump - 90 gpm	Pump - 150 gpm
Ozone system 120 g/hr	10.6 mg O_3/L	5.9 mg O_3/L	3.5 mg O_3/L
Ozone system 140 g/hr	12.3 mg O_3/L	6.9 mg O_3/L	4.1 mg O_3/L

According to the lab-scale results in, an ozone dose of 10 mg O_3/L applied to 25% of the volume in the bioreactor led to higher biomass growth and COD reduction. This ozone dose was used a basis for the selection of the ozone system and pump. From **Table 14**, it can be seen that the ozone generator with a production rate of 120 g/hr and the pump with a 50 gpm pumping capacity would generate an ozone dose of 10.6 mg/L in the ozonated portion.

5.1.1 Determination of the CAPEX and OPEX costs for a shared ozonation unit

Using the ozone dose as selection criteria for the equipment along with the costs from **Table 13**, the capital expenditure can be determined, as shown in **Table 15**. An important factor to consider is that access to electricity is not always available on lagoon sites. For this reason, a diesel generator was included in the list of items contributing to the capital expenditure.

Table 15. CAPEX summary

CAPEX*	Items	Cost ($)
	Diesel generator	2,500
	Ozone generator	32,000
	Oxygen system	10,000
	Pump	4,000
	Total	***48,500***

*This doesn't include the costs of the trailer or enclosure or infrastructure for integration to lagoon.

The operational expenditure depends on the volume that needs to be ozonated, which in turn determines the amount of time that it will take to pump the water from a lagoon and ozonate it, or the runtime. For simplification purposes, the term ozonation session will include the runtime of the ozonation process along with the time allocated to the installation of the ozonation unit. This "session" approach is highly practical considering that the short duration of operation yearly, which would not justify a permanent infrastructure at each location. As shown in **Figure 31**, the runtime is directly proportional to the ozonated volume. To obtain the total time of an ozonation session, another four days should be added to the pumping runtime since it takes two days to install and two days to uninstall the unit.

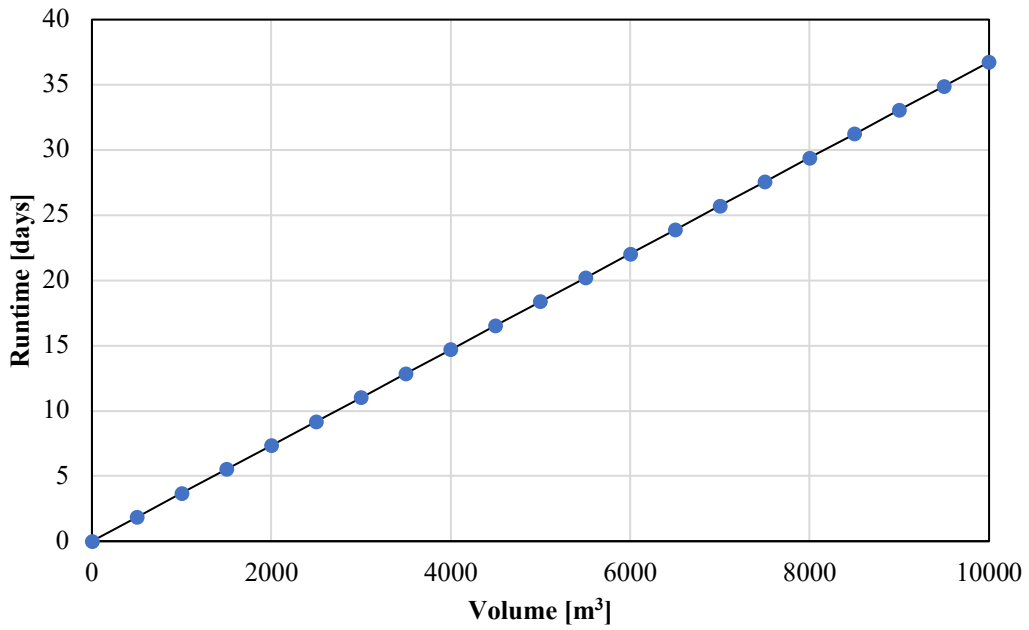

Figure 31. The runtime of an ozonation session based on the fraction of a lagoon volume to be ozonated using a 50 gpm pumping capacity.

The runtime of the ozonation process will then dictate the amount of electricity or, in the case that power to the unit is supplied by a diesel generator, the fuel consumption that will be required. The power required by the oxygen system, the ozone generator with a production rate of 120 g/hr and the pump with a 50 gpm pumping capacity is calculated to be approximately 7.3 kW. **Figure 32** shows the electricity required and the equivalent diesel consumption in the event that a generator is used. Additional information on the costs of these two forms of energy are also provided based

on the Quebec electricity cost of 0.073 $/kWh and on the diesel cost of 1 $/L (Aclarus, 2020). It can be seen that the operational costs of using a diesel generator are higher than those of using electricity.

