Impacts of Microbial Inoculator Generators (MIGs) on Nitrogenous Compounds in Septic Systems

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Abstract

The purpose of this paper is to investigate the fate, transportation, and transformation of nitrogen compounds through a microbial inoculator generator (MIG), which is a modification of an integrated fixed film activated sludge (IFAS) system. The research goal was to determine whether or not a MIG system in a commercial setting is capable of meeting the Wisconsin Department of Natural Resources (WDNR) groundwater discharge total nitrogen (TN) permit level. The current permit level is less than 10 mg/L. The research methodology first entailed a review of relevant literature. The results include comparisons between activated sludge (AS), fixed film (FF), IFAS, and MIG systems. An IFAS system is similar to a MIG, except an IFAS does not require a bacteria stock to be added to the system to reach operational objectives. The research includes information in regard to nitrogen removal involving the ammonification, nitrification, and denitrification process. The research was used to create operation parameters for a MIG pilot system at the Oconomowoc Wastewater Treatment Facility (OWWTF). The parameters involved maintaining flow rate and dissolved oxygen (DO) concentrations within the pilot system. To evaluate the performance of the MIG system, influent and effluent wastewater data samples were obtained during two collection periods. Data were analyzed using Composite Sampling techniques and Monte Carlo simulation. In this project, it was found that the system requires a lower flow rate and extreme zones for DO concentration to reach a lower total nitrogen (TN) concentration in the effluent. The current parameters feature TN concentrations between 25.4 and 26.7 mg/L TN.

Keywords: nitrogen removal, microbial inoculator generator (MIG), integrated fixed film, activated sludge, nitrification process, nutrient removal, denitrification, White Knight™, wastewater treatment, septic systems, domestic wastewater, statistical analysis, composite sampling, Monte Carlo simulation, Wisconsin Department of Natural Resources (WDNR)

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Nomenclature

Symbols

$\hat{p}$	Proportion
$s_{\bar{y}}$	Standard Error of the Mean
$\bar{y}$	Overall Composited Sample Mean
μ	Population Mean
μ_{AOB}	Specific growth rate of ammonium oxidizing bacteria
$\mu_{max,AOB,DO}$	Maximum specific growth rate of ammonium oxidizing bacteria correct for DO concentration
A_{MIG}	Surface area of the MIG
b	Skewness
b_{AOB}	Specific endogenous decay rate of ammonium oxidizing bacteria
C_e	Effluent concentration
C_i	Influent concentration
F_I	Inlet Flow
F_{IR}	Internal Recycle Flow
F_{IR}/F_I	Internal Recycle
H_1	Alternative hypothesis
H_o	Null hypothesis
k	Composite Population Size
K_{NH}	Half-velocity coefficient for ammonia
$K_{o,AOB}$	Half-velocity coefficient for DO for AOB
m	Individual Population Size

n	Number of Composited Samples
Q_i	Flow rate
s	Sample Standard Deviation
s^2	Sample Variance
S_{NH}	Ammonia concentration
S_o	DO concentration
SRT	Solids retention time
t	T-Statistic
V.L.	Violation Limit
α	Significance Level
α_1	Shape
β	Scale
θ	Threshold
σ	Population Standard Deviation
υ	Degrees of freedom

Abbreviations

Anammox	Anaerobic Ammonium Oxidizing
AOB	Ammonium Oxidizing Bacteria
AS	Activated Sludge
BOD	Biochemical Oxygen Demand
BOD_5	Five-Day Biochemical Oxygen Demand
CBOD	Carbonaceous Biochemical Oxygen Demand

CMAS	Complete Mix Activated Sludge
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
F/M_r	Food to Microorganism Ratio
FF	Fixed Film
GPD	Gallons per Day
GPM	Gallons per Minute
H_2O	Water
IFAS	Integrated Fixed Film Activated Sludge
MBBR	Moving Bed Biofilm Reactor
MIG	Microbial Inoculator Generator
MLVSS	Mixed Liquor Volatile Suspended Solids
N_2	Nitrogen Gas
N_2O	Nitrous Oxide
NH_3/NH_4^+	Ammonia/ammonium ion. The symbols are used interchangeably for ammonia in this document. The predominant form present is determined by the pH of the solution.
NO_2^-	Nitrite
NO_3^-	Nitrate
NOB	Nitrite Oxidizing Bacteria
O_2	Elemental Oxygen/Oxygen Gas
ORP	Oxidation Reduction Potential

OWWTF	Oconomowoc Wastewater Treatment Facility
Permit	Wisconsin Department of Natural Resources Groundwater Discharge Permit
PFD	Process Flow Diagram
RAS	Return Activated Sludge
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TSS	Total Suspended Solids
U.S. EPA	United States Environmental Protection Agency
Unit	Pilot Unit
WDNR	Wisconsin Department of Natural Resources
WEF	Water Environmental Federation

Impacts of Microbial Inoculator Generators (MIGs) on Nitrogenous Compounds in Septic Systems

In Wisconsin, for many occupied buildings (including residences) that are not connected to sewer systems, septic systems are often deployed to store and to treat domestic wastewater. A basic septic system design typically consists of a large underground tank into which wastewater and sewage flows. In the tank, an anaerobic (i.e., oxygen-less) bacterial environment develops, which reduces the solid and organic material in the wastewater, resulting in sludge, which is periodically removed, as well as a liquid – referred to as an effluent – which is typically discharged into a nearby seepage or leach drain field. In septic system designs, the effluent is first treated in the underground storage tank before it is released into the drain field, and subsequently, into the environment. In the drain field, additional impurities are trapped and eliminated in the soil, and the treated liquid eventually enters the environment – typically, into groundwater systems and surface water systems (such as lakes and rivers).

The liquid effluent from a septic system contains both nitrogen and phosphorus. One reason for this state of affairs is that wastewater entering a septic system features high levels of nitrogen and phosphorus, because human urine contains both chemical elements, but septic systems are unable to remove both elements. This scenario presents an environmental challenge. Although nitrogen and phosphorus in the proper amounts can serve as nutrients for plants – and thus are found in fertilizers – too much nitrogen and phosphorus leads to deleterious and even toxic environmental problems, such as excessive growth of algae in water bodies. For this reason, septic systems are regulated by the Wisconsin Department of Natural Resources (WDNR). The WDNR limits the amount of nitrogen and phosphorus that septic systems can release into the environment.

This project report features the results of an investigation into the effectiveness of a nitrogen-reducing method for septic systems. Nitrogen-reducing technology has been designed and deployed in septic systems to address the problem of high nitrogen levels in septic effluent. One new example of this technology is a Microbial Inoculator Generator (MIG), which employs a proprietary mixture of bacteria to reduce the amount of total nitrogen (TN) in septic system effluent (Nelson & Rawson, 2010; Wickham, 1996). The purpose of the project described in this report was to determine if MIG technology can reduce the total amount of nitrogen in the effluent so that it complies with WDNR requirements for safe discharge into the environment. A systematic investigation was conducted, in which the null hypothesis (H_0) was that MIG technology would not reduce the TN, and the alternative hypothesis (H_1) was that the MIG would be effective in reducing the TN so that a septic system can meet the WDNR's discharge regulations. Influent and effluent data samples from a MIG pilot unit at the Oconomowoc Wastewater Treatment Facility (OWWTF) were systematically collected each week from October to December 2017 and from March to April 2018 (i.e., the collection period) on TN, as well as several other parameters, such as flow rates, temperature, and alkalinity. Other parameters were included because they can affect the amount of nitrogen in the effluent. Composite sampling statistical technique was employed to evaluate the H_0 and H_1. The analysis revealed that the MIG, as utilized in this study, does not reduce TN in septic effluent to a level that meets WDNR standards.

In order to explain the project investigation, this report is organized in the following manner. An overview and a technically detailed discussion of common wastewater treatment methods for the removal of TN are first presented, with a focus on biological treatment systems, including MIG technology. Next, the methods employed in the project are described, including

site details, data collection, and data analysis. The results of the analysis are then presented, indicating that MIG technology is not a feasible nitrogen-reducing solution for septic systems.

Wastewater Treatment Methods for Total Nitrogen Removal: An Overview

The two primary nutrients regulated in wastewater treatment are nitrogen and phosphorus (Water Environmental Federation [WEF], 2009). Nitrogen can be present in the environment in four forms depending on the oxidation state: organic nitrogen, ammonia (NH_3), nitrite (NO_2^-), and nitrate (NO_3^-) (WEF, 2009). Nitrogen removal from wastewater treatment plants is measured in TN, which categorizes all forms of nitrogen into one group (WEF, 2009).

Nitrogen is typically removed from wastewater by a biological treatment process, which reduces the nutrients in water bodies. The biological nitrogen removal process is completed in three stages. The first stage is ammonification, during which the organic nitrogen is transformed to NH_3. The second stage is nitrification, where the NH_3 is oxidized to NO_2^-, and then to NO_3^- in an oxygen-rich, aqueous environment, or an aerobic process (Bitton, 2011; Weaver, n.d.). The final stage is denitrification, in which NO_3^- is converted to nitrogen gas (N_2) in an environment without oxygen, or an anoxic process (Bitton, 2011; Weaver, n.d.).

Throughout the three nitrogen-removal stages, microorganisms play a significant role. Before the nitrogen stages begin, the wastewater goes through aerobic biological decomposition, which involves the organic waste in the wastewater being consumed. The organic waste is consumed through three steps: oxidation, synthesize reaction, and endogenous respiration. First, some organic waste is oxidized to produce end products (carbon dioxide [CO_2], water [H_2O], NH_3, and other end products) and energy for cell maintenance and to synthesize new cell tissue. The rest of the organic waste uses the energy produced in oxidation to create new cell tissue; this is called the synthesis reaction. Once the organic matter is consumed, the endogenous

respiration step begins. The cells begin to consume their own cell tissue to obtain energy. The end products of this stage result in CO_2, H_2O, and NH_3 (Abu-Orf, Bowden, Burton, Pfrang, Stensel, Tchobanoglous, Tsuchihashi, Metcalf & Eddy, 2014). After this step the nitrogen removal stage begins. The microorganisms, specifically bacteria, that convert NH_3 to NO_2^- in the aerobic nitrification process are referred to as autotrophic nitrifying bacteria, utilizing NH_3 as an electron donor. On the other hand, the bacteria that convert NO_3^- to N_2 in the anoxic denitrification process are referred to as heterotrophic denitrifying bacteria, utilizing NO_3^- as an electron acceptor in the absence of oxygen (Fu, Yang, An, & Xue, 2009).

The common wastewater treatment system for TN removal is an activated sludge (AS) system (Boltz, Freudenberg, Gellner, Gunsch, Kim, & Schuler, 2010). An AS system treats the wastewater through three stages, as demonstrated in Figure 1. The inflow to the AS system typically passes through a primary clarifier, where sludge from the wastewater will settle to the bottom of the tank. The primary clarifier is not a part of the AS treatment system. The AS treatment begins once the primary clarifier effluent enters the aeration tank. The aeration tank reduces the biochemical oxygen demand (BOD) and total suspended solids (TSS) in the wastewater. This step is omitted if there are few settleable solids. The wastewater then goes on to the secondary clarifier to settle out any of the remaining solids and bacteria. A portion of the settled solids in the secondary clarifier typically are returned to the aeration tank. This process stream is referred to as the return activated sludge (RAS) because it recycles the microorganisms on the bottom of the secondary clarifier through the aeration tank. The portion of the settled solids not recycled are discharged to the solids treatment process as waste activated sludge (WAS) (Abu-Orf et al., 2014).

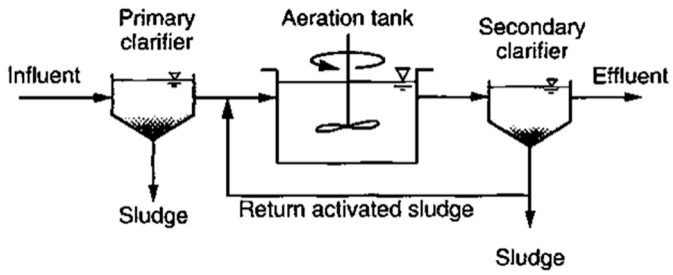

Figure 1. Typical activated sludge process for complete-mix. Adapted from *Wastewater Engineering Treatment and Resource Recovery* by M. Abu-Orf et al., 2014, Boston: McGraw-Hill, p. 702.

One alternative for the AS system is to replace the aeration tank with a bioreactor tank. The bioreactor tank takes the water through biological treatment zones to remove the nitrogen. The biological treatment zones consist of anoxic and aerobic processes; these zones can be seen in Figure 2.

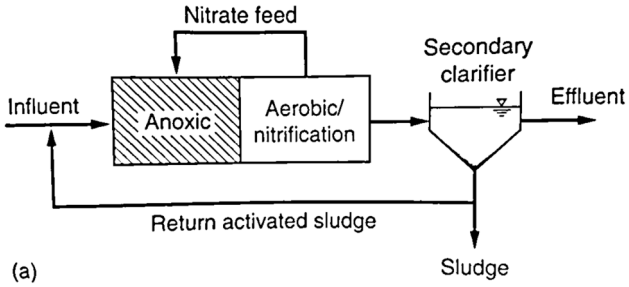

Figure 2. Typical activated sludge progression. Adapted from *Wastewater Engineering Treatment and Resource Recovery* by M. Abu-Orf et al., 2014, Boston: McGraw-Hill, p. 798.

The AS system can be prone to poor settling rates, causing the system to be inefficient, and reducing its ability to remove nitrogen. The poor settling rates can be offset by oversizing the system to ensure the regulations are being reached (Boltz et al., 2010). Alternatively, the integrated fixed film activated sludge (IFAS) system can be used. This system uses the same biological treatment processes as an AS, but the IFAS includes media in the final zone to increase the microbial growth within the system (Malovanyy, Trela, & Plaza, 2015). A schematic of an IFAS system is presented in Figure 3. Microbial growth increases because the media provides extra surface area for the microorganism to grow on. The increase in microbial growth increases the residual time for treatment, which enhances the biological treatment. This allows for the size of the system to be reduced (Qiao, Nishiyama, Fujii, Bhatti, & Furukawa, 2012; Sriwiriyarat, Randall, & Sen, 2005).

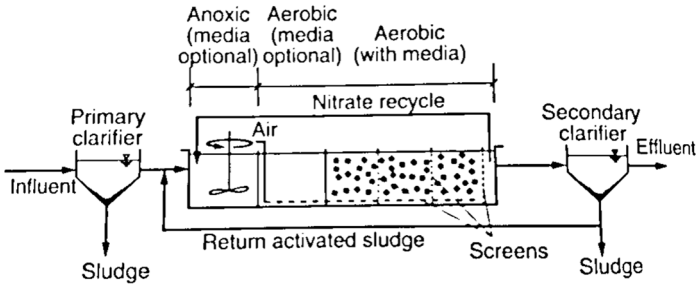

Figure 3. Schematic of an IFAS system. The IFAS has a suspended plastic biofilm carrier. Adapted from *Wastewater Engineering Treatment and Resource Recovery* by M. Abu-Orf et al., 2014, Boston: McGraw-Hill, p. 998.

The IFAS system has been modified into a new system designed for on-site wastewater treatment plants with septic tanks. The modified design is a MIG. The MIG is designed to

enhance the treatment process by transforming the septic tank from an anaerobic to an aerobic process by aerating the tank. The addition of air in the tank promotes aerobic bacteria. The bacteria grow as fixed film, or biofilm, on the media and suspended growth in the septic tank. The suspended growth discharges into a leach field. The MIG system is demonstrated in Figure 4 (Septic Preservation Services, Inc., n.d.). The access covers presented in Figure 4 have vents to the atmosphere. The design is similar to the IFAS system, but instead of having different zones within the system, all zones are blended in one area. One of the main differences a MIG includes is a “tea bag.” The "tea bag” is filled with microbial organisms to improve the wastewater treatment process by promoting nitrification and denitrification to take place (Knight Treatment Systems, 2014; Wickham, 1996). Inoculation of the tea bag occurs quarterly until the microbial community stabilizes; at this point, the inoculation of the tea bag can be reduced (Knight Treatment Systems, 2014). In order to treat the water to meet regulatory requirements, there may be multiple MIG units within one process tank based on the flow rate of the system.

