SB-MBBR Technology: Treatment Strategies for Nitrification in Elevated Carbon Concentration Industrial Wastewater

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ABSTRACT

The dairy processing industry, in specific cheese processing, is a growing sector of the economy world wide. For example, China's demand for milk products projected an increase of 3.2-fold by 2050. While domestically in Canada, an annual increase of 5% has been reported since 2015 projected to continue to 2018. An increase in the demand for milk and dairy products translates to an increase in the produced wastewater. For every 2 L of milk processed 5 L of wastewater is produced, meanwhile, for every 1kg of cheese processed 9 kg of cheese whey is produced requiring the use of an estimated 10 kg of milk. Cheese whey is a by-product generated during the cheese manufacturing processes, with cheese processing wastewater defined as a combined stream of all wastewaters from a cheese processing facility.

Cheese processing wastewater can be characterized as medium to high strength wastewater with COD concentrations of above $1000 \text{ mg}\cdot\text{L}^{-1}$. In specific, cheese effluent can display COD concentrations in the range of $2500\text{-}5000 \text{ mg}\cdot\text{L}^{-1}$ and ammonia concentration in the ranges from $100\text{-}140 \text{ mg}\cdot\text{L}^{-1}\text{-N}$, and total phosphorus is in the range of $50\text{-}60 \text{ mg}\cdot\text{L}^{-1}\text{-P}$. Due to the high concentrations of carbon and nutrients in cheese processing wastewater, current stringent provincial regulations applied to municipal wastewater treatment plants restrict the discharge to sewers without prior treatment; leading to an increased demand for decentralized treatment units. Decentralized treatment units like sequencing batch reactors (SBR) gained popularity in cheese wastewater treatment due to their simple construct, reduced building expense and smaller footprint compared to conventional systems. The SBR however, has the potential of discharging floating or settled sludge during the draw or decant stages. The aerobic granular SBRs (AG-SBRs) promoted the formation of granules decreasing the potential of discharge. However, AG-SBRs were found to have problems regarding granular formation. The larger granules in an AG-SBR tended to

destabilize due to endogenous respiration within the granule resulting in its collapse and washout. While granule size affected nitrogen removal, smaller granules removed more nitrogen than larger granules. Replacing the granules with plastic carriers in an SBR introduced the sequencing batch moving bed biofilm reactor (SB-MBBR). The carriers eliminated the washout problem encountered in AG-SBRs as the biomass is retained on the carriers and remains in the reactor. It was postulated that an anaerobic, aerobic, anoxic (AOA) stage sequence would be ideal for dairy treatment using an SB-MBBR. AOA stage sequence allows for C, N, and P removal from a single reactor by selecting for the phosphorus accumulating organisms (PAOs), and nitrifiers. To date, however, the operation of an SB-MBBR with an AOA stage sequence treating wastewater from the food processing industry, in specific cheese wastewater has not been reported.

Wastewater from a cheese making facility in eastern Ontario was modelled in a laboratory setting. The initial treatment was conducted using three 2.3 L SB-MBBR reactors operating an AOA react stage over 8-hours of operation. The purpose of the cycle was to remove C, N and P in the same reactor. Although each of the reactors (SB-MBBRs -A, -B and -C) were operated with different AOA stage times, the results showed the removal of organic carbon (of up to $90\% \pm 5\%$) and phosphorus (of up to $65\% \pm 6\%$). The ammonia concentration over the 8-hour cycle did not drop below $100 \text{ mg} \cdot \text{L}^{-1}$, in SB-MBBR-B, and -C there is a noted release of ammonia in the anaerobic stage of the cycle. As such the aim of this thesis was to investigate operational strategies implemented to achieve nitrification in high carbon concentration wastewater. To achieve this aim two operational strategies were investigated. The first operational strategy extended the duration of the aeration stage aiming to achieve nitrification in the same reactor. The second operational strategy added a nitrification reactor in series to the first SB-MBBR reactor, separating the carbon removal from the ammonia removal.

The operational strategies were first tested using three 280 ml batch assay reactors (BARs). The test for strategy1 was conducted using the carriers from SB-MBBR-A, -B and -C placed in BAR1-A, -B and -C. Each of the BARs were fed the effluent from the SB-MBBR corresponding to the carriers origin. The testing continued for 33 days (810 hours) before nitrification was achieved in the BARs and the concentration of ammonia dropped below $50 \text{ mg}\cdot\text{L}^{-1}$. Once acclimatized to nitrification, the BARs removed 89-93 % of ammonia with an SARR of $0.90 \text{ gN}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. Due to the time that is taken to achieve nitrification in the BARs, strategy 1 was deemed unfeasible.

The second operational strategy of adding a nitrification reactor was initially tested in BAR2: rep1, -rep2, -rep3, which are replicates of the same testing condition. The BARs tested over a 30-hour testing period resulted in an ammonia removal in the range of 81-83%, with a SARR of $0.8\text{-}1.0 \text{ gN}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. The acclimatization period was significantly shorter than strategy 1. Hence, separating the carbon removal from the ammonia removal was deemed a feasible strategy for this setup. As such, the carriers from the BARs were then used to seed a 2.3 L reactor in series to SB-MBBR-A that was designed using the SARR value obtained from the BAR2 tests. Characterization of the nitrification reactor was conducted over a 16-hour run by sampling the water treated as it flowed from SB-MBBR-A to SB-MBBR-nit. The results showed a 100% removal of ammonia and 98% of organic carbon in the two reactors in series.

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LIST OF ABBREVIATIONS

MBBR	Moving Bed Biofilm Reactor
SB-MBBR	Sequencing Batch Moving Bed Biofilm Reactor
SBR	Sequencing Batch Reactor
AG-SBR	Aerobic Granular Sequencing Batch Reactor
ANAMMOX	Anaerobic Ammonia Oxidation
AOB	Ammonia-Oxidizing Bacteria
NOB	Nitrite Oxidizing Bacteria
COD	Chemical Oxygen Demand
bCOD	Biodegradable Chemical oxygen demand
BOD	Biological Oxygen Demand
DO	Dissolved Oxygen
FA	Free Ammonia
HRT	Hydraulic Retention time
NLR	Nitrogen Loading Rate
TSS	Total Suspended Solids
FSS	Fixed Suspended Solids
PAOs	Phosphorus Accumulating Organisms
AOA	Anaerobic, Aerobic, Anoxic
CAS	Conventional Activated Sludge
AF	Anaerobic Filter
UAF	Upflow Anaerobic Filter

Chapter 1: Introduction

1.1 Background

Industrial wastewater is broadly defined as the effluent resulting from human activity which is associated with the processing and manufacturing of goods from raw material (Ng, 2006). The point source discharge of industrial wastewater is among the leading pollution threats to freshwaters in Canada (Semeniuk, 2017). Handling and discharge of industrial wastewater as such is important due to the various impacts constituents in the wastewater can cause. There are several constituents of concern such as suspended solids, nutrients and priority pollutants (organic and inorganic components), refractory organics, heavy metals, and dissolved inorganics, (like calcium, sodium, and sulphate). Depending on the industry addressed, the constituents may vary in concentration. The focus of this thesis is food processing wastewater and in particular cheese processing wastewater.

The dairy processing industry is considered one of the most important industries in developing countries. In recent years the industry has seen a boom in demand resulting in increased production levels of dairy products in specific cheese. For example, China's demand for milk products is projected to increase 3.2-fold by 2050 with supplies being generated regionally and globally. China is a leading milk importer, with 12 Tg fresh milk imported in 2013, this is an increase of 123 times from 1961, and represents 25% of domestic consumption in 2013 (Bai et al., 2018). The Canadian cheese industry has also experienced an increase in popularity with exports growing yearly, in 2016 the market grew by 11% (Canadian Dairy Information Centre, 2017). It is estimated that to produce 2 L of milk 5 L of wastewater are generated (Pereira et al., 2018). Thus, as the demand for milk increases so does the associated need for wastewater treatment.

The Cheese processing wastewater is characterized by a high concentration of organic carbon, nitrogen, and phosphorus. Cheese processing wastewater exhibits chemical oxygen demand (COD) concentrations in the range of $0.8\text{--}102\text{ g}\cdot\text{L}^{-1}$ and biological oxygen demand (BOD) values in the range of $0.6\text{--}60\text{ g}\cdot\text{L}^{-1}$. The nutrient concentration in a typical cheese processing wastewater is in the range from $0.01\text{--}1.7$ and $0.006\text{--}0.5\text{ g}\cdot\text{L}^{-1}$ of TKN and TP respectively (Janczukowicz et al., 2008; Rivas et al., 2010). The range of the cheese processing wastewater would highly depend on the facility and the type of cheese processed. Some facilities would choose to discard their cheese whey, as such the cheese processing wastewater concentration would be in the higher range. Other facilities that process the cheese whey further would tend to have cheese processing wastewater concentrations in the lower range presented.

The effluent of cheese processing facilities is most commonly discharged to sewers across Canada. However, due to the high concentrations and intermittent discharge, the wastewater can interfere with the efficiency of downstream wastewater facilities. As such, due to current stringent provincial regulations on municipal wastewater treatment facilities in some locals, treatment is required prior to discharge (Government of Canada, 2016). For example, the city of Ottawa does not allow discharge or deposits that have a pH either lower than 5.5 or greater than 9.5. It also specifies that the sewage should not have a BOD exceeding $300\text{ mg}\cdot\text{L}^{-1}$, $10\text{ mg}\cdot\text{L}^{-1}$ of phosphorus, or $100\text{ mg}\cdot\text{L}^{-1}$ of Kjeldahl nitrogen (City of Ottawa, 2018).

Other more remote facilities lacking access to sewer systems treat their wastewater before discharge depending on the location of discharge. If not treated properly, the release of wastewater containing elevated concentrations of nutrients can cause eutrophication depending on the location and the size of the basin discharged to (Prazeres et al., 2012). The release of organics, on the other hand, can lead to a decrease in the dissolved oxygen concentration in the water (Janczukowicz et

al., 2008). The federal wastewater discharge regulations to rivers are currently limited to a COD concentration of $25 \text{ mg}\cdot\text{L}^{-1}$. Furthermore, the concentration of unionized ammonia should be less than $1.25 \text{ mg}\cdot\text{L}^{-1}$ as N, at $15^{\circ}\text{C} \pm 1$ to prevent acute toxicity of the receiving waters (Government of Canada, 2016). The focus of the treatment as such was for the removal of organic carbon and for the reduction of ammonia from the wastewater to discharge to the sewer. These lead to an increase in the demand for decentralized treatment units.

The effluent of a typical cheese processing facility in eastern Ontario was tested and replicated as synthetic wastewater for this work. The results showed the cheese processing wastewater to contain $2000 \text{ mg}\cdot\text{L}^{-1}$ COD, $120 \text{ mg}\cdot\text{L}^{-1}$ of ammonia, and $50 \text{ mg}\cdot\text{L}^{-1}$ of phosphorus. This research included the operation of a sequencing batch moving bed biofilm reactor (SB-MBBR), to remove carbon, nitrogen and phosphorus using an anaerobic, aerobic, anoxic (AOA) stage sequence. In particular, this research is founded on the assumption that an AOA sequence is ideal for dairy and in specific cheese wastewater treatment. AOA stage sequence allows carbon, nitrogen and phosphorus removal from a single reactor by selecting for the phosphorus accumulating organisms (PAOs) and nitrifiers. Previous research in other industries has reported relatively high levels of carbon, nitrogen and phosphorus removals using the AOA stage sequence with SB-MBBR (Schwarzenbeck et al., 2005). To date, however, the operation of an SB-MBBR with an AOA stage sequence treating wastewater from the food processing industry, in specific cheese wastewater has not been reported. The initial operation of the AOA SB-MBBR lacked any removal of ammonia. As such, the focus of this particular research is the operation strategy of SB-MBBR systems to achieve nitrification of high carbon strength wastewater mimicking a food processing effluent.

1.2 Aim of Study

The objective of this study is to investigate the nitrification in an SB-MBBR treating high carbon industrial wastewater from the food and dairy industry. This study analyzed the post-carbon removal wastewater produced from an existing SB-MBBR. The SB-MBBR operating an anaerobic, aerobic, anoxic stage cycle (AOA) did not remove ammonia from the cheese processing wastewater treated. As such two strategies were investigated to achieve nitrification. The first operational strategy investigated the length of time required to achieve nitrification in a single reactor by extending the aeration stage. The second operational strategy investigated separating the organic removal from the ammonia removal by operating two SB-MBBR in series.

The specific objectives of this research are as follows:

- (i) Investigate the nitrification rates of the operational strategy of extending the duration of the aeration cycle;
- (ii) Investigate the nitrification rates of the operational strategy of adding a second aerated SB-MBBR reactor to achieve nitrification;
- (iii) Compare the performance of both operational strategies by determining their respective surface area removal rates (SARR) and compare the two operational strategies.
- (iv) Characterize the operation of the second aerated SB-MBBR system for nitrification performance.

1.3 Thesis Organization

The thesis presented is comprised of six chapter. Chapter 1 is an introductory chapter that includes background information, the aim of the research, thesis organization and contribution of the authors for the article presented in Chapter 4. Chapter 2 includes the literature review of the treatment of food and beverage industry industrial wastewater, moving bed biofilm reactor (MBBR) and nitrification in industrial wastewater. Chapter 3 is the material and methods section and includes the research methodology, reactors design, operational strategies, and testing protocols. Chapter 4 (presented as an article), discussed different operational strategies utilized to achieve nitrification in high carbon industrial wastewater. This chapter specifically focuses on the batch test assay experiments (BAR) and their results. Chapter 4 has been prepared for submission to the journal Environmental Technology. Chapter 5 written as a result and discussion chapter, discusses the operation of the two-stage SB-MBBR system utilized to remove carbon, nitrogen, and phosphorus from synthesized cheese wastewater. The system was designed based on the investigation carried out in Chapter 4. Finally, Chapter 6, contains an overall review of the research findings and study conclusion.

1.4 Contribution of Authors

Article 1: SB-MBBR Technology: Treatment Strategies for Nitrification in Elevated Carbon Concentration Industrial Wastewater

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To be Submitted to: Journal of Environmental Technology

Nour Al-Ghussain: Conducted Literature review, performed sampling and analytical procedure for batch assay reactors (BARs), analyzed results and wrote the manuscript.

Alexandra Tsitourous: Operated and tested the laboratory reactors SB-MBBRs -A, -B and -C.

Robert Delatolla: Provided expertise, supervision and guidance in the analysis of the results and the development of the experimental procedure and reviewed the manuscript.

Onita Basu: Provided supervision and guidance and revised the manuscript.

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Semeniuk, I., 2017. Charting Canadas troubled waters: Where the danger lies for watersheds nationwide.

Chapter 2: Literature Review

2.1 Industrial Wastewater Treatment

Industrial wastewater is a broad word encompassing the wastewater produced by humans activity associated with the manufacturing of goods from raw material (Ng, 2006). A more specific definition would then depend on the type of industry. There are several sources of the industrial wastewater, pulp and paper industry, iron and steel industry, mines and quarries, food industry, complex organic chemical industry and so forth. Wastewater from industries may contain high concentrations of suspended solids, nutrients, and various priority pollutants. In addition to these constituents, refractory organics, like surfactants, phenol and agricultural pesticides can also be present in the water, as well as different concentrations of heavy metals and dissolved inorganics like calcium, sodium, and sulphate. Other industrial wastewater physical attributes like total solids (TS), odours, temperature, colour, and turbidity require treatment before discharge. Further, industrial wastewater can contain pathogenic organisms, for example, slaughterhouse wastewater, or mold and fungi like the wastewater from starch and yeast factories. Industrial wastewater could also be nutrient deficient as in the case of mining wastewater. The focus of this work is food processing wastewater. Food processing wastewater is characterized as high strength wastewater, containing high concentrations of fats, oils, and grease or other organic and inorganic compounds. In specific, this work looks at the treatment of dairy and in particular cheese processing wastewater.