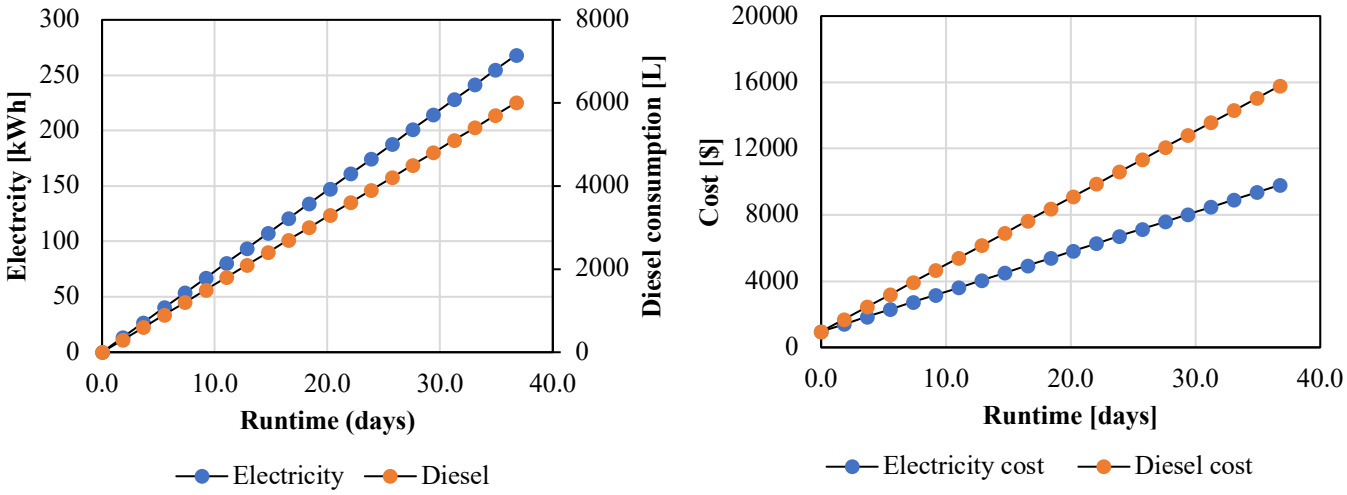

Figure 32. Electricity (kWh) and diesel consumption (L) based on runtime -left. Cost of electricity and diesel based on runtime.

In order to get an estimate for the operational expenditures, a minimum lagoon volume was selected based on the design criteria of 100 m^3/day, for which the new WSER applies. The total volume would be approximately 36,500 m^3. Additionally, it can be assumed that this lagoon station has two cells, as did the ones in the pilot test, each with a volume of 18,250 m^3. Therefore, ozonating 25% the second cell (i.e. 4562.2 m^3) would result in a runtime of 17 days. Along with the time required for installation, an ozonation session for this lagoon would last 21 days. It is assumed that the power is supplied by a diesel generator. **Table 16** shows the items that contribute to the operational expenditure. It should be noted the maintenance expense was given on a yearly basis by Aclarus (2020), as shown in **Table 13**, but it is assumed that the lagoon cannot be ozonated for about six months a year due to ice formation. The maintenance is therefore distributed over the remaining six months, resulting in a cost of 11 $/day. As for the labour, it is assumed that an operator works 8 hours/day and is paid a salary of 28 $/hour (Indeed, 2020).

Table 16. OPEX summary

OPEX*	Items	Cost ($)
	Diesel fuel	2,780
	Maintenance	231
	Labour (2 people)	9,408
	Total	***12,419***

*This doesn't include the costs of transportation, and the plumbing or infrastructure necessary for integrating the ozonation unit to the lagoon.

5.2 Lagoon A Case Study

The study done on the upgrading of Lagoon A provides useful insight into various alternatives for improving the performance of the existing lagoons coupled with some rough financial estimates. This case is an opportunity to compare the integration of ozonation with other alternatives on an economic level.

5.2.1 General information on the existing lagoon A system

The community is currently serviced using a gravity sewage collection system as well as sewage force mains that deliver the sewage to the community sewage treatment lagoon. In the core area, there are approximately 80 service connections to the gravity sewage. The lagoon is a two-cell facultative lagoon, with a total volume of approximately 23,050 m^3.

The lagoon system was originally designed to provide one year of storage capacity (i.e. 23 050 m^3) equivalent to an average daily flow of 63 m^3/day. This is considerably less than the estimate of the current (2017) average daily flow of 129 m^3/day, assuming the entire population of the community has wastewater that is treated at the lagoon. Therefore, the existing lagoon is severely undersized and needs to be expanded or replaced by a different technology, to meet the current and the projected flows from the community. **Table 17** shows the 20-year population projections along with its associated wastewater flow rates.

Table 17. Projected Wastewater Production

	Year 0 (2017)	**Year 5 (2022)**	**Year 10 (2027)**	**Year 15 (2032)**	**Year 20 (2037)**
Per capita wastewater production (L/day)	180	180	180	180	180
Per capita infiltration and extraneous flow (L/day)	90	90	90	90	90
Projected Population	478	545	619	701	789
Total wastewater production (m^3/day)	129	147	167	189	213
Year production (m^3)	47,107	53,710	61,002	69,084	77,756

5.2.2 Upgrade strategies proposed in 2017

In response to the population projections and the need to upgrade the existing wastewater treatment, the following alternatives were proposed: (1) expansion of the existing facultative lagoon, and (2) conversion of the existing facultative lagoon to an aerated lagoon and submerged attached growth reactor system.

The overall capital and annual operational expenditures of the proposed alternatives are presented in **Table 18**. It is already evident that in the case of this lagoon station, the cost of integrating ozonation is much lower when compared to the other options proposed. However, due to the increased projected wastewater production at the lagoon A, the integration of ozonation alone may not provide sufficient treatment or the capacity of treatment might have to be increased. This

particular lagoon could consider discharging more than once a year, and in this case, ozonation could provide assistance to reach the discharge regulations in shorter amount of time. Nevertheless, this cannot be a long-term solution as the population using lagoon A continues to grow, and the lagoon is already currently undersized. It is also important to note that note all aspects of treatment have been evaluated in the pilot studies and the integration of ozonation to an existing lagoon has not yet been proven to offer the same level of treatment as the one offered by the other proposed upgrades.