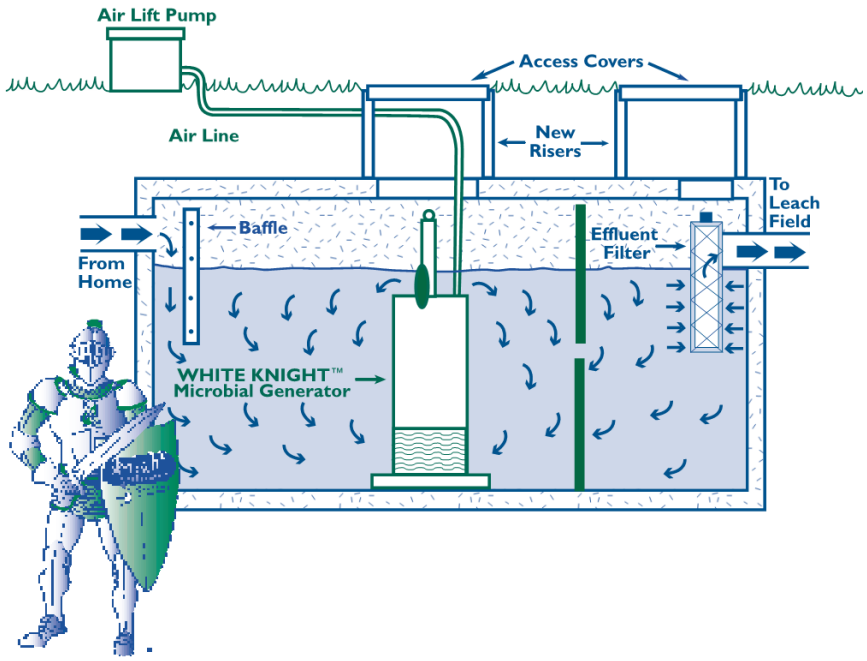

Figure 4. MIG placed into a traditional septic treatment system. Adapted from "White Knight™ Microbial Inoculator/Generator" [Owner's Manual] by Septic Preservation Services, Inc., n.d., p. 2. Retrieved from http://septicpreservation.com/wp-content/uploads/2015/03/White-Knight-Manual.pdf.

The MIG treatment system operates by placing a MIG within a process tank between the inlet of wastewater and the outlet for the treated water. The wastewater flows through the bottom of the MIG and exits through the top of the MIG, as seen by the flow arrows in Figure 4. The water is lifted through the MIG by the force of an external air pump, which runs constantly to provide the required oxygen for the bacteria to feed. The air flow in the MIG unit creates a circulation for the contents in the process tank to be treated (Septic Preservation Services, Inc., n.d.). The MIG has multiple vertical tubes within the unit where microorganisms can grow. The vertical tubes allow microorganisms to grow on them, which enhances the nitrogen removal process by creating a biofilm layer on the tubes. The air passing through the MIG creates an aerobic zone on the outer biofilm layer where aerobic nitrifying bacteria thrive, and an

anoxic/anaerobic zone on the inner biofilm where anoxic denitrifying bacteria thrive (Knight Treatment Systems, 2014). The aerobic and anaerobic zones -- featuring the highest activity zones of microbial growth -- can be seen in Figure 5. Figure 5 demonstrates the two types of microbial growth that can occur in the system, either a media bed (tube) or suspended growth.

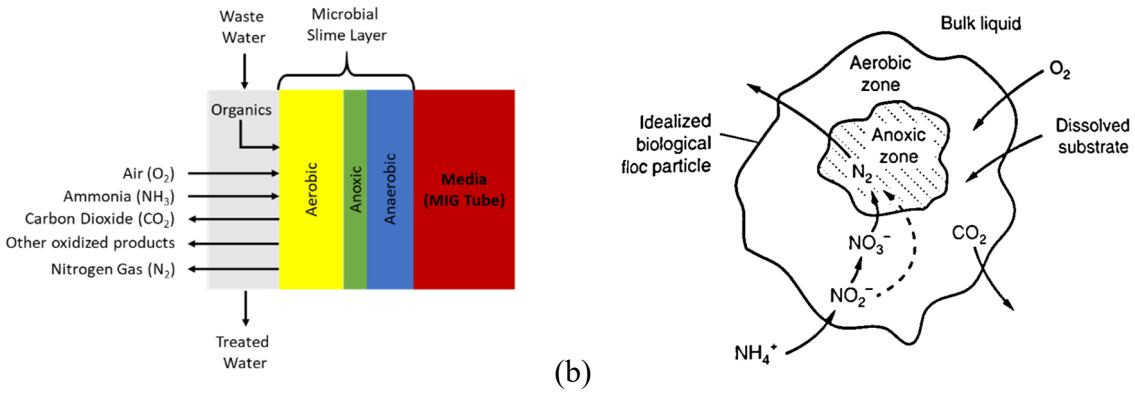

Figure 5. Layers of microbial growth on media and suspended solids. (a) Adapted by Jill Vande Boom (b) Adapted from *Wastewater Engineering Treatment and Resource Recovery* by M. Abu-Orf et al., 2014, Boston: McGraw-Hill, p. 799.

The water is then recirculated through the MIG, which is caused by the air flow, until the treated water and some bacteria leave the system and go to the leach fields.

Background

Wastewater Treatment Systems: Technical Details

Three common biological wastewater treatment methods include AS, fixed film (FF) and IFAS. The technical details of the three systems are described below, along with an integrated wastewater treatment method of all three designs that was analyzed for nitrogen removal.

Activated sludge. In history, the most common wastewater treatment process for removing suspended, colloidal, and dissolved solids from wastewater has been the AS system (Abu-Orf et al., 2014). A typical schematic of an AS system can be seen in Figure 2. According to Abu-Orf et al. (2014, p. 701), AS is defined as follows:

> By definition, the basic activated sludge treatment process consisted of the following three basic components: (1) a reactor in which the microorganisms responsible for treatment are kept in suspension and aerated; (2) liquid solids separation unit, usually in a sedimentation tank; and (3) a recycle system for returning solids removed from the liquid-solids separation unit back to the reactor.

AS usually follows a pretreatment process to remove large and settleable solids, often completed by primary sedimentation tanks. Primary sedimentation removes a substantial portion of settleable solids. The most common operational problem for AS relates to the sedimentation tank having poor solids settling rates (Abu-Orf et al., 2014; Boltz et al., 2010). This problem leads to increases in effluent solids concentration, decreasing the disinfection capabilities, and therefore leading to an increased risk in the health of the public and the environment (Boltz et al., 2010). The AS system's effectiveness is reduced when it is exposed to high hydraulic and organic loading rates. Another disadvantage is that expandability of the system is difficult. In order to meet higher loading demands, additional building structures and larger bioreactors are required (Azimi, Hooshyari, Mehrdadi, & Bidhendi, 2007).

Fixed film. One of the next treatment processes introduced into the wastewater treatment industry was FF. The process consisted of using synthetic media to promote growth in the reactors (Jenkins & Sanders, 2012). A FF system uses media made up of rocks, sponge, plastic or other synthetic material, the latter being used more often as the biofilm carrier (Abu-Orf et al.,

2014; Jenkins & Sanders, 2012). A limiting factor to the media are the biofilm layers. The biofilm layers require the substrate to diffuse into the biofilm in order to make the substrate available for use by the attached microorganisms (Jenkins & Sanders, 2012). The environmental and kinetic characteristics of the fully development biofilm varies in a FF diffusion process (Jenkins & Sanders, 2012). According to Jenkins and Sanders (2012, p. 3), "the advantages of a FF process includes reduced operating and energy costs, smaller reactor volumes, minimized need for settling capacity, operational simplicity and reduced sludge." One design consideration that was taken into account for FF systems is excessive growth on the media. The excessive growth could clog the media system or cause free-floating media to sink (Jenkins & Sanders, 2012).

Integrated fixed film activated sludge. In order to improve the AS and FF systems, a modified system was designed, called the IFAS system, as depicted in Figure 3. The definition of an IFAS, according to Abu-Orf et al. (2014, p. 997), is:

> an integrated fixed film activated sludge (IFAS) process, or hybrid process, consists of an activated sludge system in which a material to support attached biomass growth has been added in addition to the suspended biomass growth in an activated sludge reactor.

Biomass consists of a variety of many different microorganisms. The addition of the biomass carriers, also referred to as media, increases the capacity of the treatment system within the aeration tank. Biomass carriers can be broken down into two categories: (1) the moving bed biofilm reactor (MBBR), and (2) fixed media AS system (Singh & Kazmi, 2016a). A MBBR carrier moves freely inside the reactor, whereas a fixed media is fixed inside the reactor (Singh & Kazmi, 2016a). The media for the system is usually synthetic and varies between systems.

IFAS systems are a common process for TN removal, using the biological nitrification-denitrification process, as part of the AS treatment processes. The IFAS systems can be used where space is limited for treatment (Abu-Orf et al., 2014). Biological nitrification-denitrification is the removal of nitrogen using microorganisms, similar to the traditional AS process. The media added in the system enhances the environment for nitrification to take place because of the increase in surface area for microbial growth (Sriwiriyarat et al., 2005). This design maintains effective treatment during organic and hydraulic shock loading (Singh & Kazmi, 2016a). IFAS systems usually require dissolved oxygen (DO) concentrations between 4 to 6 mg/L, which is slightly higher than other treatment systems (Abu-Orf et al., 2014). The higher DO concentration is needed to provide a sufficient aerobic environment to promote nitrification in the biofilm.

IFAS systems provide a larger treatment capacity at a smaller size compared to AS processes because the fixed media creates a more stable environment for nitrification. A disadvantage of the media is that some media must be removed when conducting maintenance on the diffusers, which may decrease the effectiveness of the biological process (Abu-Orf et al., 2014). One advantage is that, based on the DO conditions and loading rate of the system, IFAS systems allow a possible opportunity for simultaneous nitrification-denitrification. Simultaneous nitrification-denitrification is where both nitrification and denitrification happen within one zone (Abu-Orf et al., 2014). Nitrification can be completed through an addition of DO to the system. The addition of DO limits the TN removal, because in order for full denitrification to take place within the system, DO needs to be less than 0.2 mg/L (Urbini, Gavasci, & Viotti, 2015). However, the media allows simultaneous nitrification-denitrification, because DO is not able to reach the inner portion of the biofilm which creates an anoxic zone, thereby providing an

environment for denitrification (Downing & Nerenbery, 2008). Figure 5 demonstrates the zones within the biofilm.

Microbial inoculator generator. A MIG is a new and emerging design for biological rehabilitation of treatment systems (Knight Treatment Systems, 2014). One proprietary model of the MIG is patented under United States Patent # 7,658,851, which describes a simple, low-cost system for residential and small commercial designs that includes nutrient removal for nitrogen, sulfur and carbon waste (Nelson & Rawson, 2010). The standards for the invention were based on the typical secondary treatment standards for previous residential and small commercial projects, where the discharge limits from the system are 30 mg/L BOD and 30 mg/L suspended solids (Nelson & Rawson, 2010). A MIG is similar to the IFAS system in regard to the treatment process, but a MIG is designed to reduce the amount of sludge produced and to reduce the biomat layer in the leach fields associated with septic tank systems. The leach fields are where the filtering action of the soil removes any remaining suspended solids. The bacteria that are discharged with the treated water will help to unclog any biogrowth, or biomat layer, in the soil by consuming the organic material, which will help the soil filter the treated water (Septic Preservation Services, Inc., n.d.).

A typical concern for septic systems is the clogging of soil by the biomat layer in the leach field. The biomat layer can cause clogging, which can cause effluent to backup into the septic tank. The promotion of bioremediation within the soil is reached by growing aerobic and anaerobic bacteria that can grow and reproduce in suspended growth and fixed film treatment zones (Nelson & Rawson, 2010). The typical treatment system usually does not contain the necessary microorganism to perform at the level needed, so a periodic feed bag, or “tea bag” was added to the design. The tea bag consists of a microbial community to enhance the performance

of the system, which is based on United States Patent #5,531,898 (Wickham, 1996). The tea bag is located above the cylindrical unit, as seen in Figure 4.

The proprietary blend consists of a microbial community including: (1) an active enzyme mixture (amylase, lipase, protease, and cellulase), (2) active bacteria mixture (*Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus thuringiensis*, *Starkeya novella*, *Pseudomonas fluoresens*, and sulfur metabolizing bacteria), and (3) a nutrient source from mushroom compost with 10,000 mg/kg or less of chemical oxygen demand (COD) (Nelson & Rawson, 2010; Wickham, 1996). *Pseudomonas spp.* and *Bacillus ssp.* have been shown to stimulate the predation of the biomat and are known to be significantly involved in nitrification. Besides reducing the biomat, *Pseudomonas spp.* are known to be dominant denitrifiers and occur in both the nitrification and denitrification process. Within the *Pseudomonas spp.* and *Bacillus ssp.*, there are specific soil bacteria species (*Pseudomanas fluorescens*, *Bacillus subtilis* and *Bacillus licheniformis*), which are included in the tea bag, and which are superior degraders of carbon and nitrogen compounds. These bacteria are more aggressive than the standard forms occurring in humans (Nelson & Rawson, 2010). The combination of these microorganisms in the tea bag enhances the performance by reducing the organic solids without increasing the residence time for the treatment. The tea bag is located on a tube above the cylindrical unit for ease of re-inoculating. This location allows the microorganisms to grow, reproduce, and blend into the tank. The microorganisms are mixed into the tank by the air bubbles rising from the bottom of the cylindrical unit. The air bubbles allow for the microorganisms to slough within the unit as biofilm or suspend in the tank as suspended growth (Knight Treatment Systems, 2014).

The overall treatment of the MIG was designed to reduce the BOD, TSS, and TN (Nelson & Rawson, 2010; Wickham, 1996). The effluent results for the MIG, according to Knight Treatment System (2014), are as follows:

- Greater than 80% reduction in organic waste content (BOD)
- Greater than 80% reduction in TSS
- 50% or greater reduction in nitrogen

In order to use the system on a larger commercial project the nitrogen removal efficiency must be increased to 65% or greater or must be capable of reducing the TN concentration to below 10 mg/L (WDNR, 2017; WDNR, 2018).