Dairy wastewater depending on the dairy product processed can be characterized as medium to high strength wastewater with COD concentrations of above $1000 \text{ mg} \cdot \text{L}^{-1}$ (Sabrina et al., 2012). In specific, cheese effluent defined as the stream containing all wastewater from a processing facility can display COD concentrations in the range of $2500\text{-}5000 \text{ mg} \cdot \text{L}^{-1}$ and ammonia concentration in

the ranges from 100-140 mg·L⁻¹-N, and total phosphorus is in the range of 50-60 mg·L⁻¹-P (Britz and van Schalkwyk, 2006). Typically, the molar ratio of carbon to nutrients (C:N:P) for a dairy processing wastewater is 100:2.3:0.8 (Sparchez et al., 2015). On the other hand, the optimal molar ratio for biological wastewater treatment is around 100:5:1, indicating that dairy processing wastewater is highly loaded with organic carbon (Sparchez et al., 2015). As such, the most common approach to treating dairy and cheese processing wastewater is using biological wastewater treatment, most commonly activated sludge (AS) processes due to the high organic content (Orhon, 2009; Surmacz-Gorska et al., 1996). The basics of biological carbon and nutrient removal from food processing wastewater are presented in the following sections, as well as approaches to treating food processing wastewater in specific cheese processing wastewater.

2.2 Biological Treatment of Industrial Wastewater

In food processing wastewater treatment, the primary objective is the removal of organics and nutrients as well as physical attributes like TSS to meet standards for sewage discharge. Achieving the treatment would be done using either a suspended growth system or an attached growth system. A suspended growth system is characterized by free-floating microorganisms in the reactor, while an attached growth system sees the microorganisms attached to an inert packing material within the reactor (Metcalf and Eddy, 2003). The following sections introduce the biological removal of carbon, nitrogen and phosphorus from food processing wastewater.

2.2.1 Carbon Removal

Organic carbon may be divided into two fractions, biodegradable and non-biodegradable. The biodegradable fraction can be further subdivided to readily and slowly biodegradable content. The readily biodegradable carbon is consistent of smaller molecules like volatile fatty acids and other forms of dissolved or soluble carbon that assimilate into the mass of the microorganisms. The

slowly biodegradable content is consistent with larger more complex molecules, and longer chains of carbon that are broken down before uptake (Metcalf and Eddy, 2003). The measure of the organic content in wastewater is quantified by two values: chemical oxygen demand (COD), and biological oxygen demand (BOD). The typical ratio of BOD/COD is 2.0-2.2 a higher ratio would indicate that the wastewater is industrial, such water is characterized by a higher concentration of refractory or nonbiodegradable constituents (Brown et al., 2005).

2.2.2 Nitrogen Removal

Biological nitrogen removal is broken into two steps nitrification and denitrification. In nitrification, ammonia is oxidized to nitrate, and in denitrification, the nitrate is reduced to nitrogen gas. Nitrification is further broken into two processes, nitritation and nitrataion. Nitritation is defined as the oxidation of ammonia to nitrite by the action of ammonia-oxidizing bacteria (AOB), while nitrataion is the oxidation of nitrite by the action of nitrite-oxidizing bacteria (NOBs). In each of the processes, the substrate of the process inhibits it (Abeling and Seyfried, 1992; Balmelle et al., 1992; Surmacz-Gorska et al., 1996). Stoichiometrically the reaction that shows the oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) by *Nitrosomonas* can be limited by the ammonia and oxygen concentrations in the wastewater (Equation 2.1).

The oxidation of nitrite to nitrate (NO_3^-) by *Nitrospira* is limited by the concentration of nitrite in the wastewater (Equation 2.2).

Nitrifiers responsible for the nitritation and nitrataion are *Nitrosomonas* and *Nitrospira* respectively (Henze, 1997). The nitrifiers have a lower cell yield than the heterotrophic bacteria,

the cell mass yield of 0.06-0.20 g VSS/g NH_4^+ -N oxidized is typical. Besides nitrification, there are several pathways for the removal of ammonia from wastewater. Assimilation and stripping are two such pathways. Assimilation is the uptake of ammonia into cell mass as a nitrogen source. The assimilation rate at which the bacteria take up ammonia nitrogen for cell synthesis occurs at a rate of 0.087g-N/g of COD produced (WEF, 2011). Ammonia stripping, on the other hand, occurs at pH between 8.5-9.0 or higher, where the concentration of ammonia increases and is stripped out of the water (Brown et al., 2005).

Several factors affect the nitrification rate; organic load in the influent stream, dissolved oxygen (DO) concentration, ammonia concentration, temperature, as well as pH and alkalinity (Bassin et al., 2011). The optimal pH range for nitrifying bacteria is between pH 7.5-8.6. If the pH drops, the nitrification rate decreases, and at around pH 6 nitrification stops (WEF, 2011). Alkalinity, on the other hand, is affected by the oxidization and reduction of the nitrogen species; nitrification consumes alkalinity at a rate of 7.14 mg Alkalinity/mg NH_4 -N (Brown et al., 2005). While, denitrification returns alkalinity to the water at a rate of 3.75 mg Alkalinity/mg NO_3^- -N reduced (Brown et al., 2005).

Denitrification can be accomplished by the action of a variety of heterotrophic microorganisms, in contrast to nitrification (Brown et al., 2005). For denitrification, a consumption of 1.91 g C/g NO_3^- -N converted is required. As such, most times an external carbon source is added for denitrification, however, the requirement can be met by recycling the feed (Brown et al., 2005). The addition of an external carbon source leads to rapid denitrification, which occurs at a rate of 0.03-0.11 kg NO_3^- -N/kg VSS·d. Without a carbon source, the denitrification rate is slower at 0.03 kg NO_3^- -N/kg VSS·d. The denitrifiers in a low carbon environment would depend on the by-products of microbial decay and lysis as carbon sources (WEF, 2006).

Typically, denitrification occurs under anoxic conditions, but the occurrence of denitrification under aerobic conditions is also possible by the action of heterotrophic nitrifiers. The processes utilizing such bacteria is called simultaneous nitrification and denitrification (SND). SND theoretically saves 40% of cellular energy needs by decreasing the need for external carbon addition due to both processes occurring in the same reactor (Abeling and Seyfried, 1992; Münch et al., 1996; Turk and Mavinic, 1986). The SND processes also reduce the biomass production compared to conventional AS processes and require a lower footprint than conventional processes. Several factors are thought to control the SND, the temperature, solid retention time (SRT), DO, ammonia concentration, and pH (Zeng et al., 2003). DO is thought to be the most important factor according to (Ruiz et al., 2003) who found at a low DO level of about $0.7 \text{ mg}\cdot\text{L}^{-1}$ it was possible to accumulate more than 65% of the loaded ammonia nitrogen as nitrite with a 98% ammonia conversion.

Another type of microorganism that can shortcut the process of nitrification and denitrification is anaerobic ammonium oxidation process (ANAMMOX). The ANAMMOX bacteria use ammonium as the inorganic electron donor. Nitritation in combination with ANAMMOX bacteria has prime usage for industrial wastewater with high ammonia concentrations (Zekker et al., 2012). ANAMMOX requires the stable accumulation of nitrite to achieve a controlled 1:1 ratio between ammonia and nitrite for the processes to be achieved (Equation 2.3).

Combining both leads to savings in aerations costs as well as decreasing in the external carbon requirement, and aeration requirements. The process further reduces the production of WAS (Waste Activated Sludge) and the CO_2 emissions that occur during denitrification.

2.2.3 Phosphorus Removal

Biological removal of phosphorus is achieved by incorporating phosphorus into cell biomass by the action of phosphorus accumulating organisms (PAOs). Typical microbial biomass contains 1.5% to 2.5% (w/w) phosphorus per volatile solids (WEF, 2011). PAOs can accumulate phosphorus in excess to that ratio thus removing phosphorus from the wastewater. The enhanced biological phosphorus removal (EBPR) systems select and proliferate PAOs by using a configuration consisting of an anaerobic zone followed by an aerobic zone. In the anaerobic cycle, the PAOs convert the readily available organic material to energy-rich carbon polymers called PHA (polyhydroxyalkanoates). The energy used for the breakdown of carbon is taken from the breakdown of the polyphosphate molecules resulting in an increase of the concentration of phosphorus in the water. During the aerobic cycle, the PAOs oxidize previously stored PHAs to obtain energy, by uptaking the phosphorus and building energy reserves (WEF, 2006). The phosphorus is removed from AS systems by sludge wasting from the settling tank. The biological removal of phosphorus can remove up to 97% of the total phosphorus in the influent, but this process can be highly variable due to the difficulty of cultivating PAOs and maintaining them in the reactor (Wang et al., 2006).

2.3 Suspended Growth Systems in Industrial Wastewater

2.3.1 Activated Sludge System

Activated sludge (AS) systems are widely used for the treatment of sewage wastewater; a typical configuration would consist of an aeration tank followed by a sedimentation tank (Figure 2.1). AS systems have also found applicability in the treatment of industrial wastewaters with different concentrations of organic carbon, nutrients and other contaminants. For the treatment of food processing wastewaters, AS systems, although applicable, can have operational difficulties. Food processing wastewater is highly loaded with organic carbon with an intermittent discharge pattern and varied discharge concentrations depending on the day and the time of day. The variation and high concentrations create a shock load effect, in turn affecting the microorganisms and reducing the removal rates (Yu et al., 2003). The elevated concentrations of organic carbon increase the growth rate of the microorganisms and can cause problems like sludge bulking (Yu et al., 2003). The bulking was addressed by several studies via modifying the AS processes by mainly reducing the loads treated or physically enhancing the sedimentation process. Combining the treatment of dairy wastewater with domestic wastewater helped dilute the concentration of organic carbon to the system decreasing the shock load effect (Sparchez et al., 2015). To enhance the settleability, magnetically charged activated sludge which is a modification of the AS process improved the solid-liquid separation characteristics, reducing both the time required for settling and the production rate of excess sludge (Ying et al., 2010). These improvements, however successful, required operational sophistication that resulted in inaccessibility to farms and small processing plants looking for a more decentralized process with fewer maintenance requirements. Most of the

application of AS in the studies reviewed were focused on the removal of organic carbon, lacking in reporting removals of nutrients from the wastewater treated.

Figure 2.1: Conventional wastewater treatment train used to treat domestic sewage and can apply to the treatment of food processing wastewater in specific dairy and cheese (Ionics Freshwater, 2017).

2.3.2 Sequencing Batch Reactor

The sequencing batch reactor (SBR) is an activated sludge (AS) process that combines a biological reactor and a settler. In contrast to the continuous flow systems, the SBR fills in the beginning of a cycle and decants at the end of it. SBR systems typically have five stages of operation: fill, react, settle, draw, and idle (Figure 2.2) (Lobo et al., 2016). The react cycle can have different stage conditions controlled to achieve the removal of one or more constituents. The stage conditions can be aerobic, anaerobic, or anoxic or a combination of one or more of those stage conditions within the react stage. The SBR is used in the application of several processes like carbon removal via an aeration stage, SND via a sequence of anoxic and anaerobic stages, ANAMMOX with an anaerobic

stage, nitrification, and phosphorus removal via a sequence of aerobic and anaerobic stages. Its diverse applicability makes the SBR a flexible process in the food processing industry.

In the food industry, the implementation of SBR in the treatment of wastewater from a winery removed COD of up to 95%, with a noted lower sludge production compared to conventional AS (Mace and Mata-Alvarez, 2002; Torrijos and Moletta, 1997). To treat cheese processing effluent, the SBR was used with a rotating anaerobic-aerobic cycle. The SBR was further used to treat synthetic dairy water resulting in varied removal which was dependent on the volume load to the reactor, and the organic load tested. The performance ranged between 65 to 96% for varying volume load tested, while for varying organic load tested for the SBR the range was between 90-99% (Yahi et al., 2014). SBRs were also used as polishing reactors after other processes, in one study, brewery wastewater coming from a UASB was polished for ammonia removal of up to 97% (Rodrigues et al., 2015). In another study, the SBR was used to polish wastewater coming from an up-flow downflow reactor; the SBR operated under an anoxic, anaerobic, aerobic cycles. The SBR with different stage conditions removed up to 90% of COD, with removals of both nitrogen and phosphorus of over 90% (Malaspina et al., 1995). A later study conducted a similar treatment of aerobic-anaerobic conditions to assess the potential of performing psychrophilic anaerobic digestion; the obtained removal was up to 97% COD removal with a residual of $33 \text{ mg} \cdot \text{L}^{-1}$ COD in the wastewater in an SBR reactor (Frigon et al., 2009). In addition to the brewery and winery wastewater, the SBR has also been used to treat dairy wastewater and fruit-juice wastewater (Dugba and Zhang, 1999; Kushwaha et al., 2013; Tawfik and El-Kamah, 2012). Enhancement to the treatment was implemented to treat more highly loaded wastewater, by combining municipal and dairy wastewater to remove both COD and ammonia (Neczaj et al., 2008, 2007). Although the SBR has an advantage over conventional activated sludge, offering operational flexibility and a

smaller footprint, the SBR is prone to washout events where floating or settled sludge during the draw or decant phase can be discharged (Moghaddam and Sargolzaei, 2015).

Figure 2.2: The SBR has five stages, starting with a fill, react, settle, decant and idle as shown in the figure (Dohare and Bocharé, 2014).

2.3.3 Granular Treatment of Food Processing Wastewater

Granular reactors have been widely used for the treatment of food processing wastewater in specific, cheese and dairy wastewater. Several granular processes operate either under aerobic, anaerobic or anoxic or a combination of one or more. Processes like the aerobic granules (AG), up-flow anaerobic sludge blanket (UASB), static granular bed reactor (SGBR), have been used for the treatment of food processing effluent (Castillo et al., 2017).

The AG was first observed in a lab scale SBR bubble column inoculated with AS (Nancharaiiah and Reddy, 2018). The AG attracted much interest after that due to its ability to improve the efficiency of biological wastewater treatment. The granules have high sedimentation velocity, and high methanogenic activity in anaerobic conditions (Hulshoff Pol et al., 2004). In contrast to AS aggregates which have a floc size between 30-200 μm , the AG has a larger floc size in the range of 200 to 2000 μm (Barr et al., 2010). Due to the granule size, the AG has several zones; the inner core is an anaerobic zone, the oxygen concentration decreases from outer to inner layers forming

both an anoxic and aerobic region. In the food processing industry, the application of the AG in an SBR was studied for palm oil effluent treatment; the reactor removed both COD and ammonia at a rate of 91.1% and 97.6% respectively (Abdullah et al., 2011). The AG has also been implanted in the treatment of dairy wastewater with high measured removals of carbon, phosphorus and nitrogen (Arrojo et al., 2004; Mañas et al., 2012; Meunier et al., 2016). AG, although very successful in the treatment of food processing wastewater, the more conventionally applied granular reactors to the treatment of dairy wastewater is the UASBs and SGBR (Baloch et al., 2008; Karadag et al., 2015a; McHugh et al., 2003).

The UASB has an advantage of simple construction, low operational cost and high organic removal (Karadag et al., 2015a). UASB has been used to treat food wastewater with a COD removal efficiency ranging between 70-90% (Diamantis and Aivasidis, 2010). The processes were also applicable to the treatment of several wastewaters like slaughterhouse wastewater, piggery wastewater, dairy and fishery (Erdirencelebi, 2011; Gutiérrez et al., 1991; Latif et al., 2011; Nadais et al., 2005; Passeggi et al., 2012). Drawbacks of the processes are a longer start-up period compared to AS processes, low performance at low loading rates, and the need for post-treatment after discharge due to the increase in TSS (Lim and Kim, 2014). Further, anaerobic technology is not adept at the removal of nutrients, most times the recorded removal is a by-product of maintaining the growth of anaerobic biomass (Jędrzejewska-Cicińska and Krzemieniewski, 2010).