Table 18. Costs associated with the proposed alternatives

Alternative	CAPEX ($)	Annual OPEX ($)
Facultative Lagoon Expansion	5 854 000	43 140
Conversion to Aerated Lagoon and SAGR System	3 201 400	122 300
Integrated ozonation	*48,500*	*12,419 (per ozonation session)*

It is therefore evident that the estimated costs associated with integrating ozonation to lagoon are considerably lower than those of the proposed alternatives. However, for lagoons that require significant improvement in treatment efficiency or that are severely undersized, such as lagoon A, it seems that this strategy, although very economical, may not be sufficient. Based on the results obtained, there is an indication that low dose ozonation may improve biomass growth. If supported by more experiments, low dose ozonation could potentially be used as a short-term solution in smaller lagoons, in case there is difficulty in reaching compliance.

Conclusion

The present thesis research has led to the development of a methodology to investigate the integration of ozonation to lagoon treatment. The method developed allowed to obtain reproducible results, to maintain bacterial growth in the system and to have flexibility in the operation to assess the impact of several variables: time of ozonation, wastewater matrix including the volume of wastewater ozonated, ozone dose and temperature, which were however not thoroughly investigated in the present study due to time constraints. An important improvement relative to work that was previously done was the use of a consortium of microorganisms, obtained through the pre-incubation of sludge collected at a full-scale lagoon and used within six days. However, the methodology developed did not allow to elucidate the lack of nitrification and the absence of effect on the biodegradation of the selected CECs, which had been observed in our earlier work.

Based on the experiments conducted and the results obtained, there is an indication that low dose ozonation may enhance biomass growth and consequently facilitates organic matter removal or transformation. Experiments in various wastewater matrices showed that an ozone dose of 10 mg O_3/L applied to a 25% fraction of the volume can lead to an increase in specific growth rate of lagoon biomass of 11.3 – 43.5% and a subsequent improved removal of organic matter 5-8%. However, these results cannot yet confirm that the integration of ozonation to lagoons would be sufficient to obtain the increase performance required to ensure that FN communities would meet regulations without having to consider other upgrades of their treatment systems. Further work is still required to optimize the integration by considering a wider range of ozone doses, portions of the lagoon volume ozonated, types of wastewaters and timing of the ozonation in the season.

As for the preliminary economic analysis, based on the current information available, the costs associated with the use of a shared ozonation unit between lagoon stations is significantly cheaper than some full-scale technologies available for increasing the treatment efficiency. Integrating ozonation to lagoons requires an approximate CAPEX of $48,500 and an OPEX of $12,419 as opposed to the several millions of dollars of investment essential for a lagoon expansion or for the conversion to an aerated lagoon and SAGR system. However, as pointed out earlier, due to the lack of sufficient evidence that the integration of low dose ozonation to lagoons would provide the same increases in treatment performance, it is not possible to perform a direct comparison of these options.

Limitations

The major limitation in this thesis is the fact that the ozone dose of 10 mg/L was not tested on the other growth phases (i.e. exponential and stationary). Conducting these additional experiments would allow to conclude which one of the two factors (ozone dose or timing) contributed to the increase in biomass growth and organic matter uptake.

Another considerable limitation that affected the way all experiments were conducted was the time restraint in terms of sample collection and monitoring the biomass growth. Due to the duration of the different growth phases of the biomass, it was difficult to capture the full performance of the biological treatment occurring inside the bioreactor. This became increasing difficult when the experiments were conducted in conditions where the lag phase was prolonged, such as in the case of decreased temperature. Connecting the bioreactor to an automatic UV spectrophotometer could be a solution to continuously monitor the biomass growth.

Aside from the long experiments, the preparation required before each experiment, specifically the sludge collection and conditioning, was time consuming and limited the amount of experiments that could be conducted. Furthermore, due to the freezing of the lagoon in the winter, it was not possible to collect sampled during the wintertime, further limiting the time during which experiments could be conducted. Developing a method to conserve conditioned lagoon sludge over a prolonged period of time (i.e. weeks or months) would decrease the time allocated for the preparation of each experiment and increase the number of experiments.

Another limitation was the use of synthetic wastewater instead of raw wastewater. Although efforts were made to use 100% raw wastewater, the biomass growth could not reach detectable optical density levels. The use of synthetic wastewater does not represent the complexity of the wastewater matrix that is encountered in municipal sewage and may lead to an overestimated efficiency of ozone, as discussed in previous sections. The methodology will have to be further refined in order to be able to sustain biomass growth in the small-scale bioreactor using 100 % wastewater.

Although the use of lagoon sludge provides a much more complex inoculum than individual bacterial cultures mixed in the lab, it is possible that due to the natural layers forming in facultative lagoons (i.e. aerobic, facultative, anaerobic), there is still a lack of microbial diversity in the experiments presented here. To capture the complexity of a full-scale lagoon system, the use of bioreactor in series can be implemented to mimic these different populations.

Finally, the use of optical density for biomass growth monitoring, although very simple, made it impossible to include suspended solids in the bioreactor and take into account in the study their possible interaction with ozone. To resolve this issue, the biomass growth can potentially be measured using other indirect methods such as the production of gas in the bioreactor. The bioreactor setup enables the installation of a pressure sensor.