Nitrogen Removal

Influent to domestic wastewater treatment systems is typically considered to contain up to 50 mg/L of TN, with up to 80% of that being in the NH_3 form. The combined total of organic nitrogen and NH3 in water is commonly expressed as total kjeldahl nitrogen (TKN) (WEF, 2009). TN removal is achieved by going through three stages known as ammonification, nitrification, and denitrification, as shown in Figure 6 (Abu-Orf et al., 2014). Ammonification is the initial stage in the process, referred to as bacteria decomposition and hydrolysis. The process transforms the organic nitrogenous compounds into inorganic forms, i.e. NH_3. The process is mainly driven by a wide variety of microorganisms including bacteria, actinomycetes, and fungi (Bitton, 2011). The nitrification stage converts NH_3 into NO_3^-. The process requires an aerobic environment, which involves having DO within the water. The DO in the water is used as an electron acceptor allowing the autotrophic bacteria to oxidize NH_3 into NO_2^- and then to NO_3^-. The denitrification stage converts NO_3^- to N_2. The process requires anoxic conditions where heterotrophic bacteria anaerobically convert the NO_3^- into N_2 (Urbini et al., 2015; Wen, Ren,

Wei, Li, Lin, & Chen, 2010). According to Wen et al. (2010) and Bitton (2011), the process can be represented as:

Ammonification	Nitrification	Denitrification
$\text{Proteins} \rightarrow \text{amino acids} \rightarrow NH_3$	$NH_3 \rightarrow NO_2^- \rightarrow NO_3^-$	$NO_3^- \rightarrow NO_2^- \rightarrow N_2$

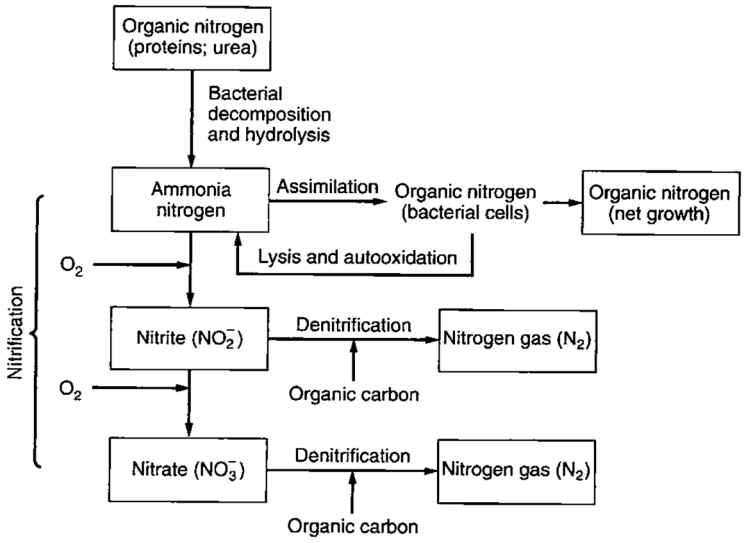

Figure 6. Nitrogen transformations in biological treatment process. Adapted from *Wastewater Engineering Treatment and Resource Recovery* by M. Abu-Orf et al., 2014, Boston: McGraw-Hill, p. 632.

Microbiology. Biological nutrient removal, specifically for TN removal, cannot be understood completely without an awareness of the microbiological processes occurring. When treating wastewater, there are multiple mixtures of microbial communities that need to be considered. These microbial communities can consist of bacteria, fungi, algae, protozoa, helminths, viruses, and other microscopic plants and animals (Abu-Orf et al., 2014).

The factors considered when designing a treatment system are linked to the environmental conditions that promote the specific microorganisms needed to meet regulatory discharge requirements. The environmental factors must be controlled to select for and promote the growth of microorganisms required for nitrogen removal in wastewater. Some of the main environmental factors for microorganisms are temperature, pH, and water chemistry of the wastewater. The optimal growth range for temperature and pH are narrow for specific microorganisms, but many can survive in a wide range of temperature and pH conditions. In regard to temperature, the growth rate will double for every increase of temperature by 10°C until the optimal temperature is reached (Abu-Orf et al., 2014). Most bacteria will grow well between 8° to 30°C, but the optimal range for nitrification is between 25° to 30°C, and for denitrification, it is between 20° to 30°C (Rungkitwatananukul, Nomai, Hirakata, Pungrasmi, Puprasert, Hatamoto, & Yamaguchi, 2016). The range for the pH of the environment in order for most bacteria to grow is between 4.0 and 9.5. A pH between 6.5 and 8.5 is considered optimal (Abu-Orf et al., 2014; Bitton, 2011).

Another factor is water chemistry, which involves the makeup of the water. This can affect the competition between microorganisms. Competition is usually over food that is available to the microorganisms, relating to carbon, nitrogen or oxygen substrates, which would cause some microorganisms to go dormant or active (Rungkitwatananukul et al., 2016; Wen et al., 2010). The ability of the microorganisms to grow in the different environments will influence the efficiency of the treatment process (Wen et al., 2010). These factors affect the selection, survival, and growth of the specific microorganisms needed to enhance treatment (Abu-Orf et al., 2014; Rungkitwatananukul et al., 2016; Wen et al., 2010).

Microorganisms that are important to the TN removal process are bacteria and protozoa. The main bacteria found in the biofilm and in suspension include autotrophic bacteria (nitrifying bacteria), heterotrophic bacteria (denitrifying bacteria), nitrite oxidizing bacteria (NOB) (*Nitrobacter* and *Nitrospira*), and *Proteobacteria* (Rungkitwatananukul et al., 2016; Wen et al., 2010; Zhang, Zhang, Peng, Han, & Gan, 2015). Typically, autotrophic bacteria nitrify, and heterotrophic bacteria denitrify (Wen et al., 2010). A specific species of bacteria is capable of performing both processes, *Thiosphaera pantotropha* (*T. pantotropha*). These bacteria perform heterotrophic nitrification and aerobic denitrification to produce N_2 (Wen et al., 2010). There are several other bacteria that have been found to heterotrophically nitrify and aerobically denitrify, besides *T. pantotropha*. *Paracoccus denitrificans*, *Alcaligenes faecalis*, *Comamonas sp.* and *Diaphorobacter sp.* have been found to act similarly (Wen et al., 2010). *Beta-* and *Gamma-proteobacteria* are dominant microorganisms in the role of NO_3^- reduction (Rungkitwatananukul et al., 2016). The dominant microorganisms are influenced by the change of COD:NO_3^- ratios. When the COD:NO_3^- ratio is low, *Acidovorax* and *Chlorobi* are abundant, but at high COD:NO_3^- ratios, *Spirochaetes*, *Rhizobium*, and *Zoogloea* are dominant (Rungkitwatananukul et al., 2016). Ciliated protozoa are utilized as a final clean for the water, as they feed on the suspended bacteria within the treatment system (Madoni, 2011; Singh, Kazmi, & Starkl, 2016b). The environmental factors that affect protozoan populations include changes in the operating conditions and DO, which can reduce their growth rates (Singh et al., 2016b).

Another form of nitrogen removal that does not use the three-stage process is the anaerobic ammonium oxidizing (anammox) process. The bacteria involved in this process are similar to the three-stage process but use anammox bacteria to chemoautotrophically oxidize NH_3 using NO_2^- to produce N_2 and NO_3^- (Zhang et al., 2015). The process operates best when

there is a low organic carbon source and low aeration. The dominant bacterial genes in the process are ammonium oxidizing bacteria (AOB) and anammox bacteria. The AOB are dominant in the AS portion of the system, whereas the anammox bacteria are dominant in the biofilm portion of the system (Zhang et al., 2015). AOB prefer to grow in flocculent sludge or small granular sludge where there is high oxygen and NH_3 concentration for the bacteria to use. Anammox bacteria prefer the inner portion of the biofilm. The biofilm has a biomass retention time that is sufficient for the bacteria and is resistant to DO as they prefer low O_2 concentrations (Zhang et al., 2015). During this process, the NOB is suppressed, because AOB and anammox bacteria dominate the system. The AOB and anammox bacteria's dominance over NOB result in an improved nitrogen removal efficiency (Zhang et al., 2015).

The design parameters that need to be taken into account in order to keep the above microorganisms in an optimal environment include solids retention time (SRT), biofilm thickness, DO concentrations, temperature, and pH (Abu-Orf et al., 2014; Bitton, 2011; Rungkitwatananukul et al., 2016; Singh et al., 2016b). The biofilms and SRT work hand-in-hand with each other. The media provide an increased surface area for the microorganisms to grow on, therefore increasing the amount of biomass contained in the treatment tank. The biomass being fixed instead of suspended biofilm helps prevent washout during hydraulic shock loads. This design helps with continuing the high treatment potential because of the increase in biomass (Singh et al., 2016b; Zhang et al., 2015). The increase in biomass may increase the SRT, because there is a higher number of solids forming in the system (Zhang et al., 2015). The growth on the biofilm creates layers of microorganisms, which create different microbiology and community dynamics (Bott, Regmi, Rutherford, Schafran, & Thomas, 2011). The biofilm growth concept is for the bacteria on the surface, where oxygen is abundant, to consist of AOB

or autotrophic bacteria that feed on the NH_3 in the influent water, which oxidizes the NH_3 to NO_2^-. The inner surface of the biofilm, where oxygen decreases as depth into the biofilm increases, then consists of anammox bacteria or heterotrophic bacteria, which use the NO_2^- that the AOB or autotrophic bacteria produce and chemoautotrophically oxidize NO_2^- to produce NO_3^-, and then N_2 (Wen et al., 2010; Zhang et al., 2015).

DO concentration is a parameter that will affect the performance of nitrogen-transforming microorganisms. AOB and NOB both require an aerobic environment, but AOB are able to perform in low DO environments, whereas NOB do not perform as well in low DO environments (Downing & Nerenburg, 2008). The DO levels play a major role in bacteria selection, as well as in pollution removal efficiencies, i.e., removal of BOD for treatment (Singh et al., 2016b). Therefore, the MIG system design accommodates these different parameters by including high surface areas to promote microbial growth and zones of appropriate O_2 concentrations. The increase in microorganisms will then enhance the treatment process in relation to nitrification and denitrification rates, as well as help to stabilize organic and hydraulic shock loads (Singh & Kazmi, 2016a).

Ammonification. The first stage in the three-stage nitrogen removal process is ammonification. Ammonification transforms organic nitrogenous compounds to inorganic forms, e.g., NH_3. A wide variety of bacteria, actinomycetes, and fungi microorganisms drive the process (Bitton, 2011). The amino portion of organic to inorganic nitrogenous compounds occur when proteins are converted by extracellular proteolytic enzymes to peptides and amino acids. Amino acids are then converted into NH_3 through deamination. Deamination is the process in which the amino portion of an amino acid is oxidized or reduced (Bitton, 2011).

Nitrification. Following ammonification in the nitrogen-removal process is nitrification. The nitrification stage is accomplished by utilizing two categories of microorganisms--known as AOB and NOB--to convert NH_3 to NO_3^- through an aerobic microbial process (Bitton, 2011). AOB utilize the NH_3, from the ammonification stage, and convert it into NO_2^-. AOB are a part of the *Beta-* and *Gamma-* subdivisions of the proteobacteria group. The dominant genus of the AOB category is *Nitrosomonas*, while other AOB in the system are *Nitrosospira*, *Nitrosococcus*, *Nitrosolobus*, and *Nitrosovibrio* (Bitton, 2011). This step is the limiting factor for nitrogen removal as AOB have a slow growth rate and poorly compete with heterotrophic bacteria. NOB utilize the NO_2^- AOB produces and convert the NO_2^- to NO_3^-. NOB belong to the *Alpha-proteobacteria* group and they complete the conversion autotrophically. The dominant NOB genus in the system is *Nitrospira*. Other chemolithotrophic NOB are *Nitrospina*, *Nitrospira*, and *Nitrococcus* (Bitton, 2011).

The nitrification process generates energy during the oxidation of NH_3 to NO_3^-. The microorganisms use the energy to convert CO_2, biocarbonate or carbonate into organic carbon (Bitton, 2011). Environmental factors that will affect the growth and metabolic activity of the process, along with the kinetics of nitrification, include reactor configuration, DO concentration, alkalinity, SRT, organic loading, hydraulic loading, and AS recycling rates (Azimi et al., 2007; Bitton, 2011; Noguera, Park, Reusser, & Whang, 2006). The nitrification process favors the presence of oxygen. Optimum operation of nitrification then requires a DO concentration typically around 2 mg/L or greater to achieve a complete and stable nitrification process (Noguera et al., 2006). Nitrification is often oxygen limited. To ensure high nitrification rates, the O_2/(NH_3 to NO_3^-) mass ratio should be maintained above four (Azimi et al., 2007; Bitton, 2011). Unfortunately, increased DO concentration will lower the effectiveness of the

denitrification stages. In addition, nitrification rates are less affected by changes in temperature, which may be attributed to greater oxygen solubility in colder environments, or the heat associated with the biofilm (Bott et al., 2011). As a result, IFAS systems are often selected in cold climates as a nitrogen removal process over AS (Bott et al., 2011; Di Trapani, Christensson, Torregrossa, Viviani, & Ødegaard, 2013). Alkalinity should be at a sufficient level to prevent the pH from increasing from 7.2 to 7.8 by neutralizing the hydrogen ions being produced during the oxidation process (Bitton, 2011).

AS systems, for an effective nitrification process, were designed for long SRT, which lowers the organic loading rate, also referred to as the food-to-microorganism ratio (F/M_r) (Weaver, n.d.). This ratio relates to a model completed by Baeza, Gabriel, and Lafuente (2004) showing that as the availability of NH_3 increases, the nitrification rate increases, until the optimal condition of NH_3 is reached and the nitrification rate becomes steady. This result can be seen in Figure 7. The three lines in the figure represent an internal recycle ratio which is based on the internal recycle flow (F_{IR}) to the inlet flow (F_I). The recycle flow is leaving an oxic zone to an anoxic zone in the system.

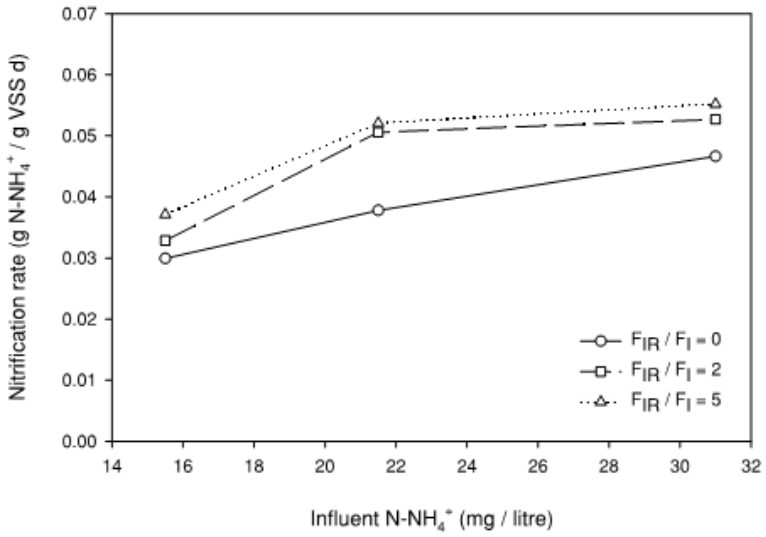

Figure 7. Specific nitrification rate in the oxic reactors versus influent N-NH4+. These results were obtained in an experiment with different internal recycle ratios (F_{IR}/F_I). Adapted from "Effect of Internal Recycle on the Nitrogen Removal Efficiency of an Anaerobic/Anoxic/Oxic (A2/O) Wastewater Treatment Plant (WWTP)," by J.A. Baeza et al., 2004, *Process Biochemistry, 39*(11), p 1620. http://dx.doi.org/10.1016/S0032-9592(03)00300-5

The same model by Baeza et al. (2004) was created as a 3D graph to show the internal recycle ratio aspect. Figure 8 presents the 3D model comparing the influent NH_3 and internal recycle (F_{IR}/F_I) to the nitrogen-removal rate. The ideal conditions to reach the highest nitrogen-removal rate is to have a high internal recycle and a high influent NH_3 concentration.