The SGBR is a granular process used commonly for the treatment of low to midrange strength wastewater. The water coming to the SGBR has a downward flow; the granules form within the reactor due to the shear stress acting on the sludge. Notably, the processes were used for the treatment of dairy wastewater; the effluent displayed high removals of COD and TSS, nutrients concentration did not show a notable decrease (Oh et al., 2015).

Granular processes effluent generally requires post-treatment to fulfil effluent standards. For example, a chemical phase (coagulant) to reduce TSS or remove phosphorus from wastewater treated (Rivas et al., 2010; Vlyssides et al., 2012). Further, granular formation and maintenance are challenging, the size of the granule is an essential factor in its stability, the larger the granule, the more unstable the granule can become (Liébana et al., 2018; Zheng et al., 2006). This instability comes from the endogenous respiration within the granule which destabilize the inside core of the granules and results in their collapse (Zhang et al., 2015). Also, anaerobic granules experience a prolonged startup due to the slow growth of anaerobic microorganisms (Liu and Liu, 2006). The nitrogen removal was also affected by granular size in AG, the smaller granules ranging in size between 0.5-0.9 mm removed nitrogen at a higher rate than a larger sized granule this is attributed to the rate of oxygen penetration into the zones of the granule (Zhang et al., 2015).

2.4 Attached Growth Systems in Industrial Wastewater

2.4.1 Biofilms

Biofilms are a consortium of microorganisms enveloped in an extracellular polymeric substance (EPS) and attached to a surface (Donlan, 2002; WEF, 2006). The biofilm is a highly diverse ecosystem and contains a variety of microorganisms, for example, a micrograph of an industrial water biofilm showed the biofilm to contain corrosion products, clay material and freshwater diatoms with filamentous bacteria (Donlan, 2002).

Figure 2.3: A graphical representation of the cycle of biofilm formation (Puiu, 2017).

The biofilm formation consists of three main stages, attachment, maturation, and dispersion (Figure 2.3). The first step in biofilm formation is surface conditioning; the surface is coated with a proteinaceous material that incites a transition from planktonic status into an attached form (Donlan, 2001). The next step after conditioning is attachment; the microorganisms adhere to the pioneering bacteria, specific microorganism features like pili assist in the attachment to the growing biofilm. The production of EPS by the microorganisms matures the biofilm and further supports the growth of it. The EPS helps the community interact using signalling. After maturation; the biofilm becomes too vast for maintenance reaching a dispersion point. There are several methods of dispersal, shedding of daughter cells from actively growing cells, detachment due to low nutrient levels, quorum sensing, and shearing of the biofilm aggregates. For biological wastewater treatment, the biofilm is encouraged to grow on a support material. The support material could be either static or moving depending on the technology used. There are several

types of support material like saponite, sand, granular activated carbon (GAC), and synthetic plastic carriers (Karadag et al., 2015b). The support material offers compactness in treatment; the media used, depending on the kind, can offer a high specific surface area for the biofilm to grow on (Rodgers & Zhan, 2003). For example, a K5 AnoxKaldness carrier have a specific surface area of $800 \text{ m}^2/\text{m}^3$.

In the treatment of food processing wastewater and specifically dairy wastewater biofilms eliminated the washout events encountered by both AS and granular processes as the biofilm remains in the reactor on the carriers (Biswas and Turner, 2012). However, due to the very high organic loads in some food processing industry effluents, aerobic reactors required a higher oxygen supply which increased the cost of the process. Further, aerobic reactors incited increased growth of microorganisms on biofilms causing some of the same problems encountered in AS processes like clogging and an increase in the concentration of TSS (Karadag et al., 2015b; Kushwaha et al., 2013). The following sections present attached growth processes commonly used in the treatment of dairy and cheese wastewater.

2.4.2 Trickling Filter and Anaerobic Filter

The trickling filter is an attached growth process consisting of a bed with highly porous media supporting the biofilm. The wastewater is percolated or trickled from the top and treated water is extracted from the bottom. The media filling of a trickling filter can either be a plastic packing material or rock. The rock bed filters are circular, and the wastewater is distributed over the top of the bed by a rotary distributor. Conventionally, the effluent of a trickling filter is sent to a clarifier after the treatment due to an increase in TSS concentration within the reactor. The trickling filter was shown to handle shock loads of organic carbon (Aziz and Ali, 2017). Despite this, the trickling filter did not find popularity in the treatment of food processing wastewater. Most times the effluent wastewater did not meet required effluent regulations, and the processes itself was not sufficient to remove organics. As such, the trickling filter is found in combination with another process. The addition to the treatment train requires space requirements as well as cost and energy needs. The trickling filter displayed varied treatment efficiency when treating food processing wastewater. In one study when treating brewery wastewater, the trickling filter removal rate of COD was between 60-92% depending on the loading rate to the filter (Lemji and Eckstadt, 2015).

The Anaerobic Filter (AF), a fixed bed biological reactor with the same operational concept as a trickling filter. Up-flow AF (UAF) characterized by the wastewater flowing from the bottom to the top, found application in the treatment of food processing wastewater. In a study, the treatment performance of the UAF was measured for three wastewaters simulated after fruit canning, winery and cheese dairy wastewater. Results showed removals of over 90% for organic carbon but noted that due to the high loading rates there was clogging that required recirculation that increased liquid mixing (Rajinikanth et al., 2009). The clogging was attributed to various parameters, the

liquid mixing or the amount of dispersion, fraction of dead space, channelling and quantity of biomass accumulation. Other uses of AF included using it as a pre-treatment step treating cheese processing wastewater before anaerobic digestion, with removal rates of about 50% for COD and 60% of TSS (Gannoun et al., 2008). AF is further used with a modification of adding slag grains to enhance the treatment for dairy effluent. The COD removal achieved was above 80%, with no significant removal of TN recorded in the reactor (Jo et al., 2016). Although the AF shows excellent removals, it has several disadvantages. The AF requires constant upkeep and has a more complex design. Further, AF reactors have a low reduction of pathogens and nutrients from the treated wastewater. To add, the effluent from the process requires more treatment ergo its usage as a pretreatment step. There are also reported problems with clogging and subsequent maintenance requirement as such there is a need for cleaning. Also, the AF removes a high percentage of BOD and COD from the wastewater but has limited removals of TN.

2.4.3 Moving Bed Biofilm Reactors

The moving bed biofilm reactor (MBBR) was developed in Norway by Kaldness Miljøteknologi. The first MBBR reactor was installed in the US in 1995, and since then over 400 installations have been made worldwide (Borkar, 2013). The initial development of the MBBR carriers was for nitrification in 1980 (Hem et al., 1994). Later the technologies application expanded to include carbon removal and phosphorus removal (Helness and Ødegaard, 1999). The carriers used are small cylindrical shaped polyethylene elements with a specific gravity of around 0.96 g/cm³ (Metcalf, 2003). The carriers can be used in anaerobic and aerobic basins to support the growth of the biofilm. The carriers have various sizes; for example, a K1 carrier has a cylindrical shape with a diameter of 10 mm and a height of 7 mm with a surface area of 500 m²/m³. The carrier allows both heterotrophic and autotrophic bacteria to grow on it within the biofilm; depending on the

operational conditions of the reactor the carriers have been shown to achieve high removals of nitrogen and COD from wastewater (Figure 2.4), as well as operating at different loading rates (Borkar, 2013; Odegaard, 1998).

Figure 2.4: The MBBR is shown in A under aerobic conditions and B under anoxic or anaerobic operation (Odegaard, 1999).

Due to the compactness of the carriers, MBBR reactors reduce the footprint needed compared to conventional AS systems in terms of both area and energy, especially under anaerobic conditions. The carriers can also be integrated into existing facilities reducing the cost of installation (Barwal and Chaudhary, 2014; Rodgers and Zhan, 2003). Other advantages include lower maintenance, no required backwash, no washout, and withstanding fluctuating loading rates (Helness and Ødegaard, 1999; Hem et al., 1994). The carriers were noted for their resilience to pH changes, high nutrient conditions, toxic substances, as well as temperature fluctuations (Hosseini and Borghei, 2005). In one study the carriers were operated at low temperatures; the carriers were shown to nitrify at temperatures below 1°C (Young et al., 2016).

The MBBR was used for the treatment of dairy wastewater using carriers with a specific surface area of $400 \text{ m}^2/\text{m}^3$ in a single stage reactor. The MBBR removed over 90% of COD in the wastewater with a carbon loading rate of $500 \text{ g COD} \cdot \text{m}^{-3} \text{ h}^{-1}$ (Rusten et al., 1992). Later studies

focused on anaerobic MBBRs rather than operating a fully aerobic reactor. Anaerobic MBBR application to the treatment of dairy wastewater achieved removals of TCOD between 80-95 % (Wang et al., 2009). Following the studies presented, other studies looked at operating a single reactor (sequence batch moving bed biofilm reactor (SB-MBBR)) under different conditions to remove not only the COD in the food processing wastewater but also TN and TP. The following two sections introduce nitrification in food processing wastewater treatment and the SB-MBBR in the treatment of dairy and cheese wastewater.

2.4.3.1 Nitrification in Food Processing Wastewater

Nitrification in biofilm processes is affected by several factors, carbon loading rate, dissolved oxygen concentration, ammonia concentration, wastewater temperature, pH and alkalinity (WEF, 2010). An increased organic loading rate increases the rate of growth of heterotrophs suppressing the function of nitrifiers in the same biofilm. In an established nitrification biofilm in addition to the organic load, oxygen can be rate limiting if the molar ratio between O_2 to NH_4-N is below 2 (Hem et al., 1994; WEF, 2010). Ammonia concentration can be inhibitory to the process of nitrification; a high concentration can lead to nitrite accumulation. For the treatment of food processing wastewater containing high concentrations of C, N, and P several studies looked at the application of MBBR carriers (Andreottola et al., 2002; Luostarinen et al., 2006). The primary concern is the organic carbon concentration in the water as in effect it interferes with the processes of nitrification. A study investigated the MBBR reactors effect of COD on nitrification and denitrification operated in series with an anaerobic reactor followed by an aerobic reactor. The study noted the effect of high COD rates on the nitrification rate stability, the higher the COD, the lower the nitrification rate detected (Jonound et al., 2003). For the treatment of dairy wastewater, a study using an MBBR removed 90% to 97% of carbon and ammonia removal in the range of

13.3 % to 96.2% (Andreottola et al., 2002). The operating system used was an equalization tank followed by an aeration MBBR, and then a settling tank. The ammonia removal from the wastewater was attributed to assimilation due to the fluctuating nature of the removal rate. The studies presented focused on organic carbon removal from dairy and cheese wastewater, nitrification using an MBBR in cheese wastewater has not been previously investigated.

Furthermore, nitrification under high carbon conditions in a one stage reactor operating an anaerobic, aerobic, anoxic cycle was not previously investigated using an MBBR carrier.

2.4.3.2 SB-MBBR with AOA Operation

Anaerobic, aerobic, anoxic sequence of operation (AOA) is postulated to enable the simultaneous nitrogen and phosphorus removal from a single reactor. The AG-SBR operating such a sequence of stages treating municipal wastewater was studied and found that both nitrogen and phosphorus was removed of up to 83% and 92% respectively (Soejima et al., 2008; Tsuneda et al., 2006). Another study using an AOA stage configuration was successful at removing C, N and P in the same reactor. The removal rates were as high as 99% for COD, 98% for ammonia and 99% for phosphate (Zhang et al., 2015). The AG as previously stated had a significant drawback in its operation, washout of the granules. In addition to washout, the formation and later stability preventing breakdown are further challenges. The sequencing batch moving bed biofilm reactor (SB-MBBR), is a combination of MBBR carriers within the single SBR reactor. The carriers retain the biomass within the reactor eliminating the washout issue encountered in AG-SBRs, further eliminating the need to form granules and maintain them.

The use of the SB-MBBR for the treatment of fishery wastewater was able to achieve removals of 97%, 74%, and 70% of COD, total Kjeldahl nitrogen (TKN) and total phosphorus (TP) respectively using a fill, anoxic, aerobic and decant sequence of operation (Nguyen and Nguyen,

2013). The SB-MBBR has also shown success in the removal of phosphorus from municipal wastewater (Helness and Ødegaard, 1999), and a combination of phosphorus and nitrogen removal (Helness and Ødegaard, 2001). Although the SB-MBBR is utilized for the treatment of high carbon loaded wastewater, the treatment of cheese wastewater has not been previously investigated. The SB-MBBR operating an AOA stage condition is utilized to investigate the nitrification strategies in high loaded wastewater coming from a cheese processing facility.

2.5 References

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Chapter 3: Material and Methods

3.1 SB-MBBR Reactor Operation

Wastewater from a cheese processing facility was analyzed and used to create a synthetic wastewater recipe. Synthetic wastewater was fed to a sequencing batch moving bed biofilm reactor (SB-MBBR). The cheese wastewater, characterized with high C: N: P ratio, contained $3000 \text{ mg}\cdot\text{L}^{-1}$ chemical oxygen demand (sCOD), $150 \text{ mg}\cdot\text{L}^{-1}$ of total nitrogen (TN), and $127 \text{ mg}\cdot\text{L}^{-1}$ of total phosphorus as $\text{PO}_4^{3-}\text{-P}$ (TP). The synthetic wastewater fed the SB-MBBR reactor throughout the experimental run.

Three SB-MBBRs, -A, -B and -C were operated with a sequence of stages starting from anaerobic, aerobic and ending with anoxic during the 8-hour run. Each of the three reactors had different staging times (Figures 3.1 and 3.2). The reactors had each a volume of 2.3 L and a 40% fill ratio. The carriers used were K5 carriers harvested from the full scale integrated fixed-film activated sludge system (IFAS) (Hawkesbury wastewater treatment plant, Ontario, Canada). The IFAS system operated as a secondary treatment system with intermittent nitrification. The system operated at a carbon load of $3200 \text{ g COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ and a nitrogen load of $220 \text{ g N}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. In the operation of the SB-MBBR reactor the surface area loading rate (SALR), temperature and operation times were kept constant with the staging times for the anaerobic and aerobic stages varying for the three reactors.

The constituent concentrations of the influent synthetic wastewater used in this work was modified from (Forrest et al., 2016; Hoang et al., 2014; Young et al., 2017a) and contained the following: $\text{NH}_4\text{Cl}: 298 \text{ mg}\cdot\text{L}^{-1}$, $\text{NaHCO}_3: 812.8 \text{ mg}\cdot\text{L}^{-1}$, $\text{MgCl}_2: 58.4 \text{ mg}\cdot\text{L}^{-1}$, $\text{CaCl}_2\cdot 2\text{H}_2\text{O}: 29 \text{ mg}\cdot\text{L}^{-1}$, $\text{KH}_2\text{PO}_4: 175.6 \text{ mg}\cdot\text{L}^{-1}$, $\text{FeCl}_3: 4.8 \text{ mg}\cdot\text{L}^{-1}$. Carbon source: $\text{C}_6\text{H}_{12}\text{O}_6: 1160 \text{ mg}\cdot\text{L}^{-1}$, $\text{NaAc}: 650 \text{ mg}\cdot\text{L}^{-1}$,

Peptone:1160 $\text{mg}\cdot\text{L}^{-1}$. Trace nutrients; $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$:200 $\text{mg}\cdot\text{L}^{-1}$, $\text{NaMoO}_4\cdot 2\text{H}_2\text{O}$:49.62 $\text{mg}\cdot\text{L}^{-1}$, $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$:205.1 $\text{mg}\cdot\text{L}^{-1}$, $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$: 2 $\text{mg}\cdot\text{L}^{-1}$, $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$:59.82 $\text{mg}\cdot\text{L}^{-1}$.