Future work

In order to capture the full potential of low dose ozonation integration to a lagoon system, it would be beneficial to conduct the experiments presented in this thesis at more ozone doses, volume fractions and in various environmental conditions, preferably in raw wastewater. These aspects were not investigated as much as initially planned in the thesis proposal due to time constraints, especially in the context of Covid-19 and the closure of the laboratories.

Additional techniques for verifying the partial oxidation of organic matter should be implemented in order to assess if ozone is preferentially breaking down contaminants into compounds of lower molecular weight or mineralizes them. As discussed in the literature review, another aspect that is essential to the function of a lagoon is the presence of algae. Therefore, it would be valuable to develop experiments that would assess the growth bacteria in the presence of algae, and their combined interaction with low dose ozonation.

Additionally, several other parameters should be analyzed and monitored such as the relative abundance of dominant bacterial communities. This could help pinpoint if low dose ozonation affects the diversity of microorganisms and if the lack of biodegradability of some contaminants is due to the absence of specific bacteria. In conjunction, it would also be interesting to assess the effect of low dose ozonation on the EPS (i.e. extracellular polymeric substance) produced by bacteria and verify the findings put forward by Fu et al. (2019). They reported that low dose ozonation alters the EPS fraction surrounding bacteria and increases the contact between cells and DOM (i.e. dissolved organic matter). This enhances the mass transfer of DOM to microorganisms without comprising their resistance to ozone toxicity.

Finally, upon gathering a better understanding of the factors and conditions affecting the combination low dose ozonation and biological treatment, additional pilot tests would be necessary in case the concept proves to be fully working at bench-scale.

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Appendix I – Determination of chemical oxygen demand, COD

Oxygen Demand, Chemical

DOC316.53.01099

USEPA[1] Reactor Digestion Method[2] **Method 8000**

0.7 to 40.0[3] mg/L COD (ULR); 3 to 150 mg/L COD (LR); 20 to 1500 mg/L COD (HR); 200 to 15,000 mg/L COD (HR Plus)

Scope and application: For water and wastewater. Digestion is required.

[1] Ranges 3 to 150 mg/L COD and 20 to 1500 mg/L COD are USEPA approved for wastewater analyses (Standard Method 5220 D), Federal Register, April 21, 1980, 45(78), 26811-26812.
[2] Jirka, A.M.; Carter, M.J., Analytical Chemistry, 1975, 47(8), 1397.
[3] The ULR is only available with spectrophotometers that can measure at a wavelength of 350 nm.

Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows adapter and light shield requirements for the instruments that use them.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for test tubes

Instrument	Adapters	Light shield
DR 6000, DR 5000	—	—
DR 3900	—	LZV849
DR 3800, DR 2800, DR 2700	—	LZV646
DR 1900	9609900 (D[1])	—
DR 900	4846400	Cover supplied with the instrument

[1] The D adapter is not available with all instrument versions.

Before starting

Install the instrument cap on the DR 900 cell holder before ZERO or READ is pushed.
DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before this test is started.
The reagent that is used in this test is corrosive and toxic. Use protection for eyes and skin and be prepared to flush any spills with running water.
The reagents that are used in this test contain mercury. Collect the reacted samples for proper disposal.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Run one blank with each set of samples. Run all tests (the samples and the blank) with the same lot of vials. The lot number is on the container label. Refer to Blanks for colorimetric determination on page 4.
Store unused (light sensitive) vials in a closed box.
If the samples contain high concentrations of chloride, refer to the Alternate reagents section.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

Description	Quantity
Beaker, 250-mL	1
Blender	1
COD Digestion Reagent vials	varies
DRB200 Reactor	1
Light shield or adapter (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	1
Magnetic stirrer and stir bar	1
Opaque shipping container for storage of unused, light-sensitive reagent vials	varies
Pipet, TenSette, 0.1- to 1.0-mL, with pipet tips (for use with the 200–15,000 mg/L range)	1
Pipet, volumetric, 2.00-mL	2
Pipet filler safety bulb	1
Test tube rack	2

Refer to Consumables and replacement items on page 7 for order information.

Sample collection and storage

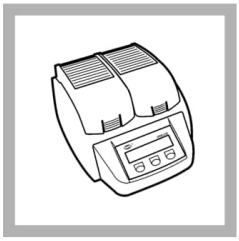
- Collect samples in clean glass bottles. Use plastic bottles only if they are known to be free of organic contamination.
- Test biologically active samples as soon as possible.
- Homogenize samples that contain solids to get a representative sample.
- To preserve samples for later analysis, adjust the sample pH to less than 2 with concentrated sulfuric acid (approximately 2 mL per liter). No acid addition is necessary if the sample is tested immediately.
- Keep the preserved samples at 2–6 °C (36–43 °F) for a maximum of 28 days.
- Correct the test result for the dilution caused by the volume additions.

Reactor digestion procedure

1. Put 100 mL of sample in a blender. Blend for 30 seconds or until homogenized.

For samples with large amounts of solids, increase the homogenization time. If the sample does not contain suspended solids, go to step 3.

2. For the 200–15,000 mg/L range or to improve accuracy and reproducibility of the other ranges, pour the homogenized sample into a 250-mL beaker and gently stir with a magnetic stir plate.



3. Set the DRB200 Reactor power to on. Preheat to 150 °C.

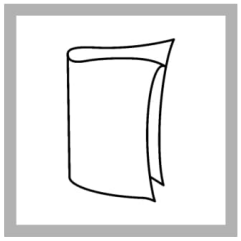
Refer to the DRB200 User Manual for selecting pre-programmed temperature applications.