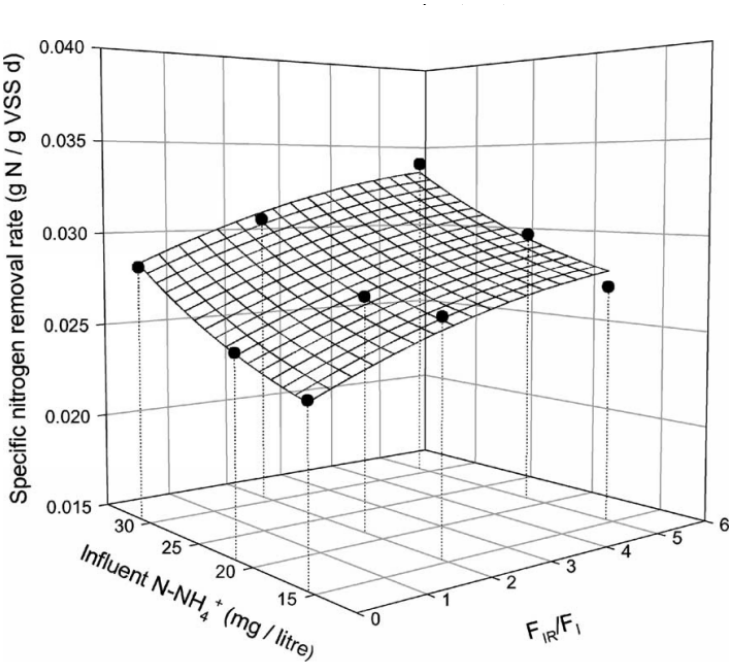

Figure 8. Nitrogen removal rate versus influent ammonium concentration and internal recycle ratio. These results were obtained in an experiment with different internal recycle ratios (FIR/FI). Adapted from "Effect of Internal Recycle on the Nitrogen Removal Efficiency of an Anaerobic/Anoxic/Oxic (A2/O) Wastewater Treatment Plant (WWTP)," by J.A. Baeza et al., 2004, *Process Biochemistry, 39*(11), p 1623. http://dx.doi.org/10.1016/S0032-9592(03)00300-5

Another impact on the nitrification stage involves the selection of the media. Media with high specific surface area will improve the nitrification rate. Azimi et al. (2007) indicated that this result appears to be caused by reducing competition between heterotrophic (COD elimination) and autotrophic (nitrification) organisms.

Denitrification. Denitrification typically follows the nitrification process. Denitrification is the reduction of NO_3^- to N_2 (Abu-Orf et al., 2014; Bitton, 2011). Microorganisms responsible for the denitrification process are heterotrophic or autotrophic microorganisms. When NO_3^- is present as an electron acceptor, the microorganisms can switch to anaerobic growth. There is a variety of physiological and taxonomic groups for denitrifiers which can use various energy

sources, e.g., organic, inorganic chemicals, or light, but the domains are with the Bacteria and Archaea groups. A few genera of denitrifying microorganisms are *Pseudomonas*, *Bacillus*, *Spirillum* and *Rhizobium* (Bitton, 2011).

The process is completed by NO_3^- being the electron acceptor and the soluble organic substrates are the electron donors (Abu-Orf et al., 2014; Bitton, 2011). A high soluble carbonaceous biochemical oxygen demand (CBOD) concentration is required for denitrification to occur. The high CBOD concentration creates an anoxic condition for denitrification to occur -- thereby enhancing TN removal to nearly 100 percent (Downing & Nerenberg, 2008; Weaver, n.d.). In order for the reduction to occur, the electron acceptor cannot be oxygen, as oxygen releases more free energy, making oxygen a more desirable reaction than NO_3^-. The environment required for denitrification to occur requires the absence of oxygen (Abu-Orf et al., 2014; Downing & Nerenberg, 2008; Weaver, n.d.). The N_2 produced escapes the system through gas bubbles, because it has low water solubility (Bitton, 2011). Some nitrogen is not completely converted to N_2 and is left as nitrous oxide (N_2O). N_2O gas is an air pollutant. The conditions which favor the incomplete reaction are low COD/NO_3, short SRT, and low pH (Bitton, 2011).

The most common biological denitrification designs for attached growth processes are characterized into three types: denitrification filter, suspended media denitrification, and fluidized bed denitrification (Abu-Orf et al., 2014). The typical environmental factors to enhance suspended media denitrification include temperatures between 20° to 30°C, pH between 7 and 9, and low oxidation reduction potential (ORP) values, along with low DO concentrations (Urbini et al., 2015; Rungkitwatananukul et al., 2016; Weaver, n.d.). Denitrification can occur within AS flocs or biofilms even at a high concentration of oxygen in the bulk liquid (Bitton, 2011).

The conventional AS treatment system uses a recycle stream for the biological nitrification-denitrification process. The recycle stream is required for the system to provide NO_3^- to create an anoxic unit with high CBOD and low DO concentration conditions for the denitrification stage (Downing & Nerenberg, 2008; Fu et al., 2009). The process flow diagram (PFD) for this process is similar to Figure 2. Experiments conducted on different recycle ratios have determined that as the recycle ratio increases, the denitrification efficiency also increases (Fu et al., 2009). Experiments conducted by Baeza et al. (2004) demonstrated 12.3% improvement when the recycle ratio, the internal recycle flow to influent flow, went from 0 to 5, which was caused by the NO_3^- loading rate increasing the denitrification rate. The internal recycle would then improve the TN removal rate of the system (Baeza et al., 2004).

Oxidation-Reduction Potential. A parameter used to measure the performance of nitrification and denitrification is oxidation-reduction potential (ORP) (Bishop & Li, 2002). ORP is defined by Bishop and Li (2002, p. 35) as "the electromotive force developed when oxidizers or reducers are present in aqueous solution." The higher the ORP, the more oxygen is available for certain microorganisms. The typical ORP value to show that nitrification is operating effectively should be greater than 100 millivolts (mV), whereas for the organic substrate, in the form of COD, ORP should have a value around 250 mV (Bishop, & Li, 2002; Prein, 2012). For denitrification, the typical ORP values to operate effectively need to be less than 50 mV (Prein, 2012; Weaver, n.d.).

Methods

In this project, the MIG was investigated to determine if the effluent TN concentration from the system would meet the WDNR groundwater discharge Permit (Permit). The Permit requirements were to be in accordance with WDNR (2017) Chapter NR 140: Groundwater

Quality, in order for the system to be used on larger commercial projects. Chapter NR 140 provides restrictions on TN concentrations that are allowed to be discharged to the groundwater from municipal wastewater effluent streams (WDNR, 2017). In order for the MIG to meet the Permit, the effluent stream's TN concentration must be reduced by 65% or greater or its TN concentration must be reduced to below 10 mg/L (WDNR, 2017; WDNR, 2018). In order to reduce the TN concentration, the system needs to reduce the NH_3, NO_3^-, and NO_2^- concentrations in the effluent. These nitrogen compounds are reduced through the system if the ammonification, nitrification, and denitrification processes are performing well. In order to investigate the MIG's performance, a pilot unit (Unit) was installed at the OWWTF. Two trials were completed to evaluate the performance of the MIG. The first trial (Trial 1) began October 12, 2017 and ended December 6, 2017. The second trial (Trial 2) began March 8, 2018 and ended April 5, 2018.

Description of Pilot Unit

The Unit was placed between the aerated grit chamber and the primary clarifiers. This location was selected because it had the closest values to raw wastewater before treatment took place and the distance to pipe the wastewater to the Unit was minimum. The site plan showing how the system was connected to the original OWWTF can be seen in Figure 9. The raw wastewater was pumped from the aerated grit chamber to the Trash Tank (influent stream) of the Unit. The Unit discharged the treated wastewater (effluent stream) by gravity to the primary clarifier. The effluent was discharged to the primary clarifier as a precaution if the quality of the effluent was not to regulations. The primary clarifier assured the water was treated to the quality it needed to be before discharging to the OWWTF outfall.

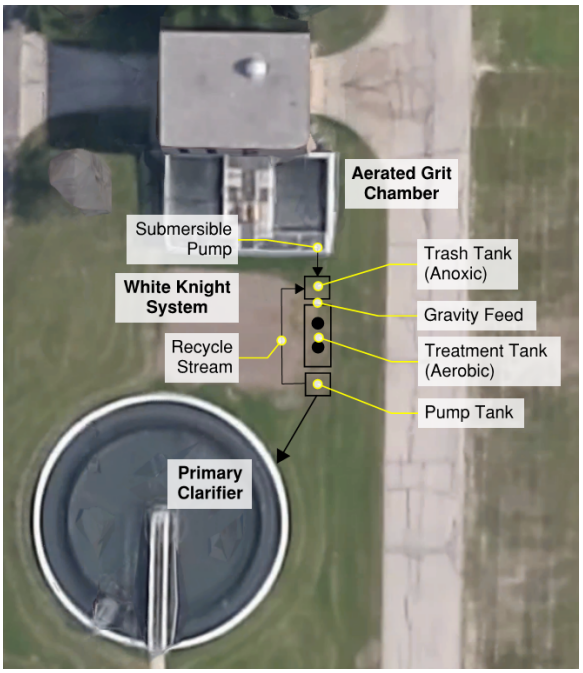

Figure 9. Pilot unit site plan.

The Unit contained three precast concrete tanks: an 800-gallon tank (Trash Tank), a 2,000-gallon tank (Treatment Tank), and an 800-gallon tank (Pump Tank). The influent pump was located in the OWWTF grit chamber and pumped into the Trash Tank. The wastewater flowed by gravity through the remainder of the system. A WK-78 unit was installed in the Treatment Tank. Model WK-78 is a MIG unit designed to have two columns with each receiving approximately 3.4 CFM of airflow through the columns (Knight Treatment Systems, Inc., 2008). The cross-section view of the WK-78 model is presented in Figure 10. The wastewater flowed through the inlet into the Treatment Tank. Air was introduced into the tank at the bottom of the MIGs. The wastewater was circulated in the tank by the air bubbles rising to the top of the tank. After the detention time, the treated water flowed through the effluent filter and into the treatment tank to be discharged.

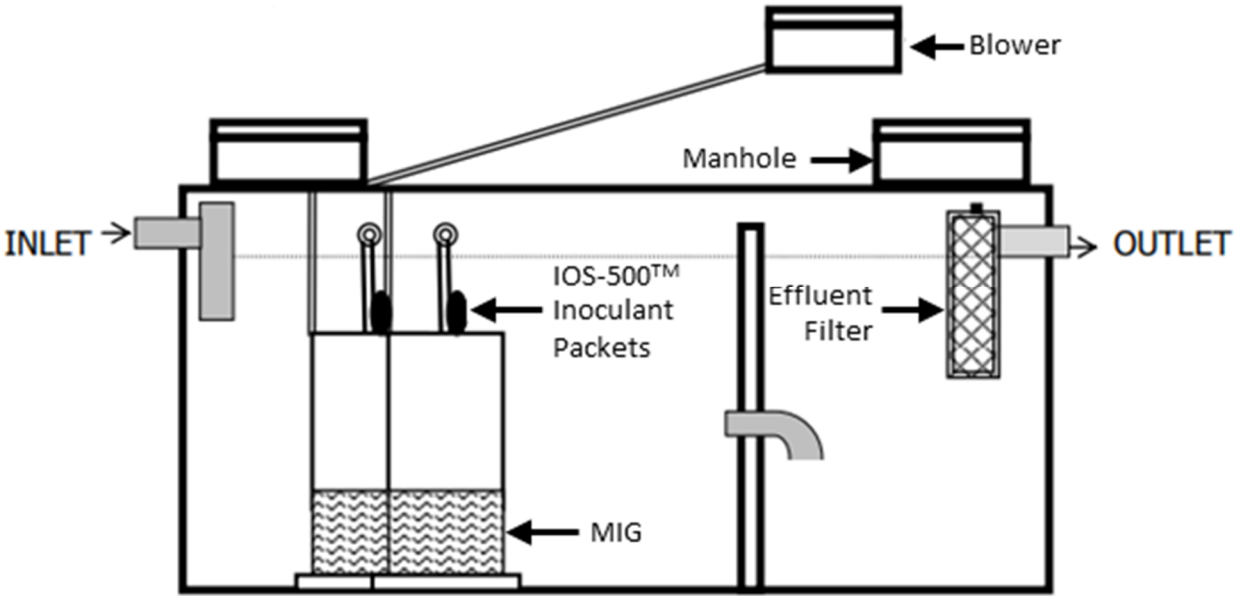

Figure 10. Cross-section of WK-78 treatment tank. Adapted from *Design, Installation, Operation & Service Manual* by Knight Treatment Systems, Inc., 2008, p.7. Retrieved from http://septicpreservation.com

The traditional design parameter for the WK-78 system was to allow for a detention time of 24 hours for the treatment section with the MIGs. This indicates a flow of less than 2,000 gallons per day (GPD) for the WK-78. Flow was initially set to 2,000 GPD using the traditional design parameters, and was later reduced by half. The system had 80% of the flow delivered over 12 hours to match the typical flow represented in residential or small commercial raw wastewater production. Once the wastewater was treated, the water moved to the Pump Tank. A Recycle Stream was pumped from the Pump Tank and discharged into the influent end of the Trash Tank to promote a more complete denitrification of the wastewater -- see Figure 11. Traditional WK-78 units are not installed with a recycle system. The water quality samples of the Recycle Stream were pulled from the Pump Tank. It was assumed that the effluent, pump tank, and recycle stream had the same parameters.

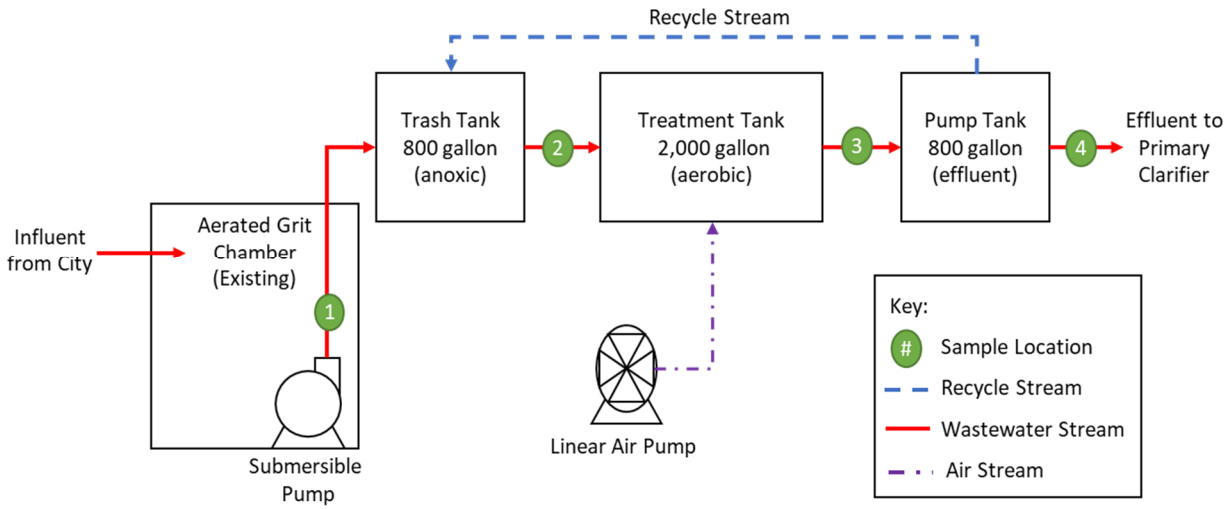

Figure 11. Process flow diagram (PFD) of pilot unit system.

Trial 1 had two pumps operating on timers. The flow rate of the influent feed pump was set up to present a diurnal flow, which would be similar to the daily flow a traditional system would treat. The feed pump was calibrated to approximately 5 gallons per minute (GPM). The feed pump was set to operate at every other 15-minute interval between 7 AM to 5 PM, and then the system operated every two hours for a 15-minute interval for the remainder of the day. This flow rate appeared to limit nitrification; therefore, the feed pump settings were changed to operate every hour for a 15-minute interval. The recycle pump was set to operate continuously at a flow rate of approximately 3 GPM. The aeration blower was set to operate continuously.

Trial 2 had a similar operation as Trial 1. The system continued with two pumps operating on timers and the feed pump following a diurnal flow. The change between Trial 1 and Trial 2 was the flow rate of the feed pump and the timing of the aeration blower. The flow rate was reduced to approximately 3 GPM and was increased to 3.5 GPM after a week. The purpose of starting at a lower flow rate was to create a higher detention time for the microbial

community to create biofilms and stabilize. The assumption in the first trial was the high flow rate in the beginning did not allow for the microbial community to create a stabilized biofilm. The aeration blower was changed from a continuous flow throughout the day to operating continuously from 8 AM to 6 PM, then turning off for 15-minutes every 2.5 hours. The reduction in flow from the feed pump at night served to increase the DO concentration in the tank overnight.