Figure 3.1: The SB-MBBR setup consisting of SB-MBBRs -A, -B and -C respectively from left to right. The reactors were fed wastewater modelled after an eastern Ontario cheese wastewater effluent.

Figure 3.2: The SB-MBBRs eight-hour operational cycle with stage times for SB-MBBRs -A, -B and -C.

3.2 Nitrification Strategies

Figure 3.1: Experimental plan used to investigate the nitrification strategies.

Table 3.1: Description of the phases of each strategy. The description includes naming, carrier origin and the purpose of the operation.

	Phase	Reactor Name	Carrier Origin	Purpose of operation
Strategy 1	1	BAR1-A, -B, -C	SB-MBBR-A, -B, -C	Observe how long it will take for the carriers to nitrify if the aeration stage was extended
	2		Acclimated carriers from Strategy 1, Phase 1	Test the kinetics of the Acclimated carriers
Strategy 2	1	BAR2 -rep1, -rep2, rep3	IFAS system partially nitrifying municipal wastewater	Acclimate the carriers using the effluent from SB-MBBR-A with the best removal of C and P
	2		Acclimated carriers from Strategy2, Phase 1	Measure the kinetics of the triplicated test over 30 hours. The data was meant to be used to design the SB-MBBR-nit in Phase three
	3	SB-MBBR-nit	30 carriers were used to seed the reactor in combination with the IFAS carriers harvested	Test and characterize the kinetics of the two reactors in series.

The SB-MBBRs failed to remove ammonia during the 8-hour cycle, which lead to this work that focused on investigating possible nitrification strategies in the system. The first operational strategy to achieve nitrification in high carbon concentrated wastewater investigated is extending the aeration stage of the SB-MBBRs. The purpose of the first strategy was to investigate the length of time needed to achieve nitrification in the same reactor after the consumption of organic carbon. The second investigated strategy is the addition of a second aerated SB-MBBR in series for the

purpose of nitrification. The second operational strategy focused on separating the organic removal from the ammonia removal. The following sections present the methods used to investigate each of the two strategies (Figure 3.3). A detailed table is presented to describe the strategies phases (Table 3.1). The table indicates the carrier origin and the purpose of the phase.

3.2.1 Extended Aeration Stage

The operational strategy of extending the aeration stage was investigated using three 280 ml batch assay reactors (BAR1-A, -B, -C) (Figure 3.4). Each BAR1 housed ten carriers harvested from a different source. In the first operational strategy, the carriers came from SB-MBBRs -A, -B, and -C and were placed in BAR1-A, -B, -C. In phase 1 BAR1-A, -B, -C were fed with effluent wastewater coming from the SB-MBBR corresponding to the carrier origin. The operation of the experiment ceased when detected nitrification decreased the concentration of ammonia to below $50 \text{ mg} \cdot \text{L}^{-1}$. Strategy1 aimed to test the length of the aeration stage required to achieve nitrification. The sCOD, $\text{NH}_4^+/\text{NH}_3\text{-N}$, $\text{NO}_3^- \text{-N}$, $\text{NO}_2^- \text{-N}$, and Alkalinity (as CaCO_3), were measured over the

Figure 3.4: The figure shows the BARs set-up used to conduct the experiments (left) and the schematic diagram of the process (right). From right to left, the BARs are numbered as BAR1-A, -B, -C for strategy 1, and BAR2-rep1, -rep2, -rep3 for strategy 2.

experimental period. Note that $\text{NH}_4^+ \text{-N}$ is used to denote the sum of $\text{NH}_4^+ \text{-N}$ and $\text{NH}_3 \text{-N}$ in the

water. Deionized water was added to supplement any aeration losses that occurred over the operation due to evaporation. Aeration to the BARs was supplied at a rate of $16 \text{ ml} \cdot \text{min}^{-1}$ in all experimental runs and was kept constant throughout the experiment. The addition of either NaOH or HCl maintained the pH between 7 -8.5. The optimal pH for nitrification is between 7.5 and 8.5 (Metcalf and Eddy, 2003; WEF, 2010). Before filling the BARs, the tested alkalinity is adjusted accordingly by the addition of sodium bicarbonate. The TSS and VSS were measured before the start of the experiment and at the end of the thirty-three days (810 hours). After the thirty-three days (810 hours) the kinetics of the carriers acclimated were tested (second phase strategy1). This was done by the addition of the effluent from SB-MBBR-A to the BAR1-A, -B, C. SB-MBBR-A is selected as the reactor with the highest removal of both COD and phosphorus. The experiment lasted for thirty hours over which the same constituents mentioned above are measured.

3.2.2 Second Aerated SB-MBBR in series

The second experimental strategy (BAR2) used the same setup of the BAR experiments used in the first experiment (BAR1), the difference being the carrier's origin. The carriers used in the setup were harvested from the IFAS system and used in the operation of BAR2 -rep1, -rep2, and -rep3 (Figure 3.4). The carriers were harvested from an IFAS system operated as a secondary treatment system with intermittent nitrification. The system operated at a carbon load of $3200 \text{ g COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ and a nitrogen load of $220 \text{ g N} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$. The effluent of the SB-MBBR can be characterized as low C and high N, as mentioned in section 3.1 the SB-MBBR showed success in the removal of organic carbon and phosphorus but lacked any ammonia removal. The BARs took three weeks to a month to acclimate to the low carbon high ammonia wastewater (phase 1). The system mirrored the steady state operation of the second phase of the first operational strategy by operating using the effluent from SB-MBBR A. After the acclimation period, three sets of full triplicated data

recorded over a 21, 29 and 30-hour periods were collected for the operational strategy. The data for the 30-hour period is presented in article 1 as it was the run with the most data points (phase 2). The other runs are presented in a separate table as supplemental material in Appendix A. The COD, $\text{NH}_4^+/\text{NH}_3$ -N, NO_3^- -N, NO_2^- -N, and Alkalinity (as CaCO_3) were measured throughout the experimental runs. Note that NH_4^+ -N is used to denote the sum of NH_4^+ -N and NH_3 -N in the water. The aeration, pH and alkalinity adjustment remained the same as described for the first strategy. Distilled water was added to supplement any evaporation.

3.2.2.1 Characterization of Two SB-MBBRs in Series

The data from strategy 1 phase 2 was plotted and processed to obtain an average surface area removal rate (SARR) value (Appendix A, Table A1.). The average SARR value used to design the reactor was $1.07 \text{ gN}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. The value obtained was then used to design the nitrification sequencing batch moving bed biofilm reactor (SB-MBBR-nit). The SB-MBBR-nit is a 2.6 L (0.3 L carrier volume and 2.3L water volume) reactor with a 33% fill ratio (Figure 3.5). The carriers for the SB-MBBR-nit reactor were harvested from the BARs (30 carriers) and used in combination with K5 carriers taken from the full scale, Hawkesbury IFAS system. The IFAS system operated as a secondary treatment system with intermittent nitrification. The system operated at a carbon load of $3200 \text{ g COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ and a nitrogen load of $220 \text{ g N}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. The SB-MBBR-nit reactor is placed in series to SB-MBBR-A reactor, with both reactors operated on an 8-hour cycle (Figures 3.5 and 3.6). The reactor was operated for a month to acclimate to the new operating conditions prior to testing. The two reactors in series were characterized over a 16-hour period, tracking the change in the concentration of constituents from SB-MBBR A to the nitrification SB-MBBR. The constituents measured over the 16-hour cycle are COD, NH_4^+ -N, NO_3^- -N, NO_2^- -N, and Alkalinity

(as CaCO_3). In addition to those constituents, TP and TN are measured over the 16-hour cycle. The pH and DO were monitored at 5 min intervals for R1 and R2.

Figure 3.2: Image of the nitrification reactor (SB-MBBR-nit).SB-MBBR-nit operated on an 8-hour cycle.

Figure 3.3: The Set-up consisting of two reactors in series, the first reactor is the SB-MBBR-A and the second reactor is the nitrification SB-MBBR-nit.

3.3 Statistical Design

The statistical analyses for the batch assay reactors and the SB-MBBRs were conducted using the paired t-test between data sets, p-values less than 0.05 were considered significant. Data points were triplicated every other measurement for all tests conducted. The correlation analysis used the Pearson correlation with $R > 0.85$ to indicate significant correlation.

3.4 Analytical Methods

3.4.1 Ammonium/Ammonia

The ammonia concentration of the samples was measured using the HACH TNT 832 (HACH Method 10205) test ranging from 2 to 47 $\text{mg}\cdot\text{L}^{-1}$ kit (HACH USA, 2017a). The sample filtered using first a 1.5 μm glass fibre 691 filters (Millipore Corporation, Darmstadt, Germany), then

further filtered with 0.45 μm Millipore G filter (Millipore Corporation, Darmstadt, Germany). The sample diluted by a 1:5 dilution ratio after filtration was adjusted incrementally in correlation to the previous point tested during the experimental run. A 1000 μL pipette is then used to aliquot 0.2 ml of the diluted sample into the vial. The vial is closed and inverted to release the powder into the vial. After mixing the vials 2 to 3 times the vial is left to react for 15 minutes. The vial is placed after cleaning with a delicate task wipe in the HACH DR6000 spectrophotometer (HACH, USA). The Spectrophotometer set program is used to read the vials. Reading of both the absorbance and the concentration was done from the designated program window. The data is further processed using a calibration curve to correct for any interference constituents could cause. The calibration for the ammonia was constructed using synthetic wastewater influent to the reactors excluding the ammonium chloride. The calibration curve is constructed by adding ammonium chloride to the synthetic wastewater at different concentrations. The calibration curve and consequently the dilutions during the test period used DI water. Samples throughout the run were kept at 4°C and tested within 24 hours of the sampling period to avoid deterioration. The HACH TNT 832 tests are stored in the fridge at 4°C as outlined by the instructions in the operation manual.

3.4.2 Nitrite

The concentration of Nitrite was measured using the standard method 4500- NO_2^- B (distributed by Thermo Fisher Scientific, Waltham, MA). All the samples are filtered using first a 1.5 μm glass fibre 691 filters (Millipore Corporation, Darmstadt, Germany), then further filtered with 0.45 μm Millipore G filter (Millipore Corporation, Darmstadt, Germany). In accordance with the method established in (Young et al., 2017) the final filtrate was then diluted incrementally from 1:5 dilution factor to as high as 1:45 dilution factor, to account for the nitrite generation and change during the testing period. The range of the method used is between 0.01 to 1.00 $\text{mg}\cdot\text{L}^{-1}$ NO_2^- . After the

dilution, 10 ml of the sample was pipetted using a 10-ml pipette into a 25 ml Erlenmeyer flask, followed by 0.4 ml of nitrite colour reagent. The sample was stirred and then left for 20 minutes for the reaction to take place. After 20 minutes, 2 to 3 ml of the sample is pipetted to the HACH Co. glass cuvette to rinse it. The cuvette is emptied and filled again with the sample; the exterior is wiped before placing it into the HACH DR6000 Spectrophotometer (HACH, USA). The absorbance is read at an optical wavelength of 543 nm. A calibration curve was constructed using synthetic wastewater influent to the set-up with different concentration of nitrite standard added. The calibration curve is used to read the concentration from absorbance. Samples throughout the run were kept at 4°C and tested within 24 hours of the sampling to avoid deterioration. The nitrite colour reagent was stored in a dark glass container in the refrigerator at 4°C as best practice measure.

3.4.3 Nitrate

The soluble nitrate concentrations in all samples were measured using the HACH TNT 835 (HACH Method number 10206) (Hach, 2005). The TNT vial measuring range is from 0.23 to 13.5 mg·L⁻¹. The sample filtered using first a 1.5 µm glass fibre 691 filters (Millipore Corporation, Darmstadt, Germany), then further filtered with 0.45µm Millipore G filter (Millipore Corporation, Darmstadt, Germany). The filtrate was then taken and diluted incrementally depending on the measurement of the previous sample to accommodate the change of concentration during the testing period. The calibration curve constructed show little deviation from the sample concentration measured on the Spectrophotometer, indicating there is a little interference during this test using the current method. 5ml of diluted sample is placed into a 10 ml beaker and mixed with 50 mg of sulfamic acid. The solution stirred until no crystals remain, is left for 10 minutes. After the reaction time, 1 ml of the sample is placed in the vial. The kit provides a solution A, 0.2

ml of the solution is added to the vial. The vial is capped and shaken two to three times, at which point it is set aside for a 15-minute reactions time. The outside of the vial is cleaned using a delicate task wipe then the vial is inserted into the HACH DR6000 Spectrophotometer (HACH, USA), and the readings obtained are recorded. The samples throughout the run were kept at 4°C and tested within 24 hours of the sampling to avoid deterioration. The kit was stored at room temperature, and the sulfamic acid is kept stored in the chemicals closet as per safety measures.

3.4.4 Soluble Chemical Oxygen Demand

The soluble chemical oxygen demand concentration was measured using the HACH method 8000 LR (a kit version of standard methods 5220D). The range of this method is 30 to 150 mg·L⁻¹ COD. A HACH DRB200 digester (HACH, USA), was preheated to 150C° before the experiment. The sample filtered using first a 1.5 µm glass fibre 691 filters (Millipore Corporation, Darmstadt, Germany), then further filtered with 0.45µm Millipore G filter (Millipore Corporation, Darmstadt, Germany). In accordance with the method established in Forrest et al., 2016, the 2 ml of the filtrate was pipetted from the beaker to the HACH COD LR vial. The vial was then sealed and shaken several times to ensure proper mixing. The vials were then immediately added to the digester for a reaction time of 140 minutes. After the reaction time, the vials were left in the digester until the temperature decreased to 120 C°. The vials were then placed on the rack to allow them to reached room temperature. The vials were cleaned using delicate task wipes before placing them into the HACH DR6000 spectrophotometer (HACH, USA). The program COD LR 430 was then used to obtain the readings for the concentration. The samples throughout the run were kept at 4°C and tested within 24 hours of the sampling to avoid deterioration. The kit was stored at room temperature.

3.4.5 TN

The HACH total nitrogen reagent LR reagent set was used to measure TN in this work (HACH USA, 2017b). The test is applicable in the following range of $0.5\text{--}25.0\text{ mg}\cdot\text{L}^{-1}\text{ -N}$ (HACH method number: 10071). Before the start of the test, the wastewater is diluted to fit the range of the method. The DRB200 (HACH, USA), is heated to $105\text{ }^{\circ}\text{C}$ before the experiment begins. the TN persulfate reagent powder pillow is added to one TN hydroxide reagent vial. The cap is tightly closed and then shaken for 30 seconds. The vial is placed in the digester for 30 minutes afterwards. The vial is removed and left to cool using heat resistant gloves. The TN reagent A powder pillow is then emptied into the vial and shaken for 15 seconds. The vials left for 3 minutes had the TN reagent B powder pillow added to it. After shaking the vial for 15 seconds, a pipette is used to take 2 ml of the digested vial and placed into a new TN reagent C vial. The vial is then inverted ten times and left to sit for another five minutes. The DR6000 spectrophotometer (HACH, USA), is used to read the vial using the stored program. Both absorbance and concentration are recorded for every sample tested.