4. Prepare the sample: Remove the cap from a vial for the selected range. Hold the vial at an angle of 45 degrees. Use a clean pipet to add 2.00 mL of sample to the vial.

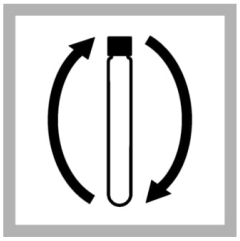
For 250–15,000 mg/L vials: Use a TenSette Pipet to add 0.20 mL of sample to the vial.

5. Prepare the blank: Remove the cap from a second vial for the selected range. Hold the vial at an angle of 45 degrees. Use a clean pipet to add 2.00 mL of deionized water to the vial.

For 250–15,000 mg/L vials: Use a TenSette Pipet to add 0.20 mL of deionized water to the vial.



6. Close the vials tightly. Rinse the vials with water and wipe with a clean paper towel.

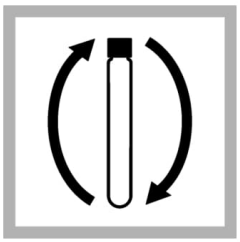


7. Hold the vials by the cap, over a sink. Invert gently several times to mix. **The vials get very hot during mixing.**

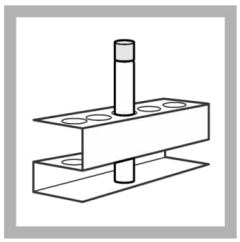
8. Put the vials in the preheated DRB200 reactor. Close the lid.

9. Heat the vials for 2 hours.

10. Set the reactor power to off. Let the vials cool in the reactor for approximately 20 minutes to 120 °C or less.

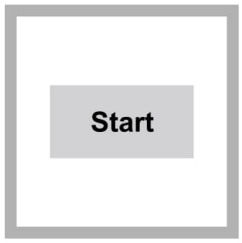


11. Invert each vial several times while it is still warm.



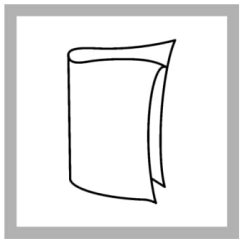
12. Put the vials in a tube rack to cool to room temperature.

Colorimetric procedure



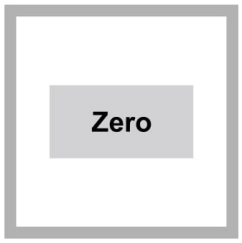
1. Start program **431 COD ULR**, **430 COD LR** or **435 COD HR**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.

Note: *Although the program name can be different between instruments, the program number does not change.*

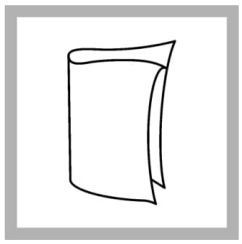


2. Clean the blank sample cell.

3. Insert the blank into the cell holder.



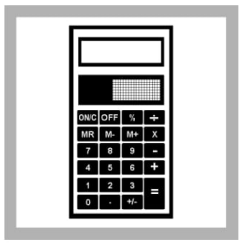
4. Push **ZERO**. The display shows 0 or 0.0 mg/L COD.



5. Clean the prepared sample cell.

6. Insert the prepared sample into the cell holder.

7. Push **READ**. Results show in mg/L COD.



8. If using High Range Plus COD digestion reagent vials, multiply the result by 10. For the most accurate results with samples near 1500 or 15,000 mg/L COD, repeat the analysis with a diluted sample.

Blanks for colorimetric determination

The blank vial can be used again and again for measurements that use the same lot of reagent vials. Measure the absorbance of the blank vial over time and prepare a new blank vial when the absorbance changes.

1. Put the instrument in the absorbance mode at the applicable wavelength. Refer to Table 3 on page 7.
2. Add 5 mL of deionized water into an empty vial.
3. Put the vial in the instrument and zero the instrument.
4. Put the blank vial that is used in the test procedure into the instrument and record the absorbance value.
5. Keep the blank vial in the dark.
6. Prepare a new blank when the absorbance has changed by approximately 0.01 absorbance units.

Appendix II – Determination of ammonia

Nitrogen, Ammonia

DOC316.53.01080

Salicylate Method[1] **Method 10023**

0.02 to 2.50 mg/L NH_3–N (LR) **Test 'N Tube™ Vials**

Scope and application: For water, wastewater and seawater.

[1] Adapted from Clin. Chim. Acta, 14, 403 (1966).

Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows adapter and light shield requirements for the instruments that use them.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for test tubes

Instrument	Adapters	Light shield
DR 6000, DR 5000	—	—
DR 3900	—	LZV849
DR 3800, DR 2800, DR 2700	—	LZV646
DR 1900	9609900 (D[1])	—
DR 900	4846400	Cover supplied with the instrument

Before starting

Install the instrument cap on the DR 900 cell holder before ZERO or READ is pushed.
DR 3900, DR 3800, DR 2800 and DR 2700: Install the light shield in Cell Compartment #2 before this test is started.
The reagents that are used in this test contain sodium nitroferricyanide. **Keep cyanide solutions at pH > 11 to prevent exposure to hydrogen cyanide gas.** Collect the reacted samples for safe disposal.
Keep the samples sealed at all times to prevent ammonia contamination from the air.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

Description	Quantity
Light shield (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	1
Nitrogen Ammonia, Reagent Set, Low Range Test 'N Tube™ AmVer™	2
Funnel, micro, poly	1
Pipet, TenSette®, 1.0–10.0 mL	1
Pipet tips, for TenSette® Pipet, 1.0–10.0 mL	2

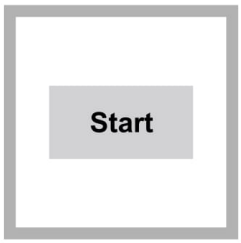
[1] The D adapter is not available with all instrument versions.