Data Collection

The inlet of the Trash and outlet of the Pump tanks were the measuring points for influent and effluent parameters, respectively. Mid-stream sample points were added between the Trash and Treatment Tank and the Treatment and the Pump Tank to measure the effect of denitrification and nitrification, respectively, within the system. The four sample points measured for the following parameters: flow rate, temperature, five-day biochemical oxygen demand (BOD_5), TKN, NH_3, NO_3^-, NO_2^-, pH, mixed liquor volatile suspended solids (MLVSS), TSS, and DO. The sample locations can be seen in Figure 11.

The data collection was completed by grab samples for Trial 1, which was collected three times per week during the collection period. The sample parameters were analyzed by the OWWTF laboratory technician and a laboratory company. In Trial 2, data were acquired through a hand-held probe (probe), which collected data four times per week during the collection period. In order to keep consistency with measurements for the probe, each sample was collected by having the probe submerged two feet and waiting a minute before recording the NH_3, NO_3^-, temperature, and DO concentration measurements from the probe. The probe was calibrated once a week or unless the probe was taking a long time to stabilize the measurements.

Project's Statistical Analysis Method

The samples were composited by mathematically averaging the three or four sample values per week, which resulted in weekly composited sample data sets for the data collection period. The weekly composited samples were used in a statistical analysis to see if the Unit met the Permit requirement. A statistical analysis investigated the H_0 that assumed the mean TN concentration was equal to or exceeded 10 mg/L, whereas the H_1 proposed that the mean TN concentration was less than 10 mg/L. The summation of NO_3^- and NH_3 represented the sample TN concentration. The acceptance of the alternative hypothesis, at a significance level of 1/60, would demonstrate that the Permit criterion was fulfilled. The significance level for a Type I error, or a false positive, is when the probability is less than 1/60. The false positive indicates that, if the test accepts the H_1 (that is, the TN concentration was less than 10 mg/L), then there is a chance of 1/60 or 1.67% that the true or actual result was equal to or exceeds 10 mg/L.

The significance level was set at 1/60, which means one month out of 60 months can feature a violation of the Permit limit of exceeding a 10 mg/L TN concentration. The Permit was based on a monthly average, which was usually set in place for five years before the Permit was renewed. The significance level was formed based on the Permit being in place for five years with a sample average taken monthly. Ideally, all 60 months of the samples would be below 10 mg/L, but a rare event, like an act of God, may cause a sample to violate the Permit. Therefore, the significance was set to allow one month to exceed the Permit limit.

The statistical technique that was used to test the H_0 and H_1 was composite sampling featuring Monte Carlo – a probability method – simulation. Monte Carlo was able to extend the data set to a larger population, which was used to see how often the Permit requirement for TN

concentration would be exceeded. The statistical technique is here presented with a numerical example to create a graph that can be used to see if the MIG will meet the Permit requirements.

Composite Sampling. Composite sampling is a data collection technique, which combines several individual samples into one homogenous sample (United States Environmental Protection Agency [U.S. EPA], 2002). The homogenous sample can be physically combined or the values can be averaged into a combined sample. The physically combined sampling technique was not used in this project. The composite sample instead consisted of an average of the individual sample values. Specifically, the composite sample for the statistical analysis consisted of three samples that were averaged as a weekly sample TN concentration. The three samples were analyzed in the laboratory separately, so the data were characterized as replicate individual samples compared to physical composite samples. Replicates have their individual values, which can be averaged, whereas a composite sample that is physically mixed will have one combined average value. By having replicates, the variance for the weekly average could be calculated, versus a physical composite sample with which the variance would be lost.

The composite sampling analysis was used to estimate where the mean of the data would need to be, given a range of standard deviations. The parameters of the mean and standard deviation were used to randomly generate data sets to compare the proportion of individual samples to the composited samples. A positive test would consist of a TN composited sample concentration that was below 10 mg/L. A negative test would consist of a TN composited sample concentration that was equal to or above 10 mg/L.

Numerical Demonstration. Composite samples were calculated with Microsoft Excel to determine the proportion that would result in a negative test. A negative test would occur when the TN composited sample average concentration was above 10 mg/L. Composite

sampling for the investigation focused on the proportion of negative test results in composited samples compared to individual samples. The analysis was based on the individual samples following a gamma distribution with a skew of 1.0. The distribution of the individual samples was based on the field-collected data for the WK system, which was characterized by the K-S test to follow a gamma distribution. The data had a skew of 0.77, but the sample size was small. Therefore, the skew was set at 1.0, as it was assumed that with more data, the skew would become right skewed. When the individual samples were composited, it was assumed the composited samples would follow normal distribution. The estimating of proportional composite sampling was modified from Garner, Stapanian, Yfantis and Williams (1989) and Gonwa (2017) for gamma distributions.

The number of individual samples (m) and the number of samples in a composite group (k) were set at 2,400 samples and 4 samples per group, respectively. The number of composited samples (n) would be equivalent to $n=m/k$, which was 600 composited samples (Garner et al., 1989). The investigation looked at the probability of a one-month violation in the 60-month life of the Permit. For the composite groups, this meant that out of 600 composited samples, an expected number of 10 could exceed the 10 mg/L Permit limit.

In order to test the relationship of the individual samples to composited samples, multiple data sets were randomly generated with different means (μ) and standard deviations (σ). The following variables were assumed:

- violation limit (V.L.): 10 mg/L
- individual population size (m): 60 samples
- composite population size (k): 4 individual samples in one composite sample
- significance level (α): 1/60

Assuming that the composited samples are normally distributed, Equation (1) was used to generate the required means for the individual samples in order to achieve the goal of an expected 10 mg/L exceedance in the composited samples with a probability of 1/60:

$$\mu = V.L. - t_{\alpha,m-1}\frac{\sigma}{\sqrt{k}} = 10 - |T.INV(0.0167,60-1)|\left(\frac{\sigma}{\sqrt{4}}\right) = 10 - 1.09\sigma. \quad (1)$$

Equation (1) resulted in an nonconservative model, as the composited samples do not have a normal distribution criterion and instead were skewed to the right. Because of the positive skew, the observed frequency of violation of composited samples exceeded the probability of 1/60. The model was modified to account for the skew by changing 1.09 in Equation (1) to 1.30, which yielded the desired probability of violation. The value of 1.30 came from multiple Monte Carlo simulations plugging in values from 1.00 to 1.50 to get the desired exceedances.

In order to show a better understanding of the relationship between the standard deviation and the mean, a graph of the standard deviation versus the mean is presented in Figure 12. The graph shows that the relationship between the two descriptive statistics, mean and standard deviation, is linear.

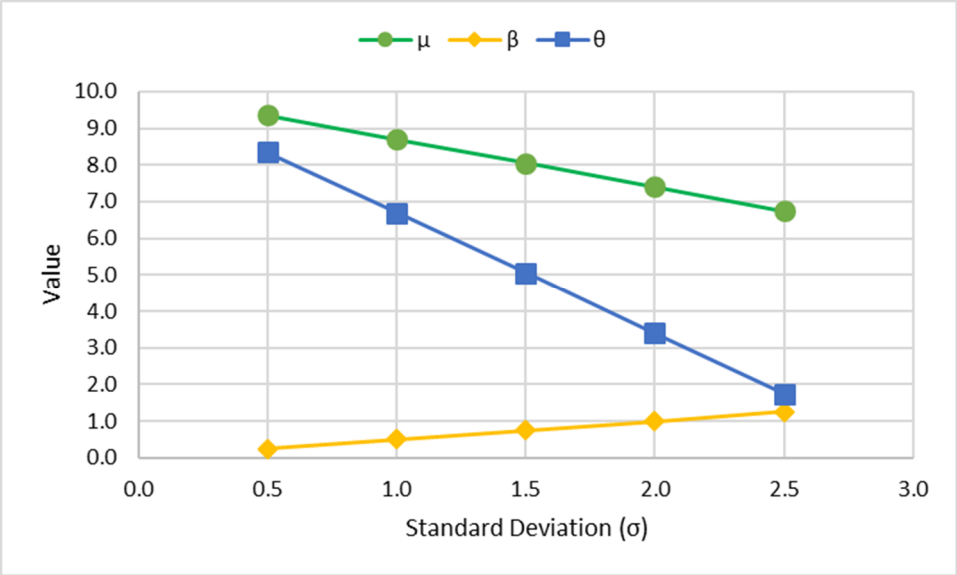

Figure 12. Individual standard deviation versus mean, scale, and threshold.

The individual samples were generated by using the three-parameter gamma distribution. The three-parameter gamma distribution parameters were determined using method of moments, which are shape (α_1), scale (β), and threshold (θ). The threshold presents the minimum value that can be achieved in the gamma distribution for each mean and standard deviation. The shape parameter was limited because the individual samples needed to have a skew (b) of 1.0. The shape parameter for a gamma distribution was given by Equation (2), which yielded a constant shape parameter of 4.0:

$$\alpha_1 = \left(\frac{2}{b}\right)^2. \tag{2}$$

Equation (3) and (4) were used to compute θ and β:

$$\theta = \mu - \sqrt{\alpha_1 \sigma^2} = (10 - 1.30\sigma) - \sqrt{4\sigma^2} = 10 - 3.30\sigma, \quad (3)$$

$$\beta = \frac{\sigma^2}{\mu - \theta} = \frac{\sigma^2}{(10 - 1.30\sigma) - (10 - 3.30\sigma)} = \frac{\sigma}{2}. \quad (4)$$

The method was repeated using Equations (1) through (4) for the following standard deviations: 0.5, 1.0, 1.5, 2.0 and 2.5. The gamma distribution parameters for the specified standard deviations and a skew of 1.0 are summarized in Table 1 and Figure 12.

Table 1

Parameters for Gamma Distribution

σ	μ	b	α_1	β	θ
0.5	9.350	1.0	4.00	0.250	8.350
1.0	8.700	1.0	4.00	0.500	6.700
1.5	8.050	1.0	4.00	0.750	5.050
2.0	7.400	1.0	4.00	1.000	3.400
2.5	6.750	1.0	4.00	1.250	1.750

The 2,400 individual samples of the three-parameter gamma distribution were generated based on the parameters listed in Table 1. Each data set was comprised of 600 composited samples with four individual samples in each composited sample. The descriptive statistics for the generated data sets can be seen in Table 2. The information from Table 2 shows that the composited samples have about half the skew of the individual samples, indicating that the distribution of composited samples is more symmetric than the distribution of individual

samples. The change in the distribution for the σ = 0.5 and σ = 2.5 simulations can be seen in Figure 13.

Table 2

Generated Data Set Descriptive Statistics

Variable		N	Mean	StDev	Variance	SE Mean	Minimum	Median	Maximum	Skewness
St Dev 0.5	Individual	2400	9.341	0.496	0.246	0.0101	8.467	9.253	12.109	1.10
	Composited	600	9.341	0.249	0.062	0.0102	8.776	9.318	10.156	0.45
St Dev 1.0	Individual	2400	8.692	0.993	0.986	0.0203	6.858	8.533	13.395	0.98
	Composited	600	8.692	0.501	0.251	0.0204	7.535	8.630	10.341	0.57
St Dev 1.5	Individual	2400	8.043	1.524	2.322	0.0311	5.243	7.792	15.162	0.98
	Composited	600	8.043	0.798	0.636	0.0326	6.140	7.976	10.979	0.61
St Dev 2.0	Individual	2400	7.407	2.004	4.014	0.0409	3.496	7.078	17.215	1.05
	Composited	600	7.407	0.983	0.966	0.0401	4.932	7.367	11.084	0.51
St Dev 2.5	Individual	2400	6.815	2.539	6.447	0.0518	1.917	6.367	21.357	1.10
	Composited	600	6.815	1.290	1.664	0.0527	3.083	6.750	11.608	0.52

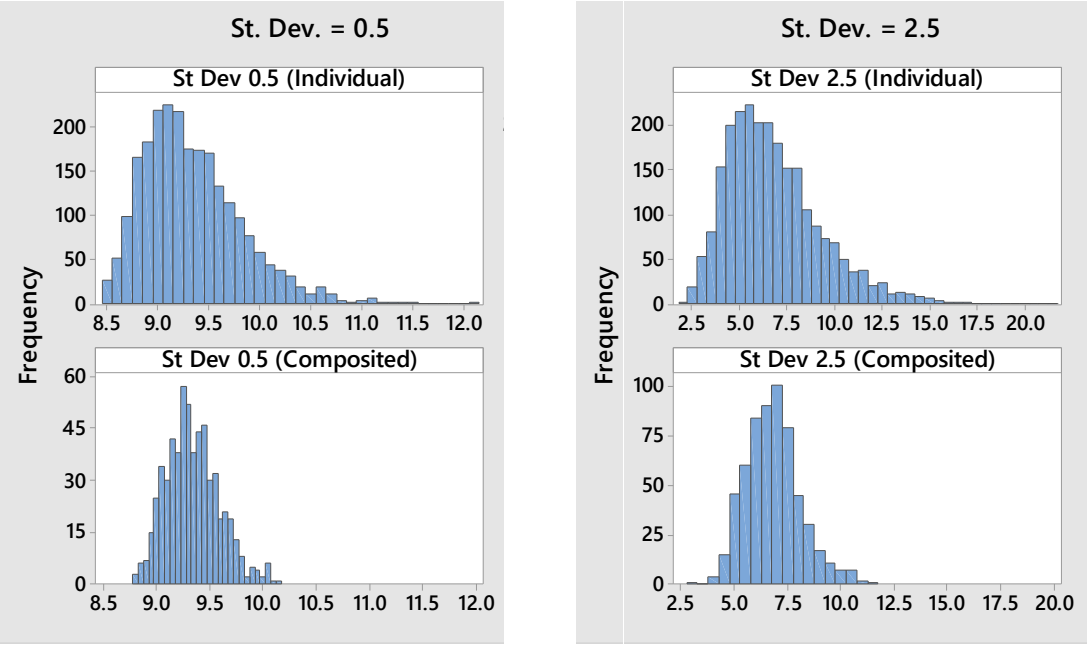

Figure 13. Histograms of the generated data set.

In each data set, the number of negative test results, where composited samples was greater than 10, were counted. The proportion for each data set was found by using Equation (5):

$$\hat{p} = \frac{no.of\ values \geq 10}{no.of\ values\ in\ data\ set} = \frac{COUNTIF(dataset,>=10)}{COUNT(dataset)} = \frac{228}{2400} = 0.095. \quad (5)$$

The proportions for the individual samples were compared to the proportions of the composites. Figure 14 represents the relationship between the proportions and the selected standard deviations in Table 1. The figure shows that the proportions for composites are smaller compared to the individual samples, which means the amount of negative test results are reduced. For example, when the mean is 9.34 with a standard deviation of 0.50, the individual samples have 228 negative test results, but the composites have nine negative test results. This changes the proportion of 0.095, which does not meet the significance level of 0.0167, to 0.015, which does meet the significance level. The relationship of proportion of negative results between the individual samples and the composites for each specified standard deviation can be seen in Figure 14. The figure shows that as the standard deviation increases, there is an increase in negative test results amongst individual samples, but when they are composited together, the proportion of the negative test results remain constant at about 1/60.