3.4.6 TP

The HACH total phosphorus low range method has a measuring range of $0.06\text{ to }3.5\text{ mg}\cdot\text{L}^{-1}\text{ PO}_4^{3-}$ was used (HACH USA, 2017c). The samples collected were diluted by a 1:5 dilution factor and later by a 1:10 dilution factor. Before the start of the test the DRB 200 digester (HACH, USA), is preheated to $150\text{ }^{\circ}\text{C}$. 5ml of the sample is pipetted and placed in the total and acid hydrolyzable test vial, also, 0.1 g of the potassium persulfate powder pillow is further added to the test vial. The amount needed is premeasured using a scale and weighing paper to add the required amount of 0.1 g consistently. The contents of the vial are mixed, and the vial is placed in the digester for 30 minutes. The digested vial is placed on a rack until it reaches room temperature. After the cooling

period, 2 ml of NaOH standard solution provided in the kit is added to the vial. The vial contents are emptied to a 5cm cuvette, and the DR6000 spectrophotometer (HACH, USA) is zeroed. The contents of the cuvette are returned to the vial by using a funnel, and 0.32 g of the PhosVer3 Phosphate reagent pillow is premeasured and added to the vials. The vial is then mixed for 20 to 30 seconds and left for 2 minutes before it is emptied into the cuvette again. The reading is taken from the spectrophotometer as $\text{mg}\cdot\text{L}^{-1}$ of PO_4^{3-} .

3.4.7 Alkalinity

The HACH TNT 870 with a measuring range from 25 to 400 $\text{mg}\cdot\text{L}^{-1}$ of CaCO_3 was used to determine the alkalinity of the water samples (distributed by Thermo Fisher Scientific, Waltham, MA). The sample was filtered using first a 1.5 μm glass fibre 691 filters (Millipore Corporation, Darmstadt, Germany), then further filtered with 0.45 μm Millipore G filter (Millipore Corporation, Darmstadt, Germany). The sample was then diluted after filtration by a 1:5 dilution ratio using DI water. 0.5 ml of the diluted sample was added to the TNT vial after the addition of 2ml of solution A in the kit. After leaving the vial for five minutes after mixing, the vial is wiped and placed in HACH DR6000 Spectrophotometer (HACH, USA). The Samples were kept at 4°C and tested within 24 hours of the sampling to avoid deterioration.

3.4.8 Dissolved Oxygen

The dissolved oxygen concentration was measured using a HACH HQ40d optical DO meter (HACH, USA). Luminescent dissolved oxygen (LDO) probe model number 10101 was attached to the DO meter. The unit was calibrated and maintained using manufacturer guidelines and recommendations to maintain measurement accuracy and precision. The probe is calibrated by adding ¼ inch of reagent water to a narrow neck BOD bottle and mixing it. The probe is then put

in the bottle and measured. The range of measurement was from 0.01 to 20 mg·L⁻¹, and the resolution of the device was 0.01 mg·L⁻¹.

3.4.9 pH

The pH of all samples was measured using a VWR symphony pH meter (VWR, PA, USA). Before measurements, the pH was regularly calibrated using three buffer solutions with standard pH of 4, 7, and 10. The electrode was rinsed with DI water and dried in between measurements. The electrode was then stored in a pH seven buffer solution and was rinsed and dried following each measurement and before placing in the storage solution. The range of measurement was from -2.00 to 19.99 pH and the resolution was ±0.01 pH units.

3.4.10 Solids Analysis

Solida measurements were conducted in accordance with methods outlined by the standard methods (APHA, 2012). The 2540 D-Total Suspended Solids (TSS) (dried at 103-105 °C), 2540 E- Volatile suspended solids (VSS) (ignited at 550 °C). Three samples were taken from each batch assay reactor and processed; this was done for the first operational strategy. The two SB-MBBRs in series also were processed in the same method outlined.

3.4.11 Microscopic Image Analysis

The Digimizer (MedCalc Software, Ostend, Belgium) image analysis software was used to make measurements of the biofilm thickness. The image was acquired using the ZEISS STEMI 305 microscope (ZEISS, Hamburg, Germany) and later processed using the Digimizer. Five images from carriers representing one condition, each with 50 individual length measurements were used to obtain the thickness of the biofilm in each of the conditions.

3.4.12 Microscopy

Five carriers from BAR1 -A, -B, -C were taken and imaged. The ZEISS STEMI 305 (ZEISS, Hamburg, Germany) was used to obtain the images. The front optic was set to 1x with a forward working distance (FWD) of 110; the lens magnification range was between a min zoom of 8x and a maximum zoom of 40x. The ZEN blue (© Carl Zeiss Microscopy GmbH, 2011) program was used to capture and store the images for later processing by Digimizer.

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Chapter 4: SB-MBBR Technology: Treatment Strategies for Nitrification in Elevated Carbon Industrial Wastewaters

4.1 Setting the Context

The article presented in Chapter 4 is titled *SB-MBBR Technology: Treatment Strategies for Nitrification in Elevated Carbon Industrial Wastewaters* by N. AlGhussain, O. Basu, A. Tsitourous, R. Delatolla. This article is in preparation for publication in the Journal of Environmental Technology. This article discusses nitrification strategies applied to cheese wastewater characterized by high carbon and nutrient concentrations. Three SB-MBBR with different AOA stage cycle times removed both organic carbon and phosphorus, but no removal of ammonia was seen throughout the 8-hour cycle. Two nitrification strategies are proposed in this article; the first nitrification strategy is investigating the length of time required to extend the aeration stage of the AOA cycle to achieve nitrification. The second nitrification strategy is the addition of a nitrification reactor in series to the SB-MBBR separating the carbon removal from the ammonia removal. This article describes the characterization of each of the strategies.

4.2 Graphical Abstract

Figure 4.1: Graphical abstract.

4.3 Abstract

High carbon and nutrient conditions characterize wastewater from the process of cheese making. The research aims to investigate the design and operational strategies of a sequencing batch moving bed biofilm reactor (SB-MBBR) with an anaerobic, aerobic, and anoxic (AOA) stage sequence to achieve nitrification in a high carbon concentrated wastewater from the food industry. The potential to utilize an SB-MBBR has not been previously investigated for the treatment of cheese making effluent. Carbon removal was feasible in the SB-MBBR (99% removal) directly with two operational strategies further investigated for nitrification. The two strategies investigated were extending the aeration cycle and secondly utilized a second SB-MBBR in series to achieve nitrification. The first operational strategy required 33 days (810 hours) for the system to adapt and for nitrification to occur, achieving 89-93% of NH_4^+ -N removal efficiency. The time required to achieve nitrification rendered this option infeasible. The second operational strategy confirmed that separating the carbon and ammonia removal resulted in the high removal of both compounds. The 30-hour run resulted in an 81-83% NH_4^+ -N removal efficiency, with a SARR of 0.83-1.31 $\pm$ 0.08 gN $\cdot$ m⁻²·d⁻¹.

Keywords.: Nitrification, Sequencing Batch Moving Bed Biofilm Reactor (SB-MBBR), Cheese wastewater, Food processing wastewater treatment

4.4 Introduction

Wastewater from food processing is classified as high strength wastewater which contains high amounts of fats, oils, grease, organic and inorganic compounds and nutrients. The concentration of each of those constituents in the wastewater depends mainly on the production process, and the type of food processing effluent examined. The food processing industry includes several sectors that manufacture different types of food; examples include meat product processing, processing and preserving fruits and vegetables, manufacturing of vegetable and animal oils, manufacturing of dairy products. Considering the wide range of manufacturing processes, the food industry is considered one of the most important industries worldwide and in particular developing countries. Of note is the increasing demand for dairy products in the milk processing industry. For example, China's demand for milk products is projected to increase 3.2-fold by 2050 with supplies being generated regionally and globally. China is a leading milk importer, with 12 Tg fresh milk imported in 2013, this is an increase of 123 times from in 1961, and represents 25% of domestic consumption in 2013 (Bai et al., 2018). It is estimated that to produce 2 L of milk, 5 L of wastewater are generated (Pereira et al., 2018). Thus, as the demand for milk increases so does the associated need for wastewater treatment.

With a growing demand for milk, there is also an accompanying increase for cheese products, this, in turn, increases the production of cheese processing wastewater. To produce 1 kg of cheese, 10 kg of milk is required. This process produces 9 kg of cheese whey (Prazeres et al., 2012). Cheese whey is a by-product generated during the cheese manufacturing process. The concern over the production of cheese whey is due to the high organic loads and volume of wastewater generated; this volume worldwide is disposed of most times on land or in water bodies (Rico et al., 2015). Cheese processing wastewater, which is a combination of cheese whey with water used in the

processes and facility and exhibits a chemical oxygen demand (COD) concentrations in the range of $0.8\text{--}102\text{ g}\cdot\text{L}^{-1}$ and biological oxygen demand (BOD) values in the range of $0.6\text{--}60\text{ g}\cdot\text{L}^{-1}$. The nutrient concentration in a typical cheese processing effluent is in the range of $0.01\text{--}1.7$ and $0.006\text{--}0.5\text{ g}\cdot\text{L}^{-1}$ total Kjeldahl nitrogen (TKN) and total phosphorus (TP) respectively (Janczukowicz et al., 2008; Rivas et al., 2010). The type of cheese manufactured, and the process used to manufacture it affects the cheese processing wastewater concentrations of COD, TKN and TP, for example, the lower limit of the range of concentration presented would indicate the facility processes cheese whey decreasing the concentrations mixed with the effluent water from the facility. Despite the wide range of concentrations found in cheese processing effluent, the impact caused by the constituents remains. Organics can cause depletion of oxygen leading to septic conditions when released to sewers, while the nutrients in the wastewater can lead to eutrophication and affect the downstream efficiency of wastewater treatment plants (Janczukowicz et al., 2008).

Due to the high organic content of dairy effluent biological treatment is the most common method of treatment. Activated sludge processes are commonly used, these processes include conventional activated sludge (AS), sequencing batch reactors (SBR) and granular sludge reactors. Granular sludge reactors consist of aerobic granules (AG), up-flow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB), all of which have been used to treat dairy processing wastewater (Castillo et al., 2017). With regards to the cheese wastewater treatment, the most applied biological treatment methods are UASBs and SBRs (Carvalho et al., 2013; Kushwaha et al., 2011). The UASBs simple construction, low operational cost and high organic removal attracted many installations and research for its usage in the treatment of cheese processing wastewater (Karadag et al., 2015a). The UASB effluent generally requires post-treatment to fulfil

discharge standards, more recent research focused on enhancing the process by adding SBRs before UASBs or after, or combining the whey with dairy manure or domestic wastewater as ways to enhance the treatment (Rico et al., 2015; Tawfik et al., 2008). The challenge in UASBs is granular formation and maintenance (Liébana et al., 2018). For instance, the start-up of anaerobic granules is prolonged due to the slow growth of anaerobic microorganisms and can lead to effective treatment delays (Liu and Liu, 2006). For a more rural setting, SBRs rather than UASBs have found an application. The rural cheese-making industries have intermittent discharge and require a more localized smaller footprint treatment (Carvalho et al., 2013).

More recently aerobic granular SBRs (AG-SBRs) were used in the treatment of dairy effluent; the granules removed carbon, nitrogen and phosphorus from the effluent in the same reactor (Angela et al., 2011; Mañas et al., 2012; Meunier et al., 2016). The one stage process presented removals of up to 90% of total COD, 80% of the total nitrogen (TN), and 67% of total phosphorus (TP). The SBR achieving these removals had a stage sequence of anaerobic and aerobic cycles over an 8-hour testing period (Schwarzenbeck et al., 2005). However, the AG-SBR was found to have similar problems to the UASBs when it comes to the stability and maintenance of the granules. The shape of the granules and the size are considered important factors in the removal of nitrogen and carbon from the wastewater (Zhang et al., 2015). The larger size granules tend to destabilize due to endogenous respiration within the granule resulting in their collapse and washout. The nitrogen removal is also affected by the size of the granules, a smaller granule size ranging between 0.5-0.9 mm removed nitrogen at a higher rate than a larger size granule. Further, high organic loads can incite the overgrowth of filamentous bacteria, destabilizing the structure of the granules (Schwarzenbeck et al., 2005).

Replacing granules with plastic carriers in an SBR introduced the sequencing batch moving bed biofilm reactor (SB-MBBR) to the treatment of several domestic and industrial wastewaters. The carriers eliminated the need to maintain granules as well as any problems resulting from washout and dissolution of granules encountered in both UASBs and AG-SBRs. The carriers retain the biomass within the reactor. The SB-MBBR used for the treatment of fishery wastewater was able to achieve removals of COD (97%), TKN (74%) and TP (70%) in the same reactor (Nguyen and Nguyen, 2013). The SB-MBBR has also shown success in the removal of a combination of phosphorus and nitrogen in the same reactor (Helness and Ødegaard, 2001).

For the removal of C, N and P in the same reactor, the SBR reactor stage required different operational conditions. In particular, it is postulated that an anaerobic, aerobic, and anoxic (AOA) sequence is ideal for dairy wastewater treatment. AOA stage sequence allows for the simultaneous nitrogen and phosphorus removal from a single reactor as well as carbon by selecting for the phosphorus accumulating organisms (PAOs), and nitrifiers. Previous research in other industries has reported relatively high levels of carbon, nitrogen and phosphorus removals using the AOA stage sequence with SB-MBBR (Schwarzenbeck et al., 2005). To date, however, the operation of an SB-MBBR with an AOA stage sequence treating wastewater from the food processing industry, in specific cheese wastewater has not been reported. This research investigates the operation of the SB-MBBR treating high strength wastewater mimicking a food processing effluent. In particular, this study examines an AOA sequence for carbon removal and nitrogen removal which has received limited attention in the cheese wastewater industry.

4.5 Materials and Methods

4.5.1 SB-MBBR Reactor Operation

Three SB-MBBRs: A, B, and C with varying AOA stage times were fed synthetic wastewater mimicking the wastewater from a typical cheese processing industry (Figure 4.2). The 2.3 L reactors used were AnoxKaldness © K5 carriers with a bulk specific area of $800 \text{ m}^2 \cdot \text{m}^{-3}$ and had a 40% fill ratio. The system acclimated to the respective AOA cycle times two months before conducting the experiments. The carriers were initially seeded from a secondary IFAS treatment plant located in Hawkesbury, Ontario, Canada. The IFAS system was partially nitrifying wastewater operated at a carbon load of $3200 \text{ g COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ and a nitrogen load of $220 \text{ g N} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$.

Figure 4.2: The SB-MBBRs eight-hour operational cycle with stage times for Reactors A, B and C.

AOA stage times were initially modelled from (Schwarzenbeck et al., 2005), the aeration times were then adjusted to reflect the anaerobic time used by (Yin et al., 2015). Times were adjusted to accommodate for phosphorus removal by PAOs. The stage times reflected a shorter anaerobic stage

in SB-MBBR A, while a longer anaerobic stage was tested in SB-MBBR-C. SB-MBBR B operated 120 mins anaerobic stage in an eight-hour cycle. The aim was to investigate cycle timing for the removal of carbon, ammonia and phosphorus from high loading wastewater.

The constituent concentration of synthetic wastewater was as follow: NH_4Cl :298 $\text{mg}\cdot\text{L}^{-1}$, NaHCO_3 :812.8 $\text{mg}\cdot\text{L}^{-1}$, MgCl :58.4 $\text{mg}\cdot\text{L}^{-1}$, $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$:29 $\text{mg}\cdot\text{L}^{-1}$, KH_2PO_4 :175.6 $\text{mg}\cdot\text{L}^{-1}$, FeCl :4.8 $\text{mg}\cdot\text{L}^{-1}$. Carbon source: $\text{C}_6\text{H}_{12}\text{O}_6$:1160 $\text{mg}\cdot\text{L}^{-1}$, NaAc :650 $\text{mg}\cdot\text{L}^{-1}$, Peptone:1160 $\text{mg}\cdot\text{L}^{-1}$. Trace nutrients; $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$:200 $\text{mg}\cdot\text{L}^{-1}$, $\text{NaMoO}_4\cdot 2\text{H}_2\text{O}$:49.62 $\text{mg}\cdot\text{L}^{-1}$, $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$:205.1 $\text{mg}\cdot\text{L}^{-1}$, $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$:2 $\text{mg}\cdot\text{L}^{-1}$, $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$:59.82 $\text{mg}\cdot\text{L}^{-1}$.