Refer to Consumables and replacement items on page 5 for order information.

Sample collection and storage

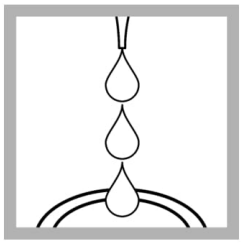
- Collect samples in clean glass or plastic bottles.
- If the sample contains chlorine, add one drop of 0.1 N sodium thiosulfate to 1 liter of sample to remove each 0.3 mg/L of chlorine.
- To preserve samples for later analysis, adjust the sample pH to less than 2 with concentrated hydrochloric acid (about 2 mL per liter). No acid addition is necessary if the sample is tested immediately.
- Keep the preserved samples at or below 6 °C (43 °F) for a maximum of 28 days.
- Let the sample temperature increase to room temperature before analysis.
- Before analysis, adjust the pH to 7 with 5.0 N sodium hydroxide standard solution.
- Correct the test result for the dilution caused by the volume additions.

Test 'N Tube procedure



1. Start program **342, Ammonia LR TNT**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.

Note: *Although the program name can be different between instrumentss, the program number does not change.*

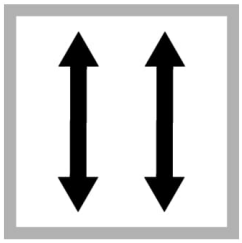


2. Prepare the blank: Add 2.0 mL of ammonia-free water to one AmVer™ Diluent Reagent Test 'N Tube for Low Range Ammonia Nitrogen.

3. Prepare the sample: Add 2.0 mL of sample to one AmVer™ Diluent Reagent Test 'N Tube for Low Range Ammonia Nitrogen.

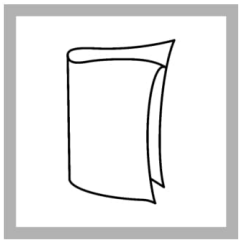
4. Use a funnel to add the contents of one Ammonia Salicylate Reagent Powder Pillow to each vial.

5. Use a funnel to add the contents of one Ammonia Cyanurate Reagent Powder Pillow to each vial.



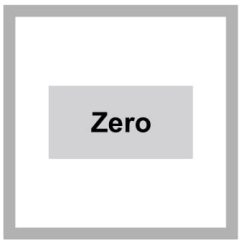
6. Close the vials tightly. Shake thoroughly to dissolve the powder.

7. Start the instrument timer. A 20-minute reaction time starts.

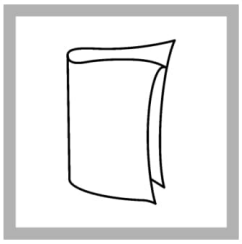


8. After the timer expires, clean the blank vial.

9. Insert the blank vial into the cell holder.



10. Push **ZERO**. The display shows 0.00 mg/L NH_3–N.



11. Clean the sample vial.

12. Insert the sample vial into the cell holder.

13. Push **READ**. Results show in mg/L NH_3–N.

Interferences

Interfering substance	Interference level
Calcium	2500 mg/L as $CaCO_3$
Iron	All levels. Correct for iron interference as follows: **1.** Use one of the Iron, Total procedures to measure the iron concentration of the sample. **2.** Use an iron standard solution to add iron to the deionized water blank so that the blank has the same iron concentration as the sample. The iron interference will be zeroed out from the test result.
Magnesium	15,000 mg/L as $CaCO_3$
Monochloramine	Monochloramine that is in chloraminated drinking water interferes directly at all levels and gives high results. Use a Free Ammonia and Monochloramine method to determine free ammonia in these sample matrices.
Nitrate	250 mg/L as NO_3^-–N
Nitrite	30 mg/L as NO_2^-–N
pH	Adjust acidic or basic samples to approximately pH 7. Use 1 N sodium hydroxide standard solution for acidic samples and 1 N hydrochloric acid standard solution for basic samples.
Phosphate	250 mg/L as PO_4^{3-}–P
Sulfate	300 mg/L as SO_4^{2-}

Appendix III – Ozonation system setup

Figure 33. Triogen ozone generator (left) and Wedeco ozone detectors (right) (Schlageter, 2018)

Appendix IV – CECs matrix interaction

A side experiment was conducted to verify if the calibration curves for the chromatographic quantification can be prepared using the typical 10% methanol solvent. It was found that there was a decrease in signal for several compounds when using synthetic wastewater (COD of approx. 190 mg/L) as the solvent for the calibration curve preparation. There is a 27% decrease for atrazine, 21% for carbamazepine and 28% for ibuprofen. Since there are no suspended solids present in the synthetic wastewater solution (i.e. it was filtered through 0.45 μm nylon filter), this finding indicates that there is some adsorption occurring between the compounds and the dissolved organic matter (i.e. DOM) in the wastewater. There no adsorption observed for the remaining compounds (i.e. caffeine, naproxen and gemfibrozil). The matrix interaction of all the selected CECs can be seen in **Figure 34**.