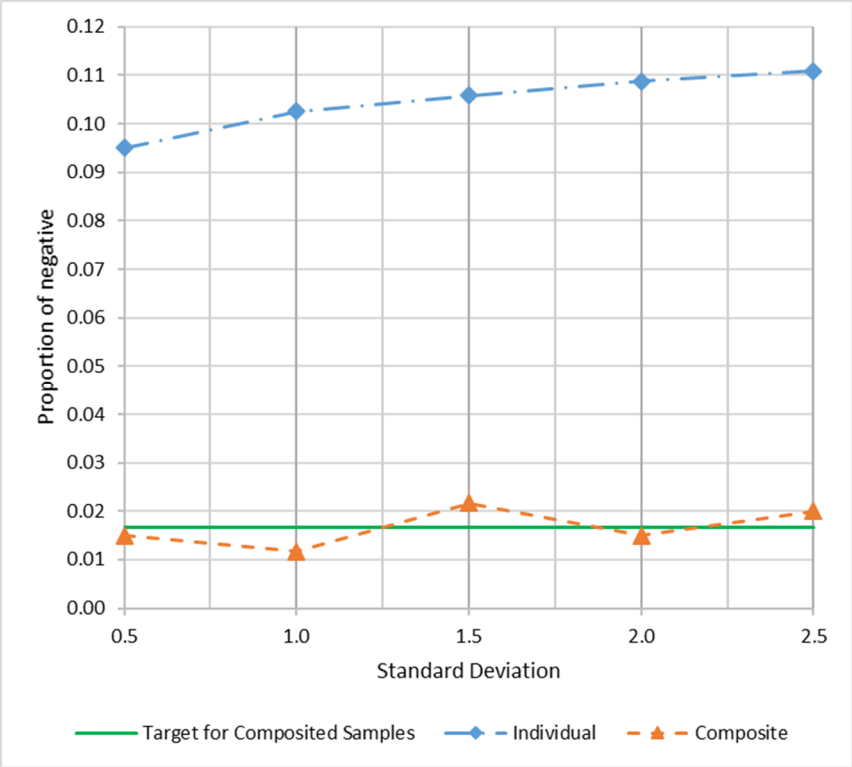

Figure 14. Observed probability of exceeding 10 mg/L.

Results

The wastewater characteristics for the OWWTF during the Trial 1 and Trial 2 pilot systems are provided in Table 3 and Table 4. The data collected for the two trials are in Appendix A and Appendix B.

Table 3

Trial 1 OWWTF Wastewater Characteristics

	TKN (mg/L)	Ammonia (NH_4^+ mg/L)	Nitrate (NO_3^- mg/L)	Alkalinity (mg $CaCO_3$/L)	BOD_5 (mg/L O_2)	TSS (mg/L)
Influent	43.8	32.9	1.7	459	204	257
Effluent	32.9	26.4	0.3	449	54	41

Note. Adapted from influent values from data collection. Average values from October 2017 to December 2017.

Table 4

Trial 2 OWWTF Wastewater Characteristics

	Ammonia (NH_4^+ mg/L)	Nitrate (NO_3^- mg/L)	TN (N mg/L)
Influent	22.2	9.3	31.5
Effluent	17.9	7.6	25.4

Note. Adapted from influent values from data collection. Average values from March 2018 to April 2018.

Pilot System Trial 1

The influent feed and recycle pump daily flow rates are shown in Figure 15 over the trial period. The daily flow rate for the feed pump started at approximately 2,500 GPD. The flow rate was reduced, because it seemed the retention time was too low to promote nitrification. The flow rate was reduced to approximately 1,400 GPD. During the middle of the trial period, the feed pump was inadvertently shut off for a day, which caused the recycle ratio to increase dramatically. The recycle ratios are presented in Figure 15.

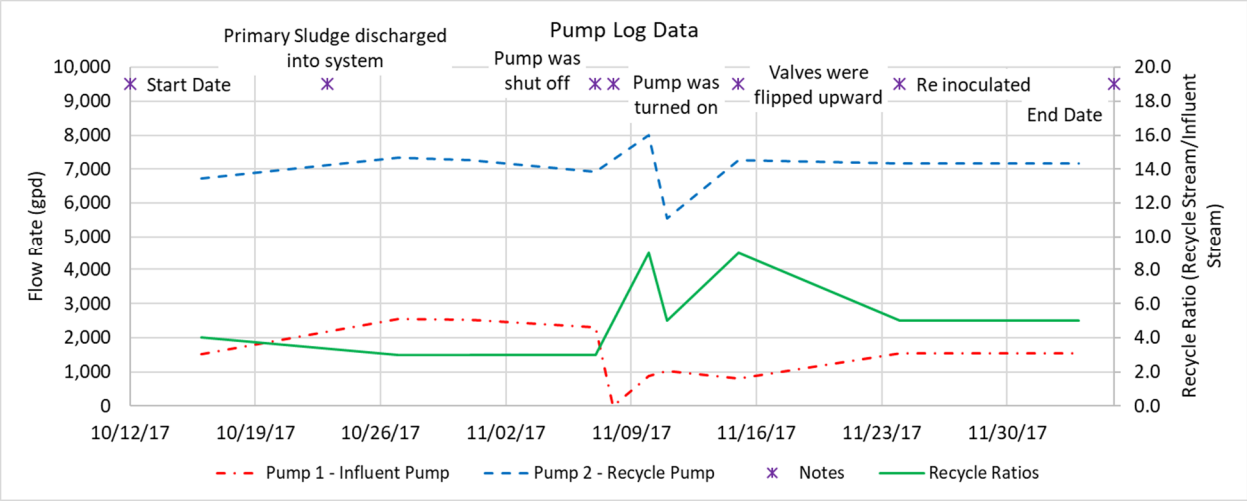

Figure 15. Trial 1 influent and recycle stream flow rates and recycle ratio.

In mid-November, the temperatures began to decrease below freezing at night. The system was shut down in the beginning of December as the temperature did not improve. The system was shut down as there was (1) the risk of pipes freezing because the system was above ground without heat trace, and (2) the microbial community was not reaching steady state and the cold environment was not promoting a stabilized state.

The influent and effluent data collected between the start and end data of the trial are presented in Figure 16 and Figure 17. The data collected during the experiment included the following parameters: TKN, NH_3, NO_3^- (NO_3^-/NO_2^-), and alkalinity. The TKN concentration was collected to compare to the NH_3 concentration entering the plant. Theoretically, TKN and NH_3 should be relatively close and in a consistent ratio. The two concentrations should be close, as it is assumed the organic nitrogen is converted into NH_3 following the ammonification process when the wastewater enters the treatment plant. In the beginning of the trial, there was a large difference between the TKN and NH_3 concentration, but over time, the difference began to decrease. The large organic concentration in the beginning was assumed to be caused by the

unsteady flow rate. Once the flow rates were steady, the difference decreased. The peaks in TKN seem to be linked to when the flow rates were changed.

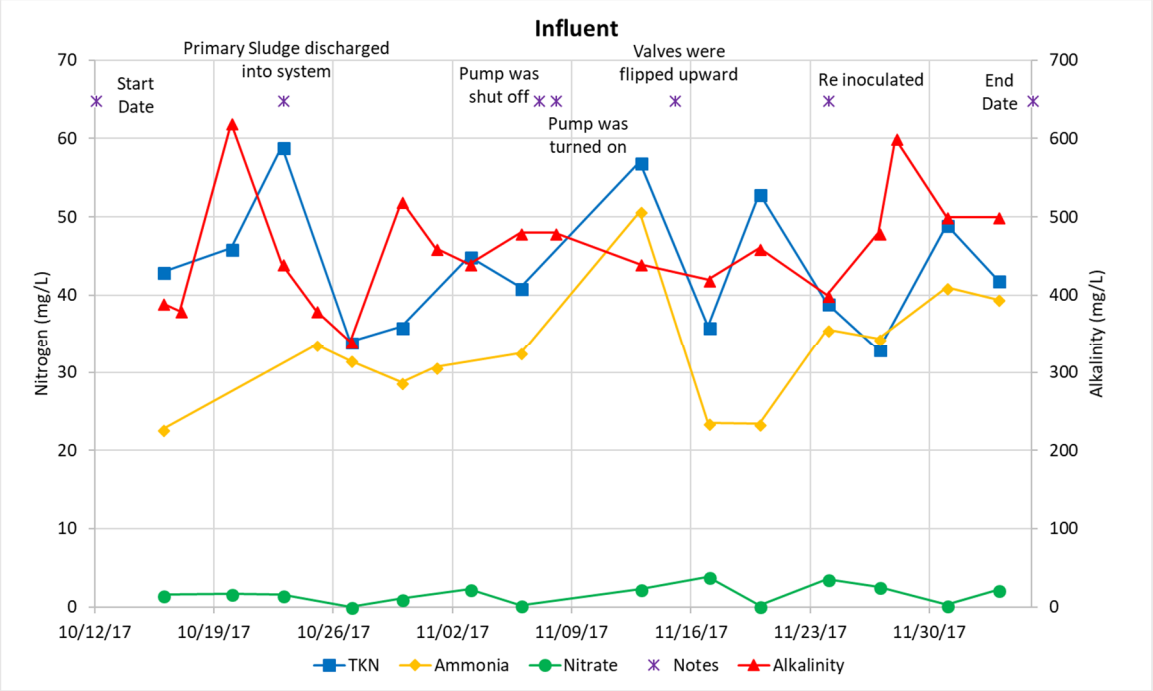

Figure 16. Trial 1 influent data collection.

Figure 16 presents peaks in the influent NH_3 and TKN concentrations on several occasions. As the wastewater proceeds through the MIG treatment process, the effluent NH_3 and TKN concentration, presented in Figure 17, were relatively constant, even though there were peaks in the influent. The consistency of the effluent concentrations suggests that the system was operating to its capacity with the current operating settings. The current settings indicated that the nitrogen removal process may be taking place, but one or more conditions required to increase the removal rate may be absent.

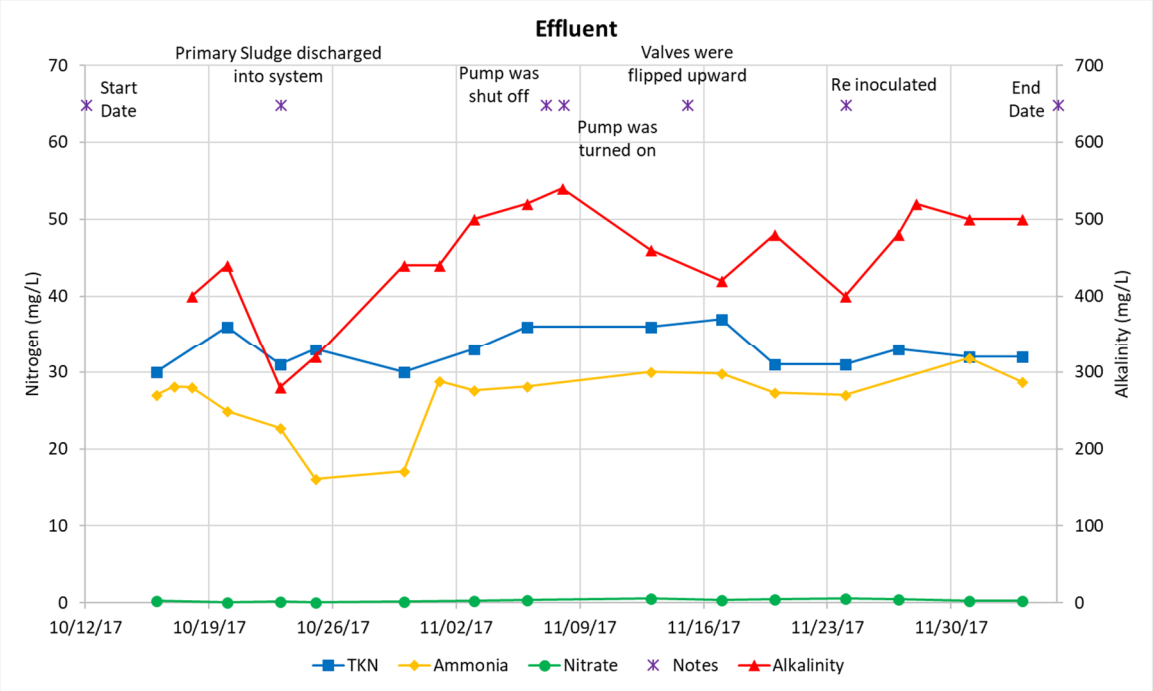

Figure 17. Trial 1 effluent data collection.

In the beginning of the trial, the ammonification process was not completely converting all the organic nitrogen to inorganic nitrogen (i.e., NH_3). A reduction in inorganic nitrogen prevents a complete reaction of nitrification and dentification. As the trial continued, ammonification appeared to improve. The next process considered was nitrification. Nitrification can be seen if the alkalinity concentration in the system decreases, as the process consumes alkalinity to convert NH_3 to NO_3^-. The comparison of Figure 16 and Figure 17 demonstrates a decrease in alkalinity in the beginning of the trial, but does not follow the same trend for the end of the trial.

The next process is dentification. Denitrification converts NO_3^- to N_2. The comparison of Figure 16 and Figure 17 demonstrates that a majority of the NO_3^- was reduced. It could be assumed that the denitrification process was complete. Based on the data collected in Figure 16

and Figure 17, it seemed the nitrification process was the limiting condition to achieve a higher nitrogen removal rate. The excess NH_3 in the effluent may have been a result of incomplete nitrification. The incomplete nitrification process could have resulted in excess NH_3 that was not converted to NO_3^-. The lower NO_3^- concentration could limit the denitrification process as there is only a low concentration of NO_3^- to convert into N_2.

Another consideration is that anammox or assimilation was taking place. Assimilation would be caused by an organism absorbing the NH_3 for nutrients. The influent NH_3 was being reduced when compared to the effluent NH_3. The decrease in NH_3 concentration should relate to an increase in the NO_3^- concentration, as nitrification would convert NH_3 to NO_3^-, but the NO_3^- concentration decreased. The decrease can be seen when comparing Figure 16 and Figure 17. For anammox to take place, the reaction of NH_3 and NO_2^- need to take place to produce N_2. Assuming this process was taking place, it could explain why NH_3 was being reduced at the rate desired, because the concentration of NO_2^- was too low. This shows that the NO_3^- concentration was the limiting factor for anammox to occur.

For Trial 1, the flux removal rates for TKN, NH_3, and NO_3^- were computed. The flux removal rates were computed using Equation (6). The variables in the equation are flow rate (Q_i), influent concentration (C_i), effluent concentration (C_e), and surface area of the MIG (A_{MIG}):

$$F_{Removal} = \frac{Q_i(C_i - C_e)(3.785)}{A_{MIG}x1000}, \tag{6}$$

$$= 1{,}524gpd(43 - 30)\frac{mg}{L}\left(\frac{1}{12.3m^2}\right)\left(\frac{1\,g}{1000mg}\right)\left(\frac{3.785L}{gal}\right),$$

$$= 6.1\frac{g}{m^2d}.$$

The flux removal rates are presented in Figure 18 to show how they changed over the trial period. The flux removal rates seem to have followed the change in flow rate. When the flow rate was high, the removal rates were high and vice versa. The NO_3^- and NH_3 removal rates also seem to have worked together, which suggests the possibility that anammox may be the process taking place over the traditional nitrification/denitrification process.

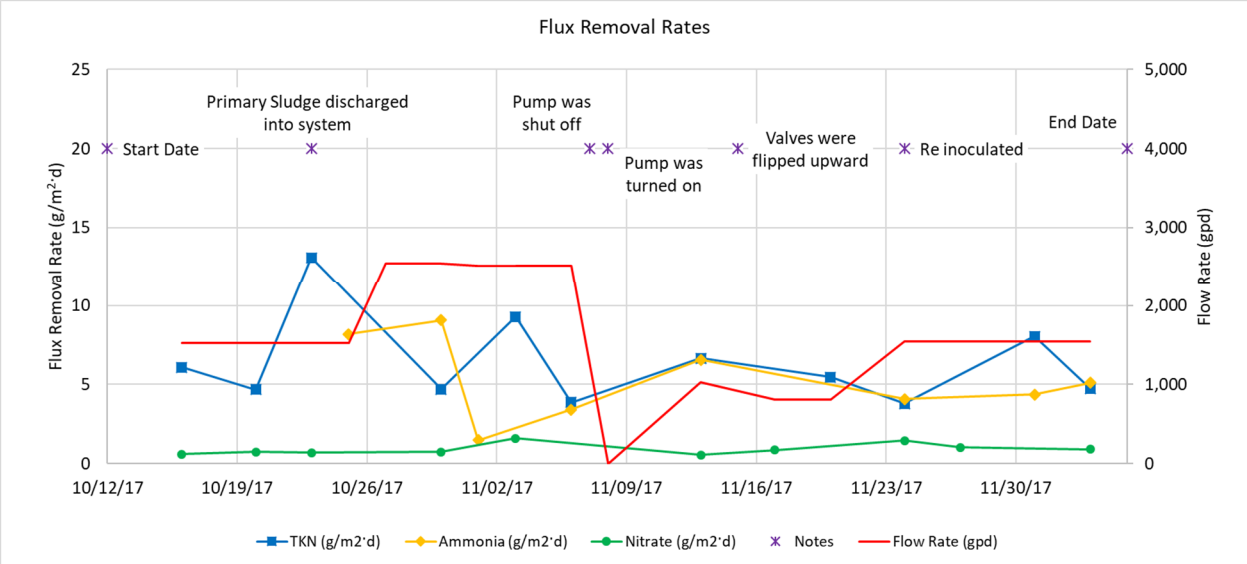

Figure 18. Trial 1 flux removal rates.