4.5.2 Nitrification Strategy Batch Assay Reactors

The initial setup did not remove ammonia as such nitrification in the SB-MBBR reactor was investigated via two operational strategies. Strategy 1 extended the duration of the aeration stage until nitrification was detected. The purpose of extending the aeration stage length was to remove carbon first from the reactor, as carbon is an inhibitory constituent to the function of nitrifiers. Strategy 2 placed a second SB-MBBR reactor in series to the first to allow for separate carbon and nitrogen removal.

Strategy 1: For the first operational strategy, three batch assay reactors (BAR1-A, BAR1-B and BAR1-C) were used. Each BAR had a volume of 280 ml and contained 10 K5 MBBR carriers. The carriers used in the first operational strategy were harvested from SB-MBBRs -A, -B, and -C described above. The BARs were initially filled by using the effluent from the SB-MBBRs corresponding to the carrier origin. For example, carriers from SB-MBBR-A were placed in BAR1-A and fed the effluent from of the same reactor. The operation of the BAR1 consisted of an acclimation period which lasted 33 days (810 hours). After the 33 days operation (810 hours), the BARs showed the removal of ammonia and a corresponding generation of either nitrate or

nitrite indicating nitrification. At the end of the 33 days (810 hours), the kinetics of the carriers were tested. To conduct the kinetics testing of the BARs after the 33 days (810 hours) of acclimation, BAR1-A, -B and -C were replenished with effluent coming from SB-MBBR-A which had the highest removal of COD and phosphorus (Table 4.1). Characterization of the after-acclimatization operation was conducted by measuring the concentration change over a period of 30 hours.

Strategy 2: For operational strategy two, the carriers were harvested from Hawkesbury, Ontario, Canada. Ten MBBR carriers were placed in BAR2-rep1, -rep2 and -rep3; the carriers were acclimated for one month before testing the kinetics of the system. The BARs in this strategy were fed the effluent from SB-MBBR-A (Table 4.1).

Table 4.1: The influent characteristics coming from the SB-MBBR A and the influent to the BAR1-A, -B, -C and BAR2-rep1, rep2, rep3 used for kinetic testing.

Constituent	Value
Temperature	21.66±1.15°C
Ammonia (NH ⁺ ₄ -N)	110±8 mg·L ⁻¹
Nitrite (NO ₂ ⁻ -N)	0.031±0.027 mg·L ⁻¹
Nitrate (NO ₃ ⁻ -N)	0.21 ±0.06 mg·L ⁻¹
Total Phosphorus (PO ₄ ⁻³ -P)	14±2.6
TSS	0.513±0.200 g·L ⁻¹
sCOD	70.0 ± 9.6 mg·L ⁻¹
Alkalinity (CaCO ₃)	889± 10 mg·L ⁻¹
pH	8.39 ±0.08

The effluent from SB-MBBR-A has a range of COD concentration between 60-100 mg·L⁻¹ and ammonia concentration between 100-120 mg·L⁻¹ (Table 4.1). The measured constituents for both strategy one and two are sCOD, NH₄⁺-N, NO₃⁻-N, NO₂⁻-N, and alkalinity (as CaCO₃). Surface area removal rates (SARR) were also calculated to characterize the performance of the biofilm on the carriers (EQ1). The SARR for a typical nitrification system is 1 gN·m⁻²·d⁻¹ (WEF, 2011).

$$SARR = Q \left(\frac{\text{concentration influent ammonia} - \text{concentration effluent ammonia}}{V * \%Fill * SA (k5)} \right) \quad (1)$$

Deionized water was added to make up for any evaporation losses that occurred. Aeration to the BARs was supplied at a rate of 16 ml·min⁻¹ in all experimental runs. The addition of either NaOH or HCl maintained the pH between 7-8.5, throughout all experimental runs. Alkalinity was tested before filling the BARs. The addition of sodium bicarbonate was used to adjust the alkalinity. Alkalinity was added to the influent water to account for nitrification requirements of 7.14 mg alkalinity per mg ammonia oxidized (WEF, 2011).

4.5.3 Analytical Methods

All samples were filtered with a 1.5 µm glass fibre 691 filters (Millipore Corporation, Darmstadt, Germany), followed by 0.45µm Millipore G filter (Millipore Corporation, Darmstadt, Germany). The filtered samples were tested for sCOD, NH₄⁺-N, NO₃⁻-N, NO₂⁻-N, and Alkalinity (as CaCO₃). Soluble COD was measured using the HACH vials method 8000 LR (30-150 mg·L⁻¹). Soluble nitrite (4500-NO₂⁻ - B) was measured in accordance with the standard methods (APHA, 2012). Total ammonia nitrogen was measured using the TNT 832 plus test vials (HR:2-47 mg·L⁻¹NH₄⁺/NH₃ -N) (HACH, USA). The ammonia (NH₄⁺-N) used in this text refers to the sum of NH₄⁺-N and NH₃-N that exist in the water. The soluble nitrate was measured using the TNT 835 plus test vials (LR:0.2-13.5 NO₃⁻ -N) with sulfamic acid addition (HACH, USA). The alkalinity (as CaCO₃) was analyzed using the TNT 870 vials (25-400 mg·L⁻¹). Total suspended solids (TSS) and volatile

suspended solids (VSS) were measured using standard methods (APHA, 2012). All samples were analyzed using the HACH DR6000 spectrophotometer (HACH, USA). pH was measured using the VWR pH probe, while DO was measured using a HACH probe (HACH, USA). The Biofilm thickness was measured using Digimizer (MedCalc Software, Ostend, Belgium). Microscopy images were taken using ZEISS Stemi 305 (ZEISS, Hamburg, Germany). To obtain the thickness five images are taken from each BARs carrier by using the microscope, the images are later analyzed using Digimizer. In total, fifty measurements are taken per image, for a total of 250 measurements per BAR.

4.5.4 Statistical Analysis

Data were analyzed using a paired t-test between data sets, p-values less than 0.05 were considered significant. Data points were triplicated every other measurement. The correlation analysis used the Pearson correlation with $R > 0.85$ to indicate significant correlation. The value was chosen to correlate the trends with ammonia production and uptake in the BAR tests per time unit.

4.6 Results and Discussion

4.6.1 SB-MBBR Performance

SB-MBBRs -A, -B and -C operation was tested with three different AOA stage durations. The highest organic carbon removal was obtained in SB-MBBR-A. The removal in all three SB-MBBRs was between 98-99% indicating the length of the aerobic stage was not significant to the removal of organic carbon (Table 4.2). The longer anaerobic stage was meant to enhance the phosphorus removal from the wastewater (Zhang et al., 2015); however, the results showed a decrease in the phosphorus removal overall as the anaerobic stage time increases. The percent removals of phosphorus (Table 4.2) in SB-MBBR-A,-B and -C are consistent with an assimilation rate of $1 \text{ mg} \cdot \text{L}^{-1}$ per $100 \text{ mg} \cdot \text{L}^{-1}$ of BOD removed (WEF, 2011). But, as the anaerobic stage length

increased, the removals of both carbon and phosphorus decreased, while ammonia increases during the anaerobic stage. Due to the high concentration of organics and the presence of peptone in the influent, the concentration of ammonia is increased due to ammonification in the anaerobic stage.

Table 4.1: The removal rates for total ammonia nitrogen, phosphorus and carbon in the three SB-MBBRs over an eight-hour cycle.

Reactor	COD	NH ₄ ⁺ -N	PO ₄ ⁻³ -P
%Removal			
SB-MBBR A	99±5%	-1±0.04%	72±4%
SB-MBBR B	98±5%	-47±2%	59±3%
SB-MBBR C	98±5%	-25±1%	63±3%

During the anaerobic stage, the microorganisms in the biofilm on the carrier's experience death as well as regeneration as part of biofilm maintenance. A longer anaerobic cycle would affect the rates at which the death of microorganisms happens. Exposure to low oxygen conditions for a more extended period causes a death event which in turn releases ammonia through the microbial break down as part of endogenous respiration (Katipoglu-Yazan et al., 2012). As such the longer anaerobic stages in SB-MBBR-C and -B exhibited a higher ammonia production, with SB-MBBR B exhibiting a higher concentration of ammonia production. Although the results show a difference, the trend in the production and consumption of ammonia in the three SB-MBBRs showed no significant difference over the anaerobic and aerobic stages. The decrease in the aerobic region followed the ammonia generation in the anaerobic region due to ammonification. The results show the least overall ammonia production coupled with an acceptable range of COD and phosphorus removal in SB-MBBR-A this could be attributed to the length of the aerobic stage (Figure 4.3).

SB-MBBR-A

Figure 4.3: Constituent concentration measured over an 8-hour cycle in SB-MBBR A. The figure shows the concentration change of ammonia, nitrate, nitrite, COD, and TP with time.

The reduction of ammonia initially expected throughout the stage configuration was not observed in contradiction of research by others operating AOA stage cycling (Schwarzenbeck et al., 2005; Yin et al., 2015; Zhang et al., 2015). The high COD concentration is expected to have led to high heterotrophic growth that suppressed the nitrifying community development which prevented the removal of ammonia in the system (J. KellerK. and Greenfield, 1997; Roy et al., 2010). The observed pattern of increasing ammonia concentration and the subsequent decrease in the aerobic region indicated uptake through cell activity (Figure 4.3). The reduction was consistent with an ammonia assimilation rate of 0.0875g-N per g of particulate COD formed (Grady et al., 1999). The reduction shows that the removal was not due to nitrifiers activity in the aerobic region. As

such, the lack of ammonia removal in the initial study period necessitated further investigation via the two nitrification strategies.

4.6.2 Strategy 1: Extending the Duration of the Aeration Stage

Extending the duration of the aeration stage in the SB-MBBR setup was achieved by placing the carriers harvested from the SB-MBBRs into BAR1s-A, -B and -C. The BARs subsequently were fed the wastewater from the SB-MBBRs. By doing so, several trends were observed over the testing period that lasted 33 days (810 hours). The influent sCOD to $\text{NH}_4^+\text{-N}$ ratio in the BARs was an average of 0.53, while the influent to the SB-MBBRs had an average sCOD to $\text{NH}_4^+\text{-N}$ ratio of 32. The SB-MBBR can be characterized as a high carbon loaded system while the BARs can be classified as carbon deficient.

Over the first 20-36 hours, all BAR systems had a noticeable drop in Ammonia (Figure 4.4). The most significant decrease occurred in BAR1-A where ammonia concentration dropped to $27 \pm 7 \text{ NH}_4^+\text{-N mg}\cdot\text{L}^{-1}$ from an initial ammonia concentration of $96 \pm 1 \text{ mg}\cdot\text{L}^{-1}\text{NH}_4^+\text{-N}$. The decrease was expected in terms of nitrification; however, no nitrification was observed as neither nitrate nor nitrite concentration changed over this period. Also, interpreting the decrease as assimilation into the biofilm is unlikely, as the rate of ammonia decrease did not correspond to previously presented research at 0.0875g-N per g of particulate COD formed. Thus, this initial loss may be due to stripping. The wastewater pH was around 8.5-9 as recorded in the initial 20-36 hours despite the pH adjustment.

Figure 4.4: The figure shows the acclimatization period of carriers coming from SB-MBBR-A, -B and-C, placed in BAR1s-A, -B, -C for strategy 1. After 36 hours of operation, an increase of

ammonia was observed in BAR1s-A, -B, and -C. Ammonification is thought to be the cause of the increase due to the breakdown of organic nitrogen in the wastewater. The slow degradation of organic material releases organic nitrogen by the action of heterotrophic bacteria on the carriers; organic nitrogen is converted later to ammonia (Grady et al., 1999). In the three BARs, there was a pattern after the initial spike in ammonia concentration. The spike was followed with a flattening followed by a second spike. The trend observed is thought to be due to a lag in ammonification. The initial spike was attributed to the exposure to starvation conditions after which there is a distinct lag in ammonia production. The lag is thought to be an acclimation period to the increased production of organic carbon due to cell death occurring in the batch, after which the production continues to spike. The ammonia concentration increases peaked at approximately 288 hours and then was observed to decrease through the remainder of the test runs. BAR1-A reached the highest ammonia concentration of $406 \pm 4 \text{ mg} \cdot \text{L}^{-1} \text{ NH}_4^+ \text{-N}$ (Figure 4.4). Production of nitrate and nitrite accompanied the decrease in ammonia indicating that this decrease was due to the function of nitrifiers in the biofilm.

Between 672-810 hours (days 28-33) of the testing period, the three BARs had a different nitrate and nitrite production trends. The nitrate production was significantly higher in BAR1-C compared to BAR1 -A. The nitrate concentration at the end of the 33 days (810 hours) reached $293 \text{ mg} \cdot \text{L}^{-1}$ of $\text{NO}_3^- \text{-N}$ in BAR1-C, versus $81.4 \text{ mg} \cdot \text{L}^{-1}$ in BAR1-A. Conversely, the concentration of nitrite rather than nitrate was higher in BAR1-A at $252 \text{ mg} \cdot \text{L}^{-1}$ compared to $23 \text{ mg} \cdot \text{L}^{-1}$ in BAR1-C. The nitrite accumulation in BAR1-A indicated a lag in the recovery of the nitrite oxidizing bacteria (NOBs). The ammonia-oxidizing bacteria (AOB) has a lower specific growth rate than NOBs (Im et al., 2014; Rezaee et al., 2015). The increase in production of nitrite indicated a recovery of the AOB community in the biofilm. The observed nitrite accumulation can be attributed to the inability

of the NOBs to establish themselves on the carriers within the testing period. Although the ammonia trends showed similarity, the trend in NOB and AOB activity manifested again within the same BAR tests when the kinetics of the system was tested. A linear equation best models the ammonia drop in BAR1-A, -B and -C with an average slope of -0.64 ± 0.03 (Figure C1, Appendix C). The decrease between hours 288-810 follows a linear pattern in the three BARs with BAR1-A as the steepest slope. Followed by both BAR1-B, and BAR1-C is indicating that in BAR1 there was a faster recovery of AOB activity than in -B and -C. Data from BARs showed a similar trend in ammonia concentration over the operation period of 33 days (810 hours). The correlation over the entire run comparing BARs -A, -B, and -C showed an $R > 0.96$.

After the 33-day (810 hours) testing period, the BARs carrier's kinetics were tested. The BARs were refilled with effluent from SB-MBBR-A with the best removal of COD and phosphorus. The results obtained over the 30-hour testing period showed BAR1 -A, -B and -C nitrifying with the ammonia concentration decreasing to $10 \pm 2 \text{ mg} \cdot \text{L}^{-1} \cdot \text{NH}_4^+ \cdot \text{N}$ (Appendix B, Fig.B1). The steady state surface area removal rates (SARR) obtained was $0.89 \pm 0.02 \text{ gN} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$. In a previous study using the same type of carriers used in this study, a maximum SARR of $2.13 \text{ gN} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ was obtained with an ammonia concentration of the influent not exceeding $25.5 \text{ mg} \cdot \text{L}^{-1}$ (Young et al., 2017b). In this experiment, the influent ammonia to the BARs tested was as high as $106 \text{ mg} \cdot \text{L}^{-1} \text{ NH}_4^+ \cdot \text{N}$. The high ammonia concentration coupled with a pH ranging between 8-9, can lead to inhibition due to free ammonia (FA). The concentration of nitrate and nitrite over time mirrored the behaviour observed in the period from 672-810 hours (28-33 days) at the end of strategy 1 testing. BAR1-A showed an accumulation of nitrite of up to $75 \text{ mg} \cdot \text{L}^{-1} \text{ NO}_2^- \cdot \text{N}$, while BAR1-B and BAR1-C showed complete nitrification with nitrate as the end product when the kinetics of the system was tested. The effluent concentration of 66 and $61 \text{ mg} \cdot \text{L}^{-1} \text{ NO}_3^- \cdot \text{N}$ for BAR1-B, -C

respectively was observed. Statistically, there was no significant difference between the nitrite concentration compared at $p < 0.05$ in the three BARs of strategy 1. The significance was observed in the ammonia and nitrate concentration trends over the testing period, which directs us to conclude that the activity of the NOB is the difference between the three BARs after the acclimation period. The similarity in behaviour between the observed results at the end of the acclimation period and the kinetics tested enforced the belief of a connection to the carrier origin. The carriers took from differently operated AOA stage times in SB-MBBRs -A, -B and -C experienced several changes over the 33-day (810 hours) testing period in terms of TSS, VSS and biofilm thickness (Figure 4.5).