In the synthetic wastewater recipe shown in **Table 6**, the DOM comes primarily from the peptone, meat extract and urea. In raw wastewater and natural waters, DOM is a heterogenous mixture of dissolved aggregated organic molecules derived from decaying biomass, biomolecules and their degradation products (Hernandez-Ruiz, Abrell, Wickramasekara, Chefetz, & Chorover, 2012). Due to the varying composition and physicochemical properties of DOM, it can exhibit reactivity towards a multitude of contaminants. It is generally characterized by the hydrophobic-hydrophilic properties of its materials (Maoz & Chefetz, 2010). In the case of the synthetic wastewater used in these experiments, it is possible that only a certain fraction of DOM was present with which ibuprofen, atrazine and carbamazepine may have interacted. In fact, all three were reported to undergo sorption on the hydrophobic acid fraction of DOM (Hernandez-Ruiz et al., 2012; Maoz & Chefetz, 2010; Seol & Lee, 2000). It is possible that the raw wastewater from the lagoon might have had a different DOM composition onto which sorption of caffeine, naproxen and gemfibrozil could have occurred. Nevertheless, the interaction with DOM can affect the bioavailability of CECs and consequently, alter their accurate detection and quantification (Lajeunesse & Gagnon, 2007).

This experiment confirmed that each calibration curve had to be prepared in the same media as the one used for the experiments themselves. This would prevent detecting an overestimated amount of the compounds mentioned above, especially ibuprofen, atrazine and carbamazepine, leading to lower removal efficiencies. In other words, as organic matter is being biodegraded, the CECs that are adsorbed onto it are released back into the wastewater leading to higher concentrations. The desorption of CECs from DOM masks their potential decrease caused by biodegradation and/or ozonation. Therefore, using a calibration curve with the same medium as the experiment, the adsorption of the analytes onto DOM is accounted for.

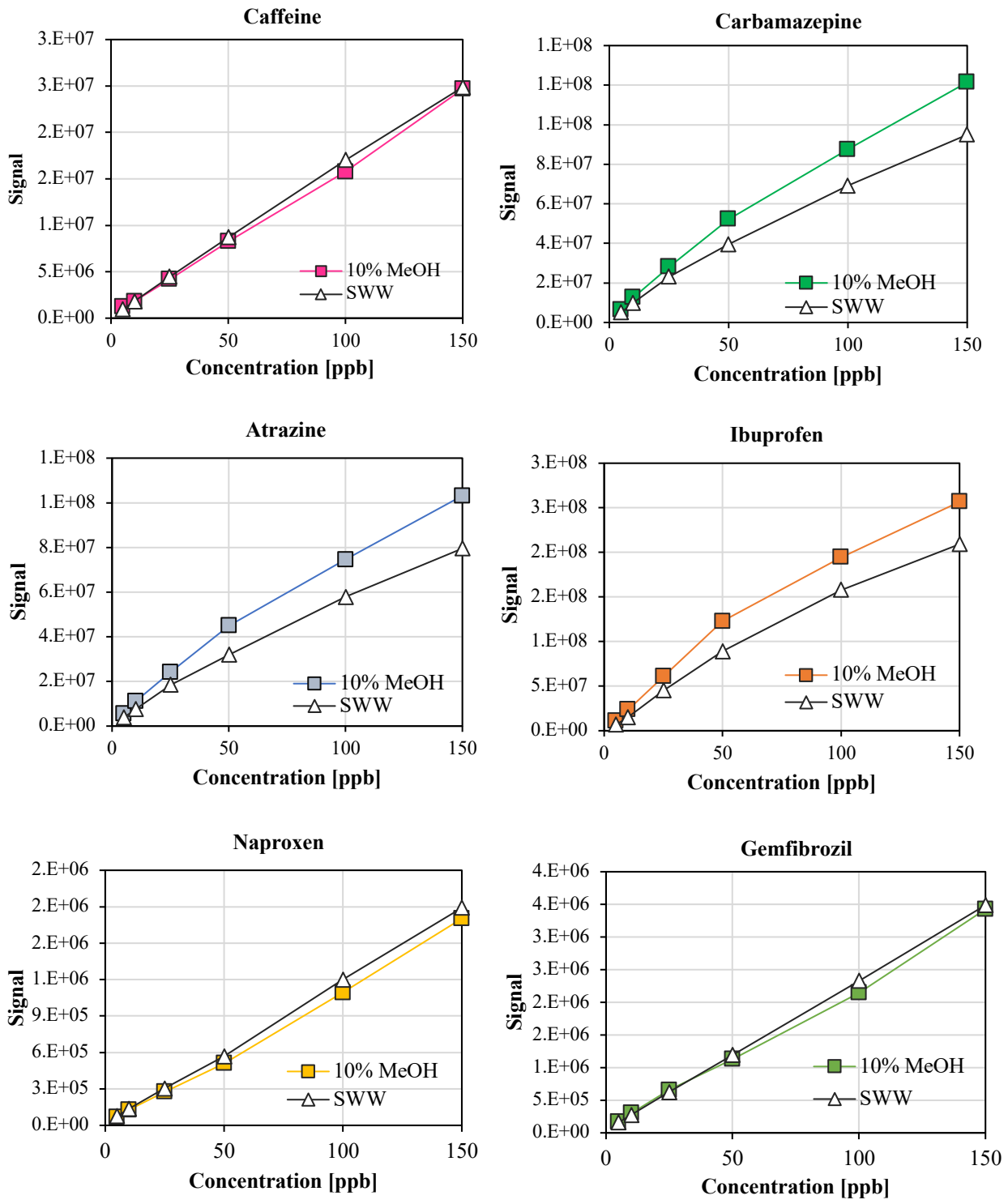


Figure 34. Interaction of selected CECs with the dissolved organic matter present in the synthetic wastewater.