Pilot System Trial 2

For Trial 2, an attempt was made to provide a higher detention time for the system in the beginning to increase the microbial community. The flow rates began at 3 GPM at approximately 800 GPD. During the first week, the temperatures were still below freezing (32°F), which resulted in the influent pipe freezing. The flow rate was increased to 3.5 GPM at

approximately 1,200 GPD to try to prevent freezing by having a larger volume of water in the pipes. The influent feed and recycle pump daily flow rates are shown in Figure 19 over the trial period, along with the recycle ratios.

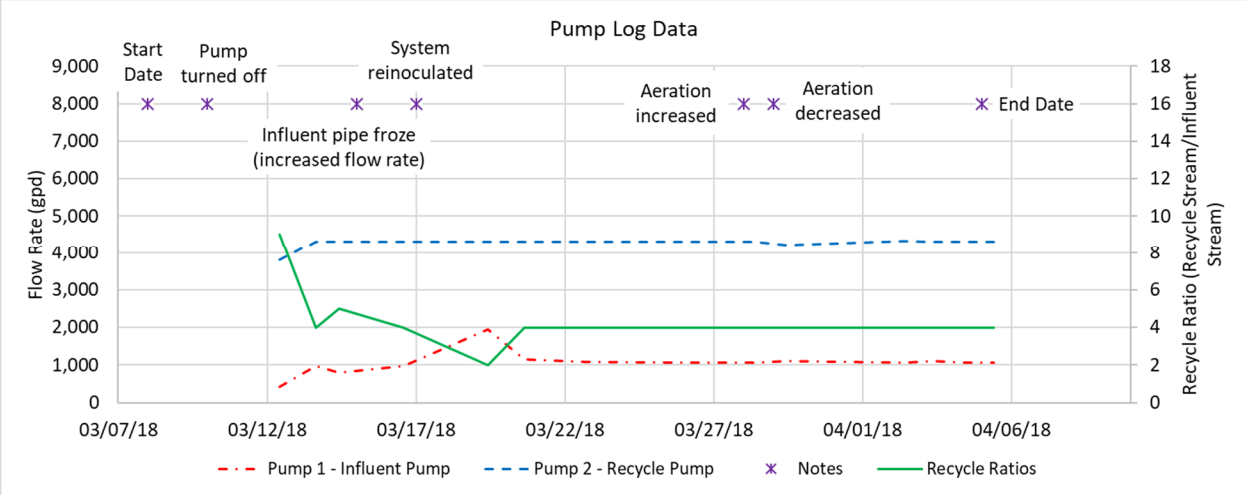

Figure 19. Trial 2 influent and recycle stream flow rates and recycle ratio.

After four weeks of data collection, the system was shut down. The data collected seemed to present a stabilized system. The effluent TN concentration discharged from the treatment system was consistent. The outside temperature was not increasing at the rate required to show a potential reduction in the TN concentration for the time period. An increase in temperature would assist the microbes to remove TN concentration at a higher rate.

The influent and effluent data collected during the time period are presented in Figure 20 and Figure 21. The measurement parameters to characterize the wastewater were different from Trial 1, because the method of collecting the data was different. The hand-held probe allowed

for data parameters to be known sooner. The parameters recorded were NH_3, NO_3^-, and DO concentrations. The TN concentration was the combination of NH_3 and NO_3^-.

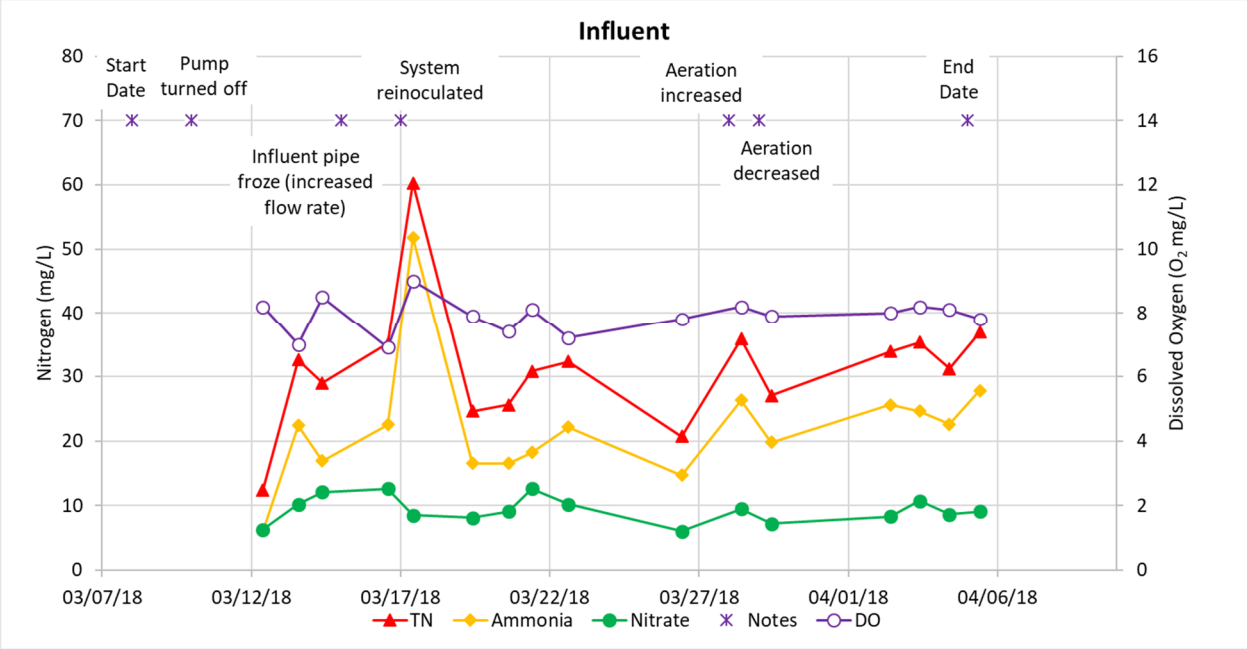

Figure 20. Trial 2 influent data collection.

The data collected during the first week suggested nitrification was taking place at the lower flow rate. Nitrification was suggested because the NH_3 concentration was reduced, and the NO_3^- concentration was increased. This change in concentrations suggested that the nitrification reaction was taking place. Another factor that could have led to the increase in nitrification was an increase in DO concentrations. The DO concentrations in the effluent were between 7.8 and 11.1 mg/L O_2. Once the DO concentration dropped to below 1 mg/L O_2, the data indicate that denitrification took over. In the third week of the trail, the Treatment Tank DO

concentration was low (approximately 1.5 mg/L O_2). The aeration rate was increased to achieve approximately 2 mg/L O_2 or slightly greater to promote nitrification in the tank. After the adjustment to the aeration rate, there was no evident improvement in TN concentration reduction. It was assumed the nitrification process was limited by available O_2 in the system. Once the nitrification was lost, it could not be recovered. The microorganisms are slow-growing and do not favor cold temperatures. The temperatures for the experiment were below freezing on a majority of the nights, which did not help to promote microbial growth.

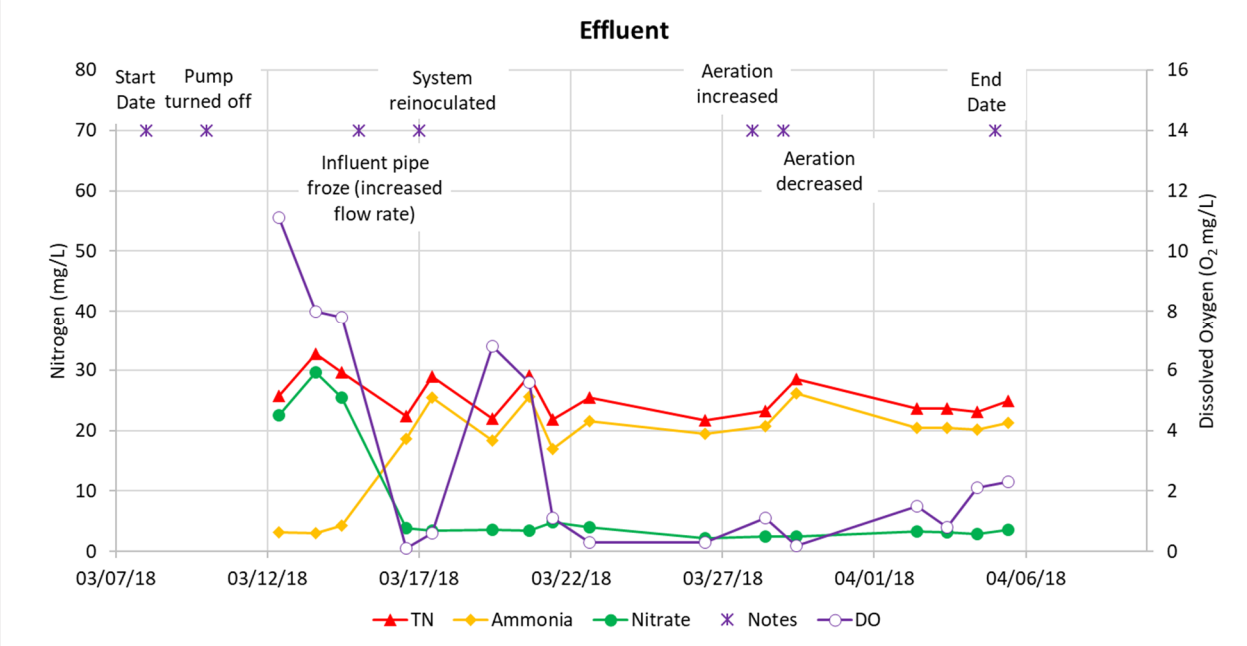

Figure 21. Trial 2 effluent data collection.

The flux removal rates were calculated for Trial 2 from Equation (6). The flux removal rates for Trial 2 are presented in Figure 22. When the system was reinoculated with the

proprietary blend of microorganisms, the removal rate of NH_3 increased. Throughout the collection period, the NO_3^- removal rate for Trial 2 was greater than Trial 1. The NH_3 removal rates in Trial 2 were half the removal rates in Trial 1, whereas the NO_3^- removal rates in Trial 2 doubled the removal rates in Trial 1.

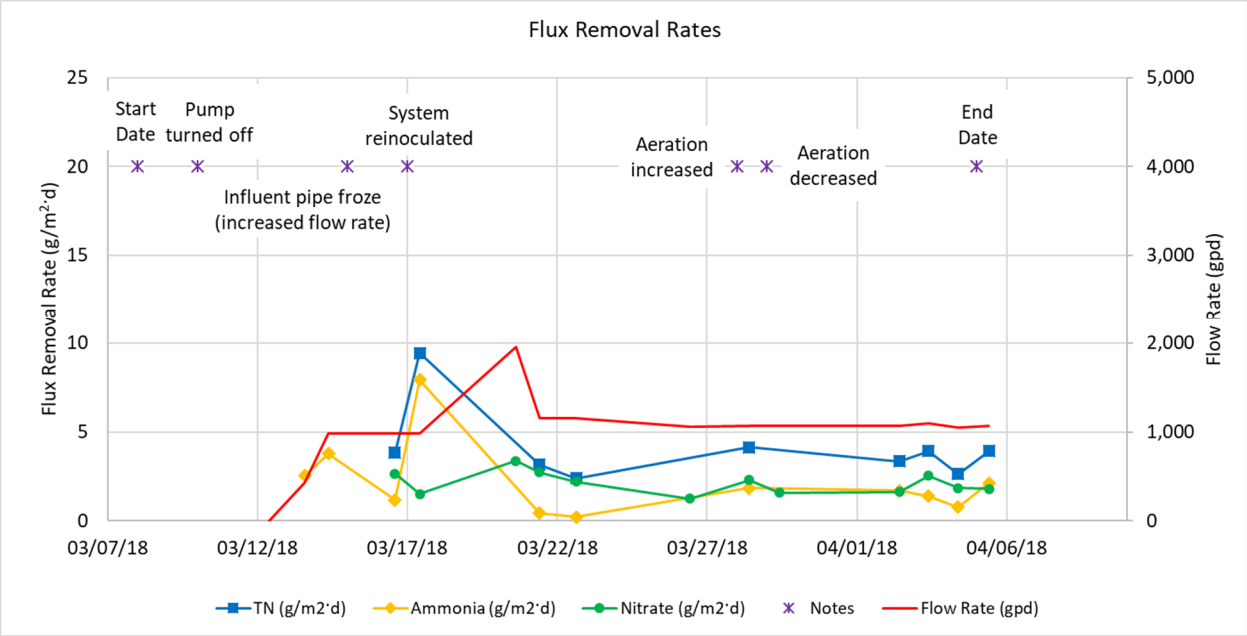

Figure 22. Trial 2 flux removal rates.

The removal percentages of TN, NH_3, and NO_3^- concentrations were calculated. Figure 23 presents the removal percentages in relation to the Permit limit of reducing 65 percent or greater of TN concentration throughout the system. NO_3^- was removed near the percentage required, but Figure 23 demonstrates the limiting process. The limiting process is nitrification. The NH_3 is not being completely converted to NO_3^- to reduce the TN concentration to 65 percent

or greater. At moments in the collection period, the concentration of NH_3 was increasing. This state of affairs suggests that there is an issue with the TKN. During this trial, TKN was not collected throughout the trial, but for two days. The data collected indicate that only half the TKN was being converted to NH_3. This result is evidence as to why NH_3 is not being reduced at the rate desired -- because TKN was continuously being converted to NH_3.

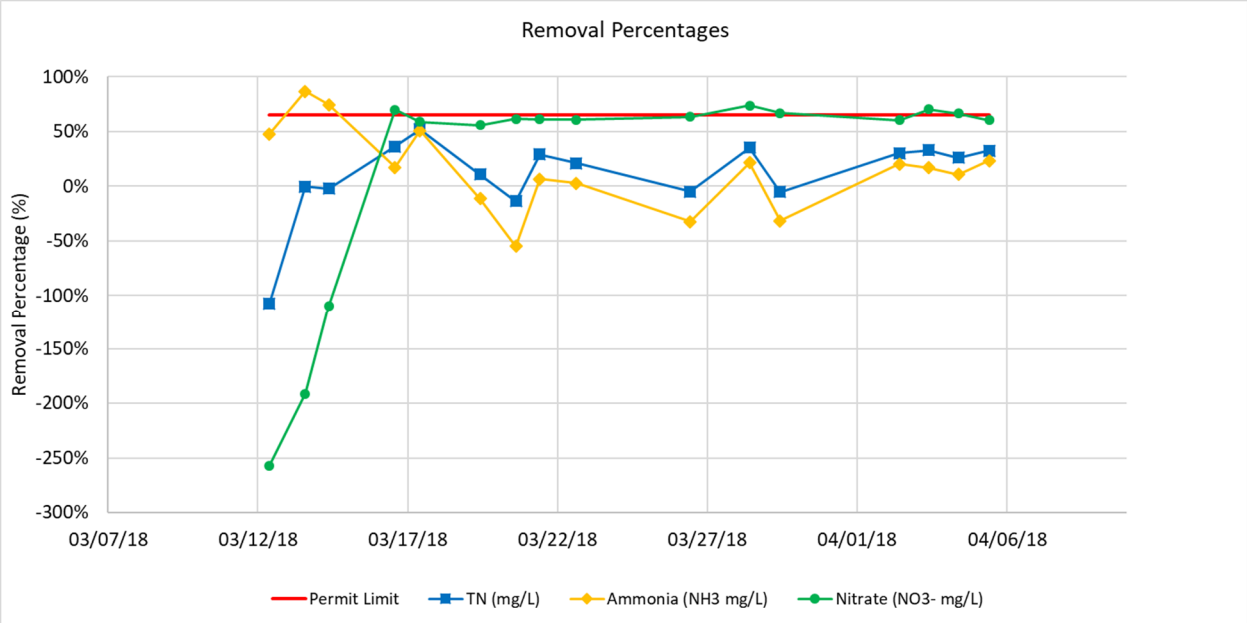

Figure 23. Trial 2 TN removal percentages.