Figure 4.4: The biofilm thickness, VSS, TSS and Ammonia consumption in BARs at time zero days till the end of the experiment at time 33 days (810 hours).

TSS and VSS experienced an increase from day 0 at the start of the experiment, until day 33(810 hours) at the end of strategy-1 testing (Figure 4.5). The increase in TSS and VSS accompanied by

the spike in ammonia concentrations in BAR1 -A, -B and -C indicate the occurrence of a significant death and decay event over the initial period of 12 days (288 hours). The death event was also accompanied by a change in biofilm thickness, in turn explaining the increase in the TSS and VSS concentration over the testing period of 33 days (810 hours). The most likely affected microorganism are the heterotrophs, as with time the concentration of the sCOD in the water is insufficient to sustain cell activity with an influent concentration of $51 \pm 2 \text{ mg} \cdot \text{L}^{-1}$. The change of TSS and VSS is increasingly related to a change in the biofilm thickens on the carriers (Appendix B, Fig.B2). The least change in TSS and VSS was observed in BAR1-A, with a TSS and VSS change of 30% and 20% increase respectively from the start to the end of the testing period. The most significant increase was observed in BAR1-C, which showed a TSS and VSS increase of 124% and 93% respectively (Figure 4.5). The increase in BAR1-B in terms of TSS and VSS is 73% and 89% respectively. Similarly, the carriers previously operated in SB-MBBR-C with the longest anaerobic stage, experienced the most significant loss in the biofilm thickness ($71\% \pm 4\%$) (Figure 4.5). While carriers from SB-MBBR-A and -B in BAR1-A, -B experienced a loss of ($29\% \pm 1\%$), ($41\% \pm 2\%$) respectively.

The results suggested that the carrier's exposure to previous operational conditions, affected the carriers biofilm morphology and in turn, the change in thickness and subsequent behaviour expressed during the 33 days (810 hours) acclimation period. The carries exposed to a longer aerobic cycle (carriers from SB-MBBR-A) had a distinctly thinner but rough biofilm (Appendix B, Fig.B1). In contrast, carriers exposed to the longest anaerobic stage (carriers from SB-MBBR-C) were almost clogged, indicative of the carriers being exposed to less shear stress from aeration. The change in biofilm thickness in BAR1-C allowed room for the growth and recovery of the NOBs in the biofilm. While the carriers from BAR1-A persisted in producing nitrite, this indicated

that the NOB community did not recover. BAR1-B showed a slightly better recovery and had similar intake trends to BAR1-C of ammonia and nitrite. At a significance $P < 0.05$ the ammonia concentration change in the three BARs is significant although the trend has a high correlation. The highest ammonia concentration was reached in BAR1-A with the lowest TSS production over the same period. On the other hand, the lowest production of ammonia was in BAR1-C with the highest change in thickness. The difference in the ammonia concentration change could be explained as a product of the TSS in the 280 ml BARs. BAR1-A was able to ammonify to a higher degree due to less TSS in the water. Comparing this pattern with BAR1-B with a moderate biofilm thickness loss we observe a higher production of ammonia over the first 12 days (288 hours). In terms of nitrate production, there was no evident difference between the nitrate production between BAR1-C and -B indicating that both recovered the NOB community activity. The nitrite concentration changes over the 33 days (810 hours) for the three BARs show no significant difference between the time series at $P < 0.05$. Further suggesting that the activity of the NOB community in the BAR is the cause of the observed differences. Testing the kinetics after the 33 days of operation, supported the findings presented. It was evident due to observing no concentration changes of either nitrate and nitrite in the respective BARs.

The BARs after acclimation were capable of ammonia removed of up to 93%. The time needed to reach this point was deemed unfeasible. Extending the aeration stage for nitrification lead us to operate the BARs for 33 days (810 hours), while the carriers underwent a change in the biofilm thickness and capabilities. The idea of a single SB-MBBR reactor capable of treating wastewater characterized by an influent concentration of $3000 \pm 160 \text{ mg} \cdot \text{L}^{-1}$ of COD and ammonia concentration of $100 \pm 7 \text{ mg} \cdot \text{L}^{-1}$ is not feasible on an industrial scale.

4.6.3 Strategy 2: Addition of a Nitrification Reactor in Series

Nitrification strategy two consisted of separating the carbon and ammonia removal steps into two reactors in series. The post-carbon removal of the carriers tested over a 30-hour period that resulted in an ammonia removal efficiency of 84%, 83%, and 81% in BAR2s -rep1, -rep2, and -rep3 respectively (Figure 4.6). The BARs presented are triplicates of the same conditions. The BARs surface area loading rate (SALR) of ammonia was an average of $1.12 \pm 0.12 \text{ gN}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$; this corresponded to a SARR of $0.93\pm0.08 \text{ gN}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. The conversion of ammonia to nitrate in the BARs was complete with less than $0.1 \text{ mg}\cdot\text{L}^{-1} \text{ NO}_2^- \text{-N}$ and less than 10% mass balance error.

Figure 4.6: Strategy2: The 30-hour kinetic testing of BAR2-rep1, -rep2, and -rep3. The figure shows the concentration change of ammonia, nitrate and nitrite over the 30-hour testing period.

The effluent from SB-MBBR A fed to the three BARs had an initial ammonia concentration of $96 \pm 1.2 \text{ mg} \cdot \text{L}^{-1} \text{NH}_4^+ \text{-N}$ and a COD concentration of $57 \pm 1 \text{ mg} \cdot \text{L}^{-1}$. At the end of the 30 hours run the concentration in the effluent from the BARs was $16.8 \pm 1.1 \text{ mg} \cdot \text{L}^{-1} \text{NH}_4^+ \text{-N}$ with nitrate as the dominant effluent species with a concentration of $77.5 \pm 6 \text{ mg} \cdot \text{L}^{-1} \text{NO}_3^- \text{-N}$ (Figure 4.6). The conditions were kept constant over the testing period, alkalinity was initially adjusted from $770 \text{ mg} \cdot \text{L}^{-1}$ to $1485 \text{ mg} \cdot \text{L}^{-1}$, while the pH was adjusted to prevent stripping.

The difference between the three BARs was in the nitrite concentration intake trend. Statistical analysis showed a low correlation of $R=0.4$ between the data from BAR2-rep1, -rep2 and -rep3. The difference could indicate that the NOB community on the carriers are behaving differently although they have been exposed to the same conditions. In contrast to the carriers in strategy 1 and the kinetic testing following the end of the 33 days (810 hours) acclimatization period, the carriers in strategy 2 did not show signs of nitrite accumulation. Indicating that the cause of the low correlation is due to another factor other than the conditions the carriers are exposed to before testing. The lag in uptake may indicate that the high concentration of ammonia in the influent to the BARs may affect the function of NOBs. Inhibition of NOBs can be due to FA, a concentration between $0.1\text{-}1 \text{ mg} \cdot \text{L}^{-1}$ FA can cause the inhibition. Whereas the AOB are less sensitive and are inhibited in the range of $10\text{-}150 \text{ mg} \cdot \text{L}^{-1}$ FA (Di Trapani et al., 2015). The pH in the BARs started at an initial value of 8.5 and was adjusted to 7.5, the FA in the BARs could have been an inhibitor. Nitrification using the carriers seeded from a partially nitrifying IFAS system (Hawkesbury, Ontario, Canada), showed no nitrite accumulation. Contrary to the steady state behaviour of the carriers seeded from SB-MBBR-A, -B and -C, the carriers in the BAR2s-rep1, -rep2, and -rep3 in strategy 2 displayed complete nitrification with nitrate as the final product. Results of the BARs in strategy 2 infer that the nitrite accumulation observed in the kinetics test operation was a result

of the carrier's exposure to AOA conditions in the SB-MBBRs as well as the change in the biofilm thickness. The values obtained were consistent with the kinetic test of strategy 1 indicating that the SARR was affected by the high ammonia concentration in the influent.

4.7 Conclusion

Cheese wastewater was treated using an SB-MBBR operating an AOA cycle. The SB-MBBRs operated different stage times to remove carbon, phosphorus and ammonia in the same reactor. The operation of the SB-MBBRs showed carbon and phosphorus removals but lacked any ammonia removal. The objective of this research was to investigate the nitrification in high loaded wastewater via two operational strategies. The first operational strategy of extending the aeration stage resulted in a testing period lasting 33 days (810 hours). The length of the strategy ruled out the feasibility of removing carbon, ammonia, and phosphorus in the same reactor. The second operational strategy of separating the ammonia removal from the carbon removal had a faster start-up period and was deemed feasible.

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Chapter 5: Characterization of the Second Aerated SB-MBBR

5.1 Setting the Context

The operation of a single SB-MBBR to remove both carbon and nutrients in the same reactor using wastewater from a cheese making process was found to be infeasible. The length of time required to drop the concentration to below $50 \text{ mg}\cdot\text{L}^{-1}$ in the same reactor eliminated the first operational strategy. Chapter 4 results in regard to strategy 2 phase 2 showed that by separating the carbon removal from the ammonia removal stages into separate reactors, the startup was sped. Phase 3 of strategy 2 incorporated the results of BAR2-rep1, -rep2, and -rep3 (Appendix A, Table A1), into the design of a nitrification reactor to be placed in series to SB-MBBR-A presented in Chapter 4. The carries from the BAR2 testing were then taken to seed SB-MBBR-nit. Both SB-MBBR-A and SB-MBBR-nit were then tested over a 16-hour cycle to characterize the two reactors in series. The following chapter discusses the results obtained.

5.2 SB-MBBR-A

The SB-MBBR-A operation consisted of an anaerobic, aerobic, and anoxic (AOA) cycle sequence with cycle times of 1.2 hours anaerobic, 6.37 hours aerobic, and 0.23 hours anaerobic. The influent wastewater consisted of a C: N ratio of 10.6, with an organic carbon load of $35.4 \text{ g COD}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, the wastewater was treated over an 8-hour cycle in SB-MBBR-nit. In the first 1.2 hours of operation under anaerobic conditions, the COD experienced a decrease of 23% from an influent concentration of $2600\pm 16 \text{ mg}\cdot\text{L}^{-1}$ (Figure 5.1), while the TP concentration, on the other hand, remains unchanged. An AOA stage cycle was thought to promote an enhanced biological phosphorus removal and the selection of PAOs. However, the lack of release of any phosphorus in the anaerobic region indicated no presence of PAOs in SB-MBBR-A. In the following aerobic

stage, the primary constituent consumed is COD. The concentration of COD decreases by 95% in the aerobic stage of the cycle (Figure 5.1). Carbon was the prevalent constituent removed; for SB-MBBR-A the organic carbon surface area removal rate (SARR) of the K5 carriers was 34.6 ± 1.7 g COD·m⁻²·d⁻¹, equivalent to 97.7% removal of influent carbon concentration (Table 5.1).

Figure 5.1: COD, TP, TSS and VSS concentration across the 8-hour cycle in SB-MBBR-A.

The TP concentration during the aerobic stage decreased by 69%; this represents a decrease of $22 \pm 1 \text{ mg} \cdot \text{L}^{-1} \text{ PO}_4^{3-}\text{-P}$. The decrease seen over the aerobic stage was inconsistent with assimilation, as the rate of assimilation for heterotrophic bacteria is $1 \text{ mg} \cdot \text{L}^{-1}$ per $100 \text{ mg} \cdot \text{L}^{-1}$ of BOD removed (WEF, 2011). For SB-MBBR A the assimilation is $1.5 \text{ mg} \cdot \text{L}^{-1}$ per $100 \text{ mg} \cdot \text{L}^{-1}$ of BOD removed which represents a slightly higher value.

Looking at the results in terms of the TSS and VSS in SB-MBBR-A, we observe a TSS increase from $277 \pm 41 \text{ mg} \cdot \text{L}^{-1}$ to $773 \pm 47 \text{ mg} \cdot \text{L}^{-1}$. Correspondingly the VSS increased from a concentration of $240 \pm 43 \text{ mg} \cdot \text{L}^{-1}$ to a final concentration of $670 \pm 41 \text{ mg} \cdot \text{L}^{-1}$ over the 8-hour cycle (Figure 5.1). Calculating the ratio between P sequestered to VSS produced in SB-MBBR-A gives us a ratio of $0.05 \text{ mg P per mg VSS}$. The P incorporated in a strictly aerobic sludge can reach a theoretical value of $0.02 \text{ mg P per mg VSS}$, while in an enhanced biological phosphorus removal systems (EBPR) mixed liquor can contain between 0.06 to $0.15 \text{ mg P per mg VSS}$ (WEF, 2011). The results show the ratio obtained in terms of P consumed per VSS produced is lower than in an EBPR system, on the other hand, assimilation alone does not account for the decrease in concentration observed although it is the most likely pathway of removal.

Figure 5.2: The concentration of TN and ammonia, alkalinity and pH/DO in SB-MBBR-A over the 8-hour cycle.

Ammonia concentration in SB-MBBR-A increases over the anaerobic stage, followed by a slight decrease in the aerobic stage resulting in a 3% removal. While total nitrogen (TN) decreases by 59% over the 8-hour cycle (Figure 5.2). In the anaerobic stage of the reactor, the TN concentration increases from 245 to 270 $\text{mg}\cdot\text{L}^{-1}$ corresponding to a 9% increase, the ammonia follows the same increasing trend with a 30% increase. The TN concentration increase can likely be attributed to ammonification during the anaerobic stage. Biofilm on the carrier experiences death and regeneration as part of biofilm maintenance. Exposure to low oxygen conditions for a more extended period causes a death event which in turn releases ammonia through microbial break down (Katipoglu-Yazan et al., 2012).

Following the anaerobic stage, during the 6.37 hours of the aerobic stage, the TN experiences a decrease in concentration. Since no ammonia was removed and no significant production of nitrate or nitrite was observed in the reactor, the decrease could have only been due to organic nitrogen uptake by cellular activity. The TN concentration displayed a percent loss of 56%, decreasing by 137 $\text{mg}\cdot\text{L}^{-1}$ TN-N (Figure 5.2). Considering the uptake by heterotrophic bacteria is in the ratio of 100:5:1 the displayed decreases TN can be related to the heterotrophic activity. Using the ratio, we can estimate the required nitrogen of 127 $\text{mg}\cdot\text{L}^{-1}$, considering a 10% error, the data show that the resultant decrease measured in TN can be attributed to heterotrophic growth. The increase in VSS during the cycle also further points to cell growth within the reactor further indicating that the decrease is due to cell uptake. Alkalinity in the reactor increases with the highest concentration at observed at 5.58 hours during the aerobic stage. The increase in alkalinity observed is possibly due to the breakdown of carbohydrates and fatty acids in the water. The increase indicated that there was a lack of oxygen, the supply of oxygen was readily consumed as soon as it is supplied to the reactor. At 5.58 hours the DO concentration starts to increase to a maximum of 6.11 $\text{mg}\cdot\text{L}^{-1}$

¹, accompanying the increase in DO is a similar decrease in alkalinity indicating that the change in alkalinity is related to the breakdown of organics under an oxygen-limited aerobic stage (Figure 5.2). In the anoxic stage following the aerobic stage, a small decrease in COD and TN (5% and 7% respectively) is observed but no change to the concentration of TP.