Appendix V – Summary of experiments

Table 19. Summary of experiments

Experiment	Type	Sludge/WW sampling	Sludge sampling date	Type of wastewater	Temperature (°C)	Duration	Ozone dose (mg O_3/L)	Time of ozonation	Max OD_{600} (nm)	Initial- Final pH	Notes
1	Control	lagoon 1	2019-05-14	synthetic	25	4 days	-	-	0.329	7.47 - 6.89	First trial
2	Control (25% SWW volume change at 48 hrs)	lagoon 3	2019-05-28	synthetic	25	6 days	-	-	0.113	6.91 - 7.88	Change in volume to check bacteria's ability to recover from an exchange in volume that will occur during ozonation
3	Control (25% SWW volume change at 45 hrs)	lagoon 3	2019-06-13	synthetic	25	9 days	-	-	0.172	7.16 - 8.26	Longer duration to check for nitrification - none observed
4	Control (25% SWW volume change at 22 hrs)	lagoon 3	2019-08-08	synthetic	25	123 hours (6 days)	-	-	0.171	7.2-?	Change in volume performed in the stationary phase of cell growth - very quick bounce-back of OD of cells
5	Ozonation	lagoon 3	2019-08-27	synthetic	25	120.5 hours (6 days)	15	25 hours (stationary)	0.202	7.21 - 8.34	Supposed to ozonate in the stationary phase - no peak in OD observed afterwards
6	Ozonation	lagoon 2 * (high levels of nitrifying bacteria)	2019-09-12	synthetic	25	98 hours (5 days)	20	26 hours (stationary)	0.193	7.18 - 8.24	Increase in ozone dose
7	Ozonation	lagoon 3	2019-10-03	synthetic	25	26 hours (over 2 days)	20	8 hours (exponential phase)	0.127	7.02 - 7.81	Ozonate during exponential phase
8	Control	lagoon 3	2019-10-03	synthetic	25	26 hours (over 2 days)	-	-	0.127	6.89 - 7.74	Control run in the same time frame as the two previous ozonation experiments
9	Control	lagoon 3	2019-11-04	synthetic	25	12 hours	-	-	0.196	7.08 - 7.81	Rate calculations - lag phase ozonation shows promise
10	Ozonation	lagoon 3	2019-11-04	synthetic	25	13 hours	10	12.5 (stationary)	0.191	7.06 - 7.86	Decrease the ozone dose (suspecting a higher dose might impede bacterial growth rather than promote it)
11	Ozonation	lagoon 3	2019-11-04	synthetic	25	11 hours	10	5.5 (lag - start of exponential)	0.195	7.09 - 7.70	Ozonating at the beginning of the exponential phase
12	Control	lagoon 3	2019-11-04	synthetic	25	12 hours	-	-	0.117	-	OD 50% lower than usual

13	Ozonation	lagoon 3	2019-11-04	synthetic	25	12 hours	10	5.5 (lag - start of exponential)	0.156	-	Longer lag phase and lower OD for the same time frame
14	Control	lagoon 3	2019-12-06	synthetic	25	12 hours	-	-	0.203	7.03 - 7.72	Remake of exp12
15	Ozonation	lagoon 3	2019-12-06	synthetic	25	12 hours	10	5.3 (lag - start of exponential)	0.203	7.04 - 7.75	Remake of exp13
16	Control	lagoon 3	2019-11-04	synthetic	25	-	-	-	-	-	SWW was cloudy, contaminated
17	Ozonation	lagoon 3	2019-11-04	synthetic	25	12 hours	10	5.3 (lag - start of exponential)	0.201	7.09 - 7.77	Replicates of exp 15
18	Ozonation	lagoon 3	2019-11-04	synthetic	25	12 hours	10	5.3 (lag - start of exponential)	0.205	7.18 -7.85	Replicates of exp 15
19	Control	lagoon 3	2020-05-26	50:50 synthetic: raw	25	12 hours	-	-	0.043	-	Very little growth was observed
20	Ozonation	lagoon 3	2020-05-26	50:50 synthetic: raw	25	12 hours	10	5.3 (lag - start of exponential)	0.092	8.41 - 8.54	Slower growth but seems to be boosted by ozonation
21	Ozonation	lagoon 3	2020-05-26	50:50 synthetic: raw	25	12 hours	10	5.3 (lag - start of exponential)	0.081	8.37 - 8.54	Slower growth but seems to be boosted by ozonation
22	Ozonation	lagoon 3	2020-05-26	50:50 synthetic: raw	25	12 hours	10	5.3 (lag - start of exponential)	0.100	8.38 - 8.49	Slower growth but seems to be boosted by ozonation
23	Control	lagoon 3	2020-06-16	50:50 synthetic: raw	15	12 hours	-	-	0.037	8.26 - 8.40	No growth observed
24	Ozonation	lagoon 3	2020-06-16	50:50 synthetic: raw	15	12 hours	10	5.3 (lag - start of exponential)	0.009	8.35 - 8.48	No growth observed
25	Ozonation	lagoon 3	2020-06-16	50:50 synthetic: raw	15	12 hours	10	5.3 (lag - start of exponential)	0.015	8.34 - 8.46	No growth observed
26	Ozonation	lagoon 3	2020-06-16	50:50 synthetic: raw	15	22 hours	10	15.5 (lag - start of exponential)	0.039	8.35 - 8.43	Allow 12 hours for lag phase