Trial 1 and 2 Overall Data Analysis

The results presented above suggest the overall concern with the trials was that the NH_3 concentration was not reducing at the rate desired. For both trials, the temperature was near freezing (32°F), which does not promote the growth of nitrifiers to reduce NH_3 to NO_3^-. Trial 2 featured a limitation in the DO concentration, which presents another factor that does not

promote the growth of nitrifiers. The two factors indicate the kinetics of the nitrification process were being negatively affect.

According to Abu-Orf et al. (2014, p. 625), Equation (7) addresses the variables that can affect the kinetics of the nitrification process in a complete mixed activated sludge (CMAS) treatment system. This is not the same as the pilot system, but the kinetics between the pilot system and the CMAS are assumed to be similar. Equation (7) can be employed to determine the kinetics of the nitrification process based on the NH_3 concentration (S_{NH}):

$$S_{NH} = \frac{K_{NH}[1 + b_{AOB}(SRT)]}{SRT\left(\mu_{max,AOB,DO} - b_{AOB}\right) - 1.0}. \quad (7)$$

The variables that affect the S_{NH} are the SRT, specific endogenous decay rate of AOB (b_{AOB}), half-velocity coefficient for NH_3 (K_{NH}), and maximum specific growth rate of AOB correct for DO concentration ($\mu_{max,AOB,DO}$). The specific growth rate of AOB (μ_{AOB}) is based on the DO concentration (S_o), S_{NH}, K_{NH}, and half-velocity coefficient for DO for AOB ($K_{o,AOB}$), which is presented in Equation (8) (Abu-Orf et al., 2014, p. 624):

$$\mu_{AOB} = \mu_{max,AOB}\left(\frac{S_{NH}}{S_{NH} + K_{NH}}\right)\left(\frac{S_o}{S_o + K_{o,AOB}}\right) - b_{AOB}. \quad (8)$$

Equation (7) and (8) feature the main factors that were affecting the nitrification rate. These factors include SRT, temperature, and DO concentration. Equation (7) suggests that the SRT for the treatment system was not long enough, the media in the system was not enough, or the temperature of the environment was too low for the rate of reduction needed. All three of these factors affect the growth rate of AOB in the system to convert NH_3 to NO_3^-. When the

flow rate was reduced from 5 GPM in Trial 1 to 3.5 GPM in Trial 2, the effluent concentration of NH_3 was reduced from 26.4 mg/L to 17.9 mg/L. The temperatures were approximately the same during both periods and the media was the same. This result suggests that if the temperatures were higher, and if the SRT would have stayed the same as in Trial 2, the concentration of NH_3 could have been reduced to desired levels. There are other considerations as to why the growth rate was below desired levels. For example, the concentration of DO could be too low, which would affect the growth rate as presented in Equation (8).

Statistical Analysis

The descriptive statistics needed for the statistical investigation were the μ and σ of the individual samples. The descriptive statistics are presented in Table 5. Individual samples had a standard deviation greater than the statistical technique considered, but the analysis presented a linear relationship. Individual samples in Table 5 were compared to Figure 12 to see if the system would meet the Permit requirements. In order to meet the Permit requirements with a standard deviation of 3.37, the mean would need to be less than 6.0 mg/L TN. It is fairly obvious the mean TN concentration is greater than 6.0 mg/L TN, so the system does not meet the Permit requirements.

Table 5

Sample Descriptive Statistics of Field Samples

Sample Type	Individual	Composite
Sample Size (n)	16	4
Mean (y bar)	25.4	25.2
Standard Deviation (s)	3.37	1.82
Variance (s^2)	11.39	3.33
Error of the Mean ($s_{y\ bar}$)	0.84	0.91
Skewness (b)	0.77	1.79

Conclusion

Nitrogen compounds enter the environment through human activities, including domestic wastewater discharges into surface and groundwater (Azimi et al., 2007; Urbini et al., 2015). High concentrations affect plant life and the environment. The environment needs nutrients for the growth of algae and aquatic plants, but excess nutrients in water bodies result in a significant growth of algae (U.S. EPA, 2016; WEF, 2009). Significant algae growth is referred to as algae blooms, which in turn can be harmful to humans because the same algal species can produce elevated toxins and promote bacterial growth, which can result in illnesses for humans (U.S. EPA, 2016).

Domestic wastewater discharges nitrogen and phosphorus into the environment. Nitrogen and phosphorus are nutrients, which at high concentrations can cause nutrient-rich environments in water bodies. Nutrient-rich water bodies have an excess amount of NH_3 and carbonaceous waste, which allows the NH_3 to oxidize to NO_3^-. The high levels of NO_3^- can cause a toxic environment for aquatic life (Azimi et al., 2007). Therefore, nitrogen is one of the

nutrients regulated in domestic wastewater discharge to prevent possible harmful environmental effects.

Steps were made in the early 1970s to help improve water quality, including the development of attached growth biological denitrification. Over time, the processes continue to be improved to meet the more stringent nitrogen removal effluent discharge regulations (Abu-Orf et al., 2014). New processes include FF, IFAS, and MIG, all of which promote TN removal. TN removal involves two stages that are needed during the treatment process. The two stages are nitrification and denitrification, which both need different environments for the microorganisms to complete the process.

The MIG pilot system that was operated for the project described in this report presented factors that need to be improved in order to meet the stringent discharge limits for TN concentrations. The main environmental factors that affected the TN removal in the MIG was the DO concentration, temperature, and SRT. At the current design and operating conditions, the MIG was discharging TN concentrations between 25.4 and 26.7 mg/L. This discharge concentration of TN is significantly greater than the discharge Permit limit of 10 mg/L TN concentration. In order for the system to meet the discharge limits, the environmental temperature would need to be higher, the DO concentration would need to be increased, the SRT would need to be increased, and more media would need to be deployed.

Recommendations

Further experiments need to be conducted on the MIG to determine the design conditions that will promote simultaneous nitrification and denitrification to reduce TN concentrations to 10 mg/L in the effluent stream. Based on Trial 1 and 2, the recommendations for operating the MIG include warmer temperatures, increased SRT, and the deployment of extreme zones of DO

concentrations for the anoxic and aerobic zones in the system. The next trial should consist of moving the MIGs in the treatment tank to have both MIGs at the front end of the tank. This relocation should increase the DO concentration without increasing the air flow rate going into the tank. As the water progresses through the tank, the DO concentration should be reduced by nitrifiers. The effluent of the treatment tank should have low levels of DO concentration; therefore, the recycle stream would not be impacted by the higher DO concentrations in the front of the treatment tank. The reduced DO in the recycle stream would keep the trash tank in an anoxic zone. The extreme zones of DO should promote both nitrification and denitrification processes. During this trial, the flow rate would need to remain the same or reduced further to increase the SRT.

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Appendix A – Pilot System Trial 1 Data Collection

Table A.1

Influent Data Collection

Date	pH	DO (mg/L)	TKN (mg/L)	Ammonia (NH_4^+ mg/L)	Nitrate (NO_3^- mg/L)	Alkalinity (mg $CaCO_3$/L)	BOD_5 (mg/L O_2)	TSS (mg/L)
10/16/17	7.46		43	22.7	1.6	390	189	276
10/17/17		4.72				380		
10/18/17							175	588
10/20/17		4.28	46		1.7	620	258	230
10/23/17		3.38	59		1.6	440		210
10/25/17		3.36		33.6		380	195	180
10/27/17		0.89	34	31.5	0.076	340	177	260
10/30/17		0.96	36	28.7	1.1	520	199	212
11/01/17		0.39		30.7		460	177	164
11/03/17	7.9	2.64	45		2.3	440	238	194
11/06/17		0.49	41	32.5	0.27	480	239	428
11/08/17		3.97				480	249	302
11/13/17		6.68	57	50.7	2.3	440	319	250
11/17/17			36	23.5	3.9	420	145	130
11/20/17			53	23.4	0.22	460	110	198
11/24/17			39	35.6	3.6	400	166	
11/27/17			33	34.4	2.6	480		
11/28/17						600		
12/01/17		1.76	49	41	0.3	500	220	232
12/04/17			42	39.5	2.2	500		

Table A.2

Effluent Data Collection

Date	pH	DO (mg/L)	TKN (mg/L)	Ammonia (NH_4^+ mg/L)	Nitrate (NO_3^- mg/L)	Alkalinity (mg $CaCO_3$/L)	$CBOD_5$ (mg/L O_2)	TSS (mg/L)
10/13/17							30	
10/16/17	7.66		30	27	0.32		41	39
10/17/17		1.45		28.1				
10/18/17		1.18		28		400	156	124
10/20/17		0.7	36	24.9	0.12	440	67	94
10/23/17		2.63	31	22.7	0.15	280	66	32
10/25/17		2.27	33	16.1	0.095	320	56	62
10/30/17		3.26	30	17.1	0.17	440	34	42
11/01/17		3.66		28.8		440	35	42
11/03/17	7.96	6.55	33	27.6	0.25	500	29	14
11/06/17		1.79	36	28.1	0.34	520	48	20
11/08/17		6.24				540	30	10
11/13/17		6.68	36	30	0.6	460	39	18
11/17/17			37	29.8	0.43	420	84	54
11/20/17			31	27.3	0.45	480	46	20
11/24/17			31	27	0.55	400	54	26
11/27/17			33		0.45	480		
11/28/17						520		
12/01/17		3.24	32	31.8	0.3	500	53	22
12/04/17			32	28.7	0.29	500		

Table A.3

Pump Log Data Collection

	Pump 2 - Recycle Pump			Pump 1 - Feed Pump			Timer (minutes)		
Date	EC131 (cycle)	ETM129 (cycle)	Δ EC (starts)	EC135 (cycle)	ETM133 (cycle)	Δ EC (starts)	Right	Mid	Left
10/13/17	999814	50.53		999867	13.57				
10/16/17	999814	117.77	0	999920	28.81	53	1	435	1440
10/27/17	999815	386.55	1	1000251	122.22	331	1	435	1440
10/31/17	999815	483.33	0	1000369	155.84	118	1	435	1440
11/07/17	999815	644.81	0	1000559	209.80	190	1	270	1440
11/10/17	999816	724.86	1	1000585	215.76	26	1	270	1440
11/11/17	999816	743.32	0	1000597	219.20	12	1	270	1440
11/15/17	999817	840.20	1	1000637	229.98	40	1	270	1440
11/24/17	999817	1055.21	0	1000798	276.26	161	1	270	1440

Appendix B – Pilot System Trial 2 Data Collection

Table B.4

Influent Data Collection

Date	DO (mg/L)	Ammonia (NH_4^+ mg/L)	Nitrate (NO_3^- mg/L)	TN (N mg/L)	Temperature (°C)
03/12/18	8.2	6.1	6.3	12.4	10.1
03/13/18	7	22.4	10.2	32.6	10
03/14/18	8.5	16.9	12.1	29	10.1
03/16/18	6.9	22.5	12.6	35.1	10.1
03/17/18	9	51.8	8.5	60.3	10
03/19/18	7.9	16.5	8.1	24.6	10.5
03/20/18	7.4	16.5	9.1	25.6	10.2
03/21/18	8.1	18.2	12.6	30.8	10.3
03/22/18	7.2	22.1	10.2	32.3	10.6
03/26/18	7.8	14.7	6	20.7	10.5
03/28/18	8.2	26.3	9.5	35.8	10.5
03/29/18	7.9	19.8	7.2	27	10.5
04/02/18	8	25.6	8.3	33.9	10.5
04/03/18	8.2	24.6	10.7	35.3	10.7
04/04/18	8.1	22.6	8.6	31.2	10.6
04/05/18	7.8	27.8	9.1	36.9	10.7

Table B.5

Effluent Data Collection

Date	DO (mg/L)	Ammonia (NH_4^+ mg/L)	Nitrate (NO_3^- mg/L)	TN (N mg/L)	Temperature (°C)
03/12/18	11.1	3.2	22.5	25.7	7.1
03/13/18	8.0	3.0	29.7	32.7	8.4
03/14/18	7.8	4.3	25.4	29.7	8.3
03/16/18	0.1	18.6	3.8	22.4	9.5
03/17/18	0.6	25.5	3.5	29.0	9.6
03/19/18	6.8	18.4	3.6	22.0	11.0
03/20/18	5.6	25.6	3.5	29.1	10.1
03/21/18	1.1	17.0	4.9	21.9	9.8
03/22/18	0.3	21.5	4.0	25.5	10.2
03/26/18	0.3	19.5	2.2	21.7	9.9
03/28/18	1.1	20.7	2.5	23.2	10.5
03/29/18	0.2	26.1	2.4	28.5	11.1
04/02/18	1.5	20.4	3.3	23.7	9.9
04/03/18	0.8	20.5	3.2	23.7	10.2
04/04/18	2.1	20.2	2.9	23.1	9.8
04/05/18	2.3	21.3	3.6	24.9	9.6

Table B.6

Pump Log Data Collection

	Pump 2			Pump 1			Timer (minutes)		
Date	EC131 (cycle)	ETM129 (cycle)	Δ EC (starts)	EC135 (cycle)	ETM133 (cycle)	Δ EC (starts)	Left	Mid	Right
03/08/18	999820	1509.20		1001024	502.96				
03/12/18	999820	1602.41	0	1001063	513.36	39	1	270	1440
03/13/18	999820	1631.53	0	1001086	519.95	23		270	1440
03/14/18	999820	1650.18	0	1001098	523.37	12		270	1440
03/16/18	999820	1702.17	0	1001140	535.17	42		270	1440
03/19/18	999820	1770.52	0	1001183	561.67	36		270	1350
03/20/18	999820	1799.32	0	1001206	568.24	23		270	1350
03/22/18	999820	1847.61	0	1001242	578.70	36		270	1350
03/26/18	999820	1938.55	0	1001309	597.87	67		270	1350
03/28/18	999820	1986.10	0	1001344	607.93	35		270	1350
03/29/18	999820	2010.20	0	1001363	613.34	19		270	1350
04/02/18	999820	2106.08	0	1001434	633.49	71		270	1350
04/03/18	999820	2129.55	0	1001452	638.61	18		270	1350
04/04/18	999820	2153.41	0	1001469	643.59	17		270	1350
04/05/18	999820	2177.98	0	1001488	648.78	19		270	1350

Appendix C – Copyright Letter of Authorization

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March 5, 2018

Ms. Jill Vande Boom
C/O Milwaukee School of Engineering
1025 North Broadway
Milwaukee WI. 53202

Re: Letter of Authorization

To whom it may concern:

Ms. Jill Vande Boom, Master's Candidate at the Milwaukee School of Engineering, is duly authorized to make use of, reproduce and/or copy all relevant images and documents that are the property of Knight Treatment Systems Inc. with regard to the White Knight Microbial Inoculator Generator™ (MIG) for inclusion within her Master's Thesis Project.

Respectfully,

Mark C. Noga, President

Cc: D Nelson MSOE
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