5.3 SB-MBBR-nit

The effluent of SB-MBBR-A into SB-MBBR-nit is characterized as carbon deficient with influent C: N ratio of 0.56. The COD concentration at the beginning of the cycle was $57 \pm 1.25 \text{ mg} \cdot \text{L}^{-1}$ (Table 5.1). SB-MBBR-nit operated an aerobic react stage and had a fill ratio of 33% of K5 carriers. The SALR of ammonia to the reactor was $0.95 \text{ gN} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$. COD concentration during the second 8-hour cycle increased by $56 \text{ mg} \cdot \text{L}^{-1}$. Meanwhile, the concentration of TP increased. The COD concentration increase was attributed to the lysis of the suspended growth bacterial cells carried in the influent from SB-MBBR-A to SB-MBBR-nit. Due to the limited supply of organic carbon in SB-MBBR-nit, the cells experience a death event. The TSS concentration in the influent to SB-MBBR-nit is $643 \text{ mg} \cdot \text{L}^{-1}$ with a VSS concentration of $583 \text{ mg} \cdot \text{L}^{-1}$, over the cycle duration both TSS and VSS experience a reduction of 17% and 13%, respectively. The TP concentration over the cycle doubled increasing to a concentration of $21.8 \text{ PO}_4^{3-}\text{-P} \text{ mg} \cdot \text{L}^{-1}$ (Figure 5.3). In SB-MBBR-A the assimilation of phosphorus was postulated to be the primary pathway, while an increased uptake pointed to the possibility of the growth and presence of suspended growth PAOs in the system. The P release yield during the SB-MBBR-nit operation cycle was 0.13 mg P per mg VSS reduced. A release of $1.73 \text{ PO}_4^{3-}\text{-P} \text{ mg} \cdot \text{L}^{-1}$ is expected relative to the decrease in VSS over the testing cycle if the microbial community in the reactor was heterotrophic without the presence of PAOs. Contrary to the theoretical release of P calculated, the observed release in SB-MBBR-

nit of $11 \text{ PO}_4^{3-}\text{-P mg}\cdot\text{L}^{-1}$ was measured. Hence, the concentration profile in the SB-MBBR-nit reactor is indicative of a suspended growth PAO population in the SB-MBBR-A reactor.

Figure 5.3: The concentration of COD, TP, TSS and VSS over the second 8-hour cycle in SB-MBBR-nit.

The TN in SB-MBBR-nit contrary to SB-MBBR-A remained constant over the 8-hour cycle, while the ammonia concentration decreased to $0.23 \pm 0.02 \text{ mg} \cdot \text{L}^{-1} \text{ NH}_4^+ \text{-N}$ (Figure 5.4). The decrease in ammonia was accompanied by an increase in nitrite rather than nitrate as the constituent with the highest concentration at the end of the cycle. The nitrate concentration over the 8-hour cycle did not exceed $6.30 \pm 0.29 \text{ mg} \cdot \text{L}^{-1} \text{ NO}_3^- \text{-N}$ (Table 5.1), while the concentration of nitrite is measured at a maximum of $96.80 \text{ mg} \cdot \text{L}^{-1} \text{ NO}_2^- \text{-N}$ by hour 16. The build-up of nitrite in the reactor was an indication that the nitrification step of nitrification was inhibited. Nitritation is the first step of nitrification and mediated by the action of ammonia-oxidizing bacteria (AOB). Nitritation is mediated by the action of *Nitrosomonas*, while nitrification, the second step of nitrification, is done by the action of *Nitrospira* (Henze, 1997). The cell mass yield of nitrifiers from both steps is less than heterotrophic bacteria yield; they range from 0.06-0.20 g VSS per g $\text{NH}_4^+ \text{-N}$ oxidized. Further, the two steps of nitrification are known to be inhibited by their substrates (Abeling and Seyfried, 1992; Balmelle et al., 1992; Surmacz-Gorska et al., 1996). As such, it can be stipulated that the nitrite build up can be due to the high concentration of FA in the SB-MBBR-nit in the pH range recorded (Anthonisen et al. 1976). The pH in the reactor affects the FA concentration, during the 8-hour cycle, the pH remains between 8-8.5 without any pH adjustment (Figure 5.4). The equilibrium between ammonia (FA) and ammonium shifts increasing the concentration of FA at pH concentrations above 8 (Shannon, 2014). A concentration between 0.1- 1 $\text{mg} \cdot \text{L}^{-1}$ FA can cause the inhibition to NOBs. AOBs are less sensitive and are inhibited in the range of 10-150 $\text{mg} \cdot \text{L}^{-1}$ FA (Di Trapani et al., 2015). As the ammonia concentration decreases we observe a slight increase of nitrate, the increase can be attributed to the removal of the inhibitory effects of FA as the final concentration of ammonia is $0.23 \text{ mg} \cdot \text{L}^{-1}$. The alkalinity in the influent wastewater over the 8-hour cycle decreases by 76% (Figure 5.4). The decrease in alkalinity is consistent with nitrification

consumption rate of 7.14 mg Alkalinity per mg NH_4^+ -N (Brown et al., 2005). The DO concentration throughout the 8-hour cycle in SB-MBBR-nit remained between 7.5-8 mg L^{-1} . As such with both DO and alkalinity requirements met the factor most likely affecting the concentration of nitrate is FA.

Figure 5.4: The concentration of TN and ammonia, alkalinity and pH/DO in SB-MBBR-nit over the 8-hour cycle.

5.4 Overall System Performance

The two SB-MBBR in series achieved removal of carbon in SB-MBBR-A (98%) and ammonia removal in SB-MBBR-nit (100 %). The heterotrophic community in SB-MBBR-A is thought to have dominated the biofilm on the carriers in this reactor preventing any significant removal of ammonia from the wastewater. While in SB-MBBR-nit with the influent wastewater was devoid of elevated carbon concentrations, hence restricting the overgrowth of the heterotrophic community and enabling the activity of the nitrifying population. Ammonia was removed solely in SB-MBBR-nit, indicating that the biofilm on the carriers was relieved of heterotrophic competition. Overall, the carriers over the 8-hour cycle in SB-MBBR-nit displayed a SARR of $0.95 \text{ gN} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ resulting in an ammonia removal rate of 100% (Table 5.1). Separating the organic carbon and ammonia removal was also performed by (Pastorelli et al.,1997), the system displayed similar removals to the presented system while treating primarily settled wastewater. Overall, SB-MBBR-A achieved removal of both carbon and phosphorus, but the major drawback encountered in SB-MBBR-nit is the release of half of the assimilated quantity of phosphorus. The release of phosphorus in SB-MBBR-nit indicated that the system was carrying a high percentage of TSS and VSS from SB-MBBR-A to SB-MBBR-nit and in particular a presence of PAOs population in SB-MBBR-A in suspended form rather than attached.

Table 5.1: Constituent effluent and influent concentrations over the 16-hour cycle in both reactors SB-MBBR-A and SB-MBBR-nit.

Species	SB-MBBR-A			SB-MBBR-nit		
	Influent Concentration $\text{mg}\cdot\text{L}^{-1}$	SD	Effluent Concentration $\text{mg}\cdot\text{L}^{-1}$	SD	Effluent Concentration $\text{mg}\cdot\text{L}^{-1}$	SD
TN	244.77	2.39	100.90	1.49	105.00	2.02
NH₄⁺-N	98.33	2.36	95.50	3.08	0.23	0.02
NO₃⁻-N	0.34	0.02	0.25	0.01	6.30	0.29
NO₂⁻-N	0.00	0.00	0.01	0.00	96.80	0.02
PO₄³⁺	32.32	2.16	10.16	1.70	21.18	1.64
COD	2600.00	16.33	57.00	1.25	113.33	2.05
TSS	276.67	41.10	773.33	46.96	643.33	47.84
VSS	240.00	43.20	670.00	41.43	583.33	28.67

5.5 References

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Chapter 6: Conclusion

Treating cheese wastewater effluent in an SB-MBBR with an AOA stage sequence tested with different times resulted in the removal of both carbon and phosphorus. However, the concentration of ammonia showed no decrease in the system. Two nitrification strategies were tested for nitrification in high carbon concentration wastewater. The first nitrification strategy was extending the aeration stage of the AOA stage sequence. The second nitrification strategy separated the carbon removal from the ammonia removal. The main conclusions drawn from this work are the following:

- 1- Extending the aeration stage required 33 days (810 hours) of operation. At the end of the 33 days (810 hours), the ammonia concentration drops to below $50 \text{ mg} \cdot \text{L}^{-1}$. Strategy 1 was found unfeasible due to the length of time (33days) it required to achieve nitrification in the BAR tests.
- 2- For nitrification to be achieved via strategy 1, the carriers had to undergo an acclimation period. During that period the thickness of the biofilm on the carriers decreased. The decrease was correlated to the length of the anaerobic stage the carriers were exposed to before testing. Carriers exposed to a more extended anaerobic stage displayed a more substantial loss in biofilm thickness.
- 3- The behaviour of the biofilm on the carriers after the acclimation period was further correlated to the thickness change. Carriers that experienced a higher reduction in thickness did not show any nitrite accumulation compared to carriers with a lower reduction.
- 4- Strategy 2 separated the carbon and ammonia removal resulting in a faster start-up period. Nitrification in the BAR took place over a 30-hour experimental run. The removals

obtained from the BAR test resulted in an 81-83% $\text{NH}_4^+\text{-N}$ removal efficiency, with a SARR of $0.83\text{-}1.31 \pm 0.08 \text{ gN}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$.

The results of the first part of the experiments prompted testing of strategy 2 with a second nitrification SB-MBBR (SB-MBBR-A) in series of SB-MBBR-A (SB-MBBR-nit). As such the two reactors in series were characterized over a 16-hour period. The main conclusions drawn from this work are the following:

- 1- SB-MBBR-A organic carbon SARR of the K5 carriers was $34.6 \pm 1.7 \text{ gCOD}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, equivalent to 97.7% removal of influent carbon concentration.
- 2- TP in SB-MBBR-A was thought to be assimilated into the cells due to biological activity initially. A yield of 0.05 mg P/mg VSS indicated that another pathway of removal is present. The release of TP in SB-MBBR-nit indicated the presence of PAOs in suspended form in SB-MBBR-A.
- 3- In SB-MBBR-A TN concentration decreased due to the decrease in organic nitrogen as no ammonia, nitrate, or nitrite reduction or production was observed. The organic nitrogen is consumed due to biological activity in correlation to the decrease in organic carbon.
- 4- In SB-MBBR-nit TN concentration remains constant, while the ammonia concentration decreases indicating nitrification is the primary driver of the decrease.
- 5- Nitrite accumulation in SB-MBBR-nit is attributed to the FA concentration in the reactor, when the concentration of FA decreases towards the end of the experimental run nitrate concentration increases slightly. The influent ammonia concentration to SB-MBBR-nit was $98 \text{ mg}\cdot\text{L}^{-1}$.
- 6- The nitrification reactor (SB-MBBR-nit) resulted in an ammonia removal of 100%, with a SARR of $0.95 \text{ gN}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. The separation of the carbon removal from the ammonia

removal afforded the nitrifiers less competition in the biofilm on the carriers in SB-MBBR-nit.

6.1 Future Research

The SB-MBBR technology with and AOA stage sequence was not previously investigated for the treatment of cheese wastewater characterized by high carbon concentration. The initial SB-MBBR setup did not remove ammonia from the wastewater inciting further investigation into strategies to achieve nitrification in the setup. The results showed that for cheese processing wastewater characterized as medium strength wastewater achieving the removal of organic carbon, ammonia and phosphorus in one reactor is not feasible. Using two reactors in series removed both organic carbon and ammonia of up to 99%. But the concentration of TSS and phosphorus in the effluent indicated that further investigation into a setup that would reduce both TSS and phosphorus from the effluent of the two SB-MBBR in series is required. Both of those constituents prevent the discharge of this wastewater in some locals. For example, in Ottawa the phosphorus concentration should not exceed $10 \text{ mg}\cdot\text{L}^{-1}$ preventing the discharge of the effluent from the setup investigated in to the sewers. Furthermore the concentration of COD in the effluent from SB-MBBR-nit ($100 \text{ mg}\cdot\text{L}^{-1}$) prevents discharge in rivers according to the federal regulations. As such the technology would require more investigation for its applicability to rural facilities lacking access to the sewer.

APPENDIX A

The value obtained from the BAR2 -rep1, -rep2, -rep3 experiments were used to obtain the percent fill required to obtain 99% removal in SB-MBBR-nit. The values obtained were plotted to obtain max, min and avg slopes. The average SARR value is $1.07 \text{ g NH}_4^+\text{-N}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. Using the SARR value obtained the percent fill used for the reactor was 33% Equation A1.

$$SARR = Q \left(\frac{\text{concentration influent } \text{NH}_4 \frac{\text{mg}}{\text{L}} - \text{concentration effluent } \text{NH}_4 \frac{\text{mg}}{\text{L}}}{V * \% \text{Fill} * SA (k5)} \right) \quad \text{Equation A1}$$

Table A1: The summary of the runs conducted for the strategy 2. The table includes the ammonia slopes, the DO, C: N ratio of the influent water and the PH. The table also contains the SARR data calculated for each of the runs.

		Slope (Ammonia)						
Time (h)	BAR2	Avg.	Min	Max	Average DO	sCOD: NH_4^+	Average pH	SARR ($\text{g/m}^2 \cdot \text{d}$)
21	-rep1	-4.74	-4.34	-5.40	8.82	0.69	7.74	1.28
21	-rep2	-4.08	-2.86	-4.71	8.81	0.69	7.71	1.31
21	-rep3	-3.59	-2.08	-4.85	8.84	0.69	7.66	1.34
29	-rep1	-3.84	-3.19	-4.93	8.87	0.42	7.96	0.96
29	-rep2	-4.62	-3.77	-5.12	9.24	0.42	8.41	1.05
29	-rep3	-4.13	-2.96	-4.72	8.89	0.42	8.57	0.93
30	-rep1	-2.65	-1.58	-3.74	8.32	0.59	7.81	0.83
30	-rep2	-2.85	-0.95	-3.79	7.95	0.59	7.55	0.92
30	-rep3	-2.74	-2.90	-3.06	8.15	0.59	7.92	1.03
Average SARR								1.07

APPENDIX B

Figure B1: The figure shows the acclimated MBBRs from SB-MBBRs A, B and C placed in SB-MBBR-A, -B, -C respectively. The figure shows the ammonia, nitrate and nitrite concentration over the testing period.

SB-MBBR A

a

SB-MBBR B

b

SB-MBBR C

c

d

e

f

Figure B2: The spectroscopy images show the carriers taken from SB-MBBRs-A, -B, and-C, before (a, b and c) and after (d, e, and f) the thirty-three-day (810 hours) acclimation period.

APPENDIX C

The pattern of uptake between 288 hours to 810 hours was fitted by a linear equation; the slopes were then averaged (Figure C1). The average of the three linear fitted lines is -0.65 ± 0.03 .

Figure C1: Ammonia Uptake between the hours 288-810 (12-33 days), the decrease in ammonia concentration is fitted with a linear equation in BAR1-A, -B, -C.